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Role of sperm morphological parameters in the selection of fertilization methods

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Abstract

To investigate the role of *in-vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in cases with isolated teratozoospermia, and to provide reference for infertile patients to choose a reasonable insemination program. From January 2018 to December 2022, 433 couples received assisted reproductive assistance in the Department of Reproductive Medicine of our hospital. Among them, 81 patients presented isolated teratozoospermia (other sperm parameters were normal). These underwent IVF/ICSI, and differences in embryological data were compared between groups. 60 patients with moderate teratozoospermia went IVF, 11 needed rescue ICSI (R-ICSI, 18.33%), and 49 developed up to the blastocyst stage. 14 patients with severe teratozoospermia went IVF, 2 needed R-ICSI (14.29%), and 12 developed up to the blastocyst stage. There were no statistically significant differences in the proportion of R-ICSI between the two groups. Embryological outcomes were not significantly different between groups. The present results suggest that patients with moderate and severe teratozoospermia can be effectively treated with IVF, being R-ICSI performed when necessary.

Keywords

Sperm morphological parameters; *In vitro* fertilization; Intracytoplasmic sperm injection; Teratozoospermia; Male infertility

Papel de los parámetros morfológicos del esperma en la selección de los métodos de fecundación

Resumen

Investigar el papel de la fertilización *in vitro* (FIV) o la inyección intracitoplasmática de espermatozoides (ICSI) en casos con teratozoospermia aislada, y proporcionar una referencia para que los pacientes infértiles elijan un programa de inseminación razonable. Desde enero de 2018 hasta diciembre de 2022, 433 parejas recibieron asistencia reproductiva asistida en el Departamento de Medicina Reproductiva de nuestro hospital. Entre ellas, 81 pacientes presentaron teratozoospermia aislada (otros parámetros espermáticos fueron normales). Estos fueron sometidos a FIV/ICSI, y se compararon las diferencias en los datos embriológicos entre los grupos. 60 pacientes con teratozoospermia moderada se sometieron a FIV, 11 necesitaron ICSI de rescate (R-ICSI, 18.33%), y 49 evolucionaron hasta el estadio de blastocisto. 14 pacientes con teratozoospermia grave se sometieron a FIV, 2 necesitaron ICSI-R (14.29%) y 12 alcanzaron la fase de blastocisto. No hubo diferencias estadísticamente significativas en la proporción de R-ICSI entre los dos grupos. Los resultados embriológicos no fueron significativamente diferentes entre los grupos. Los presentes resultados sugieren que los pacientes con teratozoospermia moderada y grave pueden ser tratados eficazmente con FIV, realizándose R-ICSI cuando sea necesario.

Palabras Clave

Parámetros morfológicos espermáticos; Fecundación *in vitro*; Inyección intracitoplasmática de espermatozoides; Teratozoospermia inyección intracitoplasmática de espermatozoides; Teratozoospermia; Infertilidad masculina

1. Introduction

In China, according to the data from the National Bureau of Statistics, the infertility rate of couples of childbearing age has climbed to about 12%–18% in 2022. There are more than 50 million infertile patients in China, and the male factor is a primary cause in about 50% of couples [1–3]. With the development of assisted reproductive technology (ART), especially the appearance of ICSI, some patients who could not have children because of male factors have the chance to conceive [4–6]. Because it can treat almost all forms of infertility, ICSI has become the most common laboratory technique in ART, accounting for almost 70% of ART cycles, according to global estimates [7, 8].

However, the use of ICSI varies considerably by region, having its highest in the Middle East (around 100% of cases) and Latin America (85%), followed by North America (74%), Europe (70%), and Asia (55%) [7].

On the contrary, as of 27 March 2022, the Chinese Society of Reproductive Medicine (CSRM) Data Reporting System reported that ICSI accounted for 19.20% in 2019 [9]. Because of its invasiveness, concerns have been raised of whether the broad use of this technique leads to adverse outcomes for the conceived child's wellbeing [8, 10]. Therefore, considering the cost and potential risks of ICSI, it is necessary to seriously explore the indications of ICSI for male infertility and formulate the most appropriate treatment plan.

