A simple non-invasive method of spermatozoa retrieval from the bladder (SPERB) in patients with retrograde ejaculation

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Abstract Retrograde ejaculation is very commonly seen in men presenting with aspermia. Despite having normal spermatogenesis, these men present with infertility as the ejaculate is retrograded into the urinary bladder, due to various causes. Diagnosing this condition involves thorough history taking followed by examination of post-masturbatory/coital urine. Retrieval of spermatozoa from the bladder maintaining its vitality is crucial in providing fertility treatment. Various methods employed in retrieving motile spermatozoa from the bladder in literature is either invasive or cumbersome causing inconvenience to the couple. Here, a simple and non-invasive spermatozoa retrieval from the bladder (SPERB) technique is described in detail. Case report of the couple who conceived using spermatozoa retrieved by this method is discussed.

Keywords: Anejaculation, aspermia, dry orgasm, non-invasive sperm retrieval, retrograde ejaculation, sperm retrieval, sperm retrieval from bladder, sperm recovery

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INTRODUCTION

Retrograde ejaculation (RGE) is the commonest cause of Aspermia.^[1] It is a type of ejaculatory dysfunction where semen is pushed retrograde into the urinary bladder instead of the normal antegrade flow into the urethra during ejaculation. The absence of antegrade ejaculation causes male infertility. It is a disheartening condition in men and distress to the couple as there is no antegrade ejaculation despite normal and sustained erection followed by the perception of ejaculation. Parnham and Serefoglu^[2] quoted the incidence of RGE as 0.3% to 2% of male infertile population. Many cumbersome and

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invasive methods were used in managing infertility in these men. We use a simple and non-invasive method for spermatozoa retrieval in these men from urinary bladder. Herein, we describe the detailed methodology and the case reports of spermatozoa retrieval from the bladder in patients diagnosed with retrograde ejaculation.

PHYSIOLOGY OF MALE SEXUAL FUNCTION

Male sexual function is a series of complex events that takes place involving psychological, neurogenic, vascular, and hormonal factors.^[3] With good libido and sexual

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stimuli in men, arousal and penile erection is achieved. This is followed by the two phases of events known as emission and ejaculation.

The process of emission takes places with the involvement of distal epididymis, vas deferens, seminal vesicles, prostate gland, prostatic urethra, and bladder neck. Stimuli mediated by the innervations form sympathetic nervous system initiates emission by the closure of bladder neck. Contents from the epididymis, vas deferens, seminal vesicles, prostate, and bulbourethral glands are then deposited into the prostatic urethra.^[4]

Ejaculation, also known as seminal emission is initiated by the presence of semen in the prostatic urethra. It is controlled by the coordinated neurological stimuli from T10-L2 (sympathetic fibres) to S2-S4 (somatic fibres).^[5] Highly coordinated neurological and muscular events aid in the process of ejaculation. The pelvic floor muscles around the urethra upon rhythmic contractions propels the ejaculate via the penile urethra and external meatus.^[6]

PATHOPHYSIOLOGY IN RETROGRADE EJACULATION

The events of sexual function like libido, arousal, and erection are normal in patients with retrograde ejaculation. The process of emission take place but due to inadequate closure of bladder neck, semen flows into the urinary bladder.^[7]

The semen deposited into the bladder is exposed directly to the acidic urine, which makes the spermatozoa either immotile or non-vital. In order to treat infertility, it is essential to isolate the spermatozoa from urine and retain spermatozoa motility and viability.

Etiology of retrograde ejaculation is given in Table 1.

DIAGNOSIS

High index of suspicion of retrograde ejaculation is raised when a male partner has good erection and achieves the sense of orgasm and ejaculation but there is aspermia/no antegrade ejaculation – dry orgasm. Eliciting the patient's medical [Table 1] and surgical history is very essential in confirming the diagnosis with further investigations. History should also include the details of surgeries in bladder neck, spinal cord, urethra, neoplastic excision with irradiation in the pelvic floor.^[4] The chances of RGE is ruled out in men with painful antegrade

Table 1: Etiology of retrograde ejaculation in men^[10,11]

