

# NONINVASIVE TECHNIQUES FOR IMPROVING FERTILITY POTENTIAL OF RETROGRADE EJACULATES

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## INTRODUCTION

Several noninvasive techniques have been employed for insemination of sperm recovered from the bladder following retrograde ejaculation, and at least 33 pregnancies have been reported [3, 4, 5, 9, 17, 18]. The main objective of the various procedures is to quickly separate the spermatozoa from the toxic urine and/or to mitigate the adverse bladder environment [12].

## METHODS

*Older Methods.* A previously used but invasive method included replacing the urine with a few milliliters of an isotonic buffer solution by catheterization before masturbation [13]. An early noninvasive technique was described in 1954 by Fischer and Coats [12] in which the urine was neutralized by the oral ingestion of alkalinizing agents ( $\text{NaHCO}_3$ ) given because the normal urine is acidic. With this technique, the urine voided after masturbation is centrifuged and the wife is inseminated with the sperm recovered. Unfortunately, the technique has yielded variable quality of the recovered semen because the osmolality of urine deviates from the normal osmolality of human semen.

*Cryopreservation.* The problem of variable semen quality with this noninvasive method resulted in reduced likelihood of a good quality specimen at the time of ovulation. For this reason, cryopreservation of multiple samples has been attempted so that a good thawed specimen may be employed at the time of ovulation [1]. Some pregnancies have been reported following the insemination of cryopreserved semen recovered from the bladder [1, 14].

*Sperm Washing Techniques.* Another method has been described to circumvent the problems of the Fischer and Coats [12] technique. This involves centrifugation of the urine and suspension of the sediment in Ham's F-10 medium. However, attempts are first made to adjust the urine osmolality and pH [15]. Urry et al. [18] also reported recovered retrograde sperm of good quality and subsequent

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pregnancies, again after inseminating the washed retrograde specimen in Ham's F-10 medium. They found 7 of 12 patients with retrograde ejaculation candidates for the sperm wash procedure because they had demonstrated at least occasional samples with reasonable sperm numbers and motility. The wives of 6 of these 7 patients became pregnant within six cycles of exposure to sperm samples meeting minimum criteria. Mahadevan et al. centrifuged at  $500 \times g$  for 5 min, whereas Urry et al. centrifuged at  $250 \times g$  for 5 min. A similar technique with achievement of pregnancy was reported after using Biggers, Whitten, and Whittingham (BWW) medium instead of Ham's F-10 [5].

*Donor Seminal Plasma.* Although pregnancies have been reported using the above techniques, the recovered specimen frequently demonstrates asthenospermia. Recently, some data were presented showing that the small antegrade fraction of 3 men with retrograde ejaculation demonstrated no progressive motility after 40 min following suspension in Ham's F-10 media; however, following suspension in donor seminal plasma, motility significantly improved and all 3 wives became pregnant in 1, 3, and 5 cycles respectively [8]. Another wife whose husband did not have an antegrade fraction also conceived in her first cycle after the insemination of the retrograde semen suspended in donor seminal plasma.

Brassesco et al. [3] found 7 of 15 patients with retrograde ejaculation suitable candidates for sperm wash and insemination. Pregnancies were reported in 6 of the 7 patients by the fourth month of insemination and a pregnancy in the other patient in the sixth month of insemination. All 7 cases resulted in normal healthy children. One couple achieved a second pregnancy. The pellet in all these cases had been suspended in normal seminal plasma.

#### *Sperm Preparation*

*Alkalinization of the Urine.* All methods for collection of retrograde specimens involve alkalinization of the urine. This is based on several previous observations including those of Makler et al. [19], who found that the average pH of semen is 7.65 (range of 7.2-8.2), but also demonstrated that spermatozoa remain motile, at least for short periods, at a wider range of pH. In fact, although motility is better maintained at higher pH levels, a rise in pH above 9 is considered spermicidal, whereas some of the diminished motility seen in an acid environment ( $\text{pH} < 6.0$ ) appears to be partially reversible [16, 20].

