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Sperm banking for male reproductive preservation: a 6-year retrospective multi-centre study in China

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Abstract

Sperm banking can preserve male fertility effectively, but the current conditions of sperm cryopreservation in China have not been investigated. This retrospective investigation was based on data collected at multiple centres in China from January 2003 to December 2008. The collected data included urogenital history, indication for cryopreservation, semen parameters, use rate, type of assisted reproductive technique (ART) treatment and pregnancy outcome. The study population included 1 548 males who had banked their semen during the study period at one of the clinics indicated above. Approximately 1.9% (30/1 548) of the cryopreserved semen samples were collected from cancer patients; about 88.8% (1 374/1 548) of the patients had banked their semen for ART and 8.6% (134/1 548) had a male infertility disease (such as anejaculation, severe oligozoospermia and obstructive azoospermia). The total use rate of cryopreserved semen was 22.7% (352/1 548), with 119 live births. The cancer group use rate was 6.7% (2/30), with one live birth by intracytoplasmic single sperm injection (ICSI). The ART group use rate was 23.2% (319/1 374), with 106 live births. The reproductive disease group use rate was 23.1% (31/134), with 12 live births. The semen parameters in each category varied; the cancer patient and infertility disease groups had poor semen quality. *In vitro* fertilization (IVF) and ICSI were the most common ART treatments for cryopreserved sperm. Semen cryopreservation as a salvage method is effective, but in many conditions it is underutilized, especially in cancer patients. Lack of awareness, urgency of cancer treatment and financial constraints are the main causes of the low access rate. The concept of fertility preservation should be popularized to make better use of this medical service in China.

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1 Introduction

With the application of intracytoplasmic single sperm injection (ICSI), the success rate of assisted reproductive techniques (ARTs) using cryopreserved sperm has been considerably improved. Sperm banking, which is an increasingly more acceptable method for men to preserve their fertility for the future, is considered under a number of circumstances, such as in the case of patients who anticipate undergoing cancer-related therapy or an ART procedure, those with high-risk occupational exposure and those with oligospermia.

For male cancer patients with fertility plans after chemotherapy or radiation therapy, sperm storage is an efficient and essential procedure. According to current literature, testicular cancer accounts for 26.1% of all cancers diagnosed in men aged 20–24 years and Hodgkin's disease accounts for an additional 15.2% of cancers in the same age group [1]. The advances in chemotherapy have greatly improved the cure rate of these malignancies: the 5-year survival rate for testicular cancer is 95% and that of Hodgkin's disease is 80% [2, 3]. The malignancy itself (especially testicular cancer, Hodgkin's lymphoma and leukemia) impairs spermatogenesis. Chemoradiotherapy is potentially gonadotoxic because the germinal epithelium of the testis is very sensitive to a range of drugs [4–7]. About 15%–30% of cured cancer patients remain sterile in the long term [8]. In light of this finding, sperm preservation, as a simple, accessible and effective way to preserve fertility potential, should be recommended to all men preparing to undergo potentially sterilizing cancer treatment [9].

In addition, regardless of their cancer status, men who want to preserve their fertility for the future can rely on sperm cryopreservation. Men can benefit from sperm cryopreservation in the following circumstances: (1) the male partner in an infertile couple who is too nervous to produce an ejaculated semen sample on the day of oocyte retrieval; (2) men who plan to undergo a vasectomy as a form of contraception; (3) men with high-risk careers who desire fertility in the future; and (4) men with a reproductive disease, such as anejaculation, severe oligozoospermia and obstructive azoospermia.

Although sperm banking can successfully preserve the male reproductive capacity, the use rate of this medical service is extremely low, especially in the Chinese population. To our knowledge, little data are available about the current condition of homologous sperm cryopreservation on the large scale. We have performed the first retrospective review of several centres in China and explored the variation in findings and limitation factors. This study aims to illustrate the current condition of homologous sperm cryopreservation on a large scale and review the importance and application capacity of such costly medical services in China.

