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Acrosin Profiles of Human Spermatozoa Recovered from the New Sperm Prep™II Filtration Column

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SOFIKITIS, N., MIYAGAWA, I., ZAVOS, P.M., INAGA, S., IINO, A., TODA, T., HARADA, T., MIO, Y. and TERAKAWA, N. *Acrosin Profiles of Human Spermatozoa Recovered from the New Sperm Prep™II Filtration Column*. Tohoku J. Exp. Med., 1992, 166 (4), 451-457 —— Twenty semen samples were collected and two aliquots of 1.5 ml were prepared from each sample. One sample of each pair (fraction-one) was used for evaluation of semen parameters and total acrosin activity of spermatozoa. The other sample of each pair (fraction-two) was mixed with an equal volume of Ham's F-10 medium and filtered through the Sperm Prep™II. At the end of the filtration, the filtrate was pooled, sperm parameters were evaluated and total acrosin activity was assessed. Percentage of normal spermatozoa, sperm motility, and total acrosin activity of spermatozoa were significantly higher in the postfiltered fraction-two than in the fresh fraction-one. Considering the great importance of acrosin for fertilization, it is suggested that Sperm Prep™II filtration method may be beneficial in preparing spermatozoa from infertile men with low acrosin profiles for assisted reproduction programs. Further studies will be necessary, however, in order to confirm this, since the present study concerned men with known fertility. ———— human; spermatozoa; acrosin

Acrosin, a serine proteinase associated with the acrosome of spermatozoa, appears to be involved in the acrosome reaction as well as the capability of spermatozoa to bind to and penetrate the zona pellucida (Rogers and Bentwood 1982; Zaneveld et al. 1990). Most of acrosin is present in the acrosome as a precursor, proacrosin. Proacrosin becomes activated during the fertilization process (Green 1978; Goodpasture et al. 1981). In vitro, proacrosin rapidly

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converts to acrosin at pH 8.0. There is much evidence that acrosin is important in the fertilization process (Zaneveld et al. 1990). Most recent evidence for the role of acrosin in human fertilization was provided by proving that 4-acetamidophenyl 4'-guanidinobenzoate, an acrosin inhibitor, prevents the penetration of human spermatozoa through the human zona pellucida (Zaneveld et al 1990). Acrosin levels in individual ejaculates are significantly correlated with their capacity to fertilize human oocytes in vitro (Van der Ven et al. 1987). A relationship between acrosin levels and fertility was also found in cattle (Pace et al. 1981). Separate measurement of proacrosin and the non-zymogen (free) acrosin is probably not important from a clinical standpoint (Zaneveld et al. 1990). However, measurement of the total acrosin activity may be an useful test in the male infertility clinic because it measures the activity of an enzyme that is vitally important in fertilization.

A number of semen manipulative techniques are currently available to remove the undesireable spermatozoa, debris, and other factors and to increase sperm quality before performance of the various assisted reproductive techniques. For assisted reproductive procedures, sperm separation is essential for two additional reasons. The first is to remove the seminal plasma, because substances in the plasma can cause severe cramps when used for artificial insemination ; additionally it is believed that long exposure to seminal plasma can decrease sperm fertilizing potential. The various sperm separation techniques include the swim-up, or sperm rise methods (Russell and Rogers 1987 ; Haris et al. 1981), and the swim-down, or sedimentation-type, methods (Erickson 1977 ; Dmowski et al. 1979). Less popular methods include Ficoll centrifugation (Kaneko et al. 1980), Percoll density gradient (McClure et al. 1989), and Sephadex filtration (Quinlivan et al. 1982). Zavos (1990, 1992) recently developed new filtration methods (Sperm Prep™ filtration method and Sperm Prep™II filtration method) which are less time consuming than the swim-up procedure (Zavos and Centola 1990). Additionally, the Sperm Prep™ and Sperm Prep™II filtration methods are recently considered the methods of choice for isolating high numbers of motile spermatozoa (Zavos and Centola 1990). Zavos and Centola (1990), comparing the swim-up procedure and the Sperm Prep™ filtration procedure found that the latter yielded significantly greater numbers of total sperm in a much shorter time interval than the swim-up.

