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Research Article

Clinical Pregnancy and Live Birth Outcomes after Intracytoplasmic Injection of Fresh versus Frozen Testicular Sperm

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This study aimed to investigate the potential benefits of frozen testicular sperm and to retrospectively analyze the clinical pregnancy and live birth outcomes following intracytoplasmic injection of fresh or frozen testicular sperm. A total of 468 infertile couples undergoing intracytoplasmic sperm injection cycles utilizing either fresh or frozen testicular sperm extracted between January 2017 and December 2021 were included in this analysis. Participants were categorized into two groups: those utilizing fresh testicular sperm ($n = 324$) and those utilizing frozen testicular sperm ($n = 144$). Outcome measures encompassed fertilization rate, embryo development, clinical outcomes, and birth status of infants. Statistical analysis revealed no significant differences in 2PN fertilization rate, Day 3 (D3) available embryo rate, high-quality embryo rate, blastocyst formation rate, clinical pregnancy rate, live birth rate, and preterm birth rate between the two groups. Although the preterm birth rate in the frozen testicular sperm group was lower compared to the fresh testicular sperm group, the difference was not statistically significant (5.6% vs. 15.1%). Notably, there were no discernible distinctions in clinical pregnancy and birth outcomes, as well as infant birth parameters, between the fresh and frozen testicular sperm groups. These findings suggest that frozen testicular sperm holds practical value and warrants consideration for clinical application.

1. Introduction

Assisted reproductive technology (ART) has revolutionized the prospects of parenthood for infertile couples by enabling the conception of biological offspring. Male infertility contributes significantly to the complexity of infertility factors, accounting for up to 50% of cases [1]. Recent studies in murine models have implicated various antihypertensive medications in male fertility reduction [2, 3], underscoring the need for comprehensive human studies to elucidate the potential reversible or permanent effects of these drugs on male fertility [4]. Therefore, it becomes imperative to meticulously select antihypertensive therapies, taking into account their implications for male reproductive health and fertility desires. Additionally, males with diminished concentrations of total testosterone often exhibit a higher incidence of male accessory gland inflammation (MAGI) and compromised sperm quality [5]. MAGI manifests in inflammatory and microbial forms [6], with elevated leukocyte levels in ejaculated semen adversely affecting sperm structural integrity, including the acrosome, nucleus, and tail. Notably, treatment with transdermal testosterone has demonstrated a propensity to improve sperm morphology [5]. Azoospermic, characterized by the absence of identifiable sperm in ejaculated semen samples, accounts for approximately 5%–10% of male infertility cases [7], representing an extreme form of infertility [8].

The integration of intracytoplasmic sperm injection (ICSI) with testicular sperm extraction (TESE) has emerged as a promising approach for azoospermic patients to achieve conception [7]. Simultaneous performance of TESE and oocyte pick-up (OPU) during an ICSI cycle, utilizing fresh sperms obtained through TESE, offers a pragmatic strategy to preserve any surplus sperms for future cycles, thus obviating the need for repetitive testicular biopsies.

Autologous sperm cryopreserve primarily serves as a cornerstone for male fertility preservation, aligning with the concept of "reproductive insurance." Indications for male fertility preservation encompass scenarios such as diseases or medical treatments posing a threat to fertility, environmental exposures affecting fertility, anticipated declines in semen quality, and the proactive preservation of sperm for future fertility needs. Moreover, ART implementation may necessitate cryopreserved sperm in situations where fresh semen is unavailable on the day of oocyte retrieval due to retrieval difficulties or logistical constraints, or when individuals exhibit severe semen abnormalities, necessitating alternative sperm retrieval methods such as surgical retrieval or assisted methods [9, 10].

Despite the growing body of evidence suggesting comparable outcomes between fresh and frozen sperms from TESE in ICSI cycles [11], existing studies lack comprehensive neonatal birth parameters, and some fail to provide detailed embryological parameters beyond top-quality embryos [7]. Thus, the present study aims to address this gap by comparing clinical pregnancy and live birth outcomes in ICSI cycles utilizing fresh versus frozen testicular sperm.

2. Materials and Methods

2.1. Study Population. Data pertaining to patients undergoing ICSI cycles utilizing either fresh or frozen sperms from TESE between January 2017 and December 2021 were compiled. The inclusion criteria were as follows: (i) males diagnosed with azoospermia; (ii) males diagnosed with severe oligozoospermia or asthenozoospermia; (iii) males diagnosed with oligozoospermia or asthenozoospermia; (iv) all cycles, including repeated cycles. Involving ICSI combined with either fresh or frozen sperms from TESE; (v) autologous fresh testicular sperm; (vi) utilization of autologous frozen testicular sperm. Exclusion criteria included: (i) patients undergoing conventional in vitro fertilization, short-time fertilization, half-ICSI or preimplantation genetic testing cycles; (ii) cycles involving in vitro maturation; (iii) cycles involving oocytes vitrification freezing; (iv) previous cycles experiencing complete fertilization failure in conventional IVF for undetermined reasons; (v) previous cycles exhibiting general abnormalities in the zona pellucida of oocytes, such as serrated/waxy features; (vi) utilization of donor fresh sperm; (vii) utilization of donor frozen sperm. A total of 468 cycles were analyzed, with 324 assigned to the fresh testicular sperm group and 144 to the frozen testicular sperm group.