2 Materials and methods

This study was approved by the Reproductive Ethics Committee of Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. This is a retrospective study, as patients' records were reviewed to determine the diagnosis and cause of sperm banking, pre-freeze semen parameters, use rate, type of infertility treatment and pregnancy outcome. From January 2003 to December 2008, a total of 1 548 men cryopreserved their semen in three sperm banks and one reproductive centre (Shanghai Human Sperm Bank, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai; Institute of Human Reproduction and Stem Cell Engineering, Human Sperm Bank, Central South University, Changsha; Zhejiang Institute of Planned Parenthood Research & Zhejiang Human Sperm Bank, Hangzhou; Jinghua Hospital, Shenyang East Group, Shenyang Reproductive Medicine Centre, Shenyang). All patients underwent a detailed fertility evaluation, including medical history, physical examination, sexually transmitted disease screening (hepatitis B virus [HBV], hepatitis C virus [HCV], human immunodeficiency virus [HIV], syphilis, gonorrhea, mycoplasma, chlamydia), and semen analysis pre- and post-cryopreservation. Routine semen analysis, performed in accordance with the guidelines in the World Health Organization (WHO) laboratory manual on semen analysis (1999 edition), was performed on each sample before and after cryopreservation [10]. Briefly, the semen analysis included determination of the volume, pH, viscosity, sperm concentration, motility and morphology. Samples were frozen according to a standard sperm cryopreservation protocol and stored in liquid nitrogen. A vial of each sample was retrieved for a post-thaw test.

For anejaculation patients, electroejaculation (EEJ) was performed under general anaesthesia while the patients were placed in the lateral decubitus position. Prior to electrostimulation, the bladder was flushed with 10% $NaHCO_3$ solution; a volume of 20 mL was instilled for collection of retrograde-emitted sperm. Multiple electrical stimulations ranging from 5 to 20 V and lasting for 2–4 s were administered. The urethra was manually milked into a sterile container both during and upon completion of electrostimulation to obtain the maximum volume of antegrade semen. After EEJ, bladder catheterization was routinely performed for collection of retrograde specimens.

Patients with obstructive azoospermia were subjected to percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE) based on their explicit diagnosis. If motile sperm were detected after the post-thaw test, the sample would be recommended for banking to avoid repeated invasive procedures during ICSI treatments. Some epididymal fluid was collected during the vasoepididymostomy or vasovasostomy procedure as a precaution in case of failure of the operation.

If no motile sperm were detected, the sample would not be banked after notification of the patients. As only a few sperm are required for IVF with ICSI, any sample containing motile sperm was banked after proper consent for cryopreservation was obtained.

The samples were delivered to the reproductive centres in liquid nitrogen. Density gradient centrifugation of the sperm samples was carried out to prepare spermatozoa for intrauterine insemination (IUI) or IVF. Briefly, density gradients were prepared by pipetting 1 mL of 80% Isolator (Irvine Scientific, Santa Ana, CA, USA) into a sterile 15-mL conical centrifuge tube and overlaid with 1 mL of 40% Isolator (Irvine Scientific). An aliquot of thawed semen was placed on top of the upper layer with a transfer pipette and centrifuged for 20 min at 300 × *g*. The supernatant was aspirated and the pellet was resuspended in 2–3 mL warmed mHTF (37ºC, Irvine Scientific). The sample was centrifuged for 5 min at 300 × *g* and the supernatant was removed. The resuspension and centrifugation steps were repeated, and the final pellet was resuspended in about 0.3 mL of warmed mHTF (Irvine Scientific) for further analysis. Sperm motility and female factor of infertility were taken into account when determining the optimal ART procedure.

3 Results

From January 2003 to December 2008, 1 548 males banked their semen in the above-listed four health organizations, which are licensed by the Chinese Ministry of Health. Cancer, ART and reproductive diseases were the main reasons why the men resorted to sperm banking. The use of cryopreserved sperm from these men resulted in a total of 119 live births. Table 1 gives the clinical data of the men, including the reasons for sperm banking, age and seminal parameters at the pre-freeze stage. The majority of cancer patients and infertility patients have poor semen quality. Table 2 summarizes data from previous antineoplastic treatments, the use rate, ART treatment and pregnancy outcome. About 86.7% (26/30) of the cancer patients banked their semen prior to chemo- or radiotherapy. One testicular

Table 1. Characteristics of the subjects, seminal parameters and use rate for sperm banking.

