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Original Article

Severe teratozoospermia associated with severe oligospermia affects clinical outcome in human ICSI cycles

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Abstract: This study retrospectively investigated the effects of obvious head shape defect in sperm on embryo development and pregnancy outcome. Couples with severe oligospermia (total sperm count was less than several hundred, thus not enough for morphological assessment) were divided into two groups based on morphological observation of sperm head. Group 1 (N=45): sperm with obvious head shape defects (large or small, tapered, pyriform and amorphous head defects). Group 2 (N=166): sperm with "good looking head shape". The parameters of embryo development and pregnancy outcome were compared between the two groups. There were lower fertilization rates, availablity rate, high-quality embryo rate and cumulative live birth rate and higher miscarriage rate in Group 1 when compared with Group 2. No birth defects in the two groups were reported in the clinic. Embryo development and pregnancy outcome with fresh versus frozen-thawed sperm were similar in two groups. However, poor embryo development and pregnancy outcome were found in the obvious head shape defect sperm group regardless of using fresh or frozen-thawed sperm. While embryo development was apparently divergent in OAT/OA/NOA groups, the clinical outcomes were similar. Sperm with obvious head shape defects did affect embryo development and clinical outcome in OAT groups, but not in the OA/NOA group. All above results may supply a reference for clinical outcome when using these severe teratozoospermia associated with extreme oligospermia samples.

Keywords: Teratozoospermia, intracytoplasmic sperm injection, embryo development, pregnancy outcome

Introduction

The introduction of intracytoplasmic sperm injection (ICSI) in 1992 [1], was followed by reports with high fertilization and implantation rates using this method [2], and it had opened a new era in the treatment of male factor infertility. Until now, ICSI has been widely used for various male infertility factors, including oligo-astheno-teratozoospermia (OAT), obstructive azoospermia (OA) and non-obstructive azoospermia (NOA). We can safely say that most server cases of OAT with less than 10 sperms can be treated with ICSI if the available sperm can be retrieved. With the development of sperm cryopreservation technology, frozen-thawed sperm is widely used in ICSI, avoiding repeated testicular sperm extraction surgery, thus reducing the expense and the risk of procedural complications such as damage to the already suboptimal testicle [3]. Nowadays, even the most rare spermatozoa can be found after frozen-thawed. In our recent study, four healthy babies were successfully delivered using the cryopreserved rare spermatozoa stocked with novel cryopiece [4]. After some initial skepticism, it has been confirmed that there is no difference in pregnancy rates using fresh sperm versus cryopreserved-thawed surgically retrieved sperm from men with azoospermia with ICSI [5]. The wide application of ICSI in severe male factor infertility and the deep understanding of sperm biology has lead to more detailed investigation of potential paternal effects on embryo development and clinical outcome. It is widely accepted that poor morphology sperm leads to infertility. Usually we emphasize the shape defect of sperm head,

although the sperm neck, midpiece and tail defects are also considered. However, sperm morphology detection as a predictor of ICSI outcomes and is still a considerable controversy. Numerous reports suggested that abnormal morphology was not an accurate predictor of clinic outcome [6-10]. A possible reason is that during ICSI the embryologist can microscopically select individual sperm that appear to have "good looking head shape" morphologically from the severest impaired specimens (Kruger strict morphology of zero). Thus the sperm for fertilization may not be representative of the entire sperm sample population, making the initial semen morphology assessment irrelevant. Furthermore, many studies excluded the subgroup of men with rare sperm sample, owing to logistical problems performing morphology assessments on a small number of spermatozoa [6]. However, no motile sperm with "good looking head shape" was occasionally found in some extremely rare specimens (total sperm count was less than several hundred, thus not enough for morphological assessment), yet these patients refused to use the donor sperm and insisted on using their own head defect sperm after the full informed consent, which made embryologists have no choice but to inject obvious abnormal head shape sperm with ICSI. The embryologist selected the sperm with the head shape looking a little better among all the specimens with obvious head shape defects. The aim of this study is to investigate the embryo development and pregnancy outcome using the little bit of teratospermia if there is no other normal sperm that are available.

