

## Preparation of human frozen-thawed seminal specimens using the SpermPrep\* filtration method: improvements over the conventional swim-up method†

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**Objective:** To examine the qualitative and quantitative characteristics of frozen-thawed spermatozoa recovered through the SpermPrep (ZBL, Inc., Lexington, KY) filtration method or the swim-up technique for the purpose of intrauterine insemination (IUI) or other techniques for assisted reproduction.

**Design, Setting, Patients:** Thirty pairs of frozen specimens purchased from three commercial semen suppliers were used in this study. Each pair consisted of two aliquots from the same semen specimen.

**Main Outcome Measures:** Spermatozoa recovered via the SpermPrep filtration and swim-up processes were evaluated for sperm numbers recovered, sperm motility, grade of sperm motility, percentage of morphologically normal spermatozoa, the response of spermatozoa to a hypo-osmotic environment (hypo-osmotic swelling test), and the amount of debris present.

**Results:** Application of the SpermPrep filtration method resulted in recovery of significantly greater numbers of spermatozoa ( $P < 0.01$ ) than were recovered with the swim-up method ( $31.1 \pm 3.2 \times 10^6$  versus  $10.2 \pm 1.8 \times 10^6$  spermatozoa, respectively). This represents a mean recovery of approximately one half (49%) of all spermatozoa applied to the filter, whereas for the swim-up method, it was only 15%. The overall quality of recovered spermatozoa was virtually identical between the two methods ( $P > 0.05$ ). The percent motile sperm improved by a mean of 18% to 20%, the grade of motility improved by a mean of 0.4 points (scale 0 to 4), the percent of morphologically normal spermatozoa increased by a mean of approximately 10%, the percent of spermatozoa reactive to a hypo-osmotic medium test increased by a mean of approximately 9%, and the debris score decreased by a mean of 0.2 to 0.3 points (scale 0 to 4). Most importantly, the mean total number of motile, morphologically normal spermatozoa after filtration through the SpermPrep column was  $20.2 \pm 1.1 \times 10^6$ , representing a mean recovery of 73% of the normal spermatozoa originally applied to the column. This was 316% greater than the yield obtained with the swim-up method ( $6.4 \pm 0.8 \times 10^6$ ), which was significantly greater ( $P < 0.01$ ) than that recovered via the swim-up method. Also, the time required to harvest sperm through SpermPrep filtration was 20 to 25 minutes versus 80 minutes required for the swim-up method ( $P < 0.05$ ).

**Conclusion:** Considering that the effectiveness of frozen-thawed semen is already limited when compared with fresh semen, SpermPrep filtration is the method of choice over the swim-up technique of sperm selection because the former provides significantly greater numbers of high quality sperm. It should be considered as an adjunct in semen preparation for IUI or other forms of assisted reproduction. Fertil Steril 1992;57:1326-30

**Key Words:** SpermPrep, semen filtration, semen preparation, frozen-thawed semen

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Improvements in rates of conception could be realized if frozen-thawed spermatozoa are selected on the basis of their motility, progressive motility, and morphological characteristics. Such selection of spermatozoa could be properly applied at the time of intrauterine insemination (IUI) or other forms of assisted reproductive techniques (ART) because the seminal plasma, cryoprotective agents, and other

background materials and debris should be removed from the spermatozoa before these procedures are performed.

A number of manipulative techniques for fresh semen are currently available to remove the undesirable spermatozoa, debris, and other factors and to increase sperm quality. These various techniques include the most popular ones, swim-up or sperm rise methods (1) and swim-down or sedimentation type methods (2). Less popular methods include Ficoll centrifugation (3), Percoll density gradient (4), and Sephadex (5), or glass wool fiber filtration (6). It should be emphasized, however, that with many of these manipulative techniques often the increase in sperm quality is achieved at the expense of numbers of recovered spermatozoa that may not be especially advantageous for patients with various seminal deficiencies such as oligozoospermia. Equally important, none of these techniques have been employed successfully with frozen-thawed semen because of the low quality and quantity of sperm recovered, from a sample that is already compromised in quality. Also, the relatively lengthy time period required to perform these procedures is additionally disadvantageous because the life expectancy of post-thaw spermatozoa may be limited (7).

