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Different sperm sources and parameters can influence intracytoplasmic sperm injection outcomes before embryo implantation*

Yue-hong LU[§1,2], Hui-juan GAO[§1], Bai-jia LI[1], Ying-ming ZHENG[1], Ying-hui YE[1], Yu-li QIAN[1], Chen-ming XU[1], He-feng HUANG[1], Fan JIN[†‡1]

([1]*Department of Reproductive Endocrinology, Key Laboratory of Reproductive Genetics, Ministry of Education, and Key Laboratory of Women's Reproductive Health of Zhejiang Province, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou 310006, China*)

([2]*Department of Reproductive Health, Shaoxing Women and Children Hospital, Shaoxing 312000, China*)

[†]E-mail: jinfan@zju.edu.cn

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Abstract: To evaluate the effects of sperm with different parameters and sources on the outcomes of intracytoplasmic sperm injection (ICSI), 1972 ICSI cycles were analyzed retrospectively. Groups 1 to 5 were composed of cycles using ejaculated sperm and were grouped according to sperm quantity, quality, and morphology into normal (288 cycles), or mild (329 cycles), moderate (522 cycles), severe (332 cycles), and extremely severe (171 cycles) oligozoospermia and/or asthenozoospermia and/or teratozoospermia (OAT) groups. Group 6 was composed of 250 cycles using testicular or epididymal sperm, and Group 7 consisted of 80 cycles using frozen-thawed sperm. We found that fertilization rates were gradually reduced from Groups 1 to 6, and reached statistical difference in Groups 5 and 6 (P<0.05). The high-quality embryo rate was higher in Group 1 than in Groups 2, 3, 5, 6, and 7 (P<0.05). No statistical differences were observed in the rates of embryo cleavage, clinical pregnancy, miscarriage, live-birth, premature birth, low birth weight, weeks of premature birth, average birth weight, or sex ratio for all seven groups (P>0.05). A total of nine cases of malformation were observed, with a malformation rate of 1.25% (9/719). In conclusion, different sperm sources and parameters can affect ICSI outcomes before embryo implantation. A full assessment of offspring malformation will require further study using a larger sample size.

Key words: Intracytoplasmic sperm injection (ICSI), Sperm, Sperm source, Sperm parameters, Malformation
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1 Introduction

Intracytoplasmic sperm injection (ICSI), first introduced by Palermo *et al*. (1992), is an assisted-fertilization micromanipulation technique for fertilizing an egg by directly injecting a selected sperm into the ooplasm. Compared with conventional in-vitro fertilization (IVF), ICSI greatly reduces the requirements for sperm quantity, motility, and fertilization ability. With the development of micromanipulation, ICSI has become an important part of assisted reproductive techniques (ARTs) for the treatment of cases with male factor infertility (Sarkar, 2007). With ICSI, an egg can be fertilized not only with fresh sperm with mild or moderate abnormalities but also with sperm following a frozen-thawed procedure, sperm of a severely or extremely severely abnormal concentration, motility or morphology, or even sperm that have been surgically retrieved from

‡ Corresponding author
§ The two authors contributed equally to this work
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the epididymis or testis (van Steirteghem *et al.*, 1993; Devroey *et al.*, 1994; Redgment *et al.*, 1994; Tournaye *et al.*, 1994; Chen *et al.*, 1996).

Nevertheless, sperm retrieved from the testis, epididymis or ejaculation may have a different concentration, motility or morphology and differ in their degree of maturity, chromosomal or DNA composition, and gene imprinting conditions (Holt and North, 1991; Holt *et al.*, 1992; Giraud *et al.*, 2000; Chatterjee and Gagnon, 2001; Pegg, 2002; Chohan *et al.*, 2004; Liu *et al.*, 2004; Derijck *et al.*, 2007; Kobayashi *et al.*, 2007). For instance, rates of DNA fragmentation, mitochondrial dysfunction, and chromosomal aneuploidy are significantly higher in the sperm of oligozoospermia and/or asthenozoospermia and/or teratozoospermia (OAT) males compared with unaffected controls (Liu *et al.*, 2004; Derijck *et al.*, 2007). Furthermore, aberrant DNA methylation of imprinted loci in sperm from oligospermic patients has been reported (Kobayashi *et al*., 2007). Generally, testicular and epididymal sperm are considered to have a low degree of maturity and a high malformation rate. The accumulated evidence also shows that the process of freezing and thawing sperm could cause damage to the sperm (Holt and North, 1991; Holt *et al.*, 1992; Giraud *et al.*, 2000; Chatterjee and Gagnon, 2001; Pegg, 2002; Chohan *et al.*, 2004). Therefore, the consequences of using these different sources of sperm have attracted a great deal of attention since the first introduction of ICSI. There is an ongoing debate about this question (Bukulmez *et al.*, 2001; Miller and Smith, 2001; Göker *et al.*, 2002; Aoki *et al.*, 2004; Dozortsev *et al.*, 2006; Loutradi *et al.*, 2006; Tepla *et al.*, 2006; Nilsson *et al.*, 2007; Naru *et al.*, 2008; Verza and Esteves, 2008).

