Original Research Article

Percutaneous epididymal sperm aspiration in combination with intracytoplasmic sperm injection as a treatment of choice in Obstructive azoospermia

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Abstract

Intracytoplasmic sperm injection (ICSI) has proved a boon in the field of male infertility. ICSI is unique procedure in which a small quantity of progressively motile and morphologically normal spermatozoa is required and a single best-looking motile spermatozoon is injected into the oocyte. This procedure can overcome the drawbacks of poor semen quality in terms of low count, poor motility and morphology of spermatozoa. Percutaneous epidydimal sperm aspiration (PESA) is a simple procedure of retrieving spermatozoa from epididymis in cases of obstructive azoospermia. The aim of the study was to find out the cumulative rates of sperm retrieval after 2nd PESA attempt and the feasibility of percutaneous epididymal sperm aspiration in obstructive azoospermia. A total of 100 patients were included in the study which was carried out over a period of 2 years. PESA was performed under general anaesthesia on the day of ovum pick up procedure. PESA was repeated at a different site of the same epididymis or at the contralateral site till motile spermatozoa were retrieved and processed. Sperm count motility percentage was calculated using Maklers counting chamber. In 52% of the cases (52/100), motile spermatozoa were retrieved after first PESA attempt. In cases of failure to retrieve spermatozoa after first attempt, a repeat procedure was done. In the second PESA attempt, 33%cases (16/48) reported motile spermatozoa making an overall success rate of 68%(68/100) of surgically retrieving the motile spermatozoa. The PESA remains a simple and effective technique of surgically retrieving the spermatozoa in obstructive azoospermia.

Keywords: Male infertility, Azoospamia, PESA, Intracytoplasmic sperm injection.

Introduction

Parenthood is a dream of every couple and when this dream is shattered, the resulting infertility is devastating. Coupled to the social stigma attached to this condition, the uncertainties of diagnosis and low success rates of assisted reproductive techniques, the management and counselling of infertile couples remains a challenging task.About one third of all cases of infertility are due to male infertility.

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The assisted reproductive investigation and techniques focus mainly on the female partner. The evaluation of the male partner is cursory based mainly on 2 or 3 semen analysis, semen culture and screening of virological markers in HIV, HBsAg and HCV apart from routine blood tests. The first human pregnancies from Intracytoplasmic sperm injection (ICSI) performed by Palermo GD were successful in 1992 and since then this procedure has proved a boon for treatment of male infertility [1]. ICSI has made the dream of parenthood possible even in severe and difficult forms of male infertility with poor quality of semen. Azoospermia characterised by occasional presence of spermatozoa in semen. Technically azoospermia is defined as absence of sperm in the centrifuged pellet of two separate semen samples and

presents a challenging and difficult situation. However, with the application of percutaneous epididmal sperm aspiration in combination with intracytoplasmic sperm injection, pregnancy can be achieved in such difficult cases of male infertility. Two types of azoospermia have been reported i.e obstructive and nonobstructive azoospermia. Obstructive azoospermia is characterised by the mechanical obstruction in the genital tract which may be congenital or acquired. Infections, surgeries, trauma and failure of vasectomy reversal are important causes of obstructive azoospermia (Fig 1& table 1).

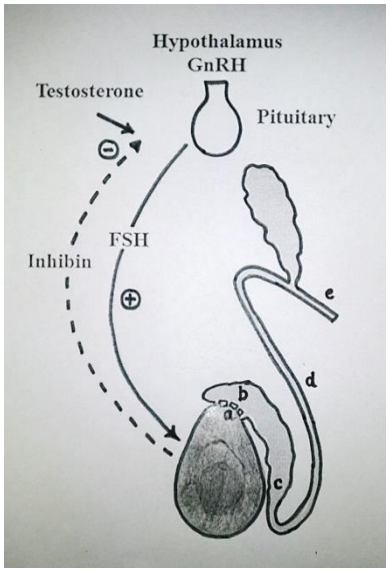

Fig 1:Aetiology of Obstructive Azoospermia (reproduced from Tom Connoly's ACE presentation2016, ICSI outcomes using surgical retrieved sperm-Dissecting the details)

Table 1:Aetiology of Obstructive Azoospermia

Congenital	CBAVD Cystic fibrosis
Acquired	Chlamydia Gonorrhoea Tuberculosis
Iatrogenic	Vasectomy Herniorraphy Hydrocoele repair

As a matter of fact, the process of spermatogenesis in the obstructive azoospermia is normal and the hormonal profile of FSH, LH, and testosterone is within normal limits [2]. Non obstructive azoospermia, on the other hand is further divided into central NOA and testicular NOA. Significant elevation in FSH suggests testicular failure whereas low levels of gonadotropins and low to normal testosterone suggest central NOA. The aetiology of the azoospermia can be found by taking a detailed history, physical examination, semen analysis and endocrine evaluation of the male. Testicular biopsy is not done routinely but can be done as a confirmation procedure in some selected cases.

