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Intracytoplasmic sperm injection with fresh versus cryopreserved testicular sperm in azoospermic patients

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Abstract

Background: The purpose of this study is to compare the outcome of intracytoplasmic sperm injection (ICSI) using fresh sperm versus frozen-thawed sperm in both obstructed and non-obstructed azoospermias. This retrospective study included 159 ICSI cycles from 126 couples. In 91 obstructed azoospermia cases, 66 cycles were treated with fresh testicular sperm and 25 cycles were treated with frozen-thawed testicular samples. In 68 non-obstructed azoospermia cases, 32 cycles were treated with fresh testicular sperm and 36 cycles were treated with frozen-thawed testicular sperm, and the main measure and outcomes calculated are fertilization rate, clinical pregnancy, and live birth rate.

Results: In case of obstructed azoospermia, there were no statistically significant differences between fresh sperm and frozen-thawed testicular sperm used for ICSI regarding fertilization rate, clinical pregnancy rate, and live birth rate as shown (57%, 47%, 0.093 *p* value; 23.7%, 17.4%, 0.54 *p* value; and 11.9%, 8.7%, 0.68 *p* value, respectively). Non-obstructed azoospermia cases also show no significant differences in fertilization rate (37%, 36%, 0.91 *p* value), clinical pregnancy rate (20%, 14.3%, 0.58 *p* value), and live birth rate (4%, 3.6%, 0.93 *p* value).

Conclusion: Cryopreservation of testicular sperm is reliable if carried out before ovulation induction especially in cases with non-obstructive azoospermia

Keywords: Obstructive azoospermia, Non-obstructive azoospermia, ICSI, Fresh testicular sperm, Frozen-thawed testicular sperm

Background

Intracytoplasmic injection with testicular sperm since its introduction in 1993 [1, 2] has become a routine treatment procedure for patients with azoospermia, whether they suffer from obstructive azoospermia (OA), which is defined as the absence of spermatozoa in the ejaculate despite normal spermatogenesis (OA is a common urologic condition and accounts for 6.1% [1] to 13.6% [2] of patients presenting for fertility evaluation [3, 4]), or non-obstructive azoospermia (NOA) which is defined as no sperm in the ejaculate due to failure of spermatogenesis and is the most severe form of male infertility. Testicular failure affects approximately 1% of the male population and 10% of men who seek fertility evaluation [5].

High fertilization rates, pregnancy rates, and implantation rates are obtained in obstructive patients [6–8].

In the population of patients with NOA, however, the probability of finding sperm is only 50% in a non-selected population [9], but fertilization and pregnancy rates can reach acceptable levels [9–13].

Surgically retrieved spermatozoa from azoospermic patient are a routine procedure used for ICSI in IVF centers. However, the recovery of fresh testicular biopsies at the day of oocyte retrieval is highly stressful for the couple as it implies a 50% risk of pointless ovarian stimulation of the female partner. Moreover, repeated testicular surgery in subsequent ICSI cycles may cause testicular devascularization and possibly permanent injury [14, 15].

Several centers initiated the use of frozen-thawed surgically retrieved spermatozoa in addition to freshly retrieved spermatozoa to achieve ICSI pregnancy, and the

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outcome of cycles using fresh or frozen-thawed retrieved spermatozoa is the source of much debate. The majority or reports suggest no significant worsening in outcome with the use of cryopreserved gametes after surgical retrieval [13, 16–18]. Others, however, have reported a significantly lower FR [19, 20], clinical pregnancy rate (CPR) [10, 20], and implantation rate [10, 21] using cryopreserved spermatozoa.

The aim of our study is to present our data on ICSI cycles using surgically retrieved sperm from azoospermic men, to assess embryo development, and to compare the outcomes of ICSI for fresh sperm versus frozen-thawed testicular sperm in both OA and NOA.

Methods

This retrospective study included 159 ICSI cycles from 126 couples, with 98 cycles treated with fresh testicular sperm and 61 cycles treated with frozen-thawed testicular samples. Before assisted reproduction, 126 males were evaluated by clinical history, physical examination, and hormonal assay. Female partners were also assessed by full history and infertility investigation, and informed consent was taken from all patients.

The etiology of azoospermia was classified as obstructive azoospermia OA (91 cycles) and non-obstructive azoospermia NOA (68 cycles).

