Retrograde ejaculation: Preparation of spermatozoa for insemination from retrograde ejaculates using the new SpermprepTM filtration method

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abstract

Twelve urine-voided specimens were collected from the same paraplegic individual after coitus and voiding within 5 min into 30 ml modified Ham's F-IO buffer following production of each retrograde ejaculate (RE). After collection and assessment each voided specimen was centrifuged and the spermatozoa were resuspended in 1.0 ml modified Ham's F-10 (Zavos/WiIson, 1984). Following assessment, each resuspended specimen was filtered using the newly developed SpermPrep^{TM}II method according to the manufacturer's specifications (ZBL, Inc., Lexington, KY 40523 USA). The semen parameters assessed after voiding of specimen were: % motility, 32+3.8; grade of motility, 1.9+0.2 (0-4); % normal morphology, 51±6; % intact omes, 52+7%; and debri presence, 4.0+0.0 (0-4). The specimens were filtered for 10-12 min recovering a filtrate volume of 4.7 ml. The filtered aliquots were centrifuged/resuspended in O.S ml modified Ham's F-10 and ed. Sperm counts (x10^6) were performed after resuspension. There were significant differences ($P < 0.05$) in all parameters assessed following the recovery of sperm filtered. The post-filtered values of sperm recovered were 41% which was quite efficient/adequate. The new SpermPrep^{TM}U method also provides additional features such as speed/ease for use that enables the recovery of high quality sperm from RE's for WI and IVF. The time, speed and ease for use (simplicity of technique) features, encompassed in the SpermPrep^{TM}II are important during the recovery of voided specimens as it is the case with RE.

Introduction

The ejaculatory reflex control is a rather complex and well-synchronized process which involves simultaneous/subsequent sympathetic and parasympathetic stimulaüon of different organs and structures in the male genital tract (Kimura et al, 1977; Rieser, 1961). Alterations of neurologic stimulus affecting seminal emission/bladder neck competence can adversely affect a man's ability to ejaculate semen effectively. Traumatic or surgical injury to the sympathetic nerves may result in either retrograde ejaculation (RE) or a complete lack of seminal emission.

Destruction or alteration of the nerves involved with emission and antegrade ejaculation may occur as a sequence of several surgical procedures. Those procedures include a) retroperitoneal lymph node

dissection on patients with nonseminomatous testicular tumors or testicular cancer (Narayan et al, 1982); b) abdominal aortic aneurysmectomy (Weinstein/Machleder, 1975); and
c) anterior resection of the rectum abdominal-perineal approach, causing nerve injury in the area of the inferior hypogastric plexus (Williams et al, 1951).

In addition to ejaculatory dysfunction due to neurogenic origin, RE can also be of myogenic or neuromuscular origin. Neuromuscular causes involve destruction or alteration (scarring) of the muscles during the performance of surgical procedures in the area of the bladder neck, urethra, or accessory glands. These procedures include a) transurethral resection of the prostate, which is one of the most common causes of vesical neck incompetence yielding RE (Virupannavar/Tomera, 1982); b) prostatectomy, which may result in a 50% incidence of RE (Rieser, 1961); and c) scarring and possibly disrupting elastic/muscular fiber functionality of the vesical neck (Gute et al, 1968). RE is suspected in patients with a history of bladder neck surgery as a child.

Retrograde Ejaculation/Treatment.

Depending on the etiology and localization of the disturbance, infertile men can be classified into diagnostic groups on the basis of algorithmic schemes. The diagnosis of RE is easily made by examining the postejaculatory urine and finding sperm present. RE is not a common male infertility, but has increased in incidence recently due to surgical aggressiveness in pelvic/genital malignancies (Schram, 1976). However, RE is the most common cause of azoospermia (Girgis et al, 1968) associated with absence of ejaculate at orgasm. The most common cause of RE is transurethral resection of the prostate (Virupannavar/Tomera, 1982), but nearly all cases of RE have resulted from surgical or medical illness, including diabetes mellitus, or interference with sympathetic nervous function (Narayan et al, 1982; Gute et al, 1968).