Congenital

- \bullet Abnormal ejaculatory duct opening into the bladder proximal to the sphincter $\ensuremath{\textbf{Acquired}}$
 - \bullet Lesions/ injury to the spinal $\mbox{cord}^{[8]}$

• Type II diabetes mellitus^[4]

latrogenic

- Surgeries in the retroperitoneal region,
- Colorectal surgeries,
- Surgeries involving bladder neck and urethra,
- Non-nerve sparing retroperitoneal lymph node dissection,^[4]
- Neoplastic and open pelvic floor surgeries^[9]

Idiopathic Drugs induced^[10,11]

- α-receptor antagonists
- Diuretics
- Antidepressants
- Classical neuroleptics

Table 2: ART outcome

Parameters	Numbers
Oocytes retrieved	09
Metaphase II oocytes	08
Zygotes (two pronuclei seen 16 hours after ICSI)	06
Cleavage stage (Day 3) embryos	05

ejaculation, as it could be due to pathologies in the ejaculatory duct.^[12]

The spermatogenesis is intact despite which the male remains infertile as there is only defective spermatozoa transportation.^[13,14] Final diagnosis of retrograde ejaculation can be elicited in men by detailed history taking and examination of post coital or post masturbatory urine for the presence of spermatozoa. The voided urine, upon macroscopic examination will appear cloudy,^[2] and plenty of immotile spermatozoa.^[15] can be seen when viewed under the microscope.

Though spermatogenesis is usually unaffected in these men, exposure to the acidic pH of the urine in the bladder makes the spermatozoa immotile/non-vital.^[16] Hence, if the time of exposure of spermatozoa to urine is minimized, motile spermatozoa can be obtained and can be used for fertility treatment procedures which has evolved over a period of time. Cloudy urine has to be differentiated from milky urine (chyluria), which occurs in filariasis or trauma to the lymphatics of the genital tract.

Many methods have evolved in an attempt to recover motile spermatozoa in patients with retrograde ejaculation and one such method is spermatozoa retrieval from bladder (SPERB), which is described below. A detailed literature review pertaining to fertility management in these men is mentioned in Table 3.

S. No Author	Year of publication	Patient details n	Type of management	Outcome	Pros	Cons
Hotchkiss RS, Pinto AB, Kleegman S ^[18]				Achieved 2 consecutive pregnancies and live births in case 1 and achieved a pregnancy in case 2.	Obtained pregnancies by simple insemination methods as the spermatozoa parameters were normal.	 Restricting fluid intake.2. Involves catheterization of bladder.3. Increased risk of urinary tract infections (UTI).4. Insemination of
		Case 2: A 36-year-old male, diagnosed with intraurethral lateral lobe intrusion of the prostate and retrograde ejaculation. Female partner was apparently normal	bladder is first rinsed (180 cm ³) and then is filled (2 cm ³) with Ringer glucose solution3. The contents of the bladder is either voided or obtained by re-catheterization after manually inducing ejaculation. Case 1: Out of 7.5 cm ³ obtained from the bladder with plenty of motile spermatozoa, 1.5 cm ³ was injected into the cervix and 6 cm ³ was inseminated over the cervix. Case 2: Sample obtained by the above- mentioned method was used for intra vaginal insemination and achieved			more volume makes it cumbersome for the female partner.
Bourne RB,	1971	Male partner, a known case of long-standing		First case of insemination done	1. Improved spermatozoa	1. Restricting fluid intake.2. Involves
Kretzschmar WA, Esser		diabetes presented with infertility and an ejaculation. Female partner was apparently	failed to obtain pregnancy after 6 inseminations.2. Observed poor motility of	in patient with diabetes and retrograde ejaculation.	motility by administration of oral sodium bicarbonate.2.	catheterization of bladder and increases the risk of UTI
JH ^[19]		normal	spermatozoa due to acidic nature of urine and hence sodium bicarbonate was given orally.3. 25 to 50 motile spermatozoa/high power field was obtained before oral administration of sodium bicarbonate and obtained 50 to 100 motile spermatozoa was obtained later.		Centrifuged sample was used thereby reducing the volume of insemination	
Abrahams JI, Solish GI, Boorjian P, Waterhouse RR(^{20]}	1975	Two male patients developed retrograde ejaculation post Y-V plastic surgery of the bladder.	Surgically managed by reconstructing the internal vesicular sphincter.	 Restored normal antegrade ejaculation in both patients.2. One patient had fathered a child 	Aids in conception without any medical intervention.	Surgically managed
Schram JD ^[2 1]	1976	Male partner underwent bladder neck surgery at the age of 23 and when tried for pregnancy, he was diagnosed with retrograde ejaculation. Female partner was apparently normal.	 6-8 hours fluid restriction, followed by voiding post masturbatory urine in the vagina 	Pregnancy was achieved	No bladder catheterization and alkalinization of urine was employed	 Fluid restriction2. Voiding post ejaculatory urine in the vagina is not a welcoming method to practise
Glezerman M,	1976					
Lunenfeld B, Potashnik G, Oelsner G, Beer R. ^[22]		Case 1: Male partner, hemiplegic followed by an accident presented with secondary infertility and was diagnosed with retrograde ejaculation. Female partner was apparently normal.	Case 1: 1. Post ejaculatory sample was mixed with 15 ml of Eagle's solution and centrifuged for 1500 rpm, 10 minutes. Then insemination was done.2. Trimethoprin and sulfamethoxazole given orally to maintain	Case 1 and 2: 1. Acidic nature of the urine was neutralized using Eagle's solution2. Achieved pregnancy and healthy live birth	Case 1 and 2: No bladder Case 1: 1. catheterization was employed antibiotics. Case 2: 1. sodium bic	Case 1: 1. Administration of antibiotics. Case 2: 1. Administration of oral sodium bicarbonate.
		Case 2: A 24-year-old male with the history				