The methods of alkalinization vary among the different authors. Mahadevan et al. [18] in 1987 recommended 4 gm of  $\text{NaHCO}_3$  4  $\times$  daily, beginning 3 days before collection. However, we have found that many men complain about the side effects of the  $\text{NaHCO}_3$  when taken this way. We altered the dosage to just twice—5 gm each at 2 h and 1 h prior to ejaculation—and found a consistent pH between 7.3 to 7.6. Similarly, Braude et al. [4] were able to maintain the pH between 7.6 and 8.1 by giving one dose of 5-10 gm  $\text{NaHCO}_3$  2 to 3 h before voiding for spermatozoa collection. Perhaps the slightly higher pH reported by Braude et al. [4] is related to their adjustment of the medium with NaOH in order to raise the pH to 7.6-8.0 (we do not make this adjustment).

*Preparation of the Urine Containing Semen (Fig. 1).* The urine is immediately checked for a semen clot. The clot, if present, is removed and diluted 1 : 2 with Ham's F-10 media. If no clot is present, the entire urine sample is diluted 1 : 2 with Ham's F-10 media. The diluted specimen is then centrifuged at  $300 \times g$  for 5 min. The pellet is resuspended with 0.5 ml to 1 ml Ham's F-10. Another option is to suspend in 0.5 to 1 ml of sperm-free donor seminal plasma instead of the Ham's F-10.

*Osmolality.* The osmolality of normal human seminal plasma has been found to average  $366 \pm 16$  mosmol/kg [19]. The quality of the semen recovered from the bladder depends upon the osmolality of the urine [10]. Adverse effects of hypo-osmotic solutions have been reported, specifically affecting the tail [11]. Irreversible tail damage may occur following exposure below 200 mosmol/kg. The method we use frequently leads to a high urine osmolarity, which is reduced by equal parts of Ham's F-10 media,

TABLE 1 Summary of Preparation for Retrograde Ejaculation

## 1. Methods of alkalization prior to collection

| <i>Author</i> | <i>Method</i>   |
|---------------|---|
| Mahadevan     | 4 gm NaHCO <sub>3</sub> , 4 times daily for 3 days                |
| Check         | 5 gm NaHCO <sub>3</sub> at 2 h and 5 gm NaHCO <sub>3</sub> at 1 h |
| Braude        | 5 to 10 gm NaHCO <sub>3</sub> , 2-3 h—adjust urine with NaOH      |
| Hotchkiss     | replace urine with isotonic buffer by catheterization             |

## 2. After orgasm void urine specimen.

## 3. pH of urine to support sperm viability.

| <i>Author</i> | <i>Range</i> |
|---------------|--------------|
| Makler        | 7.2-8.2      |
| Braude        | 7.6-8.0      |
| Check         | 7.3-7.6      |

If the pH is high then adjust with HCL. If the pH is low then adjust with NaOH.

## 4. Osmolality of urine to support sperm viability.

| <i>Author</i> | <i>Range</i>                                    |
|---------------|---|
| Check         | 200-300 mosmol/kg, adjust with media to 270-300 |
| Braude        | > 300 mosmol/kg                                 |
| Brassesco     | 300-500 mosmol/kg                               |

If the osmolality is not within range then adjust with a medium that is at the proper pH and osmolality.

## 5. Centrifugation of urine

- diluted with media
- undiluted

## 6. Resuspend sperm pellet for insemination.

- medium; Ham's F-10, BWB, or Hepes buffered Hank's solution.
- seminal plasma

usually adjusted to an osmolality of 280-285 mosmol/kg. If this is inadequate, extra water is added. We prefer the levels of the sperm insemination mixture to be between 270 and 300 mosmol/kg, whereas Braude et al. [4] prefers slightly over 300 mosmol/kg. Brassesco et al. [3] required voiding every 15 min and requested ejaculation only when the osmolality was between 300 to 500 mosmol/kg.

*Preparation of the Donor Seminal Plasma.* The donor must have a minimum of 70% motile sperm (minimum grade 3 or 4 quality). After liquefaction or within 1 h of ejaculation, the semen is centrifuged at 16,000 rpm until it is completely devoid of sperm. The seminal plasma is then ready for immediate use. We have found the best results with donor seminal plasma when the sample is fresh and not frozen, used within 2 h of collection, and when it comes from a patient demonstrating prior excellent quality of motility.