The No. 176 document "Technical Specification for Human Assisted Reproduction" issued by the former Ministry of Health clearly stipulates the indications for ICSI: severe oligozoospermia, asthenozoospermia and teratozoospermia; irreversible obstructive azoospermia; spermatogenic dysfunction (excluding genetic defects); immune infertility; IVF failure; abnormal sperm acrosome; preimplantation embryo genetic testing.

However, in our IVF laboratory, we observed that patients with severe teratozoospermia did not need ICSI for fertilization. Therefore, the purpose of this study was to investigate the role of sperm morphology in the selection of ICSI, and to provide reference for infertile patients to choose a reasonable insemination program.

2. Materials and methods

2.1 Patient selection

Retrospective analysis of 433 infertile couples who underwent IVF/ICSI in the Maternal and Child Health Hospital of Changde city, Department of Reproductive Medicine from January 2018 to December 2022.

Inclusion and exclusion criteria: male patients with primary infertility; female patients with secondary infertility; absence of abnormal karyotypes; normal hormone levels; presence of isolated teratozoospermia (normal sperm volume, viscosity, total count, concentration, motility and vitality) and absence of leucocytes and sperm agglutination. Semen evaluation followed the guidelines of the World Health Organization (WHO, 2021–20).

2.2 Sperm examination method

Semen was collected after 2–7 days of abstinence. Sperm motility was evaluated using the Computer-aided Semen Analysis System (CASA, BEION V4.20, BEIONMED, Shanghai, China). Sperm morphology was determined using the modified Papanicolaou staining protocol. Teratozoospermia (<4%) was classified in different degrees: ≤3%–<4% as mild teratozoospermia, ≤2%–<3% as moderate teratozoospermia, ≤1%–<2% as severe teratozoospermia and <1% as extremely severe teratozoospermia. In the present report, cases with moderate and severe teratozoospermia were treated using IVF, with R-ICSI when fertilization failed. In addition, severe teratozoospermia patients were transferred to ICSI because the sperm count was less than 1×10^6 on the day of fertilization.

2.3 Controlled ovarian stimulation protocols

Controlled Ovarian Stimulation (COS) included the long gonadotrophin-releasing hormone (GnRH) agonist protocol, which was suitable for the generally of patients, mainly those with normal ovarian function; the luteal phase ovarian stimulation protocol, which was suitable for patients with poor ovarian reserve function or who could not obtain usable embryos by other protocols; the GnRH antagonist protocol, which was often used in patients with polycystic ovarian syndrome (PCOS), high response and low ovarian response; and the minimal stimulation protocol, which was suitable for patients who could not undergo ovarian stimulation due to illness, or in patients with low ovarian response, repeated poor embryo quality, basal follicle-stimulating hormone (bFSH) levels >15 U/L, and low antral follicle count (AFC) due to conventional superovulation protocol.

The trigger of oocyte maturation was chosen based on patient age, body mass index (BMI), anti-Müllerian hormone (AMH) and bFSH levels, and AFC. When two of the dominant follicles were ≥18 mm or 3 were >17 mm in diameter, 5000–10,000 IU of human chorionic gonadotropin (HCG) was given in combination with estrogen level determination. Follicle retrieval was carried out under the guidance of transvaginal ultrasound 35–36 hours after HCG injection.

2.4 IVF procedure

The appearance of the second polar body was used as the criterion for early fertilization. If the proportion of oocytes with early fertilization was <30%, R-ICSI was performed. After insemination, embryos were cultured in an incubator at 37 °C, 6% CO_2 and 5% O_2. Embryo development was recorded daily up to day 6, according to Istanbul Consensus [11]. The score of cleavage stage embryos [12] and blastocysts [13] followed published standards.