2.2. Testicular Sperm Preparation. Partial testicular tissues were obtained via percutaneous testicular sperm aspiration and placed in a culture medium-filled dish. The process of testicular sperm preparation involved the following steps: (i) Preparation of testicular tissue suspension: Testicular tissue was disrupted using a 1 ml syringe, and motile sperm were under a microscope. Subsequently, the dish was placed in an incubator for a minimum of 30 min. (ii) Sedimentation: The entire testicular tissue suspension was transferred into a conical bottom centrifuge tube and allowed to stand upright. After 8–10 min, the upper layer of liquid was carefully aspirated into a new conical bottom centrifuge tube. (iii) Centrifugation: The retained upper layer liquid was centrifuged at 300 g for 10 min. Following centrifugation, the supernatant liquid was discarded, leaving approximately 0.1 ml of sediment. This sediment was thoroughly mixed and placed in the incubator for later use.

2.3. Freezing and Thawing of Testicular Sperm. Micro-vitrification was employed to freeze fresh testicular sperm obtained from testicular biopsy or surplus after ICSI. The procedure proceeded as follows: (i) The front end of a sterile straw (CASSOU straw 005565, 005569) was sharpened. (ii) A foam box containing liquid nitrogen was positioned at a 5 cm distance from the foam box mouth. (iii) An equal volume of sperm freezing solution (SAGE, USA) was added to the 0.1 ml of sediment and thoroughly mixed. (iv) Approximately 20 μl of the aforementioned mixture was dripped onto the front end of the sterile straw and exposed above the liquid nitrogen. (v) After approximately 40 s, the mixture turned white and solidified. Subsequently, it was placed into liquid nitrogen and enclosed in a casing pipe for storage. Frozen testicular sperm were preserved in liquid nitrogen for future use.

Thawing procedures were conducted as follows: (i) The straw containing frozen testicular sperm was swiftly removed from the liquid nitrogen and immersed in oil at 37°C for approximately 10 s. (ii) The frozen testicular sperm were gently extricated into a droplet on the ICSI dish using a syringe. (iii) After approximately 15–20 min, motile sperm could be observed under the microscope for subsequent use in ICSI procedures.

2.4. Fertilization, Embryo Culture, and Evaluation. ICSI utilizing either fresh or frozen testicular sperm was conducted subsequent to OPU. Normal fertilization was defined by the presence of two pronuclei and the second polar body in zygotes observed 16–20 hr postinjection. Zygotes were cultured in G1-plus medium (Vitrolife, Sweden) on Day 1 (D1), followed by transfer to G2-plus medium (Vitrolife, Sweden) on Day 3 (D3), maintained under conditions of 5% O_2 and 6% CO_2.

Evaluation of cleavage-stage embryos was performed according to the criteria established by the Istanbul Consensus [12]. The scoring format comprised three numerical components representing cell number, stage-specific cell size, and fragmentation, respectively. For example, 8-cell grade I embryos were classified as "811," while 8-cell grade II embryos with uneven cell size were designated as "821," and 8-cell grade II embryos with less than 10% fragmentation were denoted as "812." Day 3 (D3) high-quality embryos were defined as those meeting the criteria of "811, 812, 821, 711, and 911."

Table 1: Baseline characteristics of the two study groups.

Characteristics	Fresh testicular sperm ($N=324$)	Frozen testicular sperm ($N=144$)	Wilcoxon	
			Z value	P value
Female age (year)	32.00 (28.00, 36.00)	31.00 (28.00, 35.00)	0.529	0.597
Male age (year)	34.00 (30.25, 39.00)	34.00 (30.00, 39.00)	0.499	0.618
Infertility factors	—	—	1.786	0.181
Only male factors	283 (87.35%)	130 (90.28%)	—	—
Male and female factors	41 (12.65%)	14 (9.72%)	—	—
No. of oocytes retrieved	12.00 (7.00, 17.00)	13.00 (6.00, 18.00)	0.075	0.940
FSH (mIU/ml)	7.60 (6.47, 8.83)	7.55 (6.33, 9.22)	0.370	0.711
LH (mIU/ml)	4.08 (3.02, 5.73)	4.19 (3.31, 5.53)	0.600	0.549
E_2 (pg/ml)	42.00 (32.00, 56.00)	42.15 (32.00, 61.25)	0.390	0.697
Endometrial thickness (mm)	11.20 (9.90, 13.60)	10.85 (9.00, 13.00)	1.848	0.065

FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol.