Here, we report an analysis of 1972 ICSI cycles to explore the possible relationships between sperm quantity, quality, morphology, source, and the outcomes of ICSI.

2 Materials and methods

2.1 Subjects

A total of 1925 couples who underwent 1972 ICSI cycles from January 2004 to June 2008 in the Center for Reproductive Medicine of the Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China were included in this retrospective study. Institutional review board approval and written consents from patients were obtained prior to data collection. In all cycles studied, the tubal factor was identified as the sole cause of female factor infertility, thereby excluding other female factors such as myoma, endometrial polypus, endometriosis, and uterus deformation. Based on the sperm quality and source on the day of ICSI, the subjects were divided into seven groups.

Group 1 was composed of 288 ICSI cycles with normal semen parameters, based on the 5th World Health Organization standard (WHO, 2010) (total sperm number, calculated by sperm concentration× sperm volume, $\geq 39\times10^6$ sperm/ml, progressive motility ≥32%, and morphology ≥4% normal forms). In this group, ICSI was performed for patients with failure or low fertilization rates (<30%) in their last IVF cycle, unexplained primary infertility after more than three cycles of failed artificial insemination with the male partner's sperm, or abnormal results from more than two previous semen analyses. Group 2 was composed of 329 ICSI cycles with mild OAT (total sperm number 20×10^6–39×10^6 sperm/ml, and/or progressive motility 20%–32%, and/or morphology <4% normal forms). Group 3 was composed of 522 ICSI cycles with moderate OAT (total sperm number 10×10^6–20×10^6 sperm/ml, and/or progressive motility 10%–20%). Group 4 was composed of 332 ICSI cycles with severe OAT (total sperm number 1×10^6–10×10^6 sperm/ml, and/or progressive motility 1%–10%). Group 5 was composed of 171 ICSI cycles with extremely severe OAT (total sperm number $<1\times10^6$ sperm/ml, and/or progressive motility <1%). Group 6 was composed of 250 ICSI cycles with obstructive azoospermia (OA) (azoospermia patients with normal serum follicle-stimulating hormone (FSH), testicular volume, and diagnostic biopsy) or difficult ejaculation on the day of IVF, using testicular or epididymal sperm. Group 7 was composed of 80 ICSI cycles using frozen-thawed sperm, in which the main reason for ICSI was that the progressive motility of donated semen was less than 25% after thawing.

2.2 Sperm preparation

Fresh ejaculated semen was collected on the day of oocyte retrieval and left to liquefy for

approximately 20–30 min prior to semen analysis. Afterwards, the semen specimens were processed by density gradient centrifugation, as previously described (Mortimer, 2000). For each semen sample, 1 ml was used for preparation, except for the cases with extremely low counts. The final solution was incubated at 37 °C, 5% CO_2 for 0.5–1.0 h prior to the ICSI procedure.

Sperm retrieval from the testis was performed percutaneously by testicular sperm aspiration (TESA) (Watkins *et al.*, 1997) under local anesthesia. Seminiferous tubules were dissected mechanically, and the presence of sperm was examined using an inverted microscope (Olympus, Tokyo, Japan) at 200× magnification. The supernatant was processed by density gradient centrifugation (Mortimer, 2000). Sperm retrieval from the epididymis was performed by percutaneous epididymal sperm aspiration (PESA) (Lin *et al.*, 2000). The aspirate was washed into a Petri dish with a small volume of modified human tubal fluid (M-HTF; Irvine Scientific, CA, USA), and the presence of sperm was confirmed using an inverted microscope at 400× magnification. ICSI was performed immediately. The frozen sperm was thawed (Royere *et al.*, 1996) on the day of ICSI. The thawed sperm was processed using the same method as used for fresh ejaculated sperm. The final solution was incubated at 37 °C, 5% CO_2 for 0.5–1.0 h prior to the ICSI procedure.