Materials and methods

Ethical approval

All the clinical information was obtained from the Reproductive Medicine Center, Shanghai General Hospital, Shanghai JiaoTong University and the clinical analysis was approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine.

Patients and semen parameters

Patients with severe rare sperm have been enrolled in this study. We retrospectively analyzed embryo development and pregnancy outcome of their IVF cycles. This study involved data collected from patients undergoing treatment at Shanghai General Hospital ART Center from January 2015 to December 2016. All female infertility factors were excluded. Only women ≤38 years were enrolled in this study to minimize the contribution of maternal age as a confounding variable. All male patients suffered from severe azoospermia or OAT. Inclusive criteria: ejaculated liquefied semen specimens and frozen-thawed semen specimens were placed on equilibrated IVF medium and centrifuged at 500 g for 5 min, then supernatant was removed and about 100 μl was left for precipitation. The presence of sperm was confirmed with 10 μl out of the 100 μl mixed sample under an inverted microscope at 10×20 magnification. Only ≤5 sperm observed in fifty 10×20 magnifications or ≤20 sperms observed in all magnifications were included in the study. As a result, 211 ICSI cycles were enrolled for analysis. IVF cycles were further divided into two groups based on sperm head morphological observation.

Group 1 (N=45): sperm with obvious head shape defects (large or small, tapered, pyriform and amorphous head defects). No motile sperm with "good looking head shape" were found in all specimens when ICSI was performed. The embryologists had no choice but to inject the motile sperm with head shape looking a little better among all the sperm with obvious head shape defect in ICSI.

Group 2 (N=166): sperm with "good looking head shape". Enough motile sperm with good looking head shape was selected for ICSI.

IVF protocol

Oocyte retrievals were performed after using rFSH (Gonal-F, Meck), HMG (HMG, Lizhu) and GnRH antagonist (Cetrotide, Merck). Transvaginal sonography (TVS) guided follicular puncture was processed 36-38 hours after the hCG (Mochida Pharmaceutical) injection. Oocytes were cultured in IVF medium (vitrolife, Sweden) at 37°C in 6% CO_2, 5% O_2 and 89% N_2. The oocytes were stripped of the surrounding cumulus cells using hyaluronidase (80 IU/mL, Medicult, DK) and ICSI was performed 38-42 h after the hCG injection. The sperm with/without good looking head shape mentioned above were injected into the MII oocytes. Fertilization was checked by the presence of pronuclei 18-20 h after ICSI. Fertilized embryos were cultured in G1plus medium (vitrolife, Sweden). Cleavage and embryo quality assessment was performed on Day 2 and Day 3 based on mor-

Table 1. Comparison of sperm with obvious head shape defect (Group 1) and good looking head shape (Group 2) on pregnancy outcome and embryo development in the cases of severe rare sperm

	Group 1 (n=45)	Group 2 (n=166)	*P*
Age (female)	28.42±3.18	28.63±3.72	0.730
Age (male)	30.64±4.96	30.92±5.65	0.765
No. of oocytes	14.09±10.34	13.89±6.88	0.876
No. of oocytes injected	11.20±7.59	11.29±6.15	0.935
Normal fertilization rate	66.9% (337/504)	74.1% (1389/1874)	<0.001
Fertilization rate	70.4% (355/504)	78.0% (1461/1874)	0.000
Cleavage rate	95.8% (340/355)	97.6% (1426/1461)	0.059
Available rate	55.3% (188/340)	63.0% (899/1426)	0.008
High-quality embryos rate	34.7% (117/337)	43.9% (610/1389)	0.002
No. of embryos transfer	1.89±0.31	1.91±0.28	0.656
Implantation rate	25.9% (28/108)	32.6% (149/457)	0.178
Clinical pregnancy rate	40.4% (23/57)	45.7% (111/243)	0.466
Miscarriage rate	34.8% (8/23)	11.7% (13/111)	0.006
Cumulative live birth rate	33.3% (15/45)	59.8% (98/164)	0.002
Total deliveries (Singleton + Twins)	15 (10+5)	98(66+32)	
Birth defect	None	None	

Data are presented as mean (M) ± standard deviation (SD). Cumulative live birth rate included all live births. Non-pregnant patients were not counted before utilization of all fresh and frozen embryos.

phologic parameters. Embryo transfers were performed on Day 3 using a Wallace catheter. Clinical pregnancy was confirmed by vaginal ultrasound 30 days after embryo transfer showing the presence of an intrauterine gestational sac. The status of newborns was investigated by hospital record reviews and patient telephone interviews.

