International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Sandeep SP et al. Int J Reprod Contracept Obstet Gynecol. 2024 May;13(5):1289-1294
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20241082

Case Series

Male factor infertility with azoospermia: our experience in a tertiary care centre

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Received: 29 February 2024
Revised: 17 April 2024
Accepted: 18 April 2024

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ABSTRACT

Azoospermia is the absence of spermatozoa in two separate centrifuged semen samples. It is one of the major contributors of male factor infertility. Usually detected in the course of infertility evaluation. Many forms of azoospermia are amenable to medical treatment. Authors report a case series of 16 cases of infertility with Azoospermia which were managed in the Assisted reproductive technology centre of a tertiary care teaching hospital of the Indian Navy in Mumbai between Jun 2022 to Jul 2023. Out of the 16 cases 13 cases underwent surgical sperm retrieval by PESA/TESE. We could retrieve sperms in 11 cases. In 5 cases the retrieved sperms were motile and in 6 cases immotile sperms were obtained. ICSI done with both motile and immotile sperms resulted in fertilization. Our study shows that IVF-ICSI can produce successful fertilization even with Immature sperms or sperms obtained directly from the testicular tissue and IVF-ICSI allows for pregnancy in couple where the man has irreversible azoospermia as long as it is possible to recover sperm from the testes.

Keywords: Azoospermia, Male factor infertility, Surgical sperm retrieval

INTRODUCTION

Infertility is defined as the inability to achieve pregnancy after 12 months of unprotected intercourse. Factors affecting the male are crucial contributors to infertility amongst infertile couples. Approximately 15% of couple trying to get conceived are unsuccessful at 1 year, with a male factor solely responsible in 20% of cases and contributing along with a female factor in 30-40% cases.[1] Male infertility in its most severe form is known as azoospermia. Azoospermia is defined as the complete absence of spermatozoa in two separate centrifuged semen specimens. It affects nearly 1% of the male population and about 10%-15% of all males with infertility.[2,3] Many untreatable testicular disorders result in azoospermia.[4]

The exact pathophysiology of azoospermia is not always known. Azoospermia can be due to pre-testicular, testicular, and post-testicular causes. It is also classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA). It is important to differentiate between obstructive and nonobstructive azoospermia. With the advanced assisted reproductive technologies, various fertility options are available for couples having difficulties in conception due to male infertility, even with azoospermia.

Amongst azoospermia patients, 40% will have obstructive azoospermia.[5,6] Obstructive azoospermia may be due to the following reasons: congenital bilateral absence of the vas deferens, obstruction of ejaculatory and epididymal

ducts, atresia of the seminal vesicles, various infections of the genitourinary tract resulting in obstruction or pelvic and inguinal procedures leading to a complete blockage such as a bilateral vasectomy.[7,8] In obstructive azoospermia, spermatogenesis is often normal. Therefore, treatment options for obstructive azoospermia often include the surgical correction of the blockage in addition to other assisted reproductive techniques.

The majority of azoospermic men, about 60%, will have nonobstructive azoospermia making it the most common type of azoospermia. Nonobstructive azoospermia is most often due to severe defects in spermatogenesis, which are frequently due to primary testicular failure or dysfunction. It can also result from dysfunction of the pituitary or hypothalamus. The exact pathology of nonobstructive azoospermia is often idiopathic. Advanced assisted reproductive techniques can often treat nonobstructive azoospermia (primary testicular failure).[9]

Testicular biopsies of patients suffering from severe spermatogenic failure often show various areas of normal spermatogenesis.[10] These sperm can be retrieved using testicular sperm extraction (TESE) or testicular sperm aspiration (TESA) techniques and used in advanced assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI). Sperm retrieved from the testes in these ways and used for in-vitro fertilization with ICSI generally result in healthy offspring.[11,12]

Health care professionals face many challenges in providing care to infertile men with spermatogenic failure. Diagnostic modalities used for patients with azoospermia are hormonal assessment, biomarkers in semen, ultrasonography, testicular biopsy, and vasography. The best tool for diagnosing distal male reproductive system obstruction is transrectal ultrasound.[13,14]

CASE SERIES

This study was conducted in the Assisted Reproductive Techniques (ART) Centre of a tertiary care teaching hospital of the Indian Navy in Mumbai between Jun 2022 to Jul 2023. During this time period we have managed 16 cases of primary infertility with Azoospermia. The diagnosis was established on the basis of two semen analysis evaluations done at separate occasions. The investigations included thorough history, physical examination including evaluation of the scrotum and testes, laboratory tests and ultrasound imaging.

