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Azoospermia – Novel Management In The Current Era

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ABSTRACT

Around 15 % couples are unable to conceive even after 1 year of unprotected intercourse. A male factor is solely responsible in about 20 % of infertile couples. The prevalence of Azoospermia is seen in around 10–15% of men being evaluated for infertility and seen around 1% of general population. Three main causes have been identified for Azoospermia. They can be classified into Pretesticular, testicular and post testicular causes. Pretesticular causes account for around 2-3 % cases of azoospermia. This can be due to Pituitary disorder, craniopharyngioma or Pituitary tumour. Testicular causes accounts for 55-60% causes of azoospermia denoting intrinsic disorders of testis, wherein the testis is abnormal, atrophic or absent. Post testicular causes also known as Obstructive azoospermia accounts for around 35-38% cases where sperms are produced but not ejaculated. It can due to the obstruction in the reproductive ductal system or ejaculatory disturbances. Operative sperm retrieval with Intracytoplasmic sperm injection (ICSI) has revolutionized the management of azoospermia in ART clinics worldwide. The management of azoospermia should consider the differential diagnosis of azoospermia, stricter selection criteria for surgical sperm retrieval, identification of men who can benefit from other interventions before surgery, best method of surgery likely to benefit the patient with least complications and the availability of IVF/ICSI laboratory services. While planning surgical sperm retrieval important objectives by clinicians are to retrieve adequate number of sperms to be used for ICSI as well as for cryopreservation if needed to be used in future, try to find morphologically better sperms and to minimize complications and limit testicular damage. Surgical sperm retrievals can be as epididymal sperm aspiration or Testicular sperm extractions. Men with Non obstructive azoospermia at times benefit more from Microsurgical Testicular sperm extraction (MicroTESE). The availability of the latest technology of freezing & vitrification of single /few sperms has been a boon to men with nonobstructive azoospermia. Yet, the biggest challenge in men with Non obstructive azoospermia is the uncertainty of sperm retrieval by any of the known surgical retrieval techniques. None of the factors studied like the cause of NOA, hormonal profile of the patient, testicular volume or the histology if previous FNA done in the past can predict the probability of finding the sperm accurately.

Introduction

Around 15 % couples are unable to conceive even after 1 year of unprotected intercourse. A male factor is solely responsible in about 20 % of infertile couples. Azoospermia is defined as absence of sperms from at least 2 separate centrifuged semen samples.[1] According to the WHO 2010 guidelines, at least two semen samples obtained more than two weeks apart should be examined before labeling a sample as azoospermia.

The prevalence of Azoospermia is seen in around 10–15% of men being evaluated for infertility and

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seen around 1% of general population.[2] Men with azoospermia are followed in infertility clinic with a thorough history, physical exam and investigations. It is important to establish the etiology and the type of azoospermia before devising any treatment strategy. Some patients may be successfully treated medically using hormonal manipulation while others will ultimately require attempts at surgical sperm retrieval to be used for ICSI.

Etiology of Azoospermia

Three main causes have been identified for Azoospermia. They can be classified into Pretesticular, testicular and post testicular causes.

Pretesticular causes account for around 2-3 % cases of azoospermia. The main underlying cause is the inadequate stimulation of otherwise normal testicles & genital tract. This can be due to Pituitary disorder, craniopharyngioma or Pituitary tumor. It can be acquired due to some disease, tumor, trauma or can be congenital (e.g. Kallmann's syndrome). 3Hypogonadotropic hypogonadism (HH)is the most common cause in pretesticular azoospermia and one of the treatable causes of azoospermia. Kallmann's syndrome is the most common cause of congenital Hypogonadotropic hypogonadism due to deficiency of Gonadotropin-releasing hormone (GnRH) from hypothalamus.4 It is due to the failure of migration of GnRH secreting neurons to the olfactory lobe. Men with Kallmann's syndrome present clinically with anosmia along with features of Hypogonadotropic hypogonadism. The incidence of Kallmann's syndrome, is in between 1 in 8000 to 1 in 80,000 births.5,6 This disorder is related to genetic mutation on KAL 1 gene with X-linked recessive inheritance pattern. Intake of anabolic steroids can also lead to HH. This is mainly due to the negative feedback loop exerted by exogenous testosterone on Gonadotropins. This in turn leads to decrease in FSH and intratesticular testosterone leading to suppression of spermatogenesis. 7

Testicular causes accounts for 55-60% causes of azoospermia denoting intrinsic disorders of testis, wherein the testis is abnormal, atrophic or absent. In this there is primary testicular failure and sperm production is severely disturbed to absent.[3]Various causes of testicular azoospermia lead to Non obstructive azoospermia ranging from testicular torsion, tumor, genetic causes (e.g Yq microdeletion, Klinefelter's syndrome XXY), mumps orchitis, undescended testis, gonadotoxic effects from cancer& other medications.

