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Original Research Article

Intracytoplasmic sperm injection outcomes using ejaculated versus testicular sperm in severe male factor infertility: a retrospective study

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ABSTRACT

Background: Intracytoplasmic sperm injection (ICSI) using testicular sperm is commonly performed in severe male factor infertility. However, differences in sperm maturity and function between ejaculated and testicular sperm may influence reproductive outcomes.
Methods: This retrospective cohort study included 92 ICSI cycles from 84 couples treated between January 2018 and December 2024 at the Institute of Reproductive Medicine, Madras Medical Mission. Cycles were grouped according to sperm source: fresh ejaculate (n=61), fresh testicular sperm aspiration (fresh TESA) (n=25), and frozen testicular sperm aspiration (frozen TESA) (n=6). Outcomes assessed included fertilization, embryo arrest, embryo transfer (ET), and clinical pregnancy rate (CPR).
Results: Baseline characteristics were comparable among groups. The number of normally fertilized oocytes (2PN) was significantly higher in the fresh ejaculate group than in fresh and frozen TESA groups (7.08±4.4 vs 5.12±3.1 vs 3.17±2.9; p=0.021). The number of frozen embryos was also significantly higher with ejaculated sperm (p=0.02). Embryo arrest was highest in frozen TESA cycles (33.3%) compared to fresh TESA (8%) and ejaculated sperm cycles (3.3%) (p=0.02). Clinical pregnancy rate per cycle was higher with ejaculated sperm (50.8%) than fresh TESA (36%) and frozen TESA (0%) (p=0.042).
Conclusions: ICSI cycles using ejaculated sperm were associated with better embryological outcomes compared to testicular sperm cycles. Frozen testicular sperm cycles demonstrated relatively poorer embryo developmental outcomes; however, interpretation is limited by the small sample size. Despite lower overall success, testicular sperm remains an important option when ejaculated sperm is unavailable.

Keywords: Intracytoplasmic sperm injection, Testicular sperm aspiration, Male infertility, Embryo development, Clinical pregnancy rate, Assisted reproductive technology

INTRODUCTION

Infertility affects approximately 10% of the global population, with male factor infertility contributing to 30% of these cases.[1] Azoospermia, defined as absence of sperm in ejaculate, occurs in 1% of men.[2] This condition can be due to either bilateral obstruction of the male genital tract (obstructive azoospermia) or non-obstructive causes.[3] The introduction of intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of severe male factor infertility by enabling successful fertilization even in cases with markedly impaired semen parameters or azoospermia.[3] In azoospermia men, sperm retrieval techniques such as testicular sperm aspiration (TESA) allow the use of surgically retrieved sperm for assisted reproduction. However, testicular spermatozoa differ from

ejaculated sperm in terms of maturity, motility, chromatin packaging, and functional characteristics, which may influence fertilization, embryo development, and pregnancy outcomes following ICSI. In addition, cryopreservation of testicular sperm may further affect sperm viability and developmental potential.

Previous studies comparing ejaculated and testicular sperm in ICSI cycles have reported conflicting results regarding fertilization, embryo quality, and pregnancy outcomes. Therefore, the present study aimed to compare ICSI outcomes using fresh ejaculated sperm, fresh testicular sperm (fresh TESA), and frozen testicular sperm (frozen TESA) in patients with severe male factor infertility.

METHODS

Study design and participant selection

The present study was designed as a retrospective cohort study conducted over a period of seven years, from January 2018 to December 2024. The study included couples who underwent ICSI treatment for subfertility at the Institute of Reproductive Medicine, Madras Medical Mission.

Participants were selected based on predefined inclusion and exclusion criteria. The inclusion criteria comprised couples undergoing ICSI for severe male factor infertility. Severe male factor infertility was defined by sperm concentration less than 5 million/ml and/or the presence of severe abnormalities in sperm motility and morphology, including cases of azoospermia. The ejaculated sperm group included men with sperm concentration <5 million sperm/ml, while the testicular sperm groups included azoospermic men undergoing TESA.

Couples in whom donor sperm was used for fertilization were excluded from the study to maintain uniformity in evaluating reproductive outcomes associated with the male partner's sperm parameters and sperm retrieval methods. Y chromosome microdeletion analysis was carried out for all patients, and no microdeletions were detected in the study population.

A total of 92 ICSI cycles were assessed in the present study. Based on the sperm source used during the treatment cycle, the participants were categorized into three groups. Group A included cycles using fresh ejaculated sperm (61 cycles), group B consisted of cycles using fresh testicular sperm (fresh TESA) samples (25 cycles) and group C included cycles using frozen TESA samples (6 cycles).