Although it may be possible to correct RE either by surgical means or by drug therapy (Stockamp et al, 1974; Andaloro/Dube, 1975), it may not be possible to regain normal antegrade ejaculation in all patients (Girgis et al, 1968). The standard procedure for treatment for RE in patients in whom surgical means or drug therapy do not yield positive results (Amelar, 1966). This technique involves the use of artificial insemination (Al) of bladder contents after manually induced ejaculation (masturbation). Other techniques involve postcoital-voiding insemination (Marmar et al, 1977) or postcoital voiding with Al after the voided specimen is gently centrifuged and concentrated (Schram, 1976).

A new technique has been introduced which can dramatically improve the recovery of motile sperm from a variety of ejaculates with different characteristics. This technique encompasses several attributes (Zavos/Centola, 1990a, 1991; Zavos, 1991). The SpermPrep$^{n'4}$11 technique has significant effects, in the manner that either fresh, cryostored (stored at 5 ^{0}C), frozen-thawed specimens, or even oligozoospermic and/or asthenozoospermic specimens (Zavos/Centola, 1991; Ohashi et al, 1992) are prepared and improved for IVF.

The lack of good-quality, low-risk techniques for the recovery and reconstitution of spermatozoa for Al following RE has led us to develop/employ new techniques in RE collection, filtration via the SpermPrep^{TM} technique. The main objective in this study was to determine whether

rapid transfer of spermatozoa (after RE) to a buffered solution might reduce the time of exposure of such spermatozoa to the detrimental effects of urine such as acidity, hypertonicity, and contamination, and allow recovery of good-quality spermatozoa for SpermPrep filtration/reconstitution for use in Il-JV or IVF.

Materials / Methods

Voided Specimen Recovery.

Twelve voided-urine specimens were collected from one patient within 5 min following production of each specimen (self-masturbation). The patient was instructed to take an alkalizing agent (one teaspoon of bakng soda in water) twice daily before the treatment and to void just prior to each semen production. Following self-masturbation (mean sexual abstinence time, 110 h), the urine was voided into a clean glass container (250 Ml) containing 30 ml of Ham's F-IO buffer (pH 7.2; osmotic pressure, 325 mOsm/L). Immediately after collection (after voiding), routine semen analysis performed. Voided specimens were centrifuged for 10 min at 500 xg. The sperm pellet recovered during the centrifugation was resuspended in 1.0 ml, volume of Ham's F-IO buffer. The resuspended sperm wetæ assessed/filtered via the SpermPrep™II method. All the results obtained were statistically analyzed by the variance techniques of the Statistical Analysis System (SAS; 1979).

SpermPrep™n Filtration Procedure.

The SpermPrep™II was used very similarly as previously for the SpermPrep technology (Zavos/Centola, 1990b; Zavos, 1992) with some simplified modifications (ZBL, Inc. , Lexington, KY USA). The proper standard laboratory techniques were employed in our laboratory during the whole filtration process: complete sterility/maintenance of all semen diluents, the SpermPrep™II filter and all other materials within a temperature range of 30-35^{0}C. At the end of the filtrate was centrifuged for 10 min at 400 xg, resuspended in 0.5 mL Ham's F-IO medium and assessed as previously described.

