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Original Research Article

Does the oocyte quality impact intracytoplasmic sperm injection outcomes in severe male factor infertility: a retrospective study

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ABSTRACT

Background: ICSI has transformed the approach in treating severe male factor infertility, however the quality of oocyte plays a significant role in compensating for sperm abnormalities and enhancing the clinical outcomes. The study aims to evaluate the impact of oocyte quality on fertilization rates, embryo development and pregnancy outcomes in couples undergoing intracytoplasmic sperm injection (ICSI) in two distinct groups- cryptozoospermia and azoospermia.
Methods: A retrospective observational study was conducted at a fertility centre in a tertiary hospital. A total of 61 couples with male infertility were included in the study. Group 1 includes 42 patients diagnosed with cryptozoospermia and Group 2 includes 19 patients diagnosed with azoospermia. Controlled ovarian stimulation was done using gonadotrophins to induce follicular development followed by ICSI procedure. All the retrieved oocytes were categorized based on the morphology criteria outlined in the Istanbul consensus into two distinct groups: oocytes with normal morphology and oocytes with abnormal morphology. Clinical outcomes including fertilization rate, implantation rate, pregnancy rate and miscarriage rate were evaluated.
Results: The observed fertilization rate in oocytes with normal morphology and oocytes with abnormal morphology was 84.03% vs. 78.26% in the cryptozoospermia group and 80.76% vs. 78.57% in the azoospermia group. The overall pregnancy rate was 26.19% in group 1 and 31.58% in group 2 with no statistical significance. Oocytes with normal morphology exhibited higher pregnancy rates, while oocytes with abnormal morphology showed very low or no pregnancy in both groups.
Conclusions: The study concludes oocyte quality has a greater impact on the ICSI outcomes irrespective of the type of sperm used. Future studies with a large cohort size are needed to validate these findings.

Keywords: Azoospermia, Cryptozoospermia, ICSI, Oocyte quality, Pregnancy rate

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technique developed to improve pregnancy outcomes in couples, particularly when the male partner exhibits poor semen characteristics or when couples have experienced poor or no fertilization rates after conventional in vitro fertilization. ICSI entails the precise microinjection of sperm into mature oocytes.[1] ICSI has transformed the approach in addressing severe male factor infertility, including conditions such as cryptozoospermia (sperm observed only in pellet), azoospermia (absence of sperm in ejaculate), asthenozoospermia (reduced sperm motility), teratozoospermia (abnormal sperm shape), and oligozoospermia (low sperm count).[2-4] The technique greatly improves the likelihood of a successful pregnancy by avoiding several of the natural inherent obstacles to fertilization. For males, this approach is a viable way to overcome their infertility issues.[5]

The quality of metaphase II oocytes has been identified as a crucial factor for fertilization in patients undergoing

ICSI.[6] To produce high-quality embryos, both male and female gametes must exhibit superior quality. Although both gametes possess the ability to potentially repair DNA breaks, this ability is significantly diminished in the sperm cell. Consequently, the oocyte must address these DNA damages following fertilization and before the initial cell division in the early stages of embryo development. Thus, the quality of the oocyte holds particular significance for men experiencing severe male factor issues, such as cryptozoospermia or azoospermia, as the subpar quality of sperm in these cases is associated with increased sperm DNA damage.[7,8]

Nevertheless, reports regarding the effect of oocyte quality on ICSI outcomes were conflicting. Setti et al found that the mature oocytes exhibiting normal morphology were associated with improved embryo development and higher implantation rates compared to abnormal oocytes with smooth endoplasmic reticulum in the patients undergoing ICSI procedure.[9] Oocytes with intracytoplasmic and extracytoplasmic abnormalities had a negative impact on fertilization rate, cleavage-stage embryo and blastocyst formation, implantation, pregnancy and miscarriage rates during an ICSI cycle.[10,11] However, few studies have shown that the quality or morphology of oocytes has minimal or no effect on the development and implantation potential of embryos.[12-14]

Hence, the current study aims to evaluate the impact of oocyte quality on fertilization rates, embryo development and clinical outcomes in couples undergoing ICSI with male infertility in the cryptozoospermia group by using ejaculated sperm and azoospermia male partners by using surgically retrieved sperm.

