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ORIGINAL ARTICLE

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Influence of sperm morphology on pregnancy outcome and offspring in *in vitro* fertilization and intracytoplasmic sperm injection: a matched case-control study

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Sperm morphology was once believed as one of the most predictive indicators of pregnancy outcome in assisted reproductive technology (ART). However, the impact of teratozoospermia on *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) outcomes and its offspring remains inconclusive. In order to evaluate the influence of teratozoospermia on pregnancy outcome and newborn status after IVF and ICSI, a retrospective study was conducted. This was a matched case-control study that included 2202 IVF cycles and 2574 ICSI cycles and was conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya in Changsha, China, from June 2013 to June 2018. Patients were divided into two groups based on sperm morphology: teratozoospermia and normal sperm group. The pregnancy outcome and newborn outcome were analyzed. The results indicated that couples with teratozoospermia had a significantly lower optimal embryo rate compared to those with normal sperm morphology in IVF ($P = 0.007$), while there were no statistically significant differences between the two groups in terms of the fertilization rate, cleavage rate, implantation rate, and pregnancy rate (all $P > 0.05$). Additionally, teratozoospermia was associated with lower infant birth weight in multiple births after IVF. With regard to ICSI, there was no significant difference in both pregnancy outcome and newborn outcome between the teratozoospermia and normal groups (both $P > 0.05$). Furthermore, no increase in the risk of birth defects occurred in the teratozoospermia group after IVF/ICSI. Consequently, we believe that teratozoospermia has limited predictive value for pregnancy outcomes in IVF/ICSI, and has little impact on the resulting offspring if multiple pregnancy is avoided.

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INTRODUCTION

Infertility is defined as the failure to conceive after 12 months of unprotected sexual intercourse. It is a major public health problem, and the World Health Organization (WHO) reports that approximately 10%–15% of couples are subfertile, and a male factor is involved in about half of these couples.[1] For infertile men without sexual dysfunction, the diagnosis, for which morphological evaluation is an integral part, is mainly based on abnormal semen parameters, and the diagnosis would provide valuable information about male fertility and clinical management.[2]

In the early 1990s, researchers considered sperm morphology as the most significant indicator of subfertility,[3] and revealed the relationship between teratozoospermia and poorer fertilization outcomes during *in vitro* fertilization (IVF).[4] Subsequent studies validated a worse outcome for those patients, including impaired embryonic development, lower potential for implantation, and lower clinical pregnancy rate.[5,6] Thus, sperm morphology was once believed to be one of the most informative indices for discriminating between fertile and infertile males as well as for predicting the pregnancy rate associated with assisted reproductive technology (ART). However, its clinical utility is currently under reconsideration because studies have shown controversial results of pregnancy outcomes in patients with teratozoospermia undergoing ART. Evidence from recent researches have suggested that there was no difference in the IVF/intracytoplasmic sperm injection (ICSI) outcome among men with teratozoospermia.[7,8] A meta-analysis concluded that isolated teratozoospermia was not associated with decreased clinical pregnancy rates with IVF/ICSI.[9] Thus far, the role of sperm morphology in IVF remains open to debate.

For patients with extremely impaired semen parameters or previous IVF failure, the application of ICSI can result in better clinical outcome. This technique neutralizes the impact of male factor, but also diminishes the relevance of sperm morphology to fertility. Numerous studies have suggested that teratozoospermia patients exhibit similar fertilization and pregnancy rates as males with normal sperm morphology.[10,11] However, other researchers argued that abnormal sperm morphology can adversely affect the developmental capacity of embryos during ICSI, resulting in diminished blastocyst development and quality.[12] In addition, the invasive procedure and

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the bypass of fertilization leads to an increased risk of malformations and chromosomal abnormalities in ICSI-derived embryos, eventually causing birth defects in offspring.[13] Therefore, the clinical management of teratozoospermia is usually empirical, and there is no consensus on the influence of sperm morphology in ART procedure and the optimal treatment for such patients.

Consequently, considering the uncertain role of sperm morphology in ART, we attempted to evaluate the impact of teratozoospermia on pregnancy outcomes and newborn status after IVF/ICSI, as well as to assess the risks associated with IVF/ICSI, in order to gain a better understanding of the influence of sperm morphology in ART.

