Original Article

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Comparing the Clinical Outcomes of Intrauterine Insemination by Two Different Density Gradient Preparation Methods

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Background. Sperm preparation has play an integral part in the success of in-vitro fertilization. The aim of this study was to compare 2 different density gradient preparations for sperm separation in respect to sperm recovery, motility, motion parameters and clinical outcome after intrauterine insemination.

Methods. One-hundred and 21 women who received intrauterine insemination due to ovulation dysfunction were randomly allocated into 2 groups, using either the Percoll (Amersham, Pharmacia Biotech AB, Sweden) or the PureSperm (Nidacon, Göteborg, Sweden) density gradient method for sperm preparation. The characteristics of sperm before and after separation and the clinical outcome of intrauterine insemination were compared between the 2 groups.

Results. PureSperm and Percoll demonstrated comparable ability to recover the sperms with progressive motility. There was no difference in motion parameters and the number of sperm recovered with progressive motility between the Percoll and the PureSperm density gradient preparations. The clinical pregnancy rate was also comparable between the 2 groups, 12.5% (7/56) in the PureSperm group compared to 13.8% (9/65) in the Percoll group, ($p > 0.05$).

Conclusions. Despite using different density composition and volume, PureSperm demonstrated clinical effect comparable to that of Percoll in preparing sperm for intrauterine insemination.

Purification of sperm from the semen is one of the most important procedures in assisted reproductive procedures. Several methods have been used to select motile spermatozoa. Among them, Percoll (Amersham, Pharmacia Biotech AB, Sweden) density gradient centrifugation has been widely performed in many IVF centers since it was first introduced into the market.[1] Several reports have proved that using the Percoll solution can recover sperm with good fertilization ability.[2-4] However, some think the Percoll particles retained after sperm washing may act as tissue irritants when intrauterine insemination (IUI) is carried out.[5] Moreover, the possible deleterious effects of centrifugation with Percoll gradients on sperm longevity have also been raised.[6] In 1996, Svalander *et al.* suggested that the Percoll procedure be abandoned from human clinical use partly due to its content of higher endotoxin level.[7] Since then, several new products proclaimed with reduced endotoxin levels have been marketed as potential replacements for Percoll in density gradient semen preparation.[8,9] Of these, PureSperm (Nidacon, Göteborg, Sweden) was one of the most widely used products available on the market. Yet, in the past few years, there have continued to be doubts regarding this new product as an efficient substitute for the Percoll. Several published reports have compared their differences in regards to sperm recovery, motility, motion parameters.[10,11] However, none of these studies have really compared their clinical outcomes in IUI. The aim of this study was to compare the Percoll and PureSperm sperm preparation methods not only in respect to sperm recovery, percent motility, and motion parameters but also in their clinical outcomes. To our knowledge, this is the first paper that discusses the clinical outcome of IUI by the 2 different density gradient preparations.

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METHODS

From January 2002 to October 2002, women who received IUI in our hospital due to ovulation dysfunction were enrolled in this study. These women had previously taken clomiphene citrate for several cycles but were unable to conceive. Patients were selected on the basis of their agreement to join this study (expressed in a written consent). All patients were superovulated with the concurrent use of clomiphene citrate (Clomid, Shinogi, Taiwan) and recombinant follicle stimulating hormone (r-FSH, Puregon, Organon, Oss, Netherlands). Only patients with at least 2 follicles and measured at least 18 mm on the day of human chorionic gonadotropin (HCG, Pregnyl, Organon, Oss, Netherlands) injection were included. Patients with total follicle number more than 10 (high responders) were excluded from the study. Semen samples were collected, with 3-5 days of abstinence, and processed within 60 minutes of ejaculation. The specimen was randomly prepared by the gradient separation method with either PureSperm (n = 56) or Percoll (n = 65) as the separation medium.

In the Percoll preparation, solution was diluted with synthetic human tubal fluid (HTF) to 95% and 47.5%. The density gradient was prepared by layering 1.5 mL of 47.5% Percoll over equal amount of 95% Percoll into a 15 mL conical Falcon tube. Lastly, 0.5 mL of ejaculate was layered on the top. In the PureSperm preparation, density gradient was prepared by utilizing ready-to-use solutions of 80% and 40% PureSperm, respectively. The preparation method was the same as for Percoll preparation except in which only 0.5 mL of each concentration was used. Only the upper layer of sperm was taken for later usage.