Aims & Objectives

To study the feasibility of percutaneous epididymal sperm aspiration in obstructive azoospermia. To calculate the cumulative rates of sperm retrieval after second PESA attempt. To find out the percentage of

motile spermatozoa in percutaneous epididymal sperm aspiration

Material and methods

The study was conducted in assisted reproductive technology centre of a tertiary care referral university teaching hospital for infertility during the period April 2016 to March 2018. All patients were screened for inclusion and exclusion criteria. The inclusion criteria were patients undergoing treatment for infertility with male partner having following characters; Normal physical examination, Testes with normal volume, absence of varicoceles and Negative semen cultures and clinical features of obstructive azoospermia. The exclusion criteria were patients of Non obstructive Azoospermia based on endocrine evaluation.The type of study was observational study in which a total of hundred cases of infertile men with diagnosed obstructive azoospermia were included over a period of two years.

A written informed consent was taken from all the participants and from their spouses. The study was approved by the hospital ethical committee. The data was entered in MS Excel and analysed. After detailed history taking, through physical examination was carried out. Three semen analysis tests were done on separate occasions to finally arrive at the diagnosis of azoospermia. Other routine tests like haemoglobin estimation, Total and differential white blood cell count, Blood grouping and crossmatching and blood sugar levels were done. Hormonal evaluation included serum FSH, LH, testosterone and prolaction levels. Serological markers for HIV, HBsAg and HCV were done as part of overall evaluation.Percutaneous epididymal sperm aspiration (PESA) was performed under general anaesthesia in association with intravenous anaesthesia using propofol on the day of ovum pick up procedure. A 26 gauge needle was attached to a 5ml syringe and inserted into the epididymis through the scrotum (Fig 2).

Fig 2: Insertion of needle into the Epididymis

Negative suction was created and the needle was moved in and out within the epididymis until the fluid enters the syringe. The needle was withdrawn from the epididymis and the aspirate was flushed into 0.5 ml sperm medium kept at 37^0C. Simple washing of the aspirated fluid was done in the sperm medium to a volume of 1.5ml (Fig 3).

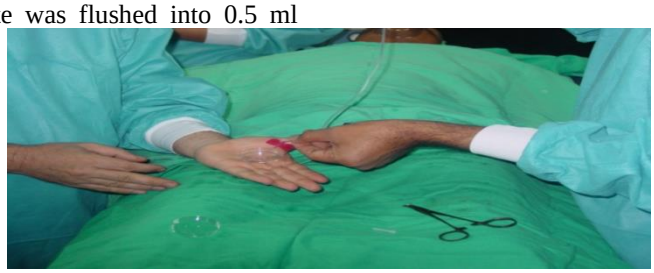

Fig 3: Retrieval of Seminal fluid after aspiration

The resulting mixture was centrifuged at 300g for 5 minutes. The supernatant was discarded and the pellet resuspended in 0.3ml of medium. The tube containing the epididymal aspirate was transferred to the incubator in the IVF laboratory. PESA was repeated at a different site of the same epididymis or at the contralateral site till motile spermatozoa were retrieved (Fig 4). In case of failure to retrieve motile spermatozoa even from both the epididymis, the testicular sperm aspiration was the option but was not done due to technical difficulties.

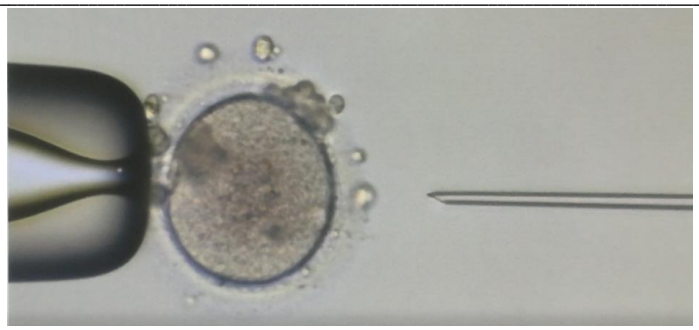

Fig 4. Sperms retrieved by PESA used for ICSI

Results

In 52% of the cases (52/100), motile spermatozoa were retrieved after first PESA attempt. In cases of failure to retrieve spermatozoa after first attempt, a repeat procedure was done. In the second PESA attempt, 33%cases (16/48) reported motile spermatozoa making an overall success rate of 68 %(68/100) of surgically retrieving the motile spermatozoa. In four cases, insufficient spermatozoa were retrieved. (Graph 1)

