

## INTRACYTOPLASMIC SPERM INJECTION (ICSI) OUTCOME IN MEN WITH AZOOSPERMIA

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### ABSTRACT

**Background:** The management of patients with azoospermia involves percutaneous testicular sperm aspiration (PTSA) or testicular sperm extraction (TESE) according to the type of azoospermia combined with intracytoplasmic sperm injection (ICSI). Our study have investigated the effect of the male age, the cause of azoospermia, the type of sperm used, on the ICSI cycle outcome in men with azoospermia. **Objective:** To assess the effect of: male age, the cause of azoospermia (obstructive or non-obstructive azoospermia), the type of sperm used (fresh or frozen-thawed) on the icsi cycle outcome in men with azoospermia. **Methods:** A retrospective analysis of 54 icsi cycles performed at Baghdad Specialized Fertility Center between 2012 and 2014,

involving men with azoospermia to assess its effect on icsi cycle outcome. **Results:** Analysis of the results did not show any statistically significant differences in the fertilization ,embryo cleavage, clinical pregnancy, live births and miscarriage rates in relation to the male age , ,but statistically significant differences in relation to cause of azoospermia and type of sperm used. **Conclusion:** The findings of the present study suggest that the ICSI outcome in patients with azoospermia is not influenced by the age of the male, but affected by the type of azoospermia (better outcome for OA in comparison to NOA) but has better pregnancy and live birth rates with lower miscarriage rate when use fresh type compared to frozen-thawed sperms.

**KEYWORDS:** Azoospermia, ICSI cycles, cycles outcome.

## INTRODUCTION

Azoospermia is the medical condition of a man not having any measurable level of sperms in his semen. It is associated with very low levels of fertility or even sterility. In humans, azoospermia affects about 1% of the male population.<sup>[1]</sup>

The first successful surgical sperm extraction in combination with intracytoplasmic sperm injection (ICSI) was done in 1994.<sup>[2]</sup>

The use of surgically retrieved sperm from the testis or epididymis with ICSI has now made it possible for patients with non-obstructive azoospermia and patients with obstructive azoospermia which is not suitable for surgical reconstruction to have children, as before this, the only management options was donor insemination or adoption.

The aim of this study was to assess the effect of: male age, the cause of azoospermia (obstructed or non-obstructed), the type of sperm used (fresh or frozen-thawed) on the ICSI cycle outcome in men with azoospermia

## METHODS

A retrospective analysis of testicular sperm extraction (TESE) and percutaneous sperm aspiration (PTSA)-ICSI cycles, carried out between 2012 and 2014 in our center (BSFC). The study included 54 cycles involving men with azoospermia, couples admitted for icsi cycles.

The outcome of the ICSI cycles was studied to evaluate the effect of: male age, the cause of azoospermia, the type of sperm used (fresh vs. frozen-thawed) on the fertilization, embryo cleavage, and clinical pregnancy, live birth, and miscarriage rates.

The diagnosis of obstructed or non-obstructed azoospermia was based on clinical history and evaluation to include testicular size and consistency, epididymal distension, the presence of the vasa, serum FSH levels and genetic study. The presence of normal sized testes ± epididymal and vasal dilatation, normal FSH, LH and testosterone levels, and normal karyotype with the absence of Y chromosome microdeletions suggested obstructed azoospermia. The final confirmation was made at the time of TESE and after review of the testicular histopathology. The presence of 'normal spermatogenesis' in the histopathology specimen supported and confirmed the clinical diagnosis of obstructed azoospermia

**Criteria used to diagnose obstructed azoospermia**

Testicular size <4cm length, dilated epididymis, absent or dilated vas, normal FSH, LH, Testosterone are normal, histopathology normal spermatogenesis.

**Surgical Sperm Retrieval**

In PTSA sperm aspiration attempt was done using 21 gauge butterfly needle, if no sperm found, TESE was done by four random, transverse, 1-cm incisions were made in the tunica albuginea, in different quadrants of the testis, and one piece of testicular tissue was taken from each incision, the specimens were then examined by an embryologist, where all the tubules were teased and analyzed for the presence of sperm. Any viable sperm present was either prepared for a fresh ICSI cycle or cryo-preserved for future use in ICSI. During the same surgical procedure, a specimen was taken for histological analysis.

**Freezing of TESE Sperm**

Each labelled cryogenic vial was filled with 1 mL sperm-freezing medium (Midcult, Denmark), which was allowed to equilibrate at room temperature for at least 1 h before use. The dissected pieces of tissue,  $\approx 2/3$  pieces were immersed in each vial containing the cryoprotectant solution. These were incubated at room temperature for 10–15 min and frozen using a controlled rate freezer. The samples were frozen at  $-8^{\circ}\text{C}/\text{min}$  to  $-30^{\circ}\text{C}$ , then  $-2^{\circ}\text{C}/\text{min}$  to  $-150^{\circ}\text{C}$ . The vials were immersed in liquid nitrogen.

