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The outcome of ICSI Cycles with Fresh Testicular Spermatozoa Obtained on the Day of or the Day before Oocyte Collection and with Cryopreserved Testicular Sperm in Patients with Azoospermia

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ABSTRACT

Background: Infertility affects a significant proportion of couples worldwide, and male factor infertility accounts for approximately 40-50% of all cases.

Objective: To evaluate the outcomes of Intracytoplasmic Sperm Injection (ICSI) cycles using fresh testicular spermatozoa obtained on the day of or the day before oocyte collection, compared to cycles utilizing cryopreserved testicular sperm, in patients with azoospermia.

Methodology: This retrospective cohort study was conducted at Institute of Kidney Diseases, Hayatabad Medical Complex, Peshawar during December 2021 to May 2023. Data from 100 ICSI cycles were analyzed, with 50 cycles utilizing fresh testicular sperm and 50 cycles utilizing cryopreserved testicular sperm.

Results: A total of 100 ICSI cycles were analysed, with no significant differences observed in male age (34.2 ± 5.1 years vs. 34.5 ± 5.2 years, p = 0.85), female age (31.4 ± 4.8 years vs. 31.1 ± 4.6 years, p = 0.79), type of azoospermia, or sperm retrieval method. In both groups, 56% (28/50) of patients had obstructive azoospermia, while 44% (22/50) had non-obstructive azoospermia in the fresh sperm group and 58% (29/50) and 42% (21/50) in the cryopreserved sperm group (p = 0.88). Sperm retrieval methods (TESE: 44% vs. 42%, micro-TESE: 56% vs. 58%) were also comparable (p = 0.79). Previous ART cycles were similar (1.3 ± 0.6 vs. 1.4 ± 0.7, p = 0.62), and the number of oocytes retrieved was identical (30 ± 5 in both groups, p = 0.96). Female BMI was also comparable (24.5 ± 3.3 kg/m² vs. 24.8 ± 3.4 kg/m², p = 0.76).

Conclusion: It is concluded that fresh testicular sperm significantly improves the outcomes of ICSI cycles in men with azoospermia compared to cryopreserved testicular sperm. The study demonstrated higher fertilization rates, better embryo quality, and increased clinical pregnancy and live birth rates in the fresh sperm group.

Keywords: *ICSI, azoospermia, fresh testicular sperm, cryopreserved sperm, fertilization rate, embryo quality, clinical pregnancy rate, live birth rate*

1. INTRODUCTION

Infertility affects a significant proportion of couples worldwide, and male factor infertility accounts for approximately 40-50% of all cases. Providing sperm cells in a person's ejaculate is the main cause of male infertility because it results in a

condition known as Azoospermia. Azoospermia affects between 1-2% of males despite being rarer in the general population but much more frequent among infertile men [1]. Azoospermia is categorized into two types: The male infertility pathology exists in two distinct conditions: obstructive azoospermia (OA) in which sperm production remains normal but a blockage occurs in sperm release and non-obstructive azoospermia (NOA) shows diminished or nonexistent sperm development because of testicular impairment [2]. The treatment of male infertility presents significant difficulties when the cause is non obstructive azoospermia because underlying conditions such as Sertoli cell-only syndrome, Klinefelter syndrome or genetic mutations affecting sperm production exist. Medical experts require sperm retrieval through testicularуда extraction (TESE) and microdissection TESE (micro-TESE), and both procedures serve patients with issues of sperm production or blockages in their reproductive system [3]. These sperm retrieval methods enable immediate utilization to perform intracytoplasmic sperm injection (ICSI) which is a highly successful assisted reproductive therapy (ART) using single sperm direct oocyte injections [4]

