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# Asian Journal of Andrology

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## The Proceedings of the Third Asia-Pacific Forum on Andrology

10–13 October 2009, Nanjing, China



Shanghai Institute of Materia Medica  
Chinese Academy of Sciences  
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## *Asian Journal of Andrology initiate its new logo*

**W**ith the abbreviation of Asian Journal of Andrology—"AJA" being the basic design element, a strong masculine body structure is created by traditional Chinese calligraphy. The man covering the blue globe symbolizes the consistent tenet of AJA—"Located in Asia, Facing the World." Asian Journal of Andrology, with this fresh image, is determined to create a brand new chapter in the history of international andrological research, to improve men's health and to promote scientific communication and cooperation among scientists and doctors in Asia, as well as in the world!

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**Andrology**

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The Proceedings  
of  
the Third Asia-Pacific Forum on Andrology

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Environment, Life Style & Genetic/Epigenetic Factors  
and Men's Health



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## Welcome Message from Chairman of 3APFA

Dear Colleagues,

On behalf of the Asian Journal of Andrology (AJA) and the Organizing Committee, I wish to extend to everyone with great concerns about men's health my cordial invitation to attend the Third Asia-Pacific Forum on Andrology (3APFA), in conjunction with the Tenth Anniversary Celebration of AJA, to be held on October 10-13, 2009, Nanjing, China.

The theme of this Forum is "Environment, Life Style & Genetic/Epigenetic Factors and Men's Health", endeavoring to explore the profundity and diversity of the contributing factors to men's health.

The Scientific Committee, under the guidance of the Advisory Committee, designed a comprehensive scientific programme concentrating on one vital issue and embracing a large range of important aspects including:

- 1 Men's Health: Global and Regional Perspectives
- 2 Sperm Biology and Fertilization
- 3 Male Infertility and Assisted Reproductive Technology
- 4 Male Sexual Dysfunction
- 5 Male Ageing, Androgen Deficiency and Therapy
- 6 Prostate Diseases and Prevention, Diagnosis & Treatment of Prostate Cancer
- 7 Environment Hazards to Men's Health
- 8 Prevention of Life-threatening STDs/HIV Infections
- 9 Prevention of Overpopulation and Male Contraception
- 10 Alternative/Traditional Medicine and Andrology

With an aim of bridging the communication between the lab and the clinic, we deeply believe that the Forum deserves the attention from not only basic scientists but also clinicians in the field of andrology.

Besides the traditional plenary lectures and symposia we have innovatively introduced in the programme "Panel Discussion on Holistic Concept on Men's Health, Health Determinants and Health Promotion" and "Public Consultation" sessions. "Panel Discussion" session allows an all-dimensional discussion among scientists, clinic doctors, public, media, and policy makers. "Public Consultation" session brings doctors out of the clinics to the public hall and allows the public to touch more knowledge on men's health in a relaxed and friendly atmosphere. In its Tenth Anniversary Celebration, AJA will express a great deal of gratitude to people who support and contribute to its development in various ways, and will review the past, summarize the present and shape the future.

In addition, there are several satellite workshops with topics on male infertility, erectile dysfunction, prostate disease, andrology in ARTs, LOH, *etc.* A specific session for WHO standard semen analysis manual (5th edition) will also be organized during the Forum.

We look forward to welcoming you to the 3APFA, Nanjing, China, which no doubt will be a memorable occasion for fruitful interchanges of ideas and the development of long-lasting friendship in the coming years.

With Best Regards,



Prof. Yi-Fei Wang  
Chairman of Third Asia-Pacific Forum on Andrology  
Executive President of Asian Society of Andrology  
Editor-in-Chief of Asian Journal of Andrology



## Welcome Message from Chairman of Local Organization Committee of 3APFA

Dear Colleagues:

On behalf of the Local Organizing Committee, I sincerely invite you to attend the Third Asia-Pacific Forum on Andrology (3APFA), in conjunction with the Tenth Anniversary Celebration of Asian Journal of Andrology, which will be held in Nanjing, China on October 10-13, 2009.

The theme of the forum is "Invironment, Lifestyle & Genetic/Epigenetic Factors and Men's Health". Ten topics covering hot issues in current research field of men's health have been set up and several internationally reputable experts in related fields will be invited to present their views on these aspects. During the Forum, several satellite meetings on andrological surgery, laboratory research of Assisted Reproductive Technology (ART) and other popular andrological aspects will take place and Chinese Session will be held particularly for Chinese andrologists. Since 2009 is the 10th Anniversary of Asian Journal of Andrology (AJA), the celebration ceremony will be held during the Forum. As the host, we are honored to take this opportunity to co-organize this forum with Shanghai Institute of Materia Medica, CAS and Shanghai Jiao Tong University. We do believe that this meeting will offer a platform for fruitful exchanges and communication among researchers all over the world.

Nanjing, locating at the foot of Zhongshan Mountain, near Yangtze River, is one of China's historical and cultural cities. October is the clear and crisp days of autumn in Nanjing, with the orange osmanthus blossoming with fragrance. During the meeting, the Local Organizing Committee will carefully arrange rich and colorful social activities, allowing you to enjoy the beautiful natural scenery and city culture landscape besides the academic exchanges. We believe Nanjing, as the ancient capital of the Six Dynasties, will give you an indelible impression.

The whole Local Organizing Committee of 3APFA are looking forward to meeting you in Nanjing, China!

With Best Regards,



Prof. Jia-Hao Sha  
Chairman of Local Organizing Committee of Third Asia-Pacific Forum on Andrology  
Director of Laboratory of Reproductive Medicine, Nanjing Medical University





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## Short CV of Keynote Speakers (In alphabetic order)



**Arif Adimoelja** is the Professor and Head of Study Centre—Services for Men's Health, Reproduction, Sex and Aging annex Andrology Clinic School of Medicine, Hang-tuah University/Naval Teaching Hospital Surabaya, Indonesia. He is Secretary General of Asian Society of Andrology (ASA), and President Elect of the XI AOFSS (Asian Oceanic Federation of Sexology). He serves on several editorial board of Andrology

Journals, IJA (*International Journal of Andrology*), AJA (*Asian Journal of Andrology*), *Andrologia* (First European Andrology Journal), *Indonesian Journal Andrology*. Written three handbooks for andrology and spermatology, with more than 342 prominent projects in International and National Journals in Medicine. His research interests include Andrology, Men's Health, Sexual Health, Reproductive Endocrinology, Early Aging Process Prevention, and Herbals to Phyto-pharmacy.



**Jens Peter Bonde** is the Medical doctor from Aarhus University 1979, PhD in Public Health Aarhus University 1991. He specializes in occupational medicine and public health in 1993, Medical Dissertation (University of Aarhus) on the impact of the occupational environment on male fertility in 1993. He has been head of the department of Occupational Medicine at Aarhus University Hospital.

He chaired the project management group of a European Concerted Action on Occupational Hazards to male reproductive capability 1993-1997 (The Asclepius project) and initiated and chaired a national prospective study on fertility in first pregnancy planners. He's research has from the mid 80'es mainly been devoted epidemiologic studies on environment and reproductive health. He has taken part in several international research projects and he has published some 250 original papers, reviews, editorials, book chapters and reports of which 80 during past 5 years. He is member of the editorial board of several international scientific journals and consultant of governmental committees.



**Arthur (Bud) Burnett** is the Director of the Basic Science Laboratory in Neurourology of the James Buchanan Brady Urological Institute and Director of the Male Consultation Clinic. He specializes in sexual medicine, major pelvic reconstruction, voiding dysfunction, female urology, and prostate cancer. Dr Burnett has served in multiple professional capacities with medical organizations

and advisory committees and now is the Treasurer of the Sexual Medicine Society of North America. He has written more than 150 original peer-review articles and 30 book chapters, along with numerous additional editorials and publications relating to his biomedical research and clinical activities. His work has appeared in many prominent journals such as *Science*, *Nature*

*Medicine*, *Proceedings of the National Academy of Sciences*, *Journal of Urology*, *Urology* and *Journal of Andrology*. Dr Burnett also holds journal editor appointments such as Co-Editor-in-Chief of the *Journal of Andrology*, Assistant Editor of the *Journal of Urology*, and Reviews Editor of the *Journal of Sexual Medicine*.

**Chawnschang Chang's** research focuses on the Androgen Receptor (AR). His pioneer cloning of human and rat Androgen Receptor (Science-1988) represents the landmark discovery in the androgen-AR field that allows Andrologists for the first time to monitor the AR status in androgen related diseases. Dr Chang made a great achievement in discovering of more than 100 AR coregulators and their function in the modulation of Androgen-AR functions in various diseases.

By knockout AR in individual cell of prostate, Dr Chang's lab then discovered AR could function as suppressor to CK5-basal intermediate epithelial cell, as survivor to CK-8 luminal epithelial cell and as proliferator to stromal cell. These findings of differential AR function in individual cells of prostate not only help to explain why androgen deprivation therapy via systematic suppression androgen would fail, it also help to develop new drugs to target AR in selective prostate cell to battle androgen related diseases including prostate cancer. Dr Chang published 300 papers in the AR-Urology field and trained more than 100 Ph.D. students/post Drs.; 60 of his trainees are now professors in various Universities. Dr Chang is the Regional Coordinator in North America of *Asian Journal of Andrology*.



**Trevor G. Cooper** is the Scientist, Head Andrology Laboratory, Centre of Reproductive Medicine and Andrology of the University, Münster, Germany; Advisory Board member of *Asian Journal of Andrology*. He is also the Organiser QuaDeGA (external quality control scheme for semen analysis). His main scientific interests include semen analysis, spermatogenesis, epidymis, male infertility. He made over 250 scientific contributions including 217 scientific papers, with 81 as the first author.



**David de Kretser** is a reproductive endocrinologist and was the Director of the Monash Institute of Medical Research in Melbourne. He is currently Governor of Victoria but retains a strong interest in andrology. Professor de Kretser initiated and directed Andrology Australia, the Federal Government's program in male reproductive health providing public and professional education and research in this field.



His research in the field of reproductive biology, infertility and

endocrinology is internationally recognised. His research led to the isolation and elucidation of the biology of the inhibin-related proteins in reproductive biology, inflammation and fibrosis and interactions with other TGF $\beta$  family proteins. He retains interests in the genetic causes of male infertility. Prof De Kretser is the Chairman of Advisory Board of *Asian Journal of Andrology*.



**Martin Dym** is a Professor in Department of Biochemistry and Molecular & Cellular Biology. He worked in National Institute of Child Health and Human Development, NIH and had been Research Fellow of Harvard Medical School and professor and chairman of Department of Cell Biology, Georgetown University. He was reviewer for NICHD and NIA Center Grants and Program Projects and reviewer

for funding agencies in Germany and England. He is interested in molecular biology aspects of testis development.



**Timothy M. M. Farley** is a Scientist with the World Health Organization Department of Reproductive Health and Research in Geneva. He currently focuses on HIV prevention and the interface with sexual and reproductive health, including male circumcision, microbicides to reduce the risk of HIV infection by women, and research on the prevention of mother-to-child transmission of HIV. Dr Farley has a PhD in statistics from the University

of Kent, United Kingdom. He worked as a statistician in international contraceptive research and development, and on the safety evaluation of contraceptive methods particularly for users in developing countries for over 20 years. As part of his work with WHO, Dr Farley has coordinated the Organization's work on the safety and effectiveness of contraceptive methods and the control of sexually transmitted and reproductive tract infections. He now concentrates on research and programmatic support for accelerating HIV prevention.



**Aleksander Giwercman** is working at Scanian at Malmö University Hospital since 1999. He was chairman of the Fertility Centre 2001-2006 and since 2007 of the Reproductive Medicine Centre at the same hospital. He is adjunct professor in andrology at the University of Lund and leader of research group in molecular reproductive medicine and main supervisor of 7 completed and 4 ongoing PhD theses.

His areas of research interest are gene-environment interaction and male reproductive function; cancer, cancer treatment and reproduction; clinical aspects of sperm DNA damage. He is the Advisory Board Member of *Asian Journal of Andrology*.

**Goh Hng Hang Victor** is the Professor of Medicine, Division of Endocrinology at UCLA and, concurrently the Director of the Core Laboratory of the General Clinical Research Center at LA BioMed, Research Institute. He graduated with a BSc(Hons). Dr Goh had served as a consultant to the World Health Organisation from 1980 to 2003 and had held many positions including Chairman of the Regional Advisory



Panel for Asia and the Pacific, Member of the Matched Reagent Global programme, and Rapporteur of the In Depth Review Panel of WHO. Dr Goh was also an Adjunct Principal Research Fellow at the Defence Medical and Environmental Research Institute of the Defence Science Organization (DMERI/DSO) as was Chairman of the Institution Review Board of DSO since 1996. Dr Goh has published and presented more than 480 articles in various fields. His current research interests include aging research in men and women, restoration of cyclicity in women with menstrual dysfunctions, research in shift work and circadian rhythm and the establishment of LCMSMS methods for small molecules. Prof Goh is the Editorial Board Member of *Asian Journal of Andrology*.

**Louis Gooren** is the emeritus Professor of endocrinology at the hospital of the Vrije Universiteit of Amsterdam, the Netherlands and is the Former head of Andrology Unit, Division of Endocrinology, Department of Internal Medicine. His scientific interests include the role of androgens/estrogens in 1) male health, particularly with regard to aging, 2) male sexual functioning, 3) disorders of sexual differentiation including transsexualism. He holds an honorary professorship in male health at Hang Tuah University, Surabaya, Indonesia. Prof Gooren is Dr Chang is the Regional Coordinator in Europe of *Asian Journal of Andrology*.



**J. Anton Grootegoed** is President of the International Society of Andrology and director of the Junior Science and the Master of Science Molecular Medicine programs. He had been full professor of biochemical endocrinology, and chair of the Department of Reproduction and Development at Erasmus University Medical Center. The research of his department is focused on gametogenesis and early embryonic development in mammalian species. Current studies address chromatin structure and gene expression in gametogenesis, X chromosome inactivation in embryonic development, and gene expression in stem cells. His interest in research related to development of new methods for contraception targeting the male was catalyzed by his



membership, 1986–1992, of the WHO Steering Committee of the Task Force on Methods for the Regulation of Male Fertility (Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization). Prof. Grootegoed is the Advisory Board Member of *Asian Journal of Andrology*.



**David Handelsman** is Australia's first professor of Andrology (1996 University of Sydney), Head of the first Andrology Department (Concord Hospital, 1999) and inaugural Professor/Director, ANZAC Research Institute (1998). After training in medicine (FRACP Endocrinology) and research (NHMRC & Wellcome Research Fellow) he worked in USA and Germany and served many research

and health policy bodies. He was awarded the RACP's Susman Prize (1994), the inaugural AMA Men's Health Award (2003) and Honorary Life Member, Endocrine Society of Australia (2008). Over 30 years, he has published 350 scientific papers, served 12 Editorial Boards and been reviewer for 97 scientific journals, supervised 17 PhD students and 10 other graduate students while maintaining continuous peer-reviewed and industry grant funding. Prof. Handelsman is the Deputy Editor-in-Chief of *Asian Journal of Andrology*.



**Tim Hargreave** is a Senior Fellow, Department of Clinical Sciences in Edinburgh University, Member Ethics Review Committee of World Health Organisation Geneva, Technical Adviser WHO UNAIDS HRP Male Circumcision Africa HIV prevention program and chairs Science and Ethics Review Group UNDP UNFPA World Bank WHO Special Program of Research. He is Editor of the WHO No Scalpel Vasectomy video featuring Prof Li. He Co-Authored the Manual

for the Standardised investigation of the Infertile couple WHO Geneva and the WHO Manual for male circumcision under local anaesthesia WHO Geneva. He gave the First report of Y Microdeletions as a cause of male infertility Clinician Myself. This work has been followed by more than 300 publications and testing for Y microdeletions is now part of the standard assessment in many clinics.



**Ralf Henkel** is the Deputy Chairperson and Research Coordinator, Department of Medical Biosciences, University of the Western Cape, Regional coordinator in Africa of *Asian Journal of Andrology*. His current research interests focus on the impact of reactive oxygen species on sperm functions, fertilization and pregnancy. Moreover, he is interested in the biochemistry of the outer dense fibres and their involvement in the generation of

motility as well as the investigation of traditional remedies in

andrology and their impact on male reproductive functions.

**Sae-Chul Kim** is a Professor of the Department of Urology at the Chung-Ang University College of Medicine, Seoul, Korea. Prof. Kim is Past President of the Korean Urological Association, The Korean Society of Andrology, The Korean Society of Fertility and Sterility, the Korean Society for Sexology, and the Asian Pacific Society of Sexual Medicine, and has actively involved as a Council member of the Urological Association of Asia. He chaired Local Organizing Committee of the 8th Congress of the International Society of Andrology. He is also Editor of the *Journal of Sexual Medicine* and the *Asian Journal of Andrology*, and Scientific Advisor of the *Adrologia*. His recent main research interests are male sexual dysfunction, testosterone deficiency and metabolic syndrome and lower urinary tract symptoms in male.

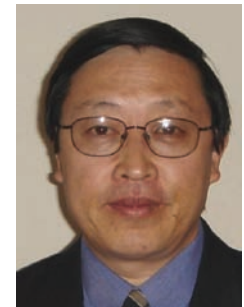


**Philip S. Li** is an Associate Research Professor of Urology, as well as Associate Research Professor of Reproductive Medicine; and Director of Microsurgical Research and Training Program at the Center for Male Reproductive Medicine and Microsurgery, Cornell Institute for Reproductive Medicine, and Department of Urology, at Weill Medical College of Cornell University.

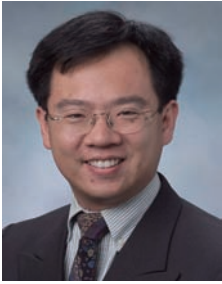


Dr Li is an internationally recognized expert in the no-scalpel vasectomy (NSV) and a leading researcher in microsurgery and male reproductive physiology. Since 2005, he has been served as an Honorary Professor of Urology and Reproductive Medicine at the Andrology Center of Peking (Beijing) University, West China Hospital of Sichuan University and the Renji Hospital of Shanghai Jiao Tong University. Dr Li has authored or co-authored 62 papers in peer-reviewed journals, books and book chapters on topics related to male reproductive medicine and microsurgery. He has presented over 74 abstracts on topics of male infertility and microsurgical techniques at national and international medical conferences.

**De-Yi Liu** is currently a Senior Research Fellow in Department of Obstetrics & Gynaecology, University of Melbourne, Australia. His main research interests are human sperm function, assisted reproductive techniques (ART) and male infertility. Over the last 20 years, he has developed various sperm function tests, particularly sperm-zona pellucida (ZP) binding, sperm-oolemma binding, ZP-induced acrosome reaction and sperm-ZP penetration for screening sperm defects causing male infertility. These new sperm tests are able to diagnose subtle sperm defects which can not be detected by conventional semen analysis. His research is now focused on



the study of the mechanism of causes of defective sperm-oocyte interaction in infertile men. He has published extensively in this field with over 70 scientific papers, 5 book chapters and over 100 abstracts presented at national and international conferences. He has been frequently invited as speaker to international symposia and workshops. He was an Associate Editor for *Human Reproduction* (2003-2006) and a current Editorial Board Member for the *Asian Journal of Andrology* and the *Reproduction and Contraception*. He is a regular reviewer for several reproductive medical journals.



**Peter Y. Liu** is NHMRC Career Development Fellow, Department of Andrology, Concord Hospital and ANZAC and Woolcock Institutes, University of Sydney. He received medical (1993) and doctoral (2003) degrees from the University of Sydney, and clinical and research training from Concord and Royal Prince Alfred Hospitals (Sydney), Mayo Clinic (Rochester, MN) and Harbor-UCLA

Medical Center (Los Angeles, CA). He has received numerous awards: Young Investigator Award, Endocrine Society of Australia (2004); NHMRC (Australia) Career Development Award (2007); NIH Trainee Merit Award, American Society of Andrology (2005); Outstanding Reviewer Award, Journal of Clinical Endocrinology and Metabolism (2007) and continuous personal or project funding from the NHMRC (Australia) since 1999. His research interests include male fertility and its regulation, the endocrinology of sleep and sleep disordered breathing, metabolic actions of androgens and gonadal axis physiology. Prof Liu is the Editorial Board Member for the *Asian Journal of Andrology*.



**Premendu P. Mathur** is the Professor of Department of Biochemistry & Molecular Biology and Co-ordinator, Centre for Excellence in Bioinformatics, Pondicherry University, India. He has served on the Minorities Affairs Committee of the Society for Study of Reproduction (SSR), and is currently serving on the Committee on Reproduction and the Environment of the SSR and is an elected member of the Executive Council of the Indian

Science Congress Association. He is Vice-Chairman of Scientific Committee of Third Asia-Pacific Forum on Andrology, China, 2009. His major research interests focus on male reproductive system. He has significantly contributed to the effects of natural and man-made compounds on male reproductive system. His other research areas include blood testis barrier and male contraception. He has published more than 85 articles in high impact journals and chapters in books. He has served as ad hoc reviewer for various journals like *Reproduction* (UK), *Journal of Andrology* (USA), *Journal of Urology* (USA), *Toxicology, Food and Chemical Toxicology, Reproductive Toxicology, Asian Journal of Andrology, Environmental Toxicology and Pharmacology, Regulatory Toxicology and Pharmacology, Toxicology Letters*. He is the Regional Coordinator in Asia of *Asian Journal of Andrology*.

**Robert McLachlan** is an NH&MRC Principal Research Fellow at Prince Henry's Institute and consultant endocrinologist at the Monash Medical Centre with interests in male reproductive medicine, especially in male fertility and androgen physiology. He is Consultant Andrologist to the Monash IVF program with research interests in the genetics of male infertility. He is a past President of the Fertility Society of Australia, and a consultant to the WHO on male fertility regulation. He is Director of the Andrology Australia, a Federal Government initiative committed to research and community & professional education in male reproductive health.



**Wolf-Bernhard Schill** is Professor emeritus, Center of Dermatology and Andrology, Justus Liebig University. He was Fellow in Reproductive Biology, University of Chicago, President of the German Society of Fertility and Sterility (1987-1991), President of the German Society of Andrology (1991-2001) and Committee member of the Bavarian AIDS Foundation. He is Editor-in-Chief of the international journal—*Andrologia* since 1995 and is co-editor of several other scientific journals. He is the Advisory Board Member for the *Asian Journal of Andrology*. He has 1 145 scientific contributions including original manuscripts, short communications, abstracts, review articles, book contributions, CD-ROM, and conducted supervision of 74 doctoral theses. He is interested in Andrology, reproductive biology, reproductive biochemistry and immunology, cryobiology, membrane biochemistry, male contraception, drug effects on potency and fertility, hormone substitution therapy in aging men.



**Suresh C. Sikka** works in Department of Urology, Tulane University Health Sciences Center. He is very active in Andrology research, participates in many teaching/training, fund raising, and administrative activities and was elected to Tulane University senate. He has been actively involved in many study sections for grant reviews and is the Principal Investigator or Co-investigator of many federal and privately funded research grants. He has established a comprehensive Standardized Semen Analysis Training Program with quality control management at Tulane University and since has trained more than 120 technicians all over USA and Canada. This program caters to the needs of many major pharmaceutical companies for their drug safety studies in the male reproduction area in a multicenter clinical trials format as per the requirement by FDA. He has authored



over 150 peer reviewed manuscripts, book chapters, and review articles, and over 270 abstracts. He has made many presentations and lectures at various National and International scientific and professional meetings. His current research interests focus on understanding the role of oxidative stress and aging as related to many Urological disorders (mainly erectile dysfunction, infertility and prostate inflammation/cancer); their early detection and prevention, and involve many state of the art proteomic and genomic technologies.



**B Mohd Ismail Bin Mohd Tambi** is consultant clinical andrologist from the Damai Service Hospital in Kuala Lumpur and chief consultant of the Specialist Reproductive Research Centre at the National Population and Family Development Board. Dr Mohd Ismail is an MBBS holder from Madurai, India, who joined the Malaysian medical service in 1979 as a houseman at the Johor Baru General Hospital. A stint as a

research and medical officer with the National Population in Family Development followed, and this was what caught the doctor's interest on the subject of men's health. He went on further to pursue clinical training in andrology, the science of men's health. Once he completed his training and became a certified andrologist, Dr Mohd Ismail returned to Malaysia to introduce men's family planning, as well as to branch out into fertility.



**Yi-Fei Wang** is the Chairman of the Third Asia-Pacific Forum on Andrology and the Editor-in-Chief of *Asian Journal of Andrology*. Prof. Wang is the senior advisor of president of Shanghai Jiaotong University. Nationally, he serves as the member of Scientific Discipline Review Group, Scientific Degree Evaluating Committee, State Council of China and member of National Strategic Advisory Committee

for Scientific and Technological Development, Ministry of Science and Technology, China. Internationally, he worked at Geneva, Switzerland as the Medical Officer of Department of Reproductive Health and Research, World Health Organization from 1995 to 2001. He won the award of La Medaille de Chevalier de la Legion D'Honneur by the Government of the Republic of France in 1995, and National Excellent Teacher of China in 1996 and 2004. Now he assumes many important duties in China, such as the editor-in-chief of *Asian Journal of Andrology*. His research interests include structure and function of gametes, capacitation and fertilization, reproductive immunology, infertility, reproductive medicine, family planning.

**Wei Yan** is a Associate Professor of Physiology, Molecular and Cellular Biology in Department of Physiology and Cell Biology, University of Nevada School of Medicine. Dr Yan's research is focused on Molecular Mechanisms of Germ Cell Development and Fertility. He has published over 60 peer-reviewed research articles on topics of reproductive biology since 1993, which include more than 20 papers published after he started his own lab. His work has been published in prestigious journals, including Nature Genetics, PNAS, Nucleic Acids Research, RNA, Molecular and Cellular Biology, Developmental Biology, *etc.* Dr Yan received the Nevada High Education System Regents' Rising Researcher Award and the SSR Young Investigator Award in 2009. He has served as an ad-hoc reviewer for more than 30 biomedical journals and the NIH study sections. Dr Yan has obtained two NIH grants and one pilot grant from the Sanford Center for Aging. His research focus on late spermiogenesis-specific genes is based upon his belief that malfunction of these genes is involved in many forms of sperm defects in idiopathic male infertility patients.



#### **Ching-Hei Yeung (Yang Jingxi)**

is a research scientist in the Centre of Reproductive Medicine and Andrology of the University Clinic, Muenster, Germany. She was Visiting Scientist in Beijing Family Planning Research Institute, and in Max Planck Society Clinical Research Unit for Reproductive Medicine, Muenster, Germany, and also in Institute of Reproductive Medicine of the University, Muenster, Germany. She has over 160 publications in international journals and as book chapters. She is Editorial Board member of *Asian Journal of Andrology* since 2005. Her research interests include Epididymis of mammals including primates and human, structure, function and cell biology, sperm maturation: interaction with the epididymis, physiology and cell biology, sperm analysis methodology and functional tests and post-testicular male contraception.



**Yong-Lian Zhang** is the Academician of Chinese Academy of Sciences, Head of Sex Hormones Action Laboratory, State Key Laboratory of Molecular Biology, Shanghai Institute of Biochemistry. She is also the Chairman of Advisory board of *Asian Journal of Andrology*. She is interested in the research of sperm maturation related epidymis-specific programmatically expressed novel genes.



# 3APFA Agenda

Time	Day 1 Fri		Day 2 Sat		Day 3 Sun		Day 4 Mon		Day 5 Tue						
	Lobby	Lobby	Lilac Room	Peace Hall	Peace Hall (A)	Peace Hall (B)	Lilac Room	Peace Hall (A)	Peace Hall (B)	Peace Hall	Violet Room	Lilac Room	Magnolia Room		
8:00-9:30				Peace Hall						Plenary 2					
9:30-9:50			Pre-course 1 (In Chinese)		Symposium 1	Symposium 2	/	Symposium 5	Symposium 6	Closing Ceremony	/	/	/		
9:50-10:00					Coffe Break & Poster 1			Coffe Break & Poster 2		Award Ceremony					
10:00-10:10						Symposium 2					Coffe Break & Poster 3				
10:10-10:20															
10:20-10:30					Symposium 1	Lilly Satellite Workshop	/	Symposium 5	Symposium 6	Special Session for Chinese (Panel Discussion)	/	/	/		
10:30-10:50					MSD Satellite Workshop				Shering-Plough Satellite Workshop						
10:50-11:10															
11:10-11:30															
11:30-12:00															
12:00-13:00			Lunch				Lunch				Lunch for Chinese Attendees				
13:00-15:00					Symposium 3	Symposium 4	/	Symposium 7	Symposium 8	/	Special Session for Chinese 1	Special Session for Chinese 2	Special Session for Chinese 3		
15:00-15:10					Coffe Break & Poster 1			Coffe Break & Poster 2			Coffe Break & Poster 3				
15:10-16:40	Pre-course Registration	On-Site Registration			Symposium 3	Symposium 4	AJA Meeting ASA Meeting				Special Session for Chinese 1	Special Session for Chinese 2	Special Session for Chinese 3		
16:40-17:10						Pfizer Satellite Workshop			Symposium 7	Symposium 8					
17:10-18:00															
18:00-18:30				Opening Ceremony	Chair Invitation Banquet (Humanity Hall)										
18:30-18:40			Plenary I												
18:40-19:30															
19:30-21:00			Welcome Reception												

Poster 1: Pt1- (Male Infertility), Pt3- (Sperm Function/Semen Analysis);  
 Poster 2: Pt2- (Environment and Life Style), Pt4- (Prostate), Pt6- (Male sexual function/Male Ageing);  
 Poster 3: Pt7- (Sperm Biology), Pt8- (Male Contraception and Others).



**Third Asia-Pacific Forum on Andrology  
in conjunction with  
the Tenth Anniversary Celebration of AJA**  
(10–13 October 2009, Nanjing, China)

**“Environment, Life Style & Genetic/Epigenetic Factors and Men’s Health”**

**Programme**

**9-Oct International Conference Hotel**

13:00–18:00 Pre-course Registration

**10-Oct Lobby**

8:00–18:00 Registration

**10-Oct Lilac Room**

8:00–10:00 Pre-course 1 (in Chinese): WHO Standard Semen Analysis Manual (5th edition)  
10:00–12:00 Pre-course 2 (in Chinese): Scientific Article English Writing  
12:00–13:00 Lunch  
13:00–16:30 Pre-course 3 (in Chinese): Microsurgery on Andrology

**10-Oct Outside Activity**

13:00–16:30 Site-visit to Labs or Hospitals

**10-Oct Peace Hall**

**Opening Ceremony**

Chair: Prof. Jia-Hao Sha (China)

18:00–18:05 **Welcome Address**  
Prof. Qi Chen (China)  
18:05–18:25 **Opening Remark**  
Prof. Yi-Fei Wang (China)  
18:25–18:35 **AJA 10th Anniversary Video**

**Plenary 1. Men’s Health: Global and Regional Perspectives**

Chair: Prof. David J Handelsman (Australia)

18:35–19:20 **PL1-1:** Biomedical and social determinants of men’s health  
Prof. David de Kretser (Australia)  
19:30–21:00 **Welcome Reception (Standing Buffet)**

**Morning, 11-Oct Peace Hall (A)**

**Symposium 1. Male Infertility and Assisted Reproductive Technology**

Chairs: Prof. J. Anton Grootegoed (The Netherland), Prof. Yi-Xin Wang (China)

8:00–8:30 **KS1-1:** Clinical management of male infertility  
Prof. Robert McLachlan (Australia)  
8:30–9:00 **KS1-2:** Understanding sperm function  
Dr Trevor G. Cooper (Germany)  
9:00–9:15 **S1-01:** The concerns on genetic risk during assistant reproductive technology application  
Prof. He-Feng Huang (China)



- 9:15–9:30 **S1-02:** The outcome after intracytoplasmic injection of fresh and frozen-thawed epididymal and testicular sperm from different cause  
*Prof. Ling-Bo Cai (China)*
- 9:30–9:40 **S1-03:** Beneficial effects of polydeoxyribonucleotide (PDRN) on spermatogenetic activity in experimental varicocele  
*Dr Letteria Minutoli (Italy)*
- 9:40–9:50 **S1-04:** Defective hMLH1 expression in patients with non-obstructive azoospermia  
*Dr Hui Tian (China)*
- 9:50–10:00 **S1-05:** Medium containing caffeine can stimulate motility of epididymal or testicular sperm and can better facilitate motile sperm selection for ICSI  
*Dr Jian-Chi Ding (China)*
- 10:00–10:10 Coffee Break / Poster I
- 10:10–10:20 **S1-06:** Effect of different sperm treatments in porcine oocytes activation by ICSI  
*Dr Carmen Matas (Spain)*
- 10:20–10:30 **S1-07:** Initial analysis on clinical effects of ICSI with frozen-thawed epididymal and testicular sperm  
*Dr Peng Xu (China)*
- 10:30–10:40 **S1-09:** Effect of obstruction to sperm egress on the male testis and epididymis  
*Dr Anil Kumar Sarda (India)*
- 10:40–10:50 **S1-10:** FSH and HCG combined therapy in hypogonadotropic hypogonadism  
*Dr Yi-Xin Wang (China)*
- 10:50–11:00 **S1-11:** The roles of meiotic regulator BOLL and its downstream substrate CDC25A in male infertility  
*Dr Chia-Ling Chung (Taiwan, China)*
- 11:00–11:10 **S1-13:** ROS originated from leukocytes: impact on movement, viability and morphology of cultivated human spermatozoa  
*Dr Ting-Yan Shi (China)*
- 11:10–11:20 **S1-14:** Comparison of chromatin status and fertilizing ability between epididymal and ejaculated human spermatozoa  
*Dr Mohammad Reza Sadeghi (Iran)*
- 11:20–11:30 **S1-15:** Tubal hydration  
*Dr M. A. Bashed (Bangladesh)*
- 11:30–12:00 **Satellite workshop 1: —MSD sponsor**
- 12:00–13:00 Lunch

## Morning, 11-Oct Peace Hall (B)

### Symposium 2: Environment and Life Style to Men's Health

*Chairs: Prof. Aleksander Giwercman (Sweden), Prof. Xin-Ru Wang (China)*

- 8:00–8:30 **KS2-1:** Androgens, obesity, sleep and sleep disordered breathing  
*Prof. Peter Y. Liu (Australia)*
- 8:30–9:00 **KS2-2:** Physical exercise and sleep are important lifestyle factors that can modulate age-related changes in sex steroids, physiological and sexual functions in Asian men  
*Prof. Victor Goh (USA)*
- 9:00–9:20 **S2-01:** Higher incidences of cryptorchidism, hypospadias, spontaneous abortion, and lower sperm qualities in industrialized areas with petrochemical complexes than in a non-industrialized area  
*Prof. Sae Chul Kim (Korea)*
- 9:20–9:30 **S2-02:** Testicular Dysgenesis Syndrome: Effects of Phthalates of Leydig cells  
*Dr Ren-Shan Ge (USA)*
- 9:30–9:40 **S2-03:** Persistent organohalogen pollutants repress androgen receptor function in a cag-dependent manner  
*Dr Yvonne Lundberg Giwercman (Sweden)*
- 9:40–9:50 **S2-04:** Relationship between urinary phytoestrogens and idiopathic male infertility  
*Dr Yan-Kai Xia (China)*
- 9:50–10:00 **S2-05:** Effects of 4-nitrophenol isolated from diesel exhaust particles on reproductive functions in immature male rats  
*Dr Kazuyoshi Taya (Japan)*
- 10:00–10:10 Coffee Break / Poster I
- 10:10–10:20 **S2-06:** Mono-(2-ethylhexyl) phthalate affects the steroidogenesis in Leydig cells through provoking ROS perturbation  
*Dr Yun-Hui Zhang (China)*

10:20–10:30	<b>S2-07:</b> Male reproductive toxicity, testicular oxidative stress and health hazard impact of delta-9-tetrahydrocannabinol and cannabinoids estrogenic drugs <i>Dr Tapas Kumar Mandal (India)</i>
10:30–10:40	<b>S2-08:</b> Alterations of anandamide metabolism in fertile and infertile human sperm <i>Dr Sheena E. M. Lewis (UK)</i>
10:40–10:50	<b>S2-09:</b> Effect of tobacco and psychotropic substance smoking on reproductive health of men <i>Dr Monis Bilal Shamsi (India)</i>
10:50–11:00	<b>S2-10:</b> Influence of temperature of floor surface on fertility in rats <i>Dr Gook-Sup Song (Korea)</i>
11:00–11:40	<b>Satellite workshop 2: —Lilly sponsor</b>
12:00–13:00	Lunch

### Afternoon, 11-Oct *Peace Hall (A)*

#### Symposium 3: Sperm Function and Semen Analysis

*Chairs: Prof. Jens Peter Bonde (Denmark), Prof. Yi-Qun Gu (China)*

13:00–13:30	<b>KS3-1:</b> Sperm preparation: state-of-the-ART <i>Prof. Ralf Henkel (South Africa)</i>
13:30–14:00	<b>KS3-2:</b> Evaluating sperm function <i>Dr De-Yi Liu (Australia)</i>
14:00–14:30	<b>KS3-3:</b> WHO5 Semen Manual and reference values <i>Dr Trevor G. Cooper (Germany)</i>
14:30–15:00	<b>KS3-4:</b> Tulane andrology semen analysis training program and quality control management for multicenter clinical trials <i>Dr Suresh C. Sikka (USA)</i>
15:00–15:10	Coffee Break / Poster I
15:10–15:25	<b>S3-01:</b> Assessment of seminal estradiol and testosterone levels as predictors of human spermatogenesis <i>Dr Qiu-Fang Zhang (China)</i>
15:25–15:35	<b>S3-02:</b> Leukocytes and oxidative stress may compromise sperm chromatin condensation <i>Dr Ralf Henkel (South Africa)</i>
15:35–15:45	<b>S3-03:</b> Which factors help to predict the IUI outcome: sperm DNA integrity, sperm-cervical mucus penetration or sperm-hyaluronan binding capacity? <i>Dr Ping Ping (China)</i>
15:45–15:55	<b>S3-04:</b> Clinical utility of sperm DNA fragmentation measures <i>Dr Donald P. Evenson (USA)</i>
15:55–16:05	<b>S3-05:</b> Impact of the atmospheric temperature on human semen parameters <i>Dr Kang-Shou Yao (China)</i>
16:05–16:15	<b>S3-06:</b> Changes in sperm morphology and semen parameters following varicocelectomy <i>Dr Anurag Mishra (India)</i>
16:15–16:25	<b>S3-07:</b> Declining quality of donated semen: a 10-year historical comparative cohort study <i>Dr Ronit Haimov-Kochman (Israel)</i>
16:25–16:35	<b>S3-08:</b> Factors affecting fecundity among sperm donors: a multivariate analysis <i>Dr Wen-Bing Zhu (China)</i>
16:35–16:45	<b>S3-09:</b> The analysis of semen parameters and bacterial culture results in epididymal fluid from 36 patients with obstructive azoospermia <i>Dr Ju-Fen Zheng (China)</i>
16:45–16:55	<b>S3-10:</b> Analysis of the acrosome reaction in human sperm induced by recombinant ZP3 expressed in different Chinese hamster ovary cells with altered glycosylation <i>Dr Manuel Avilés (Spain)</i>
16:55–17:30	Discussion

### Afternoon, 11-Oct *Peace Hall (B)*

#### Symposium 4: Prostate Diseases and Prevention, Diagnosis & Treatment of Prostate Cancer

*Chairs: Prof. Ismail Tambi (Malaysia), Prof. Shu-Jie Xia (China), Prof. Zhong-Cheng Xin (China)*

13:00–13:30	<b>KS4-1:</b> New roles of androgen receptor in the future andrology: 20 years after cloning androgen receptor Prof Chawnshang Chang (USA)
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- 13:30–15:30 **Debate Topic: Is there any relationship between prostatitis and BPH?**  
 Debaters: Affirmative — *Dr Zhong Wang (China), Dr Chun-Hua Deng (China), Dr Qiang Wei (China)*  
 Negative — *Dr Jun Lu (China), Dr Xiang Wang (China), Dr Yu-Tian Dai (China)*
- 15:30–15:45 **S4-01:** Development of a second-generation antiandrogen for treatment of castration resistant prostate cancer  
*Dr Charlie Degui Chen (China)*
- 15:45–16:00 **S4-02:** AKT/p70S6K1 signaling pathway in prostate tumorigenesis and angiogenesis  
*Dr Bing-Hua Jiang (China)*
- 16:00–16:10 **S4-03:** Prostate cancer screening in General Practice - knowledge, practice and uptake of evidence  
*Dr Dragan Ilic (Australia)*
- 16:10–16:20 **S4-04:** Inhibition of tumor growth and induction of apoptosis in prostate cancer cell lines by overexpression of tissue inhibitor of matrix metalloproteinase-3  
*Dr Xue-Jian Zhao (China)*
- 16:20–16:30 **S4-05:** Sex function after radical prostatectomy  
*Dr Ding-Wei Ye (China)*
- 16:30–16:40 **S4-06:** Stem cell properties of prostate tissue-derived CD133+MDR1+ cells and Oct-4 expression  
*Dr Lakshmi Narasu Mangamoori (India)*
- 16:40–16:50 **S4-07:** The detection of TMPRSS2:ERG fusion in Chinese prostate cancer and the relationship between TMPRSS2:ERG and the pathological grade  
*Dr Fa-Xian Yi (China)*
- 16:50–17:00 **S4-08:** Testosterone and dihydrotestosterone synthesized from DHEA in stromal cells of prostate cancer affect androgen receptor of prostate cancer cells  
*Dr Atsushi Mizokami (Japan)*
- 17:00–17:10 **S4-10:** Is it necessary to change the PSA threshold for screening prostate cancer in China?  
*Dr Dao-Hu Wang (China)*
- 17:10–17:20 **S4-11:** A rapid screening model of steroid 5 alpha-reductase inhibitor *in vitro*  
*Dr Jian-Hui Wu (China)*
- 17:20–17:30 **S4-12:** Extracorporeal shock wave therapy for chronic pelvic pain syndrome  
*Dr Hong Heng Chong (China)*
- 17:30–18:10 **Satellite workshop 3: —Pfizer sponsor**

### Afternoon, 11-Oct Lilac Room

- 15:00–16:30 AJA Editorial Board Meeting  
 16:30–17:00 ASA Executive Meeting

### Afternoon, 11-Oct Humanity Hall

- 18:30–20:30 Chairman Invitation Banquet

### Morning, 12-Oct Peace Hall (A)

#### Symposium 5. WHO Symposium--HIV and Circumcision

*Chairs: Prof. David de Kretser (Australia), Prof. Yi-Fei Wang (China)*

- 8:00–8:10 **Opening Remark**  
*Prof. Yi-Fei Wang*
- 8:10–8:40 **KS5-1:** Evidence on male circumcision and prevention of HIV and other STIs  
*Dr Tim Farley (Sweden)*
- 8:40–9:10 **KS5-2:** Male circumcision in resource-limited settings -- development of WHO normative guidance  
*Dr Timothy Hargreave (UK)*
- 9:10–9:30 **Live video showing a simple male circumcision using “Shang Ring”**
- 9:30–10:00 **KS5-3:** The need for high-quality training and surgical standard for adult male circumcision in Asia  
*Dr Philip S. Li (USA)*
- 10:00–10:10 Coffee Break / Poster II
- 10:10–10:30 **KS5-4:** WHO's perspective and strategy to promote male circumcision for HIV prevention  
*Dr Tim Farley (Sweden)*

10:30–12:00 **Panel Discussion: Needs and Demands for Circumcision in Asia and Pacific Regions**  
 12:00–13:00 Lunch

### Morning, 12-Oct *Peace Hall (B)*

#### Symposium 6. Erectile Dysfunction / Male Ageing

*Chairs: Prof. Zhou-Jun Shen (China), Prof. Sae-Chul Kim (Korea)*

- 8:00–8:30 **KS6-1:** Androgens and male ageing: current evidence of safety and efficacy  
*Prof. Louis J Gooren (the Netherland)*
- 8:30–9:00 **KS6-2:** Erectile dysfunction management for the future  
*Dr Arthur Burnett (USA)*
- 9:00–9:20 **S6-01:** TDS as marker for precocious aging to men's health issues  
*Prof. Arif Adimoelja (Indonesia)*
- 9:20–9:35 **S6-02:** Efficacy and safety of Maxpol T for tissue engineering penoplasty on small penis syndrome  
*Dr Zhong-Cheng Xin (China)*
- 9:35–9:50 **S6-03:** VIP enhance erectile function in rats through an androgen-independent pathway  
*Dr Min-Guang Zhang (China)*
- 9:50–10:00 **S6-04:** The survey of quality of erection questionnaire (QEQ) of Chinese outpatients with erectile dysfunction (ED) (multiple centers experience)  
*Dr Hui Jiang (China)*
- 10:00–10:10 Coffee Break / Poster II
- 10:10–10:20 **S6-05:** Efficacy and prognostic factors predicting successful response to sildenafil after radical prostatectomy  
*Dr Hesham Helmy (Egypt)*
- 10:20–10:30 **S6-06:** Experimental study of arginase gene and protein expression and its activity in the penis of diabetic rats  
*Dr Jun-Ping Shi (China)*
- 10:30–10:40 **S6-07:** Association between erectile dysfunction and metabolic syndrome in aging men: Hallym aging study  
*Dr Sang Kon Lee (Korea)*
- 10:40–10:50 **S6-08:** Partial penile sensory axon resection for treatment of premature ejaculation  
*Dr Aref El-Seweifi (Germany)*
- 10:50–11:00 **S6-09:** Gene expression study of seminal vesicle in men with ejaculatory dysfunction  
*Dr Pei-Yu Lin (Taiwan, China)*
- 11:00–11:10 **S6-10:** The Research in detecting extracellular calcium concentration of corpus cavernosum smooth muscle *in vivo* on erectile dysfunction  
*Dr Fei-Xiang Wang (China)*
- 11:10–11:50 **Satellite workshop 4: —Schering-Plough sponsor**

### Afternoon, 12-Oct *Peace Hall (A)*

#### Symposium 7: Sperm Biology and Fertilization

*Chairs: Prof. De-Yi Liu (Australia), Prof. Chen Xu (China)*

- 13:00–13:30 **KS7-1:** The unlimited potential of human spermatogonial stem cells  
*Prof. Martin Dym (USA)*
- 13:30–14:00 **KS7-2:** Small RNAs in epididymis  
*Prof. Yong-Lian Zhang (China)*
- 14:00–14:30 **KS7-3:** Roles of testicular microRNAs  
*Dr Wei Yan (USA)*
- 14:30–15:00 **KS7-4:** Aquaporins in testis and spermatozoa  
*Dr Ching-Hei Yeung (Germany)*
- 15:00–15:10 Coffee Break / Poster II
- 15:10–15:25 **S7-01:** Roles of microRNA-449 in mammalian spermatogenesis  
*Dr Chen Xu (China)*
- 15:25–15:40 **S7-02:** Integrated functional genomics research of normal human epididymis  
*Dr Jian-Yuan Li (China)*



- 15:40–15:50 **S7-03:** Gonadotropin-regulated testicular helicase (GRTH/DDX25): a master post-transcriptional regulator of spermatogenesis  
*Dr Maria Dufau (USA)*
- 15:50–16:00 **S7-05:** Characterization of SPAG4L, a new member of SUN gene family, involved in the specific stage of human spermatogenesis  
*Dr Xiao-Wei Xing (China)*
- 16:00–16:10 **S7-06:** Curcumin affects the dynamics of chromatin associated proteins in spermiogenesis  
*Dr Xiao-Yu Xia (China)*
- 16:10–16:20 **S7-07:** Role of p73 in mammalian germ cell apoptosis during the first round of spermatogenesis in the rat  
*Dr Ricardo Moreno (Chile)*
- 16:20–16:30 **S7-09:** Antimicrobial activity of the cystatin-related epididymal spermatogenic (CRES) protein  
*Dr Qing Yuan (China)*
- 16:30–16:40 **S7-10:** Does Y-bearing spermatozoa undergo capacitation faster than X-bearing spermatozoa?  
*Dr Jin Kobayashi (Japan)*
- 16:40–16:50 **S7-11:** Modified technique for spermatogonial stem cell transplantation into the seminiferous tubules in mouse model  
*Dr Xiang Wang (China)*
- 16:50–17:00 **S7-12:** Differential potency of induced pluripotent stem cells into male gametes *in vitro*  
*Dr Peng Li (China)*
- 17:00–17:10 **S7-14:** Xeno-free culture of human spermatogonial stem cells supported by human embryonic stem cell-derived fibroblast-like cells  
*Dr Bin Chen (China)*
- 17:10–17:20 **S7-15:** Transplantation of spermatogonial stem cell suspension through rete testis of azoospermic mice  
*Dr Arefeh Jafarian (Iran)*
- 17:20–17:30 **S7-16:** Endoplasmic reticulum protein 29 (ERp29), a protein related to sperm maturation is involved in sperm-oocyte fusion in mouse  
*Dr Zhi-De Ding (China)*
- 17:30–17:40 **S7-17:** Purification and characterization of testicular relaxin-like factor in boars  
*Dr Tetsuya Kohsaka (Japan)*
- 17:40–18:00 Discussion

### Afternoon, 12-Oct *Peace Hall (B)*

#### Symposium 8: Male Contraception, Alternative Medicine and Others

*Chairs: Prof. FX Arif Adimoelja (Indonesia), Prof. Premendu P. Mathur (India)*

- 13:00–13:30 **KS8-1:** Progress and Prospects for Male Hormonal Contraception  
*Prof. Peter Y. Liu (Australian)*
- 13:30–14:00 **KS8-2:** Effect of natural and synthetic products on the testis  
*Prof. Premendu P. Mathur (India)*
- 14:00–14:30 **KS8-3:** Importance of andrology as a clinical discipline  
*Prof. Wolf-Bernhard Schill (Germany)*
- 14:30–15:00 **KS8-4:** Nutrients and botanicals for optimizing men's health. Examining the evidence for *Eurycoma longifolia* jack, the Malaysian Ginseng in men's health  
*Prof. Mohd Ismail Tambi (Malaysia)*
- 15:00–15:10 Coffee Break / Poster II
- 15:10–15:25 **S8-01:** Proteomic analysis of testis biopsies in men treated with transient scrotal hyperthermia as potential male contraceptive  
*Dr Xue-Jiang Guo (China)*
- 15:25–15:35 **S8-03:** The contraceptive effect of filtering-type nano-copper complex/polymer composites intra-vas device in the male animals  
*Dr Xun-Bing Huang (China)*
- 15:35–15:45 **S8-04:** Evaluation of microsurgical two suture longitudinal intussusception technique of vasoepididymostomy—the indian experience  
*Dr Srivathsan Ramani (India)*
- 15:45–15:55 **S8-06:** Effects of vasectomy at different sites on spermatogenesis in rabbits  
*Dr Bing Peng (China)*
- 15:55–16:05 **S8-08:** Repair and functional reconstruction of the penis (report of 62 cases)  
*Dr Hao Wang (China)*



16:05–16:15	<b>S8-09:</b> Ethical issues in penile transplantation <i>Dr Li-Chao Zhang (China)</i>
16:15–16:25	<b>S8-10:</b> Endocervical response to human semen and <i>C. trachomatis</i> : insights into increased heterosexual HIV transmission in co-exposed women <i>Dr Danny Schust (USA)</i>
16:25–16:35	<b>S8-12:</b> Effects of GnRH agonist on annexin 5 and 3 $\beta$ -HSD expression in rat Leydig cell <i>Dr Bing Yao (China)</i>
16:35–16:45	<b>S8-13:</b> Extracellular matrix metalloproteinase inducer: a novel poor prognostic marker for human seminomas <i>Dr Xue-Cheng Bi (China)</i>
16:45–16:55	<b>S8-14:</b> The predictive value of age, body mass index and increment of plasma testosterone on the effects of testosterone administration to hypogonadal men <i>Dr Farid Saad (Germany)</i>
16:55–17:05	<b>S8-15:</b> Regulatory actions of estrogen receptors on GnRH neuronal function <i>Dr Kevin Catt (USA)</i>
17:05–17:30	Discussion

### Morning, 13-Oct *Peace Hall*

#### Plenary 2. Environmental and Genetic Factors and Men's Health

*Chairs: Prof. Martin Dym (USA), Prof. Fei Sun (China)*

8:00–8:30	<b>PL2-1:</b> Post-meiotic gene expression in spermatogenesis <i>Dr J. Anton Grootegoed (the Netherland)</i>
8:30–9:00	<b>PL2-2:</b> Gene-environment interaction and male reproductive function <i>Prof. Aleksander Giwercman (Sweden)</i>
9:00–9:30	<b>PL2-3:</b> Environmental risk to male reproductive health <i>Prof. Jens P. E. Bonde (Denmark)</i>

#### Closing Ceremony

*Chair: Prof. Yi-Fei Wang (China)*

9:30–10:00	<b>Closing Speech</b> <i>Prof. David J. Handelsman (Australia)</i>
10:00–10:20	<b>Scientific Presentation Award Ceremony</b>
10:20–10:30	Coffee Break / Poster III

#### Special Session for Chinese Panel Discussion

*Chair: Prof. Yi-Fei Wang (China)*

10:30–12:00	<b>Topic: The Future of Andrology in China</b> <i>Prof. Chawnshang Chang (USA), Prof. Ying-Lu Guo (China), Prof. Yi-Ran Huang (China), Prof. Philip S. Li (USA), Prof. De-Yi Liu (Australia), Prof. Jia-Hao Sha (China), Prof. Yong-Lian Zhang (China), Prof. Can-Quan Zhou (China)</i>
12:00–13:00	Lunch for Chinese Attendees

### Afternoon, 13-Oct (*Violet Room*)

#### Special Session for Chinese (I)

*Chairs: Prof. He-Feng Huang (China), Prof. Zheng Li (China), Prof. Zuo-Ming Zhou (China)*

13:00–13:20	<b>C1-01:</b> What we know the androgen receptor roles in reproductive medicine, 20 years after cloning androgen receptor <i>Prof. Chawnshang Chang (USA)</i>
13:20–13:40	<b>C1-02:</b> Sperm-specific genes and male infertility <i>Dr Wei Yan (USA)</i>
13:40–13:50	<b>C1-03:</b> Cloning and identification of a sperm binding protein HEL-S61 expressed in the human testis <i>Dr Jun Liu (China)</i>
13:50–14:00	<b>C1-05:</b> Expression of hsa-miR-135a in sperm with low motility <i>Dr De-Yu Chen (China)</i>
14:00–14:10	<b>C1-06:</b> Proteome analysis of infertility and high-fertility sperm by two-dimensional difference gel electrophoresis and mass spectrometry <i>Dr Zhen Xiang (China)</i>



- 14:10–14:20 **C1-07:** Evaluating sperm DNA integrity by labeling nick-end of the diffused chromatin using TdT-mediated dUTP  
*Dr Li-Hong Zhang (China)*
- 14:20–14:30 **C1-08:** The expression research of ZNF265, IL-1 $\alpha$ , IL-6, TGF- $\beta$  and FasL during the Sertoli cell infected by UU  
*Dr Wei-Yi Li (China)*
- 14:30–14:40 **C1-09:** Attenuated *Salmonella typhi* carrying the plasmid-co-expressed GRIM-19 and Survivin-specific shRNA plasmid for prostate cancer therapy  
*Dr Yan-Bo Liu (China)*
- 14:40–14:50 **C1-11:** The effect of Smad4 Sumolation modulated by PIAS family proteins on the proliferation of prostate cancer  
*Dr Xiao-Meng Li (China)*
- 14:50–15:00 **C1-12:** Distinctive gene expression of stromal cells from transitional zone and peripheral zone of prostate  
*Dr Fu-Jun Zhao (China)*
- 15:00–15:10 Coffee Break / Poster III
- 15:10–15:20 **C1-13:** Effect of endothelin-1 on cyclooxygenase-2 expression in human hormone refractory prostate cancer cells  
*Dr Rui-Peng Jia (China)*
- 15:20–15:30 **C1-14:** Prenatal exposure to nanoparticle-rich diesel exhaust suppressed testicular function in immature male rats  
*Dr Chun-Mei Li (China)*
- 15:30–15:40 **C1-16:** Combined effect of nonyl phenol and di-n-butyl phthalate in rat sertoli cells *in vitro*  
*Dr Dong-Mei Li (China)*
- 15:40–15:50 **C1-17:** Seasonal changes in spermatogenesis and immunodetection of cytochrome P450 aromatase in wild male ground squirrels  
*Dr Qiang Weng (China)*
- 15:50–16:00 **C1-18:** Androgen levels (T and DHT) and 5 $\alpha$ -reductase activities in the androgen target tissues of male individuals at different developmental stages  
*Dr Yu-Gui Cui (China)*
- 16:00–16:10 **C1-19:** Antioxidant effect of tetrandrine on cultured rabbit corpus cavernosum endothelial cells injured by hydrogen peroxide  
*Dr Jun Chen (China)*
- 16:10–16:20 **C1-20:** Hypoxia on SD rat corpus cavernosum smooth muscle cell apoptosis  
*Dr Xiao-Jun Huang (China)*
- 16:20–16:30 **C1-21:** Experimental research of using small intestinal submucosa for corporal body grafting in the rabbit  
*Dr Fa Sun (China)*
- 16:30–16:40 **C1-23:** Male germ-cell transplantation from normal Mongolia sheep to the testes of azoospermic poll Dorset sheep results in production of donor's offspring  
*Dr Ying-Ji Wu (China)*
- 16:40–17:30 Discussion

### Afternoon, 13-Oct (Lilac Room)

#### Special Session for Chinese (II)

*Chairs: Prof Yi-Ran Huang (China), Prof. Philip S. Li (USA), Prof Jia-Yin Liu (China)*

- 13:00–13:20 **C2-01:** What's new for male adult circumcision and HIV prevention in China  
*Prof. Philip S. Li (USA)*
- 13:20–13:40 **C2-02:** To be determined  
*Dr Guo-Min Wang (China)*
- 13:40–13:50 **C2-03:** Shorten exposure of eocytes to spermatozoa improves embryo quality  
*Dr Bu-Fang Xu (China)*
- 13:50–14:00 **C2-04:** The comparing of therapy effect of different operation method of varicocele with 685 cases  
*Dr You-Sheng Yao (China)*
- 14:00–14:10 **C2-05:** Investigation research of 177 cases of patients with azoospermism or oligospermism infection of *Ureaplasma urealyticum*  
*Dr Yi-Feng Peng (China)*
- 14:10–14:20 **C2-07:** The diagnosis and surgical treatment for obstructive azoospermia  
*Dr Xiang-An Tu (China)*
- 14:20–14:30 **C2-09:** Azoospermia: a laboratory diagnosis and research of 758 cases  
*Dr Jiu-Lai Wu (China)*



- 14:30–14:40 **C2-10:** Clinical characterization of oligospermic or azoospermic men with AZF region microdeletion of Y chromosome  
*Dr Xiao-Bin Zhu (China)*
- 14:40–15:50 **C2-12:** Guidance for the identification of the KY health status (KY3H) in men with low sexual desire  
*Dr Guang-Chong Qi (China)*
- 15:00–15:10 Coffee Break / Poster III
- 15:10–15:20 **C2-13:** The differential diagnosis of primary and secondary venogenic erectile dysfunction  
*Dr Bin-Zhang (China)*
- 15:20–15:30 **C2-14:** The relationship between premature ejaculation and central lumbar intervertebral disc herniation  
*Dr Xin-Dong Zhang (China)*
- 15:30–15:40 **C2-15:** Sexual practice and attitude of adults in Guangzhou China: a community based survey  
*Dr Xin Tao (China)*
- 15:40–15:50 **C2-16:** Correlation research between benign prostate hyperplasia and erectile dysfunction  
*Dr Jun-Hong Deng (China)*
- 15:50–16:00 **C2-17:** Clinical study of curing premature ejaculation by combined application of SSRIs and PDE5 inhibitor  
*Dr Jun-Long Wang (China)*
- 16:00–16:10 **C2-18:** Dorsal penile nerve in the treatment of primary premature ejaculation clinical significance  
*Dr Chun-Ying Zhang (China)*
- 16:10–16:20 **C2-20:** The risk factors of erectile dysfunction for the aged patients with BPH under different treatments  
*Dr Han-Jian Shen (China)*
- 16:20–16:30 **C2-21:** Transrectal high-intensity focused ultrasound combined with endocrine therapy in treating prostatic carcinoma  
*Dr Jun Lv (China)*
- 16:30–16:40 **C2-22:** A retrospective study on the learning curve of 1122 cases of TUVRP by one surgeon  
*Dr Ai-Hua Li (China)*
- 16:40–16:50 **C2-23:** Correlation of chronic prostatitis with psychological analysis  
*Dr Li-Wen Deng (China)*
- 16:50–17:00 **C2-24:** Clinical study the relationship between sexual function in men with symptomatic benign prostatic hyperplasia with chronic prostatitis  
*Dr Shi-Xin Chen (China)*
- 17:00–17:10 **C2-26:** Influence of health education on the life quality of sequela after vasectomy  
*Dr Ming-Xun Huang (China)*
- 17:10–18:00 Discussion

### Afternoon, 13-Oct (Magnolia Room)

#### Special Session for Chinese (III)

Chairs: Prof. Zhen-Wen Chen (China), Prof. Jie Qiao (China)

- 13:00–13:20 **C3-01:** Standardization of clinical laboratory on andrology  
*Dr Yi-Qun Gu (China)*
- 13:20–13:40 **C3-02:** The recommendations for andrology laboratories  
*Dr Li-Qing Fan (China)*
- 13:40–13:50 **C3-03:** Sperm banking for timely reproductive insurance: multiple centers' investigation in China  
*Dr Ping Ping (China)*
- 13:50–14:00 **C3-04:** Polymorphisms of nucleotide-excision repair genes in relation to sperm DNA fragmentation and male infertility  
*Dr Ai-Hua Gu (China)*
- 14:00–14:10 **C3-05:** Study on correlation between seminal plasma elastase and semen major parameters and sperm function  
*Dr Ya-Feng Liu (China)*
- 14:10–14:20 **C3-06:** The influence of cigarette smoking on human sperm quality and DNA integrity  
*Dr Zhi-Hong Niu (China)*
- 14:20–14:30 **C3-07:** Effect of Bcl-2 genetic variants on spermatogenesis failure in a Han-Chinese population  
*Dr Shou-Lin Wang (China)*
- 14:30–14:40 **C3-08:** Study of sperm sorting using microfluidic chip  
*Dr Wei Wang (China)*
- 14:40–14:50 **C3-09:** Determination fructose and  $\alpha$ -glycosidase of seminal plasma in fifty patients with azoospermia oligospermia or asthenospermia  
*Dr Qiang Li (China)*



14:50–15:00	<b>C3-11:</b> Study on the relationship sperm DNA damage <i>Dr Xiao-Hong Bai (China)</i>
15:00–15:10	Coffee Break / Poster III
15:10–15:20	<b>C3-12:</b> Semen characteristics of 5704 subfertile men in Guangxi region <i>Dr Feng Liu (China)</i>
15:20–15:30	<b>C3-13:</b> Determination of the main population of sperm: treating oligo-asteno-teratozoospermia syndrome with self-designed Chinese traditional and western medicine <i>Dr Jiang-Ping Du (China)</i>
15:30–15:40	<b>C3-14:</b> The outcome of ICSI with frozen-thawed sperm obtained by PESA on azoospermia patients <i>Dr Zheng-Jie Yan (China)</i>
15:40–15:50	<b>C3-17:</b> The effect of sperm DNA fragmentation on the rate of fertilization, cleavage and high quality embryo in conventional IVF <i>Dr Ai-Jun Zhang (China)</i>
15:50–16:00	<b>C3-19:</b> The spermatogenesis process rather than sperm retrieval approaches play an important role in the clinical outcome of ICSI for azoospermic patients <i>Dr Xiao-Jun He (China)</i>
16:00–16:10	<b>C3-20:</b> Comparison of the outcome and safety of ICSI by different origin of sperm <i>Dr Yue-Hong Lu (China)</i>
16:10–16:20	<b>C3-21:</b> Experience of sperm retrieval in non-obstructive azoospermic men with maturation arrest <i>Dr Ming-Chun Tsai (Taiwan, China)</i>
16:20–16:30	<b>C3-22:</b> The phenomenon and solutions of non-specific results of MAR test with high viscosity semen <i>Dr Yu Liu (China)</i>
16:30–17:00	Discussion

**P.S.**

Poster board (height 1.20 m, width 0.80 m, in size) will be available in Peace Hall (B).

**Poster 1:** Pt1- (Male Infertility), Pt3- (Sperm Function/Semen Analysis);

**Poster 2:** Pt2- (Environment and Life Style), Pt4- (Prostate), Pt6- (Male Sexual Function/Male Ageing);

**Poster 3:** Pt7- (Sperm Biology), Pt8- (Male Contraception and Others).



# The Proceeding of

## The Third Asia-Pacific Forum on Andrology

in conjunction with the 10th Anniversary Celebration of Asian Journal of Andrology

Environment, Life Style & Genetic/Epigenetic Factors and Men's Health

10-13 October, 2009, Nanjing, China

## Section I

Plenary and Symposium Lectures & Oral Presentation in Chinese Session



### PL1-1

#### Biomedical and social determinants of men's health

David M. de Kretser

Governor of Victoria and Emeritus Professor Monash University, Melbourne, Victoria, Australia

Understanding the factors that determine the health of men are critical to assist in the development of strategies designed to prevent disorders that can cause premature death or disability. Both the biomedical and social determinants are critical parameters and must be considered together.

A man's genome, constituted at fertilisation from contributions from his parents, forms his genetic template that determines his sex. The subsequent testicular development, through the production of androgens, causes differentiation of the male genitalia. Androgen production by the testis occurs in both fetal and post-pubertal stages and, the latter influences secondary sexual development and the adult male phenotype.

The expression of a man's genome is influenced by the intrauterine environment during pregnancy resulting in outcomes that may become manifested in adult life impacting on cardiovascular disease, diabetes and the metabolic syndrome. Termed the Barker hypothesis, the mechanisms are likely to involve epigenetic processes. Direct environmental exposure to alcohol, through maternal drinking in pregnancy may result in the fetal alcohol syndrome.

Family, education and religious beliefs are important determinants of "behavioural conditioning" that can impact on a man's genome influencing such factors as what constitutes a healthy diet. Dietary patterns can influence the subsequent development of obesity. Behavioural patterns are established that may minimise or maximise the impact of psychological stresses later in life, resulting in depression, anxiety and suicide.

The impact of the combination of lifetime experiences, environmental exposure and social factors such as employment and socio-economic status on a man's genetic template, all combine to ultimately influence patterns of disease.

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### PL2-3

#### Environmental risk to male reproductive health

Jens Peter Bonde

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Male reproductive disorders that are of interest from an environmental point of view include sexual dysfunction, infertility, cryptorchidism, hypospadias and testicular cancer. Several reports indicate declining sperm counts and increase of these reproductive disorders in some areas during some time periods past 50 years. Except testicular cancer this evidence is circumstantial and needs cautious interpretation. However, the male germ line is one of the most sensitive tissues to the damaging effects of ionizing radiation, radiant heat and a number of known toxicants. So far occupational hazards are the best

documented risk factors for impaired male reproductive function and include physical exposures (radiant heat, ionizing radiation, high frequency electromagnetic radiation), chemical exposures (some solvents as carbon disulfide and ethylene glycol ethers, some pesticides as dibromochloropropane, ethylendibromide and DDT/DDE, some heavy metals as inorganic lead and mercury) and work processes such as metal welding. Improved working conditions in affluent countries have dramatically decreased known hazardous workplace exposures, but millions of workers in less affluent countries are at risk from reproductive toxicants. New data show that environmental low-level exposure to biopersistent pollutants in the diet may pose a risk to people in all parts of the world. For other noxious agents the evidence is only suggestive and further evaluation is needed before conclusions can be drawn. Whether compounds as phthalates, bisphenol A and boron that are present in a large number of industrial and consumer products entails a risk remains to be established. The same applies to psychosocial stressors and use of mobile phones. Finally, there are data indicating a particular vulnerability of the fetal testis to toxicants – for instance maternal tobacco smoking. Time has come where male reproductive toxicity should be addressed from entirely new angles including exposures very early in life.

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### PL2-2

#### Gene-environment interaction and male reproductive function

Aleksander Giwercman

Reproductive Medicine Centre, Malmö University Hospital, Lund University, Malmö, Sweden

According to the hypothesis proposed by Skakkebaek and co-workers, poor semen quality, testis cancer, undescended testis and hypospadias are symptoms of one underlying entity, the so called testicular dysgenesis syndrome (TDS). TDS was suggested to be a result of disruption of embryonal programming and gonadal development during foetal life and as aetiological factor, an impact of adverse environmental factors such as hormone disruptors, probably acting upon a susceptible genetic background, was suggested.

Extensive studies considering the risk of TDS related diseases in Denmark compared to Finland, showed higher sperm counts and lower risk of cryptorchidism and testicular cancer among Finns. Although second generation immigrant studies clearly indicate an impact of environmental or life-style related factors in the aetiology of TGCC, certain influence of discrepancy in genetic background, can not be excluded. A more obvious example of the impact of genetic factors on the risk of TDS concerns Afro-Americans having significantly lower incidence of testicular cancer as compared to Caucasians living in the USA.

A yet unexplored scenario is a possible interaction between genetic and environmental/life-style-related factors, certain genotypes making individuals more susceptible to adverse exogenous exposures. In epidemiological studies we have provided some evidence for modifying effects of polymorphisms in the androgen receptor gene on the impact of pesticides

organohalogen pollutants on male reproductive parameters. These *in vivo* findings have also been seen *in vitro*. Studying such interactions has biological, epidemiological and public health related implications. It will help us to understand the background for the defects in male reproductive organs, facilitate proper design of epidemiological studies and add to identifying individuals susceptible to certain environmental and life-style related hazards.

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## PL2-1

### Post-meiotic gene expression in spermatogenesis

J. Anton Grootegoed

Department of Reproduction and Development Erasmus MC–University Medical Center, Rotterdam, The Netherlands

Cytodifferentiation of mammalian spermatids, developing towards spermatozoa in the post-meiotic part of spermatogenesis named spermiogenesis, includes acrosome and tail formation, marked changes in volume and structure of cytoplasm and organelles, and reorganization of the nuclear chromatin. This is followed by release of fully developed spermatids from Sertoli cells, referred to as spermiation, and the acquisition of fertilizing capacity during transit of the maturing spermatozoa through the epididymis. Transcriptional silencing of the post-meiotic haploid genome, by histone-to-protamine transition and chromatin compaction, precedes the final steps of spermiogenesis when new proteins are still being synthesized. This ongoing protein synthesis is guaranteed by increased stability of mRNAs and developmental control of their translation.

Studying spermatogenesis in the mouse, it is possible to look at the effect of targeted mutation of specific genes (gene knockout). If the targeted genes are highly conserved between mouse and human, the generated mouse models can provide information about possible causes of human male infertility, and perhaps may also reveal opportunities for development of new contraceptives targeting the male. In recent years, quite many knockout mouse models have been generated by various research groups. For genes expressed post-meiotically, during spermiogenesis, reported defects concern, among other things, dysregulation of acrosome biogenesis, tail formation, nuclear condensation, and cytoplasm removal. We will discuss two genes in more detail: *Tssk1* and *Tssk2*, encoding the testis-specific serine/threonine kinases TSSK1 and TSSK2 which are found in the cytoplasm of elongating spermatids. In early elongating spermatids, we detected accumulation of the kinases TSSK1 and TSSK2, and the substrate protein TSKS, in a ring-shaped structure around the base of the flagellum and in a cytoplasmic satellite, both corresponding to structures which have been described to originate from the chromatoid body (CB). In mammalian round spermatids, the cytoplasmic CB organizes mRNA metabolism and small regulatory RNA pathways, in relation to haploid gene expression. However, little is known about fate and functions of the CB at later steps of spermatogenesis, when elongating spermatids undergo chromatin compaction and transcriptional silencing. Targeted deletion of the tandemly arranged genes *Tssk1* and *Tssk2* in mouse resulted

in loss of the CB ring around the flagellum, and male infertility with elongating spermatids showing a collapsed mitochondrial sheath. The results point to TSSK1/TSSK2-dependent functions of a transformed CB in post-meiotic cytodifferentiation of spermatids. This will be discussed in the context of other mouse knockout models possessing defects in spermiogenesis.

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## KS1-1

### Clinical management of male infertility

Robert I. McLachlan

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Male reproductive dysfunction is the sole or contributory cause of infertility in half of couples. Remarkable advances in assisted reproductive treatments (ART), particularly intracytoplasmic sperm injection (ICSI), now provide fertility in previously sterile men and have led to a reduction in the demand for donor insemination. However male reproductive health is not defined only by the availability of motile spermatozoa: clinicians in fertility practice have an obligation to evaluate and care for infertile men. For example natural fertility may be restorable and even when specific treatment is not possible, providing the man with a reason for his infertility will assist him in coming to terms with his disability. In addition, some health issues are more prevalent in infertile men and must be sought (e.g. androgen or gonadotropin deficiency, testicular cancer) and the opportunity taken to assess and improve general and sexual health. Finally the genetic basis of infertility must be considered as it may have implications for the health of offspring: a close relationship with genetic counsellors is now an essential part of modern ART practice.

Primary spermatogenic failure is a collective term for a heterogeneous group of disorders that present with abnormal sperm number, motility and/or structure/function. It affects ~5% of the population: most cases are unexplained (idiopathic) but increasingly genetic factors are recognised and their identification is essential in informing couples about the prospect for normal pregnancy, transmission of infertility and/or non-gonadal disease in offspring. Karyotypic anomalies (numerical, structural) increase in prevalence as sperm densities fall and are seen in ~14% of azoospermic men. Routine karyotyping is widely recommended for ICSI candidates with sperm densities < 10 million mL<sup>-1</sup>. An increased prevalence of aneuploidy in the ejaculated or testicular sperm of severely infertile men with a normal peripheral blood karyotype probably underlies the 3-fold higher rate of apparently de novo abnormalities in ICSI offspring.

Yq chromosomal deletions are evident in ~5% of severely oligo/azoospermic men and are routinely assessed prior to ART in men with sperm densities < 5 million mL<sup>-1</sup>. Care must be taken in performing this PCR-based testing so as to avoid false detection; external quality assurance should be used. Extensive Yq deletions carry a poor prognosis for sperm recovery by testicular sperm extraction. Vertical transmission via ICSI is



inevitable and will be associated with fertility defects in these boys. The gr/gr sub-deletion within the AZFc region appears to be a risk factor for infertility in some genetic backgrounds.

Expansions of the CAG repeats in exon 1 of the androgen receptor number is weakly associated with oligospermia in some populations but is not recommended for routine testing. Sperm DNA quality shows some relationship to reproductive outcomes however currently this assessment lacks sufficient predictive power to effectively inform or modify clinical treatment pathways and that further research is required.

In the azoospermic man, distinguishing spermatogenic failure from obstruction is essential as this has major implications for management and is usually evident based on testis size, semen volume/pH and serum FSH levels, but diagnostic testicular biopsy may be required. Obstruction may follow infection, trauma or vasectomy, or result from congenital absence of the vas in which setting cystic fibrosis (CFTR) gene mutations are common in some populations, especially Caucasian wherein 85% of otherwise healthy men are heterozygotes or compound heterozygotes for CFTR mutations making evaluation of female partner's CFTR gene status essential.

Natural fertility may be restored through withdrawal of spermatogenic toxins, cessation of anabolic steroid usage, gonadotropin treatment in pituitary disease, surgery in suitable obstructive cases and even through management of erectile or ejaculatory dysfunction of diverse aetiologies. Background natural fertility rates in sub-fertile men requires that evidence be obtained from placebo-controlled randomised clinical trials for proposed interventions; such supportive data are lacking for many common treatments such as empirical vitamin or antibiotic treatment, or varicocele ligation. In obstructive azoospermia, the decision to attempt surgical correction, as opposed to sperm retrieval and ICSI, is affected by the underlying anatomy, surgical access & skill, and the presence of female co-factors.

Conventional IVF and ICSI offer excellent fertility prospects (similar to that of female factor couples) in almost all cases of male infertility in which viable sperm or mature spermatids can be isolated. In severe spermatogenic failure (e.g. Klinefelter's syndrome), testicular sperm can be isolated in 30%-60% of men via needle aspiration or random open biopsy but higher recovery rates have been reported using microdissection testicular sperm extraction. Thorough and realistic discussion is required regarding the prospects for live birth, androgen deficiency as a result of testicular damage, potential health issues in offspring and the donor sperm alternative.

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## KS1-2

### Understanding sperm function

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This talk will summarise the life history of a spermatozoon once released from the germinal epithelium in the testis, and examine the changing environments it encounters, and its responses to them, on its passive way through the epididymis

(increasing osmolality, potassium, organic osmolytes, potential volume decrease, membrane depolarisation, decreasing sodium, ionic strength, surface and membrane-inserted proteins, changes in morphology, potential for motility, quiescence), through the tumultuous changes upon ejaculation (rapid osmotic and ionic changes, potential volume increase, activation of motility, loss of cytoplasmic droplets) and active transport through the female tract (lower osmolality, decreasing potassium, increasing sodium Na, membrane hyperpolarisation, ionic strength, loss of decapacitation factors, potential swelling, hyperactivation, acrosome reaction). Many functions of spermatozoa are displayed only at certain times and locations and they can not easily be examined routinely.

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## KS2-2

### Physical exercise and sleep are important lifestyle factors that can modulate age-related changes in sex steroids, physiological and sexual functions in Asian men

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This review shows that age is a major determinant of most health compartments in men. Gonadal, somatotrophic, adrenal and peripheral endocrine axes, cognition, physical functions, coital frequency, masturbation, erectile functions, body composition, lipids and lipoprotein profiles, metabolic parameters, and sleep duration changed with increasing age. However, studies in a group of Asian men showed clearly that sleep duration, independent of age, body fat, and BMI was positively associated with T and BioT, but not with DHEAS or any of the sexual activities studied. Men who masturbated had higher levels of both T and BioT. But DHEAS was significantly associated with coital frequency, desire for sex and rigidity of morning penile erection. Regular exercises of sufficient duration and intensity were shown to be associated with beneficial changes in multiple compartments including increases in lean and bone mass, and decreases in fat mass. Serum total T was significantly increased, while insulin levels were decreased. Increases in HDL-cholesterol were in tandem with decreases in the HDL/TC ratio. Asian men who exercised regularly were able to fall asleep better and had better sense of well being. **Conclusion:** The present study showed that besides age, sleep duration and physical exercise were associated with higher androgen levels, improved metabolic and cardiovascular parameters and bone and muscular health in men. These two lifestyle factors appear to be able to modulate some age-related decline in functions. Therefore, the active promotion of good sleep hygiene and regular exercise as lifestyle habits is an important adjunct modality for the management of aging in men.

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**KS3-1****Sperm preparation: state of-the-ART**

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Since the birth of Louise Brown on 25 July 1978 and the subsequent onset of assisted reproduction technology (ART), scientists and clinicians were urged to improve sperm separation techniques as the percentage of andrological cases increased rapidly. While the first *in vitro* fertilization (IVF) cases were performed to treat tubal infertility, the increasing number of men showing poor semen quality prompted the development of a wide array of different techniques focusing on the selection and enrichment of motile and functionally competent spermatozoa from the ejaculate. The first sperm separation methods only comprised washing procedures with subsequent re-suspension of the male germ cells. Subsequently, more sophisticated methods were developed to obtain sufficient amounts of motile, functionally competent spermatozoa. These conventional strategies for sperm preparation used for IVF were mainly based on motility and include any type of swim-up method. Even the commonly used methods for density gradient centrifugation are based on the sperm cell's own ability to move through the inter-phases between the different layers. In filtration techniques like glass wool filtration, the separation procedure is based on the sperm cells' own motility but is supplemented by adhesion and filtration processes. In order to increase the total number of motile sperm after the separation process, attempts have been made to stimulate sperm motility by means of various stimulants to the medium like pentoxifylline, hyaluronic acid or platelet activating factor.

However, with the advent of intracytoplasmic sperm injection (ICSI), assisted reproduction faced new challenges since by employing this method all physiological barriers for sperm entry into the oocyte are bypassed and the selection process is entirely dependent on the selection the embryologist is doing for a single sperm that is injected. The basis for this selection is viability, for which the criteria are again sperm motility as well as morphology. However, spermatozoa from men diagnosed with severe teratozoospermia are known to have elevated percentages of damaged DNA, which is promutagenic. Since genetic and biological protection mechanisms do not necessarily preclude further embryonic development and cannot always be repaired, such damage might even manifest in the germ line. Considering that in ICSI no biological sperm selection process is involved but single man-selected sperm are injected into oocytes, this risk of injecting sperm with damaged DNA might be even higher and reports on increased chromosomal abnormalities, birth defects or childhood cancer in babies raised serious concerns about this technique. A recent meta-analysis estimated the risk of increased birth defects following assisted reproduction between 30%–40%. In addition, on the one hand, sperm are extraordinary sensitive to oxidative stress and centrifugation. On the other hand, in most conventional sperm separation strategies, centrifugation steps are involved, which can damage mitochondrial function and remove seminal antioxidants protecting sperm. Therefore, it was

imperative to develop advanced strategies which address these serious problems and result in a selection of “healthy” sperm to be used for ART.

While the basis for sperm selection in the conventional strategies is sperm motility, in the advanced strategies the selection principles are based on (i) advanced morphological evaluation, (ii) electrical charges and (iii) molecular binding.

## a) Selection based on morphology

New computer-enhanced sperm morphology evaluation using high power magnifications make it possible that the fine nuclear morphology, particularly of the sperm head and nucleus, of motile sperm can be examined in real time without staining the sample and is called ‘motile sperm organellar morphology examination’ (MSOME). This method lead to the establishment of a new ICSI procedure called intracytoplasmic morphologically selected sperm injection (IMSI). Sperm selected by means of MSOME have not only been shown to have high quality morphological features and significantly lower DNA fragmentation rate, but also significantly higher clinical pregnancy and lower miscarriage rates after IMSI.

## b) Electronegative charges

During epididymal transit, sperm acquire CD52, a highly sialated, lipid-anchored glycoprotein which causes sperm to obtain negative surface charges ranging between -16 and -20 mV. This electrical charge has been called the zeta potential (electrokinetic potential). Using the zeta potential, attempts have been made to separate sperm by charging positively test tubes by rubbing them with latex gloves. Sperm selected by the zeta potential method revealed significantly higher percentages of normal sperm morphology, nuclear maturity, acrosome intactness and less DNA damage. However, due to the fact that the recovery of functional sperm using this method currently is relatively low, the zeta method has not been implemented for use in an ICSI program. Conversely, in a more sophisticated attempt applying the zeta potential with the aim not only to isolate functional sperm but also to separate these sperm from leukocytes that also have a negative surface charge, an electrophoresis method through a perforated membrane with pore sizes allowing only sperm to pass through. Using this electrophoretic method, a normal birth after ICSI has been reported.

## c) Molecular binding

Other alternative approaches are based on the fact that dysfunctional sperm exhibit certain specific surface molecules that bind respective binding molecules like annexin V or hyaluronic acid. Correspondingly, the binding of these molecules has been shown to be significantly correlated with motility, acrosomal function or DNA damage.

## a. Annexin V binding

Annexin V binds to phosphatidyl serine which is externalized from the inner to the outer leaflet of the plasma membrane in case the sperm cells start inducing apoptosis. Such apoptotic sperm can then be identified and eliminated by means of flowcytometric cell sorting using magnetic beads (magnetic-activated cell sorting; MACS) or even activated glass wool. Studies showed that the MACS approach resulted in pregnancies and births after ICSI.

## b. Hyaluronic acid (HA)

The use of HA for sperm separation has already a more



than 20-year history. Initially, however, the focus of the use of HA was on improving sperm motility. More recently, the ability of sperm to bind HA is utilized to select functional sperm for fertilization with ICSI. This approach is promising as HA is non-toxic and reduces genetic complications and it appears that this strategy seems to be the most “physiologic” approach. Principally, two different approaches have been designed, a dish with microdots of HA (PICSI), or a viscous medium containing HA.

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### KS3-2

#### Evaluating human sperm function

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Assessment of human sperm function is essential for evaluating the status of male fertility. Conventional semen analysis provides basic information on sperm count, viability, motility and morphology, but the clinical predictive value of these semen parameters for male fertility is still limited. This is mainly because conventional semen analysis can not detect many other specific sperm defects such as defective sperm-oocyte interaction, immature nuclear chromatin, DNA fragmentation or abnormalities. In order to improve clinical diagnosis of sperm defects causing male infertility, many other sperm function tests have been developed in the past decade, and clinical value of these tests has been evaluated. These tests include computer aided semen analysis (CASA), hyperactivation (HA), sperm nuclear immaturity (toluidine blue or acidic aniline blue stains), chromatin DNA normality (Acridine Orange stain or sperm chromatin structure assay, SCSA, sperm chromatin dispersion, SCD) or DNA fragmentation (TUNEL or COMET assay), sperm capacitation (protein tyrosine phosphorylation), the acrosome reaction (AR) induced by progesterone, calcium ionophore A23187 or the human zona pellucida (ZP), sperm-ZP binding and sperm-hyaluronic acid binding assay (HBA). These tests are likely to detect specific sperm defects that are unable to be revealed by conventional semen analysis. However, the frequency of these sperm defects contributing to a failure of fertilization *in vitro* or male infertility has not been well established. Some of these tests appear to be useful clinically but others are still inconclusive and require further investigation.

Today, assisted reproductive technologies (ART) such as conventional *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the most effective treatment procedures for human infertility. Clinical IVF program provides a useful tool to study the relationship between sperm function tests results and fertilization rate, since the same ejaculate can be used for clinical treatment and sperm tests. Oocyte factors such as immaturity or morphological abnormalities can be detected and excluded from the data analysis. As many sperm test results are usually correlated with each other, a multivariate logistic regression analysis should be used in the data analysis to determine which one or group of sperm tests is most

independently and significantly related to fertilization rate.

In clinical IVF, defective sperm-ZP binding and penetration are the major causes of failure of fertilization. Simply, sperm are unable to pass the barrier of human ZP to fertilize oocytes. With ICSI treatment, a single sperm is injected into the cytoplasm which bypasses the barrier of the ZP. Thus, most patients with previous low or zero fertilization rates in IVF can achieve high fertilization rates with ICSI. Usually a sperm morphological defect is a major contributor to poor sperm-ZP binding and penetration. Although some infertile men with severe teratozoospermia have normal sperm-ZP binding, there is a very high frequency of defective ZP-induced AR which impairs sperm-ZP penetration. Defective ZP-induced AR is also found in about 25% of unexplained infertile men with normal semen analysis and normal sperm-ZP binding. Human ZP plays an important role as a biological barrier to eliminate abnormal sperm. Sperm bound to the ZP have much better morphology and almost all sperm have normal chromatin DNA. Thus there is a biological selection for normal functional sperm during sperm-ZP interaction. Sperm with abnormal morphology and abnormal (immature) chromatin or denatured DNA will be eliminated through the process of sperm-ZP interaction.

Our recent study showed that protein tyrosine phosphorylation, a marker for sperm capacitation, is highly correlated with sperm-ZP binding capacity. While protein kinases are unlikely to be involved as co-receptors of sperm for binding to the ZP, the level of protein tyrosine phosphorylation of capacitated sperm is likely to be a useful marker for normal sperm function including sperm-ZP binding capacity. Furthermore, HA of capacitated sperm assessed by CASA is highly correlated with the ZP-induced AR of ZP bound sperm. Thus Assessment of HA by CASA in capacitated sperm may be able to predict the ability of sperm undergoing the ZP-induced AR after binding to the ZP.

In fertile men, the average ejaculate contains  $300\text{--}400 \times 10^6$  sperm of which about 50% have progressive motility. However, only an average of 14% (range 8%–25%) of motile sperm is able to bind to the human ZP and about 50% of ZP-bound sperm undergoes the AR. Thus there is a very small proportion (average < 7%) of motile sperm in the ejaculate in fertile men that have the capacity to bind to and penetrate the ZP. In other words, the majority of motile sperm in the human ejaculate have no fertilising ability as they are incapable of interacting with the oocyte. This may be the main reason why most sperm tests do not accurately predict sperm fertilizing ability. In future, new sperm functional tests should be developed to detect the sub-population of fertile sperm which is capable of interacting with the ZP of oocytes rather than the whole sperm population in ejaculate.

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### KS3-3

#### WHO standard semen analysis manual (5th edition)

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This workshop will involve audience participation in the form





of answers to questions based on the soon to be published 5th WHO manual: are the laboratories employing WHO methods? Where and how are the samples collected? How is semen volume measured? Do they use WHO-recommended counting chambers, sperm stains and methods of membrane integrity? Do they make replicate measurements? If so, how do they compare the results of the replicates? Do they count sufficient spermatozoa for precise estimations of sperm values? When is azoospermia recorded? How do they interpret reference values? WHO5-recommended answers, and reasons for them, will be provided.

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#### KS3-4

##### **Tulane andrology semen analysis training program and quality control management for multicenter clinical trials**

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**Background:** The assessment of conventional semen analysis parameters such as sperm concentration, motility, volume, morphology, total sperm count, WBC evaluation, as well as other parameters remain problematic even for the experienced technologist. Considerable inter- and intra-laboratory variability exists with regard to the assessment of these semen parameters. The major reasons why the basic assessments of semen samples have variable predictive value (predictive for fertility potential or for the success of drug safety studies) is due to a high degree of variability in the techniques, use of variety of counting chambers and other equipments, training experience of personnel involved in such evaluations. The use of standardized laboratory protocols and techniques are clearly desirable for the success of such studies. **Aim:** The Tulane Andrology Training Program is designed to enable the laboratory technologists to perform a comprehensive semen analysis mainly for drug safety studies using uniform standardized protocols upon satisfactory completion of the training. Emphasis is placed on the elimination of error and assessment of fundamental seminal parameters (volume, sperm concentration, total sperm count, progressive motility, morphology, and round cells/WBC analysis) in order to provide accurate, reproducible and meaningful information. Each participant receives descriptive information and hands-on training on proper semen analysis techniques. Their performance is subject to quality checks not only at the time of training and during post-training certification, but throughout the study duration. **Methods:** Each trainee receives a training manual describing all the protocols and procedures (based on WHO lab manual). Additional updates, depending upon any alteration in original protocols are provided as needed and communicated to each training participant and study site. Two sets of quality control processes are followed: (i) Q/C counts using standardized vials, and (ii) Q/C motility using pre-recorded video format. Each participant receives instructions on overview of training, goals of training, actual hands-on training process in the laboratory; one on one practice session; proficiency and Q/C program testing and assessment; trouble-shooting; emphasis on critical factors that determine the

success of the training as well as the study. The training program requires less than 15% coefficient of variation for its successful quality control management. **Conclusion:** The participants undergo vigorous training to learn: 1) Basic concepts on semen production and sperm parameters. 2) Step-by-step procedures for semen analysis – video demonstration followed by specific discussion. 3) Importance of practice sessions and standardized procedures using donor samples. 4) Importance of Q/C samples so as to minimize the intra-assay and inter-assay variations. 5) Abnormal values, troubleshooting logic, and steps for corrective actions. 6) Competency checks with training quiz, feedback and re-training when indicated. 7) Pass/Fail status at the completion of the training at both Tulane and at respective study sites.

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#### KS4-1

##### **New roles of androgen receptor in the future andrology: 20 years after cloning androgen receptor**

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Cloning of human and rat Androgen Receptor (Science-1988) represents the landmark discovery in the androgen-AR field that allows Urologists for the first time to monitor the AR status of progressing prostate cancer and dissect the mechanisms for failure of androgen deprivation therapy. Later discovery of the first AR coregulator (1996-PNAS) that led to subsequent findings of more than 100 such coregulators and their function in the modulation of Androgen-AR functions in various diseases. In 2002 the generated the first floxed AR mouse (PNAS-2002) that could knockout AR in a selective cell, which then led to the discoveries of AR pathophysiological function in SBMA Neuro disease (Nature Medicine-2007), Neutropenia (JEM-2009), bladder cancer (JNCI-2007), breast cancer (JEM-2003) and liver cancer (Gastroenterology-2008). Knockout AR in individual cell of prostate, then led to discover AR can function as suppressor to CK5-basal intermediate epithelial cell, as survivor to CK-8 luminal epithelial cell and as proliferator to stromal cell. These findings of differential AR function in individual cells of prostate not only help to explain why androgen deprivation therapy via systematic suppression androgen would fail, it also help to develop new drugs to target AR in selective prostate cell to battle prostate cancer.

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#### KS5-1

##### **Evidence on male circumcision and prevention of HIV and other STIs**

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From the 1980s and 1990s several authors had noted the markedly lower prevalence of HIV in countries where circumcision was commonly practiced compared with countries



where circumcision was rare. These ecological associations were supplemented by cross-sectional and prospective studies among men in the general population as well as those at high risk of HIV infection which pointed to a 40%-70% lower incidence of HIV in circumcised compared with uncircumcised men. While the difference in incidence was not likely explained by confounding or other characteristics of circumcised and uncircumcised men, it was not until the results of three randomized controlled trials of men allocated to immediate or delayed circumcision became available that the scientific community and policy makers were convinced. The three trials conducted in East and Southern Africa between 2003 and 2006 showed a consistent 60% reduction in incidence. Potential biological mechanisms for a protective effect of circumcision include lower numbers and accessibility of Langerhans cells, and greater keratinisation and less vulnerability of the shaft and glans penis to minor injuries or tears during intercourse. The picture with regard to the effects of circumcision on other sexually transmitted infections is less clear – little or no effect on gonorrhoea or chlamydia, but some protection against herpes simplex virus type 2 and human papilloma virus.

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#### KS5-2

##### **Male circumcision in resource-limited settings -- development of WHO normative guidance**

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There is now grade 1 evidence that Male Circumcision (MC) reduces the risk of a man acquiring HIV. Modelling studies indicate MC could in the next 10 years save upto 2 million lives in those African Countries with high HIV prevalence. Several African Countries are now scaling up public health male circumcision programmes. The most effective immediate public health MC programmes in Africa will need to target 18–20 years old men. In the longer term there is a need for infant circumcision programmes. In order to implement more widespread MC there is a need to make the surgical procedures as simple as possible so that safe operations can be performed by paramedical staff. The World Health Organisation manual of male circumcision under local anaesthetic was written with these objectives in mind. Included in the manual are three adult techniques and four paediatric procedures. The adult procedures are the dorsal slit, the forceps guided and the sleeve resection methods. Paediatric methods included are the plastibell technique, the Mogen and Gomco shield method and a standard surgical dorsal slit procedure. Each method is described in a step by step manner with photographic and line drawing illustrations. In addition to the WHO manual of surgical technique a teaching course has been developed and using this course it has been possible in one week to train a circumcision surgeon who has had no or minimal previous surgical experience. Further scaling will require training of circumcision surgeons, monitoring

performance, training the trainer workshops as well as advocacy at national, international and government meetings. In addition to proceeding with standardised methods work is in progress to assess novel techniques in adults such as stay on ring devices and policies are being formulated as to how to assess new devices. Also work is in progress to explore efficiencies in surgical processing by task sharing. Proper informed consent and safety remain paramount and great care has to be taken as programmes in Africa scale up. In continental China where the HIV epidemic is at a much earlier stage there may be a case for considering infant circumcision but great care will be needed to ensure that there is no harm.

(For an e-copy of the WHO Manual contact thargreave@urologyedinburgh.co.uk including WHO MC Manual in the subject bar of your email.)

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#### KS5-4

##### **Strategy to promote male circumcision for HIV prevention**

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Following announcement of results in 2005 from the first randomized controlled trial that showed a 60% reduction in risk of HIV infection in circumcised men, WHO and UNAIDS developed technical tools and policy guidance to help countries expand safe circumcision services. Within ten days of the publication of the remaining two randomized trials in early 2007, a WHO/UNAIDS technical consultation made eleven key policy and programmatic recommendations for countries or districts with generalized HIV epidemics and low circumcision prevalence. Since then intensified technical support has been given to countries to conduct situation analyses of circumcision practices and facilities available, assess the costs and cost effectiveness of different scale-up strategies, and develop national circumcision scale-up strategies. Such programmes have been supported by a technical manual on different circumcision technique, training tools, quality assurance and quality improvement resources, and a monitoring and evaluation framework. Following a slow start, rapid progress is now being made. For example, Kenya is implementing a national programme for voluntary medical male circumcision, Botswana has started a national programme to offer circumcision to all adult men within 5 years, and South Africa is putting in place its policy and strategy through the National AIDS Council. Particular challenges for countries include the interface with traditional circumcisers, potential risk compensation if circumcised men believe themselves immune to HIV infection and thus forgo other HIV risk-reduction behaviours, diversion of scarce resources from other high priority interventions, as well as potential negative impacts on women's health programmes.

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**KS6-1****Androgens and male ageing: current evidence of safety and efficacy**

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The endocrinology of the aging male, started by pioneers like professor Alex Vermeulen in Gent /Belgium in the early 1970-ies, has become a hot topic. The finding of a decline of total and free testosterone (T) with aging is well documented. It also has been found that low T predicts mortality in aging populations. It is, however, of note that poor health is associated with a depression of plasma T. But low T, as observed in men receiving androgen deprivation for prostate cancer, induce /worsen the metabolic syndrome and diabetes mellitus type2. Pivotal in this discussion is that only men with (often uncharacteristic) symptoms of hypogonadism who have subnormal plasma T should receive T treatment.

**Efficacy:** Several studies have found an improvement of body composition: reduction of fat mass/waist circumference, increase of lean body mass, improvement of lipid profiles, of fasting insulin and HbA1c, of cytokines. These results are encouraging but longer-term studies investigating clinical endpoints are needed. T administration improves bone mineral density. T administration improves sexual functioning and has an add-on effect in men unsuccessfully treated with phosphodiesterase inhibitors. T administration improves muscle mass and maybe muscle strength. T administration may improve mood. A common denominator of the above studies is that the effects are most prominent in men with true hypogonadal values of T. Further the increment in plasma T following T administration should aim for midnormal plasma T.