Statistical analysis

The Statistics Package for Social Sciences (SPSS, Chicago, IL, USA) was applied for statistical analysis. Data of continuous variables were presented as means (M) ± standard deviation (SD) and Student's *t*-test was used to assess the differences between the two groups. The ANOVA test was appropriately used for analyzing the differences among more than two groups. Chi-squared test or Fisher's exact test was used for analyzing categorical variables represented as frequency and percentage. Values of $P<0.05$ were considered to be statistically significant.

Results

Sperm with obvious head shape defect affects embryo development and pregnancy outcome

There is no significant difference between the above two groups in female age, male age, mean number of oocytes, injected oocytes per cycle and mean number of embryos per transfer (**Table 1**). The main parameters of embryo development were correlated with sperm head morphology other than cleavage rate. As shown in **Table 1**, sperm with obvious head shape defect resulted in a lower fertilization rate (including normal fertilization rate), availability rate and high-quality embryo rate, compared with sperm with good looking head shape (66.9% vs. 74.1%; $P=0.001$ (70.4% vs. 78.0%; $P<0.001$), 55.3% vs. 63.0%; $P=0.008$, 34.7% vs. 43.9%; $P=0.002$, respectively).

In Group 1, there was a slightly but insignificantly lower implantation rate compared with Group 2 (25.9% vs. 32.6%; $P=0.178$). A similar result was observed in clinical pregnancy rate (40.4% vs. 45.7%; $P=0.466$) (**Table 1**). However, the miscarriage rate in Group 1 was sharply higher than Group 2 (34.8% vs. 11.7%; $P=0.006$). As shown in **Table 1**, cumulative live birth rate in Group 2 was excellent (59.8%), but the data in Group 1 was nearly 50% lower than Group 2 (33.3% vs. 59.8%; $P=0.002$). In total 150 live births (76 Singleton and 37 Twins) in the two groups were delivered, among which no birth defects were reported to the clinic. Follow-up beyond the neonatal period was not conducted.

Table 2. Evaluation of fresh versus frozen-thawed sperm on embryo development and pregnancy outcome between obvious head shape defect sperm (Group 1) and good looking head shape sperm (Group 2)

	Group 1 (n=45)		Group 2 (n=166)	
	Fresh (n=32)	Frozen-thawed (n=13)	Fresh (n=92)	Frozen-thawed (n=74)
Age (female)	27.78±2.88	30±3.44	28.55±3.53	28.73±3.97
Age (male)	29.31±4.00	33.92±5.69	31.08±6.06	30.73±5.14
No. of oocytes	13.50±10.59	15.54±9.95	13.77±6.46	14.03±7.40
No. of oocytes injected	10.72±7.46	12.38±8.07	11.11±5.73	11.51±6.67
Normal Fertilization rate	64.1% (220/343)	72.7% (117/161)	73.3% (749/1022)[a]	75.1% (640/852)
Fertilization rate	66.5% (228/343)	78.9% (127/161)	77.0% (787/1022)[a]	79.1% (674/852)
Cleavage rate	96.5% (220/228)	94.5% (120/127)	96.4% (759/787)	99% (667/674)[b,c]
Available rate	54.5% (120/220)	56.7% (68/120)	64.4% (489/759)[a]	61.5% (410/667)
High-quality embryos rate	34.1% (75/220)	35.9% (42/117)	47.8% (358/749)[a]	39.4% (252/640)
No. of embryos transfer	1.90±0.31	1.89+0.32	1.92±0.28	1.90±0.30
Implantation rate	25.6% (19/74)	26.5% (9/34)	30.5% (83/272)	35.8% (69/193)
Clinical pregnancy rate	38.5% (15/39)	44.4% (8/18)	43.0% (61/142)	49.5% (50/101)
Miscarriage rate	33.3% (5/15)	37.5% (3/8)	13.1% (8/61)	9.8% (5/51)[b]
Cumulative live birth rate	31.3% (10/32)	38.5% (5/13)	58.2% (53/91)[a]	61.6% (45/73)
Total deliveries (Singleton + Twins)	10 (6+4)	5 (4+1)	53 (37+16)	45 (29+16)