Post testicular causes also known as Obstructive azoospermia accounts for around 35-38% cases where sperms are produced but not ejaculated. It can due to the obstruction in the reproductive ductal system or ejaculatory disturbances. Congenital bilateral absence of the vas deferens (CBAVD) can be seen in up to 2-6% of men with obstructive azoospermia. This condition is manifested mainly due to the mutation in cystic fibrosis transmembrane conductance regulator (CFTR gene). According to the European association of Urology guidelines, men with CBAVD should be offered genetic counseling and testing for CFTR gene mutations. It is the most common genetic disease of Caucasians and 4% are carriers of gene mutations involving CFTR gene located on chromosome 7p .8Another common cause of obstructive azoospermia is elective vasectomy. One of the most common indication for reversal operations is the need for reproduction in a new relationship. In many Low- & Middle-Income countries (LMIC), obstruction may result from ependymitis following Sexually transmitted infections such as gonorrhea or chronic infections such as Tuberculosis. Epididymal obstruction is also seen in Young's syndrome, which presents as a triad of chronic sinusitis, bronchiectasis and obstructive azoospermia.[9]

It is imperative to establish the etiology of azoospermia which further enables the clinicians towards appropriate management options and helps them to individualize treatment plan.

Table 1. Conditions requiring surgical sperm retrieval

Obstructive Azoospermia (OA)	Non Obstructive Azoospermia (NOA)
Congenital bilateral absence of vas deferens (CBAVD)	Genetic causes- Klinefelter's, Yq microdeletion
Young's Syndrome (chronic sinusitis, bronchiectasis, OA)	Cryptorchidism/ Testicular dysgenesis
Ejaculatory ductal atresia	Maturational arrest
Ejaculatory duct cysts	Germ cell aplasia
seminal vesicle duct cysts	
Midline prostatic cysts	
Acquired causes-	**Acquired causes-**
Trauma	Trauma, Torsion
Infections(STI and tuberculosis) epididymitis, prostatitis, seminal vesiculitis	Post infections (e.g. Mumps orchitis)
Post vasectomy	Post chemoradiation
Iatrogenic- Injury during surgeries involving hernia, scrotal, bladder neck, prostate.	Exogenous factors (steroid intake, irradiation, heat)
	Systemic diseases (liver cirrhosis, renal failure)

Physical Examination of Azoospermia

A careful physical exam with an emphasis on the genitalia also provides important information on the potential etiology of azoospermia. The general physical exam should assess for degree of virilization, obesity, gynecomastia, anosmia (consistent with Kallmann's Syndrome), bilateral hemi-anopsia (suggestive of pituitary tumor), and abdominal or inguinal scars. Urogenital exam should include close inspection of the phallus looking for abnormalities of the urethral meatus and penile curvature. Testicular examination should assess for testicular size (using orchidometer), consistency, and the presence of masses. Both epididymis and vasa deferens should be palpated for their presence, consistency, and nodularity which may suggest obstructive or infectious etiologies. Finally, palpation of the spermatic cords with and without Valsalva may reveal a clinically palpable varicocele, while a digital rectal examination may help identify dilated seminal vesicles or cysts causing ejaculatory duct obstruction.

