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Original Research Article

Reproductive outcome after surgical sperm retrieval and intracytoplasmic sperm injection in couples with male factor sub fertility: a 10-year retrospective observational study

Nikhil Sebastian*, Jayalakshmi Suraj

Department of Reproductive Medicine, Dream Flower IVF Centre, Kasaragod, Kerala, India

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***Correspondence:**
Dr. Nikhil Sebastian,
E-mail: nikhilsebastian571@gmail.com

ABSTRACT

Background: To evaluate surgical sperm retrieval (SSR) rate in men with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) and to compare clinical pregnancy rate (CPR) following ICSI. It was a retrospective observational study done at Dream Flower IVF Centre, Kasaragod, Kerala, India.
Methods: The 124 azoospermic men who underwent SSR along with intra cytoplasmic sperm injection (ICSI) between June 2013 to May 2024 were included in the study. OA and NOA were classified depending on the history, physical examination, ultrasonography and laboratory findings. Analysis of data on SSR rate and reproductive outcome following ICSI was done.
Results: A total of 124 male patients presented with azoospermia of which 52 patients (41.93%) had OA while 72 patients (58.06%) had NOA. The SSR rate was significantly higher in OA (100%) when compared to NOA (48.6%; $p<0.05$). The follicle stimulating hormone (FSH) level was significantly lower in participants with OA (5.2±2.0) when compare to NOA (25.1±15.2; $p<0.05$). ICSI using surgically retrieved spermatozoa was done in 90 couples. We failed to retrieve sperms in 34 (28.41%) patients, of which 27 couples opted for donor sperm (DS) for ICSI. The CPR was 55.8% and 39.5% in OA and NOA respectively. No significant difference in CPR ($p=0.286$), miscarriage rate ($p=0.056$) or live birth rate ($p=0.904$) were observed among the three groups.
Conclusions: Azoospermic men can be counselled about the probability of successful sperm retrieval as well as their likelihood to father their biological child following ICSI without the need of donor spermatozoa.

Keywords: Infertility, Azoospermia, Intra cytoplasmic sperm injection, Testicular sperm extraction, MicroTESE

INTRODUCTION

Azoospermia, defined as complete absence of sperm from the ejaculate is present in about 1 percent of all men and in 10 to 15 percent of infertile men.[1,2] Men diagnosed with azoospermia are broadly categorized as having a mechanical obstruction along the seminal tract (Obstructive azoospermia [OA]) or an intrinsic testicular impairment of sperm production NOA.[3] SSR is a means of extracting sperm in men with azoospermia.[2] A number of methods have been proposed to surgically retrieve sperm, including percutaneous epididymal sperm aspiration (PESA), micro epididymal sperm aspiration (MESA), testicular fine needle aspiration (TeFNA), testicular sperm extraction (TESE) and micro dissection TESE (Micro TESE) depending on etiology (OA vs NOA).[4]

Sperm retrieval "rates" (i.e. the percentage of post procedural sperm retrieval) vary according to the technique used, the patient population and the skill of the surgeon. Micro TESE has become popular because spermatogenesis in men with NOA is often found in small foci.[5,6] Despite being well preserved in OA, spermatogenesis is either minimal or absent in men with

testicular failure, thus lowering the success rate of sperm retrieval in the latter.[7]

Since its introduction in 1993, ICSI of oocytes using testicular and epididymal sperm has become a routine treatment procedure for patients with Azoospermia as male infertility factor.[8,9] The type of Azoospermia can also affect the outcome of ICSI procedure. Some studies have found no difference in outcomes of patients with OA vs NOA but most studies show that sperm harvested from NOA patients resulted in lower fertilization rates as compared to patients with normal spermatogenesis.[10-14]

There is a need to evaluate the SSR rate in men with OA and NOA as well as to compare the clinical outcomes of ICSI with surgically retrieved sperms taking into account the type of azoospermia and SSR procedure (TESA/TESE/Micro TESE). To address these questions, we conducted a large retrospective analysis of data at dream flower IVF centre, Kasaragod, Kerala over a period of 10 years.