PATIENTS AND METHODS

Patients

The study was approved by the Medical Ethics Committee of the Reproductive and Genetic Hospital of CITIC-Xiangya in Changsha, China (approval number: LL-SC-2019-014), and all patients gave informed consent. The initial sample included 9313 cycles of IVF (734 cycles of teratozoospermia and 8579 cycles of normal sperm) and 2960 cycles of ICSI (1673 cycles of teratozoospermia and 1287 cycles of normal sperm), which had been performed at the Reproductive and Genetic Hospital of CITIC-Xiangya from June 2013 to June 2018. Using the analytical method propensity score matching (PSM), the number of IVF/ICSI cycles was determined using predefined variables, including female and male age; sex hormone levels such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E2), anti-Müllerian hormone (AMH); and female body mass index (BMI). The IVF cycles had a 1:2 match, whereas the ICSI cycles had a 1:1 match. As a consequence, this matched case-control study finally included 2202 IVF cycles (734 cycles of teratozoospermia and 1468 cycles of normal sperm) and 2574 ICSI cycles (1287 cycles of teratozoospermia and 1287 cycles of normal sperm). Couples who underwent their first and/or second IVF/ICSI cycle due to teratozoospermia or isolated female tubal infertility were selected for this study. Teratozoospermia was defined as <4% sperm with normal morphology according to the WHO strict criteria. Patients were divided into a teratozoospermia group and a normal sperm group based on the sperm morphology.

The inclusion criteria were as follows: (1) the teratozoospermia group comprised patients with isolated teratozoospermia and teratozoospermia accompanied by mild asthenospermia and oligospermia, whereas patients in the normal sperm group comprised cases of female tubal infertility without any other pathological conditions and male factors; (2) couples with a normal chromosome karyotype; (3) female patients < 35 years of age and the ovarian stimulation protocol was varied with the individuals; and (4) female patients in whom at least four oocytes had been retrieved. Exclusion criteria for this study were cases in which sperm were collected with testicular sperm aspiration and microepididymal sperm aspiration (MESA) and the presence of gynecological diseases such as polycystic ovarian syndrome (PCOS), endometriosis, intrauterine adhesion, and recurrent abortion; infectious diseases; malignancies; and autoimmune diseases.

Semen analysis

Semen samples were collected by masturbation after 3–5 days of sexual abstinence, and analyzed according to the WHO guidelines 5th edition.[14] After complete liquefaction of the ejaculated semen specimen at 37°C, ejaculated semen volume was determined by weighing, and the sperm concentration and motility were measured in a Makler Counting Chamber (Sefi Medical Instruments, Haifa, Israel), with a magnification of ×200 (Olympus BX43, Tokyo, Japan). Briefly, at least 200 sperm were counted with one replicate, and the average results were documented. Sperm morphology was assessed using the modified Papanicolaou staining method using WHO 5th edition criteria, which has a cutoff of 4% for normal.[14] At least 200 sperm were evaluated, and sperm morphology was verified by averaging the results from two different technicians. All the technicians were well trained and participated in an internal quality control (QC) procedure for standard morphological assessment of sperm.

IVF/ICSI procedure

After the oocytes had been retrieved, cumulus-oocyte complexes (COCs) were identified and collected in 1 ml of G-IVF medium (Vitrolife, Gothenburg, Sweden), and cultured for 3–6 h to induce the full maturation of oocytes. High-quality motile sperm were enriched using a swim-up method or a 45%–90%-discontinuous gradient method with G-IVF™ PLUS (Vitrolife). The oocytes and at least 1×10^5 motile sperm were co-incubated in 1 ml of G-IVF culture medium containing 10% human serum albumin (Vitrolife). After an additional 16–18 h, the oocytes were examined for the presence of a second polar body to confirm fertilization.

Before micromanipulation of ICSI, sperm were prewashed and transferred to a conical test tube for incubation at 37°C for at least 1 h. An ICSI dish (Falcon, New York, NY, USA) containing ten 5-μl microdrops of G-MOPS handling medium and 3 PVP (Vitrolife) microdrops was prepared, and the dish was covered with 4 ml of paraffin oil (Vitrolife). The COCs were briefly rinsed in 80 IU ml^{-1} hyaluronidase (Vitrolife), and the cumulus cells were removed by repeated gentle aspiration. Denuded oocytes were washed and incubated in fresh G-IVF medium (Vitrolife). If motile spermatozoa were found, the denuded oocytes were transferred to blank microdrops, and ICSI manipulation was performed according to standard protocol.[15] To ensure minimal manipulation exposure (<15 min per dish), no more than four mature oocytes were placed per ICSI dish during manipulation. The injected oocytes were cultured in G-IVF medium overnight before checking whether they were fertilized.