Year of Patient details publication bladder neck fit		Patient details bladdar nack fibrosis nessanted with an	Type of management bicarbonata (1 a OID) was administered	Outcome	Pros	Cons
biadder neck hurorsk presented with ejaculation and infertility. Retrograde ejaculation was diagnosed. Female pa was apparently normal. 1978 A 42-year-old male developed retrogr ejaculation and infertility due to bladd	pladger neck inprosi- ejaculation and infe ejaculation was diag was apparently norr A 42-year-old male ejaculation and infe	bladder neck hlorosis presented with an ejaculation and infertility. Retrograde ejaculation was diagnosed. Female parther was apparently normal. A 42-year-old male developed retrograde ejaculation and infertility due to bladder nocv surrarv. Female narther was identified	picarbonate (1 g ucu) was administered orally and pH of 7 was achieved.3. Above mention method was used and insemination was done. 1. Post ejaculatory urine was retrieved from the bladder by using Hotchkiss technique with modifications 2 To achieve multiple	Pregnancy was achieved in second insemination	Avoided multiple bladder catheterization by freezing the somen samele	 Catheterization of bladder was performed2. Semen freezing and thawing was done
to have oligomenorrhoea.	to have oligomenorr	to partice was rectrined	with mounteentries. To extrave mutuple inseminations and avoid urinary tract infection, semen freezing was done 3. Multiple insemination was done followed by ovulation induction.			aron sew Sumer
1978 A 27-year-old male with infertility was diagnosed with retrograde ejaculation to radical inguinalorchiectomy and retroperitoneal node dissectionfor a teratocarcinoma of the left testis	A 27-year-old male w diagnosed with retro; to radical inguinalorc retroperitoneal node teratocarcinoma of th	A 27-year-old male with infertility was diagnosed with retrograde ejaculation due to radical inguinalorchiectomy and retroperitoneal node dissectionfor a teratocarcinoma of the left testis	 Alpha adrenergic closure of the bladder was aimed and drug was prescribed 1-2 hours prior to coitus.2. A low volume antegrade ejaculate was obtained (0.1-0.2 ml with 32 million spermatozoa)3. Insemination was done along with Clomiphene citrate to time the ovulation 	Achieved pregnancy and healthy live birth in third insemination	Urine alkalinization and catheterization was not performed	Ejaculatory volume was very minimal
 1982 A 30-year-old male complaining an ejaculation and infertility was diagnosed with retrograde ejaculation. 1998 	A 30-year-old male co ejaculation and infertil with retrograde ejacul:	mplaining an ity was diagnosed ation.	The method proposed by Glezerman <i>et al.</i> ^[22] was employed with modifying the bicarbonate dose to OD and insemination was performed.	Fourth insemination resulted in pregnancy and healthy live birth.	No bladder catheterization was employed -	Administration of oral sodium bicarbonate
Case 1: A 31-year-old male with absence of antegrade ejaculation and infertility was diagnosed to have retrograde ejaculation. Female partner with the same age was apparently normal. Case 2: A 32-year-old male was diagnosed with retrograde ejaculation. Female partner was apparently normal.	Case 1: A 31-year-old antegrade ejaculation diagnosed to have rett Female partner with tt apparently normal. Case 2: A 32-year-old with retrograde ejaculi was apparently norma	male with absence of and infertility was rograde ejaculation. The same age was male was diagnosed ation. Female partner I.	Case 1 and 2: 1. Low electrolyte solution like glucose solution is introduced into the bladder by catheterization2. Patient is advised to masturbate with full bladder3. Re-catheterization is performed and the contents from the bladder is retrieved4. Intra uterine insemination (IUI) was performed with ovulation induction for female partner to time the ovulation	Case 1: Pregnancy and healthy twin delivery were achieved when seventh IUI was performed Case 2: Achieved pregnancy and healthy live birth when first cycle IUI was done		Case 1 and 2: 1. Multiple catheterizations were performed in retrieving motile spermatozoa. This increases the chances of UTI.2. Masturbation with full bladder is not convenient for the patient
1999 A systematic review of 224 studies on the diagnosis, medical and surgical management, outcome for patients with retrograde ejaculation are tabulated in detail.	A systematic review of diagnosis, medical and management, outcome retrograde ejaculation detail.	224 studies on the surgical for patients with are tabulated in	Conclusion: More clinical trials and comparative studies are required.			
2015 1. Retrograde ejaculation and semen freezing was done in 63 men. 2. Spermatozoa were retrieved by modified Hotchkiss technique	 Retrograde ejacula freezing was done in Spermatozoa were re Hotchkiss technique 	tion and semen 63 men. 2. trieved by modified	Modified Hotchkiss method: 1. Catheterization was performed and 40 ml of sterile culture medium at room temperature was introduced into the bladder.2. Fluid restriction was advised for 4 hours prior to the spermatozoa retrieval.3. Patient is then advol to mentuche and then advised to		 Spermatozoa retrieved from bladder can withstand freezing and resulted in pregnancies 	 Restricting fluid intake2. Involves catheterization of bladder
						(Continued)

