Comparison of Sperm Motility and Sperm Recovery Rate After 40%/80% and 50%/100% Discontinuous Percoll Gradients Preparation

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Abstract : *Two discontinuous Percoll gradients (40%/80% and 50%/100% Percoll gradients) were assessed for efficacy of selection of motile sperm from 55 normozoospermic semen samples. Each semen sample was assessed for basic semen parameters before and after preparation by both techniques. The preparation of sperm on both two step Percoll gradients allowed about one third of the progressively motile sperm to be recovered. Sperms prepared by the 50%/100% Percoll gradients had higher percent progressive motility and percent normal morphology but there were no statistically significant differences between both techniques. Sperm preparation by 40%/80% Percoll gradients seems to be better than 50%/100% Percoll gradients because total sperm number, total motile sperm number, and total sperm recovery rate after preparation by 40%/80% Percoll gradients were greater than 50%/100% Percoll gradients significantly. (Thai J Obstet Gynaecol 1995;7:25-32.)*

Key Words : Discontinuous Percoll gradients preparation/ Normal semens

Short Title : Discontinuous Percoll gradients for normal semens

Percoll, a medium composed of colloidal silica particles coated with polyvinyl-pyrrolidone, has been found to improve the isolation of motile spermatozoa, generally free of contamination from other seminal constituents. Percoll gradients was found to return a high proportion of functional sperm. The buoyant density apparently protects the sperm from the trauma of centrifugation.[1] The use of Percoll to create buoyant density gradients in the preparation of human was firstly described using continuous self-generating gradients produced by high-speed centrifugation.[2] The production of continuous Percoll gradients is time-consuming and requires a high-speed centrifuge; therefore, a two-step discontinuous gradient which is more simple to prepare was intro duced.[3] Subsequently,

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discontinuous Percoll gradients has been widely used in many laboratories for separating human sperm. This method is suitable for sperm preparation because it has the advantage of being a comparatively rapid and effective method.

The two-layer Percoll density gradients concentration that have been used and recommended in many reports are 40%/80% Percoll gradients[4-7] and 50%/100% Percoll gradients.[8,9] No comparative study of sperm morphology, sperm motility and sperm recovery rate after both sperm preparation techniques has been reported. This study was conducted to compare sperm morphology, sperm motility and the recovery of motile sperm form normozoospermic semen samples.

Materials and Methods

Semen Collection

This study was based upon 55 samples collected from the 45 volunteers, all of whom possessed normal semen profiles according to WHO guideline.[4] The semen samples were obtained by masturbation after at least 48 hours of abstinence and collected into sterile plastic containers. After liquefaction, samples were assessed for conventional parameters (volume, morphology, motility, sperm concentration, and round cell concentration) using the procedures laid down by the World Health Organization.[4] The remainder was divided into equal portions and then recovered by the 40%/80% and 50%/100% Percoll centrifugation separation technique.

Percoll Gradient Preparation

Isotonic Percoll was created by supplementing 10 ml of 10 x concentrated medium 199 (Flow Laboratories, Irvine, UK) with 300 mg bovine serum albumin (BSA), 3 mg sodium pyruvate and 0.37 ml sodium lactate syrup, and adding 90 ml of Percoll (Pharmacia, Uppsala, Sweden). This preparation was designated 100% percoll[10] and subsequently diluted to 40%, 50%, 80% with medium BWW.[11]

40%/80% Percoll Gradient Preparation

For each semen sample, a two-layer Percoll discontinuous gradient comprising 3 ml 80% Percoll overlaid with a further 3 ml 40% Percoll was prepared in a 15-ml(17x120 mm) sterile conical test tube.

50%/100% Percoll Gradient Preparation

For each semen sample, a two-layer Percoll discontinuous gradient comprising 3 ml 100% Percoll (isotonic Percoll) overlaid with a further 3 ml 50% Percoll was prepared in a 15-ml(17x120 mm) sterile conical test tube.