It has been reported that selected sperm populations via the swim-up method averaged approximately twice in acrosin activity than in the spermatozoa in the raw ejaculate (Van der Ven et al. 1987). There are no reports however, comparing the acrosin content of filtrated spermatozoa via the Sperm Prep™ method. Considering that the Sperm Prep™II filtration method has been widely applied in assisted reproduction programs, it is therefore of interent in evaluating the acrosin content of spermatozoa recovered via the Sperm Prep™II filtration method. Differences in acrosin profiles between fresh and filtered samples may result in

differences in sperm fertilizing capacity because total sperm acrosin activity, as previously noted, is significantly correlated with sperm fertilizing capacity (Van der Ven et al. 1987). The objective of the present study was to compare the total acrosin content of spermatozoa filtered via the Sperm Prep™II method with that of the original, unprocessed ejaculate.

Materials and Methods

Twenty semen samples were collected via masturbation from 20 fertile men after three days of sexual abstinence. After liquefaction at 37°C, semen parameters were evaluated according to standard procedures recommended by the World Health Organization (1980) with a phase-contrast microscope. Motility was also graded according to an arbitrary grading system on a scale from 0 to 4 (Sofikitis et al. 1991) with 0 representing no movement, 1 meaning weak forward progression of some sperm, 2 indicating steady forward progression of most motile sperm, 3 meaning strong forward progression, and 4 representing very vigorous and rapid forward progression. To evaluate sperm morphology, the samples were observed by a laser microscope (Iino et al. 1989). Each sample had a volume of seminal plasma greater than 3 ml, sperm concentration greater than 100 million per ml, and percentage of motile spermatozoa greater than 20%. Two aliquots of 1.5 ml were prepared from each sample (20 sample pairs). One sample of each pair (fraction-one) was used for evaluation of total acrosin activity as described by Zaneveld and co-workers (1990). In brief, the spermatozoa were washed free of seminal plasma by centrifugation over Ficoll (1,000 × g for 30 min) to remove the soluble proteinase inhibitors in semen that can interfere with acrosin activity. The sperm pellet was subsequently suspended in a buffer that had (a) a detergent which facilitated disruption of the sperm acrosomes and released the acrosomal enzymes (Triton X-100; Sigma Chemical Company, St Louis, MO, USA); (b) a basic pH which allowed activation of proacrosin into enzymatically active acrosin; and (c) a synthetic arginine amide substrate (*N*-α-benzoyl-DL-arginine-*p*-nitroanilide; BAPNA; Sigma Chemical Company) which, when hydrolyzed, released a chromophoric product. Finally the total amount of color developed after 3-hour incubation period was measured spectrophotometrically. The acrosin activity was expressed in μIU/10^6 spermatozoa. One IU of acrosin activity was defined as the amount of enzyme that hydrolyzes 1 μmol of BAPNA per minute at 23°C. A change in absorbance of 9.9 at 410 nm corresponds to the hydrolysis of 1.0 μmol of BAPNA. The other sample of each pair (fraction-two) was mixed with an equal volume of Ham's F-10 medium (Gibco, Grand Island, NY), properly liquefied and filtered through the Sperm Prep™II (ZBL, Inc. Lexington, KY, USA). The Ham's F-10 medium was supplemented with 3% of human serum albumin (albuminar-25; Armour Pharmaceutical Co., Kankakee, IL), adequate levels of antibiotics, a pH balance of 7.2 to 7.4, and a balanced osmotic pressure of 320 to 325 mOsm (using D-glucose). The Sperm Prep™II was used similarly as previously described for the Sperm Prep™ (Zavos and Centola 1990) and Sperm Prep™II technology (Zavos, 1992), with some simplified modifications. Filtration was begun by placing the well mixed, liquefied semen into the filter. No prefiltration sperm wash was performed. Sperm Prep™II comes with an extension funnel which is attached to the column during the preparation steps. It should be emphasized also, that proper standard laboratory techniques were employed in our laboratory including complete sterility and maintenance of all semen diluents within a temperature range of 30-35°C during the whole filtration process. Approximately, 0.8-1.1 ml of filtrate was recovered during each minute of filtration. At the end of the filtration period (five minutes) the filtrate was pooled, sperm parameters were evaluated and total acrosin activity was assessed as noted above.

Statistical analysis

Student's t-test for paired observations was used to compare sperm parameters and total acrosin activity of the filtered spermatozoa (fraction-two) with those of the fresh, non-filtered samples (fraction-one). A probability less than 0.05 was considered to be statistically significant.

RESULTS

Sperm concentration

The mean total sperm content in the filtered fraction was 53.3×10^6 spermatozoa, which represented a mean recovery of approximately one third (33%) of the original population, since the mean total sperm count in fraction-one or the prefiltration fraction-two was 162.4×10^6 spermatozoa (Table 1).