METHODS

Study design

This retrospective observational study was conducted at the Krishna Institute of Medical Sciences (KIMS) Fertility Centre, KIMS, Hyderabad, India, between 2016 and 2019. The study was approved by the ethical committee (KIMS/IEC-BHR/2025/102-06).

Patient selection

A total of 61 patients diagnosed with severe male infertility in our centre were included in the study. The patients were categorized into two groups Group 1 includes 42 patients diagnosed with cryptozoospermia and Group 2 includes 19 patients diagnosed with azoospermia, in whom sperms were obtained using surgical sperm retrieval techniques (SSR). Patients' baseline characteristics and hormone profiles were recorded

Collection and processing of sperms

Semen samples for patients with cryptozoospermia (Group 1) were collected via masturbation on the day of oocyte retrieval, assessed for sperm count, and subsequently centrifuged or processed without delay. Viable sperms from semen precipitation were cultured in G-MOPS medium at room temperature for 1-2 h before ICSI. In azoospermic patients (Group 2), SSR methods including testicular sperm extraction (TESA/TESE/MESA) or percutaneous fine-needle aspiration (PESA) were performed. The seminiferous tubules were microdissected under the microscope and suspension containing sperms was released into the culture media. Following microscopic observation of the sperm, the complete sample was suspended in 3 ml of GMOPS medium and centrifuged at 1200 rpm for 10 minutes. The resulting pellet was frozen. On the day of the ICSI procedure, the frozen sperm pellet was thawed and subsequently used for ICSI.

Controlled ovarian stimulation, oocyte retrieval and quality analysis

Controlled ovulation stimulation was initiated on day 2 of the menstrual cycle with the administration of gonadotrophins for a duration of 8 to 10 days. Follicular monitoring was performed with regular transvaginal ultrasound examinations to assess ovarian response. Once the leading follicle reached 18 mm, ovulation was triggered with recombinant HCG or GnRH agonist. Oocytes were aspirated 34-36 hours post-hCG administration through transvaginal ultrasound-guided puncture using a 17 gauge, 35 cm double-lumen aspiration needle (Vitrolife, Germany) and examined for oocytes under a stereozoom microscope. Post collection, oocytes were washed and incubated in GIVF medium for 2 h. Further, the oocytes were denuded with Hylase (Vitrolife, Germany) and examined under an inverted microscope to determine their morphology and level of maturation.

The Istanbul consensus defines oocytes with normal morphology as those exhibiting characteristics, including transparent cytoplasm with homogeneous fine granularity, a spherical or ovoid first polar body featuring a smooth surface, and a colorless zona pellucida that maintains a normal shape. Oocytes with abnormal morphology had either cytoplasmic or extracytoplasmic abnormalities or both. Cytoplasmic abnormalities include dark granulated cytoplasm, the presence of smooth endoplasmic reticulum, refractile bodies and vacuolization. Extracytoplasmic abnormalities include a large or granulated perivitelline space and fragmented polar bodies.[15]

Treatment procedure

Intracytoplasmic sperm injection was performed on MII oocytes following the methodology outlined by Habermann et al.[16] Fertilization was evaluated 16–18 h post sperm injection using a microscope. The presence of two pronuclear zygotes was deemed indicative of fertilization. Embryo cleavage was evaluated 24 h post-fertilization and again at the 48 h mark following fertilization assessment. The evaluation of the embryos

involved analyzing blastomere size, symmetry, and the fragmentation of nuclear debris, whereas the scoring of blastocysts was conducted using the Gardner scoring system. The embryos were transferred either at the cleavage stage i. e., on day 2 and day 3, or at blastocyst stage on day 5. To confirm pregnancy, the blood β-HCG level was measured 10-12 days after embryo transfer.

Outcome variables

Oocyte quality, fertilization rate, embryo implantation rate, pregnancy rate, miscarriage rate and ongoing clinical pregnancy rate at 10-weeks gestation were analyzed. It is our practice to discharge patients from our care at 10-weeks gestation for further antenatal care elsewhere.

