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INTRACYTOPLASMIC SPERM INJECTION (ICSI) WITH FRESH VERSUS CRYOPRESERVED TESTICULAR SPERMS IN EGYPTIANS NON-OBSTRUCTIVE AZOOSPERMIC PATIENTS

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ABSTRACT

Objective (s): To compare fertilization rate, grading of embryos, blastocyst formation rate and pregnancy outcome during ICSI with fresh versus frozen–thawed testicular sperms in Egyptian patients with non-obstructive azoospermia.

Design: Prospective study.

Setting: Assisted Reproduction Unit at the ELITE Fertility Care Center, Cairo, Egypt between October 2017 and September 2019.

Subject: Evaluate testicular sperm selection technique using fresh and thawing in intracytoplasmic sperm injection (ICSI). The study population consisted of 97 couples.

Results: The study population of 97 couples who referred to ICSI divided into 2 groups, fresh testicular sperm and frozen–thawed testicular sperm, including complete dissecting analyses, flow up and comparison between the changes between two groups during ICSI outcome.

Conclusion: The present study show significant differences in fertilization rate who is better between two groups, and no significant differences in grading of embryos, blastocyst formation rate and clinical pregnancy rate.

Keywords: Non-obstructive azoospermia, ICSI, Fresh testicular sperm, Frozen-thawed testicular sperm, Embryo quality, Pregnancy outcome.

1. INTRODUCTION

Intracytoplasmic sperm injection (ICSI) with testicular sperm consider [1, 2] a routine methods in for treatment in of male human who are azoospermia. Also, who suffered from non-obstructive azoospermia (NOA) which is known as there is no sperm in the ejaculate because failure in the process of sperm formation and is the common cases of male infertility. Testicular failure acts as 1% of the male population and 10% of men who have problem in fertility evaluation [3].

On the other hand recovered spermatozoa using surgically methods from patient are not preferred procedure used for ICSI in *in-vitro fertilization* (IVF) cases. In spite of, the regenerate of fresh testicular samples at the day of oocyte retrieval is more overwrought for the couple as a 50% risk was indicated of useless

ovarian stimulation is for the cases. Moreover, repetition of testicular surgery in subsequent ICSI cycles may be lead to testicular devascularization and possibly permanent injury [4, 5]

A lot of IVF centers started using of frozen-thawed surgically retrieved spermatozoa and freshly retrieved spermatozoa to obtain ICSI pregnancy, and the outcome of cycles using fresh or frozen-thawed retrieved spermatozoa is the source of much discussion. More studies illustrated that there is no significant advances in outcome with using cryopreserved gametes after surgical retrieval [6, 7–8]. Others, however, have reported a significantly lower fertilization rate [9, 10], clinical pregnancy rate (CPR) [11, 12], and implantation rate [11, 12] using cryopreserved spermatozoa.

The present study aimed to use ICSI cycles with surgically retrieved sperm in Egyptians non-obstructive azoospermic men, to estimate embryo growth, and to compare the outcomes of ICSI for from fresh sperm against or from frozen-thawed testicular sperm.

2. SUBJECTS AND METHODS

2.1. Subjects:

The study population consisted of 97 Egyptians couples who were referred to Assisted Reproduction at ELITE Fertility Care Center, Cairo, Egypt between October 2017 and September 2019. Among 97 couples with non-obstructive azoospermia who underwent controlled ovarian hyperstimulation (COH) and testicular sperm extraction (TESE), (67.0%) fresh spermatozoa had a testicular biopsy, and (33.0%) frozen-thawed spermatozoa had a testicular biopsy, undergo a consecutive cycle of ART ICSI. Patients were selected for surgery if their ejaculate free from spermatozoa on two centrifuged semen analyses evaluated by a certified andrologist.

2.2. Testicular sperm extraction (TESE) Collection and Preparation

TESE was performed under general anesthesia. On the day of the oocyte retrieval, the male partners were asked to produce fresh ejaculates, and by extended sperm preparation [13], the absence of spermatozoa in the specimen was verified.

2.2.1. TESE Collection

TESE was performed according to the technique published by [14]. After stabilization of the testicle, a small incision in the testicle's mid portion was performed, cutting through the scrotal skin, tunica vaginalis and the tunica albuginea. A substantial piece of the extruding testicular tissue was cut with small scissors, washed by medium to remove blood traces and placed in a petri dish containing nearly 1–3 ml Earle's balanced salt solution (EBSS) (Gibco BRL, Life Technologies, Paisley, UK) with heparin (Sigma Chemical Co., St Louis, MO, USA).

2.2.2. TESE preparation

In the laboratory, a wet preparation was carried out [15], for examination. Testicular tissue was vigorously fragmented and minced using two glass slides and immediately examined under the inverted microscope (Diaphot 300; Nikon Corp., Tokyo, Japan) at 3200 and 3400 magnification to detect presence of any spermatozoa. Once spermatozoa were found the surgical procedure was terminated. If spermatozoa were not observed up to three biopsies were taken from different areas of the same testicle and also from the contralateral testicle. TESE can be done on the opposite testicle. The testicle was closed by layers using 3-0 vicryl or plane stitches. Part of the testicular specimen was sent for histological analysis.