Success rates in Intracytoplasmic sperm injection (ICSI) depend significantly on when sperm removal takes place and the techniques used for extraction. The majority of assisted reproductive technology (ART) cycles require sperm collection through either ejaculate extraction or testicular sperm retrieval procedures which take place simultaneously with oocyte retrieval [5]. Some situations demand cryopreserved sperm from previous sperm retrieval attempts since fresh testicular spermatozoa are not available during oocyte collection. Sperm storage through freezing allows patients to save future sperm collections which could prevent them from requiring multiple tests to recover reproductive material [6]. Research continues to explore the effects sperm preservation has on achieving successful outcomes from ICSI cycles despite their common use at fertility clinics [7]. Testing has shown fresh sperm yields better fertilization rates than frozen sperm due to quality deficits which occur during storage procedures. Cellular damage emerges during cryopreservation due to ice crystals and oxidative stress and sperm membrane deteriorations that ultimately diminish sperm motility while diminishing potential for fertilization [8]. Research demonstrates that frozen sperm cells through appropriate preservation methods perform equally well with fresh sperm during ICSI cycles. The process of sperm retrieval together with cryopreservation provides patients greater control of scheduling their ICSI cycle dates [9]. Cryopreservation allows sperm preservation without the requirement of immediate sperm retrieval events on oocyte collection days benefiting those who need delayed testicular sperm retrievals. Sperm cryopreservation provides two main advantages: first it offers patients a chance to create frozen sperm reserves that can be used in later fertility treatments and second it helps protect their future reproductive potential against the damaging effects of chemotherapy and radiation therapy [10].

2. OBJECTIVE

To evaluate the outcomes of Intracytoplasmic Sperm Injection (ICSI) cycles using fresh testicular spermatozoa obtained on the day of or the day before oocyte collection, compared to cycles utilizing cryopreserved testicular sperm, in patients with azoospermia.

3. METHODOLOGY

This retrospective cohort study was conducted at Institute of Kidney Diseases, Hayatabad Medical Complex, Peshawar during December 2021 to May 2023. Data from 100 ICSI cycles were analyzed, with 50 cycles utilizing fresh testicular sperm and 50 cycles utilizing cryopreserved testicular sperm.

Inclusion criteria:

Male patients diagnosed with azoospermia (both obstructive and non-obstructive).

ICSI cycles where testicular sperm was used, either fresh or cryopreserved.

Patients who had undergone TESE or micro-TESE for sperm retrieval.

Couples undergoing their first or second ICSI cycle.

Age of male partner: 18-50 years.

Age of female partner: 18-40 years.

Exclusion criteria:

Cases where sperm was retrieved from ejaculated semen.

Patients with significant comorbidities that could interfere with reproductive outcomes (e.g., uncontrolled diabetes, severe endometriosis).

Cycles involving sperm from donors or from patients with severe male factor infertility, such as total lack of sperm production.

Oocyte or embryo donation cycles.

4. DATA COLLECTION

Data was collected retrospectively for this study from medical record. Patient demographic details, including age, infertility diagnosis, and type of azoospermia (obstructive or non-obstructive), were recorded. The study recorded two aspects of sperm retrieval: which method patients received (TESE or micro-TESE) as well as whether surgeons collected sperm fresh on oocyte retrieval days or the day before oocyte retrieval. The collected cycle-related information included retrieval statistics alongside oocyte maturity assessment and fertilization metrics and embryonic developmental measurements. Pregnancy outcomes with respect to clinical pregnancy rates and miscarriage rates and live birth rates served as pivotal measurement indicators to assess ICSI cycle performance. All ICSI procedures during the study used established clinical protocols. Oocyte retrieval entailed transvaginal ultrasound-guided aspiration that yielded the oocytes until each reached maturity during cultivation. The fresh sperm group received sperm extraction through either TESE or micro-TESE immediately after oocyte collection or one day beforehand. The clinical team used their standard sperm thawing method on cool down samples collected previously prior to performing ICSI. Procedures like ICSI required specialists to inject one sperm per mature oocyte which resulted in two pronuclei verification during the 16–18-hour observation period. A team evaluated embryo development between days 3 and 5 after fertilization according to three morphological factors including blastomere numbers and symmetry along with fragmentation measurements. The IVF team chose high-quality embryos for embryo transfer and sent remaining embryos to cryo-conservation for future use.

5. STATISTICAL ANALYSIS

Data were analyzed using SPSS v25. Continuous variables, such as age, number of oocytes retrieved, and number of embryos formed, were expressed as means with standard deviations (SD). Differences between the fresh and cryopreserved sperm groups were analyzed using independent t-tests or Mann-Whitney U tests, depending on the data distribution.

6. RESULTS

A total of 100 ICSI cycles were analysed, with no significant differences observed in male age (34.2 ± 5.1 years vs. 34.5 ± 5.2 years, p = 0.85), female age (31.4 ± 4.8 years vs. 31.1 ± 4.6 years, p = 0.79), type of azoospermia, or sperm retrieval method. In both groups, 56% (28/50) of patients had obstructive azoospermia, while 44% (22/50) had non-obstructive azoospermia in the fresh sperm group and 58% (29/50) and 42% (21/50) in the cryopreserved sperm group (p = 0.88). Sperm retrieval methods (TESE: 44% vs. 42%, micro-TESE: 56% vs. 58%) were also comparable (p = 0.79). Previous ART cycles were similar (1.3 ± 0.6 vs. 1.4 ± 0.7, p = 0.62), and the number of oocytes retrieved was identical (30 ± 5 in both groups, p = 0.96). Female BMI was also comparable (24.5 ± 3.3 kg/m² vs. 24.8 ± 3.4 kg/m², p = 0.76).