Newer concepts in the management of infertile men with nonobstructive azoospermia

Operative sperm retrieval with Intracytoplasmic sperm injection (ICSI) has revolutionized the management of azoospermia in ART clinics worldwide. Even in men with nonobstructive azoospermia there are small foci of areas of normal spermatogenesis which can yield sperms for ICSI ,thus giving these men a chance to father a child. In fact, in a study it was observed that despite the fact that non obstructive azoospermia can be caused by vast spectrum of causes and long considered untreatable, yet in around 50 % men small foci of area of spermatogenesis can be found.[12] There are two ways for retrieving the sperms – it can be percutaneous or open. In men with Obstructive azoospermia (OA) both methods of percutaneous or open testicular biopsy can be used with 100 % sperm retrieval rates. However, in Non-obstructive azoospermia the sperm retrieval rates range between 40-50% even with multiple testicular biopsies.[10] In 2008 , a Cochrane meta-analysis on surgical sperm retrieval techniques concluded that there is insufficient evidence to recommend any specific surgical sperm retrieval technique for azoospermia but all efforts should be made to undertake a technique which is least invasive and minimizes the complications related with the surgical intervention.[11]

The management of azoospermia should consider the differential diagnosis of azoospermia, stricter selection criteria for surgical sperm retrieval , identification of men who can benefit from other interventions before surgery, best method of surgery

likely to benefit the patient with least complications and the availability of IVF/ICSI laboratory services .[15] With newer developments ,one can also counsel the patients regarding the availability of techniques to cryopreserve even single or few sperms. To optimize the management for azoospermia it is imperative to have a multidisciplinary Team of urologists/andrologists, IVF specialists, reproductive endocrinologists, geneticists, embryologists and counselors.

While planning surgical sperm retrieval important objectives by clinicians are to retrieve adequate number of sperms to be used for ICSI as well as for cryopreservation if needed to be used in future, try to find morphologically better sperms & to minimize complications and limit testicular damage.12

Fig 1: Systematic approach towards deciding management of azoospermia

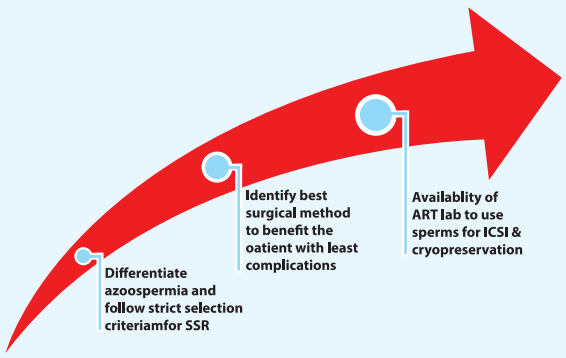


Fig 2 : Objectives when planning surgical sperm retrieval for ICSI

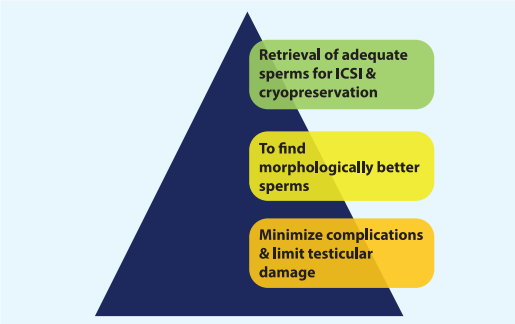


Table 2 : SURGICAL SPERM RETRIEVAL(SSR) TECHNIQUES & INDICATIONS:

Sperm retrieval technique	Acronym	Indications
Percutaneous epididymal sperm aspiration	PESA	Obstructive Azoospermia (OA)
Microsurgical epididymal sperm aspiration	MESA	Obstructive Azoospermia (OA)
Testicular sperm aspiration	TESA	Obstructive Azoospermia/ Failed Epididymal retrievals
Testicular sperm extraction	TESE	Obstructive Azoospermia (OA)/ Failed Epididymal retrievals Failed TESA attempts Non-Obstructive Azoospermia (NOA)
Microsurgical Testicular sperm extraction	Micro TESE	Non-Obstructive Azoospermia (NOA)

Epididymal Sperm retrieval:

This is done for obstructive azoospermia. Epididymal fluid rich with sperms can be obtained by open surgery or percutaneous method (direct aspiration by puncturing the scrotal skin). Open surgery can be done with or without the aid of microsurgery.

a. Percutaneous epididymal sperm aspiration (PESA): It is an simple, cost-effective method which is less invasive than microsurgical epididymal sperm aspiration (MESA). PESA

can be performed under local anesthesia or mild conscious sedation. Epididymis is first stabilized between thumb and the index finger and epididymal sperm aspiration is done using a fine needle and the fluid aspirated is sent to the IVF lab for the search for sperms, preferably motile. As post PESA fibrosis can create difficulties in subsequent microsurgery, it is best avoided, if one is planning to perform a surgical procedure like microsurgical vaso-epididymal anastomosis (VEA), at a later date.

