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Effect of swim-up, Percoll and Sephadex sperm separation methods on the hypo-osmotic swelling test

J.H.Check[1], D.Katsoff, J.Kozak and D.Lurie

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden and Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Camden, NJ, USA

[1]To whom correspondence should be addressed at: 8002 E.Greentree Commons, Marton, NJ 08053, USA

The separation of spermatozoa from the seminal plasma is required to prepare the spermatozoa for intrauterine insemination, in-vitro fertilization and sex selection. This study evaluated the effects of sperm preparation techniques on the functional integrity of the sperm membrane, as measured by the hypo-osmotic swelling test (HOS). Thirty-four semen specimens obtained from the male partner of infertile couples were evaluated. A semen analysis and HOS test were performed on each specimen. The remainder of the specimen was divided into three equal aliquots, the first prepared using Percoll, the second using a swim-up method and the third using a Sephadex column. After the preparation, a semen analysis and HOS test was performed on each aliquot. The mean and standard deviation for the HOS test was 72.9 ± 8.5% initially, 71.2 ± 13.1 after Percoll, 75.2 ± 15.1 after swim-up and 62.4 ± 14.5 after Sephadex. Analysis of variance showed that the mean HOS score was the same after Percoll and swim-up as it was initially but significantly lower after preparation with Sephadex. There was also a higher proportion of abnormal semen specimens (HOS <50%) after preparation with Sephadex than after the other preparation methods. We recommend the use of the HOS test as part of a screening panel for sperm separation.

Key words: Hypo-osmotic swelling test/Percoll/Sephadex/swim-up

Introduction

The separation of spermatozoa from the seminal plasma is important for several therapeutic indications. Most importantly it is required in order to perform intrauterine insemination (IUI), since introduction of prostaglandins from seminal plasma directly into the uterine cavity may result in severe cramps and even shock; in addition, reduction of bacterial content is needed. Other indications for separation of spermatozoa include capacitation and acrosome reaction for in-vitro fertilization (IVF) and for sex selection, even when intracervical insemination (ICI) is performed.

Several techniques have been developed for sperm separation including swim-up (Berger *et al.*, 1985; Gellent-Mortimer *et al.* 1988), Percoll discontinuous gradient (Gorus *et al.*, 1981; Forster *et al.*, 1983; Arcidiacono *et al.*, 1983; Dravland and Mortimer 1985; Hyne *et al.*, 1986; Akenlof *et al.* 1987; Iizuka *et al.*, 1988; Gellent-Mortimer *et al.*, 1988), Sephadex gel filtration (Steeno *et al.*, 1975), glass wool filtration (Paulson *et al.*, 1979), glass bead columns (Daya *et al.*, 1987) and albumin gradients (Ericsson 1977; Broer and Danker, 1978).

Several studies have been performed comparing the different techniques, especially concerning final motile density and bacterial content (McDowell, 1983; Hull *et al.*, 1986). For example, Sun *et al.* (1987) found the highest motile density with a 'fall-down' method but the best morphology and least bacterial contamination after Percoll. Katayama *et al.* (1989) found a higher number of motile spermatozoa and better in-vitro fertilizing capacity with glass wool separation compared to swim-up. Punjabi *et al.* (1990) recently concluded that it is important to individualize the sperm separation procedure to find that best suited to a given individual.

The hypo-osmotic swelling test (HOS) is a simple method of evaluating sperm fertilizing capacity (Jeyendran *et al.*, 1984; Van der Ven *et al.*, 1986; Check *et al.*, 1988). In this study, we used the HOS test to evaluate the effects of sperm separation techniques on the integrity of the sperm membrane. HOS scores were evaluated for sperm specimens prepared with swim-up, Percoll discontinuous gradient and Sephadex gel filtration (McClure *et al.*, 1989; Check *et al.*, 1989a, 1991).

Materials and methods

Thirty-four specimens obtained from the male partners of infertile couples were evaluated. Only men with motile sperm concentrations $\geq 10 \times 10^6$/ml were included in the study. Each semen specimen was evaluated initially by performing a semen analysis and the HOS test. The routine semen analysis was performed using a Makler counting chamber and the motile concentration was calculated. The HOS test was performed as previously described (Jeyendran *et al.*, 1984). The specimen was then divided equally into three aliquots. The first aliquot was put onto a modified two-layer discontinuous Percoll gradient (Check *et al.*, 1989a) including a 90% bottom layer instead of a 95% layer. The second aliquot was subjected to multi-tube swim-up preparation (Check *et al.*, 1991). The third aliquot was put through a modified Sephadex gel filtration column (Steeno *et al.*, 1975; Check *et al.*, 1991). A centrifuged washed aliquot would have provided a useful second control but the specimens had inadequate volumes to divide into four aliquots and still sum the procedures. After preparation, a semen analysis and HOS test was performed on each aliquot.

Differences in the semen parameters between the initial measurement and following the three sperm preparation methods were analysed using analysis of variance for repeated measures (a randomized block design). The level of statistical significance used was $P \leq 0.05$. When the analysis of variance demonstrated differences between the preparations, multiple comparisons were performed to analyse further differences.

The sperm specimen was classified as follows: normal, if the HOS score was $\geq 60\%$; grey-zone, if HOS was between 50 and 60%; and abnormal, if HOS was $< 50\%$. A comparison of the proportion of abnormal scores obtained after each preparation was made using Cochran's Q-test for related proportions with $P \leq 0.05$ as the level of statistical significance. An alpha level of 0.05 was used.

