Easiness of sperms retrieval in non-obstructive azoospermia is a strong predictor of clinical pregnancy after intracytoplasmic sperm injection

Original Article

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ABSTRACT

Background: Testicular sperm extraction followed by Intracytoplasmic Sperm Injection (TESE-ICSI) is the only route to genetically father a child in couples with Non-Obstructive Azoospermia (NOA).

Objective: The aim was to identify the effect of severity of NOA on clinical pregnancy following ICSI.

Patients and Methods: This retrospective study included 643 patients who underwent 712 ICSI cycles at a specialized In-Vitro Fertilization center between 2014 and 2019. We classified the patients into easy (445) and difficult NOA (267) based on the difficulty of microdissection TESE procedure, number of sperm retrieved, and the number of surgical attempts.

Results: The clinical pregnancy rate is significantly higher in the easy NOA group [167/299 (55.8%)] as compared with the difficult NOA group [68/194 (35.1%)]. The mean age of the females and the mean follicle stimulating hormone (FSH) levels were significantly lower in the easy NOA group compared with the difficult group [27.5±4.3 vs 29.9±4.6 years, *P*=0.0001, and 6.8±2.1 vs 7.4±2.5 mIU/ml, *P*=0.004, respectively]. There were no significant differences between the 2 groups regarding the mean male age, FSH level, and testicular size. The cumulus mass and the number of mature oocytes injected correlated significantly with the clinical pregnancy (*r*=0.122, *P*=0.012 and *r*=0.145, *P*=0.003, respectively). The sperm source whether fresh or frozen, the testicular pathology, and the identification of round spermatids in the pathology specimens did not correlate with the clinical pregnancy. We used the binary logistic regression to identify the final predictors. Only the NOA severity could be used to predict ICSI outcome. Easy NOA is more likely to have positive outcomes with ICSI (Odds ratio=1.70; Confidence interval=1.069–2.71; *P*=0.025). The model showed that neither female age nor FSH level could predict ICSI outcome in those patients.

Conclusion: Easy sperm retrieval is an independent factor that is associated with positive ICSI outcomes in couples with NOA.

Key Words: Difficult NOA, easy NOA, ICSI outcome, micro-TESE.

INTRODUCTION

Infertility was ranked by the WHO (2011) as one of the major health problems associated with disability among people younger than 60 years[1]. Remarkably, 9% of heterosexual couples complain of infertility[2]. Approximately half of them affected are males, where azoospermia is the severest form[3]. Currently, testicular sperm extraction followed by Intracytoplasmic Sperm Injection (TESE-ICSI) is the only route to genetically father a child in couples with non-obstructive azoospermia (NOA)[4]. This therapeutic modality has a cumulative

chance of 25% of resulting in a live birth. Spermatozoa are successfully retrieved in 50% of men with NOA, and the subsequent use of these spermatozoa in one or more ICSI treatments results in a live birth rate of 50%[4]. Currently, two methods are used to recover testicular sperms, random biopsy testicular sperm extraction (TESE) and microdissection TESE (micro-TESE). The procedure of micro-TESE performed with an operative microscope is generally considered to be the best method with higher spermatozoa recovery rates and minimal tissue loss in patients with NOA[5,6]. Repeated TESE may be needed after failed ICSI cycles with fresh and cryopreserved testicular

sperms or following an unsuccessful attempt to retrieve testicular sperms. The role of repeated testicular biopsies to retrieve sperms in azoospermic men was assessed by several studies[7,8]. Sperm recovery rate in repeated TESE was reported as between 74.7 and 96% when the previous recovery procedure was successful[7].

However, testicular spermatozoa were found only in 11.1–33% of azoospermic men with a previous sperm-negative procedure[7].We aimed in this study to identify the effect of the easiness of sperm retrieval – based on the difficulty of micro-TESE procedure, number of spermatozoa retrieved, and the number of surgical attempts – on clinical pregnancy following ICSI.

