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Perspectives

Male fecundity prognosis and infertility diagnosis in the era of personalised medicine

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1 Semen analysis: the opportunities in China

In daily clinical practice, we attempt to classify diseases according to patients' symptoms and reference values for certain tests. A minimal evaluation is performed following the diagnostic standard, and the patient is treated according to the diagnosis. In this 'classification medicine' model, a patient's individual characteristics are more or less ignored.

Semen analysis is one element of the initial clinical evaluation for an infertile couple [1]. It is important because it provides laboratory evidence that enables andrologists to predict male fecundity, diagnose infertility and select an appropriate assisted reproductive technology (ART) for treatment. The WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction [2] was first published in 1980 in response to a growing need for the standardisation of procedures for the examination of human semen. Over the past 30 years, the manual has been updated to the fifth edition [3] and has always been recognised as providing global standards and has been used extensively by research and clinical laboratories throughout the world. There are several methods of semen analysis, but those recommended by WHO are the most comprehensive and robust. The manual, which remains the andrologist's 'Bible', has a vital continuing role in raising the standards of andrology laboratories [4]. However, evidence suggests that the current WHO recommendations for semen analysis are poorly followed in China and Europe [4–6], and unreliability of semen analysis results is still the main complaint of fertility clinicians. The procedure recommended by WHO is complex and time-consuming and requires more experience and skills than other techniques. Most andrology laboratories—even hospitals with ART centres—therefore use other, simpler methods, such as computer-assisted sperm analysis and the Makler chamber.

Obviously, there is an urgent need in China to introduce and promote the standardisation of semen analysis procedures according to the WHO recommendations. The imminent appearance of the fifth edition of the manual [3] would be an excellent opportunity to improve semen analysis methods here. Fortunately, some forward-looking experts in China have been engaged in extending the influence of this edition, for example, by sponsoring a seminar or a training course or translating the fifth edition into Chinese. A monograph, "Special Issue on Semen Analysis in the 21st Contury Medicine" for this new edition of the WHO manual has been published in *Asian Journal of Andrology* and the Chinese translation in *Journal of International Reproductive Health/Family Planning* in 2010. Now most experts in reproductive medicine in China recognised the importance of applying the fifth edition to their daily clinical services.

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Another important cause of the unreliability of semen analysis in China is the deficiency of reliable quality assurance (QA) and quality control (QC) systems in the country's andrological laboratories. QA/QC for semen tests are described in detail in the new edition of the manual, and these should be routine in every semen test. To achieve standardisation of semen analysis in andrological laboratories throughout the country, a prerequisite for the establishment of a centre should be that it utilises external quality assessment.

The reference values in the new edition have been changed dramatically from those in the fourth edition [7]. For example, the proportion of motile sperm as an indicator of progressive motility (A + B) has decreased from 50% to 32%. This change would be helpful in modifying technological standards for donor screening in Chinese sperm banks in the future, to increase the screening rate of donors and promote the development of sperm banks. It is estimated that only 20%–30% of volunteers in China are qualified to be donors according to The Technological Specifications of Human Sperm Banks [8], issued by the Ministy of Public Health of China.

2 Semen analysis: the challenge

Across the previous four editions of the WHO manual, the reference value of normal sperm morphology rate decreased from 30% to 15%, and it is dramatically reduced to 4% in the new version. However, the normal morphology evaluation has become increasingly captious. In our opinion, it is difficult to accept that more than 90% of sperm are abnormal in a healthy man. How can we choose an appropriate ART treatment according to such a high threshhold for normal sperm morphology? What criteria should be used to define normal sperm? This is a problem that needs to be discussed.

In fact, the result of semen analysis is not the best evidence a doctor can use to predict a man's fertility, diagnose infertility and make a choice of ART treatment, even if the analysis is carried out according to WHO recommendations. A semen analysis evaluates certain characteristics of semen and the spermatozoa contained in it. The characteristics measured are only some of the factors that influence semen quality. In addition, it offers only a diagnosis of symptoms, not indications of the underlying causes of sub-fertility. Stony Brook University (NY, USA) [9] in 2007 estimated that 30% of men with a normal semen analysis according to the reference values in the fourth edition of the WHO manual actually have abnormal sperm function. Conversely, men with poor semen analysis results may go on to father children.