Statistical analysis was used to analyze the differences in comparison with fresh versus frozen-thawed sperm of Group 1 (panel 1 and panel 2) and Group 2 (panel 3 and panel 4) or Group 1 versus Group 2 of fresh (panel 1 and panel 3) and frozen-thawed sperm (panel 2 and panel 4). No comparisons were made among other groups for statistical analysis. Data are presented as mean (M) ± standard deviation (SD). [a]P<0.05, compared with panel 1 (n=32); [b]P<0.05, compared with panel 2 (n=13); [c]P<0.05, compared with panel 3 (n=92). Cumulative live birth rate included all live births. Non-pregnant patients were not counted before utilization of all fresh and frozen embryos.

These results suggested that sperm with obvious head shape defect did affect embryo development. Although clinical pregnancy rate seemed not to be affected by these sperm, they greatly increased miscarriage rate and decreased cumulative live birth rate.

The effect of the head shape defects on sperm cryopreservation in pregnancy outcome and embryo development

Female age, male age, mean number of oocytes, injected oocytes per cycle and mean number of embryos per transfer were not significantly different between fresh and frozen-thawed sperm groups (**Table 2**). In the group of head shape defect sperm, the main parameters of embryo development were not correlated with fresh versus frozen-thawed sperm. Implantation rate was similar (25.6% vs. 26.5%; *P*=0.930) between the two sub-sets. Moreover, there was no significant difference in clinical pregnancy rate (38.5% vs. 44.4%; *P*=0.669), miscarriage rate (33.3% vs. 37.5%; *P*=0.842) and cumulative live birth rate (31.3% vs. 38.5%; *P*=0.642) (**Table 2**, left two panels). In the group of good looking head shape sperm, the main parameters except cleavage rate of embryo development were not associated with fresh versus frozen-thawed sperm. Implantation rate (30.5% vs. 35.8%; *P*=0.236), clinical pregnancy rate (43.0% vs. 49.5%; *P*=0.313), miscarriage rate (13.1% vs. 9.8%; *P*=0.586) and cumulative live birth rate (58.2% vs. 61.6%; *P*=0.659) were not altered by the cryopreservation procedure (**Table 2**, right two panels).

When focusing on the two fresh sub-sets (**Table 2**, panel 1 and 3), the group with sperm with obvious head shape defect was observed with lower fertilization rate (including normal fertilization rate), availability rate and high quality embryo rate compared with the group with good looking head shape sperm (64.1% vs. 73.3%; *P*=0.001 (66.5% vs. 77.0%; *P*<0.001), 54.5% vs. 64.4%; *P*=0.008, 34.1% vs. 47.8%; *P*<0.001, respectively). Although obvious head shape defect sperm did not negatively affect clinical pregnancy rate and miscarriage rate (38.5% vs. 43.0%; *P*=0.614, 33.3% vs. 13.1%;

P=0.062, respectively), we did find that cumulative live birth rate in fresh sperm sub-set of Group 1 was significantly lower than that in Group 2 (31.3% vs. 58.2%; *P*=0.009). When comparing the two frozen-thawed sub-sets (**Table 2**, panel 2 and 4), the key parameters except cleavage rate of embryo development were not related to sperm head morphology. There were slightly but insignificantly lower implantation rates (26.5% vs. 35.8%; *P*=0.293), clinical pregnancy rates (44.4% vs. 49.5%; *P*=0.692) and cumulative live birth rates (38.5% vs. 61.6%; *P*=0.119) in sub-set of frozen-thawed in Group 1 compared with those in Group 2. However, miscarriage rate in the sub-set of frozen-thawed in Group 1 was significantly higher than that in Group 2 (37.5% vs. 9.8%; *P*=0.033).