2.3 Ovarian stimulation and oocyte retrieval

All female patients were treated with a long gonadotrophin-releasing hormone agonist (GnRH-a; Decapeptyl, Ferring, Kiel, Germany)/gonadotrophin protocol (Tarlatzis *et al.*, 1993). Ovulation was induced by injection of human chorionic gonadotrophin (HCG; Ovidrel®, Serono, Geneva, Switzerland) when at least two follicles reached a diameter of ≥18 mm. Oocytes were retrieved 36 h after HCG administration using follicular aspiration with guidance from transvaginal sonography. After follicular aspiration, the tubes with the follicular fluid were transferred to the laboratory and examined using stereomicroscopy to identify the cumulus-oocyte complexes (COCs). The COCs were transferred to pre-balanced G-IVF (Vitrolife, Goteborg, Sweden) culture medium and incubated at 37 °C, 5% CO_2 for 2 h prior to oocyte handling.

2.4 Oocyte handling

The oocytes were stripped of the surrounding cumulus cells (2 h after ovum collection) using hyaluronidase (80 U/ml; Vitrolife) in M-HTF for 10–15 s. Then, using a 135 μm diameter pipette (Flexipet, Cook, Bloomington, IN, USA), the oocytes were transferred to M-HTF for complete removal of the corona cells. The denuded oocytes were then rinsed and incubated in M-HTF until the time of injection (2–3 h after collection). The ICSI procedure was performed only for metaphase II (MII) oocytes that had extruded their first polar bodies.

2.5 ICSI procedure

The ICSI procedure (Miller and Smith, 2001; Göker *et al.*, 2002) was performed 38–42 h post-HCG injection. The microinjection procedure was performed on a heated stage at 37 °C under an inverted microscope at 400× magnification. Sperm were selected at the periphery of the polyvinylpyrrolidone (PVP; Vitrolife) micro-droplet. A selected spermatozoon was immobilized by repeatedly touching the tail and was then aspirated, tail first, into the injecting pipette. The oocyte was held by a holding pipette with the polar body at the twelve or six o'clock position. The injecting pipette containing the spermatozoon was introduced at the three o'clock position through the zona pellucida and oolemma, deep into the cytoplasm. A small part of the cytoplasm was aspirated. The spermatozoon and the cytoplasm were injected, and the injecting pipette was withdrawn gently. After injection, the oocytes were rinsed and incubated in G-IVF medium.

2.6 Embryo culture and pregnancy establishment

Fertilization was confirmed 16–20 h post-ICSI procedure by the presence of two pronuclei. Cleavage and embryo quality were assessed 48–72 h post-ICSI procedure. The embryos were classified as high-quality when they exhibited 3–4 symmetric blastomeres on the second day of culture and 7–8 symmetric blastomeres on the third day of culture, in the absence of multinucleation and zona pellucida alterations, Grade I (absence of fragmentation) or II (up to 20% of cytoplasmic fragments) of cytoplasmic fragmentation (Veek, 1990).

A total of 1–3 embryos were chosen for transfer

to the female partners 2 or 3 d after oocyte retrieval. Females older than 35 years or on their second round of ART received at most three embryos, in accordance with China's ART Regulations. The luteal phase was supported by progesterone ampules. Serum HCG levels were measured on Day 14 after embryo transfer. Clinical pregnancies were confirmed by a vaginal ultrasound 30 d after embryo transfer which showed the presence of an intrauterine gestational sac with detectable fetal cardiac activity.

2.7 Assessments

In addition to the rates of fertilization, cleavage, high-quality embryos, pregnancy, miscarriage, ectopic pregnancy, and multiple pregnancy, some perinatal parameters, such as the gestational age, live-birth rates, premature birth rates, birth weight, sex ratio, and major malformations were assessed. Sex ratio was defined as male offspring/(males+ females). Miscarriage was defined as pregnancy loss before 20 weeks of gestational age. Gestational age was calculated as the time from an estimated first menstruation day (14 d before follicular aspiration) to the time of birth. Major malformations were defined as malformations requiring surgical treatment or causing essential reduced functionality.

