Original Articles/นิพนธ์ต้นฉบับ

# Changes in Sperm Quality after Two Different Techniques of Sperm Preparation

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## Abstract

**Introduction:** Sperm preparation is an important step in Assisted Reproductive Technology (ART). The two commonly used sperm preparation techniques in ART laboratory are the swim-up technique and density gradient centrifugation. The objective of this study was to compare the sperm qualities after sperm preparation between the swim-up technique and density gradient centrifugation.

**Materials & Methods:** A total of 25 semen samples were obtained from men who attended infertility clinic, Department of OB-GYN, Faculty of Medicine Ramathibodi Hospital, Mahidol University during February 2012 and March 2012. Semen samples were divided into two equal aliquots for sperm preparation by using the swim-up technique and density gradient centrifugation. Sperm qualities were evaluated as the sperm concentration, total motile sperm, the percentage of sperm motility, and the percentage of normal sperm morphology. The comparison between groups was done by paired t-test with significant level at $\alpha < 0.05$.

**Results:** The swim-up technique gave a better result in sperm motility and sperm normal morphology than the density gradient centrifugation technique. Density gradient technique resulted in higher sperm concentration and total motile sperm.

**Conclusions:** The swim-up technique should be a more suitable technique for sperm preparation in ART laboratories because it is a very simple, fast, effective, and less expensive.

**Keywords:** Sperm preparation technique / Swim-up technique / Density gradient centrifugation

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## Introduction

Various techniques of sperm preparation in ART aim to improve qualities sperm and separate all of the unwanted cells - seminal plasma, bacteria, and cell debris - that may reduce the fertilizing ability of spermatozoa[1]. These techniques can be divided into four types: migration techniques, density gradient separation, filtration techniques, and washing[2]. Although many sperm preparation techniques are available, the swim-up and density gradient centrifugation are the two common techniques used for sperm preparation. Briefly, the swim-up technique allows motile sperm to swim in the culture medium, whereas, density gradient centrifugation selects spermatozoa according to their density. Sperm preparation is considered to be an important step for treatment of infertility couples wanting to receive treatment such as intrauterine insemination (IUI), *in vitro* fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI)[3]. The objective of this study is to compare the sperm qualities which included sperm concentration, total motile sperm, percentage of sperm motility, and percentage of normal sperm morphology between two techniques of sperm preparation i.e. swim-up technique and density gradient centrifugation.

## Materials and Methods

### Semen analysis

A total of 25 normozoospermic semen samples were obtained from men who attended infertility clinic, Department of OB-GYN, Faculty of Medicine, Ramathibodi Hospital during February 2012 and March 2012. The present study has been approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects, the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand. (Document no. MURA2012/37 February 7, 2012.) Routine semen analysis was performed according to World Health Organization (WHO) guidelines[4]. Each ejaculate was split into two equal aliquots and prepared for swim-up technique and density gradient centrifugation.

### Swim-up technique

1 ml of semen aliquot was transferred to a 15 ml conical centrifuge tube, and then mixed gently with 1 ml of Ferticult flushing medium (FertiPro, N.V., Beernem, Belgium). The semen aliquot mix was then centrifuged at 300 g for five minutes, and the supernatant was discarded, leaving the sperm pellets in the conical centrifuge tube. 0.5 ml of Ferticult flushing medium was gently layered onto the pellets and the conical centrifuge was positioned at an angle of 45° for 45 minutes in 37°C[5]. The supernatant at a depth of 0.5 ml was then removed for evaluation.

### Density gradient centrifugation

Sil-Select Plus(tm) 40/80 (FertiPro, N.V., Beernem, Belgium) was prepared. By performing density gradient centrifugation, the lower layer was 1 ml of Sil-Select Plus™ 80 which was over layered by 1 ml of Sil-Select Plus™ 40. This two layer gradients is stable for up to two hours. 1 ml of semen aliquot was then gently placed on top of the two layers. The semen aliquot mix was then centrifuged at 350 g for seven minutes. The supernatant was removed and discarded, and the pellets were transferred to another new conical centrifuge tube and mixed gently with 1 ml of Ferticult flushing medium and centrifuged at 300 g for five minutes. This step of discarding the supernatant, mixing 1 ml of Ferticult flushing medium, and centrifuging it at 300 g for five minutes was performed for a second time. Finally, 0.5 ml of Ferticult flushing medium was gently layered onto the pellets and mixed well[5]. The uppermost layer to a depth of 0.5 ml is then removed for evaluation.

### Statistical analysis

The results obtained during the experiment were reported by the comparisons of quantitative variables

which were done by paired t- test. The level of significance was set at $\alpha < 0.05$. Data were analyzed using Computer program SPSS for Microsoft Windows version 18 (SPSS Inc., Chicago, IL, USA), license from Mahidol University.