METHODS

The study retrospectively analysed 124 azoospermic men who underwent SSR along with ICSI as the treatment for infertility at Dream Flower IVF centre, Kasaragod, Kerala from June 2013 to May 2024 over a period of 10 years. The objectives of the study were to evaluate SSR in men with OA and NOA and to compare CPR between OA and NOA after ICSI with surgically retrieved spermatozoa.

Inclusion criteria

All azoospermic men undergoing SSR for ICSI were included.

Exclusion criteria

Couples using donor oocytes for ICSI and men with hypogonadotropic hypogonadism and those with Y chromosome micro deletion (YCMD) involving AZFa or AZF_b sub regions were excluded from the study.

Six patients with karyotype abnormalities which included 3 patients with Klinefelter syndrome were included in the study. Only the first treatment cycle of each patient using fresh spermatozoa for ICSI were included.

The partners of azoospermic men had been investigated appropriately. Azoospermia was confirmed on at least two different centrifuged ejaculates. A thorough evaluation including demographic data, history, physical examination, hormonal profile (FSH, LH, Testosterone), genetic testing (YCMD and Karyotype), ultrasound scanning of the scrotum was done. Data on total number of oocytes, mature oocytes (MII) and fertilization rate and CPR was also collected.

Sperm acquisition

TESA/TESE or micro TESE was done depending on the type of azoospermia. Patients with OA underwent either TESA or TESE while NOA patients underwent TESE or micro TESE. After spinal anaesthesia TESA was performed using 21-gauge butterfly needle that was longitudinally inserted into the superior pole of the testicles avoiding the epididymis. Forward and backward movements of the needle as well as slight change in needle orientation were performed to ensure parenchymal disruption to allow needle aspiration. Open TESE was performed through a transverse incision on the scortum. 3 to 4 3mm biopsies were taken around the testis. The wound was closed with 5/0 PDS and 3/0 monocryl. All cases of micro TESE were performed by the same senior urologist at Dream Flower IVF centre. The aspirated materials from all the three surgical procedures were collected in a petridish and washed with minimum volume of modified human tubal fluid medium (HEPES, Irvine Scientific™, Santa Ana, California, USA) at 37°C. The recovered material was checked for the presence of spermatozoa and centrifuged at 300×gm for 8 minutes. The fraction was diluted or concentrated as necessary.

Stimulation protocol

Injection menotropin was used for ovarian stimulation along with either gonadotropin releasing hormone agonist or antagonist for LH Surge suppression. Oocytes retrieval was performed prior to but on the same day of SSR. ICSI and embryo culture were carried out in a clean IVF laboratory. Ultrasound guided embryo transfer was performed on third day of embryo development (Fresh transfer) or the embryos were frozen and transferred on a later month (Frozen transfer) depending on the condition of the patient. Pregnancy was confirmed by serum beta HCG test after 15 days of transfer. Clinical pregnancy was confirmed by presence of cardiac activity on ultrasound at 5 to 7 weeks. Miscarriage was determined by the presence of non-viable clinical pregnancy within 20 weeks. Live births were recorded for pregnancy more than 24 weeks.

Statistical methods

Data were analyzed using SPSS version 25 for Windows (Version 25, 2017, IBM corporation, Armonk, New York, United State). Data presented as mean±SD or frequency (Percentage). Cross tabulations were computed for categorical variables and compared using chi-square test/ Fisher's exact test. Normality of continuous data was assessed using ShapiroWilk Test. Continuous parameters were compared using independent sample T Test, Mann Whitney U test or one-way ANOVA. $P<0.05$ Was considered statistically significant.

