

Comparative Analysis of Surgically Retrieved Sperms in Cases of Obstructive and Nonobstructive Azoospermia Treated for Infertility

Deepa Talreja, Chirag Gupta¹, Hrishikesh Pai, Nandita Palshetkar, Rupin Shah¹

Bloom IVF Center, Lilavati Hospital and Research Center, ¹Department of Urology, Lilavati Hospital and Research Center, Mumbai, Maharashtra, India

ABSTRACT

Background: Azoospermia is a highly evolving subject in the last few decades. In the past, use of donor sperm was the only option providing a realistic chance of conception for couples affected by azoospermia. Introduction of sperm retrieval techniques and assisted reproductive technologies, especially intracytoplasmic sperm injection (ICSI), has provided these men a chance to father their genetically own child and changed the management approach significantly. **Objective:** The objective was to compare the sperm retrieval rate (SRR) and ICSI outcomes of surgically retrieved sperms in cases of obstructive and nonobstructive azoospermia (NOA) as well as to evaluate the efficacy of sperm retrieval techniques. **Materials and Methods:** A total of sixty azoospermic patients were included in the study. The patients were divided between OA (16) and NOA groups (44). A retrospective outcome analysis was done on SRR and ICSI results among them. **Results:** The overall SRR in patients with NOA and OA was 47.7% and 100%, respectively ($P < 0.001$). On subgroup analysis, higher serum follicle-stimulating hormone has shown significantly decreased sperm retrieval. The size of testes was not found to be related to sperm retrieval. Fertilization and embryo formation rate were found to be higher in OA cases in comparison to those of NOA cases. Clinical pregnancy rate showed no significant difference. **Conclusion:** Various sperm retrieval techniques can provide new dimensions for successful ICSI and managing azoospermia patients. Although SRRs as well as ICSI outcomes are lower in NOA patients than patients with OA, still they are potentially fertile. A systematic approach especially in patients with NOA is an important step. Microdissection testicular sperm extraction is an attractive option for NOA patients in order to increase the chances of successful sperm retrieval.

KEYWORDS: Fertilization rate, nonobstructive azoospermia, reproductive outcomes, sperm retrieval

INTRODUCTION

Azoospermia, defined as “Absence of sperms in Ejaculate after centrifugation,” is a relatively common cause of male infertility, affecting approximately 1% of the general population and observed in 10%–15% of infertile men.^[1] In the past, the use of donor sperm was the only option providing a realistic chance of conception for couples affected by azoospermia. However, introduction of sperm retrieval techniques and assisted

reproductive technologies, especially intracytoplasmic sperm injection (ICSI), has provided these men a chance to father their genetically own child.

Azoospermia can broadly be categorized as obstructive azoospermia (OA) where men are

Address for correspondence: Dr. Deepa Talreja, C-303, Siddharth Nagar, Near Jawahar Circle, Jaipur - 302 017, Rajasthan, India.
E-mail: drchirag845@gmail.com

Received: 19-04-2019
Accepted: 04-06-2020

Revised: 19-03-2020
Published: 27-10-2020

Access this article online

Quick Response Code:



Website:
www.jhrsonline.org

DOI:
10.4103/jhrs.JHRS_41_19

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Talreja D, Gupta C, Pai H, Palshetkar N, Shah R. Comparative analysis of surgically retrieved sperms in cases of obstructive and nonobstructive azoospermia treated for infertility. J Hum Reprod Sci 2020;13:201-8.

having normal spermatogenesis, but there is a mechanical obstruction (OA) along the seminal tract, or as nonobstructive azoospermia (NOA) due to the intrinsic impairment of testicular sperm production.^[2] Although in patients with OA, sperm retrieval is nearly 100% successful, the probability of finding sperm is around 50% in an unselected population of NOA patients.^[3]

Although surgical correction may be possible in selected cases of OA, treatment options for most couples with azoospermia-related infertility will ultimately include assisted reproductive techniques (ARTs).^[4] Over the years, several methods of epididymal and testicular sperm retrieval have been described to be used in conjunction with ART for men with azoospermia. Sperm retrieval techniques for OA include epididymal aspirations (MESA, percutaneous epididymal sperm aspiration [PESA]), whereas testicular sperm aspiration (TESA) and testicular sperm extraction (TESE or micro-TESE) are the procedures of choice in NOA patients for sperm retrieval. With the introduction of these sperm retrieval techniques, approach to managing azoospermic patients has changed significantly. ICSI has not only improved pregnancy rates in cases using sperm from ejaculated semen, but also provided new chances for achieving pregnancy with sperm retrieved from the epididymis or testis.