Following vigorous tissue shredding, collected and transferred to a 6 ml conical tube (Falcon; Becton Dickinson Labware, NJ, USA) and centrifuged at 300 g for 10 min. Purification of the cell suspensions was executed by lysis of erythrocytes (by a 5 min suspension in erythrocyte-lysing buffer, containing 155 mM NH₄Cl, 10 mM KHCO₃, 2 mM EDTA, pH 7.2). Cell separation after red cell lysis, by discontinuous Percoll gradient, was not performed in all cases. However, occasionally, when a lot of cell debris was seen, the resuspended pellet was layered on a mini-Percoll gradient (90–45%) and centrifuged at 300 g for 20 min. The original pellet after red cell lysis or the sperm-containing fraction following Percoll separation (90% Percoll) was washed twice, by addition of 6 ml of human tubal fluid (HTF)–HEPES–albumin medium supplemented with 7.5% synthetic serum (Irvine Scientific, Santa Ana, CA, USA) and centrifuged at 250 g for 5 min. The final pellet was incubated until the sperm injection (between 3 and 6 h). Prior to the injection, the final pellet was examined under the inverted microscope for the presence of spermatozoa using a petri dish containing multiple droplets (up to 50) of 10 µl each. If spermatozoa were identified, they were transferred to drops of 10% polyvinyl pyrrolidone (Irvine) covered by pre-equilibrated embryo-tested paraffin oil.

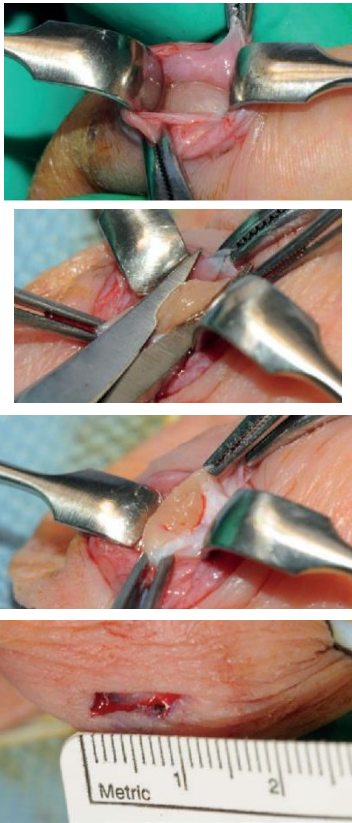


Fig.1:Collect testicular tissue sample by surgery



Fig. 2:Petri dish for TESE specimen collection.



Fig. 3: Petri dish contain washing media



Fig.4: Petri dish contain tissue scratching by needle and **Fig.5:**Testicular tissue after prepared cover by mineral oil to investigated under the inverted microscope.

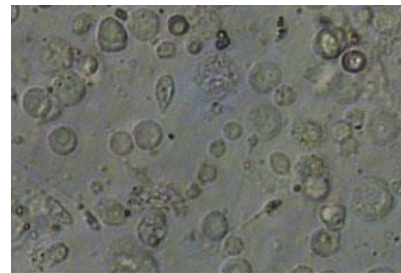
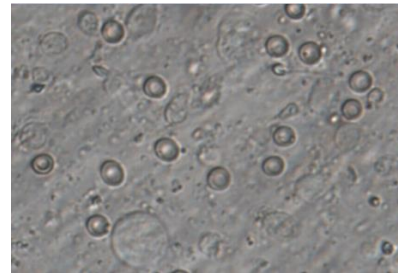


Fig.6 &7: Testicular biopsy after processing

2.2.3. TESE cryopreservation

Fresh TESE was cryopreserved using special cryoprotectant (Sperm Freeze LifeGlobal® Media) it's a single step, HEPES-buffered, cryoprotectant media used for the cryopreservation of sperms by calculation of total volume required 1/3 sperm freeze and 2/3 processed sample then loaded inside straw storage then gradually stored in liquid nitrogen started on vapor for 15 minutes until fully merged under the surface. Then transfer quickly into the liquid nitrogen and store at -196°C .

2.2.4.-Thawing of TESE

Thoroughly centrifuge it for 15 minutes at 300-350g. We resuspended the pellets in sperm wash media for 15 minutes Minute and then used for ICSI.

The straw was removed from the liquid nitrogen and thawed at 37°C for 1 minute. The content of straw in drained in a conical bottom

tubefollowed by slowly adding of sperm wash media then centrifugation at 1100 rpm for 10 minutes and supernatant removed 0.1 or 0.2 ml concentration remain for using in ICSI.

3. Ovarian stimulation

At the ART clinic preliminary evaluations including general and local vaginal examination ultrasound evaluation was done by ob/Gyn consultants using SONOACE X4 – MEDISON Korea. Hormonal profile including estradiol (E2), prolactin (PRL), luteinizing hormone (LH) and follicular stimulating hormone (FSH) were measured.

According to the ART protocols, women get ovarian gonadotropin stimulation drugs consisted of human menopausal gonadotropins (Menotrophin HMG such as Merional or Fostimon 75 I.U, from IBSA and Menogon 72 I.U from Ferring) which contain equal concentrations of LH and FSH. Until recently, all available human FSH pharmaceutical preparations were extracted from postmenopausal women urine. While HMG may be used as a source of FSH, it has low specific activity and contains significant amounts of LH (as well as other proteins), which is thought to be associated with poor oocyte quality, reduced fertilization rates, lower embryonic viability, and early pregnancy wastage[16]. The number of ampoules of initial gonadotropin dose used for ovarian stimulation is 75-300 IU/ml adjusted according to:

- 1 - The patient's age.
- 2 - Body mass index.
- 3- Baseline serum FSH concentrations on day 2 or 3 of menstruation.
- 4- Previous response to ovarian stimulation.

3.1. Oocyte retrieval

Oocytes were aspirated under either general or spinal anesthesia using transvaginal ultrasonographic guidance timing depends on protocol for stimulation, approximately between 34-36 hours after the human chorionic gonadotropin (HCG) injection. Follicles were aspirated under a negative pressure of 180-200 mm Hg with a single or double lumen needle

(Ovum Aspiration Needle Single Lumen – OPS, or Double Lumen Aspiration Needle-Cook medical) (Figs. 8&9).