RESULTS

There was a total of 124 male patients who presented with azoospermia at our clinic.52 patients (41.93%) had OA

while 72 patients (58.06%) had NOA. The SSR rate was significantly higher in OA (100%) when compared to NOA (35 patients i.e. 48.6%) (p<0.05). FSH level was significantly lower in participants with OA (5.2±2.0) when compared to NOA (25.1±15.2; p<0.05) (Table 1). Sperm retrieval was done either by TESA, TESE or micro TESE. When the patients were classified according to the type of surgery FSH was significantly higher in participants who underwent TESE and micro TESE as compared to TESA (p<0.05) (Table 2). FSH was also significantly higher in participants who underwent micro TESE as compared to TESE. Sperm retrieval rate was higher in patients who underwent TESA and TESE as compared to micro TESE (p<0.05). This could be due to the fact that all the men with OA in the study underwent TESA which had 100% SSR when compared to NOA patients (who had comparatively lower SSR rate) majorly underwent micro TESE as the SSR procedure. Out of 72 patients with NOA 28 patients underwent TESE while 44 underwent micro TESE. Even though the sperm retrieval rate was higher in NOA patients who underwent TESE when compared to MTESE, this difference was not significant (p=0.147) (Figure 1).

ICSI using surgically retrieved spermatozoa were performed in 90 couples. We failed to obtain sperms for 34 (28.41%) patients. Of these men 27 used donor sperms for ICSI while the remaining 7 patients had their treatment cancelled. In the latter, the retrieved oocytes were frozen in 5 patients and discarded in 2 patients. There was no difference in mean maternal (p=0.412) or paternal (p=0.809) age of patients in the three groups (OA, NOA, donor sperm). Similar results were observed regarding the mean number of oocytes retrieved (p=0.270), the mean number of MII oocytes (p=0.544), the mean fertilization rate after ICSI (p=0.341) and the mean number of transferred embryos (p=0.671) (Table 3).

Table 4 gives the pregnancy related outcomes for patients who decide to undergo ICSI. No significant difference in clinical pregnancy (p=0.286), miscarriage rate (p=0.056) or live birth rate (p=0.904) was observed among the three groups. The CPR in OA was 55.8% and NOA was 39.5%. A total of 23 and 22 infants were delivered after ICSI with surgically retrieved sperms in OA and NOA respectively. The live birth rates as well as the sex ratio of these children were comparable to those reported in donor sperm ICSI group.

Table 1: Comparison of participant baseline profile when classified according to type of azoospermia.

Variables	Obstructive azoospermia	NOA	P value
Number of patients	52	72	-
Male age (in years) (Mean±SD)	39.3±4.5	39.2±4.9	0.867
Male endocrine			
FSH (Mean±SD)	5.2±2.0	25.1±15.2	0.001
Testosterone (Mean±SD)	397.1±161.8	270.2±2106	0.005
Successful sperm retrieval N (%)	52 (100%)	35 (48.6%)	0.001

Data analyzed using Independent Sample T test or Fisher's Exact test

Table 2: Comparison of participant's baseline profile when classified according to type of SSR technique.

Variables	TESA	TESE	MTESE	P value
Number of patients	23	46	44	-
Male age (in years) (Mean±SD)	39.8±5.4	39.1±4.7	39.2±4.6	0.809
Male endocrine				
FSH (Mean±SD)	5.2±2.0	11.9±10.0*	29.2±16.5*#	0.001
Testosterone (Mean±SD)	450.0±116.2	368.1±186.6	252.9±205.0*#	0.004
Successful sperm retrieval N (%)	23 (100%)	46 (80.7%)	18 (40.9%)	0.001

Data analyzed using Independent Sample T test or Mann Whitney U test or Fisher's Exact test. *Significantly different from TESA at p<0.05. #Significantly different from TESE at p<0.05.

Table 3: Outcome of oocyte retrieval in females whose partner had obstructive azoospermia vrsus NOA versus failed sperm retrieval.

Variables	Obstructive azoospermia	NOA	Failed sperm retrieval	P value
Number of patients	52	38	34	-
Maternal age (in years) (Mean±SD)	31.1±4.2	30.9±5.1	31.7±4.6	0.412
Male age (in years) (Mean±SD)	39.3±4.5	38.5±5.0	40.0±4.9	0.716
Number of oocytes (Mean±SD)	11.4±6.5	9.3±5.3	10.3±6.2	0.270
MII (Mean±SD)	8.4±4.7	7.3±4.1	7.9±4.8	0.544

Data analyzed using one-way ANOVA test.