Although a Cochrane meta-analysis^[5] on surgical sperm retrieval techniques concluded that there is insufficient evidence to recommend any specific technique and that the least invasive technique should be used, recent studies mainly focusing on NOA concluded that microsurgical TESE may be associated with a higher recovery rate.^[6]

Till date, several studies have reported the outcomes of ICSI using nonejaculated sperm,^[7,8] but still, minimum data are available comparing sperm retrieval and ICSI results, providing a clear distinction between the types of azoospermia.^[9,10]

The aim of our study was to compare the sperm retrieval rate (SRR) and ICSI outcomes of surgically retrieved sperms in cases of OA and NOA as well as to evaluate the efficacy of these sperm retrieval technique in men treated for infertility.

MATERIALS AND METHODS

Patient selection

A retrospective observational study was performed at a tertiary-level *in vitro* fertilization (IVF) center over a period of 1 year (from August 2015 to July 2016). A written consent was obtained from all the patients

participating in the study. Ethical clearance was taken from the institutional review board.

A total of sixty azoospermic patients were included in the study. All patients were diagnosed with azoospermia, based on the WHO criteria, i.e., the complete absence of spermatozoa in the ejaculate in at least two semen samples including high-speed centrifugation with examination of the entire pellet.^[11] The patients were divided between OA and NOA groups on the basis of history, physical examination (i.e., bilateral testicular volume, condition of epididymis, and spermatic cord), endocrinological assessment (serum follicle-stimulating hormone [FSH], luteinizing hormone, and total testosterone), genetic testing (Yq microdeletions and karyotyping), and on the basis of histopathological types from previous biopsies, when available. In OA group, patients in whom surgical reconstruction was not possible or failed or they did not opt for it were included in study. Female partners for all males included in the study were having normal fertility or potentially correctable form of infertility. Only the first treatment cycle of each patient using fresh sperm for ICSI was included. In patients with testicular failure, spermatozoa from donors with proven fertility were offered, as a backup for ICSI, before the starting of treatment.

Retrieval of sperms

In all cases, surgical retrieval was performed on the day of oocyte retrieval in the female partner. A step-wise approach of sperm retrieval was used in all cases. In the case of OA, PESA was the initial technique of choice. In case of failure to retrieve sperms, TESE was considered. For patients with NOA, the initial procedure of choice was TESE by “Needle aspiration biopsy” (NAB) using 18G scalp vein needle, a percutaneous method which acquires a proper piece of testicular tissue, equal to an open biopsy, and not just an aspirate. In case of failure to retrieve sperms by percutaneous method, open TESE techniques such as single seminiferous tubule (SST) mapping or microdissection-TESE using operating microscope ($\times 25$ magnification) were used. Patients with very small testicular volume (< 5 ml) and very high FSH value (> 25 mIU/ml) were subjected to open microdissection TESE techniques only. On failure to retrieve sperm in one testis, the same procedure was used on the contralateral side in the same operation. Sperm retrievals were performed under local anesthesia in case of percutaneous procedures and under general anesthesia for open procedures. The extracted testicular tissues were placed in a dish with buffered medium and were immediately examined in the IVF laboratory. In

cases where clinical differentiation between OA and NOA was uncertain, a random biopsy was taken from one testis for histological examination.