**Safety:** the two main complications of T administration are 1) erythrocytosis which is dose-dependent, particularly in elderly men 2) prostate: LUTS does not appear to be a problem. Prostate cancer has not been related to circulating T. A saturation model of the prostate with regard to circulating T has been proposed implying that when plasma T rise over a certain threshold there is no further androgenic effect on prostate (patho-)physiology. Age is the strongest predictor of prostate carcinoma, so elderly men receiving T must be checked periodically for the development of prostate cancer.

**Conclusion:** There is increasing evidence that elderly men with hypogonadal values of plasma T benefit from T administration, particularly in conjunction with changes in lifestyle and diet. Provided there is adequate surveillance, it is acceptably safe.

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**KS6-2****Erectile dysfunction management for the future**

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The management approach for erectile dysfunction (ED) has undergone significant evolution over the past few decades. Only in the recent past has the condition been accurately defined clinically and understood in terms of disease states and pathologic factors involving not only the basic physiology but increasingly the molecular science of the erectile response. Management of ED has similarly become increasingly advanced with a trend toward therapies which are scientifically established and geared toward patient and partner informed decision-making and preferences for therapy. At the same time, progress in the field is moving forward conceptually with respect to what constitutes “ideal therapy”. Current therapy for ED has served patients with minimally invasive, simply administered and effective treatments, although many options remain repetitive in nature and achieve temporary erectile responses, e.g., oral phosphodiesterase type 5 inhibitors. New concepts in therapy for ED are evolving with aims to produce more durable responsiveness and enable natural erectile function restoration and preservation. Exciting areas of investigation that may realize this potential as applied to the penis include novel pharmacotherapies, growth factor therapy, gene and cell-based therapy and regenerative medicine. This presentation serves to explore and discuss scientific foundations for future therapies in ED management.

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**KS7-1****The unlimited potential of human spermatogonial stem cells**

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**Aim:** This study was initially designed to isolate, characterize, and culture human spermatogonial stem cells. We next carried out experiments to reprogram the spermatogonial stem cells and/or their progenitors back to embryonic stem (ES)-like cells. **Methods:** Immunohistochemistry; enzymatic dispersion of germ cells; magnetic activated cell sorting; proliferation assay; cell culture; Western blots; RT-PCR. **Results:** Using immunohistochemistry on testis sections, we localized GPR125 to the plasma membrane of a subset of the human spermatogonia. Immunohistochemistry also showed that MAGEA4 was expressed in all spermatogonia (Adark, Apale, and type B) and possibly preleptotene spermatocytes, whereas KIT was expressed in late spermatocytes and round spermatids, but not in spermatogonia. UCHL1 was found in the cytoplasm of spermatogonia, whereas POU5F1 (also known as OCT-4) was not detected in any of the human germ cells. GFRA1 (a receptor for GDNF) and ITGA6 (also known as 6 integrin, CD49f) were localized to the plasma membrane of the spermatogonia. Next, we isolated GPR125-positive spermatogonia from adult human testes using a 2-step enzymatic digestion followed by magnetic activated cell sorting. The isolated GPR125-positive cells co-expressed putative stem cell markers such as GPR125, ITGA6, THY1 (also known as CD90), and GFRA1.



Using defined media, GPR125-positive cells were cultured as spermatogonial stem cells for short periods of time and exhibited a marked increase in cell number as shown by a proliferation assay. Immunocytochemistry of putative stem cell genes after 2-weeks in culture revealed that the cells were maintained in an undifferentiated state. Western blots showed that ERK1/2 phosphorylation was increased after 2-weeks of culture of the GPR125-positive spermatogonia compared to the freshly isolated cells. Recently, it was demonstrated that spermatogonial stem cells from adult mice are capable of de-differentiating to ES-like cells. The ES-like cells then differentiated into derivatives of the three embryonic germ layers *in vitro*. We and others now demonstrate the startling ability of human spermatogonial stem cells and/or their progenitors to spontaneously de-differentiate to ES-like cells, followed by their differentiation into various cell lineages of the three germ layers. In the system described here, there is neither addition of genes or retroviruses to the germ cells, nor any of the ethical problems associated with the use of human eggs to obtain ES-like cells. In the endodermal pathway we showed that large quantities of insulin-producing cells could be obtained from the ES-like cells. **Conclusion:** These results indicate that human spermatogonia share some but not all phenotypes with spermatogonial stem cells and progenitors from other species. GPR125-positive spermatogonia are phenotypically putative human spermatogonial stem cells and retain an undifferentiated status *in vitro* after several weeks in culture. The reprogramming studies strongly suggest that human spermatogonial stem cells and/or progenitors have great potential for cell-based, autologous organ regeneration therapy for various diseases. This study also provides novel insights into the molecular characteristics, isolation, and culture of human spermatogonial stem cells and/or progenitors and suggests that the ERK1/2 pathway is involved in their self-renewal.

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### KS7-2

#### Small RNAs in epididymis

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Identification, detection, and use of small RNA molecules have displayed incredible importance in the life science. Here, we showed two interrelated lines of this very research in a sperm maturation related organ-epididymis. 1) Small double-stranded RNAs (dsRNAs) called small interfering RNAs (siRNAs) have been used successfully to silence the expression of epididymis-specific genes *in vivo* by a pathway known as RNA interference (RNAi). 2) A series of known and novel small regulatory RNA molecules, referred to as microRNAs (miRNAs) in rat epididymis have been identified. An epididymis-specific non-coding RNA has been identified as the precursor of a 24nt miRNA. An epididymis-specific new member of the carboxyl-esterase family has been identified as its target gene *in vitro*.

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### KS7-3

#### Roles of testicular microRNAs

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MicroRNAs (miRNAs) represent a subclass of small noncoding RNAs that are believed to mainly function as post-transcriptional regulators of gene expression. Genetic ablation of Dicer, a key enzyme required for the production of mature miRNAs, in spermatogonia or Sertoli cells has revealed that Dicer-dependent miRNAs are essential for the development and normal function of these two testicular cell types. Our recent work on selective inactivation of Dicer in pachytene spermatocytes and round spermatids demonstrated that miRNAs were indispensable for the meiotic and haploid phases of spermatogenesis. Using the deep sequencing technology, we have defined the miRNA transcriptome of major testicular cell populations including Sertoli cells, Leydig cells, primitive type A spermatogonia, pachytene spermatocytes, round spermatids and elongated spermatids. Large-scale of expression profiling analyses identified many testis-specific miRNAs. Bioinformatic and reporter assays of mRNA targets of these testis-specific miRNAs revealed that 1) testis-specific miRNAs tend to target testis-specific mRNAs, 2) one miRNA can target multiple mRNAs, and 3) one mRNA can be targeted by numerous miRNAs. The interwoven relationship between miRNAs and their target mRNAs appear to act as a fail-safe mechanism, suggesting important roles of these miRNAs in spermatogenesis. ~20% of the testicular miRNAs are encoded by X chromosome and most of the X-linked miRNAs escape the meiotic sex chromosome inactivation (MSCI) and the post-meiotic sex chromatin (PMSC). This finding represents the first observation of X-linked genes escaping the pan-chromosomal silencing effects, implying possible critical roles of these X-linked miRNAs. Using the pachytene spermatocytes- round spermatid-specific Dicer knockout mice, we have demonstrated that miRNAs were involved in the regulation of translational status of haploid mRNAs by confining those transcripts to the ribonuclear protein particles (RNP) through interactions with the RNA-induced silencing complexes (RISCs). The role of miRNAs in initiation and maintenance of MSCI and PMSC is under extensive investigation in my laboratory.

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### KS7-4

#### Aquaporins in testis and spermatozoa

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Since spermatozoa leaving the testis encounter increasing osmolality in the epididymis during maturation and storage and are challenged by the decrease in osmolality in the female tract



fluids upon ejaculation, cell volume regulation is a crucial sperm function in natural fertilisation. The present work has identified at physiological and molecular levels the water channels aquaporin (AQP) involved in this process.

Studies on mRNA and proteins of testis and spermatozoa limit sperm aquaporins to AQP7, AQP8 and AQP11. In sperm from Aqp7 knockout mice, water efflux and influx occurred faster than the wild type, in accordance to an up-regulation of Aqp8 expression. As AQP8 is present all along the sperm tail whereas AQP11 only at the tip of the tail, and swelling in sperm with blocked RVD (regulatory volume decrease) occurs at the mid-piece/principal piece junction causing angulation of the tail, it is most likely that AQP8 is the major aquaporin responsible for water fluxes during volume regulation. This could explain the present clinical finding that AQP8 expression by ejaculated sperm was inversely correlated with sperm coiling, which is a swelling phenomenon. On the other hand, correlation of AQP7 expression with progressive motility suggests its role in sperm glycerol metabolism. The association of AQP11 with the residual cytoplasm of elongated spermatids and the distal tail of spermatozoa support the hypothesis of a role in the turnover of surplus cellular components and the maintenance of a functional germinal epithelium. These novel findings provide basis for further study of the role of AQPs in testicular and sperm function as well as clinical significance.

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#### KS8-1

##### Progress and prospects for male hormonal contraception

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Finding a life partner and starting a family are near-universal human desires. Fulfilling these expectations requires effective planning because child rearing imposes major life demands; consequently fertile couples practice family planning in order to avoid unintended pregnancies and to space and limit desired pregnancies to best fit their personal desires and financial resources. For these reasons, broadening contraceptive choice to allow men and women the option to share family planning responsibilities satisfies important individual and societal needs. To this end, generating male-directed contraceptive methods is an important objective because the two currently available methods are not widely acceptable because vasectomy is not easily reversible whereas condoms have limited efficacy.

The most comprehensively researched male-directed contraceptives in humans are hormonally based methods of which combined androgen-progestin treatment regimens are the most promising. Male hormonal contraceptive (MHC) methods exploit negative sex hormone feedback suppression of pituitary gonadotropin secretion analogous to inhibition of ovulation by combined estrogen-progestin contraceptives. WHO-sponsored proof of concept studies showing that testosterone treatment profoundly suppressed spermatogenesis and prevented pregnancies are now decades old these observations later confirmed with a longer acting testosterone formulation and

in combination with a progestin to allow more physiological androgen dosing. More recently, it has been demonstrated that androgen-progestin contraceptive regimens (i) can be widely applied in men of differing age, ethnicity and other baseline characteristics; (ii) are superior to androgens administered alone and compare favorably with vasectomy in terms of time of onset of contraceptive action; and, (iii) allow spermatogenesis to fully recover to levels consistent with normal male fertility in all men following cessation of androgen +/- progestin treatments.

Ultimately, pharmaceutical industry needs to be engaged in the process. Unfortunately, disengagement has occurred, at least in the short term, probably due to the perceived profitability of male hormonal contraception in the context of a shrinking global market and an increasingly adversarial medicolegal climate. Despite these setbacks, progress is continuing. Support for male-directed hormonal methods continues to be fostered through internationally focused organizations, such as the World Health Organization and CONRAD who have jointly sponsored the first large-scale androgen-progestin contraceptive efficacy study, involving 400 couples in seven countries (but none in the USA) that will commence shortly, as well as the final publication of results from an important large efficacy study conducted in China. However, what is needed is to re-engage the pharmaceutical industry.

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#### KS8-2

##### Effect of natural and synthetic products on the testis

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For centuries, plants and plant-based products have been used as a valuable and safe natural source of medicines for treating various ailments. The therapeutic potential of most of these plants could be ascribed to their anticancer, antidiabetic, hepatoprotective, cardioprotective, antispasmodic, analgesic and various other pharmacological properties. Many indigenous plant species are being used by tribal communities to cure gynecological disorders and to improve fertility. However, several commonly used plants have been reported to adversely affect male reproductive functions in wildlife and humans. The effects observed with most of the plant and plant-based products have been attributed to the antispermatogenic and/or antisteroidogenic properties of one or more active ingredients. A few of the important natural products that merits discussion as fertility regulators have been extracted from plants such as Cotton plant (*Gossypium barbadense*), Neem (*Azadirachta indica*), Aswagandha (*Withania somnifera*), Carica papaya, Garlic (*Allium sativum*), Pepper (*Piper longum*), Tulsi (*Ocimum sanctum*), Tripterygium wilfordii, Bacopa monnieri, Hibiscus (*Hibiscus rosa sinensis*) and Bitter gourd (*Momordica charantia*).

Gossypol, a phenolic compound isolated from the seed, roots, and stem of the cotton plant, has been shown to perturb the normal course of spermatogenesis and cause immobilization of spermatozoa. Ultrastructural changes in Leydig cells and



Sertoli cells, and alterations in Sertoli-Sertoli cells and Sertoli-germ cells associations have been reported in rodent models on administration with neem leaf extracts. The seed extracts of *Carica papaya*, particularly the chloroform extract and its methanol and ethyl acetate sub-fractions, have been reported to inhibit sperm motility in rats and langur monkeys, and cause azoospermia in rabbits. Chronic administration of garlic powder at a dose of 50 mg has been shown to suppress spermatogenesis and impair cholesterol synthesis leading to androgen depletion. Similarly, antispermatogenic effects accompanied with a marked increase in serum gonadotropins and a decrease in intratesticular testosterone concentration have also been reported in rats exposed to piperine, a major alkaloid present in piper species. Oxidative changes in testis and induction of apoptosis has also been observed in rats exposed to piperine at dose levels of 10 and 100 mg/kg body weight. The benzene extracts of tulsi leaves has been reported to reduce spermatogenesis by retarding the Sertoli cell activity without affecting the germ cells. Long-term oral administration of triptolide, a diterpene triepoxide isolated from the Chinese medicinal plant, *Tripterygium wilfordii*, has been shown to cause germ cell degeneration, multinucleated giant cell formation and tubular atrophy in rodents. Significant alterations in the histoarchitecture of the testis such as intraepithelial vacuolation, loosening of germinal epithelium and exfoliation of germ cells have been reported in mice exposed to *Bacopa monnieri*. The alcoholic extracts of the flowers of hibiscus and seeds of bitter gourd have been demonstrated to exhibit antispermatogenic, antisteroidogenic and androgenic activities in rodent models. Similarly, several synthetic chemicals such as organochlorines, dioxins, phthalates and plasticizers are also known to exhibit estrogenic/ anti-androgenic properties and thereby interfere with the endocrine regulation of male reproduction. A deeper insight into the molecular mechanisms of action of these natural and synthetic compounds could pave way for developing therapeutic strategies against their toxicity.

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### KS8-3

#### Importance of andrology as a clinical discipline

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Andrology is a young interdisciplinary medical speciality that deals with the male and is mainly concerned with the physiology and pathophysiology of male reproductive functions. Thus, its clinical focus is the diagnosis and therapy of male fertility disturbances. Andrology is thought to be the male equivalent to gynecology and therefore takes care of disorders of the male reproductive organs. Often andrology is closely connected to *in vitro* fertilization centers. This supports its significance as an integral part of reproductive medicine. According to a broader definition by the WHO, andrology is engaged in all aspects of male reproductive health.

In addition, andrology is concerned with problems of erectile dysfunction. Approximately, 10%-15% of andrological patients seek advice because of sexual dysfunctions. The

diagnosis of penis problems includes lack of firmness of erection or bending and deformity of the erect penis (Peyronie's disease).

Another important issue is the engagement with the aging male. Due to dramatic changes in the demographic development there will be a tremendous change of the ratio old to young men within the next 20 years. Therefore in the future a great request for consultations in older men will arise particularly from the aspect of health prevention and hormone dysfunction.

Further areas of andrological activities are the diagnosis and management of testicle problems and prostate disorders, for example prostate enlargement, inflammation or cancer (the latter being mostly a genuine problem of urology) prevention of infertility, primary and secondary hypogonadism, delayed puberty, adverse drug side effects and environmental pollutants with regard to fertility, cryopreservation of semen and testicular tissue, paternity problems in forensic medicine, family planning, male contraception and basic andrological research. All these issues are of increasing importance for the further development of clinical andrology as an independent medical discipline, however, working in close interdisciplinary cooperation with urology, endocrinology, dermatology, gynecology, neurology, sexology, psychosomatic medicine, psychiatry, molecular genetics, environmental medicine and veterinary medicine as well as with basic sciences such as reproductive physiology, biochemistry, immunology, virology or epidemiology.

Both history taking and clinical examination of the male patient are essential for the andrological workup and diagnosis of a male factor in the case of infertility. Often only the clinical examination of the patient allows the correct interpretation of the semen parameters leading to an etiopathologically orientated therapy. Therefore, to improve the management of andrological problems, formal training and performance of training courses in clinical andrology are required. This is emphasized by the fact that presently only a few text books and WHO guidelines on the subject of andrology are currently available (Rowe *et al.* 1993, 2000; WHO 1999; Nieschlag and Behre, 2000; Schill *et al.* 2006). In conclusion, further training and education in all aspects of clinical andrology are urgently needed.

The activities of the International Society of Andrology (ISA) together with the national societies and the European Academy of Andrology (EAA) are of particular importance for the proclamation of andrology to receive more attention and awareness by the national health politicians. Progress has been made in that andrology as a medical specialty was accepted by the medical associations of Indonesia (2002), and Germany (2003). The qualification andrologist may be acquired in Germany by dermatologists, endocrinologists and urologists, but not by gynecologists. A medical skills certified by the medical association allows the identification of the specialist by the patient, guarantees quality control and efficiency assurance and attracts more medical professionals to get into the field. It is the merit of the WHO expert group within the special program of research development and research training in human reproduction by introduction of the WHO laboratory manual to achieve the goal to establish andrology as a basic and clinical speciality. Thereby, a worldwide acknowledgement of andrology as an essential partner of reproductive medicine was accomplished.

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## KS8-4

**Nutrients and Botanicals for optimizing Men's Health. Examining the evidence for *Eurycoma longifolia* jack, the Malaysian Ginseng in men's health**

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The tropical rainforest in Malaysia showed its species richness that is unique where many of the flora species have been found to contain value as food, medicine, therapy resources and many others usage. The Malay Peninsula early literature of modern botany started in 1511 under Portuguese rule which became more established with the coming of the British colonies with their professional botanists who wrote about the flora, vegetations, taxonomy, systematic, and ethno botany in Malay Peninsula. Malay traditional medicine manuscript published in 1930 by Garden Bulletin as "The Medical Book of Malayan Medicene" edited by Dr J. D. Gimlett and I. H. Burkill glorified plants among which is the Simaroubaceae species (specifically *Eurycoma longifolia* jack) that have special role in men's health. That's how *Eurycoma longifolia* came into the picture as the botanical that potentiate men's health and since then much studies have been done and documented and the standardized extract being patented and sold worldwide as active botanical for men.

The Malaysian Ginseng or *Eurycoma longifolia* jack known scientifically or Long Jack as it is internationally known, is a common herbal shrub found along the slopes of hilly terrains of the Malaysian rainforests. Its medicinal value discovered were unique to the ground where it is found. The aphrodisiac phyto-compound found in the Malaysian Peninsular species is more in concentration when compared to the species found elsewhere. It contains all sorts of alkaloids of the Quassinoid variety which have the properties of healing Malarial fever, being antischistosomal, in managing allergies and common fevers, cytotoxic, inducing apoptosis and anti-tumors. The standardized water-soluble extract contains phenolic compounds, tannins, high molecular weight polysaccharides, polypeptides with 30-39 amino acids and 4 300 Daltons (labelled as Eurypeptides), glycoprotein and mucopolysaccharides. The Eurypeptides and glycoprotein complex exert anti-cancer, pro-fertility, aphrodisiacs and anti-aging properties. The water-soluble extract portion is non-toxic even at higher dosage. The standardized water-soluble extract has been patented in 2004(US2004/0087493 A1) after having gone extensive animal and human clinical evaluation.

**Animal studies:** early studies in 1980-1990 on *Eurycoma longifolia* jack on the mouse animal model with organic extract and water-soluble extract done by the late Dr Ang Hooi Hoon and co-workers from School of Pharmaceutical Sciences, University Science Malaysia, Minden, Penang and Dr Johari Saad and co-workers of University of Malaya reported increase levels of testosterone and increase fertility based on sperm motion and activation profiles using computer assisted motion analyzer. In 2002 Dr Azimahtol, School of Biosciences and Biotechnology, Faculty of Science and Technology, National University of Malaysia, evaluate the effect of water-soluble extract comparing

with sildenafil citrate (Viagra) in triggering penile erection. The Standardized extract increases and enhances the levels of cGMP and cAMP, which ultimately leads to penile erection. In an earlier study on cancer cells Prof Azimahtol found the water-soluble extract has anti proliferative effect on human breast cancer cell line, MCF-7 and induced apoptosis of cancer cells. The ethanol extract has been found to be toxic to liver and the kidneys and only the standardized water-soluble extract is used.

**In-vitro studies:** Dr Harniza and Dr Johari from the University of Malaya isolated the bioactive complex polypeptides from the standardized water-soluble extract, labeled as Eurypeptides and tested its effect on the biosynthesis of various androgens. The Eurypeptides works by activating the CYP17 (17  $\alpha$ -hydroxylase/17, 20 lyase) enzyme to enhance the metabolism of pregnenolone and 17-OH pregnenolone to yield more dehydroepiandrosterone (DHEA) as well as the metabolism of progesterone and 17-OH progesterone to 4-androstenedione. Excess DHEA is aromatized into testosterone to be utilized at target sites.

**Early human trials on *eurycoma longifolia*:** the landmark studies on human testicular cells incubated with standardized water-soluble extract of *Eurycoma longifolia* and with control pregnenolone incubated with the same herb showed the herb potentiate steroidogenesis in the testicular cells with 4 times increase level of testosterone and testosterone precursors. There was no increase in Dehydrotestosterone which is associated with cancer of prostate. In a double blind, placebo controlled study conducted in 2000 by Dr Sareena from the Department of Sport Science at the University of Malaya, provided 14 healthy adult males with either 100 mg day<sup>-1</sup> of the Standardized extract or placebo for 8 weeks. Both groups performed an intensive strength-training program for 8 weeks. The group on the Standardized extract showed improved body mass index as well as muscle strength. In a non comparative non placebo controlled study conducted in 2001 by Dr Ismail Tambi at the Reproductive Research Center in Kuala Lumpur, Malaysia, where 30 married men, aged 31–52 years old, took 200 mg of the standardized extract regularly for three consecutive weeks. Interviews indicated that 73% felt healthy, active and energized, 82 improved psychological wellbeing and 62% felt increased sexual desire. The subjects showed an increased free testosterone index. Increased testosterone levels help improve libido, energy and muscle mass for bodybuilding. Their levels of DHEA, another critical steroid hormone, gradually increased from 26% after one week to 47% after three weeks.

**Extended clinical trials:** a double-blind placebo controlled human clinical trial conducted in 2004 by Dr. Ismail Tambi where 20 male volunteers of various health conditions from the ages of 38 to 58 randomly given 200, 400, or 600 mg of the standardized extract or placebo for 2 months showed elevated SHIM scores showing improvement in sexual desire and performance as well as improved AMS score showing improvement in sexual, physical, psychology, and vasomotor domains for majority of the volunteers. Testosterone and DHEAS levels showed high normal levels when compared to baseline. Diabetic volunteers showed an improved level of blood glucose. The cortisol levels were fluctuating suggesting the extract modulates the release of cortisol. The majority of volunteers have high normal levels



of thyroxin and IGF-1 compared to the placebo group. There were no changes to the tumor markers, liver function, renal function, blood profile and lipid profile, though majority showed elevation of HDL. The study by S. Talbott, *et al.*, to determine the effects of the standardized extract on cortisol and testosterone levels during high-intensity exercise on men who were taking part in a 24-h mountain bike event where the subjects provided a saliva sample prior to and after each of four 14.91-mile laps; the eight samples of each participant underwent an enzyme immunoassay (Salimetric, State College of PA) to determine and compare levels of cortisol and testosterone. This was compared to placebo samples. Both placebo and supplement were taken approximately 30 minutes prior to exercise. It was found that cortisol levels in the standardized extract group were significantly lower—33 percent—than in the placebo group. Also, testosterone levels in the supplement group were 16.4 percent higher than that of the placebo group indicating that standardized extract of *Eurycoma longifolia* is a potent anti-stress as well as androgenic even within 24 h of taking. Study by Dr Ruxiang Wang *et al* from Liaoning Sport Science and Technology Institute, China in 2007, studied standardized extract of *Eurycoma longifolia* on elderly male cyclists to explore ergogenic and fatigue recovery effect, found the extract enhances muscular strength and facilitates fatigue recovery besides enhancing free testosterone, increase IGF-1 and reducing SHBG level. Recent male fertility studies done in a Fertility Centre in Kuala Lumpur, Malaysia using standardized extract, given to males with Idiopathic Oligospermia, Asthenospermia and Teratospermia improves sperm profiles with three to six months of continuous usage which resulted in achieving 17% spontaneous pregnancy in an otherwise non productive marriage. The rest achieved pregnancies through ART since the post herbal extract sperm samples were sufficient for such procedures, which were not before taking the herb.

**Conclusion:** standardized water-soluble extract of *Eurycoma longifolia* jack, the Malaysian Ginseng has undergone numerous Clinical Trials and evaluations locally and internationally and has been found to be a beneficial botanicals optimizing men's health. The Standardized extract has been patented as a supplement for men's health specifically in enhancing testosterone and improving male fertility of idiopathic causes. The extract has been found to be non-toxic even at a high dose of 600mg to the body vital functions. Extended studies on the same extract has proven the herb to be an energizer, an aphrodisiac, an ergogenic botanicals which also facilitate muscle fatigue recovery and anti stress. It is a potent adaptogen as well. Overall effects on the various hormones are also positive. It enhanced total testosterone and DHEA (Dehydroepiandrosterone) which also modulate testosterone level, ensuring enough free testosterone for the body's need. Its other adaptogenic effects include positive effect on body cortisol levels as well as on growth hormone as it modulates the released of IGF-1. With all these ample scientific and clinical findings, Standardized patented water-soluble extract of *Eurycoma longifolia*, the Malaysian Ginseng has emerged as a potent botanical supplement in men's health.

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### S1-01

#### The concerns on genetic risk during assistant reproductive technology application

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Assistant reproductive technology (ART) is an evolving field where new technologies are currently introduced without sufficient knowledge of their potential long-term effects. Some studies have suggested that the potential risks of ART including genetic risk on offspring should be paid attention to. Firstly, the average pregnancy ages of the patients with ART treatment usually are older than the normal pregnancy women. Secondly, some ART patients have medical problems such as reproductive system diseases and genetic defect which often result in abortion. Thirdly, the technologies in assistant reproduction such as control ovarian hyperstimulation (COH), gamete maturation *in vitro*, embryo culture, micromanipulation and frozen-thawed embryo, may interfere the genetic information and induce unexpected problems. Our laboratory now is interested in evaluation of genetic risks during ART procedure.

There are some factors which may affect the genetic information of embryo and offspring. These factors include elder age, reproductive system diseases and genetic diseases. The pregnancy age of women may play an important role in the genetic safety of offspring as well as in infertility. The women with elder age usually have higher risk in outcome of pregnancy. The diseases of reproductive system include PCOS, endometriosis and ovarian premature senility in women, and oligospermia, aspermia and teratospermia in men. These diseases are the common causes of infertility. The genetic diseases include abnormal euchromosome, abnormal sex chromosome and single gene disease which often result in infertility or abortion. The genetic diseases are also the base of PGD technology. In our laboratory we now can examine some genetic diseases including single gene disease using PGD.

COH, gamete maturation *in vitro*, embryo culture, micromanipulation and frozen-thawed embryo may affect the development of gamete and embryo. Some studies have shown that ART might affect genetics or epigenetics, resulting in the changes in the development of gamete and embryo. Our study demonstrated that after culture of embryo with medium some genetic imprints of infant and placenta were changed. We also found that the abnormal expression of methylase and acetylase in ART embryo might result in the abnormal methylation of genes and acetylation of proteins. Furthermore, our study on proteomics in ART placenta showed that there was different expression of some proteins with different functions. We also found that the rate of new microdeletion of Y chromosome in ART offspring was higher than that in natural pregnancy offspring. Genomics study showed that some ART offspring had different gene expression. Part of these different genes is imprint genes and had the difference in imprint gene.

ART is rapidly evolving and helping a lot of infertile couples. As for any medical procedures, there possible unexpected effects including genetic risks which need to be



envisaged to make sure that the balance between benefits and risks are clearly on the benefit side. The further study is needed to evaluate the risk including genetic risk during ART procedure and increase the safety when we treat patients with ART.

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### S1-02

#### The outcome after intracytoplasmic injection of fresh and frozen-thawed epididymal and testicular sperm for different cause

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**Aim:** To assess whether the outcome of intracytoplasmic sperm injection (ICSI) using sperm surgically retrieved from men with obstructive azoospermia (OA) or nonobstructive azoospermia (NOA) depends on the cause and to compare the outcome of ICSI with fresh and frozen-thawed epididymal spermatozoa. **Methods:** Sperm was retrieved by percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA) and ICSI was performed. We analyzed our data to compare the outcome of ICSI with sperm from epididymus in terms of congenital bilateral absence of vas deferens (CBAVD, in 136), acquired causes (76), idiopathic (10) and ejaculatory dysfunction (10). The rates of fertilization, cleavage, implantation and pregnancy were evaluated. **Results:** Overall, 232 PESA procedures were performed in the husbands, followed by ICSI procedures with either fresh ( $n = 209$ ) or frozen-thawed ( $n = 23$ ) epididymal spermatozoa. Thirty-two TESA procedures followed by ICSI were all fresh spermatozoa. There was no difference in outcome, whether the sperm was fresh or frozen, or whether epididymal and testicular. The fertilization, cleavage, quality embryo, implantation (24% vs. 19.4%) and clinical pregnancy (38.5% vs. 33.3%) rates were comparable with sperm from testicular among the normal and impaired spermatogenesis groups. The fertilization, cleavage, quality embryo, implantation and clinical pregnancy rates were comparable among the four groups with sperm from epididymus, apart from implantation rate on acquired cause and ejaculatory dysfunction (33% vs. 15%,  $P < 0.05$ ). **Conclusion:** The clinical outcome of ICSI with fresh and frozen-thawed spermatozoa after retrieval by PESA was similar. Sperm origin (epididymal vs. testicular), status (fresh or thawed), cause (CBAVD, acquired causes, idiopathic, ejaculatory dysfunction) had no significant effect upon fertilization implantation rate or pregnancy rate. Epididymal retrieval and thereafter cryopreservation should remain the first choice for OA men.

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### S1-03

#### Beneficial effects of polydeoxyribonucleotide (pdrn) on spermatogenic activity in experimental varicocele

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**Aim:** Varicocele affects male fertility and is the most common known cause of infertility which can be surgically treated. Varicocele activates the inducible hypoxic factor 1 alfa (HIF-1 alfa) and the vascular endothelial growth factor (VEGF) with neoangiogenesis. Experimental evidences seem to recognize a protective role of VEGF that could improve the spermatogenic activity. VEGF exerts its biological function by binding to tyrosine kinase associate receptor, namely VEGF-R1 (flt-1) and VEGF-R2 (flk/KDR), which are also located on the surface of Leydig and Sertoli cells. However, the mechanisms that regulate the alterations of varicocele have not yet been fully clarified and as a consequence there is still a strong need to identify specific pharmacological treatments to limit the damage. It has been proposed that adenosine may influence the fertility in spermatozoa through interaction with A2 receptors. In this regard A2 receptor could be of strategic importance in the treatment of the infertility. Polydeoxyribonucleotide (PDRN) is the active fraction of a preparation used in therapy as a tissue repair stimulating agent. PDRN is a compound that, acting through adenosine receptors, is able to stimulate VEGF production. In this study we evaluated the effect of PDRN on experimentally induced varicocele in the rat. **Methods:** Rats were anesthetized with an intraperitoneal injection of 50 mg kg<sup>-1</sup> of pentobarbital sodium and were subjected to partial ligation of the left renal vein to produce reflux in the left spermatic vein and create a varicocele in the pampiniform plexus for 28 days (Varicocele group). Sham operated rats underwent to the same surgical procedures except for renal vein partial occlusion. The animals were randomized to receive after 28 days: 1) PDRN (8 mg kg<sup>-1</sup> ip<sup>-1</sup>) for 7 or 14 days; 2) PDRN (8 mg kg<sup>-1</sup> ip<sup>-1</sup>) plus DMPX (0.1 mg kg<sup>-1</sup> ip<sup>-1</sup>) concomitantly for 7 or 14 days. The animals for each group were killed after 7 and 14 days respectively. VEGF messenger RNA and protein expression were evaluated by RT-PCR and Western blotting analysis. Furthermore, histological damage, immunohistochemistry analysis for VEGF and VEGF receptor-1 and spermatogenic activity were assessed from testis samples. **Results:** Varicocele caused an increase in VEGF mRNA and its protein. Furthermore, histological examination revealed a marked damage and reduced spermatogenic activity. PDRN administration increased significantly VEGF mRNA and its protein, decreased histological damage and ameliorated spermatogenic activity. The concomitant administration of DMPX reverted the protection exerted by PDRN. **Conclusion:** Our results demonstrated that PDRN might represent a new potential therapeutic approach in patients with varicocele.

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### S1-04

#### Defective hMLH1 expression in patients with non-obstructive azoospermia

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**Aim:** To identify the expression of hMLH1 and its methylation status in the testes of patients with non-obstructive azoospermia (NOA). **Methods:** The expression level of hMLH1 mRNA and the localization of hMLH1 protein in the testes of controls and patients with NOA were examined by the reverse transcription-PCR and immunohistochemistry, respectively. Methylation specific-PCR was used to study whether the promoter regions of hMLH1 were methylated. The genome DNAs of both normal and NOA patients' testes were modified by bisulfate and the CpG islands (region B or region C) of hMLH1 promoter were amplified with unmethylation or methylation primers. Patients undergoing orchiectomy for prostate carcinoma or testicular biopsy for obstructive azoospermia) were recruited for this study as controls. The pathological types of NOA patients were subdivided as sertoli cell only syndrome (SCOS), hypospermatogenesis and germ cell arrest. Informed consent was obtained from all patients, and this study received ethical approval from the institutional review boards of the University of Science and Technology of China. **Results:** The levels of hMLH1 mRNA were decreased in testes of patients with germ cell arrest and SCOS compared to that in normal controls, however, no obviously decreased levels of hMLH1 mRNA were found in patients with hypospermatogenesis. Immunohistochemical results indicated that hMLH1 proteins were exclusively expressed in primary spermatocyte. The expression levels of hMLH1 were also found to be significantly reduced in patients with germ cell arrest. Complete absence of hMLH1 expression was observed in SCOS, since these testes have no any meiotic cells. Similar to the expression levels of hMLH1 mRNA in patients with hypospermatogenesis, no significant alteration in hMLH1 levels in these patients. **Conclusion:** Decreased numbers of meiotic recombination hMLH1 foci have been demonstrated in infertile men. However, little is known about the mRNA and protein expression of hMLH1 in patients with non-obstructive azoospermia (NOA). During this study, defective hMLH1 expression was identified in patients with NOA, which was strongly related to their testicular pathological types. Abnormal expression of hMLH1 may lead to male infertility.

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### S1-05

#### Medium containing caffeine can stimulate motility of epididymal or testicular sperm and can better facilitate motile sperm selection for ICSI

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**Aim:** Caffeine has been known to stimulate motility of human and animal sperm. The aim of this study is to evaluate if caffeine can substantially stimulate motility of epididymal and testicular sperm obtained by percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA), Testicular Sperm Extraction (TESE), or testicular sperm aspiration (TESA). Furthermore, pregnancy and delivery rates of transferred embryos were evaluated when epididymal or testicular sperm treated with caffeine were used for intracytoplasmic sperm injection (ICSI). **Methods:** Motility of fresh or frozen-thawed

epididymal or testicular sperm was evaluated before and after treatment with a medium containing caffeine. A stock medium (10X) of HTF (SAGE *In vitro* Fertilization, Inc, Trumbull, CT, USA) containing 3.88 mg mL<sup>-1</sup> caffeine-sodium benzoate (Sigma, St. Louis, MO, USA) was made, aliquoted, and then stored in a -20°C freezer until use. Fresh or frozen-thawed epididymal or testicular sperm samples with poor motility were treated with medium containing caffeine (one part caffeine medium mixed with nine parts sperm sample). Motile (including tail twitching) and immotile sperm were counted before and after caffeine treatment. Motility was calculated and statistically compared (paired *t*-test) between samples before and after caffeine treatment. Initial test showed substantial increase of motility of epididymal or testicular sperm after caffeine treatment. Therefore, sperm treated with caffeine were used for ICSI in such cycles. Briefly, epididymal or testicular sperm with poor motility were treated with caffeine. Samples were not washed after caffeine treatment and directly placed in the ICSI dishes adjacent to the 7% PVP drop (SAGE *In vitro* Fertilization, Inc, Trumbull, CT, USA). The sperm were picked up with ICSI needle, moved to PVP drop, immobilized, washed four times and then aspirated into the ICSI pipet for injection into mature oocytes. After ICSI, oocytes were cultured in Quinn's advantage media series (fertilization, cleavage, and blastocyst media) for fertilization and embryo development. Embryos were transferred on day-3 (cleavage stage) or on day-5 (blastocyst stage). **Results:** Eight fresh or frozen-thawed epididymal or testicular sperm samples were evaluated for motility before and after caffeine treatment. Mean percent of motile sperm was 6.31% (1.55% to 18.6%) before treatment and was increased to 23.5% (7.3% to 49.1%) after caffeine treatment which was statistically significant ( $P = 0.0052$ ). Nineteen (19) ICSI cycles were performed using sperm treated with caffeine. Out of a total of 250 oocytes injected, 188 fertilized (fertilization rate: 75.2% with a range from 50% to 100%); and 97 (97/188, 51.6% per fertilized eggs) transferable embryos were obtained, of which 35 (6 cleavage stage and 29 blastocyst stage) were transferred fresh and 62 expanded blastocysts were cryopreserved. Out of 19 cycles, 16 had embryo transfer, resulting in 12 ongoing pregnancies (63.1% per cycle and 75% per transfer) with an implantation rate of 42.8% (15/35, cardiac activity). Ten patients delivered 13 babies (seven singletons and three sets of twins), one pregnancy is still ongoing with a singleton and one was spontaneously miscarried. All babies delivered appear to be healthy. Out of three cycles with no embryo transfer, two patients had embryos cryopreserved. One of which underwent a frozen embryo transfer, resulting in a singleton pregnancy with live birth. **Conclusion:** Caffeine can substantially stimulate motility of fresh or frozen-thawed epididymal and testicular sperm and this treatment can greatly assist the finding of live sperm for ICSI. Oocytes injected with caffeine treated epididymal or testicular sperm can yield good fertilization rates (75% with a range from 50% to 100%). The embryos developed had high implantation potential (42.8%) and produced viable pregnancies and healthy babies. This technique has potential to significantly improve chances for pregnancy with sperm recovered by PESA, MESA, TESA and TESE.

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**S1-06****Effect of sperms of different treatments on porcine oocytes activation by ICSI**

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**Background:** Activation of the oocyte is a fundamental developmental event that includes such processes as cortical granule exocytosis, zygotic genome activation, prevention of polyspermy and the release of the oocyte from meiotic arrest (Whitaker, 2006). The clinical technique called intracytoplasmic sperm injection (ICSI) has been of great importance for treating infertility. However, it is known that 2%–3% of ICSI cycles fail as an apparent consequence of the oocyte failing to activate (Mahutte and Arici, 2003). The mechanism of oocyte activation during fertilization has been an unresolved issue in reproductive biology for many years (Parrington *et al.*, 2007). Identifying the correct mechanism is important in terms of furthering our understanding of a fundamental biological process, as in many cases where ICSI fails, a key reason appears to be failure of the injected sperm to activate the oocyte (Araki *et al.*, 2004; Heindryckx *et al.*, 2005; Moaz *et al.*, 2006). **Aim:** To determine how different treatments of spermatozoa affects the oocyte activation by ICSI. **Methods:** Ejaculated (EJ) and epididymal (EP) spermatozoa were divided into three groups: 1) spermatozoa without any treatment (control = C); 2) spermatozoa washed through Percoll® gradient (P); and 3) spermatozoa washed through Percoll® and incubated with porcine oviductal fluid protein (50 µg mL<sup>-1</sup>) for 30 min (P-POF). Oocytes were matured *in vitro* 44h in NCSU-37 media, and microinjected with sperm from the different groups. Four h after injection, the putative zygotes were stained with Hoechst and classified as: (i) metaphase II; (ii) activation. A total of 461 oocytes was injected in 6 replicates. **Results:** Oocyte activation (categorical data) was modeled using a binomial model of parameters and analyzed by ANOVA. The results showed that the oocyte activation was higher in EJ sperm (P and POF groups, 52.5% ± 4.9% and 57.3% ± 5.0%, respectively) than in EJ-C (32.9% ± 5.1%) and EP sperm (C: 13.4% ± 4.8%; P: 10.7% ± 5.0%; and P-POF: 26.3% ± 5.0%). **Conclusion:** The modification of EJ sperm membrane by washing sperm through Percoll® gradient or incubating with POF induces higher oocyte activation.

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**S1-07****Initial analysis on clinical effects of ICSI with frozen-thawed epididymal and testicular sperm**

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**Aim:** To discuss whether diagnostic epididymal and testicular biopsy followed by cryopreservation for intracytoplasmic sperm

injection (ICSI) was feasible to clinical treatment. **Methods:** This retrospective study involved 46 treatment cycles of 46 patients with obstructive azoospermia. Cryopreserved group (five cycles) was scheduled with frozen-thawed epididymal and testicular sperm from diagnostic epididymal and testicular biopsy. Control group (41 cycles) was scheduled with fresh epididymal and testicular sperm at the day of oocyte retrieval. Fertilization, embryo quality, pregnancy and implantation rates were evaluated. **Results:** A total of 78 471 matured MII oocytes were used for ICSI in cryopreserved group and control group respectively. Fertilization rate, cleavage rate and high quality embryo rate were 74.7% ± 19.0%, 88.5% ± 3.3%, 67.9% ± 18.5% in cryopreserved group, and 78.3% ± 17.3%, 90.5% ± 18.1%, 48.4% ± 26.3% in control group. There was no significant difference between the two groups. In the cryopreserved group, four clinical pregnancies were achieved including one ectopic pregnancy and three ongoing pregnancies. In the control group, 17 clinical pregnancies were achieved including two miscarriages and 15 ongoing pregnancies. A significant higher clinical pregnancy rate and implantation rate were observed in cryopreserved group than in control group (80.0% vs. 43.6%; 50.0% vs. 26.3%, *P* < 0.05), but the differences between the two groups were of no statistical significance. **Conclusion:** Our results indicated that diagnostic epididymal and testicular biopsy followed by cryopreservation for ICSI was feasible and efficient for patients with azoospermia. The clinical outcomes were similar to that of ICSI with fresh epididymal and testicular sperm.

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**S1-09****Effect of obstruction to sperm egress on the male testis and epididymis**

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**Background:** The effects of obstructive azoospermia on the testis and epididymis have been studied in vasectomized subjects. However, effects of obstruction to sperm egress in primary obstructive azoospermia on the testes and epididymis have not been extensively reported. The present study on fifty patients of primary obstructive azoospermia focuses on the local immune response and its effect on the testis and epididymis. This was a prospective study in a tertiary hospital setting comparing epididymal histology and epididymal fluid antisperm antibody in men with primary obstructive infertility with those in men with proven fertility. **Aim:** To study the effect of local immune response due obstruction on the testis and epididymis in 50 patients undergoing vaso-epididymostomy. **Methods:** Fifty men with primary obstructive infertility in the study group and ten subjects with proven fertility as control were included in the study. While performing vasoepididymostomy

on such patients, testicular and epididymal tissues were taken, and epididymal fluid was aspirated for estimation of antisperm antibodies. Testicular and epididymal histologies were studied under light microscopy for evidence of cellular immune response to obstruction. Antisperm antibodies in epididymal fluid were assayed by ELISA. **Results:** No effects of obstruction were evident on spermatogenesis or the testicular histology. Epididymal epithelial flattening with local or diffuse loss of cilia ( $n = 25$ ); pigment in the epithelial cells as a remnant of sperm ingestion ( $n = 20$ ); intraductal macrophages ( $n = 29$ ) with sperm ingestion in 62%; breach of epithelium with sperm extravasation ( $n = 20$ ) were considered as evidence of effects of obstruction leading to proximal ductal dilatation and damage. Presence of plasma cells and lymphocytes ( $n = 31$ ) and macrophages ( $n = 11$ ) in the interstitium were considered effects of antigenic sperm extravasation in the interstitium. These effects could be a local cell mediated immune response. Significant ELISA for antisperm antibodies was found in 29 patients; all patients with inflammatory cell infiltrate in the interstitium had significant ELISA for antisperm antibodies in the epididymal fluid. The cellular immune response as assessed by the presence of interstitial inflammatory cells and macrophages in epididymal histology was found in 12/20 patients. Testicular biopsies in all patients were normal. Humoral immune response as assessed by significant titres of antisperm antibodies in epididymal fluid was found in 14/20 patients. Evidence of local immune response (cellular or humoral or both) was found in 17/20 patients. **Conclusion:** We believe that intermittent exposure to small amounts of extravasated sperms in the epididymal interstitium would be sufficient to produce a local cell mediated immune response with its attendant effects on the epididymis, the primary site of sperm maturation, producing irreversible changes in its structure. These changes in the epididymis are in addition to those due to increased intraductal pressure as a result of obstruction to sperm egress.

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#### S1-10

##### **FSH and HCG combined therapy for hypogonadotropic hypogonadism**

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**Aim:** To assess the safety and efficacy of follicle stimulating hormone (FSH) and human chorionic gonadotrophin (HCG) combined therapy in patients with hypogonadotropic hypogonadism (HH syndrome). **Methods:** An open-label self control study was conducted in 43 male patients aged from 14 to 36 years old (average age 21 years old). All these patients were diagnosed with HH syndrome, including seven cases of Kallmann syndrome, two cases combined with bilateral cryptorchidism, one case after cryptorchidopexy. Patients underwent combined intramuscular administration of FSH (75IU three times a week) and HCG (2000IU twice a week) at least for 3 months. **Results:** After 3 months of therapy, all the patients felt stronger and more

vigorous; 27 (79%) patients out of 34 patients rated as Tanner stage 1 before treatment developed to stage 2, with growing of beard, pubic hairs and/or axillary hairs; four (44%) patients out of nine patients rated as Tanner stage 2 before treatment developed to stage 3. Testicular volume by palpation increased from  $2.88 \pm 1.79$  mL to  $4.66 \pm 3.12$  mL ( $P < 0.05$ ); testicular volume by B-ultrasonography increased from  $2.10 \pm 1.37$  mL to  $3.94 \pm 2.97$  mL ( $P < 0.05$ ). After 3 months of therapy, sperm was found in one (3%) patients out of 39 azoospermia patients before treatment. After 9 months of therapy, sperm was found in five (13%) patients out of the 39 azoospermia patients. Compared to the serum sex hormone level before treatment, serum follicle stimulating hormone (FSH) and testosterone (T) increased ( $P < 0.05$ ), while luteinizing hormone (LH) decreased ( $P > 0.05$ ) after 1.5 months of therapy. Serum FSH and T increased ( $P > 0.05$ ), while LH decreased after 3 months of therapy ( $P > 0.05$ ). Two patients combined with bilateral cryptorchidism (the positions of cryptorchidism varied from annulus inguinalis internalis to upside of scrotums) were already 14 and 16 years old, after 3 months of therapy, their bilateral testis descent to scrotums and enlarged. One patient underwent cryptorchidopexy responded well to FSH and HCG combined therapy. Two (33%) out of 6 patients aged from 26 to 28 years old responded well to the FSH and HCG combined therapy; four patients over 30 years old showed no improvement over base line before treatment except for increased libido after 3 months of therapy. There was no impairment of liver or kidney functions in all patients, even after nearly 2 years therapy. **Conclusion:** Long time combined intramuscular administration of FSH and HCG is safe and has no obvious side effects; for patients with HH syndrome less than 28 years old, FSH and HCG combined therapy can promote secondary sex characteristics development and enhance testicular functions of testosterone secretion and spermatogenesis.

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#### S1-11

##### **The roles of meiotic regulator BOLL and its downstream substrate CDC25A in male infertility**

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**Aim:** Boll is well-known as meiotic regulator in fly and exerts its function by activation of CDC25 phosphatase. Of the CDC25 family in mammalian, CDC25A is abundantly expressed in testicular germ cells and is considered as a potential RNA target of BOLL. Our objective was to clarify the relationships between the BOLL and CDC25A expressions in the testes of infertile men and to investigate the presence of mechanism by which BOLL modulates CDC25A expression. **Methods:** The protein expression of BOLL, mRNA and protein expressions of CDC25A as well as spermatocyte number in the testes of 32 infertile men were measured. The interaction between BOLL protein and CDC25A mRNA was studied by co-immunoprecipitation, RNA-electrophoretic mobility shift assay, RNA stability assay and translation assays in HeLa cells. **Results:** The protein expression levels of BOLL and CDC25A were significantly decreased in

patients with maturation arrest at the spermatocyte stage. A significant and positive correlation between BOLL and CDC25A protein expressions was noted, and both their expressions were significantly correlated with spermatocyte numbers. Correlation analysis between upstream (BOLL) and downstream (CDC25A) expressions suggests a cause and effect relationship between BOLL and CDC25A in humans. In HeLa cells, BOLL directly binds to CDC25A mRNA via a 21-nucleotide region of the 3' UTR, and a poly (U) region is critical for BOLL binding. This 21-nucleotide region is highly conserved across several species. BOLL increases the CDC25A translation, and this effect does not involve alteration of mRNA stability. **Conclusion:** CDC25A is subject to translation control by BOLL, and a decreased CDC25A expression caused by lack or decrease of BOLL may contribute to meiotic failure and male infertility. The obtained BOLL-CDC25A relationship in human cells represents an important reproductive pathway that is evolutionarily conserved in model organism and humans.

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### S1-13

#### ROS originated from leukocytes: impact on movement, viability and morphology of cultivated human spermatozoa

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**Background:** Accumulated data suggest that high level of reactive oxygen species (ROS) in sperm impairs sperm functions, even causes infertility. Giving that leukocyte is the major source of ROS, the inflammation in both male and female genital system might contribute to the change of ROS level in the process of sperm maturation and sperm-egg interaction. **Aim:** To evaluate the influence of inflammation-originated ROS on spermatozoa. **Methods:** Phorbol myristate acetate (PMA)-stimulated human polymorphonuclear leukocyte (PMN) cultivating system was reconstituted. Human spermatozoa with normal physiological parameters were selected from fertile donor using swim-up technique. Endogenous and exogenous ROS were generated by PMN and hypoxanthine-xanthine oxidase (HX-XO), respectively. The former group was treated by adding to PMN with the final density of  $1 \times 10^6 \text{ mL}^{-1}$  and PMA with the final concentration of  $50 \text{ ng mL}^{-1}$ . The positive control with exogenous ROS was dealt with hypoxanthine and xanthine oxidase of the individual final concentrations of  $0.2 \text{ mmol L}^{-1}$  and  $10 \text{ mU L}^{-1}$  respectively. Human spermatozoa in different groups was cultured for 5 days at  $25^\circ\text{C}$  in a  $5\% \text{ CO}_2$  incubator. Chemiluminescence assay (CL) and computer-assisted sperm analysis system (CASA) were employed in ROS measurement and sperm motility. Fluorescent probes were applied in the analysis of viability and mitochondrial function of spermatozoa. **Results:** Our results showed that sperm viability had been kept at more than 60% during five days culture, although motility and mitochondrial function dropped to 40% on day five. ROS level was inversely correlated with sperm functions, including motility, viability, mitochondrial function and percentage of normal morphology. Sperm functions

remarkably decreased from day three in PMA-PMN group, compared to the negative control ( $P < 0.001$ ). **Conclusion:** The inflammation-originated ROS is significantly associated with sperm functions; therefore, measurement of ROS in both semen and cervix mucus is highly recommended as a useful indicator of the infertility management. Further studies on the mechanism of oxidative damage to female genital tract is crucial for a better treatment of infertility.

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### S1-14

#### Comparison of chromatin status and fertilizing ability between epididymal and ejaculated human spermatozoa

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**Background:** Percutaneous epididymal sperm aspiration (PESA) is a simplified technique for retrieval of spermatozoa in men with obstructive azoospermia, in which spermatozoa are aspirated from the epididymis. The epididymis plays an important role in male fertility, not only as a conduit for sperm transport, but also as a place for sperm maturation and storage. Therefore, it is concerned that retrieved epididymal sperms are not as mature and functional as ejaculated spermatozoa. **Aim:** The present study was conducted to compare sperm chromatin integrity and ICSI outcomes between retrieved sperm from epididymal aspirate and ejaculated semen. **Methods:** 113 samples containing epididymal aspirate of 57 obstructive azoospermic men and 56 ejaculated semen of normozoospermic men of infertile couples submitted for ICSI program at Avicenna Infertility Clinic (AIC) were included in this study. Female factor infertility was excluded from the study. Each semen sample was divided in two aliquots for the evaluation of chromatin integrity and swim-up processing to be used for ICSI. Oocytes were retrieved according to a long-term pituitary down-regulation stimulation protocol using GnRH, FSH and hCG. Sperm chromatin integrity was evaluated by using three cytochemical staining methods including Chromomycin A3 (CMA3) to evaluate protamine deficiency, Aniline blue (AB) to assay abnormal persistence of nuclear histones, and Toluidine blue (TB) to study DNA susceptibility for denaturation. Fertilization rate (PN) and embryo quality was analyzed for each patient. Finally, Correlations of sperm chromatin integrity results and ICSI outcomes were assessed between two groups. **Results:** Significant differences were found in sperm chromatin integrity between azoospermic and normospermic men using three cytochemical staining methods. In obstructive azoospermic men the percentage of sperm stained with AB, CMA3 and TB were  $35.4 \pm 16.2$ ,  $41.5 \pm 13.4$  and  $52.6 \pm 16.3$  respectively. Compare to normospermic men with  $27.4 \pm 12.5$ ,  $30 \pm 12.5$ , and  $36 \pm 11.9$ , respectively ( $P < 0.05$ ). There was also significant increased in fertilization rate of oocytes fertilized by ejaculated spermatozoa ( $74.04\% \pm 23\%$ ), compared with epididymal spermatozoa ( $60.6\% \pm 27.2\%$ ) ( $P < 0.05$ ). However, no significant difference of embryo quality were found



between two groups ( $P > 0.05$ ). **Conclusion:** Sperm chromatin integrity is essential for protection of sperm genome from *in vivo* and *in vitro* damaging factors. Sperm fertilizing ability and chromatin integrity is acquired during transit and storage through epididymis. Our results showed higher level of sperm with abnormal chromatin and lower fertilizing ability in epididymal aspirate in comparison with ejaculated sperm that may further confirm the important role of epididymis in sperm maturation.

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## S1-15

### Tubal hydration

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Tubal hydration is a noninvasive method to reproduce the tubal patency due to various reasons or tubal spasm. Nearly 25% unfortunate couples are childless due to tubal block. Currently the following two methods like IVF & ICSI are being needed for the treatment of infertile couples. These methods are completely invasive, costly and temporary. In economically mid level countries like Bangladesh, childless couples cannot afford luxury IVF or ICSI procedure. More over socio-religion factors are working against these procedures. The most common causes of tubal blocks are infection, M.R (repeated) D&C, unsafe delivery, legation, etc. Moreover, the percentage of tubal blocks is rising fast. Considering all these issues, the Infertility Treatment & Research Center (ITRC) is working with tubal hydration since 2001. We found that pregnancy rate with take-home baby is quiet encouraging. To my opinion, tubal hydration practice should be one of the options for the treatment of tube block. More studies are needed to bring invaluable smiles back to the lips of parents.

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## S2-01

### Higher incidences of cryptorchidism, hypospadias, spontaneous abortion, and lower sperm qualities in industrialized areas with petrochemical complexes than in a non-industrialized area

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**Aim:** This study was undertaken to determine whether the incidences of cryptorchidism, hypospadias, and spontaneous abortion, and sperm quality in industrialized areas with petrochemical complexes and in a non-industrialized area exhibit spatial heterogeneity. **Methods:** Newly diagnosed cases of cryptorchidism or hypospadias in men aged 0–4 years registered from January 2000 to December 2005 were investigated nationally and regionally, in Chuncheon (CC) (a non-industrialized area) and in Yecheon (YC) and Ulsan (UL) (industrialized areas with petrochemical complexes). Incidence

of spontaneous abortion using a questionnaire, sperm quality using light microscopy and transmission electron microscopy, and blood levels of reproduction-related hormones were compared between workers in the petrochemical complexes ( $n = 66$ ) and those in the non-industrialized area ( $n = 85$ ). **Results:** The national incidences of cryptorchidism and hypospadias increased from 0.50 to 1.54 and from 0.14 to 0.25 over the study period, respectively. For cryptorchidism, the incidence in YC was 4.57 in 2000 and this abruptly increased to 16.37 in 2004, and in UL the corresponding figures were 0.59 in 2000 and 1.19 in 2004, whereas in CC they were 0.23 in 2000 and 0.78 in 2005. The incidences of cryptorchidism in YC were higher than national incidences throughout the study period, while those in CC were lower in 2001, 2002, and 2004. For hypospadias, UL and YC showed increased incidences in 2002 and 2004, respectively, but it was difficult to compare annual and regional incidences because of small sample numbers. The workers of petrochemical complexes comparing to those in the non-industrialized area showed a higher incidence of spontaneous abortion (26.4% vs. 11.5%) ( $P < 0.01$ ), higher blood levels of TSH (1.67 vs. 1.31  $\mu\text{U mL}^{-1}$ ), T3 (140.77 vs. 97.50  $\mu\text{g dL}^{-1}$ ), and T4 (7.07 vs. 5.88  $\mu\text{g dL}^{-1}$ ) ( $P < 0.05$ ), decreased sperm motilities (42.1% vs. 34.6%) ( $P < 0.01$ ), and higher rates of cytoplasmic residues (28.4% vs. 14.9%) ( $P < 0.001$ ), absent acrosome (16.6% vs. 13.4%) ( $P < 0.05$ ), and abnormal nucleus chromatins of sperms (33.1% vs. 25.5%) ( $P < 0.05$ ). Those who showed normal microscopic findings on semen analysis showed the same differences in ultrastructural findings between the two groups. **Conclusion:** The higher incidences of cryptorchidism, hypospadias, and spontaneous abortion, and more likely impaired sperm qualities in industrialized areas with petrochemical complexes suggest that exposure to petrochemical pollutants detrimentally affects reproductive organ development during fetal stage, sperm function, and conception.

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## S2-02

### Testicular dysgenesis syndrome: effects of phthalates of Leydig cells

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**Aim:** Phthalates are the most abundantly produced plasticizers for PVC plastics. Studies have demonstrated there is an increased incidence of cryptorchism and hypospadias in male boys as well as testis cancer and decreased male fertility in male adults, a syndrome referred to be testicular dysgenesis syndrome (TDS). **Methods:** When male rats are exposed to phthalates in utero, they developed TDS. Given the prevalent uses of phthalates, and their tendency to leach out from PVC plastics, concern of human exposures has arisen. Phthalates have androgen-interfering effects critically dependent on the dosage and stage of the life cycle during which exposure occurs. In

particular, low-dose exposure of phthalates that approximates documented human exposure level (e.g., 10 mg kg<sup>-1</sup>) increases, not inhibits, serum testosterone levels when rats were exposed in utero. These low-dose effects were in striking contrast to the suppression of testosterone production observed at high doses. The mechanistic basis of the distinct low-dose and high-dose effects of phthalates, and in particular how in utero exposures can result in testicular toxicity that is manifest in the adult, must be understood in order to understand phthalate-mediated TDS. We will discuss the mechanisms by which prenatal exposure to phthalates, both low and higher doses, affect Leydig cell function in postnatal testes. Two populations of Leydig cells may be affected in utero, whether directly or indirectly: the fetal Leydig cells, which differentiate in rat testes by day 12.5 of gestation and produce high levels of testosterone; and the stem cells of adult Leydig cells, which are present in the testes prior to birth but do not begin to differentiate until day 11 postpartum. **Conclusion:** These populations of Leydig cells could be the target of phthalates thus causing TDS.

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## S2-03

### Persistent organohalogen pollutants repress androgen receptor function in a cag-dependent manner

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**Background:** Adequate androgen signalling is an absolute requirement for normal male sex differentiation, puberty and sperm output thereafter. Persistent organohalogen pollutants (POPs) can act by mimicking or inhibiting steroid hormones, like estrogens and androgens, by directly binding to the hormone receptor, either as agonists or antagonists. A disruption of the normal hormonal signals can in turn modify the organization and future functioning of the reproductive system. All androgen actions are mediated by the androgen receptor (AR), which contains a polymorphic glutamine repeat commonly referred to as the CAG repeat. The mean number of CAG repeats in a given population varies with ethnicity, the normal range being approximately 10–30, with a mean length of 22 in Caucasians, slightly shorter in African Americans and longer in Asians. In the European Union-supported INUENDO project, where semen and blood samples from Greenland Inuit, Polish, Ukrainian and Swedish men were collected, no correlation between sperm counts and sperm concentration was found (Toft 2006). However, when the genetic variant also was taken into account, the CAG repeat length seemed to modify the effect of the endocrine disruptor so that high POP exposure in combination with a short CAG repeat (< 20) was associated with 40% lower sperm

concentration. **Aim:** Our aim was to investigate if three different ARs, with CAG repeat lengths in upper and lower normal range as well as the median length, differ in activity by the effect of the two POP markers, *p,p'*-DDE and CB-153, separately and in a mixture. **Methods:** Three AR vector harbouring the CAG repeats 16, 22 and 28, respectively, were co-transfected into COS-1 cells together with a firefly-luciferase androgen responsive reporter plasmid and a renilla transfection efficiency control plasmid. AR activity was induced with 10 nmol L<sup>-1</sup> 5 $\alpha$ -dihydrotestosterone in the absence or presence of a mixture of *p,p'*-DDE and CB-153 in concentrations corresponding to what was found in three populations in Europe and the Inuit. The luciferase and renilla activities were measured by a dual-luciferase reporter system and normalised for total protein content. Six experiments were done in duplicates. Friedman's test was used for overall comparison of AR function and Wilcoxon non-parametric paired test was used to compare the difference in transactivating capacity within each CAG-length as well as between different lengths in the absence or presence of pollutant. **Results:** The AR containing CAG22 was set as reference (100%) and other constructs compared to this. For cells treated with high concentration of *p,p'*-DDE, as previously assessed in Inuit and Ukrainian men, AR activity was significantly inhibited for 16 CAG with a median 23% (min: -33; max: -1%, *P* = 0.02), when compared to the same CAG repeat length without *p,p'*-DDE. With *p,p'*-DDE in low concentration (0.525 ng mL<sup>-1</sup>), as measured in serum of men from Poland and Sweden, no statistically significant differences were found. When the POP-mixture was added, the transactivating capability was repressed overall (CAG16 14%, CAG22 6% and CAG28 26%), with a statistically significant decrease for CAG28 (*P* = 0.03). **Conclusion:** The negative influence of *p,p'*-DDE on short CAG lengths was in agreement with the previous *in vivo* finding regarding lower sperm number in individuals with few CAG repeats and high POP concentration in the circulation. When a mixture of the two compounds *p,p'*-DDE and CB-153 was used, the longest CAG repeat length tested displayed a significantly decreased activity, both when compared to non-exposed cells and to the two shorter repeats. With respect to future human risk assessments of environmental pollutants, it seems as an important issue to further investigate possible gene-environment interactions.

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## S2-04

### Relationship between urinary phytoestrogens and idiopathic male infertility

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**Aim:** To investigate the relationships between urinary phytoestrogens (PEs) levels, idiopathic male infertility and semen quality in Chinese adult males. **Methods:** Through eligibility screening procedures, 608 idiopathic infertile men and 469

fertile controls were recruited. Based on their semen volume, sperm concentration, sperm number per ejaculum and sperm motility, subjects with idiopathic infertility were further divided into “normal” and “abnormal” semen quality groups. By using UPLC-MS/MS, individual exposures to PEs were evaluated by urinary concentrations of eight PEs or their metabolites (secoisolariciresinol, SEC; enterolactone, ENL; enterodiol, END; daidzein, DAI; equol, EQU; genistein, GEN; naringin, NAR; coumestrol, COU), which were adjusted by urinary creatinine (CR). **Results:** Most of the adult males in our study demonstrated high exposure levels of PEs. The median CR-adjusted isoflavone concentrations were higher than those in the U.S reports, but the lignans concentrations were lower than those in the U.S reports. The CR-adjusted urinary concentrations of SEC, GEN and DAI of infertile group were significantly higher than those of control group. With the increasing SEC, GEN and DAI levels (assessed as quintiles), the odds ratios (ORs) of semen volume, sperm concentration, sperm number per ejaculum and sperm motility showed the similar trends. From the first quintile to the fourth quintile, there were no significant variations about the ORs, whereas from the fourth quintile to fifth quintile, the ORs increased significantly. Subjects with higher urinary concentrations of SEC, GEN and DAI were more likely involved in idiopathic male infertility with abnormal semen quality ( $P < 0.01, 0.05$  and  $0.05$ , respectively). **Conclusion:** High level urinary concentrations of SEC, GEN and DAI were found associated with increased risk of male idiopathic infertility. Furthermore, the idiopathic infertile subjects with abnormal semen might be in higher risk. These findings should be of concern because of the ubiquitous exposure of PEs.

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#### S2-05

##### Effects of 4-nitrophenol isolated from diesel exhaust particles on reproductive functions in immature male rats

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**Aim:** To investigate the *in vivo* effects of 4-nitrophenol (PNP) isolated from diesel exhaust particles on reproductive function in immature male rats. **Methods:** Twenty-eight-day-old rats were injected subcutaneously with PNP (0.01, 0.1, 1, or 10 mg kg<sup>-1</sup>) daily for 14 days. **Results:** Plasma concentrations of luteinizing hormone (LH) were significantly lower in all PNP dosage groups than in the control group, and follicle-stimulating hormone (FSH) significantly decreased in rats treated with 0.1, 1, or 10 mg kg<sup>-1</sup> PNP. However, plasma concentrations of testosterone

obviously increased in 10 mg kg<sup>-1</sup> PNP treatment group, and plasma concentrations of immunoreactive (ir)-inhibin also significantly increased in the 0.1, 1, and 10 mg kg<sup>-1</sup> PNP groups. Plasma concentrations of prolactin significantly increased in 10 mg kg<sup>-1</sup> PNP group, and plasma concentrations of corticosterone significantly increased in all treatment groups. **Conclusion:** These results clearly show that PNP influences the hypothalamic-pituitary-gonadal axis in immature male rats, with decreased secretion of LH and FSH and increased secretion of testosterone and inhibin. PNP therefore appears to disrupt endocrine activity in the immature male reproductive system.

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#### S2-06

##### Mono-(2-ethylhexyl) phthalate affects the steroidogenesis in Leydig cells through provoking ROS perturbation

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**Aim:** Di-2-ethylhexyl phthalate (DEHP), the most environmentally abundant phthalate, is considered an anti-androgen based on its ability to suppress Leydig cell testosterone (T) production. Recent *in vivo* studies have revealed different effects of low and high level of phthalate on T production. We asked whether the oxidative stress in DEHP-exposed Leydig cells might account for the response of the compound on T production *in vivo*. An *in vitro* study was designed to test whether mono-(2-ethylhexyl) phthalate (MEHP), the main metabolite of DEHP and mediator of its toxicity, causes oxidative stress in progenitor, immature and adult rat Leydig cells (LCs), and how T production is affected. **Methods:** PLCs, ILCs and ALCs were purified from the testes of 21, 35, 49-day-old Long-Evans rats. LCs were incubated at 34°C in a humidified atmosphere of 5% air: 5% CO<sub>2</sub>, and exposed to medium containing luteinizing hormone (LH, 0.1 ng mL<sup>-1</sup>) and 0, 50 nmol L<sup>-1</sup>, 1, 20, 400 μmol L<sup>-1</sup>, and 8 mmol L<sup>-1</sup> of MEHP (the Leydig culture medium, phenol red-free DMEM:F12, served as the 0 control) for 24 h. Reactive oxygen species (ROS) and levels of testosterone production were measured by 2', 7'-dichlorofluorescein diacetate (DCFDA) uptake and radioimmunoassay (RIA), respectively, after 1, 3, 6, 12, 18, 24 and 40 h of MEHP treatment. Activities of enzymes related T production in Leydig cells, including P450<sub>sc</sub>, 3β-HSD, CYP11A1, 17β-HSD and STAR, were determined by radiometric conversion assay and immunofluorescence assay, after 3 and 24 h of 20, 100 and 400 nmol L<sup>-1</sup> MEHP treatment *in vitro*. All experiments were conducted in duplicate and repeated at least twice. **Results:** The purities of isolated LCs were above 95%. Only in adult Leydig cells (ALCs), were the responses to phthalate exposure consistent with results of *in vivo* studies: T concentrations in spent media were higher at the 1 μmol L<sup>-1</sup> and 400 μmol L<sup>-1</sup> doses and lower in 8 mmol L<sup>-1</sup> MEHP. In the next two experiments, MEHP was added to the cells, and at time points from 3 h to 40 h, T production,



and fluorescent intensity were measured and found to be time-dependent, T production was greater at each time increment up to 18 h. Longer incubations of 24 and 40 h diminished the capacity of cells to produce T by 2 to 6-fold. A peak of fluorescent intensity at 3 h showed that phthalate exposure induced formation of ROS and that oxidative stress had occurred in the cells. After several h of quiescence, ROS emission increased 8-fold over the next 6 h. As the amount of fluorescent intensity was inversely correlated with T-production, we inferred that MEHP caused oxidative damage and resulted in lowered capacity for T production. There was a dose-response relationship between T production and P450 $\alpha$ , 17 $\beta$ -HSD and STAR activities in MEHP-treated ALCs. **Conclusion:** Low doses of DEHP (10 mg kg<sup>-1</sup> DEHP *in vivo* or 20  $\mu$ mol L<sup>-1</sup> MEHP *in vitro*) could enhance testosterone biosynthesis if activities of T biosynthetic enzymes are potentiated by smaller scale changes in redox potential associated with mild oxidative stress. However, if the oxidative stress reaches an overload at high doses (750 mg kg<sup>-1</sup> DEHP *in vivo* or 8 mmol L<sup>-1</sup> MEHP *in vitro*), we postulate that it becomes damaging to the Leydig cell. This may be a primary cause of decreased T production underlying the anti-androgenic effects of phthalates.

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#### S2-07

##### Male reproductive toxicity, testicular oxidative stress and health hazard impact of delta-9-tetrahydrocannabinol and cannabinoids estrogenic drugs

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**Aim:** To examine the testicular oxidative stress, testicular damages of delta-9-tetrahydrocannabinol and cannabinoids estrogenic drugs and to investigate male infertility in mouse testis. **Methods:** Histological, biochemical analyses of lipid peroxidation (MDA assay), lipid-protein content and assays of antioxidant enzymes activities profiles, glutathione content, serum testosterone and FSH and LH hormones levels etc. at low doses and high doses of the drugs and at the withdrawal of the drugs (recovery dose) were performed. **Results:** At lower doses, there was an adverse effect on the testis (as revealed from the histopathological changes in testis showing detrimental changes in various germ cell layers of seminiferous tubules). Significant shrinkage of tubuli with maximum reduction of tubular diameter, and biochemical changes (as revealed by increase in the MDA value and decrease in the lipid content) were noticed. But these effects were not so much pronounced at higher dose or at the recovery dose. Similarly, marked decrease in antioxidant enzyme systems (SOD, CAT and GSH-PX) and glutathione content were observed at low doses. However, the effects were slightly higher at high dose and at recovery dose of drugs. Similarly, low dose treatments caused significant shrinkage of tubular diameter and tissue degenerations of testis, leading the testicular

atrophy resulting in lowered plasma testosterone, pituitary FSH and LH levels. But at high dose and at the withdrawal of the drug (recovery dose), regression of seminiferous tubules and recovery of various germ cell layers of testes through restoration of corrective measure of antioxidant enzyme profiles and the revival of the testosterone, FSH and LH levels were observed.

**Conclusion:** The testicular damages and adverse effect resulting in the testicular atrophy at low doses impairs male infertility. On the contrary, high dose and the recovery dose of drugs have a recovery effect on the various germ cells, which might be possible due to extra synthesis of endogenous protein-antioxidant defense systems as protective defense function.

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#### S2-08

##### Alterations of anandamide metabolism in fertile and infertile human sperm

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**Aim:** To determine whether or not any differences in the expression and/or activity of endogenous endocannabinoid system (ECS) components were present in fertile and infertile human sperm. **Methods:** Sperm from ten fertile donors and ten infertile men attending for investigations were analysed to characterise the complete ECS system. The synthesis of [<sup>3</sup>H]AEA through the activity of NAPE-PLD was assayed in sperm homogenates (200  $\mu$ g/test), by using 100  $\mu$ mol L<sup>-1</sup> [<sup>3</sup>H]NAPE and reversed phase-high performance liquid chromatography (RP-HPLC), coupled to online scintillation counting. The hydrolysis of 10  $\mu$ mol L<sup>-1</sup> [<sup>3</sup>H]AEA by FAAH was assayed in sperm extracts (50  $\mu$ g/test), by measuring the release of [<sup>3</sup>H]ethanolamine. For studies of CBR binding, membrane fractions (50  $\mu$ g/test) from sperm were used in rapid-filtration assays with the synthetic cannabinoid [<sup>3</sup>H]CP55,940 (400 pmol L<sup>-1</sup>). FAAH and NAPE-PLD protein contents were quantified by enzyme-linked immunosorbent assay (ELISA), performed on sperm fertile and infertile homogenates (20  $\mu$ g/well). Protein contents of CB1 and CB2 receptors were measured by densitometric analysis of Western blots. **Results:** FAAH activity, responsible for AEA degradation, was significantly reduced in sperm from infertile men compared with those from fertile men ( $P < 0.05$ ). In contrast, the protein content of FAAH was not changed between the two groups. AEA synthesis, by NAPE-PLD activity, was significantly decreased in infertile versus fertile men's sperm ( $P < 0.0001$ ), although NAPE-PLD protein content was

the same in both groups. There were no significant changes in protein content of CB1 and CB2 receptors. Yet, a slight, but not statistically significant, decrease of pan-CBR binding was observed in infertile versus fertile men's sperm. **Conclusion:** In conclusion, our data highlight a down-regulated mechanism of AEA synthesis and degradation in infertile versus fertile men's sperm. It will be useful to characterise the other ECS components, with particular emphasis on the involvement of 2-AG metabolism in human infertility.

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## S2-09

### Effects of tobacco and psychotropic substance smoking on male reproductive health

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**Introduction:** Germinal cells are vulnerable to smoking. Smoking can lead to disorders in microtubular polymerization and assembly, then affecting the movement of chromosome from equator to pole, thus leads to chromosomal aneuploidies. Smoking tobacco only or mixed with psychotropic substances can produce alkaloids (predominantly nicotine), poly phenols, phytosterols, cadmium, cotinine, benzo[a]pyrene and about 30 kinds of metallic compounds. Cadmium, nicotine, cotinine and benzo[a]pyrene derived from smoking have carcinogenicity and binds covalently to DNA forming its adduction thus disrupting the integrity of DNA. Reactive Oxygen Species (ROS) present in smoke interferes with the Oxidative Stress (OS) homeostasis mechanism and an increased ROS leads to pro/antioxidant imbalance. All these alterations cumulatively manifest themselves affecting the reproductive outcome of an individual. **Aim:** The affect of smoking tobacco alone or mixed with psychotropic substances on the reproductive potential are analyzed in this study. **Methods:** Twenty-five chronic smokers [16 is tobacco smoking only and nine is tobacco smoking mixed with psychotropic substance] and 15 healthy controls were analyzed for semen parameters (WHO, 1999), seminal Total Antioxidant Capacity (TAC) by antibody based commercially available kit, ROS by chemiluminescence, sperm DNA integrity by comet assay, apoptosis by flow cytometry and morphological defects by Transmission and Scanning Electron Microscopy, respectively. **Results:** Mean duration of smoking was  $8.56 \pm 2.32$  years. The mean sperm count in smokers was  $13.28 \pm 4.67$  million per ml as compared to  $28.10 \pm 5.13$  million per ml in controls. Progressive motility was  $42.04 \pm 5.08$  in smokers and  $54.56 \pm 2.32$  in controls. Percentage of abnormal morphology sperm in smokers was  $47.67 \pm 7.04$  and  $30.21 \pm 5.92$  in controls. ROS in smokers was significantly higher as compared to controls ( $P = 0.026$ ). The difference in TAC levels in both the study groups was non significant ( $P = 0.052$ ). Number of apoptotic spermatozoa was significantly higher in smokers group than in

the control ( $P = 0.031$ ). Smokers also exhibited greater number of spermatozoa with higher DNA damage as compared to controls ( $P = 0.021$ ). Increased axonemal defects of microtubular assembly were found in smokers by TEM. Sperms of smokers revealed higher number of cytoplasmic droplets in the midpiece and tail defects predominantly the coiled tails. **Conclusion:** The spermatozoa are more vulnerable to DNA damage because of impaired repair capacity and increased free radical production by tobacco and testicular inflammation induced by it. The effect is more lethal in case of smokers and drug abusers, which leads to higher mutation load, increased DNA breaks, oxidative stress and pathological sperm parameters. Smoking tobacco only or mixed with psychotropic substance undoubtedly are a threat to fertility of an individual.

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## S2-10

### Influence of temperature of floor surface on fertility in rats

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**Aim:** The hypothesis of this investigation is that the floor surface temperature may affect the development of reproductive organs, growth and survival in experimental rats. **Methods:** A total of 6 floor systems were constructed with maintaining the surface temperature at 12, 18, 23, 28, 33 and 40°C, respectively. Simultaneously the ambient temperature was sustained at 20°C in all floor systems. A total of 60 Sparque-Dorge rats were divided into six groups by the floor temperature ( $t_f$ ). Each group consists of 10 rats (five males and five females) and they were cultured at the designed floor surface temperatures from December 22, 2008 to March 20, 2009. The changes of body weight, feed intaking and water consumption were measured. The fertility was compared within each group by the time for breeding and the number of the young. **Results:** The biggest increase in the body weight was 299 g in the  $t_f$  23°C environment, whereas the least increase was 257 g in the  $t_f$  40°C environment. The feed intaking condition in the  $t_f$  12°C environment was the best, and in the  $t_f$  40°C environment the water consumption was the most. In terms of the time for breeding, the shortest group was  $t_f$  23°C as 57.4-day, whereas the longest group was  $t_f$  40°C as 74.8-day. The highest fertile group was the  $t_f$  28°C environment (born: 71/ perished: 4), and followed by the  $t_f$  18°C (b: 60/p: 9),  $t_f$  33°C (b: 59/p: 6),  $t_f$  23°C (b: 50/p: 5),  $t_f$  40°C environment (b: 45/p: 3) and the worst fertile group was the  $t_f$  12°C environment (b: 37/p: 21). **Conclusion:** Even though ambient temperature is similar, the floor surface temperature affects the time for breeding ( $P < 0.01$ ), the number of the young ( $P < 0.05$ ) and the survival rate ( $P < 0.05$ ).

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## S3-01

### Assessment of seminal estradiol and testosterone levels as predictors of human spermatogenesis

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**Aim:** To investigate whether seminal testosterone/estradiol levels and/or their ratios may be a good indicator for predicting normal spermatogenesis. **Methods:** The concentrations of estradiol and testosterone in seminal fluid were measured using competitive immunoassay techniques in specimens collected from 192 infertile patients and 103 normospermic men. Infertile patients were subdivided into three groups according to their semen analysis results and testicular biopsy: oligozoospermia, obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). **Results:** Results showed that seminal testosterone levels in the infertile groups were lower than that in the normospermic individuals ( $P < 0.01$ ), while seminal estradiol levels in the OA group were significantly higher than those in normospermic and NOA groups ( $P < 0.01$ ). Testosterone/estradiol ratios in the seminal plasma from the infertile groups were significantly lower than that in the normospermic group ( $P < 0.01$ ). However, seminal estradiol levels among normospermic and NOA groups showed no significant differences. **Conclusion:** This study suggests that the local balance between androgen and estrogen, or their ratios, may play an important role in maintaining normal spermatogenesis. Also, decreased seminal testosterone/estradiol ratio may be a good indicator for identifying the absence of sperm production in NOA patients.

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### S3-02

#### Leukocytes and oxidative stress may compromise sperm chromatin condensation

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**Aim:** Considering that the final protection of the DNA against major assaults in terms of chromatin condensation is finalized in the epididymis, it is not known how sperm production of reactive oxygen species (ROS) and inflammatory processes can contribute to protamine deficiency that is predetermined in the testes. Therefore, the objective of this study was to investigate the relationship between compromised sperm chromatin condensation, sperm morphology, the intrinsic ROS production, sperm DNA damage as well as the impact of the presence of leukocytes on chromatin condensation. **Methods:** In 70 patients attending the Jena assisted reproduction unit, sperm DNA status was determined by means of the TUNEL (Promega) and chromomycin A3 (CMA3) assays (Sigma). Sperm ROS-production was determined by means of dihydroethidine (DHE; Molecular Probes) and sperm were analyzed using fluorescence

microscopy. Normal sperm morphology was evaluated according to strict criteria. All statistical calculations were performed using the MedCalc statistical software (Version 10.4; Mariakerke, Belgium). After testing for normal distribution by means of the Kolmogorov-Smirnov test appropriate statistical tests, either parametric or non-parametric, were performed. For non-parametric correlations, apart from the Spearman rank correlation ( $\rho$ ), Kendall's  $\tau$  was calculated, which is even less parametric than Spearman's  $\rho$ . The main advantages of using Kendall's  $\tau$  are that its distribution has slightly better statistical properties and there is a direct interpretation of Kendall's  $\tau$  in terms of probabilities of observing concordant and discordant pairs. **Results:** The percentage of CMA3-positive sperm and leukocyte concentration (Spearman's  $\rho$ :  $r = 0.220$ ,  $P = 0.0803$ ; Kendall's  $\tau$ :  $r = 0.178$ ,  $P = 0.0377$ ) as well as percentage of ROS-positive sperm ( $r = 0.3010$ ;  $P = 0.012$ ) correlated significantly. Non-leukocytospermic patients showed a tendency ( $P = 0.0611$ ) towards a lower protamine deficiency as indicated by a mean of  $35.4\% \pm 15.3\%$  CMA3-positive spermatozoa compared to the leukocytospermic group with  $43.7\% \pm 19.3\%$ . However, if a lower cut-off value of  $0.5 \times 10^6$  leukocytes  $\text{mL}^{-1}$  was applied, this difference was significant ( $P = 0.0059$ ) with mean percentages of CMA3-positive sperm of  $30.3\% \pm 12.5\%$  for those ejaculates containing  $< 0.5 \times 10^6$  leukocytes  $\text{mL}^{-1}$  and  $43.5\% \pm 18.4\%$  for those with  $\geq 0.5 \times 10^6$  leukocytes  $\text{mL}^{-1}$ , respectively. There was also a weak, but significant positive correlation between the presence of seminal leukocytes and CMA3-positive spermatozoa (Spearman's  $\rho$ :  $r = 0.220$ ,  $P = 0.0803$ ; Kendall's  $\tau$ :  $r = 0.178$ ,  $P = 0.0377$ ). On the other hand, poor chromatin condensation in terms of protamine deficiency (CMA3-positivity) correlated significantly with sperm ROS production ( $r = 0.301$ ;  $P = 0.0120$ ). No association was found between CMA3-positivity, TUNEL-positivity and sperm morphology. While P- (poor prognosis: 0%–4% normal morphology) and G-pattern (good prognosis: 5%–14% normal morphology) morphology did not differ regarding chromatin condensation, P-pattern patients had a significantly higher percentage of DNA-fragmentation ( $P = 0.0323$ ). **Conclusion:** As oxidative stress is associated with disturbed chromatin condensation and the results of this study suggest that the idea that under-protamination of sperm DNA will automatically lead to DNA-fragmentation might have to be revisited.

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### S3-03

#### Which factors help to predict the IUI outcome: sperm DNA integrity, sperm-cervical mucus penetration or sperm-hyaluronan binding capacity?

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**Aim:** About 40% of infertility diseases are due to male factor. Besides semen analysis including sperm concentration, motility and morphology, other tests may help predict the outcome of intrauterine insemination (IUI) treatment which is adopted for minimum cost and less invasiveness. The objective of this study was to investigate the relationships of sperm DNA integrity, sperm-cervical mucus penetration or sperm-hyaluronan binding ability with IUI outcome. **Methods:** The semen samples were collected from donors of Shanghai Human Sperm Bank. The semen parameters of donors should meet the following criteria: 1) before freezing, density  $\geq 60 \times 10^6 \text{ mL}^{-1}$ , motility (grade a + b)  $\geq 60\%$ , normal morphology rate  $\geq 30\%$ ; 2) post-thaw, density  $\geq 40 \times 10^6 \text{ mL}^{-1}$ , motility  $\geq 40\%$ . The candidates were divided into two groups. 1) High-fertilization rate group (HF group): 22 donors, age ranging from 20–36 years (mean age  $23.5 \pm 6.2$  years), five women have already obtained pregnancy within 20 AID cycles by these donors' semen; 2) low-fertilization rate group (LF group): 17 donors, age ranging from 20–38 years (mean age  $26.1 \pm 8.3$  years), none woman has already obtained pregnancy within 20 IUI cycles by these donors' semen. After post-thaw semen analysis was performed, sperm chromatin dispersion test, sperm-hyaluronan-binding assay (HBA), sperm-cervical mucus penetration tests were adopted to evaluate sperm DNA integrity, sperm-cervical mucus penetration and sperm-hyaluronan binding capacity respectively. Parametric tests (*t*-test) were used for comparisons between groups when the data followed a normal distribution as demonstrated by the Kolmogorov–Smirnov test. Non-parametric tests were used to compare parameters in fertile and infertile males when the data did not follow a normal distribution. **Results:** The average parameters of fresh semen of HF group were  $109 \times 10^6 \text{ mL}^{-1}$  (density), 63% (motility), 49% (normal forma rate), and that of LF group were  $79 \times 10^6 \text{ mL}^{-1}$  (density), 61% (motility), 51% (normal forma rate); the average parameters of post-thaw semen of HF group were  $45 \times 10^6 \text{ mL}^{-1}$  (density), 46 % (motility), and that of LF group were  $41 \times 10^6 \text{ mL}^{-1}$  (density), 42 % (motility); it did not achieve statistical significance. SCD test of the two groups showed that the fragment rate were  $14.7\% \pm 5.2$  (HF group) and  $17.9\% \pm 6.5\%$  (LF group), the difference was not statistically significant. 83% of HF group had normal HBA scores ( $> 60\%$ ), while 57% of LF group had normal HBA scores, and the difference was statistically significant. 89% of HF group had normal cervical mucus penetration result, while 43% of LF group had normal HBA scores, and the difference was statistically significant. **Conclusion:** Conventional semen analysis including sperm concentration, motility and morphology is widely used as a fundamental indicator for male fertility. However, the results do not provide accurate diagnostic or prognostic information about human fertility either *in vivo* or *in vitro*. HBA for testing of sperm maturity and fertility and sperm-cervical mucus penetration tests which reflects sperm motility and morphology may be helpful to evaluate the outcome of IUI. DNA integrity, as measured by the SCD test, does not correlate with pregnancy outcome in IUI.

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### S3-04

#### Clinical utility of sperm DNA fragmentation measures

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**Aim:** To evaluate the usefulness of sperm DNA fragmentation data related to pregnancy outcomes and toxicology. **Methods:** Updated literature review on sperm DNA fragmentation data derived by terminal deoxynucleotidyl transfer-mediated dUTP nick end-labeling (TUNEL), COMET and Sperm chromatin structure assay (SCSA) tests on semen samples obtained from couples attending an infertility clinic or sperm donors not exposed or exposed to environmental pollution. Current thought is that COMET and TUNEL measure actual DNA strand breaks while the SCSA measures the susceptibility of DNA to denaturation of double stranded DNA to single stranded DNA at the sites of DNA strand breaks. SCSA also measures simultaneously the % of immature sperm (lack of exchange of histones for protamines). The results of most assays correlate highly with each other. Flow cytometric measures by TUNEL or SCSA provide statistical robustness over light microscope TUNEL or COMET. **Results:** Interpretations of the data from the different assays are based on the hypothesis that the increase in the amount of DNA fragmentation is pathologic. The clinically significant threshold for SCSA has been determined to be 27%–30% DFI (DFI =DNA Fragmentation Index) of sperm with measurable DNA fragmentation for natural insemination. Couples with no known fertility problems were 7.0 X more likely to achieve a pregnancy/delivery if the DFI was  $< 30\%$  ( $n = 362$ ,  $P = 0.0001$ ). TUNEL thresholds have had wide variations from 8 to 36% DFI. COMET data are the most difficult to define with regard to thresholds. Sperm DNA damage is associated with lower rates of natural insemination and intrauterine insemination (IUI) pregnancies. A meta-analysis shows a strong association between DNA damage and failure of natural pregnancy and low IUI pregnancies. The Bungum et al study showed that infertility clinic patients using IUI had OR of 9.9 (95% confidence interval: 1.18–2.07;  $P = 0.05$ ) for pregnancy when the SCSA defined %DFI was  $< 27\%$ . A number of studies have been done investigating the relationship between sperm DNA damage and IVF/ICSI outcomes. The general conclusion is that the DNA damage is weakly related with lower IVF pregnancy rates with OR of about 1.5. The relationship between sperm DNA damage and ICSI pregnancy outcomes is weaker than that of IVF and in the range of about OR 1.15. However, data show that a very high %DFI is negative for ICSI outcome. Likely the selection of highly motile and good morphology sperm by the ICSI technician mitigates the potential adverse effects as well as the selection of the best looking or fastest growing embryos. It is noted however, that a high level of sperm DNA fragmentation is related to an increased level of ICSI pregnancy loss with OR in the range of 2.5. **Conclusion:** It is not known whether DNA strand breaks are random or are in part targeted to hot spots. Some sites are likely clinically significant while others may be clinically insignificant. At least some single strand DNA breaks may be repaired by the fertilized oocyte but if the damage is extensive it may overwhelm the repair system. Double strand

breaks are not repairable. Meta analysis publications on the utility of sperm DNA fragmentation tests for concluding the clinical utility for clinical usefulness are, to this point, often misleading due to the great heterogeneity of laboratory and clinical methods, skill and experience of laboratory technicians and biased interpretations, even so, the greatest value of sperm DNA fragmentation tests appears to be the diagnosis of the idiopathic infertile couple where the female tests and the semen analysis are in the normal range but the sperm DNA fragmentation level is above a threshold that is inhibiting normal pregnancy. Such couples can then be advised to perhaps skip IUI procedures and go directly to ICSI. Furthermore, the couples with a high %DFI should be advised of the increased risk of miscarriage. Also, high %DFI values can pinpoint lifestyle and potential environmental exposures that cause sperm DNA fragmentation and with such knowledge the couple can circumvent damaging factors. The SCSA has shown a very high correlation between exposure to environmental toxicants (that prevent or cause loss of pregnancy) and levels of exposure.

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### S3-05

#### Impact of the atmospheric temperature on human semen parameters and the cryosurvival of human spermatozoa

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**Aim:** To investigate the impact of the atmospheric temperature on human semen parameters and the cryosurvival of human spermatozoa. **Methods:** A total of 10 829 semen samples from 1 866 donors, whose ages ranged from 20 to 42 years in Zhejiang Human Sperm Bank, China, were analyzed. They were divided into two groups according to the atmospheric temperature when ejaculating in Hangzhou, China: Group A, < 20°C; Group B, the temperature > 20°C. The semen parameters were assessed by the World Health Organization criteria. **Results:** When comparing the different semen parameters, the semen volume, sperm concentration and the sperm morphology in group A were significantly better than those in Group B. However, the progressive motility in group A was significantly lower than that in group B ( $P < 0.05$ ). The cryosurvival of spermatozoa in group A was significantly higher than that in group B ( $P < 0.05$ ). **Conclusion:** The atmospheric temperature would affect human semen quality and the cryosurvival of spermatozoa. It is suggested that the semen quality would be better when the atmospheric temperature was below 20°C. These days would be suitable for semen donating.

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### S3-06

#### Changes in sperm morphology and semen parameters following varicocelectomy

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**Aim:** To evaluate the changes in sperm count, sperm motility, sperm function tests and sperm morphology following varicocelectomy. **Methods:** The study included 112 subjects of which 71 subjects were of varicocele (46 with and 25 without infertility) and 41 subjects were controls (20 fertile adults and 21 adults with infertility without varicocele). All subjects underwent detailed semen analysis (including volume, pH, sperm counts, motility, morphology and sperm function test), FNAC of bilateral testes and hormonal analysis (LH, FSH, testosterone). Bilateral low ligation (under loupe magnification) of varicocele was done in Doppler confirmed cases and changes in above parameters were recorded post operatively. The subjects were followed up for a period of 6 months. The parameters were compared and analyzed among the four groups and also with post operative values in subjects of varicocele using *t*-test or Chi-square test as applicable. Some data showed wide variation, so Mann Whitney's test was also used where applicable. Statistical significance was defined if the  $P < 0.05$ . **Results:** On pre-operative evaluation subjects with varicocele were found to have significantly low sperm counts, low sperm motility, deranged sperm function and higher proportion of sperms with abnormal morphology in semen as compared to controls. The semen parameters improved markedly within 3–6 months after bilateral varicocele ligation with significant increase in sperm counts, motility and also functional capacity of sperm (morphology and sperm function test). There was significant improvement in the subjects' hormonal status and grade of testicular histology after surgery. The extent of impairment and post operative improvement of all parameters was related to the grade of varicocele. **Conclusion:** Varicocele has an adverse effect on male fertility by affecting semen parameters. Bilateral varicocele ligation causes a progressive and significant improvement of semen parameters and thus increases the chances of conception. Therefore, though the indication of surgery for symptomatic varicocele is well established, we also recommend surgery in all cases of varicocele associated with infertility or oligospermia.

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### S3-07

#### Declining quality of donated semen: a 10-year historical comparative cohort study

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**Background:** Several studies suggested that global semen quality is declining. Systematic review of literature demonstrated a significant log decline in the average sperm concentration in 50 years (1940–1990) among men without infertile history. During the last decade in Israel, the prevalence of malformations of the male reproductive system as well as testicular cancer is increasing. **Aim:** To compare semen parameters of sperm

donated recently vs. 10 years ago in the sperm bank of Hadassah. **Methods:** The study group included individuals who donated semen between 2004 and 2008 ( $n = 19$ ), whereas the control group consisted of men donating semen between 1995 and 1999 ( $n = 18$ ). Each donor provided 4–5 semen samples per month for 12 to 24 consecutive months (range: 48–120 semen samples). The sperm concentration and motility in each event of semen donation were recorded. The average sperm parameters were monthly calculated. **Results:** All semen donors were young ( $< 37$  years, mean age:  $25.3 \pm 3.5$  years), healthy, fertile, unmarried, non-smoker and university educated. The ongoing criteria adopted for a qualified donation is 2 to 3 semen samples, with a sperm concentration of 50 million  $\text{mL}^{-1}$  and 60% progressive motility. The previous criteria for qualified donation was stricter with a sperm concentration of 70 million  $\text{mL}^{-1}$  and 70% progressive motility. These criteria were proposed based on certain attrition rate of the sperm in the process of freezing, thawing and preparation for intra-uterine insemination. In spite of the decrease in sperm parameters qualified for donation, 2/3 (52/79) of semen donation applicants were rejected based on semen quality whereas only 1/3 of applicants failed to donate 10 years ago. The profile of the sperm donors did not change along the decade. However, the average sperm parameters dropped from a concentration of  $106 \pm 25$  million  $\text{mL}^{-1}$  to  $67 \pm 15$  million  $\text{mL}^{-1}$  and sperm motility rate declined from  $79\% \pm 4.5\%$  to  $68\% \pm 4\%$ . ( $P < 0.0001$ ,  $P < 0.0001$ , respectively). When the previous criteria was implemented to analyze the latest group of sperm donors, only 35% of the semen donation samples were qualified, with an average concentration of 83 million  $\text{mL}^{-1}$  and 72% motile sperm, still significantly lower than the semen quality 10 years ago ( $P = 0.03$ ,  $P = 0.002$ , respectively). The total pregnancy rate (94%) did not differ between the groups in spite of the significant decline in sperm quality. **Conclusion:** Decrease in sperm concentration and motility among fertile semen donors within such a short duration is alarming. Investigation of the possible association between endocrine disrupter exposure in Israel and human semen quality may provide a plausible environmental etiology for male infertility.

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### S3-08

#### Factors affecting fecundity among sperm donors: a multivariate analysis

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**Aim:** The objective of the present research is to evaluate the factors influencing the quality of donors' semen in artificial insemination. **Methods:** Based on clinical histories from the computer register database in our hospital, 50 sperm donors were involved between January 2002 and December 2006 in this retrospective study. Pregnancy rate (PR) was used as a parameter

to determine donor fertility potential. We evaluated the relationship between factors such as sperm routine parameters, plasma membrane integrity, DNA fragmentation index (DFI) and PR. **Results:** The percentage of normal morphology, post-thaw motility, cryosurvival rate, phosphatidylserine translocation and DFI were correlated with donor fecundity ( $P < 0.01$ ). Among them, both normal morphology and post-thaw progressive motility were proportional to donor fecundity, and fertility potential was notably higher when the percentage of propidium iodide-negative spermatozoa was  $> 48\%$  and DFI was  $< 7\%$  in post-thaw samples. Routine seminal analysis cannot serve as a reliable predictor of male fertilization ability. **Conclusion:** The present data suggest that the integrity of both plasma membrane and DNA are crucial factors affecting the fecundity of sperm donors. Therefore, the addition of some of these new sperm tests to routine semen analysis could significantly improve the recruitment of sperm donors.