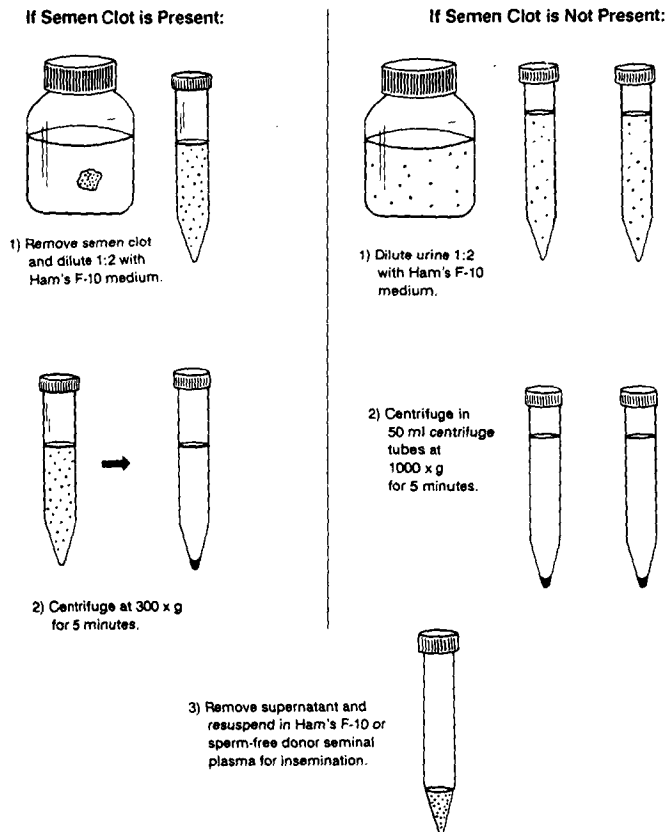
*Use of Adrenergic Drugs to Try to Achieve Antegrade Ejaculation.* The use of Ornade® in the 3 cases reported by Check et al. [8] resulted in a small antegrade fraction ranging from 0.05 to 0.4 ml. The sperm counts ranged from  $< 1 \times 10^6$ /ml to  $137 \times 10^6$ /ml. It is frequently assumed that even if one can achieve a small antegrade flow, the first portion (and usually the best fraction) will go retrograde. But in these cases, because the retrograde fraction exposed to urine did not do as well in donor seminal plasma

as did the antegrade fraction, the latter was used for insemination. It is therefore reasonable to at least attempt to get an antegrade fraction, evaluate both, and choose the best fraction for insemination.

*Intrauterine Versus Intracervical Insemination.* There is no evidence to indicate that a higher pregnancy rate would be achieved by intrauterine versus intracervical insemination. The latter provides another test of sperm quality function by determining sperm progression in the mucus. Intracervical insemination is also safer in that there is less risk of infection. Nevertheless, if intrauterine insemination is needed because of a concomitant cervical factor problem, then antibiotics such as penicillin and streptomycin should be added to the media. If the specimen contains debris and bacteria, it should be layered onto Percoll gradients with subsequent centrifugation.

The timing of the female's insemination is based on waiting until a mature follicle of at least 18mm diameter is attained by pelvic sonography and the serum estradiol is at least 200 pg/ml per mature follicle

### Preparation of the Urine Containing Semen



**FIGURE 1** Protocol for sperm preparation of urine containing semen from men with retrograde ejaculation.

[6]. Two h following intracervical insemination, the number of sperm with progressive forward motion per high powered field is noted [7].

## CONCLUSIONS AND PRACTICAL CONSIDERATIONS

The data support employing newer methods of separating sperm from urine by washing, with attention given to osmolality and pH. There is no data to support a superiority of one collection medium over another. Thus, Ham's F-10, BWW, or Hepes buffered Hank's solution are interchangeable. However, our data support the possibility that donor seminal plasma may improve motility in some cases, at least compared with Ham's F-10 [8]. Brassesco et al. [3] do not mention the reason for their use of donor seminal plasma but they do report one of the more successful series. The possibility exists that some motility-enhancing factor or a suppressor of a sperm motility inhibiting factor may be deficient in these other supporting media. Indeed, a seminal plasma substance known as the epididymal motility protein appears to induce motility in bovine sperm [2].

Because of the theoretical risk of AIDS from fresh seminal plasma, it would be preferable to first try one of the standard supporting media and only if the results are poor try donor seminal plasma.

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