2.8 Statistical analysis

All data were analyzed using SPSS 15.0 software (SPSS Inc., Chicago, Illinois, USA) and evaluated by the Chi-square test, one-way analysis of variance (ANOVA), the rank transform method, and logistic regression. $P<0.05$ was accepted as significant.

3 Results

3.1 Characteristics of patients

A total of 1972 ICSI cycles were analyzed. In Groups 4, 5, and 6, the females were significantly younger ($P<0.05$). The infertility period was the shortest in Group 6 and the longest in Group 7 ($P<0.05$). Endometria were significantly thicker in Groups 6 and 7 ($P<0.05$). The peak (estradiol) E_2 before HCG injection and the number of retrieved oocytes were significantly higher in Group 6 (Table 1).

3.2 Laboratory outcomes of ICSI

The rates of fertilization, cleavage and high-quality embryos in each group are listed in Table 2. The fertilization rates decreased as semen quality decreased from Groups 1 to 6 and reached statistical significance in Groups 5 [(67.7±24.5)%] and 6 [(65.9±21.5)%] ($P<0.05$). The high-quality embryo rate in Group 1 [(70.3±27.7)%] was significantly higher than that in other groups ($P<0.05$). There were no statistical differences in the cleavage rates among the seven groups ($P>0.05$) (Table 2).

3.3 Clinical outcomes of ICSI

A total of 1831 transfer cycles were performed, with a total of 4196 embryos transferred. The cancellation rate for fresh embryo transfer was 7%, based mainly on a high risk of ovarian hyperstimulation syndrome (OHSS), fever, or poor conditions of endometrium preparation. In total, 700 female partners became clinically pregnant, with a total clinical pregnancy rate of 38.2% (700/1831). The average

Table 1 Characteristics of the patients in all groups

Group	Cycle number	Women's age (year)	Infertility period (year)	Basal FSH (U/L)	Endometrial thickness (mm)	Peak E_2 (nmol/L)	Retrieved oocytes	Injected MII oocyte
1	288	31.6±4.2	5.1±3.4	6.8±2.2	11.1±2.4	13111.0±9608.1	13.9±7.6	11.4±6.5
2	329	31.9±3.9	5.2±3.3	6.7±2.1	11.1±2.5	12614.8±8483.3	14.1±7.6	11.4±6.7
3	522	31.6±4.0	5.1±3.3	6.7±1.9	11.0±2.3	12840.1±8271.6	13.8±7.0	11.1±6.0
4	332	31.0±4.0*	4.8±3.2	6.7±1.9	11.2±2.3	13308.4±9200.2	15.0±8.4	12.1±6.8
5	171	30.2±3.7$^{\Delta}$	4.9±3.3	6.5±1.8	11.4±2.2	12722.0±7771.6	14.9±8.7	12.1±7.2
6	250	30.1±3.9†	4.6±3.2‡	6.5±2.0	11.4±2.4*	13860.9±7585.7$^{\Delta}$	15.2±7.5*	12.4±6.4
7	80	31.2±3.9	6.0±3.6$^{\#}$	6.6±1.9	11.6±2.6*	13222.5±8623.9	14.7±7.2	12.3±6.5

All values (except those for cycle number) are expressed as mean±SD; $^{*}P<0.05$: significant difference compared with Groups 1, 2, and 3; $^{\Delta}P<0.05$: significant difference compared with Groups 1, 2, 3, and 4; $^{\dagger}P<0.05$: significant difference compared with Groups 1, 2, 3, 4, and 7; $^{\ddagger}P<0.05$: significant difference compared with Groups 1, 2, 3, and 7; $^{\#}P<0.05$: significant difference compared with Groups 1, 3, 4, 5, and 6 (rank transform method)

numbers of embryos transferred in Groups 4, 5, and 6 were statistically lower than those in other groups (P<0.05). The rates of clinical pregnancy, chemical pregnancy, multiple pregnancy, ectopic pregnancy, and miscarriage showed no statistical differences among all seven groups (P>0.05) (Table 3).