b. Microsurgical sperm aspiration (MESA): In this procedure a fine incision is made on the epididymis and with the help of operating microscope single tubule of the epididymis is identified, contents aspirated and sent to the IVF lab for the search of motile sperms. One can combine this procedure with a VEA. There have been studies which have shown that with MESA enough sperms are retrieved which can be used for ICSI as well as for cryopreservation as sperms are highly concentrated in epididymal fluid in obstructive azoopsermia.[13]

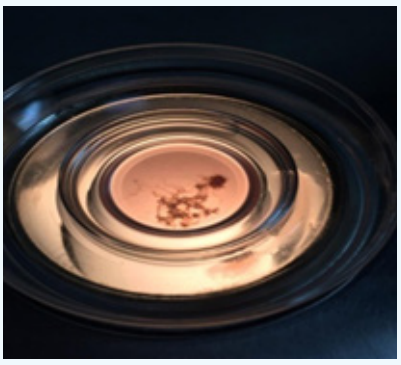
Testicular sperm retrieval:

a. Testicular sperm aspiration (TESA): It can be done in certain cases for obstructive azoospermia where there is a previous failed PESA/ MESA attempt. or in cases with absent/ fibrosed epididymis. TESA can done under local anesthesia or mild sedation. In TESA a wide bore needle (20-22 G scalp vein needle) attached to a 10- or 20-ml syringe is inserted through the scrotal skin into the testis, preferably along the anteromedial or anterolateral side of the testis at an oblique angle to avoid any injury to the blood vessels under tunica albuginea. 12The fluid is aspirated after creating negative pressure and sent for examination to the IVF laboratory. If insufficient numbers are obtained even after multiple needle biopsies then the procedure is repeated on the contralateral testis.

b. Testicular sperm extraction (TESE): Conventional TESE is an open surgical method wherein the testicular tissue is excised without the help of operating microscope. Biopsy sample, approximately around 450-500gm, is put in the petri dish with culture media and sent to the lab. Many centers prefer needle TESE (nTESE) over conventional multiple biopsies before proceeding for micro TESE as conventional TESE can lead to extensive testicular damage in men with NOA who have a very small localized foci of spermatogenesis.14

Needle TESE (nTESE) is a percutaneous method where 18-20-G scalp vein needle attached with 10 ml syringe is inserted into the testis. Negative pressure is applied and scalp vein tubing is clamped. As the needle is withdrawn out, a strand of seminiferous tubule seen is grasped gently with microsurgical forceps and put in the petri dish with culture media and sent to the IVF Lab. Multiple such biopsies can be taken with adequate amount of tissue obtained for examination and use.[14]

Fig 3 : Seminiferous tubules collected in the fluid obtained from TESE



c. Microsurgical Testicular sperm extraction (micro TESE): Microdissection testicular sperm extraction has completely revolutionized the management of non-obstructive azoospermia. It was first described in 1999, that on using an operating microscope there was a much higher likelihood of

sperm retrieval from a dilated seminiferous in cases of non-obstructive azoopsermia.[21] In micro TESE, the scrotum is incised and the testis is exteriorized. A transverse incision is taken and testicular parenchyma is exposed. With the help of Operative stereo microscope of upto 14-25 X magnification, seminiferous tubules are examined and more turgid tubules which look healthier with increased diameter are selected and microsurgical multiple biopsies taken. These biopsies are then sent to the lab where they are shredded with sterile slides or teased with help of fine sterile needles. The resultant tissue is suspended in droplets of culture media, covered with oil. These droplets are meticulously scanned under high magnification inverted microscope, for as much as 2 to 4 hours. Scanning can be done my multiple teams of embryologist. If sperms are obtained, they are immediately used for ICSI or frozen for later use. Hemostasis is better achieved as vessels can be easily identified under the microscope and injury avoided. The tunica is then closed with 5-0 or 6-0 polypropylene sutures. [12]The main drawback seen with micro TESE technique is the need of operating microscope, microsurgical instruments, required expertise and increased cost associated with the procedure.