Our techniques of TESE by NAB, SST mapping, or microdissection-TESE are described briefly below:^[12]

Needle aspiration biopsy

It is a percutaneous method for obtaining a testicular biopsy. An 18G scalp vein needle is introduced into the testis and with a 10-mL syringe, suction is applied. The needle is moved back and forth in one direction only. The scalp vein tubing is then clamped near the syringe to prevent the tissue from being sucked into the syringe and the needle is slowly withdrawn. When the needle exits the testis, a thick strand of testicular tissue is seen. This is grasped with a pair of microsurgical nonserrated forceps, and more tissue is pulled out from the testis. The clamp is then released, and the tubing is flushed with air to deliver another piece of tissue from the needle. Thus, a large piece of testicular tissue, equal to an open biopsy, is obtained. Hence, this NAB procedure is also termed needle TESE, as compared to open conventional (cTESE).

Single seminiferous tubule mapping

The scrotum is opened and the testis is exposed. A puncture is made in an avascular area of tunica with a 22G needle. With the help of a prong of a micro-forceps, puncture hole is stretched so that a loop of seminiferous tubule pops out. This tubule is grasped with the micro-forceps and pulled out of the testis and inspected under the operating microscope. If the tubule looks healthy, then more of it is pulled out until a sizeable length is delivered. It is then cut and transferred into the medium.

This technique allows a large piece of testicular tissue to be harvested without a cut or suture on the tunica. As the procedure is atraumatic, it can be repeated all over the testis. We usually take 20–30 (depending on the size of the testis) such biopsies from all over the testis, thus a very comprehensive mapping is done.

Micro-testicular sperm extraction

Following exposure of testis, tunica is incised along the entire transverse axis of the testis. The protruding parenchyma is inspected for dilated tubules, which are biopsied and checked for sperm. If no sperm are found, then the testis is bivalved and the two cut halves are explored. Blood vessels can be seen radiating from the hilum, and the parenchyma is dissected between these vessels. Eventually, the entire parenchyma can be everted over a finger placed on the outer surface of the tunica. This allows the entire testis to be inspected; most of it would be a mixture of atretic and thin tubules.

Tubules that are larger or “healthier looking” than the neighboring parenchyma are mainly selected and biopsied. For cases where often the entire parenchyma is uniform (e.g., in men with maturation arrest), multiple random micro-biopsies are taken from all over. If no sperm is found on one side, then the other side is explored. Careful hemostasis with a micro-bipolar cautery is important. The tunica is closed with 5–0 or 6–0 polypropylene sutures (Prolene; Ethicon EndoSurgery, Cincinnati, OH, USA).

Ovarian stimulation

After a systematic pre-ART evaluation, all female partners underwent ovarian stimulation using recombinant FSH or highly purified HMG in a flexible antagonist protocol. Injection human chorionic gonadotropin (HCG) 10,000 IU was given intramuscularly to trigger final oocyte maturation when at least three follicles reached 17 mm in diameter. In case the number of follicles on the day of trigger was more than 14, injection triptorelin 0.2 mg subcutaneous was given in place of HCG. Transvaginal ultrasound-guided oocyte retrieval was done after 34 h of injection HCG administration under anesthesia. Oocyte retrievals were performed on the same day of sperm retrieval but prior to it.

Sperm processing and injection

The excised testicular tissue was placed in a dish with buffered medium and mechanically teased with needles, and the suspension was directly assessed under the inverted microscope at $\times 200$ or $\times 400$ magnification. If sperms were easily identified, the suspension was transferred to a test tube, mixed with sperm preparation medium, and further processed by centrifugation at 300 g for 8–10 min. Following that supernatant was removed, the pellet was resuspended and 10 μ l droplets were smeared on the bottom of an ICSI dish and overlaid with oil to be used further for ICSI. During ICSI, best looking sperms, i.e., sperms with normal morphology and preferably motile sperms were used. In patients with failed sperm retrieval, ICSI was carried out using donor sperms, as per the prior acceptance and consent by the couple for the same. Excess testicular tissue containing sperms was cryopreserved for future use.

Fertilization and embryo transfer

Fertilization of oocytes was checked after about 16–17 h postfertilization for pronuclei formation (first check), and those displaying presence of two pronuclei and two polar bodies were considered to be normally fertilized and cultured further. Fertilization rates (FRs) were described as the percentage of oocytes with two distinct pronuclei per injected metaphase II oocytes. Embryos were evaluated on day 3 morphologically and assessed for development and quality. Good-quality

day-3 embryos were defined as six to eight cells and <10% fragmentation and were selected for transfer. In all our cases, two good-quality day-3 embryos were transferred. Supernumerary good-quality embryos were cryopreserved for future use.