PATIENTS AND METHODS

Study design and settings

This was a retrospective study conducted from 2014 to 2019 and included 643 patients who underwent 712 Intracytoplasmic Sperm Injection (ICSI) cycles at a specialized in-vitro fertilization (IVF) center (Adam International Hospital, Giza, Egypt). We classified the patients into easy NOA (445) and difficult NOA (267) based on the difficulty of micro-TESE procedure, number of spermatozoa retrieved, and the number of surgical attempts. The patients signed written informed consent regarding the outcome and the risk associated with micro-TESE. Moreover, they were assured that confidentiality would be maintained during and after the study and information given would be used only for research purpose. Approval of the Ethical Committee was obtained and the study conforms to Helsinki declaration (1964).

Inclusion criteria

All the recruited participants experienced primary infertility of any duration and repeatedly azoospermic ejaculate with alkaline pH and with high or normal serum follicle stimulating hormone (FSH). We repeated micro-TESE at least after 6 months from the last attempt[9].

Exclusion criteria

All participants with obstructive azoospermia, hypogonadotrophic hypogonadism, bilateral cryptorchidism, azoospermic factor microdeletion a or b, or abnormal karyotyping that contraindicates ICSI were excluded from the study. Moreover, patients' wives who produced less than three oocytes in response to controlled ovarian hyperstimulation were excluded from the study. Moreover, patients' wives who had anti-Müllerian hormone less than 0.7 ng/ml were excluded from the study as they had significantly lower fecundability compared with their counterparts with higher anti-Müllerian hormone levels[10].

All the participants were subjected to the following: full clinical evaluation (history and examination) was done. At least two semen analyses with centrifugation and pellet analysis were conducted for each patient.

Basal hormonal profile (follicular stimulating hormone, luteinizing hormone, total testosterone, prolactin, and estradiol) was measured for the patients. All the patients with NOA had their serum hormone levels measured using chemiluminescence immunoassay (CLIA) technique, with values in the range of 1.5–14mIU/ml for FSH, 1.5–8 mIU/ml for Luteinizing hormone, 2.5–17 ng/ml for prolactin, 2.4–8.3 ng/ml for total testosterone, and 20–47 pg/ml for estradiol taken as normal. Early follicular phase FSH was measured for their wives who provided a blood sample on either day 2, 3, or 4 of the menstrual cycle and then the serum was stored frozen at −80ºC until analyses where FSH was measured by a direct immunochemiluminometric assay using the automated Immulite system (Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania, USA) (3–51 mIU/ml)[11,12]. Peripheral blood karyotyping and azoospermic factor microdeletion by PCR were offered for each patient with NOA.

Evaluation of prior histopathology reports and/or slides for patients who underwent previous micro-TESE was done. Testicular spermatozoa retrieval for each patient was done using micro-TESE technique[5,6]. Notably, we determined the total numbers of retrieved, injected (M2), and fertilized oocytes; numbers and quality of embryos formed; chemical pregnancy; clinical pregnancy; and miscarriage for their wives. Two protocols were used for controlled ovarian hyperstimulation. In brief, the long GnRH agonist protocol was based on the administration of daily leuprorelin (Lucrin; Abbvie, North Chicago, Illinois, USA) or triptorelin (Decapeptyl; Ferring, Saint-Prex, Switzerland) in the mid-luteal phase before the stimulation cycle. The administration of recombinant FSH (Gonal F; Merck, Kenilworth, NJ, USA), highly purified hMG (Menopur; Ferring Saint-Prex, Switzerland), or urinary FSH (Fostimon; IBSA, Lugano, Switzerland) was started when pituitary desensitization was achieved (7–10 days after the initiation of GnRH agonists), as evidenced by the absence of ovarian follicles more than 10mm, endometrial thickness less than 4mm on transvaginal ultrasound examination, and serum E2 less than 30. Alternatively, in the GnRH antagonist protocol, Cetrorelix (Cetrotide; Merck, Kenilworth, NJ, USA) was started daily 0.25 mg/days subcutaneously on day 5 or 6 of stimulation. We used transvaginal ultrasound with a frequency of 7MHz (Medison Digital Color MT; Medison Co. Ltd, Seoul, Korea). When two or more follicles reached a diameter of 18mm, human chorionic gonadotrophins (Choriomon, 10000 IU, intramuscular; IBSA, Lugano, Switzerland)) were administered 36h before recovery of large ovarian follicles by transvaginal needle-guided ultrasound aspiration. Modified Ham's F-10 with HEPES (99168; Irvine Scientific, Santa Ana, California, USA) was used to flush the ovarian follicles in case of difficult retrieval or if few follicles were seen.