Based on number of expected oocytes or previous trial patient had number lower than expected. This lumen linked to aspirator (aspirator 3 Labotect Germany). The aspirated follicular fluid containing oocyte cumulus complexes (OCC) which collected in pre-warmed 37C rounded bottom tubes (14 ml falcon). The tubes were heated in thermo block (G73 Block Warmer, K-Systems).

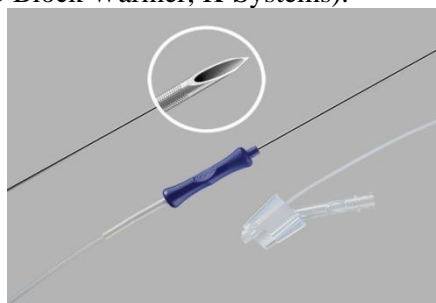


Fig.8: Aspiration Needle Single

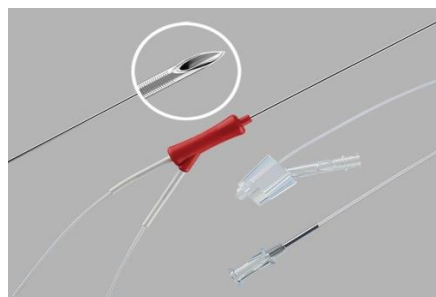


Fig.9: Double Lumen Aspiration

Tubes were spread on to sterile polystyrene dish (3003 Falcon) and checked in IVF work station (L126 IVF Dual, K-Systems) with heated stage and fixed microscope (Olympus SZ61) and (Galaxy 14S, Eppendorf, USA). (OCC) identified was transferred into buffered media (Multipurpose Handling Medium (MHM) with Gentamicin, Irvin Scientific). After finishing retrieval, (OCC) transferred into culture media (Global total for fertilization, lifeglobal group) and placed in bench top CO2 non humid (G85, K-Systems Denmark).

3.2. Denudation and identification

Using IVF work station, with heated stage and fixed microscope (SZ61 Olympus japan), two mechanisms were involved:

a. Biochemicalin which buffered Hyaluronidase Solution 80 IU (90101, Irvin Scientific) in 100 μ l for 30-45 seconds.

b. Mechanicalin which flexible pipettes used for denuding via gently multiple movements with appropriate diameter 170 μ l (Cook Flexipet Pipette, Cook medical).

Morphological analysis of denuded oocytes according to their maturity and normality as the following: Metaphase II, Metaphase I, Germinal Vesicle, Abnormal, Necrotic, and Emptyzona. Finally oocytes cultured again at the same previous culture media waiting for ICSI. While those planned for IVF denudation didn't applied.

4. Intracytoplasmic Sperm Injection (ICSI) procedure

On anti-vibration table, inverted microscope it has 20X and 40X lens, with Hoffman optics, and a 37 °C heating plate surface (IX53, Olympus). Three dimensions movable micromanipulators; one on the left to control the holding micropipette, and the other one on the right for the injection micropipette. In addition to an hydraulic manipulator for the fine movements, and electric one for largest movements. Mineral oil used to and connected to the microinjection pipettes by a flexible tube in order to aspirate and inject the spermatozoa. After male specimen processing and oocytes denudation, ICSI was undergo. Samples were incubated until time of injection.

Injection dish (1006 Falcon) micro drops of buffered media (Multipurpose Handling Medium (MHM) with Gentamicin, Irvin Scientific) polyvinyl Pyrrolidone (PVP), (Polyvinyl pyrrolidone Solution with HAS 10% 90123, Irvin Scientific) for mechanically immobilizations of sperm were used, dish was covered with mineral oil (Irvin scientific) to protect media from evaporation and changes in osmolality and pH. Individual sperm subjected to ICSI was examined and evaluated.

The needle carefully inserted through zona-pellucida, puncturing cytoplasm membrane into cytoplasm of the oocyte. The sperm injected into the cytoplasm, and the needle is

carefully removed (Figs.10 & 11). Injected oocytes were cultured in single step media (Globo TOTAL, life global group), culture dish placed in large CO₂ humid incubator (forma water jacket Thermo fisher scientific, USA), or (C200 Labotect incubator, Germany), for 5 or 6 days included 2 observations.

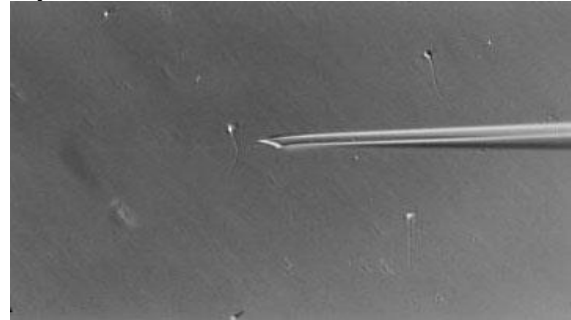


Fig.10: Sperm selection for ICSI



Fig.11: Micromanipulation of oocytes

5. Assessment of fertilization, embryo cleavage rates, embryo grading and embryo transfer:

5.1. Fertilization check

After 16-18 hours pronucleus (PN) fertilization checked. At the same time, IVF oocyte-cumulus complex denuded to enable embryologist to check fertilization. One, two, or three PN were seen, 3 PN were excluded. (SZ61 Olympus, Japan) was used in PN check except for vacuolated cytoplasm or 3PN confirmation, inverted microscope was used.

5.2. Cleavage rate and decision day of transfer:

Second observation on day two reveals one of the following: rapid division more than 4 cells, normal division 4 cells, slow division less than 4 cells, or PN arrest stage. Routinely 4 cells without fragmentations were elected to day five or six.

