Intracytoplasmic Sperm Injection Fertilization Rate Does Not Depend on the Proportion of Round-headed Sperm, Small-acrosomal Sperm, or Morphologically Normal Sperm in Patients with Partial Globozoospermia

Ling-Ying Jiang, Ling-Yun Yang, Xiao-Mei Tong, Hai-Yan Zhu, Ya-Mei Xue, Wen-Zhi Xu, Yang Yang, Song-Ying Zhang

Department of Obstetrics and Gynecology, Assisted Reproduction Unit, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310016, China

Abstract

Background: Generally, intracytoplasmic sperm injection (ICSI) may be the preferable method to treat partial globozoospermia, but whether there exist some correlations between ICSI fertilization rate and the proportion of round-headed sperm or morphologically normal sperm remains open. This study was to explore the correlation between ICSI fertilization rate and the sperm morphology in patients with partial globozoospermia.

Methods: Thirty-four patients diagnosed with partial globozoospermia accepted the following assisted fertilization treatments – 2 cases accepted in-vitro fertilization (IVF) alone, 26 cases accepted ICSI alone, and 6 accepted split IVF/ICSI. Detailed morphological characteristics were described using Diff-Quik rapid staining. Sixty cases accepting IVF or ICSI treatment in our reproductive center were considered as the control group after being matched by relevant criteria. Fertilization rate, embryo quality, embryo implantation rate and clinical pregnancy rate were calculated.

Results: Besides very high proportion of round-headed sperm, partial globozoospermia also showed very high proportion of small-acrosomal sperm and very low proportion of morphologically normal sperm. Fertilization rate of IVF (IVF alone plus split IVF) was very low in partial globozoospermia (25.4% ± 17.4%), but ICSI (ICSI alone plus split ICSI) achieved satisfying fertilization rate compared with the control group (66.2% ± 22.5% vs. 68.8% ± 29.4%, P > 0.05). In patients with partial globozoospermia, there were no correlations between ICSI fertilization rate and the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm.

Conclusions: There was high proportion of small-acrosomal sperm in partial globozoospermia. For patients with partial globozoospermia, ICSI is more preferable than IVF. ICSI fertilization rate does not depend on the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm.

Key words: Fertilization Rate; Globozoospermia; Intracytoplasmic Sperm Injection; In-vitro Fertilization; Sperm Acrosome

Introduction

Different from total globozoospermia, which is characterized by 100% round-headed sperm,[1,2] partial globozoospermia has a large, but not total, proportion of round-headed sperm and even has morphologically normal sperm in one sperm sample.[3-6] In daily clinical practice, partial globozoospermia is more commonly found than total globozoospermia,[6] and it might be correlated with a larger number of couples’ infertility.

So far, total globozoospermia has been well-studied, especially its morphological characteristics,[7,8] fertilizing capacity,[9-11] oocyte activation capacity,[12-15] and even genetic background.[16-18] For partial globozoospermia, current studies have mainly focused on structural characteristics and fertilizing capacity and the general view is that intracytoplasmic sperm injection (ICSI) is a more suitable method than in-vitro fertilization (IVF) to obtain an acceptable fertilization rate.[4,6,19]

In total globozoospermia, ICSI cannot achieve an ideal fertilization rate because of the very low oocyte activation capacity of round-headed sperm. However, there are different proportion of acrosomeless sperm and even different proportion of morphologically normal sperm in partial globozoospermia. Whether the different proportion of acrosomeless sperm or different proportion of morphologically normal sperm implies different oocyte activation capacity? Or whether there exists some correlation between ICSI fertilization rate and the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm?
fertilization rate and the sperm morphology? These questions remain open.

In this study, we investigated the fertilization rate of IVF or ICSI in 34 cases that were diagnosed with partial globozoospermia according to the criteria of more than 25% round-headed sperm in the ejaculate. The proportions of round-headed sperm and morphologically normal sperm were calculated. Simultaneously, 60 cases accepting IVF or ICSI treatment in our reproductive center in November 2013 were considered as the control group after being matched for confounding conditions. Fertilization rate, embryo quality, embryo implantation rate, and clinical pregnancy rate were calculated. This study was to describe the morphological characteristics of partial globozoospermia and to demonstrate if there is any correlation between ICSI fertilization rate and the sperm morphology in patients with partial globozoospermia.