2.5 Monitoring index

The following embryological parameters were determined: number of oocytes retrieved, total fertilization rate (includes abnormal fertilized oocytes), normal fertilization rate (appearance of two pronuclei and two polar bodies), high quality day-3 embryos, and blastocyst rate.

2.6 Statistical analysis

The data were analyzed using the SPSS 25.0 (IBM, NY, USA) program. Means were compared by the *t*-test for independent samples. Categorical variables were analyzed using descriptive statistics and Chi-square test, with continuity correction. In the presence of cells with expected value <5 in contingency tables, the Fisher exact Test was used. All statistical tests were two-tailed, with a significance level of 0.05 ($p < 0.05$).

3. Results

From January 2018 to December 2022, 433 couples received assisted reproductive assistance in the Department of Reproductive Medicine of our hospital. Among them, 81 patients presented isolated teratozoospermia (other sperm parameters were normal). These underwent IVF/ICSI, and differences in embryological data were compared between moderate and severe teratozoospermia groups. 60 patients with moderate teratozoospermia (mT) went IVF, 11 needed R-ICSI (18.33%). 14 patients with severe teratozoospermia (sT) went IVF, 2 needed R-ICSI (14.29%). There were no statistically significant differences in the proportion of R-ICSI between the two groups (Fig. 1).

Forty-nine patients with mT successfully completed conventional IVF, and 58.1% developed up to the blastocyst stage. 12 patients with sT successfully completed conventional IVF, and 64.8% developed up to the blastocyst stage. There were no statistically significant differences in the embryological outcomes between two groups (Table 1).

Twelve patients with sT successfully completed conventional IVF, and 64.8% developed up to the blastocyst stage. 6 patients with sT successfully completed ICSI, and 68.4% developed up to the blastocyst stage. There were no statistically significant differences in the embryological outcomes between two groups (Table 2).

Forty-nine patients with mT successfully completed conventional IVF, the number of oocytes retrieved was 11.8. 11 patients with mT need R-ICSI, the number of oocytes retrieved was 14.7. There were statistically significant differences in the number of oocytes retrieved between two groups. But other embryological outcomes were not significantly different between groups (Table 3).