Statistical analysis

The statistical analysis was conducted using SPSS version 21. Categorical variables were described using percentages and frequencies. A Chi-square test (χ^2) was applied to evaluate the relationship between groups and variables. A p value less than 0.05 is considered statistically significant, while a p value >0.05 is considered insignificant.

RESULTS

Study baseline characteristics

A total of 61 couples diagnosed with male infertility were included in the study. Group 1 includes 42 male partners diagnosed with cryptozoospermia and group 2 includes 19 male partners diagnosed with azoospermia (group 2). The average age of the male partner in both groups was 34 to 35 years. Mean female age was slightly higher in group 1 (31.07±5.75) compared to group 2. In female partners, controlled ovarian stimulation was performed using gonadotrophins for a mean duration of 9 days (p=0.28). The total gonadotropin dosage used was slightly higher in group 2 (3513.33±1373.98). Average endometrium thickness was 8.2±1.4 mm in cryptozoospermia and 8.8±1.2 mm in azoospermia groups (p=0.06) (Table 1). No significant statistical difference was observed in baseline characteristics between both groups.

Table 1: Baseline characteristics of the study.

Variables	Cryptozoospermia (Group 1) (n=42)	Azoospermia (Group 2) (n=19)	P value
Male age (years)	35.73±5.18	34.05±5.91	0.13
Female age (years)	31.07±5.75	28.94±4.32	0.07
Days of stimulation (days)	8.88±1.86	9.15±1.60	0.28
Total dosage of gonadotrophins	3137.83±1280.11	3513.33±1373.98	0.17
AFCs in both ovaries	9.92±3.93	11.06±4.63	0.18
Endometrium thickness (mm)	8.2±1.4	8.8±1.2	0.06

Table 2: Cryptozoospermia group treatment outcomes.

Variables	Cryptozoospermia (n=42)		P value
	Oocytes with normal morphology (n=29)	Oocytes with abnormal morphology (n=13)	
Male (years)	35.62±5.26	36±5.22	0.41
Female (years)	31.31±5.60	30.53±6.29	0.34
Days of stimulation	8.58±1.84	9.53±1.80	0.06
Total gonadotrophins dosage (IU)	3011.12±1206.88	3401.83±1439.62	0.19
Endometrium thickness (mm)	8.18±1.57	8.38±1.04	0.22
Total no.of oocytes retrieved	10.17±4.82 (295)	9.30±3.61 (121)	0.28
No. of MII oocytes	8.20±4.10 (238)	7.07±2.84 (92)	0.18
Fertilization rate	84.03% (200/238)	78.26% (72/92)	0.11
Cleavage rate	99% (198/200)	95.83% (69/72)	0.09
No. of embryos transferred	2.31±0.76 (67)	2.84±0.68 (37)	0.01
Implantation rate	20.89% (14/67)	2.7% (1/37)	0.04
Pregnancy rate	34.48% (10/29)	7.69% (1/13)	0.03
Miscarriage rate	40% (4/10)	0%	-
Clinical ongoing pregnancy rate at 10 weeks gestation	20.6% (6/29)	7.69% (1/13)	0.15

Cryptozoospermia (group 1) treatment outcomes

In cryptozoospermia group, 29 female partners had oocytes with normal morphology and 13 had oocytes with abnormal morphology. Duration of ovulation stimulation was (9.53±1.8 days). The total gonadotrophin dosage given (3401.83±1439.62 IU) was marginally higher in the oocytes with abnormal morphology compared to oocytes with normal morphology with no statistical significance. The total number of metaphase II oocytes retrieved in the oocytes with normal morphology was 238 (8.20±4.10) and in the oocytes with abnormal morphology was 92 (7.07±2.84) (p=0.11). Similarly, the fertilization rate was 84.03% in the oocytes with normal morphology and 78.26% in the oocytes with abnormal morphology with no statistical significance. Significantly higher implantation rate (20.89%), pregnancy rate (34.48%) and clinical ongoing pregnancy rate at 10 weeks gestation (20.69%) were observed in the oocytes group with normal morphology compared to the oocytes with abnormal morphology group with a statistical significance. Miscarriages were observed only in the oocytes with normal morphology sub-group (Table 2).