**Methods**

**Subjects**

Thirty-four infertile couples in which the male partners had partial globozoospermia accepting IVF or ICSI treatment in our reproductive center from January 2009 to November 2013 were recruited as our subjects. Among the 34 female partners, 9 had obstruction of fallopian tube, 3 had polycystic ovarian syndrome, 2 had endometriosis, 1 had obesity (body mass index > 30 kg/m²), and other 19 had no obvious abnormalities. There was an average of 4 years of infertility duration in these couples. Five couples achieved spontaneous pregnancy in the past, and the other 29 were diagnosed with primary infertility. Ten out of the 34 couples (five from secondary infertility and the other five from primary infertility) had accepted at least two cycles of intrauterine insemination treatment before, but none had achieved successful pregnancy.

Sixty cases accepting IVF or ICSI treatment in November 2013 were chosen as the control group based on the following matching criteria: Female age between 31 and 33 years; a standard long protocol; the first IVF or ICSI cycle without fertilization or intracytoplasmic sperm injection. All the 34 infertile couples accepted the standard long protocol criteria of WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition). Two trained technicians individually evaluated at least 200 consecutive sperm cells per patient sample. Acrosomeless sperm, abnormal-acrosomal sperm, and morphologically normal sperm were separately recorded. We calculated the percentage of round-headed sperm, abnormal-acrosomal sperm, or morphologically normal sperm according to the following formula:

Percentage of round-headed sperm = Number of round-headed sperm/200 × 100%.

Percentage of abnormal-acrosomal sperm = Number of abnormal-acrosomal sperm/200 × 100%.

Percentage of morphologically normal sperm = Number of morphologically normal sperm/200 × 100%.

**In-vitro fertilization or intracytoplasmic sperm injection treatment**

All the 34 infertile couples accepted the standard long protocol. The standard long protocol involved down-regulation with subcutaneous injection of 0.05 mg GnRH agonist Triptorelin (Ferring, Wittland, Germany) starting on day 20 of the previous cycle, followed by recombinant gonadotrophin stimulation with either injection of Puregon (Organon, Oss, The Netherlands) or injection of Gonal F (Merck Serono, Geneva, Switzerland). When a group
of follicles reached 16 mm in diameter, ultrasound-guided transvaginal ovum pick-up (OPU) was performed 36 h after 5000 IU urinary HCG Pregnyl (Organon, Oss, The Netherlands) administration. Finally, oocytes were cultured in BD Falcon dishes (Becton Dickinson, NJ, USA), and the culture density was three oocytes per drop (volume of each drop was 75 µl). The culture medium for oocytes was G-IVF (Vitrolife, Göteborg, Sweden) plus 10% (v/v) Serum Substitute Supplement (IrvineScientific, California, USA) and the incubation system was carbon dioxide incubator (Thermo Fisher Scientific, Ohio, USA) at 37°C, with 6% CO₂.

On the day of OPU, semen sample of the male partner was collected by masturbation into sterile plastic container and then centrifuged with gradient liquid Isolate (InvineScientific, California, USA). Finally, the sediment was diluted into suspension with a sperm concentration of 15 × 10⁶/ml and then the suspension was cultured in an incubator at 37°C with 6% CO₂ for IVF or ICSI use.

Of the 34 cases, IVF alone was carried out for asking of 2 couples with secondary infertility, and split IVF/ICSI was carried out for asking of 6 couples who met two preconditions – more than 10 oocytes achieved and more than 1 × 10⁶ progressive sperm in the stripped suspension. Split IVF/ICSI meant that for the same infertile couple IVF was carried in several oocytes while ICSI was carried in the remaining oocytes. The other 26 cases accepted ICSI treatment alone.

For IVF, 30,000 sperm were added to one 75 µl culture drop that meant the sperm concentration in the fertilizing drop was 4 × 10⁵/ml. For ICSI, about 1 µl sperm suspension was added into polyvinylpyrrolidone (InVitroCare, Maryland, USA) and then ICSI was performed as described previously by Palermo et al.[5] ICSI instrument was NARISHIGE micromanipulation system (NT-88NE-N2, Nikon, Japan). Indications of ICSI in the control group include: Progressive motility was under 10%; normal morphology rate was below 1%; sperm total number after washing process was below 1 × 10⁶.