Reasons for sperm banking	Number	Age (years, mean, range)	Seminal parameters		
			Density ($\times 10^6$)	a + b (%)	Normal morphology rate (%)
Carcinoma	30				
Testicular tumour	21	28.2 (18–37)	47.7	29.9	11.6
Hodgkin's disease	3	30.4 (21–33)	28.2	26.7	20.5
Digestive system tumour	1	28.0	19.8	24.5	12.0
Nasopharyngeal carcinoma	2	30.0 (27–33)	39.5	35.6	17.5
Brain carcinoma	2	26.5 (25–28)	136.8	57.1	18.5
Bone sarcoma	1	27.0	73.0	24.5	16.0
ART	1 374				
Insurance for no ejaculation	627	31.6 (25–51)	62.1	43.8	26.7
Fertile plan during separation	747	34.1 (24–49)	60.2	41.1	22.3
Reproductive diseases	134				
Anejaculation	9	35.2 (28–47)	57.6	13.5	16.7
Severe oligozoospermia	66	37.3 (26–51)	3.1	12.3	9.6
Obstructive azoospermia	59	33.9 (25–52)	10.7	5.9	10.2
High–risk career	7	26.5 (22–35)	27.8	20.3	35.2
Before vasectomy	3	34.7 (32–41)	95.6	70.1	27.6

Abbreviation: ART, assisted reproductive technique.

Table 2. Previous treatment, use rate, and ART procedure and pregnancy outcome.

Number of patients	Pre-/post-treatment		Use rate (%)	ART procedure			Live births
	Pre-	Post-		IUI	IVF	ICSI	
Carcinoma							
Testicular tumour (21)	19	2	9.5 (2/21)	0	1	1	1
Hodgkin's disease (3)	3	0	0	0	0	0	0
Digestive system tumour (1)	1	0	0	0	0	0	0
Nasopharyngeal carcinoma (2)	1	1	0	0	0	0	0
Brain carcinoma (2)	2	0	0	0	0	0	0
Bone sarcoma (1)	0	1	0	0	0	0	0
ART							
Insurance for no ejaculation (627)	–	–	27.1 (170/627)	9	103	58	59
Fertile plan during separation (747)	–	–	19.9 (149/747)	6	97	46	47
Reproductive diseases							
Anejaculation (9)	–	–	55.6 (5/9)	0	0	5	2
Severe oligozoospermia (66)	–	–	3.0 (2/66)	0	0	2	1
Obstructive azoospermia (59)	–	–	40.7 (24/59)	0	0	24	9
High-risk career (7)	–	–	0	0	0	0	0
Before vasectomy (3)	–	–	0	0	0	0	0

Abbreviations: ART, assisted reproductive technique; IUI, spermatozoa for intrauterine insemination; IVF, *in vitro* fertilization; ICSI, intracytoplasmic single sperm injection .

cancer patient had his sperm cryostored prior to chemoradiation therapy after a one-sided testectomy; the use of this cryopreserved sample later resulted in the birth of a healthy boy through a single ICSI cycle. All the pregnancies (13 live births) obtained using cryobanked semen in patients with cancer and/or reproductive disease resulted from ICSI. In the ART group, pregnancies were obtained by IVF (70 live births) and ICSI (36 live births).

4 Discussion

The first clinical cryobank was established in 1953 by Jerome K. Sherman in Iowa City, Iowa, USA. In the same year, the first pregnancy conceived with frozen sperm was reported. Over the past 50 years, the technique has been refined and standardized, allowing men to preserve their fertility potential efficiently. The indications for cryopreservation have expanded greatly with the development of ICSI. The first human sperm bank in China, located in Changsha, was established in 1981. To date, a total of 10 human sperm banks have been established in China. Reproductive medicine has improved rapidly over the past decade, with approximately 300 reproductive clinics performing ART treatments. Although the technique is already well established, sperm storage became available in China relatively recently. The general population and some medical professionals remain unfamiliar with the technique, its applications and its limitations.

According to our study, cancer is the primary reason for sperm cryopreservation. Sperm storage for cancer patients preserves their reproductive potential for the future and helps to alleviate the future emotional burden among survivors [11].

Another major group that required sperm cryopreservation included men in an infertile couple as part of ART. Even when sperm is of low quality, sperm banking should be encouraged because ICSI requires only a few spermatozoa for a successful outcome [12, 13]. Importantly, an increase in genetic or phenotypic abnormalities has not been demonstrated in infants conceived with the use of cryopreserved sperm. Unfortunately, only a small portion of male cancer patients resorted to sperm cryopreservation. A retrospective study showed that 1 372 910 people were diagnosed with cancer in 2003, and about 4% (approximately 55 000) of them were under the age of 35 [14]. In other words, although there are a large number of young cancer patients (aged 20–40 years) in China, only 30 young cancer patients (< 0.1%) cryopreserved sperm during the past 6 years in three sperm banks and one reproductive centre. This rate is far below that of other countries, indicating that reproductive insurance is severely underutilized in China.