Recently, a new technique (SpermPrep; ZBL, Inc., Lexington, KY) has been introduced that yields a higher level of sperm recovery and is rapid and reproducible (8). Because of these advantages, the SpermPrep technique could have significant effect, in the manner that frozen-thawed specimens are prepared and improved before their use in artificial (noncoital) reproduction procedures. The present study was undertaken to compare the use of the SpermPrep filtration column and the conventional swim-up method for processing frozen-thawed specimens.

## MATERIALS AND METHODS

Thirty pairs of frozen specimens, purchased from three commercial semen suppliers, were used in this study. Each pair consisted of two similar aliquots from the same ejaculate processed identically by the supplier. The specimens were thawed according to the suppliers' specifications and were gently diluted using modified Ham's F-10 medium (GIBCO, Grand Island, NY) as the diluent to avoid any adverse osmotic side effects to the spermatozoa (9). The final dilution was five to six times the original frozen-thawed volume over a period of 10 to 15 minutes. The Ham's F-10 medium was supplemented with

3% human serum albumin, adequate levels of antibiotics (150 U/mL of penicillin-streptomycin), a pH of 7.2 to 7.4, and an osmotic pressure of 320 to 325 mOsm, balanced with D-glucose.

## Seminal Specimen Evaluation

After semen samples were properly thawed and before dilution, each sample was evaluated (within 15 minutes) according to standard procedures recommended by the World Health Organization with a phase-contrast microscope (10). Semen measures included volume, sperm count per milliliter, percentage sperm motility, grade of sperm motility (11), hypo-osmotic swelling test (12), sperm morphological features and presence or absence of debris (13). All seminal samples were evaluated under blind conditions by the same technician. The post-thaw seminal characteristics (means  $\pm$  SE) of the 30 pairs of specimens used before application of SpermPrep filtration or swim-up methods were: volume 0.5 mL, total sperm numbers  $67.8 \pm 7.2 (\times 10^6)$ , motility  $55.2\% \pm 6.1\%$ , grade of sperm motility  $3.2 \pm 0.2$  (0 to 4), normal sperm forms  $74.3\% \pm 5.6\%$ , hypo-osmotic test  $63.1\% \pm 3.1\%$ , and debris presence  $2.2 \pm 0.2$  (0 to 4). After semen evaluation and dilution, each sample was centrifuged at  $360 \times g$  for 7 minutes at room temperature. The final washed sperm pellets were processed further, and one aliquot was used for the swim-up procedure and the other for the SpermPrep filtration procedure. Data for the two techniques applied were compared with each other using one-way ANOVA followed by either the Student's *t*-test or the paired Student's *t*-test where appropriate.

## Swim-up Procedure

The swim-up procedure was performed by gently overlaying 2.0 mL of Ham's F-10 modified medium over the centrifuged sperm pellet that contained  $67.8 \pm 7.2 \times 10^6$  spermatozoa. The centrifuge tube (15-mL polystyrene; Fisher Scientific, Pittsburgh, PA) was held at a  $5^\circ$  angle for 60 minutes at  $37^\circ\text{C}$  to allow the maximal number of motile spermatozoa to swim up into the overlaid medium. After incubation, 1.8 mL of the medium was carefully removed without disturbing the pellet at the bottom of the tube, and the harvested spermatozoa were assessed as previously described.

## SpermPrep Filtration Procedure

The SpermPrep was used according to the manufacturer's specifications and instructions (ZBL,

Inc.). It should be emphasized that proper standard laboratory techniques were employed in our laboratory during the whole filtration process and were applied similarly during the swim-up. Those techniques included complete sterility and maintenance of all semen diluents, the SpermPrep filter, and all other materials within a temperature range of 30 to 35°C.