Fertilization rates were scored 18–22 h after ICSI or IVF procedure. Embryo transfer was performed on day 3 or day 5 after OPU only if the conditions were suitable for transfer, otherwise transfer was canceled and the embryos were frozen. Blood β-HCG was measured on day 15 after OPU, and an ultrasound was made 3–5 weeks after embryo transfer. Indication of a successful clinical pregnancy was one or more intrauterine gestational sacs with fetal heartbeat.

**Statistical analysis**

Statistical analyses were undertaken using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). The normally distributed data were expressed as means ± standard deviation, and the skewed data were expressed as the median (quartile range). χ² test was used to compare the rate between two groups, and the one-way ANOVA test was used to compare multiple samples. The Pearson correlation test was used to determine correlations. Scatterplots and R were used to describe the changing trend. All P values were based on two-sided comparisons, and P < 0.05 was considered as statistically significant.

**RESULTS**

**Sperm morphological characteristics under the oil immersion lens**

Under the oil immersion lens, we found in partial globozoospermia not only a large number of acrosomeless sperm, but also lots of sperm with small acrosomes. The head of acrosomeless sperm looked like a ball (red arrow, Figure 1), and the head of small-acrosomal sperm was thin and pointy, which made it look like a sunflower seed (yellow arrow, Figure 1). Morphologically normal sperm was also seen in partial globozoospermia (green arrow, Figure 1).

**Proportion of acrosomeless sperm, small-acrosomal sperm, or morphologically normal sperm in partial globozoospermia and nonglobozoospermia**

Both the percentage of acrosomeless sperm and the percentage of small-acrosomal sperm in partial globozoospermia were significantly higher than nonglobozoospermia – normozoospermia, oligozoospermia, asthenospermia, teratozoospermia and oligoasthenoteratozoospermia (P < 0.001). The percentage of morphologically normal sperm in partial globozoospermia was the lowest at about 0.3%, which was significantly lower than normozoospermia, oligozoospermia and asthenospermia (P < 0.001), lower than teratozoospermia (P < 0.05), and had no difference compared with oligoasthenoteratozoospermia (P > 0.05). All the detailed parameters are shown in Table 1.

![Figure 1: Morphological characteristics of normozoospermia, partial globozoospermia, and total globozoospermia under the oil immersion lens.](image-url)

(a and b) Showed that round-headed sperm, small-acrosomal sperm, and morphologically normal sperm could be seen simultaneously in normozoospermia and partial globozoospermia (red arrow meant round-headed sperm, yellow arrow meant small-acrosomal sperm, and green meant morphologically normal sperm); (c) Showed partial globozoospermia with no morphologically normal sperm; (d) Showed that all the sperm were round headed.
In-vitro fertilization or intracytoplasmic sperm injection results of partial globozoospermia and the control group

Of the 34 cases of partial globozoospermia, 2 cases accepted IVF alone, 26 cases accepted ICSI alone, and the other 6 cases accepted split IVF/ICSI. For split IVF/ICSI, 56 oocytes accepted IVF, and 60 oocytes accepted ICSI. Total IVF failure happened in two cases (one from IVF alone, and the other from split IVF/ICSI). Fertilization rate of IVF (IVF alone plus split IVF) in partial globozoospermia was significantly lower than that of the control group \( (P < 0.01) \), but ICSI (ICSI plus split ICSI) achieved a satisfying fertilization rate compared with the control group \( (P > 0.05) \) [Table 2].

Correlations between the fertilization rate of in-vitro fertilization or intracytoplasmic sperm injection and the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm

Pearson correlation test showed that there were no significant correlations between the fertilization rate of IVF or ICSI and the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm \( (P > 0.05) \) [Figure 2].

Discussion

It is well-known that large, but not total, proportion of round-headed sperm is the basic feature of partial globozoospermia. In this study, we investigated 34 cases of partial globozoospermia and further demonstrated two features of partial globozoospermia’s fertilizing capacity – satisfying fertilization rate of ICSI and unsatisfying fertilization rate of IVF. In addition, we also found a high proportion of small-acrosomal sperm in partial globozoospermia.