100

Table 3: (Continued)

duration of success of ante grade duration of success of ante grade Surgical management2. The 1. Surgical management2. The ejaculation not mentioned. ejaculation not mentioned. Cons ejaculation.2. Psychological ejaculation2. Psychological 1. Regaining antegrade 1. Regaining antegrade penefits to the patient penefits to the patient Pros ⁻our weeks follow up showed ę 1. Antegrade ejaculation was conception was achieved one antegrade ejaculation (after masturbation) with volume achieved.2. Spontaneous 2.8 ml, 25 million/ml of spermatozoa with 26% progressive motility. after surgery Dutcome month a acid copolymer - 8 ml was injected into the Retrieved spermatozoa bladder neck collagen injections at various void.4. The contents are centrifuged and a 1. Cystoscopy was done and transurethral bladder neck.3. Dextranomer/hyaluronic patient did not respond to the drugs.2. pseudoephedrine were prescribed and 1. For 4 weeks amitriptvline and oral Cystoscopy revealed wide opening of is cryopreserved and later used for bladder neck at different sites Type of management pellet is obtained.5. ites were given. treatment. A 30-year-old male presented with infertility had anejaculation since puberty. Female A 28-year-old male presented with dry ejaculate and was diagnosed with partner was apparently normal. retrograde ejaculation. Patient details Year of publication 2017 2020 Table 3: (Continued) Liang X, Lei H, Han H, Liu S, Li G, Zhanj J et al^[30] ы́. Sinanoglu C Arda E^[29] Cakiroglu No Author 12 3