**Preparation of Sperm for ICSI**

For fresh sperm ICSI, the tubules were dispersed using two hypodermic sterile needles and milked with blunt ended sterile glass pipettes. The supernatant was centrifuged once in 3 mL of pre-warmed Sperm Washing Medium (Quinn's (R) Sperm Washing Medium, Sage media, USA) at 500 g. The pellet was re-suspended in 50  $\mu\text{L}$  of the fertilizing medium (FM; Quinn's Advantage (R) Fertilization Medium, supplemented with 10% Quinn's Advantage Serum Protein Substitute, SAGE Media). A drop was put on a slide and observed for the presence of motile and non-motile spermatozoa. The final suspension was incubated for at least 1 h before use for ICSI to enable any sperm to attain motility.

For ICSI cycles using frozen/thawed sperm. The vials were thawed by incubation at  $37.5^{\circ}\text{C}$  for 5 min. The tissue was milked and centrifuged via the same method as for fresh TESE samples.

## ICSI

Ovarian stimulation was done with either recombinant FSH, human menopausal gonadotropin or urinary FSH. When the follicles reached 18–22 mm, 10 000 IU of HCG was administered. Oocytes were aspirated using trans-vaginal ultrasonographic guidance 35–36 hs after hCG administration. The picked oocytes were placed in Hyase for 1 min (80 U/mL) (ICSI-Cumulase, Origio, Denmark) and the cumulus cells stripped by aspiration with a Pasteur pipette. The oocytes were then transferred to the FM with hydroxyethylpiperazine-ethane-sulfonic acid buffer (HEPES, SAGE Media, USA) and further stripped using a 134  $\mu$ m sterile denudation pipette (SweMed Denudation Pipette 0.134–0.145 mm or 0.130–0.133 mm, VitroLife). Oocytes were then transferred to the FM and equilibrated for 45–60 min before ICSI.

ICSI was carried out at 41 h after hCG administration. The oocytes were washed in a drop of HEPES medium to remove traces of FM. Oocytes were injected with a single sperm, washed and transferred to a fresh dish of equilibrated medium (Quinn's Advantage (R) Cleavage Medium). Pronuclei were examined the next day. Embryo transfer was performed on day 2 or day 3 using a soft catheter with trans-abdominal US guidance.

Most female partners (80%) had 2-3 embryos transfer. Sometimes one embryo transfers were performed in female partners when just one embryo was normally grow . All patients received progesterone 400 mg pessaries as a supplement throughout the luteal phase with 3500 i.u low molecular weight heparin was given subcutaneously until a pregnancy test was performed 2 weeks later. A positive clinical pregnancy was evidenced by the detection of a fetal heartbeat at week 6 by trans-vaginal US.

## RESULTS

The mean (range) age of the men was 41 (20–63) years. There were no statistically significant differences in the fertilization, embryo cleavage, clinical pregnancy, live birth, and miscarriage rates between the different male age groups.

Of the 54 ICSI cycles included in the study, 9 (16.6%) involved patients with obstructed azoospermia and 45 (83%) involved patients with non obstructed azoospermia.

Statistical studies to compare results were done using the statistical significance t-test (if t – value was more than 2.0 this means the difference is statistically significant).

The results are shown in (Table 1) which showed a statistically significant differences regarding fertilization rate, embryo cleavage rate, clinical pregnancy rate, live birth rate and miscarriage rate between OA and NOA, the better outcome was noticed in the OA group.

**Table 1. ICSI outcome in OA and NOA TESE-ICSI cycles.**

ICSI outcome measure	Azoospermia			Total n = 54
	NOA cycles n = 45 (83.3%)	OA cycles n = 9 (16.6%)	t value	
Fertilisation rate, %	39 (86.6%)	9(100%)	114.52	
Embryo cleavage rate, %	59.6	88.5	192.66	
Clinical pregnancy rate, n (%)	57.1	66.6%	64.54	
Live birth rate, n (%)	57.1	66.6	54.59	
Miscarriage rate, n (%)	42.8	33.3	53.3	

In the 54 ICSI cycles, frozen –thawed sperm was used in 20 cycles (37.1%) and fresh sperm was used in 34 cycles (62.9%). There were no statistically significant differences in the embryo cleavage, while there were higher fertilization rate, pregnancy rate and live birth rate in the fresh sample group with lower miscarriage rate. (Table 2).