Prognostic factors for sperm retrieval success

Genetics

Genetics play a very important role in predicting the success of micro-TESE. The prevalence of microdeletions in azoospermia men was found to range from 10%-15%[16]. Microdeletions most frequently occur on the long arm of the Y chromosome, Yq, and there are three known deletions, namely Azoospermia Factor a (AZFa), Azoospermia Factor b (AZFb) & Azoospermia Factor c (AZFc). The deletions in this region are specifically related to failure of spermatogenesis. The sperm retrieval success in men with NOA exhibiting deletions on AZFa & AZFb is nil [17], while men showing AZFc deletions still show some areas of sparse spermatogenesis.[18,19]. It has been seen that in around 50–60% of men with Yq microdeletion at AZFc, sperms can be retrieved by microTESE.[24]

Klinefelter syndrome (KS) is the most common genetic abnormality seen in men with infertility. Men with KS have successful micro-TESE rates similar to or better than all men with NOA. In Klinefelter's syndrome the probability of finding the sperm on TESE / Micro TESE is around 30-70%. [22]

Few men with denovo genetic mutations have a predisposition to various cancers. It is now known that there is a 50% chance of retrieving sperms in cancer patients, who have taken chemotherapy or radiotherapy in the past.[23]

Histology

As far as testicular histology is concerned, highest SRR is seen in hypo spermatogenesis (SRR of 73–100%).[25] Histological features of maturational arrest have lesser SRR of around 40% and the least SRR is seen in Sertoli-cell-only syndrome (SRR of 20%)[26] In a recent systematic review examining sperm retrieval rate, higher success was seen with micro TESE as compared to conventional TESE in patients of Non-obstructive azoospermia . (63 versus 42.9%). [20]

Cryptorchidism

Undescended testis (Cryptorchidism) is a common problem affecting 3% of full-term male babies and upto 30% of preterm male babies. It is associated with decreased fertility, increased incidence of testicular germ cell tumors, testicular torsion and inguinal hernias. Few studies in the past found correlations between sperm recovery rate and the age at orchidopexy was done. Men with a history of

orchidopexy done before ten years of age had better sperm recovery rates when compared to men who had orchidopexy after ten years of age. [27] Recent studies have reported no statistical differences between the men who had orchidopexy before or after ten years of age and their sperm recovery. They also did not find any correlation between positive sperm recovery rate and testicular volume. [28]

Varicocoele

There is a debate whether there is a need for varicocelectomy in every patient of non obstructive azoospermia (NOA) with varicocele. Some studies have suggested that varicocelectomy in men with clinical varicocoele and NOA prior to microTESE improves the sperm retrieval rates.[29] There have also been few studies which did not observe in any improvement in sperm recovery in treated or untreated varicocoele with NOA men undergoing microTESE.[30]

Age

Though advanced paternal age is associated with adverse pregnancy outcomes in terms of lower pregnancy rates and live birth rates, but there is limited data to show that there is so far no association between paternal age and outcome of microTESE.

FSH

Many studies in the past also found positive correlation between different levels of FSH and predictability of sperm retrieval rate. In one a prospective study it was seen that in men with FSH levels more than 19.4mIU/ml had a poor probability of sperm retrieval when compared with men with less FSH than the cut off mentioned. [31] It was also seen that probably predominance of hypospermatogenesis in the successful group of patients contributed to the positive findings. With the current evidence it is now understood that FSH is a good marker of the global testicular function, does not serve itself as a strong predictor of successful microTESE.

Testicular volume and size

Men with NOA have predominantly small atrophic testis. Few studies in the past have found that testicular long axis of less than 4.6cms have higher probability of NOA. [32] Though large testis are normally considered a sign of normal spermatogenesis, yet testicular volume alone is a poor marker of sperm recovery rate in microTESE. Recent data suggest that use of testicular volume or size alone to predict the outcome of successful sperm retrieval in microTESE is very limited. [33]

The biggest challenge in men with Non obstructive azoospermia is the uncertainty of sperm retrieval by any of the known surgical retrieval techniques. None of the factors studied like the cause of NOA, hormonal profile of the patient, testicular volume or the histology if previous FNA done in the past can predict the probability of finding the sperm accurately.[15]