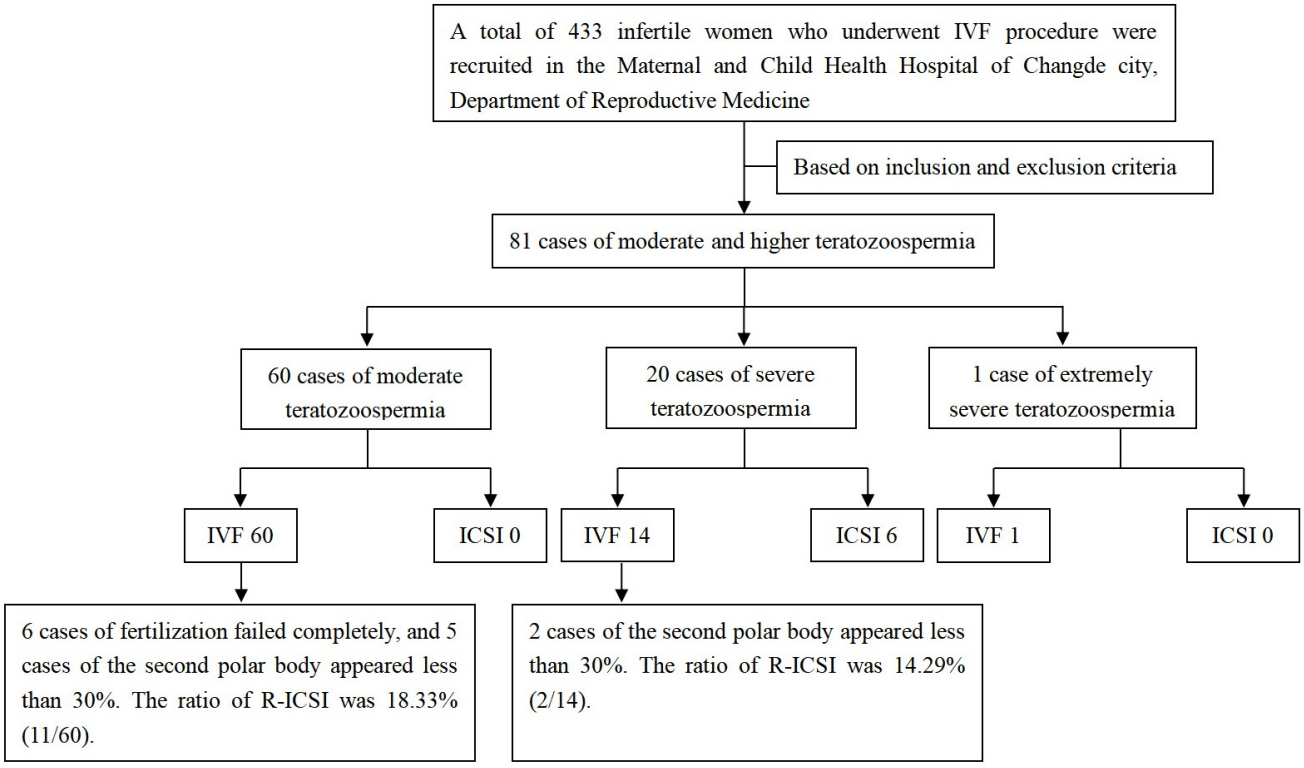

FIGURE 1. Patient selection flowchart. Four hundred thirty-three couples received assisted reproductive assistance. Among them, 81 patients had moderate or higher teratozoospermia. Among the 60 patients with moderate teratozoospermia, all underwent conventional IVF, of these, eleven patients were treated with rescue ICSI. In 20 cases of severe teratozoospermia, 14 were treated with one-generation fertilization and 6 with ICSI. In 14 patients with first-generation fertilization, two patients were treated with rescue ICSI. One patient with extremely severe teratozoospermia underwent conventional *in vitro* fertilization. IVF: *in-vitro* fertilization; ICSI: intracytoplasmic sperm injection; R-ICSI: rescue intracytoplasmic sperm injection.

TABLE 1. Comparison of IVF data between moderate and severe teratozoospermia.

Laboratory data	mT IVF (n = 49)	sT IVF (n = 12)	$t(\chi^2)/p$ value
Number of oocytes retrieved	11.80 ± 6.49	10.75 ± 8.21	−1.853/0.064
Total fertilization rate	80.45%	73.64%	0.207/0.837
Normal fertilization rate	65.05%	54.26%	0.845/0.404
Day-3 good quality embryo rate	51.86%	51.43%	0.483/0.633
Blastocyst formation rate	58.11%	64.76%	−0.774/0.448
Top quality blastocyst formation rate	17.56%	22.45%	−0.787/0.468

IVF: in-vitro fertilization.

TABLE 2. Comparison of IVF and ICSI data in patients with severe teratozoospermia.

Laboratory data	sT IVF (n = 12)	sT ICSI (n = 6)	$t(\chi^2)/p$ value
Number of oocytes retrieved	10.75 ± 8.21	11.67 ± 8.29	−0.700/0.944
Total fertilization rate	73.64%	63.80%	−0.271/0.791
Normal fertilization rate	54.26%	62.07%	−1.432/0.190
Day-3 good quality embryo rate	51.43%	61.11%	−1.111/0.318
Blastocyst formation rate	64.76%	68.42%	0.199/0.853
Top quality blastocyst formation rate	22.45%	26.32%	1.191/0.253

IVF: in-vitro fertilization.

TABLE 3. Comparison of IVF and R-ICSI data in patients with moderate teratozoospermia.