5.3. Day five/six embryo grading:

Embryo scoring on day 5 or day 6. Blastocysts were graded according to Gardner Grading system. While Pre-blastocyst stage, 3 stages aren't included in Gardner grading system; compacting cells, morula, and morula with cavitations. Cells that fuse; boundaries aren't totally compacted was named in the study as compacting cells. Complete compaction (fusion) was named morula, and morula with cavitations was called cavitating morula. Gardner grading system includes 38 grades differentiated according to expanding, and quality of trophectoderm (TE), and inner cell mass (ICM). Abnormal cavity and contract embryos plus 3 pre-blastocyst so total (43) grades are included.

Gardner grading system/ Gardner-Schoolcraft grading system. [17]

This system includes different numbers, and letters. Numbers written first while letters second. The number expresses the expansion of blastocyst. First letter expresses the quality of ICM, and second expresses the quality of TE.

5.4. Embryotransfer

Embryo transfer was performed on day 5 of development using a soft catheter. Selected embryos were loaded using (MHM, Irvin Scientific) in soft catheter (classic Wallace) (Fig. 12), connected to syringe with special tip (BD LuerLok, BD). All embryo transfers were performed under abdominal ultrasound guidance.

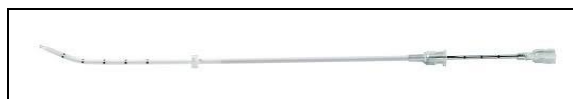


Fig.12: Catheter (classic Wallace)

Ongoing Pregnancy

By abdominal probe of ultrasound sonar after more than 5 weeks, signs of pregnancy were detectable.

- **Ethical Aspects:**
- **Informed consent was taken from all patients. Ethical approval from the ethics committee in Al-Azhar University, Faculty**

of Medicine, Egypt. The protocol of the study is in agreement to the principles of ethical medical research of Helsinki declaration [last updated in Brazil, 2013]. All participating women have to verbally consent for participating in this study. All eligible women have the right to decline participating or withdraw from this study at any phase without being adversely affected regarding the medical service she should receive.

6. Statistical Analysis:

Data were analyzed using SPSS Statistics version 23 (IBM© Corp., Armonk, NY) and MedCalc version 18.2.1 (MedCalc© Software bvba, Ostend, Belgium).

Continuous numerical variables were presented as mean and SD and inter-group differences were compared using the unpaired t-test. Categorical variables were presented as number and percentage and differences were compared using the Pearson chi-squared test. Ordinal data were compared using the chi-squared test for trend. Multivariable regression analysis was used to examine the relation between sperm type and fertilization rate, cleavage rate, blastocyst formation rate or 2PN arrest rate as adjusted for the effect of wife's age. For pregnancy rate, multivariable binary logistic regression analysis was used. Two-sided p-values <0.05 were considered statistically significant.

RESULTS

The present study showed the outcome of non-obstructive azoospermic patients which investigated of testicular biopsy (fresh or thawed biopsy) and assessment of the early embryo development. This outcome was investigated on: fertilization rate (PN morphology), cleavage, compactation. Also, embryo morphology was assessed on the mornings of days 1, 2, 3 and 5 of embryonic development and the ability of embryos reached to blastocyst stage. Table 1 and figure 13, showed the total number of patients undergoing testicular biopsy n=97, which divided into two groups: group (A) fresh testicular sperms, n=65 and group (B) frozen or thawed testicular sperms, n=32.

Table 1: Proportion of patients undergoing ICSI using fresh or frozen sperms

Variable	Groups	N	%
Sperm type	Fresh sperms (group A)	65	67.0%
	Frozen sperms (group B)	32	33.0%
	Total	97	100.0%

Data are number (N) and percentage (%).

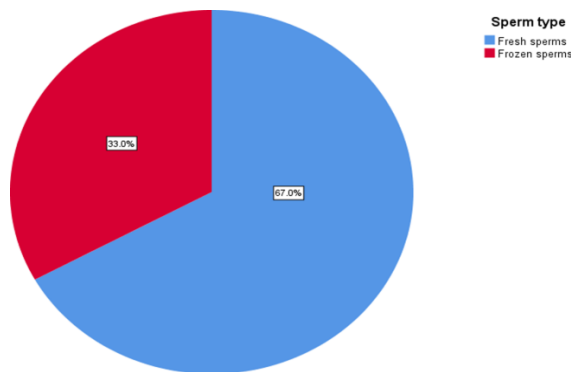
**Figure 13:** Proportion of patients undergoing IVF using fresh or frozen sperms.

Table (2), showing the mean of female age (years) of patients undergoing investigated in group A was 32.6 ± 7.0 , which include less than 35 years 35 women represent 53.8% and more than 35 years 30 women represent 46.2%. mean age of female in group B was 29.9 ± 6.1 , which include less than 35 years 26 women represent 81.3%, and more than 35 years 6 women represent 18.8%. In the table (3), showed the mean number of retrieved oocytes in group A was 10.4 ± 6.3 , mean number of MII oocytes were 8.2 ± 5.4 and the mean number of fertilized oocytes were 5.9 ± 4.6 , mean number of arrested 2PN 0.2 ± 0.6 , mean number of cleaved embryos were 5.6 ± 4.7 , mean number of blastocysts were 4.4 ± 2.5 , mean number of transferred embryos were 2.5 ± 0.9 , and the mean number of frozen embryos were 0.3 ± 0.4 .