Table 3: Azoospermia group treatment outcomes.

Variables	Azoospermia (n=19)		P value
	Oocytes with normal morphology (n=15)	Oocytes with abnormal morphology (n=04)	
Male (years)	34.27±6.68	33.25±0.5	0.38
Female (years)	29.47±4.37	27±4.08	0.16
Days of stimulation	9.09±2.07	9.5±1	0.35
Total gonadotrophin dosage (IU)	3902.2±1332.03	2443.7±903.09	**0.03**
Endometrium thickness (mm)	9.1±1.45	8.32±0.47	0.16
Total no. of oocytes retrieved	9±2.53 (135)	9.75±3.86 (39)	0.32
No. of MII oocytes	6.93±1.9 (104)	7±3.55 (28)	0.47
Fertilization rate	80.76% (84/104)	78.57% (22/28)	0.46
Cleavage rate	96.42% (81/84)	95.45% (21/22)	0.44
No. of embryos transferred	2.36±0.89 (35)	1.75±1.5 (7)	0.16
Implantation rate	17.14% (6/35)	0	-
Pregnancy rate	40% (6/15)	0	-
Miscarriage rate	50% (3/6)	0	-
Clinical ongoing pregnancy rate at 10 weeks gestation	20% (3/15)	0	-

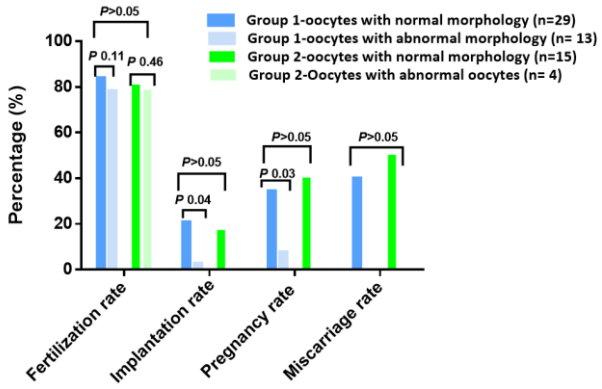

Figure 1: Comparison of treatment outcomes between Group 1 (Cryptozoospermia) and Group 2 (Azoospermia).

Azoospermia (group 2) treatment outcomes

Out of 19 female partners, 15 had oocytes with normal morphology and 4 had oocytes with abnormal morphology. The average duration of ovulation stimulation was 9 days. A total gonadotrophin dosage (3902.2±1332.03 IU) given to the patients with oocytes of normal morphology was marginally higher compared to oocytes with abnormal morphology (2443±903.09 IU), demonstrating statistical significance (p= 0.03). In oocytes with normal morphology, 40% of female partners were pregnant and 50% of pregnant women had miscarriages. In oocytes with abnormal morphology, a total of 28 M II oocytes were retrieved (mean 7±3.55). Despite achieving a satisfactory fertilization rate (78.57%) and cleavage rate (95.45%), implantation or pregnancy was not observed in all 4 patients (Table 3).

Comparison of outcomes between cryptozoospermia and azoospermia groups

A comparative analysis between the two groups showed no significant statistical correlation (p>0.05). In the Cryptozoospermia group (group 1), the pregnancy rate was 26.19% and in the azoospermia (group 2) was 31.58%. Miscarriages were observed only in oocytes with normal morphology in both groups. However, in group 2 patients with oocytes with abnormal morphology, implantation and pregnancy were not observed (Figure 1).

DISCUSSION

ICSI is the only available treatment for azoospermic and cryptozoospermic men.[17] The decision to use either ejaculated or testicular sperm in cases of cryptozoospermia varies in different clinical settings, however, the effectiveness of assisted reproductive technology in these cases is also greatly influenced by the quality of the oocyte. The pre-selection of oocytes exhibiting the highest developmental potential, determined through morphological criteria, holds significant importance for enhancing the efficiency of assisted reproduction technology. Consequently, many studies have documented the correlation, or lack thereof, between oocyte morphological abnormalities and ICSI outcomes.[12,14,18,19] The current study is the first comparative analysis to study the impact of oocyte quality on embryo development and pregnancy outcomes, specifically examining cases using ejaculated sperm from cryptozoospermia versus sperm obtained from testicular biopsy from azoospermia male partners.