Each equal portion of remaining semen samples after initial assessment was carefully layered on the top of each prewarmed (37° C) gradients.

Centrifugation and Collection of Sperm from Gradients

The Percoll gradients were centrifuged at room temperature in a swinging bucket rotor at 500g for 20 minutes and the seminal plasma was then discarded and the cells collected into two fractions derived from (1) the base of the 100% Percoll fraction (100% fraction), (2) the base of the 80% Percoll fraction (80% fraction). The cells from each fraction were resuspended in 5 ml of BWW, centrifuged at 500g for 5 minutes and finally resuspended in approximately 0.5 ml BWW.

Each sperm suspension from 80% fractions and 100% fractions were assessed for percent normal morphology, percent progressive motility, total recovered sperm number, total recovered motile sperm number.

The total sperm recovery rate was calculated as:

(Final recovered volume x Final sperm concentration) / (Initial semen volume x Initial sperm concentration)

The sperm motility recovery rate was calculated as:

(Final recovered volume x Final sperm concentration x Final motility) / (Initial semen volume x Initial sperm concentration x Initial motility)

And expressed as a percentage. The sperm motility recovery rate reflected the proportion of motile spermatozoa from the initial sample that were effectively recovered by this preparation method.

Statistical Analysis

Results are experssed as the mean ± standard error of means (SEM). The significance of differences between mean results of percent motile sperm, percent normal morphology, total sperm recovery rate, and motile sperm recovery rate from both techniques were compared by paired t-test. A probability of 0.05 was assumed to denote a significant difference. Statistical analyses were carried out using the STATVIEW programme on an Apple Mcintosh SE.

Results

The population of donors used in this study exhibited the mean semen characteristics shown in Table 1.

Comparison of the semen parameters measured before and after sperm preparation by both discontinuous Percoll gradients, the per cent progressive motility, per cent normal morphology after sperm preparation by both techniques increased significantly ($p<0.001$), but total sperm number, total motile sperm number and total round cell number reduced significantly ($p<0.001$). The preparation of sperm on both two-step Percoll gradients allowed about one-third of the progressively motile sperm to be recovered.

Sperms prepared by the 50%/100% Percoll gradients had better progressive motility and more normal morphology but the difference's were not statistically significant.

Table 1 *Semen parameters before discontinuous Percoll gradient preparation*

Semen Parameters	Mean ± SEM
Volume (ml)	3.3 ± 0.2
Normal Morphology (%)	36.7 ± 2.4
Progressive Motility (%)	69.1 ± 1.4
Semen Concentration (x 10^6/ml)	116.3 ± 11.2
Round Cell* Concentration (x 10^6/ml)	1.9 ± 0.3

*Round cells include polygonal epithelial cells from the urethral tract, spermatogenic cells, and leucocytes. As a general guide, a normal ejaculate should not contain more than 5 x 10^6 round cells/ml.

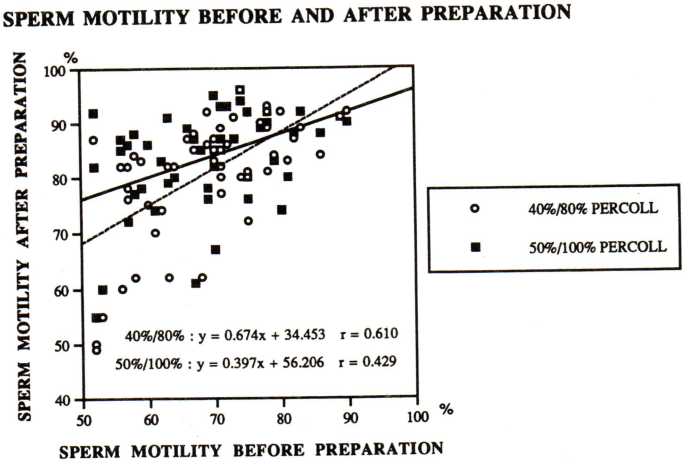


Fig. 1 Comparison of the percent progressive motility of sperm before and after sperm preparation by 40%/80% (open circles) and 50%/100% (solid squares) discontinuous Percoll gradients preparations. The correlation between percent progressive motility before and after sperm preparation by 40%/80% (broken line) and 50%/100% (solid line) Percoll gradients were demonstrated.

