TESA/ICSI Outcome among Obstructive and Non-Obstructive Azoospermia

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Objective: To evaluate the Intracytoplasmic Sperm Injection (ICSI) cycle outcome for azoospermic in non-obstructive azoospermia (NOA) compared to obstructive azoospermia (OA) patients undergoing TESA procedures.

Setting: IVF Unit, Prince Sultan Military Medical City, Riyadh, KSA.

Design: A Retrospective Study.

Method: The data of couples that underwent ICSI with fresh sperm retrieval using TESA and reached the stage of embryo transfer were documented from November 2012 to March 2015. A total of 85 patients were included in this study. Personal characteristics, laboratory data, TESA data, stimulation parameter and pregnancy outcome were documented.

Result: Fifty-six males had OA and 29 had NOA. Female characteristics including age, FSH, BMI and the parity were similar. Male characteristics including age, smoking, and TESA motility and count were similar. Cycle characteristics including cycle number, protocol type, stimulation drug and duration, and estradiol and progesterone on the day of human chorionic gonadotropin (hCG) trigger were similar. Stimulation outcome including the number of collected, mature, and fertilized oocytes, embryo transferred, the day of embryo transfer and number of grade 1 embryo were similar. There was significantly better quality oocytes and higher number of frozen embryos in NOA group, P-value=0.03 and 0.04, respectively. Pregnancy, implantation, and miscarriage rate were also similar with no significant difference between both groups.

Conclusion: ICSI cycle outcome for azoospermic patients in NOA compared to OA undergoing TESA procedure was similar in both groups and no factors were affected the final cycle outcome.

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Approximately 10-15% of infertile males have absent sperm in the ejaculate, called azoospermia; it constitutes approximately 1% of all males[1,2]. Azoospermia is further categorized into obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) according to the underlying cause. In OA, there is a mechanical block in the genital tract between the epididymis and the ejaculatory duct, or there is an absence of the vasa deferentia[3]. On the other hand, NOA is defined by the failure of sperm detection in the centrifuged semen in conjunction with primary testicular failure[1,4].

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Intracytoplasmic sperm injection (ICSI) is indicated for the treatment of azoospermia with surgically retrieved sperm. Surgical retrieval of sperm is successful in most cases of OA and in approximately 50% of NOA cases[3]. Sperm retrieval techniques include ultrasound-guided fine needle aspiration (FNA), conventional testicular sperm extraction (c-TESE) and microdissection TESE (micro-TESE)[5].

Testicular sperm aspiration (TESA) is a common surgical procedure for sperm retrieval in assisted reproductive technology (ART) that enables azoospermic males to father their genetic offspring. It is a blind procedure usually performed under local anesthesia, or mild sedation in which a wide-bore needle is introduced to the skin of testis and suction is applied. The content of the needle is examined by embryologist to identify sperm with a stereomicroscope[6]. Testicular aspiration biopsy was first reported in 1965, but the first viable pregnancy was reported in 1995 by Yemini et al[7].

TESA enables the operator to reach more testicular sites without extensive testicular damage and minimal side-effects. In addition, TESA is recommended to be the first option of testicular biopsies as it is efficient, easy, safe and well tolerated by patients[6,7]. The success of sperm detection was evaluated in several studies. Lewin et al and Khadra et al reported a sperm retrieval rate of 58.8% and 53.6%, respectively[7,8].

TESA is considered less successful compared to other sperm retrieval procedures[6,9]. A study comparing the efficacy of TESA and TESE in NOA revealed that TESE was more efficient in detecting sperms and was recommended to be the first choice for sperm retrieval in NOA cases[9].

The aim of this study is to evaluate the ICSI cycle outcome for azoospermic in NOA compared to OA patients undergoing TESA procedure.

METHOD

Eighty-five patients who underwent TESA for sperm retrieval after reaching the stage of embryo transfer from November 2012 to March 2015 were reviewed. The following personal characteristics were documented in both groups: female age, female BMI, parity, AFC (antral follicle count), male age, and male smoking, female FSH, E2 and progesterone on day of trigger, TESA motility, TESA count; in addition to the stimulation parameters and the pregnancy outcome.

The criteria of acceptance in our IVF unit were as follows: male nationals, female $\leq$35 years of age, BMI of $\leq$30, and day 2 FSH of $\leq$13 IU/L at the time of referral.

Controlled ovarian stimulation cycle was initiated by subjecting the female partner to one of the different stimulation protocols using GnRH agonist (short or long) with Decapeptyl 0.1 mg/day (IPSEN), or GnRH antagonist with Cetrotide 0.25 mg/day (Merck). Ovarian stimulation was performed using rFSH (Gonal f or Puregon, Merck) or human menopausal gonadotrophin (HMG) (Menogon, Ferring). If a minimum of two follicles reached 18 mm or more or three follicles reached 17 mm or more, human chorionic gonadotrophin (HCG) trigger (5000 - 10,000 iu) (Pregnyl, Merck) injection was given 36 hours prior to oocytes retrieval.

