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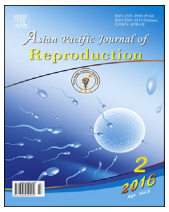

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Pregnancy outcomes of using ICSI with frozen-thawed spermatozoa in Riyadh, Saudi Arabia

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ABSTRACT

Objective: To assess the pregnancy outcomes of intracytoplasmic sperm injection (ICSI) in a sample of Saudi men with obstructive (OA) and nonobstructive (NOA) azoospermia.
Methods: A retrospective cohort study was undertaken between August 2004 and December 2012. The study was conducted in the Reproductive Endocrinology and Infertility unit at the department of Obstetrics and Gynecology, King Abdul-Aziz Medical City and King Fahd National Guard Hospital. A total of 136 ICSI cycles (6 in OA group and 130 in NOA group) with thawed-frozen spermatozoa was included in the study. Data on demographic and clinical characteristics of couples were collected such as age, number of cycles, clinical pregnancy, fertilization, and implantation.
Results: 43.4% of the cycles performed had the first ICSI attempt, and 72.8% had primary infertility. Male gender was the most common cause of infertility (96.3%). It was noted that the pregnancy rate was 10.8% in the NOA group, while none (0.0%) of the OA group had a positive pregnancy. Of 14 pregnancies in the NOA group, 8 (57.1%) had successful live birth. Primary infertility was more common in NOA than in OA (74.6% *vs.* 33.3%), conversely secondary infertility was less common in NOA than in OA (66.7% *vs.* 25.4%, *P* value = 0.047). The fertilization rate was not different between the two groups.
Conclusions: This is the first hospital-based examinations of ICSI outcomes in Saudi Arabia. The study failed to present statistical evidence that the ICSI outcomes, including fertilization rate and clinical pregnancy, differ between the two types of azoospermia in the Saudi population.

1. Introduction

Intracytoplasmic sperm injection (ICSI) is a very common assisted-reproduction technique which was launched in 1992 [1]. It is especially indicated to treat male factor infertility [1], where conventional IVF (*in vitro* fertilization) and other techniques for micromanipulation, including subzonal insemination (SUZI) and partial zonal dissection (PZD) did not obtain satisfactory results [2]. The evident ability of ICSI to achieve high fertilization and pregnancy rates applied less to the severity of oligospermia and led to its application for azoospermic patients [3]. Azoospermia is found in 1% of men, and in most cases comes from obstructive causes [4]. In 20% of azoospermia cases, the etiology is a bilateral obstruction of the male genital tract [5]. The most common causes of obstructive azoospermia (OA) include vasectomy and congenital absence of the vas deferens [4], which is found in 1–2% of the infertile male population [5]. The second group of azoospermia men are those with nonobstructive causes (NOA) [4]. Patients of this group often have small testis size and elevated follicle stimulation hormone (FSH) levels, which could be characterized as having testicular failure [4]. The common causes of NOA include Klinefelters's syndrome and mumps orchitis [4].

Fertilization and pregnancy can be achieved with spermatozoa retrieved not only from the ejaculate, but also from the epididymis (in patients of OA) or seminiferous tubules in the testis [6]. Spermatozoa can be successfully retrieved from testicular biopsies in approximately 60% of the cases [6]. Testicular spermatozoa never attain full normal function because maturation occurs in the epididymis [6]. However, in some patients with OA, microsurgical epididymal spermatozoa aspiration (MESA) fails to retrieve any sperm, either due to fibrosis or to the complete absence of the epididymis [7]. Nonetheless, most recent publications have reported acceptable

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fertilization and pregnancy rates for patients diagnosed with primary testicular failure [8,9], since there is clear evidence that there is an increase in the chromosomal aneuploidy rate in these patients' spermatozoa [10].

The outcome of ICSI using non-ejaculated sperm depends on many factors [11]. These include the etiology of azoospermia, the origin of the retrieved sperm, and the sperm status being fresh or cryopreserved [12]. The use of thawing cryopreserved sperm could be advantageous over the use of freshly retrieved sperm [13]. One reason for this is the avoidance of hormonal stimulation of the female partner and the risk of having failed sperm retrieved, as it accounts for 50% of male patients suffering from NOA [13]. Moreover repeated testicular surgery in subsequent ICSI cycles may cause testicular devascularization and possibly permanent injury [13]. In order to overcome these problems, diagnostic biopsies can be performed and cryopreservation of the spermatozoa can be performed.