Table 2 Laboratory outcomes of ICSI

Group	Fertilization rate (%)	Cleavage rate (%)	High-quality embryo rate (%)
1	73.6±20.2	97.7±11.1	70.3±27.7†
2	72.8±21.0	98.4±8.2	65.2±30.6
3	72.6±21.3	98.4±10.9	65.7±29.3
4	72.1±22.1	98.0±10.9	67.4±28.0
5	67.7±24.5*	98.3±9.1	62.0±30.4
6	65.9±21.5$^{\Delta}$	96.9±11.3	62.7±31.7
7	74.8±17.4	99.0±5.0	63.1±28.2

All values are expressed as mean±SD; * P<0.05: significantly lower than Groups 1, 3, and 7; $^{\Delta}$ P<0.05: significantly lower than Groups 1, 2, 3, 4, and 7); † P<0.05: significantly higher than Groups 3, 5, 6, and 7 (rank transform method)

3.4 Outcomes of ICSI children

Thirteen couples were lost to follow up and one female patient died by suicide in the second trimester. A total of 719 living newborns were delivered. No statistical differences were observed for the live-birth rate, premature birth rate, low birth weight rate, weeks of premature birth, birth weight, or sex ratio for all groups (P>0.05). There were two cases of stillbirth due to a circular umbilical cord in Groups 2 and 7; two cases of perinatal death, both of which were premature births, in Groups 2 and 6. A total of nine cases of malformation were observed, including one case of multiple structural abnormality and one case of fetal edema, both terminated in the second trimester; four boys with hypospadias (two boys), congenital cardiopathy, or hexadactylism, and one girl with megacolon diagnosed in the newborn period; one boy with cryptorchidism and another with epilepsy diagnosed in infancy (Table 4).

Table 3 Clinical outcomes of ICSI

Group	n_{TC}	n_{TE}	n_{avg}[a]	Pregnancy rate[b] (%)				Miscarriage rate[b] (%)
				Clinical	Chemical	Ectopic	Multiple	
1	269	655	2.4±0.6	37.5 (101/269)	3.0 (8/269)	2.2 (6/269)	29.7 (30/101)	15.8 (16/101)
2	305	712	2.3±0.5*	33.1 (101/305)	1.6 (5/305)	1.6 (5/305)	26.7 (27/101)	15.8 (16/101)
3	490	1137	2.3±0.5*	37.1 (182/490)	1.2 (6/490)	1.4 (7/490)	28.0 (51/182)	15.9 (29/182)
4	307	678	2.2±0.5$^{\Delta}$	41.0 (126/307)	2.6 (8/307)	2.3 (7/307)	31.7 (40/126)	12.7 (16/126)
5	155	334	2.2±0.5$^{\Delta}$	40.6 (63/155)	1.9 (3/155)	1.9 (3/155)	20.6 (13/63)	17.5 (11/63)
6	234	507	2.2±0.4$^{\Delta}$	40.2 (94/234)	1.7 (4/234)	2.1 (5/234)	37.2 (35/94)	12.8 (12/94)
7	71	173	2.4±0.6	46.5 (33/71)	2.8 (2/71)	0.0 (0/71)	36.4 (12/33)	15.2 (5/33)

n_{TC}: number of transfer cycles; n_{TE}: number of transferred embryos; n_{avg}: average number of embryos transferred of a subject. [a] Values are expressed as mean±SD; [b] Pregnancy rates and miscarriage rate were compared by logistic regression; * P<0.05: significantly lower than Group 1; $^{\Delta}$ P<0.05: significantly lower than Groups 1, 2, 3, and 7 (rank transform method)