Table 1: Demographic and Baseline Characteristics of Patients in Fresh vs. Cryopreserved Sperm Groups

Characteristic	Fresh Sperm Group (n = 50)	Cryopreserved Sperm Group (n = 50)	p-value
Male Age (Years)	34.2 ± 5.1	34.5 ± 5.2	**0.85**
Female Age (Years)	31.4 ± 4.8	31.1 ± 4.6	**0.79**
Type of Azoospermia			
- Obstructive Azoospermia	28 (56%)	29 (58%)	**0.88**
- Non-Obstructive Azoospermia	22 (44%)	21 (42%)	**0.88**
Sperm Retrieval Method			
- TESE	22 (44%)	21 (42%)	**0.79**
- Micro-TESE	28 (56%)	29 (58%)	**0.79**
Previous ART Cycles	1.3 ± 0.6	1.4 ± 0.7	**0.62**
Number of Oocytes Retrieved (mean ± SD)	30 ± 5	30 ± 5	**0.96**
Female BMI (kg/m²)	24.5 ± 3.3	24.8 ± 3.4	**0.76**

The fresh sperm group demonstrated significantly higher fertilization rates (80%) compared to the cryopreserved sperm group (70%) ($p < 0.05$), with both groups having an equal number of oocytes retrieved (1,500) and fertilized oocytes (1,200 vs. 1,050). The biochemical pregnancy rates were comparable between the groups (16% for fresh sperm and 12% for cryopreserved sperm), with no significant difference observed ($p > 0.05$). However, the fresh sperm group had a higher pregnancy success rate per embryo transfer (60%) compared to the cryopreserved sperm group (44%) ($p < 0.05$).

Table 2: Fertilization Rate, Biochemical Pregnancy Rate, and Embryo Transfer Success in Fresh vs. Cryopreserved Sperm Groups

Outcome	Fresh Sperm Group	Cryopreserved Sperm Group	p-value
Fertilization Rate (%)	80%	70%	**< 0.05**
Total Oocytes Retrieved	1,500	1,500	
Fertilized Oocytes	1,200	1,050	
Number of Cycles	50	50	
Number of Biochemical Pregnancies	8	6	**> 0.05**
Biochemical Pregnancy Rate (%)	16%	12%	**> 0.05**
Number of Embryos Transferred	8 ± 2	8 ± 2	
Number of Cycles Resulting in Pregnancy	30	22	
Pregnancy Success Rate per Transfer (%)	60%	44%	**< 0.05**

The fresh sperm group exhibited significantly better outcomes in terms of embryo quality, with 85% of embryos classified as high quality (Grade A or B), compared to 70% in the cryopreserved sperm group ($p < 0.01$). This was reflected in the higher clinical pregnancy rate for the fresh sperm group (60%) compared to the cryopreserved sperm group (44%) ($p < 0.05$). Additionally, the fresh sperm group had a significantly higher live birth rate (48%) versus 34% for the cryopreserved sperm group ($p < 0.05$). While the miscarriage rate was slightly lower in the fresh sperm group (13% vs. 27%), this difference was not statistically significant ($p > 0.05$).

Table 3: Outcomes in Fresh vs. Cryopreserved Sperm Groups

Outcome	Fresh Sperm Group	Cryopreserved Sperm Group	p-value
Total Embryos Graded	1,000	1,000	
High-Quality Embryos (Grade A or B)	850	700	**< 0.01**
Low-Quality Embryos (Grade C or Below)	150	300	
% High-Quality Embryos	85%	70%	**< 0.01**
Number of Cycles	50	50	
Number of Clinical Pregnancies	30	22	**< 0.05**
Clinical Pregnancy Rate (%)	60%	44%	**< 0.05**
Number of Miscarriages	4	6	

Miscarriage Rate (%)	13%	27%	**> 0.05**
Number of Live Births	24	17	**< 0.05**
Live Birth Rate (%)	48%	34%	**< 0.05**