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### S3-09

#### The analysis of semen parameters and bacterial culture results in epididymal fluid from 36 patients with obstructive azoospermia

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**Aim:** To analyze the semen parameters and bacterial culture results in epididymal fluid from 36 patients with obstructive azoospermia caused by different causes. **Methods:** Thirty-six patients diagnosed with obstructive azoospermia, who underwent diagnostic percutaneous epididymal sperm aspiration (PESA) and were found to have sperm in the epididymal fluid from July 2008 to November 2008, were divided into three groups according to the causes: group A (14 cases), Inflammatory epididymal obstruction with a definite history of epididymitis; group B (seven cases), Reproductive duct obstruction without a definite history of reproductive duct inflammation; group C (15 cases), Congenital bilateral absence of the vas deferens (CBAVD). The epididymal fluids obtained by PESA were simultaneously sent to laboratories for semen analysis, routine bacterial culture, and tuberculosis bacilli culture. **Results:** Patients with sperm count  $\geq 20 \times 10^6 \text{ mL}^{-1}$ ,  $1-20 \times 10^6 \text{ mL}^{-1}$  and  $\leq 1 \times 10^6 \text{ mL}^{-1}$  in the epididymal fluid accounted for 33.3% (12/36), 7% (15/36) and 25% (9/36) respectively; there was no significant difference in the sperm count among groups ( $P > 0.05$ ); patients with grade A + B sperm, with only grade C sperm and only grade D sperm in the epididymal fluid accounted for 41.7% (15/36), 36.1% (13/36) and 22.2% (8/36) respectively; there was no significant difference in the sperm motility among groups ( $P > 0.05$ ); macrophages were found in 16 cases and a few red blood cells were found in the epididymal fluid; the routine bacterial culture and tuberculosis bacilli culture of the epididymal fluid specimens from all the patients were negative. **Conclusion:** In our study, the sperm count and motility in the epididymal fluid had no significant difference among the different causes of reproductive

duct obstruction and the semen parameters in the epididymal fluid from most patients with obstructive azoospermia were qualified enough for intracytoplasmic sperm injection (ICSI); no active bacteria were detected in the epididymal fluid from patients with obstructive azoospermia who were not in the acute phase of reproductive duct inflammation and the epididymal fluid specimens from PESA could be used for ICSI safely.

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### S3-10

#### Analysis of the acrosome reaction in human sperm induced by recombinant ZP3 expressed in different Chinese hamster ovary cells with altered glycosylation.

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**Aim:** Acrosome is an organelle present in the sperm anterior head. During fertilization, the outer acrosomal membrane fuses with the sperm plasma membrane at different points, which is known as acrosome reaction (AR). This process is responsible for the release of the acrosomal enzymes that together with the sperm motility allowed the zona pellucida (ZP) penetration and fertilization. Progesterone and ZP are natural agonists of the sperm AR. The human ZP is formed by four glycoproteins called ZP1, ZP2, ZP3 and ZP4. It was previously reported that the AR in capacitated human sperm is mainly induced by ZP3. Different studies suggest that the carbohydrate chains contained in the ZP glycoproteins may play a key role in the sperm-ZP binding; however, a controversy is still present about the carbohydrate implication and the type of carbohydrate involved in this process. Studies using native human ZP are scarce due to the difficulty to get enough samples. An alternative approach to study the function of the ZP glycoproteins in AR is to use recombinant proteins produced in different cell systems. In this study, the role of the carbohydrate residues in the ZP3 was evaluated using AR assays. **Methods:** Human ZP2 and ZP3 cDNA (kindly provided by Dr Dean, NIH, USA) was subcloned in the plasmid pEGFP-N1 (Clontech). Different constructions were electroporated into Chinese hamster ovary cells (CHO) to obtain stable transfected cells. ZP3 were also expressed in CHO lec1 and CHO lec8. Both types of cells have an altered glycosylation. CHO lec1 cells are defective in N-acetylglucosaminyltransferase I (GlcNAc-TI) activity and therefore cannot convert the oligomannosyl intermediate (Man5GlcNAc2Asn) into N-linked complex carbohydrates; however, CHO lec8 cells are defective in a galactose transporter and consequently produce glycoproteins with deficiency in galactose residues. Ten different semen samples were obtained from eight volunteers. Informed consent was obtained from all subjects participating in the study as

approved by the ethics committee in the University of Murcia. The semen samples were washed and capacitated by swim-up protocol during 1 hour incubation at 37°C and 5% CO<sub>2</sub> atmosphere in HTF medium supplemented with human serum albumin. AR assays were performed using 10 × 10<sup>6</sup> sperm mL<sup>-1</sup> incubated with the different ZP glycoproteins (1 µg µL<sup>-1</sup>) secreted by CHO cells, progesterone (10 and 3.2 µmol L<sup>-1</sup>) and calcium ionophore (10 µmol L<sup>-1</sup>). The AR was evaluated using the Pisum sativum lectin conjugated with FITC. The percentage of viable sperm was assessed using the eosin-nigrosin staining. **Results:** Recombinant human ZP3 expressed by CHO, CHO lec1 y CHO lec8 cells has an apparent molecular weight of 60 kDa, 39 kDa and 42 kDa respectively. ZP3 contained in the cell culture supernatant produced by CHO, CHO lec1 and CHO lec8 cells induced 46.4%, 29.13% and 28.9% sperm AR respectively. The AR is only statistically significant in the case of the ZP3 produced by CHO cells. A 50.5% and 52.1% of AR was obtained with 3.2 and 10 µmol L<sup>-1</sup> of progesterone respectively. AR induced by recombinant ZP2 (27.7%) is similar to the spontaneous AR (27.5%). The higher AR (84.8%) was induced using the calcium ionophore. In the different experiments the percentages of viable sperm were close to 90% except in the case of calcium ionophore treated sperm. In the latter case the percentage of viable was greatly reduced (13%). **Conclusion:** In conclusion, the recombinant ZP3 produced in CHO cells has a similar biological activity compared to progesterone. However, recombinant ZP3 produced in CHO lec1 and CHO lec8 cells are not able to induce the AR. These results suggest that a change in the glycosylation pattern of the ZP3 expressed by CHO cells produces a lost in the biological activity. Additionally, both intact N-linked and O-linked oligosaccharide chains contained in the ZP3 are probably responsible for the induction of the acrosome reaction.

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### S3-13

#### Clinical evaluation of three sperm counting chambers fitting computer-assisted sperm analysis system for semen analysis

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**Aim:** To compare three sperm counting chambers by the computer-assisted sperm analysis (CASA) system and to evaluate the differences of the main parameters and same item after semen analysis. To assess the different counting chambers on the sperm count test results and the impact of the differences between each other. **Methods:** The sperm samples of 50 infertile male patients were selected randomly from the out-patients in our hospital. Each sample was analyzed with Makler, Macro and Cell-VU counting chambers. Each time twenty sets of data were collected, and sperm density, motility rate and the rate of forward sperm movement were analyzed with CASA system. At the same time, Sperm densities were manually tested with

WHO recommended cytometer chamber (CC) as the controls. Inter-related parameters were analyzed, and all means were expressed with ( $x \pm s$ ). The parameters between samples were compared with *t*-test, and  $P < 0.05$  was considered statistically significant. **Results:** The sperm density tested with the three kinds of sperm counting chambers with the CASA system were: Makler chamber,  $(23.48 \pm 3.27) \times 10^6 \text{ mL}^{-1}$ , Macro chamber,  $(24.05 \pm 3.53) \times 10^6 \text{ mL}^{-1}$ ; Cell-VU chamber,  $(30.91 \pm 4.24) \times 10^6 \text{ mL}^{-1}$ . There were significant differences between the average sperm densities tested with the three chambers, with the result of Cell-VU chamber higher than the other two. All the three results were significantly higher than that of CC [ $20.16 \pm 2.94) \times 10^6 \text{ mL}^{-1}$ ,  $P < 0.05$ ]. The sperm viability and the rate of forward sperm movement were  $52.20\% \pm 14.45\%$ ,  $55.62\% \pm 15.51\%$ ,  $40.52\% \pm 13.48\%$ , and  $39.83\% \pm 13.85\%$ ,  $40.60\% \pm 14.22\%$ ,  $32.35\% \pm 13.78\%$  tested with Makler, Macro and Cell-VU counting chambers, respectively. The results of Makler and Macro were significantly higher than that of Cell-VU ( $P < 0.05$ ), but there were no significant differences between the two results ( $P > 0.05$ ). **Conclusion:** On semen analysis, there are systematic errors between the results with different testing chambers, affecting the results of sperm density and viability. So the test results are significantly different between laboratories. The present study shows that the sperm density tested by Cell-VU plate is higher than the other two counting board, but the sperm motility and the rate of forward sperm movement were lower than that of the other two counting chambers, which is different with the results of other literatures. Makler and Macro chamber has a good consistency on the boards of various parameters, which indicates the importance and necessity of standardization of semen analysis.

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#### S4-01

##### Development of a second-generation antiandrogen for treatment of castration resistant prostate cancer

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**Background:** Metastatic prostate cancer is treated with drugs that antagonize androgen action, but most patients progress to a more aggressive form of the disease commonly known as castration-resistant prostate cancer, which is driven by elevated expression of the androgen receptor. Currently, there is no effective drug for castration-resistant prostate cancer. **Aim:** to identify compounds that can treat castration resistant prostate cancer and to study their mechanism. **Methods:** High throughput screening and medicinal chemistry were used to identify compounds that can inhibit the androgen receptor function in castration resistant prostate cancer. Preclinical studies and clinical studies were used to study the efficacy and safety of a drug candidate. **Results:** Through high throughput screening and medicinal chemistry, we identified a series of compounds that inhibit the androgen receptor function in castration resistant

prostate cancer. We further characterize two of the compounds in preclinical and clinical settings. The compounds bind to the androgen receptor with greater relative affinity than the clinically used antiandrogen bicalutamide, reduce the efficiency of its nuclear translocation, and impair both DNA binding to androgen response elements and recruitment of coactivators. The compounds are orally available and induce tumor regression in mouse models of castration-resistant human prostate cancer. Of the first 30 patients treated with one of the compounds in a Phase I/II clinical trial, 13 of 30 (43%) showed sustained declines (by  $> 50\%$ ) in serum concentrations of prostate-specific antigen, a biomarker of prostate cancer. **Conclusion:** These compounds appear to be promising candidates for treatment of castration resistant prostate cancer.

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#### S4-02

##### AKT/p70S6K1 signaling pathway in prostate tumorigenesis and angiogenesis

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**Aim:** To study the roles of AKT, p70S6K1, and their effectors expressed in human prostate cancer cells and human endothelial cells in regulating tumor growth and angiogenesis, and to investigate the potential mechanisms. **Methods:** Alter the expression of AKT, p70S6K1, and their effectors in human prostate cancer cells and human endothelial cells *in vitro*, and then study the changes of these signaling molecules and interactions in animals in mediating tumor growth and angiogenesis. **Results:** PI3K/AKT signaling is commonly activated in human prostate cancer. Our previous study initially demonstrated that PI3K signaling plays an important role in angiogenesis. In this study, we found that PI3K and AKT activate p70S6K1 to regulate expression of VEGF, MDM2, and hypoxia-inducible factor 1 (HIF-1) in prostate cancer cells. The inhibition of AKT and p70S6K1 activation decreased prostate tumor growth and angiogenesis. To test the downstream targets of p70S6K1 in tumorigenesis, we found that the inhibition of HIF-1 expression by HIF-1 siRNA decreased prostate tumor growth and angiogenesis. To assay the roles of AKT and p70S6K1 expression in human endothelial cells in prostate tumor growth and angiogenesis, we altered the expression of AKT and p70S6K1 expression in human endothelial cells, and established the chimeric tumor model to study endothelial AKT and p70S6K1 expression in affecting tumor angiogenesis. We showed that endothelial AKT and p70S6K1 expression significantly affected tumor growth and angiogenesis. **Conclusion:** These results demonstrated the important roles of AKT and p70S6K1 in both human prostate cancer cells and endothelial cells in mediating prostate tumorigenesis, and HIF-1 and VEGF are downstream effectors for regulating tumor growth and angiogenesis.

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## S4-03

**Prostate cancer screening in General Practice – knowledge, practice and uptake of evidence**

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**Background:** Currently there is insufficient evidence to either support, or refute, screening for prostate cancer. It is anticipated that data from ongoing trials will provide a definitive answer on this important issue. Although many Australian males are concerned about being diagnosed with prostate cancer, few research the benefits and limitations of screening themselves. Rather, the majority prefer to act upon the advice and information given to them by their General Practitioner (GP). **Aim:** The overall aim of this project is to identify the current knowledge, practice and uptake of evidence of GPs on screening for prostate cancer. **Methods:** A mixed methods approach using qualitative and quantitative methods was used. GPs were recruited through advertisements placed through their local division of general practice. Focus group interviews, and follow-up surveys, were conducted with GPs working in both a rural and urban setting. Qualitative data was analyzed using thematic analysis, with supporting quantitative data analyzed descriptively. **Results:** A total of 77 GPs participated in the study, with 39 urban GPs and 38 rural GPs. Several principal issues were identified through the interviews. Few were aware of recommendations on screening offered by governing medical bodies, or the trials investigating the merits of prostate cancer screening currently being conducted in Europe and the USA. The majority of GPs preferred to use a combination of prostate specific antigen (PSA) test and digital rectal examination (DRE) when screening men for prostate cancer. GPs were evenly split in their practice of screening, with some always screening men aged over 45, whilst others preferred the patient to ask for a PSA test or DRE. Few GPs had access to patient education materials on prostate cancer screening, and fewer utilized such materials when discussing the merits of prostate cancer screening with their patients. GPs were uncertain as to whether results from the two trials in Europe and USA would alter their behavior when deciding whether to screen men for prostate cancer. **Conclusion:** Australian GPs have a good understanding of the benefits and limitations of screening men for prostate cancer. Further research is needed to explore (i) how GPs can get greater access to evidence on prostate cancer screening, (ii) how to best implement this evidence in practice, and (iii) what impact, if any, such evidence will have on the patient/doctor relationship and how men are screened for prostate cancer.

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## S4-04

**Inhibition of tumor growth and induction of apoptosis in prostate cancer cell lines by overexpression of tissue inhibitor of matrix metalloproteinase-3**

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**Aim:** The destruction of extracellular matrix by matrix metalloproteinases is a key event in cancer progression. The tissue inhibitors of metalloproteinases can restrain tumor growth by inhibiting these enzymes. We sought to determine whether overexpression of tissue inhibitor of metalloproteinase 3 (TIMP-3) could suppress the malignant phenotype of human prostate cancer cell line PC-3M *in vitro* and *in vivo*. **Methods:** Immunohistochemistry was used to examine the expression of TIMP-3 in prostate cancer cell lines and prostate cancer tissues. Semi-quantitative RT-PCR and Western blots were performed to analyze the expression of TIMP-3 mRNA and protein after transfection with pcDNA-TIMP-3 vectors. Cell proliferation was assayed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining kit. Acridine orange/ethidium bromide staining and flow cytometry analysis were used to detect the apoptosis of the TIMP-3 overexpressing cells. Cell Adhesion Assays, cell wound healing assay and Transwell Invasion assay were performed to examine the effect of TIMP-3 on cell invasion and adhesion. To evaluate the tumorigenic properties of the TIMP-3 overexpressing cells *in vivo*, stable overexpression of TIMP-3 cells were transplanted into nude mice, and the tumor growth was observed. To determine if TIMP-3 could inhibit tumor growth, parental PC-3M cells were injected into the right flank of mice. After palpable tumors had formed, the tumors were injected with the pcDNA-TIMP-3 vector. Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay was used to confirm the apoptosis of tumors. **Results:** Stable overexpression of TIMP-3 inhibited cell proliferation significantly by MTT assay. Both early and late apoptosis were observed in TIMP-3 overexpressing cells and flow cytometry analysis showed S-phase blocking of the cell cycle. Monolayer invasion assay and transwell invasion assay demonstrated significantly decreased invasive potential in TIMP-3 overexpressing cells compared with control cells. Cell adhesion and motility were also lower after TIMP-3 was overexpressed. *In vivo*, cells stably overexpressing TIMP-3 completely lost the ability to form tumors following being injected into nude mice. Transfection of TIMP-3 into established tumors by electroporation also had a significant antitumor effect. TIMP-3 treated tumor tissues had significant apoptosis by TUNEL assay. **Conclusion:** These results showed that overexpression of TIMP-3 inhibits invasion and proliferation of prostate cancer cells *in vitro* and inhibits tumor growth *in vivo*. The experiments suggest a potential use for TIMP-3 in the gene therapy of prostate cancer.

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**S4-05****Sex function after radical prostatectomy**

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Radical prostatectomy (RP) is a standard surgery operation for the treatment of clinically localized prostate cancer and sex dysfunction is still a commonly complication after RP. How to recovery the sex function after RP will become an important problem for us. We analyze some possible factors associated with the recovery of sex function after RP. Three aspects may explain how to give a satisfactory recovery after RP. First, patient selection: we should assess patient's age, sex function before RP, diseases correlated with sex disorder, the patients' expectancy for the sex function after RP and the clinical stage of prostate cancer. Second, surgical technique: It is including the reservation of neurovascular bundles (the nerve sparing radical prostatectomy should aim in maintaining sexual function), understanding of the anatomy of the bladder neck and prostate—this is the base for the operation, application for the electromyography with blunt dissection and hematischesis—this is one of the key points for a perfect prostatectomy. Third, drug and follow-up: Urologists should assess the recovery of patients' sex function during the follow-up. 5-phosphodiesterase inhibitor may help the recovery of sex function. In a word, a satisfactory functional recovery is based on the preoperative, intraoperative, and postoperative aspects above.

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**S4-06****Stem cell-properties of prostate tissue-derived CD133+MDR1+ cell and its Oct-4 expression**Satyanarayana Rentala<sup>1</sup>, Prameela Devi Yalavarthi<sup>1</sup>, Lakshmi Narasu Mangamoori<sup>2\*</sup><sup>1</sup>Centre for Biotechnology, Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad 500072, India<sup>2</sup>Institute of Science and Technology, Jawaharlal Nehru Technological University, Hyderabad 500072, India

**Background:** CD133 (prominin-1), a 5-transmembrane glycoprotein, has recently been considered as an important marker that represents the subset population of cancer stem-like cells. **Aim:** The purposes of the present study are to isolate cancerous stem-like cells from normal healthy volunteers and prostate cancer patients (CD133+) who also express MDR1 and to ascertain the effect of Oct-4 on 'stem-ness' and its role in the process of these CD133+ cells differentiating towards epithelium. **Methods:** CD133+ cells were isolated using magnetic beads from normal healthy volunteers and prostate cancer patients (NV-CD133+ and PC-CD133+), respectively. The isolated cells were analyzed for the expression of CD133, MDR1 and Oct-4 by flow cytometry and Western blot technique. CD133+MDR1+ cells were cultured with the presence or absence of antihuman Oct-4 blocking antibody. **Results:** PC-CD133+ cells displayed higher

Oct-4 expression accompanying with the ability of self-renew. Furthermore, PC-CD133+ cells were highly co-expressed with the multiple drug-resistant marker MDR1. The treatment with Oct-4 blocking antibody could specifically block the capability of PC-CD133+ cells to differentiate into prostate epithelial cells which bearing CD57. **Conclusion:** The higher Oct-4 expression of PC-CD133+ cells may represent a reservoir of differentiation potential for progression of prostate cancer. The MDR1 expression of PC-CD133+ cells *in vitro* and *in vivo* is partially due to preferential activation of Oct-4 gene expression.

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**S4-07****The detection of TMPRSS2: ERG fusion in Chinese prostate cancer and the relationship between TMPRSS2: ERG and the pathological grade**Fa-Xian Yi<sup>1</sup>, Qiang Wei<sup>2</sup>, Xiang Li<sup>2</sup>, Kun-Jie Wang<sup>2</sup>, Hong Li<sup>2\*</sup>, Ya-Guang Yuan<sup>1</sup><sup>1</sup>Department of Urology, The First Affiliated Hospital of Inner Mongolia Medicine College, Hohhot 010050, China<sup>2</sup>Department of Urology, Huaxi Hospital, Sichuan University, Chengdu 610041, China

**Aim:** To detect the incidence of the fusion gene—TMPRSS2: ERG in Chinese patients with prostate cancer and study the associations between TMPRSS2: ERG and pathologic grade of prostate cancer. **Methods:** Sixty-two patients performed prostate biopsy with a positive result were recruited into our study in Huaxi Hospital of Sichuan University (Chengdu, China). Ten patients with benign prostatic hyperplasia (BPH) and nine human cancer cell lines were involved as the control. The findings of the pathologic features of every patient were recorded. Nest RT-PCR was applied to examine TMPRSS2: ERG and the positive results were cloned and the sequence was measured. Eventually, the Gleason scores were compared between TMPRSS2: ERG-positive group and TMPRSS2: ERG-negative one. The association was analyzed between the fusion and the pathologic features with the method of logistic regression. **Results:** The incidence of TMPRSS2: ERG in this group of Chinese patients with prostate cancer was 45.2% (28/62). TMPRSS2: ERG was not found in BPH and the human cancer cell lines. The specificity and sensitivity of TMPRSS2: ERG in the diagnosis of prostate cancer were 100% and 45.16% respectively. The Gleason scores between the fusion-positive group and fusion-negative group had no significant difference ( $Z = -0.609$ ,  $P = 0.542$ ). But the major Gleason scores between the fusion-positive group and fusion-negative group had significant difference ( $Z = -2.600$ ,  $P = 0.009$ ). TMPRSS2:ERG was respectively associated with 3 pathological features including cribriform growth pattern, foamy gland morphology and signet-ring cell features with the analysis of univariate logistic regression (OR = 6.250,  $P = 0.002$ ; OR = 6.666,  $P = 0.023$ ; OR = 3.240,  $P = 0.035$ ). Through the method of multivariant logistic regression, only cribriform growth pattern was associated with TMPRSS2: ERG (OR = 3.750,  $P = 0.033$ ). The account of the pathological features of the fusion-positive group was significantly more than that of the fusion-negative group ( $Z = -4.565$ ,  $P = 0.000$ ). **Conclusion:** TMPRSS2: ERG

occurs in almost half of Chinese patients with prostate cancer. It was suggested that TMPRSS2: ERG is associated with higher pathological grades of prostate cancer in the present study.

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#### S4-08

##### Testosterone and dihydrotestosterone synthesized from DHEA in stromal cells of prostate cancer affect androgen receptor of prostate cancer cells

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**Background:** One of the mechanisms by which advanced prostate cancer (PCa) usually relapses after androgen deprivation therapy (ADT) is the adaptation to residual androgens in PCa tissue. It has been observed that androgen biosynthesis in PCa tissue, the so-called ‘intracrine’ androgen formation, plays an important role in this adaptation. **Aim:** To investigate how stromal cells derived from normal prostate and prostate cancer affect adrenal androgen DHEA and to explore its metabolism in androgen-sensitive PCa LNCaP cells. **Methods:** We cocultured the LNCaP cells transfected with a luciferase reporter (pGL3PSAp-5.8) driven by PSA promoter and commercially available normal prostatic stromal cells (PrSC) or stromal cells from prostate cancer obtained at prostatic needle biopsy (PCaSC). Subsequently, we added DHEA and examined the PSA promoter activity 24 h later by a luciferase assay. Also, we cocultured them using Boyden chamber to examine the effect of DHEA under the coculture on LNCaP growth 4 days after DHEA addition. For knockdown of AR in PrSC, we transfected AR siRNA; for knockdown of the AR in LNCaP cells, we transfected shRNA-hAR plasmid. We measured the concentration of androgens (T, DHT, androstenedione, and androstenediol) in culture medium by LC-MS/MS. **Results:** DHEA alone had little effect on PSA promoter activity and the proliferation of LNCaP cells. However, when LNCaP cells were treated with DHEA in the presence of prostate-derived stromal cells, especially PCa-derived stromal cells (PCaSC), stromal cells accelerated DHEA-induced PSA promoter activity via androgen receptor activation. Moreover, PCaSC stimulated the proliferation of LNCaP cells under physiological concentrations of DHEA. Biosynthesis of testosterone or DHT from DHEA in stromal cells was involved in this acceleration of LNCaP cell proliferation. Androgen biosynthesis from DHEA depended upon the activity of various steroidogenic enzymes present in stromal cells. **Conclusion:** this coculture assay system provides new insights into ‘paracrine’ and ‘intracrine’ androgen biosynthesis under the microenvironment of PCa cells before and after ADT, and offers a model system for the identification of important steroidogenic enzymes involved in PCa progression and for the development of the corresponding inhibitors of androgen biosynthesis.

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#### S4-10

##### Is it necessary to change the PSA threshold for screening

##### prostate cancer in China?

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**Aim:** PSA > 4 ng mL<sup>-1</sup> is widely used for prostate cancer screening in most of the western countries to guide prostate biopsy though PSA > 10 ng mL<sup>-1</sup> is still adapted in china. In this study we try to evaluate the role of PSA > 4 ng mL<sup>-1</sup> in prostate cancer screening in Chinese. **Methods:** 137 patients with PSA > 4.0 ng mL<sup>-1</sup>, aging from 51 years to 86 years old, were enrolled in this study from May 2008 to April 2009. All the patients received TRUS-guided prostate biopsy. The patients received DRE measurement before biopsy and 40 patients was found with abnormal DRE. Sextant biopsy and extended 12-cores prostate biopsy were used in our study. The sextant biopsy was mainly used in patients with PSA > 50 ng mL<sup>-1</sup> and extended 12-cores prostate biopsy was used in patients with PSA < 50 ng mL<sup>-1</sup>. **Results:** Among these 137 patients, 112 patients with the final pathology were followed up and 25 patients were missed during follow-up. Totally, prostate cancer was detected in 44 patients (39.2%, 44/112) in our study. Among these patients, 11 prostate cancer patients were confirmed in the 42 patients with PSA < 10 ng mL<sup>-1</sup> with a positive rate of 26.19%, three prostate cancer were found in 23 patients with PSA between 10 ng mL<sup>-1</sup> and 20 ng mL<sup>-1</sup> (13%), 24 prostate cancer in 39 patients with PSA > 20 ng mL<sup>-1</sup>. Comparing sextant biopsy with extended 12-cores biopsy, the complication was not increased with the increased cores. **Conclusion:** the PSA threshold > 4 ng mL<sup>-1</sup> was suitable for prostate cancer screening in Chinese patients.

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#### S4-11

##### A rapid screening model of steroid 5 alpha-reducase inhibitor *in vitro*

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**Aim:** To establish a rapid screening model of steroid 5 alpha-reducase inhibitor that is usually used in the treatment of benign prostatic hyperplasia (BPH). **Methods:** 1) Model establishment: A series of concentration of NADPH was added into a 96-well plate, incubated at 37°C, and the OD340nm value was detected with microplate reader for ELISA at 0 and 10 min, then standard curve of NADPH was prepared. We derived steroid 5 alpha-reducase from liver of SD rats, added a series of concentrations of testosterone (0–40 μmol L<sup>-1</sup>) and 20 μL 5 alpha-reducase into a 96-well plate, incubated together at 37°C, detected OD340nm value at 0 and 10 min, then analyzed enzyme activity of 5 alpha-reducase. 2) Model test: eight rows of 96-well plate were divided into four groups, two rows each group. Groups were 0, 30 and 60 nmol L<sup>-1</sup> epristeride and 0, 30 and 60 nmol L<sup>-1</sup> finasteride. 0–40 μmol L<sup>-1</sup> testosterone, 22 μmol L<sup>-1</sup> NADPH,



20  $\mu\text{L}$  steroid 5 alpha-reductase were added to each well, and the total volume was adjusted to 200  $\mu\text{L}$  with Tris-HCl buffer, and then incubated together at 37°C, OD340nm value was detected at 0 and 10 min. **Results:** 1) The  $K_m$  value of steroid alpha-reductase was 3.794  $\mu\text{mol L}^{-1}$ , its  $V_{max}$  was 0.271  $\mu\text{mol L}^{-1}$  per min; 2) The  $K_i$  of epristeride was 148.24  $\text{nmol L}^{-1}$ , its  $IC_{50}$  was 31.52  $\text{nmol L}^{-1}$ , and the curve diagram suggested that epristeride was uncompetitive enzyme inhibitor; The  $K_i$  of finasteride was 158.71  $\text{nmol L}^{-1}$ , its  $IC_{50}$  was 13.62  $\text{nmol L}^{-1}$ , the curve diagram suggested that finasteride was competitive enzyme inhibitor. Both results were consistent with published data. **Conclusion:** The rapid screening model of steroid 5 alpha-reductase inhibitor could effectively screen drugs of anti-BPH *in vitro*.

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#### S4-12

##### Extracorporeal shock wave therapy for chronic pelvic pain syndrome

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**Background:** Prostatitis is one of the most common urological diseases. Chronic pelvic pain syndrome (CPPS) has an incidence of 1.5%–10% among male populations all over the world. It is accompanied by many symptoms like voiding disorders and pains in perineum, testis, penis, bladder and the back. Quality of life of the affected men is often poor. NIH classification holds that many prostate affections, especially those in which no infection can be detected, are associated with patho-physiological processes outside the prostate (neurological, neuromuscular, perineal or intrapelvic causes). The origin of CPPS is still unknown and an efficient therapy for it is still missing. Shock wave has been widely used in ESWL. Shortly after its establishment in urology, ESWL was modified and also applied as extracorporeal shock wave therapy (ESWT) with weaker energy sources in orthopaedics for the treatment of degenerative and painful arthropathies and chronic pain in soft tissue. Good results were achieved especially in the treatment of pain. Otorhinolaryngology has discovered ESWT for the treatment of sialolithiasis. The applicability of ESWT was also postulated for the treatment of Peyronie's disease. ESWT should reduce the degree of deviation and the pain of erection. Furthermore, there is clinical evidence that shock wave can relax muscles with chronic hypertension. **Aim:** To investigate whether ESWT is an effective therapy for CPPS. **Methods:** A total of 186 patients (age 16–45, mean 26.4) with CPPS for a minimum of 6 months (average duration of CPPS complaints 21.2 months [7–143 months]) without inflammation signs in urine or seminal fluid or clinical evidence of prostate cancer were included into the study. The patient is positioned on his back and the therapy head is coupled on the perineum. At each session, 3 000 pulses of focused SW are applied at a frequency of 3.5 Hz. The treatment is effected at an energy level of 0.25 mJ per  $\text{mm}^2$ . The time between individual treatments is one week. A total of four

sessions in the course of four weeks is planned. Follow up was performed after 4 weeks. Specific complaints including the average urinary symptoms score, the average pain score, and the average score of quality of life were investigated by NIH-CPSI before and after the treatment. All patients completed the treatment course and were followed up. **Results:** The duration of ESWT was each 13 min. No side effects occurred. NIH-CPSI improved markedly. The average NIH-CPSI score was 34.1 before treatment and 17.9 after ESWT. The average pain score of 15.8 was reduced to 8.2 after treatment. The average urinary symptoms score was 7.2 down to 3.8. The average score of quality of life was increased by 46.8%, i.e. 11.1 down to 5.9 after ESWT. **Conclusion:** ESWT in the prostate region can be a safe and effective treatment chronic pelvic pain syndrome. It was an easily performable treatment showing no side effects at all. ESWT achieved a reduction of CPPS related pain and complaints followed by better quality of life.

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#### S6-01

##### TDS as marker for precocious aging to men's health issues

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In developing countries booming of aging population towards the year 2025 has been predicted. Indonesia is one of those countries where old population will increase to 414% (WHO). Diabetes is currently reported to affect 246 million people worldwide. By 2025 diabetes may affect men at all ages over the 380 million. ED may be affected to 80 % of men at all ages (MMAS, 1994). Currently not only ED affects men at younger-age but also possible other co-morbidities such as: metabolic syndrome, obesity, hyper-cholesterol, hypertension and cardio-vascular-diseases (CVD). Aging in men is promoted by the decrease production of testosterone known as Testosterone Deficiency Syndrome (TDS).

TDS, ED and Precocious Aging: Current researches have proven that TDS significantly correlates with ED, medical option treatments for ED, such as the use of PDE-5 inhibitors are currently preferred over the majority of men. Unfortunately PDE-5 treatment failures may still occur. In our study of 30 young-age men revealed that PDE-5 treatments failures are due to TDS. Testosterone supplement treatments in this group of young-age men with ED with PDE-5i treatment failures have credited to improve hardness of their erection. PDE-5i treatment failures in young-age men with ED can predict precocious aging and the development of possible co-morbidities herewith.

Conclusions and remarks: Early detection of TDS in ED men with PDE-5i treatment failures is of extreme important. A more detailed research on ED and the occurrence of co-morbidities such as metabolic syndrome, obesity, hyper-cholesterol, hypertension and cardio-vascular-diseases (CVD) should therefore be encouraged. Efforts to prevent and correct TDS are of paramount important to prevent precocious aging and promote men's health issues. TDS is still poorly understood as

marker for precocious aging.

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#### S6-02

### Efficacy and safety of Maxpol T for tissue engineering penoplasty on small penis syndrome

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**Aim:** To evaluate the safety and efficacy of PLGA Biodegradable Scaffold (Maxpol T) for tissue engineering penoplasty, the effect of autologous fibroblast cell (AFBC) seeded Maxpol T implantation for penile girth enlargement was investigated.

**Methods:** Sixty-nine patients (mean aged: 34.40 ± 9.34 years) with small penis syndrome with informed consent were enrolled in this study from two medical centers. Scrotal skin (2 × 2 cm) was attached under local anesthesia and AFBC were cultured and stored in nitrogen tank. AFBC were seeded Maxpol-T and cograft was implanted under the Buck's fascia of penile shaft by circum incision. Patients were followed up 1, 3, 6 months to evaluate the effects on penile girth changes with ruler and sonography. Patient's satisfaction ratio was evaluated by VAS and IIEF5 pre- and post-operation. **Results:** The mean penile girth in placed state and erected state before operation were 8.18 ± 0.83 cm and 10.26 ± 1.22cm. At 6 months after operation, the mean penile girth in placed state and erected state were significantly increased to 12.19 ± 1.27 cm and 13.18 ± 1.31 cm ( $P < 0.001$ ). Sixty-five (94.2%) patients satisfied with the penile girth increased more than 2 cm without sexual dysfunction and severe side effects according to VAS and IIEF5 evaluation. Six cases (8.7%) revealed prolonged edema, and three of them showed pinpoint erosion and were treated by local medication.

**Conclusion:** Tissue engineering penoplasty by implantation cografting AFBC seeded Maxpol T cograft on penile shaft might be safe and effective methods for penile girth enlargement in patients with small penis syndrome.

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#### S6-04

### The survey of quality of erection questionnaire of Chinese outpatients with erectile dysfunction: multiple centers experience

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**Background:** Quality of erection questionnaire (QEQ) is an effective tool for assessing the quality of erectile function in erectile dysfunction (ED) patients. At present, numbers of international studies have confirmed that QEQ is an effective tool to evaluate erectile function. But in China, there were less data

about it. **Aim:** Therefore, we use QEQ for Chinese ED patients and survey the relationship with QEQ score, International Index of Erectile Function (IIEF) score, erectile function (EHS). **Methods:** Lead by Chinese Society of Andrology, since June 2008 to June 2009, we conducted investigation with ED patients in 46 centers of 11 Chinese big cities (Beijing, Shanghai, Guangzhou, Hangzhou, Nanjing, Shenyang, Dalian, Wuhan, etc.). The investigation questionnaire includes age, ED degree, duration of ED, medication, data of sex satisfaction, IIEF, and QEQ ratings before and after treatment, etc. The ED patients defined by the IIEF score filled the first part of the questionnaire, and then completed it after four weeks treatment with PDE5 inhibitor. Excluding criteria: IIEF score  $\geq 26$  or not available; not taking PDE5; having no sex activities during treatment; not completing the questionnaire. A total of 4 507 questionnaires were collected during this survey, while 4 252 valid. The data were stated by the Peking University Health Science Center School of Public Health using SPSS 13.0. **Results:** 1) The basic situation of the patients: patients of 30–50 years old were the majority of the ED patients (60%). A lot of patients had a history of smoking (60.2%) and drinking alcohol (58.7%). Patients suffered from hypertension, hyperlipidemia, diabetes were 15.4%, 12.9% and 11.3%, respectively. The rate of mild, moderate and severe degree of ED patients were 30%, 43.5% and 26.5%, respectively. The duration of ED were less than 5 years in 74% patients, 5-10 years and more than 10 years were 20.9% and 5.1%, respectively. Compared to the erection time, patients pay more attention to the hardness of erection (56.3%). There were 64.4% of ED patients with no medication, and the majority (54.8%) of the medication patients took the traditional Chinese medicine. 2) Treatment effect: 95.5% of patients have efficacy with PDE5 inhibitors therapy. 39.5% became normal. Mild ED ratio increased from 30% to 51%, while moderate and severe degree of ED patients decreased from 43.6% to 7.6% and 26.5% to 1.8% ( $P \leq 0.01$ ) respectively. Frequency, satisfaction and enjoyment of the sexual life were improved ( $P \leq 0.01$ ). Many patients had more sexual intercourse post therapy. Before treatment, the patients not enjoying sex life accounted for 77.4%, while enjoy part accounting for only 0.6%. After treatment, patients who have a high degree enjoyment reach to 47.7%, and the extremely enjoyable part also reached to 3.5%; Satisfaction of sex life had greatly improved. After treatment, 74.9% of the not satisfied changed to 58.8% satisfied and 13.5% very satisfied; The total score of QEQ and IIEF increased from 13.57 and 27.38 to 23.39 and 69.33 ( $P \leq 0.01$ ). 3) Hardness of erection: After treatment, patients had a significant change in hardness. 1-level hardness declined from 17.3% to 0.5%, while 4-level hardness rose from 1.2% to 63.6% ( $P \leq 0.01$ ); 93.4% of 1, 2-level hardness patients were improved to 3 and 4 level ( $P \leq 0.01$ ). 4) Benefits of 3, 4-level hardness: Compared with 3-level hardness, 4-level hardness can significantly improve sexual intercourse frequency. In the 5-6 times in the 7–10 times and 11 times sex life, while the ratio of 3-level hardness patients were 31%, 15.7% and 1.4%, the ratio of 4-level hardness were 34.5%, 21.3% and 6.1%; For the satisfaction and enjoyment of sexual life, the 4-level hardness was significantly better than 3-level hardness ( $P \leq 0.01$ ). QEQ score of 3-level hardness was 54.92, while the 4-level hardness was as high as 79.66 ( $P \leq 0.01$ ); IIEF score



of 3-level hardness was 20.09, while the 4-level hardness was as high as 25.78. **Conclusion:** 1) Although the number of ED patients with long duration and the proportion of elderly patients were increased, short duration patients and middle-aged patients were still the majority of the patients who seeking for treatment. So there need more education about the sexual and reproductive health in order to make more patients come for treatment in the early stage of the disease. 2) Unhealthy living habits and associated diseases have a significant negative impact on ED and the treatment. 3) Erection hardness plays an important role in sexual life, which can significantly affect the frequency of sexual intercourse, satisfaction and enjoyment. 4) PDE5 inhibitors are the first-line treatment for ED treatment, which can give effective treatment, improving erectile function, especially the hardness, which giving a sexual quality of life.

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### S6-05

#### Efficacy and prognostic factors predicting successful response to sildenafil after radical prostatectomy

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**Aim:** To assess the efficacy and safety of sildenafil citrate in the management of erectile dysfunction (ED) followed by radical prostatectomy (RP) and to analyze different prognostic factors predicting the response to sildenafil in such a challenging group of patients. **Methods:** One hundred patients with ED followed by RP participated in an open-labelled, non-randomized, prospective and dose-escalation study. The median age of the patients was 56 years and the mean period after RP was  $38.6 \pm 11.4$  months. The study duration was 12 weeks, comprised a 4-week run-in period followed by two active treatment periods of 4 weeks with 50 and 100 mg of sildenafil, respectively. Patients were assessed by means of the International Index of Erectile Function (IIEF) questionnaire at baseline and after each treatment period. At the end of the study, the Global Efficacy Assessment Question was used to evaluate treatment satisfaction. Factors affecting the patient's response to sildenafil were assessed by means of uni- and multivariate analysis. **Results:** The entire study group was suffering from severe ED at baseline, with a mean erectile function (EF) domain score of  $6.5 \pm 0.9$ . EF scores improved to  $11.8 \pm 8.4$  and  $17 \pm 11$  with 50 and 100 mg of sildenafil, respectively. Sildenafil therapy significantly improved the ability of many patients to achieve and maintain an erection. The mean scores for question 3 of the IIEF were  $1.0 \pm 0.1$ ,  $2.1 \pm 1.4$  and  $3.0 \pm 1.8$  at baseline, with 50 mg of sildenafil and 100 mg of sildenafil, respectively, while the corresponding scores for question 4 were  $1.0 \pm 0.1$ ,  $1.9 \pm 1.4$  and  $3.0 \pm 1.8$ . The satisfaction rate was 54%. The response was dose-dependent but the incidence of adverse effects increased from 6% with 50 mg of sildenafil to 34% with 100 mg of sildenafil. In univariate analysis, histology and grade of tumor and postoperative partial tumescence were found to significantly impact the patients' response to sildenafil. In multivariate analysis, postoperative partial tumescence was the

only independent predictive variable. **Conclusion:** Sildenafil was found to be a safe and satisfactory treatment for ED post-RP. The effect was dose-related. Patients with postoperative partial tumescence were the best responders.

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### S6-06

#### Experimental study of arginase gene and protein expression and its activity in the penis of diabetic rats

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**Aim:** To investigate the pathogenesis of penile erectile function in diabetes mellitus rats. **Methods:** 30 Sprague-Dewley rats were injected intraperitoneally with streptozotocin (STZ) to make experimental models of DM. Rats were divided randomly into diabetes group ( $n = 9$ , two dead excluded), STZ group ( $n = 9$ ) and control group ( $n = 10$ ). Injected with apomorphine, penile erection was observed. The protein and mRNA expression of arginase was detected by Western blot and RT-PCR respectively. Arginase activity in the penis was detected with ultraviolet spectrophotometry. **Results:** Diabetes mellitus group showed a marked decrease in erectile function compared to control group ( $P < 0.01$ ). DM group showed higher expression levels of arginase II protein and mRNA, and were respectively 1.51 and 1.48 fold than that in normal. In contrast, arginase was not significantly different in diabetic group ( $P < 0.01$ ), Arginase activity in the erectile tissues of diabetic rats was 2.2 fold of that in normal; STZ group showed no significant change in erectile function. **Conclusion:** The increased level of arginase II mRNA and protein expression and activity of Arginase in diabetic cavernosal tissue may contribute to the erectile dysfunction (ED) associated with diabetes mellitus. It may play an important role in pathogenesis of the ED of diabetic mellitus.

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### S6-07

#### Association between erectile dysfunction and metabolic syndrome in aging men: hallym aging study

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**Aim:** The aim of this study was to investigate the relationship between erectile dysfunction (ED) and metabolic syndrome (MS) in a population-based cohort study, Hallym Aging Study (HAS). **Methods:** Among the 1 520 participants in HAS, 278 men aged  $\geq 50$  years, who underwent detailed health evaluations, including health-related questionnaires, evaluation of the medical history and various life style factors as well as clinical measurements,

were included in the study. ED and MS were assessed by using a 5-item version of the International Index of Erectile Function (IIEF-5) and the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), respectively and the relationship between ED and MS was investigated. **Results:** Of the 278 men, 120 (43.2%) had MS. Ninety percent of men with MS and 78.5% of men without MS had ED ( $P < 0.05$ ) and chi-square analysis revealed significant differences in the rate of MS between groups when stratified by ED severity ( $P < 0.001$ ). The multivariate logistic regression analysis, controlling for age and comorbidities, showed that men with MS were 3 times more likely to have ED than men without MS and among the metabolic risk factors, only waist circumference (WC) was significantly associated with the presence and severity of ED ( $P < 0.05$ ). **Conclusion:** The presence of MS was independent risk factors for ED and WC was the most significant metabolic risk factors predicting the risk of ED. These results highlight the clinical importance of evaluating ED in patients with MS, especially in patients with abnormal WC.

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#### S6-08

##### Partial penile sensory axon resection for treatment of premature ejaculation

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**Aim:** Partial penile sensory axon resection (PSAR) is performed to produce hyposensitivity of the glans penis as a management of premature ejaculation (PE). **Methods:** Resection of the lateral and the ventral faning distal twigs of the glandular axons of the dorsal nerve of the penis proximal to the corona glandis has resulted in reduced sensation of the glans leading to improvement of premature ejaculation. Seventeen patients suffered from secondary PE with a mean age of 35.5 years, were treated during the last 24 months. All patients had ejaculatory latency ranging from 2–3 s to 2–4 min. **Results:** All patients were free of major post-operation complications. Three of them (17.6%) did not respond to the therapy while the other 14 (82.4%) had responded with a variable latency extending from 7–20 min. One patient (5.9%) suffered from slight coldness of the glans while one (5.9%) complained about a feeling of anaesthesia at the tip of the glans. Both patients had reported on a longer latency for ejaculation. The two non responders returned to sildenafil 20 mg tablets in one case while the other continued on Xylocain local anaesthetic gel. No affection of erection was observed. No correlation was found between the amount of the resection and the post operation ejaculatory latency. The results were evaluated through a questionnaire. **Conclusion:** Partial penile neurectomy (PSAR) has achieved a goal in the treatment of PE and the technique must be further evaluated on a large number of patients. It overweighs the results achieved using Psychosexual counselling and behavioural treatment, administration of selective serotonin reuptake inhibitors, application of anaesthetic topical ointments, management with phosphodiesterase inhibitors and lastly local injection of hyaluronic acid gel.

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#### S6-09

##### Gene expression study of seminal vesicle in men with ejaculatory dysfunction

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**Aim:** To better understand the genetic basis of ejaculatory dysfunction (EjD), we examined genes differentially expressed in seminal vesicle tissues in elderly men with and without EjD using cDNA microarray analysis and Ingenuity Pathways Analysis (IPA). **Methods:** A total of six patients with bladder or prostatic carcinoma undergoing radical surgery were enrolled in this study. Three of them had normal ejaculatory function, whereas another three men had EjD. Seminal vesicle tissues not invaded by tumor were obtained for RNA extraction and subsequent cDNA microarray analysis (Agilent Human Whole Genome Oligo 4 × 44 K Microarray). Genes with significant differential expressed between two groups were identified by the *t*-test analysis and more than 2-fold changes. IPA software was used to analyze the database. Quantitative real time RT-PCR was used to verify the microarray data. **Results:** A total of 1664 genes were significantly up-regulated and 1 096 genes were significantly down-regulated in samples with EjD. These target genes were overrepresented in several IPA networks in which the top five networks include (1) nervous system development and function, cell-to-cell Signaling and interaction, (2) inflammatory response, (3) post-translational modification, connective tissue disorders (4) cellular assembly and organization, cancer, cellular growth and proliferation and (5) cardiovascular system development and function. For canonical pathway analysis, the top five canonical pathways are (1) circadian rhythm signaling, (2) hepatic fibrosis / hepatic stellate cell activation, (3) p53 signaling, (4) role of pattern recognition receptors in recognition of bacteria and viruses and (5) airway pathology in chronic obstructive pulmonary disease. Quantitative real time RT-PCR confirmed the expressions of genes. **Conclusion:** Genes dysregulated in the seminal vesicle tissue of EjD men suggest that EjD results from combination of neuron and vascular degeneration, aging stress related hormonal change, apoptosis and inflammation activity. These data provide the basis for elucidating the causes of EjD in humans.

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#### S6-10

##### New technique: detecting extracellular calcium concentration of corpus cavernosum smooth muscle *in vivo*

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**Aim:** To detect the extracellular calcium concentration of corpus

cavernosum smooth muscle *in vivo* using needle-type calcium sensor, and to discuss its correlation with erectile dysfunction. **Methods:** 48 experimental SD rats were randomized into the injury group ( $n = 24$ ) and the control group ( $n = 24$ ), bilateral cavernous nerves were cut in injury group, and nothing was done in control group. One month later, using needle-type calcium sensor, we detected extracellular calcium concentration of corpus cavernosum smooth muscle *in vivo* and compared the results of the two groups. Data was expressed as mean  $\pm$  SD. Result: The extracellular calcium concentration was much lower in the injury group ( $0.07 \pm 0.38$  mV) than the control group ( $-0.22 \pm 0.45$  mV) ( $P < 0.05$ ). **Conclusion:** Needle-type calcium sensor would be a new technique in evaluating erectile dysfunction in future.

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### S7-01

#### Roles of microRNA-449 in mammalian spermatogenesis

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**Aim:** To dissect the roles of microRNA-449 in mammalian spermatogenesis. **Methods:** Testicular expression profiles of miRNAs in prepubertal (7 days of age) and adult (60 days of age) C57BL/6 mouse were examined to identify potential miRNAs implicated in spermatogenesis. Cellular localization and transcriptional regulation of a miRNA cluster (miR-449 cluster) was analyzed using *in situ* hybridization and a reporter assay, respectively. The gene structure of miR-449a and its transcriptional regulation were investigated. The effects of miR-449a ectopic expression on GC-1-spg cells were also investigated. By homologous recombination, we have inactivated the miR-449 gene cluster in a mouse embryonic stem cell line and miR-449 cluster knockout mice will be generated soon.

**Results:** There was a significant difference between prepubertal and adult testicular microRNA expression profiles. miRNA-Gene and miRNA-GO network analyses indicated a number of microRNAs including miR-449a appeared to be pivotal in spermatogenesis. miR-449a, along with miR-449b and c, were processed from a common primary transcript (miR-449 cluster) which is located within the second intron of Cdc20b gene. The miR-449 cluster and Cdc20b shared the same promoter under the control of transcriptional factors CREM-tau and SOX5. miR-449a over-expression decreased the proliferation rate of GC-1-spg cells compared to control, which might result from down-regulation of some miR-449a target genes involved in cell cycle G1/S transition. **Conclusion:** The miR-449 cluster displays a testis-preferential expression pattern. miR-449a over-expression causes a decreased proliferation rate in GC-1-spg cells. The physiological role of the miR-449 cluster including miR-449a in spermatogenesis will be analyzed further using the knockout mice.

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### S7-02

#### Integrated functional genomics research of normal human epididymis

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**Aim:** To comprehensively demonstrate the human epididymal gene expression profile involved in spermatozoa maturation. **Methods:** We first constructed a fertile human epididymis cDNA library, 20 000 clones from the library were sequenced. Meanwhile, we applied 2D-PAGE, 2D-DIGE and mass spectrometry (The 4800 Plus MALDI TOF/TOF™ Analyzer) on the analysis of the human epididymis proteome, including the epididymal protein profiling of the different age groups (new-born, young and old), the protein profiling of the epididymal luminal fluids, and the difference protein profiling of the epididymis of different regions (caput, corpus or cauda). Furthermore, epididymal specific genes and epididymal luminal proteins were further confirmed and studied by RT-PCR, Western Blot, immunohistochemistry, indirect immunofluorescence (LSCM) and sperm function analysis. **Results:** A global normal human epididymal gene expression and protein profile datasets library were constructed. Through large scale proteome analysis of normal male epididymis, epididymal tissue protein spots (1 720) and luminal protein spots (405) were identified. The number of unique epididymal and luminal proteins was 485 and 248, respectively. Bioinformatics analyses were performed on the data to assess predicted subcellular locations and involvement in metabolic pathways. Furthermore, 40 proteins were cloned and polyclonal serum was raised against recombinant proteins. Other 600 polyclonal antibodies were produced against the specific polypeptide. Tissue and cellular localization were identified through RT-PCR, Western blot and immunofluorescent. Immunofluorescent analysis showed that about 220 proteins were located on the different site of mature sperm. Functional analysis showed that 60 proteins may be involved in the process of motility and fertilization. **Conclusion:** A comprehensive human epididymal gene expression profile was generated in our study, which creates a reference map for further investigations into epididymal gene and protein functions. It displays a global epididymal sperm-binding protein family that shows unimaginable binding and functional polymorphism. These datasets can be applied to male infertility diagnosis and treatment, as well as to the development of contraceptive drug.

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### S7-03

#### Gonadotropin-regulated testicular helicase (GRTH/DDX25): a master post-transcriptional regulator of spermatogenesis

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**Aim:** To elucidate the role of gonadotropin-regulated testicular helicase (GRTH) in germ cell development and maturation, the



mechanism of its post-transcriptional regulation of genes during the progress of spermatogenesis, and its relevance to human reproduction. **Methods:** Studies on GRTH cellular and species characterization and functions utilized wild type, GRTH+/-, GRTH-/- male mice generated in our laboratory. Genomic DNA samples from 143 Japanese infertile patients (non-obstructive azoospermia) and 100 fertile controls (Kanazawa University, Kanazawa, Japan) were evaluated for GRTH/DDX25 gene mutations. GRTH peptide polyclonal antibodies (12-23 and 466-479) and to germ cell proteins, and to phospho-Ser and -Thr were utilized for evaluation of expression by Western blots, immunofluorescence and EM localization. Mutations were determined dHPLC analysis and DNA sequencing. Purification of testicular germ cells and Leydig cells was performed by centrifugal elutriation. Subcellular fractions from these cells were prepared to assess GRTH and associated mRNAs at these sites. Transfection of COS-1 cells with GRTH wild type or phospho-deficient mutant constructs was performed to characterize the expressed GRTH species. Other techniques used were immunoprecipitation (IP) and (co-IP). GRTH-mRNA complexes were isolated and RNAs were characterized by RT-PCR. *In-situ* hybridization, immunocytochemistry, confocal and electron microscopy were performed to localize GRTH at cellular and subcellular levels. **Results:** Male germ-cell maturation is orchestrated by a cascade of temporally regulated factors. Gene expression during spermatogenesis requires temporal uncoupling of transcription and translation. GRTH, a testis-specific member of the DEAD-box family of RNA helicase discovered in our laboratory, is a target for gonadotropin-induced androgen action and a post-transcriptional regulator of key spermatogenic genes. This helicase, which is expressed in germ cells (spermatocytes and round spermatids) and Leydig cells, has ATPase and bidirectional RNA-unwindase activities and increases the translation of messages. GRTH is the only helicase known to be regulated by hormones. It is developmentally regulated and its expression is dependent on gonadotropin via cAMP and androgen, directly in Leydig cells and indirectly in germ cells. GRTH null male mice generated in our laboratory are sterile due to spermatogenic arrest at step 8 of round spermatids and their failure to elongate. Blood testosterone is normal, excluding androgen reduction as the cause of the arrest. The chromatoid body (CB-mRNA storage organelle) in spermatids is markedly reduced in size in GRTH null mice. GRTH is necessary to maintain the structure and function of this organelle. GRTH is a binding protein and an integral component of messenger ribonucleoprotein particles (mRNP) in germ cells, whose proteins are expressed at different steps of spermatogenesis (ie. TP1 and TP2, Prm1, Prm2, tACE and others). The 56-kDa GRTH nuclear species transports target mRNAs to the cytoplasm for storage in chromatoid bodies of spermatids to be released for translation during spermatogenesis. A 61-kDa phosphorylated species is present in polyribosomes, where it regulates the translation of mRNAs encoding spermatogenesis factors. Also, as a component of mRNP particles GRTH acts as a negative regulator of tumor necrosis factor receptor 1 and caspase pathways, and promotes NF- $\kappa$ B function to control apoptosis in spermatocytes of adult mice. A unique heterozygous SNP Arg242 His (exon 8) was found in azoospermic Japanese

men (5%). This mutation impacted on the post-transcriptional modification of the expressed GRTH, since expression of the cytosolic 61-kDa species was absent and only the 56-kDa nuclear species was present. SNPs of exons 8 (missense) and 11 (silent) found in our studies were not apparent in infertile patients from West China (Mol Human Reprod 2007; 12: 887. Hum Reprod 2006; 21: 775; Human Reprod 2008; 23: 278). Also, the exon 10 silent mutation reported in the infertile Chinese patients, although present in the Japanese infertile patients was not significantly different in the allele change from control. The SNPs of the GRTH gene might be associated with an ethnic background of male infertility among Asian men. The absence of the phospho-GRTH species of the mutant protein could be of relevance to functional aspects of the protein (translational events and cytoplasmic functions) that impact on germ cell development and/or function. **Conclusion:** These studies have demonstrated that GRTH is a multifunctional RNA helicase, and its association with some cases of male infertility underlines its importance as a central, post-transcriptional regulator of spermatogenesis. Moreover, GRTH links androgen action and spermatogenesis. These studies on GRTH function and regulation provide insights into the intrinsic requirements of spermiogenesis, and open new avenues to studies of spermiogenesis regulation, infertility and male contraception.

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#### S7-05

#### Characterization of SPAG4L, a new member of SUN gene family, involved in the specific stage of human spermatogenesis

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**Aim:** To study the expression pattern, cellular location and subcellular location properties of SPAG4L gene, a new member of SUN gene family. **Methods:** Northern blot and reverse transcription polymerase chain reaction (RT-PCR) were performed to explore the expression pattern of SPAG4L mRNA in different tissues. Aborted fetal testes ( $n = 3$ ) and testicular biopsy samples ( $n = 6$ ) were collected and used to analyze the expression of SPAG4L by RT-PCR and *in situ* hybridization to investigate its spatial and temporal expression in human testis during development. Finally, GFP tagged SPAG4L and a series of SPAG4L mutants were constructed to clarify the subcellular location properties of SPAG4L. **Results:** Northern blot and RT-PCR results showed that SPAG4L was enriched in adult testis and the fragment size was about 1.7 Kb. SPAG4L mRNA was expressed in adult testis, but not in fetal testis by RT-PCR, indicating that the expression of SPAG4L may regulate sexual maturation. *In situ* hybridization results revealed that SPAG4L

mRNA was abundantly detected in spermatocytes and round spermatids, but no obvious positive signal was observed in spermatogonium. These data suggested that *SPAG4L* was mainly expressed in the meiotic phase of spermatogenesis. Subcellular localization analysis indicated that green fluorescent protein-labeled full-length *SPAG4L* and two deletion mutants (1-220 and 52-230), which contained the TM and coiled-coil region, localized to the nuclear membrane and endoplasmic reticulum after transfecting for 24 h *in vitro*. In contrast, the C-terminal region of *SPAG4L* (221-379), which included the SUN domain, was found throughout the cells. This finding suggests that the TM and coiled-coil region were sufficient for *SPAG4L*'s localization to the nuclear membrane and endoplasmic reticulum, whereas 221-379 are not involved in the localization of *SPAG4L*. **Conclusion:** *SPAG4L* was enriched in adult testis and mainly expressed in the meiotic phase of spermatogenesis. *SPAG4L* protein was located in the nuclear membrane and ER, and TM and coiled-coil are necessary for its localization. These data indicated that *SPAG4L* may play an important role in the meiotic phase of spermatogenesis.

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#### S7-06

##### Curcumin affects the dynamics of chromatin associated factors in mouse spermiogenesis

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**Background:** Hyperacetylation of histones is considered as a necessary process for spermatid transformation in mammalian spermiogenesis, which is decided by the balance between the HATs and HDACs. It is observed that an extensive range of chromatin associated factors (CAFs) are removed from chromatin around Step 9, just before the histone replacement, inferring the relationship between these events. **Aim:** In this research, we investigated the effects by Curcumin, which is a specific inhibitor on HAT activity of p300/CBP, on the dynamics of CAFs in mouse spermiogenesis. **Methods:** Testicular cell (TCs) suspension was made by two-step enzyme digestion. Then TCs were cultured in DMEM/F12 added 50  $\mu\text{mol L}^{-1}$  Curcumin at 34°C for 48h. After that, TCs were dried on the slides, TUNEL and IHC essays were executed. **Results:** Incubation in 50  $\mu\text{mol L}^{-1}$  Curcumin for 48 h did not induce the apoptosis. However, the dynamics of CAFs was obviously changed by the treatment. The signals of TAF1, AP2a, TOP2 $\beta$ , H3K4-Me3 and H4K20-Me3 disappeared much earlier, at about Step 5. Acetylated H4 could not be detected after Step 9 instead of the normal hyperacetylated pattern. At the same time, the HDAC1 signal was retained until Step 15. **Conclusion:** By studies on spermatids *in vitro*, HAT inhibitor treatment regulated the HDAC1, exhibited the close interactions between HATs and HDACs. Particularly, the acetylation status of histones might play as a trigger of the disassociation of other CAFs and the following programs. HATs and HDACs might be important during the reprogramming of the mouse spermiogenesis.

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#### S7-07

##### Role of p73 in mammalian germ cell apoptosis during the first round of spermatogenesis in the rat

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**Background:** p73 belongs to a small but important family of p53-related proteins (p53, p63, p73). Genotoxic stimulus, like the anticancer drug etoposide induces the activation of p73 by promoting its phosphorylation by the tyrosine kinase c-Abl. Our own studies showed that p53 increases during physiological and etoposide-induced germ cell apoptosis. Previous studies have shown that p73 is expressed in mammalian testis, but its role during genotoxic stimuli, or physiological apoptosis, has never been studied, making this apoptotic factor an enticing target as the missing link controlling germ cell demise. **Aim:** To determine if p73 is involved in germ cell apoptosis. **Methods:** Immunohistochemistry was used to localize the phosphorylated form of p73 in rat testis. Western blots was used to determine the levels of total and phosphorylated p73 and immunofluorescence. **Results:** In rat testis, p73 is expressed throughout all the first wave of spermatogenesis and its protein levels increased in 25-day-old rats, which was coincident with the peak of physiological apoptosis observed during this age. c-Abl and p73 showed a similar distribution pattern in histological testes sections of rats from 5 days old up to adulthood (2 months old). Both proteins were mainly found in the cytoplasm of germ and Sertoli cell in all studied ages. However, the cytoplasm of meiotic spermatocytes lining the lumen of seminiferous tubules of 25-day-old rats showed a clear increase in the label of both p73 and c-Abl. Interestingly, apoptotic germ cells, which can be easily identify as round pycnotic cells, showed a strong staining of p73 and c-Abl. Isolated apoptotic germ cell also have an increased levels of total p73 and its phosphorylated forms, as compared to non apoptotic germ cells. Pharmacological inhibition of the tyrosine kinase c-Abl using STI (Gleevec), prevented the physiological apoptosis during the first round of spermatogenesis, and also prevented the increase in total and phosphorylated p73. Interestingly, STI prevented the increase in the levels of p73 (total and phosphorylated), but it had not effect on p53 or in its phosphorylated (active) form at Ser-15. We have previously shown that an intratesticular injection of 200  $\mu\text{mol L}^{-1}$  Etoposide in 21-day-old rats, induces apoptosis of meiotic pachytene spermatocytes, as evaluated morphologically and by caspase-3, -8 and -9 activation. We show here that etoposide induced an increase at the protein level of p73, and its phosphorylated form. Interestingly, apoptosis induced by etoposide (caspase activation and the number of apoptotic spermatocytes) was prevented when this drug was injected along with STI. Total and phosphorylated p73 remained similar to control testis when etoposido was injected along with STI. **Conclusion:** Our results show for the first time a role of p73 during physiological and etoposide-induced germ cell apoptosis during spermatogenesis. In addition, our results suggest that similar mechanisms are shared during

physiological and etoposide-induced apoptosis.

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### S7-09

#### Antimicrobial activity of the cystatin-related epididymal spermatogenic (CRES) protein

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**Background:** Cystatin-related epididymal spermatogenic (CRES) protein, a member of the cystatin superfamily of cysteine protease inhibitors, exhibits highly restricted expression in mouse testis and epididymis with an age-dependent pattern, suggesting roles in male reproduction. Previous studies have revealed that other cystatin family members, cystatin 3 and cystatin 11, showed antibacterial activity *in vitro*. **Aim:** To investigate the antimicrobial activity of the CRES protein. **Methods:** Colony forming unit (CFU) assay and spectrophotometric method were examined respectively to investigate the effect of recombinant CRES protein on *Escherichia coli* (*E. coli*) or *Ureaplasma urealyticum* (Uu) *in vitro*. The cysteine residues of CRES protein were mutated to determine whether the antimicrobial activity of CRES is dependent on its disulfide bonds. The active center of CRES was also determined by functional analysis of three overlapping CRES polypeptides started from its N-terminus. Furthermore, the antimicrobial mechanism was also investigated by means of electron microscopy. **Results:** After incubation of 103 CFU of *E. coli* with 100 ng mL<sup>-1</sup> CRES recombinant protein fused with Glutathione-S-transferase (GST) for 4 h, a substantial loss of CFU was observed, and the effect was dose and time dependent. Furthermore, it took 36 h for Uu to grow to plateau stage when incubated with GST-CRES recombinant protein, while only 18 h was needed when incubated with GST. The antibacterial and anti-mycoplasma activities were not impaired when the cysteine residues of CRES protein were mutated, indicating the antimicrobial effect was not dependent on its disulfide bonds. Functional analysis of three overlapping CRES polypeptides started from its N-terminus showed that, the N-terminal 30 residues (N30) loss the antimicrobial activity completely, while N60 showed similar activity as full-length CRES protein. These results indicate the active center of CRES protein resided between the 31st to 60th amino acid residues from its N-terminus. *E. coli* treated with GST-CRES for 4 h visualized by scanning electron microscope showed a smooth continuous membrane structure, suggesting the antibacterial activity of CRES was not accomplished by permeabilization of bacterial membranes. **Conclusion:** The antimicrobial activity of CRES protein suggests it may be involved in protecting the male reproductive tract against invade pathogens.

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### S7-10

#### Do Y-bearing spermatozoa undergo capacitation faster than X-bearing spermatozoa?

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**Aim:** To evaluate the ratio of X- and Y-spermatozoa in the capacitated state and cholesterol depletion pattern of spermatozoa using fluorescent *in situ* hybridization (FISH), and to find out the influence of sex chromosome on the capacitated state of spermatozoa. **Methods:** The experiments were carried out using fresh and frozen semen from three different Japanese black bulls. A part of Sperm were suspended with Brackett and Oliphant's medium (BO medium), which contained 10 mg mL<sup>-1</sup> BSA and 10 mmol L<sup>-1</sup> caffeine and 5 Unit mL<sup>-1</sup> heparin for promoting capacitation and incubated at 39°C in an atmosphere of 5% CO<sub>2</sub> in air. The capacitation state of sperm was analyzed by chlortetracycline (CTC) staining. Other sperm were evaluated cholesterol depletion from the sperm membrane by staining with fillipin fluorescence. After CTC or fillipin staining, the Y-bearing sperm were subsequently determined by the FISH technique. The Y chromosome was stained by the Y chromosome specific probe labeled with digoxigenin and anti-digoxigenin FITC and were applied to analyze the ratio of Y-bearing spermatozoa. The FISH staining slides were analyzed under an epifluorescence microscope. About 1 000 spermatozoa were counted per slide. **Results:** We identified three distinct CTC patterns (F: uncapacitated, B: capacitated, AR: acrosome-reacted cells). After CTC staining, Y chromosome signal could be detected in CTC staining sperm by FISH. In CTC staining sperm, the total Y-bearing sperm ratio was 50.7% and there was no difference from theological ratio (50:50). Signal of Y-chromosome on the sperm was observed distinctly, but the ratio of Y-sperm was altered during incubation time, especially in the 120 min (0 min/120 min, total 51.8%/51.2%, F pattern 51.8%/45.0%, B pattern 55.0%/48.3%, AR pattern 38.7%/51.7%). The statistical analysis showed that there were significant differences ( $P < 0.05$ ) among CTC patterns in the ratio of Y-bearing spermatozoa. Fillipin staining sperm were detected by UV fluorescence with fillipin sterol complexes. A subpopulation of negative fillipin labeling sperm that were considered as capacitated, indicated significant higher Y-bearing sperm ratio than positive fillipin labeling sperm (54.8% vs. 42.5%,  $P > 0.05$ ). **Conclusion:** In bulls, Y-bearing spermatozoa could acquire capacitation certainly faster than X-bearing spermatozoa.

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### S7-11

#### Modified technique for spermatogonial stem cell transplantation into the seminiferous tubules in mouse model

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**Aim:** To develop a modified technique for spermatogonial stem cell (SSC) transplantation with aid of operating microscope in an infertile mouse model. **Methods:** Male neonatal C57BL/6 (B6) mice served as the SSC donors. SSCs labeled with PKH26-GL marker were detected by flow cytometry to verify purity. Adult B6 males were rendered infertile by busulfan treatment as the recipient. Microinjection of SSC suspension under microscope was employed respectively one month later via efferent ducts, rete testis, and seminiferous tubules. Recipients received manual microinjection via seminiferous tubules were subjected for Hematoxylin and eosin staining, confocal laser scanning microscopy, and real-time fluorescent PCR at 30, 60 and 90 days post-transplantation. Positive controls received neither busulfan treatment nor transplantation. Negative controls were injected with an equal amount of transplant medium. **Results:** For manual microinjection under 100 × magnifications, it took 10 min per testis for a complete delivery of 50 µL SSC suspension per testis. However, SSC transplantation via recipient efferent duct, rete testis resulted inefficient injection volume, averaged at 10 to 15 µL per testis averaged at 15 to 20 min. Transplantation via seminiferous tubules by mechanical micromanipulator took 30 min for 30 µL per testis. Donor SSCs showed the earliest transference and colonization in recipient testes 7 days after transplantation. The new generated germ cell layers appeared to be intact. No morphological abnormalities were observed during spermatogenesis, or in the elongation phase of spermatogenesis 90-days post-transplantation. **Conclusion:** Manual injection under microscope could successfully deliver the SSCs into the recipient seminiferous tubules.

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### S7-12

#### Differential potency of induced pluripotent stem cells into male gametes *in vitro*

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**Background:** Derived from a founder population of primordial germ cells (PGCs), the occurrence of sperm cells is a unique process in reproductive biology. Induced pluripotent stem cells (iPS) acquire properties similar to those of embryonic stem cell (ES), which are capable of maintaining pluripotency, have the capacity of self-renewal and can differentiate into cell types from all three germ cell layers. Furthermore, the *in vitro* totipotency of mouse ES cells has been demonstrated to give rise not only to PGCs but also sperm-like cells. **Aim:** Thus, in the present study, the iPS cells was cultured through the process of embryoid body (EB) differentiation, then induced by all-trans retinoic

acid (RA), in order to find the differential potency of induced pluripotent stem cells into male gametes and the expression profile of male germ cell-associated genes, such as: *Oct-4*, *c-kit*, *VASA*, *DAZL*, *SCP3*, *Prm1* and *TP2*, *et al.* **Methods:** From the Stem Cell Research Center, Shanghai Jiao Tong University School of Medicine, we got the established cell lines called Tg-GFP-miPS4.1& Tg-GFP-miPS11.1, *in vitro* cultured in ES cell culture medium from passages 40 to 50, then transferred to EB media condition for the initiate of spontaneously development. Upon termination of the cultures, total RNA and protein in different time course, such as D0, D3, D5 and D7 was collected and the expression profiles of Oct-4, c-kit, VASA and DAZL were examined using real-time PCR and Western blot. The panel of genes examined represents markers from iPS to PGCs. When the expression of PGCs markers were confirmed, the adherent cultures of EBs were exposed to daily treatment with 2 µmol L<sup>-1</sup> all-trans retinoic acid (RA). Upon termination of the treatment, cells were washed with PBS and total RNA was still isolated using TRIzol reagent. The adult female and male mice were used as controls. **Results:** The temporal pattern of expression of the germ cell and sperm cell genes revealed by real-time PCR could be divided into two groups: genes whose expression declined progressively (*OCT-4*); genes whose expression was initially low and then increased (such as DEAD-box protein 4-*DDX4*, Primordial Germ Cell Marker [*VASA*], and *DAZL*). These data demonstrated that cultures derived from differentiating EBs express some genes that are markers of PGCs proliferation and differentiation from iPS. Thus, after 2 µmol L<sup>-1</sup> RA were added to the EBs culture media, the altered expression of the target genes such as *SCP3*, *TP2* and *Prm1* resemble markers of male germ cells could be found. **Conclusion:** iPS cells are considered to be pluripotent cells as ES cells, with the ability to produce all cell types in the body, including germ cells. The process of EB differentiation shows a spontaneous differentiation of iPS into cells of all three germ layers, and it is from these differentiating aggregates of cells that putative PGCs can be identified. While the PGCs respond to RA, RA can stimulate mitotic proliferation of PGCs and induced PGCs to give rise to embryonic male germ cells. Our research indicated that the derivation of mammalian germ cells is possible *in vitro* from induced pluripotent stem cells. But the process of spontaneous differentiation of iPS is inefficient and unpredictable suggesting that the expression pattern of male germ cell associated genes does not mimic the *in vivo* profiles of the genes. And RA is a multipurpose factor, which can regulate the development and differentiation of neural cells, heart cells, and many other tissues. The appropriate concentration of RA and how long it to differentiate stem cell to sperm cell should be explored. Thus, the defined pathway of germ cell development and differentiation need to be further investigated.

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### S7-14

#### Xeno-free culture of human spermatogonial stem cells supported by human embryonic stem cell-derived fibroblast-like cells

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**Aim:** To establish a new method for the isolation and identification of human spermatogonial stem cells (SSCs) from testicular tissue, as well as the culture conditions required to expand SSCs on human embryonic stem cell-derived fibroblast-like cells (hdFs). **Methods:** Testicular cells were isolated from normal human fetal testicular tissues by a sequential two-step enzymatic treatment, followed by magnetic-activated cell sorting (MACS) based on the expression of surface CD49f in SSCs, which was also evaluated by flow cytometric analysis. CD49f<sup>+</sup> cells were cultured on hdFs which were derived from differentiated human embryonic stem cells (hESCs). The SSCs were identified by the expression of alkaline phosphatase (AKP), immunological markers and genes that have been used routinely to characterize SSCs. **Results:** Large-scale cultures of SSCs were maintained on hDF feeder layers, and expanded in the presence of a combination of cytokines and glial cell line-derived neurotrophic factor (GDNF) for at least 2 months. Cell surface marker analysis showed that SSCs retained high levels of AKP activity and were stained strongly for SSEA-1, OCT4 and CD49f. They also expressed the genes OCT4, SOX3 and STRA8 as detected by reverse transcription polymerase chain reaction (RT-PCR) analysis. **Conclusion:** These data clearly illustrate a novel approach for the growth of human SSCs using hdFs as feeder and thereby could eliminate xenogeneic contaminants. This system will provide a new opportunity for the study of regulatory mechanism of the “niche” that governs SSCs self-renewal, and will be a valuable source of SSCs for their potential clinical application.

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### S7-15

#### Transplantation of spermatogonial stem cell suspension through rete testis of azoospermic mice

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**Background:** Spermatogenesis is a biological process that results in the conversion of the relatively undifferentiated germ cell into the highly differentiated spermatozoa. The loss of spermatogonia following chemo- or radiotherapy leading to temporary or permanent infertility of the patient is a well known and unwanted side effect of many oncological therapies. Testis cell transplantation from a mouse into a recipient mouse rete testis results in donor-cell-derived spermatogenesis in nearly all the hosts' testes. This requires the transfer of cells from a donor animal into the testis of a recipient animal, in which the spermatogonial stem cells will colonize and initiate donor-

derived spermatogenesis. **Aim:** To study the transplantation of spermatogonial stem cell suspension through rete testis of azoospermic mice. **Methods:** To isolate germ cells, a 2- to 4-day-old mouse testis cells were dissociated enzymatically. Busulfan treatment was used to eliminate proliferating cells in the testes of recipient mice. The donor cells, suspended in DMEM, were introduced into the rete testis of recipient mice via microinjection method. To distinguish the progeny of the transplanted donor stem cells from endogenous germ cells, BrdU-labeled cells were used. In addition, reverse-transcriptase polymerase chain reaction (RT-PCR) was performed to determine levels of c-kit and cyclin B1 expression in spermatogonial stem cells after transplantation. **Results:** Transplantation of stem cells into rete testis of the recipients left testis was done and cryosection of the transplanted testis showed a significant increase in the number of spermatozoa in the left epididymal lumen compared with that of the control (right testis). It is interesting that cells that had been cultured on feeder layers were able to colonize busulphan-emptied recipient testes after spermatogonial stem cell transplantation. Spermatogonial stem cell was not alkaline phosphatase activities but C-kit gene was expressed in spermatogonial stem cells. Cyclin B1 expression was reduced after busulfan treatment compared with untreated mouse. The expression of this gene was however increased after germ cell transplantation. **Conclusion:** Elimination of differentiating germ cells is believed to provide the necessary environment for donor spermatogonial stem cells to migrate to the basement membrane and establish a stem cell niche. BrdU-labeled testis cells were successfully transplanted into recipient mouse rete testis. These cells remained in all recipient testes up to two months after transplantation. Culture of spermatogonial stem cells before transplantation helps proliferation and improves stem cell transplantation efficiently. The present study confirms that regeneration after cytotoxic treatment is based on morphological criteria. We demonstrate the increase in stem cell numbers during regeneration and after transplantation. The results of this study provided functional data in support of stem cell self-renewal, and demonstrated the increase in the number of stem cell suspension through rete testis of azoospermic mice considerably enhances the efficiency of the rete testis injection in this species.

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### S7-17

#### Purification and characterization of testicular relaxin-like factor in boars

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**Background:** Relaxin-like factor (RLF), also known as insulin-like peptide 3 (INSL3), is a novel member of insulin-relaxin gene family originally discovered by the screening of a boar testicular cDNA library and seems to be expressed mainly in gonadal tissues in several mammalian species. RLF is essential for testis descent, as shown in rodents, but the physiological significance in adults remains unclear. This peptide is biosynthesized as a single large precursor molecule consisting of the A-B-C heterotrimeric structure, in which the A- and B-domains are covalently linked by two disulfide bonds. Unlike relaxin, however, very little is known whether RLF undergoes proteolytic processing to dissociate C-domain, and then matures to A-B heterodimer. **Aim:** To report the purification and characterization of boar testicular RLF and shows the retention of its high bioactivity and to demonstrate the expression profile of RLF during the testicular development. **Methods:** Testes from Duroc and its crossbred boars were used in this study. Purification of RLF was performed according to the method of Kohsaka *et al.* (1993). Purified RLF was digested and the resultant peptide mixtures were directly analyzed by LC-Maldi Tof/Tof MS. The bioactivity was evaluated by showing a dose-dependent increase in cAMP production in HEK 293T cells transfected with a mouse RXFP2/LGR8-expression construct. The expression profile of RLF during the testicular development was analyzed by Western blotting. **Results:** Using an extraction procedure that minimized proteolysis, followed by gel filtration, ion-exchange FPLC, and reverse-phase HPLC, RLF was isolated as a single peak with the molecular size of about 12 kDa as revealed by SDS-PAGE. MS/MS analysis with the ProteinPilot database search engine indicated that distinct A-B-C heterotrimeric structure was identified with 60% protein coverage. RLF retained almost full bioactivity in the HEK 293T cells transfected with RXFP2/LGR8 gene. In addition, Western blot analysis found that the RLF was up-regulated during puberty onwards but is then down-regulated in the aging males. **Conclusion:** The present findings suggest that RLF is stored as the A-B-C heterotrimeric proform structure with biological activity in the boar testis.

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### S8-01

#### Proteomic analysis of testis biopsies in men treated with transient scrotal hyperthermia as potential male contraceptive

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**Aim:** Mild testicular heating safely and reversibly suppresses

spermatogenesis in several mammalian species and the histological changes in testes after heat treatment is marked by accelerated stage- and germ cell-specific apoptosis. In this study, we attempted to clarify the underlying molecular mechanism(s) involved in heat-induced increase of germ cell apoptosis and further understand the mechanism of spermatogenesis suppression induced by exogenous heat administration.

**Methods:** Two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-TOF) were used to assess the protein changes in human testicular biopsies at different time after heat treatment (Week 2 and 9 post-treatment, representing the stages of seminiferous epithelia began damaged and recovered respectively, were selected as the investigated points). Then a network of those proteins showing changes and their related cellular processes were constructed by bioinformatics methods. A key protein, HNRNPH1, among the network was selected for the further functional study using immunohistochemistry, flow cytometry and RNAi methods. **Results:** 1) Thirty-one and 27 known proteins were identified with significant differential expression at 2 and 9 weeks after heat treatment respectively, and used to characterize the cellular and molecular events in the testes at these two stages. Bioinformatics analysis indicated “proliferation”, “differentiation”, “apoptosis”, and “cell survival” were the predominant events both at these two stages. At 2 weeks post-treatment, the changed expression of a series of proteins could promote apoptosis or suppress proliferation and cell survival. At 9 weeks post-treatment, the changed expression of proteins could promote cell proliferation, differentiation and survival, but resist cell apoptosis. 2) HNRNPH1 protein, presumed to be the key protein involved in the suppression of spermatogenesis induced by heat treatment, was selected for the further functional study using the mouse model. Localization analysis of HN(n)RNPH1 protein in the mouse and human testis indicated it was gradually expressed in the germ cells and mainly in the nucleus of spermatocytes along with the initiation of spermatogenesis. Using the study model of mouse spermatocyte cell line (GC2-spd cells), we found along with the increase of cell apoptotic rate, the expression of HnRNPH1 protein decreased remarkably ( $P < 0.05$ ). Furthermore, the apoptotic rate of HnRNPH1-targeted siRNA cells was higher than that of control cells markedly both at baseline and after heat treatment ( $P < 0.01$ ). These results indicated HnRNPH1 protein was an anti-apoptosis factor. **Conclusion:** Heat treatment induced the reversible suppression of spermatogenesis by changing the expression of a series of proteins related to proliferation, differentiation, apoptosis, and cell survival. These differentially-expressed proteins were the key molecular targets of heat treatment and highly related to spermatogenesis, just as we have proved in the functional study on HNRNPH1 protein.

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### S8-02

#### Experience of inline vasectomy

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**Aim:** To evaluate the feasibility of inline vasectomy (ILV) with NSV instruments in Indian setup and to compare ILV with LEFI (ligation excision with fascial interposition) methods of vas occlusion. **Methods:** Using standard NSV instruments for vas isolation, 100 patients were randomized into two groups: the LEFI group ( $n = 50$ ) and the ILV group ( $n = 50$ ). Standard NSV steps were followed for the LEFI group and ILV was performed as described by Marmar (2001) using short bladed curved scissor and thermal cautery. Operating time, failure rates and complications rates of hematoma, granuloma, pain and infection of two groups were evaluated for comparison. **Results:** There was follow up of 81% in our study. Mean operation time in LEFI group was 8.64 min and 7.95 min in ILV group ( $P = 0.007$ ). Significant difference in post-vasectomy pain assessment at 15 days and 2 months follow-up ( $P = 0.001$  and  $P < 0.001$  respectively) were found between the two groups. Similarly, significant increase in incidence of granuloma formation in LEFI group was found as compared to that of the ILV group (69.2% vs. 4.8%,  $P < 0.001$ ). Furthermore, granuloma formation was positively correlated to post-vasectomy pain ( $r^2 = 0.1, 0.34$  and  $0.03$  at 15 days, 2 months and 3 months, respectively). None of the clients in both groups developed hematoma or infection. At 3 months follow-up semen analysis, all had sperm negative result. **Conclusion:** ILV can safely be performed with NSV instruments. The most significant outcome was reduction in time taken for the procedure. Another major outcome of this study was reduction in granuloma formation and post-vasectomy pain in the ILV group. There is a definite positive co-relation between granuloma formation and post-vasectomy pain. ILV is safer and equally successful as LEFI. ILV can be safely included in the national program for permanent male contraception with the additional requirement of thermal cautery.

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### S8-03

#### The contraceptive effect of filtering-type nano-copper complex/polymer composites intra-vas device in the male animals

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**Aim:** To investigate whether the filtering-type nano-copper complex/polymer composites Intra-vas Device is an efficacious, safe and reversible male contraceptive device. **Methods:** Twenty male adult beagle dogs and forty male rabbits were respectively assigned to four groups (sham-operation, IVD, reversibility and vasectomy groups). Dogs' semen parameters, concentration of  $\alpha$ -glucosidase, copper and zinc ions were tested at preoperation and at 1, 3, 6, 12 months postoperation. The pregnant percentages were evaluated by male and female mating trials for the rabbits after the operations were done in four groups. The histological changes in morphology of testes, epididymes and vas deferens of the animals were evaluated both

in optical and electron microscopes, while the apoptosis of the cells in these organs were detected by TUNEL method. **Results:** No sperm was detected in the semen of the dogs which had been inserted IVD and experienced vasectomy, at 1, 3, 6 and 12 month postoperation. The concentration of  $\alpha$ -glucosidase in IVD group, reversibility group and sham-operation group were not significantly different before or after operation. The pregnant percentages of female rabbits in vasectomy group, IVD group and reversibility group were all zero and before IVD was taken out, in the reversibility group, but after it was taken out, the pregnant percentages in reversibility group and the sham operation group reached 60% and 80% respectively. The cellular morphology and ultra-structures of the testes, epididymes and vas deferens of the male animals (both male dogs and rabbits) in IVD, reversibility group and sham-operation group were in normal ranges compared with the vasectomy group, which were present with obvious apoptosis of cells. No significant changes in the quantities of copper and zinc ions were found in semens of the male dogs before or after the operation. **Conclusion:** Our studies validate that the filtering-type nano-copper complex/polymer composites intra-vas device is an efficacious, safe and reversible male contraceptive device.

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### S8-04

#### Evaluation of microsurgical two suture longitudinal intussusception technique of vasoepididymostomy – the indian experience

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**Aim:** To evaluate the efficacy of microsurgical two suture longitudinal intussusception technique of vasoepididymostomy (LIVE) in idiopathic primary infertility. **Methods:** Patients with evidence of obstructive azoospermia with normal FSH, LH and testosterone; normal scrotal USG and normal spermatogenesis by FNA were included in this study. The two suture longitudinal intussusception technique of vasoepididymostomy was performed in all these cases. Note is made of the fact that a total of 70 scrotal explorations had to be done in order to complete the given number of cases. **Results:** In our study of 25 patients, 68% of the anastomosis was at the level of corpus, 24% at the caput and 8% being at the cauda. The mean operating time was  $70.4 \pm 20.56$ . The success rate of the study was evaluated by the presence of sperm in the semen either at the end of 3 months or at the end of 6 months. During this study the success rate at the end of 3 months and 6 months was calculated to be 44% and 56%, respectively. The mean semen volume was 3.15 mL. The average sperm count was 32.8 millions. The mean percentage of motility was 45%. At the end of 6 months, no patency was found in the patients in whom anastomosis were constructed at the head. Eighty-five percent of the success was when the anastomosis had been done at the corpus and the rest were in the patients where the anastomosis had been done at the tail. **Conclusion:** Surgery is more cost effective than assisted reproductive techniques and should be considered if a surgically



amenable cause is suspected. There is no specific preoperative indicator of epididymal tubular status. The site of anastomosis is of significance as there is a good success rate if the anastomosis is done as distal as possible. Tubular intussusception technique is superior to the other techniques and should be preferred.

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#### S8-06

##### Effects of vasectomy at different sites on spermatogenesis in rabbits

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**Aim:** To compare the spermatogenic effects of vasectomy at different sites. **Methods:** The left or right vas deferens was ligated through a longitudinal ventral midline incision near the epididymal head (unilateral proximal vasectomy, 12 animals) or via the inguinal canal (unilateral distal vasectomy, 11 animals) in adult male Japanese white rabbits aged 6 months, with a sham operation being performed on the contralateral side. Six months after operation, testes, epididymides and vasa deferentia were removed and methacrylate resin-embedded sections prepared to evaluate spermatogenesis by histological (qualitative) and stereological (quantitative) studies of the organs. **Results:** The juxta-epididymal segment of the vasectomized vas deferens was severely distended (filled with sperm) in 10 of the 11 animals with distal vasectomy, moderately or slightly distended in 9 of the 12 animals with proximal vasectomy. Severe spermatogenic damage (atrophy of seminiferous tubules, without any spermatids in the seminiferous epithelium of most tubular profiles) occurred in seven animals with proximal vasectomy (the juxta-epididymal vas deferens moderately or slightly distended), in only one animal with distal vasectomy (the juxta-epididymal vas deferens not severely distended). **Conclusion:** The distal vasectomy did not have significant effects on spermatogenesis as the occluded reproductive tract was still distensible and long (or large) enough to store all the sperm and testicular fluid produced by the testis. With the proximal vasectomy, however, the occluded reproductive tract was not long (or large) enough to store all the sperm and testicular fluid produced by the testis, which, probably due to the back pressure from sperm and testicular fluid, resulted in spermatogenic damage, thus reducing the production of sperm and testicular fluid and the distension of the reproductive tract. In other words, the long-term effect of vasectomy on spermatogenesis is pressure-mediated.

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#### S8-08

##### Repair and functional reconstruction of the penis: report of 62 cases

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**Aim:** To investigate the effects of surgery treatment to serious penile lesions and malformation. **Methods:** Sixty-two patients, aged 19 to 63 years (mean 35), were collected. Among these patients, four patients were suffered from penis partial defect and they were respectively treated with defect penis, penis lengthening and urethroplasty; three patients' penis were completely missing who were treated with penis reconstruction surgery; twenty-two cases with serious penile bending were treated with the "16 point" bending surgery; of fifteen cases of penile fracture, one case got conservative treatment, fourteen patients were patch penis, corpus spongiosum, deep penile dorsal vein ligation; five cases of post-operative complications of three pieces of penile prosthesis implantation were treated. The complications included the prosthesis perforating to the urethra, water pump failure, broken connection tube, erection angle < 60° and failure to expansion the corpus cavernosum. The patients were treated by taking out prostheses, urethral repair cracks, replacement of the prostheses, excision of fibrosis scar and re-implantation prostheses respectively. Four cases of penis complete amputation were treated with the penis replantation; three cases of avulsion injury were treated with the replantation and free flap skin; six Paget's disease of penises were treated with the lesion free skin buried in the scrotum and penis. **Results:** This group was followed up for 3 months to 4 years, with the average of 9 months. Among the four cases of penis partial defect, two patients were satisfied with the penile appearance and sexual function; one got some satisfactory and one was unsatisfied. Three cases of loss of the penis completely were satisfied with both the postoperative appearance and urination, and 1 was not satisfied. Twenty-two cases of penile curvature deformities were corrected, one case was recurrence. Fourteen of the fifteen penile fracture patients were followed, all of which got the restoration of sexual function, one case was missing; five cases of post-operative complications included mild bending, algopareunia, subcutaneous induration, poor hardness and poor sexual pleasure, not for further treat. Five cases of post-operative complications of three pieces penile prosthesis were successful, and four of their spouses were satisfied with their sexual function after operation, only one of their spouse was not satisfied. Of four cases of complete amputation of penis, two cases were successful replanted while two cases were necrosis. Three cases of skin grafting to treat avulsion were successful. All those cases of penile Paget's disease were follow-up for 2–4 years. Free skin graft was taken if they were survival. One patient died of brain metastases 18 months after operation and five cases were disease-free survival. **Conclusion:** Those cases should be treated based on procedure of andrological and urological surgery, together with microsurgical, flap or skin graft technique. The urologist should design personalized surgical procedure. Most of the patients' penis shape and erectile dysfunction can be reconstructed by our procedures, but some patients can not achieve the desired appearance or function of penis. New approaches of the treatment ought to be developed to restore both of the shape and function for those with severe injury to the



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### S8-09

#### Ethical issues in penile transplantation

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This review provides an overview of the ethical issues associated with penile transplantation, a form of composite tissue allografting. Human penile transplantation, an experimental technique, has been performed only once in China all over the world. Although Koga reported experimental allogenic penile transplantation in 2003, it was performed on animal. Ethical issues of penile transplantation involve both the graft donor and the graft recipient. With regard to the recipient, significant concerns include surgical risks and benefits, informed consent, body image (including surgical expectations and outcomes), and compliance. Donor issues include family consent and privacy, graft harvesting as well (leaving the donor without a penis). Many of the ethical issues can be explored during the individual's assessment and consent process. Because no medium-term or long-term outcome data are available and only one case has been done, the burdens and ethical problems of penile transplantation remain unknown.

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### S8-10

#### Endocervical response to human semen and *C. trachomatis*: insights into increased heterosexual HIV transmission in co-exposed women

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**Background:** Women infected with *C. trachomatis* are more likely to be infected with HIV. The mechanism for this effect is likely complex and remains incompletely defined. Human semen may play an important role in helping the female reproductive mucosal cells negotiate the delicate balance between tolerance to foreign sperm and control of pathogen invasion. This balance is frequently challenged, since sexual pathogens and sperm are often delivered simultaneously. Despite common heterosexual delivery of *C. trachomatis* and HIV-1 to the human female reproductive surfaces, *in vitro* and animal model investigations of these infections seldom involve use of semen or seminal plasma for pathogen delivery. The anatomic site chosen for studies on pathogen transmission across the female genital tract also needs better definition. The endocervix differs from the highly-studied vagina, ectocervix and endometrium in its cellular composition. It is a single cell mucosal barrier to pathogen invasion and has a relatively high surface area compared to the transformation zone and ectocervix. The endocervix may, in fact, represent

the major site for both *C. trachomatis* and HIV transmission across the female genital tract. Our endocervical cell models are non-transformed and polarizable and support co-infection with HIV-1 and *C. trachomatis*. **Aim:** To investigate the immune response of primary, immortalized endocervical epithelial cells to human semen and the effects of human semen on endocervical transmission of *C. trachomatis*. We also address the effects of *C. trachomatis* on epithelial permeability in polarized endocervical cells. **Methods:** A2EN cells are primary, immortalized endocervical cells that are kept at low passage and can be polarized. Non-polarized A2EN cells were exposed to seminal plasma for 24 h and processed for cytokine secretion analyses using PCR arrays. These findings were verified by ELISA and expanded to include a dose response to human seminal plasma. In separate experiments, A2EN cells were seeded onto filter inserts coated with human extracellular matrix and kept in culture at conditions that promote polarization. Daily transepithelial resistance measurements (TEM) demonstrated polarization by day 6 in culture. Cells were infected with *C. trachomatis* serovar D on day 5 of culture. TEM was measured daily up to 72 h post-chlamydial infection. To study the effects of human seminal plasma on *C. trachomatis* infection of epithelial cells, semen was collected from non-infected fertile male volunteers and seminal plasma separated using standard centrifugation. TZM-bl cells, a cervical carcinoma cell line important in assaying productive HIV-1 infections, were exposed to *C. trachomatis* elementary bodies for two h in the presence or absence of varying concentrations of human seminal plasma. *C. trachomatis* elementary bodies (EB) and infection media were then removed and cells were assayed using western immunoblotting at 24 and 48 h post-infection to quantitate the level of *C. trachomatis* infection. **Results:** Using PCR arrays, a 2-fold increase in IL-8, a 3-fold decrease in IL-8 receptor and a 30% increase in CCL20 transcription was noted in endocervical cells exposed to seminal plasma. MCP-1 levels were relatively high in seminal plasma and an effect on A2EN secretion of this chemokine could not be detected (data not shown). Using ELISA, GM-CSF and CCL20 secretion by A2EN cells was significantly increased in the presence of seminal plasma, in a dose-dependent fashion (Figure 1). Beginning 24 h post-infection and continuing through 48 h post-infection, transepithelial resistance in *C. trachomatis*-exposed polarized A2EN cells was decreased when compared to mock-infected cells. Transepithelial resistance returned to mock-infected levels by 72 h post-infection. Experiments on the effects of human semen on infection of TZM-bl cervical cells by *C. trachomatis* demonstrated that exposure to human semen had biphasic effects on *C. trachomatis* infectivity. The presence of human semen decreased *C. trachomatis* infection in a dose-dependent fashion at levels greater than 1% (Figure 2). Exposure to 0.05% SP resulted in a small but significant enhancement of *C. trachomatis* infection. **Conclusion:** Primary, immortalized endocervical epithelial cells represent a unique model for the study of HIV and *C. trachomatis* transmission across the female genital tract. These models must, however, include exposure to pathogens in the presence of human semen if we hope to improve our approach to rational transmission prevention. Semen-induced endocervical inflammation and chemokine production may combine with increased inflammation- and *C. trachomatis*-

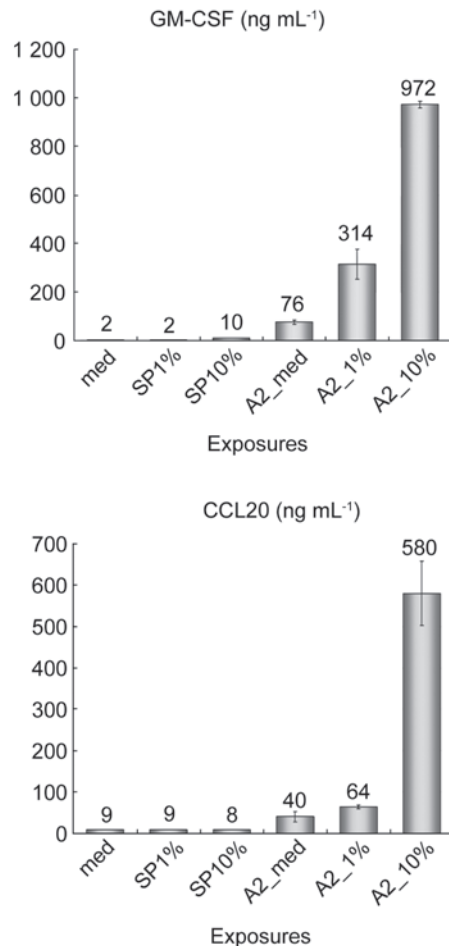


Figure 1. (A): Human endocervical cells were exposed to seminal plasma (SP) for 24 h. (B): GM-CSF and CCL20 were determined by ELISA. Standard deviations are represented by error bars. Experiments were repeated three times in triplicate. A2-A2EN cells, med-media alone.

induced endocervical epithelial permeability to support HIV transmissibility despite semen-induced decreases in endocervical infection with *C. trachomatis*.