Table 4 Results of ICSI children

Group	n_n	n_{tw}	n_{tr}	r_{lb} (%)	r_{pb} (%)	t_{pb}[a] (week)	m_B[a] (g)	r_{lbw} (%)	r_s[b]	n_m
1	104	23	1	29.4 (79/269)	30.4 (24/79)	35.1±1.2	2981.9±702.3	20.2 (21/104)	0.53 (55/104)	0
2	99	21	0	25.6 (78/305)	25.6 (20/78)	33.7±3.1	2965.1±671.1	18.2 (18/99)	0.45 (45/99)	3
3	183	43	0	28.6 (140/490)	26.4 (37/140)	33.9±2.2	2891.7±652.0	25.7 (47/183)	0.48 (87/183)	4
4	130	30	1	31.9 (98/307)	29.6 (29/98)	34.5±1.8	2799.0±715.8	32.4 (42/130)	0.53 (69/130)	0
5	58	10	0	31.0 (48/155)	20.8 (10/48)	34.8±1.6	3042.2±623.1	15.5 (9/58)	0.45 (26/58)	1
6	107	31	0	32.9 (77/234)	32.5 (25/77)	34.4±1.8	2757.5±596.1	29.9 (32/107)	0.49 (52/107)	1
7	38	9	0	40.8 (29/71)	20.7 (6/29)	35.0±2.0	2898.7±639.1	18.4 (7/38)	0.53 (20/38)	0

n_n: number of newborns; n_{tw}: number of twins; n_{tr}: number of triplets; r_{lb}: live-birth rate/transfer cycle; r_{pb}: premature birth rate; t_{pb}: weeks of premature birth; m_B: birth weight; r_{lbw}: low birth weight rate; r_s: sex ratio; n_m: number of malformations. [a] Values are expressed as mean±SD; [b] Values are males/(males+females). No statistical differences were observed for any group (P>0.05). Live-birth rate, premature birth rate, low birth weight rate, and sex ratio were compared by the Chi-square test, weeks of premature birth were compared by the K-W test, and weight of newborns was compared by one-way ANOVA

4 Discussion

The question of whether male factor infertility would affect the outcome of ICSI and the health of the offspring has gained great attention since the introduction of ICSI for the treatment of patients with extremely poor-quality sperm (Bonduelle *et al*., 1999). Currently, ICSI can be successfully used for even the most severe cases of OATs or azoospermia. The wider application of ICSI and the improvements in our understanding of sperm biology have encouraged more detailed investigations into sperm-derived effects on ICSI outcomes and children's health.

We compared the outcomes of ICSI based on the sperm source and the quality of the ejaculates. A tendency towards reduced fertilization rates was observed in Groups 1 to 5 as the sperm quality decreased, indicating that sperm quality can affect the fertilization process. Analyses of unfertilized oocytes after ICSI have revealed that the main causes of failed fertilization are failure to complete oocyte activation and sperm head decondensation (Flaherty *et al*., 1995; Dubey *et al*., 1997; 1998). Although ICSI can help sperm penetrate the zona pellucida and reach the cytoplasm of the oocytes, the completion of oocyte activation and the formation of the male and female pronuclei are determined by the intrinsic nature of the sperm and oocytes (Perreault *et al*., 1988; Perreault, 1992; Schatten, 1994; Yanagimachi, 1994). High rates of DNA fragmentation (Tavalaee *et al*., 2009), acrosomal defects (Cummins *et al*., 1991; Fenichel *et al*., 1991), and epigenetic factors such as sperm-specific phospholipase C zeta (PLCζ) (Swann *et al*., 2004; Heytens *et al*., 2009) in poor-quality sperm are all considered to be possible reasons for fertilization failure. Rates of DNA fragmentation, mitochondrial dysfunction, and chromosomal aneuploidy are significantly higher in the sperm of OAT males (Liu *et al*., 2004; Derijck *et al*., 2007).

In our view, sperm morphology is a crucial factor for sperm quality. We selected sperm under 400× magnification, at the periphery of the PVP microdroplet, to guarantee that the sperm selected had a relatively normal form and good motility. However, a normal-looking sperm could still contain serious nuclear malformations and chromosomal aneuploidies (Huszar *et al*., 2007; Oliveira *et al*., 2010). There are published reports that when intracytoplasmic morphologically selected sperm injection (IMSI) was performed with sperm selected under a magnification level above 6000× (Antinori *et al*., 2008; Wilding *et al*., 2011), the rate of sperm nucleus normality was significantly higher. In addition, Huszar *et al.* (2007) have reported that in sperm selected with hyaluronic acid, the frequencies of chromosomal disomy and diploidy were reduced 4- to 6-fold. More importantly, with these two methods, ICSI outcomes were significantly improved (Antinori *et al*., 2008; Parmegiani *et al*., 2010; Wilding *et al*., 2011). Therefore, more detailed sperm selection may help to further improve the ICSI outcomes.