Pregnancy follow-up

Pregnancy was confirmed with blood test (serum beta HCG) after 14 days from embryo transfer. A clinical pregnancy was defined as presence of gestational sac on ultrasound 5 weeks after embryo transfer. All patients were followed up till at least 12 weeks of gestational age.

Statistical analysis

SPSS software (SPSS version 16, IBM®, Chicago, IL, USA) was used for data analysis. Chi-square test was used for comparing categorical data, and *t*-test was used for comparing the means, when appropriate. $P < 0.05$ was considered to be significant.

RESULTS

A total of sixty patients were studied. Out of these, 44 were included in nonobstructive azoospermic group and 16 were in OA group. Baseline characteristics were compared in both the groups [Table 1]. The mean age of the patients in both the groups was 35.1 ± 4.7 and 34.5 ± 4.9 with no statistically significant difference. The mean volume of testis in both the groups was analyzed. In the NOA group, the mean left testicular volume was 11.23 ± 4.1 and the mean volume of the right testis was 11.44 ± 4.5 , whereas in the OA group, the mean left and right testicular volumes were 23.25 ± 2.6 and 24.75 ± 2.37 , respectively. Hence, there was obvious and significant difference in bilateral testicular volumes between both the groups ($P < 0.001$). In the NOA group, the mean serum testosterone and serum FSH were 316.54 ± 110.3 and 16.71 ± 8.9 , respectively. In the OA group, the mean serum testosterone and serum FSH were 495.85 ± 99.36 and 5.35 ± 1.1 , respectively. This difference in serum testosterone and serum FSH levels among both the groups was found statistically significant ($P < 0.001$). Age of the female partners and duration of infertility were comparable in both the groups.

In patients with NOA, the overall SRR calculated was 47.7%, as in 21 out of the 44 patients, sperm retrieval was successful using a step-wise approach. In case of OA, sperm retrieval was successful in all 16 cases, resulting in overall 100% SRR with a highly statistically significant difference among both the groups ($P < 0.001$) [Table 2].

In the NOA group, on subgroup analysis, on the basis of serum FSH levels, Group 1 (i.e., serum FSH > 15) showed

Table 1: Baseline characteristics of patients in both the groups

	NOA	OA	P
Male patients			
Number of patients	44	16	-
Mean age (years)	35.1±4.7	34.5±4.9	0.58
Hormonal profile			
Serum FSH (mIU/ml)	16.71±8.9	5.35±1.1	<0.001
Serum testosterone (ng/ml)	316.54±110.3	495.85±99.36	<0.001
Testicular volume (ml)			
Left	11.23±4.1	23.25±2.6	<0.001
Right	11.44±4.5	24.75±2.37	<0.001
Female partners			
Mean age (years)	31.4±4.3	32.1±5.2	0.6
Duration of infertility (years)	4.2±3.6	5.1±4.7	0.43

OA: Obstructive azoospermia, NOA: Non-OA, FSH: Follicle-stimulating hormone

Table 2: Comparison of sperm retrieval rates in men with obstructive azoospermia and nonobstructive azoospermia

Parameters	Results	P
Overall SRR (%)		
OA (%)	100 (16/16)	<0.001
NOA (%)	47.7 (21/44)	
Subgroup analysis		
OA		
Epididymal (PESA) (%)	68.7 (11/16)	-
Testicular (NAB technique) (%)	100 (5/5)	-
NOA		
Testicular (NAB technique) (%)	36.5 (15/41)	-
Testicular (SST/mTESE) (%)	20.6 (6/29)	-
SRR on the basis of testicular volume in NOA (ml)		
<14	48.6 (18/37)	1.0
>14	42.8 (3/7)	
SRR on the basis of serum FSH in NOA (mIU/ml)		
>15	20 (3/15)	0.01
<15	62.07 (18/29)	