The situation changed dramatically in the late 1970s, when *in vitro* fertilisation (IVF) and embryo transfer was introduced, as investigators interested in male infertility were able to assess the human oocyte–sperm interaction. It is also possible to discriminate donor sperm functions by insemination of different recipients in cases of artificial insemination with donor (AID) treatment. Studies suggest that the semen parameters evaluated according to the WHO criteria are not good predictors of IVF outcome [10–12]. Our sperm bank recruits donors according to The Technological Specifications of Human Sperm Banks [8], which specifies that the semen volume, sperm concentration and progressive motility must be > 2 mL, > 60×10^6 mL^{-1} and > 60%, respectively. In a retrospective analysis of 1 551 donor and 18 064 AID-ICI (intracervical insemination) treatment cycles from our bank, the clinical pregnancy rate per cycle was 20.5%. Of the 1 551 donors, 181 (11.7%) did not initiate a pregnancy after 12 cycles of insemination with frozen semen from different recipients for each treatment cycle. These results clearly indicate the dissociation between the results of semen analysis and outcomes of clinical treatment. Obviously, clinicians need a better diagnostic system for fecundity prognosis as well as for infertility diagnosis and treatment.

The advent of ART has highlighted the necessity for clinicians to recognise the role that genetics plays in cases of male factor infertility because new technologies, such as intracytoplasmic sperm injection (ICSI), allow men with sub-optimal sperm quality to overcome natural selection mechanisms and produce a viable zygote. Many studies show that genetics contributes to infertility by influencing a variety of physiological processes, including hormonal homeostasis, spermatogenesis and sperm quality [13]. Some andrologists have voiced concern about the concealment of reproductive defects through ART that might have negative consequences at the epigenetic level. Although the majority of children conceived through ART seem normal, in one study major congenital abnormalities were observed at birth in 9/150 (6.0%) children; the total malformation rate was 6.5% [14]. In comparison with a general neonatal population, there was a slight but significant increase in the prevalence of aneuploidy in the sex chromosomes of ICSI children (from 0.2% to 0.6%).

In addition, there were increased autosomal chromosome abnormalities (from 0.07% to 0.4%) [14, 15], but it may be more worrisome that other abnormalities show up only later in life. Accurate detection of underlying reproductive abnormalities may help to guide individual management decisions, maximise ART treatment outcomes and safeguard the health of subsequent ART-derived generations. Developing techniques and methods to detect genetic abnormalities or unfavourable polymorphisms before performing ART is also critical.

3 Sperm function analysis: the future alternative

Human sperm bank is an ideal platform for developing new tests of spermatozoa function because it contains extensive stores and long-frozen sperm samples. More importantly, compared with a couple, a sperm bank can supply one male's sperm to different women under ART treatment. The negative effects for women will be minimal. This would be the gold standard for defining the fecundity of sperm.

Advances in our understanding of the causes of infertility and evidence-based medicine have facilitated the development of increasingly complex diagnostic tools and prognostic models. Male infertility has already become a common disease, and most causes of infertility are spermatogenesis-related. In addition to environmental factors, lifestyle and diseases, more than 3 000 genes (about 4% of human genes are testis-specific) and complicated epigenetic mechanisms may be involved in the regulation of spermatogenesis. More than 400 mutant mouse models with specific reproductive abnormalities have been produced, and numerous associations between these mouse mutants and human infertility have been discovered [16]. Abnormality of the altered proteins could lead to male infertility. Obviously, male infertility is a complicated disease with high heterogeneity.

Biomarkers are frequently used for disease diagnosis and stratification, treatment selection, monitoring disease progression, and establishing patients' responses to therapy (efficacy or adverse events) [17]. Semen analysis is still recognised as a surrogate endpoint in male fertility evaluation. Spermatogenesis-related genes and the mouse model-related loci could be new potential biomarkers for male infertility diagnosis, but classic single-gene-based molecular diagnosis has a limited role in clinical applications because of the heterogeneous nature of male infertility. Therefore, moving from 'classic' diagnosis to molecular-network screening might result in more rapid and efficient identification of clinical implications [18].

Recently, scientific and technological advancements have seen an exponential growth of biomarkers derived from proteomics, epigenomics, and genomics. Combined with high-throughput technology, proteomic biomarkers could potentially be related to the clinical manifestation of male infertility and to patients' responses to treatment. Epigenomic and genomic molecular biomarkers could enable us to understand the germline basis of disease development and of differential clinical responses to a specific medical treatment.

The variability in patient characteristics of infertility necessitates proven, personalised diagnostic approaches to optimised efficacy and safety outcomes. Personalised management strategies, based on individual patient characteristics, have been proposed, and the further development of these strategies may represent real progress towards individually tailored fertility treatment.