SPERMATOZOA RETRIEVAL FROM THE BLADDER (SPERB) - A SIMPLE NON-INVASIVE TECHNIQUE

This novel technique involves a simple method of spermatozoa retrieval from the bladder in patients diagnosed with retrograde ejaculation. We coined the term "SPERB – Spermatozoa Retrieval from the Bladder." SPERB is a simple, non-invasive and effective method of spermatozoa retrieval in men with retrograde ejaculation which we routinely perform in Department of Andrology & Reproductive Medicine, Chettinad Super Specialty Hospital, Chennai since 2009. The main principle of this technique is to reduce the time of contact of the spermatozoa with urine in the bladder, thereby retaining spermatozoa motility.

Methodology

Step 1: In a sterile non-toxic wide mouth semen collection container, sperm wash medium of around 30 to 40 ml is incubated at 37°C for 45 minutes.

Step 2: The patient is initially advised to void urine to empty the bladder. This is done to reduce the volume of residual urine in the bladder and in turn minimize the exposure of spermatozoa to the acidic pH of urine.

- (1) He is instructed to either masturbate (preferable) or have coitus until he achieves orgasm.
- (2) The patient is then instructed to void the post masturbatory/post coital urine into a sterile semen collection container which contains preincubated sperm wash medium. The sperm wash medium acts as a buffer and thereby the acidic pH of urine containing spermatozoa is immediately neutralized.

A mixture of pre-incubated sperm wash medium, semen, and the residual urine from the bladder is now obtained in the semen collection container.

Step 3: To isolate spermatozoa, the sample is then aliquoted by transferring to test tubes and subjected to centrifugation at 1500 rpm for 10 minutes.

Step 4: As a result, pellet will be obtained in each aliquot. Pellet is resuspended in 0.5 ml of sperm wash medium and is examined for spermatozoa concentration, motility, and morphology.

Step 5: Based on the sperm parameters thus obtained, the choice of treatment of the female partner is made. Informed consent was obtained from the patients.

CASE REPORT

A 32-year-old gentleman and his wife, a 27-year-old lady, married for around 3 years visited us with history of primary infertility. The female partner had regular menstrual cycles. Upon eliciting detailed sexual history, male partner revealed that he has no antegrade ejaculation and no night emission despite having orgasm. Uro-genital examination was found to be normal.

Considering the history, provisional diagnosis was made as retrograde ejaculation. Our method of SPERB was performed to confirm the diagnosis. Examination of post masturbatory urine revealed the presence of occasional motile and immotile spermatozoa. The test was repeated and the later showed similar spermatozoa parameters.

Since the spermatozoa numbers were very less, ICSI (Intra Cytoplasmic Sperm Injection) was the treatment of choice for this couple. Controlled ovarian hyperstimulation was done and oocyte retrieval was performed. Spermatozoa were recovered by the above-described method on the day of oocyte retrieval, and the motile spermatozoa obtained was used for ICSI. Details of the ART cycle are mentioned in Table 2.

On the third day after ICSI, 05 cleavage stage embryos (03–8 cell grade 1 and 02–6 cell grade 1) were obtained. The rate of fertilization and cleavage was normal. Considering the age of the female partner, type of infertility and quality of embryos, a fresh transfer of 02–8 cell grade 1 embryos was performed. The remaining embryos are cryopreserved. Two weeks from the embryo transfer, serum β hCG levels was assessed and was found to be 579.2 mIU/ml. A healthy live baby boy was born at term by elective Caesarean section.

LITERATURE REVIEW OF MANAGEMENT OF RGE

Upon reviewing the literature in detail (Table 3), it is clear that many methods have evolved over a period of time in managing patients with retrograde ejaculation. But, most of these methods are invasive, cumbersome, and have lot of drawbacks and a majority of them have been withdrawn from current practice.

DISCUSSION

The percentage of men with retrograde ejaculation among the infertile men ranges from 0.3% to 2%.^[2] The

incidence of azoospermia and aspermia in our center is 10% and 0.3% respectively.