In all cases, surgical sperm was retrieved by the surgeon, and either testicular sperm aspiration under local anesthesia or testicular sperm extraction under general anesthesia was done for them.

Testicular specimens placed in petri dish with HEPES-buffered culture medium were processed by mechanical shredding [22], and the suspension was explored under an inverted microscope at × 400 magnification, when spermatozoa found in specimens was used either freshly for ICSI or when done for diagnostic specimens cryopreserved for next cycle.

Ovarian stimulation and oocyte retrieval

In all patients, short agonist protocols were used to stimulate follicular development. The short protocol includes pituitary desensitization with gonadotropin-releasing hormone agonist (Decapeptyl 0.1 mg, Ferring GmbH, Germany) at day 2 of the cycle and ovarian stimulation with follicle-stimulating hormone (fostimon, IBSA, Lugano 3, Suisse; Gonal-f, Laboroteries Serono S.A, Switzerland; Puregon, Schering-Plough, NV Organon, Oss, Netherlands) or combined LH and FSH (Merional, IBSA, Lugano 3, Suisse; Menegon, Ferring GmbH, Germany) starting at day 3 of the cycle. Measuring serum E2 and performing transvaginal ultrasound monitored the follicular development. Ovulation and final maturation of the ova were induced with human chorionic gonadotropin (hCG) (Choriomon, IBSA, Lugano 3, Suisse) as a single dose of 10,000 IU, when the leading follicle reached 18 mm in average diameter in addition to the presence of at least two other follicles of more than 16 mm in size and E2 > 500 pg/ml, then the oocytes were retrieved. Oocytes were aspirated 34–36 h after hCG administration. Oocyte retrieval was performed by transvaginal ultrasound-guided puncture using 16-gage, 35-cm double lumen aspiration needle (William A. Cook, Australia Pty Ltd.) with a negative pressure of 20 mmHg.

Oocytes denudation and evaluation

Removal of the surrounding cumulus cells was accomplished by a combined enzymatic and mechanical treatment carried out under a stereoscopic dissecting microscope. Oocytes were denudated from cumulus oophorus by exposure to 80 IU/ml hyaluronidase enzyme in HEPES-buffered medium (Hyase, FertiPro N.V., Belgium) followed by mechanical removal of the corona radiate with the use of plastic pipette stripper tips (EZ strip, Research Instruments Ltd, UK) with decreasing inner diameters of 290 and 135 μm.

Oocytes are assessed for their maturation and for their morphology under an inverted microscope (intgera Ti, R.I., Olympus, IX51/IX70, Tokyo, Japan) at × 400 magnification. Metaphase II oocytes were separated from the immature oocytes (metaphase I oocytes and germinal vesicle) just before sperm injection (3–4 h after retrieval).

Sperm preparation

In the case of azoospermia, sperms are retrieved by the urologist, under local anesthesia, either by percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA).

The technical procedure for PESA involved the insertion of a needle attached to a syringe through the scrotal skin into the epididymis. Originally, the use of a larger butterfly needle was described. Currently, most experts use a fine needle (26 gage) attached to a tuberculin syringe containing sperm washing medium. The epididymis is stabilized between the index finger, thumb, and forefinger. After creating negative pressure by pulling the syringe plunger, the tip of the needle is gently and slowly moved in and out of the epididymis until fluid is aspirated. If motile sperm are not obtained, PESA may be repeated at a different site (from the cauda to caput epididymis) until an adequate number of motile sperm is retrieved.

In TESA, the testis is divided into three poles: upper, middle, and lower poles. A needle was inserted through the scrotal skin into the anteromedial or anterolateral portion of the upper pole at an oblique angle toward the medium and lower poles. These aspirations are usually

carried out using either fine (testicular fine-needle aspiration (TEFNA)) or a scalp butterfly cannula gauge 23 attached to a syringe. The testicular parenchyma is aspirated by creating negative pressure, and the tip of the needle is moved within the testis to disrupt the seminiferous tubules; the specimen was sent to the laboratory for microscopic examination. TESA can be carried out in the contralateral testis if no sperms are obtained during the first attempt.