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### S8-12

#### Effect of GnRH agonist on annexin 5 and 3β-HSD expression in rat Leydig cells

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**Aim:** To investigate the effect of GnRH analogue on annexin 5 and 3β-HSD expression in rat Leydig cells. **Methods:** To establish the method on separation, purification and identification of the primary

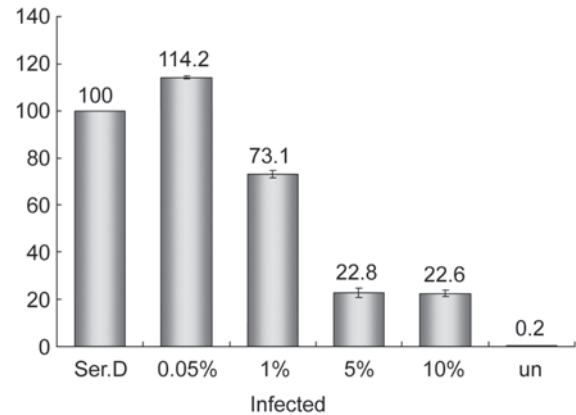


Figure 2. TZM-bl cells were exposed to *C. trachomatis* EB in the presence or absence of human seminal plasma and lysed 48 h post-infection. Extent of chlamydia infection was determined using Western immunoblotting and normalized to seminal plasma unexposed infected.

Leydig cell culture *in vitro*. The identification of the different stage cells was carried out by 3β-HSD immunocytochemistry method. Different concentrations of GnRH analogue were administrated on the Leydig cells for 24 h. Finally, the changes of annexin 5 and 3β-HSD expression were investigated by the way of Western blotting. **Results:** We found that GnRH could stimulate the annexin 5 and 3β-HSD expression. Treatment with the GnRH agonist 10–5 mol L<sup>-1</sup> has a 69.2% ( $P < 0.01$ ) significant increase in annexin 5% and 25.2% ( $P < 0.05$ ) in 3β-HSD expression compared with those of control group. There were 61.5% ( $P < 0.05$ ) and 16.64% ( $P > 0.05$ ) increase with treatment of GnRH (10–6 mol L<sup>-1</sup>, 10–7 mol L<sup>-1</sup>) in annexin 5 expression. There were 7.7% ( $P > 0.05$ ) and 2.22% ( $P > 0.05$ ) increase changes in 3β-HSD expression while with no significant change. **Conclusion:** GnRH agonist can regulate the annexin 5 and 3β-HSD expression in primary Leydig cell culture.

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### S8-13

#### Extracellular Matrix metalloproteinase inducer: a novel poor prognostic marker for human seminomas

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**Background:** Extracellular matrix metalloproteinase inducer (EMMPRIN) is a glycosylated member of the immunoglobulin superfamily whose function in human seminomas is unknown. We have recently determined that EMMPRIN possesses the ability to stimulate fibroblast and endothelial cell matrix metalloproteinases production, and that its expression was frequently up-regulated in several tumors of urinary system. Thus, EMMPRIN expression might be associated with the progression of human seminomas. **Aim:** To investigate whether the presence of EMMPRIN in seminoma tissues might help to predict the patients' prognosis. **Methods:** Sixty-five patients diagnosed with primary seminomas were tested with immunohistochemical staining and assessment of EMMPRIN. **Results:** EMMPRIN were detected in cancerous tissues of 53 patients with seminoma, but not normal testes. Thirty-five and 18 patients showed weakly to moderately and intensively positive expression, respectively. Moreover, positive EMMPRIN staining correlated significantly with various clinicopathological factors (increased TNM stage and higher histological differentiation type) as well as decreased tumor-specific survival (log-rank,  $P = 0.02$ ). In particular, EMMPRIN expression was an independent prognosticator as shown by Cox regression analysis ( $P < 0.001$ ). **Conclusion:** EMMPRIN expression in primary tumor predicts an unfavorable prognosis in human seminoma, suggesting its crucial role in the progression of this tumor.

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**S8-14****The predictive value of age, body mass index and increment of plasma testosterone on the effects of testosterone administration to hypogonadal men**Farid Saad<sup>1\*</sup>, Ahmad Haider<sup>2</sup>, Louis Gooren<sup>3</sup><sup>1</sup>Bayer Schering Pharma, Scientific Affairs Men's Healthcare, and Gulf Medical University, Ajman, UAE<sup>2</sup>Private Urology Practice, Bremerhaven, Germany<sup>3</sup>VUmc, Amsterdam, the Netherlands

**Background:** It is now clear that low testosterone (T) levels are associated with the metabolic syndrome and its sequels such as diabetes mellitus type 2 and cardiovascular disease, and vice versa. There is increasing evidence of the beneficial effect of T treatment on features of the metabolic syndrome, but a better definition of elements which determine the success of administration of T is needed. **Aim:** In this study we aimed to test 1) whether age of the subjects receiving T treatment has impact on the improvements of features of the metabolic syndrome; 2) whether indicators of obesity (BMI) has any predictive value for the improvements of the metabolic syndrome; 3) whether the increment in plasma T following T administration were a determinant of its effects on variables of the metabolic syndrome. **Methods:** One hundred and twenty-two hypogonadal men (18–83 years, mean age  $59.6 \pm 8.0$  years), with T levels between  $5.8\text{--}12.1$  nmol L<sup>-1</sup> (mean  $\pm$  SD:  $9.3 \pm 1.7$ ) were treated with parenteral testosterone undecanoate for 2 years as the sole intervention upon which plasma T rose from  $9.3 \pm 1.7$  nmol L<sup>-1</sup> to  $14.9 \pm 4.5$  nmol L<sup>-1</sup> ( $P < 0.01$ ) at 3 months, then stabilized at  $19.2 \pm 4.6$  nmol L<sup>-1</sup> after 6 months. We further

subdivided our cohort into four subgroups each according to their baseline age, BMI, and testosterone levels. **Results:** 1) Baseline BMI had no effect on plasma testosterone achieved with T administration; 2) the higher the baseline BMI, the higher percentual change of the studied variables but less often recovery from the metabolic syndrome compared to men with lower BMI at baseline. Age had no effect on the plasma T achieved with T administration; the higher the age, the higher baseline BMI, waist circumference, glucose, cholesterol, triglycerides and LDL and frequency of the metabolic syndrome. The effects of T administration on cholesterol and triglycerides were most favorable in the oldest. Percentual changes in HDL and LDL were similar in all ages. The older the patients, the less often they recover from the metabolic syndrome. The increase in plasma T from baseline following T administration: The greater the increase in plasma T, the greater the change in cholesterol, HDL, LDL, and triglycerides and the more often recovery from the metabolic syndrome. **Conclusion:** Higher age and BMI predict a less favorable outcome of administration of T but favorable changes are still considerable, particularly with regard to effects on cholesterol and triglycerides. The increase in T following T administration is predictive of a favorable outcome and deserves more attention in future studies. It should be studied what the optimal dose/achieved plasma levels of T are in relation to its favorable effects.

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**S8-15****Regulatory actions of estrogen receptors on GnRH neuronal function**Kevin Catt<sup>\*</sup>, Lian Hu, Lazar Krsmanovic

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**Aim:** Estradiol (E2) exerts both positive and negative regulatory actions on gonadotropin releasing hormone (GnRH) synthesis and secretion in hypothalamic GnRH neurons. However, the extent to which these actions are mediated by the individual estrogen receptors (ERs) expressed in GnRH neurons has not been well defined. **Methods:** Single-cell RT-PCR revealed the expression of both ERalpha and ERbeta in cultured fetal and adult rat hypothalamic GnRH neurons. **Results:** ERalpha and ERbeta, or individual ERs, were expressed in 94% of cultured fetal GnRH neurons. In adult female rats at diestrus, 68% of GnRH neurons expressed ERs, followed by 54% in estrus and 19% in proestrus. The individual ERs are expressed in 24% of adult male GnRH neurons. ERalpha had marked G(i)-mediated inhibitory effects on spontaneous action potential (AP) firing, cAMP production, and pulsatile GnRH secretion, consistent with its negative regulation of GnRH neuronal activity. Conversely, increased E2 concentration and ERbeta agonists increased the rate of AP firing, GnRH secretion, and cAMP production, consistent with ERbeta-induced positive regulation of GnRH secretion. Due to the coupling of ERalpha to pertussis toxin-sensitive G(i/o) proteins, E2 also activated G protein-activated inwardly rectifying potassium (GIRK) channels, decreased



membrane excitability and slowed the firing of spontaneous APs in hypothalamic GnRH neurons. **Conclusion:** These findings demonstrate that the actions of E2 on GnRH neuronal membrane excitability, cAMP production, and GnRH secretion are mediated by the dose-dependent activation of ERalpha and ERbeta receptors expressed in GnRH neurons.

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#### C1-03

##### Cloning and identification of a sperm binding protein HEL-S61 expressed in the human testis

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**Aim:** The HEL-S61 protein is a adenylate kinase. Studying its function can not only help elucidate the mechanisms of sperm maturation but also aid in finding new candidate molecules for diagnosing and treating male infertility as well as developing novel contraception alternative. **Methods:** The HEL-S61 gene was identified by analyzing a human epididymis cDNA library, and its structure and function were predicted and analyzed with bioinformatics tools. Polyclonal serum was raised against synthesized peptides. The gene expression pattern was analyzed by RT-PCR and immunohistochemical staining. The effect of the HEL-S61 on sperm motility and fertilization was analyzed by blocking spermatozoa with anti-HEL-S61 polyclonal serum. **Results:** The human HEL-S61 gene was located on chromosome 9 encoding 195 amino acids and had a 100% homology to the adenylate kinase 1 (AK1). At the transcriptional level, HEL-S61 was mainly expressed in human epididymis and testis. However, at the translational level, it was detected only in the testis, with no expression in epididymal epithelial cells. Immunofluorescent staining showed that HEL-S61 protein bound the midpiece of sperm tail. The motile (%) of sperm declined after blocking with anti-HEL-S61 polyclonal serum, and no effect was observed on fertilization. **Conclusion:** HEL-S61 is a sperm binding protein expressed in testis and plays an important role in sperm motility.

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#### C1-05

##### Expression of hsa-miR-135a in sperm with low motility

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**Aim:** To analyze the expression of hsa-miR-135a in sperms with low motility. **Methods:** Thirty semen samples from asthenospermia patients and 20 semen samples from the healthy normal adult men were collected. Real-time RT-PCR was used to analyze the expression of hsa-miR-135a; the expression of the target genes of hsa-miR-135a were searched from gene-expression profiles in asthenospermia patients' sperms. **Results:** The expression level of hsa-miR-135a in low motility sperms

was 0.62 fold of that in high motility sperms, and the expression level of IFT20 in low motility sperm was 0.286 fold of that in high motility sperm. **Conclusion:** hsa-miR-135a can inhibit the expression of IFT20 and decrease sperm motility.

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#### C1-06

##### Proteomic analysis of infertile and high fecundity spermatozoa of donors for AID treatments

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**Aim:** To compare the proteomic differences between infertile and high fecundity spermatozoa of AID donor. **Methods:** Twenty donors were selected according to the fecundity of their frozen semen used for AID treatment. Ten donor were assigned to infertile group as no pregnancy was obtained after 12 AID treatment cycles. The other 10 donors were assigned to high fecundity group as they had five clinic pregnancies after 10 or less AID treatment cycles. Two-dimensional difference gel electrophoresis (2-D DIGE) coupled with mass spectrometry (MS) was used to analyze the proteomic differences of the two groups of spermatozoa. **Results:** Total 45 protein spots (fold difference of spot volume ratio  $\geq 1.5$ ,  $P < 0.05$ ) were found, and among which, 21 spots were up-regulated and 24 spots were down-regulated in infertile spermatozoa by the 2-D DIGE/DeCyder analysis. Forty-one proteins corresponding to 45 spots were identified using MS/MS analysis and peptide mass profiling matched to the databases. According to the associated property, these proteins fell into five categories: 1) protein with sperm energy metabolism, 2) cell skeleton, 3) sperm motility, 4) sperm maturatin and 5) other functional proteins. Sixty-six percent (27 proteins) of the proteins identified in this study were corresponding to sperm energy metabolism, cell skeleton and sperm motility. **Conclusion:** Proteins corresponding to sperm movement seem to play important roles in the fecundity of spermatozoa for AID treatments.

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#### C1-07

##### Evaluating sperm DNA integrity by labeling nick-end of the diffused chromatin using TdT-mediated dUTP

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**Aim:** To set up a new method which use terminal deoxynucleotidyl transferase (TdT) to transfer labeled nucleotide to a broken DNA strand from dispersed sperm chromatin for evaluating sperm DNA integrity based on sperm chromatin

dispersion (SCD) test and TdT-mediated dUTP nick end labeling (TUNEL) assay. **Methods:** In 12 volunteer, the SCD test was performed firstly, and then the labeled nucleotide was transferred to the 3'-OH group of a broken DNA strand from dispersed sperm chromatin with the use of TdT. After stained by Diff-Quik solution, sperm DNA dispersed halos could be observed to evaluate sperm DNA integrity further. Semen processing was performed before testing: an aliquot of the washed sperm sample was mixed with 1% low melting point agarose (to obtain a 0.7% final agarose concentration) at 37°C. Aliquots of 50 µL of the mixture were pipetted onto the glass microscopic slides precoated with 0.65% agarose, covered with a cover slip, and left to solidify at 4°C for 5 min. Cover slips were carefully removed from slides before the SCD test and TUNEL assay were performed. **Results:** Sperms which show dark brown signal under the light-field microscope indicate DNA strand breaks. The nuclei with large to medium size halo were considered sperm with non-fragmented DNA whereas nuclei with small size halo or without halo were considered sperm with fragmented DNA. When the DNA fragmentation of a sperm was detected by SCD test and TUNEL assay simultaneously, the sperm with DNA fragmentation showed brown signal with small size halo or without halo whereas the sperm with DNA no-fragmentation showed red signal with large halo or medium halo. **Conclusion:** Sperms with DNA fragmentation determined by SCD test were those determined by TUNEL. Using TdT to transfer labeled nucleotide to a broken DNA strand from dispersed sperm chromatin is effective in testing the sperm DNA fragmentation to determine semen quality.

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#### C1-08

##### The expression of ZNF265, IL-1 $\alpha$ , IL-6, TGF- $\beta$ and FasL during the sertoli cells infected by UU

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**Aim:** To investigate the relationship among ZNF265-a potential immune regulator in the sertoli cells, pro-inflammatory and anti-inflammatory, analyzing the latent immunoregulation mechanism of inflammation in the testis *in vitro* and *in vivo*. **Methods:** Using the well-characterized *Ureaplasma urealyticum* (UU) inducing model, we tested the expression of IL-1 $\alpha$ , IL-6, TGF- $\beta$ , FasL and ZNF265 in the 1st, 2nd, 3rd week both *in vitro* and *in vivo*, respectively. **Results:** As the inflammation recovered and with the help of negative regulation by cytokine TGF- $\beta$  and ZNF265, the high levels of IL-1 $\alpha$  and IL-6 expression in the 1st week decreased gradually in the following weeks both *in vitro* and *in vivo*. **Conclusion:** The variation trend of ZNF265 was similar to TGF- $\beta$  and FasL *in vitro* and *in vivo* when the Sertoli cells were infected by UU.

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#### C1-09

##### Attenuated *Salmonella Typhi* carrying the plasmid-co-expressing

##### GRIM-19 and Survivin-specific shRNA for prostate cancer therapy

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**Aim:** To explore whether the pGRIM-19-siRNA-Survivin co-expression plasmids have synergic effect in anti-prostate cancer and to confirm the feasibility of attenuated *Salmonella Typhi* as a delivery carrier for prostate cancer xenografts therapy.

**Methods:** The recombinant plasmids were transfected into human androgen-independent DU145 prostate cancer cell line with lipofectamine 2000. Cell proliferation was measured by MTT at different time. Cell cycle and apoptosis were analyzed with flow cytometry (FCM), Annexin V-FITC kit, acridine orange staining and Tunel method. Survivin protein distribution was detected with immunofluorescent analysis. The expression of Survivin, GRIM-19, Stat3, c-Myc, CyclinD1, Bcl-xL, caspase3 and VEGF were evaluated by RT-PCR and Western blot. The nude prostate cancer xenografts were hypodermically constructed and attenuated *Salmonella Typhi* carrying different plasmids was injected through peritoneal cavity to the tumor. The tumor volumes, tumor growth rates and tumor weights were compared between groups at the same time. **Results:** The co-expressing pGRIM-19-siRNA-Survivin plasmid was successfully constructed. The plasmid has two promoters to express si-RNA-Survivin and GRIM-19 at the same time. *In vitro* experiments showed that the Survivin expression was down-regulated at gene and protein levels after transfection of psi-Survivin, pGRIM-19 and pGRIM-19-siRNA-Survivin plasmids, but GRIM-19 expression in pGRIM-19 and pGRIM-19-siRNA-Survivin groups was up-regulated. Cell proliferation experiments showed that co-expression plasmid pGRIM-19-siRNA-Survivin inhibited cell growth more than single gene therapy group. By FCM and Annexin V-FITC detection, G1 phase arrest and apoptosis cells were observed in psi-Survivin, pGRIM-19 and pGRIM-19-siRNA-Survivin groups. There were more G1 phase arrest cells and apoptosis cells in co-expressing pGRIM-19-siRNA-Survivin plasmid group. The results from acridine orange and TUNEL staining analysis showed co-expression plasmid could promote apoptosis significantly. The immunofluorescence results showed that Survivin gathered in nucleus and the cytoplasm in the transfected psi-Survivin, pGRIM-19 and pGRIM-19-siRNA-Survivin groups. RT-PCR and Western analysis further revealed the pGRIM-19-si-Survivin expression plasmid could down-regulate Stat3, c-Myc, cyclinD1, Bcl-xL expression and increase the expression of caspase3. *In vivo* experiments proved that si-Survivin and GRIM-19 could inhibit the prostate cancer xenografts growth with synergism. The average tumor weight and average tumor volume in attenuated *Salmonella Typhi* carrying pGRIM-19-si-Survivin plasmid group were lower than



those in the two single gene therapy groups. The apoptosis occurred obviously attenuated Salmonella Typhi carrying pGRIM-19-si-Survivin plasmid group. It is found that the co-expression plasmid inhibited prostate xenografts growth by down-regulating Stat3, c-myc, cyclin D1, Bcl-xL and VEGF but up-regulating caspase3. **Conclusion:** The co-expression pGRIM-19-si-Survivin plasmid showed synergic anti tumor effect not only for hormone-independent DU145 prostate cancer cells *in vitro* but also for the subcutaneous xenografts of DU145 cells *in vivo*. The anti-tumor mechanism of the co-expression pGRIM-19-si-Survivin plasmid was to promote apoptosis in prostate cancer cells. Attenuated Salmonella Typhi as the delivery carrier of co-expression plasmid can be applied well in gene therapy.

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### C1-11

#### The effect of Smad4 sumolation modulated by PIAS family proteins on the proliferation of prostate cancer

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**Aim:** To investigate the effect of Smad4 sumolation modulated by PIAS family proteins on the proliferation of prostate cancer cells. **Methods:** We adopted wild-type Smad4 or mutant-type Smad4, which had mutation at 113 and 159 lysine to arginine and resulted in the Sumolation-deficiency of Smad4 protein. The wild-type or mutant-type Smad4 was transfected with each four PIAS family (PIAS1, PIAS3, PIASx or PIASy) or Sumo-1 plasmids into prostate cancer pc3 cells. Their impacts on the cell proliferation were analyzed by MTT method. **Results:** The effects of PIAS family members on pc3 cell proliferation were not significant. But when they were co-transfected with Sumo-1 and wild-type Smad4 by enhancing Smad4 sumolation, the very significant effects were showed up on inhibiting cell proliferation. The mutant-type Smad4, which could not be modulated by PIAS family, could not inhibit pc3 cell proliferation. When co-transfected with wild-type Smad4 and Sumo-1, PIAS family members showed significant inhibition effects, with 31.7% inhibition for PIAS1, 66.1% inhibition for PIAS3, 67.2% inhibition for PIASx $\beta$ , and the highest inhibition of 82.2% for PIASy. **Conclusion:** PIAS family proteins regulating Smad4 sumolation play important roles in pc3 cells proliferation inhibition.

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### C1-12

#### Distinctive gene expression of stromal cells from transitional zone and peripheral zone of prostate

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**Background:** Different diseases generate in peripheral zone (PZ) and transitional zone (TZ) of prostate. Interaction of epithelium-stroma is one of main mechanisms of prostatic diseases. Stroma plays an important role in the growth, differentiation and malignant transformation of epithelium. It is important to research biological character of stroma in PZ and TZ. **Aim:** This study is to investigate the distinctive gene expression of stromal cells of prostate from TZ and PZ. **Methods:** Specimens of normal prostate were obtained from dead body, primary culture of stromal cell derived from TZ and PZ. Distinctive gene expression of prostatic stromal cells from TZ and PZ was screened and analyzed by SBC-Human cDNA microarray. **Results:** We screened the stromal cells from TZ and PZ in 20-year-old men (TZ20 and PZ20), and found distinctive gene expression, including 1 387 up-regulation genes and 1 319 down-regulation genes. Thirty-eight types of up-regulation genes and 28 types of down-regulation were classified by Go-analysis. There were 15 up-regulations and two down-regulations because their functions were significant after Dif-Path analysis. **Conclusion:** We demonstrated that there are distinctive genes expression of stromal cells from TZ and PZ of prostate. We believe that the difference in gene expression is the important reason for pathogenesis of zonal difference in diseases of prostate.

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### C1-13

#### Effect of endothelin-1 on cyclooxygenase-2 expression in human hormone refractory prostate cancer cells

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**Aim:** To explore the effects and possible mechanism of recombinant human endothelin-1 (ET-1) on cyclooxygenase-2 (COX-2) expression in human hormone refractory prostate cancer cells. **Methods:** PC3 cells were treated with 100 nmol L<sup>-1</sup> ET-1 for indicated h (3, 6, 9, 12, 24 h) and the indicated concentration (0.1, 1, 10, 100 nmol L<sup>-1</sup>) for 24 h. Besides, 100 nmol L<sup>-1</sup> ET-1 were used to treat PC3 cells alone or in combination with BQ-123 (endothelin receptor A antagonist, ETAR antagonist, 1  $\mu$ mol L<sup>-1</sup>), BQ788 (endothelin receptor B antagonist, ETBR antagonist, 1  $\mu$ mol L<sup>-1</sup>). COX-2 mRNA and protein expression in PC3 cells was detected by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. **Results:** ET-1 can significantly induce time-dependent up-regulation of COX-2 mRNA in PC3 cells. RT-PCR analysis indicated that the steady-state COX-2 mRNA levels increased in ET-1 treated cells compared with control by 2-, 2.3-, 2.6-, 3- and 2.9-fold at 3, 6, 9, 12 and 24 h, respectively. Moreover, ET-1 treatment evoked a time-dependent increase in COX-2 protein levels. Western blots exhibited low expression

of COX-2 proteins in untreated PC3 cells but showed a 1.5-fold increase in COX-2 protein by 3h and a 1.9-, 2.2-, 2.5-, 2.3-fold increase in COX-2 protein after 6, 9, 12 and 24 h of ET-1 stimulation, respectively. ET-1 also increased COX-2 mRNA and protein levels in a dose-dependent fashion. Treatment of PC3 cells with 0.1 and 1 nmol L<sup>-1</sup> ET-1 for 24 h revealed 1.5- and 2-fold increases in COX-2 protein expression respectively, and reaching maximum responses (2.5-fold) at 10 and 100 nmol L<sup>-1</sup> ET-1. COX-2 mRNA levels increased 1.2-fold at 0.1 nmol L<sup>-1</sup> ET-1, reaching maximum levels (2.1-fold) at 100 nmol L<sup>-1</sup> ET-1. Moreover, BQ123 was able to completely block ET-1-induced COX-2 expression, whereas BQ 788 did not. **Conclusion:** ET-1 could induce the up-regulation of COX-2 in PC3 cells. The possibility of blocking their activity may have relevant implication in the prevention and treatment of this malignancy. Targeting COX-2 and related signaling cascade via ETAR blockade may be therapeutically advantageous in the treatment of HRPC. In this regard, combination treatment with COX-2 inhibitors and ETAR antagonist may be warranted to design newer therapeutic approaches to HRPC.

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#### C1-14

##### **Prenatal exposure to nanoparticle-rich diesel exhaust suppressed testicular function in immature male rats**

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**Aim:** To investigate the effects of in utero exposure to nanoparticle-rich diesel exhaust (NR-DE) on reproductive function in male rats. **Methods:** Pregnant F344 rats were exposed to NR-DE (148.86 µg/m<sup>3</sup>, 1.83 × 10<sup>6</sup> particles/cm<sup>3</sup>, 3.40 ppm CO, 1.46 ppm NO<sub>x</sub>), filtered diesel exhaust (F-DE; 3.10 µg/m<sup>3</sup>, 2.66 particles/cm<sup>3</sup>, 3.30 ppm CO, 1.41 ppm NO<sub>x</sub>), or clean air (as a control) from gestation day 1 to 19 (gestation day 0 = day of sperm-positivity). Male offspring were examined on postnatal day 28. **Results:** The relative weights of the seminal vesicle and prostate to body weight were decreased after exposure to NR-DE or F-DE compared with controls. Serum concentrations of testosterone, progesterone, corticosterone, and follicle stimulating hormone and testicular concentrations of steroidogenic acute regulatory protein and 17β-hydroxysteroid dehydrogenase mRNA were decreased after exposure to NR-DE or F-DE compared with control levels. In contrast, serum concentrations

of immunoreactive inhibin were increased after exposure to NR-DE or F-DE compared with control levels, whereas transcription of follicle stimulating hormone receptor mRNA was increased in the NR-DE exposure group only. **Conclusion:** These results suggest that prenatal exposure to NR-DE or F-DE leads to endocrine disruption after birth and suppresses testicular function in male rats.

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#### C1-15

##### **Purification of rat fetal Leydig cells: actions of diethylhexyl-phthalate**

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**Aim:** To explore a new method of fetal Leydig cell (FLC) enrichment and investigate whether the in utero diethylhexyl-phthalate (DEHP) exposure affects the steroidogenic functions of FLCs. **Methods:** Pregnant dams of Long Evan rats were treated with 0,750 mg kg<sup>-1</sup> body weight of DEHP from gestational day (GD) 12 to 21. Pup testes were removed for purification of FLCs. Testes from postnatal 90-day-old Long Evan rats were also isolated for purification of adult Leydig cells (ALCs) as control. Leydig cells were identified by 3β-hydroxysteroid dehydrogenase histochemical staining. After digested by collagenase, Prenatal and adult testicular interstitial cells were separated with seminiferous tubes by nylon mesh and centrifugated with Percoll density gradient sedimentation. The cell pellets (containing ~5% of FLCs and ALCs) were incubated with polyclonal rabbit anti-luteinizing hormone receptor (LHR, the Leydig cell specific surface protein) antibody and then LHR-positive cells were immunoselect by magnetic beads. Characteristics of FLCs were compared with ALCs. **Results:** The purity of FLCs we enriched was more than 98%. The gene and protein differences between FLCs and ALCs were detected. After the in utero DEHP exposure, FLCs in 750 mg kg<sup>-1</sup> DEHP group had significant decreased LH and 22-hydroxycholesterol-stimulated testosterone production. **Conclusion:** In conclusion, a new method of FLC purification has been conducted, and FLCs in 750 DEHP mg kg<sup>-1</sup> day<sup>-1</sup> rats had notably low testosterone production.

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#### C1-16

##### **Combined effect of nonyl phenol and di-n-butyl phthalate in rat sertoli cells *in vitro***

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**Aim:** To investigate the combined toxicity effect of nonyl phenol (NP) and di-n-butyl phthalate (DBP) on rat sertoli cells. **Methods:** Male rat testicular Sertoli cells were isolated, purified, cultured and identified with FSHR fluorescence staining. The purity of Sertoli cells was > 95%. The purified sertoli cells were then treated with the toxicants at different doses, NP (0, 0.1, 1, 10  $\mu\text{mol L}^{-1}$ ), DBP (0, 0.1, 1, 10  $\mu\text{mol L}^{-1}$ ) and their combination NP + DBP (0.1 + 0.1, 1 + 1, 10 + 10  $\mu\text{mol L}^{-1}$ ). The viability of sertoli cells was determined by Cell Counting Kit-8 (CCK-8). After staining with fluorescein diacetate (FDA) and propidium iodide (PI), flow cytometris analyses were used. **Results:** As compared with control, the viability of sertoli cells in 0.1, 1  $\mu\text{mol L}^{-1}$  NP and 0.1, 1, 10  $\mu\text{mol L}^{-1}$  DBP were significantly increased at 6 h, whereas that in 10  $\mu\text{mol L}^{-1}$  NP group and that in NP + DBP (10 + 10  $\mu\text{mol L}^{-1}$ ) were significantly decreased. At 12 h, all groups were of no statistical difference. At 24 h and 48 h, that in 10  $\mu\text{mol L}^{-1}$  NP group and NP + DBP (1 + 1, 10 + 10  $\mu\text{mol L}^{-1}$ ) were significantly decreased. The following Annexin V/PI staining demonstrated that NP (10  $\mu\text{mol L}^{-1}$ ) and NP + DBP (10 + 10  $\mu\text{mol L}^{-1}$ ) treatments led to increased proportion of cell apoptosis and necrosis, which were also evidenced by the detection of condensation and marginal changes of Hoechst stained chromatins. **Conclusion:** Low dose and short-term NP and DBP treatments can promote the proliferation. But, at high doses, the proliferation of Sertoli cells are inhibited. The combined effect of NP and DBP is synergistic.

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#### C1-17

##### Seasonal changes in spermatogenesis and immunodetection of cytochrome P450 aromatase in wild male ground squirrels

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**Aim:** Estrogen is clearly involved in numerous processes in male, and it plays an important role in the production and function of spermatozoa during spermatogenesis especially. The purpose of the present study was to investigate seasonal changes of testicular tissue, localization and immunoreactivity of P450 aromatase (P450arom) in testes of wild male ground squirrels during the breeding and non-breeding seasons. **Methods:** Testicular samples were dehydrated in ethanol series and embeded in paraffin wax. Serial sections (4  $\mu\text{m}$ ) were mounted on slides coated with poly-L-lysine. Some sections were stained with hematoxylin-eosin (HE) for observation of general histology. The serial sections of testes were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections were then incubated with primary

antibody raised against human placental P450arom for 12 h at room temperature. Thereafter, the sections were incubated with the second antibody goat anti-rabbit IgG conjugated with biotin and peroxidase with avidin, using a rabbit ExtrAvidin<sup>TM</sup> staining kit, followed by visualization with 30 mg 3,3'-diaminobenzidine solution in 150 mL of 0.05 mol Tris-HCl buffer, pH 7.6, plus 30  $\mu\text{L}$   $\text{H}_2\text{O}_2$ . Finally, the reacted sections were counterstained with hematoxylin solution. The control sections were treated with normal rabbit serum instead of the primary antisera. Testicular tissue was weighed and diced into small pieces using a clean razor blade. Tissue was homogenized in a dounce homogenizer containing 300  $\mu\text{L}$  of 10 mg  $\text{mL}^{-1}$  PMSF stock and incubated on ice for 30 min, maintaining temperature at 4°C throughout all procedures. Homogenates were centrifuged at 12 000  $\times$  g for 10 min at 4°C. Protein extracts (25  $\mu\text{g}$ ) were mixed with an equal volume of 2  $\times$  Laemmli's sample buffer. Equal amounts of each sample were loaded and run on a 15% SDS-PAGE gel at 18V  $\text{cm}^{-1}$  and transferred to nitrocellulose membranes using a wet transblotting apparatus. The membranes were blocked in 3% BSA for 1 h at room temperature. Primary incubation of the membranes was carried out using a 1:1 000 dilution of rabbit anti-P450arom antibody for 60 min. Secondary incubation of the membrane was then carried out using a 1:1 000 dilution of goat anti-rabbit IgG tagged with horseradish peroxidase for 60 min. Finally, the membrane was colored with 25 mg 3,3'-diaminobenzidine solution in 25 mL TBS-T buffer plus 3  $\mu\text{L}$   $\text{H}_2\text{O}_2$ . **Results:** There were marked variations in testicular weight and size from breeding season (April) to non-breeding season (September). On the basis of morphology of the testes, spermatogonia, primary spermatocytes, second spermatocytes, spermatozoa were identified in breeding season. Immunoreactivity for P450arom was found in Leydig cell, Sertoli cell, spermatogonia, kinds of spermatocytes and spermatozoa in the breeding season. The immunohistochemistry for the P450arom was not observed in testicular tissues during non-mating season. With that P450arom antibody, the bands of the Western blot of P450arom were only detected in breeding season. **Conclusion:** Seasonal changes in testicular weight and size in wild male ground squirrels are correlated with spermatogenesis and immunoreactivity of P450arom, and estrogen produced by Leydig cells, Sertoli cells and germ cells may play an autocrine or paracrine role in spermatogenesis and testicular recrudescence and regression process.

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#### C1-18

##### Androgen levels (T and DHT) and 5 $\alpha$ -reductase activities in the androgen target tissues of male individuals at different developmental stages

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**Aim:** To document the physiological variation of testosterone (T) and dihydrotestosterone (DHT) levels and 5 $\alpha$ -reductase activities in androgen target tissues: foreskins of male individuals of Chinese Han people at different developmental stages, and to investigate the effect of androgen on male pubertal development and sexual maturation. **Methods:** The foreskins were acquired from 78 subjects due to phimosis or redundant prepuce. Tissues were homogenized; the T and DHT levels in supernatant were measured by RIA. 5 $\alpha$ -reductase activities of two types were measured by method modified by our group. The concentration unite was transformed into pmol per mg protein per mL. Androgen levels of all subjects, aged 9 months to 37 years, were analyzed by the polynomial curvilinear regression. The selected subjects were grouped into three groups: eight individuals aged 4 to 6 years as child group, eight individuals aged 14 to 16 years as pubertal group, and six individuals aged 24 to 26 years as adult group. **Results:** Based on the physiological curves of the polynomial curvilinear regression and comparison of three groups at different developmental stages, the levels of T and DHT in target tissues remained low in childhood [(0.368  $\pm$  0.229) pmol mg<sup>-1</sup> protein mL and (0.589  $\pm$  0.375) pmol mg<sup>-1</sup> protein mL], T/DHT ratio was 0.752  $\pm$  0.405. At the pubertal stage, these volumes raised and reached peaks at 14 to 16 years [(1.120  $\pm$  0.278) pmol mg<sup>-1</sup> protein mL and (2.394  $\pm$  0.813) pmol mg<sup>-1</sup> protein mL], T/DHT ratio 0.376  $\pm$  0.161. After completion of pubertal development, T and DHT trended to keep at more stable and higher levels [(1.184  $\pm$  0.295) pmol/mg protein mL and (2.250  $\pm$  0.666) pmol mg<sup>-1</sup> protein mL], T/DHT ratio 0.511  $\pm$  0.164. The activities of 5 $\alpha$ -reductase isozymes in target tissues remained low in children from 2 to 5 years of age. After 6 years of age these values would rise during the stages of pubertal development and reach new peaks at age of 14 to 16 years. After completion of pubertal development, 5 $\alpha$ -reductase activities tended to keep at more stable and high levels. **Conclusion:** We documented the physiological curves of androgen and 5 $\alpha$ -reductase in the target tissues of human men at the different developmental stages. Androgen (T, also DHT) in the target tissues plays key roles in controlling male pubertal development and sexual maturation.

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#### C1-19

##### Antioxidant effect of tetrandrine on cultured rabbit corpus cavernosum endothelial cells injured by hydrogen peroxide

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**Aim:** To investigate the antioxidant effect of tetrandrine (Tet) on cultured corpus cavernosum endothelial cells (CCECs) injured by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). **Methods:** The CCECs were cultured *in vitro* and divided randomly into three groups: contrast group, H<sub>2</sub>O<sub>2</sub> group, and Tet + H<sub>2</sub>O<sub>2</sub> group. The effects of Tet on the viability of cell injured by H<sub>2</sub>O<sub>2</sub> were assessed by 4,5'-simethylthiazaoxy colorimetric assay. The level of malondialdehyde (MDA), superoxide dismutase (SOD) activity and lactate dehydrogenase (LDH) release of CCSMC injured by H<sub>2</sub>O<sub>2</sub> were detected by chemical colorimetry, respectively. **Results:** After treatment with 1 mmol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> for 4 h, the cell viability and SOD activity of CCECs were decreased ( $P < 0.01$ ), respectively. While LDH release and MDA content were increased ( $P < 0.01$ ), respectively. However, treatment with different concentrations of Tet (10<sup>-7</sup>–10<sup>-4</sup> mol L<sup>-1</sup>) could inhibit the damaging effects of H<sub>2</sub>O<sub>2</sub>, with increased cell viability and SOD activity ( $P < 0.01$ ), and decreased LDH release and MDA content ( $P < 0.05$  or  $P < 0.01$ ), respectively. **Conclusion:** Tet has protective effect on CCECs injured by H<sub>2</sub>O<sub>2</sub>, which might be related to enhancing the antioxidant abilities of CCECs, and decreasing the injury of cell membrane by lipid peroxidation.

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#### C1-20

##### Hypoxia on SD rat corpus cavernosum smooth muscle cell apoptosis

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**Aim:** To explore the hypoxia and SD rat corpus cavernosum smooth muscle cell apoptosis. **Methods:** CCSMC was cultivated *in vitro*, immunohistochemical method was used to identify cell. The duration of hypoxia (1% O<sub>2</sub> concentration) lasts for 12 h, 24 h, 48 h, and 72 h respectively and conventional oxygen concentration was chose as control. Cell cycle and apoptosis was determined by flow cytometry. **Results:** *In vitro* cultured CCSMC grew well, anti-smooth muscle  $\alpha$ -actin monoclonal antibody proved positive by immunohistochemical staining. Flow cytometry showed that CCSMC in G<sub>0</sub>/G<sub>1</sub> cells in 48 h in the proportion gradually increased, then decreased; S phase cells and the proportion of G<sub>0</sub>/G<sub>1</sub> phase showed the opposite trend, G<sub>2</sub>/M phase cells had no significant proportion of the laws. **Conclusion:** The degree of CCSMC apoptosis increases with the extension of hypoxic duration, with maximum is 48 h. Further extension of time can not increase the apoptosis.

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**C1-21****Experimental research of using small intestinal submucosa for corporal body grafting in the rabbit**

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**Aim:** We evaluated the morphological, immunological and functional response to small intestinal submucosa grafting for corporal body to determine its potential as a grafting material for penile surgery. **Methods:** A total of 20 male New Zealand White rabbits underwent implantation of 10 × 5 mm small intestinal submucosa graft following excision of an ellipse of tunica albuginea and 20 control animals underwent tunical excision with reimplantation of this autologous segment. The animals were euthanized, and the penis was sectioned and histologically studied at intervals of 2, 6, 12, and 24 weeks. Before death an erection was induced by a rapid intracavernosal injection with saline except of animals of postoperation at 2 weeks. After death the area under the graft was evaluated for stromal collagen by H&E, Masson's trichrome stain and Sirius stain. Protein expression of the inflammatory markers inducible nitric oxide synthase (iNOS) and transforming growth factor-β1 (TGF-β1) was evaluated by immunohistochemical methods. **Results:** There were no identifiable gross differences between experimental groups and control groups. Histologically, slight inflammation at 2 week precipitously decreased to a normal appearing tunica albuginea at 6, 12 and 24 weeks. Collagen I and III mostly occupied the grafting site 2 weeks postoperation. After that, the collagen I increased evidently and collagen III decreased gradually until Collagen I mostly occupied the grafting site at 24 weeks. The two groups showed middle expression of iNOS and TGF-β1, which were induced at 2 week and precipitously decreased to a normal appearing tunica albuginea at 6 and 12 and 24 weeks with no significant difference between them. At 6 weeks, six of 10 had normal erections by a rapid intracavernosal injection with saline and four slight penile deviation, two in control group, two in small intestinal submucosa group. Only one of 10 slight penile deviation was observed in control at 24 weeks. **Conclusion:** The small intestinal submucosa demonstrate tissue specific regeneration properties in the New Zealand White rabbits tunica albuginea. In addition, small intestinal submucosa grafting of the tunica albuginea preserves the duration and magnitude of the erectile response to rapid intracavernosal injection with saline. And this type of tunical grafting does not stimulate a significant inflammatory response of rejection, or cause corporeal fibrosis. It is off-the-shelf material, not only safe, but also technically easy to use and readily available.

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**C1-22****The study of eight classic Chinese traditional recipes on spermatogenic stem cells of the mouse aspermia model**

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**Aim:** The present project was aiming at introducing stem cell theory into research of traditional Chinese medicine to verify the efficacy of Chinese medicine on proliferation, differentiation of spermatogenic stem cells. **Methods:** Four animal models were established including three for oligospermia and one for aspermia. In aspermia model using Busulfan, eight Chinese traditional recipes involving nine kinds of herb medicine were evaluated. By using gene chips the effects of Jin Gui Shen Qi Pill and Wu Zi Yan Zong Pill on expression of genes for spermatogenesis were explored. **Results:** 1) Except Sheng Jing Zan Yu Pill, other seven recipes involving eight kinds of herb medicine exhibited the effects of protection of damaging spermatogenic cells and stimulating and initiating spermatogenic action of the cells. The most effective one was combined effects of Jin Gui Shen Qi Pill and Wu Zi Yan Zong Pill, which was 2.5 folds higher than placebo groups and 70% higher than control. 2) The efficacy of improvement action for each recipe were as follows: Wu Zi Yan Zong Pill, Jin Gui Shen Qi Pill, Liu Wei Di Huang Pill, You Gui Pill, Ren Shen Lu Rong Pill, were within the range of 75%–25% higher than placebo with the highest being Wu Zi Yan Zong Pill, Zuo Gui Pill, Zi Yin Zhong Zi Pill were 15% and 10% higher than placebo respectively. 3) The mechanism of Jin Gui Shen Qi Pill and Wu Zi Yan Zong Pill actions were different, with the former mainly acted on Sertoli cell and the latter directly acted on spermatogenic cells. 4) The genechip analysed that 200–300 genes among 30 000 genes inspected were found to exhibit complex effects on spermatogenesis. **Conclusion:** The classic traditional Chinese medicine has the strong efficacy on proliferation, differentiation of spermatogenic stem cells. This study is supported by a grant from the National Natural Science Foundation of China (No.90409016).

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**C1-23****Male germ-cell transplantation from normal Mongolia sheep to the testes of azoospermic Poll Dorset sheep results in production of donor's offspring**

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**Aim:** To rescue fertility of an azoospermic Poll Dorset sheep (male recipient sheep) by transplantation of germ cells from normal Mongolia sheep (male donor sheep) into its testes, and mating the Poll Dorset sheep with female Mongolia sheep via sexual intercourse or artificial fertilization techniques, to result in production of F1 offspring. **Methods:** Testis irradiation in male recipient sheep; preparation of the single cell suspension

from donor testis and enrichment of the spermatogonia via differential cell attachment; transplantation of the enriched spermatogonia cells into recipient testes; analysis of sperm; comparison of phenotype between F1 offspring and their sire. **Results:** The donor Mongolia sheep was castrated and, the enriched spermatogonial cells were transplanted into rete testis of the recipient Poll Dorset sheep under ultrasound guidance. Six months after germ cell transplantation, the sperm motility in the previously azoospermic Poll Dorset sheep increased from 9.5% to 40.5%. The recipient was bred to seven female Mongolia sheep. One female was mated by naturally, resulting in the birth of twin lambs; six females were mated by artificial insemination, one aborted, one didn't become pregnant, and four successfully produced one offspring each. From the total of six offspring, five were phenotypically similar to the male donor Mongolia sheep with regard to ears, fur, and tail, whereas one was similar to the recipient Poll Dorset sheep. **Conclusion:** This is the first report of donor cell-derived sperm production and production of donor-derived progeny after germ-cell transplantation in sheep. These results demonstrate that germ cell transplantation is feasible for rescuing a male infertility phenotype between unrelated sheep.

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#### C2-03

##### Shorten exposure of oocytes to spermatozoa improves embryo quality: possible role of reactive oxygen species

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**Aim:** To evaluate the relationship of embryonic quality with different time of oocytes exposed to spermatozoa in conventional IVF and explore the possible mechanisms. **Methods:** From Nov. 2007 to Jun. 2008, a total of 106 patients younger than 35 years old with infertility because of tube obstruction, 117 cycles in total underwent conventional IVF. Totally 1 384 sibling oocytes were obtained and assigned to three groups randomly. In group A ( $n = 35$ ), 495 oocytes were cultured with spermatozoa for 3 h before denudated completely. In group B ( $n = 35$ ), 446 oocytes were exposed to spermatozoa for 3 h while the granular cells were partially denudated, and then denudated completely after 13–15 h. In group C ( $n = 36$ ), 443 oocytes were cultured with spermatozoa for 16–18 h before denudated completely. The insemination media was HTF supplemented with 10% SSS. The oocytes of group A and B were transferred into the same media and the media with spermatozoa were discarded. On D1, about 16–18 h after insemination, all of the oocytes were transferred into P-1 media supplemented with 10% SSS. The media of the three groups were collected as samples, and the HTF supplemented with 10% SSS without spermatozoa and oocytes were collected as control sample. ROS levels in the media were measured by chemiluminescence assay using luminol as the probe. Then the oocytes without PN on D<sub>1</sub> and abandoned embryos on D<sub>3</sub> were collected for the follow tests. The intensity of H<sub>2</sub>O<sub>2</sub> concentrations was measured using 29,79-dichlorodihydrofluorescein diacetate by Quanti

cell 500 fluorescence imaging. Damage of mitochondria and DNA fragmentation was observed via transmission electron microscopy, and TUNEL was performed to determine the DNA fragmentation. **Results:** The ROS levels in media of group A was much lower than that in other two groups ( $P < 0.05$ ). Correspondingly, the H<sub>2</sub>O<sub>2</sub> concentration in oocytes and embryos of group A were the lowest ( $P < 0.05$ ). There was no statistic difference in DNA fragmentation rate among the three groups ( $P > 0.05$ ). However, via TEM, the damage of mitochondria was found much more frequently in group C than those in other two groups ( $P < 0.05$ ). **Conclusion:** As mentioned in one of our previous experiments, the high quality embryo rate in short co-incubation group (group A) was much higher than that in other two groups. As all well known, ROS causes damage to DNA fragmentation in somatic cells, this research was performed to confirm the hypothesis that ROS generated in culture media by spermatozoa after insemination may be a harmful factor for early embryonic growth and explore the possible mechanism. From the results, ROS level in the media of group A was much lower than that in other two groups, which indicated that long time exposure of oocytes to spermatozoa may induce high ROS level in culture media, and the result was positively correlation with the H<sub>2</sub>O<sub>2</sub> concentration in oocytes/embryos. High ROS level and H<sub>2</sub>O<sub>2</sub> concentration may lead to mitochondria damage of oocytes and embryos, which may be one of the mechanisms that exposure of oocytes to spermatozoa long time decreases high embryo quality rate.

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#### C2-04

##### The therapeutic effects of different operation methods for varicocele: a study of 685 cases

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**Aim:** To investigate the therapeutic effects of three operation methods for varicocele and to explore which is the best choice in the management of patients with primary varicocele. **Methods:** From January 1990 to November 2006, 1 075 patients with VC grade II or III were treated in the Second Affiliated Hospital, Sun Yat-Sen University. A total of 685 patients were followed-up. We recruited these patients with primary varicocele above grade II for this study. These patients were divided into three group according to three kinds of operation methods of varicocelectomy: group A ( $n = 369$ ) were treated with highly selective high ligation of varicocele; group B ( $n = 218$ ) were treated with transinguinal canal operation; and group C ( $n = 98$ ) were treated by Laparoscopic of renovated polomo operation. The complications, quality of semen and natural impregnation rate were studied in three groups to compare the therapeutic effect of the three methods. **Results:** The recurrence rate of group A was lower than that of group B with obvious statistical difference ( $P < 0.05$ ) group B and C had more possibility of scrotal edema than group A ( $P < 0.05$ ); and group A and C had less pain of testicle and skin than group B ( $P < 0.05$ ). The



testicular atrophy rate of group A was lower than that of group B and C after three months ( $P < 0.05$ ). The increasing rate of the quality of semen and natural conception rate were higher in group A than in other groups if patients' ages were below 30 years old ( $P < 0.05$ ). The increasing rate of the quality of semen of patients below 30 was also higher than those above 30 ( $P < 0.05$ ). No difference was found between three groups in natural conception rate in patients above 30 ( $P > 0.05$ ). **Conclusion:** Highly selective high ligation of varicocele would be the best choice in the management of patients with primary varicocele due to lower rate of scrotal edema, testicular atrophy, hydroceles and higher quality of semen and higher natural conception rate.

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## C2-05

### Investigation of *Ureaplasma urealyticum* infection in 177 cases of azoospermia or oligospermia

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**Aim:** To investigate *Ureaplasma urealyticum* (Uu) infection in the male patients with azoospermia or oligospermia, in order to give further causal evidence for the prevention of Uu infection and study the etiology of male infertility. **Methods:** The comparison was conducted between 177 cases of male infertility and 40 cases of normal fertility. According to the World Health Organization (WHO), the infertility were for sperm density of less than  $20 \times 10^6$  and sperm motility of  $a < 25\%$  or  $a + b < 50\%$  under the computer-assisted semen analysis (CASA). Uu was detected by culture solution. It is positive for Uu if the color of solution changes from yellow to red, which indicates Uu growth, otherwise negative. Antisperm antibodies (AsAb) in semen were detected by mixed anti-globulin reaction, it is positive if the ratio of immobile sperm to active sperm under the microscope is more than 10%, otherwise negative. All the data were registered by trained investigators. **Results:** The Uu infection rate in infertile male is 35%, which is higher than that of the normal control group (10%) ( $P < 0.05$ ). AsAb positive rate in Uu-infected patients is 35%, which was significantly higher than that of the group without Uu infection (8%) ( $P < 0.05$ ). The Uu infection was related with the age, the sex life frequency, the education level, the environment, as well as redundant prepuce. **Conclusion:** The Uu infection can cause decrease in sperm quality and the emergence of AsAb. Redundant prepuce is one of the main causes of Uu infection. Frequent sex life, poor living conditions, and low level of education are all risk factors of Uu infection. It also suggests the benefits of circumcision for men of child-bearing age.

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## C2-07

### The diagnosis and surgical treatment for obstructive azoospermia: a report of 56 cases

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**Aim:** To evaluate the diagnosis and surgical treatment for obstructive azoospermia. **Methods:** The clinical information of 56 cases of obstructive azoospermia was analyzed from October 2004 to November 2008 in our hospital. Among them, 43 cases were obstructive azoospermia with ejaculatory duct (EDO), and 13 cases were obstructive azoospermia with suspected epididymal level. The diagnostic methods included semen analyses, fructose and neutral  $\alpha$ -glucosidase measurement in seminal plasma, transrectal ultrasonography (TRUS), and vasography necessarily. Forty-three patients with EDO were treated by transurethral resection of ejaculatory duct (TURED), and 13 patients of obstructive azoospermia with suspected epididymal level underwent scrotal exploration, of them 11 patients who were confirmed to have epididymal obstruction underwent either bilateral or unilateral vasoepididymostomy. Postoperative semen assay, postoperative patency rate and postoperative impregnation rate were followed. **Results:** Semen analyses of the 43 cases of EDO showed azoospermia in low semen volume (0.2–3.4 mL, average 1.0 mL), low PH (5.8–7.6, average 6.7), absent or low semen fructose (0–51.4  $\mu\text{mol}$ , average 5.65  $\mu\text{mol}$ ). TRUS showed pure dilated ejaculatory duct in 10 cases, prostatic cyst in 4 cases, pure dilated seminal vesicles on both sides in five cases, one dilated seminal vesicles in four cases, dilation of both ejaculatory duct and seminal vesicles in eight cases, dilated seminal vesicles with prostatic cyst in three cases, one dilated seminal vesicles with another hypoplasia or absence in two cases, dilated seminal vesicles with dilated ejaculatory duct and calcification in three cases, one dilated seminal vesicles with another hypoplasia or absence and dilated ejaculatory duct in four cases. Thirteen cases of obstructive azoospermia with suspected epididymal level showed azoospermia, low semen neutral  $\alpha$ -glucosidase (2.1–45 mU, average 16.18 mU). TRUS showed normal seminal vesicles, ejaculatory duct and prostate. Among all the cases followed up more than 3–51 months after operation, 36 patients (83.7%) had improved semen parameters. Of the 43 patients treated by TURED, 11 (25.6%) had pregnancies. Of the 43 patients treated by vasoepididymostomy six (54.5%) demonstrated sperm in the semen assay and three (27.3%) had pregnancies. **Conclusion:** Semen analyses, fructose and neutral  $\alpha$ -glucosidase measurement in seminal plasma, TRUS and vasography are major diagnostic methods for obstructive azoospermia. TURED and vasoepididymostomy may respectively be the effective methods for the treatment of azoospermia with EDO and obstructive azoospermia with epididymal level.

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## C2-09

### Azoospermia: a laboratory diagnosis and research

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**Aim:** To explore the cause of azoospermia by experimental analysis. **Methods:** This paper conducts an analytical research of 758 cases of azoospermia, diagnosed in our hospital from March 2005 to July 2009. Our methodologies include CASA with centrifugal smears; seminal fructose; seminal neutral- $\alpha$ -glucosidase; BUS; CFTR gene, epididymal puncture; immediately urine test after ejaculation; semen Leukocyte peroxidase or seminal plasma PMN-Elastase; endocrine hormones; cytogenetics genetic testing (Analysis of chromosome karyotype, Y chromosome microdeletions, SRY) and testicular biopsy, etc. **Results:** Of all the 758 samples, we found 151 (19.9%) cases of reproductive tract abnormalities, 98 (12.9%) of which were caused by congenital malformations and 53 (7%) by acquired deformities; 98 (12.9%) cases of reproductive tract infections; 110 (14.5%) cases of endocrine disorders; 181 (23.9%) cases of cytogenetics and genetic abnormalities, of which 67 (8.8%) were caused by 46,xy, Klinefelter syndrome, 14 (1.8%) by Kallmann syndrome and 46,xy,y<18/46,xy,y<21/46,xy,1qh+/46,xy,inv(9)(p11;q13) respectively accounted for three cases (0.4%), 46,xy,dely(q11)/46,xy,t(11;14) (q25;q22)/46,xy,t(14;21)/46,xy,t(2;9)/45,xy,t(13;18)/48,xxxy respectively accounted for two cases (0.3%), 46,xy, t(12:15) I (16)/46,xy,y = 18/46,xx, SRY +/46xy y = 21/46,xy, var (1qh+)/46,xy, var (1qh+)(16 qh+)/46,xy,t (1:20)/46,xy, yqh+/47,xyy/46,xy, t (2:11)/ 46,xy,t(18;21) (q11;q22)/46,xy,t(1;15)(q21;q23)/46,xy,del(y)(q11)/46,xy,inv(9)/46,xy,t(x;6)(p12q12) respectively accounted for one case (0.1%). In addition, 61 (8%) cases of Y chromosome microdeletions, including eight (1.1%) cases of AZFa, 21 (2.8%) cases of AZFb, 30 (3.8%) cases of AZFc; two (0.3%) cases of AZFb+c; testicular biopsy in 92 (12.1%) cases demonstrated no sperm production, and 126 (16.6%) cases of unknown causes. **Conclusion:** Through experiments, this paper gives a clear etiological diagnosis of azoospermia, providing valuable groundings for clinical practice.

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## C2-10

### Clinical characterization of oligospermic or azoospermic men with AZF region microdeletion of Y chromosome

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**Aim:** To Analyze clinical characterization of 35 men who were oligospermic or azoospermic with AZF region microdeletion of Y chromosome. **Methods:** According to the techniques recommended by WHO, we collected 367 oligospermic or azoospermic men. Using modified multiplex PCR to make

molecular diagnosis of Y chromosomal microdeletion. All the cases with Y chromosomal microdeletions were divided into two groups: severe oligospermia group and azoospermia group, and then into two subgroups according to deletion types: pure AZFc microdeletion and other types. Data were collected about marriage age, infertility history, sperm, testis volume, paracentesis of epididymis/testis, karyotype and hormone test. **Results:** There were one case in AZFa, one case in AZFb, 29 cases in AZFc, two cases in AZFa + b + c, two cases in AZFb+c. All but two patients tested were 46, XY with no identifiable karyotypic anomaly. FSH, LH, testosterone and testis histology could not differentiate those with oligospermia or azoospermia, nor could they predict whether sperm could be found in harvested testis tissue. **Conclusion:** Severe oligospermic or azoospermic men with normal phenotype and karyotype may have Y chromosomal microdeletion, whose rate is about 10%, and the most common type is AZFc deletion. Merely AZFc microdeletion has less influence on spermatogenesis than other deletion types. With regard to azoospermic men only with AZFc deletion, testis puncture biopsy may be helpful to find useful sperm, but it will not be suitable for other types.

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## C2-11

### AZF microdeletions on the Y chromosome among azoospermia or severe oligozoospermia men in Jilin Province, northeast China

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**Background:** Y chromosome microdeletions are the most frequent genetic cause of male infertility. In different countries and areas, the published frequencies of microdeletions are usually inconsistent, and the frequency varies from 1.0% to 55.5%. **Aim:** To establish the frequency of Y chromosome microdeletions in patients with azoospermia or oligozoospermia in Jilin Province, northeast China. **Methods:** A total of 135 infertile men (37 with severe oligozoospermia and 98 with non-obstructive azoospermia) from the male infertility clinics of the Institute of Reproductive Medicine of Jilin Province were studied. Genomic DNA was extracted from peripheral blood samples. Multiplex polymerase chain reaction (PCR) by nine Y-specific sequence-tagged sites (sY84, sY86, sY127, sY134, sY143, sY157, sY254, sY255 and sY152) of AZF region was performed to screen the microdeletions in the AZF region of Y chromosome. **Results:** Microdeletions of the AZF region on Y chromosome were observed in 17 patients (17/135, 12.6%). Of the 17 cases with microdeletions, 11 patients were azoospermic (64.7%), and six were oligozoospermia (35.3%). Frequency of microdeletions in azoospermic men was 11.2% (11/98). Frequency of microdeletions in men with oligozoospermia was 16.2% (6/37). None of the patients showed isolated microdeletion in the AZFa region, but three azoospermic men had deletion in the AZFb region. All patients were detected to

have the AZFc region microdeletions. Eight patients (four with oligozoospermia and four with azoospermia) were detected to have microdeletions in the AZFd region (sY152). Of the 17 patients with microdeletions, seven patients (two with oligozoospermia and five with azoospermia) were detected to have AZFc deletion, two azoospermic patients had AZFb + c deletion, one azoospermic patients had AZFb + c + d deletion, and seven patients (four with oligozoospermia, three with azoospermia) had AZFc + d deletion. sY255 (AZFc) was deleted in all patient (17/17, 100%), and its frequency was the highest among all microdeletion sites. **Conclusion:** The incidence of Yq microdeletions in the studied population in Jilin Province, northeast China is consistent with those published in other countries and areas, but frequencies of deletions in the AZFc and AZFd region are higher than those that have been published.

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### C2-13

#### The differential diagnosis of primary and secondary venogenic erectile dysfunction

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**Aim:** To explore the value of penile base compression test in the differential diagnosis of primary and secondary venogenic erectile dysfunction (VED). **Methods:** 18 patients (group B) with VED and six normal adult males (group A) for control were studied. The method went as follows: Firstly, pediatric blood pressure cuff was used to encircle the penile base, and Doppler Flowmetry was employed to hear the flow sound of penile dorsal artery (PDA). Secondly, the cuff was pressurized, and then the pressure of PDA was measured again. Thirdly, papaverine was injected into the corpus cavernosum in a dose of 40 mg using 26G syringe and the pressure of PDA was measured subsequently. Fourthly, the penis was decompressed and the erection condition was recorded ten minutes later. Beside, the blood flow of glans penis (GP) was measured with Laser Doppler Flowmetry PF2 concurrently. **Results:** All the six patients in group A developed penile erections with an average increased PDA pressure of 15.3 mmHg and an average increased GP microcirculatory blood flow of 3.3 folds. According to the erection condition during the compression test, the patients in Group B could be divided into B1 (four cases) and B2 (12 cases). The four patients in B1 developed erections when compressing the penile base, but the penis became flaccid after decompression, which showed an average increased PDA pressure of 18.8 mmHg and an average increased GP microcirculatory blood flow of 1.9 folds. The 12 patients in group B2 never developed erection during the test with an average increased PDA pressure of 13.2 mmHg and an average increased GP microcirculatory blood flow of 2.1 folds. **Conclusion:** After papaverine was injected into the corpus cavernosum, the cases developed erections when compressing penile base, but showing flaccid after decompression should belong to primary VED. Meanwhile, the other cases never showing erection during the test should consider as secondary VED.

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### C2-14

#### The relationship between premature ejaculation and central lumbar intervertebral disc herniation

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**Aim:** To investigate the relationship between premature ejaculation (PE) and central lumbar intervertebral disc herniation (CLIDH). **Methods:** A total of 263 outpatients of PE, all coming from Research Institute of Andrology, Nanjing University of Traditional Chinese Medicine, were involved in our study between May 2007 and March 2009. They were all examined by CT to detect CLIDH. The patients diagnosed with CLIDH were then equally randomized into two groups: the treatment group ( $n = 180$ ), treated by lumbar traction; and the control group ( $n = 60$ ), given sertraline hydrochloride tablets, both for 4-8 weeks. CIPE-5 scores of all patients were analyzed, and the ejaculation latency and sexual satisfaction were recorded, before and after the treatment.

**Results:** After the treatment, CIPE-5 scores, ejaculation latency and sexual satisfaction of treatment group were significantly improved compared with pre-treatment, showing remarkable statistic difference ( $P < 0.01$ ). However, no statistical difference was found in control group before and after treatment. There are also statistic difference between two groups after treatment ( $P < 0.05$ ). **Conclusion:** CLIDH is one of the most possible reasons for PE, which has no-confirmed reason. Lumbar traction is a non-traumatic, simple and highly effective method to deal with this kind of PE patients with CLIDH. In our study, a new possible etiology and practicable therapeutic method of PE were proposed for the first time. It provides a new way and new method for diagnosis and treatment for PE, deserved to be tried and popularized.

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### C2-15

#### Sexual practice and attitude of adults in Guangzhou China: a community based survey

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**Aim:** To measure the sexual behavior and attitude of adults in Guangzhou China in the new era when western culture conflict with traditional Chinese culture, still we compared the similarities and differences between the two genders, besides the connection between education level and the two genders' sexual practice. **Methods:** From 2005 to 2007, a cross sectional survey was carried out among 300 male and 300 female adults, a total 600 adults in Guangzhou, China. Among these, 255 female and 229 male adults completed the whole interview and fulfilled the questionnaires. Information involves sexual attitude and sexual behavior, including masturbation, sexual foreplay, oral sex,

intercourse, coordination with sexual partner, self-evaluation of one's sexual function, and so on. The information related to sociodemographics, health status were also included. **Results:** The response rate is 73.33% (229/300) in male and 85% (255/300) in female. All of them are above 18 years old, and have had sex behaviors. Nearly half (48.8%; 124/255) of the female adults admitted of masturbation experiences, while more than two thirds (68.8%; 158/229) of the male adults admitted. More than half women (59.9%; 153/255) need passionate touch before sex, while half men (63.2%; 145/229) need more moderate touch. One third of the women (33.3%; 85/255) could accept oral intercourse, while two thirds of the men (61.1%; 141/229) couldn't accept oral intercourse. The frequency of sex life per month is less than 10 times in most women (72.5%; 185/255) and men (77.7%; 178/229). A little more men (72.9%; 167/229) than women (54.9%; 140/255) would like to frequently change the body position in sex life. Still, more men (72.9%; 167/229) than women (54.9%; 140/255) would like to coordinate with the sexual partner when he/she is required for sex. Regarding their own cognitive of the sexual function, 74.1% (189/255) women thought they were in general level. Only 45.0% (103/229) men thought the same, while 39.7% (91/229) and 15.3% (35/229) felt self-confidence or self-abasement about their sexual function respectively. **Conclusion:** The data provide insight into the sexual life condition of adults in Guangzhou China. Although masturbation and oral sex are two kinds of the common sex activities, part of the adults in South China still had negative attitude to the two activities, and especially in women. Most men and women could enjoy their sex lives through the most common way of sexual intercourse. Men were more active than women in the leading position of sex life. Moreover, compared to their counterpart with lower education level, female with higher education has been reported to require more passionate foreplay, and more male with higher education felt self-confident and less self-abase to their sexual function. To propagandize and promote healthy sexual education are necessary to prepare people to grasp sufficient sexual knowledge, practice it healthily and enjoy it.

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## C2-16

### Correlation between benign prostate hyperplasia and erectile dysfunction

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**Aim:** To explore relationship between benign prostate hyperplasia (BPH) and erectile dysfunction (ED) from five aspects. **Methods:** Totally 108 patients with BPH were divided into two groups according to score: group A (IIEF-5 score  $\leq 12$ , with weaker sexual function) and group B (IIEF-5 score  $\geq 13$ , with better sexual function). I-PSS, prostate volume measurement, serum PSA value, serum testosterone, and

Q<sub>max</sub> assay were carried out in both groups. Calculated by SPSS13.0. Statistical significance existed at  $P < 0.05$ . **Results:** The incidence of BPH concurrent with ED was 49.07%. IPSS scoring: group A  $23.23 \pm 6.05$ , group B  $19.98 \pm 7.16$ , with statistical difference ( $P < 0.05$ ). There was a weak correlation between IPSS scoring and IIEF scoring ( $r = 0.115$ ). Prostate volume was  $63.19 \pm 35.38$  mL in group A, and  $58.91 \pm 34.99$  mL in group B, and there was no statistical significance ( $P > 0.05$ ). Mean serum PSA value was  $5.25 \pm 4.48$  ng dL<sup>-1</sup> in group A, and  $4.48 \pm 4.46$  ng dL<sup>-1</sup> in group B, with no statistical significance ( $P > 0.05$ ). Q<sub>max</sub>: Group A  $10.28 \pm 5.11$  mL s<sup>-1</sup>, group B  $10.64 \pm 5.00$  mL s<sup>-1</sup>. There was no statistical significance ( $P > 0.05$ ). Testosterone value: mean value in group A was  $352.37 \pm 226.32$ , and group B  $445.09 \pm 224.70$ . There was statistical significance ( $P < 0.05$ ). There was a weak correlation between IIEF score and testosterone value ( $R = 0.24$ ). In addition, correlation analysis was performed between IIEF-5 score and IPSS score, IIEF-5 score and plasm testosterone respectively. There was a certain correlation ( $r = 0.115$ ,  $r = 0.24$ ). **Conclusions:** BPH patients always have a higher incidence of ED. There was a certain correlation between serum PSA value and IPSS scoring, but also between serum PSA value and prostate volume. Patients with severe lower urinary tract symptoms and low serum testosterone values are usually at a higher risk of ED occurrence.

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## C2-17

### Clinical study of curing premature ejaculation by combined application of SSRIs and PDE5 inhibitor

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**Aim:** To explore the clinical effects of curing premature ejaculation by combined application of selective serotonin reuptake inhibitors (SSRIs) and phosphodiesterase type 5 (PDE5) inhibitor. **Methods:** Paroxetine hydrochloride 20–40 mg daily was given at the breakfast, and vardenafil 10–20 mg was given 15–60 min before sexual life, with two weeks for a course of treatment. Further enhanced sexual ability and sexual tolerance of adjuvant therapy were recorded. **Results:** After 1–6 courses of treatment, the average ejaculation latency extended from less than 1 min before treatment to an average of 6.3 min after treatment. After three courses of treatment, the ejaculation latency extended to 6–10 min or more in most of the patients. Almost all patients within 1 week found that the ability of control ejaculation was in varying degrees improvement: cured in 12 cases (20.0%), excellent in 19 cases (33.3%), effective in 24 cases (40.0%) and invalid in five cases (8.3%). The total excellence rate was 51.7%, and the total effective rate was 91.7%. **Conclusion:** The combined application of SSRIs and PDE5 inhibitor is safe, practical and effective to curing premature ejaculation.

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## C2-18

### The clinical significance of Dorsal penile nerve in the treatment of primary premature ejaculation

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**Aim:** To explore the value of studying the normal dorsal penile nerve number, course, distribution and the dorsal penile nerve sective neurectomy in the treatment of primary premature ejaculation. **Methods:** Thirty-eight adult male anatomy of the penis body, dorsal penile nerve to show that record numbers of nerve of the penis back and running, and distribution. 314 patients with primary premature ejaculation sective neurectomy of the dorsal penile nerve, age 20–45 years old, course 1–22 years. **Results:** Thirty-eight bodies of dorsal penile nerve distributed in parallel on both sides and dorsal surface, four bodies dorsal penile nerve branches to the distribution side of the abdomen; 38 bodies dorsal penile nerve is  $3.6 \pm 1.2$  branches: seven branches in one case; six branches in one case; five branches in six cases; four branches in nine cases; three branches in 14 cases; two branches in seven cases. Three hundred and fourteen cases of dorsal penile nerve numbers in with primary premature ejaculation patients is  $7.0 \pm 1.9$  branches: five branches in 64 cases, six branches in 56 cases, seven branches in 52 cases, eight branches in 40 cases, nine branches in 33 cases, 10 branches in 28 cases, 11 branches in 25 cases, 12 branches in 11 cases, 13 branches in five cases. Vagina ejaculation latency  $4.31 \pm 1.87$  min post-operation, and sexual satisfaction  $61\% \pm 17\%$ . Before and after surgery vagina ejaculation latency, sexual satisfaction, compared with a significant difference  $P < 0.01$ . **Conclusion:** Exceptional increase in the dorsal penile nerve number is possibly the pathology foundation for primary premature ejaculation. Dorsal penile nerve sective neurectomy is safe and effective for the treatment primary premature ejaculation.

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## C2-20

### The risk factors of erectile dysfunction for the aged patients with BPH under different treatments

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**Aim:** To identify the risk factors of erectile dysfunction (ED) for the aged patients with benign prostatic hyperplasia (BPH) under different treatment. **Methods:** The history of 128 male patients who were diagnosed as BPH in our hospital during January 2006 to October 2007 was collected. According to the treatment, they were allocated into two groups: transurethral resection of prostate (TURP) ( $n = 62$ ) and medication ( $n = 66$ ). The IIEF-5 assessment was repeated at a 6-month follow-up. Statistic methods were used to analyze the 128 patients' data. **Results:** The morbidity rate of ED was 68.60% for all 128 patients. Six months after TURP, the rate of patients reporting ED increased 12.90% ( $69.36\%$  vs.  $82.26\%$ ). All the patients whose prostate volume was larger than 60 mL before operation and who had intraoperative capsular perforation were reported ED after

TURP. The rate of retrograde ejaculation was 90.91%. The rate of ED increased 14.70% for patients who used Finasteride, and decreased 12.50% for patients who used  $\alpha 1$ -AR antagonists only. **Conclusion:** The incidence of newly reported ED after TURP was 12.90%. Risk factors for its occurrence were prostate volume and intraoperative capsular perforation. The rate of retrograde ejaculation was 90.91%. Finasteride was associated with a negative effect on sexual functioning. In contrast,  $\alpha 1$ -AR antagonists had no negative effect on sexual functioning, so  $\alpha 1$ -AR antagonists can be considered as the first-line medicine for the treatment of LUTS/BPH with the demand of sexual function.

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## C2-21

### The experimental and clinical study of high intensity focused ultrasound in the treatment of benign prostatic hyperplasia and prostate cancer

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**Background:** HIFU (high intensity focused ultrasound) has been widely used in the clinical treatment of hepatocarcinoma, bone tumor, breast cancer and soft tissue sarcoma *et al.* However, study on HIFU in the treatment of diseases of urinary system such as benign prostatic hyperplasia (BPH) and prostate cancer (PCa) have not carried out widely. **Aim:** The objective of this study was to determine the efficiency and safety of this non-invasive technique. On the basis of these studies, the preliminarily clinical application was researched. **Methods:** The third-generation HIFU device called the Sonablate-500™ (Focus Surgery, Indianapolis, IN, USA) was used. In the experiment, twenty canines were randomly divided into five groups. Sixteen canine prostates were treated with transrectal HIFU device. Serial study was performed at 30 min, 30 days, 60 days and 180 days after therapy. Two dogs were sacrificed in these periods of time. The rectum, periprostatic tissue, and prostate were excised en bloc. Tissues were fixed for gross and histological analysis. In the clinical study, 130 patients with high risk benign prostatic hyperplasia were treated by transrectal HIFU. International prostate symptom score (IPSS), quality of life (QoL), maximum urinary flow rate ( $Q_{max}$ ), post void residual urine volume (PVR) and the change of the prostatic volume through transrectal ultrasounds were employed for the evaluation of curatives effect on 30th, 60th, 90th, 180th and 360th day after the treatment. A total of 57 prostate cancer patients underwent transrectal HIFU with the Sonablate-500. The patients with localized prostate cancer underwent transrectal HIFU treatment except biochemical failure. Advanced prostate cancer cases were treated by transrectal HIFU combined with androgen ablation treatment. T-lymphocyte subsets CD4, CD8, CD4/CD8, NK cells and cytokines IL-2 and SIL-2R in 6 mL of peripheral venous blood were examined before and 2 weeks after HIFU treatment. **Results:** Histologically, there was a clear demarcation between necrotic treated tissue and the unaffected surrounding



tissue. For the patients with benign prostatic hyperplasia, the more frequent complications were urinary retention, frequency and hematuria. No rectal injury occurred during the treatment. Three patients had epididymitis. QoL score became lower, while  $Q_{max}$  improved, PVR decreased ( $P < 0.01$ ) and volume of prostate reduced ( $P < 0.05$ ) significantly after the HIFU prostate ablation treatment. The biochemical disease-free rates at 1, 2 and 3 years in localized prostate cancer patients were 86%, 81% and 79%, respectively. Patients with prostate cancer show hypimmunity when compared with the controls. There were significant differences between the values of immunity related indexes before and after HIFU treatment ( $P < 0.05$ ). **Conclusion:** HIFU is capable of destroying prostatic tissue, bordering the urethral sharply without injury to the involved and surrounding tissue. It has become apparently that volume of prostate is condensed while the prostatic urethral average maximal diameter is increased after the ablation. There is a low incidence of post-operative complications associated with HIFU treatment. For many reasons, HIFU appears highly attractive as a new optional, safe and efficacious minimally invasive treatment for prostatic diseases. The data on longer follow up are keenly awaited. Besides, HIFU therapy can enhance the immune function of the patients with prostate cancer, and play an important role in the antitumor effect.

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## C2-22

### A retrospective study on the learning curve of 1 122 cases of TUVRP by one surgeon

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**Background:** In recent years, due to the aging of the Chinese population and the popular application of drugs to benign prostatic hyperplasia (BPH), the composition of patients who received treatment of transurethral vaporization resection of prostate (TUVRP) has been significantly changed. Since 1995, TUVRP as a kind of improved surgery means from transurethral resection of prostate (TURP), has been performed to BPH for more than ten years in China. **Aim:** To evaluate the alteration about composition of patients with BPH in recent ten years and the effect of surgeon's skill in performing TUVRP. **Methods:** The clinical data were gained from 1122 patients who underwent TUVRP by one surgeon from 1995 to 2007. Group A was the earlier 100 cases; Group B was the late 170 cases. The composition such as age, prostate volume, BPH complications, accompanied diseases with impact on urinary function, surgical method, operating time, American Society of Anesthesiologists (ASA) grade, surgical complications, IPSS score, maximum urine flow rate (MFR) and so on were analyzed (Table 1). **Results:** In group A, 38 cases (38.00%) were admitted to surgery for complications associated with BPH such as urinary retention, bladder calculi, hematuria, urinary infection, urethrostenosis, hydronephrosis, renal insufficiency and so on; in group B, 152 cases (89.41%) were admitted for complications associated with BPH,  $P < 0.001$ . In group A, 6 cases (6.00%) were accompanied with diseases with impact on urinary function, and in group B, 26 cases (15.29%) were accompanied with diabetes, bladder diverticulum, kidney ureteral stones, bladder cancer, neurological and urinary bladder, and other diseases with impact on urinary function. In group A, postoperative complications occurred in 12 cases (12.00%), four external orifice stricture of urethra, three hematuria, two cerebral infarction, one TUR syndrome, one intestinal obstruction, one mortality (for cerebral infarction) and two were given reoperation. In group B, complications occurred in nine cases (5.29%), four external orifice strictures of urethra, four hematuria

Table 1. The composition of surgical patients with BPH and the efficiency of YUVRP in two groups.

	Group A	Group B
Age (year)	71.36	75.71*
Prostate volume (g)	39.74 (10-175)	63.09 (10-224)*
Open operation (%)	39 (39.00)	—
Operating time (min)	51.98 (25-145)	33.44 (14-105)***
Preoperative hemoglobin (g L <sup>-1</sup> )	124.54 ± 19.86	133.67 ± 17.46
Postoperative hemoglobin (g L <sup>-1</sup> )	133.67 ± 17.46	128.88 ± 15.62
Postoperative Bladder irrigation (%)	75 (75)	25 (8.28)*
Bladder irrigation time (day)	2.11 (1-5)	1.52 (1-3)
Application of hemostatic agent (%)	100 (100)	13 (14.71)
Perioperative blood transfusion (%)	6 (6.00)	1 (0.58)
Preoperative IPSS score	21.91 ± 6.56	25.78 ± 7.76
Postoperative IPSS score	9.17 ± 7.36	8.09 ± 5.98
Preoperative QOL index	4.08 ± 0.84	5.18 ± 0.87
Postoperative QOL index	2.48 ± 1.54	1.74 ± 1.42*
Preoperative MFR (mL s <sup>-1</sup> )	8.82 ± 3.98	8.91 ± 3.99
Postoperative MFR (mL s <sup>-1</sup> )	16.19 ± 7.49	16.64 ± 5.25

Compared between the two groups, \* $P < 0.001$ .

and one deep vein thrombosis of lower limb, with no mortality and reoperation. **Conclusion:** Composition of patients with BPH has been significantly changed in recent ten years, the tendency of aging is presented and most cases receive surgery only when severe complications associated with BPH appears. TUVRP is an effective therapeutic method for BPH and is suitable for popularized application. The surgeon's skill will be continuously improved as the performed cases increase; as a result, better efficacy can be gained.

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## C2-23

### Correlation of chronic prostatitis with psychological analysis

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**Aim:** Study the psychological status of chronic prostatitis patients, and the correlation of mental symptoms with the chronic prostatitis (CP). **Methods:** A total of 600 CP patients were randomly sampling in our hospital. Control group had 40 examples, for 18–46-year-old normal male volunteers. The routine urine test was normal, NIH-CPSI was smaller than for 5 min. Included in questionnaire were: 1) age, occupation, course of disease, education background, body status; 2) score of the National Institute of the Health Chronic Prostatitis Symptom Index (NIH-CPSI); 3) score of the International Index of Erectile Function-5 (IIEF-5); 4) grade of hard of Erectile Function; 5) Symptom Checklist 90 (SCL-90). Compared with various symptoms relevance, counts the different formation rate. The data were analyzed by SPSS 10.0. **Results:** Of the total 600 patients, there are significant relation of NIH-CPSI and mental anxiety symptoms ( $P < 0.01$ ), the NIH-CPSI and mental depression symptoms ( $P < 0.05$ ). There also are significant relation between ED and mental anxiety symptoms ( $P < 0.05$ ), the ED and mental depression symptoms ( $P < 0.01$ ). **Conclusion:** NIH-CPSI of CP patients has relations with mental symptom gradings (anxiety and depression), this indicates that there may exist mutual influence between CP and mental symptoms (anxiety and depression).

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## C2-24

### Clinical study on sexual function of men with symptomatic benign prostatic hyperplasia accompanied by chronic prostatitis

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**Aim:** To evaluate the prevalence of sexual dysfunction in the population of men with benign prostatic hyperplasia (BPH) accompanied by chronic prostatitis (CP)-BPH/CP and lower urinary tract symptoms (LUTS), and to study the relationships

among the following parameters, sexual dysfunction, urinary symptom, age, WBC and lecithin in expressed prostatic secretion (EPS), international prostatic symptoms (IPSS), maximum urinary flow rate ( $Q_{max}$ ), total prostate volume (TPV), quality of life (QoL), postvoid residual urine (PVR) and chronic prostatitis symptoms index (NIH-CPSI). **Methods:** A total of 160 men with symptomatic BPH/CP were investigated using the IPSS, WBC, lecithin in EPS, international index of Erectile Function-5 (IIEF-5), ejaculatory function score, libido and the measurement of  $Q_{max}$ , TPV, QoL, PVR, NIH-CPSI. The correlation and regression analysis was used to determine the relationships among the variables. Statistical analysis was performed between the symptomatic parameters of BPH and sexual function. **Results:** The mean age of the patients was  $65 \pm 6.2$  years, the mean course of BPH/CP was  $8.02 \pm 3.11$  years, the mean IPSS score was  $21.03 \pm 5.35$ , the mean IIEF-5 score was  $15.23 \pm 3.75$ , the mean libido score was  $2.45 \pm 0.87$  and mean ejaculatory function score was  $2.15 \pm 0.67$ . The mean NIH-CPSI score was  $20.69 \pm 7.06$ . There were 135 cases of erectile dysfunction (84.25%), 128 cases of poor sexual drive (80.06%) and 112 cases of poor ejaculation (70.06%). Significant correlation existed among age  $Q_{max}$ , QoL, PVR, TPV and IIEF-5-scores ( $P = 0.002$ ), ejaculatory function ( $P < 0.001$ ), libido score ( $P = 0.002$ ). The statistical significance of the correlation between the quality of life and the patients' ill course, WBC and lecithin in EPS was not remarkable. **Conclusion:** Age and CPSI are the main risk factors of sexual function. There are high incidences of sexual dysfunction in patients with BPH accompanied by CP. The degree of ED, libido, ejaculatory function is related to the degree of LUTS, TPV, and PUV. The decline in the quality of life and sexual life caused by BPH/CP is of certainty and significance.

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## C2-26

### Influence of health education on the life quality of sequela after vasectomy

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**Aim:** To discuss the influence of health education on life quality of sequela after vasectomy. **Methods:** Twenty-eight patients were divided into two groups with randomized allocation, only the experimental group combined with health education. Through the questionnaire investigation, two groups of patients with knowledge about reproductive health and life quality situation were compared. **Results:** The experimental group was obviously better than the control group in knowledge of reproductive health ( $P < 0.01$ ). They were also better in constitution, mental function and life quality ( $P < 0.01$ ). **Conclusion:** Health education can improve obviously sequela self-healthcare consciousness and life quality.

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## C3-03

### Sperm banking for timely reproductive insurance: multiple centers' investigation in China

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**Aim:** Assisted reproduction techniques (ART) especially the introduction of ICSI has totally changed the reproductive prospects for men who suffer from diseases (such as cancer patients, severe oligozoospermia, dry ejaculation, azoospermia, etc.) or for other reasons that the semen need to be stored for future use. The study intended to explore the current condition of sperm banking for the purpose of fertility salvage and the use rate of cryopreserved sperm according to different categories. **Methods:** The retrospective study was based on the data collected by three human sperm banks and one reproduction center of four different areas in China. There were total 1 548 males who had their semen cryopreserved in these health organizations from January, 2003 to December, 2008. The investigation included urogenital history, indication for cryopreservation, semen parameters and use of semen or not. **Results:** The data showed that there were 1 584 males banking their semen during the past 6 years in the health organizations above. 1.9% (30/1 548) of the cryopreserved semen were from cancer patients, among which testicular tumor and hodgkin's disease the most common cancers; 88.9% (1 374/1 548) banking their semen for ART because of anxiety about dry ejaculation or unstable semen quality on the day of oocytes retrieval; 8.6% (134/1 548) had andrologic diseases (anejaculation, severe oligozoospermia and obstructive azoospermia were the common reasons). The total use rate of cryopreserved semen was 22.67% (351/1 548), among these, cancer group was 6.7% (2/30), the ART group was 23.2% (319/1 374), andrologic diseases group was 23.1% (31/134). **Conclusion:** Cancer, ART and andrologic diseases were the main reasons of sperm banking. But according to the large number of young patients with cancer in China, the number of those who had the semen cryopreserved was too low. ART males banking their sperm beforehand could lessen the anxiety and help to collect semen on the day of oocyte retrieval. So sperm cryopreservation should be recommended to all young patients with cancer. As to infertile couples, sperm banking could help to assure the success of ART. For those who suffer from andrologic diseases (anejaculation and severe oligozoospermia), sperm banking could actually preserve their reproductive potential and make fatherhood realized with the help of ART.

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### C3-04

#### Polymorphisms of nucleotide-excision repair genes in relation to sperm DNA fragmentation and male infertility

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**Aim:** Nucleotide-excision repair is believed to play an important role in spermatogenesis, and its dysfunction might be related to male infertility. To explore the biomarker of genetic susceptibility for male infertility, we investigated the associations between the variants in NER genes and sperm DNA damage as well as the semen quality. **Methods:** Genotypes were determined by PCR-RFLP, and sperm DNA damage was evaluated by TUNEL assay. **Results:** Based on a case-only study consisting of 620 infertile men, we found a significant association of XPA(-4) polymorphism with sperm DNA fragmentation. Individuals carrying the XPA(-4) A allele showed higher sperm DNA fragmentation and lower sperm concentration compared with the G allele carriers. Furthermore, we explored the contribution of this polymorphism to the risk of male infertility in a case-control study including 620 patients and 385 controls, which revealed a 1.52-fold (95% CI, 1.08–2.02) risk for developing male infertility of the XPA(-4) A carriers compared with noncarriers. By luciferase assay we verified that the XPA(-4) A allele had a lower transcriptional activity than the XPA(-4) G allele. **Conclusion:** All these data provided first evidence that genetic polymorphisms in XPA promoter altered its transcriptional activity and, thus, might be a marker for genetic susceptibility to sperm DNA damage and male infertility in Chinese populations.

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### C3-05

#### Study on correlation between seminal plasma elastase and semen major parameters and sperm function

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**Aim:** To study the relationship between seminal plasma elastase and semen major parameters. **Methods:** The level of seminal plasma elastase was detected by Enzyme-linked immunosorbent assay (ELISA). Semen major parameters, such as abnormal morphological sperm rate, sperm acrosin reaction, AsAb and *Ureaplasma urealyticum* (UU) infection were measured and analyzed in 209 infertile men according to WHO laboratory manual about human semen analysis. **Results:** Forty-three cases of 209 male infertile men whose level of seminal plasma elastase was equal to or higher than 290 ng mL<sup>-1</sup>, set up into inflammation group; the other (< 290 ng mL<sup>-1</sup>) 166 cases were set up into non-inflammation group. Semen concentration, motility, a + b grade motility, and sperm acrosin reaction rate in inflammation group were lower than those of non-inflammation group ( $P < 0.05$ ); Higher abnormal morphological sperm, UU infection rate and AsAb positive rate were observed in inflammation group ( $P < 0.05$ ); Sperm volume, pH value and liquefying

time were not significantly different between inflammation and non-inflammation group ( $P > 0.05$ ). **Conclusion:** The level of seminal plasma elastase is associated with sperm quality, which can be considered one of the important reasons resulting in male infertility.

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### C3-06

#### The influence of cigarette smoking on human sperm quality and DNA integrity

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**Aim:** To evaluate the effects of cigarette smoking on human sperm quality and DNA integrity. **Methods:** The study group, consisting of infertile male smokers, was evaluated from 2008 to 2009 ( $n = 291$ ) against the control group, consisting of non-smoking infertile men ( $n = 401$ ) during the same time span. The patients were 22 to 45 years old. Four hundred and twenty-nine were primary infertile and 263 were secondary infertile. Sperm parameters such as sperm concentration, motility, viability and morphology were measured according to the WHO criteria. Sperm DNA integrity was analyzed with sperm chromatin structure assay (SCSA) and flow cytometry analysis. The degrees of DNA fragmentation and spermatozoa immaturation were expressed by DNA fragmentation index (DFI) and high DNA stainability (HDS) respectively. **Results:** Compared with others, the patients who consumed  $\geq 20$  cigarettes/day or smoked  $\geq 10$  years ejaculated decreased volume (2.16 mL vs. 2.67 mL, 2.35 mL vs. 2.62 mL,  $P < 0.05$ ) and their sperm parameters dropped, including lower progressive motility (33.8% vs. 40.8%, 32.4% vs. 41.5%,  $P < 0.05$ ) and higher malformation rate (79.8% vs. 64.9%, 75.9% vs. 68.5%,  $P < 0.05$ ). Spermatozoa from Smokers had a significantly higher DFI (12.5%  $\pm$  9.2% vs. 9.6%  $\pm$  8.4%,  $P < 0.05$ ) and HDS (14.6%  $\pm$  11.8% vs. 10.8%  $\pm$  9.1%,  $P < 0.05$ ) than those of non-smokers. Furthermore, DFI was negatively correlated to semen concentration ( $r = -0.26$ ,  $P < 0.05$ ) and HDS was negatively correlated to progressive motility ( $r = -0.18$ ,  $P < 0.05$ ); both DFI and HDS are positively correlated to sperm malformation rate ( $r = 0.31$  and  $r = 0.39$ ,  $P < 0.05$ ). **Conclusion:** Cigarette smoking may play deleterious roles on semen volume and sperm parameters including motility, vitality, morphology and nuclear integrity.

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### C3-07

#### Effect of *Bcl-2* genetic variants on spermatogenesis failure in a Chinese Han population

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**Background:** Apoptosis is very common during various stages of mammalian germ cell, and the *Bcl-2* gene is one of the most important apoptotic regulators. Although its genetic variants were reported to convert its normal functions and be involved in cancers and autoimmune diseases, little information is obtained on *Bcl-2* polymorphisms in male spermatogenesis. **Aim:** To investigate the critical role of *Bcl-2* polymorphisms in human spermatogenesis and their mechanisms. **Methods:** The SNPs in coding regions of *Bcl-2* gene was scrutinized in a hospital-based case-control study including 274 infertile patients with idiopathic azoospermia or severe oligozoospermia and 183 fertile controls, and their potential risk in spermatogenesis was analyzed with the multivariate logistic regression model. In addition, the HeLa cells were used to establish the transfectant cells expressing wild-type or variant *Bcl-2* protein, and then the difference was compared in cytotoxicity and apoptosis induced by paclitaxel, and the mechanisms were also discussed. **Results:** Three SNPs were found in exon 2, including rs1801018: A > G, rs1800477: G > A, and rs61733416: C > T which was the first report in Chinese-Han population. The frequency of rs1800477: G > A (GA + AA) in azoospermic patients was significantly lower than that in fertile controls as well as in the oligozoospermic patients ( $P = 0.01$ ). It appeared to be correlated with low risk of idiopathic azoospermia (adjusted OR = 0.45; 95%CI = 0.23–0.89). In addition, the HeLa cells expressing the *Bcl-2* Ala43Thr variant, similar to the control cells, were more sensitive to paclitaxel-induced cytotoxicity and apoptosis than the cells expressing wild-type *Bcl-2*. Consistently, the cleaved-PARP and p-Bcl-2 protein were subsequently increased after the paclitaxel treatment, which were also predicted with bioinformatics analysis. **Conclusion:** Due to the decreased anti-apoptotic function in the Ala43Thr variant of *Bcl-2*, it appeared to confer resistance to azoospermia in Chinese Han population, which might provide some helpful evidences of susceptibility to azoospermia.

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### C3-08

#### Study of sperm sorting using microfluidic chip

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**Aim:** To study a new sorting method for motile sperm using passively polydimethylsiloxane (PDMS) microfluidic chip. **Methods:** Forty sperm specimens were sorted by the homemade polydimethylsiloxane (PDMS) microfluidic chip device, and the semen parameters (sperm motility and morphology) were compared before and after the experiment. Meanwhile, the sperm motility and morphology in these sperm samples were analyzed by both microfluidic chip and density gradient centrifugal separation. Meanwhile, thirty motile sperm samples were prepared by density gradient centrifugal separation in order to assess sperm motility using PDMS microfluidic chip. **Results:** The experimental results showed there were significant

differences between pre-sorting and post-sorting in terms of sperm motility and morphology using microfluidic chip. Sperm motility increased from  $29.4\% \pm 8.1\%$  to  $94.2\% \pm 3.3\%$  ( $P < 0.001$ ), the percentage of morphologically normal sperm increased from  $10.5\% \pm 8.0\%$  to  $30.0\% \pm 5.5\%$  ( $P < 0.01$ ). Comparing with density gradient centrifugal separation method, sperm motility by microfluidic chip sorting device significantly increased ( $93.5\% \pm 2.5\%$  /  $63.3\% \pm 13.2\%$ ,  $P < 0.01$ ), but in terms of sperm morphology there was no significant difference ( $30.0\% \pm 5.5\%$  /  $28.2\% \pm 11.3\%$ ,  $P > 0.05$ ). Meanwhile, there was no effect on sperm motility between spermatozoa with and without exposure to PDMS ( $P > 0.05$ ). **Conclusion:** Microfluidic chip sorting device in the selection of sperm can obtain better qualitative sperm, which is a simple, fast, causing small injury to sperm. The microfluidic chip sorting device provides a novel method for sorting motile, morphologically normal spermatozoa from semen without centrifugation. It is also better than the density gradient centrifugation. This technology may be useful in sorting motile spermatozoa, insemination and *in vitro* fertilization to improve the birth health quality.

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### C3-09

#### Determination fructose and $\alpha$ -glycosidase of seminal plasma in fifty patients with azoospermia, oligospermia or asthenospermia

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**Aim:** To assess the fructose and  $\alpha$ -glycosidase of seminal plasma in infertile male with azoospermia, oligospermia or asthenospermia. **Methods:** The seminal plasma was kept at  $-20^{\circ}\text{C}$  after centrifugation under 1 000 r/m. The fructose and  $\alpha$ -glycosidase were assessed by colorimetric method within one week. **Results:** The data of fructose and  $\alpha$ -glycosidase of semen plasma of the three groups were shown as follows (Table 2). **Conclusion:** Our results demonstrated that seminal plasma fructose and  $\alpha$ -glycosidase are valuable for the diagnosis and treatment of the infertile male with azoospermia, oligospermia or asthenospermia, especially for the diagnosis of azoospermia and oligospermia. The rates of abnormality of seminal plasma fructose and  $\alpha$ -glycosidase were 93.3% and 81.8% in azoospermia and oligospermia respectively (Table 3). We concluded that this assessment could replace vasography in the diagnosis of simple azoospermia due to obstruction and dysgenesis of epididymis or deferent duct. For the infertile males with azoospermia, low level of seminal plasma fructose and  $\alpha$ -glycosidase indicates dysfunction of epididymis or vesicular seminalis, which impeded the production and maturation of sperm. The high rate of lower seminal plasma fructose in male with asthenospermia indicates that lower fructose may be one reason for asthenospermia, which would be valuable for the treatment.

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### C3-11

#### The association between sperm DNA damage and outcome of *in vitro* fertilization

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**Aim:** To study the effects of sperm DNA damage on fertilization rate, embryo development and pregnancy outcome of *in vitro* fertilization (IVF). **Methods:** Semen samples were obtained from males who underwent IVF ( $n = 52$ ) and adult healthy fertile men ( $n = 24$ ) as control group. The level of sperm DNA damage was determined by improved sperm chromatin dispersion (SCD) test. The relationships among the level of sperm DNA damage, semen parameters, fertilization rate, cleavage rate, good embryo rate and pregnancy rate were analyzed. **Results:** The IVF group had significantly higher DFI than the control group ( $12.8\%$  vs.  $5.4\%$ ,  $P < 0.05$ ). No correlation between conventional semen parameters (sperm concentration, motility and morphology) and sperm DNA damage ( $P > 0.05$ ) was observed. The patients who underwent IVF were divided into group I (DFI  $< 15\%$ ) and group II (DFI  $\geq 15\%$ ), the group I had significantly higher fertilization rate and cleavage rate than group II ( $69.22 \pm 21.17\%$  vs.  $51.42 \pm 27.34\%$ ,  $P < 0.05$ ). The pregnancy group had significantly lower DFI than no pregnancy group ( $12.6\%$  vs.  $13\%$ ,  $P < 0.05$ ). By using Binary logistic regression analysis, the probability of pregnancy decreased 8.1% when DFI increased 1% (OR = 0.919,  $P = 0.034$ ,  $\beta = -0.085$ ). **Conclusion:** The sperm DNA damage was more sensitive than traditional semen analysis when used for estimating the function of sperm and can be used as a predictor for male fertility.

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### C3-12

#### Semen characteristics of 5 704 subfertile men in Guangxi region

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Table 2. Disposition of seminal plasma fructose and  $\alpha$ -glycosidase.

<i>n</i>	Low fruc. (%)	Low $\alpha$ -gly. (%)	Low fruc. and $\alpha$ -gly. (%)
Azoo.(15)	2 (13.3)		6 (40.0)
Oligo.(22)	2 (9.1)	6 (27.2)	5 (22.7)
Astheno.(13)	7 (53.8)	2 (15.4)	

Table 3. The abnormal ratio of seminal plasma fructose and  $\alpha$ -glycosidase

<i>n</i>	Low fructose (%)	Low $\alpha$ -glycosidase (%)
Azoo. (15)	8 (53.3)	6 (40.0)
Oligo. (22)	7 (31.8)	11 (50)
Astheno. (13)	7(53.8)	2 (15.4)



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**Aim:** To analyze semen characteristics of 5 704 subfertile men who attended to our infertility clinic in Reproductive Medical Centre, Second People's Hospital, Nanning, China between January 2006 and December 2008. **Methods:** Conventional semen analysis was performed according to methods of 2001 World Health Organization manual. All men diagnosed with azoospermia were confirmed by examining at least two separate ejaculates. Semen characteristics were classified as follows: azoospermia (no sperm detected after centrifugation of semen), severe oligospermia (sperm concentration  $< 5 \times 10^6$  mL<sup>-1</sup>), oligospermia (sperm concentration  $5\text{--}19 \times 10^6$  mL<sup>-1</sup>), asthenospermia (sperm motility grade a + b < 25%), teratospermia (normal sperm morphology < 15%) and severe teratospermia (normal sperm morphology < 5%). For non-obstructive azoospermic men, testicular biopsy was performed to determine presence of mature sperm in testis. **Results:** Mean age of these men was 32.8 (range 21–46) years old. Semen characteristics of all 5 704 subfertile men were summarized as follows: azoospermia 33% ( $n = 1\ 893$ ), severe oligospermia 3.5% ( $n = 200$ ), oligospermia 17.3% ( $n = 985$ ), asthenospermia 36.7% ( $n = 2\ 093$ ), teratospermia 54% (1 359 of 2 485 which morphology was performed) and severe teratospermia 3.5% (87 of 2 485). Men with normal semen characteristics were only 12.9% ( $n = 733$ ). Among 1 893 azoospermic men, over 83% of them were obstructive, about 50% of which were able to find sperm in testicular biopsy, and non-obstructive azoospermia whereas other 8% had mixed causes. When sperm characteristics compared between each year from 2006 to 2008, both the mean sperm concentration and normal sperm morphology progressively decreased by 29% to 50%. **Conclusion:** Besides azoospermic, only very small proportion of men with severe oligospermia (3.5%), oligospermia (17.3%) and severe teratospermia (3.5%) may be diagnosed as male infertility associated with poor sperm quality. The majority of men have mild to moderately reduced semen quality against 13% of them with normal semen parameters. Therefore, routine semen analysis can not make clear diagnosis of male infertility. Thus, further specific sperm function assays are necessary to improve clinical diagnosis of male infertility.