This issue is important in advanced countries as well, although to a lesser extent. A survey regarding cryobanked semen in the United Kingdom found that only 9% of oncological units have relevant information leaflets available [15]. A recent survey in Ireland corroborates these findings [16].

According to a recent study, seven main factors have an impact on sperm banking: (1) severity and (2) personal risk of infertility, (3) the importance that patients place on having children, (4) awareness of the benefits of sperm banking, (5) cost, (6) misperceptions, and (7) cultural attitudes [17].

Based on our current understanding, the main three reasons for the severe underutilization of sperm banking in China are as follows. First, there is a lack of awareness. More specifically, oncologists and patients may not clearly comprehend the negative effects of cancer treatments on fertility and are not fully aware of new developments in reproductive medicine. Therefore, sperm storage was not recommended to patients at their first visit. A lack of relevant information and knowledge is a major limiting factor in the referral of semen cryostorage to male cancer patients. Second, underutilization is ascribed to the urgency of cancer treatment. After a diagnosis of cancer, the cancer treatment is generally the patient's top priority. Therefore, they may ignore fertility planning since it is not an immediate concern. In addition, there is usually little time to preserve sperm prior to cancer treatment. When patients come to our clinics, semen analysis generally shows azoospermia or severe oligospermia with zero post-thaw motility. The third factor, which is equally important, is financial. Most medical facilities charge for sperm storage. In Australia, the service is provided without additional charge to patients under the hospital-based national health scheme [18]; however, in China, sperm storage is not covered by provincial insurance plans as part of a fertility treatment. The cost per year is about 100–200 US dollars, which is a heavy burden for the low-income population. To improve the current conditions in China, we suggest that the concept of fertility preservation should be established through the training of medical professionals, especially staff in radiation oncology and haematology departments. The government should also offer financial support for sperm cryopreservation to patients without existing offspring. In our study, only one testicular cancer patient utilized his specimen, and a healthy infant boy was born by ICSI. A recent American study reported that the use rate of a banked semen sample was about 4.7% for cancer patients [19]. There are many different reasons for not using the stored semen and/or discontinuing its storage; for example, recovery of fertility potential, death, and altered fertility plans [20]. Although the use rate is low, semen cryopreservation is an invaluable service, as a suitable treatment for non-obstructive azoospermia is not yet available.

According to the present study, 88.8% (1 374/1 548) of the men who cryopreserved semen did so to guarantee a potential chance of conception by ART. Banking of this kind is usually short term (less than one year). Men may be too anxious to ejaculate successfully on the day of oocyte retrieval. In that case, sperm banking could alleviate their anxiety and ensure that ART proceeds. Some couples undergo ART for family planning because the husband will physically be away for unavoidable reasons; in such cases, sperm banking and semen cryopreservation might be the only alternatives.

Some men resorted to sperm banking because of reproductive diseases, such as anejaculation, severe oligozoospermia and obstructive azoospermia. Anejaculation patients could cryopreserve the semen retrieved by EEJ. Previous studies demonstrated that anejaculation due to a neurogenic or psychogenic cause results in sperm with low motility and poor quality in both the antegrade and retrograde fractions, whether in the first or in the later procedures. These findings are consistent with our study, which found that spontaneous semen emission was very rare in these men. Stasis of seminal fluid leads to low motility even in fresh semen samples [21]. Thus, ICSI is most widely used among various ART treatments in anejaculation patients.

A cryopreservation program is appropriate for severe or extreme oligozoospermia. Severe oligozoospermia is characterized by sperm count fluctuations that may result in an insufficient quantity of motile sperm for ICSI on the day of oocyte retrieval. To alleviate the risk of TESE, which can lead to complications such as haematoma, inflammation and fibrosis [22], we propose that patients with transient azoospermia or repeatedly low sperm counts should generate a pool of frozen spermatozoa before attempting ICSI [23]. At present, storage and subsequent pooling of ejaculate from oliozoospermic patients for later combined use has little benefit, as poor post-thaw survival is the limiting factor. Vitrification with a cryoloop or straw is a revolutionary procedure due to the high cryosurvival rate of sperm banking and will become the mainstream approach for rare sperm cryopreservation.