All patients underwent standard ovarian stimulation, oocyte retrieval and ICSI procedures according to institutional protocols. For TESA samples, retrieved testicular tissue was mechanically processed in the embryology laboratory to isolate viable spermatozoa. Sperm selection for ICSI was performed by experienced embryologists based on the presence of motility and/or morphological viability under high-magnification microscopy. Fertilization assessment was performed approximately 16-18 hours after ICSI by identification of two pronuclei (2PN). Embryo development was subsequently monitored until embryo transfer or cryopreservation. Cycles with total fertilization failure or complete embryo arrest were considered as negative outcomes and were included under no-pregnancy outcomes based on intention-to-treat (ITT) analysis. In cycles where viable embryos were obtained, embryo transfer details were recorded and analysed for pregnancy-related outcomes.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac detected on transvaginal ultrasonography. Clinical pregnancy rate (CPR) per cycle was calculated as the number of clinical pregnancies divided by the total number of initiated ICSI cycles, expressed as a percentage. Clinical pregnancy rate per transfer was calculated as the number of clinical pregnancies divided by the total number of embryo transfer cycles, expressed as a percentage.

Statistical analysis

Statistical analysis was performed using SPSS version 30.0. Continuous variables were expressed as mean±standard deviation and categorical variables as frequency and percentage. Comparisons between groups were performed using one-way ANOVA for continuous variables and chi-square test or Fisher's exact test for categorical variables. A p value<0.05 was considered statistically significant.

RESULTS

A total of 84 patients undergoing 92 ICSI cycles were included in the study and were divided into 3 groups (A, B and C) of 61, 25 and 6 cycles respectively. Among these, total fertilization failure (TFF) was observed in 2 cycles, accounting for 2.2% of the study population. Embryo arrest occurred in 6 cycles, representing 6.5% of the total cycles assessed. These cycles were included as negative outcomes according to intention-to-treat analysis. Embryo transfer (ET) was successfully performed in 84 cycles, which constituted 91.3% of the total study cycles.

Baseline demographic, hormonal, and ovarian response characteristics were comparable among the three groups. The mean number of MII oocytes showed a decreasing trend across the groups with values of 8.34±4.7 in group A, 6.52±4.02 in group B and 4.67±2.8 in group C. However, this difference was not statistically significant (p=0.061). The mean number of normally fertilized oocytes (2PN) was significantly higher in the ejaculated sperm group compared to fresh and frozen testicular sperm groups (p=0.021). Similarly, the number of frozen embryos was significantly higher in the ejaculated sperm

group (p=0.02) (Table 1). The proportion of embryo arrest differed significantly among the groups, with the highest proportion observed in the frozen testicular sperm group (33.3%) compared to fresh testicular sperm (8%) and ejaculated sperm groups (3.3%) (p=0.02) (Table 2). The fresh ejaculated sperm group demonstrated the highest clinical pregnancy rate (CPR) per cycle (50.8%), followed by the fresh TESA group (36%), while no clinical pregnancies were achieved in the frozen TESA group. Similarly, CPR per transfer was higher in the ejaculated

sperm group (53.4%) compared to the fresh TESA group (40.9%), whereas no pregnancies were observed in the frozen TESA group. The differences in both CPR per cycle and CPR per transfer among the groups were statistically significant (p=0.041 and p=0.042, respectively) (Figure 1).

Table 1: Baseline characteristics and embryological outcomes among study groups.

Variables	Group A (fresh ejaculate) (n=61)	Group B (fresh TESA) (n=25)	Group C (frozen TESA) (n=6)	P value
Wife age (years)	32.54±4.9	30.48±4.3	29.83±2.1	0.104
Husband age (years)	37.90±3.9	36.64±5.6	34.17±3.5	0.108
Husband serum FSH	6.01±3.9	5.73±4.1	5.15±3.8	0.863
Husband testosterone	9.63±5.2	9.62±3.9	9.43±4.9	0.995
Total oocytes	11.28±6.05	9.28±5.7	7.83±3.7	0.188
Number of MII	8.34±4.7	6.52±4.02	4.67±2.8	0.061
Number of 2PN	7.08±4.4	5.12±3.1	3.17±2.9	0.021
Number of frozen embryos	6.44±4.4	4.36±3.1	2.5±3.39	0.02

Data are presented as mean±standard deviation (SD).

Table 2: ICSI outcomes among study groups.

	Group A (fresh ejaculate) (n=61)	Group B (fresh TESA) (n=25)	Group C (frozen TESA) (n=6)	P value
TFF*	1 (1.6%)	1 (4%)	0	0.738
Embryo arrest	2 (3.3%)	2 (8%)	2 (33.3%)	0.02
ET done*	58 (95.1%)	22 (88%)	4 (66.7%)	0.05
Pregnancy outcome				
No pregnancy	28 (45.9%)	16 (64%)	5 (83.3%)	0.041
Biochemical	2 (3.3%)	0	1 (16.7%)	

Data are presented as frequency (%),*TFF-Total fertilization failure; ET- Embryo transfer.