Assessment of fertilization and embryo quality

Fertilization was assessed 16–18 h after insemination or microinjection, and the cleavage stage and embryo morphology were documented prior to the embryo transfer. All the embryos were transferred into fresh G1.5 medium (Vitrolife), and cultured at 37°C in air containing 6% CO_2 and 5% O_2 with 95% humidity. On day 3 (66–68 h postinsemination), embryo morphology was assessed. Normal fertilization was defined as the formation of two pronuclei (2PN) and second polar body extrusion. Abnormally fertilized oocytes exhibiting 1PN or 3PN were excluded. The embryo quality was evaluated according to the number and size of the blastomere and the percentage of anucleate fragments. High-quality embryos were selected for subsequent transfer.

Outcome measures

Laboratory indices, including the fertilization rate, cleavage rate, optimal embryo rate, and implantation rate, were documented. A clinical pregnancy was diagnosed based on normal intrauterine pregnancy by detecting the fetal heartbeat activity at 28–35 days postretrieval. In addition, maternal and fetal outcomes were assessed, including pregnancy complications, newborn status, as well as major birth defects. Infant birth weight was measured after delivery, and any abnormalities were documented (low birth weight <2500 g; extreme low birth weight <1500 g; fetal macrosomia >4000 g). Birth defects were also noted at the time of delivery. Before the child turned one year

of age, a routine follow-up examination was conducted by contacting the couples via phone to obtain neonatal information, mainly for birth defects and neonatal diseases.

Statistical analyses

Statistical analysis was performed using SPSS software 23.0 (IBM Corp, New York, NY, USA). PSM analysis was conducted with SPSS software 23.0 and STATA software 15.0 (Stata Corp, College Station, TX, USA). Data were presented as the mean ± standard deviation (s.d.) and percentage. Difference between the teratozoospermia and normal sperm groups was determined by independent Student's *t*-test, Chi-square test, and Fisher's exact test. A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Teratozoospermia and IVF outcome

To analyze the impact of teratozoospermia on IVF outcome, a total of 2202 cycles were included, of which 734 cycles were from the teratozoospermia group and 1468 cycles from the normal sperm group. Baseline information of the patients, such as age, infertility duration, serum sex hormone, and retrieved oocytes, was comparable between the two groups (all $P > 0.05$; **Table 1**). There was a significant difference between the two groups in terms of the source of sperm: frozen semen usage was higher in the teratozoospermia group than that in the normal sperm group, but prewashed semen volume and total motile sperm count did not significantly differ between the two groups (both $P > 0.05$).

The teratozoospermia group showed slightly decreased fertilization rate and normal fertilization rate after IVF; however, there was no statistical difference compared to the normal sperm group (both $P > 0.05$; **Table 2**). It is important to note that a significantly lower optimal embryo rate was observed in couples with teratozoospermia than in those with normal sperm morphology (57.86% *vs* 59.98%, $P < 0.05$). Furthermore, despite the slightly lower pregnancy rate and higher abnormal pregnancy rate, including abortion and heterotopic pregnancy, in the teratozoospermia group, the observed differences had no statistical significance (all $P > 0.05$). With regard to the maternal outcome, we assessed four different pregnancy complications including gestational diabetes, gestational hypertension, gestational edema, and gestational anemia. The likelihood of developing pregnancy complications was slightly higher in the teratozoospermia group than that in the normal sperm group, of which only the incidence of gestational hypertension increased significantly (4.04% *vs* 1.95%, $P < 0.05$).

To further evaluate the influence of sperm morphology on the neonatal outcome in IVF, we divided neonates into the following three subgroups: singleton, twin, and triplet births according to the number of babies born (couples were informed of the potential risks, and a small percentage of IVF pregnancies still resulted in triplet birth). The results revealed that when singletons and twins were delivered, there were no statistical difference in terms of baby gender, weeks of gestation, birth weight, and premature birth rate between the teratozoospermia and normal groups (**Table 3**). However, teratozoospermia may be associated with adverse postnatal outcomes in triplet pregnancies, which can be manifested as lower birth weight (mean ± s.d.: 1033.33 ± 76.38 g *vs* 1958.33 ± 323.14 g, $P < 0.05$) and a higher rate of extreme low birth weight cases (3 of 3 *vs* 0 of 6, $P < 0.05$). In addition, the subgroup comparison in both single and multiple births showed no increased risk of birth defects in the teratozoospermia group compared to that of the normal group.