Overall, these results showed that the differences between embryo development and pregnancy outcomes with fresh versus frozen-thawed sperm were not found to be significant in the obvious head shape defect sperm group, and good looking head shape sperm group. However, poor embryo development and pregnancy outcomes were found in the obvious head shape defect sperm group in comparison with the good looking head shape sperm group regardless of using fresh or frozen-thawed sperm.

The embryo developmental potentiality and pregnancy outcome in OAT and OA/NOA patients who underwent ICSI with head shape defective sperm

Female age, male age, mean number of oocytes, injected oocytes per cycle and mean number of embryos per transfer were not significantly different among three OAT/OA/NOA groups (**Table 3**). In the OAT group, the main parameters except cleavage rate of embryo development were correlated with sperm head morphology. As shown in **Table 3** (left two panels), the obvious head shape defect sperm group was observed with a significantly lower fertilization rate (including normal fertilization rate), availability rate and high quality embryo rate compared with the good looking head shape sperm group (64.8% vs. 70.5%; *P*=0.039 (68.6% vs. 74.1%; *P*=0.039), 56.3% vs. 65.8%; *P*=0.006, 36.8% vs. 46.6%; *P*=0.007, respectively). There were slightly but insignificantly lower implantation rates (26.5% vs. 35.4%; *P*=0.137) and clinical pregnancies (39.5% vs. 50.0%; *P*=0.237) between the two sub-sets. However, miscarriage rates in the sub-set of OAT in Group 1 was significantly higher than that in Group 2 (35.3% vs. 13.1%; *P*=0.035). More than two times statistical difference in cumulative live birth rate was observed between the two sub-sets (30.6% vs. 65.4%; *P*<0.001).

In the OA group, the main parameters except available rate of embryo development were not found to be significantly different between the two sub-sets (**Table 3** middle two panels). The lower implantation rate (22.2% vs. 34.4%; *P*=0.304), clinical pregnancy rate (40.0% vs. 45.7%; *P*=0.734) and cumulative live birth rate (40.0% vs. 56.9%; *P*=0.469) were not significantly different in the two sub-sets. Despite the huge differences in values, there was no statistical significant in miscarriage rate (50.0% vs. 9.4%; P=0.084) in the two sub-sets.

In the NOA group, there was a significantly lower cleavage rate (87.0% vs. 98.6%; *P*<0.001) in the obvious head shape defect sperm group in comparison with the good looking head shape sperm group (**Table 3**, right two panels). The outcomes of clinical pregnancy had no potent variety.

The parameters of embryo development were mostly different in the three OAT/OA/NOA sub-sets of obvious head shape defective sperm group (**Table 3**, panel 1, 3 and 5) and good looking head shape sperm group (**Table 3**, panel 2, 4 and 6). All clinical outcome parameters were not found to be significantly different.

Taken together, these results showed that the obvious head shape defective sperm in the OAT groups did affect embryo development and clinical outcome, which led to higher miscarriage rates and lower cumulative live birth rates. In the OA/NOA group, obvious head shape defect sperm seemed not to affect embryo development and clinical outcomes. While embryo development was apparently divergent in the groups of OAT, OA and NOA, the similar clinical outcomes were observed between the obvious head shape defective sperm group and the good looking head shape sperm group.

Table 3. Comparison of sperm with (Group 2) and without good looking head shape (Group 1) on embryo development and pregnancy outcome in the cases of OAT, OA and NOA etiologies