OA: Obstructive azoospermia, NOA: Non-OA, FSH: Follicle-stimulating hormone, SRR: Sperm retrieval rate, PESA: Percutaneous epididymal sperm aspiration, NAB: Needle aspiration biopsy, SST: Single seminiferous tubule, mTESE: Microdissection testicular sperm extraction

SRR of 20%, whereas in Group 2 (i.e., serum FSH <15) SRR was found 62.07%. This difference in SRR in correlation with FSH levels between two subgroups was statistically significant ($P = -0.01$), which indicates that FSH can be used as a predictor of sperm retrieval in patients with NOA. When subgroup analysis was done on the basis of testicular volume, SRR calculated was

48.6% in Group 1 (i.e., testicular volume <14 ml) and 42.8% in Group 2 (i.e., testicular volume >14 ml). In our study, this difference in SRR in correlation with testicular volume levels between the two subgroups was not found statistically significant ($P = 1.0$).

On comparing the techniques of sperm retrieval in both the groups, in patients with OA, PESA was successful in 11 out of 16 patients, resulting in SRR of 68.7% by PESA alone. In the remaining five cases where PESA was failed, TESE with NAB technique was done which resulted in successful retrieval in all cases (SRR 100%).

In the NOA group, on analyzing the various techniques, TESE by NAB technique alone succeeded in retrieving sperms in 15 out of 41 patients (36.5%). In the remaining 26 patients where sperms were not found by NAB technique, further procedures (SST mapping/micro dissection TESE) were performed. Three out of the 41 patients of NOA with very small testicular volume (<5 ml) and very high FSH value (>25 mIU/ml) were subjected to open TESE techniques directly. Out of these 29 patients in whom micro-TESE was done, sperms were successfully retrieved in six patients, i.e., 20.6% of micro-TESE patients.

When laboratory and clinical outcomes were compared [Table 3], no significant differences were found in the number of oocytes retrieved among both the groups. The number of oocytes available for ICSI, i.e., mature oocytes (MII), was also comparable. In case of NOA, sperms were present for ICSI in 21 patients as we failed to obtain sperms in 23 patients. FR in these patients was 55.6 ± 32.8 (mean \pm standard deviation [SD]). On the other hand, in the OA group, FR calculated was 76.3 ± 34.5 (mean \pm SD), which was significantly higher than that of the NOA group ($P = 0.04$).

Similarly, when we compared cleavage stage embryo formation rate, it was found to be lower in patients

with NOA than patients with OA (45.3 ± 27.2 and 63.9 ± 30.3 , NOA vs. OA, respectively). This difference was also statistically significant ($P < 0.02$).

In patients with NOA, out of 21 cases where sperms were found and ICSI was done, 10 patients showed a positive beta HCG after 14 days of embryo transfer and 11 patients came out to be negative. Out of the positive patients, one patient showed fall in beta HCG levels, later on resulting in biochemical pregnancy. Nine patients showed the presence of cardiac activity in ultrasonography at 6–7 weeks, suggesting a clinical pregnancy rate of 42.9%. On comparing clinical pregnancy rate in patients with OA, out of 16 patients, 11 showed a positive result with serum beta HCG and all patients reached up to sonography suggestive of presence of cardiac activity, with no recorded biochemical pregnancy. Hence, the clinical pregnancy rate was 42.9% in the NOA group and 68.8% in the OA group ($P = 0.18$).

DISCUSSION

Our study compared the SRR and clinical and laboratory outcomes of surgically retrieved sperms in cases of OA and NOA.

Azoospermia is defined as “Absence of sperms in ejaculate after centrifugation.” In men with azoospermia, two distinct clinical diagnoses are usually seen in the form of NOA or OA. Currently, ART has become an established option for these men to achieve parenthood with consistent results. In contrast to the patients with OA where surgical correction is possible in selected cases, majority of patients with NOA need retrieval of testicular sperms for ICSI as an only treatment option. However, in comparison to men with OA, in NOA cases, lower success rates of pregnancy are achieved.

