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Selection of single-stranded deoxyribonucleic acid spermatozoa via the SpermPrep* filtration column

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The fluorochrome acridine orange (AO) test was described by Tejada et al. (1) and provides chromatin heterogeneity information on the DNA content of a spermatozoal population. The test is based on the principle that the fluorochrome AO when bound to the double-stranded normal DNA fluoresces green, whereas when bound to denatured, single-stranded DNA, fluoresces red. An abnormally high percentage of denatured DNA-containing spermatozoa that fluoresce red have been associated with decreased male fertility, whereas a high percentage of green fluorescing spermatozoa indicates a normal semen sample (2). It has also been shown that when the ratio of green fluorescent sperm is <45%, the probability of oocyte fertilization is lower, whereas a ratio of green fluorescent sperm > 45% indicates a higher probability of fertilization (2).

A number of semen manipulative techniques are currently available to remove the undesirable spermatozoa, debris, and other factors and to increase sperm quality before the performance of various assisted reproductive technologies (ART). Recently, a new method (SpermPrep; ZBL, Inc., Lexington, KY) that recovers a high ratio of motile, morphologically normal spermatozoa and is quite rapid and reproducible has been introduced (3). Although there are many studies evaluating the qualitative aspects of the retrieved sperm population among the various manipulative techniques, there are no studies dealing with DNA abnormalities of the retrieved spermatozoa. As known, DNA-abnormal spermatozoa are considered undesirable in ART, and it seems to be important to evaluate the retrieved spermatozoa for DNA abnormalities after employment of sperm separation techniques. The SpermPrep filtration method has been recently considered to be the treatment of choice for sperm manipulation when compared with other methods (3, 4). Therefore, the present study was designed to evaluate the percentage of single-stranded DNA spermatozoa before and after SpermPrep filtration.

MATERIALS AND METHODS

Eighteen semen samples were collected via masturbation from 18 fertile donors who regularly contribute to our semen bank. The obtained specimens were evaluated according to standard procedures recommended by the World Health Organization (5). Two, 1-mL aliquots were prepared from each sample that contained $98.5 \pm 4.6 \times 10^6$ spermatozoa. The 18 pairs of samples were washed in modified Ham's F-10 medium (GIBCO, Grand Island, NY) as previously described (4). After centrifugation at $400 \times g$ for 7 minutes, the washed pellet was resuspended in 2 mL of the same medium. The first aliquot of each pair was evaluated for sperm motility and sperm morphology, and the AO staining procedure was performed as previously described (1). A fluorescence microscope with an excitation filter of 490 nm and

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a 530-nm barrier filter was used to evaluate 200 spermatozoa per sample. The results were expressed as a proportion of red to green spermatozoa. Most of the red spermatozoa did not stain clearly red, but a wide range of yellow, orange, and red was observed. We have employed extremely strict criteria, and these spermatozoa were all classified as red because denaturation, which had already started, was considered abnormal. The second aliquot of each pair was filtered via the SpermPrep method. The SpermPrep was used according to the manufacturer's specifications (ZBL, Inc.). At the end of the filtration, the filtrate was pooled, centrifuged, and resuspended in 1 mL of modified Ham's F-10 medium. Sperm motility and morphology were assessed, the AO test was similarly performed, and the proportion of red to green spermatozoa was calculated and expressed as means ± SD. Student's *t*-test for paired observations was used to compare the data for the two techniques applied.

RESULTS

Application of the SpermPrep filtration method resulted in recovery of a large proportion of the original number of spermatozoa. The mean of 48.6 ± 3.7 $\times 10^6$ total spermatozoa with the SpermPrep filter represents a mean recovery of approximately one half (49%) of all spermatozoa applied to the SpermPrep filter. The proportion of red to green spermatozoa was significantly higher ($P < 0.001$) in aliquot one than in the postfiltered, aliquot two (Table 1). Application of the SpermPrep filtration method as applied in the current study also resulted in recovery of greater numbers of motile, morphologically normal spermatozoa ($P < 0.001$) as compared with the nonfiltered aliquot one (Table 1).

Table 1 Proportion of Single-Stranded (Red) to Double-Stranded (Green) DNA Spermatozoa and Other Qualitative Sperm Parameters Before and After Processing the Spermatozoa via the SpermPrep Filtration Method*

		Sperm parameters considered		
Treatment	Aliquot	Red:Green	Motility	Normal morphology
			%	%
Prefiltered	1	0.80†	48.3 ± 4.4	54.2 ± 5.2
Postfiltered	2	0.44	65.7 ± 5.3	83.4 ± 6.1

* Values are means ± SD.
† Means are different between all prefiltered and postfiltered parameters considered; ($P < 0.001$).

DISCUSSION

The present study confirmed the previous findings indicating that sperm motility and percentage of morphologically normal spermatozoa were significantly higher ($P < 0.001$) in the SpermPrep-filtered samples than in the nonfiltered samples (3, 4). Additionally, the data presented in this study revealed, for the first time, that the proportion of red to green spermatozoa in the SpermPrep-filtered samples was significantly lower than in the nonfiltered samples. These data suggest that the SpermPrep filtration method increased the percentage of double-stranded DNA spermatozoa and simultaneously decreased the percentage of single-stranded DNA spermatozoa in the final filtrate. Considering that a high percentage of single-stranded DNA spermatozoa is associated with decreased male fertility (2), it appears that the SpermPrep filtration method, by entrapping the single-stranded DNA denatured spermatozoa, may increase the fertilizing capacity of the overall postfiltered sperm sample. The SpermPrep filtration method is the first semen manipulation technique that showed an increase ($P < 0.001$) in the percentage of double-stranded DNA spermatozoa recovered after filtration. Additionally, the SpermPrep filtration procedure has increased sperm motility and percentage of normal spermatozoa parameters, which are known to correlate positively with the sperm's fertilizing capacity (6). Furthermore, the SpermPrep filtration method is less time consuming than other methods because it can be completed in only 20 minutes. Therefore, this study using the SpermPrep method is the first to achieve significant clinical and statistical improvements in percentage of healthy, double-stranded DNA spermatozoa and should be considered as an excellent method for selecting spermatozoa for use in the various ART including intrauterine insemination. Further studies using other semen preparation methods should be carried out to establish the pattern of selection of healthy, double-stranded DNA spermatozoa among these techniques and compare the results with those generated via the SpermPrep method.

SUMMARY

Eighteen semen samples were collected from 18 normospermic men. Two aliquots (1 mL) were prepared from each ejaculate, washed with Ham's F-10, and each washed sperm pellet was reconstituted in 2 mL volume of Ham's F-10 medium. Each aliquot one was stained using the AO-staining method. Each

aliquot two was filtered via the SpermPrep II method, and the recovered spermatozoa were stained similarly. The proportion of single-stranded DNA (red) spermatozoa to double-stranded (green) spermatozoa was significantly higher in aliquot one than in the postfiltered sample (aliquot two), suggesting that the SpermPrep filtration procedure selectively entrapped the spermatozoa with abnormal DNA.

Key Words: SpermPrep filtration, human spermatozoa, single-stranded DNA, double-stranded DNA.

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