The days of embryos transfer in each group and the number of embryos transfer in day 2

Table 2: Wife's age in both study groups

Variable	Fresh sperms	Frozen sperms	t (df) / χ^2 (df)	P-value
Wife's age (years)	32.6 ± 7.0	29.9 ± 6.1	-1.824 (95)	0.071*
Wife's age				
≤35 years	35 (53.8%)	26 (81.3%)	6.900 (1)	0.009#
>35 years	30 (46.2%)	6 (18.8%)		

Data are mean \pm SD or number (%). *Unpaired t-test. $t = t$ -statistic $\chi^2 =$ chi-squared statistic, df = degree of freedom, # Pearson chi-squared test.

Table 3: Number of retrieved oocytes, fertilized oocytes, number of cleaved embryos and number of formed blastocysts in both study groups

Variable	Fresh sperms (n=65)	Frozen sperms (n=32)	Difference	95% CI	P-value*
	Mean \pm SD (GA)	Mean \pm SD (GB)			
Total oocyte number	10.4 ± 6.3	13.1 ± 8.5	2.7	-0.4- 5.7	0.084
Number of MII oocyte	8.2 ± 5.4	10.7 ± 6.7	2.4	-0.1- 4.9	0.059
Number of fertilized oocytes	5.9 ± 4.6	5.6 ± 3.9	-0.3	-2.2- 1.6	0.766
Number of cleaved embryos	5.6 ± 4.7	5.3 ± 3.9	-0.4	-2.3- 1.5	0.692
Number of blastocysts	4.4 ± 2.5	3.4 ± 1.5	-1.0	-2.7- 0.7	0.250
Number of arrested 2PN	0.2 ± 0.6	0.3 ± 1.2	0.1	-0.3- 0.5	0.598
Number of transferred embryos	2.5 ± 0.9	2.4 ± 0.9	0.0	-0.4- 0.4	0.845
Number of frozen embryos	0.3 ± 0.4	0.3 ± 0.5	0.0	-0.2- 0.2	0.839

Data are mean and standard deviation (SD). *Unpaired t-test. 95% CI = 95% confidence interval.

were 0 embryos 0.0%, and day 3 were 43 embryos 66.2%, and day 5 were 22 embryos 33.8% in group A, while the number of embryos transfer in day 2 were 2 embryos 6.3%, and day 3 were 19 embryos 59.4%, and day 5 were 11 embryos 34.4% in group B as shown in table 4

The fertilization rate was 71.1 % in group A and fertilization rate was 56.7 % in group B, as shown in table 5 and figure 14. Cleavage rate was 95.6% in group A while the cleavage rate

was 94.6% in group B, as shown in table 5 and figure 15. Blastocyst formation rate was 46.2% in group A and the blastocyst formation rate was 39.9% in group B, as shown in table 5 and figure 16. Table 5 and figure 17. showed that 2PN arrest rate in the group A was 4.4%, while 2PN arrest rate in the group B was 5.4.

Table 4: Day of embryo transfer in both study groups

Variable		Fresh sperms (n=65)		Frozen sperms (n=32)		χ^2 (df,1)	P-value*
		N	%	N	%		
Day of embryo transfer	D2	0	0.0%	2	6.3%	0.060	0.806
	D3	43	66.2%	19	59.4%		
	D5	22	33.8%	11	34.4%		

Data are number (n) and percentage (%) df = degree of freedom
 χ^2 = chi-squared statistic *Chi-squared test for trend.

Table 5: Fertilization rate, cleavage rate, blastocyst formation rate and 2PN arrest rate in both study groups

Variable	Fresh sperms (n=65)		Frozen sperms (n=32)		Difference	95% CI	P-value*
	%	SD	%	SD			
Fertilization rate (%)	71.1	26.4	56.7	25.0	-14.4	-25.6 to -3.3	0.012
Cleavage rate (%)	95.6	11.0	94.6	17.0	-1.0	-6.7 to 4.7	0.726
Blastocyst formation rate (%)	46.2	24.4	39.9	16.5	-6.3	-23.2 to 10.6	0.453
2PN arrest rate (%)	4.4	11.0	5.4	17.0	1.0	-4.7 to 6.7	0.726

Data are mean and standard deviation (SD). *Unpaired t-test.
 95% CI = 95% confidence interval.

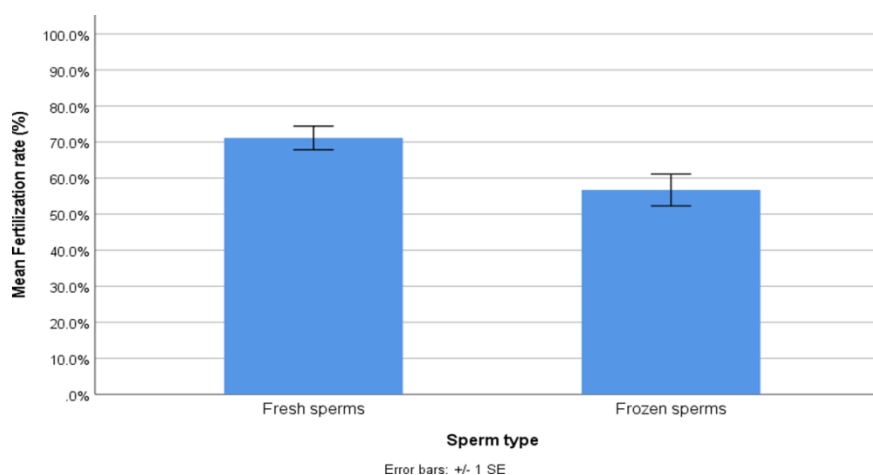


Figure 14: Mean fertilization rate in both study groups. Error bars represent the standard error (SE).