Obstruction of the seminal ducts is the cause of infertility in about 5% of infertile males. The most up-to-date methods of microsurgical vasal and epididymal reconstruction (vasovasostomy and vasoepididymostomy), transurethral resection of the ejaculatory ducts, has been generally successful [24–26]. Although the success rate of reconstructive operations is high, there still exists the need to bank motile sperm in epididymal fluid during certain operations or diagnostic procedures (microsurgical epididymal sperm aspiration [MESA] or PESA for congenital bilateral absence of the vas deferens) as a precaution in the case of failure and need for additional invasive procedures during ICSI treatment.

In China, the most popular birth control measure is the intrauterine device. A study in Shanghai found that 65.49% of ever-bearing women had adopted that method [27]. The percentage of males who had undergone a vasectomy is low compared with that in western countries, and so fertility insurance by cryopreservation prior to vasectomy comprises only a small portion.

Semen is routinely contained in cryovials that are stored in a large liquid nitrogen tank. In China, approximately 120 000 000 persons are HBV carriers. A method to guarantee the security of samples is crucial. In order to prevent possible cross-contamination of semen samples, high-security ionomeric resin straws (Cryo Bio System, France), which effectively heat-seal the vial and will not open or crack at −196ºC, are used for HBV-positive samples.

Our retrospective study included 352 couples who underwent ART (IUI: 15 couples, IVF: 201 couples, ICSI: 136 couples), which resulted in 119 live births, mainly from IVF and ICSI. A previous study of ICSI using cryopreserved sperm from men with cancer reported a pregnancy rate of 56.8% per retrieval [28]. The decision to use IVF or ICSI may depend on female factor infertility or abnormal sperm function (such as sperm DNA fragmentation, diminished maturity and physiological function), which are detectable by specialized tests. For cases that repeatedly fail, tests of sperm function may be needed to select the optimal ART approach.

5 Conclusion

Sperm cryopreservation is a precautionary measure that may be taken to extend the possibility of experiencing fatherhood. For example, cancer patients may have their sperm preserved prior to chemo- or radiotherapy, men who have a reproductive disease may wish to preserve their sperm, and ART patients may find comfort and hope by preserving their semen. Although the use rate of cryopreserved semen is generally low, it may be helpful and should be made available to men in the above-mentioned groups.

This study was the first to illustrate the current condition of homologous sperm cryopreservation on a large scale. The findings may be useful in further investigation of the current use rate and in the development of medical training and practice policy in China. The possibility of utilizing unused samples for research or as donor sperm samples for other infertile patients with proper consent should be explored in an effort to make better use of such a precious resource.

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References

1 Wu XC, Chen VW, Steele B, Roffers S, Klotz JB, *et al.* Cancer incidence in adolescents and young adults in United States1992–1997. J Adolesc Health 2003; 32: 405–15.
2 Schover LR, Brey K, Lichtin A, Lipshultz Li, Jeha S. Oncologists' attitudes and practices regarding banking sperm before cancer treatment. J Clin Oncol 2002; 20: 1890–7.
3 Jemal A, Clegg LX, Ward E, Ries LA, Wu X, *et al.* Annual report to the nation on the status of cancer, 1975–2001, with a special feature regarding survival. Cancer 2004; 101: 3–27.
4 Relander T, Cavallin-Stahl E, Garwicz S, Olsson AM, Willén M. Gonadal and sexual function in men treated for childhood cancer. Med Pediatr Oncol 2000; 35: 52–63.
5 Viviani S, Santoro A, Ragni G, Bonfante V, Bestetti O, *et al.* Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP *vs.* ABVD. Eur J Cancer Clin Oncol 1985; 21: 601–5.
6 Clark ST, Radford JA, Crowther D, Swindell R, Shalet SM. Gonadal function following chemotherapy for Hodgkin's disease: a comparative study of MVPP and a seven-drug regime. J Clin Oncol 1995; 13: 134–9.
7 Ortin TT, Shostak CA, Donaldson SS. Gonadal status and reproductive function following treatment for Hodgkin's