In our study, a step-wise approach was used for sperm retrieval in both the groups. The overall SRR calculated in patients in the NOA group was 47.7% as compared to 100% in patients with the OA group ($P < 0.001$). Our results are consistent with other studies also. Friedler *et al.* found a SRR of 100% in the OA and 40.6% in the NOA group.^[13] Vloeberghs *et al.*^[14] and Cissen *et al.*^[15] reported successful sperm retrieval in 40.5% and 43.7% of cases of NOA cases, respectively. This lower retrieval rate in the NOA group compared with the OA group can be explained by etiological factors as NOA can result from intrinsic (e.g., primary testicular failure) or extrinsic (secondary testicular failure) defect in testis function. The rationale of using testicular sperms is based on the fact that testicular sperms can be retrieved from some NOA men despite the absence of ejaculated spermatozoa in their semen because of the existence of isolated foci of active spermatogenesis.^[16]

Table 3: Comparison of outcomes in men with obstructive azoospermia and nonobstructive azoospermia

	NOA	OA	P
Number of retrieved oocytes	12.4 \pm 3.1	11.7 \pm 4.2	0.49
Number of oocytes for ICSI (i.e., MII oocytes)	10.7 \pm 2.5	9.8 \pm 2.8	0.24
Fertilization rate (%)	55.6 \pm 32.8	76.3 \pm 34.5	0.04
Embryo formation rate (cleavage stage) (%)	45.3 \pm 27.2	63.9 \pm 30.3	0.02
Bio chemical pregnancy	1	Nil	-
Clinical pregnancy rate (%)	42.9 (9/21)	68.8 (11/16)	0.18

OA: Obstructive azoospermia, NOA: Non-OA, MII: Metaphase II, ICSI: Intracytoplasmic sperm injection

When the different techniques were compared, TESE by NAB technique alone succeeded in retrieving sperms in 15 out of the 41 patients (36.5%). In 26 patients in whom sperm retrieval was not successful by bilateral TESE, a step-wise comprehensive technique using SST mapping and micro-TESE was performed. Apart from these 26 patients, three patients were directly subjected to micro-TESE in view of very small testis and high FSH. Sperms were successfully retrieved in six patients, i.e., 20.6% of micro-TESE patients. This shows that patients with NOA should be counseled for TESE as well as for micro-dissection TESE prior to the surgery as it helps in increasing the probability of finding sperms further in comparison to TESE alone. In a review analysis of surgical recovery of sperms in patients with NOA, Ishikawa concluded that micro-TESE, performed with an operative microscope, is widely considered to be the best method for sperm retrieval in NOA, as larger and opaque tubules, presumably with active spermatogenesis, can be directly identified, allowing the higher spermatozoa retrieval rates with minimal tissue loss and low postoperative complications.^[16]

In our study in case of OA, PESA was performed in all the 16 cases as initial procedure. Sperm retrieval was successful in 68.7% (i.e., 11 out of 16) of patients by PESA alone. In the failed five cases, testicular extraction by TESE with NAB technique was done, which resulted in successful retrieval in all cases (SRR 100%). This showed that the percutaneous procedures for sperm retrieval in OA are alone successful with 100% retrieval rates when percutaneous epididymal and testicular retrievals are combined. Consistent with our results, in a recent study, Esteves *et al.*^[2] reported a cumulative sperm retrieval success rate (SRR) of 97.9% using PESA, with or without TESA, in men with OA, regardless of the cause of obstruction. PESA alone was able to retrieve sperm in more than 80% of the cases. Similarly, Glina *et al.*^[17] performed a series of 58 men with OA treated with ICSI who underwent percutaneous epididymal sperm retrievals (with rescue TESA whenever needed). The authors reported 100% recovery of motile sperm using these combined techniques.

In our study, we correlated serum FSH levels and testicular volumes as factors affecting sperm retrieval in NOA patients. On subgroup analysis, on the basis of serum FSH levels, we found a reciprocal correlation between FSH levels and successful sperm retrieval. Consistent with our results, a retrospective cohort study by Cissen *et al.* including data from 1371 TESE procedures found that lower levels of FSH were predictive for successful sperm retrieval.^[18] However, in contrast to our results, a retrospective study

by Friedler *et al.* failed to find a correlation of FSH level to predict the presence or absence of testicular sperm after TESE.^[19] In few studies, it has been suggested that FSH level predicts better sperm retrieval by the conventional method of TESE but not by micro-TESE, as FSH levels reflect overall testicular function but it may not be reflective of patchy spermatogenesis.