Role of Optimizing Testosterone levels prior to MicroTESE

Many etiological factors can contribute to the spermatogenic failure seen in NOA. It can be caused due to hypothalamic and pituitary

causes leading to Hypogonadism and also can be due to testicular or post testicular reasons. In Hypogonadism, due to gonadotropin deficiencies serum testosterone levels are lower than 300 ng/dl. These patients represent a small proportion of men with NOA. In majority of men with NOA no specific cause of spermatogenic failure is recognized. Hence, various empirical medical therapeutic options can be tried for low testosterone but with limited success. [34]

The rationale behind optimizing testosterone levels is that spermatogenesis is positively correlated with optimal intratesticular testosterone production. Various medical therapies are directed to increase the endogenous testosterone production.

Clomiphene citrate is a selective estrogen receptor modulator that blocks the negative feedback and increases the gonadotropin levels. HCG is a LH analog that stimulates testosterone production from the Leydig cells. Aromatase inhibitors like anastrozole and letrozole block the conversion of testosterone to estradiol by aromatase. Thereby, increasing the endogenous testosterone production.

Aromatase inhibitors prevent peripheral conversion of testosterone to estrogen which is increased in obese men with NOA. Normal fertile men have a testosterone/estradiol ratio (µg/dl testosterone, pg/µl estradiol more than 15 and men with a ratio less than 10 can present with abnormal spermatogenesis. In a study of 140 men with KS who had T/E ratio< 10, when treated with 1 mg Anastrozole daily responded with an increase in serum testosterone to greater than 250 ng/dl. These subsets of patients also had a higher sperm retrieval rate on microTESE.[35]

In a study conducted to evaluate the role of optimizing testosterone levels, hormonal treatment was started 2-3 months prior to microTESE in men with NOA. Men with low serum testosterone <300 ng/dl were treated with aromatase inhibitors (50 to 100 mg testolactone orally twice daily or 1 mg anastrozole daily) when T/E2 ratio was found to be less than 10. Clomiphene was initiated in the group of men who had low testosterone levels but normal T/E2 ratio. HCG injections were added to the regimen at a dose of 1500-2000IU 2-3 times a week if there was an inadequate response to oral medications. No difference in SRR between men with NOA who presented with normal serum testosterone, and those who presented with serum testosterone less than 300 ng/dl and were treated to optimize testosterone.[36] It was observed that the success of outcome of micro-TESE is based on the presence of focal regions of the most advanced stage of spermatogenesis. It is also noted by many authors that it is the intratesticular androgenic bioactivity and intratesticular testosterone, which are approximately 40 times higher than serum testosterone, which play a key role in the control of spermatogenesis but can rarely be measured.[37]

Single/few Sperm freezing using Sperm Vitrification Device (Sperm VD) for Cryptozoospermia/Non obstructive azoospermia

The availability of the latest technology of freezing of single sperm (Sperm VD) has been a boon to men with nonobstructive azoospermia. This cryopreserved sperm can be used for the ongoing ICSI cycle as well as

future fertility use. In one of the studies with the largest cohort of 44 cases using Sperm VD, after thawing recovery rate of 96% of retrieved spermatozoa and thawed motility rate of 33%.,was obtained.[38]The tissue is obtained either by high speed centrifugation of ejaculated sperm or dissected testicular tissue obtained by TESE or micro TESE .This tissue is processed twice and most of the liquid is removed and the remaining 100 µl tissue suspension is mixed and distributed into 10 µl droplets on a petri dish. A 4 µl droplet of polyvinyl pyrrolidone (PVP) 10% solution for filling the microcapillary and several collection droplets of 0.6 µl of Quinn's Sperm Washing Medium are then added to the plate. The plate is covered with light paraffin oil. The 10 µl droplets are thoroughly searched for the presence of spermatozoa under ×200 magnification using an inverted phase contrast microscope. Any spermatozoa found is transferred to a collection droplet and cryopreserved.

Conclusion

In conclusion, surgical sperm retrieval techniques available today in conjunction with ICSI have revolutionized the treatment of azoospermia. In order to optimize the treatment for azoospermia it is imperative to understand the etiology of azoospermia, select the surgical retrieval technique best suited for the patient and effectively implement the ICSI technology. which will give an individualized personalized treatment to patients suffering from azoospermia.

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