The ICSI procedure was performed after the preparation of the oocytes according to the guidelines

of Palermo *et al.*[13]. The injected ova (metaphase II oocytes) were incubated for fertilization and further cleavage. Fertilization was confirmed after 16–18h by the observation of two distinct pronuclei (2PN) and two polar bodies. Embryo quality was assessed on days 2 or 3 after oocyte retrieval using a four-point score as described by Racowsky *et al.*[14].The score depended on cell number, as 4 cell stage on day 2 and 8 cell stage on day 3 having the highest score, and additional points were added for each of the following features : Absence of fragmentation or fragmentation involving less than 25% of embryonic surface and absence of irregularities in blastomere size or shape[14]. Immature, deformed and postmature oocytes were excluded. Blastocyst grading was done according to Gardner *et al.*[15].Thus, embryos with the best morphology and the most advanced stage of development were selected and transferred, by the same clinician, using a K-Jet embryo transfer catheter (Cook Echo-Tip catheter, Brisbane, Queensland, Australia). All the female patients received luteal supplementation by intravaginal administration of 400mg natural-micronized progesterone twice daily (Prontogest; Marcyrl, El Obour City, Egypt. Chemical pregnancy was identified as a positive β-human chorionic gonadotrophin assay,whereas clinical pregnancy was identified by the presence of at least one gestational sac with fetal heart beat detected by transvaginal ultrasound examination, and miscarriage was identified as pregnancy loss before 24 weeks of gestation[16].