## Results

Before sperm preparation, sperm qualities of all semen under study were within normal range by WHO criteria (Table 1). Reference values for normal semen quality in man compatible with normal fertility according to WHO (2010) were as follows; sperm concentration ≥15 x106/ml, percentage of sperm motility ≥40%, and percentage of normal sperm morphology ≥4%.

After sperm preparation, sperm qualities were improved in both techniques. Swim-up technique gave a better result in the percentage of motility in comparison with density gradient centrifugation (98.30±1.79% VS 94.77±1.79%, respectively) (p<0.001). Swim-up technique was also resulted in significantly higher percentage of normal sperm morphology than density gradient centrifugation (15.44±2.00% VS 11.52±2.00%, respectively) (p<0.001). Sperm concentration decreased from 43.80±7.42x$10^6$/ml before sperm preparation to 37.18±9.13x$10^6$/ml after swim-up preparation (p <0.001). On the contrary, sperm concentration after density gradient centrifugation increased with no significant difference. Sperm concentration after preparation was also significantly difference between two techniques. Total motile sperm after sperm preparation significantly decreased in both techniques, significantly more in swim-up technique than density gradient centrifugation (18.28±4.56x$10^6$ VS 21.34±7.07 x$10^6$, respectively) (p <0.001).

## Discussion

The important of sperm preparation in ART is to improve the quality of the sperm. Many techniques of sperm preparation gave different results in sperm concentration, total motile sperm, sperm motility, and sperm normal morphology. These techniques compared of at least four techniques i.e. migration techniques, density gradient separation, filtration techniques, and washing[2,6]. Each technique has its own strength. Migration is mainly focused on “swim-up” techniques which can select highly motile sperm with normal morphologically sperm. Density gradient

**Table 1**

| Semen analysis parameters | (1) Before Mean (S.D.) (95% CI) | (2) Swim-up Mean (S.D.) (95% CI) | (3) Density gradient centrifugation Mean (S.D.) (95% CI) | (1) & (2) | | (1) & (3) | | (2) & (3) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | t | p-value | t | p-value | t | p-value |
| Concentration ($10^6$/ml) | 43.80 (7.42) (40.74, 46.86) | 37.18 (9.13) (33.41, 40.95) | 44.95 (14.57) (38.93, 50.97) | 3.44 | <0.01 | -0.41 | 0.686 | -4.89 | <0.001 |
| Total motile sperm ($10^6$) | 70.77 (16.15) (64.11, 77.43) | 18.28 (4.56) (16.39, 20.16) | 21.34 (7.07) (18.42, 24.26) | 19.50 | <0.001 | 20.81 | <0.001 | -4.17 | <0.001 |
| Motility (%) | 68.90 (6.09) (66.39, 71.42) | 98.30 (1.79) (97.56, 99.04) | 94.77 (1.79) (94.03, 95.51) | -29.22 | <0.001 | -24.52 | <0.001 | 14.49 | <0.001 |
| Morphology (%) | 17.16 (2.33) (16.19, 18.13) | 15.44 (2.00) (14.61, 16.27) | 11.52 (2.40) (10.53, 12.51) | 4.62 | <0.001 | 9.98 | <0.001 | 11.33 | <0.001 |

separation classifies sperm according to density using commercial gradient materials such as Nycodenz® (Nyegaard & Co., Oslo, Norway), Ficoll® (Pharmacia, Quebec, Canada), IxaPrep® (MediCut, Copenhagen, Denmark), Sil-Select Plus™ (FertiPro, N.V., Beernem, Belgium), SpermGrad™ (Vitrolife, Gothenburg, Sweden), PureSperm® (NidaCon Laboratories AB, Gothenburg, Sweden) or Isolate® (Irvine Scientific, Santa Anam CA,USA)[7-9]. Filtration is performed by many kinds of designed filter such as glass wool column filtration, glass beads, sephadex columns, and transmembrane migration, in order to separate the good quality sperm. These columns are expensive and not very common[2]. Washing is mainly focused on centrifugation steps which only used in patients with oligozoospermia[10].

Swim-up technique and density gradient centrifugation are two common techniques used in ART. In our study, Sil-Select Plus™ (FertiPro, N.V., Beernem, Belgium) was a medium that were used while performing density gradient centrifugation technique because of its cost and availability. Previous study indicated that it gave a lowest percentage of sperm DNA damage[11].

The important parameters that indicate the success rate of ART are the percentage of sperm motility and the percentage of sperm normal morphology[12-15]. Our study showed that swim-up technique gave better results than density gradient centrifugation in sperm motility and sperm normal morphology. Density gradient centrifugation gave better semen qualities in sperm concentration and total motile sperm.

Swim-up technique is a very simple, fast, effective, and less expensive sperm preparation technique. Density gradient centrifugation is more expensive due to the cost of commercial gradient media. In conclusion, swim-up techniques may be more suitable than density gradient centrifugation in standard laboratories in developing countries.

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