	OAT		OA		NOA	
	Group 1 (n=36)	Group 2 (n=82)	Group 1 (n=5)	Group 2 (n=52)	Group 1 (n=4)	Group 2 (n=32)
Age (female)	28.31±3.25	28.66±3.27	28±2.74	28.77±3.74	30±3.37	28.34±4.77
Age (male)	30.11±4.73	31.48±5.86	32.4±3.21	30.56±5.55	33.25±8.26	30.09±5.27
No. of oocytes	14.03±10.69	14.73±7.28	18.60±9.40	13.58±5.97	9.00±6.93	12.22±7.03
No. of oocytes injected	11.06±7.79	11.95±6.47	15.00±7.25	10.60±5.30	7.75±5.12	10.72±6.58
Normal fertilization rate	64.8% (258/398)	70.5% (691/980)[a]	76.0% (57/75)	79.1% (436/551)[b]	71.0% (22/31)	76.4% (262/343)[b]
Fertilization rate	68.6% (273/398)	74.1% (726/980)[a]	78.7% (59/75)	82.8% (456/551)[b]	74.2% (23/31)	81.3% (279/343)[b]
Cleavage rate	96.3% (263/273)	97.0% (704/726)	96.6% (57/59)	96.6% (447/456)	87.0% (20/23)[a]	98.6% (275/279)[e]
Available rate	56.3% (148/263)	65.8% (463/704)[a]	42.1% (24/57)	56.6% (253/447)[b,c]	80.0% (16/20)[a,c]	66.5% (183/275)[d]
High-quality embryos rate	36.8% (95/258)	46.6% (322/691)[a]	24.6% (14/57)	36.7% (160/436)[b]	36.4% (8/22)	48.9% (128/262)[d]
No. of embryos transfer	1.93±0.26	1.94±0.23	1.80+0.42	1.86±0.35	1.75+0.50	1.90±0.30
Implantation rate	26.5% (22/83)	35.4% (84/237)	22.2% (4/18)	34.4% (45/131)	28.6% (2/7)	23.7% (23/97)[b]
Clinical pregnancy rate	39.5% (17/43)	50.0% (61/122)	40.0% (4/10)	45.7% (32/70)	50.0% (2/4)	35.3% (18/51)
Miscarriage rate	35.3% (6/17)	13.1% (8/61)[a]	50.0% (2/4)	9.4% (3/32)	0% (0/2)	11.1% (2/18)
Cumulative live birth rate	30.6% (11/36)	65.4% (53/81)[a]	40.0% (2/5)	56.9% (29/51)	50.0% (2/4)	50.0% (16/32)
Total deliveries (Singleton + Twins)	11 (6+5)	53 (34+19)	2 (2+0)	29 (21+8)	2 (2+0)	16 (11+5)

Statistical analysis was used to analyze the differences in comparison with Group 1 versus Group 2 of OAT (panel 1 and panel 2), OA (panel 3 and panel 4) and NOA (panel 5 and panel 6) or three etiologies of Group 1 (panel 1, panel 3 and panel 5) and Group 2 (panel 2, panel 4 and panel 6). No comparisons were made among other groups for statistical analysis. Data are presented as mean (M) ± standard deviation (SD). [a]P<0.05, compared with panel 1 (n=36); [b]P<0.05, compared with panel 2 (n=82); [c]P<0.05, compared with panel 3 (n=5); [d]P<0.05, compared with panel 4 (n=52); [e]P<0.05, compared with panel 5 (n=4). Cumulative live birth rate included all live birth. Non-pregnant patients were not counted before utilization of all fresh and frozen embryos.

Discussion

The relationship between sperm morphology and IVF outcome has been a research hot spot in the last two decades. A meta-analysis of sperm morphology and IVF clinical outcomes in 49 different studies using conventional IVF concluded that sperm morphology assessment may be valuable as a diagnostic reference in evaluating male infertility [11]. This, however, is still an area of considerable debate. Numerous other reports suggest that abnormal morphology is not an accurate predictor for IVF clinical outcomes [6-10]. There are several potential limitations when considering the value of sperm morphology to predict pregnancy outcome. First, the embryologist can microscopically select individual sperm that appear morphologically "normal" from even the most severely impaired specimens (Kruger strict morphology of zero) when preform the ICSI. Thus, the sperm for fertilization may not be representative of the whole sperm population, making the initial semen morphology assessment irrelevant. Then, sperm morphology in many studies is assessed strictly in outpatient examination before performing IVF. It is possible that the semen sample used on the actual day of fertilization is not consistent with the sample assessed before, and even the results of morphological assessment may be totally opposed to the sperm used on the day of fertilization.