Statistical analysis

We used SPSS version 26 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) in the analysis. Descriptive data presented as mean ±SD, frequencies, and percentages. We used the independent Student t-test to compare between easy and difficult NOA. Contingency table analysis was used to compare ICSI outcome using χ^2 or Fisher's exact test as appropriate. Potential clinical predictors including difficulty of micro-TESE were correlated with the clinical pregnancy rate using Pearson correlation. Only significant predictors in the correlation were used as independent variables in the regression analysis. *P* value less than 0.05 is considered to be significant, with all values at two-tailed until otherwise stated.
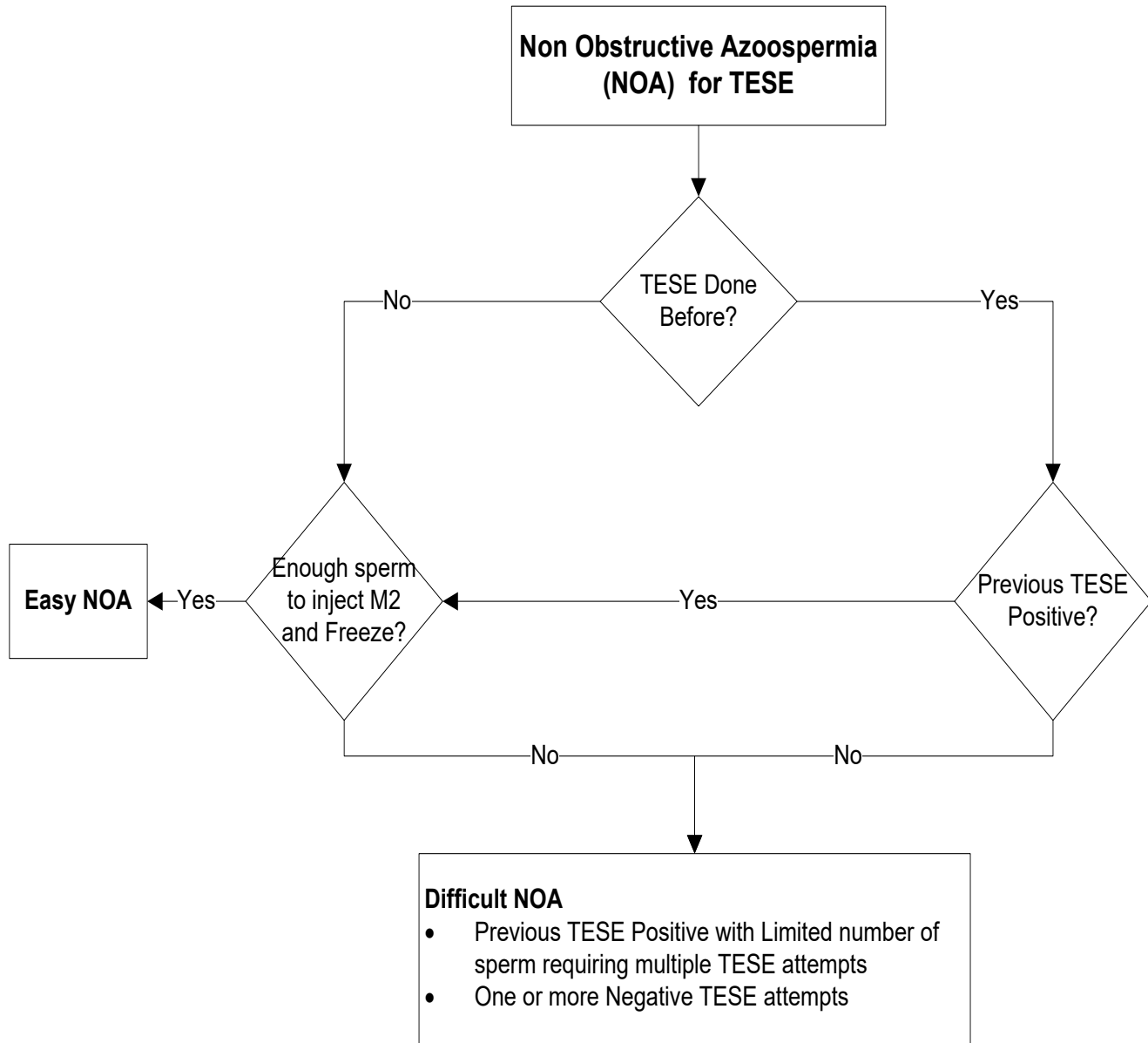
RESULTS

The retrospective analysis included 643 men with azoospermia who underwent 712 TESE/ICSI attempts. The mean age of males was 37.4±7 years, whereas it was 28.4±4.5 for the wives. The patients' demographics, the clinical characteristics, and the ICSI findings are shown in Table 1. We classified the ICSI attempt according to the difficulty of surgical testicular spermatozoa retrieval, number of spermatozoa harvested, and the repetition of TESE attempts into easy(445) and difficult (267) NOA, as shown in Fig.1. Testicular histopathology was similar in both groups. Sertoli cell-only (SCO) picture prevailed in both groups (35 and 37% in easy and difficult NOA, respectively), and hypospermatogenesis was the second most common finding (27 and 21% in easy and difficult NOA, respectively). Detailed histopathological pictures are shown in Table 2.

Table 1: Patients demographics and characteristics

	Mean	SD
Male age (Y)	37.4	7.0
Female age (Y)	28.4	4.5
Male FSH (mIU/ml)	17.88	14.86
Female FSH (mIU/ml)	7.05	2.3
Cummulus Mass	16.1	8.6
Mature oocytes	12.5	6.9
Injected oocytes	12.8	7.2
Fertilized	8.6	5.6
Fertilization Rate (%)	67.7	20.2
Embryos	8.5	5.5
Blastocyst in Day 5	4.1	3.1
Total Blastocyst	4.5	3.5
Embryo Transfer Day	2.8	1.9
Embryo Transfer Number	2.3	0.73
Good Embryos Transferred	1.2	1.0
Fair Embryos Transferred	0.8	1.7
Bad Embryos Transferred	0.6	0.9

FSH, Follicle Stimulating Hormone.

Non Obstructive Azoospermia
(NOA) for TESE

TESE Done
Before?