The obtained tissues from the epididymis or the testis were placed in a special media for handling gametes. By using a scalpel or surgical blade, the tissue was sliced or dissected mechanically, and wet slides prepared were examined under phase contrast microscope (Olympus BX41 Tokyo, Japan) at × 200 magnification. It was then placed in a flushing media for washing. After that, if sperm was found then it is dissected to remove tissues and blood cells, and then the content was placed into an Eppendorf tube and kept in an incubator for 30 min under 37 °C then centrifuged for 5–10 min. The supernatant was removed. After adding 1 ml of flushing media, it was then kept in incubator for another 30 min and centrifuged for 5–10 min. The supernatant was discarded, and the remaining was used as fresh sample for ICSI when done for diagnostic specimens cryopreserved for the next cycle.

Cryopreservation of samples

After the semen is allowed to be liquefied in the room temperature, it was then mixed with freezing media 1: 0.7 ml from SpermFreeze^{TM} (FertiPro) in drops with gentle swirling, then we leave the mixture at room temperature for 10 min for equilibration. The mixture was put in cryovial then fixed in the freezing straw; leave the straw fixed in a liquid nitrogen vapor for 15 min then transfer quickly into the liquid nitrogen and store at 196 °C.

Thawing

We removed as many cryovials as required from the liquid nitrogen and placed the cryovial in tap water for 5 min. We placed the cryovial mixture into the centrifuge tube diluted with sperm wash media (FertiCult TM Flushing medium) of 3 ml per 0.5 sperm mixture mixed thoroughly then centrifuged it for 15 min at 300–350*g*. We resuspended the pellet in sperm wash media for 15 min then used for ICSI.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection was performed in MII oocytes according to the technique described by [18]. Oocytes were transferred to the ICSI dish prepared with drops of IVF media (ferticult-IVF, FertiPro N.V., Belgium) covered by mineral oil.

Assessment of fertilization, embryo cleavage, and embryo transfer

Fertilization assessments were performed 17 ± 1 h post-injection. Normally, fertilized oocytes should be spherical and have two polar bodies and two PNs. PNs should be juxtaposed, approximately the same size, and centrally positioned in the cytoplasm with two distinctly clear, visible membranes [23].

Embryo quality was evaluated under an inverted microscope. The following parameters were recorded: (1) the number of blastomeres, (2) the fragmentation percentage, (3) variation in blastomere symmetry, and (4) defects in the zona pellucida and the cytoplasm.

High-quality (grade A) embryos were defined as those having all of the following characteristics: either 4–6 cells on day 2 or 8–10 cells on day 3 of development, less than 15% fragmentation, symmetric blastomeres, colorless cytoplasm with moderate granulation with no inclusions, absence of perivitelline space granularity, and absence of zona pellucida dysmorphism. Embryos lacking any of the above characteristics were considered as low quality [24]. For each couple, 1–4 embryos were transferred, depending on the embryo quality and the female's age. Embryo transfer was canceled if no embryos were available. Embryo transfer was performed on day 2 or day 3 using a Gynetics catheter (Gynetics Medical Products N.V, Lommel, Belgium). Transfers were performed with transabdominal ultrasound guidance.

Statistical analysis

All the statistical analysis procedures are performed using SAS 9.4 statistical software (SAS institute Inc. Cary, NC, USA© 2014). The differences for continuous variables were examined using Wilcoxon-Mann-Whitney test for samples without assumed normal distribution. The chi-square test was used for categorical variables and proportions, and for categorical variables with cell counts less than five, Fisher's exact test was used. p values less than 0.05 are considered statistically significant.

Table 1 Comparison between fresh and frozen-thawed obstructive azoospermia group regarding different criteria and outcomes

Variables	Fresh OA Mean (± SD)	Frozen OA Mean (± SD)	p value
Number of patients	66	25	
Age of wife	31.1 (± 5.75)	30.7 (± 6.2)	0.75
Age of husband	36.6 (± 8.9)	37 (± 8.5)	0.69
Number of oocyte	6.6 (± 4.2)	8.7 (± 4.7)	0.04
Number of grade A embryos	1.38 (± 1.7)	1.1 (± 1.2)	0.42

Table 2 Comparison of outcomes between fresh and frozen-thawed obstructive azoospermia group

	Fresh OA %	Frozen OA %	*p* value
Fertilization rate	57	47	0.093
Clinical pregnancy	23.7	17.4	0.54
Live birth	11.9	8.7	0.68

Results

In the obstructive azoospermia (OA) group which has 91 cases, 66 of fresh spermatozoa were used for ICSI and 25 frozen-thawed spermatozoa were used for ICSI in which the result shows no significant difference in age of wife and husband and grade A embryos and significant difference in number of oocyte retrieved as shown in Table 1.