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### C3-13

#### Determination and meaning of main population in sperm during treating oligo-asteno-teratozoospermia syndrome with self-designed Chinese traditional and western medicine

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**Aim:** To evaluate the determination and meaning of main population in sperm during treating oligo-asteno-teratozoospermia syndrome with self-designed Chinese traditional and western medicine. **Methods:** The chromatin structure of sperm samples by acridine orange staining from

30 infertile male patients were analyzed using fluorescence microscope, flow cytometry and the sperm routine detection methods during treating oligo-asteno-teratozoospermia syndrome with self-designed Chinese traditional and western medicine. **Results:** Sperm chromatin structure assay using flow cytometry showed that the difference in master-group sperm signal were significant ( $P < 0.01$ ). Sperm using routine detection methods showed that the difference in activity except for activity rate and spermatid density were significant ( $P < 0.01$ ). **Conclusion:** The treatment of oligo-asteno-teratozoospermia syndrome with self-designed Chinese traditional and western medicine is efficacious. The determination of main population in sperm is meaningful to the evaluation system of male infertility therapy.

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### C3-14

#### The outcome of ICSI with frozen-thawed sperm obtained by PESA on azoospermia patients

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**Aim:** Since the report by Temple-Smith *et al.* (1985) on the use of microsurgically aspirated epididymal spermatozoa in *in vitro* fertilization (IVF), an alternative fertility treatment for patients suffering from infertility has become possible. With the introduction of intracytoplasmic sperm injection (ICSI), high fertilization and pregnancy rates have been obtained with epididymal spermatozoa (Tournaye *et al.*, 1994). However, the question arises as to whether freezing epididymal spermatozoa may be considered generally useful. In the study, we compared the results of ICSI with either fresh or frozen-thawed epididymal spermatozoa in a large retrospective case series. **Methods:** Sperm from the infertile males by PESA were frozen with conventional freezing method. The cryopreservation medium contained glycerol at a concentration of 15%, egg yolk, citrate, glycine, glucose and antibiotics. After equilibration in a 37°C water bath for 10 min, the mixture was aspirated into 2.0 mL cryogenic vials. The vials were then rapidly frozen in liquid nitrogen vapor by placing them horizontally 8–10 cm above the liquid nitrogen surface for 10 min (room temperature to  $-80^\circ\text{C}$ ; rate  $-10^\circ\text{C min}^{-1}$ ). The vials were then plunged directly into the liquid nitrogen ( $-196^\circ\text{C}$ ). For thawing, the vials were removed from the liquid nitrogen and placed at 37°C for 10 min. After ICSI process, the fertilized oocytes were cultured *in vitro* for 72 h and the high quality embryo were transferred to the wives. Fertilization rate, implantation rate, clinical pregnancy rate were compared between two groups, the 54 cycles with frozen-thawed sperm and the 282 cycles with fresh PESA sperm as control. **Results:** Although the freezing–thawing process may impair the quality, especially the motility of the epididymal spermatozoa, we observed no significant differences in quality between fresh and frozen–thawed epididymal spermatozoa. Recovering rates of frozen sperm from PESA was 65.9%. No significant differences were found in rate of fertilization, cleavage rate, high-qualified

embryo, clinical pregnancy between two groups. (72.4% vs. 74%, 98.1% vs. 96.3%, 51.7% vs. 46.8%, 42.6% vs. 39.0%,  $P > 0.05$ ). Neither vital pregnant complications nor neonate malformations were found in both groups. **Conclusion:** PESA may also be proposed as the primary sperm retrieval technique in patients with obstructive azoospermia. Patients wishing to store epididymal spermatozoa may be good PESA candidates because PESA was less invasive, less expensive than MESA. We found the embryo development and pregnant outcome were not effected by using frozen-thawed sperm obtained by PESA.

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### C3-17

#### The effect of sperm DNA fragmentation on the rate of fertilization, cleavage and high quality embryo in conventional IVF

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**Background:** Routine semen analysis provides useful information concerning sperm production by the testis, sperm motility and viability *etc.* Although the information revealed by this assay is obviously useful for the initial evaluation of the infertile male, it is not a functional test of the spermatozoon. As a result, it could not properly predict the outcome of IVF treatments. On the contrary, sperm genome quality has been emphasized for several years as playing an important role in early embryogenesis, and there are several methods to test sperm DNA damage. However, the relationship between sperm DNA damage and the outcome of IVF remains a controversy. **Aim:** In this study, we aimed to investigate the effect of sperm DNA fragmentation on the rate of fertilization, cleavage and high quality embryo in conventional IVF, and further to evaluate the necessity of testing sperm DNA damage in IVF treatments. **Methods:** Ninety-eight couples undergoing conventional IVF from June 2007 to July 2008 in Reproductive Center of Ruijin Hospital were enrolled. The parameters of sperm routine test were normal (sperm density  $\geq 20 \times 10^6 \text{ mL}^{-1}$ , grade a  $\geq 25\%$ , or grade a + b  $\geq 50\%$ ). Sperm DNA damage was measured by the sperm chromatin dispersion (SCD) assay. SCD of semen aliquot was taken from ejaculate and used for IVF. All couples were classified into two groups regarding to the DNA fractionation index (DFI) threshold value: group I  $< 15\%$  and group II  $\geq 15\%$ . Fertility, cleavage and high quality embryo rates were compared between the two groups. **Results:** There was no statistical difference in the mean age, infertility years, basic hormone level, estrogen level of HCG day between the high- and low-DFI group. In group I, median DFI was 13.26%; and was 20.48% in group II ( $P < 0.05$ ). In group I, fertility and cleavage rates were a little higher than those in group II, but there were no significant difference between the two groups ( $P > 0.05$ ). However, the high quality embryo rate was statistically higher in group I than that in group II ( $P < 0.05$ ). **Conclusion:** Low fertility rate and/or fertility failure are the puzzling problem of conventional IVF.

Clinical date indicates that routine sperm test or morphology analysis could not evaluate the fertility ability thoroughly. DNA fragmentation index (DFI) was hypothesized to be one of the useful parameters. However, Our results suggest that although DFI can not provide independent information about fertilization, cleavage, the relationship between DFI and high quality embryo rate provides preliminary evidence that sperm with high levels of DNA fragmentation ( $\geq 15\%$ ) is at greater risk for low quality embryo. The potential adverse effect of sperm DNA damage on the quality of embryo should be a concern. We consider it is necessary to test sperm DNA damage when evaluating an infertile male.

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### C3-19

#### The spermatogenesis process rather than sperm retrieval approaches play an important role in the clinical outcome of ICSI for azoospermic patients

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**Aim:** Previous and current observations show a discrepancy on the clinical outcome of ICSI with testicular and epididymal sperm for azoospermic patients. In present study, we investigated the clinical outcome of ICSI from unequal spermatogenesis progress and different surgically- retrieved sperm, tried to determine whether the different surgical sperm retrieval approaches impact the clinical outcome of ICSI for azoospermic patients. **Methods:** This retrospective study included a series of 114 cycles from 92 obstructive azoospermic (OA) patients with normal spermatogenesis and another 42 cycles of 73 nonobstructive azoospermic (NOA) patients with defective spermatogenesis. Before ICSI performed, all these patients' spermatogenesis situation was confirmed by testicular tissue pathology examination. NOA group included hypospermatogenesis and spermatogenesis maturation arrest. OA group was divided into two groups according to the spermatozoa retrieval methods. 53 percutaneous spermatozoa aspiration (PESA) cycles and 64 testicular spermatozoa aspiration (TESA) cycles. The reasons why TESA were used in those OA patients were the absence of epididymal spermatozoa or punctate mixed with many leukocytes after PESA. All the NOA patients were treated with TESA for ICSI. The fertilization rate, cleavage rate, clinical pregnancy rate and miscarriage rate were analyzed between each group. Mean values were compared by Student's *t*-test, and proportions were compared by the chi-square or Fisher exact test, depending on the sample size.  $P < 0.05$  was considered statistically significant. **Results:** Fertilization and clinical pregnancy rates were significantly higher in OA group than that in NOA-TESA group (64.1% vs. 44.9%, 37.5% vs. 23.8%,  $P < 0.05$ ), neglected



different surgical sperm retrieval approaches, whereas the miscarriage rate was significantly higher for NOA-TESA group. However, no statistical difference occurred when compared the fertilization, clinical pregnancy and miscarriage rates between OA-PESA group and OA-TESA group (65.4%, 39.2%, 15.0% vs. 63.1%, 36.1%, 18.2%,  $P > 0.05$ ). **Conclusion:** This study demonstrated that for the ICSI clinical outcomes there was no significant difference between testicular sperm and epididymal sperm group for obstructive azoospermic patients. However, it also suggested that it was spermatogenesis process rather than spermatozoa retrieval approaches affect the clinical outcome of ICSI in azoospermic patients.

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### C3-20

#### Comparison of the outcome and safety of ICSI by different origin of sperm

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**Aim:** To study the outcome and safety of ICSI by different quality and sources of the sperm. **Methods:** A total of 1 275 patients who underwent 1 295 ICSI cycles were evaluated retrospectively. Patients were divided into six groups according to the quality and sources of sperm. Group 1 is composed of 89 cycles of ICIS in 88 patients with relative normal semen parameters. Group 2 is of 209 cycles in 207 patients with mild oligo-astheno-teratozoospermia (OAT). Group 3 is of 672 cycles in 667 patients with severe OAT (SOAT). Group 4 is of 108 cycles in 103 patients with extremely severe OAT (ESOAT). Group 5 is of 177 cycles in 172 patients using testicular or epididymal sperm. Group 6 is of 42 cycles in 41 patients using frozen-thawed sperms. **Results:** 1) The fertilization rates in the six groups were 75.5%, 74.6%, 72.7%, 71.3%, 66.0% and 74.2%, respectively. It gradually reduced with decreased semen quality, and it was significantly lower in Group 4 (71.3%) and Group 5 (66.0%) ( $P < 0.05$ ). 2) The cleavage rates were 98.1%, 98.5%, 97.8%, 97.4%, 95.8% and 99.5% in the six groups respectively. It was significantly lower in Group 5 (95.8%) ( $P < 0.05$ ). 3) The good quality embryo rates were no significantly difference in all six groups. 4) The clinical pregnancy rates were 39.5%, 29.7%, 37.3%, 42.3%, 38.7% and 46.2% respectively, and the implantation rates were 20.1%, 16.9%, 20.7%, 25.4%, 26.1% and 29.2%, respectively. These were significantly lower in Group 2 ( $P < 0.05$ ), and there were no significantly differences in the other 5 groups ( $P > 0.05$ ). 5) The rates of chemical pregnancy (4.7%, 1.6%, 1.4%, 3.1%, 1.2% and 5.1%, respectively), multiple pregnancy (26.5%, 29.8%, 28.8%, 26.8%, 43.8% and 38.9%, respectively), miscarriage (14.7%, 17.9%, 16.2%, 15.4%, 9.4% and 5.6%, respectively), live-birth (25.6%, 22.4%, 27.7%, 31.9%, 33.1% and 41.0%, respectively), and premature birth (31.8%, 23.3%, 24.0%, 25.8%, 27.8% and 25.0%, respectively), as well as weeks of premature birth ( $35.3 \pm 0.8$  W,  $33.0 \pm 3.9$  W,  $34.0 \pm 2.2$

W,  $34.5 \pm 1.7$  W,  $34.4 \pm 2.0$  W and  $34.5 \pm 2.4$  W, respectively), weight of newborns ( $3\ 071.4 \pm 550.8$  g,  $2\ 995.3 \pm 692.4$  g,  $2\ 875.3 \pm 692.3$  g,  $2\ 957.9 \pm 656.7$  g,  $2\ 792.2 \pm 577.9$  g, and  $2\ 838.1 \pm 724.4$  g, respectively), low birth weight rate (17.9%, 14.8%, 25.4%, 17.9%, 22.1% and 23.8%, respectively) and sex ratios (males/males + females) (42.9, 52.8, 46.1, 38.5, 51.9 and 52.4, respectively) were no significantly difference in all 6 groups ( $P > 0.05$ ). 6) There is one boy with hypospadias in Group2; and there are three boys with congenital cardiopathy, hypospadias, hexadactylism respectively, and one girl with megacolon in Group 3. **Conclusion:** Different origin and different parameter of sperm can mainly affect the fertilization and cleavage, with no effect on the implantation, miscarriage or the newborns. The assessment of malformation of offspring deserves increased sample scale to further study.

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### C3-21

#### Experience of sperm retrieval in non-obstructive azoospermic men with maturation arrest

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**Aim:** Infertile men with maturation arrest are a unique group of non-obstructive azoospermia (NOA) in whom normal testicular volume and FHS level might be found. This study was conducted to retrospectively review the clinical characteristics of NOA men with maturation arrest and the outcome of sperm retrieval. **Methods:** A total of 24 men with NOA and maturation arrest were enrolled. These patients were identified from 456 men with azoospermia underwent sperm retrieval between November 1996 and October 2008. These 24 patients were characterized based on their clinical history, testicular volume, hormone profiles, testicular histopathology, chromosome karyotype, Y chromosome deletion test and result of sperm retrieval. **Results:** Of the 24 patients, seven were diagnosed as maturation arrest at the spermatid stage (post-meiotic arrest) and the other 17 patients were diagnosed as maturation arrest at the spermatocyte stage (meiotic arrest). Genetic anomalies were found in one out of seven (14.3%, 47, XXY) patients with post-meiotic arrest and six out of 17 of patients (35.3%, four patients AZF b + c deletion, one patient with AZFc deletion and one patient with inversion of chromosome 11) with meiotic arrest. Eleven patients presented with normal testicular volume and normal serum FSH values. No significant difference in testicular volume and hormone profiles between post-meiotic arrest and meiotic arrest patients was noted. None of all these patients had successful sperm retrieval. **Conclusion:** Maturation arrest is a definable subgroup of NOA men accounting for 5.3% of our azoospermic population. They have a higher incidence of genetic anomalies. Despite having normal testicular volume and serum FSH level in a subgroup of these patients, sperm retrieval may be difficult.

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**C3-22****The phenomenon and solutions of non-specific results of MAR test with high viscosity semen**

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**Aim:** To study the influence of semen solution of high viscosity on mixed agglutination reaction (MAR) test. **Methods:** While specific viscosities of semen samples were reduced gradually in high viscosity group ( $11.35 \pm 2.00$ ) and normal viscosity group ( $6.01 \pm 0.81$ ), the concomitant changes were traced of antisperm antibodies detected by MAR test. High viscosity seminal plasma was added into 31 normal viscosity semen samples to make up high viscosity semen specimens. The latter were treated by setting up an assay blank control, washing spermatozoa and pipetting fibrinolysin into specimens, respectively. The MAR test results of post-treated high viscosity samples and normal viscosity samples where high viscosity seminal plasma were not added were compared. **Results:** The results of MAR test were associated with semen viscosity in high viscosity group ( $r = 0.912$ ,  $P < 0.001$ ). However, no such correlation was detected in normal viscosity group ( $r = 0.120$ ,  $P > 0.05$ ). In general, MAR results were significantly higher in high viscosity group than that in normal viscosity group ( $P < 0.001$ ), but the MAR results were the same between the two groups when the viscosity of high viscosity group decreased to normal ( $P > 0.05$ ). Compared with the homogeneous sperms which high viscosity seminal plasma were not inserted, MAR results of post-treated high viscosity samples were not significantly different by pipetting fibrinolysin into high viscosity semen or by setting up an assay blank control ( $P > 0.05$ ). **Conclusion:** Semen specimen of high viscosity can result in false positive consequence of MAR test. This influence can be removed by pipetting fibrinolysin into high viscosity semen or setting up an assay blank control.

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Section II

Poster Presentations

**Pt1-01****Male infertility**

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With 50% infertility wholly or partially attributable to male factor, it is incumbent on the family physician to be knowledgeable in this field. After the initial semen analysis it is the responsibility of doctor to determine whether specialist consultation is required or not. There is still a great deal of confusion concerning what factors contribute for not forming normal semen. It is now established that 60% of pregnancies decrease when the sperm count drops below  $20 \times 10^6 \text{ mL}^{-1}$ . The technique for sperm count has an inherent error of approximately 20% even performed by expert technician. It is advised to repeat the count on three successive specimen in a same laboratory if it is below  $30 \times 10^6 \text{ mL}^{-1}$ .

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**Pt1-02****Pregnant and perinatal outcomes after the treatment of *in vitro* maturation**

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**Aim:** To observe the pregnancy outcome of oocytes matured *in vitro*. **Methods:** The data of 35 cycles of *in vitro* maturation (IVM), which were accomplished in our ART center from August 2003 to December 2007, were analyzed retrospectively. **Results:** Abortion rate of group IVM was significantly different from that of group IVF/ICSI, and new born teratogenesis rate was not significantly different from that of group IVF/ICSI. **Conclusion:** IVM of human oocytes is going to become an alternative assisted reproductive technology.

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**Pt1-03****Multifetal pregnancy reduction after artificial reproductive technology: a single-center experience**Liu-Hong Cai<sup>1</sup>\*, Can-Quan Zhou<sup>2</sup>, Bin Zhang<sup>1</sup>, Hui Lin<sup>1</sup>, Yu-Bin Li<sup>2</sup><sup>1</sup>Department of Infertility and Sexual Medicine, Third Affiliated Hospital, Sun Yet-Sen University, Guangzhou 510630, China<sup>2</sup>Center for Reproductive Medicine, First Affiliated Hospital, Sun Yet-Sen University, Guangzhou 510089, China

**Aim:** Retrospective analysis to elucidate safety of multifetal pregnancy reduction (MFPR) to better assist decisions on MFPR after artificial reproductive technologies (ART). **Methods:** A total of 250 patients underwent MFPR between January 1992 and December 2004. Patients received ART and were advised to undergo MFPR via vagina during 7–12 pregnancy weeks if

there were more than two live fetuses. MFPR finishing twins/singletons group: to reserve two/one fetus after MFPR; Started twins group: obtained two fetuses after ART without MFPR. MFPR group: patients underwent MFPR, remaining one or two fetuses. Control group: twins or singleton after ART without MFPR. **Results:** No significant difference was shown on the outcomes of pregnancy between MFPR finishing twins group and started twins group. No significant difference between MFPR group and control group was found except less small for gestational age infant (7.9% vs. 17.0%) and placenta praevia rate (1.8% vs. 3.9%) in MFPR group. MFPR finishing twins group and finishing singletons group had significant difference in abortion rate (17.6% vs. 2.8%), birth weight ( $2.41 \pm 0.5 \text{ kg}$  vs.  $2.7 \pm 0.6 \text{ kg}$ ), cesarean delivered rate (85.0% vs. 61.1%), pregnancy induced hypertension rate (38.9% vs. 16.7%), and postpartum hemorrhage rate (11.4% vs. 2.8%). **Conclusion:** MFPR could improve the pregnancy results and decrease mother complications, and it is better to reserve single fetus than two fetuses.

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**Pt1-04****Use a new concept: the oocyte to baby rate, to evaluate the biological efficiency of *in vitro* fertilization**

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**Aim:** In this study, we use the oocyte to baby rate as a new relevant standard of success in assisted reproduction technology (ART) to assess the real biological efficiency of ART. **Methods:** From Jan 2006 to Dec 2007, there were 1 865 oocyte retrieval fresh cycles available for retrospective analysis of the live birth rate in relation to total number of oocytes collected. We excluded cycles in which all embryos were frozen, or where preimplantation genetic diagnosis had been performed, or when the cycle was a complete failure of fertilization. According to the age, the patients were divided into three groups (group1, < 35 years; group 2, 35–39 years and group3,  $\geq 40$  years). Two types of analyses were carried out, firstly, we assessed the overall oocyte to baby rate for each group, and then the rate according to the oocyte yield in each group (arbitrarily subdivided in divisions of one to five oocytes, six to 10 oocytes, 11 to 15 oocytes, 16 to 20 oocytes, and > 20 oocytes). For patients whose complete oocyte utilization account was not available because of remaining frozen embryos, the additional potential yield of live birth (live babies born) from the use of these embryos was estimated according to the statistics on pregnancy rate of frozen embryos reported by our center in the years of analysis. Statistical analysis comparing live births per oocyte in relation to age and the number of oocytes collected was performed using chi-square analysis. **Results:** A total of 1 865 oocyte retrievals cycles yielded 22 861 oocytes. The total number of transferred and frozen embryos was 7 652 (a utilization rate of oocyte is 33.4%). The overall oocyte to LBB rate was 5.2% (1 183 live babies born). When analyzed by groups, there was

no increase in oocyte to LBB rate with number of the retrieved oocytes increased. In the three age groups, the oocyte to LBB rate was 5.4%, 3.8% and 0.8 %, respectively. When the oocyte to baby rate was calculated according to the number of oocytes generated, there was a trend to greater oocyte wastage in terms of the ability to achieve a live baby born when more oocytes were collected. In particular, in the less-than-35-age group, there was a statistically significantly higher final expected live babies born rate per oocyte yield in patients with less than 15 oocytes collected. In all age groups, the collection of less than 15 oocytes did not decrease the chance of achieving a live baby born from the smaller oocyte cohort. **Conclusion:** During ART cycles, only approximately 5% of fresh oocytes produce a baby. The time has come to investigate new methods of oocyte viability assessment and consider changing current ART practice to recruit fewer oocytes. Mild ovarian stimulation may be considered as a valid alternative to the current protocol.

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#### Pt1-05

##### Comparing intracytoplasmic sperm injection and *in vitro* fertilization in patients with single oocyte retrieval

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**Aim:** To compare the efficacy of intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF) in patients with single oocyte retrieval. **Methods:** The study group consisted of 95 infertile patients (133 cycles) undergoing assisted reproduction treatment between January 2007 and September 2008 in the Center of Clinical Reproductive Medicine in Jiangsu Province Hospital. Patients characterized by a poor response were included in this study when only a single oocyte was retrieved during ovum pickup. All the males met the criteria with the postwash motile sperm > 8 million on the day of egg retrieval. The data of 133 cycles was analyzed retrospectively, in order to determine whether the fertilization and pregnancy rates (PRs) differed between ICSI and IVF cycles. **Results:** Seventy-six IVF cycles and 57 ICSI cycles produced similar fertilization rates (73.7 % vs. 82.5%) and PRs (10.5% vs. 14.0%), with no significant difference ( $P > 0.05$ ). In patients  $\leq 38$  years old, IVF and ICSI produced similar fertilization rates (73.9% vs. 83.3%,  $P > 0.05$ ) and PRs (13.0% vs. 23.3%,  $P > 0.05$ ); in patients  $> 38$  years old, IVF and ICSI also produced similar fertilization rates (73.3% vs. 81.5%,  $P > 0.05$ ) and PRs (6.7% vs. 3.7%,  $P > 0.05$ ). **Conclusion:** There was no significant difference in the fertilization rates and PRs with the patients who underwent either ICSI cycles or IVF cycles with single oocyte retrieval. The possible mechanical damage to the oocyte after ICSI may have had a detrimental effect and further diminished the chances for successful fertilization and pregnancy. So, when the males met the defined threshold with the postwash motile sperm  $> 8$  million on the day of egg retrieval, conventional IVF was recommended.

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#### Pt1-06

##### Hypogonadism in relation to infertility

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The concept of hypogonadism or low testosterone is a controversial topic. The strict definition of hypogonadism is inadequate gonadal function as manifested by deficiencies in gametogenesis and/or the secretion of gonadal hormones. Only recently has the concept of treatment of low testosterone in the aging male, been shown to provide long-term physical & mental improvement. Unfortunately most physicians are not aware that long-term hypogonadism in the aging male is associated with the increased risk of osteoporosis, mood disturbance & sexual dysfunction. Not until the recent information and advertising explosion regarding treatment of sexual dysfunction did the topic of low testosterone become so widely discussed. The use of testosterone replacement therapy to treat the so-called “male menopause” or “andropause syndrome” has been gaining tremendous popularity. This popularity is no doubt a function of increased public awareness and clinician education. The baby-boomer population is now getting old enough to reap the benefits of testosterone replacement. Testosterone injections have been widely available for a long period of time, but only recently was it realized that the dosage interval should be shortened to allow a sustained serum level of testosterone. This treatment modality is complicated by the problems of dosing irregularities.

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#### Pt1-07

##### The clinical significance of psychotherapies to non-obstructive azoospermia patients

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**Aim:** To research the psychological state of non-obstructive azoospermia patients and further conduct relevant psychotherapies. **Method:** Thirty-two confirmed non-obstructive azoospermia (NOA) patients and 64 patients who planned to undergo artificial insemination with donor's semen (AID) were randomly picked up from our out-patient department from January to December, 2008, with an average age  $32.55 \pm 5.83$  (ranging from 24 to 51). Psychological evaluation was used based on our self-made psychological evaluation questionnaire (modified from SCL-90). Psychotherapies were then carried out according to individual psychological problems. **Results:** In 64 AID cases, 16 patients expressed their satisfaction with AID and did not mind their children were biologically unrelated with them. Thirty-eight patients were able to accept their NOA, while remaining unreconciled to receive AID. Still 10 patients stated they were forced to choose AID by their parents and wives, and they might be unable to accept their biologically-unrelated

children. Among 32 confirmed NOA patients, 16 were unable to accept their NOA and four would reconfirm from other hospitals. The rest 16 patients accepted their NOA, but six of them showed obvious depression and desperation. After psychotherapies, all patients had further recognition about NOA and AID. Six of the 10 patients who had been unable to accept their biologically-unrelated children stated that they would gradually try to accept. Ten of the 16 patients who had been unable to accept their NOA also expressed their acceptance. 6 patients with depression and desperation regained normal states of mind after psychotherapies. **Conclusion:** It is of vital importance to bear and raise children in traditional view of Chinese people. The NOA patients cannot have their biologically-related offspring through AID or adoption. The failure of family lineage extension frequently causes depression and desperation or anxiety at the initial stage. Some patients still get pressures from their parents and wives. The modern medical mode (biological-psychological-social mode) has called our attention to emphasize on psychotherapies, which have showed positive effects on most patients. Considering some patients' possible innocence about AID, detailed explanations shall also be made to them even if they do not mind their biological disrelation with children.

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#### Pt1-08

##### The role of integrated fertility evaluation in the diagnosis and treatment of male infertility

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**Aim:** To investigate the role of Integrated Fertility Evaluation in the diagnosis and treatment of male infertility and offer a new idea for the treatment. **Methods:** Between August 2008 and March 2009, 401 male infertility patients in our andrology department were targeted for survey by using self-made measuring scale. The scale included both sexual function and semen quality. Sexual function section covered questions referring to libido, frequency of sex life, ejaculation and refractory period of erection. Semen quality section covered questions referring to semen analysis and sperm function test. **Results:** Of all patients, 182 (45.39%) patients had normal semen analysis results, 219 (54.61%) patients had abnormal semen analysis results. The sexual function evaluation results showed only 67 patients were perfectly normal. A majority of patients had problems such as sexual hypoactivity and ejaculatio retardate. For 182 patients with normal semen analysis results, sperm function test showed 41% had abnormal results. Also 63% had sexual hypoactivity/ low frequency of sex life and 28% had ejaculatio retardate/anejaculation or erectile dysfunction during the wife's ovulatory period. Among 219 patients with abnormal semen analysis results, nearly half patients had abnormal sperm function, and 63% were not able to have multiple sex life during the wife's ovulatory period. **Conclusion:** patients with normal semen analysis results could also have abnormal sperm function.

Low frequency of sex life and sexual function during the wife's ovulatory period also contributes to the infertility. Treatment to patients with abnormal semen analysis results should focus on both the semen quality and sexual function. Therefore, an integrated fertility evaluation is of crucial importance in the diagnosis and treatment.

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#### Pt1-09

##### Clinical observation on the effect and safety of JinLeng method on oligospermia and asthenospermia

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**Aim:** JinLeng underpants is cooling device reducing scrotal temperatures since increasing scrotal temperature may influence testicular spermatogenesis. The basic principle of JinLeng method are: low temperature favored by testicle and the traditional Chinese medicine theory "external treatment for internal illness". Nocturnal scrotal cooling by using JinLeng underpants twice a day resulted in a decrease of scrotal temperature at approximately 0.9°C. The aim of this study is to investigate the effect and safety of JinLeng method on the oligospermia and asthenospermia. **Methods:** Forty-one patients with male infertility of oligospermia or asthenospermia were treated by JinLeng underpants (JinLeng method) twice a day for 3 months. Most patients have issues such as sedentary, scrotal position higher or scrotum tighten, wearing tight pants that could lead to increasing scrotal temperatures. The sperm parameters of the patients were analyzed before and after treatment, and pregnancy outcome of the patients' wives were recorded. **Results:** Of the 38 patients who accomplished the 3-month JinLeng method therapy, 33 were significantly improved in sperm concentration, semen volume, forward sperm motility, total sperm motility, total sperm count and total motile sperm count. The effective power came to 86.8%. Five wives of the patients were pregnant in the follow-up period for 2 month. No side effect took place on the patients. **Conclusion:** JinLeng method is efficacious and safe to treat infertile males with oligospermia and asthenospermia.

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#### Pt1-10

##### Is simplex teratospermia the indication of ICSI?

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**Aim:** To investigate the relation between teraospemia and fertilization rate in in-vitro-fertilization. **Methods:** To exclude the oocyte influence on fertilization, we only included couples whose fertilization rate was 100%. A total of 94 cycle were included in our study. Before and after density gradient centrifugation in IVF, sperm morphology was evaluated by Diff-Quik stain according to the WHO criterion. **Results:** The mean

number of oocyte was  $8.0 \pm 3.54$  (1–32). The density of the sperm was normal in all patient ( $\geq 20 \times 10^6 \text{ mL}^{-1}$ ). The a+b% were less than 50% in 17 patients (18.09%) and less than 30% in four patients (4.26%). The proportion of normal morphology sperm of raw semen was  $\leq 15\%$  in 82 patients (87.23%) and  $\leq 5\%$  in 12 patients (12.77%). The proportion of mean normal morphology sperm significantly increased after density gradient centrifugation ( $10.0\% \pm 6.68\%$  vs.  $17.47\% \pm 15.12\%$ ,  $P < 0.001$ ). After sperm preparation, all normal morphology sperm percentage was  $> 5\%$  and in 60 patients (63.83%) normal morphology sperm percentage was between 6% and 15%. The clinical pregnancy rate was 47.82%.

**Conclusion:** Density gradient centrifugation can increase the normal morphology sperm percentage. The raw semen with simplex teratospermia is not indicative of ICSI.

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#### Pt1-11

##### Simultaneous microsurgical subinguinal vasectomy and varicolectomy: a case report

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**Aim:** Varicoceles appear in approximately 20% of the general male population, some person request vasectomy may combine with it. After varicolectomy, the deferential veins become the main avenue for testicular venous return, varicocele, post varicolectomy hydroce, post vasectomy pain syndrome and varicocele recurrence all can cause scrotal uncomf. Under nonmicrosurgical procedure, vas and testis artery can be injured and cause testis atrophy. **Methods:** 34 years old male, bilateral varicocele (left I right II) with right testicular pain, request vasectomy. In the guide of index finger, a 2-cm transverse incision is made at the level of the external ring. The spermatic cord is then identified as it exits the external ring. The cord is mobilized at the external ring and brought out of the wound with a Babcock clamp, and then a penrose drain is placed beneath it. Under the operating microscope ( $10\times$ ), the cremasteric, internal and external spermatic fasciae are dissected bluntly, cremasteric vein were identified and ligated. Arteries is microscopically dissected free of all surrounding tissue. All internal spermatic veins are ligated with 5-0 suture, and lymphatics were preserved. The deferential vessels were carefully dissected off for a distance of 2.5 cm and preserved. The vas was then double ligated and transected, remove about 1 cm. through the scrotom approach, the left vas deferens was treated similar to the right side. **Results:** There are two testicular arteries and three lymphatics were microscopically dissected free of all surrounding tissue and preserved, 9 internal spermatic veins and one cremasteric veins were ligated. In the right vas deferens, two veins and 1 artery is dissected free and preserved. The follow-up visit occurred 3 months and 6 months respectively. No complications episodes of testicular atrophy, vasectomy failure, varicolectomy recurrences and scrotal uncomf developed. **Conclusion:** Microsurgical approach to varicolectomy allows the identification of the artery, tiny vein and lymphatic. Men presenting for vasectomy

with palpable and symptomatic varicoceles could benefit from simultaneous vasectomy-varicolectomy. This should be performed microsurgically to identify and ligate all spermatic and cremasteric veins, to preserve not only the testicular artery and lymphatics, but also the deferential vessels to minimize the risk of scrotal uncomf, testicular atrophy and hydrocele.

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#### Pt1-12

##### Transversal 2-suture intussusception vasoepididymostomy: still an effective procedure

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**Aim:** Vasoepididymostomy remains one of the most technically challenging procedures in all of microsurgery. From end-to-end, end-to-side and Intussusception, there are several technique still used. It is said that 2-suture longitudinal intussusception vasoepididymostomy is the most superior techniques in treating obstructive azoospermia. To our experience, placement two longitudinal suture in the narrow epididymal tube is relatively difficult than transversal method, and if the diameter of vas lumen is comparable with epididymal tube, forming a larger opening for anastomosis is not necessary. Then we report our results of 2-suture transversal intussusception vasoepididymostomy. **Methods:** In the period from May 2007 to December 2008, we performed vasoepididymostomies in 19 consecutive patients. Two parallel sutures are placed in the selected epididymal tubule, oriented transversal, and then the tubule is incised between the two needles. Once the epididymal tubule has been incised and the sperm were found in the epididymal tubule fluid, the sutures are pulled through. Data collected included months of follow-up, sperm density, motility and spouses pregnant rate. The follow-up period ranged from 6 to 24 months. Then patients lost the follow up. **Results:** Motile sperm were found in the ejaculates of 9 of 16 (53%), the pregnant rate is 2 of 16 (13%). **Conclusion:** Transversal 2-suture intussusception vasoepididymostomy simplifies the performance of the anastomosis; it is still the preferred and effective method in the treatment of obstructive azoospermia. Although long-term follow-up is still needed in terms of pregnant rate.

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#### Pt1-13

##### Effect of fertilization rate on embryo implantation in IVF

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**Aim:** To investigate the effect of fertilization rate on embryo implantation in IVF. **Methods:** Data were obtained from 41



IVF cycles that each transferred embryo had imbedded and 100 cycles that each transferred embryo had not imbedded. Logistic regression was used to analyze the action of age, cause of disease, Gn dose, thickness of endometrium at HCG day, number of oocytes, maturation rate, IVF or ICSI, fertilization rate, normal fertilization rate, poly spermic fertilization rate, good embryo rate, cycle day of embryo transfer, cell number of embryo transferred and fragment on embryo implantation with each 348 embryo. **Results:** Variables in the Equation were age (OR = 1.190), normal fertilization rate (OR = 0.954), cell number of embryo transferred (OR = 0.703), good embryo rate (OR = 0.639), cycle day of embryo transfer (OR = 0.715). The 95% reference values of the age, normal fertilization rate, cell number of embryo transferred, good embryo rate, cycle day of embryo transfer were 22.86–35.26 years, 45.47%–100%, 7.81–7.97 cell, 31.51%–100%, 5.98–13.94 days. Lower normal fertilization rate was associated with higher oocytes gained by Spearman correlation analysis ( $r = -1.114$ ). Otherwise, this showed that there was no statistically significant difference between the normal fertilization and age. **Conclusion:** The normal fertilization rate that reflects the ability of both oocyte and sperm, can predict the outcome of embryo implantation and pregnancy in IVF.

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#### Pt1-14

##### Comprehensive evaluation of diagnosis and treatment for reproductive ductal obstruction in infertile male

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**Aim:** To explore the comprehensive evaluation methods of diagnosis and treatment for reproductive ductal obstruction in infertile male. **Methods:** Clinical data of 70 male patients diagnosed as having reproductive ductal obstruction were collected and analyzed using observational study from October 2008 to March 2009. Semen analysis using CASA, scrotal Doppler, and hormone test were introduced as screening tools for the diagnosis, with seminal plasma  $\alpha$ -glycosidases test, TRUS, seminal vesicle aspiration, ductal patency test, and testicular biopsy as extra differential techniques if needed. The treatment measures were selectively ensued in terms of different diagnosis, including explorative microsurgical vasovasostomy, vasoepididymostomy, TURED, and ART. All the patients were periodically followed in clinic and final pregnancy rate was recorded. **Results:** Among all the patients, distal ductal obstruction (ejaculatory duct cysts in majority) was found in nine cases, vas deferens obstruction in 35 (post-hernia reversal in seven, vasectomy in seven, mass in one, CBAVD in 20), and proximal obstruction in 26 (epididymitis in 24, orchitis in two). All the patients were given the screening CASA, hormone test and scrotal Doppler. Among the distal obstruction group, all the patients were given TRUS, with six patients being given patency test before the TURED and the other three ones being given seminal vesicle aspiration. All the proximal

obstruction candidates were given TRUS and seminal plasma  $\alpha$ -glycosidases test before the explorative microsurgery, with 22 patients demonstrating positive signs in TRUS and 18 patients presenting decreased seminal plasma  $\alpha$ -glycosidases. As for the biopsy, all the vas deferens obstruction candidates were given diagnostic testicular biopsy before surgery, not including vasectomy patients. All the CBAVD patients were given PESA before ICSI and all the proximal obstruction candidates were given intra-operative testicular biopsy or MESA before the micro anastomosis. The treatment protocols included TURED in nine, vasovasostomy in 12, vasoepididymostomy in 22, and ART in 27 (ICSI+PESA in 20, ICSI+MESA in five, ICSI+TESA in two). The prognosis of each treatment is being followed. **Conclusion:** The comprehensive evaluation of diagnosis and treatment for reproductive duct obstruction in male infertility should be ensued at the beginning of intervention. With the basic CASA, results from TRUS, seminal plasma  $\alpha$ -glycosidases test, seminal vesicle aspiration, and other extra diagnostic measures could be used as helpful tools for final correct policy-making.

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#### Pt1-15

##### Logistic regression analysis on the relation between the condition of donor sperm and the pregnancy of the women with AID treatment

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**Aim:** To explore the relation between the condition of donor sperm and the pregnancy per cycle of the women with AID treatment. **Methods:** Retrospective analysis of the results of 1 903 AID cycles performed in 1 078 women is presented. According to their AID treatments using the frozen-thawed donor spermatozoa from the human sperm bank of Guangdong, univariate and multivariate logistic regression were used to analyze the relation between the condition of frozen-thawed donor spermatozoa and pregnancy of these women. **Results:** Overall Pregnancy rates per cycle of 16.4% (313/1 903) was achieved. The success rate was affected by those factors including the spermatozoa concentration and motility before freezing, the total progressive motile number after thawing and the number of operation per cycle. Except the spermatozoa concentration before freezing, three factors were found to be evidently associated with the pregnant outcome such as sperm motility before freezing (OR = 1.982,  $P = 0.042$ ), the total progressive motile number after thawing (OR = 1.699,  $P = 0.031$ ) and the number of operation per AID cycle (OR = 2.178,  $P = 0.010$ ). **Conclusion:** sperm pre-freeze motility over 70%, the total post-thaw progressive motile numbers over  $3\ 000 \times 10^6$ , two operations per AID period could increase the pregnancy rate of the women with AID treatment.

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**Pt1-16****Preliminary investigation of the relationship between sperm DNA integrity results and ART outcomes**

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**Aim:** To assess the relationship between the sperm DNA integrity and the outcomes of assisted reproductive technique. **Methods:** All 211 semen samples were detected the sperm DNA integrity by improved sperm chromatin dispersion (SCD) test (the improving contains the change of experiment procedures, reagents preparing and utensils used), of which 41 were assigned to intrauterine insemination (IUI), 117 were assigned to in vitro fertilization (IVF) and 53 were assigned to intracytoplasmic injection (ICSI). All semen samples were divided into two groups by sperm DNA integrity ratio borderline 67.4%: normal group (normal sperm DNA integrity ratio > 67.4%), abnormal group (normal sperm DNA integrity ratio < 67.4%). Fertilization rates, oocyte cleavage rates, high-quality embryo rates and the pregnancy outcomes were compared between the normal sperm DNA integrity group and abnormal sperm DNA integrity group. **Results:** There were no significant difference in the fertilization rates, oocyte cleavage rates, high-quality embryo rates and pregnancy outcomes between the normal sperm DNA integrity group and abnormal sperm DNA integrity group. **Conclusion:** Sperm DNA integrity value probably cannot be taken as an independent index to predict the ART outcomes.

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**Pt1-17****The research on the carnitine concentration in the semen plasma of the male population resorted to assisted reproduction technique**

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**Aim:** To detect the carnitine concentration in the semen plasma of the clients who are going to have the assisted reproduction by the colorimetric method, and to compare the carnitine level in the semen plasma of the normal motility and asthenozoospermia, in order to investigate the clinical meaning of the carnitine level in male infertility. **Methods:** Dividing the male clients into three groups according to the semen analysis result (WHO criteria), normal sperm motility group ( $a + b > 50\%$  or  $a > 25\%$ ), asthenozoospermia group A ( $25\% \leq a + b \leq 50\%$ ), asthenozoospermia group B ( $a + b < 25\%$ ). The carnitine level was detected by chromatometry and the carnitine level was compared in the three groups. **Results:** There was a weak correlation between the carnitine level and sperm motility ( $r = 0.124$ ,  $P < 0.05$ ). The carnitine level in normal sperm motility group was  $438.50 \pm 158.28 \mu\text{mol L}^{-1}$ , asthenozoospermia group A was  $425.25 \pm 165.17 \mu\text{mol L}^{-1}$  and asthenozoospermia group B was  $378.38 \pm 161.19 \mu\text{mol L}^{-1}$ . The result showed that carnitine level of asthenozoospermia group B was significantly lower

than that of the normal sperm motility group ( $P < 0.05$ ). There was no significant difference of the carnitine level between the asthenozoospermia group A and normal sperm motility group ( $P > 0.05$ ). **Conclusion:** The lower carnitine level may have the influence on the sperm motility. As for those who are going to have the assisted reproduction, the carnitine concentration in the semen plasma of the asthenozoospermia male ( $a + b < 25\%$ ) should be detected, and which is probably helpful to find the cause of infertility, improve the sperm quality and conception rate.

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**Pt1-18****Semen cryopreservation of oligospermia and asthenospermia of the male infertility patients applied to intrauterine insemination cycles**

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**Aim:** To explore the effects of semen cryopreservation of oligospermia and asthenospermia of the male infertility patients applied to intrauterine insemination (IUI) cycles. **Methods:** One hundred and forty-nine cycles of intrauterine insemination in 102 couples were grouped from March 2008 to June 2009. All the couples were constitutional infertile. The infertility history of the couples was 1-22 years with mean infertility age of  $3.99 \pm 3.19$  years. The cycles were divided into three groups: Group 1 (normal semen,  $n = 52$ ), Group 2 (oligospermia and asthenospermia semen,  $n = 51$ ) and Group 3 (oligospermia and asthenospermia semen combined with cryopreserved semen,  $n = 46$ ). The couples of Group 2 and Group 3 were caused by male factor infertility. Group 1 and Group 2 were treated with fresh semen in intrauterine insemination. Group 3 were treated with fresh semen combined with cryopreserved semen in intrauterine insemination. Generally, the semen of forward motile sperm count before processing in  $5-10 \times 10^6$  should be cryopreserved until exceeding  $20 \times 10^6$ . The diagnosis of oligospermia and asthenospermia followed the WHO criteria. Semen parameters and forward motile sperm count after processing were recorded by Makler chamber. Five weeks after intrauterine insemination, the clinical pregnancy with fetal heartbeat was confirmed by ultra sonography. **Results:** Sperm concentration, sperm total motility rates, and rapid forward progressive motility (Grade A) of Group 2 before processing by Puresperm gradient centrifugation were significantly higher than those of Group 3 ( $P < 0.01$ ). The slowly forward progressive motility (Grade B) of Group 2 before processing by Puresperm were also higher than that of Group 3 ( $P > 0.05$ ). The semen volume was significantly higher in Group 3 than in Group 2 ( $P < 0.01$ ). The forward motile sperm count of Group 3 after processing by Puresperm was also higher than that of Group 2 ( $P > 0.05$ ). The pregnancy rates of Group 1, Group 2 and Group 3 were 15.4%, 9.8% and 13.0%. The





pregnancy rate of Group 3 was higher than that of Group 2 ( $P > 0.05$ ). **Conclusion:** Semen after thawing combined with Fresh semen could improve the forward motile sperm count and the pregnancy rate of oligospermia and asthenospermia of the male infertility patients after intrauterine insemination. Semen cryopreservation combined with intrauterine insemination would be an ideal treatment of oligospermia and asthenospermia of the male infertility patients.

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#### Pt1-19

##### The correlation between IVF outcomes and ER $\beta$ gene RsaI polymorphism in Chinese infertile men

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**Aim:** To investigate the significance of the ER $\beta$  RsaI polymorphism in infertile men, and its impact on clinical outcomes. **Methods:** Patients were recruited from the Reproductive Center at Beijing University Third Hospital. Three groups of Chinese men were recruited: 351 oligozoospermic men with a sperm concentration less than  $0.2 \times 10^6$  spermatozoa/mL; 396 azoospermic men with no sperm in their ejaculate; and 387 healthy men with sperm concentrations higher than  $20 \times 10^6$ /mL. These last individuals served as controls. In addition, all non-obstructive azoospermic patients (NOA) were subdivided into NOA (-) and NOA (+) groups based on the results of their testicular biopsies. The ER $\beta$  RsaI polymorphism was detected by allele-specific PCR. Main clinical outcomes from IVF and ICSI included 2PN rate, early embryonic development, and pregnancy and miscarriage rates. **Results:** 1) In NOA (-) group, the heterozygous ER $\beta$  AG frequency was significantly higher than in the other groups, whereas AA frequency was more than three times than that in control group and more than two times than that in OA and oligozoospermic groups. 2) In regards to implantation, clinical pregnancy, and ectopic pregnancy rates, there were no significant differences among the various genotype subgroups. However, the homozygous RsaI AA genotype was significantly correlated to pregnancy loss in IVF and ICSI patients. **Conclusion:** The RsaI polymorphism in ER $\beta$  have potentially modulating effects on human spermatogenesis, and may also be significantly associated with an increased risk of pregnancy loss following IVF and ICSI treatments.

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#### Pt1-20

##### The study of etiological classification and clinical features of the cases with obstructive azoospermia as well as the results of ICSI treatment

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**Aim:** To summarize the etiological classification and clinical features of the cases with obstructive azoospermia as well as the results of ICSI treatment for these patients, and to explore the prevalence of  $\Delta F508$  mutations of CFRT gene in Chinese with congenital bilateral absence of vas deferens. **Methods:** From January 2003 to December 2007, 417 cases with obstructive azoospermia visited our hospital underwent history inquiry, physical examination, semen analysis, scrotal and transrectal ultrasonography. Percutaneous epididymal sperm aspiration (PESA) was employed to retrieve spermatozoa for ICSI after definitive diagnosis had made. The outcomes of ICSI were observed. The patients with obstructive azoospermia were divided into two groups, Group 1: the patients with congenital bilateral absence of vas deferens (CBAVD); Group 2: the patients with post-inflammatory obstructive azoospermia. The control group used donor sperm for ICSI. 66 cases with congenital bilateral absence of vas deferens and 5 female partners underwent the detection of  $\Delta F508$  mutations, and the situation of the children acquired by ICSI were followed up. **Results:** There were 250 cases with 290 ICSI cycles in Group 1, 146 cases with 167 ICSI cycles in Group 2; 16 cases with 20 ICSI cycles in the patients with previous vasectomy history, five cases with six ICSI cycles in those with post-hernia surgical history and 48 cases with 52 ICSI cycles in control group. Most of the patients with obstructive azoospermia had these features: normal testis volume and FSH value, normal karyotype. All of the patients with CBAVD suffered from primary infertility, 83.2% of them with bilateral vas deferens absent, 14.4% with unilateral vas deferens absent; 76.7% with semen volume less than 1.0 mL and 93.9% of them with semen pH  $\leq 6.8$ ; 97.5% with negative result of semen fructose detection. Bilateral seminal vesicles absence and unilateral absence were found in 53.2% and 35.7% patients by transrectal ultrasonography, respectively. The enlarged and hardened epididymis or nodules in the epididymis were observed in all the cases of Group 2, and 60.7% of them were with a fathering history or previous epididymitis. Sperm could be found by PESA in 92.5% of the patients who have no any history. The enlarged ducts of epididymis and hyperchogenicity nodules were observed in 83.3% and 61.1% of them by scrotal ultrasonography, respectively. The sperm used for ICSI in control group were from sperm bank. The fertilization rate was 79.0% vs. 85.1% ( $P < 0.05$ ) and 81.1% vs. 85.1% ( $P > 0.05$ ), clinical pregnancy rate per transference of ICSI cycle was 44.9% vs. 38.8% ( $P > 0.05$ ) and 42.7% vs. 38.8% ( $P > 0.05$ ), take home baby rate was 38.2% vs. 36.7% ( $P > 0.05$ ), 35.4% vs. 36.7% ( $P > 0.05$ ) and early abortion rate was 11.8% vs. 5.3% ( $P > 0.05$ ), 10.0% vs. 5.3% ( $P > 0.05$ ) in Group 1, 2 and control, respectively. No one was with the  $\Delta F508$  mutations in total 66 patients and five female partners. **Conclusion:** In this study, the common reasons lead to obstructive azoospermia (in the turn of incidence rates) are CBAVD, post-inflammatory obstruction, vasectomy, and bilateral hernia surgery. Ninety percent cases with obstructive azoospermia are due to CBAVD or post-inflammatory obstruction and present different typical clinical characteristics. There is no significant difference in the results of using sperm retrieved from epididymis or testis of the patients with obstructive azoospermia for ICSI, comparing with that using donor sperm. The prevalence of  $\Delta F508$  mutations



in Chinese with congenital bilateral absence of vas deferens is very rare. So it is still disputed that whether detecting  $\Delta F508$  mutations as routine before ICSI is necessary.

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#### Pt1-22

##### The relationship between human normal sperm morphology rate and *in vitro* fertilization outcome

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**Aim:** To study the effects of sperm morphology on fertilization rates, cleavage rates and clinical pregnancy rates, which could help to choose a best protocol before ART. **Methods:** A total of 751 patients' sperms were divided into four groups (normal sperm morphology rate  $\leq 5\%$ ,  $5\%-10\%$ ,  $10\%-15\%$ ,  $> 15\%$ ) which underwent *in vitro* fertilization (IVF) 490 cycles or intracytoplasmic sperm injection (ICSI) 261 cycles. 618 donors' sperms were also divided into four groups with normal sperm morphology rate ( $14\%-17\%$ ,  $17\%-19\%$ ,  $19\%-23\%$ ,  $> 23\%$ ) which only underwent IVF 618 cycles. Then the fertilization rates, cleavage rates and clinical pregnancy rates among each group were analyzed and discussed. **Results:** 1) During IVF (patients or donors) cycles, there was a significant difference among four groups in fertilization rates and clinical pregnancy rates ( $P < 0.05$ ), but there was no difference in cleavage rates ( $P > 0.05$ ); 2) In ICSI cycles, there was no significant difference among four groups in fertilization rates, clinical pregnancy rates and cleavage rates ( $P > 0.05$ ). **Conclusion:** 1) Sperm morphology has a significant influence on IVF fertilization rates and clinical pregnancy rates, but no significant influence on cleavage rates. That is to say, sperm morphology affects the outcome of IVF; 2) There is no significant influence of sperm morphology on outcome of ICSI, and ICSI is an optimal alternative on patients with abnormal morphological sperms in ART.

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#### Pt2-01

##### Cell phone usage and male infertility - could there be a link?

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**Background:** The problem of lack of offspring is a phenomenon concerning approximately 15% of married couples in Egypt. Infertility is defined as inability to conceive after a year of sexual intercourse without the use of contraceptives. In half of the cases, the causative factor is the male. Males are exposed to the effects of various environmental factors, which may decrease their reproductive capabilities. A decrease in male fertility is a phenomenon which occurs in recent years, which may suggest that one of the reasons for the decrease in semen parameters is

the effect of the development of technology in the environment. A hazardous effect on male fertility may be manifested by the decrease in the amount of sperm cells, disorders in their motility, as well as morphology. The causative agents may be chemical substances, ionizing radiation, stress, as well as electromagnetic waves. **Aim:** The objective of the study was to determine the effect of the usage of cellular phones on the fertility of males subjected to marital infertility therapy. **Methods:** The following groups were selected from 425 males covered by the study: Group A: 105 patients who did not use mobile phones, Group B: 185 males who have used GSM equipments sporadically for the period of 1-2 years, and Group C: 135 people who have been regularly using mobile phones for more than 2 years. Sperm parameters included volume, liquefaction time, pH, viscosity, sperm count, motility, viability and morphology. **Results:** The comparisons of mean sperm count, motility, viability, and normal morphology among three different cell phone user groups were statistically significant. Mean sperm motility, viability, and normal morphology were significantly different in cell phone user groups within two sperm count groups. After analyzing the effect of GSM equipment on the semen, it was noted that an increase in the percentage of abnormal sperm cells was associated with the duration of exposure to the waves emitted by the GSM phones. It was also confirmed that a decrease in the percentage of sperm cells in vital progressing motility in the semen is related to the frequency of using mobile phones. **Conclusion:** Usage of cell phones decreases the semen quality of men concerning deterioration performance of the sperm count, motility, viability, and normal morphology. The decrease in sperm parameters was dependent on the duration of exposure to cell phones and independent from the initial semen quality.

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#### Pt2-04

##### Body mass index in relation to semen quality and serum sex hormone profile among Egyptian men

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**Aim:** To examine the relationship between body mass index (BMI) and semen quality among Egyptian males. **Methods:** Seventy-Five Egyptian volunteer apparently healthy adults were chosen to assess the association between BMI and both hormonal and semen parameters of young Egyptian men. Physical examination was performed by physician. Body weight, height and the BMI were calculated. Fasting blood samples were collected, serum level of total testosterone and estradiol concentration were measured using competitive immunoassay quantitative colorimetric method. FSH, LH and prolactin were measured using indirect solid phase sandwich type immunoassay method. Semen quality measures for the first ejaculates were obtained at the beginning of this study. **Results:** Serum T, sex hormone-binding globulin (SHBG), and inhibin B all decreased with increasing BMI, whereas free androgen index and E(2) increased with increasing BMI. Serum FSH was higher



among slim men. After control for confounders, men with a BMI > 25 kg/m<sup>2</sup> had a reduction in sperm concentration and total sperm count of 21.6%; (95%CI: 4.0%–39.4%) and 23.9%; (95%CI: 4.7%–43.2%), respectively, compared to men with BMI between 20–25 kg/m<sup>2</sup>. Percentage of normal spermatozoa reduced, although not significantly, among men with high or low BMI. Semen volume and percentage of motile spermatozoa were not affected by BMI. **Conclusion:** The obtained results indicated that obesity had significantly negative effect on reproductive physiology and may interfere with many testicular functions. Also, it was associated with alteration in semen parameters and serum sex hormones. These results provided additional information on the ability of obesity to reduce fertility in the Egyptian males.

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#### Pt2-07

##### Vitamin E prevents adverse effect of p-onylphenol (p-NP) on biochemical factors in the rats serum during the development of testis

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**Background:** Endocrine disruptive compounds (EDC) have been shown to affect reproductive systems, liver and thyroid which ultimately cause developmental impairment of laboratory animals, suggesting their implications in the increasing incidence of reproductive abnormalities and well being of other organs in wildlife and human populations. They may have such disruptive effects by disturbing the normal function of the steroid hormones, liver enzymes and production of reactive oxygen species (ROS). p-Nonylphenol (p-NP), one of the members of alkylphenol family, is a non-ionic detergent used in detergents, cosmetics, paints, pesticides and other formulated products, is reported to have EDC activity. On the other hand, there are other investigations which report that antioxidant can prevent disruptive effect of ROS and can increase the activity of scavenger enzymes. In our previous study preventing effects of wheat germ oil (WGO) as a rich source of vitamin E on imbalancing property of p-NP affecting serum hormones and enzymes of Wistar rats was investigated. Since WGO in addition to vitamin E, contain other biological material like phytoestrogens, poly-unsaturated fatty acids and so on, thus whether these preventing effect could be related to vitamin E component of WGO has to be answered. **Aim:** To investigate the preventing effect of vitamin E on imbalance of hormone (LH, FSH, estrogen, testosterone, prolactin, TSH) and enzymes level (AST, ALT, LDH, gama-glutamyl transferase and alkaline phosphatase) caused by p-NP during testis development. **Methods:** Twenty-four female rats after conformation of pregnancy based on vaginal plugs observation were divided in four groups ( $n = 6$ ) including p-NP (250 mg/kg/day) group, p-NP + vitamin E group, vitamin E (100 mg/kg/day) group and control (no treatment of p-NP or vitamin E). The treatments were continued till the delivery and post waning, after which the male pups were similarly divided into four groups ( $n = 6$ ) as

their mothers when they reached 90 days old. The blood samples were taken and above mentioned biochemical parameters were examined using standard diagnostic kits. **Results:** The level of estrogen, TSH, AST, LDH, gamaglutamyl transferase and alkaline phosphatase increased significantly ( $P < 0.05$ ) but no changes in the level of testosterone, LH, prolactin and ALT were observed ( $P > 0.05$ ) in the rats treated with p-NP. In addition the level of T4 and FSH in rats treated with p-NP decreased significantly ( $P < 0.05$ ) compared with controls. In the group treated with vitamin E alone no differences was observed in comparison with control group with respect to all the biochemical factors, while simultaneous treatment of vitamin E and p-NP could compensate the imbalance of T4, estrogen, AST, LDH, alkaline phosphatase and gamaglutamyl transferase due to p-NP. **Conclusion:** Vitamin E is considered as an important nutritional and reproductive factor but a strong antioxidant as well. It might be considered to compensate the adverse oxidative effect of p-NP on investigated enzymes due to its preventing role on ROS.

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#### Pt2-08

##### Effects of vitamin E on sperm parameters and hormones involved in reproduction in rats treated with para-nonylphenol

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**Aim:** The aim of this study was to investigate the toxic effect of para-nonylphenol (p-NP) on sperm parameters, hormonal levels involved in reproduction and the preventing role of vitamin E (VE) on these dysfunctions in developing rats. **Methods:** In this study, adult albino male and female Wistar rats ( $250 \pm 20$  g) were used. For copulation, a female was paired with single male in individual cage ( $n = 35$ ). Mating was confirmed by vaginal plug detection and the presence of sperm in vaginal smears. This day was regarded as 0 day of gestation. Pregnant rats ( $n = 24$ ) were divided into 4 groups: control (corn oil), p-NP (250 mg/kg/day), VE (100 mg/kg/day) and P-NP+VE. Oral treatments were performed on day seven of gestation and continued during pregnancy and weaning. After lactation, male offspring ( $n = 6$ ) were divided into the same groups as the parents and oral treatments were continued until 90 days of age. After treatment, body and left testis weight were recorded and left cauda epididymis was cut in Ham's F10 medium. Released sperm were used to analyze number, motility and viability of the sperm. Blood serum was used to assess follicle stimulating hormone (FSH), luteinizing hormone (LH), estrogen and testosterone. **Results:** In p-NP-treated rats, a significant decrease was found in body and testis weight, sperm number and sperm motility compared to control group. Co-administration of p-NP+VE significantly increased testis weight, sperm number and sperm motility compared to p-NP group. A significant increase was also found in sperm viability in VE group when compared with both p-NP and control groups. Rats treated with p-NP showed a significant decrease and a significant increase in the level of FSH



and estrogen respectively. In p-NP+VE group, the change of estrogen level but not FSH was significantly reversed compared to p-NP group. In conclusion, the results suggest that VE, as a potent antioxidant, not only is able to reverse the toxic effects of p-NP on testis weight, sperm number, sperm motility and estrogen level, but also increases sperm viability in developing rat.

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#### Pt2-10

##### Low dose effects of bisphenol-A on the male reproductive system in adult rats

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**Aim:** To investigate the effects of low dose bisphenol-A (BPA), a xenoestrogen, on the reproductive system of adult male Sprague-Dawley (SD) rats through the digestive tract. **Methods:** Fifty adult male rats were randomly divided into five groups at 10 rats per group. They were administered with a 10 mL kg<sup>-1</sup> vehicle, 200 µg kg<sup>-1</sup> Tamoxifen (TAM), 10.0, 30.0 and 90.0 µg kg<sup>-1</sup> BPA via intragastric gavage for 4 weeks. Animal body weight was measured every week. Twenty-four hours after the last treatment, blood serum was collected to determine the levels of testosterone (T), estradiol (E2), prostate specific antigen (PSA) and dihydrotestosterone (DHT) with MAIA and ELISA assays. Testes and epididymides were collected for measurement. Prostates were isolated to measure wet weight, volume, prostate index (PI) and the height of epithelial cell (HEC), and acinar luminal areas (ALA) were measured with MIVNT. **Results:** 1) There were no significant differences in body weight between the control and BPA groups, while the body weight of the TAM group was lower than that of control. 2) Compared with control, organs (testes and epididymides) weight in low dose BPA and TAM group increased. 3) Between control and BPA groups, there were no differences in E2, while T of BPA groups decreased, DHT of BPA groups slightly increased, and PSA of 10.0 µg/kg BPA ( $P < 0.05$ ) and 90.0 µg/kg BPA ( $P < 0.01$ ) groups increased. In TAM group, the E2 and PSA ( $P < 0.01$ ) were higher than those of control, and the T was lower than that of control. 4) Compared to the control group, in BPA groups, the total prostate wet weight, volume and PI increased, especially the prostate volume at mid dose and high dose BPA ( $P < 0.01$ ). Between the control and BPA groups, wet weight and index of ventral prostate (VP) decreased with BPA dose increasing, while wet weight and index of dorsal prostate (DP) increased ( $P < 0.01$ ). Total prostate wet weight, volume, PI ( $P < 0.01$ ), VP wet weight, index, and DP of TAM group decreased than those of control, while DP index was increased than that of control. 5) In BPA groups, HEC of VP and DP was significantly higher than that of control ( $P < 0.01$ ), and decreased in BPA as the dosage increased. ALA of VP and DP was bigger than that of control, and the latter increased with

BPA as the dosage increased. In the TAM group, HEC of VP and DP was lower than that of control ( $P < 0.01$ ), and ALA of VP and DP was lower than that of control. **Conclusion:** Low dose BPA may promote the proliferation of male reproductive system. Additionally, the prostate is highly sensitive to BPA at a dosage below human daily intake. It suggests that low dose BPA would accelerate prostate proliferation and aggravate benign prostatic hypertrophy (BPH).

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#### Pt2-11

##### Effect of Coal-burning endemic fluorosis on apoptosis of spermatogenic cells

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**Aim:** To evaluate the effects of coal-burning endemic fluorosis on apoptosis of spermatogenic cells. **Methods:** Twenty-four healthy adult male white rats were randomly placed into four groups of six each and orally administered different dosages of coal-baking maize containing-fluoride twice a day for 120 times per the following: 9.37 mg kg<sup>-1</sup>, 69.33 mg kg<sup>-1</sup>, 69.33 mg kg<sup>-1</sup> + 340 mg kg<sup>-1</sup> AlCl<sub>3</sub>, 69.33 mg kg<sup>-1</sup> + 20% soybean. The control group consisted of eight rats. After 90 days, rat testes were isolated for morphological observation under light microscope after HE staining. Apoptosis of spermatogenic cells was detected by TUNEL assay. **Results:** 1) Typical apoptosis was observed in the spermatogenic microstructure from the experimental groups. 2) TUNEL analysis showed that positive reactions were detected in the spermatogenic cells and negative in other cells. 3) In the experimental groups, the apoptosis mainly occurred in spermatogonium and spermatocyte, however rarely in spermatids and sperm. 4) Apoptosis index of the experimental groups was significantly higher than that of the control group. 5) Both fluoride and AlCl<sub>3</sub> administration increased the apoptosis rate of spermatogenic cells, and additional nutrition reduced it. **Conclusion:** Coal-burning endemic fluorosis leads to a high apoptosis rate of spermatogenic cells.

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#### Pt2-12

##### Reproductive toxicity test of plant-derived insecticide in male rats

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**Aim:** To evaluate the safety of a bioinsecticide derived from the mixture of two plant species (*Stemona curtisii* and *Mammea*

siamensis) on male reproduction. **Methods:** Male Wistar rats (*Rattus norvegicus*), 6-week-aged and 200–240 g weight, were used as experimental model. The rats were randomized into five groups (eight each) and orally administered for 60 days with the bioinsecticide at doses of 2, 10 and 50 mg/kg/day, respectively. Negative control group received only distilled water (1 mL/day), and the positive control group was treated with a synthetic insecticide (Methomyl, 2 mg/kg/day). The male reproduction was evaluated according to the following sexual indices: sexual behavior, weight of sex organs, epididymal sperm concentration and testicular histology. **Results:** At the dose of 2 mg/kg/day, rat's reproduction show similar values, when compared with negative control group. At doses of 10 and 50 mg/kg/day, seminal vesicle's weights were found significantly lower than that of the negative control rats ( $P \leq 0.05$ ), while other parameters were similar. Nevertheless, the adverse effects of Methomyl were obviously detected, especially the increasing seminiferous tubule diameter, the loss of germ cells due to necrosis and significantly decreased epididymal sperm concentration ( $P \leq 0.05$ ). **Conclusion:** The bioinsecticide derived from *S. curtisii* and *M. siamensis* at the dose of 2 mg/kg/day did not alter male rat's reproduction while the higher doses showed a mild toxicity by decreasing seminal vesicle's weight. A synthetic insecticide, however, demonstrated high-toxic effects on almost all sexual parameters investigated. The use of this bioinsecticide at low concentration should be suggested to farm workers, the high-risk group.

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#### Pt2-13

##### Lead citrate cytotoxic effects on mice testis ultrastructure

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**Aim:** To evaluate the effects of lead on rat testis ultrastructure. **Methods:** Thirty white male Wistar mice were randomly divided into two groups. Mice in study group ( $n = 15$ ) were intraperitoneally injected with lead acetate at doses of  $9.5 \text{ mg kg}^{-1}$  of every other day, while those in the control group ( $n = 15$ ) were injected with only distilled water. After 5 weeks, testes were isolated for morphological observation under light microscope and electronmicroscope. **Results:** Severe histological changes were detected in testes of experimental group as compared with control group. **Conclusion:** Lead citrate may play deleterious roles on testis tissue at dose of  $9.5 \text{ mg kg}^{-1}$ .

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#### Pt2-14

##### Effects of continuous exposure to 60 Hz magnetic field on testicular function in mice for 20 weeks

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**Aim:** To characterize the effect of 60 Hz MF on the motility, morphology and number of sperm as well as the apoptosis of testicular germ cell in mice after continuous exposure for 20 weeks. **Methods:** The 7-weeks-aged male BALB/c mice were randomly placed into four groups (25 each). Three experimental groups were continuously exposed to 60 Hz MF of 2, 20 or 200  $\mu\text{T}$  for 24 h/day (except for 3 hours per week for management) for 20 weeks against the control group exposed to sham conditions. Their body weights were recorded every week. After the end of the exposure both testes were isolated and their weights were recorded. The apoptosis of germ cell in the testis was analyzed by H&E and TUNEL staining. The motility, morphology and concentration of sperm from epididymis were evaluated microscopically. **Results:** Compared with control, there were no significant effects on the body weight and testis weight in mice exposed to 60 Hz MF. In H&E and TUNEL staining, germ cells showed a significantly higher apoptotic rate in the mice exposed to 20  $\mu\text{T}$  ( $P < 0.05$ ) and 100  $\mu\text{T}$  ( $P < 0.01$ ). TUNEL-positive cells were mainly detected in spermatogonia. There were no significant effects on morphology of sperm from exposed groups, whereas the concentration (200  $\mu\text{T}$ ,  $P < 0.001$ ) and the motility (20  $\mu\text{T}$  and 200  $\mu\text{T}$ ,  $P < 0.05$ ) decreased. **Conclusion:** Our experimental results suggest that continuous exposure to 60 Hz MF of 20  $\mu\text{T}$  may affect testicular functions including the motility of sperm as well as the apoptosis of testicular germ cell in mice.

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#### Pt2-15

##### Treatment of infectious necrostermia with *in vitro* high-frequency radiation

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**Aim:** To investigate the effectiveness of the treatment of infectious necrostermia with high-frequency radiation *in vitro*. **Methods:** The prostate of 32 cases of infectious necrostermia was under the radiation of the HG-2000 high-frequency hyperthermia machine *in vitro*. And the sperm density, motility rate was contrasted before and after the exposure of radiation. **Results:** The average sperm density before and after the treatment was  $(67 \pm 36) \times 10^9 \text{ L}^{-1}$  and  $(65 \pm 36) \times 10^9 \text{ L}^{-1}$ , there was no significant difference ( $P > 0.05$ ). The average sperm motility rate after the treatment with  $39\% \pm 15\%$  was significantly higher than that before the treatment with  $61\% \pm 19\%$  ( $P < 0.05$ ). **Conclusion:** The high-frequency radiation on prostate *in vitro* was effective in the treatment of infectious necrostermia.

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**Pt2-16****The impact of organic hydroperoxides on rat testicular tissue and epididymal sperm**

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**Aim:** To investigate the *in vivo* effects of organic hydroperoxides on testicular tissue and epididymal sperm. **Methods:** Wistar rats (males aged 10–12 weeks) were randomly divided into nine groups and received standard rat chow and water. Animals were injected intraperitoneally with either saline (0.5 mL), t-butyl hydroperoxide (tbHP) (5  $\mu\text{mol L}^{-1}$ , 10  $\mu\text{mol L}^{-1}$ , 20  $\mu\text{mol L}^{-1}$  and 40  $\mu\text{mol L}^{-1}$ ; 0.5 mL) or cumene hydroperoxide (cHP) (2.5  $\mu\text{mol L}^{-1}$ , 5  $\mu\text{mol L}^{-1}$ , 10  $\mu\text{mol L}^{-1}$  and 20  $\mu\text{mol L}^{-1}$ ; 0.5 mL) over a 60-day period. **Results:** It was found that the epididymal sperm concentration and motility of the cHP (10  $\mu\text{mol L}^{-1}$  and 20  $\mu\text{mol L}^{-1}$ ) and tbHP (20  $\mu\text{mol L}^{-1}$  and 40  $\mu\text{mol L}^{-1}$ ) groups were significantly decreased as compared to the saline group. Furthermore, in these groups when compared to the saline group, the superoxide dismutase (SOD) concentration and glutathione (GSH) activity were also decreased, whereas the lipid peroxidation (LPO) increased in both testicular tissue and epididymal sperm. In addition epididymal sperm motility correlated negatively to LPO and positively with SOD and GSH. **Conclusion:** Organic hydroperoxides severely affect the male reproductive system *in vivo* and 60 days of 10  $\mu\text{mol L}^{-1}$  cHP or 20  $\mu\text{mol L}^{-1}$  tbHP treatment can be used to successfully induce OS in a rat model in order to target the complete process of spermatogenesis.

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**Pt2-17****The effects of methoxychlor on development and spermatogenesis of zebra fish**

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**Aims:** To investigate the toxic effects of methoxychlor (MXC) on development and reproduction of zebra fish. **Methods:** Post-fertilized eggs of zebrafish were exposed to MXC (0–30  $\mu\text{mol L}^{-1}$ ), and the hatching rate at 48 h, the malformation ratio at 72 h, the death rate at 96 h, body weight, and the length of zebrafish were measured. Seventy healthy adult male zebrafish were exposed to MXC (0–1  $\mu\text{mol L}^{-1}$ ) for 12 days, and the sperm production and sperm motility were observed. **Results:** Judged by the hatching rate and the malformation ratio, the lowest

effective concentration of MXC for development toxicity was 5  $\mu\text{mol L}^{-1}$ . The hatching rate and the malformation ratio were 36.61 and 36.61%, significantly higher than those of DMSO control group (16.96%, 1.79%, respectively) after 5  $\mu\text{mol L}^{-1}$  MXC treatment. The LD50 of MXC on the zebra fish embryos was 22.24  $\pm$  2.85  $\mu\text{mol L}^{-1}$ . At dose of 0.1  $\text{nmol L}^{-1}$ , MXC significantly reduced the spermatogenesis and immobilized sperm motility. While at dose of 1.0  $\mu\text{mol L}^{-1}$  spermatogenesis were completely inhibited, and > 1  $\text{nmol L}^{-1}$  MXC caused spermary atrophy. **Conclusion:** MXC may cause toxic effects on both development and male reproduction of zebra fish. Male sperm motility and spermatogenesis of zebra fish are more sensitive to the inhibition of MXC.

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**Pt2-18****Exposure to inhaled nanoparticle-rich diesel exhaust disrupts regulation of testicular function in adult male rats**

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**Aim:** To investigate the effects of nanoparticle-rich diesel exhaust (NR-DE) on reproductive function. **Methods:** Eight-week-old male F344 rats were divided into 12 experimental groups and exposed to either whole NR-DE at low (15.37  $\text{g/m}^3$ ,  $2.27 \times 10^5$  particles/cm<sup>3</sup>), middle (36.35  $\text{g/m}^3$ ,  $5.11 \times 10^5$  particles/cm<sup>3</sup>), or high (168.84  $\text{g/m}^3$ ,  $1.36 \times 10^6$  particles/cm<sup>3</sup>) concentrations or clean air for 4, 8, or 12 weeks (5 h/day, 5 days/week). **Results:** NR-DE exposure for 4 or 8 weeks did not affect body weight; however, body weight was significantly decreased in rats exposed to low- or high-concentration NR-DE for 12 weeks compared to the control group. Relative weights of testes, epididymis, seminal vesicles, and prostate had increased non-significantly in all NR-DE-exposed rats at 4, 8, and 12 weeks. Adrenal gland relative weights were significantly increased at 4 weeks in rats exposed to low-concentration NR-DE. Plasma luteinizing hormone and follicle-stimulating hormone concentrations did not change significantly. Plasma testosterone concentrations were significantly increased after exposure to low- or middle-concentration NR-DE for 4 or 8 weeks than in controls. Plasma immunoreactive (ir-) inhibin concentrations were significantly increased after exposure to high-concentration NR-DE for 4 weeks or middle- or high-concentration NR-DE for 12 weeks than in controls. Testicular testosterone concentrations were significantly increased at 4, 8, and 12 weeks after exposure

to low-concentration NR-DE than in controls. In contrast, with exposure to low- or high-concentration NR-DE, testicular ir-inhibin concentrations were significantly greater than in controls, but only at 4 weeks. **Conclusion:** These results suggest that NR-DE inhalation disrupts the endocrine activity of the male reproductive system.

This study was supported by Environmental Development Fund from Ministry of the Environment, Government of Japan.

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#### Pt2-19

##### Endocrine disruptive effects of 3-methyl-4-nitrophenol isolated from diesel exhaust particles in Hershberger assay using castrated immature rats

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**Aim:** To examine the endocrine disruptive effects of 3-methyl-4-nitrophenol (4-nitro-m-cresol; PNMC) in diesel exhaust particles (DEP). **Methods:** Castrated 28-day-old immature male rats were implanted with a 5-mm-long silastic tube containing crystalline testosterone and injected with PNMC subcutaneously at doses 1, 10, or 100 mg kg<sup>-1</sup> for 5 consecutive days. **Results:** The weights of the livers significantly decreased in the 10 and 100 mg kg<sup>-1</sup> PNMC treatment groups as compared with the control group. The weights of the seminal vesicles significantly increased in the 10 mg kg<sup>-1</sup> PNMC treatment group as compared with the control group. The weights of the Cowper's glands were significantly increased in 1 mg kg<sup>-1</sup> PNMC treatment group compared with the control group. The concentrations of plasma testosterone significantly increased in the 10 and 100 mg kg<sup>-1</sup> PNMC treatment groups. **Conclusion:** These results suggested that PNMC induced accumulation of bioactive testosterone released from the implanted tube in circulation. Plasma follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels significantly decreased under all the doses in the PNMC treatment groups, indicating that PNMC acts on the hypothalamus-pituitary axis.

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#### Pt2-20

##### Seminal plasma zinc level may be associated with the effect of cigarette smoking on sperm parameters

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**Aim:** To study the effects of cigarette smoking on seminal plasma zinc level and sperm parameters, and to analyze the role of seminal plasma zinc in the effect of cigarette smoking on sperm parameters. **Methods:** Semen samples were obtained from 115 patients referring to the andrology laboratory of First Hospital, Jilin University (Jilin, China), and were categorized into four groups: normal zinc nonsmoker ( $n = 34$ ), abnormal zinc nonsmoker ( $n = 28$ ), normal zinc smoker ( $n = 29$ ) and abnormal zinc smoker ( $n = 24$ ). A questionnaire was voluntarily filled out by patients to investigate cigarette smoking frequency, which showed 62 non-smokers, 7 light smokers (< 5 cigarettes/day and < 5 years), 16 moderate smokers (5–20 per day, 5–10 years), and 30 heavy smokers (> 20 per day and > 10 years). Seminal plasma zinc concentration was measured by spectrophotometer. Sperm concentration and motility were detected by computer assisted sperm analysis (CASA). Semen analysis was performed manually according to the WHO criteria (4th edition, 1999). The sperm morphology analysis using semiautomatic sperm morphology analyzer was performed manually according to the strict criteria (Kruger TF, *et al*, 1988) after Diff-Quik staining. **Results:** Statistical analysis using Mann-Whitney test showed that seminal plasma zinc level was significantly lower in smokers than in non-smokers. A significant negative correlation was observed between seminal plasma zinc level and extent of smoking ( $r = -0.273$ ,  $P < 0.05$ ). Sperm concentration, motility and morphology among smokers were significantly lower in comparison to non-smokers ( $P < 0.05$ ). Sperm concentration, motility and morphology among abnormal zinc smokers were significantly lower than normal zinc smoker ( $P < 0.05$ ), and were significantly lower than normal zinc nonsmokers and abnormal zinc nonsmokers ( $P < 0.01$ ). There is no significant difference in sperm parameters between normal zinc nonsmokers and abnormal zinc nonsmokers ( $P > 0.05$ ). **Conclusion:** These findings suggest that cigarette smoking may have the negative correlation with seminal plasma zinc level, and make sperm concentration, motility and morphology declined. Seminal plasma zinc is associated with a decrease of anti-oxidant defenses. Seminal plasma zinc level may be associated with the effects of cigarette smoking on sperm parameters.

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#### Pt3-01

##### Study on correlation between seminal plasma activity (spermatozoa lactate dehydrogenase isoenzyme) and male infertility

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**Aim:** To study the relationship between activity of seminal plasma or spermatozoa lactate dehydrogenases isoenzyme

(LDH-X) and male Infertility. **Methods:** The methods of polyacrylamide gel electrophoresis (PAGE), enzyme-linked staining, densitometry scanning, *etc.*, were used to study LDH-X activity of seminal plasma and spermatozoa obtained from 40 fertile men and 83 infertile men. Major semen parameters were measured and analyzed according to WHO laboratory manual. **Results:** The activity of seminal plasma LDH-X ( $826.0 \pm 47.3 \text{ U L}^{-1}$ ) of infertile group was much higher than that of fertile group ( $614.0 \pm 22.1 \text{ U L}^{-1}$ ) ( $P < 0.05$ ). The activity of spermatozoon LDH-X ( $8.40 \pm 3.50 \text{ mU } 10^{-6}$ ) in infertile group was significantly lower than that of fertile group ( $15.8 \pm 10.8 \text{ mU } 10^{-6}$ ) ( $P < 0.05$ ). The rates of seminal plasma LDH-X and total spermatozoon LDH-X of two groups were statistically different ( $P < 0.01$ ). There was no difference in regards of sperm concentration, motility, a + b grade motility between two groups. **Conclusion:** These results suggested that the measurement of LDH-X activity may be used as a reliable marker for testing the quality of spermatozoa and diagnosing the male sterility, which is helpful to select the ways of assisted-production technology (ART).

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### Pt3-03

#### Screening of 'Y' chromosome microdeletions in Iranian infertile males

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**Background:** It has been hypothesized that microdeletions of Yq may account for a significant proportion of men with infertility. Three non-overlapping regions, referred to as "azoospermia factors" (AZFa, b, c from proximal to distal Yq) have been defined as spermatogenesis loci, and deletions in these regions have been shown to be pathogenically involved in male infertility associated with azoospermia or severe oligospermia. **Aim:** To evaluate the frequency of Y chromosome microdeletions in Iranian population. **Methods:** Fifty infertile men were selected. Semen analysis was carried out, and on the basis of the mean sperm count, all patients were categorized into azoospermia and oligozoospermia groups. Blood samples were obtained for DNA extraction and chromosomal analysis. Genomic DNA was extracted from blood lymphocytes and amplified by sequence tagged sites-polymerase chain reaction (STS-PCR) method to determine the presence of micro deletions in AZF locus. Thirty-four STS primers including two controls were selected to identify micro deletions of Y chromosome on each subject. **Results:** Of the 50 cases, 26 (52%) showed deletion of at least one of the STS Marker, and an overall 41 microdeletions were observed. Seventeen cases (34%) had deletion in one STS. Four oligospermia cases (8%) had deletion in two STS sites, and three azoospermia cases (6%) also had deletion in two STS sites, but in different STSs. One case had three deletions in three STS sites, and one individual had seven deletions in AZF locus. **Conclusion:** The overall frequency of Y chromosome microdeletions observed in the present study was found to be 26/50 (52%). Comparison of our data with the result of other investigators world wide shows that the incidence

of Yq microdeletions in Iranian population is much higher than international frequency. Our data agrees with other studies regarding microdeletions of AZFc, but for microdeletions of AZFa (14.6%) our results is much higher and differ significantly with many studies.

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### Pt3-04

#### Implementing comprehensive quality control in human sperm bank

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**Aim:** Establish a laboratory quality management system (QMS) in human sperm bank in order to produce valid and reliable examination results and to improve the technology and management in laboratory. **Methods:** In human sperm bank, the core technology is collecting and storing human sperms from sperm donors. The QMS of human sperm bank was established in accordance with the characteristics of human sperm bank and ISO 15189 (the International Organization for Standardization, ISO). First, it was the preparation for QMS and training. All staffs in our laboratory were well trained and had ready access to those relevant parts of the management system. they must be familiar with and strictly follow the relevant SOPs; Second, the documentation of QMS were wrote out, including the Quality Manual, the Program File, standard operating procedures (SOP), and quality and technical records. Especially, internal quality assurance and quality control were well planned; Third, comprehensive quality control procedures were integrated into the routine analysis workload. The routine work of human sperm bank included: 1) examination of sperm donors; 2) collection and delivery of sperm samples; 3) labeling the sample; 4) assessment of sperm parameters; 5) records; 6) frozen and thawed procedures 7) supplying frozen sperm; 8) Follow-up; 9) archives management. Last, QMS was verified by checking, reviewing, and monitoring work activities, with documentation by experienced, qualified individuals. **Results:** Human sperm bank of Guangdong has established comprehensive QMS. We provided standardized training for technicians and ensure that all technicians remain current on the knowledge and skills needed. Internal quality control was also well performed in our laboratory, including analysis of sperm parameters, laboratory examination of genetics and infectious disease and frozen-thawed sperm technology, *etc.* **Conclusion:** QMS of human sperm bank enables to identify, measure, control and improve the various core technology processes that will ultimately improve technology performance, and ISO15189 is helpful in establishing QMS in human sperm bank.

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### Pt3-05

#### The association between the Y chromosome microdeletion and male infertility

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**Aim:** To explore the association between Y chromosome microdeletion and male infertility. **Methods:** A total of 40 patients in Wuxi (Jiangsu province, China) including nine idiopathic azoospermia, 31 severe idiopathic oligozoospermia, and 41 healthy male controls were evaluated for the microdeletion of Y chromosome. With multiplex PCR technique, an analysis of 12 sequence tag sites (STS) in AZFa, AZFb and AZFc microdeletion was performed. **Results:** Y chromosome microdeletion was observed in 6 of the 31 males with severe oligozoospermia and in three of the nine males with azoospermia, but no such deletion was observed in the 41 normal fertile men. The two patterns of microdeletion were identified including AZFa+AZFb+AZFc and AZFc Y chromosome microdeletion. **Conclusion:** Y chromosome microdeletion might be closely related to male infertility resulting from spermatogenic failure.

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#### Pt3-06

##### Experimental study on effects of PGE1 on testis function following ischemical reperfusion

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**Aim:** To explore the effects of PGE1 on the reproductive function of testis following ischemical reperfusion injury and to investigate its possible molecular mechanism. **Methods:** Ischemical reperfusion injury pathological model was constructed with SD rats. Thirty rats were randomly divided into three equal groups: control group (Group A,  $n = 12$ ), treatment group (Group B,  $n = 12$ ) and no-treatment group (Group C,  $n = 12$ ). Forty-five days later, all rats were killed and immediately weighed. Their organ coefficient was calculated and the testis length was measured. Meanwhile the diameter of contorted seminiferous tubular was detected through ocular micrometer. **Results:** In the PGE1 treatment group, the changes of the testis structure were almost the same as those in group A, but much lighter than those in group C. Under microscope: the pathological changes of group B are almost the same as those in group A, but are much lighter than those in group C. There was no significant difference between the organ coefficient of group B compared with that of group A ( $P > 0.05$ ). But it was much higher than that of group C ( $P < 0.05$ ). There was no significant difference in seminiferous tubular diameter between group B and group A ( $P > 0.05$ ), but it was much higher than that in the group C ( $P < 0.05$ ). **Conclusion:** PGE1 can relieve the pathological changes induced by ischemical reperfusion and can be applied to improve testicular function after torsion or transplantation of the testis.

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#### Pt3-08

##### The identification of microRNAs and their putative targets that are differentially expressed in fertile men and infertile men with Sertoli cell-only syndrome

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**Aim:** This study was conducted to identify the profiling of miRNAs and their putative targets differentially expressed in the testes of normal spermatogenesis and Sertoli cell-only syndrome (SCOS). **Methods:** Testicular samples with normal spermatogenesis and SCOS were used for study. Total RNA was isolated from the testicular tissue for miRNA array (Agilent microRNA Human v2) and cDNA microarray (Agilent Human 1 cDNA Microarray) analysis. miRNA or genes with significant differential expressions were identified by *t*-test analysis and more than 2-fold changes. Up-expression of miRNAs and down-expression of genes in SCOS group were evaluated. By using bioinformatics tool, the putative mRNA targets of individual miRNAs were predicted. After cross-matching of the predicted mRNA targets from miRNAs and mRNAs from cDNA microarray were performed, further validation of miRNA and their putative targets were carried out. **Results:** A total of four miRNAs were found to be up-expressed in SCOS specimen including hcmv-miR-UL70-3p, hsa-miR-136, hsa-miR-630 and hsa-miR-663. Of the four miRNAs, the latter three have been shown to have putative target genes in human. On the other hand, a total of 223 genes were found to be down-expressed in SCOS specimen by cDNA microarray analysis. A cross-matching analysis of miRNA targets and genes from microarray showed that hsa-miR-136 has eight putative targets, hsa-miR-630 has nine putative targets and hsa-miR-663 has 11 targets. Validation was done by real time RT-PCR, and significant differences were found between the fertile group and SCOS group in miRNA-136 ( $P = 0.0039$ ), miRNA-630 ( $P = 0.0005$ ) and their target genes ILF2 ( $P = 0.0023$ ), TLE1 ( $P = 0.0008$ ), FBXW5 ( $P = 0.0049$ ) and GLG1 ( $P = 0.0201$ ). **Conclusion:** Our results indicated that miRNA-136 and -630 are up-regulated and some of their putative target genes are down-expressed in SCOS testis. Aberrant expression of differentially expressed miRNAs might result in dysregulation of spermatogenic genes that may ultimately lead to SCOS.

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#### Pt3-09

##### Relation between chromosomal variation and reproductive abnormality

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**Aim:** To explore the relation between chromosomal variation and reproductive abnormality. **Methods:** We examined chromosome



of 1 856 patients who had reproductive abnormality, and then retrospectively analyzed chromosomal variation including centric region and SAT-zone adding, secondary constriction adding in D/G group, pericentric inversion of 9chr and long arm adding or reducing of Ychr. **Results:** Ninety-six patients had chromosomal variation. The detection rate was 5.2%. Among the patients, 66 had clinical situation (68.8%). The detail information were shown as following: 1) big Ychr: 32 patients, detection rate 1.7%, 22 patients had clinical situation, and the constituent ratio was 33.8%; 2) small Ychr: 12 patients, detection rate 0.6%, 10 patients had clinical situation, and the constituent ratio was 15.4%; 3) inv(9): 17 patients, detection rate 0.92%, 12 patients had clinical situation, and the constituent ratio was 18.5%; 4) ps+: 19 patients, detection rate 1.0%, 13 patients had clinical situation, and the constituent ratio was 20.0%; 5) qh+: 16 patients, detection rate 0.9%, 9 patients had clinical situation, and the constituent ratio was 13.8%. **Conclusion:** Chromosomal variation relate closely to reproductive abnormality.

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#### Pt3-10

##### Identification and functional analysis of spermatogenesis-related genes in testis

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**Aim:** To screen genes involved in testis spermatogenesis and to investigate expression pattern and function of these genes in spermatogenesis. **Methods:** The transcription profile of mouse testis was determined by comparing samples of mice at the different postnatal stage using Affymetrix Genechip mouse microarrays (430.2) and novel spermatogenesis-related genes in testis were screened. The expression pattern and function of these genes in spermatogenesis were identified by bioinformatics analysis, RT-PCR, immunohistochemistry, in situ hybridization (ISH) and western blot. **Results:** In mining the microarray data, 2 058 genes were found to be expressed in a gradually increased manner in the developmental stages of mice testis. Eight novel testis-specific genes were isolated in mice and human testis, which were named *TSC21*, *TSC24*, *TSC29*, *TSC31*, *TSC77*, *TSC279*, *TSG23*, *TSBAG* and *TSCP*, respectively. These eight genes were exclusively expressed in mice testis and their gradually increased expression began in the mice testis of around day 18. *TSC21*, *T279* and *TSG23* were mainly located in mouse testicular seminiferous spermatocytes and spermatids. The expression of *TSC21*, *T279* and *TSG23* significantly decreased in testis from the busulfan-induced azoospermia mice, cryptorchidism and azoospermic patients. **Conclusion:** These results indicated that these genes might be involved in the process of testis spermatogenesis.

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#### Pt3-11

##### The molecular mechanism and experimental treatment of azoospermia

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**Aim:** To investigate the molecular mechanism of azoospermia and to explore a possible method for the treatment. **Methods:** The semen quality of adult male and the incidence of male infertility in Shenzhen city were retrospectively analyzed. Karyotypic studies on azoospermia and character of Y chromosome microdeletion were analyzed. Genes involved in testis spermatogenesis were screened by microarray technique. Expression pattern of these genes in human and mouse testis, and their differential expression between normal male and the patients with azoospermia were analyzed by cytogenetic and biochemical techniques. Roles of these new genes in testis spermatogenesis were analyzed by gene knock-out and RNAi techniques. Spermatocyte maturation *in vitro*, the committed differentiation of stem cells, and allogeneic grafts of testis were carried out to develop new methods for the treatment of spermatogenic arrest. **Results:** The results indicated that the semen quality of adult male in Shenzhen city was lower than the average level of the country, and the semen quality of andrology outpatient was lower than the level of some parts of the country. The karyotypic studies of 3 300 male infertility patients showed that the incidence rate of chromosome abnormality was 6.6%, of which 22 cases were firstly reported in the world, and the incidence rate of Y chromosome microdeletion was 9.8%. By microarray technique, eight new testis-specific genes involved in spermatogenesis were identified, of which the expression of seven genes were not detected in azoospermic patients's testis. By *in vitro* culture systems, spermatocytes from mouse and rat testis were differentiated to elongating spermatids, human testicular round spermatids to elongating spermatids, and rat bone marrow derived mesenchymal progenitor cells to spermatogenic cells. After grafted under the skin of castrated nude mice, the grafts from neonatal mouse testis were grown well and the spermatogenesis could be observed. Spermatozoa derived from neonatal mouse testes grafted into immunodeficient mice were injected into mouse oocyte by intracytoplasmic sperm injection, and the zygote could be developed into an embryo. **Conclusion:** Spermatogenic cell *in vitro* maturation and stem cell differentiation is hopeful for the treatment of male infertility with spermatogenic arrest.

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#### Pt3-12

##### Long-term observation on blood parameters and semen characteristics in somatic cell cloned bulls

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**Background:** Since the first production of SCNT cattle in 1998, more than 550 somatic cell cloned cattle have been produced in Japan. Although so many cloned cattle have been produced in the world, concerns about health status of these animals have been stressed by consumers. **Aim:** To examine health status of somatic cell cloned bulls. **Methods:** Blood parameters and semen characteristics of two somatic cell cloned bulls were observed since August, 2003. One cloned bull was derived from ear cell (born at September, 1998) and the other was derived from intramuscular preadipocyte cell (born at December, 2000). Two of each somatic cell cloned bulls (Japanese Black) and conventionally bred bulls (Japanese Black and Holstein) were compared during the observation. Their blood was taken from caudal vein once a month. Hematology analyses concerning hematocrit, white blood cell, red blood cell, platelet and hemoglobin were carried out using particle counter (Erma Inc., PCE-170, Tokyo, Japan). Serum biochemistry parameters including glutamic oxalo acetic transaminase, creatinine, alkaline phosphatase, blood urinary nitrogen, albumin, phosphorus, calcium, serum protein and glucose were assayed by DRY-CHEM (Fuji film, 3500-V, Tokyo, Japan). Semen was collected once a week by an artificial vagina and its characteristics such as ejaculate volume, motile spermatozoa, sperm concentration and pH were evaluated. Fertility of cloned bulls was also confirmed by artificial insemination (AI). **Results:** We could not find out any anomaly in blood parameters in cloned bulls. On every semen collection, efficient intensity of libido/sexual desire and sexual behavior were observed in both somatic cell cloned bulls. Thus, no special operation of semen collection for these clones was required even in summer. Semen characteristics of cloned bulls were within the same range of conventionally bred bulls. Several AI trials demonstrated fertility of two cloned bulls. Concerning the progeny, their gestation period, birth weight and growth curve were within the normal range of conventionally bred calves. The facts observed in the present study support a hypothesis that somatic cell cloned cattle, which are surviving to adult, would be considered as physiologically normal animals. **Conclusion:** No abnormal data could be found out in two cloned bulls and their progeny during the present study. The result does not contradict with conclusions of risk assessments on the food safety of somatic cell cloned animals and their progeny released by the Food and Drug Administration of the United States of America (2008), the European Food Safety Authority (2008) and the Food Safety Commission of Japan (2009).

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#### Pt3-13

##### Prediction of sperm retrieval with artificial neural network

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**Background:** Follicle stimulating hormone (FSH), Inhibin B, seminal plasma biochemistry and leptin have been used to predict the outcome of sperm retrieval directly or indirectly, but there isn't any marker can be very reliable. **Aim:** To predict sperm retrieval results with artificial neural network (ANN). **Methods:** Data from 66 patients with azoospermia within the FSH range 1.3 to 23.6 mIU mL<sup>-1</sup> and testis volume > 5mL were analyzed. All the patients underwent multiple bilateral testicular biopsies. Factors including (1) FSH, (2) serum leptin, (3) semen volume, testis volume, PH, seminal plasma fructose and  $\alpha$ -glycosidase were also recorded. One diagnostic model with FSH formed S1, four ANN diagnostic models formed ANN1-ANN4. ANN1 takes factor (1) as input variable, ANN2 with (1)(2), ANN3 with (1)(3) and ANN4 with (1)(2)(3) as input variables respectively. Feed-forward backprop method was used to train the ANN models. Next, we tested the four ANN models prospectively using another 66 cases, the predictive accuracies of the ANN models were compared by receiver operating characteristic (ROC) curve analysis. **Results:** Of the 132 patients, 73 (55.3%) can retrieve Spermatozoa. ANN2 had the largest area under the curve (AUC), ANN4 second, then ANN1, S1 and ANN3. The sensitivity was 94%, 83%, 83%, 85%, 61%, respectively; the specificity was 93%, 93%, 90%, 68%, 90%. The AUC of ANNA2 (0.97) was significantly greater than those of S1 (0.88,  $P < 0.05$ ), ANN1 (0.90,  $P < 0.05$ ) and ANNA3 (0.84,  $P < 0.05$ ). **Conclusion:** Anyone of the factors (1)(2)(3) as a single parameter didn't have a desirable accuracy which can only be improved by combined use with artificial neural network. At 90% and 95% sensitivity, combined use of leptin and FSH had the best specificity.