Oocyte retrieval was performed using ovarian needle guided aspiration by transvaginal ultrasound probe. The retrieved oocytes were incubated in universal IVF medium (medicult) supplemented with solution. Oocytes were maintained at 37°C in a 5.5% CO_2 atmosphere. After one hour of oocyte retrieval, cumulus cell masses were removed by mechanical denudation using 0.05% hyaluronidase (hyadase enzyme for removal of cumulus complex and corona radiate surrounding the oocyte, medicult origio) then placed in a cleave medium (medicult, origio). Global medium was used for planned blastocyst transfer. If more than half oocytes appeared normal, the patient was labelled as having normal oocytes. Normal oocytes were described as round, clear zona pellucida, small pre-vitalline space containing a single non-fragmented polar body, pale, and moderately granular cytoplasm with no inclusions. Oocytes not fitting this description were labeled as “abnormal”. Combined normal and abnormal oocytes were labeled as “others”.

The male partner had to produce semen before ovarian stimulation to ensure the presence of sperms in the ejaculate. If no sperm was retrieved on two occasions, the male was diagnosed as azoospermia. Once the diagnosis was established, he would be seen by a urologist for assessment and biopsy. The patient would undergo TESA procedure to check the crop in testes and whether it was adequate for ICSI or not. TESA in our unit is usually performed under local anesthesia with the use of a wide-bore needle through the testicular skin as described by others[10].

ICSI is performed on the same day of ovum collection. Fertilization was scored after16-18 hours of the injection. On the day of embryo transfer, embryologists scored the embryos according to Sydney and Gardner’s embryo scoring for cleavage and blastocyst embryos consecutively.

Embryo transfer was performed from day two to five according to the embryologist and physician’s decision, depending on the number and quality of embryos available.

The pregnancy rate was defined as positive pregnancy test 12 days post-embryo transfer. The implantation rate was defined as the number of intrauterine gestational sacs observed by transvaginal ultrasonography divided by the number of embryos transferred. The miscarriage rate was defined as a pregnancy loss before 20 weeks of gestation.

Data were analyzed using StatsDirect statistical package. Two-sided Mann-Whitney U test was used to compare medians between two groups; two-sided Unpaired t-test was used to compare means between two groups, Chi-square test in crosstabs, Fisher- Freeman-Halton exact in crosstabs when any cells have an expectation of less than 5. P-values of less than 0.05 were considered statistically significant.

RESULTS

Eighty-five patients were included in the study and divided according to the type of azoospermia; 56 (66%) patients had obstructive azoospermia, and 29 (34%) had non-obstructive azoospermia. Female characteristics including female age, FSH level, BMI, parity, and AFC showed no significant difference between both groups, see table 1.

Table 1: Female Characteristics

	Obstructive Azoospermia N=56	Non-Obstructive Azoospermia N=29	P-value
Female age (years)	30±4.7	30.1±4.5	P=0.92*
Female FSH level	6.7±2.3	6.8±2.2	P=0.85*
AFC	17.7±9.5	21.7±11.8	P=0.09*
Female BMI	26.4±3.5	26.4±3.8	P=0.93*
Parity	0.6±1.2	0.3±0.4	P=0.18**
Unpaired t-test*, Mann- Whitney U test**			

Furthermore, there was no difference in male age and smoking between the two groups. TESA analysis confirmed similar count and motility as well, see table 2.

Table 2: Male Characteristics

	Obstructive Azoospermia N=56	Non-Obstructive Azoospermia N=29	P-value
Male age (years)	37.2±6.9	40.8±11.5	P=0.07*
Smoking			
Smoker	7 (12.5%)	3 (10.3%)	
Non-smoker	20 (35.7%)	6 (20.7%)	P=0.43****
Ex-smoker	3 (5.4%)	1 (3.5%)	
NA	26 (46.4%)	19 (65.5%)	
TESA motility			
Immotile	34 (60.7%)	19 (65.5%)	
Motile	15 (26.8%)	5 (17.2%)	P= 0.56***
NA	7 (12.5%)	5 (17.2%)	
TESA count	$4.6x10^{-6}4 \pm x10^{-6}$	$4.9x10^{-6}6 \pm x10^{-6}$	P=0.81*
Unpaired t test* Chi-square*** Fisher-Freeman- Halton exact****			

There was no difference in a number of IVF cycles, protocol type, stimulation drug and duration of stimulation. On the day of ovulation trigger, there was no difference in the level of E2 and progesterone between the two groups, see table 3.

Although the total number of oocytes collected was similar in the two groups, there were significantly better quality oocytes in the non-obstructive azoospermia group. However, the number of mature and fertilized oocytes was similar. Furthermore, the grade, number and day of embryo transferred were not statistically different between the two groups. Having better quality oocytes in the non-obstructive azoospermia group reflected in the higher number of frozen embryos, see table 4.

Pregnancy rate was higher in the non-obstructive azoospermia group but did not reach statistical significant, 48% compared to 45%. Furthermore, there were no differences in the implantation and miscarriage rates between the two groups, see table 5.