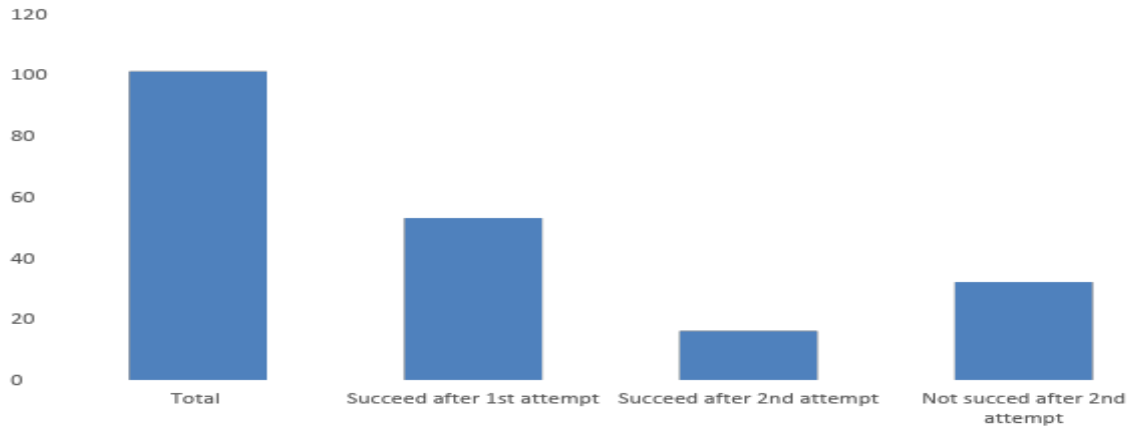

Fig 5: Success rates of sperm retrieval by PESA

Table 2:Number of PESA attempts, presence of motile sperm and retrieval rate

Number of PESA attempts	Presence of motile sperm (%)	Cumulative successful retrieval rate (%)
First PESA	52/100 (52%)	-
Second PESA	16/48 (33%)	68/100 (68%)

Discussion

The main purpose of PESA sperm processing is to obtain a clean sample containing motile spermatozoa. PESA is an effective surgical sperm retrieval method in obstructive azoospermia. Patients undergoing PESA are discharged same day and can also resume their routine activities. The complications of infection, bleeding and haematoma formation are less common though fibrosis at aspirated site is common [3]Our study found motile spermatozoa in 68% of the cases after one or two attempts of PESA procedure. Studies have shown that motile spermatozoa are retrieved commonly in obstructive azoospermia as compared to nonobstructive azoospermia [2, 4]. These findings were corroborated in our study with significant motile spermatozoa retrieved and utilized for ICSI. In a study of use of surgically retrieved epididymal spermatozoa

in ICSI showed good fertilization rates [5]. In case of repeated failed PESA attempts, testicular sperm aspiration (TESA) can be used to surgically retrieve sperm from the testis in obstructive azoospermia [6].

Surgical Sperm Recovery for Intracytoplasmic Sperm Injection: Among the many surgical methods for sperm recovery, the most widely described are microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA), testicular sperm extraction (TESE), and percutaneous testicular sperm fine-needle aspiration (TESA, also called fine-needle aspiration, or FNA). Both MESA and TESE are open surgical procedures performed with an operating microscope and general or regional anaesthesia, whereas the percutaneous procedures need only local anaesthesia. The optimal choice for surgical sperm recovery method has not been determined and certainly varies based on patient history. Hematoma risk appears to be low regardless of method. Testicular atrophy is a rare complication of TESE (Testicular sperm extraction) and TESA (testicular sperm aspiration)even when biopsies are obtained from multiple testicular sites [7]. With obstructive azoospermia, pregnancy rates using sperm retrieval and ICSI are 24% and 64%, respectively, and outcomes using either frozen-thawed or fresh sperm are comparable. Because microsurgical epididymal sperm aspiration (MESA) allows for diagnosis and possible reconstruction of ductal pathology and because it usually yields very large numbers of sperm, sperm cryopreservation and avoidance of repeat surgery may be possible. If repeat sperm retrievals are needed, the minimum interval between procedures is 3 to 6 months to allow for adequate healing [8]. Furthermore, study on birth defects after follow up of offspring's conceived by intracytoplasmic sperm injection with spermatozoa retrieved from epididymis or testis showed no significant difference as compared to offspring's born from intracytoplasmic sperm injection with ejaculated spermatozoa [9]. However, a recent study has reported comparable stillborn rates, prematurity rates and low birth weight rates in children born with intracytoplasmic sperm injection using ejaculated and non-ejaculated sperm [10]. For the evaluation of azoospermia, the hormonal evaluation includes serum FSH, LH, Prolactin, total and free testosterone and serum estradiol levels. An increased level of serum FSH more than 7.6 mIU/ml indicates an abnormality in spermatogenesis [11]. The clinical pregnancy rates, on-going pregnancy rates and the live birth rates were not calculated and this aspect remains a shortcoming of our study. However, a relevant study showed that spermatozoa retrieved from epididymis are more mature as compared to spermatozoa retrieved from testis and have been shown to provide high pregnancy rates [12].

Conclusion

Percutaneous epididymal sperm aspiration (PESA) remains a simple and effective technique of surgically retrieving the spermatozoa in obstructive azoospermia. Repeating the procedure at an interval of three to six months after an initial failure to retrieve the spermatozoa and combining it with intracytoplasmic sperm injection is the treatment of choice in some of the difficult cases of male infertility.

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