Results

The mean and standard deviation for the HOS test was 72.9 ± 8.5% initially, 71.2 ± 13.1% after Percoll, 75.2 ± 15.1% after swim-up and 62.4 ± 14.5% after Sephadex. The analysis of variance showed that the mean HOS score was the same after preparation with Percoll and with swim-up as it was initially. However, the score was significantly lower after preparation with Sephadex. Comparison of the mean values for the other semen parameters measured by sperm separation methods showed that preparation by both Percoll and swim-up lowered the count and motile concentration of the specimen but increased the motility (Table I). Preparation by gel filtration on Sephadex, however, lowered the motility and motile concentration but did not change the count.

When the specimens were classified as normal, grey-zone and/or abnormal according to their HOS score, there were no abnormal specimens initially, two abnormal specimens after preparation by Percoll, two abnormal specimens after preparation by swim-up, and six abnormal specimens after preparation by Sephadex (Table II). The proportion of abnormal specimens after each method of preparation were compared using the Cochran Q-test and showed significant differences [chi-square (3 d.f.) = 8.77]. Follow-up comparisons between the preparations showed that the proportion of abnormal specimens was higher after Sephadex, the other two preparations having the same proportions.

Of the 34 original specimens, 19 had normal HOS scores, both initially and after Sephadex; nine specimens were reduced from normal to grey-zone after Sephadex; three were converted from normal to abnormal after Sephadex; and three were reduced from grey-zone to abnormal after Sephadex, and one of these specimens also dropped to subnormal levels following Percoll. Thus, Sephadex was shown to produce the most damage to sperm membranes.

Discussion

The hypo-osmotic swelling test (HOS), which evaluates the functional integrity of the sperm membrane, has been demonstrated to be highly specific for male subfertility when the score drops below 50% (Check *et al.*, 1989). Though the only statistical difference in mean levels of HOS score was obtained following Sephadex preparation, there were two men whose specimens dropped below the critical 50% level after swim-up and also after Percoll gradient separation; an even greater number (six) dropped below 50% using Sephadex gel filtration.

The reason we chose these three techniques for comparison was based on their use for sex selection, as well as their ability to separate spermatozoa from seminal plasma (Steeno *et al.*, 1975; Kaneko *et al.*, 1983, 1984; Check *et al.*, 1989b). Since couples requesting sex selection are generally fertile, we decided to choose only men with motile sperm concentrations $\geq 10 \times 10^6$/ml. Perhaps if men with low motile sperm concentrations were used or men with antisperm antibodies, an even greater percentage of specimens may have dropped below 50% following preparation. Specimens with poor initial motility may

Table I. Comparison of mean semen parameters according to sperm separation method (data are means ±SD)

	Sperm separation method				
	Initial	Percoll	Swim-up	Sephadex	Amount
Count (10^6/ml)	74.1 ± 46.5	25.6 ± 21.6	30.2 ± 32.9	63.5 ± 53.4	$P < 0.05$[a]
Motility (per cent)	54.9 ± 12.4	64.5 ± 19.4	74.2 ± 17.0	37.0 ± 17.3	$P < 0.05$[b]
Motile concentration (10^6/ml)	39.8 ± 28.5	17.2 ± 14.1	23.2 ± 26.4	22.4 ± 20.6	$P < 0.05$[c]
HOS score	72.9 ± 8.5	71.2 ± 13.1	75.2 ± 15.1	62.4 ± 14.5	$P < 0.05$[d]

[a]Mean count was the same after Sephadex but significantly lower after Percoll and swim-up.
[b]Sephadex decreased the mean motility, while Percoll and swim-up increased the mean motility.
[c]All three preparations lowered the mean motile concentration.
[d]The mean HOS score was lowered by Sephadex only.

Table II. Distribution of sperm specimens according to HOS test criteria following sperm separation methods

HOS classification	Sperm separation method			
	Pre-wash	Percoll	Swim-up	Sephadex
Normal (HOS > 60%)	31	27	30	19
Grey-zone (50% < HOS < 60%)	3	5	2	9
Abnormal (HOS < 50%)	0	2	2	6

be even further compromised following Sephadex separation since a significant reduction in motility was found with this technique compared to the other two procedures.

It should be noted that one sample dropped to a subnormal HOS score following Percoll but not after swim-up or Sephadex and another dropped after Percoll and Sephadex but not after swim-up. A third sample was reduced after swim-up but not after Percoll or Sephadex (though this sample dropped into the grey-zone). Thus, we agree with Punjabi *et al.* (1990) that sperm preparation methods should be individualized to find the one that is best for each person. The data presented herein provide good evidence for adding the HOS test, which is a very inexpensive, simple and reproducible assay, to the screening panel for sperm separation.

These data also suggest that a better chance of achieving a pregnancy might occur if Percoll is used rather than Sephadex. However, newer techniques using Sephadex columns for sperm separation have been recently described and superiority of the final sperm preparation in terms of velocity and linearity have been claimed compared to Percoll and washing (Drobnis *et al.*, 1991). We intend to compare HOS scores to Percoll and swim-up preparation following this new procedure, which uses a shorter column length and Sephadex beads of smaller diameter.

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