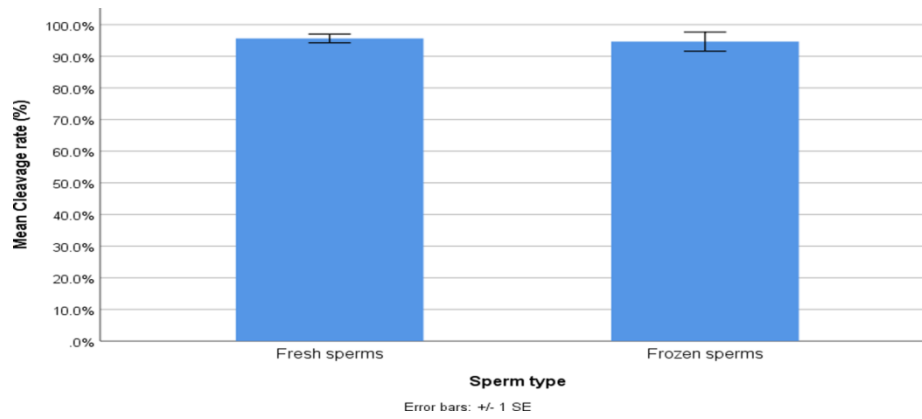


Figure 15: Mean cleavage rate in both study groups. Error bars represent the standard error (SE).

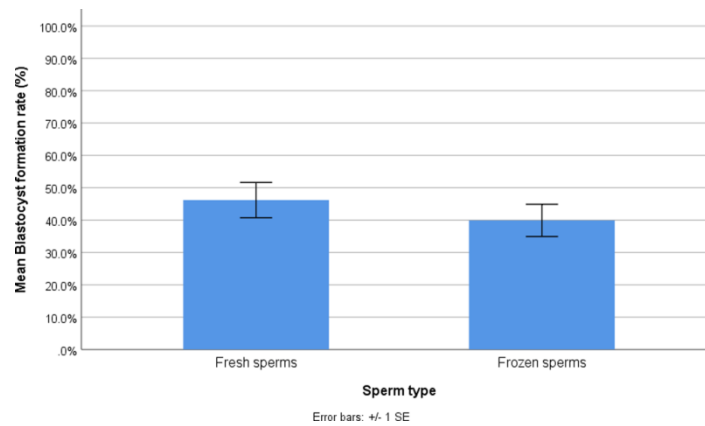


Figure 16: Mean blastocyst formation rate in both study groups. Error bars represent the standard error (SE).

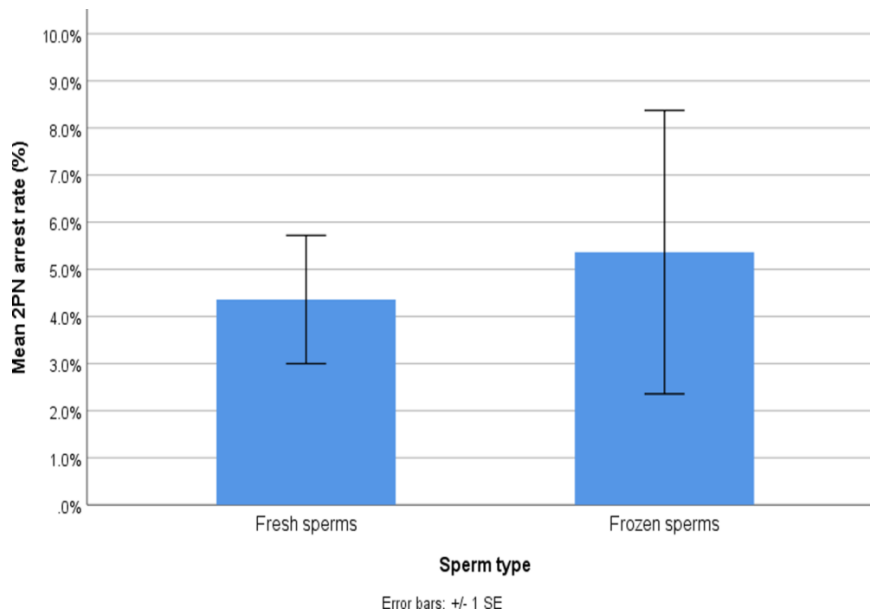


Figure 17: Mean 2PN arrest rate in both study groups. Error bars represent the standard error (SE).

Table 6: showing the clinical pregnancy rate in each group, n=65 in group A, 40 negative women and 25 positive pregnancy women, while n=32 in group B, 25 negative women and 7 positive pregnancy women, figure 18.

Table 7: showing the results of multivariable regression analysis for the relation between sperm type and fertilization rate. After adjustment for the wife's age, use of

fresh sperms was associated with significantly higher fertilization rate compared with frozen sperms (B =13.466, t = 2.357, P= 0.021).

Table 8: showing the results of multivariable regression analysis for the relation between sperm type and cleavage rate. After adjustment for the wife's age, there was no statistically significant relation between the type of sperms and cleavage rate (B = 0.384, t = 0.132, P = 0.895).

Table 6: Clinical pregnancy rate in both study groups

Variable		Fresh sperms (n=65)		Frozen sperms (n=32)		$\chi^2(1)$	P-value*
		n	%	n	%		
Clinical pregnancy	Negative	40	61.5%	25	78.1%	2.669	0.102
	Positive	25	38.5%	7	21.9%		

Data are number (n) and percentage (%).

χ^2 = chi-squared statistic, df = degree of freedom.

*Pearson chi-squared test.