All the cases were presented with primary infertility of varying durations. In 11 out of the 16 cases in the study, female factors were absolutely normal. In 3 cases female partners had associated PCOS and in 2 cases female partners had subclinical hypothyroidism. 2 cases underwent surgical sperm retrieval with PESA and 11 cases underwent TESE. We could retrieve motile sperms by PESA in both the patients who had undergone PESA. And rest of the 11 patients underwent TESE under short general anesthesia (Figure 1). 2 cases were unwilling for surgical sperm retrieval and directly opted for donor sperms. One patient who had undergone surgery for varicocele 1 year back with lower levels of serum gonadotropins and testosterone responded to medical management with gonadotropin injections. Following treatment, we could obtain occasional motile sperms in the centrifuged semen sample of the patient and then underwent ICSI and the embryos are frozen. Further details of the cases included in the case series are given in the table below (Table 1).

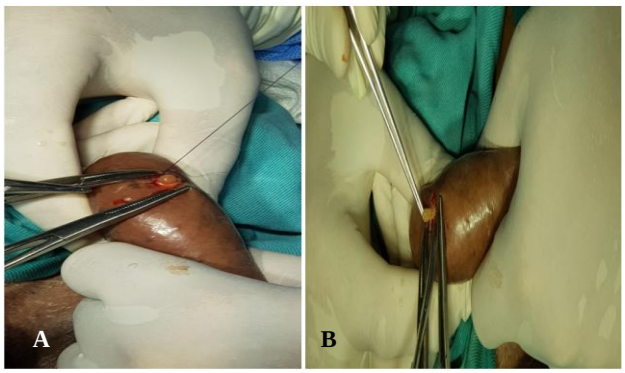

Figure 1 (A and B): Surgical procedure of testicular sperm extraction.

Table 1 describes the 16 cases in the case series one by one. It includes how the cases have been presented to us, detailed clinical examination findings, ultrasound scrotum findings, hormonal profile of the male partner, genetic studies of the male, surgical sperm retrieval procedure done for the male partner and the outcome after the assisted reproductive technology treatment.

DISCUSSION

Over the years, humans have evolved to sexual form of reproduction. The human genome is under continuous threat from microorganisms which can integrate their genetic material with it. Sexual form of reproduction involves splitting of diploid genome to haploid gametes followed by pairing of haploid gametes to form diploid genome thereby acting as a biological filter between subsequent generations to genetic impurities integrated during the lifetime of an organism. Spermatozoa contribute nearly half of the genetic material to human genome. Testis develop from indifferent gonads in presence of testicular determining factor (TDF), a product of TDF gene located on SRY region of Y chromosome. SRY in the presence of steroidogenic factor (SF-1) and Fibroblast growth factor (FGF-9) helps in expression of SOX-9 gene tilting the balance in favor of male sex differentiation.[15]

Table 1: Details of the cases included in the study.

Case no	Age (yr)	BMI (kg/m^2)	Presentation of cases	Clinical examination findings (Male partner)	USG scrotum	*FSH, LH, testosterone, prolactin, TSH	Karyotype	Y Chromosome microdeletion	PESA/ TESE	Result	Outcome after ART treatment
01	36	24.5	Primary infertility for 5yrs, Azoospermia, normal female factor.	Bilateral Varicocele, Left side thick epididymis	Gd 1 Varicocele bilateral	19.9, 8.7, 63.4, 4.4, 1.09	Normal	NEG	TESE	Immotile sperms with late maturation arrest	Embryos vitrified. Ist FET- negative awaiting next FET
02	33	22.9	Primary infertility 4yrs duration, Azoospermia, PCOS in female partner	Left testis undescended, Right- normal	Left testis undescended	28.3, 9.06, 516, 7.5, 1.3	Normal	NEG	TESE	Motile sperms	Conceived and delivered a healthy baby.
03	31	22.4	Primary infertility 3yrs, Azoospermia, normal female factor	B/L Absent epididymis	B/L Vas absent	5.4, 3.2, 421, 14, 1.2	Normal CFTR Neg	NEG	TESE	Motile sperms	Embryos vitrified and FET awaited
04	34	24.7	Primary infertility for 4yrs, Azoospermia, normal female factors	Normal	Normal	5.8, 3.8, 410, 6.2, 2.1	Normal	NEG	TESE	Motile sperms	Multiple FETs-negative
05	33	25.8	Primary infertility for 5yrs, Azoospermia, PCOS in female partner	Normal	Gd I Varicocele	5.02, 2.3, 82.1, 6.35, 1.62	Normal	NEG	Unwilling		Used donor sperms and conceived.
06	34	22.1	Primary infertility for 3yrs, Azoospermia, female factor normal	Normal	Normal	4.2, 3.6, 511, 7.1, 2.3	Normal	NEG	PESA	Motile sperms	Embryos vitrified. awaiting FET
07	32	23.9	Primary infertility for 4yrs, Azoospermia, normal female factor	Normal Varicocele (optd)	Normal	0.7, 1.5, 173, 11.8, 2.2	Normal	NEG	Medical management with Gonadotropins.	Got occasional motile sperms from the centrifuged sample.	Embryos vitrified. awaiting FET
08	33	24.9	Primary infertility for 2yrs, Azoospermia, normal female factor	Normal	Normal	2.3, 3.2, 344, 8, 5.2	Normal	NEG	TESE	Immotile sperms	FET (self)-negative. conceived after FET (donor sperm)
09	34	25.2	Primary infertility for 4yrs, Azoospermia, normal female factor	Normal	Gd I Varicocele	3.2, 2.9, 164, 10.6, 2.7	Normal	NEG	TESE	Immotile sperms +	Embryos vitrified. awaiting FET

