A Comparison of Swim-up and Simple Swim-up Methods for the Extraction of High Motility Sperm

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Abstract : **The objective of this study was to compare two methods for the extraction of high motility sperm, the swim-up and simple swim-up techniques. The results were comparable in concentration, motility and morphology.**

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ได้ศึกษาเปรียบเทียบการเตรียมเชื้ออสุจิด้วยวิธีการ swim-up และ simple swim-up technique โดยศึกษาการเคลื่อนไหวและจำนวนของเชื้ออสุจิที่มีรูปทรงปกติ ผลการศึกษาพบว่าจำนวนเชื้ออสุจิที่ได้ภายหลังการเตรียมด้วยวิธีทั้งสองไม่มีความแตกต่างกันทางสถิติ ในด้านจำนวนเชื้ออสุจิที่มีรูปทรงปกติและการเคลื่อนไหวของเชื้ออสุจิ โดยเปรียบเทียบการเคลื่อนไหวทั้ง 4 ระดับ

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INTRODUCTION

Semen preparation is an important step in the new assisted reproductive techniques. Thus, several sperm preparation techniques have been introduced to improve sperm quality[1]. Separation of spermatozoa with high rates of recovery and motility using different techniques have clinical applications,[2-5] not only for in vitro fertilization (IVF) and gamete intrafallopian transfer (GIFT), but also for intrauterine insemination (IUI). Each technique varies in complexity and length, purity of spermatozoa recovered and adverse effects of the technique on the spermatozoa. This study reports the evaluation of sperm separation procedures by using the swim-up and simple swim-up methods.

MATERIALS AND METHODS

Semen samples were collected from 51 men with suspected infertility attending the Infertility Clinic, Siriraj Hospital, which have more than 20 million sperms/ml. The ejaculates were collected by masturbation after 72 hours of sexual abstinence. Analyses of the samples, following the recommendations of the World Health Organization[6] were carried out. The samples were collected in a sterile container and allowed to liquefy for 30 minutes at 37°C and analyzed for sperm volume, counting number, motility and morphology. Each semen sample was processed by two different methods; and the results of sperm count, motility, and morphology were compared for each mothod.

1. The swim-up technique was as follows: a 0.5 ml. volume of liquefied semen was mixed with 2 ml. Ham's F 10, and the suspension was centrifuged once at 1500 rpm for 10 minutes. The supernatant was removed. The sperm pellet then was resuspended in 0.5 ml. medium and new 0.5 ml. medium was gently layered on top of the suspension. The tube was incubated at 37°C , in a 5% CO_2 in air environment for 60 minutes. The top of the medium was collected for analysis.

2. The simple swim-up technique was as follows: a 0.5 ml. volume of liquefied semen was placed at the bottom of a sterile tube and 0.5 ml. medium was gently layered onto the semen sample, followed by incubation in the same condition as described in swim-up technique.

Data Analyses

The results were analyzed using Kruskal – Wallis 1 way ANOVA and Mann – Whitney U Wilcoxon rank test.

RESULTS

The results (table 1) showed that progressive motility and normal morphology were significantly increased, compared with fresh semen; but there was no significant difference in both separations. The slow progressive motility, static motility, and no motility were decreased, compared with the fresh semen; but there was no significant difference in either separation. The sperm count after separation showed no significant difference.

Table 1. Mean (± SD) semen parameters before and after separation.

	Fresh sample	Swim-up	Simple swim-up
Concentration ($x10^6$/ml)	82.13 (45.37)	25.49 (23.51)	20.77 (20.38)
Rapid linear progression (%)	37.49 (12.51)	87.18 (10.61)	83.71 (12.96)
Slow or non-linear progression (%)	15.16 (7.97)	5.55 (7.10)	7.69 (8.41)
Non-progressive motility (%)	8.78 (3.47)	2.86 (2.86)	2.86 (3.03)
Immotile (%)	37.47 (10.63)	4.41 (4.00)	5.76 (5.58)
Morphology (% normal forms)	58.0 (12.05)	76.71 (10.89)	77.16 (12.00)

DISCUSSION

The purpose of this study was to evaluate some principle semen characteristics of men attending the clinic, comparing a widely used method for the extraction of high motility sperm from the original sample, the swim-up or washed method, with a less frequently used method, the simple swim-up method. Many methods have been used for preparing spermatozoa from normal and pathological samples for IVF, GIFT or IUI with good results. One of these is swim-up or washed procedure[7]. This technique removes seminal plasma and debris from the preparation and decreases the viscosity in the sample so forward progression of the sperm increases. The simple swim-up or layering method[3] is another method in which spermatozoa are allowed to swim upward into tissue culture medium.

We showed that both techniques used here were efficient in extracting highly motile sperm from the semen samples which had more than 20 million spermatozoa per milliliter. Keck C and colleagues found that different techniques of sperm preparation seem to be equally effective in preparing a highly concentrated sperm fraction with progressively motile, morphologically normal sperm[8]. In this study, we found the comparable motility in both methods.The procedure used in preparing the spermatozoa by the simple swim-up method was easier and lesser time consuming. The swim-up method includes a centrifugation step which has been shown to increase motility but at the expense of sublethal damage,[9] and the sperms so produced perform badly in the zona-free hamster oocyte penetration test[10-13]. This effect is thought to be due to the production by spermatozoa of reactive oxygen species (ROS) as a result of centrifugation. These mean that the simple swim-up method may be better than the swim-up method.

CONCLUSION

The swim-up and the simple swim-up methods for the extraction of high motility sperm were comparable in producing sperm of equal concentration, motility and morphology.

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