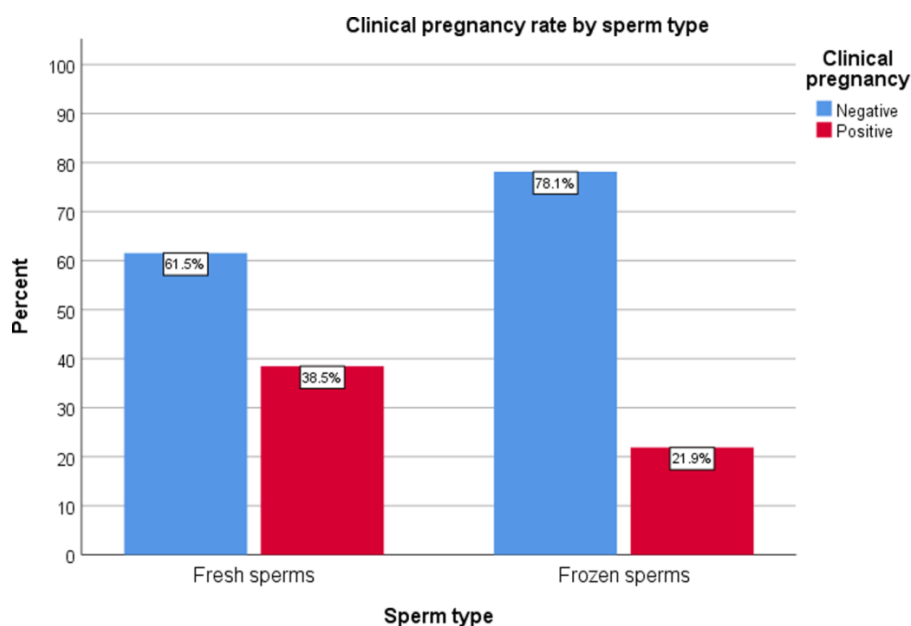


Figure 18: Clinical pregnancy rate in both study groups.

Table 7: Multivariable regression analysis for the relation between sperm type and fertilization rate as adjusted for the wife's age.

Independent variables	B	SE	T	P-value	r partial	r semipartial
Constant	45.840					
Wife's age (years)	0.363	0.397	0.915	0.363	0.094	0.091
Fresh sperms (=1)*	13.466	5.712	2.357	0.021	0.236	0.234

B= regression coefficient. SE = standard error. t = t-statistic. r partial = partial correlation coefficient.

*Referenced to Frozen sperms (=0). r semipartial = semipartial correlation coefficient.

Table 9: shows the results of multivariable regression analysis for the relation between sperm type and blastocyst formation rate. After adjustment for the wife's age, there was no statistically significant relation between the type of sperms and blastocyst formation rate ($B = 6.652$, $t = 0.792$, $P = 0.435$).

Table 10: shows the results of multivariable regression analysis for the relation between sperm type and 2PN arrest rate. After adjustment for the wife's age, there was no statistically significant relation between the type of sperms and 2PN arrest rate ($B = -0.384$, $t = -0.132$, $P = 0.895$).

Table 11A: shows the results of multivariable logistic regression analysis for

the relation between sperm type and clinical pregnancy as adjusted for the wife's age. After adjustment for the wife's age, there was no statistically significant relation between the type of sperms and clinical pregnancy rate ($B = 0.854$, odds ratio = 2.349, 95% CI = 0.856 to 6.44, $P = 0.097$).

Table 11B: shows the results of multivariable logistic regression analysis for the relation between sperm type and clinical pregnancy as adjusted for the wife's age.

After adjustment for the wife's age, there was no statistically significant relation between the type of sperms and clinical pregnancy rate ($B = 0.821$, odds ratio = 2.272, 95% CI = 0.843 to 6.121, $P = 0.105$).

Table 8: Multivariable regression analysis for the relation between sperm type and cleavage rate as adjusted for the wife's age

Independent variables	B	SE	t	P-value	r _{partial}	r semipartial
Constant	87.605					
Wife's age (years)	0.235	0.202	1.163	0.248	0.119	0.119
Fresh sperms (=1)*	0.384	2.907	0.132	0.895	0.014	0.014

B= regression coefficient. SE = standard error. t = t-statistic. r partial = partial correlation coefficient.

*Referenced to Frozen sperms (=0). r semipartial = semipartial correlation coefficient.

Table 9: Multivariable regression analysis for the relation between sperm type and blastocyst formation rate as adjusted for the wife's age

Independent variables	B	SE	t	P-value	r _{partial}	r semipartial
Constant	49.543					
Wife's age (years)	-0.33	0.684	-0.48	0.63	-0.09	0.089
Fresh sperms (=1)*	6.65	8.399	0.79	0.43	0.15	0.148

B= regression coefficient. SE = standard error. t = t-statistic. r partial = partial correlation coefficient.

*Referenced to Frozen sperms (=0). r semipartial = semipartial correlation coefficient.

Table 10: Multivariable regression analysis for the relation between sperm type and 2PN arrest rate as adjusted for the wife's age.

Independent variables	B	SE	t	P-value	r _{partial}	r semipartial
Constant	12.395					
Wife's age (years)	-0.235	0.202	-1.16	0.248	-0.12	0.119
Fresh sperms (=1)*	-0.384	2.907	-0.13	0.895	-0.01	0.014

B= regression coefficient. SE = standard error. t = t-statistic. r partial = partial correlation coefficient.

*Referenced to Frozen sperms (=0). r semipartial = semipartial correlation coefficient.

Table 11A: Multivariable logistic regression analysis for the relation between sperm type and clinical pregnancy as adjusted for the wife's age.

Variable	B	SE	Wald	P-value	Odds ratio	95% CI
Wife's age ≤35 years (=1)*	0.184	0.467	0.155	0.694	1.202	0.48 to 3.00
Fresh sperms (=1)#	0.854	0.515	2.750	0.097	2.349	0.85 to 6.44
Constant	-1.42	0.577	6.093	0.014		

B= regression coefficient, SE = standard error, Wald = Wald chi-squared statistic, 95% CI = 95% confidence interval.*Referenced to age >35 years (=0).