No — Yes

Enough sperm
to inject M2
and Freeze?

Previous TESE
Positive?

Easy NOA — Yes

Yes

No

No

Difficult NOA
- Previous TESE Positive with Limited number of sperm requiring multiple TESE attempts
- One or more Negative TESE attempts

NOA=Non Obstructive Azoospermia, TESE= Testicular Sperm Extraction

Fig. 1: Proposed criteria of easiness of sperms retrieval in Non-Obstructive Azoospermia.

Table 2: Basic differences between easy non-obstructive azoospermia (NOA) and difficult NOA

	Easy NOA	Difficult NOA	*P*
TESE Classification:			
Previous positive TESE	146 (32.8%)		
1st Time TESE	299 (67.2%)		
One Previous negative TESE		119 (44.6%)	
Two Previous negative TESE		48 (18.0%)	
Third Time TESE or more		100 (37.5%)	
Right testicular size			0.174
Small	123 (27.6%)	72 (27.0%)	
Moderate	220 (49.4%)	148 (55.1%)	
Normal	102 (22.9%)	47 (17.6%)	
Left testicular size			0.113
Small	121 (27.2%)	85 (31.5%)	
Moderate	216 (48.5%)	133 (49.8%)	
Normal	108 (24.3%)	48 (18.0%)	
Testicular Pathology			0.228
Tubular Sclerosis	4 (0.9%)	3 (1.1%)	
Sertoli Cell Only	154 (34.8%)	97 (36.6%)	
Primary spermatocyte arrest	87 (19.6%)	63 (23.8%)	
Spermatid arrest	50 (11.3%)	22 (8.3%)	
Mixed arrest	30 (6.8%)	25 (9.4%)	
Hypospermatogenesis	118 (26.6%)	55 (20.8%)	
Round Spermatids identified			0.062
No	245 (55.3%)	163 (61.5%)	
Yes	198 (44.7%)	102 (38.5%)	
Female diagnosis			0.411
No medical Issues	381 (95.3%)	203 (94.4%)	
Polycystic ovaries	15 (3.8%)	10 (4.7%)	
Polyp	3 (0.8%)	0	
Uterine anomaly	1 (0.3%)	1 (0.5%)	
Ovarian cyst	0	1 (0.5%)	
Stimulation Protocol			0.425
Fixed Antagonist	420 (94.4%)	248 (93.0%)	
Long	25 (5.6%)	19 (7.0%)	
Sperm Used			0.436
Fresh Sperm	242 (54.4%)	154 (57.7%)	
Frozen Sperm	203 (45.6%)	113 (42.3%)	
ICSI Outcome			
Fertilization Rate	3973/5938 (66.9%)	2107/3103 (67.9%)	0.345
Implantation Rate	215/729 (29.5%)	88/446 (19.7%)	0.0002*
Clinical pregnancy	167/299 (55.8%)	68/194 (25.1%)	0.0001*

ICSI, Intracytoplasmic Sperm Injection; NOA, Non-Obstructive Azoospermia; TESE, Testicular Sperm Extraction.
*Statistically significant.

The mean age of the females and the mean FSH levels were significantly lower in the easy NOA group compared with the difficult group(27.5±4.3 vs 29.9±4.6 years, *P* =0.0001, and 6.8±2.1 vs 7.4±2.5 mIU/ml, *P*=0.004, respectively). No significant differences were observed between the 2 groups regarding the mean male age, FSH level, and testicular size (Table 3). The mean cumulus mass, mature oocytes harvested and injected, the number of fertilized eggs, number of embryos, number of blastocysts in day 5, and the total number of blastocyst developed were significantly higher in the easy NOA as compared with the difficult group (Table 3). The fertilization rate was almost equal in both groups [3973/5938 (66.9%) in easy NOA compared with 2107/3103 (67.9%) in the difficult group, *P*=0.345]. However, the implantation rate and the clinical pregnancy rate were significantly higher in the easy group as compared with the difficult group [215/729 (29.5%)