Table 4: Reproductive outcomes in OA versus NOA vs donor spermatozoa.

Variables	Obstructive azoospermia	NOA	Donor sperm	P value
Number of patients	52	38	27	
Fertility	6.4±4.1	5.4±3.2	6.8±4.8	0.341
Number of embryos	2.8±0.8	2.8±0.8	6.2±0.6	0.671
Clinical pregnancy	29 (55.8%)	15 (39.5%)	12 (44.4%)	0.286
Miscarriage	10 (19.2%)	1 (2.6%)	3 (11.1%)	0.056
Live birth	20 (38.5%)	14 (36.8%)	9 (33.3%)	0.904
Delivery				
Singleton	17 (85.0%)	7 (50%)	8 (88.9%)	NA
Twin	3 (15.0%)	6 (42.9%)	1 (11.1%)	
Triplets	0 (0%)	1 (7.1%)	0 (0%)	
Sex of offspring				
Males	6 (30%)	3 (21.4%)	6 (66.7%)	NA
Females	11 (55.0%)	4 (28.6%)	2 (22.2%)	
Fraternal twins	2 (10.0%)	3 (21.4%)	0 (0%)	
Male identical twins	1 (5.0%)	2 (14.3%)	0 (0%)	
Female identical twins/ triplets	0 (0%)	2 (14.3%)	1 (11.1%)	

Data analyzed using chi-square test or Fisher's exact test.

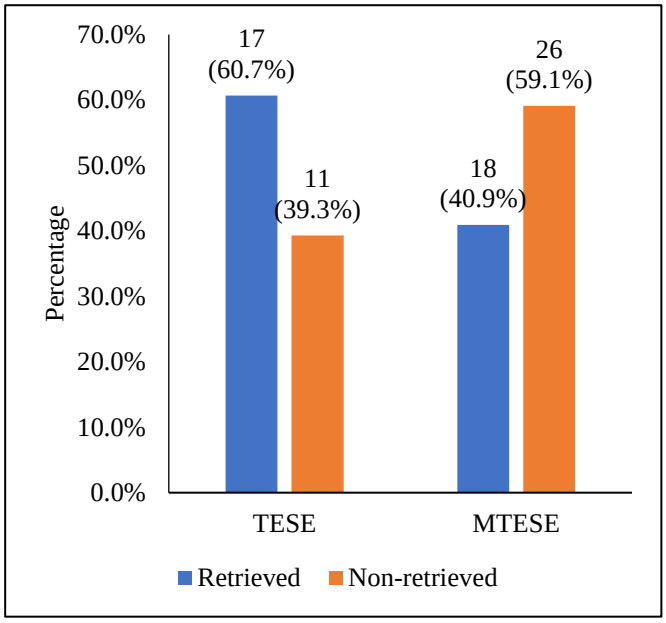

Figure 1: Sperm retrieval rate in NOA patients undergoing TESE versus micro TESE.

DISCUSSION

The development of ICSI and different techniques for spermatozoa recovery has enabled certain azoospermic males to father their biological children. There is however, limited data available that allows us to adequately counsel such couples about the success rates of spermatozoa retrieval. OA and NOA are often used to determine the probability of retrieval, but these can only be confidently diagnosed with testicular histology.[15]

NOA is characterized by hypergonadotropic hypogonadism along with bilateral small testis and elevated levels of FSH. The definitive diagnosis of testicular failure can be made only with a testicular biopsy. However testicular biopsy is not done routinely nowadays. Although the fertility might be restored in rare cases of spermatogenesis failure due to the lack of appropriate stimulation by pituitary gonadotropins the vast majority of these individuals have irreparable testicular failure.[16] Since there are no treatment options in such cases, the alternative is to attempt surgical retrieval and find viable testicular sperm for ICSI.[17] The rationale of this approach relies on the fact that rare foci of sperm production exist in up to 60% men with testicular failure.[3,17-19]