#Referenced to Frozen sperms (=0).

Table 11B: Multivariable logistic regression analysis for the relation between sperm type and clinical pregnancy as adjusted for the wife's age

Variable	B	SE	Wald	P-value	Odds ratio	95% CI
Wife's age (years)	-0.01	0.033	0.041	0.840	0.993	0.93 to 1.06
Fresh sperms (=1)*	0.821	0.506	2.633	0.105	2.272	0.84 to 6.12
Constant	-1.08	1.063	1.025	0.311		

B= regression coefficient, SE = standard error, Wald = Wald chi-squared statistic, 95% CI = 95% confidence interval.

*Referenced to Frozen sperms (=0).

DISCUSSION

After recorded the first case of ICSI trial with frozen testicular sperm, the role of cryopreserving testicular sperm for treatment of ICSI cases are well known [18-19].The important using frozen-thawed sperm for ICSI were to limit repeated surgical samples and to reduce useless female ovarian stimulation.The objective of this study was to investigate the relationship between fertilization rates, embryo quality, blastocyst morphology and clinical pregnancy rates in NOA azoospermic Egyptian patients.

There is a high probability that idiopathic non-obstructive azoospermia (NOA) is caused by genetic defects, such as gene mutation, gene polymorphism and epigenetic alterations, which lead to spermatogenic failure directly or indirectly depending on environmental factors, epigenetic modification, etc. In addition to acquired causes, the well-established genetic screening currently is only Karyotype and Y-chromosome microdeletions. Partial deletion of

Y-chromosome, DNA methylation, testis specific genes mutation may be the real causes of abnormality of spermatogenesis or meiosis[20-21-22].When sperm collection was not possible on the day of oocyte retrieval for ICSI in some cases, sperm cryopreservation in advance is preferred and has been shown to be achievable. Cryopreservation of testicular spermatozoa from MicroTESE for future ICSI cycles would avoid both unnecessary female stimulation and repetitive injuries for successive ICSI cycles [23].

1-Fertilization rates

In the case of NOA, the present study showed significant difference increase when compared fresh sperms versus frozen-thawed sperms regarding fertilization rate (71.1%, 56.7%) respectively, and this results inconsistent with the results of other studies [23,24-25-26], who concluded that the fertilization rate is nearly the same for fresh and frozen-thawed groups.

Also, several authors have demonstrated that ICSI treatments with fresh or frozen testicular spermatozoa have similar results [27-25]. Reflectreports [28-29], which involved comparison of fertilization rate, of fresh and frozen thawed MicroTESE sperm, no significant differences were observed between the two groups. [30], showed that freezing testicular not affect fertilization rate.

2- Embryo quality

In the present investigation there was no statistically significant relation between the type of sperms and embryo quality and this result was not matched with other authors who show improved embryo quality, [31], and high qualified embryo rate of fresh sperms group but was slightly lower than that of frozen thawed group [30].

3-Blastocyst and clinical pregnancy rates

The blastocyst formation rate showed no significant differences between the two groups and the present results were not matched with some investigators [32], show improved pregnancy rate in the fresh sperms in NOA and other authors concluded that higher pregnancy rate and lower abortion rate.

The present results were matched with [23-24-25-26], which concluded that no significant differences in clinical pregnancy rate and live birth rate. Our study demonstrates that the etiology is prognostic of the sperm retrieval rate in NOA patients. Among all the etiologic categories of NOA, there was no significant difference in the clinical features of NOA of different etiology.

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

Conclusion and Recommendations

We conclude that there is a trend towards better Sperm cryopreservation which has revolutionized the field of assisted reproduction. Cryopreserving sperm avoids the need for additional surgery in couples undergoing repeated in vitro fertilization with

intracytoplasmic sperm injection cycles. Moreover, it provides hope for men undergoing chemotherapy, radiation or radical surgery who once had no chance for future fertility.

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الحقن المجهري بالحيوانات المنوية المأخوذة حديثاً من الخصية مقابل الحيوانات المنوية المجمدة في الحالات التي تعاني من غياب تام غير انسدادى للحيوانات المنوية

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الملخص العربي

الهدف: تقييم معدل الإخصاب ، وتصنيف الأجنة ، ومعدل تكوين الكيسة الأريمية (البلاستيولا) ونتائج الحمل خلال عملية الحقن المجهري بالحيوانات المنوية المأخوذة حديثاً من الخصية مقارنة بالحيوانات المنوية المجمدة الذائبة في المرضى المصريين. التصميم: دراسة مستقبلية.

الإعداد: بمساعدة وحدة الإنجاب في مركز إيت لرعاية الخصوبة ، القاهرة ، مصر بين الفترة أكتوبر 2017 وسبتمبر 2019.

الموضوع: تقييم تقنية اختيار الحيوانات المنوية في الخصية والمقارنة بين الحيوانات المنوية المأخوذة حديثاً من الخصية والحيوانات المنوية الذائبة. وتألفت الدراسة من 97 زوجاً. النتائج: الدراسة لـ 97 من الأزواج الذين أشاروا إلى الحقن المجهري ينقسم إلى مجموعتين ، الحيوانات المنوية المأخوذة حديثاً من الخصية والحيوانات المنوية المجمدة ، بما في ذلك تحليلات التشريح الكاملة للنسيج، والمقارنة بين التغيرات بين المجموعتين خلال نتائج الحقن المجهري. الخلاصة: تظهر هذه الدراسة اختلافات كبيرة في معدل الإخصاب بين المجموعتين ، ولا توجد فروق ذات دلالة إحصائية في معدل إنقسامات الأجنة ، ومعدل تكوين الكيسة الأريمية (البلاستيولا) ومعدل الحمل السريري.