Table 2: Comparison of embryonic parameters between the two study groups.

Outcomes	Fresh testicular sperm ($N=324$)	Frozen testicular sperm ($N=144$)	χ^2 value	P value
Oocyte maturation rate (%)	78.1 (3268/4185)	78.3 (1420/1813)	0.041	0.840
2PN fertilization rate (%)	69.6 (2273/3268)	68.2 (968/1420)	0.889	0.346
D3 available embryo rate (%)	90.1 (1976/2192)	88.5 (814/920)	1.943	0.163
D3 high-quality embryo rate (%)	29.6 (648/2192)	27.6 (254/920)	1.201	0.273
Blastocyst formation rate (%)	51.1 (582/1139)	53.3 (255/478)	0.683	0.409

2.5. Embryo Transfer and Clinical Outcome. Fresh embryos were transferred either on Day 3 (D3) or Day 5 (D5) post-OPU. Blood human chorionic gonadotropin (HCG) level was assessed 12–14 days after embryo transfer, with an HCG concentration exceeding 10 μ/ml indicative of a positive result. Ultrasound examination was utilized to detect fetal heartbeat, confirming clinical pregnancy. Follow-up assessments were conducted until the birth of the infant. Three cases with incomplete follow-up data were excluded from analyses pertaining to early miscarriage rate and live birth rate. Premature birth was defined as gestational age greater than or equal to 28 but less than 37 weeks, while low birth weight was defined as less than 2.5 kg.

2.6. Statistical Analysis. Data were analyzed using SPSS version 25.0. Continuous variables were expressed as median (25th percentile, 75th percentile) (M (P25, P75)) and analyzed using the nonparametric rank-sum test or presented as mean ± standard deviation (SD) and analyzed using T-test. Categorical variables were presented as frequency and percentage and analyzed using the Chi-square test. A P value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics of the Two Groups. There were no significant differences observed in male or female age, the number of retrieved oocytes, female basal hormone levels (FSH, LH, and E_2), and endometrial thickness between the fresh testicular sperm group and frozen testicular sperm group ($P>0.05$) (Table 1).

3.2. Embryo Development Parameters Analysis. No significant differences were found between the fresh testicular sperm group and frozen testicular sperm group regarding oocyte maturation rate, normal fertilization rate, Day 3 (D3) available embryo rate, Day 3 (D3) high-quality embryo rate, and blastocyst formation rate ($P>0.05$) (Table 2).

3.3. Comparison of Clinical Pregnancy and Live Birth Outcomes. A total of 317 fresh embryo transfer cycles (fresh testicular sperm group: $n=235$, frozen testicular sperm group: $n=82$) were conducted. There were no significant differences observed in intrauterine implantation rate, clinical pregnancy rate, live birth rate, birth weight, and birth length between the two groups ($P>0.05$) (Table 3). Subgroup analysis further revealed that the clinical pregnancy rate of fresh Day 3 (D3) or Day 5 (D5) embryos in the fresh testicular sperm group was comparable to that of the frozen testicular sperm group ($P>0.05$) (Table 4).

4. Discussion

Male infertility can arise from various conditions such as varicocele, cryptorchidism, and hypogonadism or present as idiopathic infertility characterized by sperm parameters below the World Health Organization reference values, including oligozoospermia, asthenozoospermia, and/or teratozoospermia [13]. Myoinositol, known to regulate sperm motility, capacitation, and acrosome reaction [14], has shown promise as a supplement for males with idiopathic infertility, as evidenced by a double-blind, randomized, placebo-controlled trial [15]. Furthermore, studies have reported increased fertilization

Table 3: Comparison of clinical pregnancy and birth outcomes between the two study groups.

Outcomes	Fresh testicular sperm ($N = 324$)	Frozen testicular sperm ($N = 144$)	Median difference (95% CI)	χ^2 value	
				t Value	P value
Average number of implanted embryos	1.73 ± 0.5	1.78 ± 0.45	—	−0.85	0.398
Implantation rate	136/406 (33.5%)	47/146 (32.2%)	—	0.083	0.774
Early miscarriage rate	10/112 (8.93%)[a]	2/41 (4.88%)[b]	—	0.24	0.627
Clinical pregnancy rate	114/235 (48.5%)	42/82 (51.2%)	—	0.178	0.673
Live birth rate	95/233 (40.8%)[a]	36/81 (44.4%)[b]	—	0.333	0.564
Premature birth rate	13/95 (13.7%)	2/36 (5.6%)	—	0.994	0.319
Deformity rate	3/114 (2.6%)	1/43 (2.3%)	—	0.000	1.000
Incidence of low birth weight	28/112 (25.0%)	6/43 (13.95%)	—	2.21	0.137
Birth weight (kg)	3.10 (2.60, 3.36)	2.83 (2.50, 3.15)	0.05 (−0.15−0.24)	0.372	0.710
Birth length (cm)	50.00 (48.00, 50.00)	49.00 (48.00, 50.50)	0.00 (−1.00−1.00)	0.379	0.704

[a]Two cases were lost to follow-up after clinical pregnancy with the use of fresh sperm. [b]One case was lost to follow-up after clinical pregnancy with the use of frozen sperm.