Teratozoospermia and ICSI outcome

To further determine whether sperm morphology affects the clinical outcome of ICSI, we analyzed 2574 cycles, including 1287 cycles with teratozoospermia and 1287 cycles with normal sperm morphology. The baseline data of the two groups were comparable (all $P > 0.05$; **Table 4**). The teratozoospermia group used more frozen semen than the normal sperm group, and the prewashed semen quality of the former was comparatively worse (both $P < 0.05$).

Table 1: Demographic characteristics of the two groups undergoing *in vitro* fertilization

Parameter	*Teratozoospermia group (n=734)*	*Normal sperm group (n=1468)*	*P*
Female age (year), mean±s.d.	29.74±3.37	29.54±3.29	0.109
Male age (year), mean±s.d.	33.57±4.01	33.24±3.78	0.078
Duration of infertility (year), mean±s.d.	3.97±2.57	4.10±2.79	0.287
Female BMI (kg m^{-2}), mean±s.d.	21.15±2.20	20.98±2.24	0.087
FSH (mIU ml^{-1}), mean±s.d.	6.55±2.36	6.38±1.93	0.065
LH (mIU ml^{-1}), mean±s.d.	4.21±3.87	4.24±2.44	0.800
E2 (pg ml^{-1}), mean±s.d.	39.96±28.73	37.75±19.77	0.062
AMH (ng ml^{-1}), mean±s.d.	4.70±3.46	4.62±3.17	0.623
Thickness of the endometrium (cm), mean±s.d.	12.48±2.18	12.60±2.28	0.240
Number of oocytes retrieved, mean±s.d.	11.81±5.63	11.51±5.96	0.708
Number of transplanted embryos, *n* (%)			
0	181 (24.66)	267 (18.19)	0.000*
1	40 (5.45)	64 (4.36)	0.256
2	510 (69.48)	1128 (76.84)	0.000*
3	3 (0.41)	9 (0.61)	0.539
Source of sperm, *n* (%)			
Frozen semen	109 (14.85)	54 (3.68)	0.000*
Fresh semen	625 (85.15)	1414 (96.32)	0.000*
Semen parameters after selection, mean±s.d.			
Volume (ml)	0.22±0.21	0.23±0.23	0.171
Concentration (10^6 ml^{-1})	20.31±4.22	20.85±9.88	0.157

*$P<0.05$. s.d.: standard deviation; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estrogen; AMH: anti-Müllerian hormone

Table 2: Pregnancy outcome of the two groups undergoing *in vitro* fertilization

Parameter	*Teratozoospermia group (n=734)*	*Normal sperm group (n=1648)*	*P*
Fertilization rate, % (*n*/total)	77.33 (5742/7425)	77.84 (13875/17826)	0.382
Normal fertilization rate, % (*n*/total)	66.39 (4930/7425)	67.39 (12013/17826)	0.126
Cleavage rate, % (*n*/total)	97.54 (5601/5742)	97.40 (13515/13875)	0.574
Rate of optimal embryos, % (*n*/total)	57.86 (3241/5601)	59.98 (8107/13515)	0.007*
Implantation rate, % (*n*/total)	52.69 (558/1059)	50.49 (1185/2347)	0.234
Pregnancy rate, % (*n*/total)	53.95 (396/734)	55.79 (819/1468)	0.413
Parturition rate, % (*n*/total)	46.59 (342/734)	47.89 (703/1468)	0.566
Abortion rate, % (*n*/total)	10.86 (43/396)	11.59 (95/819)	0.703
Rate of heterotopic pregnancy, % (*n*/total)	2.78 (11/396)	2.19 (18/819)	0.535
Number of babies born, % (*n*/total)			
1	66.67 (228/342)	61.74 (434/703)	0.121
2	33.04 (113/342)	37.98 (267/703)	0.119
3	0.29 (1/342)	0.28 (2/703)	0.982
Pregnancy complications, % (*n*/total)			
Gestational diabetes	7.83 (31/396)	7.20 (59/819)	0.697
Gestational hypertension	4.04 (16/396)	1.95 (16/819)	0.033*
Gestational edema	16.92 (67/396)	14.77 (121/819)	0.333
Gestational anemia	9.09 (36/396)	8.18 (67/819)	0.593

*$P<0.05$

Unlike the impact of teratozoospermia on embryo quality in conventional IVF, teratozoospermia did not affect fertilization and embryo development in ICSI, owing to the selection of optimal sperm and the artificial fertilization step mediated through ICSI. As shown in **Table 5**, poor sperm morphology had no significant influence on pregnancy outcome, including the fertilization rate, optimal embryo rate, implantation rate, and pregnancy rate (all $P > 0.05$). Meanwhile, teratozoospermia was not associated with pregnancy complications after ICSI.