Both prepared gradients were centrifuged at room temperature for 20 minutes at 300 g. The pellet was suspended in 5 mL HTF, centrifuged at 200 g for 10 minutes. The final pellet was re-suspended in 0.5 mL of fresh HTF and ready for intrauterine insemination by the author with a Genitor catheter (Laboratory C.C.D., Paris, France).

An aliquot of 6 μL of the final suspension was loaded into a 20-μm microcell slide and subjected to a computer-assisted semen analysis (CASA) under standard set-up parameters. The CASA model is a HTM-IVOS (Hamilton Thorne-Integrated visual optics system). All analyses were conducted at room temperature, with the stage of the CASA analyzer set at the ambient temperature (approximately 25 °C).

Sperm concentration, motile sperm recovery, percent motility, and motion parameters were all measured for each semen specimen before and after separation. The outcome of IUI was reported as the pregnancy rate per cycle. Only clinical pregnancy, which was defined as a gestation sac with visible heart beat on vaginal ultrasound, was calculated. The outcomes were presented as mean ± SD. Two-tailed 2 samples *t*-test was used for statistical analysis. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Totally, there were 56 couples in the PureSperm group and 65 couples in the Percoll group. The mean age of women in the PureSperm group was 31.6 ± 4.0 years, compared to 30.7 ± 3.8 years in the Percoll group ($p > 0.05$). The mean follicle numbers in the PureSperm group were 5.1 ± 3.2 follicles and 5.0 ± 3.4 follicles in the Percoll group ($p > 0.05$). The ejaculate volume in the PureSperm group was 2.7 ± 1.0 mL, compared to 2.5 ± 1.0 mL in the Percoll group ($p > 0.05$). The sperm concentration (10^6/mL) before preparation was 71.0 ± 38.9 × 10^6/mL in the PureSperm group, compared to 77.2 ± 44.7 × 10^6/mL in the Percoll group ($p > 0.05$). The total sperm count in the PureSperm group was 107.9 ± 72.3 × 10^6, compared to 123.5 ± 91.6 × 10^6 in the Percoll group ($p > 0.05$). The percent of motile sperm was 56.9 ± 12.5% in the PureSperm group, compared to 61.5 ± 14.3% in the Percoll group ($p > 0.05$). The percent of sperm with normal morphology in the PureSperm group was 32.8 ± 7.8%, compared to 33.6.2 ± 9.7% in the Percoll group ($p > 0.05$) (Table 1). Differences in these basic characteristics between the Percoll and PureSperm groups were statistically non-significant.

The sperm concentration (10^6/mL) after PureSperm preparation was 93.2 ± 81.3 compared to 37.1 ± 29.3 after Percoll preparation ($p < 0.0001$). The percent of motile sperm with the PureSperm method was 89.0 ± 8.3%, compared to 94.2 ± 3.5% with the Percoll method

Table 1. Basic characteristics in PureSperm and Percoll groups

	Puresperm (n = 56)	Percoll (n = 65)	*p* value
Mean age of women	31.6 ± 4.0	30.7 ± 3.8	NS*
Follicle Numbers (> 18 mm)	5.1 ± 3.2	5.0 ± 3.4	NS
Ejaculate volume (mL)	2.7 ± 1.0	2.5 ± 1.0	NS
Sperm concentration (10^6/mL)	71.0 ± 38.9	77.2 ± 44.7	NS
Total sperm count ($\times 10^6$)	107.9 ± 72.3	123.5 ± 91.6	NS
Percent of motile sperm (%)	56.9 ± 12.5	61.5 ± 14.3	NS
Percent of Normal sperm morphology (%)	32.8 ± 7.8	33.6 ± 9.7	NS

*NS = non-significant.