Figure 1 shows the per cent progressive motility of sperm before and after sperm preparation on both Percoll gradients. Linear regression analysis revealed a direct positive relationship between the percent progressive motility of sperm before and after sperm preparation by both techniques.

The total motile sperm recovery rate from final Percoll fraction was calculated based on the total number of sperm in the semen and the percent progressive motile sperm found in the initial semen and the in each fraction as in the formula shown above. The percentage of sperm recovered varied widely from person to person. In this study, total sperm number and total motile sperm number after prepared by 50%/100% Percoll gradients were less than 40%/

Table 2 *Comparison of the semen parameters measured before and after sperm preparation by two discontinuous Percoll gradients*

Semen Parameters	Before Preparation	After Preparation 40%/80% Percoll	50%/100% Percoll	
Normal Morphology (%)	36.7 ± 2.4	78.5 ± 2.4	81.6 ± 3.5	NS
Progressive Motility (%)	69.0 ± 1.4	80.8 ± 1.5	83.5 ± 1.2	NS
Total Sperm Number (x10^6)	96.2 ± 10.3	47.6 ± 5.0	37.8 ± 3.8*	
Total Motile Sperm Number (x10^6)	65.5 ± 6.8	38.4 ± 4.1	31.9 ± 3.5*	
Total Round Cell Number (x10^6)	0.95 ± 0.15	0.03 ± 0.19	0.03 ± 0.02	NS
Total Sperm Recovery Rate (%)		32.8 ± 4.8	23.9 ± 1.7*	
Motile Sperm Recovery Rate (%)		37.6 ± 5.4	28.9 ± 2.3	NS

* $p<0.05$, NS : No Statistical significance

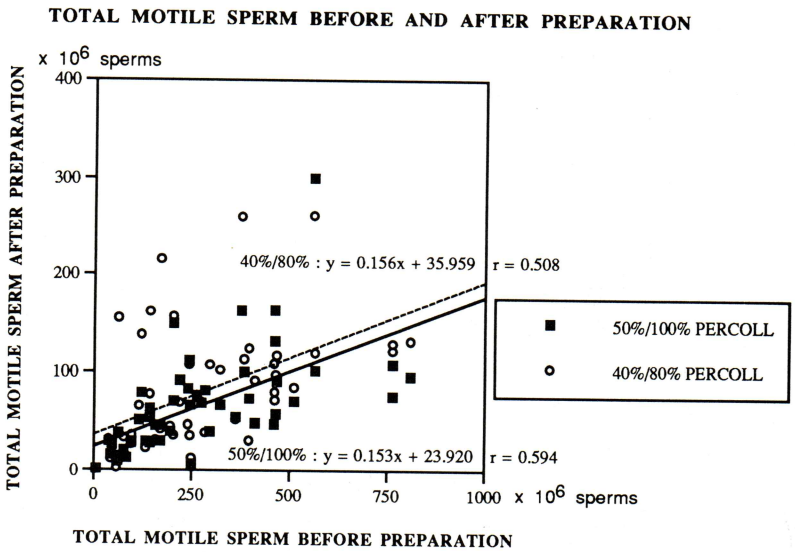


Fig. 2 Comparison of the total motile sperm number before and after sperm preparation by 40%/80% (open circles) and 50%/100% (solid squares) discontinuous Percoll gradients preparations. The correlation between total motile sperm number before and after sperm preparation by 40%/80% (broken line) and 50%/100% (solid line) Percoll gradients were demenstrated.