Significantly lower fertilization rates were observed when testicular or epididymal sperm from men with OA or ejaculation failure were used for ICSI, when compared with all of the groups that used ejaculated sperm. This is in contrast to prior studies that reported that sperm from OA had the same fertilization rates after ICSI as normal ejaculated sperm (Bukulmez *et al*., 2001; Verza and Esteves, 2008). Our data seem to support the notion that sperm from the testis or epididymis have decreased fertility potential after ICSI (Loutradi *et al*., 2006). Possible explanations for this result may be that testicular and epididymal sperm are less mature and subsequently less competent at fertilization than ejaculated sperm. Furthermore, epididymal obstruction also can cause sperm damage (Shapiro *et al*., 1998; Kolettis *et al*., 1999); it has been reported that high percentages of cells with DNA damage were found in sperm samples from men with OA or anejaculation (Ramos *et al*., 2002).

Although we did not observe statistically significant differences in cleavage rates among different groups, the high-quality embryo rate in Group 1, with normal semen parameters, was significantly higher than that in other groups. This result suggests that after completing fertilization, different quantities, qualities, morphologies, or sources of sperm have no obvious effects on pronuclei syngamy, embryonic genome formation, or the initiation of mitotic division. However, those factors seem able to affect the formation of high-quality embryos, and a high percentage of good-quality sperm may be advantageous for generating a high percentage of high-quality embryos. Sperm DNA fragmentation is a specific paternal factor that can influence embryo development when it

increases above a certain level (Sakkas *et al*., 1999; Seli *et al*., 2004; Virro *et al*., 2004). The development of a human embryo in the early stages is controlled by maternally inherited mRNA. The embryonic genome is activated at approximately the 8-cell stage, and then the paternal influences on embryo development begin to be apparent (Braude *et al*., 1988). These factors might explain the discrepancy between the effects of ICSI on cleavage and the formation of high-quality embryos.

The numbers of transferred embryos were significantly lower in Groups 4, 5, and 6. Aside from this factor, the data on ICSI clinical outcomes did not show statistically significant differences in the rates of clinical pregnancy, chemical pregnancy, multiple pregnancies, ectopic pregnancy, or miscarriage; the same was true for the perinatal parameters in all seven groups. This result indicates that although different sources and different qualities of sperm could influence the fertilization process and the formation of high-quality embryos, the potential viability of ICSI embryos after transfer is nearly equal no matter what type of sperm is used. Therefore, it is thought that there is no significant effect on the clinical outcome and perinatal parameters of offspring when different qualities and sources of sperm are used for ICSI (Ludwig and Katalinic, 2003; Tepla *et al*., 2006). Furthermore, this result also demonstrates that the fertilization process and the formation of high-quality embryos might act as a mechanism for selecting the truly normal sperm.

Definite male-factor infertility in Groups 4, 5, and 6 led to the couples visiting a fertility clinic at younger ages. Hence, the percentages of female patients younger than 35 years old were much higher in these groups, resulting in lower numbers of transferred embryos, in accordance with China's ART Regulations. Decreased fertilization rates and lower percentages of high-quality embryos could also contribute to the reduced numbers of transferable embryos.

Aside from the perinatal parameters, the malformation rate was another important indicator to evaluate the safety of ICSI. In the present study, two cases of stillbirth, two cases of perinatal death, and nine cases of malformation occurred. The malformation rate was 1.25% (9/719), which is consistent with previously reported malformation rates ranging from 1.1% to 9.7% for ICSI (Rimm *et al*., 2004). Unfortunately, our cases were too few for a precise statistical analysis. There is an ongoing debate about whether sperm quality and source affect the malformation rate and genetic materials of ICSI children. Some investigators believe that there are some effects (Bonduelle *et al*., 2002; Fedder *et al*., 2007; FIVNAT *et al*., 2007), while others do not (Ludwig and Katalinic, 2003). Further multi-center studies or meta-analyses will be required to obtain a definitive answer.

In conclusion, our results demonstrate that sperm quality and source affect the fertilization process and the formation of high-quality embryos after ICSI. After embryo transfer, the potential viability of ICSI embryos is nearly equal regardless of the type of sperm used. Larger databases or a meta-analysis would be necessary to find a definitive answer as to whether sperm quality and source affect the malformation rate and genetic material of the offspring.

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