Laboratory data	mT IVF (n = 49)	mT R-ICSI (n = 11)	$t(\chi^2)/p$ value
Number of oocytes retrieved	11.80 ± 6.49	14.73 ± 7.46	−4.505/<0.001
Total fertilization rate	80.45%	84.21%	0.542/0.462
Normal fertilization rate	65.05%	77.19%	3.497/0.061
Day-3 good quality embryo rate	51.86%	48.86%	0.180/0.671
Blastocyst formation rate	58.11%	52.04%	0.992/0.319
Top quality blastocyst formation rate	17.56%	14.29%	0.595/0.440

IVF: in-vitro fertilization.

4. Discussion

Since the birth of the first ICSI baby in the 1990s [14], the ICSI technology has been carried out for more than 30 years. At first, ICSI was mainly used for the treatment of male infertility patients caused by severe oligozoospermia, asthenozoospermia and teratozoospermia. However, with developments in ART, such as immature oocyte vitro maturation (IVM) [15], oocyte freezing [16], preimplantation genetic testing (PGT) [17], and testicular sperm [18], the proportion of ICSI use has been greatly increased. Studies have shown that the fertilization rate is closely related to semen parameters, with sperm morphology having important predictive value for fertilization results [19–21]. Several authors observed that decreased normal sperm morphology led to decreased oocyte fertilization [22, 23]. Other studies indicated that natural conception is difficult to attain when normal sperm morphology is <4% [24]. Up to the present, most reproductive medicine centers in China rely on the reference index of normal sperm morphology [25] when deciding whether to perform IVF or ICSI. Therefore, the present study focused on the need for ICSI in patients with moderate, severe, and extremely severe teratozoospermia.

In the present report all cases with moderate teratozoospermia (mT) underwent IVF, with 18.33% needing R-ICSI, and all cases with severe teratozoospermia (sT) were also treated with IVF, with 14.29% needing R-ICSI. No significant differences were found between groups regarding the R-ICSI rate. The present results also indicate that the use of IVF in cases with severe teratozoospermia did not negatively affect the total fertilization rate and blastocyst rate such as that observed in moderate teratozoospermia cases (80.45% mT *vs.* 73.64% sT; 58.1% mT *vs.* 64.8% sT). This suggests that the option for IVF in cases with severe teratozoospermia is a good option, especially R-ICSI has low need rate and no negative effect on fertilization rate and blastocyst rate. Twelve patients with sT successfully underwent conventional IVF, while 6 patients with sT completed ICSI. The blastocyst rates for IVF and ICSI were similar (64.8% IVF *vs.* 68.4% ICSI), with no

statistically significant differences in embryological outcomes between two groups. These findings suggest that IVF for severe teratozoospermia can yield embryological outcomes that are on par with those achieved through ICSI. As we had only one case with extremely severe teratozoospermia, no comparisons could be performed. In this later case, the blastocyst rate after IVF was 60.0%.

5. Conclusions

In conclusion, we could evidence that cases with isolated moderate and severe teratozoospermia can be treated with IVF, with use of ICSI as rescue in cases with failed fertilization after IVF. Based on the findings of this study, the author suggests that the results can serve as a reference for andrologists when selecting appropriate fertilization methods for patients suffering from severe teratozoospermia. However, due to the low number of patients and limited parameters from a single center, as well as the lack of data from multiple regions and centers, the conclusions drawn from this study are preliminary. These findings need to be validated by a cohort study with a larger population.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed throughout the course of this study are accessible from the corresponding author upon submission of a reasonable request.

AUTHOR CONTRIBUTIONS

XQZ and BRL—Conceptualization. WJW and LLH—Investigation. YHW and DG—Data curation. CY and MJL—Formal analysis. DHL—Writing-original draft & Funding acquisition. DHL and BRL—Writing-review & editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The current study has been approved by the Ethics Committee of the Maternal and Child Health Hospital of Changde City (Approval Number: 2023(05)). All patients provided consent to participate in the study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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