In a subgroup analysis on testicular volume with correlation to successful sperm retrieval, no statistically significant correlation was found in our study ($P = 1.0$). In consistent with our results, Devroey *et al.*^[20] reported no statistically significant difference in testicular volume between NOA patients in whom sperm could be retrieved and patients in whom sperm could not be retrieved. In contrast to our findings, Ramasamy *et al.*^[21] in a retrospective analysis of 126 men with NOA who underwent one successful microdissection TESE attempt, found that men with a successful repeat attempt had larger testicular volume at the repeat procedure compared with men with a failed repeat attempt. On comparison of SRR between cTESE and micro-TESE, a retrospective study found the latter to be only superior in men with a testicular volume <10 ml (42% vs. 27%).^[22] One explanation to these findings could be patchy spermatogenesis in small testis, which can only be retrieved by micro-TESE.

On comparison to ICSI outcomes, no significant difference was found in total as well as number of matured oocytes among both the groups. In our study, we noted a lower FR in the NOA group in comparison to the OA group (FR [%] 55.6 ± 32.8 vs. 76.3 ± 34.5 , $P = 0.04$). Similarly, a lower cleavage stage embryo formation was also observed in NOA group (45.3 ± 27.2 and 63.9 ± 30.3 , NOA vs. OA, respectively, $P < 0.02$). Similar to our results, Ghanem *et al.* in a meta-analysis of five studies reported a significantly higher FRs and significantly higher proportion of Grade A embryos in patients with OA.^[7]

Finally, the main outcome studied was implantation potential of embryos derived from ICSI with surgically retrieved sperms in both the groups, resulting in viable pregnancies. In our study, the clinical pregnancy rate in the OA group was 68.8% and in the NOA group was 42.9%. This indicates that implantation potential of embryos derived from ICSI with surgically retrieved sperms in patients with OA was better than patients with NOA with respect to clinical pregnancy rate. However, the difference was found to be statistically insignificant ($P = -0.18$).

Such decreased reproductive potential of men with NOA seen in our study in the form of lower FRs, good-quality

embryo formation rate, and clinical pregnancy rates can be explained by the fact that testicular spermatozoa from men with severely impaired spermatogenesis have a higher tendency to carry defects related to the centrioles and genetic material, which impairs the capability of the male gamete to activate the oocyte and trigger the formation and development of a normal zygote and viable embryo.^[10]

The present study was a comparative analysis of the SRR and ICSI outcomes of surgically retrieved sperms in cases of OA and NOA. The study also evaluated the efficacy of these sperm retrieval techniques in men treated for infertility. In our study, we could not correlate our outcomes with histopathological diagnosis in NOA patients as biopsy report was available only in limited cases. Other limitations of our study were small sample size and retrospective nature. In addition, we could not confirm the live birth rate of our patients, due to limited follow-up. However, other studies have shown that live birth rate after sperm injection was lower in men with testicular failure in comparison to men with OA.^[10]