We assessed the role of sperm morphology in ICSI-associated newborn outcomes (**Table 6**). Only single and twin pregnancies were analyzed, as no triplet births occurred in the normal sperm group. When the teratozoospermia and normal sperm groups were compared, no statistically significant differences were found in the number of gestation weeks, infant birth weight, premature birth rate, or rate of birth defects (all $P > 0.05$).

DISCUSSION

Over the past few decades, there has been an overall decrease in semen quality, along with diminished male fertility.[16,17] Morphological evaluation of spermatozoa is an indispensable approach to determining semen quality. In the 5th edition of the WHO guidelines, the cutoff value of teratozoospermia diagnosis was reduced from 15% to 4%.[14] Since then, there has been an increase in the diagnosis of teratozoospermia as only few sperm with ideal shape could be defined as "normal."[18] Thus, there is an urgent need to determine whether abnormal sperm morphology would have adverse effects on the outcomes of IVF/ICSI treatment. However, the role of sperm morphology in ART outcome remains inconclusive. Considering the current controversy, we conducted this retrospective study to evaluate the impact of teratozoospermia on IVF/ICSI outcome.

To maximize the reliability of the results, we established strict selection criteria. Only couples who were undergoing their first and/or second cycle were included in this study. To minimize the influence of confounding variables from women, only couples with female tubal infertility were chosen as the control group. PSM was utilized to eliminate bias between the two groups. Furthermore, the well-trained technicians and strict internal QC procedures ensured the validity of sperm morphology assessment. In this retrospective study, the results offered more evidence that teratozoospermia can result in a significantly lower optimal embryo rate in IVF, but there was no impact on any of the other indices. This indicates that abnormal sperm morphology may be associated with impaired embryo development in IVF. Additionally, teratozoospermia was associated with lower infant birth weight in cases of multiple births after IVF. With regard to ICSI, no significant difference was observed in both pregnancy outcome and newborn outcome between the teratozoospermia and normal groups. Furthermore, there was no increase in the risk of birth defects in teratozoospermia after IVF/ICSI.

Abnormal sperm morphology can be an indicator of structural defects or dysfunction, including DNA damage, chromosomal abnormalities, and decreased motility. Thus, teratozoospermia has been implicated as the most valuable predictor of fertilization potential and pregnancy outcome in conventional IVF.[19] Unlike ICSI, IVF preserves the natural selection step of spermatozoa that occurs at the sperm–oocyte interface during penetration. Defects in sperm morphology can lead to impaired binding and penetration of the human zona pellucida.[20] Extensive research has been carried out on the role of teratozoospermia in IVF, and the majority of the results indicated that teratozoospermia can result in a lower potential of fertilization, implantation, and pregnancy.[5,21] Terriou *et al.*[22] point out that a poor pregnancy rate is mainly caused by a poor fertilization rate and the limited choice of qualified embryos for transfer. This means that sperm morphology is of importance during the fertilization stage of IVF but not during embryo development. Our results, however, were inconsistent with this finding. In this study, although lower fertilization and pregnancy rates occurred in the teratozoospermia group than that in the normal sperm group, the differences had no statistical significance; however, a significantly lower optimal embryo rate was observed in the teratozoospermia group. This finding indicated that sperm morphology defects may be associated with not only penetration but also embryonic development after fertilization. Sperm with normal acrosome function may still contain ultrastructure aberrations and dysfunction, adversely affecting embryo quality. However, the clinical