To explain the absence of acrosome in globozoospermia, Dam et al.\(^6\) concluded four possible mechanisms including acrosome being lost in the sertoli cell,\(^2,8\) acrosome being lost in the cytoplasm, acrosome degeneration and absence,\(^6\) or malfunction of the caudal manchette.\(^7\) Although the exact mechanism of acrosomal absence was not clear, one common view is that globozoospermia likely originates during spermiogenesis. Here, we found that acrosomeless sperm and small-acrosomal sperm were the overwhelming types of cells in partial globozoospermia. We postulated that the small-acrosomal sperm could be the same as those dysmorphic sperm described by Dam et al. – more or less oval head shapes with less condensed chromatin, partially present and even invaginated acrosomes.\(^6\) These morphological findings suggested that partial globozoospermia might have serious agenesis or even agenesis of acrosomes.

Very high proportion of acrosomeless sperm, very high proportion of small-acrosomal sperm, and very low proportion of morphologically normal sperm were considered as the main reason for very low IVF capacity of partial globozoospermia. As we know, normal fertilizing capacity of sperm depends on normal acrosomal reaction and normal functions of acrosomal enzymes.\(^22,23\) Round-headed sperm cannot fertilize mature oocytes because of the absence of acrosome and acrosomal enzymes. A partially present and even invaginated acrosome makes the space between the

Figure 2: Correlations between the fertilization rate of in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) and the proportion of acrosomeless sperm, small-acrosomal sperm or morphologically normal sperm. (a-c) Showed the correlations between the fertilization rate of IVF and the proportion of acrosomeless sperm, small-acrosomal sperm or morphologically normal sperm, and (d-f) showed the correlations between the fertilization rate of ICSI and the proportion of acrosomeless sperm, small-acrosomal sperm or morphologically normal sperm.
Table 1: Proportion of acrosomeless sperm, small-acrosomal sperm, or morphologically normal sperm in different types of semen samples

| Group                      | Cases (n) | Age (years) | Acrosomeless (%) | Small acrosome (%) | Morphologically normal (%) |
|----------------------------|-----------|-------------|------------------|--------------------|---------------------------|
| Partial globozoospermia    | 34        | 30.7 ± 3.5  | 37.5 ± 13.4*     | 58.3 ± 11.9†       | 0.3 ± 0.4†                |
| Normozoospermia            | 25        | 30.8 ± 5.0  | 0.4 ± 0.9        | 15.0 ± 11.1        | 8.7 ± 3.4                 |
| Oligozoospermia            | 25        | 32.1 ± 4.3  | 2.5 ± 2.2        | 20.8 ± 16.5        | 5.9 ± 2.6                 |
| Ashtenospermia             | 25        | 31.3 ± 3.9  | 1.6 ± 2.8        | 18.0 ± 17.3        | 6.8 ± 1.8                 |
| Teratozoospermia           | 25        | 31.7 ± 4.6  | 1.5 ± 2.4        | 19.2 ± 10.9        | 1.9 ± 1.1                 |
| Oligoaasthenoteratozoospermia | 25   | 33.1 ± 3.1  | 3.4 ± 3.3        | 23.0 ± 21.3        | 1.2 ± 0.9                 |

*Proportion of acrosomeless sperm in partial globozoospermia was significantly higher than five types of non-globozoospermia (P < 0.001).
†Proportion of small-acrosomal sperm in partial globozoospermia was significantly higher than five types of nonglobozoospermia (P < 0.001).
‡Proportion of morphologically normal sperm in partial globozoospermia was significantly lower than normozoospermia, oligozoospermia, and asthenospermia (P < 0.001), lower than teratozoospermia (P < 0.01), and had no difference compared with oligoaasthenoteratozoospermia (P > 0.05).