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#### Pt3-14

##### The expression of Omi in the sperm of infertile patients and its relationship with apoptosis

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**Aim:** To explore the pro-apoptosis effect on the sperm of varicocele and leukocytospermic patients by determining the level of Omi and to analyze the relationship between apoptosis and Omi. **Methods:** Forty-two varicoceles and 40 leukocytospermic patients' sperm samples were obtained as pre-operation or pretherapy group, and 30 IVF sperm samples were taken as control. Samples were re-collected three months after varicocelectomy and three months after the elimination of the WBC. The Omi in sperm were detected by Western-blot. Sperm apoptosis rate was assessed using AnnexinV/PI staining. **Results:** In pre-operation varicocele group, the Omi increased significantly compared with control ( $0.88 \pm 0.54$  vs.  $0.18 \pm 0.07$ ,  $P = 0.006$ ). And after operation it decreased significantly ( $0.47 \pm 0.28$ ,  $P = 0.013$ ). In pretherapy leukocytospermic group, the Omi increased significantly compared with control ( $1.73 \pm 1.18$  vs.  $0.18 \pm 0.07$ ,  $P = 0.000$ ). And after treatment it decrease significantly ( $1.12 \pm 0.55$ ,  $P = 0.037$ ). In pre-operation varicocele group,

the sperm apoptosis rate increased significantly compared with control ( $11.78\% \pm 5.21\%$  vs.  $3.35\% \pm 1.33\%$ ,  $P = 0.031$ ). After operation it decreased significantly ( $8.23\% \pm 2.29\%$ ,  $P = 0.047$ ). In pretherapy leukocytospermic group, sperm apoptosis rate increased significantly compared with control ( $14.83\% \pm 7.56\%$  vs.  $3.35 \pm 1.33\%$ ,  $P = 0.008$ ). And after treatment it decreased significantly ( $9.64\% \pm 3.31\%$ ,  $P = 0.041$ ). There was a significant correlation between the Omi and sperm apoptosis rate in pre-operation varicoceles group ( $r = 0.787$ ,  $P = 0.004$ ), which was maintained after operation ( $r = 0.648$ ,  $P = 0.025$ ). The Omi and sperm apoptosis rate in pretherapy leukocytospermic group also had a significant correlation ( $r = 0.861$ ,  $P = 0.001$ ), so did after treatment in leukocytospermic group ( $r = 0.672$ ,  $P = 0.025$ ). There was no significant correlation between the Omi and sperm apoptosis rate in control group ( $r = 0.423$ ,  $P = 0.052$ ). **Conclusion:** The Omi in sperm increases significantly both in varicocele and leukocytospermic patients. The Omi level has significant correlation with sperm apoptosis rate and maybe an important initiator of sperm apoptosis.

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#### Pt3-15

##### The serum and seminal plasma leptin levels in athenospermia

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**Aim:** To analyse the differences of serum and seminal plasma leptin levels between those with athenospermia and control, and to explore the relationships between leptin level and sperm motility. **Methods:** Thirty-four men assessed with athenospermia and 15 men as control were enrolled in this study. All of those with athenospermia underwent a thorough investigation of clinical history, physical examination, and accessory examinations necessary. All the participants were confirmed without definite cause such as chronic prostatitis or varicocele. After sexual abstinence for 2–7 days, each participants provided a semen specimen into a sterile plastic container by masturbation. The diagnosis standard of the athenospermia was according to the standard of WHO. The semen specimens underwent computer assisted semen analysis (CASA) after liquefaction. The cell-free seminal plasma was obtained after centrifugation ( $3\ 000\ g\ min^{-1}$  for 10 min) and stored at  $-80^{\circ}C$  until assay. Blood samples were collected and serum was separated after centrifugation and stored at  $-80^{\circ}C$  until analysis. The serum and semen leptin were measured using commercial RIA kits obtained from Fu Rui biotechnology corporation. **Results:** Patients with and without athenospermia had no significant difference of body mass index (BMI) between groups ( $P = 0.346$ ). There was no significant difference between the serum leptin levels of the patients with athenospermia and the control ( $P = 0.084$ ). The seminal plasma leptin levels were significantly higher in those with athenospermia ( $P = 0.013$ ). The serum plasma leptin level in patients with athenospermia was inversely related to the semen plasma leptin level ( $\gamma = -0.455$ ,  $P = 0.007$ ), but there was no

correlation between the serum plasma leptin level and the semen plasma leptin level in the control ( $P = 0.722$ ). **Conclusion:** The seminal plasma leptin level is significantly higher in those with athenospermia. Maybe the seminal plasma leptin level can be used to explore the abnormality of sperm motility. Leptin may have some local effects in regulating the sperm motility with a complex pathway. It is still a problem whether the risk factors are the environment of the sperm storage or factors in the mature progress of the sperm.

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#### Pt3-16

##### Cloning of Cashmere goat synaptonemal complex protein 3 and time determination of first round meiosis in testes development

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**Aim:** To cloning and sequencing the synaptonemal complex protein 3 gene (*Scp3*), and explore the first round meiosis occurred in male Cashmere goats via detecting *Scp3* gene expression, which will pave the way for further study on Cashmere goat prepubertal testes development. **Methods:** Preparation of total RNA from Cashmere goat testes; RT-PCR for amplification of the goat *Scp3* gene; T-A cloning for construction of recombinant DNA; analyzing the nucleotide homology by sequence alignment; RT-PCR techniques were used to clone the coding sequence of the gene; transcription analysis of the *Scp3* gene in the Cashmere goats prepubertal testes were performed by using testicular tissue on postnatal days 45, 56, 66, 69, 73, 86, 95, 102, 110 and 122. **Results:** Cashmere goat *Scp3* cDNA was cloned and its nucleotide sequence was determined. Sequence alignment suggested that *Scp3* gene of Cashmere goat was 98% homologous to that of bovine. The tissues from postnatal days 56 and 73 to 122 were detected for *Scp3* transcription, whereas the tissues from postnatal days 45, 66 and 69 were not. These results illustrate that the first round meiosis in the Cashmere goat testes occurs mostly behind postnatal day 73. **Conclusion:** we could not define a specific time point for the first round meiosis in Cashmere goat testis because of the difference of ontogeny. The results suggest that the first round of meiosis in analyzed Cashmere goat testes occurs mostly on postnatal day 73. This time period can be used for future studies on Cashmere goat prepubertal testes development. It is the first step towards understanding of spermatogenesis in prepuberty of Cashmere goats.

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#### Pt3-17

##### Cloning of sheep tnp2 gene and transcription analysis of round spermatid cells in the *in vitro* co-culture system

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**Aim:** To cloning the transition protein-2 gene (*tnp2*) transcribed in the Mongolia sheep testes, which have been reported as the round spermatid-specific transcriptional marker gene. And to analyse its transcription in round spermatid cells in the testicular cell co-culture system *in vitro*. **Methods:** Preparation of total RNA from Mongolia sheep testis; RT-PCR for amplification of the partial CDS of *tnp2* gene; T-A cloning for construction of recombinant DNA; bioinformatics methods for analyzing the nucleotide homology; co-culture of the Mongolia sheep testis cells; RT-PCR for detection of the *tnp2* gene expressed in the co-cultured testicular cells. **Results:** After amplification of the partial CDS of *tnp2* gene, the PCR fragment was inserted into the T-A cloning vector pMD19-T. According to DNA sequencing, the length of the cloned partial ORF fragment of *tnp2* was 340 bp, encoding 113 aa residues. The *tnp2* sequence was compared with the counterpart of *Bos taurus*, and the nucleotide homology between them was 95.3%. The primers was designed based on the sheep *tnp2* sequence and used for detection of *tnp2* gene transcription in the co-cultured cells derived from four-month-old sheep testis. It was found the *tnp2* gene transcription occurred in the co-cultured cells from 2 weeks to 10 weeks *in vitro*. **Conclusion:** The partial CDS fragment of the sheep *tnp2* gene has been cloned and analyzed the sequence. This gene was conserved during the evolutionary process, especially in ruminants. The *tnp2* gene transcribes in the sheep testicular cells during co-cultured period *in vitro* until ten weeks. The results demonstrate that the round spermatid cells are generated in the co-cultured system derived from four-month-old sheep testis during the cultured period.

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### Pt3-18

#### Evaluating effective causes in sperm DNA damage: abnormal packing due to underprotamination as a key reason

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**Background:** Sperm DNA integrity is critical for male fertility and its damage contributes to poor embryo development, *etc.* So far, four causes have been indicated for DNA damage in spermatozoa: 1) abortive apoptosis, 2) oxidative stress, 3) poor chromatin packaging or abnormal packing, 4) impairment in recombination during spermiogenesis resulting in cell apoptosis. Among these causes, mostly oxidative stress, poor sperm nuclear protamination and lately, hormones have been addressed. To our knowledge, no human researches have assessed these causes simultaneously. **Aim:** To assess the effective causes of sperm DNA damage collectively. **Methods:** In this study, samples of semen and blood were collected from men undergoing

evaluation for infertility in Avicenna Infertility Clinic (AIC). Sperm analysis was done according to WHO criteria. The men were divided in two groups according to semen analysis **Results:** a male factor infertility group (group 1) ( $n = 81$ ) and a non-male factor group (group 2) ( $n = 52$ ). Serum LH, FSH and Testosterone were measured by chemiluminescence method; in addition, serum DHEA and estradiol were measured by RIA. Sperm DNA damage was examined by Toluidine blue (TB) staining method. Amount of sperm DNA protamination was examined indirectly by chromomycin A3 (CM A3) staining method. Total antioxidant capacity (TAC/oxidative stress index) was measured by spectrophotometry assay. Data were collected and analyzed by SPSS software. **Results:** The FSH, LH, TB and CMA3 were significantly higher in group 1 compared with group 2 ( $9.43 \pm 6.39$  vs.  $5.74 \pm 4.25$  mIU mL<sup>-1</sup>;  $6.53 \pm 3.5$  vs.  $9.73 \pm 4.8$  mIU mL<sup>-1</sup>;  $33.78\% \pm 15.03\%$  vs.  $19.91\% \pm 10.97\%$  and  $46.51\% \pm 16.24\%$  vs.  $31.47\% \pm 12.71\%$ , respectively,  $P < 0.001$ ). Testosterone, DHEA, estradiol and TAC did not show significant difference between the two groups ( $P > 0.05$ ). In preliminary bivariate analyses TB were positively associated with LH ( $r = 0.237$ ,  $P < 0.05$ ), FSH ( $r = 0.237$ ,  $P < 0.05$ ) and CMA3 ( $r = 0.648$ ,  $P < 0.001$ ) but inversely associated with count ( $r = -0.36$ ,  $P < 0.001$ ) and motility ( $r = -0.21$ ,  $P < 0.05$ ). However, Multivariate linear regression for variables showed sole significant relationship between TB and CM A3 ( $B = 0.62$ ,  $P < 0.001$ ). **Conclusion:** Recent studies have mainly focused on oxidative stress and reproductive hormones as the factors involved in damaging sperm DNA, however, the present work illustrates that sperm nuclear underprotamination is the key factor playing role in sperm DNA damage. In addition, entering confounding causes in models *e.g.* sperm concentration is necessary for statistics analysis because with entering confounding causes in models are adjusted their effect. However; additional studies are needed to investigate whether there are relationships between sperm DNA integrity with reproductive hormones and oxidative stress in infertile men.

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### Pt3-19

#### GSTM1 and GSTP1 genes polymorphisms and glutathione S-transferase activity in Iranian infertile men

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**Background:** Pi-GST and Mu-GST classes of glutathione S-transferase (GST) are present on human sperm surface and they play an important role against oxidative stress. Therefore, any defects in the enzyme's activity may be associated with sperm functions defects and male infertility. **Aim:** The present study was conducted to evaluate association between GSTM1 and GSTP1 polymorphisms and enzyme activity with sperm

parameters in Iranian oligoasthenoteratospermic (OAT) and normospermic men. **Methods:** This case-control study involved 95 OAT men and 26 normospermic men as control group. Semen analyses were carried out according to WHO guidelines. DNA was extracted from peripheral blood by salting out procedures. Polymorphisms at GSTM1 gene were evaluated by multiplex PCR and polymorphisms at 5 and 6 exons of GSTP1 gene were determined by PCR-RFLP. Glutathione S-transferase enzyme activity was measured by spectrophotometric method at 340 nm. Sperm parameters, GST genes polymorphisms and enzyme activity were compared between two groups. **Results:** Sperm concentration, morphology and motility were significantly lower in the OAT men compared with individuals on the control group ( $P < 0.001$ ). Frequencies of GSTM1 null genotype in OAT and normospermic groups were 52.1% and 53.8% respectively. There were no statistically significant differences in spermiogram parameters and enzyme activity in GSTM1 null and positive genotypes in the two groups. There were no statistically significant differences at GST activity in OAT and normospermic men,  $7.43 \pm 2.84$  and  $7.07 \pm 1.68$  nmol/min/ $10^6$  sperm, respectively. All the 121 men in this study had Ile/Ile genotypes at 105 codon of GSTP1. Frequency of normal homozygote (114Ala/Ala), heterozygote (114Ala/Val) and mutant homozygote (114Val/Val) genotypes in OAT men were 81.1%, 17.9% and 1.1% respectively. However in control group they were 88.5%, 11.5% and 0% respectively. **Conclusion:** Total GST activity and spermiogram parameters are not affected by deficient GST activity in GSTM1 null genotype. Compensate activity of other GST isozymes in sperm, like GSTP1, may justify the cause.

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#### Pt3-20

##### The b2/b3 subdeletion shows higher frequency of complete AZFc deletion than the gr/gr subdeletion in a Chinese population

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**Aim:** To further investigate the roles of partial deletions in spermatogenic failure and the relationship between the complete and partial AZFc deletions. **Methods:** Y chromosome AZFc deletion typing and Y chromosome haplogrouping in 756 idiopathic infertile Han-Chinese and 391 healthy Han-Chinese were performed. **Results:** We found that both the b2/b3 partial deletion and the DAZ3/41CDY1a deletion pattern were associated with spermatogenic failure. We also confirmed that two previously reported fixations, the b2/b3 deletion in haplogroup N1 and the gr/gr deletion in haplogroup Q1. Remarkably, the frequency of the complete AZFc deletion in haplogroup N1 was significantly higher than that in the haplogroup Q1. **Conclusion:** These results suggest that the b2/b3 partial deletion was associated with a higher risk of complete AZFc deletion compared with the gr/gr partial deletion.

Compared with the gr/gr deletion, the b2/b3 deletion presents a shorter distance among recombination targets and longer recombination substrates, which may be responsible for the increased incidence of subsequent recombination events that can lead to the complete AZFc deletion in this Chinese study population. The susceptibility of the b2/b3 partial deletion to the complete AZFc deletion deserves further investigation in larger and diverse populations, especially those with a relatively high frequency of b2/b3 and gr/gr partial deletions.

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#### Pt3-21

##### Effect of the Estradiol and FSH hormones on regeneration of spermatogenesis after chemoradiotherapy

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**Aim:** To restore spermatogenesis by Estradiol and FSH hormones treatment after chemoradiotherapy. **Methods:** Busulfan is a chemotherapy drug that induced sterility, azoospermia and testicular atrophy. So 20-day-old male C57Bl/6 mice were randomly divided into four equal groups and treated with 30 mg  $\text{kg}^{-1}$  busulfan. After 5 weeks this animals had daily injections (10 days) of 7.5 IU FSH (FSH group), and 12.5  $\mu\text{g}$  estradiol benzoate (EB group), FSH and EB given together (FSH+EB group) and the control group. Then the animals were killed with cervical dislocation and blood samples were taken by cardiac puncture. One testis was fixed and processed for histopathology and the other removed for DNA flow-cytometry to count haploid cells. Expression of c-kit and cyclin B1 genes analyzed after hormonal treatment with RT-PCR. All analyses were performed with the SPSS 13. **Results:** FSH increased number of spermatogonia and serum level of testosterone but it's not significant. EB has given alone induced stimulatory effects on spermatogenesis, increased 2-fold the level of serum testosterone and 2-fold increase of the number of spermatogonia. FSH given with EB multiplied EB stimulatory effects on spermatogenesis up to 4-times of control values. Expression of c-kit and CyclinB1 genes increased in E and FSH+E group and decreased in FSH only treated group in compare with control group. **Conclusion:** These findings suggest that FSH can only exert its direct action on Sertoli cells and regulated Sertoli cell genes. EB regulates spermatogonial stem cells via FSH. FSH given with EB has synergistic effect on regeneration of spermatogenesis. An alternative hypothesis to explain this effect would be the interaction of both hormones with receptor  $\beta$  in Sertoli cells and in spermatogenic cells.

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#### Pt3-22

##### Sperm DNA integrity in men whose partners with history of recurrent pregnancy abortion

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**Aim:** To analyze the relationship between sperm DNA integrity and recurrent spontaneous abortion (RSA). **Methods:** DNA integrity was evaluated by sperm chromatin dispersion (SCD) test in 56 male partners with recurrent pregnancy abortion and 20 healthy fertile men. The patients were subdivided into three subgroups, depending on their reproductive outcome during the 2-year-following-up study: pregnancy group which includes 29 patients who achieved a successful pregnancy; abortion group which includes 16 patients who experienced further abortions; and infertile group which includes 21 patients who experienced infertility during the follow-up period. **Results:** No significant differences in percentage of sperms with DNA fragmentation were observed between the RSA males ( $15.6\% \pm 7.3\%$ ) and fertile controls ( $13.9\% \pm 4.2\%$ ). The percentage of sperms with DNA fragmentation in pregnancy group, abortion group and infertile group was  $12.7\% \pm 2.8\%$ ,  $14.6\% \pm 6.3\%$  and  $18.7\% \pm 9.7\%$  respectively. Significant differences were observed between infertile group and control fertile ( $P < 0.001$ ), while no significant differences were observed among pregnancy group, abortion group and the fertile men ( $P > 0.05$ ). **Discussion:** The etiologies of RSA are multi-factorial. However, except for cytogenetic abnormalities in peripheral blood karyo-typing being the one clear factor, the impacts of the other male factors on RSA still remains uncertain and inadequate researches have been done. Among the reported studies on biochemical markers of male infertility, much attention has been given to sperm DNA fragmentation. DNA damage may be caused by defective chromatin packaging during spermiogenesis, apoptosis during either spermatogenesis or sperm transport through the male genital tract, or as a consequence of oxidative stress. The SCD test is a relatively new technique to assess sperm DNA fragmentation. It was demonstrated that the halo size, which may represent the degree of nuclear DNA damage, could be estimated with a conventional bright-field microscope using the Wright stain. Our data showed that there was no significant difference in sperm traditional parameters such as concentration, forward motility, and normal morphology in RSA men with respect to controls. We demonstrate that there was no significant difference in sperm DNA integrity between RSA men and health men. This result is inconsistent with the results from other researches on relationship between sperm DNA fragmentation and RSA. Patient selection criteria may be one of the reasons to explain these contradictory conclusions. And another reason probably is that the SCD test is more accurate than other sperm DNA fragmentation assays which used in other studies. In present study, we demonstrate that the patients who experienced infertility had significantly lower values of sperm motility, sperm morphology, and sperm DNA integrity than that of health men. Study of DNA integrity should be done in the evaluation of cases of RSA. **Conclusion:** The abnormal of sperm DNA integrity may correlate to further infertility in RSA patients.

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### Pt3-23

#### Study on sperm Y-chromosome microdeletions of patients with idiopathic oligospermia

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**Aim:** To explore the reason of gene idiopathic oligozoospermia by investigating the Y-chromosome microdeletions with idiopathic oligozoospermia patient. **Methods:** A total of 120 men were involved in the study and divided into two groups: 60 consecutive men who presented with clinical and laboratory data indicating idiopathic oligospermia, mean male age was  $32.1 \pm 2.8$  (rang 29–36 years) (group 1); the control group (group 2) included 60 men having normal seminal parameters was  $31.0 \pm 2.2$  years (rang 28–35 years). The semen and lymphocytes Y-chromosomal microdeletions of blood was performed and checked by PCR amplification of selected regions of the Y-chromosome. Multi-PCR and PCR-RFLR were used to analyze the sperm deletion of DAZ gene copies in the AZFc region of Y-chromosome. Chromosoma quantity and construction were detected by G-band in the two groups. **Results:** In the severe oligozoospermic patients and fertile males, the rates of AZFc deletion was 15%, and no other type Y-chromosomal microdeletions was found, but no deletion was detected in the normal control group. There are significantly differences in the semen Y-chromosomal microdeletions between the two groups. The rates of oligospermia AZFc microdeletion (15%) were higher than lymphocytes. **Conclusion:** The frequency of sperm Y-chromosomal microdeletions is higher than that of serum lymphocytes Y-chromosomal microdeletions in patients with oligospermia, which suggests that Y-chromosomal microdeletions might be important genotic causes sperm density abnormal and male spermatogenesis failure.

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### Pt3-24

#### The relationship between sperm preparation and sperm morphology

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**Aim:** To evaluate the relationship between sperm preparation and the novel sperm morphology indices, including sperm deformity index (SDI) and teratozoospermia index (TZI). **Methods:** Sperm morphology indices from 79 infertile husbands (experimental group) and 20 fertile men (control group) were analyzed by modified Papanicolaou staining according to the criteria recommended by the World Health Organization before and after sperm preparation. Furthermore, the experimental group were divided into four groups respectively according to the rates of abnormal sperm morphology ( $\leq 85\%$ ,  $86\%–90\%$ ,  $91\%–95\%$  and



96%–100%), SDI ( $\leq 1.20$ , 1.21–1.40, 1.41–1.60 and  $> 1.60$ ) and TZI ( $\leq 1.20$ , 1.21–1.40, 1.41–1.60 and  $> 1.60$ ) of the unprepared semen. **Results:** Our data show that there were no significant differences in the rates of abnormal sperm morphology, SDI, and TZI between before and after sperm preparation in the group which the rates of abnormal sperm morphology is  $\leq 85\%$  ( $P > 0.05$ ). In the group of TZI  $\leq 1.20$ , no significant differences were found in the rates of abnormal sperm morphology and TZI between before and after sperm preparation ( $P > 0.05$ ). Similarly, significant differences were not observed between before and after sperm preparation in the rates of abnormal sperm morphology in the group of TZI  $> 1.60$  ( $P > 0.05$ ). In all the other groups, after sperm preparation, the rates of abnormal sperm morphology, TZI and SDI including the control group were significantly decreased ( $P < 0.05$ ). Importantly, the two novel sperm morphology indices, SDI and TZI showed statistically significant differences between before and after sperm preparation ( $P < 0.05$ ). **Conclusion:** Sperm preparation can improve sperm quality greatly and it will help to increase the success rate of assisted reproductive technology. The novel sperm morphology indices, including sperm deformity index (SDI) and teratozoospermia index (TZI) are useful predictors in identifying infertile men.

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#### Pt3-25

##### A novel sperm cells separation system isolating the best sperm cells population for immediate insemination

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**Background:** Seaforia™ Sperm Separation System is a complete system intended for sperm preparation procedure in a natural way without mechanical handling, with no need of any additional laboratory equipment/technician, which can be used in the doctor's office, in clinics and in laboratories. The system is simple to operate, requires no preliminary training, and the procedure can be performed by doctors and healthcare professionals. The system is based on the original swim-up technique, with the culture medium layered over liquefied semen. During an incubation of 30 min, the motile spermatozoa migrate from the lower semen layer into the upper culture medium, collected in a fraction suitable for immediate insemination. **Aim:** To introduce the novel Seaforia™ Sperm Separation System and to evaluate its efficacy. **Methods:** The laboratory test plan for evaluating the efficacy of the novel Seaforia™ Sperm Separation System was controlled by Lotus Bio™ staff. Twenty-one volunteers of random population donated semen samples. Microscopic spermatozoa examinations of the pre- and post-separation fractions, was performed according to the World Health Organization criteria, and included evaluation of spermatozoa concentration, percentage of motile spermatozoa, percentage of progressively motile spermatozoa and a morphology evaluation. A *t*-test was performed to analyze the spermatozoa parameters pre- and post-separation. **Results:** About 50% of the inserted motile spermatozoa were isolated

using Seaforia™ Sperm Separation System. About 90% of the isolated spermatozoa were motile, with 84% having progressive motility. Normal spermatozoa morphology in general, and spermatozoa head morphology specifically, were significantly improved post separation. A significant decrease in round non sperm cells and a non significant result of the total number of progressively motile spermatozoa in the post separation fraction compared to the original semen were demonstrated. **Conclusion:** At the end of the separation procedure, the medium, which initially contained no spermatozoa, becomes spermatozoa enriched. The isolated spermatozoa population is characterized by progressive motility and a high percentage of normal morphology, separated in a simple to operate, cost effective system. These features demonstrate that the novel Seaforia™ Sperm Separation System is an excellent vehicle for selectively isolating in a natural way the best spermatozoa population with the highest potential to fertilize the ovum using insemination. Since the procedure can be performed in the doctor's office, the Seaforia™ sperm separation procedure can be conjugated to a following insemination.

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#### Pt3-26

##### Multi-center evaluation of sperm quality in China

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**Aim:** To obtain routine parameters of sperm of males from representative areas of China and evaluate the sperm quality. **Methods:** In all the representative areas a uniformed setting was prepared including detecting instruments, reagents, detection criteria and staff training. Fertile males were required to cease sex activity 2–7 days before sperm collection with reference to WHO guidelines. Fertility was defined as the ability to cause the female pregnant, expressed as a pregnancy probability of 90% within 100 days for the Han ethnic males and 85% within 6 months for the males of Minority ethnics. The data were collected and preserved in form of digital video. To control the quality of detection, each sample was re-analyzed by two technicians independently before the final report was presented. **Results:** In 15 areas, a total of 1 884 samples were collected. The volume ranged between 2.11–4.02 mL. The proportion of sperm of the a, b, c, and a + b levels were 7.62%–11.73%, 14.32%–19.75%, 20.15%–26.59%, and 21.25%–31.19%, respectively. The sperm count was  $29.23\text{--}85.37 \times 10^6 \text{ mL}^{-1}$  and sperm total number was  $63.58\text{--}305.48 \times 10^6$  per ejection. Sperm of normal morphology accounted for 37.46%–48.88% of the total. **Conclusion:** The data of sperm quality of fertile males of China, uniquely preserved in the form of digital video for each volunteer, will provide a baseline for further comparative studies and evaluations.

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**Pt3-27****Frequency of single nucleotide polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene in oligoasthenoteratozoospermic (OAT) and normozoospermic men**

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**Background:** The folate cycle is necessary for DNA synthesis and normal cell function which are important for spermatogenesis. Folate deficiency occurs frequently, and known as a risk factor for various diseases, including infertility. Methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes in the folate metabolism and DNA synthesis pathway. MTHFR activity is nearly five times higher in the adult testis than other tissues. A common polymorphism of MTHFR gene is resulting from a cytosine to thymine substitution (C677T). **Aim:** The present study aimed to analyze the association of MTHFR gene C677T polymorphism with male infertility. **Methods:** A total of 266 men with oligoasthenoteratozoospermia (OAT) and 77 normozoospermic men who attended Avicenna Infertility Clinic (AIC) were included in this study. Semen analysis was performed according to WHO guideline. Genomic DNA was extracted from peripheral blood leukocytes. The SNP analysis was performed by PCR-RFLP method using HinfI restriction enzyme. Finally, the results of two groups were compared. **Results:** Analysis of MTHFR gene polymorphism in OAT and normozoospermic men showed that the frequencies of the 677C/C, 677T/T and 677C/T genotype among the OAT group were 57.4%, 6.5% and 36.3%, respectively. Also the frequencies of the 677C/C, 677T/T and 677C/T genotype among the normozoospermic group were 42.9%, 10.4% and 46.8%, respectively. According to the statistical analysis, there was no significant difference in C677T SNP polymorphism of MTHFR gene between normozoospermic and OAT groups ( $P > 0.05$ ). **Conclusion:** There was no significant difference in the frequency of C677T genotype between OAT and normozoospermic men, which indicates that the C677T polymorphisms are not a risk factor for male factor subfertility. The result may reflect that the effect of MTHFR SNP depends on ethnic origin. Studying the effects of other SNP of MTHFR gene and also the gene(s) other than the MTHFR in the DNA methylation pathway is suggested in infertile men.

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**Pt3-28****Comparing seminal plasma markers in normospermic and azoospermic men**

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**Background:** Azoospermia affects more than 10%–15% of infertile male subjects attending to infertility clinic. Presently, testicular biopsy is the gold standard procedure to evaluate spermatogenesis status in azoospermic men. The semen is a non-invasive sample and has proven to be valuable in the evaluation of spermatogenesis. Identification of such marker in semen has great value in treatment of azoospermia. **Aim:** To find these markers by comparing classical and new seminal plasma markers in normospermic and azoospermic men. **Methods:** Semen samples were collected from 200 men of infertile couples attending to Avicenna Infertility Clinic (AIC), Tehran, Iran. Semen samples were analysed according to WHO guideline. The men were divided into two groups: normospermic ( $n = 100$ : group1) and azoospermic men ( $n = 100$ : group 2) based on semen analysis results. Seminal plasma was separated by high speed centrifugation and stored in  $-20^{\circ}\text{C}$  until measurement of markers. Four markers of fructose, neutral alpha glucosidase (NaG), Inhibin B and anti-Müllerian hormone (AMH) were determined in semen. The semen markers for fructose and NaG were measured by spectrophotometric method, while Inhibin B and AMH were assessed by ELISA method. Spermatogenesis status were evaluated by testicular sperm extraction (TESE, both cytological and histopathological evaluation in azoospermic candidates. **Results:** Fructose levels showed no difference between the two groups. However, it was significantly correlated with sperm count ( $P < 0.01$ ,  $r = -0.408$ ). Seminal plasma inhibin B (OR: 1.01, 95% CI: 1.005–1.016), AMH (OR: 1.63, 95% CI: 1.17–2.28) and NaG (OR: 1.07, 95% CI: 1.04–1.10) levels were higher in normospermic subjects compared to azoospermic men. The seminal inhibin B concentration significantly differentiated ( $P < 0.01$ ) between positive and negative outcomes of TESE due to the presence of mature sperm. Inhibin B concentration correlated positively with sperm count and morphology in normospermic group, however, NaG levels correlated with sperm count of normospermic men ( $P < 0.01$ ,  $r = 0.345$ ), and the subjects' age in the two groups. **Conclusion:** Our findings in this study suggest that seminal plasma markers and their correlation with spermatogenesis status may represent a non-invasive marker in azoospermic men who desire to be biologic parent include TESE with ICSI. Also of the four studied markers only inhibin B is correlated with the presence of sperm in testicular tissue. According to variations of inhibin B in other failures of spermatogenesis, high-through techniques such as proteomics is recommended for identification of molecular markers of seminal plasma to definitely evaluate status of spermatogenesis.

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**Pt3-29****Measurement of DNA adducts by Comet assay as a potential prognostic test for intra cytoplasmic sperm injection**

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**Background:** Infertility affects one in six couples in the UK with dysfunctional sperm being one of the most common causes. Conventional semen analysis lacks power as a prognostic test for assisted reproductive treatment (ART). Sperm DNA damage is closely associated with reproductive outcomes so it has the potential to be a useful prognostic test for ART. The alkaline Comet assay is a sensitive method to detect DNA strand breaks in individual sperm. Although this and other sperm DNA fragmentation tests show promise, as yet few clinical thresholds have been established. The importance of oxidative stress as a primary indicator of sperm DNA damage is well established. In addition to discrete strand breaks, oxidants can induce other types of DNA damage, including base loss and modification or adduct formation, the most common of which is 7,8'-dihydro-8-oxy-2'-deoxyguanosine. Potentially mutagenic DNA modifications can also be measured using formamidopyrimidine-DNA glycosylase (fpg), an enzyme which recognizes and converts oxidised purines into DNA strand breaks to be measured by the Comet. **Aim:** To quantify sperm DNA fragmentation and DNA adducts using alkaline Comet assay and determine their relationship with assisted conception outcomes of couples undergoing ICSI. **Methods:** In this study, native semen and sperm prepared by density centrifugation from men attending for ICSI were analysed to detect DNA strand breaks ( $n = 90$ ) and also DNA adducts ( $n = 51$ ). Fully frosted slides were first layered with 150  $\mu$ L normal melting agarose (1.5%) then with a mixture of 75  $\mu$ L low melting agarose (0.75%) and sperm ( $6 \times 10^4$ ). The cells were then lysed in freshly prepared cold lysis solution [2.5 mol  $\text{mL}^{-1}$  NaCl, 100 mmol  $\text{mL}^{-1}$  Na<sub>2</sub>EDTA, 10 mmol  $\text{mL}^{-1}$  Tris, 1% Triton X 100, pH 10] for 1h and dithiothreitol (10 mmol  $\text{mL}^{-1}$ ) for 30 min at 4°C and lithium diiodosalicylate (LIS, 4 mmol  $\text{mL}^{-1}$ ) in room temperature for 90 min to allow DNA unfolding. The enzyme treatment was performed by adding fpg (1  $\mu$ g  $\text{mL}^{-1}$ ) to LIS at 37°C. The slides were placed in a horizontal gel electrophoresis tank filled with fresh electrophoresis buffer (300 mmol  $\text{mL}^{-1}$  NaOH, 1 mmol  $\text{mL}^{-1}$  Na<sub>2</sub>EDTA, pH 13.0) for 20 min to allow DNA unwinding and electrophoresis were performed for 10 min at 25 V/cm and 300 mA. The slides were then rinsed three times with neutralization buffer (0.4 mol  $\text{mL}^{-1}$  Tris, pH 7.5) 5 min each to remove alkali and stained with 50  $\mu$ L of ethidium bromide (20  $\mu$ g  $\text{mL}^{-1}$ ). Fifty images were analysed by an image analysis system using the programme Komet 5.5 (Kinetic Imaging Ltd, Nottingham, UK). **Results:** Sperm from partners of couples who failed to achieve a pregnancy tended to have more DNA fragmentation but it was not significantly greater than in sperm from pregnant couples (net increase of +6.5% semen,  $P = 0.449$  and +4.5% prepared sperm,  $P = 0.259$ ). In contrast, when including the DNA lesions that were converted into strand breaks by the fpg enzyme treatment step, the total level of strand breaks made the two groups markedly different (with a

net increase of +16.0% semen,  $P = 0.016$  and +14.3% prepared sperm,  $P = 0.043$ ). **Conclusion:** This study shows the usefulness of sperm DNA damage testing in predicting pregnancy rates in ICSI couples. The significant additional damage observed by converting DNA adducts to strand breaks indicates the impact of oxidative stress on sperm DNA. By measuring both the DNA strand breaks and the fpg sensitive sites in human sperm, one may obtain a more sensitive prognostic test for assisted conception success in couples with male infertility.

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### Pt3-30

#### The analysis of Y chromosome microdeletions and testicular pathology in patients with azoospermia

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**Aim:** To discuss the meaning of sex hormone evaluation before testicle biopsy by Y chromosome microdeletions in azoospermia factor (AZF) region screen and sex hormone levels test for azoospermia patients. **Methods:** According to the guidelines recommended by WHO, we collected 272 cases with azoospermia. Samples were analyzed using modified multiplex PCR-electrophoresis. 158 patients received testicle biopsy. The following clinical datas were collected for analyzed: age, reproductive history, hormonal levels (total testosterone, FSH and LH) and ultrasound examinations, and physical examination. The 158 cases were divided into two groups according to AZF deletions: A group with AZF deletions and B group with no AZF deletions. group B was further divided into two subgroups according to the result of testicle biopsy: B1 group with spermatozoa and B1 group with no spermatozoa. **Results:** 28 out of 272 cases were found (10.29%) with Y chromosomal microdeletions, in which, 15 patients received testicle biopsy and two patients with AZFc deletion were found with sperm. One hundred and forth-three cases with no AZF deletion received testicle biopsy, and found sperm were found in 83 cases, in which 43 were confirmed with congenital spermatid duct hypoplasia, and 15 had affiliated gonads infection history. In terms of the age, years of infertile, volume of testis, LH, FSH, T, there were no statistics difference in group A and B1; in term of testis volume, LH and FSH. statistics significance was found between group A and B2 and the two subgroup of group B. **Conclusion:** Azoospermia patients with normal clinical phenotypes and chromosome karyotype may have Y chromosome microdeletions, with an incidence rate of 10% or so, and the most common deletion type of AZFc microdeletion. Simple AZFc deletion had less influence on spermatogenesis than other types, On the whole, there would be no sperm in patients with AZFa, AZF or AZFa+b+c microdeletion, testicle biopsy should not be carried

out. We conclude that testicle size and hormone levels can not provide instruction for whether patients with AZF deletions need to carry out testicle biopsy, but may be useful to patients with no AZF deletion.

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### Pt3-31

#### Azoospermia: a laboratory diagnosis of 200 cases

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**Aim:** To explore the cause of azoospermia by experimental analysis. **Methods:** Two hundred samples out of a total number of 303 cases of azoospermia diagnosed in our hospital from March 2005 to May 2009 were reviewed. Our methodologies include CASA with centrifugal smears; seminal fructose, seminal neutral- $\alpha$ -glucosidase with BUS or CFTR gene, epididymal puncture, immediately after ejaculation urine; seminal plasma PMN-Elastase; endocrine hormones; genetic testing (Analysis of chromosome karyotype, Y chromosome microdeletions, SRY) and testicular biopsy, etc. **Results:** Of all the 200 samples, we found 36 (18%) cases of reproductive tract abnormalities, 26 (13%) of which were caused by congenital malformations and 10 (5%) by acquired deformities; 25 (12.5%) cases of reproductive tract infections; 29 (14.5%) cases of endocrine disorders; 61 (30.5%) cases of genetic abnormalities, of which 31 (15.5%) were caused by Klinefelter syndrome, 2 (1%) by Kallmann syndrome and each of following twelve cases accounted for 0.5%: 46xy t(12:15) i(16), 46xy y = 18, 46xy y < 21, 46xx SRY+, 46xy var(1qh+), 46xy var(1qh+)(16 qh+), 46xy t(2:9), 46xy t(1:20), 45xy t(13:18), 48xxxy, 46xy yqh+, 46xy y = 21 respectively accounted for one case (0.5%). In addition, 16 (8%) cases of Y chromosome microdeletions, including two (1%) cases of AZFa, six (3%) cases of AZFb, eight (4%) cases of AZFc; testicular biopsy in 16 cases no sperm production, 8%, and 33 (16.5%) cases of unknown causes. **Conclusion:** Through experiments, this paper gives a clear etiological diagnosis of azoospermia, providing valuable groundings for clinical practice.

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### Pt3-32

#### Effects of Antioxidants and ROS on sperm DNA integrity in Idiopathic infertile men of Indian origin

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**Background:** Abnormalities in the sperm genome integrity are a potential reason for infertility. Sperm DNA maintains its high degree of inertness, stability and integrity by processes as

absence of transcription, replacement by protamine, presence of DNA repair genes and presence of an antioxidant mechanism. Reactive oxygen species (ROS) in pathological concentrations is an important factor challenging the antioxidant and DNA repair machinery of the sperm. ROS damages the lipid in the sperm membrane affecting the motility and morphology and can also induce mutations in the nuclear and mitochondrial genome. Sperms subjected to increased free radical concentrations, have decreased energy production and motility defects which reduces the chance of reaching the female gamete, more over the sperm oocyte interaction is also affected by the oxidation induced changes in the sperm membrane structure. The antioxidant machinery comprising of both the enzymatic and non enzymatic components regulate the free radical concentration in physiological levels. The imbalance between the anti oxidant and free radicals generated either due to increased free radical or lower activity or decreased expression of antioxidants produces an oxidative environment in the sperm. The structural biomolecules as well as the nuclear and mitochondrial genome in this oxidative stress are prone to oxidation which leads to disruption in their functions. Oxidation of nucleotides produces single strand and double strand DNA breaks, abasic sites and result in inaccurate transmission of genetic material by compromising genetic integrity. **Aim:** The current study was designed to study the effects of ROS and antioxidant levels on the sperm DNA integrity. **Methods:** Semen samples from 97 idiopathic infertile patients and 52 normozoospermic healthy controls were examined. Level of DNA damage was assessed by Comet assay, ROS by chemiluminescence and seminal antioxidants (Superoxide Dismutase-SOD, Catalase-Cat, Glutathione-GSH) by biochemical methods. Semen analysis was done according to WHO 1999 guidelines. Statistical analysis was done using Mann-Whitney test. **Results:** On the basis of sperm parameters the infertile men were divided into two groups- one with the abnormal sperm pathology (Group 1) and the other with normal sperm parameters (Group 2). In group 1 the mean sperm concentration is  $6.7 \pm 2.19$  million/ml, progressive motility is  $38.65 \pm 4.37$ , abnormal sperm morphology is  $42.37 \pm 3.18$ . In group 2, mean sperm concentration is  $23.03 \pm 1.34 \times 10^6 \text{ mL}^{-1}$ , progressive motility is  $57.39 \pm 3.71$ , abnormal sperm morphology is  $20.59 \pm 4.29$ . ROS levels were significantly higher in group 1 and 2 as compared to controls ( $P = 0.018$ ,  $P = 0.053$ , respectively). Seminal SOD and catalase was significantly lower in both group 1 and 2 ( $P = 0.012$ ,  $P = 0.037$ , respectively) as compared to controls. There was no significant difference in the levels of seminal glutathione in group 2 patients as compared with controls ( $P = 0.062$ ) whereas group 1 patients had significantly lower glutathione ( $P = 0.029$ ). The number of sperms with high DNA damage was significantly greater in both the patients groups as compared to controls ( $P = 0.009$ ,  $P = 0.017$ ). **Conclusion:** There is accumulating evidence linking sperm nuclear DNA anomalies to elevated ROS, lowered antioxidants and poor reproductive outcome. Antioxidant supplementation may help to protect spermatozoa from oxidative stress induced damage and thereby help to improve the rate of conception and successful pregnancy. Oxidative stress induced paternal DNA damage leads to pathological sperm parameters, poor quality blastocysts (during assisted conception), less than

optimal pregnancy rates, and increased chances of miscarriages, which gives emotional and psychological trauma to the couples experiencing it. Therefore assessment of oxidative stress should be included in the examination of men with idiopathic infertility.

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### Pt3-33

#### Study of azoospermia factor microdeletions in the patients whose Y chromosome are shorter than 22 chromosome

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**Aim:** To observe the microdeletions of azoospermia factor on Y chromosome in the patients whose Y chromosome is shorter than 22 chromosome. **Methods:** The subjects of this study are 47 patients with Y chromosome no longer than 22 chromosome. They are divided into two groups: 12 cases in group A whose sperm concentration are less than  $10 \times 10^6 \text{ mL}^{-1}$  and 35 cases in group B whose sperm concentration are more than or equal with  $10 \times 10^6 \text{ mL}^{-1}$ . By multiplex PCR technique, an analysis of 15 sequence tag sites was performed. **Results:** In the total 47 cases, four cases showed AZF microdeletions, the deletion rate was 8.51%; All the four cases had been found in A group. The total deletion rate was 33.33%, and the prevalence rates of microdeletion in AZFa, AZFb, AZFc and AZFd were 0%, 25%, 33.33% and 33.33%, respectively. Three of the four microdeletion cases were azoospermia, and the deletion rate was 60%. The other one was severe oligozoospermia, and the deletion rate was 14.29%. No microdeletion had been detected in group B, and the deletion rate was 0%. **Conclusion:** In the male with Y chromosome shorter than or equal with 22 chromosome, the rate of AZF microdeletion in those whose sperm concentration was more than  $10 \times 10^6 \text{ mL}^{-1}$  was very low. But the azoospermia and severe oligozoospermia patients had a great opportunity with AZF microdeletion. Especially in the azoospermia, the risk of accompanying with multiple areas of AZF microdeletion is notable.

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### Pt3-34

#### The use of the swim-up method to separate spermatozoa with DNA fragmentation

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**Background:** Previous studies observed that the degree of sperm DNA damage has negative association with various indices of fertility such as fertilization rate, embryo cleavage rate, implantation rate, pregnancy and live birth rate. The swim-up technique is frequently used for sperm preparation. Previous studies have addressed the variations in DNA

integrity of sperm in swim-up fractions. Several techniques are available to determine sperm DNA integrity, such as terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL), Comet assay and sperm chromatin structure assay (SCSA). Most of these methods require complex or expensive instrumentation such as flow cytometry and fluorescence microscopy. Sperm chromatin dispersion (SCD) test is a simple method which was developed recently in detecting DNA integrity of sperm. SCD test is performed by conventional bright-field microscopy. The SCD test is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA following acid denaturation and removal of nuclear proteins. It is confirmed recently that the results of SCD test is highly correlated with TUNEL assay and SCSA. **Aim:** To investigate the efficiency of swim-up preparation technique in eliminating spermatozoa with DNA fragmentation detected by sperm chromatin dispersion (SCD) test for infertile patients in intrauterine insemination (IUI) treatment. **Methods:** Analysis of sperm parameters including sperm concentration, sperm motility, and morphology was performed both before and after swim-up in 32 infertile men undergoing an IUI cycle. The SCD test was performed to evaluate the DNA fragmentation of sperms both before and after swim-up. The SCD test was performed following the procedure of Fernandez, with some changes in the lysing solution and in test procedure. The relationship between sperm DNA fragmentation and IUI outcome was assessed. **Results:** Compared with original semen, there was a significant ( $P < 0.05$ ) decrease in sperm DNA fragmentation after swim-up procedure. DNA fragmentation was  $22.8\% \pm 6.9\%$  in original semen samples and decreased to  $9.23\% \pm 4.95\%$  after swim-up. In the 32 patients, eight achieved clinical pregnancy, four had pregnancy loss, while 20 did not get pregnant. The DNA fragmentation of sperm in original semen of pregnant patients was lower than that of non-pregnant patients and pregnancy loss patients. However, no significant difference was detected in DNA fragmentation of sperm after swim-up procedure between pregnant patients and non-pregnant patients and pregnancy loss patients. **Conclusion:** The DNA fragmentation of sperm in original semen is correlated to pregnancy outcome in IUI, while the DNA fragmentation of sperm after swim-up procedure is not associated with pregnancy outcome in IUI. Swim-up procedure could separate and eliminate spermatozoa with DNA fragmentation in IUI treatment for infertile patients.

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### Pt3-35

#### Comparative study of two types of sperm treatment

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**Aim:** To compare two types of sperm treatment. **Methods:** Sperm samples came from Zhejiang Human Sperm Bank from March 2008 to July 2008. The 55 semen samples were chosen, which never meet the standards of the Chinese Ministry of Health of frozen semen samples. The volume of the ejaculate

was about 3 mL, sperm density  $> 60 \times 10^6 \text{ mL}^{-1}$ , sperm motility (a + b)  $< 60\%$  or sperm density  $< 60 \times 10^6 \text{ mL}^{-1}$ , sperm motility (a + b)  $> 60\%$  were selected for the study. The 55 semen samples were randomly divided into two groups: centrifugal immediacy group (30 samples) and centrifugal with sperm washing group (25 samples). After frozen and thawed, the semen of two groups were routinely analyzed accordance with WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, including pH, volume, sperm density, and sperm motility (a + b). The cryosurvival of spermatozoa = (a + b)% after thawing/(a + b)% before freezing. **Results:** In centrifugal immediacy group, sperm density raised from  $(46.52 \pm 15.01) \times 10^6 \text{ mL}^{-1}$  to  $(86.50 \pm 30.91) \times 10^6 \text{ mL}^{-1}$ , and sperm motility (a + b) descended from  $(58.00 \pm 14.18)\%$  to  $(44.33 \pm 16.39)\%$  after centrifugal; In centrifugal with sperm washing group, the sperm density raised from  $(44.44 \pm 12.31) \times 10^6 \text{ mL}^{-1}$  to  $(85.96 \pm 28.09) \times 10^6 \text{ mL}^{-1}$ , and sperm motility (a + b) descended from  $(57.76 \pm 13.59)\%$  to  $(46.48 \pm 17.49)\%$  after centrifugal. There was no significant difference between the two groups ( $P > 0.05$ ). After frozen and thawed, in centrifugal immediacy group, sperm density was  $(56.48 \pm 25.80) \times 10^6 \text{ mL}^{-1}$ , sperm motility (a + b) was  $(19.71 \pm 11.45)\%$  and sperm motility (a + b) recovery rate was  $(0.42 \pm 0.15)\%$ ; in centrifugal with sperm washing group, sperm density was  $(54.96 \pm 24.90) \times 10^6 \text{ mL}^{-1}$ , sperm motility (a + b) was  $(19.04 \pm 13.52)\%$ , sperm cryosurvival was  $(0.39 \pm 0.21)\%$ . There was no significant difference between the two groups ( $P > 0.05$ ). **Conclusion:** After centrifugal, both group can achieve the same effects: a marked improvement of sperm density, but a decline in sperm motility, which may be due to the damage of the sperm by centrifuge. There was no significant difference between the two groups. The two approach to achieve the same result, and centrifugal immediacy do not need to spend sperm washing liquid, low-cost, so we prefer to use this method for processing. It should be noted that this approach more suitable for low density and high activity of the semen samples for achieving a good density and sperm motility after freezing.

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### Pt3-36

#### Relative analysis of Follicle stimulating hormone (FSH) and pathology of testis biopsy in 267 patients with azoospermia

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**Aim:** To evaluate the significance of FSH for judging the spermatogenesis function of testes and to study the correlation between Johnsen's score and FSH level through testis biopsy in 267 azoospermatic patients. **Methods:** A total of 267 azoospermatic patients with single testis volume no less than 10 mL were retrieved and their serum value of FSH, luteinizing hormone (LH) and total testosterone (TT) were measured before testicular sperm aspiration (TESA). We drew tight the skin of

scrotum and fixed the testis with thumb, index finger and middle finger of left hand. The skin of scrotum, superficial fascia and albuginea testis were anesthetizing with 1% lidocaine at first and the testis was punctured with 20 mL injector. Modicus contorted seminiferous tubules were aspirated from different sites of the testis. Refined blood vessel forceps were inserted into the testis along the orifice and we clipped a bit of contorted seminiferous tubules out of the testis if they cannot be aspirated. Johnsen's score of the biopsy was evaluated after operation. According to Johnsen's score, 267 cases were grouped into A (score  $\geq 7$ ) & B (score  $< 7$ ). The cases whose Johnsen's score  $\geq 7$  can be treated with the technique of intracytoplasmic sperm injection (ICSI) or vasovasostomy. The patients's prognosis will be worse if Johnsen's score  $< 7$ . **Results:** According to Johnsen's score, 187 cases of the patients (70%) were enrolled in group A. The average age of group A was  $30.5 \pm 4.8$  years and average volume of their double testes was  $37.1 \pm 7.3$  mL. The average value of LH was  $4.4 \pm 1.8 \text{ IU L}^{-1}$ , FSH  $4.6 \pm 2.7 \text{ IU L}^{-1}$  and TT  $15.3 \pm 5.3 \text{ nmol L}^{-1}$ . The other 80 cases were in group B. The average age of group B was  $29.3 \pm 4.2$  years and average volume of their double testes was  $33.4 \pm 7.53$  mL. The average value of LH was  $5.0 \pm 1.84 \text{ IU L}^{-1}$ , FSH  $9.1 \pm 6.7 \text{ IU L}^{-1}$  and TT  $13.3 \pm 5.7 \text{ nmol L}^{-1}$ . The FSH value of group A was significantly different from that group B ( $P < 0.01$ ). There was no significant difference in the average age, volume of testes, LH and TT value between two groups ( $P > 0.05$ ). An overall of 183 cases (97.9%) of group A had a FSH level less than  $10.0 \text{ IU L}^{-1}$  while 52 cases (65%) were found in group B to had FSH level less than  $10.0 \text{ IU L}^{-1}$  ( $P < 0.01$ ). **Conclusion:** FSH value is a promising parameter in judging the spermatogenesis function of testes; however, misinformation will be presented to judge the spermatogenesis function taking into consideration with the FSH level exclusively. It is suggested that FSH value and Johnsen's score of testis biopsy by TESA are better clues to judge spermatogenesis function of testes. Judging the spermatogenesis function of the testes with the level of LH and TT is inefficient.

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### Pt3-37

#### Clinical analysis of the concentration determination of fructose, alpha glycosidase, ACP and $\text{Zn}^{2+}$ in the seminal plasma of 350 cases infertile men in Qingdao area, China

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**Aim:** To study the relationship between the concentration of fructose, alpha glycosidase, ACP and  $\text{Zn}^{2+}$  in the seminal plasma of infertile men and male semen quality and sexual gland function. **Methods:** A total of 350 cases infertile men were from male and urologic out-patient clinic of our hospital from January to December 2008, the age of patients was from 22 to 53 years old and the average age was 31.6 years old. When sexual abstinence for about 3 to 7 days, semen specimen of patients were collected. Semen analysis was performed on patients with oligospermia or astenoospermia



or azoospermia, semen samples were stored at  $-20^{\circ}\text{C}$ . The concentration of fructose, alpha glycosidase, ACP and  $\text{Zn}^{2+}$  were determined in 1 week. **Results:** Of the 350 cases plasma, 45 cases diagnosed with seminal vesiculitis were fructose decrease or lack, of which four had azoospermia and ultrasound showed seminal vesicle dysplasia or absence, implying that fructose has certain effect on the development and function of seminal vesicle. At the same time, of the 350 cases plasma, 53 cases were alpha glycosidase decrease, of which 11 cases were accompanied with fructose decrease, implying that males with oligospermia, astenoospermia or azoospermia may have seminal vesicle dysfunction accompanied with epididymis dysfunction. Twenty cases had increase in ACP activity and ultrasound showed prostatitis. Three cases had increase in  $\text{Zn}^{2+}$  concentration and this may be related to seafood rich in  $\text{Zn}^{2+}$  in Qingdao. **Conclusion:** From the analysis of the concentration determination of fructose, alpha glycosidase, ACP and  $\text{Zn}^{2+}$  in the seminal plasma, we conclude that fructose, alpha glycosidase and ACP are associated with male sexual gland development and function and affect semen quality, whether  $\text{Zn}^{2+}$  effects semen quality or not needs to be further studied.

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### Pt3-39

#### The relationship between leukocyte in semen and sperm motion parameters

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**Aim:** To study the relationship of leukocyte concentration in semen and sperm motion parameters. **Methods:** Sperm motion parameters were analyzed by computer-aided sperm analysis, and leukocytic number of Semen was measured by benzidine-peroxidase staining. **Results:** The Curvilinearvelocity (VCL), linearvelocity (VSL) and average path velocity (VAP) of nonleukocytic spermic group is  $45.3 \pm 11.5 \mu\text{m s}^{-1}$ ,  $29.7 \pm 5.6 \mu\text{m s}^{-1}$ ,  $33.9 \pm 9.3 \mu\text{m s}^{-1}$ , respectively. However, The VCL, VSL and VAP of leukocytic spermic group is  $30.4 \pm 10.7 \mu\text{m/s}$ ,  $18.3 \pm 6.4 \mu\text{m/s}$ ,  $22.8 \pm 4.1 \mu\text{m/s}$ , which is markedly lower than that of nonleukocytic spermic group, respectively ( $P < 0.01$ ). It is a negative relative between semen leukocyte concentration and VCL, VSL and VAP ( $r = -0.366$ ,  $P < 0.01$ ;  $r = -0.356$ ,  $P < 0.01$ ;  $r = -0.363$ ,  $P < 0.01$ ), respectively. **Conclusion:** Leukocyte concentration of Semen could affect sperm motion parameters.

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### Pt3-40

#### Quercetin protects embryonic chicken spermatogonial cells from oxidative damage intoxicated with 3-methyl-4-nitrophenol

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**Aim:** To investigate the protective effect of quercetin, an antioxidant flavonoid, on oxidative damage of testicular cells of embryonic chickens after treatment with 3-methyl-4-nitrophenol (PNMC) derived from diesel exhaust particles (DEP). **Methods:** Testicular cells from 18-day-old embryos were cultured in serum-free McCoys'5A medium and challenged with PNMC ( $10^{-7}$ – $10^{-5}$  mol  $\text{L}^{-1}$ ) alone or in combinations with quercetin ( $1.0 \text{ g mL}^{-1}$ ) for 48 h. **Results:** Exposure to PNMC ( $10^{-5}$  mol  $\text{L}^{-1}$ ) induced condensed nuclei and vacuolated cytoplasm, a decrease in testicular cell viability and spermatogonial cell number. Exposure to PNMC induced lipid peroxidation by an elevation of thiobarbituric acid reactive substances as well as decreasing glutathione peroxidation activity and superoxide dismutase activity. However, simultaneous supplementation with quercetin restored these parameters to the similar levels as the control. **Conclusion:** PNMC is therefore concluded to have induced the oxidative stress of the spermatogonial cells. Dietary quercetin may attenuate the negative effects of PNMC to restore intracellular antioxidant system in testicular cells. The present results support the therapeutic use of quercetin in the prevention or treatment of the reproductive toxicity by environmental toxicant PNMC.

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### Pt3-41

#### Effects of semen processing on generation of reactive oxygen species and mitochondrial membrane potential of human spermatozoa

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**Aim:** To evaluate the effects of semen processing on generation of reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) of spermatozoa, and to find some reliable indexes for the evaluation of sperm quality during sperm preparation. **Methods:** The samples were divided into three groups: oligoasthenoteratozoospermia (OAT), eukocytospermia, and normal group. Both swim-up and density gradient

centrifugation (DGC) methods were used to separate semen respectively. Before and after semen selection, the levels of ROS and MMP of the same samples were measured by flow cytometry. Relativity analysis was conducted between the measurements and routine semen parameters. **Results:** Before preparation the patients with abnormal semen parameters had significantly lower MMP, and higher ROS, and there was a negatively correlation between MMP and ROS ( $P < 0.05$ ). Apparent improvement of sperm parameters (except concentration) was noticed after processing, especially by DGC in OAT group; the levels of MMP and ROS (apart for decrease of ROS in leukocytospermia group,  $P < 0.01$ ) of selected sperms increased significantly, especially ROS when using swim-up method. Significant difference was found between the correlation of MMP and total normal motile sperm count after sperm preparation with two different methods in OAT group. And the level of ROS was also associated with the amount of white blood cells in leukocytospermia group. **Conclusion:** The MMP is a reasonable parameter to evaluate the isolated sperm quality in OAT group, while combines with the level of ROS could be used to assess the efficiency of sperm preparation in patients with leukocytospermia. These findings can guide us to choose the ideal sperm separation technique for different sperm samples.

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#### Pt3-42

##### Investigation of a spermatozoa concentration home testing kit

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**Aim:** To develop a spermatozoa concentration home testing kit for man who could conveniently obtain an assessment of fertility potential and to evaluate the accuracy and specificity of the kit. **Methods:** After sieving sperm by solid-phase of the cellulose membrane, sperm were stained by the specific blue dye which compared testing card to the standard color code as to determine the concentration of sperm. Results of 1 000 samples with this kit were compared with the results of WHO recommended reference method-microscope counting. The accuracy and specificity of the kit results were analyzed by kappa test. **Results:** Compared with microscope counting, results of the kit have higher consistency. The accuracy of the kit was about 97.6%; the specificity of the kit was about 97.4%, sensitivity of the kit was about 97.8% (Kappa = 0.9520, ASE = 0.0097,  $u = 98.351$ ,  $P < 0.0001$ ). **Conclusion:** Comparison of spermatozoa concentration of fast self testing kit and reference method of microscope counting has no significant difference. It has been shown that the kit can effectively estimate the concentration of spermatozoa of semen sample at home.

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#### Pt3-43

##### Does mountain biking affect sperm quality?

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**Background:** Biking is a high performance sport practiced in some mountainous regions in Venezuela. Although in some industrialized nations this practice has been associated with male infertility, evidence for this condition has not been reported in our country. **Aim:** The objective of this study was to evaluate the seminal quality and androgen levels in Venezuelan mountain bikers compared to a group of healthy subjects. **Methods:** Clinical and epidemiological data related to infertility and the practice of mountain biking were recorded. Testosterone was determined by ELISA and semen analysis was performed according to WHO guidelines. Chi square test were used to compare differences between groups. **Results:** Association between testicular trauma, perineal paresthesia and mountain biking was shown. There were no differences in seminal physical variables, testosterone levels, sperm and leukocyte count between groups. Grade “a” motility and sperm morphology were lower in bikers but these differences were statistically not significant. **Conclusion:** Our results indicated that mountain biking seems to affect neither sperm quality nor steroidogenesis, but could interfere in sexual health.

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#### Pt3-44

##### Isolating and cloning testis-expressed microRNAs

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**Background:** Small RNAs constitute a large family of regulatory molecules with diverse functions in eukaryotes. MicroRNA (miRNA) is one class of small RNAs. MiRNAs, which are small non-coding RNAs approximately 21 nucleotides in length have been identified as potent regulators of gene expression. Mammalian miRNAs are proposed to regulate approximately 30% of all protein-coding genes. Spermatogenesis is a complicated process by which the male gametes, spermatozoa, are produced. MiRNAs may be participated in translational repression during spermatogenesis. If miRNAs play critical roles in spermatogenesis, aberrant small RNA expression may be participated in male infertility, and agents that can mimic small RNA function may be used as future male contraceptives. Cloning testis-expressed miRNAs would be the first step toward these ultimate goals. **Aim:** We attempted to isolate and clone miRNAs from the rat testis using a new cloning method. **Methods:** Low molecular weight RNA fraction ( $< \text{or} = 200$  nt) was isolated from Sprague-Dawley (SD) rat testis using a

mirVana™ PARIS and 18- to 26- nt was further isolated by polyacrylamide gel electrophoresis (PAGE) and Electro-elution. The small RNA is dephosphorylated to allow directional ligation of an adapter sequence, first to the 3' end of the RNA, after PAGE purification of ligated products and rephosphorylation of the 5' terminus, ligation of a second adapter to the 5' end. Reverse transcription was performed to generate small RNA cDNAs, which were then amplified using PCR. The amplified product was cleaved by Not I to allow the concatamerization of multiple PCR products before cloning into plasmid. The terminal products were directly subcloned into pMD18-T vector for sequencing analyses. DNA sequences were analyzed by Vector NTI Suite v.9.0 to locate small RNA sequences in the cloning vector. The small RNA was then mapped to the rat genome using the BLAST program in the UCSC Genome Browser. Northern blot is used to confirm the *in vivo* expression of particular miRNA. **Results:** 18- to 26-nt was effectively isolated by polyacrylamide gel electrophoresis (PAGE) and Electro-elution from low molecular weight RNA fraction (< or = 200 nt). A total of 45 clones were subsequently characterized through DNA sequencing and database searching; 15 clones out of 45 clones were identified as miRNAs. Northern blot analysis confirmed miR-16 and miR-741 were stably expressed in testis. **Conclusion:** We have isolated and cloned miRNAs from rat testis and identified parts of the functional ones, which imply that miRNAs may play important role in spermatogenesis.

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#### Pt3-45

##### Expression profiling of hypoxia-inducible factor-1 in the testes of infertile men

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**Background:** Spermatogenesis is highly ordered and requires considerable oxygen consumption and glycolysis. When cells meet with hypoxia, a subset of genes involved in oxygen homeostasis is rapidly over-expressed in order to adapt the hypoxic condition. Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is one of the most important factors that responses to the change of oxygen homeostasis. **Aim:** The object of this study was to unravel the expression profiling of HIF-1 $\alpha$  in the human testis, and to determine the relationships between the HIF-1 $\alpha$  expressions and the patients' testicular phenotypes and clinical parameters. **Methods:** The protein localization of HIF-1 $\alpha$  in the testis of normal spermatogenesis was determined by immunohistochemical (IHC) staining. In human testis, two HIF-1 $\alpha$  isoforms have been identified, HIF-1 $\alpha$  and HIF-1 $\alpha$ Te. HIF-1 $\alpha$ Te is derived from an alternative promoter-first exon combination, but with a different genomic organization and a different nucleotide sequence. The mRNA transcript levels of HIF-1 $\alpha$  and HIF-1 $\alpha$ Te were determined by quantitative real-time PCR. **Results:** IHC demonstrated that HIF-1 $\alpha$  protein could be mainly detected in interstitial Leydig cells and post-meiotic germ cells in human testes. Real-time RT-PCR showed an

increasing tendency of HIF-1 $\alpha$  transcript levels in patients with spermatogenic failure (maturation arrest and Sertoli cell-only syndrome) ( $P < 0.05$ ) and a significantly decreasing tendency of HIF-1 $\alpha$ Te transcript levels in patients with spermatogenic failure ( $P = 0.003$ ). **Conclusion:** Given HIF-1 $\alpha$ Te isoform has been recognized as a dominant-negative regulator of HIF-1, our results confirmed the association of hypoxia with spermatogenic failure in infertile men.

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#### Pt3-46

##### Data acquisition and analysis of reproduction physiological variables of Chinese male twins

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**Aim:** To establish a database of reproduction physiology of Chinese male twins (5%–15% incidence) for further investigation. **Methods:** We conducted a retrospective cohort study in which basic physiological and reproductive parameters were collected and samples were preserved for further analysis. Five hundred cases were recruited during the first stage of study and data were applied to constitute the database. **Results:** We obtained the following data of the target population: indexes of physiological development, genetically related and circumstance related developmental indexes, life quality indexes, indexes associated with fertility, sex function and productivity. Additionally, samples of blood, urine, and sperm were also preserved. **Conclusion:** Investigation of twins will discriminate genetic and environmental contribution of phenotype observed and has advantages in eliminating biases caused by age and surroundings. This method proved to be a major advance in our study.

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#### Pt3-48

##### Contribution of the biochemical analysis of seminal plasma in the exploration of leucospermia in male infertility

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**Aim:** To evaluate the impact of male inflammatory syndrome on the composition of seminal plasma and to determine the contribution of the biochemical analysis of semen in the



exploration of a leucospermia in infertile men. **Methods:** Retrospective study of 84 infertile patients investigated by a semen analysis and seminal biochemistry as standard procedures of the WHO. Quantification of leukocytes was done after cytochemical staining based on the revelation of the peroxidase (leucoscreen). The biochemical study of semen included the spectrophotometric assay of  $\alpha$ -glucosidase (epididymal marker), citric acid (prostatic marker) and fructose (seminal vesicle marker). We analyzed also the data from clinical records and paraclinical explorations (sperm parameters, seminal biochemistry, spermoculture and urogenital tract ultrasound) looking for signs of genital infection. We have identified three groups of patients: group G1 ( $n = 47$ ) with a low concentration of leukocytes in semen ( $< 100\,000\text{ mL}^{-1}$ ), a second group G2 ( $n = 14$ ) with a concentration in leukocytes between  $100\,000$  and  $500\,000\text{ mL}^{-1}$  and a third group G3 ( $n = 23$ ) with a high leucospermia ( $> 500\,000\text{ mL}^{-1}$ ). Descriptive analysis of data and comparison of means between these 3 groups were processed, correlations between seminal biochemical markers and the number of leukocytes was made by linear regression using the SPSS 11.0 software and significance was considered at  $P < 0.05$ . **Results:** The mean value of fructose was significantly lower in the group G3 than in G1 and G2 ( $P = 0.01$ ) and a significant negative correlation was noted between the values of seminal fructose and the number of leukocytes in semen ( $r = -0.232$ ,  $P = 0.03$ ). The mean value of citric acid was also lower in G3 but the difference between the three groups was not significant ( $P = 0.09$ ). However, the prostate marker was disrupted in 54.4% of patients in G3 (versus 35.7% and 25% in G1 and G2,  $P = 0.06$ ). The mean values of  $\alpha$ -glucosidase were comparable in the three groups ( $P = 0.4$ ). Antecedents of urogenital infection were found in 52% of patients in the G3 group (versus 21.4% and 35.5% respectively in G2 and G1,  $P = 0.1$ ). **Conclusion:** Our results confirm that the inflammatory diseases of the male accessory genital glands are accompanied by a significant decrease of biochemical glandular markers leading to a change in the composition of seminal plasma. The biochemical study of semen in addition to the spermogram should be performed to carry out the diagnosis of infectious and inflammatory diseases of the genital tract in male infertility.

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#### Pt4-01

##### The clinical features of BPH in patients of advanced age and the efficiency of TUVRP

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**Aim:** To evaluate the clinical features of BPH in advanced aged surgical patients over 80 years old and the efficiency of transurethral vaporization resection of the prostate (TUVRP). **Methods:** The clinical procedure of 285 cases of patients treated by TUVRP was retrospectively analyzed and the patients were divided into three groups by age, Group A with 91 cases ( $< 70$  years old), Group B with 127 cases (between 71–79 years old)

and Group C with 67 cases ( $> 80$  years old). **Results:** Prostate volume was  $53.15 \pm 24.15\text{ g}$  in Group A,  $67.83 \pm 39.73\text{ g}$  in Group B and  $60.00 \pm 43.86\text{ g}$  in Group C ( $P < 0.001$ ). There were more Accompanied Systemic diseases of internal medicine in Group C than the other two groups ( $P < 0.001$ ). American Society of Anesthesiologists (ASA) grade was increased with age ( $P < 0.001$ ). Both preoperative and postoperative hemoglobin levels were decreased with age ( $P < 0.01$ ). Accompanied complications associated with BPH, accompanied diseases having impact on urinary function, operating time, IPSS score and QOL index had no significant difference among three groups. Postoperative maximum urinary flow rate and mean urinary flow rate were decreased with age ( $P < 0.01$ ). **Conclusion:** Prostate volume may increase over time in men with BPH below 80 years old but this tendency would be reversed if they were above 80 years old. We should pay more attention to the bladder detrusor function when evaluating the clinical Symptoms and making a therapeutic schedule to advanced aged patients over 80 years. The advanced age is not the surgical contraindication to patients with obvious surgical indication and does not increase difficulty in performing the procedure. However, the advanced age will reduce the physical quality and increase operative risk so that we should improve the preoperative preparation as much as possible. TUVRP can as effectively improve clinical symptoms of advanced aged patient as of younger patients.

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#### Pt4-02

##### Clinical Analysis of postoperative delirium in transurethral resection of the prostate

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**Aim:** To study the influential factors of postoperative delirium in transurethral resection of the prostate. **Methods:** A total of 110 elderly postoperative BPH patients in our hospital, file clinical parameters including age, hypoxemia, postoperative pain, and sleep reduction were investigated, and the data were processed by  $\chi^2$  test. **Results:** Of the total 110 patients, six (5.5%) experienced postoperation delirium. Many factors were significantly correlated with the problem in these elderly BPH patients, such as advanced age ( $\geq 70$  years), postoperative pain and sleep reduction ( $P < 0.05$ ). **Conclusion:** Advanced age, postoperative pain and sleep problem were important factors for delirium, and therefore postoperative analgesia and good sleep may help to prevent postoperative delirium.

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#### Pt4-03

##### A new biomarker, AMACR, high expression in prostate cancer

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**Aim:** To identify expression of alpha-methylacyl-CoA racemase (AMACR) in prostate cancer and to assess viability of AMACR as a tumor marker in clinical setting among Chinese. **Methods:** We used 1D PAGE and two-dimensional gel electrophoresis together with Western Blot to detect the expression of AMACR in prostate cancer. We used immunohistochemical staining for early diagnosis of prostate cancer. A total of 188 cases of formalin-fixed paraffin-embedded prostate biopsies were taken for immunohistochemical examination. Twenty-four prostate specimens, 10 of prostate cancer, 14 of benign prostate hyperplasia, were collected for 1D-, 2D-PAGE and Western blot. We used 2D PAGE, MALDI-TOF MS and MS/MS to detect PSA in benign and malignant prostate tissue. **Results:** Two-dimensional gel electrophoresis separated proteins extracted from needle biopsies into more than one thousand spots and immunoblot showed expression of AMACR in prostate cancer. On 1D PAGE and Western Blot, Among 10 cases with prostate cancer, seven cases showed AMACR positive. All 14 benign cases were negative. McNemar's test showed  $P > 0.5$ , non-significance compared with immunohistochemical methods. The results of Western Blot showed bands looked clear and patterns between positive and negative were definite. Micro foci of prostate cancers could be found when using immunohistochemistry for AMACR. The specialty was 100% and sensitivity was 94.7% (72/76) in immunohistochemical examination. We found PSA expressed both in benign and malignant prostate tissues. **Conclusion:** AMACR was shown to be over-expressed in prostate cancer using independent experimental methods and prostate specimens. AMACR was useful in prostate biopsy for prostate cancer pre-diagnosis among Chinese.

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#### Pt4-05

##### The expression and effects of Aquaporin 3 in human prostate

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**Aim:** To determine the expression and effects of AQP3 in transitional and peripheral zone of human prostate. **Methods:** AQP3 mRNA expression was analyzed in prostate by semi-quantitative RT-PCR. AQP3 protein expression and localization was characterized by Western blotting and immunostaining with polyclonal anti-AQP3 antibody. **Results:** In this study, we found that AQP3 was expressed in transitional and peripheral zone of prostate tissue and the transitional zone their AQP3 mRNA

was most equal to those in peripheral zone. AQP3 protein expression in transitional zone was stronger than in peripheral zone. Immunostaining indicated that AQP3 was localized at the plasma membranes of basal epithelial cells in human prostate. **Conclusion:** AQP3 was expressed in prostate which suggests that AQP3 may play a regulatory role in epithelial cell osmolality and the secretion of prostatic fluid.

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#### Pt4-06

##### The study of high intensity focused ultrasound in the treatment of advanced prostate cancer

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**Aim:** To study the effect of transrectal high intensity focused ultrasound on immune function of patients with advanced prostate cancer and to evaluate the safety and effectiveness of transrectal high intensity focused ultrasound in the treatment of patients with advanced prostate cancer. **Methods:** Forty patients with advanced prostate cancer were treated with transrectal HIFU device (model Sonablate 500). T-lymphocyte subsets CD4+, CD8+, CD4+/CD8+, and cytokines IFN- $\gamma$ , IL-2, IL-4, IL-10 in 10 mL of peripheral venous blood were examined before HIFU treatment, and they were reexamined 2 weeks and 4 weeks after treatment. Forty patients with advanced prostate cancer were treated by transrectal HIFU. The operating time, the time of hospitalization and complications on treatment were recorded. The volume of prostate, the serum PSA,  $Q_{max}$  and I-PSS were examined before and after transrectal HIFU treatment. **Results:** Two weeks and four weeks after HIFU treatment, the percentages of CD4+ and the ratio of CD4+/CD8+ were much higher than those before treatment ( $P < 0.05$ ), while the percentages of CD8+ was significantly lower than before treatment ( $P < 0.05$ ). The levels of cytokines IFN- $\gamma$ , IL-2 were much higher than those before treatment ( $P < 0.05$ ), while levels of cytokines IL-4, IL-10 were significantly lower than those before treatment ( $P < 0.05$ ). There were no significant difference between T-lymphocyte subsets (CD4+, CD8+, CD4+/CD8+) and cytokines (IFN- $\gamma$ , IL-2, IL-4, IL-10) at 2 weeks after HIFU treatment and those at 4 weeks after treatment ( $P > 0.05$ ). The median operating time was 80 min (range 60 to 130 min) and the median time of hospitalization was 7.5 days (range 5 to 18 days). The mean follow-up was 12 months (ranging 6–14 months). No serious complications occurred. After the treatment, the volume of prostate reduced, the serum PSA became lower,  $Q_{max}$  raised and IPSS improved significantly in both groups ( $P < 0.05$ ). Statistically, the difference is significant too. **Conclusion:** Transrectal HIFU therapy can improve the immune function of the patients with prostate cancer. The transrectal HIFU therapy appears to be a safe, efficacious and minimally invasive therapy for patients with advanced prostate cancer. The operating time and the time of hospitalization were very short.

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**Pt4-07****The Construction of the eukaryotic recombinant RB-1 vector and its expression in the prostate cancer cells**

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**Aim:** To construct eukaryotic recombinant RB-1 expression vector and investigate its expression in the prostate cancer cell line of PC-3. **Methods:** The coding sequence of RB-1 was amplified from the plasmid CMV-RB by PCR method. After cloned by FLAG-pcDNA3 vector, the fragments were identified by endonucleases digestion and sequence analysis. Then the recombinant was transfected into PC-3 cell line in cationic liposome, and the expression level was detected by Western blotting. **Results:** The FLAG-pcDNA3-RB expression vector was confirmed completely correct by PCR, endonucleases digestion and DNA sequence analysis, and the recombinant protein was detected in high expression level in PC-3 cell line by Western blotting. **Conclusion:** The eukaryotic expression vector for RB-1 has been successfully constructed and it can efficiently express in PC-3 cell line, which has laid the foundation of further study on the function and mechanism in the prostate cancer cells.

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**Pt4-08****Therapeutic treatment of organic selenium on cancer therapy and its mechanism**Yan-Bo Liu<sup>1</sup>, Li-Juan Zhao<sup>1</sup>, Ya-Xiong Guo<sup>1</sup>, Zuo-Wen Liang<sup>1</sup>, Li-Jing Zhao<sup>1</sup>, Hai-Tao Zhang<sup>3</sup>, Yan Dong<sup>1,3\*</sup>, Xue-Jian Zhao<sup>1\*</sup><sup>1</sup>Department of Pathophysiology, School of Bethune Medicine, Jilin University, Changchun 130021, China<sup>2</sup>Department of Pathophysiology, School of Basic Medicine, Beihua University, Jilin 132013, China<sup>3</sup>Department of Structural and Cellular Biology, Tulan University, New Orleans LA 70062, USA

**Aim:** To investigate the anti-prostate cancer effects of organic selenium. **Methods:** The prostate cancer cell line LNCaP was treated with different doses of selenium (Se). To determine the expression levels of PSA and AR in treated and control cells, the semi-quantitative RT-PCR and Western blot analysis were performed. The inhibition of cell proliferation was detected by SRB. The apoptosis rate and cellular cycles in LNCaP cells were analyzed with flow cytometry. The effect of Se on Stat3 determined with immunocytochemistry and immunofluorescence. To study the effects of Se on prostate tumor with castration *in vivo*, nude mice were imbedded with androgen-dependence prostate cancer LNCaP mass to establish prostate cancer xenografts subcutaneously. Once the tumors reached adequate volumes the mice were operated with castration. At the same time, 0 or 100 µg/20g/day was given by oral gavage for 16 weeks. Tumors were measured once a week. The growth rate and final weight of the tumors were recorded and compared between groups. In addition, the morphologic change and

apoptosis in the cells were determined by HE and TUNEL assays. The expression distribution and levels of AR, Stat3 and Hif-1α were determined with immunohistochemical analysis. In addition, RT-PCR and Western blot analysis were used to detect their expression at mRNA and protein levels. PSA concentration in serum was detected with ELASA assay. **Results:** Se could specifically reduce AR and PSA expressions in the LNCaP cells. The cell proliferation was inhibited by Se with time and dose dependence. Se could promote apoptosis in LNCaP cells. Se could also down-regulate Stat3 expression significantly. The prostate cancer xenograft model with relapse after castration *in vivo* showed that the average weights and volumes of the tumors were lower in Se group than that in controlled group. The tumor cells from Se group had more significant apoptotic cells and lower proliferation rate. The expression of PSA, AR, Stat3 and Hif-1α were down regulated in treated group. ELASA assay results showed that Se could decrease the level of PSA in serum. **Conclusion:** Se could inhibit androgen dependence prostate cancer LNCaP cell proliferation and promote the cell apoptosis *in vitro*. The mechanism might be related with down-regulated AR expression. On the one hand Se could promote prostate cancer cell apoptosis, on the other hand it could delay the genesis of hormone independent prostate cancer.

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**Pt4-09****Relationship between anxiety dysphoria and symptoms in the patients of type IIIA prostatitis**Yu-Ping Fan, Bin Chen\*, Hui-Xing Chen, Hong-Xiang Wang  
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**Aim:** To evaluate the relationship between anxiety dysphoria and symptoms in the patients of type IIIA prostatitis through the chronic prostatitis symptom index (NIH-CPSI) and self-rating anxiety scale (SAS). **Methods:** We analyzed 68 cases of type IIIA prostatitis from November 2007 to March 2008, which had been made definite diagnosis of anxiety by CCMD-3. Their age were between 21–51 (34.38 ± 7.03) years old. The results of NIH-CPSI, SAS were analyzed and their relationship was studied. **Results:** Thirty-six cases had a score of NIH-CPSI higher than nine and 27 cases (75%) had anxiety dysphoria more than mild degree. The T value of SAS are between 51–65 (56 ± 4.5), but the correlation between the T value of SAS and the score of NIH-CPSI was negative. **Conclusion:** The patients of type IIIA prostatitis may have anxiety dysphoria, but there is no correlation between the T value of SAS and the score of NIH-CPSI ( $P > 0.01$ ).

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**Pt4-10****Association between bilateral orchidectomy and metabolic syndrome among males with prostate cancer**

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**Background:** Prostate cancer is one of the most common cancers in aging men. There are lots of patients in China who have to receive bilateral orchidectomy due to the lateness in seeing doctors or economic reasons. The surgery of bilateral orchidectomy results in androgen deficiency. Androgen deficiency is a risk factor for metabolic syndrome. **Aim:** To evaluate the prevalence of metabolic syndrome in men with prostate cancer after bilateral orchidectomy. **Methods:** A total of 100 aged participants were included in this study. MS criteria was defined by modified NCEP—ATPIII. Experimental group were fifty men who received bilateral orchidectomy because of prostate cancer, control group were fifty age-matched men who lived in Weifang Community of Pudong New Area District, Shanghai, China. Height, weight, waist circumference, blood pressure, fasting blood glucose, fasting lipid, total serum testosterone, body mass index (BMI) were measured in all participants. We then analyzed if men who received bilateral orchidectomy because of prostate cancer had more hazard to the control. **Results:** Mean age was similar between the two groups. Men who received bilateral orchidectomy because of prostate cancer had high score in body mass index (BMI), waist circumference, triglyceride (TG) and fasting blood glucose in contrast with control group ( $P < 0.01$ ), but their total serum testosterone and high density lipoprotein (HDL) significantly were lower than control group ( $P < 0.01$ ), and there was no significant difference in blood pressure, cholesterol, low density lipoprotein (LDL) between the two groups ( $P = 0.2$ ). The prevalence of metabolic syndrome was higher in the experimental group compared with control group. **Conclusion:** One year after receiving bilateral orchidectomy, our data suggested that patients had more than 50% to have metabolic syndrome compared to control, predisposing them to higher cardiovascular risk. We recommend prospective studies to further delineate this association.

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#### Pt4-12

##### Effect of post-ejaculatory pain in men with category III chronic prostatitis/chronic pelvic pain syndrome

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**Aim:** To study a group of pain associated with ejaculation in patients with CPPS, and to understand its relationship with other symptoms, quality of life and the risk factors. **Methods:** According to the existence of pain ejaculation baseline and 3 months follow-up link, 490 CPPS patients in the chronic prostatitis cohort study were divided into four groups. Packet benchmarks are: always say "no" recorded as none, basically said "no", but at least have an answer "yes" or a lack of time recorded as "non-basic", always say "yes" is recorded as "have" basically said "Yes", but at least have an answer "No" or the

lack of ones to "have-basic". Symptoms and quality of life data were obtained at baseline, together with medical and sexual history, and cultures and microscopy of urine and seminal fluids. Associations among selected baseline risk factors, symptoms and post-ejaculatory pain were analyzed. **Results:** One hundred and twenty-nine patients were in grouped "No", 107 in "non-basic", 138 in a "have" and 116 in "basic-have". The chronic prostatitis symptom index score (excluding pain ejaculation) in group "No" gradually increased to 25.3 points 18.4 hours ( $P < 0.001$ ). In group "No", the mental and physical quality of life score also gradually better than those in the group "Yes" ( $P < 0.001$ ). White blood cell count or bacterial growth in urine, prostate fluid volume of semen in each group had no significant differences. Answer being "have", a group of men were usually young and were more likely to live alone, had lower income and less sexual behavior than those who do not have ejaculatory pain (no group). **Conclusion:** CPPS patients with pain associated with ejaculation normally have serious symptoms which are hard to improve by routine management.

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#### Pt4-13

##### Management and discussion of post-prostatectomy complications of two different operated methods

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**Aim:** To investigate the causes and preventive methods of post-prostatectomy complications with the necessity of reoperation in open surgery and endoscope-assistant surgery. **Methods:** A total of 536 patients with BPH underwent surgery from May 2003 to October 2007 in our hospital. The clinical data of 25 cases of post-prostatectomy complications with the necessity of reoperation were analyzed retrospectively. **Results:** Of the 25 cases, 16 case underwent open surgery, nine cases underwent endoscope-assistant surgery, and there was a significant difference between the two group ( $P < 0.01$ ). All cases were timely treated and recovered successfully. **Conclusion:** Endoscope-assistant surgery has the advantage of safety, little trauma and lower risk of complications than open surgery. But the long-term clinical results must be investigated. Postoperative hemorrhage and urethral stricture (including bladder neck contracture) are two major causes of reoperation. Strictly operation indication and skilled operation can effectively decrease the complication rate.

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#### Pt4-15

##### Analysis of misdiagnosis of small cell prostate cancer

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**Aim:** To explore the reason of misdiagnosis of small cell



prostate cancer and to investigate how to improve the level of its diagnosis. **Methods:** Study of two patients treated in our hospital. Interviews after six months were recorded. **Results:** The two cases were in-patients. After digital rectal examination (DRE) and urological ultrasound, they were diagnosed by transrectal biopsy of prostate. Then, they accepted radiotherapy and chemotherapy. However, patient 1 was 22 years old, misdiagnosed in chronic prostatitis and diagnosed after 13 months from attack. During the interview, he died in exhaustion in four months. Patient 2 was 24 years old, misdiagnosed in chronic prostatitis and colonitis. After 10 months from attack, he was diagnosed. Because of intestinal and urethral obstruction, he accepted sigmoidostomy and indwelling catheter. When he returns for a check after six months, he was in exhaustion and metastasised in lung, liver and lumbar, *etc.* **Conclusion:** Small cell prostate cancer is rare. Early diagnosis is very important. Digital rectal examination and urological ultrasound are important way of its diagnosis.

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#### Pt4-16

##### Ultramicrostructure of stromal cells from transitional zone and peripheral zone of human prostate

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**Aim:** Different diseases generate in peripheral zone (PZ) and transitional zone (TZ) of prostate. It is important to research biological character of stroma in PZ and TZ of prostate. This study is to explore the differences of ultramicrostructure of stromal cells, which are active component in prostatic stroma, from TZ and PZ. **Methods:** Specimens of normal prostate were obtained from dead body donors and tissues were obtained from TZ and PZ of prostate. HE staining and transmission electron microscope (TEM) were used to verify and observe the tissues of TZ and PZ. Primary culture of stromal cell derived from TZ and PZ of prostate by gradient centrifugation. Morphology and biological feature of stromal cells from different zones were identified and observed by inverted microscope, TEM and immunohistochemistry (IHC). **Results:** Mesenchymal of TZ was tight, whereas PZ was loose. Glandular epithelium in PZ was pycnotic and had lots of cytochondriome. Smooth muscle cell (SMC) in PZ was mature and had a few of rough endoplasmic reticulum (RER) and cytochondriome. Fibroblast was thin with a few of RER in PZ. Glandular epithelium in TZ was non-pycnotic. SMC in TZ was a secreting type, immature and there was many RER, ribosome and cytochondriome in SMC. Fibroblast was long-fusiform, with many RER in TZ. Primary culture of stromal cells from TZ and PZ were fibroblast. There were more Golgi's complex and RER in stromal cell from TZ, while stromal cell from PZ was longer than that from TZ and only many RER in intracytoplasm of stromal cell from PZ. There was difference in anabolic metabolism between stromal cells from PZ and TZ. Stromal cells from PZ and TZ express Vimentin and Androgen receptor positive, while PSA negative. Stromal cells from PZ and TZ were negative for Actin

expression, but they expressed Actin positive weakly as age increases. **Conclusion:** The ultramicrostructure of stromal cells from TZ and PZ of prostate are different. It suggests that cells morphosis, secretion and anabolism are different in the course of cells growth of TZ and PZ. Differences of ultramicrostructure between stromal cells from TZ and PZ may be the reason for zonal difference of prostate cancer.

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#### Pt4-17

##### Responsiveness of stromal cells from transitional zone and peripheral zone of human prostate to androgen and transforming growth factor in gradient

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**Aim:** To research how stromal cells from transitional zone (TZ) and peripheral zone (PZ) respond to dihydrotestosterone (DHT) and transforming growth factor (TGF- $\beta$ 1) in gradient. **Methods:** Specimens of normal prostate were obtained from dead body, primary culture of stromal cell derived from TZ and PZ. Stromal cells from TZ and PZ were cultured in various proportions, cells growth were measured at 2nd, 4th, 6th, 8th days by MTT after being treated by DHT or TGF- $\beta$ 1 in gradient. Cells apoptosis rate were measured by Flow Cytometry. Gene expression of cell proliferation (PCNA) and Bcl-2 were measured by Western blot. **Results:** The growth of stromal cells from PZ was faster than that of TZ. With the decreasing of DHT concentration, the growth of stromal cells from TZ was faster than PZ. With the decreasing of TGF- $\beta$ 1 concentration, the growth of stromal cells from TZ was slower than that from PZ. With the increasing of DHT concentration, the apoptosis rate of stromal cells from TZ was increasing, however that from PZ is decreasing. Comparing to the apoptosis rate of two groups, the difference is statistically significant. With the decreasing of TGF- $\beta$ 1 concentration, the Bcl-2 expression of stromal cells from PZ was weaker. With the decreasing of DHT concentration, the Bcl-2 expression of stromal cells from PZ increased. With the decreasing of TGF- $\beta$ 1 concentration, the Bcl-2 expression of stromal cells from TZ increased. With the decreasing of DHT concentration, the Bcl-2 expression of stromal cells from TZ20 is weaker. Neither DHT nor TGF- $\beta$ 1 influenced the PCNA expression of stromal cells from TZ20 and PZ20. **Conclusion:** There are different responses of growth between stromal cells from TZ and PZ with the change of DHT and TGF- $\beta$ 1 in gradient. Cell apoptosis rate and expression of Bcl-2 were different. These different responses to DHT and TGF- $\beta$ 1 are the molecular basis of prostatic cancer. Changes of cell growth, cell apoptosis and different expression of Bcl-2 are DHT and TGF- $\beta$ 1 dependent.

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#### Pt4-18

##### Correlation of the prognosis of chronic prostatitis with psychological analysis

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**Aim:** To investigate the role of psychological factors in the etiology and symptom of chronic prostatitis (CP), and to analyze the influence of the psychological factors and other relative factors on the curative effects of CP. **Methods:** A total of 600 CP patients and 100 normal controls were investigated in our hospital. The investigate contents include: 1) Age, occupation, course of disease, education background, body status. 2) Score of the National Institute of the Health Chronic Prostatitis Symptom Index (NIH-CPSI). 3) Score of the International Index of Erectile Function-5 (IIEF-5). 4) Grade of hard of Erectile Function; 5) Symptom Checklist90, (SCL-90). All the CP patients were treated with the same method and followed up for 6 weeks. Based on the therapeutic results, the influence of psychological and other relative factors on the prognosis of CP was analyzed with univariate and multivariate Cox regression. **Results:** All together 558 valid questionnaires were collected from the patients and 93 from the normal controls. Of the 558 CP patients, the mean scores on anxiety symptoms and depression symptoms were  $42.80 \pm 11.43$  and  $48.15 \pm 11.49$ , respectively, both significantly higher than those of the controls ( $32.12 \pm 9.68$  and  $35.12 \pm 10.18$ ,  $P < 0.01$ ). The rates of anxiety, depression and anxiety and/or depression in the CP group were 25.97%, 21.71% and 34.50%, respectively, all significantly higher than in the control group ( $P < 0.01$ ). The total effectiveness rate of treatment was 76.54% in the CP patients. Univariate and multivariate analyses with Cox regression revealed that anxiety; depression and disease course were the definite factors that negatively affected the prognosis of CP, while the other factors, such as age, CPSI, character and leukocyte count in eps had no influence. **Conclusion:** Such psychological obstacles as anxiety and depression play important roles in the occurrence, development and curative effects of CP. we should attach to the patient's psychological status and proper psychological intervention is sometimes necessary.

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#### Pt6-02

##### Impact of age on erectile function of patients with benign prostatic hyperplasia

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**Aim:** To retrospectively explore the impact of age on the erectile function of patients with BPH and of the patients undergoing transurethral vaporization resection of the prostate (TUVRP). **Methods:** Between January 2003 to December 2007, 179 patients with BPH and treated by TUVRP after medical therapy had failed, mean age being 75 years old (range 60 to 93 years old) were enrolled in our study. They were divided into three groups by age: Group A has 46 patients between 60 and 70 years old, with mean age  $65.69 \pm 4.76$  years; Group B has 89 patients

from 71 to 79 years old, with mean age  $72.21 \pm 2.29$  years; Group C has 42 patients from 80 to 93 years old, with mean age  $84.42 \pm 4.38$  years. The surgery was performed with a standard 26F continuous-flow Storz resectoscope and a wing loop. Following-up was obtained in postoperative 12 months (range 6 to 24 months) to all of them. Erectile function of patients was assessed with International Index of Erectile Function-5 (IIEF-5). It was analyzed that the relationship between the age and erectile function. Moreover, postoperative change of erectile function was observed in the following-up period. At the same time, International Prostatic Symptom Score (IPSS score), Quality of Life index (QOL index) and the maximum flow rate (MFR) were observed. The difference on the percentages of erectile dysfunction among the three groups was compared using the  $\chi^2$ -test. IPSS, QOL, MFR and IIEF-5 score were compared using Student's *t*-test.  $P < 0.05$  was considered statistically significant. All quantitative data were expressed as mean  $\pm$  standard error. **Results:** Before TURVP surgery, only 69 (38.55%) patients retained erectile function, while other 110 (61.45%) had lost erectile function. Of the 69 patients who retained erectile function, IIEF-5 score decreased in 30 (43.48%), increased in 22 (31.88%) and remained the same in other 17 (24.64%) postoperatively. Of the 46 patients in Group A, 14 (30.43%) had lost erectile function preoperatively, other 32 (69.57%) cases retained erectile function; IIEF-5 score was  $16.88 \pm 5.12$  preoperatively and  $13.19 \pm 5.48$  postoperatively ( $P < 0.01$ ). Among them, IIEF-5 score decreased in 14 (43.75%),  $21.67 \pm 1.97$  preoperatively and  $10.83 \pm 3.31$  postoperatively ( $P < 0.001$ ) and increased in 10 (31.25%),  $12.20 \pm 3.90$  preoperatively and  $16.25 \pm 4.35$  postoperatively, ( $P < 0.01$ ). Of the 89 patients in Group B, 53 (59.55%) had lost erectile function preoperatively, 36 (40.45%) retained erectile function; IIEF-5 score was  $12.28 \pm 5.95$  preoperatively and  $10.61 \pm 6.69$  postoperatively ( $P < 0.05$ ). Among the 36 patients, IIEF-5 score decreased in 16 (44.45%),  $15.22 \pm 4.47$  preoperatively and  $8.22 \pm 6.57$  postoperatively, ( $P < 0.001$ ), increased in 12 (33.33%),  $12.00 \pm 6.89$  preoperatively and  $15.20 \pm 6.76$  postoperatively ( $P < 0.01$ ), showed no change in 8 (22.22%),  $8.75 \pm 5.25$ . Of the 42 patients in Group C, 41 (97.62%) had lost erectile function preoperatively. Only one (2.38%) retained erectile function; IIEF-5 score was 22 preoperatively and had no change postoperatively. Average preoperative IIEF-5 score of patients with BPH was simultaneously decreased with age, and the difference between Group A and Group B was significant ( $P < 0.01$ ). Meanwhile, patients lost erectile function were significantly increased with aging process, and a significant difference was observed among the three groups ( $P < 0.01$ ). Among the patients retained erectile function in the three groups, IPSS score was  $23.85 \pm 6.96$  preoperatively and decreased to  $8.63 \pm 6.67$  postoperatively, which was statistically significant difference ( $P < 0.001$ ). QOL index was  $4.63 \pm 0.86$  preoperatively and decreased to  $2.11 \pm 1.48$  postoperatively ( $P < 0.001$ ). MFR was  $8.87 \pm 3.98$  ml/s preoperatively and increased to  $16.42 \pm 6.37$  ml/s<sup>-1</sup> postoperatively ( $P < 0.001$ ). No significant difference was observed between the change of postoperative IIEF-5 score and the improvement of clinical symptoms in the surgical patients who retained erectile function in different groups. Both IPSS score and QOL index were not statistically different between patients with increased IIEF-5 score and



decreased IIEF-5 score ( $P > 0.05$ ). **Conclusion:** Erectile function in patients with BPH gradually decreases with age. TUVRP have uncertain effect on erectile function in patients with BPH; furthermore, its negative impact on erectile function is much greater. Great attention should be paid to the negative impact on the erectile function when apply TUVRP to BPH in younger patients.