Continued.

Case no	Age (yr)	BMI (kg/m^2)	Presentation of cases	Clinical examination findings (Male partner)	USG scrotum	*FSH, LH, testosterone, prolactin, TSH	Karyotype	Y Chromosome microdeletion	PESA/ TESE	Result	Outcome after ART treatment
10	29	24.1	Primary infertility for 3.5yrs, Azoospermia, subclinical hypothyroidism in female	Normal	Gd I Varicocele	7.4, 5.5, 289, 11.6, 1.5	Normal	NEG	PESA	Motile sperms	Embryos vitrified. awaiting FET
11	29	23.5	Primary infertility for 5.5yrs, Azoospermia, normal female factor	B/L Epididymis thickened and tender (History of Epididymal TB treated)	Normal	1.9, 3, 190, 6.1, 3.3	Normal	NEG	TESE	Immotile sperms	Conceived and pregnancy ongoing
12	28	25.6	Primary infertility for 4yrs, Azoospermia, normal female factor	B/L testes small	Normal	23.51, 5.89, 129, 11.69, 4.06	Normal	NEG	TESE	No sperms	Used donor sperms and conceived
13	28	25.5	Primary infertility for 5yrs, Azoospermia, subclinical hypothyroidism in female	Varicocele, B/L testes small	Gd IV Varicocele	15.9, 21.84, 247, 15.5, 5.2	Normal	NEG	TESE	No sperms	Used donor sperms-conceived and delivered
14	33	29.0	Primary infertility for 5yrs, Azoospermia, normal female factor	Normal	Normal	5.1, 5.7, 297, 7.9, 0.94	Normal	NEG	TESE	Immotile sperms	Embryos vitrified. awaiting FET.
15	33	22.3	Primary infertility for 6yrs, Azoospermia, PCOS in female partner	Normal	Normal	18.35, 5.7, 240, 23, 2.1	Normal	NEG	TESE	Immotile sperms (late maturation arrest)	Conceived following FET. missed abortion at 8wks.
16	36	20.5	Primary infertility for 3yrs, Azoospermia, normal female factor	B/L testes small	Normal	17.1, 18.1, 103, 16.1, 2.8	Normal	NEG	Unwilling		Used donor sperms and conceived

*Units used for various hormonal profile is as follows: FSH and LH- IU/L, Testosterone- ng/dL, Prolactin- ng/mL, TSH- mIU/L

Testes are formed in abdomen and later descend into scrotum. It is covered by three layers tunica vaginalis, tunica albuginea and tunica vascularis from out to inside. Each testis contains nearly 600 seminiferous tubules, the primary site for spermatogenesis.[16] Sertoli cells are primary support cells, constitute blood testis barrier and concentrate the androgens, an environment essential for spermatogenesis. Leydig cells are interstitial cells which secrete testosterone. Spermatozoa are produced from spermatocytes. Primary spermatocytes are diploid cells with double the DNA content. They give rise to haploid secondary spermatocytes during meiosis 1. Secondary spermatocytes undergo second meiotic division to produce haploid spermatids with DNA content of N. Spermatozoa are formed by nuclear and cytoplasmic modifications in spermatids by process of spermiogenesis. Mature spermatozoa are released into lumen of seminiferous tubules by the process of spermiation.[17]

Epididymis connects testis to vas deferens. It is divided into caput, corpus and cauda and is a storage site for sperms. Sperm is a complex, motile cell containing tightly condensed haploid DNA packaged by histones and protamines.[18] It migrates from vagina to site of fertilization in the ampulla of fallopian tube, identify and fertilize the ovum. Structurally, a sperm is divided into head and flagellum. The flagellum consists of connecting piece, mid piece, principal piece and terminal piece. The connecting piece had capitulum which is a site of centrioles. The major functions of centrosome are nucleation of microtubules and miotic spindle formation. Only proximal centriole is seen in mature spermatozoa.[19]

The normal physiology together with pathophysiology of male infertility forms the basis in resolving the current pitfalls in diagnosis and management of infertile men. In our study we have managed 16 cases of male factor infertility with azoospermia by various modalities. The detailed discussion of our study is as follows.