vs 88/446 (19.7%), respectively, *P*=0.0002, and 167/299 (55.8%) vs 68/194 (25.1%), respectively, *P*=0.0001](Table 2 and Fig.2). Female age, serum FSH levels, the severity of NOA, cumulus mass, and M2 injected correlated significantly with the clinical pregnancy. On the contrary, male age, FSH levels, testicular histopathology, and sperm used (fresh vs frozen) did not correlate with the clinical pregnancy (Table 4). Female age, serum FSH level, and the severity of NOA were used as early independent factors that could predict clinical pregnancy after ICSI. We excluded the number of mature oocytes injected from the regression because it is a late predictor. The regression analysis showed that the severity of NOA is the only predictor of clinical pregnancy (odds ratio=1.70, 95% confidence interval=1.1–2.71, *P*=0.025). The model showed that female age, early follicular FSH level, and cumulus mass could not predict ICSI outcome in those patients.

Table 3: Compares between easy and difficult Non Obstructive Azoospermia (NOA) in terms of clinical parameters and ICSI outcome.

	Easy NOA (445)			Difficult NOA (267)			*P*
	N	Mean	SD	N	Mean	SD	
Male age (Y)	445	37.0	7.2	267	38.0	6.5	0.060
Female age (Y)	445	27.5	4.3	267	29.9	4.6	0.0001*
Male FSH (mIU/ml)	441	17.4	13.3	264	18.6	17.2	0.336
Female FSH (mIU/ml)	445	6.8	2.1	215	7.4	2.5	0.004*
Cummulus mass	399	16.9	8.8	257	14.7	8.3	0.001*
Mature Oocytes (M2)	399	13.1	7.0	257	11.4	6.7	0.003*
M2 Injected	442	13.4	7.1	257	11.6	7.4	0.001*
Fertilized	442	9.0	5.5	267	7.9	5.6	0.011*
Fertilization Rate	442	67.3	19.6	266	68.3	21.2	0.513
Embryos	442	8.9	5.4	266	7.8	5.6	0.011*
Blastocyst D5	147	4.3	3.1	38	3.1	2.9	0.025*
Total Blastocyst	261	4.7	4.2	109	4.2	3.5	0.221
Embryo transfer Number	306	2.4	0.7	198	2.2	0.7	0.051
Good Embryos transferred	290	1.3	1.0	191	1.0	1.0	0.170
Fair Embryos transferred	240	0.8	2.0	187	0.6	0.8	0.071
Bad Embryos transferred	231	0.4	1.0	184	0.4	0.9	0.954