FSH levels have been shown to be inversely proportional to the spermatogonial population and are associated with NOA.[20] It is therefore not surprising that we observed that patients with either small testes or elevated FSH had significantly worse outcome than those with normal parameters. The recommended method for SSR in men with testicular failure is TESE, which yields variable success rates of 25% to 60%.[21] Because the presence and geographic location of islets of normal spermatogenesis are unpredictable several authors have proposed Micro TESE as better method for SSR in such cases.[3,17-19,22,23-26]

Bromage et al have shown that in 21 men with small testes and elevated FSH entering ICSI protocol sperm could be harvested in just 29% and only one resulted in pregnancy. Other studies have similarly poor results in men with severe testicular failure; pregnancies, and the live birth rate are usually <10%. ICSI is largely ineffective in these couples.[13] In our study sperm could be retrieved in 35 (48.6%) patients and a clinical pregnancy was seen in 15 (39.5%) in patients with NOA.

Unlike NOA, OA is the endpoint of a mechanical blockage along the reproductive tract involving vas deferens, epididymis or ejaculatory duct.[3,27] Treatment options in

OA include microsurgical reconstruction and surgical retrieval for ICSI.[28] The SSR rate in OA is practically 100% and is not influenced by the sperm collection method and the cause of obstruction.[29,30] Our study also showed 100% sperm retrieval in OA patients. We evaluated the SSR rate and ICSI outcomes in men with NOA and OA. As we not only compared the type of surgical procedure used for spermatozoa collection, but also the reason why the patient was azoospermic (i.e. NOA vs OA) we found that odds of finding spermatozoa with aforesaid method was significantly reduced in NOA ($p>0.05$). Our results showing similar fertilization rate and similar CPR using testicular spermatozoa of NOA and OA patients were corroborated by others.[10,11] On the other hand many studies reported lower fertilization rate and impaired pregnancy rates using testicular sperms of NOA patients.[31,32]

Male and female age, hormonal profile, duration of infertility, number of oocytes retrieved and the number of embryos transferred were also considered in the study. Some of these factors reflect ovarian function and are robust predictors of pregnancy in ART.[33] In this series men with NOA or OA had similar ICSI outcomes when compared with donor sperm, thus indicating that sperm integrity is not differentially affected by NOA or OA. Several studies reported similar ICSI outcomes regardless of the type of azoospermia.[10,11,14,34] On the other hand many studies also showed that the ICSI outcomes were negatively affected by testicular failure. These findings may be related to an increase risk of gametes extracted from men with severely impaired spermatogenesis to carry deficiencies involving centrioles and genetic material, which have been associated to decrease zygote formation and embryo development.[35,36]

The strength of our study is that we not only compared the different types sperm retrieval techniques but also the type of azoospermia. There is limited availability of similar studies which compares the different SSR techniques and the type of Azoospermia associated with it. The reproductive outcome following ICSI with the surgically retrieved spermatozoa has also been done in this study which can be used as a useful tool to counsel couples regarding the probability of them to have their own biological child. The limitation of the study was that we could not analyse the histological type of NOA (i.e. maturation arrest, hypo spermatogenesis and Sertoli cell only) which would have given us a better picture about the ICSI outcomes in such cases.

Our study shows that irrespective of the type of SSR technique in azoospermic men the ICSI outcomes were similar in TESA, TESE and Micro TESE group. Azoospermic males presenting with primary infertility should be fully assessed including serum FSH and testicular size. The results of our study can be used as a counselling tool for reproductive medicine specialists while treating azoospermia related infertility. Azoospermic men should be advised that their chance of having their sperm retrieved even with micro TESE and achieving a live birth following ICSI is affected by the type of azoospermia. Those with normal FSH and testicular size can expect a higher rate of successful SSR.

CONCLUSION

In conclusion this study proposes that TESA can be used as the SSR technique in cases of OA while micro TESE is a better option in cases of NOA. Once the spermatozoa have been retrieved the ICSI outcomes are not affected by the SSR technique or the type of azoospermia.

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