The average age of the patients with azoospermia in our study was 32.2 yrs. Of which two were above 35 yrs. Four patients were in the overweight category with BMI>25 (25%). Average infertility duration was 4.21 yrs. Clinical examination and ultrasound scrotum revealed varicocele of various grades in 5 patients (31.2%). One patient had unilateral undescended testis with normal single testis in the scrotum. One case had congenital bilateral absent vas deferens (CBAVD), who was further evaluated for CFTR gene mutation and found negative for the mutation. On clinical examination 3 patients had bilateral small testes (18.75%).

Among the 16 azoospermia cases 11 of them had low testosterone levels (less than 300ng/dl) (68.75%), and 5 had normal levels of serum testosterone (more than 300ng/dl) (31.25%). Study conducted by Babu et al showed no statistically significant difference between serum testosterone levels among males with azoospermia or normospermia.[20] We observed raised levels of serum FSH in 6 cases (37.5%) and raised serum LH levels in 4 cases (25.0%). Both FSH and LH were elevated in 4 cases (25%). We observed low levels of gonadotropins in 3 cases (18.75%). Serum prolactin levels were within the normal range in all the cases. Two patients had subclinical hypothyroidism (12.5%). Karyotyping and Y chromosome microdeletion tests were normal in all the 16 cases.

Out of the 11 cases who underwent TESE, in 9 cases we could retrieve sperms. Previous studies have reported that the sperm retrieval rates for microdissection testicular sperm extraction (mTESE) as between 45-63% with standard or multibiopsy TESE.[21,22] In our study the sperm retrieval rate for TESE was 81.8%. In a recent meta-analysis, standard TESE was found to be two times more likely to result in a successful procedure.[23] In our study, in 3 cases the retrieved sperms were motile (33.3%) and in 6 cases they were immotile (66.6%). ICSI done with the retrieved sperms resulted in fertilization in all the 9 cases. Till now 3 couples have been conceived following embryo transfer using the surgically retrieved sperms. Of which one has delivered a healthy child, one couple had a missed abortion at 8wks and the third couple has an ongoing healthy pregnancy. 4 couples have their embyos frozen and they are awaiting frozen embryo transfer. 2 couples had negative results after multiple attempts of FETs and one of them proceeded further for FET with donor sperm embryo and conceived in the first FET cycle only.

Figure 2: (A) Extracted seminiferous tubules in a plate and (B) seminiferous tubules under magnification.

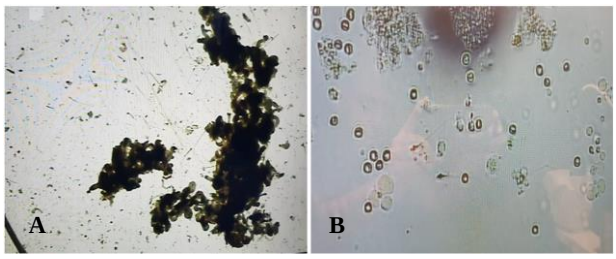

Figure 3: (A) Seminiferous tubules after mechanical disruption using needled tuberculin syringes and (B) immotile sperms visualized in the same sample.

Mechanical disruption of the retrieved seminiferous tubules after TESE was achieved by mincing using needled tuberculin syringes (Figures 2 and 3). When only

immotile spermatozoa were obtained motility stimulant – Pentoxifylline was used.[24]

CONCLUSION

The suitable treatment for OA is surgical correction if possible, and for NOA is microsurgical testicular sperm extraction followed by intracytoplasmic sperm injection (ICSI). Sperm retrieval procedures allow men with OA and NOA to father a child through assisted reproductive techniques. These options may provide the quickest avenue to achieve a pregnancy, but place the burden of treatment more heavily on the female partner as ART will be necessary. A trial to identify and correct factors that could inhibit spermatogenesis like infection, hormonal imbalance, or varicocele could help clinicians obtain even rare motile sperm in the ejaculate without the need for surgical sperm retrieval. Managing such correctable problems may also increase the chances of positive sperm retrieval at the testicular biopsy. IVF-ICSI can produce successful fertilization even with Immature sperm or sperm obtained directly from the testicular tissue and IVF-ICSI allows for pregnancy in couple where the man has irreversible azoospermia as long as it is possible to recover sperm from the testes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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Cite this article as: Sandeep SP, Sharma R, Dhawan V, Chawla S. Male factor infertility with azoospermia: our experience in a tertiary care centre: a case series. Int J Reprod Contracept Obstet Gynecol 2024;13:1289-94.