Table 3: Neonatal outcome of the two groups undergoing *in vitro* fertilization

Parameter	*Teratozoospermia group*				*Normal sperm group*			
	Singleton	*Twins*	*Triplets*	*P*	*Singleton*	*Twins*	*P*	*Triplets*	*P*
Number of babies born	228	226	3		434	534		6	
Gender of newborn, *n* (%)				0.553			0.860		1.000
Male	109 (47.81)	111 (49.12)	1 (33.33)		218 (50.23)	266 (49.81)		3 (50.00)	
Female	119 (52.19)	115 (50.88)	2 (66.67)		216 (49.77)	268 (50.19)		3 (50.00)	
Gestational age (week), mean±s.d.	38.93±1.64	36.70±1.65	29.40	0.342	39.05±1.47	36.76±1.64	0.736	35.15±1.63	0.212
Birth weight (g), mean±s.d.	3256.01±483.68	2492.35±462.66	1033.33±76.38	0.475	3285.49±512.22	2516.45±403.81	0.497	1958.33±323.14	0.001*
Rate of low birth weight, *n* (%)	9 (3.95)	94 (41.59)	3 (100)	0.902	18 (4.15)	220 (41.19)	0.920	6 (100)	NA
Rate of extreme low birth weight, *n* (%)	2 (0.88)	5 (2.21)	3 (100)	0.511	2 (0.46)	7 (1.31)	0.362	0	0.012*
Rate of fetal macrosomia, *n* (%)	7 (3.07)	0	0	0.101	26 (5.99)	0	NA	0	NA
Rate of premature births, *n* (%)	12 (5.26)	41 (36.28)	1 (100)	0.608	19 (4.38)	91 (34.08)	0.680	2 (100)	NA
Rate of birth defects, *n* (%)	8 (3.51)	6 (2.65)	0	0.720	13 (2.99)	20 (3.74)	0.450	0	NA

*$P<0.05$. s.d.: standard deviation; NA: not applicable

pregnancy rate remained unaffected after selecting "high-quality" embryos. Furthermore, we found that the incidence of gestational hypertension in teratozoospermia couples was twice as high as that in the control group. Although pregnancy complications are mainly related to maternal health, teratozoospermia-induced embryo defects may also contribute to the dysfunction of maternal-fetal interactions, resulting in maternal complications.

In contrast to the uncertain role of teratozoospermia in IVF, it is generally accepted that sperm morphology is not related to ICSI outcome because of the selection of optimal sperm and bypasses to both zona pellucida binding and penetration. Consequently, in patients who have experienced previous IVF failure or those with extremely severe teratozoospermia, ICSI is associated with higher fertilization and pregnancy rates.[23] However, inconsistencies remain: Miller and Smith[24] claimed that teratozoospermia may have a detrimental effect on blastocyst development in ICSI, which manifested as significantly more ICSI-derived embryos arrested at the 5- to 8-cell stage compared to that of conventional IVF. Nevertheless, in this study, no significant differences were observed in the ICSI pregnancy outcome and neonatal outcome between the teratozoospermia and normal sperm groups, suggesting that sperm morphology had little predictive value in the ICSI cycle. It is noteworthy that the teratozoospermia group used more frozen semen, and the prewashed semen volume and total motile sperm count in this group were worse than those in the normal sperm group; however, the pregnancy outcomes of the teratozoospermia group were unaffected. A possible explanation for this unanticipated finding may be the microscopic selection of sperm in ICSI, which allows technicians to select sperm that are morphologically normal even from a highly impaired semen sample. Thus, ICSI technique enables shifting the focus from the integral semen parameters to the quality of each individual spermatozoon. On this basis, the application of intracytoplasmic morphologically selected sperm injection offers another choice for men with severe teratozoospermia, and is a new technique that selects individual spermatozoa under high magnification, consequently improving the pregnancy outcome efficiently.[25]

Despite the long application and clinical success of ART techniques, very little has been understood about the influence of teratozoospermia on neonatal outcomes after ART. In our research, to eliminate confounding variables and specify the impact, we analyzed singleton, twin, and triplet births as three separate subgroups. The results demonstrated that teratozoospermia may affect infant birth weight in the cases of multiple births in IVF but not in ICSI. A research claimed that multiple pregnancy may be the most prevalent factor contributing to postnatal complications in ART,[26] and maternal constraints or physiological adaptations should be a potential mechanism.[27] Obviously, teratozoospermia-induced impaired embryonic development may also be responsible for the adverse postnatal outcome in the cases of multiple embryo implantation. According to a survey conducted in 2016 by the ART Management Department, Chinese Center for Disease Control and Prevention,[28] the twin delivery rates in IVF and ICSI were 27.9% and 27.22%, respectively, while our results revealed rates of >30% in both IVF and ICSI, which are higher than those reported by many other countries in the world. As a consequence, effective interventions such as single-embryo transfer should be undertaken to maintain a continuous decline in the multiple birth rate. Another significant finding in this study is that teratozoospermia showed no increased risk of major birth defect after IVF/ICSI. Thus far, the risks related to the IVF/ICSI procedure itself remain a concern, and can be mainly attributed to the bypass of penetration; the physical disturbance of the gametes; and the injection of contaminants or foreign, sperm-associated