History taking plays a significant role in suspecting and diagnosing this condition. A detailed medical, surgical, and sexual history is important to identify the cause of an ejaculation. Patient's inability to have an antegrade ejaculation despite having orgasm^[3] and passing cloudy urine^[2] after the sense of ejaculation due to mixing of semen and urine in the bladder are the key points to diagnose and to suspect retrograde ejaculation.

The final diagnosis of retrograde ejaculation is made by identifying spermatozoa in the post coital/masturbatory urine. In these patients with normal spermatogenesis, plenty of immotile spermatozoa can be seen. As there is mixture of semen with urine in the bladder, the acidic pH of urine renders the spermatozoa immotile/non-viable.^[16]

Isolation of motile spermatozoa is essential in these patients to treat infertility. Many methods have been employed for isolation of motile spermatozoa from patients diagnosed with retrograde ejaculation.

The treatment options vary largely from non-invasive to invasive methods, based on the etiology.^[17] Most of the methods used were either invasive or unpleasant to the couple seeking treatment. A detailed description of methodology used with their pros and cons are tabulated in Table 3.

SPERB, is a simple yet novel technique and is noninvasive. Spermatozoa retrieval from the bladder is done at ease just by instructing the patients to follow simple instructions. Emptying the bladder and voiding the post coital/masturbatory urine into the media acts as a buffer and reduces the acidic pH of residual urine thereby maintaining spermatozoa motility.

Among men with aspermia, SPERB method is effective in diagnosing retrograde ejaculation. The treatment method for infertility was based on the spermatozoa concentration and motility. ICSI is preferred when the recovered spermatozoa numbers are very few.

Pandiyan *et al.*^[31] introduced SPERB method in another institution and in the year 1998 presented and published the clinical outcome of using spermatozoa retrieved by this method as an abstract. Spermatozoa recovery was done in 06 patients diagnosed with retrograde ejaculation and the spermatozoa concentration and motility was suitable enough for performing intrauterine insemination. Since all the patients reported with primary infertility and had no female factor infertility, intrauterine insemination was offered and 04 pregnancies and live births were obtained.

CONCLUSION

Spermatozoa retrieval in men diagnosed with retrograde ejaculation can be performed by simple and non-invasive methods. Achieving fatherhood by natural conception is not possible in the absence of antegrade ejaculation. SPERB is a simple and easy method to retrieve motile spermatozoa and use it for fertility treatment. The choice of fertility treatment need not be restricted to higher treatment methodologies; a simple IUI should also be considered when the retrieved spermatozoa concentration and motility are favorable.

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Conflicts of interest

There are no conflicts of interest.

Commentary

Among the various causes of male infertility, the percentage of men with retrograde ejaculation ranges from 0.3% to 2%. Patient's inability to have an antegrade ejaculation despite having orgasm and passing cloudy urine after the sense of ejaculation due to mixing of semen and urine in the bladder are thekey points to diagnose and to suspect retrograde ejaculation. But the final diagnosis is made by identifying spermatozoa in the post coital/masturbatory urine. The main objective in these cases would be to obtain viable and motile spermatozoa before the acidic pH of urine renders them immotile/non-viable. In the near past, various treatment options have emerged. But there has been a constant search for a non-invasive, non-cumbersome method for the couple seeking treatment. Spermatozoa retrieval from the bladder (SPERB), described in the article, is a simple yet novel technique and is noninvasive. Emptying the bladder and voiding the post coital/masturbatory urine into the media acts as a buffer and reduces the acidic pH of residual urine, thereby maintaining spermatozoa motility. Although

this method was introduced in the year 1998, proper utilisation was limited by a smaller number of studies and the continued use of other methods of sperm retrieval like Hotchkiss method, Glezerman's methodology, etc. From the comparative analysis of all the invasive and noninvasive methods available, SPERB stands out as a promising option as it does not involve restricting fluid intake, catheterisation of bladder, increased risk of urinary tract infections (UTI) and cumbersome inseminate volume for the female partner. Achieving fatherhood by natural conception is not possible in the absence of antegrade ejaculation. As rightly quoted, the choice of fertility treatment can be a simple intrauterine insemination (IUI) when the retrieved spermatozoa concentration and motility are favourable, before proceeding to higher treatment methodologies and hence reducing the economical and psychological burden.

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