Filtration was begun by placing the 1.0-mL volume of the properly resuspended spermatozoa in the filter. The 1.0-mL aliquot contained  $67.8 \pm 7.2 \times 10^6$  washed and well-mixed spermatozoa. At the end of filtration (10 to 15 minutes), the filtrate was centrifuged and resuspended in 1.0 mL of Ham's F-10 and assessed as previously described.

## RESULTS

Application of the SpermPrep filtration method resulted in recovery of significantly greater numbers of spermatozoa ( $P < 0.01$ ) than were recovered with the swim-up method. The mean of  $31.1 \pm 3.2 \times 10^6$  total spermatozoa obtained with the SpermPrep filter represents a mean recovery of approximately one half (49%) of all spermatozoa applied to the filter. Use of the swim-up method yielded only a mean recovery of 15% of spermatozoa with the mean total recovered sperm numbers being  $10.2 \pm 1.8 \times 10^6$ . The SpermPrep column therefore yielded a recovery of spermatozoa that was 324% greater than the swim-up method.

The overall quality of recovered spermatozoa was virtually identical between the two methods (Table 1). The percent motile sperm improved by a mean of 18% to 20%, the grade of motility improved by a mean of 0.4 points (scale 0 to 4), the percent of morphologically normal spermatozoa increased by a mean of approximately 10%, the percent of spermatozoa reactive to a hypo-osmotic medium test increased by a mean of approximately 9%, and the

debris score decreased by a mean of 0.2 to 0.3 points (scale 0 to 4).

Most importantly, the mean total functional sperm fraction (TFSF = total cells  $\times 10^6 \times$  percent motility  $\times$  percent normal morphological features/ $1.0 \times 10^4$ ) was  $27.8 \pm 1.8 \times 10^6$  for post-thawed samples before processing by either method (14). After filtration through the SpermPrep column, the mean ( $\pm$ SE) TFSF was  $20.2 \pm 1.1 \times 10^6$ , representing a mean recovery of 73% of the motile, morphologically normal spermatozoa originally applied to the column. This is a 316% greater yield than achieved with the swim-up method in which the mean TFSF was  $6.4 \pm 0.8 \times 10^6$ . An average of only 23% of the TFSF included in the swim-up procedure were recovered whose values were significantly inferior ( $P < 0.01$ ) to those recovered through the SpermPrep method. Also, the time required to harvest sperm through SpermPrep filtration was 20 to 25 minutes, whereas the time required for the swim-up method was approximately 80 minutes.

## DISCUSSION

The objective of the current study was to evaluate the qualitative and quantitative characteristics of post-thawed spermatozoa recovered from the SpermPrep filtration column and the more traditional and the most extensively used method for semen preparation, i.e., the swim-up method. As known, human frozen-thawed spermatozoa are inferior to fresh spermatozoa in fertilization (15), viability, and enzymatic profile (16), cervical mucus penetration (17, 18), and other parameters. The great majority of the results have indicated that approximately 30% to 70% of spermatozoa that were motile before freezing regain their motility after thawing (7). It is of interest, therefore, to seek improved methods for spermatozoal freeze preservation and that will increase the post-thaw recovery of mo-

**Table 1** Changes in Sperm Parameters of Post-thaw Specimens After Processing via the SpermPrep and Swim-up Methods

Method employed	Sperm parameters considered				
	Motility	Grade*	Normal morphology	Hypo-osmotic swelling test	Debris*
	%		%	%	
SpermPrep (n = 30)	$18 \pm 1.2$ (132) <sup>†</sup>	$0.4 \pm 0.1$ (113) <sup>‡</sup>	$10 \pm 0.9$ (113) <sup>‡</sup>	$8 \pm 0.4$ (113) <sup>‡</sup>	$-0.2 \pm 0.0$ (-91) <sup>‡</sup>
Swim-up (n = 30)	$20 \pm 1.3$ (136) <sup>†</sup>	$0.4 \pm 0.1$ (112) <sup>‡</sup>	$9 \pm 0.7$ (113) <sup>‡</sup>	$10 \pm 0.5$ (115) <sup>‡</sup>	$-0.3 \pm 0.0$ (-89) <sup>‡</sup>

\* Scale of 0 to 4.