Azoospermia, defined by the lack of sperm in the ejaculate, is categorized into obstructive and non-obstructive azoospermia. Sperm retrieval is frequently accomplished via surgical testicular sperm extraction and may demonstrate diminished quality, characterized by heightened DNA fragmentation.[20,21] High-quality oocytes play a crucial role in these situations, as they can repair sperm DNA damage and facilitate embryonic development.[22] Research indicates that younger women with superior ovarian reserves experience increased clinical pregnancy and live birth rates in azoospermia cases, highlighting the importance of oocyte quality.[23] In the present study, in patients who had oocytes with normal morphology, 40% of female partners were pregnant whereas in the group who had oocytes with abnormal morphology, no pregnancy was observed.

In cryptozoospermia, sperms are present in very low numbers in the ejaculate. The decision between using ejaculated sperm or surgically retrieved sperm is crucial because the fertilization rate is closely related to the maturity of sperm.[24] In the present study, ejaculated sperm from cryptozoospermia male partners were used for fertilization. The oocytes with the abnormal morphology group had 78.26% fertilization, while the normal oocyte group had 84.03%. Oocytes with normal morphology had greater implantation (20.89%) and pregnancy rates (34.48%) than oocytes with abnormal morphology.

Studies indicate that utilizing testicular spermatozoa instead of ejaculated spermatozoa for ICSI is beneficial for male partners with cryptozoospermia.[25,26] Testicular sperm was reported to have lower levels of sperm DNA fragmentation than ejaculated sperm. Also, testicular sperm had higher clinical pregnancy and live birth rates than ejaculated sperm and lower miscarriage rates but fertilization rates were similar, irrespective of the type of sperm used.[27] Conversely, another study indicated that regardless of the sperm source, the fertilization capability of injected spermatozoa was affected by their motility.[28]

The present study analysis indicated that the fertilization and cleavage rates were either marginally higher or similar when ICSI was conducted using ejaculated sperm from cryptozoospermia male partners in comparison to testicular sperm (azoospermia) (82.42% vs. 80.30% and 98.16% vs. 96.22%); however, these differences lacked statistical significance. Nonetheless, the impact of oocyte quality on pregnancy was found to be significant. In both the groups, normal oocyte sub-groups exhibited comparable pregnancy (26.19% vs. 31.58%), indicating that a good quality oocytes recovered from women with good ovarian reserve and <35 years of age can mitigate the male infertility factor, thereby emphasizing the significant role of oocyte competency in the fertilization process.[29,30] However, oocytes with abnormal morphology had lower pregnancy rates in both groups. Although embryos with normal morphology developed, they were unable to advance to the robust blastocyst stage due to compromised paternal chromosomes exhibiting high DNA fragmentation. Consequently, the resulting embryos are associated with implantation failures and unsuccessful pregnancies.[31] Similar findings were reported by a meta-analysis indicating low-quality oocytes combined with low-quality sperm, can lead to a reduced success rate in fertilization and pregnancy.[32]

The findings of our study indicate that optimizing oocyte quality through suitable ovarian stimulation protocols and ensuring retrieval from women with adequate ovarian reserves, can improve ART outcomes in complex cases of male infertility. However, the study is limited because of its small sample size and single-centred study.

CONCLUSION

The findings of this study indicate that oocyte quality is crucial in cases of male infertility, significantly impacting implantation and pregnancy rates. Comparable pregnancy rates were noted in both the cryptozoospermia and azoospermia groups. This indicates that the previously documented unsatisfactory ART outcomes in male infertility cases cannot be exclusively linked to sperm quality, as the presence of high-quality oocytes significantly influences ICSI results. Study findings indicate that knowledge of oocyte quality aids in counseling patients regarding treatment outcomes and in designing tailored treatment strategies. Further multicenter studies involving larger cohorts are essential to confirm the influence of oocyte quality on patients with poor sperm quality and reproductive success in these complex situations.

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