Furthermore, cryopreservation can be made in multiple aliquots for repeated future use in ICSI cycles [14]. In 77% of the azoospermic patients, spermatozoa can be obtained from the testis by an open testicular biopsy technique and used for ICSI after freezing and thawing of the tissue [15]. The spermatozoa can be seen after thawing the tissue [15]. There were 135 ICSIs performed after thawing the testicular tissue, with a fertilization rate of 45% and a clinical pregnancy rate of 30% per oocyte retrieved [15]. The aim of this study was to evaluate the pregnancy outcomes of intracytoplasmic sperm injection using surgically retrieved sperm of azoospermic men either obstructive or nonobstructive.

2. Materials and methods

2.1. Study setting, design, and participants

A retrospective cohort study was conducted in the Reproductive Endocrinology and Infertility Unit at the Department of Obstetrics and Gynecology, King Abdul-Aziz Medical City (KAMC) and King Fahd National Guard Hospital. The study was approved by the Research and Ethics Committee at the College of Public Health and Health Informatics, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia. Written, informed consent was obtained from all participants, who agreed to share the outcomes of their cycles for research purposes.

2.2. Study selection criteria from the infertility database

Cohort of ICSI cycles performed between August 2004 and December 2012 for those who had surgically retrieved sperms in azoospermic men.

Inclusion criteria were: (1) Surgically retrieved sperm ICSI cycles including epididymal and testicular; (2) Both etiology of azoospermia included obstructive and nonobstructive. Exclusion criteria were surgically retrieved sperm for reasons other than azoospermia.

2.3. IVF cycle protocol at KAMC

2.3.1. Surgical sperm retrieval procedure

Testicular sperm retrieval was carried out under general anesthesia. Through a small vertical incision in the median scrotal raphe (2 cm), the skin, the dartos muscle, and the tunica vaginalis were opened to expose the tunica albuginea. The subtunical vessels under the surgical microscope were identified and avoided. A stay suture of 5/0 Prolene was placed into the tunica albuginea, and then a linear transverse (1 cm) incision was done. The testicular tissues were observed under optical magnification (×24). If no morphologically normal tubules could be observed, the incision was extended and blunt dissection performed between the septa of the testicular parenchyma to expose multiple areas. Copious irrigation of the field with phosphate-buffered saline solution was carried out to prevent blood from obscuring the field, and the most dilated tubule was measured using a micrometer fixed to one eyepiece of the operating microscope. Measurement was taken from edge to edge of the ST. Owing to the tortuosity of the seminiferous tubules, we measured the most dilated areas in the tubules three times and the surgeon gently rotated or reoriented the testis with his hands to render the edges of the ST in alignment with the grades of the micrometer IC. Three readings were recorded, and average diameter was calculated. This tubule was further dissected, isolated from the adjacent tissues, and examined for the presence of spermatozoa in the IVF laboratory. Another sample was taken from the adjacent tissues and was placed in Bouin's solution for histopathologic evaluation. Bipolar diathermy was applied carefully to ensure proper hemostasis. If no spermatozoa were detected, another sample was taken from the second most suspicious tubule after measuring its diameter. If no spermatozoa could be retrieved or the remaining testicular tissues were found to be homogeneous (i.e., no definite dilated tubules could be further identified under optical magnification), the same procedure was repeated by taking small samples at random from different sites of the incision up to a maximum of six samples. If no spermatozoa were detected, the contralateral testis was delivered and the same steps were followed. Testicular tissue samples were serially examined for the presence of spermatozoa until testicular sperm was detected or a maximum of six samples was obtained. The tunica albuginea was closed by Prolene 6/0, and the tunica vaginalis, dartos muscle, and skin were closed using 4/0 Vicryl in layers.

2.3.2. Seminiferous tubule and testicular tissue handling

The seminiferous tubule was minced in a Petri dish containing HEPES-buffered Ham's F10 and checked for spermatozoa under the inverted microscope. If spermatozoa were detected, we proceeded to the erythrocyte-lysing buffer (ELB) analysis. If no spermatozoa were observed, the minced tubule was removed from the Petri dish and the remaining media containing the different testicular tissue cells were placed in a tube and centrifuged for 5 min at 300 g. In positive samples, the numbers of spermatozoa were counted and motility assessed.

2.3.3. Ovarian stimulation and embryo transfer

The short GnRH-a protocol is the main protocol used in our unit. An injection of GnRH-a decapeptyl 0.1 mg SC is given to the woman at d3-4 of cycle if baseline endometrial thickness is <6 mm. The controlled ovarian stimulation was started on the same day, either by recombinant FSH (Gonal f), or human menopausal gonadotropins (menogon or merional) for seven days. On D8 repeat, estradiol level and vaginal USG were carried out. The dose of gonadotropins continued or adjusted according to these parameters. If there were two leading follicles more than or equal to 18 mm, patient was booked for ovum pick-up. Injection HCG 10000 IU was prescribed on the same day,

and she was scheduled for ovum pick-up from both ovaries after 5 h of HCG injection. The procedure was performed after obtaining written consent from the patient. It was carried out under moderate sedation (intravenous midazolam 2 mg and fentanyl 0.5 mcg/kg, maximum dose 1.5 mcg/kg). Then the patient was discharged to home. Embryo transfer was usually carried out after 2–3 days at the 4–8 cell stage. Supplementary progesterone (cyclogest) was started the day of embryo transfer and continued until 12 weeks if pregnancy test was positive and until the day of pregnancy test if it was negative.