<sup>†</sup> Values are means  $\pm$  SE. Significantly different from post-thawed specimens before processing;  $P < 0.01$ .

<sup>‡</sup> Significantly different from post-thawed specimens before processing;  $P < 0.05$ .

tile, viable spermatozoa. However, improvements in these procedures are limited, and employment of new techniques to select the most viable, motile, morphologically normal spermatozoa, which are the ones actively involved in fertilization, should be an additional goal to be met if improvements in conception rates with frozen-thawed sperm are to increase and reach satisfactory levels. It is for these reasons that most of the current semen manipulative techniques with fresh spermatozoa have as their main objective the selection of these groups of spermatozoa that have a higher fertilizing capacity than the spermatozoa present in the original ejaculate.

With the advent of in vitro fertilization (IVF) and other ART, it is considered important to use the best spermatozoa available from a frozen semen sample. Indeed, recent evidence with fresh spermatozoa has suggested that spermatozoa separated via various methods have a greater fertilizing capacity than spermatozoa in the whole ejaculate (6, 19, 20). The retrieved spermatozoa normally are selected on the basis of motility, progressive motility, and morphological characteristics. As known, motility and progressive motility are essential qualities for the spermatozoa to penetrate the various investments of the oocyte during in vitro insemination. However, although sperm motility characteristics are of considerable importance in evaluating and properly predicting the fertility potential of an ejaculate or specimen, its use as a diagnostic tool by itself is sometimes inaccurate and, in some instances, exaggerated. For example, using motility alone, neither the outcome of a human IVF (12) nor the zona-free hamster oocyte penetration test (21) can be predicted with any degree of reliability. Also, improving the percent motility alone does not cause a significant improvement in conception rates after IUI (22, 23) or in the ability of the spermatozoa to penetrate cervical mucus (24).

The data presented in this study indicated that the SpermPrep filtration method yielded spermatozoa of similar qualitative characteristics as those recovered via the swim-up method ( $P > 0.05$ ). However, the SpermPrep filtration method yielded a significantly higher number of total sperm and motile sperm ( $P < 0.05$ ) than the swim-up method. When the TFSS value was calculated for sperm recovered by both techniques, the value for SpermPrep filtration was 316% higher ( $P < 0.01$ ) than the TFSS value obtained via the swim-up method. This is of great clinical significance because the numbers of spermatozoa recovered from post-thaw specimens

could be the sole difference between success and failure of an assisted reproductive procedure.

The SpermPrep filtration procedure was also less time consuming than the swim-up procedure. The filtration procedure was completed in 20 to 25 minutes, whereas the swim-up procedure took approximately 1.5 hours to complete. This is, of course, of great importance because the SpermPrep filtration process can save precious time for the technical personnel, the clinician, and the patient. Equally important, the time savings can significantly reduce the detrimental effects on the post-thaw sperm viability and subsequent fertilization.

In conclusion, the clinically and statistically significant improvements achieved with the SpermPrep method over the conventional swim-up method in the yield of sperm numbers of good quality, together with the possibly important time savings that the SpermPrep procedure provides, demonstrates that this new technique is the method of choice for selecting motile, morphologically normal spermatozoa from frozen-thawed human semen for use in the various ART including IUI.