Table 3: Pooled analysis comparing ejaculated and testicular sperm groups.

Variables	Group A (ejaculated) (n=61)	Group B+C (testicular) (n=31)	P value
Wife age (years)	32.54±4.9	30.35±3.9	0.081
Husband age (years)	37.90±3.9	36.1±5.3	0.083
S. FSH	6.01±3.9	5.62±4.07	0.658
T. testosterone	9.63±5.2	9.58±4.1	0.961
Total oocytes	11.28±6.05	9.0±5.3	0.08
MII oocytes	8.34±4.7	6.16±3.8	0.03
No. of 2PN	7.08±4.4	4.74±3.1	0.01
No. of frozen embryos	6.44±4.4	4.0±3.2	0.008
TFF	1 (1.6%)	1 (3.2%)	0.622
Embryo arrest	2 (3.3%)	4 (12.9%)	0.096
ET done	58 (95.1%)	26 (83.9%)	0.071

Continuous variables are presented as mean±standard deviation (SD), and categorical variables as frequency (%).

A secondary pooled analysis comparing the combined fresh and frozen testicular sperm groups (group B+C) with the ejaculated sperm group (group A) demonstrated significantly higher numbers of normally fertilized oocytes (2PN) (p=0.01) and frozen embryos (p=0.008) in the ejaculated sperm group. However, differences in embryo

arrest, embryo transfer, and clinical pregnancy rate did not reach statistical significance (Table 3). In the pooled analysis, the clinical pregnancy rate (CPR) per cycle was higher in the ejaculated sperm group (50.8%) compared to the combined testicular sperm group (29%). Similarly, CPR per transfer was also higher in the ejaculated sperm group (53.4%) than in the testicular sperm group (34.6%). However, these differences did not reach statistical significance (CPR per cycle: p=0.130; CPR per transfer: p=0.273) (Figure 2).

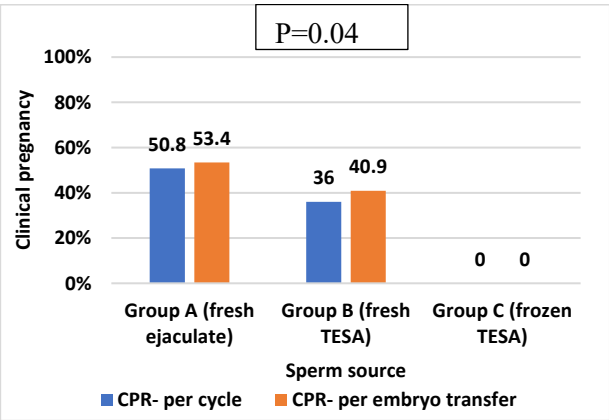

Figure 1: Clinical pregnancy outcome among study groups.

#Clinical pregnancy rates per initiated cycle and per embryo transfer were calculated using different denominators.

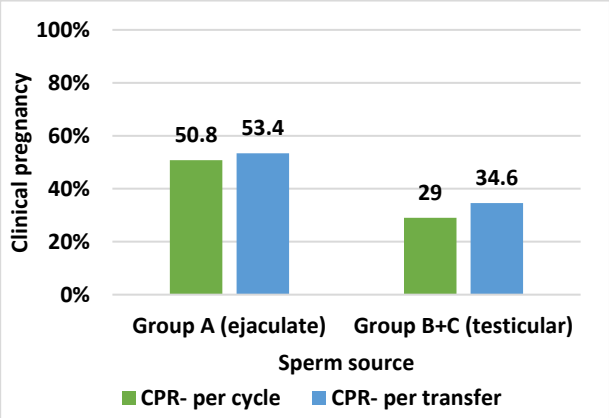

Figure 2: Clinical pregnancy outcome in pooled analysis.

#Clinical pregnancy rates per cycle and per transfer were calculated using different denominators. Differences between ejaculated and testicular sperm groups were not statistically significant (CPR per cycle: p=0.13; CPR per transfer: p=0.273).

DISCUSSION

The present study compared embryological and clinical outcomes of ICSI cycles using fresh ejaculated sperm, fresh testicular sperm, and frozen testicular sperm in severe male factor infertility. The findings demonstrated significantly better embryological outcomes in cycles utilizing ejaculated sperm, including higher numbers of normally fertilized oocytes (2PN) and frozen embryos. In contrast, frozen testicular sperm cycles showed higher embryo arrest rates and poorer embryo developmental outcomes.