**Table 2. ICSI outcome with fresh and frozen-thawed sperm cycles**

No. of cycles n = 54	Fertilization rate, %	Embryo cleavage rate, %	Clinical pregnancy rate, n (%)	Live birth rate, n (%)	Miscarriage rate, n (%)
Frozen-thawed sperm, n = 20	76.1	94.2	31	33.3	66.6
Fresh sperm, n = 34	87.3	97.2	42.2	75	25
t value	17.5	1.5	700	320.7	301.4

**Table 3 . Icsi outcome in relation to duration of sperm cryopreservation**

Duration of sperm cryopreservation	1 month (n = 11 cycles)	2 months (n = 9 cycles)
Clinical pregnancy rate, n (%)	2 (18.1)	2 (22.2)
Live birth rate, n (%)	1(9.09)	1 (11.1)
Miscarriage rate, n (%)	1 (9.09)	1 (11.1)

There were no statistically significant differences in the clinical pregnancy, live birth, and miscarriage rates in relation to the duration of cryopreservation in the cycles that used frozen-thawed sperm (Table 3), may be because at our center icsi cycles were usually carried out during the 1<sup>st</sup> or 2<sup>nd</sup> menstrual cycles hoping that we can get better pregnancy outcome.

## DISCUSSION

Advancing female age has long been established to be associated with infertility and failed assisted reproductive therapy outcomes.<sup>[3]</sup> This is caused by the gradual depletion of the

ovarian primordial follicles with advancing female age.<sup>[4]</sup> However, the present study, investigated the effect of the male age, which was shown not to have any impact on the outcome of ICSI.

The cause of azoospermia (OA vs. NOA) did not affect the fertilization, embryo cleavage, clinical pregnancy, live birth, and miscarriage rates in the present series undergoing PTSA, TESE -ICSI. Desai *et al.* 2009<sup>[5]</sup>, reported similar findings in 156 TESE-ICSI cycles of OA and NOA males. However, other studies agree with our result that suggested a significantly improved fertilization and clinical pregnancy rate in men with OA as compared with NOA due to the higher incidence of chromosomal abnormalities in sperm retrieved from men with NOA.<sup>[6]</sup>

Using frozen-thawed sperm in ICSI cycles for men with NOA has the advantage of avoiding repeated surgical sperm retrieval with each cycle and ensures the availability of sperm before beginning the ICSI cycle, which reduces the cost and avoids unnecessary cycles.<sup>[7]</sup>

Although most in vitro fertilization (IVF) laboratories frequently prefer to work with fresh rather than frozen sperm<sup>[8]</sup>, some studies suggest that there is no significant difference in the ICSI outcome between fresh and frozen-thawed sperm for men with NOA.<sup>[9,10]</sup>

In our study as well as other studies reported better ICSI outcome with the use of fresh sperm samples in ICSI, previous reports have suggested that cryopreservation is associated with lower fertilization and pregnancy rates.<sup>[11,12]</sup>

This could be explained by the fact that testicular sperm is more sensitive to cooling than ejaculated sperm.<sup>[13]</sup> Cryoinjury may occur during freezing of sperm due to swelling and rupture of the Acrosomal and plasma membranes due to the formation of intracellular ice.<sup>[14,15]</sup> It has also been shown that there is an increase in the production of oxygen free radicals during freezing and thawing of sperm, which causes injury of the plasma membrane due to lipid peroxidation.<sup>[16]</sup>

In the 54-ICSI cycles included in the present study, frozen-thawed sperm was used in 34 cycles (62.9%) and fresh sperm was used in 20 cycles (37.1%). Statistical analysis did not show any significant differences in the fertilization, embryo-cleavage, but there was significant difference and better clinical pregnancy rate, live birth rate with lower miscarriage rates between the cycles that used fresh sperm and the cycles that used frozen-thawed sperm.

There were also no statistically significant differences in the clinical pregnancy, live birth, and miscarriage rates in relation to the duration of cryopreservation, in the cycles that used frozen-thawed sperm, may be because all frozen thawed cycles were carried out during 2 months duration of freezing.

A meta-analysis study by Nicopoullou *et al.*<sup>[6]</sup> showed no difference in the fertilization or pregnancy outcome between fresh or frozen-thawed testicular sperm but reported an impaired implantation rate with frozen sperm.

On the day of oocyte collection, a second TESE may be performed if an inadequate number of sperm is found after thawing. This is usually predicted by the embryologist based on the amount of sperm retrieved in the initial TESE and the subsequent histopathology.<sup>[16]</sup> As for men with OA, sperm retrieval could be performed on the day of oocyte collection, which reduces the cost of cryopreservation.

## CONCLUSION

The findings of the present study suggest that the ICSI outcome in patients with azoospermia is not influenced by the age of the male, but affected by the type of azoospermia (better outcome for OA in comparison to NOA) but has better pregnancy and live birth rates with lower miscarriage rate when use fresh type compared to frozen-thawed sperms.

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