#### REFERENCES

1. Russell DL, Rogers BJ. Improvement in the quality and fertilization potential of a human sperm population using the rise technique. *J Androl* 1987;8:25-33.
2. Dmowski WP, Gaynor L, Rao R, Lawrence M, Scommegna A. Use of albumin gradients for X and Y sperm separation and clinical experience with male sex preselection. *Fertil Steril* 1979;31:52-7.
3. Kaneko S, Moriwaki C, Sato H. Development of multiple exposure photography method for analysis of sperm motility and preparation of washed sperm with Ficoll density gradient. *Jpn J Fertil Steril* 1980;25:491-3.
4. McClure RD, Nunes L, Tom R. Semen manipulations improved sperm recovery and function with a two-layer Percoll gradients. *Fertil Steril* 1989;51:874-7.
5. Quinlivan WLG, Preciado K, Long TL, Sullivan H. Separation of human X and Y spermatozoa by albumin gradients and Sephadex chromatography. *Fertil Steril* 1982;37:104-7.
6. Katayama KP, Stehlik E, Jeyendran RS. In vitro fertilization outcome: glass wool-filtered sperm versus swim-up sperm. *Fertil Steril* 1989;52:670-2.
7. Beck WW, Silverstein I. Variable motility recovery of spermatozoa following freeze preservation. *Fertil Steril* 1975;26:863-7.
8. Zavos PM, Centola GM. Qualitative and quantitative improvements in human spermatozoa recovered via the swim-up and a new semen filtration method. *Infertility* 1990;13:25-34.
9. Zavos PM. Opisthosmotic shock of frozen-thawed human spermatozoa. *Infertility* 1983;5:247-255.
10. World Health Organization. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge, The Press Syndicate of the University of Cambridge, 1987:1-13.

11. Zavos PM, Cohen MR. The pH of the cervical mucus and the postcoital test. *Fertil Steril* 1980;34:234-8.
12. Van der Ven HH, Jeyendran RS, Al-Hasani S, Perez-Pelaez M, Dietrich K, Zaneveld LJD. Correlation between human sperm swelling in hypoosmotic medium (HOS) test and in vitro fertilization. *J Androl* 1986;7:190-6.
13. Cohen J, Edwards R, Fehilly C, Fishel S, Hewitt J, Purdy J, et al. In vitro fertilization: a treatment for male infertility. *Fertil Steril* 1985;43:422-32.
14. Zavos PM, Goodpasture JC. Clinical use of a seminal collection device: improvements of specific seminal deficiencies through use of a seminal collection device at intercourse versus masturbation. *Infertility* 1988;11:289-304.
15. Steinberger E, Smith KD. Artificial insemination with fresh or frozen semen: a comparative study. *JAMA* 1973;223:778-82.
16. Zavos PM, Goodpasture JC, Zaneveld LJD, Cohen MR. Motility and enzyme activity of human spermatozoa stored for 24 hours at +5°C and -196°C. *Fertil Steril* 1980;34:607-9.
17. Zavos PM, Cohen MR. Bovine mucus penetration test: an assay for fresh and cryopreserved human spermatozoa. *Fertil Steril* 1980;34:175-6.
18. Katz DF, Morales P, Samuels SJ, Overstreet JW. Mechanisms of filtration of morphologically abnormal human sperm by cervical mucus. *Fertil Steril* 1990;54:513-6.
19. Forster MS, Smith WD, Lee WI, Berger RE, Karp LE, Stenchever MA. Selection of human spermatozoa according to their relative motility and their interaction with zona-free hamster eggs. *Fertil Steril* 1983;40:655-60.
20. Tanphaichitr N, Millette CF, Agulnick A, Fitzgerald LM. Egg-penetration ability and structural properties of human sperm prepared by Percoll-gradient centrifugation. *Gamete Res* 1988;20:67-73.
21. Aitken RJ, Best FSM, Richardson DW, Djahanbakhch O, Lees MM. The correlates of fertilizing capacity in normal fertile men. *Fertil Steril* 1982;38:68-76.
22. Glass RH, Erickson RJ. Intrauterine insemination of isolated motile sperm. *Fertil Steril* 1978;29:535-40.
23. Hoing LM, Devroey P, Van Steirteghem AC. Treatment of infertility because of oligoasthenoteratospermia by transcervical intrauterine insemination of motile spermatozoa. *Fertil Steril* 1986;45:388-91.
24. Tyler ET. The clinical use of frozen semen banks. *Fertil Steril* 1973;24:413-8.