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#### Pt6-03

##### Long acting testosterone added to acupuncture improves erectile function and sexual desire in psychological induced sexual dysfunction in obese men

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**Background:** Sexual dysfunction can be caused by psychological, physical and, more often, both (psychological and physical) causes. Psychological sexual dysfunction mean symptoms are erectile dysfunction (ED) and decreased sexual desire (SD). Low serum total testosterone is frequently considered as an ED physical cause. Obesity with large waist and high waist-to-hip ratio and with low-normal serum testosterone is sometimes considered as a clinical correspondence of the below normal serum testosterone hypogonadism. Acupuncture was already described to be useful in psychological induced ED, whereas testosterone supplementation has been always considered helpful in hypogonadic ED. **Aim:** Assessing erectile function and sexual desire in overweight ED men previously bad responding to PDE5 inhibitors, we aimed to watch sexual response after long acting testosterone added to acupuncture in treatment of psychological induced ED. **Methods:** Participants: 23 below 30 years old, first degree of obesity and low normal serum testosterone men having psychological induced ED, initially PDE5 inhibitors non-responders. The whole cohort was divided in two groups: Gr. A = 12 cases and Gr. B = 11 cases. Design: Gr. A has been initially treated by combined acupuncture and long acting testosterone therapy and than, after a one month washout period, with acupuncture alone. For Gr. B the design was the same but it was started by acupuncture and than followed by acupuncture plus long acting testosterone therapy, after a same one month washout period. For testosterone replacement 1 ampoule testosterone undecanoat (1 000 mg/4 mL) in intra-muscular injection was used. Acupuncture session duration was 2 months. Assessed parameters were: IIEF and its two domains: erectile function (EF) and sexual desire (SD), serum total testosterone, LH and PSA. They were evaluated at the participants recruitment (V1) and after the first (V2) and after the second type of treatments (V3). Statistics used the X2 test as significance estimation, considering P values as having mild:  $< 0.01$ , moderate:  $< 0.05$  and intense:  $< 0.001$  significance. **Results:** V1. Gr. A: IIEF score = 33.26, EF = 11.1, SD = 8.8; Gr. B: IIEF = 39.7, EF = 12.9; SD = 8.2. V2. Gr. A: IIEF = 57.4 (vs. V1:  $P < 0.001$ ); EF = 22.16 (vs. V1:  $P <$

0.001); SD = 12.54 (V1:  $P < 0.05$ ); Gr. B: IIEF = 49.1 (vs. V1:  $P = 0.025$ ); EF = 18.9 (vs. V1:  $P = 0.8$ ); SD = 8.2 (vs. V1:  $P < 0.01$ ). V3. Gr. A: EF = 20.78 (vs. V1:  $P < 0.001$ ); SD = 7.14 (vs. V1:  $P = \text{NS}$ ); Gr. B: EF = 22.62 (vs. V1:  $P < 0.001$ ); SD = 10.9 (vs. Gr. A:  $P < 0.01$ ). No serum hormones or PSA alterations were noticed during and after both, acupuncture or testosterone treatment. No treatment abandon was registered in the end of the study. There were not side effects because the acupuncture, or testosterone injections. **Conclusion:** Acupuncture mildly improves erectile function and sexual desire in psychological induced sexual dysfunction in obese men. Long acting testosterone treatment added to acupuncture enhance better than acupuncture alone erectile function and sexual desire in psychological induced sexual dysfunctional obese men, non-responding to a previous treatment with PDE5 inhibitors.

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#### Pt6-04

##### Penile augmentation surgery with injectable micronized human dermal tissues

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**Aim:** Penile augmentation surgery with tissue grafts through incision has been performed so far; however, there might be adverse effects such as wounds, scars, pains and prolonged recovery time. Penile augmentation can also be carried out through the injection of chemical filler or fat into the penis but it has the disadvantages of histological instability, migration and a high reabsorption rate of the material. As a result, current penile augmentation surgery has been pursued for rapid recovery using a simple technique and for natural appearance without any scars. We are to introduce a new method for penile augmentation using acellular human dermal tissue through injection. **Methods:** Retrospective investigation was done for this study with 27 cases from December 2007 to September 2008. Four mL of acellular micronized dermal tissues on average were used for a one-time injection and the average surgery time was 20 min. Being human derived, allogenic collagen does not require skin testing. The surgery was done for men with a small penis complex. Under the local anesthesia, according to the size of the penis and the augmentation size the patient desired, about 3–6 mL of dried acellular particulate dermal matrix (the granule of the tissue was  $500 \mu\text{m}^{-1} 000 \mu\text{m}$ ) combined with 1.5–1.8 mL of lidocaine and 0.3 mL of gentamicin per 1 mL of the tissue were injected to the three parts at the shaft of the penis in the direction of 12, 10 and 2 o'clock toward the base. **Results:** After the surgery, an augmentation effect of 3.1 cm on average around the shaft of the penis was demonstrated. One case of local skin necrosis was reported but it was treated through the conservatory treatment. No other adverse reaction occurred. There was little migration of the injected tissues after the graft. **Conclusion:** This surgical method with a minimally invasive tissue grafting has several advantages compared with preexisting techniques. It does not require incision, takes a short operation time, has rapid recovery time and has few side effects. Therefore, for the men who

experienced difficulty with penile augmentation surgery with the preexisting techniques, for example, those who had physical health problems, or were of an older age, or were on special medications, this surgical method could be recommended. It also may be considered as a new technique that can be applied to partial augmentation, correction of deformity, and reconstructive surgery as well.

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#### Pt6-05

##### Changes in erectile function and testosterone in late onset hypogonadism patients treated with long acting parenteral testosterone undecanoate

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**Aim:** Long acting parenteral testosterone undecanoate (TU) has been widely prescribed for late onset hypogonadism. This article aims to assess the effects of long-acting injectable testosterone undecanoate (TU, Nebido) in late onset hypogonadism (LOH). **Methods:** We investigated the changes in erectile function (IIEF-5, 5 Item Version of International Index of Erection) and serum testosterone concentration of patients with erectile dysfunction (ED). Of the patients who visited our clinic for partial androgen deficiency in the aging male (PADAM) symptoms and had decreased serum levels of total testosterone, men with ED were reviewed. We evaluated 38 medical records of LOH patients who have had received parenteral TU 1000 mg every 12 weeks. Compared to pretreatment, changes of serum total (free) testosterone, lipid profile, CBC, PSA and IIEF-5 score were evaluated. **Results:** Mean age and duration of therapy were 46.7 years and 217.5 days, respectively. Initial and final serum testosterone levels (ng dL<sup>-1</sup>) were 3.12 and 5.63, respectively. Plasma testosterone immediately before the following injection was evaluated. Compared to pretreatment, serum total testosterone level was significantly increased through treatment. TU also improved IIEF-5 score from 11 to 18. In this study, injectable long acting TU effectively elevated serum testosterone level. **Conclusion:** The results suggest that an optimal prescription of injectable long acting TU can effectively increase the serum concentration of testosterone and improve erectile function. For a more conclusive of findings on the effectiveness of injectable TU, large prospective studies are required.

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#### Pt6-06

##### Penile augmentation surgery using autologous dermal fat, allogenic dermis or heterogenic collagen graft

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**Aim:** To study the effectiveness of penile augmentation using autograft, allograft, and xenograft. **Methods:** The surgery was conducted on 720 patients from January 2005 to December 2007. We performed complex phalloplasty that enables simultaneous surgeries for glans augmentation, penile lengthening, and girth enhancement. Autologous dermal-fat, acellular allogenic dermal graft or heterogenic type I collagen were used. The thickness of the augmented graft was about 4–6 mm. The material was designed according to the size of the penis and then grafted. In the penile skin of the dorsal area, 1–2 cm from the subglans or circumcised scar was minimally incised at a length of 3–5 cm in a transverse direction. Enhancement tissue was placed between the dartos and Buck's fascia and fixed to the Buck's fascia in the penis. Histologic examination was conducted for patients that took penile augmentation surgery at least one year earlier. **Results:** The thickness of the augmented graft was about 3–6 mm in a few examined cases by ultrasonography. The grafted tissues well combined with the host penile stromal tissue with new vessel proliferation and did not show any abnormal histological changes in a few histologically examined cases. As for complications, in case of graft failure, the graft was removed partially or entirely. Some necrosis cases occurred but most of them were treated through conservative treatment. Most complications were transient and patients recovered through conservative treatment. **Conclusion:** Penile augmentation surgery using the above described grafted tissues showed positive results in terms of safety, effectiveness and histological findings. Autologous dermal fat, allogenic dermis or heterogenic collagen graft may be safe materials for penile augmentation surgery.

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#### Pt6-07

##### The effect of tetrandrine on the rabbit's penile erectile responses *in vivo*

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**Aim:** To investigate the effect of tetrandrine on the rabbit's penile erectile responses *in vivo*. **Methods:** Seventeen male New Zealand white rabbits were divided in two groups: Tet group (12) and control group (five). Rabbits were anaesthetized, and then the carotid artery on one side was cannulated for continuous monitoring of mean blood pressure (MBP). A 25-gauge needle was inserted into the corpus cavernosum for measuring intracavernous pressure (ICP). Cumulative concentrations of tetrandrine were injected intracavernously and normal saline in the same volumes was performed in the control group. ICP,





MBP and heart rate (HR) were determined after intracavernous administration of normal saline and tetrandrine. **Results:** The baseline ICP recorded was 14.1±3.2 mmHg. Tetrandrine increased ICP in a concentration-dependent manner. However, the MBP and HR were unchanged during the the injection periods of tetrandrine. **Conclusion:** The results of the present study suggest that tetrandrine enhances rabbit's penile erection after intracavernosal injection.

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#### Pt6-08

##### Randomized and controlled clinical investigation of sexual dysfunction in patients with chronic prostatitis

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**Aim:** To study the erectile function and the erectile dysfunction (ED) ratio of the chronic prostatitis (CP) patients. **Methods:** A total of 600 CP patients were randomly sampling in our hospital. Control group have 40 examples, for 18–46-year-old normal male volunteers. The routine urine test is normal, NIH-CPSI is smaller than for 5 minutes. Included in questionnaire were: 1) age, occupation, course of disease, education background, body status; 2) score of the National Institute of the Health Chronic Prostatitis Symptom Index (NIH-CPSI); 3) score of the International Index of Erectile Function-5 (IIEF-5). 4) grade of hard of Erectile Function. Compared with during various symptoms relevance, counts the different formation rate. The data were analyzed by the SPSS 10.0. **Results:** there were significant statistic relevance between the NIH-CPSI scores and IIEF-5 scores in two groups ( $P = 0.05$ ). There were also significant statistic relevance between ejaculation pain and ED in all in 600 patients ( $P = 0.000$ ). Patient's ED prevalence rate increase obviously along with their age's increase. **Conclusion:** 1) Various CP symptoms may be one of the causes resulting in sexual dysfunctions. 2) Ejaculation pain may be another reason resulting in sexual dysfunctions in CP patients. 3) Patient's ED prevalence rate increase obviously along with their age's increase.

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#### Pt6-09

##### Effects of vacuum aspiration on erectile function and NO-cGMP pathway in diabetic rats with erectile dysfunction

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**Aim:** To investigate the efficacy of vacuum aspiration for erectile dysfunction in diabetic rats and to explore the mechanism of action. **Methods:** Diabetic rats with erectile dysfunction induced by streptozotocin were divided into ED Model group (Group A,  $n = 9$ ), vacuum aspiration group (Group B,  $n = 9$ )

while 6 normal rats were taken as the control group (Group C,  $n = 6$ ). The Group B received vacuum aspiration of 20 KPa on their penes with a pulsed-wave mode of 1.67 Hz and 0.4 s pulse duration. The aspiration lasted 30 minutes per time and was done every other day. Twenty-four days later, erectile function of the rats in three groups was evaluated by hypodermic injection with apomorphine on the neck. NO in cavernosal tissue was measured by nitrate reductase method; NOS activity was assayed by colorimetric method and cGMP levels were detected by ELISA. **Results:** Erectile frequency of rats in Group A was increased obviously and the augment was higher than Group B ( $P < 0.05$ ). Total NOS activity in cavernosal tissue in Group A ( $1.06 \pm 0.44 \text{ U mg}^{-1}$ ) was lower than that in Group C ( $1.78 \pm 0.87 \text{ U mg}^{-1}$ ) ( $P < 0.05$ ), but higher than Group B ( $0.50 \pm 0.19 \text{ U mg}^{-1}$ ) ( $P < 0.05$ ). Activity of cNOS in Group A ( $0.64 \pm 0.26 \text{ U mg}^{-1}$ ) was significantly lower than that in Group C ( $1.23 \pm 0.59 \text{ U mg}^{-1}$ ) ( $P < 0.01$ ), but higher than Group B ( $0.18 \pm 0.06 \text{ U mg}^{-1}$ ) ( $P < 0.05$ ). NO content in Group C ( $0.60 \pm 0.30 \mu\text{mol g}^{-1}$ ) is higher than Group A ( $0.38 \pm 0.15 \mu\text{mol g}^{-1}$ ) ( $P < 0.05$ ), NO content in Group A was higher than that in Group B ( $0.19 \pm 0.06 \mu\text{mol g}^{-1}$ ) ( $P < 0.05$ ). Level of cGMP in Group A ( $2.32 \pm 0.73 \text{ pmol g}^{-1}$ ) was significantly higher than that in Group B ( $1.30 \pm 0.31 \text{ pmol g}^{-1}$ ) ( $P < 0.001$ ), and there was no significant difference between Group A and Group C ( $P > 0.05$ ). The iNOS activity had no statistical significance among three groups. **Conclusion:** The results of the present study suggest that vacuum aspiration has some effects in promoting the erectile function of diabetic rats, which is attributable to the increase of NO and cGMP by the elevation of cNOS activity.

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#### Pt6-10

##### A survey of erectile dysfunction with azoospermia

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**Aim:** To study the incidence of erectile dysfunction in patients with azoospermia and to explore the relationship between them. **Methods:** A total of 475 patients with azoospermia of the number of sex chromosome are normal, 22–49-year-old, the average age of 31.8 years old, the average time 4.9 years of infertility were involved in our study. Questionnaire surveys of IEF-5 were completed by the patients with azoospermia between April 2008 and December 2008 in the hospital for family planning specialist in Guangdong Province. **Results:** The incidence of ED was 26.7% (127/475) and the rates of the mild, moderate and severe ED were 13.7% (65/475), 10.5% (50/475) and 2.5% (12/475), respectively. It occurred mostly among the older patients, 19.0% (29/153), 27.7% (84/303) and 73.7% (14/19) in the 20–30, 30–40 and 40–49 year age groups respectively. The longer the infertility, the higher the incidence of ED, 11.2% (12/107) in the 0–3 years infertility group, 17.6% (36/204) in 3- years infertility group, 35.0% (362/103) in 6- year infertility group, 70.5% (43/61) in the 9- year infertility group. There was a significant correlation between each group. **Conclusion:** There is a high incidence of ED among patients with azoospermia. The



incidence of ED was positively correlated to both the age of the patients and the course of infertility.

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#### Pt6-12

##### The effect of combined medicine therapy and selective resection of the dorsal penile nerve of patients with premature ejaculation

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**Aim:** To investigate the effect of combined medicine therapy and selective resection of the dorsal penile nerve on the ejaculatory latency of patients with premature ejaculation (PE), sexual satisfaction of patients and sexual partners, and to study its clinical efficacy. **Methods:** A total of 24 cases were subjected to combined medicine therapy and 17 cases subjected to selective resection of the dorsal penile nerve. After 4 weeks, all patients in both groups were assessed with Chinese index of sexual function for PE and ejaculatory latency in vagina, and clinical efficacy was compared between the two groups. **Results:** The ejaculatory latency in vagina before treatment in medicine therapy group and operation group was  $0.93 \pm 0.35$  min and  $0.91 \pm 0.39$  min, respectively. After treatment the ejaculatory latency was  $4.27 \pm 1.51$  min and  $4.46 \pm 1.65$  min, respectively. The ejaculatory latency in vagina after treatment increased significantly in the two groups compared to that before treatment ( $P < 0.01$ ). The coitus satisfaction degree was  $16.81\% \pm 5.68\%$  and  $15.89\% \pm 6.47\%$  before treatment and  $59.95\% \pm 18.28\%$  and  $61.21\% \pm 15.93\%$  after treatment. One month after treatment, the effective rate of the two groups was 79.7% and 80.9% respectively. However, there was no significant difference between the two groups. **Conclusion:** The combined medicine therapy and selective resection of the dorsal penile nerve were safe and efficient in treatment of PE.

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#### Pt6-13

##### Comparison of long-term efficacy of treating venous erectile dysfunction by embedding or ligating the penile deep dorsal vein

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**Aim:** To compare the long-term efficacy of treating venous erectile dysfunction by embedding the penile deep dorsal vein (EDDV) or ligating the penile deep dorsal vein (LDDV). **Methods:** Between June 2000 and March 2006, 31 patients diagnosed with venous ED that failed in the treatment with PDE5 inhibitors, were selected, of whom 19 cases were performed with EDDV and 12 cases were performed with LDDV. All cases were available for follow up by using the abridged 5-item version of the International Index of Erectile Function (IIEF-5) scoring system. **Results:** During a 5-year follow-up, the IIEF-5 scoring

in LDDV group changed from a preoperative mean IIEF-5 score of  $8.7 \pm 2.2$  to  $22.2 \pm 4.3$ , and then decreased to  $10.4 \pm 3.1$ . However, in EDDV group, the IIEF-5 scoring changed from a preoperative mean IIEF-5 score of  $8.3 \pm 1.7$  to  $20.1 \pm 4.6$ , and then maintained to  $18.8 \pm 3.7$ , respectively. **Conclusion:** The results indicated that long-term efficacy of treating venous erectile dysfunction by EDDV is better than by LDDV.

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#### Pt6-14

##### Preparation of acellular matrix of corpus cavernosum with New Zealand white rabbits

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**Aim:** To investigate the appropriate methods for preparing acellular matrix of corpus cavernosum. **Methods:** Corpus cavernosum of adult male New Zealand white rabbits were selected and decellularized by the methods of extraction with Triton-X 100 and  $\text{NH}_3\text{H}_2\text{O}$ . Acellular effects were evaluated by HE staining of the acquired extracellular matrix. **Results:** No cells were observed with HE staining 14 days after acellular treatment. Elastic fibers arranged regularly, the gap looked bigger and structures didn't be destroyed. **Conclusion:** Acellular matrix of corpus cavernosum can be obtained successfully with the method of extraction by Triton-X 100 combined  $\text{NH}_3\text{H}_2\text{O}$ . This study may offer new clues for repairing deflection of penis in clinic.

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#### Pt6-15

##### Studies on combined treatment of functional anejaculation with Madopar and Bromocriptine

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**Aim:** To study the effects of combined treatment of functional anejaculation with Madopar and Bromocriptine. **Methods:** From October 2001 to October 2008, 49 patients aged from 26 to 37 years diagnosed with functional anejaculation in our outpatient department were involved in our study. Of the 49 patients, 17 were randomly selected as the control group with cognitive-behavioral therapy, the other 32 cases were treated with oral Madopar and Bromocriptine combined with cognitive-behavioral therapy. Initial dose of Madopar was 250 mg per day and it was increased to 125 mg per day every week until the largest dose of 750 mg per day and the dose was maintained. Initial dose of Bromocriptine was 2.5 mg per day and it was increased to 5 mg per day the next week and the dose maintained. The course of two groups was both within 3 months. The patients who had vagina ejaculation were considered as clinical cure. **Results:** Twenty-seven cases (84.38%) in combined therapy group



acquired clinical cure, whereas four cases (23.53%) in control group acquired clinical cure. There was statistical significance between two groups ( $P < 0.001$ ). **Conclusion:** Oral Madopar and Bromocriptine combined with cognitive-behavioral therapy has better therapeutic effects on functional anejaculation than cognitive-behavioral therapy alone.

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#### Pt6-16

##### Chronic Sildenafil improves erectile function and vascular endothelium function in diabetic rats

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**Aim:** Sildenafil is a widely-prescribed on-demand treatment of erectile dysfunction (ED). The aim of this study was to determine the effects of chronic therapy with sildenafil on endothelial function in diabetic ED rats. **Methods:** The diabetic ED rats were assigned randomly into four groups, consisting of a control group (vehicle only,  $n = 10$ ), a Sildenafil 2.5mg kg<sup>-1</sup> ( $n = 10$ ), a Sildenafil 5 mg kg<sup>-1</sup> ( $n = 10$ ) and a Sildenafil 10 mg kg<sup>-1</sup> ( $n = 10$ ) treatment group. An additional non-diabetic age-matched control ( $n = 6$ ) group was also allocated. The vehicle and Sildenafil were orally administered once daily from February 2009 to April 2009. Mean arterial blood pressure (MAP) of carotid and intracavernosal pressure (ICP) were measured. VEGF and endothelial nitric oxide synthase (eNOS) expression in cavernous space were evaluated. **Results:** After 10 weeks, ICP were significantly improved in the treatment groups which was the best when administrated with 5 mg kg<sup>-1</sup> d<sup>-1</sup> compared with the control group. Moreover, the improvement in erectile function was associated with increased VEGF and eNOS proteins. **Conclusion:** These results suggest that a long-term administration of Sildenafil is a useful tool for functional and structural modification in cavernous tissue from diabetic ED.

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#### Pt6-17

##### Chronic administration of vardenafil can treat erectile dysfunction

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**Aim:** To evaluate the therapeutic effect of chronic administration of vardenafil on erectile dysfunction. **Methods:** We enrolled 46 ED patients and had a chronic administration of vardenafil (10 mg d<sup>-1</sup>) for three months. The International Index of Erectile Function-5, the success ratio of insertion and maintaining erection were recorded in the patient's sexual diary before and after treatment and 6 months after drug discontinuance. **Results:**

The scores of International Index of Erectile Function-5 and the success ratios of insertion and maintaining erection were higher after treatment. There was statistical difference between the index before and after the treatment. The main index was higher than baseline 6 months after drug discontinuance and there was statistical significance ( $P < 0.05$ ). **Conclusion:** Chronic daily vardenafil can improve erectile function safely and effectively in ED patients.

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#### Pt6-18

##### Investigation on erectile function in male patients with adrenal tumors

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**Aim:** The changes of erectile function in male patients with adrenal tumors have not been clarified until recently. Our research group established this investigation to evaluate the erectile function in male patients with adrenal tumors. **Methods:** From December 2006 to December 2008, 63 male patients suffering from adrenal tumors were enrolled into this study with a mean age of  $47 \pm 10$  years, 30 with primary hyperaldosteronism, 16 with pheochromocytoma, three with Cushing's syndrome, five with adrenal malignant tumor and nine with nonfunctioning adenoma. All patients were evaluated by sex-hormone status (LH, FSH, estrogen, total and free testosterone) before the surgery. Erectile functions were evaluated before and 3 months after the surgery using International Index of Erectile Function (IIEF-5) questionnaire. **Results:** Decreasing in the IIEF scores were observed in all kind of diseases, especially those patients diagnosed as adrenal malignant tumor (Mean IIEF score was  $4.5 \pm 3.0$ ). IIEF scores in patients diagnosed as primary hyperaldosteronism, pheochromocytoma, Cushing's syndrome and nonfunctioning adenoma were  $16.1 \pm 7.2$ ,  $15.8 \pm 6.7$ ,  $19.5 \pm 4.6$  and  $16.5 \pm 7.1$  respectively. Patients' age  $< 50$  years had an increase in the IIEF scores 3 months after surgery, especially those age  $< 40$  years. In the group which patients had normal or mild hypertension, IIEF score before and 3 months after operation was  $15.4 \pm 6.9$  vs.  $18.3 \pm 7.4$ ; while in the group which patients had moderate to severe hypertension, the score was  $9.7 \pm 7.0$  vs.  $10.2 \pm 7.7$ , and no statistical significance was observed between two groups. Patients with severe complication, such as cerebrovascular accident had a severe decrease in the IIEF scores, and no increase was observed 3 months after surgery. In the group which tumor diameter was lower than 4 cm, IIEF score before and 3 months after operation was  $15.6 \pm 8.1$  vs.  $16.1 \pm 7.5$ ; while in group which tumor diameter ranked from 4 to 6cm, the result was  $14.3 \pm 8.0$  vs.  $14.8 \pm 7.7$ ; and in group with diameter more than 6cm, the score was  $15.0 \pm 7.0$  vs.  $14.7 \pm 7.3$  ( $P > 0.05$ ). **Conclusion:** 1) Our research showed that adrenal tumors do have negative effect on erectile function, no disease specify was observed in our study. 2) Erectile dysfunction was more severe in those patients who were associated with hypertension, especially in those patients who had severe complications for no



improvement were observed after surgery. 3) Patients' age < 50 years had an increase in the IIEF scores 3 months after surgery, especially those age < 40 years. 4) The relationship between level of erectile dysfunction and tumor size were not found in our study. 5) Whether the alternation of the concentration of androgen was the risk or not have nor been identified in our research.

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#### Pt6-19

##### Feasibility analysis of carrying out group sensate focus exercise in ED patients in Shanghai region

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**Background:** Although Chinese people are gradually obtaining an open attitude toward sex, China still remains as a conservative country where sexual problems could not be discussed publicly. Behavior therapy has become an important method in treating psychogenic or mixed ED, but whether this therapy could be implemented in China still remains unknown. **Aim:** To investigate ED patients' degree of understanding towards sensate focuses exercises in Shanghai region and therefore analyzes the feasibility of carrying out group sensate focus exercise in Shanghai region. **Methods:** Between October 2008 and March 2009, we investigated 368 ED patients living in Shanghai region in our andrology department, utilizing self-made questionnaire and doctors' explanation. Group sensate focus exercise was defined as follows: 10 to 15 couples were gathered in hospital and received the group sensate focus exercise training. The training was simulated using inflatable partners and couples were asked to practice at home. The contents of the self-made questionnaire includes: the degree of understanding towards sensate focuses exercises, the degree of recognition and acceptance towards group therapy. Advices from couples were also collected. **Results:** Valid questionnaire collected was 368 (100%), among which 186 (50.54%) were well educated (university degree and above) and 182 (49.46%) were poorly educated. 23% patients knew sensate focus exercise, the awareness between two groups were equal. Only less than half patients accepted the group training mood, among which 62% were well educated patients. Only 35.67% patients accepted this group sensate focus exercise, the acceptance between two groups was equal. 43.21% patients were optimistic about the implementation of this therapy in China, among which well educated patients were significantly more than poorly educated patients. 72 patients offered their advices, including the improvement of training mood, number of couples present at each training session, training time and period and charging standards. **Conclusion:** ED patients in Shanghai region marginally accepted group sensate focus exercise; well educated patients were more likely to accept the therapy. Improvements are needed to carry out the therapy and the enrollment of patients is of great importance.

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#### Pt6-20

##### Effectiveness of viagra in management of erectile dysfunction in Libya

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This is an observational study conducted in Ahlees clinic, Department of Urology and Male infertility at Benghazi, Libya during 2008–2009. It included 225 cohort who were taking Viagra for management of sexual abnormalities in the form of erectile dysfunction. The response to the drug was subjectively evaluated by patients. Accordingly, they were classified into two groups: 150 subjects comprised a Good Response Group (GRG) and 75 subjects Bad Response Group (BRG). The mean age of the two groups were  $42.7 \pm 10.0$  and  $47.3 \pm 13.0$  years with a statistical significant difference ( $P < 0.001$ ). Diabetes Mellitus was more frequently reported among the first group compared to the second group ( $X^2 = 7.6, P < 0.01$ ). Other studied factors include smoking habits, hypertension and other concomitants diseases were not reported to be significant until now. A more comprehensive analysis is going on and further results will be displayed during presentation.

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#### Pt6-21

##### Implanting China-made three-piece inflatable penile prosthesis for treating erectile dysfunction: experience with 10 cases

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**Aim:** To evaluate the results of China-made three-piece inflatable penile prosthesis (IPP) implantation on treating patients with erectile dysfunction (ED). **Methods:** China-made three-piece IPPs (YH) were implanted in 10 Chinese patients with ED who tried and failed in the first and second line treatment between May 2005 and January 2009. The etiologies of ED were neurogenic (two cases), cavernosal factor (venogenic) (two cases) and mixed type (six case), respectively. The follow-up period ranged from 2 to 45 months. **Results:** All the Implantation procedures were successful. No infection, erosion of the device or mechanical complications occurred. Three patients have complained slight penile shortening. Coitus could be performed in nine cases except one who has no sexual partner. One patient made his spouses be pregnant after implantation. **Conclusion:** Implantation of china-made three-piece IPP is an effective and safe modality in the treatment of ED, although long-term follow up is still needed.

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**Pt6-22****Diagnosis and treatment of penile fracture: report of 6 cases**

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**Aim:** To evaluate to effect of Sleeve circumcision in treating penile fracture. **Methods:** Six patients were treated within 1.5-8.0 h for penile fracture in our hospital from March 1998 to November 2007. All patients underwent Sleeve circumcision using absorbable material (2/0) after wounded rinsed with depo-heparin. **Results:** All 6 patients undergone operation were able to achieve an adequate erection and a good looking of the penis. No complications, such as deformations, penile plaque, urethral fistula or erectile dysfunction were reported (a mean IIEF-5 Scores of 22). **Conclusion:** The Sleeve circumcision is an excellent therapeutic option for penile fracture which should be treated early in order to reduce the complications.

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**Pt6-23****Effect of Pueraria tuberosa on sexual behavior of male rats**

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**Aim:** The present study was to determine the effect of Pueraria tuberosa tubers on the sexual behavior of male albino rats. **Methods:** Pueraria tuberosa tubers ethanolic extract in dose equivalent to 50, 100 and 200 mg kg<sup>-1</sup> and compared with a similar number of rat as controls. The parameters taken into account were the effect of extract on body and organ weights, change in histoarchitecture of testis, fructose level in seminal vesicles, sexual behavior and hormonal level. The change in sexual behavior was assessed by determining parameters mount frequency, intromission latency, mount latency and post ejaculatory latency. **Results:** Administration of ethanolic extract pronounced anabolic and spermatogenic effect in treated animals evidenced by weight gains in the body and reproductive organs. The treatment also markedly affected sexual behavior of animals as reflected in reduction of mount latency, an increase in mount frequency and enhanced attractability towards female. The fructose content in seminal vesicles and sperm count was significantly increased in treated groups. The level of follicular stimulating hormone, leutinizing hormone and testosterone level was significantly increased in extract treated group. **Conclusion:** Pueraria tuberosa tubers can be used in the traditional system of medicine as a Vajikaran rasayana.

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**Pt6-25****A single center experience: management of 12 patients with low-flow priapism**

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**Aim:** To report our experience in the diagnosis and treatment of low-flow priapism. **Methods:** Clinical data of 12 cases with low-flow priapism were analyzed retrospectively. The age ranged from 19 to 47 years (mean 28 years) and the time of persistent erection lasted from 6 to 168 hours (nine cases less than 6-24 h, two cases between 24-72 h and one case for 168 h). Of the 12 cases, six were due to intracavernosal injecting vaso-active agents (five in papaverine and one in prostaglandin E1), three had chronic myeloid leukemia, one took Trazodone for treatment of premature ejaculation and the other two had unknown causes.

**Results:** All the 12 patients were confirmed as having low-flow priapism according to presented history, physical examination, cavernous blood gas analysis and color Doppler ultrasonography. Among these 12 patients with low-flow priapism, one achieved complete detumescence by intracavernosal administration of 2 mg Aramine and six were cured by intracavernosal aspiration and perfusion of heparinized saline at corporal cavernosa. One patient with unknown causes achieved detumescence after glandular cavernosal shunting. The three cases caused by chronic myeloid leukemia did not resolve the symptoms through intracavernosal aspiration and perfusion and achieved detumescence by chemotherapy plus leukapheresis. Of 12 cases with low-flow priapism above, 11 cases were followed up for 3-12months and erectile dysfunction occurred in four cases (one severe ED, one moderate and two mild). **Conclusion:** Early treatment of low-flow priapism through intracavernosal aspiration and perfusing of heparinized saline at corporal cavernosa was an effective therapeutic option. Chemotherapy plus leukapheresis is recommended as a first choice treatment for low-flow priapism caused by chronic myeloid leukemia. Shunt surgery is also recommended but controvertible.

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**Pt6-26****The effect of Chuanxiongine on corpus cavernosal tissue and intracavernous pressure in rabbit**

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**Aim:** To determine whether Chuanxiongine can relax isolated rabbit corpus cavernosum strips and enhance intracavernous pressure (ICP). **Methods:** The cavernosal smooth muscle strips were separated from rabbits' corpus cavernosum. The relaxant effects of Chuanxiongine on cavernosal strips induced by phenylephrine (PE) were observed. Then preincubated with NG-nitro-L-arginine methyl ester (L-NAME), 1-H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ) and endothelium removal, the

relaxant effects of Chuanxiongzine on strips induced by PE were respectively recorded *in vitro*. The changes in rabbit ICP, duration of tumescence (DT) and mean blood pressure (MBp) were recorded after intracavernous injection of cumulative doses of Chuanxiongzine *in vivo*. **Results:** Chuanxiongzine relaxed the corpus cavernosum contraction induced by PE ( $EC_{50} 1.58 \times 10^{-4} \text{ mol L}^{-1}$ ) in a concentration-dependent manner. The relaxant activity was partially inhibited by ODQ, but not by *L*-NAME or endothelium removal. Intracavernous injection of cumulative doses (0.5, 1, 2, and 5 mg kg<sup>-1</sup>) of Chuanxiongzine induced a dose-dependent elevation in ICP. The ICP of cumulative doses increased from basal to  $19.1 \pm 3.7$  mmHg,  $24.8 \pm 2.1$  mmHg,  $30.2 \pm 4.8$  mmHg,  $39.7 \pm 6.1$  mmHg, respectively; DT ranged from  $8.5 \pm 2.8$  min to  $22.9 \pm 7.3$  min. Maximal ICP rose to  $25.8 \pm 5.9$  mmHg and DT lasted  $22.9 \pm 7.3$  min when the highest dose (5 mg kg<sup>-1</sup>) was administered. **Conclusion:** Chuanxiongzine executes a relaxant effect on rabbit corpus cavernosal tissues *in vitro* and facilitates erectile responses *in vivo*.

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#### Pt6-28

##### A study of the psychological and social factors in patients with premature ejaculation

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**Aim:** To investigate the mental health and social support in patients with premature ejaculation (PE). **Methods:** Questionnaires were performed in 136 patients with PE and 100 healthy volunteers using Symptom Checklist 90 (SCL-90) and Social Support Rating Scale (SSRS). **Results:** Somatization, depression, anxiety and phobic anxiety of the PE group were higher than those of the control group ( $P < 0.05$ ). The SSRS results of subjective and objective supports and total score of social supports of the PE group were lower than those of the control group ( $P < 0.05$ ). **Conclusion:** The disordered mental conditions and lower social support in patients with PE needs to be changed. PE patients need psychosocial interventions besides medical care.

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#### Pt6-29

##### Effects of sertraline hydrochloride on treatment of primary premature ejaculation

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**Aim:** To evaluate the effects of sertraline hydrochloride on the treatment of primary premature ejaculation (PE). **Methods:** A total of 50 patients diagnosed with primary PE were enrolled in this study. Patients received sertraline hydrochloride 50 mg d<sup>-1</sup> for 8 weeks. The outcome of PE treatment was evaluated by the intravaginal ejaculatory latency (IELT) and Chinese index of sexual function for premature ejaculation (CIPE-5) before and

after treatment. **Results:** IELT was significantly extended and there were higher scores of CIPE-5 in patients after treatment, than those before treatment ( $P < 0.05$ ). **Conclusion:** Sertraline hydrochloride is effective in the treatment of primary premature ejaculation.

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#### Pt6-30

##### Effect of tadalafil therapy against spontaneously hypertensive rats' penis cavernous tissues

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**Aim:** To observe the endothelin-1 (ET-1) concentration change in blood plasma and the histomorphology change condition in their cavernous body of penis after the continuous low dose tadalafil applied to spontaneously hypertensive rats (SHR). **Methods:** Forty SHR were divided into four equal groups at random: blank group, placebo group, continuous low dose group and intermittent dose group. Each group was treated with different doses of drug by intragastric administration respectively. The ET-1 concentration change in blood plasma and the cell morphology change condition of penile cavernous tissue in SHR were observed after four weeks. Immunohistochemistry method of SABC was adopted to detect expression of the  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) in corpora cavernous of penis of different groups.

**Results:** The serum levels of ET-1 were lower in continuous low dose group ( $12.0 \pm 1.6$ ) and intermittent dose group ( $14.3 \pm 1.7$ ) compared with blank group ( $18.0 \pm 1.9$ ) and placebo group ( $17.6 \pm 2.2$ ) ( $P < 0.01$ ), and it had significant difference ( $P < 0.05$ ) between continuous low dose group and intermittent dose group. The A-value level of absorbance in  $\alpha$ -SMA was unobvious with blank group ( $0.53 \pm 0.03$ ) and placebo group ( $0.52 \pm 0.05$ ) ( $P > 0.05$ ), and it was lower in continuous low dose group ( $0.29 \pm 0.03$ ) than intermittent dose group ( $0.38 \pm 0.03$ ) ( $P < 0.05$ ). It can be viewed under light microscope that in blank group and placebo group, endothelial cell and vascular smooth muscle cells penile cavernous tissue in SHR distributed disorderly. The disruption of continuity and integrity of endothelial cell were also found, and they had improved in continuous low dose group and intermittent dose group, especially the former. It can be viewed under transmission electron microscope that in continuous low dose group endothelial cells of penile cavernous tissue in SHR had most recovered to normal condition, and the ultrastructure indicated that morphous of tissue had improved. **Conclusion:** the vascular endothelium function and construction has improved, the impaired endothelial cell has repaired after treated with continuous low dose tadalafil, and provide the evidence for continuous low dose PDE5 inhibitor curing ED.

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#### Pt6-31

##### Treatment of penile fracture

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**Aim:** To study the diagnosis and treatment of the penile fracture retrospectively. **Methods:** Twelve cases of penile fracture registered in our hospital from January 1994 to April 2007 were retrospectively reviewed. **Results:** All of the 12 cases had typical clinical presentations, and the diagnosis was merely based on clinical findings. Ten patients were treated with surgery and two patients were treated conservatively. In the surgery group, unilateral corporeal rupture occurred in nine cases, bilateral corporeal rupture and tunica of urethral rupture occurred in one case. Eleven cases were followed up between 3 months to 5 years. Except a little absence of erect hardness and erect angle in one case, no other complications happened. **Conclusion:** The diagnosis of Penile Fracture can be made merely based on clinical manifestation. Based on appropriate incisions and skilled technique, surgery yields excellent results. Conservative therapy restricted to uncomplicated cases can lead to an equally good outcome.

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#### Pt6-32

##### Expression and meanings of SK3 and IK1 channel proteins in cavernous tissue of rat models of diabetes mellitus

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**Aim:** Endothelium-dependent hyperpolarization factor (EDHF) is involved in the relaxation of the vascular smooth muscle cells in penile arteries. In this paper, we hypothesize that EDHF-mediated relaxation is impaired in penile arteries from diabetic patients with ED, because Ca<sup>2+</sup>-activated K<sup>+</sup>-channels (KCa), in particular SK3 and IK1 are key players in EDHF mediated relaxation in small arteries. **Methods:** The diabetes model was induced in 8-week-old male SD rats by a single administration of streptozotocin (60 mg kg<sup>-1</sup> intraperitoneal injection) dissolved in citric 2 mmol L<sup>-1</sup> acid-trisodium citrate buffer, pH 4.0. After 72 h, blood samples were obtained, and the glucose concentration was measured using a one-touch glucometer. Induction of diabetes was considered successful when the glucose level was higher than 16.6 mmol L<sup>-1</sup> (300 mg dL<sup>-1</sup>). Nondiabetic control rats were injected with vehicle solution alone and kept under identical conditions. 8 weeks later, control rats with glucose higher than 11.1 mmol L<sup>-1</sup> (200 mg dL<sup>-1</sup>) were excluded from the study. Erection function of rats was examined by apomorphine injection (80 µg kg<sup>-1</sup>, neck and nape hypodermic injection), observe 30 min and record whether the penis erection and the number of penile erection. Rats were anesthetized and isolated penile tissue and the penis were store in liquid nitrogen. The expressions of SK3 and IK1 were measured by RT-PCR and Western blot, respectively. **Results:** Compared with the control group, the erection function significantly decreased in the DM group ( $P < 0.05$ ). Expression levels of both mRNA and protein of SK3 were significantly reduced in cavernous tissue of diabetic rat compared

with the control group. Relative mRNA and protein expression levels of IK1 also were significantly reduced in cavernous tissue of diabetic rats. **Conclusion:** Diabetic obviously inhibit SK3 and IKCa channel proteins mRNA and proteinum expression. The decrease of SK3 and IK1 channel proteins mRNA and proteinum expression in the cavernous tissue might play a key role in the development of ED in diabetic rats. These findings emphasize the importance of EDHF pathways for normal erectile function, suggesting that enhancement of EDHF pathways could be necessary to treat ED in many diabetic men by increasing SK3 and IK1 channel proteins expression.

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#### Pt6-33

##### Corpus cavernosum-corpora spongiosum shunt plus intracavernous tunneling for prolonged ischemic priapism

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**Aim:** To investigate the efficacy and safety of corpus cavernosum-corpora spongiosum shunt (CC-CSS) plus intracavernous tunneling (ICT) for prolonged ischemic priapism (PIP). **Methods:** Twelve patients with PIP were treated by CC-CSS plus ICT. A "T" shape incision was made on CC albuginea through glans penis for CC-CSS, and the Hagar dilator (No. 8–10) was used to establish the tunnel along the longitudinal axis of the CC for ICT. The penile hardness score (PHS) and VAS pain score were used to assess the efficacy of the surgery and the CDDU, IIEF5 and QOL were used for the assessment of penile morphology, erectile function, quality of life and the effect of PDE5i and penile prosthesis implantation (PPI). The mean duration of follow-up was 5.4 years. **Results:** The mean age of patients was 38.3 ± 9.2 years old and the mean duration of PIP was 6.6 ± 6.1 days. After CC-CSS + ICT, penis detumescence was successful, with the PHC and VAS scores 1.1 ± 0.3 and 1.1 ± 0.29, which were significantly decreased compared with pre-operation (3.8 ± 0.4 and 8.4 ± 0.79) ( $P < 0.001$ ). All of patients suffer from severe ED with CC fibrosis post treatment, the mean IIEF5 and QOL score were 11.7 ± 6.3 and 2.6 ± 1.1 respectively, which were significantly decreased compared with PIP before ( $P < 0.001$ ) and showed no response to PDE5i treatment and three cases showed satisfied sexual life with PPI. **Conclusion:** CC-CSS plus ICT is safe and effective method for PIP, and PPI is an effective method for the severe ED patients caused by PIP.

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#### Pt6-34

##### Study the influence of hyperlipemia on the male erectile function

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**Aim:** To investigate the influence of the hyperlipemia on male erectile function. **Methods:** Totally 120 male patients who had at least one item abnormal in total cholesterol (TC), triglycerides (TG), HDL and LDL were selected randomly in our hospital. And other 120 male patients who had normal level of serum lipid in the four items were selected randomly. These 240 patients' erectile function was evaluated by the IIEF-5. **Results:** The incidence of ED was 45% in abnormal serum lipid men. The incidence of ED was 30% in normal serum lipid men. There were significant differences in the incidence of ED ( $P \leq 0.005$ ). The incidence of ED among men aged 40–59 was significant different between abnormal serum lipid men and normal group. Logistic regression analysis indicated age, HDL, TC/HDL, coronary heart disease, depression or anxiety, BPH, long-term Prescription History were associated with erectile function. **Conclusion:** HDL was protective factor of erectile function; the others were risk factors of erectile function. Hyperlipidemia is an important factor of male erectile function, especially in men aged 40–59 years.

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#### Pt6-35

##### Gene transfer of endothelial nitric oxide synthase and vasoactive intestinal polypeptide to penis improves erectile responses in the diabetic rats

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**Aim:** To determine whether over-expression of endothelial nitric oxide synthase (NOS) and vasoactive intestinal polypeptide (VIP) in the penis cavernosum improves erectile function, and whether the combination of eNOS and VIP cDNA is a more effective means of augmenting erectile responses than single injection of eNOS or VIP cDNA in the diabetic rats. **Methods:** The study involved 48 STZ-induced diabetic male Sprague-Dawley rats. They were equally divided into four groups, group A (12 rats, sham operated with an intracorporal injection of 200  $\mu$ L PBS containing 20% sucrose and 100  $\mu$ g pcDNA3 vector), group B (12 rats, treated by gene therapy with the pcDNA3/eNOS cDNA), group C (12 rats, treated by gene therapy with the pcDNA3/VIP cDNA) group D (12 rats, treated by gene therapy with the pcDNA3/eNOS and pcDNA3/VIP cDNA). The intracavernous pressures (ICP), response to electrical stimulation of the cavernous nerve (15 Hz, 1.5 ms, 20 V, 1 min), were respectively measured at 3 days after injection. **Results:** The mean amplitude of ICP in the cavernosa of the eNOS or/and VIP-treated rats were greater than that of the control rats at 3 days after injection ( $P < 0.05$ ), respectively. There was significant difference in ICP between single gene therapy with eNOS or VIP and the combined therapy ( $P < 0.05$ ). **Conclusion:** The transfer of eNOS or/and VIP are capable of altering the physiologically relevant erectile response as measured by an increase in the ICP response to the stimulation of the cavernous nerve. The combination of eNOS and VIP cDNA is a more effective means of augmenting erectile responses than single injection of eNOS or VIP cDNA in the diabetic rats.

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#### Pt6-36

##### The effect of combined therapy and selective resection of the dorsal penile nerve of patients with premature ejaculation

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**Aim:** To investigate the effect of combined therapy and selective resection of the dorsal penile nerve on the ejaculatory latency of patients with premature ejaculation (PE), coitus satisfaction degree of patients, and study its influence on the clinical efficacy. **Methods:** A total of 24 cases of PE patients were subjected to combined medicine therapy. Seventeen cases of PE patients were subjected to selective resection of the dorsal penile nerve. After 4 weeks, patients in both groups were assessed with ejaculatory latency in vagina and the coitus satisfaction degree. The clinical efficacy was compared between the two groups. **Results:** The ejaculatory latency in vagina before treatment in medicine therapy group and operation group is  $0.93 \pm 0.35$  min,  $0.91 \pm 0.39$  min, respectively. After treatment they are  $4.27 \pm 1.51$  min,  $4.46 \pm 1.65$  min. The ejaculatory latency in vagina after treatment increased significantly in two groups compared to that before treatment ( $P < 0.01$ ). The coitus satisfaction degree are  $1.39 \pm 0.68$ ,  $1.31 \pm 0.77$  respectively before treatment and  $4.45 \pm 0.88$ ,  $4.51 \pm 1.03$  after treatment. A month after treatment, the effective rate of two groups was 79.2% and 82.4%, respectively. There is no significant difference between two groups. **Conclusion:** The combined medicine therapy and selective resection of the dorsal penile nerve were safe and efficient in treatment of PE.

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#### Pt7-01

##### Ultra-structure of spermatogenic seminiferous epithelium of rats with varicocele

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**Aim:** To study the cytopathological changes in the ultra-structure of testis of rats with varicocele. **Methods:** Rat model of varicocele was established with 30 male Sprague-Dawley rats, 200–250 g in weight, at  $50 \pm 10$  days of age (adolescent). They were randomly divided into 1) varicocele group (VG) ( $n = 20$ ), and 2) sham operation as control group (SoG) ( $n = 10$ ). Experimental model of rats with left varicocele was established using Saypol methods. After laparotomy, the left renal vein of rats in varicocele group were partially ligated, while that of the sham-operated control rats was isolated without ligation or narrowing. Testicular tissues of both left and right testis were obtained from the two groups. Semen samples were collected and underwent computer-aided sperm analysis (CASA) right after liquefaction at 37°C. After being broken up, the tissues in the size of about 1 mm<sup>3</sup> were labeled respectively, pre-fixed with 1.5 mL 2% of glutaraldehyde for two hours, washed with buffer,



and then fixed with 1% of osmium acid solution, dehydrated in ethanol, then the dehydration solution was gradually replaced with embedding solution of 618 epoxy resin, sliced, stained with uranyl acetate and lead citrate. **Results:** The sperm viability, motility, path velocity and straightness were significantly higher ( $P < 0.01$ ) in the SoG than in the VG. In VG group, cell structure damage was observed in bilateral testis, but the damage of right testes was relatively slight. In Sertoli cells, a large number of cytoplasmic vacuoles, broken tight junctions, lysosomes were presented, while the number of mitochondria was decreased. In spermatogenic cell, the formation of acrosome was abnormal, demonstrated by the deletion of acrosome or the small acrosome, vacuolized acrosome, abnormal sperm nuclear, excessively condensed chromatin, vacuoles in nuclear, partial deletion of mitochondrial tail sheath, or mitochondrial disorders, swelling and confused mitochondrial crista, decreased number of mitochondria in transverse section of the sperm tail. **Conclusion:** The structural damage of the spermatogenic epithelium in seminiferous is an important reason for infertility in male with varicocele.

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#### Pt7-02

##### Presence of donor-and-recipient-derived DNA microchimerism in, cell-free blood samples of living-donor renal transplant recipients

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**Aim:** To examine whether the existence of the donor-and-recipient-derived DNA chimerism in living-donor recipient's plasma can be a predictive marker for the status of transplanted organ. **Methods:** Seven female patients who had been transplanted with male living-donor kidney were enrolled in the present study. In these female recipients, the SRY1, DYZ1 1st and DYZ1 2nd genes on Y chromosome from the plasma were examined using reverse transcription polymerase chain reaction (RT-PCR). **Results:** SRY1, DYZ1 1st and DYZ1 2nd sequences were detected in the cell-free blood (plasma) of five (71.4%) of the seven female patients during six month postoperation. Two recipients encountered acute rejection. Among them, one patient was microchimerism-negative recipient, the other was microchimerism-positive recipient. At six months postoperation, serum creatinine levels in microchimerism-positive patients were significantly lower than those in microchimerism-negative patients. Among the microchimerism-positive recipients, three one around 2 weeks and another one approximately 3–4 months post-transplantation; and then a relative rate was maintained. **Conclusion:** These results suggest that plasma DNA microchimerism present in certain patients undergoing living-donor renal transplantation might be a useful predictor for the acceptance of living-donor transplanted kidneys.

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#### Pt7-03

##### The expression of Usp26 in male mouse testis and brain

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**Aim:** To study the expression of ubiquitin-specific protease 26 (Usp26) gene mRNA and protein in various mouse tissues, particularly in mouse testis, which is designed to better understand mechanism of action of Usp26 in male reproductive system. **Methods:** Male Kunming (KM) mice aged 20, 30, 35, and 45 days were obtained to study Usp26 expression pattern in testis and epididymis. Besides, Usp26 expression was also examined in brain, heart, lung, liver, kidney, adrenal gland and seminal vesicle in 35-day-old mice. Reverse transcription (RT)-PCR and immunohistochemistry analyses were performed to detect the expression of Usp26 mRNA and its protein respectively. **Results:** RT-PCR analysis showed that the Usp26 transcript was expressed in all of the above tissues. Immunohistochemistry analysis revealed that Usp26 was differentially expressed in mouse testis of different period of sexual maturation. Usp26 was not detected on postnatal day 20, while it was began to be expressed on postnatal day 30 and strongly expressed in cytoplasm of condensing spermatids (steps 9-16 spermatids) and Leydig cells in testis, as well as in nerve fibers of brain. In addition, Usp26 was detected at a moderate level in myocardial cell, the corpus of epididymis, epithelium of renal tubule and seminal gland of postnatal day 35 mice. **Conclusion:** Usp26 may play an important role in testis and brain. Further research on these issues is in progress.

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#### Pt7-04

##### Effects of freezing and thawing on sperm plasma membrane glycoconjugates: a preliminary study

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**Background:** Freezing and thawing have some detrimental effects on sperms. Molecular changes on the sperm surface affect fertility rate. **Aim:** To investigate whether freezing and thawing would affect sperm plasma membrane glycoconjugates. **Methods:** We tested wheat germ agglutinin (WGA), peanut agglutinin (PNA) and Dolichos biflorus (DBA) to detect surface glycoconjugates changes before and after freezing. Sperm smears from 45 healthy men were prepared before and after freezing and thawing. The smears were stained with the lectins and also acridin orange and were studied by fluorescents microscopy. The intensities of the reactions to lectins were measured by image analyses software. **Results:** After freezing and thawing, 46.67%,



34.09% and 73.34% of the specimens modified their reactions to PNA, WGA and DBA lectins respectively. The modification in the uptake of lectins by sperms of the different specimens was not similar. In some cases, the lectin uptakes were decreased after freezing and thawing while the others revealed increases of intensities to the same lectin. It means that there are various mechanisms that impact on the carbohydrate contents of the sperm surface. Statistical analyses showed that just 4.45% of the specimens contained more sperms with denaturated DNA after freezing and thawing. There was no correlation between the denaturated state of the sperm DNA and modification of the surface glycoconjugates. **Conclusion:** We conclude that cryopreservation affected the surface glycoconjugates at least in a subset of spermatozoa. Cryopreservation had deleterious effects on spermatozoa, especially on plasmalemma, acrosomes and tails. Induction of spontaneous acrosomal reaction, change in sperm motility and physical damage because of the crystal formation during cryopreservation may cause change in sperms surface glycoconjugates. It may have some detrimental impact on fertility of sperm that use in assisted reproductive technology.

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#### Pt7-05

##### Effect of angelica ligusticum leech mistura on the sperm producing function and sperm quality of the left testis in experimental varicocele rats

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**Aim:** To study the influence of angelica ligusticum leech mistura on the reproduction of experimental varicocele rats. **Methods:** The effect of angelica ligusticum leech mistura on the experimental varicocele rats' testis was observed by hematoxylin and eosin (HE) staining with a microscope. The rats' seminal fluid was analyzed by computer-assisted spermatozoa analysis system (CASAS). The content of MDA and the activity of SOD in different groups of rats were tested. **Results:** The injury level of constitution of treatment group was much lower than that of the control group in left testis of the rats. The activity of spermatozoa in the treatment group was better than that in the control group ( $P < 0.05$ ). The content of MDA in the treatment group was lower than that in the control group and the activity of SOD was much higher than that of the control group. The motion parameters of spermatozoa VCL, VSL, VAP in the treatment group were much faster than those in the control groups. **Conclusion:** angelica ligusticum leech mistura could protect and repair the testis' injury induced by the experimental varicocele. It could improve the activity of the experimental rats' epididymis spermatozoa. The effect may relate to antilipid-peroxidation and reinforcement of antioxidase. **Discussion:** When varicocele occurs, the oxide increases and the antioxiide decreases due to the lacking of the blood and oxygen supply and the substance of the adrenal back-streaming injure. The reactive oxygen species (ROS) include oxygen free radicals such as  $H_2O_2$ ,  $OH$ ,  $O_2^-$  and so on. The membrane of spermatozoa is easily injured by ROS

because the membrane contains plenty of unsaturated fatty acid. There are also series of antioxidase in our bodies. When the production of ROS is faster than the clearance of ROS, the lipid on the membrane of spermatozoa would be peroxidized, and the ability of metabolism would be disturbed. These series of changes lead to male infertility. The blood stream of the testis and vena spermica interna was disordered and ROS increased in varicocele. MDA is the decomposition product from the lipid peroxidation. The test of MDA could reflect the outcome of the oxygen free radicals. SOD is one of the main antioxidases in the body. SOD plays an important role in the clearance of the ROS. It's confirmed in the experiment that the angelica ligusticum leech mistura could decrease the content of the MDA in the experimental varicocele rats' left testis and improve the activity of the SOD. It could protect the injury from ROS and improve the ability of the producing spermatozoa. The study of modern medicine has proved that ferulaic acid, ligustrazine and hirudin are the main active substances in the angelica, ligusticum and leech. They all have the ability of anticoagulating blood and resisting the formation of thrombus. They could improve the microcirculation significantly. Ferulaic acid and ligustrazine have the ability to clear the ROS released by body and resist the injury by lipid peroxidation. The experiment also researched the influence of angelica ligusticum leech mistura on the activity of the varicocele rats' spermatozoa. It's confirmed that the angelica ligusticum leech mistura could improve significantly the motion parameter of the end epididymis' spermatozoa, such as VCL, VSL and VAP, but had little influence on the parameter of ALH, BCF, LIN, MAD and STR.

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#### Pt7-06

##### Y chromosome microdeletions in infertile men with azoospermia and extreme oligozoospermia

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**Aim:** To investigate the status of Y chromosome microdeletions in infertile men with Azoospermia, Cryptozoospermia and extreme oligozoospermia. **Methods:** 1) grouping: A total of 1 207 infertile men were included in the study. They were divided into two groups. Group A: Azoospermia,  $n = 1\ 000$ ; Group B: Cryptozoospermia or extreme oligozoospermia,  $n = 207$ . Azoospermia maybe related to Primary spermatogenic failure and/or obstructive azoospermia. In our study, Cases of obstructive azoospermia were discharged. 2) Screening test: multiplex Polymerase chain reaction (PCR) amplification of 18 Sequence Tagged Sites (STS), STS genomic markers from the three Y chromosome AZF encompassing all of the regions for AZF of the Y chromosome. **Results:** 160 (13.3%) microdeletions were found in 1 207 infertile men tested. Eighteen STS were tested, the general prevalence of AZF microdeletions was 4.63% (1 006 out of 21 726), the absence of STS in each infertile man averages 6.29; Y microdeletions in AZFa, AZFb and AZFc region were 13 (4%), 52 (16%) and



261 (80%) respectively; Y microdeletions in infertile men with more than one AZF regions were found in 124 (77.6%). Compared with group A (11.2%, 112/1 000), the frequency of Y microdeletions in group B (23.8%, 48/207) was significantly higher (= 21.436,  $P = 0.000$ ). **Conclusion:** Y chromosome microdeletions are the most common genetic cause of male infertility and screening for these microdeletions in azoospermic or severely oligospermic men should be standard. The infertile men showed absence of STS in more than one AZF regions. The frequency of Y deletions in infertile men with Cryptozoospermia and extreme oligozoospermia was higher than that in those with azoospermia. Detection of various subtypes of these deletions has a prognostic value in predicting potential success of testicular sperm retrieval for assisted reproduction. As there is only one Y chromosome, it may be predicted that Y microdeletions will be transmitted to male offspring. For men with azoospermia or severely damaged spermatogenesis, testing for microdeletions before ICSI is desirable.

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#### Pt7-07

##### The expression of Tesmin in mice testes development

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**Aim:** To identify the developmental expression of Tesmin in mice. **Methods:** Testes cDNA samples from Balb/c mice of different postnatal days (day 4, 9, 18, 35, 54, and 6 months) were performed with mouse whole genome 430 2.0 Array (Affymetrix Inc.) chip. The characteristics of the selected gene, Tesmin, were analyzed by RT-PCR assay. **Results:** The Affymetrix chip probe of mouse Tesmin was graduated higher expression on developmental stages of mouse testis. The scaling hybridization signal intensities of the tested testis on day 4, 9, 18, 35, 54 and 6 months of postnatal were 31.4 (Absent expression, A), 34 (A), 1292 (Present expression, P), 856.6 (P), 977.2 (P) and 830.7 (P), respectively. The expression of mouse Tesmin wasn't detected on day 4, and day 9, but was detected on days 18, 35, 54, and 6 months of mouse testis in our Affymetrix chip analysis. By combination with the RT-PCR analysis of mouse Tesmin, we observed mouse Tesmin begins to express at the age of day 18 in mouse. **Conclusion:** The Tesmin was expressed in an time-dependent manner in mouse testis, and the expression of Tesmin begins after day 18 of postnatal testis in mice, suggested Tesmin might plays an important role in mouse spermatogenesis.

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#### Pt7-08

##### Semen parameters may not be good indicators of male fecundity: a retrospective study of 1 257 cases

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**Aim:** To find out whether semen parameters is good in assessing male fecundity. **Methods:** Retrospective analysis of 1 206 cases of semen from out-patient department and 51 cases of self-matching semen with one month interval, performed with computer analyzing system, including semen parameters of density and percentage of rapid progressive sperms. SPSS 12.1 software was used for statistic analysis and two-way ANOVA for the comparison of self-matching semen parameters. **Results:** Sperm density was  $0-180 \times 10^6 \text{ mL}^{-1}$ , with an average of  $55.3 \pm 32.2 \times 10^6 \text{ mL}^{-1}$ , 73.2% were within  $20-100 \times 10^6 \text{ mL}^{-1}$ , only 13.2% were oligozoospermia, and azoospermia accounted for 1.5%; 51% of the cases had 30%-50% rapid progressive sperms; only 0.58% were normal according to the WHO standard (rapid progressive sperms > 50%). For the self-matching semen parameters, the density was  $51.8 \pm 20.7 \times 10^6 \text{ mL}^{-1}$  vs.  $53.7 \pm 18.8 \times 10^6 \text{ mL}^{-1}$ ; the volume was  $3.3 \text{ mL} \pm 1.1 \text{ mL}$  vs.  $3.6 \text{ mL} \pm 3.8 \text{ mL}$ ; the rapid progressive sperms were  $23\% \pm 12.2\%$  vs.  $26.9\% \pm 12.0\%$ , all with  $P < 0.001$ . **Conclusion:** Except for azoospermia that was obviously related to infertility, sperm parameters may not be good indicators of infertility due to the significant fluctuations.

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#### Pt7-09

##### Intrauterine insemination with partner's semen: are sperm parameters affecting pregnancy rate?

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**Aim:** To assess the effect of sperm parameters on pregnancy rate following Intrauterine Insemination (IUI) in primary and secondary infertile patients. **Methods:** A retrospective study of 97 couples underwent a total of 182 IUI cycles from January 2008 to May 2009. A detailed history and laboratory test for infertility evaluation was taken. Ovarian stimulation of all patients with Clomiphene citrate or HMG was done. Semen analysis was performed on the day of IUI before and after density gradient sperm washing. IUI was done 24 h after tracking of the dominant follicles and injection hCG was given for follicular maturation and rupture, and total rapid progressive sperms should be  $> 500 \times 10^6$  after density gradient sperm washing, or the cycle should be cancelled. **Results:** Of the 97 couples who underwent IUI cycles, 55.5% had male factor infertility and the pregnancy rate was 22.8% per cycle; 25.8% had female factor infertility, with pregnancy rate of 12.8%; and 18.7% were infertile due to both gender factors, with pregnancy rate of 17.6%. No significant difference in pregnancy rate was found among the three groups. Before density gradient sperm washing, for those successful cycles, the density was  $53.6 \pm 16.5$  million  $\text{mL}^{-1}$  and rapid progressive sperms were  $27.0\% \pm 9.9\%$ . The density was  $53.7 \pm 21.2 \times 10^6 \text{ mL}^{-1}$  and rapid progressive sperms were  $26.7\% \pm 12.1\%$  for those failure cycles. No significant difference between the two groups. After density gradient sperm washing, for the successful cycles and fail cycles, the density was  $102.3 \pm 33.9 \times 10^6 \text{ mL}^{-1}$  vs.  $98.9 \pm 57.3 \times 10^6 \text{ mL}^{-1}$ , and rapid progressive sperms were  $57.2\% \pm 5.8\%$  vs.  $43.6\% \pm 8.4\%$ ,



respectively. There is no significant difference between them. **Conclusion:** This study confirms that IUI can be applied as a first-line treatment in infertile couples. Sperm parameters do not affect pregnancy rate when total rapid progressive sperms are > 500 million after gradient sperm washing.

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#### Pt7-10

##### **Incubation of ejaculated and epididymal pig spermatozoa with oviductal fluid protein does not affect tyrosine phosphorylation pattern**

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**Background:** Capacitation, which represents the final maturational steps, renders mammalian sperms competent to interact correctly with the oocyte, and it occurs in the female genital tract *in vivo*. *In vitro*, this phenomenon can also be reproduced in defined media. During the capacitation, the membrane proteins and lipid organization change dramatically resulting in sperm binding to the zona pellucida and initiating acrosome reaction. Protein phosphorylation is known to regulate sperm function, such as motility and zona pellucida recognition. Mammalian capacitation is also probably mediated by protein phosphorylation. However, little is known about boar SP factors and porcine oviductal fluid (POF) that may influence the tyrosine phosphorylation (Tyr-P) of sperm proteins during capacitation. **Aim:** To determine if proteins from POF modify the Tyr-P pattern spermatozoa. **Methods:** POF was obtained by tubal aspiration with a micropipette from female just post-ovulation. After centrifugation, the epithelial cells was precipitated, the supernatant was collected to determine the protein concentration. Ejaculated (EJ) and epididymal (EP) spermatozoa were divided into three groups: 1) spermatozoa without any treatment (control = C); 2) spermatozoa washed through Percoll® gradient (P); and 3) spermatozoa washed through Percoll® and incubated with porcine oviductal fluid protein ( $50 \mu\text{g mL}^{-1}$ ) for 30 min (P-POF). All samples were incubated in sperm capacitation medium (TALP) after treatments. The plasma membrane was isolated according to the report (Bravo *et al.* 2005) and the soluble proteins were separated by 12% SDS-PAGE. Afterward, the proteins were transferred onto a nitrocellulose membrane and blocked with TBST-Albumin 5% overnight; after that the membranes were incubated with primary antibody (anti-phosphotyrosine antibody, 4G10 Upstate Biotechnology) (1:20 000), and then with secondary antibody (anti-mouse IgG antibody, Biorad®)(1:20 000) and revealed with ECL+Plus (Amersham®). Seven replicates were conducted for this experience. **Results:** The results showed a band of tyrosine phosphoprotein of 19 kDa in all experimental groups except the EJ-C group. A band with a range of 40-43 kDa was present in all experimental groups although the intensity of phosphorylation was different. **Conclusion:** The incubation of ejaculated and epididymal pig spermatozoa with oviductal fluid protein did not

affect protein tyrosine phosphorylation pattern in the incubation time studied.

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#### Pt7-11

##### **Increasing chromatin decondensation in boar's ejaculated and epididymal spermatozoa treated by Percoll® gradient and porcine oviductal fluid**

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**Background:** During spermiogenesis in mammals, the sperm chromatin structure undergoes replacement of nuclear histones, first by transition proteins and finally by protamines, leading to a highly packaged and condensed chromatin, which is resistant to oxidative insults. Further stabilization of the sperm chromatin structure is enhanced by the presence of seminal plasma components, such as free zinc ions and zinc-binding proteins. Moreover, the integrity of the sperm chromatin structure is an important factor in fertilization and embryo development, and can be affected by various factors. In previous studies we have showed that ejaculated spermatozoa washed by Percoll® gradient exhibited higher rates of head decondensation and pronuclei formation than epididymal spermatozoa or ejaculated spermatozoa without gradient treatment (by IVF and ICSI Matás *et al.*, 2003; Garcia-Vazquez *et al.*, 2009 respectively). **Aim:** To determine the sperm chromatin condensation after treated by Percoll® gradient and porcine oviductal fluid (POF). **Methods:** POF was obtained by tubal aspiration with a micropipette from female post-ovulation. After centrifugation, the epithelial cells were precipitated, and the supernatant was collected to determine the protein concentration. Ejaculated (EJ) and epididymal (EP) spermatozoa from different boars were washed through Percoll® gradient and incubated in sperm capacitation medium (TALP). EJ and EP spermatozoa were divided into three groups: 1) spermatozoa without any treatment (control = C); 2) spermatozoa washed through Percoll® gradient (P); and 3) spermatozoa washed through Percoll® and incubated with porcine oviductal fluid protein ( $50 \mu\text{g/mL}$ ) in TALP medium for 30 min (POF). The samples were centrifuged and the pellet was resuspended in ethanol solution and phosphate-buffered saline to make permeabilization of membranes enhanced. After that, the samples were centrifuged and the pellet was resuspended in a propidium iodide (PI) solution in PBS. Measurements were expressed as the mean red intensity fluorescence units and it was used as an index of the state of the chromatin condensation, as this is directly related to the PI uptake by DNA. **Results:** The results were analysed by two-way ANOVA. Data showed that i) chromatin condensation of the control group were lower than experimental ones (EJ-C: 2.23 and EP-C: 16.69 vs. EJ-P = 22.68, EJ-POF = 22.48 and EP-P = 21.96, EP-POF = 23.37,  $P < 0.001$ ). Although there were significant differences between EJ-C and EP-C ( $P < 0.001$ ), the chromatin condensation was similar after the spermatozoa were washed (EJ and EP). **Conclusion:** Sperm

source as well as sperm treatment appears to play an important role in the sperm chromatin condensation. The seminal plasma could be of significant benefit in improving the chromatin stabilization.

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#### Pt7-12

##### Enzymatic activity of glycosidases in boar sperm

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**Background:** The initial sperm-zona pellucida (ZP) binding is an important event that precedes sperm penetration into an oocyte. In several mammalian species, macromolecules such as lectin-like proteins and enzymes in spermatozoa are involved in the recognition of the oligosaccharides present in ZP glycoproteins. Spermatozoa glycosidases are thought to form stable enzyme-substrate complexes by binding to the oligosaccharide residues of the ZP glycoproteins with high specificity and affinity (Tulsiani *et al.*, 1998). **Aim:** The objective of this study was to analyze and compare the presence of the glycosidases ( $\alpha$ -D-Mannosidase,  $\alpha$ -L-Fucosidase,  $\beta$ -D-Glucosaminidase, and  $\beta$ -D-Galactosaminidase) in fresh ejaculated and epididymal sperm from mature boars. **Methods:** Spermatozoa were washed three times in PBS by centrifugation at  $800 \times g$  for 10 min. The pelleted sperm were resuspended in the same buffer to obtain a final concentration of  $250 \times 10^6$  spermatozoa/mL. The different enzymes were detected by incubating  $2 \times 10^6$  spermatozoa (for  $\alpha$ -D-Mannosidase,  $\alpha$ -L-Fucosidase) and  $20 \times 10^6$  spermatozoa (for the rest of enzymes) of sperm samples with the corresponding substrate conjugated to 4-methylumbelliferil for 2 h at 37°C in PBS, pH 7.3. Fluorescence was read in units of fluorescence (UF/1  $\times 10^6$  sperm) on a Fluostar Galaxy fluorimeter (BMG Lab Technologies) (using wavelengths of 340 and 450 nm for excitation and emission, respectively) and was corrected by subtracting tissue and substrate blanks. The results were analyzed using an one way ANOVA. **Results:**  $\alpha$ -D-Mannosidase and  $\alpha$ -L-Fucosidase are the enzymes of main activity present in the porcine ejaculated and epididymal sperm. A higher  $\alpha$ -D-Mannosidase and  $\beta$ -D-Glucosaminidase enzymatic activity was observed in ejaculated sperm compared to epididymal sperm ( $\alpha$ -D-Mannosidase:  $8\,810 \pm 487.6^a$ ,  $5\,363 \pm 377.4^b$  and  $\beta$ -D-Glucosaminidase:  $722.1 \pm 37.76^a$ ,  $270.0 \pm 42.36^b$  ( $P < 0.05$ ),  $\alpha$ -L-Fucosidase and  $\beta$ -D-Galactosaminidase activity was similar for both kinds of spermatozoa,  $1\,822 \pm 89.2$ ,  $1\,936 \pm 223.2$  and  $176.0 \pm 16.61$ ,  $129.4 \pm 12.46$ , respectively. **Conclusion:** This study demonstrates that different glycosidases are present in the sperm plasma membrane. The higher enzymatic activities found in ejaculated sperm compared to the epididymal sperm suggests the binding of  $\alpha$ -Mannosidase and  $\beta$ -D-Glucosaminidase coming from the seminal plasma to the epididymal spermatozoa. Supported by Seneca 08752/PI/08, AGL2006-03495 and 0452/GERM/06.

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#### Pt7-13

##### Expression of $\beta$ -chemokine RANTES in human and mouse epididymis

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**Aim:** To investigate the expression and cellular distribution of RANTES (regulated upon activation normal T cell expressed and secreted; CCL5) in male reproductive system. **Methods:** RT-PCR, in situ hybridization, immunohistochemical staining and immunofluorescence staining were employed to examine the distribution of RANTES in human and mouse epididymis. Spermatozoa were collected from different segments of epididymis and immunofluorescence staining was used to detect RANTES association. Real-time RT-PCR and Western blot were used to quantitate the levels of RANTES expression in mouse epididymis on postnatal days 7, 14, 21, 28, 35, 42, 49, 56 and 140. **Results:** The mRNA for RANTES was not detected in human testis. In contrast, the epididymis produced high amount of RANTES transcripts. In situ hybridization and immunohistochemical studies revealed RANTES-positive staining in columnar ciliated cells in efferent ductules/initial segment and in narrow cells located in the caput epididymis, in both human and mouse. RANTES-positive basal cells, located underneath the columnar cells, were only identified in the corpus and cauda epididymis in human. Immunofluorescence studies also showed obvious segment-specific expression pattern of RANTES in epididymis. We observed that RANTES was bound on the post-acrosome region of epididymis spermatozoa. Qualitative analysis of tissues from postnatal days 7, 14, 21, 28, 35, 42, 49, 56 and 140 mice indicated that the signals of RANTES were first detected on day 7 and increased during sexual maturation. **Conclusion:** These data indicate that RANTES is mainly restricted to the columnar ciliated cells and narrow cells, and is secreted into the lumen of epididymis throughout sexual maturity. The exact physiologic, pathophysiologic and molecular mechanisms involved in this process require further studies.

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#### Pt7-14

##### Expression of recombinant hamster zona pellucida glycoprotein ZP4 in mammalian cells

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**Background:** The initial interaction between gametes takes place at the sperm surface and the zona pellucida (ZP), an egg extracellular matrix in mammals. This extracellular matrix is formed by three or four glycoproteins depending on the specie (ZP1, ZP2, ZP3 and ZP4). ZP4 has been implicated in sperm

binding in porcine and bovine and in the acrosomal exocytosis in human. Recently, our group has described the presence of ZP4 in hamster ZP. **Aim:** The aim of this study was to obtain a recombinant hamster ZP4 to be used in functional assays. **Methods:** Total hamster ovarian RNA was isolated from ovaries and cDNA was synthesized with oligo-dT as primer. This cDNA was used as template to amplify hamster ZP4 using the polymerase chain reaction by means of specific primers. The ZP4 ORF was cloned into the pcDNA3 expression vector and the FLAG sequence was inserted in frame after the furin cleavage motif. FLAG epitope-labeled ZP4 construct was transiently expressed in human embryonic kidney cell line HEK 293T cells. The pattern of expression of ZP4-FLAG in transfected cells was assessed by confocal microscopy and flow-cytometric analysis. The ZP4 protein was analyzed by western-blotting, both in the cell lysates and in conditioned media. **Results:** The analysis of histograms and confocal micrographs of transfected cells expressing recombinant ZP4 localized the fusion protein at the plasma membrane and was consistent with trafficking through the biosynthetic-secretory pathway. This was also consistent with the behaviour previously described for other ZP4 proteins. On the other hand, western blot analysis demonstrated the existence of a major band in transfected cell lysates, with an apparent molecular weight of approximately 75 kDa. The broad band corresponds to the polypeptide chain posttranslationally modified by addition of complex oligosaccharide chains linked to Asn residues. The expression follows a time-dependent kinetics, with the maximal levels at 72 hours post-transfection. However, recombinant ZP4 protein was not detected in the culture supernatants, as has been described for other proteins belonging to the ZP family. Therefore, in our system, ZP4 most likely remains associated to the cell surface, either as an integral or as a tightly bound peripheral protein. **Conclusion:** Hamster ZP4 was successfully expressed in HEK 293T cells as a plasma membrane-bound highly glycosylated form. Efficient secretion of ZP4 into the culture medium was not observed. This study was supported by grants from Fundación Séneca de la Región de Murcia 0452/GERM/06 and CARM, Plan de Ciencia y Tecnología 2007/10, 464/2008.

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#### Pt7-15

##### Enzymatic activity levels of $\alpha$ -D-Mannosidase and $\alpha$ -L-Fucosidase in capacitated boar sperm

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**Background:** Recent studies have shown the presence of  $\beta$ -N-acetylglucosaminidase,  $\alpha$ -mannosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase in mammalian sperm (Tulsiani *et al.*; 2000). In preliminary experiments in our laboratory,  $\alpha$ -D-Mannosidase and  $\alpha$ -L-Fucosidase are the main enzyme activity present in the porcine ejaculated and epididymal sperm (unpublished data).

**Aim:** To analyze the  $\alpha$ -D-Mannosidase and  $\alpha$ -L-Fucosidase activity in ejaculated and capacitated sperm from mature boars. **Methods:** Fresh spermatozoa (FS) were divided into two groups: 1) FS were washed three times in PBS by centrifugation at  $800 \times g$  for 10 min. The pelleted sperm were resuspended in the same buffer; 2) FS from the same boars were washed through a Percoll® gradient and incubated in a capacitating medium (TALP) to obtain capacitated spermatozoa (CS). The final concentration for the two groups was  $25 \times 10^6$  spermatozoa  $\text{mL}^{-1}$ .  $\alpha$ -D-Mannosidase and  $\alpha$ -L-Fucosidase were detected by incubating  $2 \times 10^6$  spermatozoa of each sperm sample with the corresponding substrates conjugated to 4-methylumbelliferil for 2 h at 37°C, pH 7.3. Fluorescence was read in units of fluorescence ( $\text{UF}/2 \times 10^6$  sperm) on a Fluostar Galaxy fluorimeter (BMGLab Technologies) using wavelengths of 340 and 450 nm for excitation and emission, respectively, and it was corrected by subtracting tissue and substrate blanks. The results were analyzed using one way ANOVA. **Results:** A higher enzymatic activity was observed on  $\alpha$ -D-Mannosidase ( $15\,732.6 \pm 1\,000$  vs.  $1\,915.6 \pm 210.8$ ) and  $\alpha$ -L-Fucosidase ( $3\,765.4 \pm 263.2$  vs.  $84.5 \pm 7.0$ ) in FS than in CS, respectively ( $P < 0.05$ ). **Conclusion:** The higher enzymatic activities found in FS compared to that in CS suggest that the process produces an alteration of the integrity of the sperm plasma membrane with a loss of activity for these glycosidases. This evidence indicates that these enzymes are not strongly anchored to the sperm plasma membrane. The physiological role of this residual activity in capacitated sperm should be investigated in IVF experiments.

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#### Pt7-17

##### Purification and characterization of the relaxin-like factor and its subcellular location in the goat testis

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**Aim:** Relaxin is a 6-kDa peptide originally described as a hormone of pregnancy that is best known for its role in



parturition in most mammalian species. It is biosynthesized as the A-B-C heterotrimeric prohormone, which then matures to A-B heterodimer. Among mammals, however, domestic ruminants do not possess exceptionally relaxin. In ruminants, relaxin-like factor (RLF), also known as insulin-like factor 3 (INSL3), has been suggested to be a functional substitute instead of relaxin, but its precise function is still unknown. Goat is considered to be a useful pilot animal for studying reproductive physiology in ruminants. To date, in the male goat the cDNA coding RLF was cloned and the Leydig cells were identified as the source of RLF mRNA in the testis. This study was to purify and characterize RLF protein from the goat testis, to examine its expression profile during the testis development, and to demonstrate the subcellular localization of RLF in the testis. **Methods:** Developmental and adult testes were obtained from Saanen goats which were fed in the Nagano Station of National Livestock Breeding Center. RLF was isolated from mature testes according to the method of Kohsaka *et al.* (1993). Purified RLF was digested and analyzed by LC-MALDI-TOF/TOF mass spectrometry. The expression profile of RLF protein during testicular development was examined by Western blotting. Subcellular localization of RLF was visualized by immunoelectron microscopy. **Result:** Using an extraction procedure that minimized proteolysis, followed by gel filtration, ion-exchange FPLC, and reverse-phase HPLC, goat RLF was isolated as a single peak with the molecular size of about 12 kDa, as revealed by SDS-PAGE. MS/MS analysis indicated that the A-B-C heterotrimeric prohormone sequence was identified with 72% protein coverage. Western blot analysis of the testis during development found that the 12-kDa RLF seemed to increase during puberty onwards. Immunoelectron microscopy revealed that RLF could be seen in the Golgi apparatus of Leydig cells. **Conclusion:** This study suggests that RLF is present as the A-B-C heterotrimeric proform in the goat testis and is located in the Golgi apparatus of Leydig cell.

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#### Pt7-18

##### Isolation and purification of rat spermatogonial stem cells

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**Aim:** To isolate and purify rat spermatogonial stem cells from 22- to 24-day-old rat testes. **Methods:** Seminiferous tubules were dissected from testes of 22-24 day old rats, testicular cell suspension was isolated using two-step enzymatic digestion, germ cells were dissociated from somatic testis cells by cell culture manipulation, and spermatogonial stem cells were purified from the differentiated germ cells by selection in culture on laminin. **Results:** Using the principle that testicular cells bound to plastic or collagen matrices differently, we obtained approximately  $3 \times 10^5$  spermatogonial stem cells from five 22- to 24-day-old rats. In serum-containing medium testicular somatic cells bound to plastic well tightly, spermatogonial cells did not bind to plastic well, so we obtained spermatogonial cells first,

and then, using the rat tail collagen coated tissue culture plate, we can keep the most differentiated spermatogonial cells back in the wells. At last, during a short incubation on laminin, we obtained spermatogonial stem cells, they can form clones on feeder layer cells *in vitro*. **Conclusion:** These methods do not require special instrument, for example flow cytometry, and also can obtain enriched rat spermatogonial stem cells. The isolated spermatogonial stem cells are effective and available for further study.

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#### Pt7-19

##### Research on relationship between the abnormal sperm liquefaction and reproductive infections including Ureaplasma Urealyticum and Chlamydia Infections

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**Aim:** To investigate the relationship between the abnormal sperm liquefaction and reproductive infections including ureaplasma urealyticum and chlamydia infections. **Methods:** From January 2007 to December 2008, a total of 420 male infertility cases in our department were included and divided into abnormal sperm liquefaction group and normal sperm liquefaction group. Additional 120 cases of normal fertile male were chosen as a control group. The infection rates of ureaplasma urealyticum and chlamydia were observed in the three groups. **Results:** The infection rates of ureaplasma urealyticum and chlamydia in abnormal sperm liquefaction group was significantly higher than those of normal sperm liquefaction group and control group ( $P < 0.01$ ). And the infection rates of ureaplasma urealyticum and chlamydia in normal sperm liquefaction group and control group showed no significant difference ( $P > 0.05$ ). **Conclusion:** The occurrence of the abnormal sperm liquefaction is related to ureaplasma urealyticum and chlamydia infections.

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#### Pt7-20

##### Melatonin's in vitro effects on human sperm motility and endogenous NO and ROS production

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**Aim:** To study the effects of *in vitro* melatonin treatment on the motility of human spermatozoa, as well as the endogenous production of nitric oxide (NO) and reactive oxygen species. **Methods:** Spermatozoa were incubated with  $2\text{mmol L}^{-1}$  melatonin (120 min, 37°C, 5% CO<sub>2</sub>), motility parameters were then measured by computer aided motility analysis, and cell viability (PI), intracellular NO (DAF-2/DA) and ROS (DCFH-DA) were assessed by flow cytometry. **Results:** *In vitro* melatonin treated samples showed a significantly higher percentage of motile ( $76.81\% \pm 3.08\%$  vs.  $70.36\% \pm 2.87\%$ ;



$P < 0.01$ ), progressive motile ( $52.81\% \pm 4.03\%$  vs.  $41.51\% \pm 4.04\%$ ;  $P < 0.01$ ) and rapid cells ( $52.08\% \pm 4.07\%$  vs.  $40.55\% \pm 4.12\%$ ;  $P < 0.01$ ), and reduced number of non-viable spermatozoa compared to the control. Endogenous NO was significantly lower ( $55.07\% \pm 15.26\%$  vs.  $100\%$ ;  $P < 0.05$ ) in melatonin treated samples, but no effect was observed on ROS levels. **Conclusion:** From these results it can be concluded that melatonin was able to directly or indirectly scavenge NO, as indicated by the reduction in 4,5-diaminofluorescein-2/diacetate fluorescence, while simultaneously enhancing motility parameters. Future studies will indicate whether melatonin treatment during sperm preparation could protect sperm from excessive NO production.

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#### Pt7-21

##### The Effect of Partial and Complete AZFc Deletion on Human Spermatogenesis

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**Aim:** To study the relationship between the complete and partial AZFc deletion with spermatogenetic arrest and distribution of partial AZFc deletion in Han Chinese people was evaluated in order to reveal the unknown genetic risk factor for spermatogenesis arrest. **Methods:** We screened complete AZFc deletion and partial deletions of the AZFc region of a consecutive group of idiopathic non-obstructive azoospermia (NOA) ( $n = 458$ ) and controls (301 qualified sperm donors). Partial deletions of the AZFc region and complete AZFc deletion were analyzed by multiplex polymerase chain reaction (PCR) according to established protocols. **Results:** The rate of gr/gr deletion is 8.90% (41/458) in experimental group, while 8.6% (25/301) in control. The rate of b2/b3 deletion is 2.40% (11/458) in experimental group, while 2.3% (7/301) in control. There are four cases with complete AZFc deletion in experimental group, while no case in control. There was no significant difference in two groups on gr/gr deletion and b2/b3 deletion ( $P > 0.05$ ). And there was statistical difference in two groups on complete AZFc deletion ( $P < 0.05$ ). **Conclusion:** It is detected that gr/gr deletion and b2/b3 deletion cannot be used as one of genetic risk factors in Han Chinese males, which can be considered a kind of polymorphism of Y chromosome during the history of evolution in the human being.

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#### Pt7-22

##### Seasonal Changes in Testicular C-kit receptor and steroidogenic enzymes in Wild Male Ground Squirrels

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**Aim:** Germ cell proliferation, migration and survival during all stages of spermatogenesis are affected by stem cell factor (SCF) signaling through the c-kit receptor. The proto-oncogene c-kit has also been shown to play a role in the proliferation and survival of Leydig cells. The objective of this study was to investigate the immunolocalization of the c-kit receptor, steroidogenic enzymes P450 side chain cleavage (P450scc), 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) and 17 $\alpha$ -hydroxylase cytochrome P450 (P450c17) in the testes of the wild ground squirrels during pre-reproductive, reproductive periods and the period before hibernation. **Methods:** Testicular samples of 24 adult wild ground squirrels were collected in early April, 2009 (pre-reproductive period), late April, 2009 (reproductive period) and September, 2008 (the period before hibernation). Testicular samples were dehydrated in an ethanol series and embedded in paraffin wax. Sections (4–6  $\mu$ m) of testes were routinely stained with hematoxylin-eosin (HE) for histological observation. Serial sections of testes were mounted on slides coated with poly-L-lysine for immunohistochemistry. The serial sections of the testes were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections were then incubated with primary antibody (1:500) raised against human placental P450scc, 3 $\beta$ -HSD, porcine testicular P450c17, and c-kit receptor which raised against mouse for a whole night under 4°C. The sections were then incubated with a second antibody, goat anti-rabbit IgG conjugated biotin and peroxidase with avidin, using a rabbit ExtrAvidin™ staining kit, followed by visualization with 20 mg 3, 3'-diaminobenzidine solution in 100 mL of Tris-HCl 1:1 buffer, pH7.6, plus 16.7  $\mu$ L H<sub>2</sub>O<sub>2</sub>. Finally, the reacted sections were counterstained with haematoxylin solution. The control sections were treated with PBS instead of the primary antisera. **Results:** The histological observations showed that all types of spermatogenic cells including elongating spermatids could be found during the breeding period, and there were spermatogonia and a few primary spermatocytes in the seminiferous tubules during the period before hibernation. Immunohistochemically, localization of c-kit receptor was observed in spermatogenic cells and Leydig cells during pre-reproductive period, but only found in Leydig cells in the breeding season. Moreover, the immunolocalization of c-kit was only present in spermatogonium during the period before hibernation. In addition, P450scc, 3 $\beta$ -HSD and P450c17 were immunolocalized in the cytoplasm of Leydig cells of all testicular samples, and the samples of pre-reproductive and reproductive periods were stained more intense than the period before hibernation. **Conclusion:** The present study demonstrated that the immunodetection of the c-kit receptor was observed in spermatogonium of the wild ground squirrels during the pre-reproductive and the period before hibernation. Seasonal changes in testicular steroidogenesis suggested that the synthesis of androgen reached its peak in the breeding season. These findings indicated that the Proto-Oncogene c-kit and steroidogenic enzymes may be important regulatory factors of





the proliferation of spermatogonium in the wild ground squirrels. This study was supported by the Program for Changjiang Scholars and Innovative Research Team in Universities (IRT0607), China and the Program of State Forestry Administration, China.

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#### Pt7-23

##### **Immunodetection of P450c17, P450arom, NGF and its receptors TrkA and p75LNGFR in epididymis of the male ground squirrels during breeding season**

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**Aim:** Epididymis is a comma-shaped structure that lies along the posterior surface of each testis, and performs an important role in the maturation of spermatozoa including their acquisition of progressive motility and fertilizability. The objective of this study was the immunodetection of 17 $\alpha$ -hydroxylase cytochrome P450 (P450c17), aromatase cytochrome P450 (P450arom), NGF and its receptors trkA and p75LNGFR in epididymis of the male ground squirrels during the breeding season. **Methods:** Sixteen wild male ground squirrels thought to be adult based on their body weights (242–412 g) were captured in May (breeding season) of 2009 in Hebei province, China. Testis and epididymis were separated and samples were taken from different parts of the epididymis (caput, corpus and cauda). Samples were dehydrated in an ethanol series and embedded in paraffin wax. Serial sections (4  $\mu$ m) of epididymis were routinely stained with hematoxylin-eosin (HE) for histological observation. Some sections were mounted on slides coated with poly-L-lysine, and the serial sections were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections were then incubated with primary antibody (1:2 000) raised against human placental cytochrome P450 aromatase (P450arom), porcine testicular 17 $\alpha$ -hydroxylase cytochrome P450 (P450c17), NGF (M-20), TrkA (763), and p75LNGFR overnight under 4°C. Thereafter, the sections were incubated with the second antibody goat anti-rabbit IgG conjugated with biotin and peroxidase with avidin, using a rabbit ExtrAvidin™ staining kit (Sigma, St. Louis, MO, USA), followed by visualization with 20 mg 3,3'-diaminobenzidine (Wako, Tokyo, Japan) solution in 100 mL of 0.05 mol Tris-HCl buffer, pH 7.6, plus 16.7  $\mu$ L H<sub>2</sub>O<sub>2</sub>. Finally, the reacted sections were counterstained with hematoxylin solution (Merck, Tokyo, Japan). The control sections were treated with PBS instead of the primary antisera. **Results:** In caput epididymidis, cuboidal epithelium was composed of ciliated and unciliated cells, basal cells and few lymphocytes, but more basal cells were found in corpus epididymidis. Furthermore, some low columnar epithelia with wide lumina filled up with spermatozoa were present in cauda epididymidis. Immunolocalization of P450c17 and

P450 aromatase were located near the nucleus of epididymal epithelium and the lumen of the duct. Meanwhile, NGF and its high-affinity receptor TrkA also significantly expressed in the epididymal epithelium and luminal fluid, while its low-affinity receptor p75LNGFR showed a faint immunohistochemical signal. **Conclusion:** These results suggested that the main receptor of NGF in the epididymis of ground squirrels is TrkA instead of p75LNGFR. The immunodetection of P450c17, P450arom, NGF and TrkA suggested that it may play a regulate role in the maturation of spermatozoa in wild male ground squirrels.

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#### Pt7-24

##### **Immunolocalization of inhibin/activin subunits in testes of wild male ground squirrel during the breeding and non-breeding seasons**

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**Aim:** It is widely confirmed that transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily members, especially inhibin and activin, play crucial roles in regulating the testicular development and spermatogenesis. The objective of this study was to investigate the seasonal changes of spermatogenesis and immunolocalization of inhibin  $\alpha$  and inhibin/activin ( $\beta_A$  and  $\beta_B$ ) subunits during the breeding and non-breeding seasons in wild male ground squirrels. **Methods:** Sixteen adult males were captured in May (breeding season) and September (non-breeding season) of 2007 in Hebei Province, China. Testicular weight, size and the diameters of seminiferous tubules were measured. HE staining was used to observe the histological appearance. Immunohistochemistry (ABC method) was used to localize inhibin/activin ( $\beta_A$  and  $\beta_B$ ) subunits in testis on the two different reproductive seasons. **Results:** Testicular weight and size varied significantly between the breeding (May) and non-breeding (September) seasons (weight  $0.988 \pm 0.180$  g, size  $8.2 \pm 1.06$  mm in May and weight  $0.22 \pm 0.10$  g, size  $3.82 \pm 0.38$  mm in September). The diameters of seminiferous tubules were extremely high in the breeding seasons ( $150.56 \pm 9.12$   $\mu$ m) compared with the non-breeding seasons ( $71.4 \pm 6.8$   $\mu$ m). Obvious seasonal changes were observed in histological appearance of the seminiferous epithelium. Spermatogenesis also changed seasonally, with the entire spermatogenic cell population in May and only spermatogonia and spermatocytes in September in the seminiferous tubules. Interstitial cells presented obvious seasonal changes as well as seminiferous tubules. The inhibin  $\alpha$  and inhibin/activin ( $\beta_A$  and  $\beta_B$ ) subunits were observed in both Sertoli cells and Leydig cells on May, while only Sertoli cells in September. No immunostaining was found in the germ

cells of both reproductive seasons. Immunoreactivity of the three subunits in breeding seasons was much more intense than that in non-breeding seasons. **Conclusion:** The present results demonstrated that seasonal changes in testicular weight, size and seminiferous tubule diameters in male wild ground squirrels were correlated with changes in spermatogenesis and testicular distributions of inhibin/activin subunits. These findings suggested that inhibin and activin played important roles in spermatogenesis of wild male ground squirrels.

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#### Pt7-25

##### The comparative mechanisms study of Wuziyanzong Pills and Jinkuishenqi Pills on the function of Shenjing and Shenqi

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**Aim:** To investigate the Reinforcing Kidney mechanisms of Wu Zi Yan Zong Pills and Jin Kui Shen Qi Pills. **Methods:** In mice azoospermia model, the Wu Zi Yan Zong Pills and Jin Kui Shen Qi Pills were treated by intragastric administration for 78 days (two spermatogenesis cycles) together and respectively and compared with three control groups treated with DMSO (Solvent control) which were spontaneous recovery, and normal mice.

**Results:** The effects between the two pills on spermatogenesis function were significantly different. Wu Zi Yan Zong Pills mainly affected spermatogenic epithelium of testis and were verified the function of “Shenjing”. Jin Kui Shen Qi Pills mainly affected testicular interstitial cells and were verified the function of “Shenjing”. **Conclusion:** The two classic Chinese herbs have a corroborative and stronger spermatogenesis function.

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#### Pt7-26

##### The relationship between the mRNA levels of spermiogenesis specific genes (PRM1, PRM2&TNP2) and normal morphology of mature sperm

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**Aim:** Some studies have indicated sperm's transcripts as a molecular source of infertility research. Several reports showed

that more than 5 000 mRNAs existed in human ejaculated spermatozoa. Most of these transcripts are related to the genes expressed in spermiogenesis process. Protamines transcripts are abundant in mature sperm. Protamines are the main nuclear protein of human sperm and have an important role in normal morphology and function of mature sperm. Abnormal morphology of human sperm are accompanied with reduced fertilizing ability and functions of sperm. Furthermore, successful ART methods is closely dependent on quality of sperm's morphology. With considering the structural role of protamine in normal morphology of sperm head and abundant presence of their transcripts in mature sperm, this case-control study was designed to quantitative and compares the level of TNP2, PRM1 & PRM2 transcripts in mature sperm of normozoospermic and teratozoospermic men. **Methods:** Human ejaculates of 50 normozoospermic (mean of normal morphology: 29/1 ± 6/81) and 104 teratozoospermic (mean of normal morphology: 6/21 ± 2/54) men according to WHO guideline were analyzed. Each semen sample was divided into two aliquots for sperm chromatin staining assay (CMA3, AB staining methods) and RNA content determination. RNA was isolated from spermatozoa and then reverse-transcribed into cDNA. Using real-time RT-PCR, the content of TNP2, PRM1 & PRM2 transcripts in sperm were assessed for normozoospermic and teratozoospermic men. **Results:** PRM1&PRM2 transcripts in normozoospermic men (PRM1:  $7/49 \times 10^9 \pm 5/17 \times 10^{10}$ , PRM2:  $2/70 \times 10^7 \pm 1/90 \times 10^8$ ) was higher compared with those in teratozoospermic men (PRM1:  $2/20 \times 10^9 \pm 2/21 \times 10^{10}$ , PRM2:  $9/21 \times 10^1 \pm 7/92 \times 10$ ). However the mRNA content of TNP2 was higher in teratozoospermic men (TNP2:  $1/75 \times 10^{10} \pm 1/33 \times 10^8$ ) compared with normozoospermics (TNP2:  $4/84 \times 10^6 \pm 3/42 \times 10^7$ ). We found a significant positive correlation ( $P < 0.05$ ) between the level of TNP2 transcript and sperm head defects ( $r = 0.354$ ,  $P = 0.012$ ). The ratio of PRM1/PRM2 transcripts was negatively correlated with the sperm's tail defects ( $r = -0.846$ ,  $P = 0.008$ ) in normozoospermic men as compared to teratozoospermic men. **Conclusion:** The presence of a significant correlation between TNP2, PRM1 and PRM2 transcript contents and sperm morphology indicate that abnormal morphology of sperm may be the result of defective replacement of histones by TNP1 & TNP2 and subsequently by PRM1 & PRM2. Also our result reveals the potential applications of mRNA contents in mature sperm as diagnostic tools for human sperm functions.

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#### Pt7-27

##### Effect of reactive oxygen species on semen quality in male partner of couples experiencing recurrent spontaneous abortion (RSA)

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**Background:** Approximately 70% of all pregnancies fail to go to term and 50%–60% are lost within the first month of pregnancy and may go unnoticed. The male gamete contributes 50% of the genomic material to the embryo. The spermatozoa involve in many events, such as syngamy, cleavage and epigenetic regulation and may influence early embryo loss. Factors as occupational and environmental exposure to toxins, mutagens and carcinogens have been shown to adversely influence the number, motility and morphology and DNA quality of spermatozoa, so it may affect embryogenesis and placentation. Compared with fertile men, the patients with recurrent spontaneous abortion show a significant increase in sperm chromosome aneuploidy, abnormal chromatin condensation, DNA fragmentation and abnormal sperm morphology. Spermatozoa are particularly susceptible to damage induced by reactive oxygen species (ROS) because sperm plasma membrane is rich in polyunsaturated fatty acids, the cytoplasm contains low concentrations of the scavenging enzymes, and the sperm has limited capacity for DNA repairs. Both the seminal plasma and the sperm itself has the antioxidant mechanism that protect the spermatozoa from DNA damage and lipid peroxidation. An imbalance between the free radical concentration and antioxidants leads to oxidative stress – a cellular state in which membrane biomolecules particularly lipids and the nucleotides of the genome are prone to oxidation. This alters sperm quality and function, sperm-oocyte interaction, implantation, and early embryo development, all of which are good indicators of successful pregnancy.