Table 2. Sperm characteristics after preparation in PureSperm and Percoll groups

	Puresperm	Percoll	*p* value
Sperm concentration ($\times 10^6$/mL)	93.2 ± 81.3	37.1 ± 29.3	< 0.0001
Percent of motile sperm (%)	89.0 ± 8.3	94.2 ± 3.5	0.0005
Percent of Sperm with progressive motility (%)	35.7 ± 10.3	33.2 ± 11.6	NS
VAP	77.2 ± 15.9	74.1 ± 19.2	NS
VSL	62.1 ± 13.8	59.8 ± 18.5	NS
VCL	132.4 ± 35.1	130.9 ± 38.5	NS
ALH	5.3 ± 1.5	5.4 ± 1.6	NS
BCF	26.1 ± 3.9	25.4 ± 4.7	NS
STR	79.7 ± 7.7	80.3 ± 8.8	NS
LIN	49.8 ± 10.3	49.0 ± 11.4	NS
Pregnancy rate (%)	12.5 (7/56)	13.8 (9/65)	NS

Two-tailed 2 sample t-test.
VAP = Path Velocity; VSL = Progressive velocity; VCL = Track speed; ALH = Amplitude of lateral head; BCF = Beat cross frequency; STR = straightness; LIN = Linearity.

($p = 0.0005$). There was no significant difference in the percent of sperm with progressive motility (35.7 ± 10.3% *vs.* 33.2 ± 11.6%). The parameters for sperm motion characteristics after CASA calculation also revealed no difference between the PureSperm and Percoll preparation methods. The clinical pregnancy rate was 12.5% (7/56) in the PureSperm group compared to 13.8% (9/65) in the Percoll group ($p > 0.05$) (Table 2).

DISCUSSION

The Percoll density gradient preparation of sperm for assisted reproductive technology (ART) has been widely used for the past decades because of its ease and cost-effectiveness. However, being PVP (polyvinyl pyrrolidone)-coated silica particles, Percoll has been reported to induce detrimental effects on the prepared sperm, and limiting its use in humans was suggested by some authors.[6] Even though there is no negative report about Percoll in human use thus far, Percoll has been found to contain a higher level of endotoxin.[7] The toxin were estimated 10 to 100 times the upper limit set by the Food and Drug Administration (FDA). Moreover, the endotoxin seemingly related to increased fragmentation of human embryos and thus reduced the pregnancy rates.[11,12] Although no incidence of human anomaly linked to the usage of Percoll has been found so far, some may deem it medically unfit to continue utilizing Percoll in human semen preparation. Despite this argument, however, there is yet to be universal agreement on the use of Percoll.

Subsequently, a silane-coated silica particle called PureSperm was proposed and is now widely used as a replacement of Percoll in density gradient centrifugation for sperm preparation. However, debates persisted for the past few years in regard to the efficacy of this new

substitute for Percoll.[9] Although some reports making comparison between Percoll and PureSperm density gradient methods have been published to confirm both of them have the similar results in semen preparation, there has been no formal report concerning the outcomes in IUI between them.[9,10,13]

In this study, the mean age of patients, the total number of follicles and the pre-preparation sperm quality were comparable between the 2 groups. Since 1 technician prepared the sperm for the same physician, who used one type of catheter for IUI, the resulting pregnancy rate can be closely correlated to the separation technique. In our study, the sperm concentration by PureSperm preparation was better than by Percoll preparation (93.2 $\pm$ 81.3 *vs.* 37.1 $\pm$ 29.3 $\times$ 10^6/mL, $p < 0.0001$). On the contrary, the percent of motile sperm by Percoll preparation was better than by PureSperm preparation (94.2 $\pm$ 3.5% *vs.* 89.0 $\pm$ 8.3%, $p = 0.0005$). This difference may be attributed to the variation of gradient concentration between the 2 groups. The Percoll group utilized dilutions of 95% and 47.5%, respectively. The PureSperm used prepared stock solutions of 80% and 40%, respectively. This difference in gradient density could enable the PureSperm group to recover more but a less than ideal quality of sperm into the final product than the Percoll group. Nonetheless, the percent of sperm with progressive motility was the same in 2 groups (35.7 $\pm$ 10.3% *vs.* 33.2 $\pm$ 11.6%, $p > 0.05$). We suppose both solutions should have the same ability and efficacy to obtain the best sperm.

Density gradient preparation has maintained its reputation in semen preparation for several decades. Since Percoll was deemed unsuitable by some for the human clinical use, it may be necessary to find a more appropriate substitute. Despite differences in density composition and volume, PureSperm, a silane-coated silica, seemed in our study to emulate the role of Percoll in sperm preparation but without the proposed human hazard in our study. Further prospective randomized studies are needed to determine the most appropriate concentrations for the 2-layer gradient separation.

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