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THE SPERM PREP™ FILTRATION COLUMN SELECTIVELY ENTRAPS SINGLE-STRANDED DNA SPERMATOZOA

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Abstract: Eighteen semen samples were collected from normospermic men. Two aliquots (1 ml) were prepared from each ejaculate, mixed with Ham's F-10 medium, and centrifuged. The final washed sperm pellets were resuspended in 2 ml of the same medium. Then, the first sample (fraction-one) of each pair of aliquots was stained using the acridine orange staining. The second sample (fraction-two) was filtered via Sperm Prep™ tube and the recovered spermatozoa were similarly stained. The proportion of single-stranded DNA spermatozoa to double-stranded spermatozoa was significantly higher in the fraction-one than in the postfiltered fraction-two suggesting that Sperm Prep™ filtration procedure selectively entraps spermatozoa with abnomal DNA.

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Introduction

The fluorochrome acridine orange test was described by Tejada and co-workers and gives information on the DNA content of a spermatozoal population[1]. The test is based on the principle that acridine orange binding to double-stranded normal DNA results in green fluorescence, while binding to denatured, single-stranded DNA results in red fluorescence. An abnormally high percentage of denatured sperm heads (red) has been associated with decreased male fertility, while a high percentage of green sperm cells indicates a normal semen sample[2]. It has also been suggested that when the percentage of green cells is less than 45, the probability of oocyte fertilization is lower, while a percentage of green cells more than 45 indicates a higher probability of fertilization[2].

Human seminal plasma is composed of cells, debris, and fluids from the testis, excretory ducts, and accessory sex glands of the male. The total ejaculate in addition to the seminal plasma contains the gametes that are a mixture of motile, nonmotile, and perhaps agglutinated spermatozoa. A number of semen manipulative techniques are currently available to remove the undesirable spermatozoa, debris, and other factors and to increase sperm quality before performance of various assisted reproductive techniques. These various manipulative techniques include the swim-up method[3], Ficoll centrifugation[4], Percoll density gradient method[5], glass-wool-fiber filtration[6], and the swim down method[7]. Recently a new method has been introduced which recovers a high number of motile spermatozoa and is quite rapid and reproducible. This method is known as Sperm Prep™ flitration[8]. Although there are

many studies evaluating the retrieved sperm population, sperm motility, and sperm morphology before a semen manipulative technique and after it, there are no studies dealing with DNA-abnormalities of the retreived spermatozoa. Considering that DNA-abnormal spermatozoa are considered undesirable in assisted reproduction programs, it seems to be important to evaluate the retrieved spermatozoa for DNA abnormalities after sperm separation techniques. The Sperm Prep™ filtration has been recently considered to be the treatment of choice for sperm manipulation[8]. The present study was undertaken to evaluate the percentage of single-stranded DNA spermatozoa before Sperm Prep™ filtration and after it.

Materials and Methods

Eighteen semen samples were collected by masturbation from fertile donors. After liquefaction at 37℃ sperm parameters were evaluated according to standard procedures recommended by the World Health Organization[9]. Each sample showed sperm concentration more than 80 million per ml, percentage of motile spermatozoa more than 20%, and percentage of morphologically normal spermatozoa more than 34%. Then two aliquots of 1 ml were prepared from each sample. The 18 pairs of samples were washed in Ham's F-10 medium (Gibco, Grand Island, NY) supplemented with 30% human serum albumin (Albuminar-25; Armour Pharmaceutical Co., Kankakee, IL), adequate levels of anibiotics, a pH balance of 7.2—7.4, and a balanced osmotic pressure of 320-325 mOsm (using D-glucose). After centrifugation at 400Xg for 15 minutes the washed pellet was resuspended in 2 ml of the same medium. Thus, 18 pairs of washed sperm samples were prepared. The first sample of each pair (fraction-one) was processed for evaluation of sperm motility and sperm morphology as above. Then, the acridine orange staining was performed as described by Tejada and co-workers[1]. Finally a fluorescence microscope with an excitation filter of 490 nm and a 530 nm-barrier filter was used to evaluate the spermatozoa. A total of 200 cells were evaluated. The results were expressed as a proportion of red cells to green cells. Most of the red sperm cells did not stain clearly red, but a wide range of yellow, orange, and red was observed. These cells were all classified as red, since denaturation which had already started was considered abnormal. The second sample of each pair (fraction-two) was used for the Sperm Prep™ filtration procedure. The Sperm Prep™ was used according to the manufacturer's specifications and instructions (Fertility Technologies, Inc., Natick, MA)[8]. At the end of the filtration the filtrate was pooled, centrifuged, and resuspended in 1 ml of Ham's F-10. The sperm motility and sperm morphology were evaluated, the acridine orange test was performed as above, and the proportion of red spermatozoa to green spermatozoa was calculated.

All the values were expressed as Mean±SD. Student's t-test for paired observations was used to compare the percentage of motile spermatozoa and the percentage of morphologically normal spermatozoa between the fraction-one and the postfiltered fraction-two. Chi-square test (Yates' correction) was used to compare the proportion of red to green spermatozoa between the fraction-one and the postfiltered fraction-two. A probability less than 0.05 was considered to be statistically significant.

Results

Proportion of red spermatozoa to green spermat-

Table 1 Proportion of red spermatozoa to green spermatozoa, sperm motility, and sperm morphology[a]

Spermatozoa	Fraction	Red/Green spermatozoa	% motile spermatozoa	% normal spermatozoa
Prefiltered	1	0.80[b]	48±4[b]	54±5[b]
Postfiltered	2	0.44[c]	65±5[c]	83±6[c]

[a] Values are expressed as Mean±SD.
[b,c] Within each column, values not sharing the same superscript b,c are significantly different ; b vs c : $P<0.001$

ozoa

The proportion of red spermatozoa to green spermatozoa was significantly higher in the fraction-one than in the postfiltered fraction-two (Table 1).

Percentage of motile spermatozoa

The percentage of motile spermatozoa was significantly higher in the postfiltered fraction-two than in the fraction-one (Table 1).

Percentage of morphologically normal spermatozoa

The percentage of morphologically normal spermatozoa was significantly higher in the postfiltered fraction-two than in the fraction-one (Table 1).

Discussion

The present study confirms previous findngs indicating that sperm motility and percentage of morphologically normal spermatozoa are significantly higher in the postfiltered samples than in the prefiltered samples[8]. Additionally, the data presented in this study reveals that the proportion of red to green spermatozoa in the postfiltered samples is significantly lower than in the prefiltered samples suggesting that the Sperm Prep™ filtration method increases the percentage of double-stranded DNA spermatozoa and decreases the percentage of single-stranded DNA spermatozoa in the final filtrate. Considering that high percentage of single-stranded DNA spermatozoa is associated with decreased male fertility[2], it appears that the Sperm Prep™ filtration method entrapping single-stranded DNA denatured spermatozoa increases the fertilizing capacity of the sample. The Sperm Prep™ filtration method is the first semen manipulation method which is proven to increase the percentage of double-stranded DNA spermatozla. Additionally, the Sperm Prep™ filtration procedure increases sperm motility and the percentage of normal spermatozoa which are known to correlate postively with the sperm fertilizing capacity[10]. Furthermore, the Sperm Prep™ filtration method is not time consuming since it can be completed in 20 minutes. Therefore, we suggest usage of the Sperm Perp™ column in spermatozoal separation techniques.

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