CONCLUSION

With the advent of ICSI, even few surgically retrieved sperms can help an azoospermic patient to father his own genetic child. In this study, we have concluded that surgical sperm retrieval is possible in OA as well as NOA patients. However, SRR is less in patients of NOA due to various intrinsic or extrinsic defects in testicular function in comparison to OA patients. Similar to the SRR, the chances of achieving a successful outcome (fertilization rate, embryo formation rate, and clinical pregnancy rate) after ICSI are also negatively affected by the type of azoospermia and are reduced in men with testicular failure in comparison to patients with OA. As the cause of azoospermia is different in both the groups, sperm retrieval techniques employed are also different. In patients with OA, percutaneous sperm retrieval methods (epididymal or testicular) are sufficient in all cases irrespective of cause of obstruction, whereas in NOA, finding a functioning seminiferous tubule is not possible in all the cases and depends on the underlying cause. A step-wise comprehensive approach in the form of TESE by NAB technique followed by single seminiferous tubule mapping (SST mapping) and finally micro-TESE in a single session should be followed to get best sperm retrieval outcomes. Micro-TESE is an attractive option for NOA patients in order to increase the chances of successful sperm retrieval and thus increasing the ART success rates.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. *J Urol* 1989;142:62-5.
2. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. [corrected]. *Clinics (Sao Paulo)* 2011;66:691-700.
3. Verheyen G, Popovic-Todorovic B, Tournaye H. Processing and selection of surgically-retrieved sperm for ICSI: A review. *Basic Clin Androl* 2017;27:6.
4. Esteves SC, Agarwal A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: Case series and systematic review. *Clinics (Sao Paulo)* 2013;68 Suppl 1:141-50.
5. van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database Syst Rev* 2008;2008:CD002807.
6. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: A systematic review and meta-analysis. *Fertil Steril* 2015;104:1099-103.
7. Ghanem M, Bakr NI, Elgayaar MA, El Mongy S, Fathy H, Ibrahim AH. Comparison of the outcome of intracytoplasmic sperm injection in obstructive and nonobstructive azoospermia in the first cycle: A report of case series and meta-analysis. *Int J Androl* 2005;28:16-21.
8. Esteves SC, Lee W, Benjamin DJ, Seol B, Verza S Jr., Agarwal A. Reproductive potential of men with obstructive azoospermia undergoing percutaneous sperm retrieval and intracytoplasmic sperm injection according to the cause of obstruction. *J Urol* 2013;189:232-7.
9. Vernaev V, Bonduelle M, Tournaye H, Camus M, van Steirteghem A, Devroey P, et al. Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and nonobstructive azoospermia. *Hum Reprod* 2003;18:2093-7.
10. Esteves SC, Prudencio C, Seol B, Verza S, Knoedler C, Agarwal A. Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility. *Asian J Androl* 2014;16:602-6.
11. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed.. Geneva, Switzerland: World Health Organization; 2010. p. 45-6.
12. Shah R, Gupta C. Advances in sperm retrieval techniques in azoospermic men: A systematic review. *Arab J Urol* 2018;16:125-31.
13. Friedler S, Raziel A, Strassburger D, Schachter M, Soffer Y, Ron-El R. Factors influencing the outcome of ICSI in patients with obstructive and non-obstructive azoospermia: A comparative study. *Hum Reprod* 2002;17:3114-21.
14. Vloeberghs V, Verheyen G, Haentjens P, Goossens A, Polyzos NP, Tournaye H. How successful is TESE-ICSI in couples with non-obstructive azoospermia? *Hum Reprod* 2015;30:1790-6.
15. Cissen M, Bendsorp A, Cohlen BJ, Repping S, de Bruin JP, van Wely M. Assisted reproductive technologies for male subfertility. *Cochrane Database Syst Rev* 2016;2:CD000360.
16. Ishikawa T. Surgical recovery of sperm in non-obstructive

- azoospermia. *Asian J Androl* 2012;14:109-15.
17. Glina S, Fragoso JB, Martins FG, Soares JB, Galuppo AG, Wonchockier R. Percutaneous epididymal sperm aspiration (PESA) in men with obstructive azoospermia. *Int Braz J Urol* 2003;29:141-5.
 18. Cissen M, Meijerink AM, D'Hauwers KW, Meissner A, van der Weide N, Mochtar MH, *et al.* Prediction model for obtaining spermatozoa with testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 2016;31:1934-41.
 19. Friedler S, Raziel A, Strassburger D, Schachter M, Soffer Y, Ron-El R. Factors influencing the outcome of ICSI in patients with obstructive and non-obstructive azoospermia: a comparative study. *Hum Reprod* 2002;17:3114-3121.
 20. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, *et al.* Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod* 1995;10:1457-60.
 21. Ramasamy R, Ricci JA, Leung RA, Schlegel PN. Successful repeat microdissection testicular sperm extraction in men with nonobstructive azoospermia. *J Urol* 2011;185:1027-31.
 22. Mulhall JP, Ghaly SW, Aviv N, Ahmed A. The utility of optical loupe magnification for testis sperm extraction in men with nonobstructive azoospermia. *J Androl* 2005;26:178-81.