Sperm motility

Percentage of motile spermatozoa and motility grade were significantly higher in the postfiltration fraction-two than in fraction-one (Table 1).

Sperm morphology

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

Total acrosin activity

Total acrosin activity of spermatozoa was significantly higher in the postfiltered fraction-two than in fraction-one (Table 1).

DISCUSSION

The present study reveals that the motility grade, percentage of motile spermatozoa, and percentage of morphologically normal spermatozoa are significantly higher in the retreived cellular population after Sperm Prep™II filtration than in the fresh, non-filtered unprocessed samples. Although the filtration time was very short, the Sperm Prep™II filtration method yielded one third of all spermatozoa applied to the filter.

TABLE 1. *Characteristics of filtered and non-filtered spermatozoa recovered via the Sperm Prep™II filtration method (Means ± S.D.)*

Samples ($n=20$)	Fraction	Volume (ml)	Total sperm count ($\times 10^6$)	% normal sperm	% motile sperm	Motility grade (0–4)	Acrosin activity (μIU/10^6 sperm)
Fresh	1	1.5	162.4 ± 12	62.1 ± 4.1	48.1 ± 4.2	2.7 ± 0.2	15.2 ± 2.1
Filtered	2	4.5 ± 0.2	53.3 ± 5	71.3 ± 2.5[a]	64.3 ± 4.1[a]	3.4 ± 0.2[a]	26.8 ± 2.4[a]

[a]Comparison between fresh and filtered with in each column: $p < 0.001$.

The present study did prove that the total acrosin activity of the filtered spermtozoa was significantly higher than that assessed from the fresh spermatozoa. This increase in the total acrosin activity may be due to the recovery of higher percentage of morphologically normal spermatozoa following filtration since the presence of morphologically abnormal spermatozoa corresponds to abnormal spermatogenic function, which may subsequently lead to aberrant packing of acrosin (Goodpasture et al. 1987). Additionally, Goodpasture and co-workers (1987) found that five sperm morphologic parameters were significantly correlated with some component of the acrosin system (r-values greater than 0.35) suggesting that a reduction in acrosin content may occur in various groups of patients with high levels of morphologically abnormal spermatozoa. No such correlations were carried out in the current study and therefore the cause of the higher acrosin content in postfiltration samples may be attributable to factors others than sperm morphology.

In the present study, the total sperm acrosin activity was evaluated and not the total acrosin content, because it is the available acrosin activity and not the content that determines the ability of spermatozoa to function properly during fertilization.

Considering that low levels of acrosin activity can identify groups of infertile patients that are not recognized by the standard semen parameters (Koukoulis et al. 1988) and that acrosin is a very important enzyme for the normal fertilization process, it appears that low sperm acrosin activity may be responsible for male infertility. Therefore, it is suggested that is very important to employ sperm separation procedures in assisted reproduction programs, which isolate spermatozoa with higher spermatogenic parameters, including acrosin activity than that of the original spermatozoal population.

The present study showed very vividly that the Sperm Prep™II filtration method isolated spermatozoa with higher acrosin activity than that of the fresh spermatozoa. Any improvements in standard sperm parameters made similar to those noted in this study via using other techniques than Sperm Prep™II such as swim-up or Percoll gradient centrifugation could have similar improvements in the acrosin profiles of these spermatozoa. It was also shown that the Sperm Prep™II filtration method enabled the recovery of higher quality spermatozoa than the original ejaculate involved. Furthermore, the Sperm Prep™II filtration method was shown in other studies to be less time consuming than the swim-up and other sperm separation methods (Zavos 1992). In addition, it was found that Sperm Prep™ filtration procedure separated significantly greater numbers of motile sperm than the swim-up method (Zavos and Centola 1990, 1991). A study comparing processing techniques of donor semen for use in intrauterine inseminations showed that the clinical pregnancy rate per cycle was significantly lower in semen samples processed via the swim-up method when compared to those processed via the Sperm Prep™ filtration method (Awadalla et al. 1991). Check

and co-workers (1992) comparing the effect of Percoll discontinuous density gradients versus Sperm Prep™II filtration method on semen parameters claimed that Percoll and Sperm Prep™II seem equally effective methods for producing high quality sperm for assisted reproduction programs although Sperm Prep™II is quite faster. Thus, it is suggested that the Sperm Prep™II filtration method may be the treatment of choice in patients with various forms of spermatogenic dysfunction for sperm separation purposes to be used in the assisted reproduction programs. Further studies will be necessary to confirm these observations with other groups of subfertile patients since the present study concerned men with known fertility.

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