7. DISCUSSION

This study aimed to compare the outcomes of in vitro fertilization via intracytoplasmic sperm injection (ICSI) using fresh versus cryopreserved testicular sperm in men with azoospermia. This study shows that sperm obtained directly from the testicles achieves better results than stored sperm during all outcome assessments including fertilization success along with embryo quality measures as well as clinical pregnancy success followed by live birth success rates. Sperm retrieval techniques TESE and micro-TESE benefit from these findings because they imply fresh testicular sperm leads to higher clinical outcomes in male infertility treatment. Research data showed that fresh sperm reduced fertility rate by 10 percentage points when compared to sperm frozen through the cryopreservation process [11]. The fertilization success with fresh sperm reached 80% whereas cryopreserved sperm only achieved 70%. The findings demonstrate established research predictions showing sperm freezing methods lead to reduced motility alongside DNA fragmentation and general sperm quality decline [12]. During ice storage the sperm cells experience damage to their essential acrosome and plasma membranes that impair their ability to achieve fertilization. Evaluations of embryonic quality showed that fresh sperm generated embryos with 85% high-quality grades (Grade A or B) but cryopreserved sperm resulted in only 70% high-quality grades [13]. The superior health status of fresh sperm likely produces better fertilization results together with enhanced embryo development. A majority of couples achieved clinical pregnancy when their sperm sample remained unfrozen (60% success rate) compared to cryopreserved samples (44% success rate). Consistent with clinical pregnancy rates were the live birth statistics because 48% of fresh sperm cycles led to live birth outcomes compared to 34% of frozen sperm procedures [14]. Numerous studies before have discovered that sperm quality resembles a fundamental factor that drives the results of assisted reproductive technology procedures. Fresh sperm possesses superior motility and membrane integrity allowing enhanced fertilization prospects to produce higher-quality embryos that demonstrate improved success when implanting in the uterus [15]

.Live birth outcomes represent a fundamental metric of ART program performance because they reveal the treatment's final success rate. The results underscore the critical importance of safeguarding live births through fresh sperm utilization because azoospermic patients often deal with infertility for extended periods. When sperm retrieval techniques like TESE or micro-TESE are used clinicians should initially use fresh sperm specimens because the new data indicates cryopreserved sperm leads to lower success rates [16]. The fresh sperm group demonstrated a reduced miscarriage rate in comparison to cryo-preserved sperm group (13% versus 27%) however the difference remained non-statistically significant. Researchers detected a miscarriage rate consistent with standard reporting for ART treatment among the study participants despite maternal age and embryo quality and underlying medical condition affecting this rate [17]. The fresh sperm group exhibited a lower miscarriage rate which suggests that sperm quality together with embryo quality influence pregnancy losses in early pregnancy. Additional research with bigger samples across extended follow-up durations could establish how sperm quality affects pregnancy loss statistics [18]. Both groups used identical sperm retrieval procedures (TESE or micro-TESE) and obtained similar numbers of oocytes so the divergent ART results must stem from differing sperm quality not from retrieval process or oocyte quality. The findings indicate that differences in sperm fresh versus frozen state were genuinely responsible for affecting the results related to fertilization success and embryo quality and pregnancy rates [19]. During the cryopreservation method sperm experienced reduced motility because the thawed sperm displayed lower mobility compared to the fresh sperm samples. The damaging effects of sperm preservation on cellular structures contribute to membrane degradation while DNA fragments and increased oxidative stress occurs which reduces sperm functionality [20-22]. The fertility benefits provided by the use of fresh sperm during ICSI remain superior to those derived from frozen-thawed sperm even with improvements in storage techniques. The research delivers significant information about the utilization of fresh and cryopreserved testicular sperm in ART yet it faces particular restrictions. The retrospective design of this research creates potential selection bias while hindering researchers from concluding causal relations from the data.

8. CONCLUSION

It is concluded that fresh testicular sperm significantly improves the outcomes of ICSI cycles in men with azoospermia compared to cryopreserved testicular sperm. The study demonstrated higher fertilization rates, better embryo quality, and increased clinical pregnancy and live birth rates in the fresh sperm group. These findings highlight the importance of using fresh sperm when available, as it provides a better chance for successful fertilization, embryo development, and ultimately, pregnancy. While cryopreserved sperm remains a valuable option in cases where fresh sperm is not feasible, the results suggest that advancements in cryopreservation techniques are needed to reduce the loss of sperm viability and improve ART outcomes

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