**Methods:** Twenty-seven idiopathic infertile cytogenetically normal men with a history of Recurrent Spontaneous Abortion (three consecutive pregnancy losses prior to the 20th week of gestation). Experienced by their spouse and 15 fertile healthy controls (had fathered a child in last one year and had normal semen parameters) were included in the study. Males in which the female partners had a history of uterine, fallopian, endocrinal and immunological dysfunctions were excluded from the study. The female partner had normal karyotype (46, XX). Semen was analyzed according to WHO criterion (1999). Reactive oxygen species (ROS) was assayed in PBS washed and neat semen by chemiluminescence using Berthold Luminometer. Number of apoptotic sperms was quantified by flow cytometry. Statistics was done using Mann-Whitney test. **Results:** ROS in both washed semen and neat semen was significantly higher in the patient group as compared to controls ( $P = 0.019$ ,  $P = 0.014$ ). The washed semen had higher ROS as compared to neat semen in patients and controls ( $P = 0.032$ ,  $P = 0.040$ ). The number of apoptotic spermatozoa in patients was significantly higher in patients as compared to controls ( $P = 0.025$ ). The mean sperm count in controls is  $35.72 \pm 1.72$  million  $\text{mL}^{-1}$  as compared to patients  $21.2 \pm 2.31$  million  $\text{mL}^{-1}$ . Mean progressive motility in patients is  $45.37 \pm 5.09$  and in controls is  $59.45 \pm 3.18$ . Mean viability in patients is  $42.01 \pm 3.56$  and in controls is  $72.37 \pm 4.54$ . **Conclusion:** On analysis of the impact of ROS levels and percentage of apoptotic spermatozoa on the semen quality, it becomes apparent that they can be used as markers for sperm DNA integrity and sperm parameters can assist in understanding the aetiology of early pregnancy loss. It would also eliminate the risk of using spermatozoa with defective DNA *in vitro*. The apoptotic machinery is responsible for the removal of defective

gametes and also for the maintenance of appropriate number of the germ cells that the sertoli cells can support. Aberrant apoptotic machinery may lead to the presence of increased DNA damaged spermatozoa in the ejaculate. The diagnostic work-up adopted to determine the treatment of patients with recurrent spontaneous abortion is crucial and must include oxidative stress investigation and assessment of DNA integrity. This will aid in understanding the aetiology of RSA and provide the most adapted therapeutics and counseling to such patients.

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#### Pt7-28

#### Extracellular proteases of the ADAM family are involved in germ cell apoptosis

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**Background:** Paracrine/juxtacrine signaling between germ and Sertoli cells is of paramount importance because it regulates survival and differentiation of germ cells during spermatogenesis. In this way, the extracellular proteases belonging to the ADAM family (a disintegrin and metalloprotease) are good candidates to control the extracellular shedding of many growth factor and growth factor receptors expressed in the Sertoli and germ cells. ADAMs metalloproteases comprises a family with several members among which ADAM17, ADAM10 and ADAM9 have been shown to induce apoptosis in some cell types. It is known that germ cell apoptosis is a critical process regulating sperm production, and massive germ cell death has been associated with male infertility. During the first round of spermatogenesis in the rat, a massive wave of apoptosis took place in 25-day-old rats. Inhibition of this massive wave of apoptosis severely impairs the fertility during adulthood, but the mechanism underlying this process is still unknown. We hypothesized that trophic signaling inhibition could be a relevant pathway of apoptosis induction in germ cells. In this context, the tyrosine kinase c-kit is indispensable for maintenance of spermatogenesis and germ cell viability. Even more, in mast cell, c-kit extracellular domain shedding by ADAM17 induced apoptosis. **Aim:** To elucidate if ADAM17 induces apoptosis in germ cells. **Methods:** Immunohistochemistry was used to localize ADAM proteins. Western blot was used to determine protein levels, and Flow cytometry was used to quantify apoptosis. **Results:** Immunohistochemistry of 25-day-old rats using an antibody against the extracellular domain of c-kit showed a clear label in many spermatocytes and some spermatogonia, but apoptotic germ cells were devoid of this staining. On the contrary, using an antibody against the intracellular domain of c-kit all apoptotic cells showed a similar staining signal. Even more, a Western blot against c-kit showed that the extracellular domain of this receptor was processed in apoptotic, but not in nonapoptotic germ cells. Immunohistochemistry for ADAM17 showed low expression of this protease in spermatogonia, spermatocytes and Sertoli cells. ADAM10 showed a more cytoplasmic distribution in pachytene spermatocytes lining in the second and/or third

tier in seminiferous tubules. Some seminiferous tubules also showed staining in Sertoli cells. ADAM9 showed its presence in pachytene spermatocytes located surround the lumen of seminiferous tubules and some Sertoli cells. Interestingly, most of the apoptotic cells, evaluated by histology, were devoid of ADAM9 and ADAM10 staining, while non-apoptotic spermatocytes in the contiguous tubule, or in the same tubule, were strongly stained. On the contrary, most of the apoptotic cells showed strong staining for ADAM17. We also found an increase in the protein level, as evaluated by western blot, of ADAM17, but not ADAM10 or 9, in apoptotic germ cells. Interestingly, the mRNA level was similar between apoptotic and non-apoptotic cells. Even more, *in vivo* pharmacological inhibition of ADAM17 prevented physiological germ cell apoptosis. On the contrary, pharmacological inhibition of ADAM10 induced an increased in germ cell apoptosis. On the other hand, induction of ADAM17 by using PMA (an activator of protein kinase C) increased the number of germ cell apoptosis, which was significantly prevented when PMA was co-injected with TAPI-0, a well-known ADAM17 inhibitor. Interestingly, almost 80% of apoptotic cells after PMA treatment were also devoid of the extracellular domain of c-kit, suggesting a common signaling pathway between physiological and PMA-induced apoptosis. **Conclusion:** Our results uncover an important role of ADAM17 and ADAM10 in mammalian spermatogenesis, which regulate germ cell apoptosis in opposite ways. These results show for the first time a role for ADAM17 in germ cell apoptosis *in vivo* and shed light into the physiological regulation of this process.

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#### Pt7-30

##### Identification and characterization of the sperm-binding epididymal protein HEL-65

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**Aim:** Following ejaculation, mammalian spermatozoa require a high-energy supply. Investigations on specific and functionally active sperm protein could bring about the elucidation of the mechanisms of gamete interaction and help the search for new approaches in prognosis and regulation of fertility. **Methods:** Sperm-binding epididymal protein HEL-65 was identified from a human epididymis cDNA library. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) were used to examine HEL-65 mRNA and indirect immunofluorescence was used to localize HEL-65 protein in human epididymis and sperm. Sperm motility and function on fertilization of HEL-65 were analyzed using Computer-Aided Sperm Analysis (CASA) and zona-free hamster oocytes test, respectively. **Results:** HEL-65 has a 100 % homology to LDH-A. HEL-65 mRNA was expressed in a wide variety of tissues and the HEL-65 protein was detected at high level in the corpus regions of the mature human epididymis. It was expressed by the principle cells of epididymal epithelium, and the protein showed a checkerboard

pattern. Immunofluorescent staining showed that the HEL-65 bound on the principal and end piece of sperm tail. When the sperm were blocked with anti-HEL-65 polyclonal serum, sperm motion parameter, VAP, VCL, VAL declined, while no effect was observed on fertilization. **Conclusion:** The HEL-65 protein is a sperm-binding epididymal protein. It displays a regional-specific expression pattern in the human epididymis and bound on the principal and end piece of sperm tail. It may play an important role in sperm motility.

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#### Pt7-31

##### Human sperm binding protein HEL-38 expressed in epididymis is essential for motility and fertility of sperm

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**Aim:** To immunolocalize the HEL-38 gene product and to investigate its function in male reproduction. **Methods:** Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to examine HEL-38 mRNA. Indirect immunofluorescence and immunohistochemistry were used to localize HEL-38 protein in human sperm, testis and epididymis. The enzyme activity of semen was analyzed using a standard glyceraldehyde-3-phosphate dehydrogenase activity assay. The motility and fertility were analyzed with computer-aided semen analysis (CASA) and sperm penetration assay respectively. **Results:** The human HEL-38 gene is located on chromosome 12p13 and encodes a 335 amino acid protein. It plays roles only binding with NAD (+) coenzyme and the binding site is GXGXXG. At the transcriptional level, HEL-38 mRNA was expressed in epididymis, testis and lung. Its protein localized on the epithelium of epididymal corpus and cauda and bound to the equatorial and principle of sperm. Western blot showed that rHEL-38 has the same molecular pattern, compared with the native protein isolated from testis and epididymis. RHEL-38 has high enzyme activity. The dehydrogenase activity of semen came from azoospermia donor is lower than that from normal donor. When spermatozoa were blocked with anti-rHEL-38 polyclonal serum, sperm motility decreased but sperm penetration index increased. **Conclusion:** HEL-38 was first studied in the human epididymis and sperm. It may play an important role in motility and fertility of sperm and is a potential molecular target.

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#### Pt7-32

##### Lsh is an essential factor for the progression of meiosis in mouse spermatocytes

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**Aim:** Lymphoid specific helicase (Lsh) is a member of the SNF2-helicase family of chromatin remodeling proteins. Because Lsh null mice die at birth, a potential phenotype in male meiosis cannot be studied in these animals. Grafting of tissue from Lsh mutant mice to immuno-tolerant wild type recipients allows the study of postnatal tissue differentiation even in animals with a neonatal lethal phenotype. The aim of this study was to monitor progression of spermatogenesis in Lsh  $-/-$  male gonads and to determine whether the Lsh protein plays a role in the progression of meiosis in the male germ line. **Methods:** Testes were harvested at day 18.5 of gestation from Lsh  $-/-$ ,  $+/-$  and  $+/+$  mice of 24 litters and grafted ectopically under the back skin of 30 immuno-deficient, castrated male recipient mice (3-6 grafts/mouse). Each mouse received at least one graft from each genotype. To compare proliferative activity of germ cells between Lsh  $+/+$  and  $-/-$  testis tissue, 3 recipient mice were treated with BrdU for 1 h before grafts were harvested at 1 wk post grafting. A subset of grafts was recovered 22 days after grafting to collect pachytene spermatocytes for chromosome analysis. The stages of meiosis as well as the extent of homologous chromosome synapsis were analyzed. **Results:** Immunohistochemistry for BrdU incorporation revealed significantly fewer dividing germ cells in tissue grafts from Lsh  $-/-$  mice than in grafts from Lsh  $+/+$  animals ( $22.4\% \pm 3.8\%$  vs.  $39.2\% \pm 11.8\%$ , respectively). Analysis of meiotic configurations in control wild type ( $n = 250$ ) and heterozygous ( $n = 354$ ) spermatocytes revealed the presence of fully synapsed homologous chromosomes (20 bivalents) in the majority ( $> 80\%$ ) of cells analyzed. In contrast, lack of Lsh function induced a significant increase ( $P < 0.0001$ ) in the proportion (97.8%) of mutant spermatocytes ( $n = 670$ ) that exhibited abnormal synapsis of homologous chromosomes. The type of meiotic abnormalities most commonly observed included the presence of asynapsed chromosomes as well as the formation of axial gaps in the synaptonemal complex of one or more chromosomes per cell as detected by SYCP3 staining. Grafted tissue from the remaining mice was harvested at 4, 6 or 8 weeks after grafting ( $n = 6, 10$  or  $11$  mice, respectively) and analyzed histologically for spermatogenic differentiation. While spermatogenesis proceeded through meiosis with the appearance of round and elongated spermatids in the seminiferous tubules in grafts from Lsh heterozygote and wild type donor testes, pachytene spermatocytes were the most advanced germ cell type present in grafts from Lsh  $-/-$  mice at all analysis time points. **Conclusion:** In the absence of Lsh, proliferation of spermatogonia is reduced and germ cell differentiation is arrested at meiosis. Lsh is required for homologous chromosome synapsis in the male germ

line. These observations implicate an essential role for Lsh during male meiosis. This study also highlights the utility of testis tissue grafting to study spermatogenesis in animal models that cannot reach sexual maturity.

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#### Pt7-33

#### Decreased content of sperm mitochondrial DNA in males with POLG gene not10/10 genotype

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**Aim:** To determine the content of sperm mitochondrial DNA (mtDNA) and CAG repeat of POLG gene in males with asthenospermia. **Methods:** semen samples were classified into two groups, one with asthenospermia and the other with normal phenotype. The content of mtDNA in sperm was determined by real time PCR. We identified the CAG repeat of POLG gene by genescan. **Results:** The mtDNA/ $\beta$ -globin ratio in the asthenospermia and normal groups were 47 (2-280) and 67 (4-319), respectively. A decrease in sperm mtDNA content was detected in patients with asthenospermia. The average mtDNA copy number per sperm was 21 (4-50) and 60 (2-319) in the POLG gene not10/10 genotype groups and 10/10 genotype groups, respectively. It was apparent that mtDNA copy number per sperm with not10/10 genotype groups was remarkably less than those with 10/10 genotype groups. **Conclusion:** Our result further suggested that defects in the mitochondrial respiratory function caused by mtDNA decrease resulted in the reduction in sperm motility. The mtDNA decrease may play an important role in the pathophysiology of some male infertility. The polymorphism of the repeat length of the (CAG) in the POLG gene may affect sperm motility and have a less important role in male infertility.

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#### Pt7-34

#### Analysis of protamine & transitional protein gene mutations in infertile Indian men

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**Aim:** Sperm testicular proteins, the protamines and transitional nuclear proteins play a crucial role in sperm nuclear condensation. Mutation in any of the corresponding genes or aberrant post translation modification may lead to abnormal sperm chromatin condensation leading to male infertility.



Therefore the study was aimed to screen nucleotide changes in protamine (PRM 1 & 2) and transitional nuclear protein (TNP 1 & 2) genes in Indian oligozoospermic men. **Methods:** The study included 30 oligozoospermic men and 20 fertile controls. Semen analysis was performed according to WHO (1999) guidelines. Sperms were separated from leukocytes, epithelial cells and debris by density gradient method. Sperm DNA was isolated by phenol-chloroform method. PRM and TNP genes were amplified, and purified by guanidinium thiocyanate method. The final products were sequenced by standard PCR-DNA sequencing protocol. The nucleotides were numbered from the first nucleotide of the corresponding gene. **Results:** Sperm count ( $10.52 \pm 4.20$  vs.  $92.32 \pm 23.44$ ), and progressive sperm motility ( $28.50 \pm 12.25$  vs.  $62.5 \pm 21.5$ ) were significantly compromised in oligozoospermic men compared to controls. In total, six nucleotide changes were observed in the study out of which, 139C→A in PRM1 gene was found to be common in patients and controls, but 1083C→A in PRM2 gene was found to be present at higher frequency in patients than controls. However, a novel mutation in PRM 2 gene (311C→A) changing amino acid threonine to asparagines was observed in one patient (sperm count-17 million mL<sup>-1</sup>) with 50% abnormal head morphology and 15% forward motility. However, we found a SNP 839 T→C in TNP1 gene in 4% of the patients but not in controls. TNP2 mutation analysis showed a SNP 991C→T in TNP2 gene in 30% of the controls but not in the patients. **Conclusion:** The frequency of SNPs observed in PRM genes was similar in both oligozoospermic and controls except a novel mutation (311C→A) in the exon of PRM2 gene. This mutation might be associated with the abnormal sperm parameters, by altering the PRM2 protein conformation that could lead to infertility. This is the first of kind of study in PRM and TNP genes in Indian infertile population. Higher frequency of SNPs and rare novel mutation in Indian infertile population could be the reason for impaired fertility in this population. Further larger number of studies screening for these variants and its correlation with assisted reproductive outcome is warranted.

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#### Pt7-35

##### A novel 10, 11 translocation in an azoospermic man

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**Aim:** Reporting a case of novel 10, 11 translocation in an infertile man with azoospermia. **Methods:** Semen analysis was performed according to WHO criteria (1999). GTG banding karyotyping was done in 72 hours blood culture. Hormone estimation was done by radio-immuno assay. **Results:** Repeated semen analysis showed azoospermia even after centrifugation. Semen volume was found to be 1 mL and the liquefaction was incomplete even after 60 min. The pedigree analysis showed that his elder brother was also found to be infertile and unavailable for

the study. Patient had late virilization and appeared to be partial. Hormone analysis showed FSH-17.47 mIU mL<sup>-1</sup>, LH-22.28 mIU mL<sup>-1</sup> and testosterone -1305.86 ng dL<sup>-1</sup>. Peripheral karyotype analysis revealed 46, XY t (10; 11) (q11.2; p15) chromosome complements. **Conclusion:** To the best of our knowledge this translocation has not been previously reported in an infertile man. However, such translocation involving chromosomes 10 and 11 have been frequently observed in acute myeloid leukemia (AML) (46, XY, t (10; 11) (p12; q231[9]/46, XY[31, 46, XY, t(10; 11) (p13;q14), 46, XY, t(10; 11) (p13; q15)). However, this patient showed no symptoms of such disorder as the translocation break points are different from those reported in AML. Nil sperms in the semen and elevated testosterone levels showed completely blocked germ cell division might have resulted from impaired quadrivalent synaptic complex due to novel translocation. Also, genes that were disturbed during this translocation may also responsible for impaired fertility, which cannot be ruled out.

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#### Pt7-36

##### ROS: causal role of DNA damage in varicocele

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**Aim:** Reactive oxygen species (ROS) not only affect sperm membrane but also believed to damage sperm DNA. The study was aimed to find out the role of ROS on sperm DNA in varicocele patients. **Methods:** Thirty infertile men with bilateral varicocele attending for varicocelectomy and 50 fertile controls were included in the study. Semen analysis was performed according to standard WHO (1999) guidelines. ROS levels in the washed semen were measured by chemiluminescence assay and expressed as 10<sup>6</sup> counted photons per min/20 million spermatozoa. COMET assay was used to assess sperm DNA damage by visual scoring. Sperm with highest DNA damage was scored as D, moderate as C, lesser as B and no damage as A. **Results:** ROS levels were found to be significantly higher in varicocele patients ( $3450.57 \pm 701.20$ ) when compared to the controls ( $3.56 \pm 1.70$ ). Sperm parameters like sperm count, forward motility and normal morphology were found to be significantly ( $P < 0.01$ ) lower in patients compared to controls. Approximately 70% of the sperm samples in varicocele patient showed D grade COMETS as control showed 80% A grade COMETS. **Conclusion:** Compromised sperm parameters and subsequently impaired fertility in varicocele patients are mainly due to increased ROS production in their semen. This increased ROS levels not only affect the sperm function but also damage DNA altering genomic integrity and epigenetic mechanism resulting in impaired fertilization. Therefore earlier varicocele repair may decrease ROS production and DNA damage and improve sperm function

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**Pt7-37****Restoration of erectile function by reconstructing cavernous nerves with small intestine submucosa grafts**

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**Aim:** To investigate the restoration of erectile function by reconstructing cavernous nerves (CN) with small intestine submucosa (SIS) Grafts. **Methods:** SIS was prepared, and rat models were established and divided into three groups, including: CN injured group, sham operation group and SIS graft group. Three months after operation, all rats of every group were evaluated by Apomorphine test, and then all rats were sacrificed to detect the expression of nNOS in penis of all groups by SP method. **Results:** 1) Apomorphine test: About 72.73% (8/11) rats erected in the SIS graft group, higher than the nerve ablation group 0% (0/11) ( $P < 0.01$ ), and lower than the sham-operation group 90.91% (10/11) ( $P < 0.01$ ); the average erection number in SIS graft group was  $1.07 \pm 0.89$ , higher than that of the nerve ablation group ( $0.00 \pm 0.00$ ) ( $P < 0.01$ ), and lower than that of the sham-operation group ( $2.19 \pm 1.17$ ) ( $P < 0.01$ ). Both erectile rate and average erectile number of the SIS graft group were higher than that of the nerve ablation group ( $P < 0.01$ ), but lower than that of the sham-operation group ( $P < 0.01$ ). 2) The number of the nNOS nerve fiber: the average number of the nNOS nerve fiber of SIS group was  $70.36 \pm 10.09$ , and that of the nerve ablation group was  $22.09 \pm 4.76$ . There was obviously difference between them ( $P < 0.01$ ). It was  $90.81 \pm 5.69$  in the sham-operation group, higher than those of the SIS group and the nerve ablation group ( $P < 0.01$ ). **Conclusion:** SIS bridge technique might lead to recanalization of the severed CN and partial restore erectile dysfunction in rats after surgical injury.

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**Pt7-38****Establishment of recipient murine model for stem cell transplantation into testicular seminiferous tubules and improvements on transplantation technique**Wei Zhang<sup>1,2</sup>, Ping Liu<sup>1,2</sup>, Xiao-yu Xia<sup>3</sup>, Heng Cai<sup>3</sup>, Hui Ding<sup>1,2</sup>, Peng Li<sup>1,2</sup>, Zheng Li<sup>1,2\*</sup>, Yi-Ran Huang<sup>1,2</sup><sup>1</sup>Department of Urology, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200001, China<sup>2</sup>Stem Cell Center for Clinical Research, Renji Hospital, Shanghai Jiaotong University, Shanghai 200001, China<sup>3</sup>Key Lab on Reproductive Medicine, Shanghai Jiaotong University School of Medicine, Shanghai 200025, China

**Aim:** To establish a stable recipient model for stem cell transplantation into seminiferous tubules and improve the traditional technique for transplantation. **Methods:** Different dosages of Busulfan ( $15 \text{ mg kg}^{-1}$ ,  $30 \text{ mg kg}^{-1}$ ,  $40 \text{ mg kg}^{-1}$ ,  $0 \text{ mg kg}^{-1}$ ) were injected into four groups of mice respectively. Survival rate was recorded every day after injection, while murine testis weight, spermatogenesis of testicular tubules were investigated 4, 8, 12 weeks later. To improve the stem cell transplantation technique, new transplantation device

was designed independently. The nozzle end, syringe and the puncture needle were connected by a tee-junction in this new device. The nozzle end was used for tentative injection, and the syringe was used for piping cell suspension and then inject it out. Embryonic stem cell cultures were amplified, and then transplanted into seminiferous tubules of five model mice with the above-mentioned transplantation technique. Frozen sections were done one month after the transplantation, confocal microscope was used to detect the survival through the green fluorescence taken by embryonic stem cells and the situation of colonization in the testis. **Results:** (1) Only one mouse in group C died after injection. At the time of 4 weeks after Busulfan treatment, testis weight all decreased apparently compared with the control group ( $P < 0.05$ ). The seminiferous tubule hollow rate of group A was less than 50%, while  $> 50\%$  in both group B and C and spermatogenic cells could not be found in these hollow tubules. (2) Usually, at least 100–150  $\mu\text{L}$  cell suspension is needed for one mouse, but with this device and method, only 20–30  $\mu\text{L}$  is enough. The improved transplantation device increased the success rate, lowered the donor cell loss and shortened the process. (3) Under the confocal microscope, cells with green fluorescent can be seen in the peritubular and luminal of the seminiferous tubules. **Conclusion:** It is recommended that intraperitoneal injection of Busulfan ( $30 \text{ mg kg}^{-1}$ ) should be used to establish the recipient mice model for stem cell transplantation, which will induce a higher hollow rate of testicular tubules 4 weeks after administration. This is also confirmed by the fact that embryonic stem cells with green fluorescent can live in the seminiferous tubules. The improved transplantation device and methods will promote the efficiency and the success rate of the transplantation operation.

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**Pt7-40****Expression of HE2β1 in the testis, epididymis and spermatozoa of adults**

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**Aim:** To immunolocalize the human epididymal secretory protein 2 isoform, HE2β1, in the testis, epididymis and ejaculated sperm and to explore its characteristics of expression in the male reproductive tract. **Methods:** Immunohistochemistry was carried out to determine the expression of HE2β1 in the testis and epididymis and indirect immunofluorescence was performed to localize this protein in the ejaculated spermatozoa of the adults. **Results:** HE2β1 immunopositive staining occurred mainly in the cytoplasm of Leydig cells of the testis. In the epididymis, HE2β1 expressed mainly in the supranuclear region of the principle cells and the apical stereocilia of the duct epithelium; a weak positive staining of luminal contents was observed in some ducts. Maximum levels of HE2β1 expression were located in the initial segments. The number of HE2β1 immunopositive cells decreased along the caput to cauda. Indirect immunofluorescence



revealed that HE2β1 was concentrated on the postacrosomal head and neck region of the ejaculated spermatozoa. **Conclusion:** Immunostaining is a fairly sensitive method for determining HE2β1 expression. The localization and expression level of HE2β1, the protein with β-defensin-like motif, in the genital duct of human exhibited a region- and cell-specific expression pattern, which suggests that HE2β1 may be involved in sperm maturation, sperm-oocyte fusion and epididymal epithelial innate defense mechanisms.

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#### Pt7-41

##### Testis-specific gene, LM23, is essential for spermatogenesis in *Rattus norvegicus*

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**Aim:** To explore the function of LM23 (AF492385), a gene with testis-specific expression in *Rattus norvegicus* previously reported by our laboratory. **Methods:** We used lentivirus-mediated RNA interference (RNAi) to knock down LM23 expression in a tissue-specific manner *in vivo*. A lentiviral vector expressing a short hairpin RNA (shRNA) targeting LM23 was microinjected into one side testis of 5 weeks aged *R. norvegicus*. The scrambled RNAi was injected into the other testis as a control, using the same method. The total number of *R. norvegicus* injected was 12. **Results:** The expression of LM23 in the treated testes was significantly knocked down compared with controls. These LM23-shRNA testes contained germ cells arrested at the spermatocyte stage, and suffered from increased apoptosis and dysregulation of some meiotic genes. **Conclusion:** The results demonstrate the validity of the RNAi approach and unambiguously reveal that LM23 expression in the testis is crucial for meiosis during spermatogenesis in *R. norvegicus*.

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#### Pt7-42

##### The protective effects of α-ketoacids against oxidative stress on rat spermatozoa during the incubation

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**Aim:** To determine the effects of antioxidants including α-ketoacids (α-ketoglutarate [KET] and pyruvate [PYR]), lactate (LAC) and glutamate plus malate (GLU + MAL) against oxidative stress on rat spermatozoa during the incubation. **Methods:** We examined reactive oxygen species (ROS) level, ATP level, and protein phosphorylation in spermatozoa as well

as motility and viability when these antioxidants were added to sperm incubation medium in the presence or absence of H<sub>2</sub>O<sub>2</sub>. **Results:** Our results showed that the H<sub>2</sub>O<sub>2</sub>-induced damages, such as impaired motility, adenosine triphosphate (ATP) depletion, decreased viability and inhibition of sperm protein phosphorylation, could be prevented by the supplementation of KET or PYR in media. When there was no H<sub>2</sub>O<sub>2</sub> in medium, it was interesting to find that sperm ROS level was elevated by the addition of KET, LAC or GLU + MAL. The supplementation of KET or LAC resulted in an improvement of sperm motility and protein phosphorylation, but PYR or GLU + MAL had no significant effect. Sperm viability was not affected by the addition of these antioxidants. **Conclusion:** The present study concludes that α-ketoacids (especially KET) could be the candidate components in sperm incubation media for protecting rat spermatozoa from H<sub>2</sub>O<sub>2</sub> attack.

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#### Pt7-43

##### The effects of perfluorooctanoic acid on steroidogenesis of rat Leydig cells

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**Background:** Perfluorooctanoic acid (PFOA), also known as perfluorooctanoate, is a synthetic perfluorinated carboxylic acid, and is also thought to be a persistent environmental endocrine disruptor. PFOA has been detected in the human blood and it has been linked to infertility. One study has shown in China that PFOA and its related chemicals can reach 50 μg per liter. However, the mechanism of PFOA for reproductive toxicity is unclear. **Aim:** The objective of the present study is to inhibit Leydig cell steroidogenesis. **Method:** In Leydig cell steroidogenesis, two enzymes, 3β-hydroxysteroid dehydrogenase 1 that catalyzes pregnenolone into progesterone, and 17β-hydroxysteroid dehydrogenase 3 that catalyzes the last step of testosterone formation from androstenedione, were investigated. In Sprague Dawley rat testis microsome, radiolabeled pregnenolone (1 000 nmol L<sup>-1</sup>) and cofactor NAD<sup>+</sup> (0.2 mmol L<sup>-1</sup>) were used to measure 3β-hydroxysteroid dehydrogenase 1 activity, and radiolabeled androstenedione (100 nmol L<sup>-1</sup>) and cofactor NADPH (0.2 mmol L<sup>-1</sup>) were used to detect 17β-hydroxysteroid dehydrogenase 3 activity. **Results:** PFOA showed dose-dependent inhibition of both 3β-hydroxysteroid dehydrogenase 1 and 17β-hydroxysteroid dehydrogenase 3 activities with half maximum inhibitory concentration (IC<sub>50</sub>) of 53.2 ± 25.9 μmol for 3β-hydroxysteroid dehydrogenase 1



and  $17.7 \pm 6.8 \mu\text{mol L}^{-1}$  for  $17\beta$ -hydroxysteroid dehydrogenase 3. The inhibitions of  $3\beta$ -HSD and  $17\beta$ -HSD3 by PFOA were competitive for the substrates. **Conclusion:** PFOA inhibits  $3\beta$ -HSD and  $17\beta$ -HSD3 in rat Leydig cells.

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#### Pt7-44

##### Genetic variants in cell death pathway is associated with altered sperm apoptosis and poor semen quality

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**Aim:** The FAS/FASLG system has been proposed to play a key role in germ cell apoptosis. This study aimed to elucidate the influence of the genetic variants of cell death pathway genes on male infertility. **Methods:** The genotypes of FAS, FASLG and CASP8 were determined in 620 infertile men. Sperm apoptosis rates were measured by Tdt-mediated dUTP nick end labeling (TUNEL) assay, and the semen quality analysis was performed using computer assisted sperm analysis (CASA). **Results:** We found that polymorphisms of FAS-670A/G (rs1800682: A > G) and CASP8 -652 6N ins/del (rs3834129: -/CTTACT) were evidently associated with sperm apoptosis and semen quality. Individuals with FAS-670GG showed lower apoptosis rate and lower sperm concentration when compared individuals with the FAS-670AA genotype. Similarly, in comparison with the CASP8-652 6N ins/ins genotype, the CASP8-652 6N (ins/del+del/del) genotypes also showed significantly lower sperm apoptosis rate and poorer sperm motility. However, other polymorphisms didn't appear to affect sperm apoptosis and semen quality. **Conclusion:** This study showed for the first time that functional variants of FAS and CASP8 might contribute to the dysfunctional apoptotic mechanism in germ cells, which could result in poor semen quality of ejaculated sperm.

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#### Pt7-47

##### The oxidative stress adduct (OSA) test a novel assessment of oxidative stress, identifies a large percentage of infertile males not identified by an abnormal DNA fragmentation score: a pilot study

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**Background:** Oxidative stress (OS) has been strongly associated with adverse effects on cellular lipids, proteins and DNA, which result in defective sperm function and male infertility. However, the effects of OS on the DNA integrity of human spermatozoa have not been completely determined. We have developed a novel, proprietary oxidative stress adduct (OSA) test which measures levels of a combination of different types of molecules created from exposure to OS. **Aim:** To determine if the OSA test identifies a unique population of infertile males not identified by an abnormal sperm DNA fragmentation assay (S DFA) score. **Methods:** This is a retrospective study using discarded patient

specimens. Analysis was performed on semen specimens from 150 males from infertile couples and 20 fertile donors control males. Semen samples were divided into two aliquots to determine a) the OSA score and b) the DNA fragmentation index (DFI) using the S DFA test. **Results:** The OSA scores in the 20 fertile males were tightly clustered around a mean value of  $2.4 \mu\text{mol L}^{-1}$  with a range of 1.3 to  $3.5 \mu\text{mol L}^{-1}$ . The OSA scores in the males from 150 infertile couples were remarkably different in their distribution revealing a mean value of  $4.3 \mu\text{mol L}^{-1}$  and a range of 0.64 to  $18.15 \mu\text{mol L}^{-1}$  with 47% (71 men) being above the highest value ( $3.5 \mu\text{mol L}^{-1}$ ) in the fertile controls. In addition, the OSA assay identified 28% of the 150 males from infertile couples as having abnormal OSA scores ( $> 5 \mu\text{mol L}^{-1}$ ) but without abnormal DFI scores. Interestingly, 100% of the 10 infertile males with abnormal DFI scores also had OSA scores above  $5 \mu\text{mol L}^{-1}$ . **Conclusion:** The OSA test, a novel proprietary semen assessment of oxidative stress identified a large fraction of infertile men not identified as abnormal via DNA fragmentation testing. This may provide another means of prospectively identifying infertile males that would benefit from a clinical management change such as anti-oxidant therapy. Additional study is warranted to further explore the utility of this test in improving clinical management of male fertility.

#### Pt8-02

##### Effect of testis heat stress contraceptive underpants on sperm DNA integrity and sperm membrane damage of human male

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**Aim:** To investigate the effect of testis heat stress on male fertility by observing the changes in semen parameters, sperm DNA damages as well as sperm membrane. **Methods:** Designs and development of constant temperature controller, timer, the device of alternating current to direct current and heat stress undershirt. Eleven healthy male volunteers, who already had children, were chosen for the experiments of wearing  $43^\circ\text{C}$  testis heat stress underpants (THSU). The volunteers wore specially designed undershirts with a constant  $43^\circ\text{C}$  apparatus, two days per week, and 50 min per day, for a period of 3 consecutive months. Routine semen analysis and hypo-osmotic swelling test (HOST) as well as sperm chromatin dispersion (SCD) examination were carried out before, during and after THSU, respectively. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) were measured by chemiluminescent immunoassay. **Results:** The mean parameters of sperm density, motility, abnormality, the rate of HOST and sperm DNA integrity by SCD before THSU were  $(85.8 \pm 19.7) \times 10^6 \text{ mL}^{-1}$ ,  $46.7\% \pm 17.9\%$ ,  $(5.7\% \pm 7.9\%)$ ,  $65.1\% \pm 11.5\%$  and  $86.7\% \pm 10.3\%$ , respectively. Two months after THSU, all the parameters decreased or increased significantly, by 3 months, a dramatic decrease or increase appeared in all the parameters [ $8.7 \pm 6.6] \times 10^6 \text{ mL}^{-1}$ ,  $18.2\% \pm 7.2\%$ ,  $53.6\% \pm 5.2\%$ ,  $15.6\% \pm 8.2\%$  and  $26.2\% \pm 6.7\%$ ] were observed ( $P < 0.01$ ). During two, three



and six months recovering period after THSU, all the parameters restored gradually to the normal control levels before THSU. The THSU did not affect serum concentration of T, FSH and LH. **Conclusion:** The effect of spermatogenesis inhibition by using THSU is significant. It may be an excellent and acceptable way of THSU plus androgen to develop a new male contraception.

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#### Pt8-03

##### Morphological changes of cells of tonsils affected by trichomonas

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**Aim:** To reveal trichomonas in tonsils and the morphological changes of cells of human palatine tonsils (PT). **Methods:** Trichomonas and PT's cells were obtained by scraping from PT of male patients with iron deficiency anemia (1), remote melanoma (2), leukemia (3), lung cancer (2) and practically healthy men (2). The cell specimens were placed as smears onto microscopic slides, stained by Giemsa and observed by a light microscopy (patent No. 2 293 298 C2, 2007). **Results:** The trichomonas were revealed in the specimens of the PT of men during screening of neoplasm by a retrospective analysis of tonsil's cells, which was an unexpected discovery. The trichomonas were arranged and distributed among the epithelium with sizes from 10 to 18  $\mu\text{m}$ , small pink or red nucleuses, displayed light blue staining abundant cytoplasm with clear contours which were rounded or pear-shaped. Epithelial cells had large sizes, with a large nucleus and their cytoplasm was stained intensively blue. The epithelium displayed homogenous optically dense cytoplasm with sinuous contour. Some large epithelial cells had a large solitary vacuole or a few small vacuoles. It was clear dysplasia of epithelium. These patients did not suspect they were infected by trichomonas. **Conclusion:** These data support the idea that the test should be made in the following cases: (1) Patients in trichomoniasis and in other sexual infections. (2) Patients in neoplasm. (3) Patients in anemia. (4) Patients in pulmonary diseases. (5) Patients-perverted. The test can help to diagnosis and adequate treatment of trichomoniasis and other diseases.

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#### Pt8-04

##### Effect of dietary cowpea (*Vigna unguiculata*) on reproductive performance of sows

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**Background:** Cowpeas, as an alternative to soyabeans in animal and human nutrition contains anti-nutritional factors (ANFs) such as trypsin inhibitors, lectins, and tannins, which decrease

protein digestability. **Aim:** To confirm whether cowpea feeding has any deleterious effects on the reproductive system of pigs, mainly focusing on the reproductive performance of sows and their litters. **Methods:** Sixty large white X Landrace gilts (55–70 kg BWt) were divided into three dietary groups (control, raw cowpea and cooked cowpea) with 20 in each group. The gilts were exposed to a boar at the beginning of the study and were served in the second estrus. Five boars were selected and each served two sows per week. The gilts were fed with the diets ad lib from the duration of the gestation period until farrowing up to the weaning of the piglets at age of 5 weeks. The litter size of each group was measured, and the birth weights of the piglets were recorded. **Results:** Mean litter size at birth, mean birth weight, mean weaning weight and mean litter size at weaning were low in raw cowpea fed group. The percentage of gilts not returning to oestrous in raw cowpea fed group after two services was 83.4% compared with 87.9%, 88.6 % in control and cooked cowpea fed groups. Diet had no significant effect on the secondary sex ratio. The boars had no effect on the litter parameters. **Conclusion:** These preliminary data suggests that raw cowpea feeding has deleterious effects on the reproductive performance of female pigs and it is comparable to the effects on the rats.

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#### Pt8-05

##### Establishment and evaluation of a new multiple polymerase chain reaction to detect pathogens of sexually transmitted diseases

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**Background:** Sexually transmitted disease (STD) has become a hot point to study recently. More and more pathogens are related to STD and the number of infected patient increases rapidly. It also raises the risk of the STD patient to be infected with HIV. Multiplex PCR (M-PCR) is developed on the basis of single PCR, by which several different target fragments can be amplified simultaneously with one PCR reaction including several pairs of primers. It provides an effective solution for the diagnosis of STD with multi-agent infections. However, PCR technique needs to be well-established to avoid false-positive and false-negative results because of its high sensitivity and specificity. **Aim:** To explore epidemiological characteristics and changing trends of STD in Tianjin, simultaneously, to develop a more sensible and specific method for the diagnosis of the cases of multi-agent infections to reduce the cost and shorten the diagnosis time in clinical application. **Methods:** 1) Retrospective investigation of representative hospitals and Tianjin Centers for Disease Control was conducted to study epidemiological trends in 2006–2008. The category of infectious agents of STD, complicated infection, demographic features and sexual behavior features were statistically analyzed. 2) A new M-PCR named VG-M-PCR that can detect five pathogens of STDs simultaneously: *Neisseria gonorrhoeae* (NG), *Chlamydia*

trachomatis (CT), *Mycoplasma genitalium* (MG), *Ureaplasma urealyticum* (UU), *Trichomonas vaginalis* (TV) was established, and then M-PCR assay was done to evaluate its sensitivity and specificity. At the same time, two other multiplex PCR assays, Taq Multiplex PCR (TQ-M-PCR) and Pfu Multiplex PCR (PU-M-PCR), were performed to compare with VG-M-PCR. 3) Five confirmation-PCR(C-PCR) assays targeting different gene sequences for each of the five pathogens were established to validate the VG-M-PCR results. 4) One hundred STD specimens and 55 specimens from physical examinee were detected by VG-M-PCR, and then positive and suspicious results were verified by C-PCR. Specimens were determined as positive if the results of both M-PCR and C-PCR were positive. The 100 STD specimens were also detected by traditional methods simultaneously. NG and MG were examined with culture methods. CT and UU were examined using diagnostic kit. TV was detected by wet-mount examination. Then the results of PCR detection and traditional assays were compared. **Results:** 1) There is statistical significance between demographic features and infectious agents. During these three years, infectious diseases increased, with *Neisseria gonorrhoeae* (NG) accounting for 1 228 cases (36.70%), and complicated infections increase, especially *Chlamydia trachomatis* (CT) and *Ureaplasma urealyticum* (UU) account for the cases (16.40%). 2) VG-M-PCR readily amplified each of the DNA controls in individual and mixed formats at concentration of  $10^{-8}$  ng  $\mu\text{L}^{-1}$ . The lowest concentrations of control DNA that could be detected by TQ-M-PCR were  $10^{-7}$  ng  $\mu\text{L}^{-1}$  for MG, UU, CT, and NG, and  $10^{-6}$  ng  $\mu\text{L}^{-1}$  for TV and those could be amplified by PU-M-PCR were  $10^{-7}$  ng  $\mu\text{L}^{-1}$  for MG,  $10^{-6}$  ng  $\mu\text{L}^{-1}$  for NG,  $10^{-5}$  ng  $\mu\text{L}^{-1}$  for UU, CT, and TV. As to the controls in mixed formats at concentration of  $10^{-6}$  ng  $\mu\text{L}^{-1}$ , TQ-M-PCR could only detect CT and TV and PU-M-PCR barely amplified CT. 3) Among the total of 155 samples, VG-M-PCR indicated positive and suspicious positive specimens of 86 for UU, 53 for NG, 36 for CT, 21 for MG, and four for TV. These potential positive samples were subjected to corresponding C-PCR and the pathogen-positive specimens could be verified as following: 77 for UU, 51 for NG, 33 for CT, 19 for MG, and one for TV. The nine stochastic PCR products were sequenced using corresponding primers and the data indicated that the results were true. 4) Among the 100 specimens from STD patients, the positivity rate of pathogens detected by PCR assays were 53.0% for UU, 49.0% for NG, 31.0% for CT, 18.0% for MG, and 1.0% for TV, and 51 of them were multi-agent infections; the positive rate of pathogens detected by traditional methods were 34.0% for UU, 1.0% for NG, 22.0% for CT, 4.0% for MG, no TV was found, and 14 of them were multi-agent infections. **Conclusion:** Health education and behavior intervention should be strengthened to control the transmission and prevalence of STD. The strategy of screening samples with VG-M-PCR and then confirming the positive results with C-PCR is more accurate and reliable with low false positive rate. It can be employed in the clinical practice. In order to prevent and cure STDs actively and control its spread, it is important to examine the patients with combined infections in the early stage and detect multi-pathogens widely.

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#### Pt8-06

##### The changes of AQP2 in the graft of acute rejection rat renal transplantation model

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**Aim:** To investigate the significance and changes of AQP 2 in the graft of acute rejection rat renal transplantation. **Methods:** In this model used Wister (34) and SD (14) male rats were divided into Allograft Group ( $n = 7$ ), Isograft Group ( $n = 8$ ), Control Group ( $n = 8$ ) and Allograft + CsA Group ( $n = 7$ ) Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical studies were performed to evaluate the changes of AQP2 in the graft kidney samples of rat after kidney transplantation (TX) 3, 5, 7 d, Then the levels of mRNA and protein expression were analyzed. **Results:** Compared with the normal control, the AQP 2's expression in the graft of acute rejection (AR) rats was significantly lower ( $P < 0.01$ ), but had no significant difference between the syngeneic renal transplantation control group (sTX), and the Immunodepression group (aTX + CsA) ( $P > 0.05$ ). And its expression 5 d and 7 d after TX decreased significantly compared with that 3 d after TX ( $P < 0.05$ ). In addition, there was no remarkable difference between the expression of 5 and 7 d after TX. **Conclusion:** The level of AQP2 protein expression was decreased under the AR of renal transplantation, and the downregulation has nothing to do with the ischemic reperfusion injury and the denervation damage. When CsA was applied on the rats after kidney transplantation the downregulation would be relieved or disappeared.

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#### Pt8-07

##### Influence of Saikosaponin on sexual behavior and the level of brain monoamine neurotransmitter in rat models of depression

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**Aim:** To investigate the regulative effect and mechanism of saikosaponin on the sexual behavior of rats in depressive state. **Methods:** Thirty-five male Wister rats were divided into control group ( $n = 12$ ), model group ( $n = 11$ ) and saikosaponin group ( $n = 12$ ). The animal model of depression was established by giving chronic stress and feeding alone. The saikosaponin group rats were given Saikosaponin ( $5\text{mg kg}^{-1} \text{d}^{-1}$ ) after stable depression model was established. The frequency of licking and riding in 15 min were calculated. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical study were performed to evaluate the changes of 5-HT and BDNF in rat brain. **Results:** The frequency of licking and riding increased significantly in Saikosaponin group compared with model group ( $P < 0.05$ ). The content of brain 5-HT and BDNF increased significantly in the Saikosaponin group compared

with model group ( $P < 0.05$ ). **Conclusion:** Saikosaponin can improve the sexual behavior of rats under depressive state. The mechanism may be related to the regulation of Saikosaponin on brain monoamine neurotransmitter content, which can promote sexual function of animal models with depression.

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#### Pt8-08

##### Determination of macro- and trace elements in aqueous herbal extracts traditionally used for the treatment of male infertility

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**Aim:** Infertility affects about 15% of couples at reproductive age and 50% of these can be attributed to male infertility. Considering that in South Africa plants and herbal extracts are used as traditional remedies for various ailments and diseases, including male infertility, a thorough analysis of these traditional medicines is necessary. In view of the fact that macro elements such as Na, K, Mg and Ca (also normally referred to as electrolytes) and trace elements such as Fe, Zn and Se have been identified as important factors in male fertility and sperm functions like motility [1, 2], the aim of this study was to determine the concentrations of these elements in aqueous extracts of herbs used for the treatment of male infertility.

**Methods:** Roots of *Cissampelos capensis*, rhizomes and leaves of *Typha capensis* as well as leaves of *Hermannia ciliata* were collected in the Cape Nature Reserve. Following cleaning of the plant material was dried in a drying oven at 25°C, chopped to pieces and aqueous infusions were made with hot distilled water. Afterwards, the infusions were freeze-dried and stored in an airtight container at 4°C until further use. The determination of the macro and trace elements has at times been problematic due to various factors such as the ease of volatilisation. For this reason, different analytical techniques, namely Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Graphite Furnace Atomic Absorption Spectrometry (GFAAS), Hydride Generation (HG) and Flame Atomic Absorption Spectrophotometry (FAAS) were selected and compared. Since the speciation of the elements is at present unknown in the extracts, four different dissolution procedures were employed: HCl:H<sub>2</sub>O (I); HNO<sub>3</sub>:H<sub>2</sub>O (II); HCl:HNO<sub>3</sub>:H<sub>2</sub>O (III) and HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub>:H<sub>2</sub>O (IV). For standard, the concentrations of the elements in three certified

reference standards were determined. **Results:** The detailed data were shown in Table 4. **Conclusion:** In conclusion, this study analyzed the concentrations of elements in aqueous extracts of traditional remedies used for male fertility/infertility for the first time.

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#### Pt8-09

##### In vitro effects of bulrush extracts on sperm functions and inhibition of reactive oxygen (ROS) and reactive nitrogen species (RNS)

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**Aim:** The biodiversity in South Africa provides more than 30 000 higher plants, of which over 3 000 are used by traditional healers to treat diseases for thousands of years. In modern “Western” medicine, little is known about these traditional methods although about 25% of all prescriptions contain active ingredients derived from plants. In addition, none of these herbs has been scientifically investigated in terms of male fertility. Therefore, there is a need to test the effects of these plants on sperm function and male reproductive functions. Moreover, in South Africa, the practice of traditional healers has been legalized by an Act of Parliament. Therefore, the objective of this study was to investigate the *in vitro* effects of extracts from bulrush (*Typha capensis*) on sperm motility, viability, production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and the mitochondrial membrane potential (MMP). **Methods:** Fifty semen samples were washed with HTF Medium containing 10 mg mL<sup>-1</sup> albumin and a swim-up was performed. To 100 µL swim-up fraction, 1 µg mL<sup>-1</sup> freeze-dried aqueous extract of *Typha* rhizome was added and incubated for 1 h at 37°C. Sperm

Table 4.

	Na (ppm)	K (ppm)	Mg (ppm)	Ca (ppm)	Cr (ppm)	Ni (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Se (ppb)
<i>Typha capensis</i> (leaves)	230 ± 10	137 ± 17	42 ± 10	160 ± 17	2.35 ± 0.57	0.06 ± 0.04	< 0.01	50 ± 10	19 ± 7	2.70 ± 0.50	15 ± 10
<i>Typha capensis</i> (rhizome)	175 ± 10	8 107 ± 12	42 ± 10	1440 ± 25	2.65 ± 0.74	< 0.01	< 0.01	94 ± 4	18 ± 5	2.45 ± 0.68	< 10
<i>Cissampelos capensis</i>	130 ± 14	100 ± 15	42 ± 10	245 ± 15	0.24 ± 0.10	0.25 ± 0.13	3.85 ± 0.19	150 ± 21	0.8 ± 0.2	29 ± 5	35 ± 14
<i>Hermannia ciliata</i> .	125 ± 10	310 ± 12	162 ± 10	149 ± 25	< 0.01	< 0.01	1.39 ± 0.12	8.50 ± 1.59	0.8 ± 0.2	18 ± 7	12 ± 10



viability (Eosin test), motility, ROS production (DHT test; Molecular Probes) and the mitochondrial membrane potential ( $\Delta\psi_m$ ) (DePispher Kit; R&D Systems) were analyzed in the test samples, the controls as well as in the pellet. In order to test the inhibitory potential of the extracts of the leaves and rhizome on the production of ROS in terms of superoxide and reactive nitrogen species (RNS) in terms of peroxyntirite, concentrations of 0.01 mg mL<sup>-1</sup>, 0.05 mg mL<sup>-1</sup> and 0.1 mg mL<sup>-1</sup> extract were tested by means of a chemiluminescent Antioxidant Kit with Pholasin® (Knight Scientific Ltd.) was used. Statistical analysis was performed using the MedCalc software ver. 10.4 (Mariakerke, Belgium). **Results:** After incubation of sperm with the rhizome extract, viability (70.8% vs. 77.6%;  $P < 0.0001$ ), motility (81.4% vs. 95.3%;  $P < 0.0001$ ),  $\Delta\psi_m$  (49.0% vs. 67.5%;  $P < 0.0001$ ) and sperm ROS production (11.3% vs. 14.9%;  $P < 0.0001$ ) decreased significantly. When comparing the swim-up sperm with those from the pellet,  $\Delta\psi_m$  ( $P < 0.0001$ ) and viability ( $P < 0.0001$ ) were significantly decreased in the pellet sperm. ROS and RNS production showed a significant, dose-dependent inhibition after incubation with the rhizome and leave extract. Results also indicate a decreased MMP ( $P < 0.0001$ ) and viability ( $P < 0.0001$ ) in the pellet when compared to the swim-up. **Conclusion:** Typha capensis extracts had significant detrimental effects on various sperm functions *in vitro*. It also decreased the cells' ROS production, which could have a negative effect on cellular functions. Moreover, centrifugation not only affects sperm motility and viability but also mitochondrial functionality.

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#### Pt8-10

##### Effects of nano-silver foam spermicides on killing sperm *in vitro* and *in vivo* during menstrual cycle

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**Aim:** To evaluate the effects of nano-silver foam spermicides on killing sperm *in vitro* and *in vivo* during menstrual cycle.

**Methods:** 1) Semen samples were collected from 25 healthy male volunteers by masturbation. After liquefaction every sample was divided into three portions and 0.2 mL of semen was acquired from each portion. Nano-silver spermicides was added into one portion (group A), equivalent capacity of placebo (non nano-silver foam spermicides) into another portion (group B) and the last portion was blank group (group C). Then each sample was added with equivalent amount of 0.1% eosin staining solution and then the time-effect reaction of sperm viability was observed. 2) Ninie healthy couples who had had children were chosen as experimental objects. Ten min before coition, 2 mL of nano-silver foam were infused deeply into the vagina by female herself on ovulation day, the 5th day before and after ovulation, respectively. Mucus sample was collected from participants' cervical canals 15 min after ejaculation and sperm counts and sperm viability were determined. **Results:** 1) Observed 5 s, 1 min, 10 min and 30 min after staining, sperm viability was respectively 0.4%, 0.1%, 0%, 0% in group A, 27.9%, 23.7%,

15.5%, 3.6% in group B, and 60.1%, 59.7%, 58%, 56.2% in group C, which showed that the effect of nano-silver spermicides in the experimental group was better than that in placebo group and blank group and the difference was statistically significant ( $P < 0.001$ ). 2) Sperm counts (sperm viability) without the use of nano-silver spermicides on ovulation day, the 5th day before and after ovulation were respectively 27.7/HP (28.7%), 21.6/HP (32%), 22.3/HP (24.4%), while 5.3/HP (0%), 18.1/HP (1.8%), 25.6/HP (4.7%) with the use of the foam, which showed that the difference of sperm count in cervical canals on ovulation day had no statistical significance with and without the use of the foam ( $P > 0.05$ ). Sperm viability after the use of the foam was obviously lower than the without use of the foam, which showed a statistical significance ( $P < 0.001$ ). **Conclusion:** Nano-silver foam is a new kind of spermicide which has obvious effects on killing sperms *in vitro* and *in vivo* during menstrual cycle.

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#### Pt8-11

##### Esthetic Andrology

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Various problems and issues in andrology require great attention on both the psychological and physical management and treatment. Psycho- and physical problems and issues cannot be separated from each other, as the separation of them impairs the male patients' holistic health system and reduces quality of life. Esthetic andrology offers new perspectives in clinical andrology. It may involve serious medical examination, diagnoses, treatment and management, which may conclude to any surgical and/or non-surgical approaches. In some cases surgical approaches may provide better treatment and management, such as the cases of permanent gynecomastia in male patients. Non surgical approaches can be of advantage for skin problems such as the diversities of skin diseases of the male nipple, vitiligo, benign skin tumors, facial hyperpigmentation, alopecia and other male aging skin problems. Skin care and other esthetic modalities for the male patient is currently a new emerging life style and life behavior. Today's male also cares for an attractive fashion-look appearance as much as women will do! Esthetic andrology is a new attractive emerging scientific field in medicine, which can improve male's self esteem to manage wellness and the best quality of life. A growing need for esthetic andrology has 'tickled' "metro-sexual males" in metropolis cities world-wide.

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#### Pt8-13

##### Basal body temperature and endometriosis

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**Aim:** This investigation examined the association between

pelvic endometriosis and altered basal body temperature (BBT). **Methods:** This study population consisted of infertile couples who have been diagnosed as having endometriosis. **Results:** A significant association was found between the presence of pelvic endometriosis and the appearance of a late decline in BBT during the early follicular phase of the menstrual cycle. A temperature of 97.80°C on the first 3 days of the menses is associated with pelvic endometriosis. **Conclusion:** A relatively common problem in women is endometriosis. The association between endometriosis and infertility is clearly established. It is proposed that endometriosis has the potential to produce pathology in two ways: 1) Hypothalamic-pituitary ovarian axis and luteal phase abnormalities. 2) Peritoneal inflammatory response. The findings of this study support the clinical diagnosis of endometriosis in infertile women. The BBT chart analysis may be useful as a clinical adjunct when endometriosis is suspected.

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#### Pt8-15

##### Effect of Uremia and kidney transplantation on testicular volume and male reproductive ability

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**Aim:** To investigate the effect of Uremia and kidney transplantation on testicular volume and male reproductive ability. **Methods:** 40 Uremia patients' semen samples and 40 kidney transplantation patients' semen samples were tested. And calculate as: Fertility index (FI) = sperm concentration ( $10^6 \text{ mL}^{-1}$ )  $\times$  sperm viability  $\times$  sperm normal morphology rate. Thirty of these Uremia patients were monitored for testicular volume by color ultrasound before and after kidney transplantation. **Results:** FI in normal fertility people was 13.03 (14.26), FI in kidney transplantation patients was 7.19 (10.18), and FI in Uremia patients was 0.23 (0.76). Testicular volume of Uremia patients: which on the left side before surgery was ( $6.82 \pm 1.49 \text{ mL}$ ), and on the right side before surgery ( $7.46 \pm 1.89 \text{ mL}$ ). At 1 month, 3 months, and 1 year after kidney transplantation, the left side of Testicular volume rose to  $8.25 \pm 1.67$ ,  $9.31 \pm 1.56$ ,  $9.80 \pm 1.51$ ; and the right side of testicular volume to  $9.18 \pm 1.76$ ,  $10.41 \pm 1.43$ ,  $11.09 \pm 1.45$ . The FI and testicular volume had statistics differences ( $P < 0.01$ ) between Uremia patients and after kidney transplantation groups. **Conclusion:** Uremia reduces Male testicular volume and impairs reproductive ability, but they can be improved by successful kidney transplantation.

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#### Pt8-16

##### The suitable time for male renal transplant recipients to father pregnancy and childbirth

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**Aim:** To determine the appropriate time for male renal transplant recipients to father pregnancy after transplantation. **Methods:** A total of 212 cases of male renal transplant recipients who fathered 216 children after transplantation, including four cases of the two-child birth, among eight organ transplant centers in China between December 1981 and August 2007, were retrospectively analyzed and their children's growth and development status were investigated. **Results:** The 212 renal transplant recipients fathered 216 children 15–204 ( $46.91 \pm 26.19$ ) months after transplantation, including 115 boys and 101 girls. Among them, 20 recipients fathered 20 offspring, including three preterm birth (15.0%), 15–24 ( $21.00 \pm 2.94$ ) months after transplantation; 192 recipients fathered 196 offspring, including six preterm birth (3.1%), 25–204 ( $49.56 \pm 26.06$ ) months after transplantation. The newborn babies weighed  $1\ 950\text{--}4\ 600$  ( $3\ 253 \pm 379$ ) g. One boy suffered from a pair of soft toe. All other children were normally developed as indicated in physical examination. **Conclusion:** It would be appropriate for male renal transplant recipients with normal renal function to father pregnancy and have offspring two years after transplantation.

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#### Pt8-17

##### *Boesenbergia rotunda* (L.) Mansf. juice did not affect androgenic and estradiol levels in premature male rats.

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**Aim:** To determine if Krachai, *Boesenbergia rotunda* (L.) Mansf., juice could promote an increase of the androgenic and estradiol hormones, reproductive organ weights and epididymal sperm density, and at same time, produce toxicity in premature male rats. **Methods:** Thirty-two premature male Wistar rats (4 weeks old) were equally divided into a control and three *B. rotunda* juice-treated groups. *B. rotunda* juice was orally administered at the doses of 60, 120 and 600 mg kg<sup>-1</sup> body weight, to treated groups of male rats ( $n = 8$ ), daily for 30 days and the controls received a similar amount of distilled water. After the treatment periods, all animals were anesthetized on day 31. Their blood was collected for hematological analysis. Serum was prepared for the electrochemiluminescence immunoassay (ECLIA) to determine testosterone and estradiol levels and the radioimmunoassay (RIA) to determine androstenedione (ADD) levels. The reproductive organs were dissected and weighed and the epididymal sperm density was evaluated. **Results:** There were no significant differences in serum testosterone, androstenedione and estradiol levels, the relative weight of the reproductive organs (testis, caudal epididymis, seminal vesicle and prostate gland) and sperm density between the control and treated groups. Toxicological study revealed no significant difference of hematocrit, WBC or differential cell count. **Conclusion:** *B. rotunda* juice did not modify the testosterone,



ADD and estradiol levels, the sexual organ weights, or the epididymal sperm density during the 30 days of treatment, and high concentration of juice could not harm premature male rats.

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#### Pt8-18

##### Long toxicity of Shen Yan Ling tablet and its effect on the reproductive function in rats

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**Background:** Shen Yan Ling is an innovative Chinese traditional medicine composed with Li Gong Teng, Huang Qi, and others. This compound has conclusively curative effect on renal diseases with low side effect. **Aim:** To investigate the long toxicity of Shen Yan Ling and its effect on the reproductive function. **Methods:** Shen Yan Ling was provided by Jiangsu Kangyuan Pharmaceutical Company and the experiment was approved by the Chinese Regulation of New Drug Research before Clinical Application. Adult SD rats were divided into four equal groups (20 rats in each group with male to female ratio 10:10) as follows: low dose of Shen Yan Ling (1.25 g kg<sup>-1</sup>), middle dose (2.50 g kg<sup>-1</sup>), high dose (5.00 g kg<sup>-1</sup>) and control. The standard test of long toxicity was designed to observe the asked parameters, and to observe serum hormones and testicular and epididymis sperm. **Results:** Compared with controls, general status and body weight were normal after 3 months of treatment with Shen Yan Ling. Parameters of blood cytology and biochemistry fluctuated in the normal range, without any significant changes ( $P > 0.05$ ). Compared with control, the mass coefficient of main organs did not change significantly and a slight change in hepatic and pulmonary pathology in high dose group was found. Although serum sexual hormones did not change significantly, sperm counts in testis and epididymis reduced significantly in high dose group ( $P < 0.05$ ). **Conclusion:** Shen Yan Ling has no significant long-accumulated toxicity on rats after 3 months of treatment with the designed doses (1.25 to 5.00 g kg<sup>-1</sup>) but it exerts negative effect on the reproductive function if treated with high dose of long term.

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#### Pt8-19

##### Application of the continuous measurement of urine $\beta$ -fsh excretion in patients with pubertal disorders

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**Aim:** To evaluate the significance of urine  $\beta$ -FSH excretion

in the clinical practice and pathophysiological study by continuously measuring the urine  $\beta$ -FSH excretion in the patients with puberty disorders including precocious and delayed puberty. **Methods:** Five male volunteers (aged 5, 19, 22, 27 and 33 years) and four female volunteers (aged 5, 28, 28 and 33 years) were selected as control. Four patients with the hypogonadotropic hypogonadism (Kallmann's syndrome or IHH aged 17, 17, 19, 24 years), five patients with hypergonadotropic hypogonadism (Klinefelter's syndrome, aged 16, 16, 17, 20, 22 years), four patients with the central precocious puberty (aged 3, 5, 5, 7 years) and one patient with isosexual peripheral precocious puberty (breast development, aged 5 years) were involved to collect their early-morning urine samples for 30 to 32 days. One normal men and one normal woman collected urine samples for 63 to 64 days. The urine  $\beta$ -FSH was assayed with the method of EIA, then corrected by creatinine (Cr) concentration. **Results:** The urine  $\beta$ -FSH level of normal adult men was  $1.16 \pm 0.20 \mu\text{g mg}^{-1} \text{Cr}$  with small peak variation in their curves, while normal adult women have higher baseline ( $3.12 \pm 0.68 \mu\text{g mg}^{-1} \text{Cr}$ ) and very sharp peak variation in curves corresponding with their cycles. Patients with the hypogonadotropic hypogonadism had lower levels of urine  $\beta$ -FSH, and patients with idiopathy hypogonadism had higher levels with irregular fluctuation, than matching men. Meanwhile, patients with the central precocious puberty had much higher levels of urine  $\beta$ -FSH with irregular peaks, and patients with isosexual peripheral precocious puberty had almost normal levels, than their cotemporary children. The patterns were coincident with the clinical characteristics and serum FSH levels. **Conclusion:** The urine  $\beta$ -FSH excretion was a useful parameter for the clinical classify diagnoses and pathophysiological study on puberty disorders.

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#### Pt8-20

##### No needle no scalpel vasectomy (NNNSV): an Indian experience

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**Aim:** To test the feasibility and effectiveness of jet injector anesthesia technique i.e. "no needle no scalpel vasectomy" (NNNSV) over traditional needle anesthesia technique in no scalpel vasectomy (NSV). **Methods:** This study was conducted in: 1) Department of Surgery and Department of Family Planning, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India. 2) Civil Hospital, Amritsar, Punjab. Inclusion criteria and exclusion criteria were set according to our current practice guidelines and national standards. After informed consent 700 clients were randomized in to two groups; group A underwent NSV with jet injector anesthesia and group B underwent NSV with traditional needle injection anesthesia. For group A Medajet XL® jet injector and for group B 26" gauge needle with 5 mL syringe were used for giving local anesthesia. Medajet XL® was used as explained by Ronald S Weiss and Philip S Li. Both the groups were compared in terms of time

to anesthesia, operating time, pain, cost-effectiveness and complications rates of hematoma, accidental needle stick injuries and accidental intravascular injection. **Results:** Mean time for the onset of local anesthetic effect was 15sec and 75 sec in group A and group B respectively. Mean pain score for group A was 1.78 and that for group B was 4.51 ( $P = 0.0001$ ). Mean operation time was 8.35 min for group A and 9.35 min for group B. None of the group A clients had developed chord hematoma, whereas 20 (5.71%) clients in group B had developed chord hematoma. Similarly none of the group A clients had developed anaphylaxis but two clients in group B had developed anaphylaxis. None of our surgeons or assistants had needle stick injuries. Amount of local anesthetic required per client was 0.6 mL for group A whereas it was 5 mL for group B, a difference of rupees 20 (50 cents) per client. **Conclusion:** NNNSV is relatively painless and completely safe method of vasectomy. Jet injector anesthesia technique is the most cost effective and time effective method for application of local anesthetic with excellent client satisfaction. This study was funded by: Ministry of Health and Family Welfare. Govt. of India

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#### Pt8-21

##### **Immunolocalization of steroidogenic enzymes 3 $\beta$ HSD, P450c17, P450arom, NGF and NGFRs in adrenal glands of the male ground squirrels**

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**Aim:** To characterize the immunolocalization of steroidogenic enzymes in the breeding and non-breeding seasons in adrenal glands of male ground squirrels. **Methods:** Adrenal glands of six adult male ground squirrels were collected in the non-breeding season (July to August 2008,  $n = 3$ ) and breeding season (April 2009,  $n = 3$ ). Adrenal glands samples were dehydrated in an ethanol series and embedded in paraffin wax. Sections (4-6  $\mu$ m) of adrenal glands were routinely stained with hematoxylin-eosin (HE) for histological observation. Steroidogenic enzymes were immunolocalized using polyclonal antisera raised against human placental 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD), porcine testicular 17 $\alpha$ -hydroxylase cytochrome P450 (P450c17), human placental aromatase cytochrome P450 (P450arom), mouse NGF $\beta$ , mouse TrkA, and mouse p75, respectively. **Results:** Histologically, adrenal cortex including zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR), and medulla were observed in the breeding and non-breeding seasons. Immunohistochemically, (1) although strong immunohistochemical signal of P450arom in ZG and ZF could be found in the breeding and non-breeding seasons, immunodetection of P450arom in medulla and ZR could only be observed during the breeding season; (2) P450c17

could be immunodetected in ZF and ZR either the breeding or the non-breeding season, besides, immunodetection in medulla of P450c17 could only be identified during the breeding season; (3) immunodetection of 3 $\beta$ HSD in ZR and medulla could only be observed during the breeding season, however, ZF and ZG showed positive immunoreactivity in both the breeding and the non-breeding season; (4) Immunohistochemical signal of NGF in ZR could be identified in the breeding and non-breeding seasons, it could also be identified in medulla during the breeding season. (5) In the breeding season, immunodetection of TrkA could be observed in medulla, ZR, ZF, and ZG. Immunodetection of TrkA could not be found in the adrenal glands during the non-breeding season. (6) No immunoreaction of p75 could be identified during the breeding season. Nevertheless, ZG and ZF are slightly immunostained during the non-breeding season. **Conclusion:** The present results suggest that the immunolocalization of steroidogenic enzymes, NGF and NGFRs in adrenal glands of male ground squirrels varies between the non-breeding and breeding seasons, which possibly reflected to a physiological functional alternation promoting the ability of progesterone, estrogen and androgen-synthesizing in the breeding season; NGF may acted as an important auto- and/or paracrine regulator in seasonal functional conversion of adrenal glands of male ground squirrels, via its receptors TrkA and p75.

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#### Pt8-22

##### **Establishment of reproduction database of Chinese male**

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**Aim:** To establish a national reproduction database containing baselines of physiological variables of different ethnics. **Methods:** In the eight major administrative regions, namely, Dongbei, Huabei, Huadong, Huanan, Huazhong, Zhongnan, Xinan and Xibei, we selected representative areas where inhabited the Han ethnic and 10 minority ethnics whose population were more than 3 million in China, namely, Zhuang, Man, Hui, Miao, Weiwu'er, Xi, Tujia, Menggu, Zang, and Buyi. Male infants, children, juveniles, youths, and adults were investigated with regard to their fundamental fertility parameters and reproduction-associated physiological variables including sperm quality of adults. The data were used to constitute the database of reproduction of Chinese males. **Results:** The following data were obtained in males of different development stages: incidences of cryptorchidism, hypospadias, abnormal outer genitalia, and hermaphroditism in infants and children younger than 6 years, and their basic data of births and development as well; the juvenile development status, emergence of secondary sexual characters, the age when first spermatorrhea (by questionnaire and consecutive examination of urine



under microscope) took place, sexual hypoevolutism, sexual precocity, and the baseline of outer genita during development, in addition to the physiological constants and data of body development in the juveniles and teenagers; sexual development and activity, spontaneous fertility, disorders of fertility, and outer genita development in youths and adults. **Conclusion:** A shared national reproduction database of Chinese males with comprehensive and integrate data will be eventually constructed. Supported by the Foundation for Basic Science Research and Public Service of Ministry of Science and Technology of China (No. 2003DEB5J047 & 2006FY230400).

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#### Pt8-23

##### Relationship of hormonal status and bone state in men

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**Aim** The study was to determine the relationship of hormonal status and bone state in men. **Methods:** We have examined 96 men aged from 30 to 79 years ( $M \pm m$ ): age— $54.4 \pm 1.3$  years; height —  $1.75 \pm 0.01$  m; weight —  $84.9 \pm 1.5$  kg), divided them into age dependent subgroups 30–49 ( $n = 36$ ; age  $41.2 \pm 1.2$  years) and 50–79 years ( $n = 60$ ; age  $64.4 \pm 1.1$  years). Levels of testosterone (Test, nmol L<sup>-1</sup>) and sex hormone-binding globulin (SHBG, nmol L<sup>-1</sup>) were determined by means of chemiluminescent immunoanalysis method. The bone mineral density (BMD) was evaluated for the total body, spine (L<sub>1</sub>–L<sub>4</sub>), femur (neck, trochanter and total) and radius (ultradistal, 33% and total) using dual energy X-ray absorptiometry by the Prodigy instrument (GE Medical systems, 2005). **Results:** The correlation analysis of age dependent sub-groups: in the group of 30–49 years there is a positive correlation between Test and BMD ultradistal radius ( $r = 0.49$ ,  $P < 0.05$ ), along with the negative correlation between SHBG and Total body in the group of 50–79 years ( $r = -0.31$ ,  $P < 0.05$ ). In the group of 60–79 years ( $n = 38$ ; age  $69.7 \pm 1.0$  years) we have found a negative correlation between SHBG and Total body ( $r = -0.60$ ,  $P < 0.001$ ), SHBG and trochanter ( $r = -0.47$ ,  $P < 0.05$ ), SHBG and Total femur ( $r = -0.48$ ,  $P < 0.05$ ). Patients of 50–79 year age group with normal bone, osteopenia and osteoporosis were chosen in correspondence to the WHO criteria. For analysis' sake, we have joint the osteopenia and osteoporosis patients. Normal mineral density of lumbar spine was found in 83.3%, osteopenia and osteoporosis –17.7%, while in total femur –75% and 25% respectively. SHBG in normal femur BMD subgroup ( $41.1 \pm 2.6$ ) was considerably lower than in osteopenia and osteoporosis subgroup ( $54.4 \pm 5.6$ ,  $P < 0.05$ ). **Conclusion:** Thus, we have revealed a positive correlation between testosterone levels and ultradistal radius BMD and negative correlation between SHBG and total body BMD in patients of 50–79 year age group, trochanter and total femur in patients of 60–79 year age group.

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#### Pt8-24

##### Is laparoscope necessary in exploration for undescended testis with hypoplasia?

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**Aim:** To evaluate the necessity of laparoscope in exploration for undescended testis with hypoplasia. **Methods:** Thirty-seven patients who were diagnosed of undescended testis with hypoplasia were reviewed. **Results:** Among 37 patients, 21 hypoplastic testes had been located (intra-abdominal in one, inguinal in 18, intra-scrotum in two) by ultrasonography. Laparoscopy was applied to exploring the patients with intra-abdominal or undetermined testis. Open inguinal operation was managed for others. Confirmed by histological observation after excision, all testes in patients were found poorly developed. The positions of hypoplastic testes were mainly lower than the inner ring of the inguinal. **Conclusion:** Most hypoplastic undescended testes were located out of the abdominal cavity so that they could be easily found by the technique of open operation. Laparoscopy may not be necessary for common exploration of the undescended testes. The cause of hypoplasia of the undescended testis should be further invested.

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#### Pt8-25

##### A case report of severe penis infection by Aspergillus flavus

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**Aim:** To describe a rare severe penis infection by Aspergillus flavus. **Methods:** The objects is a 61-year-old patient presented to our urology clinic who has been suffered from balanitis for 3 years and the lesion deteriorated over time. A Glans biopsy and a series of microbiology examinations including fungus culture were performed. Medication and surgical treatment were applied. **Results:** Glans biopsy revealed a chronic inflammation with fungal infection. Fungus culture revealed a Aspergillus flavus infection at glans. Itraconazole combined with tinidazole had been administered for 20 days. One month later, the lesion had became fresh and clean. Plastic surgery was performed to close up the wound and repair the ruptured urethra with absorbable suture to achieve healing by first intention. Twenty days after operation, the penis had been completely recovered, meanwhile, natural urination and even erection had been resumed. **Conclusion:** Although Aspergillus flavus infection occurred at glans is rare, it can be correctly diagnosed by biopsy and fungus culture. Complete recovery can be attained with treatment of effective anti-fungal drugs and plastic surgery.

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**Pt8-26****Transurethral seminal vesiculoscopy with an ureteroscope: a novel technique for the treatment of persistent hematospermia**

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**Aim:** To describe a novel technique for diagnosis and management of persistent hematospermia via transurethral seminal vesiculoscopy with a ureteroscope. **Methods:** Experiences of two cases of persistent hematospermia diagnosed and treated by transurethral seminal vesiculoscopy were reported from 2008 Sep in our department. Two cases with a course of the hematospermia 6 and 48 months did not improve with medication within a period > 3 months. The cyto-logical examination and bacteriological culture of expressed prostatic secretion (EPS) by prostatic massage were negative. Definite abnormalities were not found in the imaging studies such as either transrectal ultrasonography or magnetic resonance imaging. The ejaculatory duct and seminal vesicle were observed under direct vision through the distal seminal tracts using a 7F rigid ureteroscope. **Results:** Transurethral seminal vesiculoscopy was successfully performed in the two cases and the operative time was respectively 40 and 25 min. There were no complications like injury of urethra and seminal vesicle and postoperative discomforts in the perineal region. Hematospermia in two cases disappeared within more than 6 months follow-up and they were still in follow-up. **Conclusion:** Transurethral seminal vesiculoscopy could be performed easily with minimal complications using slender rigid ureteroscope and was an effective method for the treatment of persistent hematospermia.

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**Pt8-27****Five cases report and review of replantation with microsurgical technique in penile amputation**

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**Aim:** To evaluate the efficacy of the microsurgical replantation and to improve the clinical experience in the treatment of penile amputation. **Methods:** From May 2001 to December 2007, five cases of penile amputation with a mean age of 38.5 years (range 32–52 years) were analyzed in our department retrospectively. Clinical efficacy was observed using microsurgical technique in treatment. **Results:** All patients were treated with microsurgical technique for replantation with urethra, corporal bodies, dorsal vessels and nerves anastomosis. At 1 month post-operation all patient did not find any urethral stenosis symptoms under cystourethrography examination. But the erection was weakened obviously under the nocturnal tumescence test. At 3 months post-operation the erection improved and all patients tempted to fulfill the sexual life. At 1 year

follow-up five patients had normal voiding, sensation, and erections.

**Conclusion:** Replantation with microsurgical technique is the best choice to treatment penile amputation.

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**Pt8-28****Effects of Pantothenic acid (PaA) on testicular and adrenal function in male rats**

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**Aim:** To investigate the physiological roles of PaA on testicular and adrenal function. **Methods:** Exp.1: Male rats at 3 weeks of age were fed a PaA-free diet or a 0.0016% PaA diet (control) for 7 weeks. Total body weight, as well as the weights of the liver, kidney, pituitary, testis, epididymis, seminal vesicle and prostate; sperm motility; and the plasma concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone and corticosterone were measured in rats at 10 weeks of age. Exp. 2: Male rats at 3 weeks of age were given 0.03% PaA in their drinking water for 9 weeks. Animals were decapitated, and adrenal cells were cultured in the absence or presence of rat adrenocorticotrophic hormone (ACTH;  $10^{-15}$  to  $10^{-10}$  mol L<sup>-1</sup>). **Results:** Exp.1: The relative weights of the testes were significantly higher in the PaA-deficient group compared with the control group. Several parameters of sperm motility were significantly reduced in the PaA-deficient group compared with the control group. In addition, the plasma concentrations of testosterone and corticosterone were significantly lower in the PaA-deficient group compared with the control group, whereas the plasma concentrations of FSH and LH showed no change. Exp.2: Adrenal cells in PaA-treated rats exhibited higher basal levels of corticosterone and progesterone than control rats. The response of ACTH on corticosterone and progesterone release was also higher in the PaA-treated rats than in the control rats. **Conclusion:** These results clearly demonstrate that PaA is an essential factor in testicular and adrenal endocrinology and sperm motility in male rats.

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**Pt8-29****Redesigning condoms for the prevention of STD/HIV infections**

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**Aim:** To redesign the specific condoms and to evaluate its efficacy in STD/HIV prevention. **Methods:** The latex condoms were designed for prototype to cover the foreskin of penis that usually do not encircle the foreskin that contain receptors for

virus. The studies were conducted in medical colleges/centers in India. **Results:** The newly designed latex condoms covered the foreskin there by preventing the virus entry. The specific prostate tests were conducted to support this hypothesis. The evaluative studies in men with uncircumcised penis showed positive results. The centers that participated included four across the country and the routine men's health centers. **Conclusion:** The foreskin of penis plays an important role in having the receptors for attracting the virus. Condoms could be future prevention technologies for men's health.

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#### Pt8-30

##### Icarisid II, a PDE5 inhibitor from *epimedium wanshanense*, increases cellular cGMP by enhancing NOS in rat cavernosum smooth muscle cells

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**Background:** Although PDE5 inhibitors have been extensively used in clinic, a new compound possesses PDE5-inhibitory properties that can restore erectile function also has the apparent ability to help regenerate damaged nerves is highly requested. Icarin (ICA) has been demonstrated to enhance the erectile function while its bioactive form Icarisid II (ICA-II) inhibits PDE5 enzymatic activity by binding to the active site of PDE5A1. **Aim:** To explore the effects and mechanisms of ICA-II on enhancing the cellular cGMP in rat cavernosum smooth muscle cells (RCSMCs). **Methods:** The cellular cGMP and NOS activity were assessed by enzyme immunoassay. Enzyme from RCSMCs was examined by Western immunoblot. Seventeen male Wistar rats were used for RCSMCs culture *in vitro*. The ICA-II was extracted and purified from ICA by enzymatic method. The RCSMCs were treated with ICA-II, ICA, sildenafil and DMSO at different concentrations. The intracellular cGMP basal level and effect of above medications were checked. By Western blot, the expression of nNOS, iNOS and eNOS in RCSMCs were checked. Meanwhile, effects of those medications on the NOS activity were also assayed by enzyme immunoassay *in vitro*. **Results:** ICA-II evaluated the intracellular cGMP to  $8.01 \pm 1.02 \text{ pmol mg}^{-1} \text{ min}^{-1}$ , which was much weaker than that from sildenafil ( $12.4 \pm 1.16 \text{ pmol mg}^{-1} \text{ min}^{-1}$ ) ( $P < 0.05$ ). There was no significant difference between ICA-II and ICA ( $P > 0.05$ ). With the treatment of  $10 \text{ umol L}^{-1}$  ICA-II for 24 h and 48 h, the nNOS and iNOS expression were significantly increased in RCSMCs ( $P < 0.05$ ), while the eNOS expression level was very low without any change. Notably, ICA-II increased the intracellular NOS activity and NOS expression significantly *in vitro* in RCSMCs. **Conclusion:** Except the PDE5 inhibitory effect, ICA-II increases the intracellular cGMP through the enhancement of nNOS/iNOS and NOS activity in RCSMCs *in vitro*. ICA-II implies a potential compound for neurogenic erectile dysfunction through PDE5 inhibition and NOS interference.

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#### Pt8-31

##### The effect of educational program based on PRECEDE model on depression level of patients with coronary artery disease

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**Background:** Depression is the most important barrier to proper treatment of cardiac patients causing failure in accepting their condition, decreasing their motivation in following the therapeutic recommendations and thus negatively affects the patients' function and their quality of life. **Aim:** To investigate the effect of PRECEDE Model-based educational program on depression level in coronary artery bypass grafting surgery (CABGS) patients. **Methods:** This was a quasi-experimental study which 54 post-bypass surgery patients of Isfahan Cardiovascular Research Center were investigated. The patients were randomly divided into two groups marked as intervention and control groups. The data were collected using two questionnaires. Primarily, the Cardiac Depression Scale Inventory questionnaire was used to measure the degree of depression followed by PRECEDE Model-based educational questionnaire to identify the role of educational intervention on patients. The PRECEDE Model-based intervention composed of nine educational sessions per week (60 to 90 min each). The patients were followed up for 2 months post-intervention. **Results:** Following the educational intervention, the mean score of Predisposing factors, Enabling factors, Reinforcing factors and self-helping behaviors was significantly increase in the intervention group compared to control group ( $P < 0.001$ ). Also, a significant difference associated with the mean score of depression level in patients was demonstrated between two groups following educational intervention ( $P < 0.001$ ). **Conclusion:** The findings of current study confirmed the practicability and effectiveness of PRECEDE Model-based educational program on preventing or declining the depression level in CABGS patients.

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#### Pt8-32

##### Effect of aqueous bulrush (*Typha capensis*) extract on TM4-Sertoli cell function

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**Aims:** *Typha capensis* (bulrush) has been, and is still used to this day, by African traditional healers to treat various ailments. Of special importance in male reproductive studies is the claim that a rhizome infusion is capable of enhancing what is called 'male potency' by traditional healers but rather refers to 'male infertility'



in modern medical terms. Several compounds have already been identified by researchers. These include flavones, phenols as well as hydrocarbons and triterpenoids. Despite the practice of traditional healers (Sangomas, Izinyangas or herbalists) has been legalized by an Act of Parliament in South Africa, this medicinal plant has not been scientifically investigated on its effects on male reproductive functions yet. Therefore, the aim of this study was to investigate the effects of aqueous extracts from bulrush (*Typha capensis*) on the cellular function of immortalized mouse TM4 Sertoli cells. **Methods:** Fresh rhizomes of *Typha capensis* were collected from the Cape Flats Nature Reserve at the University of the Western Cape. The rhizomes were chopped, dried at 25°C, infused with hot distilled water, freeze-dried and stored at 4°C until further use. TM4 Sertoli cells were seeded at a concentration of 4 000 cells/mL into 24 well plates. The cells were cultured in a 37°C humidified incubator with 5% CO<sub>2</sub> in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 2.5 mmol L<sup>-1</sup> glutamine adjusted to contain 15 mmol L<sup>-1</sup> HEPES, 0.5 mmol L<sup>-1</sup> sodium pyruvate and 1.2 g L<sup>-1</sup> sodium bicarbonate supplemented with 5% horse serum, 2.5% fetal bovine serum and 1% penicillin–streptomycin. The cell viability was measured with the XTT assay (Roche Cell Proliferation Kit II), the protein content with the BIO-Rad Protein assay, the pyruvate secretion with the pyruvate assay and the lactate secretion with the lactate assay. The cells were exposed to various concentrations of the *Typha capensis* extract, namely, 0 ng mL<sup>-1</sup>, 0.1 ng mL<sup>-1</sup>, 1 ng mL<sup>-1</sup>, 10 ng mL<sup>-1</sup>, 100 ng mL<sup>-1</sup> and 1 000 ng mL<sup>-1</sup> for 24, 48, 72 and 96 hours, respectively. **Results:** Cell viability showed a significant ( $P < 0.0005$ ) decline after 48 hours of exposure to a concentration of 10 ng/mL aqueous *Typha capensis* extract. After 24 and 48 hours, a decrease was observed in the protein content at 1000 ng/mL with a significant ( $P < 0.0005$ ) decline observed after 48 hours at 10 ng mL<sup>-1</sup>. There was no significant difference in pyruvate and lactate secretion observed at both 24 and 72 hours exposure at all concentrations. In contrast, a significant ( $P < 0.0005$ ) decrease in pyruvate and lactate was observed at a concentration of 10 ng mL<sup>-1</sup> at 48 hours exposure. At the same concentration, a significant ( $P < 0.0005$ ) increase in pyruvate and lactate secretion was observed at 96 hours exposure, showing a biphasic effect of the extract on Sertoli cell function. **Conclusion:** The most significant decrease in viability, protein, pyruvate and lactate were observed after exposure to 10 ng mL<sup>-1</sup> aqueous *Typha capensis* extract for 48 hours. This effect may be due to one or more compounds found within the crude *Typha capensis* extract. Therefore, more studies are needed to establish the mechanism of *Typha capensis* action as this extract may disrupt spermatogenesis by decreasing the ability of the Sertoli cell to support the germinal epithelium.

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#### Pt8-33

##### The biological characteristics of SD rat's adipose derived stem cells *in vitro*

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**Aim:** To investigate the biological characteristics of the SD rat's adipose derived stem cells (ADSCs). **Method:** The SD rat's ADSCs were isolated and cultured *in vitro*. Subcutaneous adipose tissue

was obtained from the inguinal region of SD rats. Then primary ADSCs were inoculated and cultured in Dulbecco modified Eagle medium with 10% fetal bovine serum after digestion by collagenase I. Cell morphology was observed and cell growth curve was drawn. Subsequently the cell surface markers were identified by flow cytometry or immunofluorescence. The multi-cell differentiation potential of ADSCs under specific induced environment *in vitro* was also detected. **Results:** ADSCs grew adhering to the wall as fibroblast-like cells and proliferated rapidly *in vitro*. The cell surface marker CD29, CD44 and STRO-1 were positive, while CD45 and HLA-DR were negative. Under specified conditions of differentiation, ADSCs could be induced and differentiated into smooth muscle-like cells, endothelial-like cells, fat-like cells, chondrocyte-like cell and neuron-like cells. **Conclusion:** ADSCs can express some markers for pluripotent stem cells, such as CD29, CD44 and STRO-1. Meanwhile, it has multi-differentiation capability.

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#### Pt8-34

##### Clinical study on modified Sondgrass procedure in hypospadias repair

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**Aim:** The tubularized incised-plate (TIP) urethroplasty is gaining an ever increasing popularity in hypospadias repair because of its low complication rates and favorable cosmetic and functional results published in recent years. A retrospective review of 107 patients who had TIP and its modified procedure for the primary repair of hypospadias at the General Hospital of Guangzhou Military Command was performed to assess the clinical outcomes of this technique. **Methods:** All 107 patients in an independent clinic underwent a TIP urethroplasty and its modified procedure for the primary repair of hypospadias between January 2005 and September 2008 were selected in our study. The median age at the time of surgical repair was 4.8 years old (11 months to 18 years). The position of the meatus was distal in 24 boys (38%), middle in 28 (17%) and posterior in 34 (45%) according to Barcat classification. The severity of chordee was graded according to the etiological classification on chordee without hypospadias. Cases which could not be performed with TIP or finished in one stage would be turned to other procedures or be performed in two stages. Postoperatively temporary urine diversion was carried out with suprapubic and urethral stent tube for about 8-14 days. **Results:** TIP and its modified urethroplasty were carried out in 107 patients. All patients were followed up after surgical repair for 2–26 months (median 14 months). Average operation time was 165.2 ± 28.0 min, while the average duration in hospital was 13 ± 3.5 days. Of the 107 patients, 86 (80.4%) were successfully cured, obtaining a neourethra with slit-like meatus at the tip of the glans and satisfactory voiding function. Small urethrocutaneous fistula occurred in fifteen (14.0%) cases, including nine at the coronary sulcus, one at the shaft, five at the penoscrotum. Two patients (1.8%) developed mild meatal stenosis and were successfully managed by simple treatment. Four patients (3.7%) were found with mild and persistent penile curvature



postoperatively, recurrent chordee was found in one boy a year after TIP procedure, and corrected by a second surgery. Urethral stricture and dehiscence were not encountered so far. Chordee correction was performed in twenty-nine patients (27.1%) with the dorsal plication suture. Four patients with posterior hypospadias and serious chordee were treated by alternative procedures with transection of the urethral plate in two stages, while TIP combined with Duckett urethroplasty were used for the treatment of two boys with scrotal hypospadias. **Conclusion:** Compared with traditional modus operandi of hypospadias, there are easier operative procedure, shorter operative time, lower incidence of urethral stricture and more satisfactory shape of post-op penis in TIP. But the most frequent complication of TIP is urethrocutaneous fistula. Our study suggested that with double-breasted de-epithelialized skin overjetting shaping urethra (modified TIP urethroplasty) can reduce the incidence of urethrocutaneous fistula.

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#### Pt8-35

##### The diagnosis and treatment of the Mullerian duct cyst: report of 48 cases report

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**Aim:** To study the diagnosis and treatment methods of the Mullerian duct cyst. **Methods:** The records of 48 patients with Mullerian duct cyst were reviewed and the diagnosis and treatment methods were compared. From 1993 to 1997, eight patients visited the clinic, most of whom visited us because of the chronic prostatitis symptom. They all underwent the transrectal ultrasonography (TRUS). Based on the symptoms and the size of the remnants, seven patients were treated by TRUS-guided perineal aspiration therapy. In one patient the large Mullerian duct Cyst was extirpated by open operation. From 2002 to 2007, we treated forty patients. Most of them visited us because of the obstructive azoospermia. All patients were diagnosed by the TRUS and twelve patients underwent MRI examine. Thirty-nine patients with smaller Mullerian duct Cyst were treated by transurethral cyst incision therapy. In one patient the large Mullerian duct Cyst was extirpated by laparoscopic operation. **Results:** Of the eight patients, recurrence was detected in one patient after half-year's follow-up and cured by transurethral cyst incision. Of the 40 patients, all were cured after three months postoperative TRUS follow-up. By the six months postoperative follow-up, ten obstructive azoospermia patients had made his wife pregnant successfully in all thirty-nine patients. And nine patients had sperms in semen in other twenty-nine patients. One 19-year-old patient's symptom disappeared. **Conclusion:** The Mullerian duct cyst patients often visit us because of chronic prostatitis symptom and the obstructive azoospermia. TRUS was the preferred examine method in the diagnosis of the Mullerian duct cyst. And MRI could offer more exact information. Transurethral cyst incision was effective methods for Mullerian duct cyst. Laparoscopic Mullerian duct cyst resection was a good choice for the large cysts.

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#### Pt8-36

##### Genome-wide identification of gene expression in the epididymis of alpha-chlorohydrin-induced infertile rats

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**Aim:** To establish a rat sterility model associated with epididymis and epididymal gene expression profiles of alpha-chlorohydrin-induced fertility. **Methods:** Rats were treated with 10 mg kg<sup>-1</sup> d<sup>-1</sup> alpha-chlorohydrin (ACH) for 10 consecutive days. Sperm maturation and other fertility parameters were analyzed. The sperm motility and morphology were evaluated by computer-assisted sperm analysis (CASA); sperm survival rate was assessed by SYBR-14 and propidium iodide (PI) fluorescent staining; the weights of testes, epididymides, prostates and seminal vesicles were determined by electronic balance; histological examination of above tissues were evaluated by HE staining; and serum dihydrotestosterone (DHT) and testosterone (T) were detected by ELISA. Each male rat was paired with two female rats in proestrus. Female rats were examined the next morning for the presence of sperm in vaginal smears and underwent a cesarean section on 12 day of gestation. Finally the reproductive indices were calculated as follows: copulation index (number of sperm positive females/number of pairings), pregnancy index (number of pregnancies/number of sperm positive females), and fertility index (number of pregnancies / number of pairings). Affymetrix Rat 230 2.0 oligo-microarray were used to identify epididymal special fertility-associated genes which were validated by Real-Time PCR. **Results:** Female rats mating with treated males resulted in no successful pregnancy. Spermatozoa from the cauda epididymidis of administered rats were detected a significant decrease in percentage of motile, progressive motile sperm, sperm survival rate and the morphology was also adversely affected by the treatment, while there was no significant difference in serumal androgen between the control and treated groups. Gene Spring gene ontology analysis (GO) were used to classified general functions of the epididymal chlorohydrin-induced genes, which are involved in macromolecular metabolism, transport, primary metabolism process, cell metabolism, biological process regulation, immunology regulation, ion combination, hydratase and oxidoreductase activity. Among all the different expressed genes, we analyzed and screened the down-regulated genes associated with glucose, lipid, protein and other energy metabolism which are considered as the major ACH action targets, whereas the up-regulated genes, including apoptosis, immune-related or not reported genes before, were detected and they play negative roles in sperm maturation. **Conclusion:** we established male infertile rat model with ACH (10 mg kg<sup>-1</sup> d<sup>-1</sup>, po, 10 days) through evaluating changes of sperm motility and morphology, mating index, fertility index and pregnancy index, while the ACH did not affect the major androgen (T and DHT) metabolism and sexual ability. Then we determined the down-regulated epididymal genes relation to metabolism, which can affect the epididymal sperm maturation and presumed ACH antifertility targets. Further more,

we found and analyzed the epididymal up-regulated genes associated with apoptosis and immune functions, which may be possible sites of action by ACH and other male antifertility agents.

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#### Pt8-37

##### The effect of a red palm oil supplemented diet on induced oxidative stress in rat sperm

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**Aim:** To investigate the reversal effect of a red palm oil supplemented diet on hydroperoxide-induced oxidative stress in rat sperm. **Methods:** Male Wistar rats aged 10–12 weeks were randomly distributed into three groups and fed with standard rat chow. Group 1 received no supplement while the food of group 2 and 3 were supplemented with 2 mL and 4 mL red palm oil (in 25 g/standard rat chow/day) respectively. Each group was divided into three subgroups and injected daily with 0.5 mL saline, 10  $\mu\text{mol L}^{-1}$  cumene hydroperoxide (cHP) and 20  $\mu\text{mol L}^{-1}$  t-butyl hydroperoxide (tbHP) respectively (for five consecutive days per week) over a sixty day period. **Results:** The indexes of Sperm motility, sperm concentration, superoxide dismutase concentration, glutathione and catalase activities of the sperm in rats injected with cHP and tbHP were significantly reduced compared to the control while the levels of 2,7'-dichlorofluorescein and malondialdehyde were increased. Interestingly, in the subgroups of cHP and tbHP, the amounts of dichlorofluorescein and malondialdehyde were significantly lower in the sperm of rats fed with 2 mL or 4 mL supplementation of red palm oil than in rats receiving standard rat chow only. Moreover, the indexes of superoxide dismutase concentration, glutathione and catalase activities of the sperm in these animals increased. The amounts of 2, 7'-dichlorofluorescein in the sperm of rats fed with 2 mL and 4 mL supplementation of RPO in combination to cHP and tbHP, as well as superoxide dismutase concentration, glutathione and catalase activities showed no differences when compared to control. **Conclusion:** The *in vivo* administration of cHP and tbHP have a negative effect on the rat epididymal sperm while it can be successfully reversed by the supplementation of RPO to the diet.

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#### Pt8-38

##### Replication and protein expression of hepatitis B virus S and C genes introduced into embryos by human spermatozoa

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**Aim:** Hepatitis B is a public health problem worldwide. This study

was performed to investigate replication and protein expression of HBs and HBe genes introduced into embryos via human sperm. **Methods:** Human sperm were transfected separately with recombinant plasmids pIRES2-EGFP-HBs and pIRES2-EGFP-HBe and then were fertilized with zona-free golden hamster ova. Two-cell embryos with and without the presence of green fluorescence were collected for fluorescence in situ hybridization (FISH), immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA), respectively. **Results:** FISH: the HBs- and HBe-DNA positive signals were detected in human sperm nuclei. Each nucleus in the tested two-cell embryos presented HBs- and HBe-DNA positive signals, respectively and no positive signal was detected in the control. Immunofluorescence assay: the HBsAg- and HBeAg- positive signals were detected in the cytoplasm of the tested two-cell embryos and no positive signal was observed in the control. ELISA: HBsAg amount of a single two-cell embryo was about 0.064 ng/ml and the positive result for HBeAg was detected. **Conclusion:** HBV S and C genes are able to integrate into human sperm genomes. After being introduced into embryo by spermatozoa, they are able to replicate themselves and express viral proteins, HBsAg and HBeAg, in the embryonic cells.

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#### Pt8-39

##### A possible relationship between testosterone and lower urinary tract symptoms in men

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**Aim:** The aim of this study was to search for possible associations between serum testosterone levels and the severities of lower urinary tract symptoms (LUTS) in men. **Methods:** In 278 patients of mean age 62 years, blood levels of total testosterone (TT), albumin, sex hormone-binding globulin (SHBG), fasting glucose, fasting insulin, and C-reactive protein (CRP) were measured. Free testosterone (FT), bioavailable testosterone (BT), and homeostasis model assessment-insulin resistance (HOMA-IR) were calculated. Prostate volumes were measured by transrectal ultrasonography and the severity of LUTS was assessed using the International Prostate Symptom Score (IPSS). **Results:** FT and BT were found to be negatively related to IPSS total scores and sub-scores (voiding symptoms) after adjusting for age, prostate volume, CRP, and HOMA-IR ( $P < 0.05$ ). In addition, FT and BT were found to be significantly related to the presence of severe LUTS (IPSS  $\geq 20$ ) using unadjusted and adjusted models ( $P < 0.05$ ), though the odd ratio of BT was lower than that of FT by multivariate analysis. CRP was found to be negatively correlated with serum TT ( $r = -0.128$ ,  $P = 0.038$ ) and BT ( $r = -0.126$ ,  $P = 0.041$ ), and HOMA-IR to be negatively correlated with serum TT ( $r = -0.236$ ,  $P < 0.001$ ), FT ( $r = -0.179$ ,  $P = 0.003$ ) and BT ( $r = -0.162$ ,  $P = 0.007$ ). However, no significant correlation was found between CRP, HOMA-IR, and IPSS total scores, voiding symptom scores, and storage symptom scores. **Conclusion:** The present findings support the favorable role of endogenous testosterone on lower urinary tract function, and suggest testosterone deficiency provides a pathophysiologic basis for the connection between LUTS and metabolic syndrome.

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