Table 4: Subgroup analysis based on transplant day.

Day of embryo transfer	Cycles (n)	CPR (%)	χ^2 value	P value
D3 group	—	—	3.811	0.149
Fresh	191	92 (48.2)	—	—
Frozen	73	39 (53.4)	—	—
D5 group	—	—	2.005	0.367
Fresh	29	17 (58.6)	—	—
Frozen	4	1 (25.0)	—	—

rates and improved embryo quality in ICSI cycles with myo-inositol supplementation [16].

Several investigations have demonstrated satisfactory clinical outcomes in ICSI cycles utilizing frozen testicular sperm, comparable to those with fresh testicular sperm [17–20]. Our findings corroborate these results, revealing no significant differences in clinical pregnancy rate, miscarriage rate, or live birth rate between fresh and frozen testicular sperm groups. Moreover, ICSI using frozen testicular sperm did not elevate the incidence of birth defects or impact birth weight and length. Notably, our study also revealed no disparities in normal sperm morphology and DNA fragmentation index between the micro-straw and cryo tube groups, affirming the efficacy of sperm cryopreservation with micro-straw [17]. Consequently, micro-vitrification and preservation of fresh testicular sperm from biopsy or surplus after ICSI emerge as feasible approaches in clinical settings, offering patients the advantage of avoiding repeated testicular sperm aspiration, thus mitigating patient discomfort and reducing surgical costs.

Huang et al. [17] found that the risk of premature birth in ART-assisted pregnancies is 1.72 times higher than that in natural pregnancies (OR = 1.72, 95% CI (1.60, 1.85)), so it is worthy to notice the risk of premature birth in ART. Our study observed a slightly higher preterm delivery rate in the fresh testicular sperm group compared to the frozen testicular sperm group, albeit not statistically significant (13.7% vs. 5.6%, $P = 0.319$). Similarly, the multiple pregnancy rate was comparable between the two groups (21.1% vs. 19.4%, $P > 0.05$). These findings align with prior research by Wu et al. [21], who reported no significant differences in preterm birth rate ($P = 0.224$) and multiple pregnancy rate ($P = 0.329$) between frozen and fresh testicular sperm groups. Furthermore, our study revealed a marginally higher clinical pregnancy rate (51.2% vs. 48.5%, $P = 0.673$) and live birth rate (44.4% vs. 40.8%, $P = 0.564$) in the frozen testicular sperm group, consistent with findings by Meng et al. [22], who attributed these outcomes to the selection of with high motility during frozen–thawed process.

Subgroup analysis unveiled a higher clinical pregnancy rate for Day 3 (D3) embryo transfer in the frozen testicular sperm group compared to the fresh testicular sperm group (58.6% vs. 48.2%, $P = 0.489$), further affirming the efficacy of frozen testicular sperm. However, due to the limited number of patients undergoing Day 5 (D5) blastocyst transfer in the frozen testicular sperm group, subgroup analysis was not feasible owing to sample size bias.

5. Conclusion

Micro-vitrification techniques offer a practical and accessible alternative for testicular sperm cryopreservation, circumventing the need for costly instrumentation and ethical concerns associated with other methods. Our findings demonstrate that frozen testicular sperm exhibit comparable clinical outcomes to fresh testicular sperm, with high sperm recovery rates. The simplicity and convenience of micro-vitrification preservation underscore its potential for widespread adoption in clinical practice.

Data Availability

All data relevant to the study are included in the manuscript.

Ethical Approval

The study received approval from the Institutional Ethical Committee of Sun Yat-sen Memorial Hospital (approval number: SYSKY-2023-209-01).

Consent

Written informed consent was obtained from all participants prior to their inclusion in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Yuqin Zhu and Nengyong Ouyang contributed significantly to the study design and original draft preparation. Xiaohui Ji conducted out data collection and analysis. Qingxue Zhang and Haijing Zhao supervised the project, contributed to validation, and participated in writing and reviewing the final version of the manuscript. All authors contributed to the final version of the manuscript. Yuqin Zhu and Nengyong Ouyang are the co-first authors.

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