Table 4: Demographic characteristics of the two groups undergoing intracytoplasmic sperm injection

Parameter	*Teratozoospermia group (n=1287)*	*Normal sperm group (n=1287)*	*P*
Female age (year), mean±s.d.	29.29±3.11	29.19±3.52	0.464
Male age (year), mean±s.d.	35.44±5.10	34.92±3.14	0.132
Duration of infertility (year), mean±s.d.	4.55±2.33	4.65±2.83	0.329
Female BMI (kg m^{-2}), mean±s.d.	21.10±2.48	21.04±2.09	0.474
FSH (mIU ml^{-1}), mean±s.d.	6.29±1.55	6.42±1.77	0.054
LH (mIU ml^{-1}), mean±s.d.	4.52±2.77	4.65±2.69	0.225
E2 (pg ml^{-1}), mean±s.d.	37.91±23.99	38.24±23.32	0.728
AMH (ng ml^{-1}), mean±s.d.	4.88±3.40	4.79±3.77	0.560
Thickness of the endometrium (cm), mean±s.d.	13.01±2.05	12.75±2.04	0.655
Number of oocytes retrieved, mean±s.d.	12.74±6.17	12.91±5.26	0.462
Number of transplanted embryos, *n* (%)			
0	206 (16.01)	264 (20.51)	0.003*
1	93 (7.23)	72 (5.59)	0.091
2	975 (75.76)	934 (72.57)	0.065
3	13 (1.01)	17 (1.32)	0.463
Source of sperm, *n* (%)			
Frozen semen	52 (4.04)	33 (2.56)	0.000*
Fresh semen	1235 (95.96)	1254 (97.44)	0.000*
Semen parameters after selection, mean±s.d.			
Volume (ml)	0.13±0.08	0.19±0.16	0.000*
Concentration (10^6 ml^{-1})	4.48±8.35	14.44±8.65	0.000*

*$P<0.05$. s.d.: standard deviation; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estrogen; AMH: anti-Müllerian hormone

Table 5: Pregnancy outcome of the two groups undergoing intracytoplasmic sperm injection

Parameter	*Teratozoospermia group (n=1287)*	*Normal sperm group (n=1287)*	*P*
Fertilization rate, % (*n*/total)	84.47 (10680/12644)	85.54 (7681/8979)	0.079
Normal fertilization rate, % (*n*/total)	78.95 (9983/12644)	80.39 (7218/8979)	0.060
Cleavage rate, % (*n*/total)	95.66 (10217/10680)	95.53 (7338/7681)	0.619
Rate of optimal embryo, % (*n*/total)	60.72 (6204/10217)	59.93 (4398/7338)	0.293
Implantation rate, % (*n*/total)	47.36 (986/2082)	46.82 (890/1901)	0.733
Pregnancy rate, % (*n*/total)	54.70 (704/1287)	50.89 (655/1287)	0.053
Parturition rate, % (*n*/total)	48.79 (628/1287)	45.07 (580/1287)	0.058
Abortion rate, % (*n*/total)	9.38 (66/704)	10.23 (67/655)	0.597
Rate of heterotopic pregnancy, % (*n*/total)	0.99 (7/704)	1.53 (10/655)	0.378
Number of babies born, % (*n*/total)			
1	66.40 (417/628)	66.90 (388/580)	0.855
2	33.28 (209/628)	33.10 (192/580)	0.948
3	0.31 (2/628)	0	NA
Pregnancy complication, % (*n*/total)			
Gestational diabetes	7.24 (51/704)	5.65 (37/655)	0.232
Gestational hypertension	3.27 (23/704)	3.51 (23/655)	0.803
Gestational edema	12.78 (90/704)	14.19 (93/655)	0.445
Gestational anemia	10.65 (75/704)	9.77 (64/655)	0.592

NA: not applicable

exogenous DNA.[29] Whether the use of abnormally shaped sperm would raise the risk of transmitting genetic or chromosomal abnormalities with ART remains inconclusive. It has been indicated that there is a potential relationship between sperm morphology and chromosomal aneuploidy and aberrant chromosomal integrity in ICSI.[30] However, McKenzie *et al.*[31] analyzed 54 IVF/ICSI cycles from 45 patients with profound teratozoospermia (Kruger's strict criteria of zero), in which 21 achieved pregnancy and no birth defects were noted during delivery. Our result also suggested that compared to the normal sperm group, teratozoospermia will not increase the risk of major birth defects in IVF/ICSI, regardless of whether it results in a single or multiple pregnancy. Based on this finding, ART could be considered a safe treatment for teratozoospermia patients and their offspring.