disease in childhood: the Stanford experience. Int J Radiat Oncol Biol Phys 1990; 19: 873–80.
8 Schrader M, Muller M, Straub B, Miller K. The impact of chemotherapy on male fertility: a survey of the biologic basis and clinical aspects. Reprod Toxicol 2001; 15: 611–7.
9 Trottmann M, Becker AJ, Stadler T, Straub J, Soljanik I, *et al*. Semen quality in men with malignant diseases before and after therapy and the role of cryopreservation. Eur Urol 2007; 55: 355–67.
10 World Health Organization. WHO laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.
11 Puscheck E, Philipp PA, Jeyendran RS. Male fertility preservation and cancer treatment. Cancer Treat Rev 2004; 30: 173–80.
12 Chan PTK, Palermo GD, Veeck LL, Rosenwaks Z, Schlegel PN. Testicular sperm extraction combined with intracytoplasmic sperm injection in the treatment of men with persistent azoospermia postchemotherapy. Cancer 2001; 96: 1632–7.
13 Agarwal A, Allamaneni S. Disruption of spermatogenesis by the cancer disease process. J Natl Cancer Inst Monogr 2005; 34: 9–12.
14 Tournaye H, Goossens E, Verheyen G, Frederickx V, De Block G, *et al*. Preserving the reproductive potential of men and boys with cancer: current concepts and future prospects. Hum Reprod 2004; 10: 525–32.
15 Zapzalka DM, Redmon JB, Pryor JL. A survey of oncologists regarding sperm cryopreservation and assisted reproductive techniques for male cancer patients. Cancer 1999; 86: 1812–7.
16 Allen C, Keane D, Harrison RF. A survey of Irish consultants regarding awareness of sperm freezing and assisted reproduction. Ir Med J 2003; 96: 23–5.
17 Achille MA, Rosberger Z, Robitaille R, Lebe S, Gouin JP, *et al*. Facilitators and obstacles to sperm banking in young men receiving gonadotoxic chemotherapy for cancer: the perspective of survivors and health care professionals. Hum Reprod 2006; 21: 3206–16.
18 Kelleher S, Wishart SM, Liu PY, Turner L, Di Pierro I, *et al*. Long-term outcomes of elective human sperm cryostorage. Hum Reprod 2001; 16: 2632–9.
19 Chung K, Irani J, Knee G, Efymow B, Blasco L, *et al*. Sperm cryo-preservation for male patients with cancer: an epidemiological analysis at the university of Pennsylvania. Eur J Obstet Gynecol Reprod Biol 2004; 113 (Suppl): S7–11.
20 Hallak J, Sharma RK, Thomas AJ Jr, Agarwa A. Why cancer patients request disposal of cryopreserved semen specimens posttherapy: a retrospective study. Fertil Steril 1998; 69: 889–93.
21 Mallidis C, Lim TC, Hill ST, Skinner DJ, Brown DJ, *et al*. Necrospermia and chronic spinal cord injury. Fertil Steril 2000; 74: 221–7.
22 Schill T, Bals-Pratsch M, Kupker W, Sandmann J, Johannisson R, *et al*. Clinical and endocrine follow-up of patients after testicular sperm extraction. Fertil Steril 2003; 79: 281–6.
23 Koscinski I, Wittemer C, Lefebvre-Khalil V, Marcelli F, Defossez A, *et al*. Optimal management of extreme oligozoospermia by an appropriate cryopreservation program. Hum Reprod 2007; 22: 2679–84.
24 Marmar JL, Sharlip I, Goldstein M. Results of vasovasostomy or vasoepididy-mostomy after failed percutaneous epididymal sperm aspirations. J Urol 2008; 179: 1506–9.
25 Zhang GX, Bai WJ, Xu KX, Wang XF, Zhu JC. Clinical observation of loupe-assisted intussusception vasoepididymostomy in the treatment of obstructive azoospermia (analysis of 49 case reports). Asian J Androl 2009; 11: 193–9.
26 Manohar T, Ganpule A, Desai M. Transrectal ultrasound-and fluoroscopic- assisted transurethral incision of ejaculatory ducts: a problem-solving approach to nonmalignant hematospermia due to ejaculatory duct obstruction. J Endourol 2008; 22: 1531–5.
27 Liu Y. Valuation of the service status of four kinds of contraception methods in married woman at child bearing age in five cities of China. Chin J Planned Parenthood 2004; 12: 390–3.
28 Hourvitz A, Goldschlag DE, Davis OK, Gosden LV, Palermo GD, *et al*. Intracytoplasmic sperm injection (ICSI) using cryopreserved sperm from men with malignant neoplasm yields high pregnancy rates. Fertil Steril 2008; 90: 557–63.