Comparison of Sperm Motility between The Swim-up and Swim-down Methods

Singpetch Suksompong, M.D.*
Somboon Kunathikom, M.D.*
Orawan Makemaharn, M.Sc.*
Pitak Laokirkkiat, M.D.*

Abstract : **The objective of this study was to compare the two methods for extraction of high motility sperm, the swim-up and swim-down techniques. The result showed that both methods produced greater sperm motility than the original semen. The motility of the sperm was significantly lower in the swim-down method compared with the swim-up method (65.5 ± 11.2% vs 76.5 ± 10.1%; p < 0.01).**

เรื่องย่อ : **การเปรียบเทียบเชื้ออสุจิภายหลังการเตรียมโดยวิธี Swim-up และ Swim-down**

สิงห์เพ็ชร สุขสมปอง พ.บ.*, สมบูรณ์ คุณาธิคม พ.บ.*, อรวรรณ เมฆมหรรณ วท.ม.*, พิทักษ์ เลาห์เกริกเกียรติ พ.บ.*

**ภาควิชาสูติศาสตร์-นรีเวชวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพมหานคร 10700.*

สารศิริราช 2001; 53: 296-299.

ได้ศึกษาเปรียบเทียบการเตรียมเชื้ออสุจิด้วยวิธี swim-up และ swim-down techniques โดยศึกษาการเคลื่อนไหวของเชื้ออสุจิ ผลการศึกษาพบว่าเชื้ออสุจิที่ได้ภายหลังการเตรียมด้วยวิธีทั้งสองเคลื่อนไหวดีกว่าเชื้ออสุจิก่อนการเตรียม แต่การเตรียมด้วยวิธี swim-up มีเชื้ออสุจิที่เคลื่อนไหวมากกว่าการเตรียมด้วยวิธี swim-down อย่างมีนัยสำคัญทางสถิติ (76.5 + 10.1% เทียบกับ 65.5 + 11.2%; P <0.01)

INTRODUCTION

Separation of motile spermatozoa from the entire sperm population by non-centrifugation and density gradient centrifugation are routine procedures for intrauterine insemination (IUI) and in-vitro fertilization (IVF).[1-3] Most techniques of non centrifugation are based on sperm self-migration into a sperm-free culture medium which is layered either upon or beneath a stock of either unwashed or washed spermatozoa. Though some previous studies have indicated the superiority of the swim-down technique with regard to its efficacy[4-7], the use of sperm swim-up for clinical purposes is much more prevalent.[8] This technique involves centrifugation, which may adversely affect sperm function.[9-12] This study attempted to evaluate the difference in sperm motility between the swim-up and the swim-down methods.

**Department of Obstetrics & Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700.*

MATERIALS AND METHODS

Semen samples were collected from 30 men with suspected infertility attending the Infertility Clinic, Siriraj Hospital, who had normal semen samples according to the recommendation of the World Health Organization.[13] The ejaculates were collected by masturbation after 72 hours of sexual abstinence. The samples were collected in a sterile container and allowed to liquefy for 30 minutes at 37°C. Each semen sample was processed by the swim-up or swim-down technique. After the processes, the samples were coded and sent for analysis of sperm motility (grade a + b motility) by one of our investigators who did not know which technique had been applied to each sample.

The swim-up technique was as follow: a 0.5 ml volume of liquefied semen was diluted with 1 ml 10 % HSA (human serum albumin) in HTF (human tubular fluid) in a 10-ml tube and centrifuged at 1,600 rpm (200g) for 10 minutes. The supernatant was removed. A further 1 ml of medium was gently layered on top of the pellet. The tube was incubated at 37°C, in 5% CO_2 in air for 30 minutes. The top of the medium was collected for analysis.

The swim-down technique was as follow: a 0.5 ml volume of liquefied semen was diluted with 1 ml normal saline in a 10-ml tube and allowed to stand at room temperature for 5 minutes until the debris were settled to the bottom of the tube. Therefore, 0.5 ml of sperm-containing supernatant was gently aspirated and layered on top of 1 ml of 10% HSA in HTF in another 10-ml tube. The bottom of the medium was collected for analysis after 10 minutes.

Data Analyses

The results were decoded and analyzed by SPSS for MS WINDOWS using Paired t-test.

RESULTS

Table 1 shows the ages of the couples. Mean patient age plus or minus standard deviation was 32.9 ± 4.7 years (range 25-46 years) for males, and 30.5 ± 3.1 years (range 25-38 years) for females.

Table 2 shows the semen parameters analyzed following the recommendation of the World Health Organization.

The swim-down technique increased sperm motility from 58.1 ± 8.4% to 65.5 ± 11.2% which was statistically significant (Table 3). The swim-up technique produced a sample of which 76.5 ± 10.1% of the spermatozoa were motile which was significantly greater than that yielded from the swim-down technique (Table 4).

Table 1. Ages of the couples.

Ages (years)	Number	
	Male (No.)	Female (No.)
25-29	8	10
30-34	15	16
35-39	3	4
40-44	3	-
45-49	1	-
Total	30	30

Table 2. Semen parameters of the samples.