2.4. Operational definitions

The primary outcome measure is the clinical pregnancy rate and is defined as positive serum pregnancy test (estimation of β-HCG in blood >5 mIU/mL) and a detectable intrauterine gestational sac by transabdominal ultrasonography. The secondary outcome measures include the fertilization rate which is defined as the number of oocytes injected divided by the number of embryos fertilized. The other secondary measure is the implantation rate which is defined as the number of gestational sacs seen in the ultrasound divided by the number of transfer embryos.

2.5. IVF outcome assessment

Clinical pregnancy rate, fertilization rate, implantation rate were calculated, and a comparison between the two groups of obstructive and nonobstructive azoospermia were conducted.

2.6. Statistical analysis

The data were analyzed using IBM Statistical Package for Social Sciences (SPSS®) version 22.0 (Chicago, Illinois, USA). Outcomes of patients in nonobstructive azoospermia and obstructive azoospermia were assessed using the Mann–Whitney U test and Chi-square test or Fisher's Exact test when necessary.

3. Results

A total of (n = 136) ICSI cycles were performed using testicular spermatozoa. The characteristics of 136 couples were summarized in Tables 1 and 2 using percentages (%) and mean and standard deviation (mean ± SD). One hundred thirty (n = 130) of the cycles were for nonobstructive azoospermia and 6 cycles were for obstructive azoospermia. Of 136 cycles, 59 (43.4%) had their first ICSI attempt, while 77 (56.6%) had multiple ICSI attempts with two cycles or more. It was observed that the majority of 99 (72.8%) had primary infertility, while 37 (27.2%) had secondary infertility. Male gender was the main cause of infertility (131, 96.3%). The mean age of females was (33.0 ± 5.0) years, while the mean age of males was (40.0 ± 10.0) years. The sub-group analysis show that nonobstructive azoospermia and obstructive azoospermia were similar in terms of the number of cycles, cause of infertility, and ages of both genders (P > 0.05). However, primary infertility was more common in the nonobstructive azoospermia group than the obstructive azoospermia group (74.6% *vs.* 33.3%), while secondary infertility was less common in the nonobstructive azoospermia group than the obstructive azoospermia group (25.4% vs. 66.7%), P value = 0.047 (Table 1).

The average number of each of the clinical outcomes of the ICSI cycles is calculated in Table 2. Embryos transferred had an average of 1.5 ± 1.2, oocytes retrieved had an average of 9.0 ± 5.8, oocytes injected had an average of 7.0 ± 4.8, and embryos fertilized had an average of 2.8 ± 2.7. The overall pregnancy rate was 10.3%. The clinical outcomes of ICSI cycles in both obstructive and nonobstructive azoospermia are shown in Table 2. The distribution of embryos transferred and fertilized was comparable in both groups (nonobstructive and obstructive), P value >0.05. The distribution of oocytes retrieved and injected were also comparable in both groups, P value >0.05. The percentage of Grade 1 embryos was 50% in the obstructive group and 36.9% in the nonobstructive group, and Grade 2 embryos were 33.3% and 43.1%, respectively with no significant difference. Clinical pregnancy rate was comparable in the two groups. Out of 14 pregnancies achieved in the nonobstructive azoospermia, one (7.1%) had chemical pregnancy, 5 (35.7%) had miscarriages, one (7.1%) had ectopic pregnancy and 8 (57.1%) had live birth. The implantation rate per embryo transfer was 37%, while live birth per embryo transfer was 23%.

Table 1
Characteristics of couples with etiology of azoospermia.

Characteristics	Cycles (n, %)		Type of infertility		Cause of infertility		Age of female	Age of male
	1 cycle	2 cycles or more	Primary	Secondary	Male	Both Male/Female		
OA (n = 6)	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)	6 (100.0)	0 (0.0)	34.0 ± 4.0	44.0 ± 9.0
NOA (n = 130)	57 (43.8)	73 (56.2)	97 (74.6)*	33 (25.4)	125 (96.2)	5 (3.8)	33.0 ± 5.0	38.0 ± 10.0
Total (n = 136)	59 (43.4)	77 (56.6)	99 (72.8)	37 (27.2)	131 (96.3)	5 (3.7)	33.0 ± 5.0	40.0 ± 10.0

*Fisher's Exact test significant at $\alpha < 0.05$ comparing OA with NOA.