Personalised medicine is the science of predicting, preventing, diagnosing and curing disease via the systematic use of individual patient information, including gene profiles, proteomics, metabolic status and environmental information, resulting in customised treatment protocols most suitable for the patient [19]. In fact, medicine has long been 'individualised', as traditional Chinese medicine is characterised by the four traditional methods of diagnosis: observation, listening, interrogation, and pulse taking. However, more recently, we have begun to take individualisation one level deeper. On the basis of comparative effectiveness research (CER) and high-throughput molecular technologies, personalised medicine is especially suitable for prediction, diagnosis and treatment of some complex diseases with high heterogeneity, such as cancer, heart disease, diabetes, and male infertility.

Personalised medicine based on high-throughput molecular technologies will contribute to comprehensive etiological diagnosis from data collected on genes, proteins, metabolism, tissue and environment and will provide guidelines for clinical practice. Although there are no well-established methods appropriate for individual diagnosis, many studies have shown that mutations in spermatogenesis-related genes, single-nucleotide polymorphisms (SNPs), copy-number variations, epigenetic modifications, sperm proteins and chromosomes can affect sperm function and consequently cause male in-

fertility [13]. However, it appears that such variations are not predictive when these molecular biomarkers are alone applied to clinical diagnostic practice, as positive detection rates and detection efficiency have been extremely low.

To develop a more feasible diagnosis system, we should first make full use of CER to explore the most significant sperm function–related biomarkers. Although numerous molecular biomarkers have been found, including chromosomes [20], genes [21, 22], SNPs [23, 24], transcripts [25], proteins [26–31] and epigenetic modifications [32, 33], we need more biomarkers to increase detection rate and detection efficiency. It is estimated that more than 1 000 biomarkers per test would be needed, as male infertility is highly heterogeneous. Human sperm bank would be the best platform on which to create the new system. Another topic for study is the collection of biomarkers that would optimise the test, which needs to be validated primarily by ART. There is much work to do to verify suitable biomarkers, but the hope of an optimal test for male infertility will soon be fulfilled. The next step toward this goal should be to systematically study male infertility-related biomarkers—especially those derived from proteomics, epigenomics, and genomics—to establish the specific array of proteomic, epigenomic and genomic molecular biomarkers that can be used in high-throughput platforms for male fecundity prognosis, infertility diagnosis and selection of ART treatment. This will enable us to improve therapeutic tactics and strategies and will help andrology progress to a new level.

The use of ART is restricted in China. It has generated a variety of ethical, moral, marriage/family, and legal issues. In addition, compared with natural pregnancy, ART may lead to a series of complications that may directly affect its success rate and safety, such as ovarian hyperstimulation syndrome, multiple pregnancy, damage/bleeding due to mishandling, and infection. Injecting sperm into an egg may introduce foreign genetic materials (such as bacteria and viruses) into fertilised eggs. A personalised male fecundity diagnostic system with high-throughput and aetiological analysis would facilitate the reasonable use of ART.

References

1 Devroey P, Fauser BC, Diedrich K; Evian Annual Reproduction (EVAR) Workshop Group 2008. Approaches to improve the diagnosis and management of infertility. Hum Reprod Update 2009; 15: 391–408.
2 World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 1st ed. Singapore: Press Concern; 1980.
3 World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: WHO Health Organization, 2010.
4 Ford WC. Comments on the release of the 5th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen. Asian J Androl 2010; 12: 59–63.
5 Lu JC, Zhang HY, Hu YA, Huang YF, Lü NQ. A survey on the status of semen analysis in 118 laboratories in China. Asian J Androl 2010; 12: 104–10.
6 Baker HW. Status of semen analysis-comment on "A survey on the status of semen analysis in 118 laboratories in China" by Jin-Chun Lu *et al*. in Asian Journal of Andrology. Asian J Androl. 2010; 12: 115–7.
7 World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th ed. Cambridge: Cambridge University Press; 1999.
8 Ministy of Public Health of China. The Technological Specifications of Human Sperm Banks. Available from: www.moh.gov.cn/open/uploadfile/20051128164324488.doc.
9 Understanding Semen Analysis. Stonybrook, State University of New York. 1999. Available from: http://www.uhmc.sunysb.edu/urology/male_infertility/SEMEN_ANALYSIS.html. Retrieved 2007-08-05.
10 Baker VL, Luke B, Brown MB, Alvero R, Frattarelli JL, *et al*. Multivariate analysis of factors affecting probability of pregnancy and live birth with *in vitro* fertilization: an analysis of the Society for Assisted Reproductive Technology Clinic Outcomes Reporting System. Fertil Steril. 2009 Sep 8. [Epub ahead of print]
11 Chen X, Zhang W, Luo Y, Long X, Sun X. Predictive value of semen parameters in *in vitro* fertilisation pregnancy outcome. Andrologia. 2009; 41: 111–7.
12 FIVNAT, de Mouzon J, Levy R, Mourouvin Z, Belaisch-Allart J, *et al*. Semen characteristics and quality of the conceptus in fertilization *in vitro*. Gynecol Obstet Fertil 2007; 35: 216–23.
13 O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril 2010; 93: 1–12.
14 Hindryckx A, Peeraer K, Debrock S, Legius E, de Zegher F, *et al*. Has the prevalence of congenital abnormalities after intracytoplasmic sperm injection increased? The Leuven Data 1994–2000 and a review of the literature. Gynecol Obstet Invest 2010; 70: 11–22.
15 Van Steirteghem A, Bonduelle M, Devroey P, Liebaers I. Follow-up of children born after ICSI. Hum Reprod Update 2002; 8: 111–6.
16 Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med 2008; 14: 1197–213.
17 Tesch G, Amur S, Schousboe JT, Siegel JN, Lesko LJ, *et al*.