It is well-taken that poor sperm morphology leads to infertility. Emphasis is therefore given to the shape of sperm head, although the sperm neck, midpiece and tail defects are also considered. With the development of ICSI, a method to overcome this problem of poor morphology which has been established by simply selecting only normal head shaped sperm and injecting it into each egg. However, no motile sperm with "good looking head shape" are occasionally found in some extremely rare sperm samples (total sperm count was less than hundred, thus not enough for morphological assessment). After informing the patient and obtaining the consent, the embryologists select the sperm with the head shape looking a little better among all the sperm with obvious head shape defect. The aim of this study is to investigate the embryo development and pregnancy outcome using the little bit of teratospermia if there is no other normal sperm that could be available. Results show that obvious head shape defective sperm does affect embryo development. It also greatly increases miscarriage rate and decreases cumulative live birth rate compared with good looking head shape sperm, although clinical pregnancy rate seems not to be affected (**Table 1**).

A direct link between abnormal sperm morphology and sperm function is hard to establish. Exploring the relationship between sperm morphology and sperm function, DNA damage and chromosomal status may help understand the prognostic value of strict morphology assessment. Data from several studies suggest that abnormal sperm morphology does not necessarily represent abnormal chromosomal content [12, 13]. In addition, 10% of sperms with disomic nuclei were categorized as normal by strict morphology using fluorescent in situ hybridization [14]. Sperm morphology defects may act more as a barrier to keep sperm from reaching and penetrating the oocyte zona. Sperm DNA fragmentation may affect clinical outcome and embryonic development [15, 16]. However, no clear relationship has been shown between sperm morphologic attributes and degree of DNA damage [17, 18]. Although the sperm is combined with the oocyte by "man-made" ICSI, the surface potential morphological defects such as ultrastructural abnormalities and the internal functional DNA deletion are too difficult to be assessed and improved, which affects the embryo development and even the final clinical outcome. This concept has been applied to the subcellular level in new techniques such as intracytoplasmic morphologically selected sperm injection and motile sperm organellar morphology examination (MSOME). In these techniques, investigators select sperm for ICSI using a high power 6,000 objective to magnify and make more critical assessment of sperm morphology. Sperm nucleus morphology assessed by the MSOME is positively correlated with fertilization and pregnancy rates [19]. The number of cases with these new techniques, however, is too small to draw definitive conclusions regarding efficacy and potential of increasing pregnancy rates [20, 21].

Furthermore, many studies excluded the subgroup of men with rare sperm samples, owing to logistical problems performing morphology assessments on a small number of spermatozoa [6]. In fact, teratozoospermia combined

with severe oligozoospermia may potentially affect ICSI outcomes by reducing the likelihood of locating a "normal" sperm for injection. The type of sperm defect may impact fertilization and subsequent pregnancy outcomes. Defects of the sperm head, for example, may portend a worse prognosis than midpiece or tail defects. Generally, sperm with a normal head is preferred to be injected. Studies have demonstrated normal fertilization and embryo development in humans depend on round-headed acrosomeless sperm in ICSI [22, 23]. Different types of head shape defect sperm (large or small, tapered, pyriform, round, amorphous head defects, etc.) also may affect embryo development and pregnancy outcome. The type of sperm defect seen is not classified in the present study. Further, the degree of sperm abnormalities is not mentioned either in the present study due to the shortage of standards and codes.

The controversy over the use of fresh versus frozen-thawed sperm for ICSI has been ongoing for nearly two decades. Some embryology labs hesitate to use cryopreservation for testis sperm in cases of spermatogenic dysfunction in earlier times [24]. Concerns include whether the use of cryopreserved sperm would increase the risk of IVF failure and what effect cryopreservation has on the viability and fertilization capacity of sperm. Some investigators are concerned that cryopreserved sperm is inferior to fresh sperm owing to the destructive nature of cryopreservation and inferior clinical outcomes. Studies demonstrated cryo-injury at various points in the freeze-thaw process [25]. Cryopreservation can result in rupture of the acrosomal and plasma membranes and free radical injury that can jeopardize DNA integrity [26, 27]. For a man with azoospermia due to spermatogenic dysfunction, the role of cryo-injury becomes more significant because only few sperm can be retrieved. Whether such cryo-injury could affect clinical pregnancy outcome holds a great significance. After some initial skepticism, it has become evident that pregnancy outcomes achieved with ICSI using fresh or frozen-thawed spermatozoa retrieved from men with OA are comparable to all types of azoospermia [5, 28]. Another study investigated the factors influencing ICSI outcome in men with azoospermia, and it appeared that male age, cause of azoospermia, testicular histopathology, type of sperm used, and fresh vs. frozen sperm did not make a significant difference once normal sperm was found [29]. In our present study, embryo development and pregnancy outcome with fresh versus frozen-thawed sperm were not found to be significantly different regardless of sperm morphology (**Table 2**). However, poor embryo development and pregnancy outcome were detected in the obvious head shape defective sperm group rather than the good looking head shape sperm group (**Table 2**).