Table 3: Cycle's Characteristics

	Obstructive Azoospermia N=56	Non-Obstructive Azoospermia N=29	P-value
Cycle number	2.1±1	2.2±1.2	P=0.60**
Protocol type			
Antagonist	7 (12.5%)	6 (20.7%)	
Agonist long	47 (83.9%)	21 (72.4%)	P=0.43****
Agonist short	2 (3.6%)	2 (6.9%)	
Stimulation drug			
HMG35	35 (62.5%)	16 (55.2%)	
rFSH 19	19 (33.9%)	11 (37.9%)	P=0.62****
Mixed 2	2 (3.6%)	2 (6.9%)	
Duration of stimulation days	10.7±2.2	11.2±1.9	P=0.23*
E2 levels on the day of trigger	7164±3515	8008±3163	P=0.28*
Progesterone level on the day of trigger	1.9±0.9	2±1	P=0.76*
Unpaired t-test* Mann- Whitney U test** Fisher-Freeman-Halton exact****			

Table 4: Stimulation Outcome

	Obstructive Azoospermia N=56	Non-Obstructive Azoospermia N=29	P-value
Collected oocytes	9.9±5.8	11.8±6.1	P=0.19*
Oocytes quality			
Normal	27 (48.2%)	20(69%)	
Abnormal	1 (1.8%)	2(6.9%)	P=0.03****
Others	28 (50%)	7(24.1%)	
Mature oocytes	7.4±3.3	8.7±4.3	P=0.13*
Fertilized oocytes	4.6±2.4	4.5±2.8	P=0.78*
N of embryo transferred	2.57±0.6	2.48±0.8	P=0.57**
Day of transfer	2.8±0.8	2.8±0.8	P=0.67**
N of grade 1 embryo	1.6±0.6	1.68±0.7	P=0.66*
Frozen embryos	0±0	0.4±1.4	P=0.04*
Unpaired t-test* Mann- Whitney U test** Fisher-Freeman- Halton exact****			

Table 5: Pregnancy Outcome

	Obstructive Azoospermia N=56	Non-Obstructive Azoospermia N=29	P-value
Pregnancy rate	45%	48%	P=0.8***
Implantation rate	21.1%	20.6%	P=0.95***
Miscarriage rate	3.57%	3.45%	P>0.99****
Chi-square*** Fisher-Freeman-Halton exact****			

DISCUSSION

Azoospermia is diagnosed when at least two semen samples obtained more than two weeks apart are examined, analyzed and failed to contain any sperm according to 2010 World Health Organization guidelines[1,11]. Approximately 60% of NOA cases are due to hypogonadism (primary or secondary)[12]. Our data had a higher prevalence of obstructive azoospermia, (66%). Our study revealed that male age was similar in both OA and NOA groups. Tsai et al similarly reported age 34.7±5.5 and 36.6±6.6 years consecutively (P-value=0.112)[13]. De Croo et al found that male age was significantly higher in OA than NOA 40.6±58.1 and 34.4±6.5, consecutively (P<0.0001)[14].

In our study, we found that sperm motility and count were both not significantly different in males with OA and NOA. Very few studies examined the sperm characteristics amongst OA and NOA patients. Prins et al had similar findings to our study[15]. A study of OA and NOA groups showed that motile sperms were only found in the OA group[16].

Our NOA group had better quality oocytes. The pregnancy rate was slightly higher in this group but was not statistically different from OA. That might imply a negative impact of NOA on pregnancy. In our study, the NOA group had more frozen embryos. Other studies reported a similar pregnancy rate in both groups[14,17-21]. Francisco et al reported that pregnancy rate was similar in both groups regardless of the surgical procedure used[20]. Others found higher pregnancy rates with OA group[22-28]. Mansour et al reported that acquired obstructive azoospermia associated with the high fertilization and pregnancy rates compared to the congenital absence of vas deferens (CAVD) and NOA[29].

The implantation rate in OA and NOA groups were similar. De Croo et al found similar implantation rate of 19.6% in OA and 25.8% in NOA[14]. Kahraman et al found a high implantation rate in both NOA and OA groups[21].

Vernaeve et al compared cycles of males having NOA and cycles with OA; the implantation rate was higher in males with OA (8.6% versus 12.5%)[27]. Tehraninejad et al found a higher implantation rate in OA compared to NOA, P-value=0.001[24].

Miscarriage rate in our study was comparable in OA and NOA groups. A similar finding was reported by He et al in ICSI cycles with OA and with NOA, P-value=0.433[28]. Tehraninejad et al found a miscarriage rate of 9.7% in OA and 8% in NOA, P-value=0.776[24]. Other studies reported no difference in the miscarriage rate between both groups[22,30]. Pasqualotto et al found miscarriage rate was higher in NOA compared to OA (P-value<0.05)[25].

The limitation of this study is the retrospective nature, which limited our study to what was documented in the record. The result of this study should be used with caution due to the small number of patients.

CONCLUSION

ICSI cycle outcome for azoospermic patients in NOA compared to OA undergoing TESA procedure was similar in both groups and no factor was affecting the final cycle outcome.

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