Table 2
Clinical characteristics of couples with etiology of azoospermia.

Characteristics	No. embryos transferred	No. oocytes retrieved	No. oocytes injected	No. embryos fertilized	Fertilization rate (%)	Embryos				Pregnancy per cycle
						Grade 1	Grade 2	Grade 3	Grade 4	
OA n = 6	1.3 ± 1.5	7.0 ± 3.9	5.8 ± 2.9	2.7 ± 1.8	43.1 ± 31.8	3 (50.0)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
NOA n = 130	1.5 ± 1.2	9.1 ± 5.9	7.0 ± 4.9	2.8 ± 2.7	38.6 ± 29.3	48 (36.9)	56 (43.1)	23 (17.7)	5 (3.8)	14 (10.8)
Total N = 136	1.5 ± 1.2	9.0 ± 5.8	7.0 ± 4.8	2.8 ± 2.7	38.8 ± 29.3	51 (37.5)	58 (42.6)	23 (16.9)	5 (3.7)	14 (10.3)
P value	0.633	0.503	0.706	0.629	0.640	0.671	1.000	0.589	1.000	1.000

4. Discussion

The ICSI procedure has been introduced for the treatment of severe male factor infertility [16]. A combination of ICSI and surgically retrieved sperm works to help azoospermic men to father their children. The effectiveness of this procedure is very helpful for this kind of fertility problem. In addition, it prevents repeated sperm retrieval through the microdissection procedure [12], therefore there is less general anesthesia risk and also less tissue scarring. Furthermore, efficient non-ejaculated sperm preservation allows the independence of the surgical sperm retrieval from controlled ovarian stimulation and oocyte retrieval [17–19]. Thus, unnecessary ovarian stimulation is prevented in cases where no sperm can be retrieved, and oocyte retrieval planning is possible in the others.

In this study there were no significant difference between obstructive and nonobstructive azoospermia in the numbers of cycles performed, although we did not analyze the first cycle once but repeated it, as previous studies failed to demonstrate any differences in pregnancy outcome with the analysis of only the first cycle [3,20,21]. The only significant difference we found in patient characteristics existed where the majority of our patients had primary infertility. This finding was clear in the previous study and it explained the reasons of primary infertility.

Some studies show that ICSI, in combination with surgically retrieved spermatozoa, achieves good fertilization and pregnancy rates (56.0% and 30.4%) in both obstructive azoospermia and (39.0% and 11.3%) in nonobstructive azoospermia [7], whereas some studies showed a low fertilization rate and clinical pregnancy rate [22–24]. Most of the published studies were inconsistent in their finding regarding the fertilization and pregnancy rate, and that could be explained by the source of the sperm-retrieval methods used. The sources of sperm used in this study were all surgically retrieved spermatozoa from testicular tissues. This method is considered the preferable method of retrieval in men with obstructive azoospermia because it will allow the diagnosis, reconstruction, and ability to have as much as of the sperm as possible for cryopreservation [3].

Our data shows that there were no differences in the fertilization rate between the two groups of obstructive and non-obstructive azoospermia while the majority of the retrospective analysis done previously showed there was a lower fertilization rate in nonobstructive azoospermia after performing the ICSI procedure with thawed spermatozoa. This could be explained by the fact that the causes of nonobstructive azoospermia were not researched in this study. A possible reason for the lower fertilization rates after ICSI in nonobstructive azoospermia might be the lower concentration of spermatozoa, so that the possibility of choosing a normal mature spermatozoon is reduced, as previous studies have shown. On the other hand, in this study the source of spermatozoa was from the testicles through the microdissection procedure, which explains the issue of no significant differences between the obstructive and nonobstructive azoospermia in terms of maturation in the two groups, as the source was the same in both of them [12]. Windt *et al*. have found the same in both obstructive and nonobstructive azoospermia [24].

Pregnancy and embryo implantation rates were not significantly different after the ICSI in both obstructive and non-obstructive azoospermia. De Croo *et al*. reported the same finding in both the pregnancy and embryo implantation rate after ICSI with testicular spermatozoa in obstructive and non-obstructive azoospermia [2]. A possible limitation of this study is that fewer numbers of patients in our center were found with obstructive azoospermia who also had sperm cryopreserved through microdissection surgery. Instead of that, we found most of them had testicular sperm aspiration under local anesthesia, which explained the fewer number of obstructive azoospermia in the comparison. In addition, the causes of nonobstructive azoospermia were not researched in this study, therefore further analysis is needed in future studies. This is the first hospital-based examinations of ICSI outcomes in Saudi Arabia. The study failed to present statistical evidence that the ICSI outcomes, including fertilization rate and clinical pregnancy, differ between the two types of azoospermia in the Saudi population.

Conflict of interest statement

We declare that we have no conflicts of interest.

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