The superior embryological performance observed with ejaculated sperm may be attributed to the greater functional maturity of ejaculated spermatozoa. During epididymal transit, sperm undergo maturation processes involving chromatin condensation, membrane remodelling, and acquisition of fertilizing capacity. Testicular spermatozoa are relatively immature and may exhibit altered chromatin packaging and reduced motility, which can adversely influence fertilization and embryo development following ICSI.

In addition, cryopreservation-related oxidative stress and membrane damage may further impair the developmental competence of frozen testicular sperm.[4,5] In the present study, the number of normally fertilized oocytes (2PN) and frozen embryos progressively declined from the ejaculated sperm group to the fresh and frozen testicular sperm groups. Embryo arrest rates were highest in frozen testicular sperm cycles. Although clinical pregnancy rates were lower in testicular sperm groups, acceptable pregnancy outcomes were achieved with fresh testicular sperm once embryo transfer was performed. These findings suggest that while embryological efficiency may be reduced in testicular sperm cycles, embryos that successfully progress to transfer may retain satisfactory implantation potential.

The secondary pooled analysis combining fresh and frozen testicular sperm cycles further demonstrated significantly higher numbers of fertilized oocytes, and frozen embryos in the ejaculated sperm group. However, differences in embryo transfer rates and clinical pregnancy rates did not reach statistical significance after pooling. This may indicate that sperm source predominantly influences early embryological events rather than implantation potential following successful embryo transfer.

Previous studies evaluating ICSI outcomes using surgically retrieved sperm have reported conflicting findings. Naru et al conducted a study involving 517 couples and reported no significant differences in pregnancy or miscarriage rates between surgically retrieved sperm and ejaculated sperm in azoospermic men undergoing ICSI.[6] Similarly, Vishwekar et al showed no statistically significant correlation was identified between the fertilization rate (72% versus 65%), implantation rate (58% versus 51%), and clinical pregnancy rate (CPR) (51% versus 44.82%) when comparing the ejaculated sperm group to the retrieved sperm group, respectively.[7] Jamal et al also observed no significant differences in fertilization or cleavage rates between ejaculated and surgically retrieved spermatozoa.[8]

Conversely, some studies have reported superior outcomes with testicular sperm in selected clinical situations. Mohammad et al conducted an analysis revealing that the

fertilization rates for the cohorts from which sperm were procured via ejaculation, PESA, and TESE were observed to be 72.1%, 73.6%, and 51.3%, respectively.[9] Negri et al demonstrated improved ICSI outcomes using testicular sperm in patients with persistent necrozoospermia, possibly due to reduced oxidative damage in testicular spermatozoa.[10] Similarly, Pasqualotto et al reported satisfactory reproductive outcomes with epididymal and testicular sperm in azoospermic patients.[11] A meta-analysis by Nicopoullos et al concluded that surgical sperm retrieval techniques provide acceptable reproductive outcomes in azoospermic men undergoing ICSI.[12]

Although the ejaculated sperm group demonstrated higher clinical pregnancy rates, pooled analysis showed no statistically significant difference in pregnancy outcomes once embryo transfer was achieved. This suggests that sperm source may have a greater influence on early embryological development than on implantation potential following successful embryo transfer. The poorer outcomes observed in frozen testicular sperm cycles in the present study may be related to the combined effects of sperm immaturity and cryopreservation-associated cellular damage. However, interpretation of frozen TESA outcomes should be made cautiously because of the relatively small sample size in this subgroup.

An important limitation of the present study is that the ejaculated sperm group consisted men with severe oligozoospermia, whereas the testicular sperm groups included azoospermic men undergoing sperm retrieval. Therefore, differences observed in reproductive outcomes may reflect both sperm source and the underlying severity of spermatogenic dysfunction. The study is also limited by its retrospective design, unequal distribution of study groups, and small number of frozen TESA cycles may have introduced selection bias and limited subgroup comparisons. Additionally, sperm selection for ICSI, particularly in TESA samples, may be subject to operator-dependent variability. Variations in gamete handling and embryology laboratory techniques between personnel may also have influenced embryological outcomes.

Despite these limitations, the study provides clinically relevant real-world data regarding the use of different sperm sources in severe male factor infertility and supports the continued role of testicular sperm retrieval in azoospermic patients undergoing ICSI.

CONCLUSION

ICSI cycles using ejaculated sperm were associated with better embryological outcomes compared to cycles utilizing testicular sperm. Frozen testicular sperm cycles were associated with relatively poorer embryo developmental outcomes, although interpretation is limited by the small sample size. Despite lower overall embryological outcomes, acceptable clinical pregnancy rates were achieved with fresh testicular sperm, supporting its continued role in the management of azoospermia and severe male factor infertility. Further prospective studies with larger sample sizes are required to better evaluate reproductive outcomes associated with fresh and cryopreserved testicular sperm.

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