It has been demonstrated that testicular sperm retrieved from patients with OA and NOA can be equivalent to ejaculated sperm in the fertilization, pregnancy and implantation rates after ICSI [30-32]. Another study also showed that outcomes with sperm retrieved from patients with OA and NOA were comparable to using ejaculated sperm in ICSI [33]. In the present study, no significant difference in cumulative live birth rate was observed between obvious head shape defect sperm and good looking head shape sperm when ejaculated or testicular sperm was used for ICSI, while embryo development was mostly significantly different in OAT, OA and NOA patients (**Table 3**). There were still inconsistent viewpoints about embryo quality and clinical outcome from different origins of spermatozoa for ICSI. Low fertilization and cleavage rates were also observed in testicular sperm from OA and NOA patients, significantly impairing embryo quality and blastocyst formation. A possible explanation for this may be that testicular spermatozoa are less mature and subsequently less competent to fertilize than the ejaculated ones [34, 35]. Moreover, ICSI with testicular spermatozoa is less successful in men with NOA than with OA [35]. Another study showed that pregnancy and implantation rates after ICSI in OA and NOA were significantly higher than those with ejaculated sperm in OAT [36], while fertilization rate, cleavage rate and embryo quality were not found significantly different. Furthermore, researchers investigated the percentage of DNA fragmentation between testicular and ejaculated sperm, and compared the ICSI outcomes in two sequential attempts. Interestingly, testicular sperm had a lower incidence of DNA fragmentation than ejaculated sperm. There is still an ongoing debate as to whether different the origins of sperm can influence embryo development and clinical outcome. However, it was clear that the obvious head shape defec-

tive sperm in OAT groups did affect cumulative live birth rate compared with the good looking head shape sperm. No significant difference was found in the OA/NOA group due to the number of cases being not sufficient (**Table 3**).

Despite it was confirmed the birth defects of infants conceived by ICSI were not significantly different from those with traditional IVF [37, 38], the risk of birth defects in neonates conceived by ICSI with obvious head shape defect sperm were still not fully understood. It is reassuring to note that there was no infant with birth defects detected among the 150 live births in the present study (**Table 1**), however, long-term follow-up at multiple IVF centers and a larger live birth data set will be needed to fully analyze the rate of birth defects among children conceived by ICSI with obvious head shape defect sperm.

In conclusion, microscopic selection of sperm with good looking head shape during the ICSI procedure showed about 60% cumulative live birth rate even in extremely rare sperm samples after excluding female infertility factors in this study. However, no motile sperm with good looking head shape were found in all specimens when ICSI was performed in some cases, which made embryologists have no choice but to inject obvious abnormal head shape sperm in ICSI after informing the patients who refused to use the donor sperm, and obtaining their consent. Lower fertilization rate, availability rate and high-quality embryo rate were observed in the obvious abnormal head shape sperm group. Although clinical pregnancy rate seemed not to be affected by this sperm, abnormal head shape sperm greatly increased miscarriage rate and decreased cumulative live birth rate (33.3%). Using fresh or frozen-thawed sperm results in similar embryo development and clinical outcomes in obvious abnormal head shape sperm and good looking head shape sperm groups, however, poor embryo development and clinical outcomes were found in the obvious head shape defect sperm group regardless of using fresh or frozen-thawed sperm. While embryo development was apparently divergent, similar clinical outcomes were observed in the OAT/OA/NOA groups. Sperm with obvious head shape defect in the OAT groups did associate with poor embryo development and clinical outcome, whereas in the OA/NOA group it did not. All above results may supply a reference for clinical outcome when using these severe teratozoospermia associated with extreme oligospermia samples.

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Disclosure of conflict interest

None.

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