The fertilization rate, clinical pregnancy rate, and live birth rate show no significant differences, as shown in Table 2.

In the non-obstructive azoospermia (NOA) group, we had 68 cases; in 32 of them, fresh spermatozoa were used for ICSI, and in 36 of them, frozen-thawed spermatozoa were used for ICSI, and there was no significant difference regarding the age of couple, number of retrieved oocyte, and grade A embryos as shown in Table 3.

Clinical pregnancy were recorded from five cases using fresh spermatozoa, but only one of them reached full term, as from four clinical pregnancies from the group who used frozen-thawed spermatozoa, one reached full term. Fertilization rate, clinical pregnancy, and live birth rate show no significant differences as shown in Table 4.

Discussion

After reporting the first ICSI trial with frozen testicular sperm, the advantages of cryopreserving testicular sperm for ICSI are well known [25, 26].

The main advantages of using frozen-thawed sperm for ICSI are to avoid repeated surgical biopsy and avoid useless ovarian stimulation.

However, there are several drawbacks of using frozen-thawed sperm for ICSI; first, there is a substantial risk of ~ 20% of not finding sperm suitable for injection despite extreme efforts in the case of NOA.

Table 3 Comparison between fresh and frozen-thawed non-obstructive azoospermia group regarding different criteria and outcomes

Variables	Fresh NOA Mean (± SD)	Frozen NOA Mean (± SD)	*p* value
Number of patients	32	36	
Age of wife	32.8 (± 5.8)	30.5 (± 7)	0.14
Age of husband	36.5 (± 6.6)	36.1 (± 7.35)	0.98
Number of oocyte	5.6 (± 3.1)	5.2 (± 3.3)	0.61
Number of grade A embryos	0.34 (± 0.6)	0.42 (± 0.73)	0.66

Table 4 Comparison of outcomes between fresh and frozen-thawed non-obstructive azoospermia group

	Fresh NOA %	Frozen NOA %	*p* value
Fertilization rate	37	36	0.91
Clinical pregnancy	20	14.3	0.58
Live birth	4	3.6	0.93

Second is regarding motility of the sperm as we know it is an indicator for sperm viability, although in vitro culture of frozen-thawed testicular sperm improve the motility in OA but not so promising in the case of NOA where the only immotile sperm was found 10 11 MX.

In the case of OA, our study shows no significant difference when we compared fresh sperm versus frozen-thawed sperm regarding fertilization rate (57%, 47%), clinical pregnancy rate (23.7%, 17.4%), and live birth rate (11.9%, 8.7%) as shown in previous studies [18, 27, 28].

Regarding NOA, the fertilization rate is nearly the same for fresh and frozen-thawed which was 37% and 36% respectively with no significant difference.

Also, there are no significant differences in clinical pregnancy rate and live birth rate (5%, 4%, *p* value 0.71) and (1%, 1%, *p* value = 1) respectively.

Our results similar to [18, 27–30], but unlike [31] which show improved embryo quality, there is higher pregnancy rate and lower abortion rate, and also, Mohamed and Shedeed (2008) show improved pregnancy rate in the fresh sperms in NOA [32].

The limitation in our study was due to difficulty in data collection due to missing information, which leads to small sample size, which may affect the significance of our results.

In conclusion, cryopreservation of testicular sperm is a reliable technique to do before ovulation induction especially in cases of non-obstructive azoospermia.

Conclusion

Cryopreservation of testicular sperm is reliable if carried out before ovulation induction especially in cases with non-obstructive azoospermia.

Abbreviations

ICSI: Intracytoplasmic injection; NOA: Non-obstructive azoospermia; OA: Obstructive azoospermia; SD: Standard deviation

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Authors' contributions

The author read and approved the final manuscript.

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Ethics approval and consent to participate
Informed consent was taken from all patients. Ethical approval from the ethics committee in Sulaimani University College of Medicine was obtained and are available upon request to upload.

Consent for publication
Not applicable

Competing interests
The author declares that he/she has no competing interests.

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