Parameters	Mean ± S.D.	Min. – Max.
Volume (ml)	2.7 ± 0.3	2.0 - 3.4
pH	7.5 ± 0.1	7.4 - 7.8
Sperm concentration (x 10^6 /ml)	74.4 ± 21.0	42 - 120
Total sperm count (x 10^6)	194.3 ± 54.6	96 - 290
Motility (%)	58.1 ± 8.4	44 - 73
Normal morphology (%)	38.2 ± 5.9	32 - 62
Viability (%)	79.0 ± 2.8	75 - 84
White blood cell count (x 10^6 /ml)	0.4 ± 0.2	0.0 - 0.7

Table 3. Percentages (mean + S.D.) of motile spermatozoa before and after preparation by the swim-down method.

	Sperm motility (%)	P value*
Swim-down	65.5 ± 11.2	< 0.01
Original semen	58.1 ± 8.4	

*Paired t-test

Table 4. Percentages (mean + S.D.) of motile sperms after preparation by the swim-up and swim-down methods.

	Sperm motility (%)	P value*
Swim-up	76.5 ± 10.1	< 0.01
Swim-down	65.5 ± 11.2	

*Paired t-test

DISCUSSION

The main purpose of sperm preparation for IUI and IVF is to select high quality motile sperm from the ejaculate. The motility of the sperm in the final preparation has been suggested to be important in the fertilization rate of human IVF.[14,15] In the present study, both the swim-up and swim-down techniques extracted highly motile sperm. Some studies have shown that the swim-down method is superior to the swim-up procedure during sperm separation by self-migration[4-7] and has been confirmed by Makler A and colleagues in their study on the effect of gravitation on human spermatozoa.[16] In this study, we found that the swim-up method produced more motile sperm than the swim-down technique. The difference might be due to some differences in the detail of the swim-down technique. The time used in the swim-up procedure was longer which might allow more motile spermatozoa to accumulate. In the swim-down technique, the time used could not be prolonged because the immotile sperm would sink to the bottom of the tube.[15] A newer study has shown

that the swim-up preparation resulted in a higher pregnancy rate than the swim-down technique after intrauterine insemination.[1] Although the sperm prepared by the swim-down technique had a lower motility rate than the swim-up technique, it could still extract highly motile sperm from the original semen. The procedure used in the swim-down technique is simple and needs less equipment, and can be performed in 15 minutes. This procedure may be used in rural hospitals where the swim-up technique can not be performed.

CONCLUSION

The swim-up method was better than the swim-down method in producing a greater percentage of motile sperm in the sample after preparation.

References

1. Carrell DT, Kuneck PH, Peterson CM, Hatasaka HH, Jones KP, et al. A randomized, prospective analysis of five sperm preparation techniques before intrauterine insemination of husband sperm. Fertil Steril 1998; **69:** 122-126.
2. Allen NC, Herbert III CM, Maxon WS, Rogers BJ, Diamond MP, et al. Intrauterine insemination: a critical review. Fertil Steril 1985; **44:** 569-580.
3. Mortimer D. Sperm preparation techniques and iatrogenic failures of in-vitro fertilization. Hum Reprod 1991; **6:** 173-176.
4. Kaneko S, Sato H, Kobayashi T, Iizuka R. Funnel procedure to separate human sperm with good motility. Arch Androl 1987; **19:** 107-113.
5. Kobayashi T, Kaneko S, Hara I, Aoki R, Ohno T, et al. Swim-down separation of progressively motile sperm from poor quality human semen by the modified funnel procedure. Andrologia 1991; **23:** 17-20.
6. Gonzales GF, Pella RE. Swim-down: a rapid and easy method to select motile spermatozoa. Arch Androl 1993; **30:** 29-34.
7. Ing RMY, Li DQ, Harding AM, Jones WR. A comparison of swim-down and swim-up methods for the extraction of high motility sperm. Fertil Steril 1991; **55:** 817-9.
8. Mahadevan MM, Trounson AO, Leeton JF. Successful use of human semen cryobanking for in vitro fertilization. Fertil Steril 1983; **40:** 340-343.
9. Shoham Z, Megory E, Lidor A, Madgar I, Lunenfeld B, et al. Effect of washing and suspension on penetration of human sperm into bovine cervical mucus. Arch Androl 1987; **19:** 261-267.
10. Aitken RJ. Evaluation of human sperm function. Br Med Bull 1990; **46:** 654-674.
11. Aitken RJ. Assessment of sperm function for IVF. Hum Reprod 1988; **3:** 89-95.
12. Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J Reprod Fertil 1987; **81:** 459-469.
13. WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed. Cambridge University Press, New York, 1992.
14. Mahadevan MM, Trounson AO. The influence of seminal characteristics on the success rate of human in vitro fertilization. Fertil Steril 1984; **42:** 400-405.
15. Gellert-Mortimer ST, Clarke GN, Baker HW, Hyne RV, Johnston WI. Evaluation of nycodenz and percoll density gradients for the selection of motile human spermatozoa. Fertil Steril 1988; **49:** 335-341.
16. Makler A, Stoller J, Blumenfeld Z, Feigin PD, Brandes JM. Investigation in real time of the effect of gravitation on human spermatozoa and their tendency to swim-up and swim-down. Int J Androl 1993; **16:** 251-257.