Table 2: Treatment characteristics of partial globozoospermia cases and the control group

| Items                          | Partial globozoospermia | Control group |
|-------------------------------|-------------------------|---------------|
| Cases (n)                     | 34                      | 60            |
| Age of women (years)          | 31.9 ± 2.9              | 31.7 ± 0.9    |
| Cases of IVF alone (n)        | 26                      | 10            |
| Cases of IVF/ICSI (n)         | 2                       | 45            |
| Cases of ICSI (n)             | 6                       | 5             |
| Number of oocytes for IVF (n) | 78                      | 523           |
| Number of oocytes for ICSI (n)| 258                     | 110           |
| Fertilization rate of IVF (%) | 25.4 ± 17.4* (alone plus split) | 70.3 ± 24.3 |
| Fertilization rate of ICSI (%)| 66.2 ± 22.5 (alone plus split) | 68.8 ± 29.4 |
| Good-quality embryo rate (%)  | 47.4 ± 25.2             | 45.3 ± 27.1   |
| Cases of fresh embryo transfer (n) | 11                    | 14            |
| Clinical pregnancy rate (%)   | 54.5 (6/11)             | 57.1 (8/14)   |
| Embryo implantation rate (%)  | 36.4 ± 39.3             | 32.1 ± 31.7   |

The results are expressed as the means ± SD. *Fertilization rate of IVF in partial globozoospermia was significantly lower than that of the control group (P < 0.01). SD: Standard deviation; ICSI: Intracytoplasmic sperm injection; IVF: In-vitro fertilization.

sperm head membranes smaller and then the whole acrosomal volume smaller, which makes the amount of acrosomal enzymes in partial globozoospermia less than that in sperm with normal acrosome volume. Meanwhile, as low as 0.3% of normal morphology rate might not be able to ensure ideal IVF capacity.[24,25] From the above reasons, it might explain that the IVF capacity of partial globozoospermia was significantly lower than that of the control group in our study.

Fortunately, just because that there were still some oval and even morphologically normal sperm, partial globozoospermia did not lose fertilizing capacity completely unlike total globozoospermia. This might be why there is no spontaneous fertilization occurring in total globozoospermia, but some successful spontaneous pregnancies in partial globozoospermia. Actually, although most couples with partial globozoospermia in our study suffered from primary infertility, five cases had conceived successfully before.

In total globozoospermia, ICSI is considered as the optimal method to achieve successful fertilization although the fertilization results are not good enough because of poor oocyte activation capability of round-headed sperm.[12-15] Unlike total globozoospermia, partial globozoospermia could achieve an ideal ICSI fertilization rate, which suggested that sperm from partial globozoospermia chosen for injection have normal oocyte activation capability. Therefore, we could conclude that the differences between partial globozoospermia and total globozoospermia include not only different proportion of round-headed sperm, but also different oocyte activation capability. This point of view is in consistent with Dam’s point, which stated that partial globozoospermia is a distinctive sperm malformation that exists separately from total globozoospermia.[6]

In the present study, the Pearson correlation test showed that there were no significant correlations between ICSI fertilization rate and the proportion of round-headed sperm, small-acrosomal sperm, or even morphologically normal sperm (P > 0.05). This implied that ICSI fertilization rate might not depend on the proportion of the three types of sperm cells. In an actual process of ICSI for partial globozoospermia, oval sperm or even morphologically normal sperm are preferred for injection, and this selection process might be the key to achieve an ideal ICSI fertilization rate. Because, there is a very low percentage of morphologically normal sperm in partial globozoospermia, it takes more time and needs professional skills to select normal or nearly normal sperm.

Pearson correlation test also indicated that there were no significant correlations between the fertilization rate of IVF and the proportion of round-headed sperm, small-acrosomal sperm, or even morphologically normal sperm (P > 0.05). This result needs to be clarified by further studies with a larger sample size.

In conclusion, we observed sperm morphology by Diff-Quik staining and found a very high proportion of small-acrosomal sperm in partial globozoospermia. A total of 34 cases of partial globozoospermia accepted IVF or...
ICSI treatment and achieved low IVF rate and comparable ICSI fertilization rate. We considered that ICSI might be the preferable method to treat partial globozoospermia, and found that ICSI fertilization rate might not depend on the proportion of round-headed sperm, small-acrosomal sperm, or morphologically normal sperm. Therefore, what does ICSI fertilization rate depend on? Maybe just one caped sperm is all it takes.

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