The best strategies for the management of teratozoospermia patients undergoing conventional IVF or ICSI remain controversial. The decision is usually made after the assessment of male fertility conditions, and the result of their previous ART treatments. There are no widely accepted criteria, so clinical management for couples is often empirical.[32] Several studies have shown that sibling oocytes obtained with ICSI and conventional IVF had similar rates of embryo formation, as well as similar clinical pregnancy and live birth rates.[33,34] In this research, teratozoospermia did not adversely influence the

Table 6: Neonatal outcome of the two groups undergoing intracytoplasmic sperm injection

Parameter	*Teratozoospermia group*			*Normal sperm group*			
	Singleton	*Twins*	*Triplets*	*Singleton*	*P*	*Twins*	*P*
Number of babies born	417	418	6	388		384	
Gender of newborn, *n* (%)					0.748		0.848
Male	207 (49.64)	204 (48.80)	4 (66.67)	197 (50.77)		190 (49.48)	
Female	210 (50.36)	214 (51.20)	2 (33.33)	191 (49.23)		194 (50.52)	
Gestational age (week), mean±s.d.	39.00±1.56	36.56±1.98	32.20	38.86±2.36	0.297	36.65±1.96	0.657
Birth weight (g), mean±s.d.	3312.19±477.67	2471.34±439.54	1525.00±204.33	3250.49±478.77	0.070	2474.97±460.33	0.912
Rate of low birth weight, *n* (%)	14 (3.36)	192 (45.93)	6 (100)	16 (4.12)	0.566	158 (41.15)	0.172
Rate of extreme low birth weight, *n* (%)	2 (0.48)	12 (2.87)	2 (33.33)	2 (0.52)	0.942	5 (1.30)	0.123
Rate of fetal macrosomia, *n* (%)	22 (5.28)	0	0	16 (4.12)	0.441	1 (0.26)	0.296
Rate of premature births, *n* (%)	22 (5.28)	79 (37.79)	1 (50.00)	26 (6.70)	0.393	70 (36.46)	0.781
Rate of birth defects, *n* (%)	7 (1.68)	24 (5.74)	1 (16.67)	6 (1.55)	0.882	23 (5.98)	0.881

s.d.: standard deviation

major indices of both IVF and ICSI, and showed similar pregnancy and neonatal outcomes. Thus, given the time consumption, costs, and potential risks associated with ICSI, we suggest that when initiating ART treatment, IVF may be the first choice for mild teratozoospermia patients and ICSI should only be considered in severe cases or in cases where a successful pregnancy with IVF is unlikely. However, it should be noted that a more controlled study with ICSI and IVF of sibling oocytes is needed to support the findings.

To our knowledge, this is the first large-scale retrospective study containing both pregnancy outcomes and live birth outcomes in teratozoospermia after ART. This information will help researchers gain a better understanding of the impact of sperm morphology on embryo development, clinical pregnancy, and offspring safety in ART. Due to practical constraints, this retrospective study has some limitations. First, this study does not address the influence of different types of sperm defect, even though sperm head and sperm tail defects may affect pregnancy outcomes differently. Second, inconsistencies in results from different laboratories may attribute to the heterogeneity of sperm morphology, and the subjective propensity of technicians. Thus, a stricter QC procedure and multicentric study are needed to validate the current results. Additionally, a randomized controlled trial with a larger sample size can provide more useful information about the clinical management of teratozoospermia patients.

Taken together, we believe that teratozoospermia has limited predictive value for pregnancy outcome in IVF/ICSI, and our findings offer evidence for the safety of their offspring. However, multiple embryo implantation should be avoided to protect neonatal health. Meanwhile, it should be noted that no single parameter may adequately serve as a predictor for ART outcome. In addition to sperm morphology, a comprehensive consideration is needed for the clinical treatment of teratozoospermia.

AUTHOR CONTRIBUTIONS

WJZ was responsible for data acquisition and analysis and drafted the article. CH contributed to the study conception and design. SHJ and XRJ carried out the laboratory work. FG was in charge of the reproductive center and clinical data management. LQF revised the manuscript. WBZ helped with data interpretation, conceived the study, and made critical revision of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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