FSH, Follicle Stimulating Hormone; NOA, Non-Obstructive Azoospermia.
*Statistically significant.
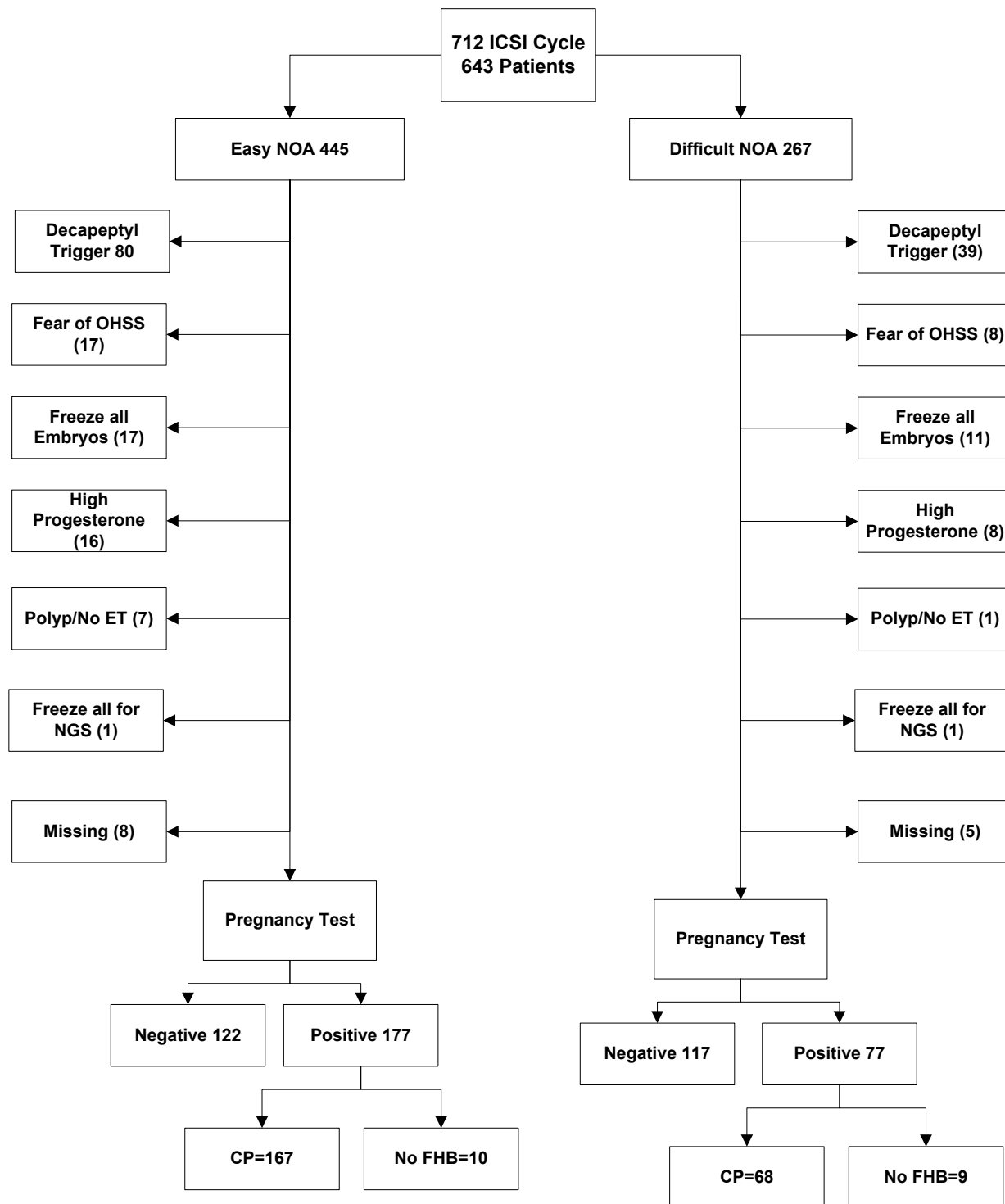
CP= Clinical Pregnancy, ET= Embryo Transfer, FHB= Fetal Heart Beat, ICSI= Intracytoplasmic Sperm Injection,NOA= Non Obstructive Azoospermia, NGS=Next Generation Sequencing, OHSS= Ovarian Hyperstimulation Syndrome,

Fig. 2: ICSI cycles in Non-obstructive Azoospermia (Easy versus Difficult).

Table 4: Correlation between the clinical pregnancy and male and female clinical parameters

| | Clinical Pregnancy | |
	r	*P*
Male Age	-.069	0.138
Female Age	-0.136	0.003*
Male FSH	-0.014	0.758
Female FSH	-0.11	0.05*
NOA (easy vs. Difficult)	-0.205	0.0001*
Testicular Pathology	-0.073	0.111
Spermatid identification	-0.062	0.182
Female Diagnosis	-0.059	0.239
Sperm Used (fresh vs. frozen)	0.059	0.23
Cumulus Mass	0.122	0.012*
MII Injected	0.145	0.003*

FSH, Follicle Stimulating Hormone; MII, Metaphase II oocytes; NOA, Non-Obstructive Azoospermia.
*Statistically significant.

Table 5: Regression analysis of the potential clinical predictors of clinical pregnancy

				95% CI for Exp(B)	
	B	Significance	OR	Lower	Upper
Female age	-0.040	0.142	0.960	0.910	1.014
FemaleFSH	-0.062	0.227	0.940	0.850	1.039
Non Obstructive Azoospermia	0.533	0.026	1.703	1.065	2.724
Cummulus mass	0.005	0.783	1.005	0.970	1.041
Constant	1.247	0.196	3.480		

CI, Confidence Interval; FSH, Follicle Stimulating Hormone; NOA, Non-Obstructive Azoospermia (Easy vs Difficult); OR, Odds Ratio.