Results

Sperm parameters assessed in the voided-urine specimen mixture recovered after RE are shown in Table l. The spermatozoan parameters assessed prior to filtration and post-filtration via the SpermPrep^{Th4}11 filtration are shown in Table 2. There were significant differences ($P < 0.05$) in all parameters assessed following the recovery of spermatozoa post-filtration. There were no differences ($P > 0.05$) in the total functional sperm fraction (TFSF; Zavos et al, 1984) values between the pre/post-filtration samples indicating that the SpermPrep™II technique selected the majority of the motile, morphologically normal spermatozoa from the pre-filtered specimen. Also, the post-filtration values of sperm recovered were 41% which was quite efficient, adequate/consistent with other studies. The new SpermPrep™II also provided

Table 1 Characteristics of specimen obtained after recovery in voided-urine mixture (mean +SEM) [1]

	Sperm Parameters				
Volume of voided urine specimen (ml)	Motility	Grade (0-4)	Morphology (% Normal)	Acrosome (% Intact)	Debri
		1.9 ± 0.2	51 ± 6.1	52 ± 7.1	4.0±0.0

[1] Urine containing the RE was voided into 3.0 ml. of Ham's F-10.

Table 2. Spermatozoan parameters assessed before filtration/postfiltration via the SpermPrep™II (mean ± SEM) [I]

Parameters Assessed	Pre-filtered	Post-filtered	
Concentration (total; x 10^6)	57.8 ± 11.2	23.6 ± 5.1*	Concentration
Motility (70)	48.9 ± 7.3	83.4 ± 6.1*	
Grade (0-4)	2.5 ± 0.2	3.6 ± 6.1*	

[1] Specimens filtered for 10-12 min; volume recovered of 4.7±0.3 mL.

Morphology (% Normal)	50.6 ± 8.4	79.7 ± 6.7*
Acrosome (% Intact)	53.7 ± 9.1	79.7 ± 7.2*
Debri present (0-4)	4.0 ± 0.0	0.3 ± 0.1*
TFSF (x10)	14.3 ± 4.7	15.7 ± 4.1

additional features such as speed and ease of use that enabled the recovery of high quality sperm from RE's for IUI or other forms of assisted reproductive techniques. The time, speed and ease of use (simplicity of technique) features, encompassed in the SpermPrep[Th4]11 method are of utmost importance during the recovery of voided specimens as is the case with RE.

Discussion

In infertile men with RE, contamination of the semen with urine cannot be avoided, but it can be minimized. Reducing the time interval that urine is in contact with the spermatozoa should reduce the detrimental effects of urine on the voided spermatozoa. Attempts were made to minimize the detrimental effects of urine via recovery of the voided spermatozoa directly into Ham's F-10 buffer. Reconstitution of spermatozoa from the voided mixture (urine Ham's F-10 buffer) into Ham's F-IO was performed to minimize the induced "urine shock". The technique employed in this study assists in the recovery/reconstitution of retrograde ejaculates fit for Il-Jl or IVF. The recovered spermatozoa, although partially shocked (urine shock), acquired normospermic qualities when filtered via the SpermPrep[Th4]11 and reconsütuted (Table 2). The acquisition of better quality spermatozoa was mainly attributed to the beneficial effects of the filtration method which enables the entrapment of dead, morphologically abnormal/sluggish sperm and allows the high quality sperm to be recovered in the filtrate.

The technique in this study has various advantages in comparison with other methods suggested in the literature (Amelar, 1966; Mamar et al, 1977). These include a) requiring very little patient preparation; b) involving low risks by not employing bladder catheterization for the recovery of the RE; c) avoiding the use of drug therapy, such as adrenergic blocking agents (Kimura et al, 1977) or sympathomimetic drugs which have detrimental side effects (Shader, 1964) to achieve antegrade ejaculation; d) avoiding the use of postcoital-voiding insemination (Schram, 1976) which could induce some psychologic misgivings (Marmar, 1977) to the couple, in addition to being a non-hygienic method; e) reducing the risk of transporting urine contaminants into the female reproductive tract at the time of Al, by the incorporation of adequate levels of antibiotics in all buffers in this study; and b) utilizing the already washed, sterile, filtered and reconstituted sperm preparations for Il-Jl or IVF.

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[1] TFSF: Total functional sperm fraction.

*Differences between the two columns ($P < 0.05$)

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