80% Percoll gradients significantly. Linear regression analysis revealed a direct positive relationship between the total motile sperm number before and after sperm preparation by both techniques. Figure 2 shows the total motile sperm number before and after sperm preparation on both Percoll gradients.

The total sperm number, total motile sperm number, and total sperm recovery rate were significantly hig-

her, when 40%/80% Percoll gradients were used. The motile sperm recovery rate from the 50%/100% Percoll gradients and the 40%/80% Percoll gradients were similar (p=0.0577), but considerably fewer motile sperms were recovered from 50%/100% Percoll gradients.

Discussion

A variety of methods have been used to separate motile sperm from semen, and each has both desirable and undesirable attributes. The ideal separation technique should: 1) be rapid, simple, and inexpensive, 2) recover most of motile sperms in the specimen. 3) result in no damage or physiologic alteration of the separated sperm, 4) remove dead sperm andother cells, including microorganisms, 5) remove toxic and bioactive substances, 6) process large volumes of semen, and 7) allow the final volume of the sperm suspension to be controlled. Depending on the application, these criteria differ in their importance.[7]

The Percoll gradient preparation has been demonstrated to yield good sperm recovery and final sperm motility[3,9,10,12-14] It is a very simple and convenient sperm preparation technique for selecting sperm with good fertilizing ability as evaluated by laboratory tests, having a longer survival[15], enhanced motility[9,15-17] and superior nuclear maturing[18]. The volume of the sperm suspensions after Percoll separation could be adjusted because both methods involve resuspension of a pellet of the desired volume. Therefore, discontinuous Percoll gradients have been widely used in many laboratories for separating human sperm and it seems to be the best method for sperm preparation.

In this study, the comparison of the efficacy of motile sperm selection by 50%/100% Percoll with that by 40%/80% was chosen because no comparative study of sperm morphology, sperm motility and sperm recovery rate after both sperm preparation techniques has been reported. The preparation of sperm on both two-step Percoll gradients allowed about one-third of the progressively motile sperm to be recovered. Both gradients gave a high yield of motile sperms. The progressive motility, normal morphology and the motile sperm recovery rate after preparation by both methods did not differ significantly. The statistically significant improvement of percent progressive motility indicated the validity of sperm selection by both methods of discontinuous Percoll gradients. The final samples were found to be fairly free of round cells (germinal cells and leucocytes).

Both Percoll gradients were very successful in eliminating morphologically abnormal sperm. In this study, the result was similar to the previous report[19], but the increase in normal forms was not shown in some reports.[18,20] These differences can be explained either by the lack of uniformity in the Percoll gradient

preparation technique, or by the classification used to describe morphology.

The results of this investigation also indicated a direct relationship between the percent progressive motility and total motile sperm number before and after preparation on discontinuous Percoll gradients. The total sperm number, total motile sperm number, and total sperm recovery rate were significantly higher, when 40%/80% Percoll gradients were used. The motile sperm recovery rate from both discontinuous Percoll gradients in this study was lower than previous reports.[10,12] Although the total motile sperms recovered from 40%/80% Percoll gradients had considerably higher number than 50%/100% Percoll gradients, the motile sperm recovery rate in the two fractions were not significantly different (p=0.0577)

Sperm preparation by 40%/80% Percoll gradients seems to be better than 50%/100% Percoll gradients because total sperm number and total motile sperm number after prepared by 40%/80% Percoll gradients were greater than 50%/100% Percoll gradients significantly. The sperm preparation by 40%/80% Percoll gradients warrant further investigation as a density gradient medium for the selection of motile human spermatozoa from poor semen samples, especially, oligozoospermia because it yielded higher total sperm number and total sperm recovery rate. However, centrifugation on both two-step Pecoll gradients are convenient and efficient ways to prepare normal morphology and motile sperm for assisted reproduction techniques from normo-zoospermic semen samples.

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