Successes Achieved and Challenges Ahead in Translating Biomarkers into Clinical Applications. AAPS J. 2010 Mar 16. [Epub ahead of print]
18 Roukos DH. Systems medicine: a real approach for future personalized oncology? Pharmacogenomics 2010; 11: 283–7.
19 Müller G. Personalized prognosis and diagnosis of type 2 diabetes–vision or fiction? Pharmacology 2010; 85: 168–87.
20 Nicopoullos JD, Gilling-Smith C, Almeida PA, Homa S, Nice L, Tempest H, Ramsay JW. The role of sperm aneuploidy as a predictor of the success of intracytoplasmic sperm injection? Hum Reprod 2008; 23: 240–50.
21 Buckman C, George TC, Friend S, Sutovsky M, Miranda-Vizuete A, *et al*. High throughput, parallel imaging and biomarker quantification of human spermatozoa by ImageStream flow cytometry. Syst Biol Reprod Med 2009; 55: 244–51.
22 Gyamera-Acheampong C, Mbikay M. Proprotein convertase subtilisin/kexin type 4 in mammalian fertility: a review. Hum Reprod Update 2009; 15: 237–47.
23 Feugang JM, Kaya A, Page GP, Chen L, Mehta T, *et al*. Two-stage genome-wide association study identifies integrin beta 5 as having potential role in bull fertility. BMC Genomics 2009; 10: 176.
24 Olshan AF, Luben TJ, Hanley NM, Perreault SD, Chan RL, *et al*. Preliminary Examination of Polymorphisms of GSTM1, GSTT1, and GSTZ1 in Relation to Semen Quality. Mutat Res 2010; 688: 41–46.
25 Lalancette C, Platts AE, Johnson GD, Emery BR, Carrell DT, *et al*. Identification of human sperm transcripts as candidate markers of male fertility. J Mol Med 2009; 87: 735–48.
26 Muratori M, Luconi M, Marchiani S, Forti G, Baldi E. Molecular markers of human sperm functions. Int J Androl 2009; 32: 25–45.
27 Zhao C, Huo R, Wang FQ, Lin M, Zhou ZM, *et al*. Identification of several proteins involved in regulation of sperm motility by proteomic analysis. Fertil Steril 2007; 87: 436–8.
28 Aitken RJ, Nixon B, Lin M, Koppers AJ, Lee YH, *et al*. Proteomic changes in mammalian spermatozoa during epididymal maturation. Asian J Androl 2007; 9: 554–64.
29 Barratt CL. The human sperm proteome: the potential for new biomarkers of male fertility and a transformation in our understanding of the spermatozoon as a machine: commentary on the article 'Identification of proteomic differences in asthenozoospermic sperm samples' by Martinez *et al*. Hum Reprod 2008; 23: 1240–1.
30 Liao TT, Xiang Z, Zhu WB, Fan LQ. Proteome analysis of round-headed and normal spermatozoa by 2-D fluorescence difference gel electrophoresis and mass spectrometry. Asian J Androl 2009; 11: 683–93.
31 Siva AB, Kameshwari DB, Singh V, Pavani K, Sundaram CS, *et al*. Proteomics-based study on asthenozoospermia: differential expression of proteasome alpha complex. Mol Hum Reprod. 2010 Mar 18. [Epub ahead of print]
32 Miller D. Spermatozoal RNA as reservoir, marker and carrier of epigenetic information: implications for cloning. Reprod Domest Anim 2007; 42 Suppl 2: 2–9.
33 Boissonnas CC, Abdalaoui HE, Haelewyn V, Fauque P, Dupont JM, *et al*. Specific epigenetic alterations of IGF2-H19 locus in spermatozoa from infertile men. Eur J Hum Genet 2010; 18: 73–80.