DISCUSSION

Our study had shown a significant correlation between female age and clinical pregnancy. This finding can be explained by the fact that woman's age imposed a significant footprint on early embryo morphological development (3 Pronuclei), as compromised meiotic spindle function occurs with increasing female age[17]. Similarly, van Loendersloot *et al.*[18] stated that female age can be considered the most powerful predictive factor for pregnancy chances in IVF. Additionally, Bocca *et al.*[19] had demonstrated the significance of female age on IVF and ICSI outcomes. However, it should be noted that Bocca *et al.*[19] had conducted their study on patients with obstructive OA and those with NOA and found significantly high fertilization rates in patients with OA than patients with NOA whose partners were younger than 35 years old. Meanwhile, our study did not show

any correlation between male age and clinical pregnancy. In agreement with our finding, Wu *et al*.[20] reported no correlation between paternal age and clinical pregnancy rate. In contrast, Shrem *et al*.[21] had shown that paternal age above 37 years was significantly adversely correlated with clinical pregnancy. Besides, serum FSH levels of the females in the easy NOA group were significantly lower and had shown a significant correlation with clinical pregnancy than those in the difficult NOA group which could be explained by the fact that patients with elevated FSH levels are associated with decline in the quantity of the ovarian reserve with subsequent reduced pregnancy and live birth rates[22]. Moreover, early follicular phase FSH has been assessed as a marker of natural fertility, especially among subfertile women[23].

Furthermore, our study revealed a significant association between the maturity of the oocytes punctured, mean number of fertilized oocytes, quality of the embryos formed on day 3, blastocysts formed on day 5, and gestational sacs determined by transvaginal ultrasound and favorable ICSI. In the same context, Lazzaroni-Tealdi *et al*.[24] had revealed that oocyte size demonstrated significant effect on embryo quality and was an independent predictor of embryo quality after adjustment for age. Consistently, Yin *et al*.[25] had shown that transfer of equal number of embryos on day 3 was significantly associated with the highest clinical pregnancy rates in women younger than 35 years old. On the contrary, Capalbo *et al*.[26] had demonstrated that the well-settled parameters of blastocyst evaluation were not reliable enough as indicators to improve the selection among euploid embryos. Thus, all poor morphology and slower growing expanded blastocysts should be biopsied and similarly considered for frozen embryo transfer cycles[26]. Moreover, neither testicular histopathology nor FSH level in patients with NOA demonstrated correlation with clinical pregnancy. In the same line, a study conducted by Guler *et al*.[27] stated that testicular histopathology did not influence pregnancy rate in patients with NOA. Furthermore, Shrem *et al*.[21] did not find any correlation between male FSH level and clinical pregnancy. On the contrary, a study conducted by Zitzmann *et al*.[28] had shown that an FSH cutoff of 20 IU predicted pregnancy. Moreover, our study did not demonstrate any correlation between fresh or frozen spermatozoa and clinical pregnancy. Similarly, a recent systematic review and meta-analysis concluded that ICSI outcome is not affected by whether the retrieved testicular sperm is fresh or frozen[29].

Interestingly, our study is one of the first to demonstrate the crucial role of the easiness of getting spermatozoa from patients with NOA whose partners are normal to achieve clinical pregnancy with respect to the difficulty of TESE procedure, number of spermatozoa retrieved, and the number of surgical attempts. Thus, easy NOA cases were considered the only independent predictor of clinical pregnancy after adjustment of the other factors.

Consistently, a study stressed on the essential need of developing new treatment options to retrieve spermatozoa from NOA cases to enable biological fatherhood[30]. Admittedly, the retrospective nature and the relatively small sample can be regarded as the major limitations of this study. Moreover, inability to determine the live birth rate can be considered another limitation.

CONCLUSION

In brief, easy testicular sperm retrieval is an independent factor that is associated with positive ICSI outcomes in couples with NOA.

CONFLICT OF INTEREST

There are no conflicts of interest.

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