Journal of Assisted Reproduction and Genetics, Vol. 18, No. 3, 2001

ANDROLOGY

Influence of Swim-Up Method on the Recovery of Spermatozoa From Different Types of Semen Samples

SATISH KUMAR ADIGA[1] and PRATAP KUMAR[1,2]

Submitted: August 7, 2000
Accepted: September 27, 2000

Purpose: *To compare the effectiveness of swim-up method, using different types of semen samples.*
Methods: *In this retrospective study undertaken in university medical college infertility centre, subfertile couples undergoing Intra Uterine Insemination were selected. A total of 600 semen samples used for the preparation of sperm samples using swim-up method were analyzed. Relative Yield was calculated from the sperm count and motility before and after swim up from each semen sample in six different groups.*
Result(s): *Statistically significant increase in relative yield was found in oligospermic samples (20.41) followed by teratospermia (16.98). However, relative yield was low in asthenospermic (11.97) and normal (>60 million/ml) samples (11.66).*
Conclusion(s)*: Semen samples with good sperm count resulted in poor recovery after swim up. Swim-up method appears to be effective for oligospermic samples. Modifications like multiple tube swim up, using appropriate incubation time based on the initial semen parameter, will enhance the sperm yield after swim up.*

KEY WORDS: IUI; relative yield; sperm; swim up.

[1] Manipal Assisted Reproduction Centre, Kasturba Medical College, Manipal 576 119, India.
[2] To whom correspondence should be addressed at Manipal Assisted Reproduction Centre, Department of Obstetrics and Gynecology, Kasturba Medical College, Manipal 576 119 India. e-mail: drpratapkumar@usa.net.

INTRODUCTION

Intra Uterine Insemination (IUI) is the most widely used medically assisted conception (MAC) technique in patients with moderate male factor, cervical mucus hostility, and unexplained infertility as a first step before undergoing more complex programs such as in vitro fertilization (1). Separation of seminal fluid and other contaminants such as dead sperms, cell debris, prostaglandins, and microorganisms from inseminate is the initial important step in the laboratory. Several methods such as swim-up and swim-down procedure, density gradient centrifugation, and ficoll columns have been used for the separation of motile spermatozoa (2,3).

The swim-up technique has become a standard step in the preparation of sperm prior to assisted fertilization. Several modifications, such as migration of sperm into medium from the undisturbed pellet (4,5), a soft or loosened sperm (6,7), a concentrated sperm suspension (2), or an aliquot of the ejaculate (8), of the swim-up technique are described. Another method of sperm separation that uses percoll is effective in the isolation of motile spermatozoa, and high pregnancy rates have been reported (9,10). However, withdrawal of Percoll for use in assisted conception in humans has led to the search of alternative methods (11) or to use the conventional swim-up method. The swim up from semen is considered as the least complex technique for obtaining populations of highly motile human spermatozoa, and it has been recommended for sperm fertilizing ability testing by WHO (12,13). Although there are a large number of publications describing

1058-0468/01/0300-0160$19.50/0

Table I. Categorization Based on the Semen Parameters of the Patients

Group		Description
I	Normal (20–60 million/ml)	>20 million sperm/ml; >50% progressive motility; >14% normal forms
II	Normal (>60 million/ml)	>60 million sperm/ml; >50% progressive motility; >14% normal forms
III	Oligospermia	<20 million sperm/ml; >50% progressive motility; >14% normal forms
IV	Asthenospermia	>20 million sperm/ml; <50 progressive motility; >14% normal forms
V	Teratospermia	<14% normal forms; >20 million sperm/ml; >50% progressive motility
VI	Oligo Astheno Teratospermia	<20 million sperm/ml; <50% progressive motility; <14% normal forms

the applications of the swim-up technique, no comparison has been made on the nature of semen sample used and the outcome of the motile sperm population in the swim-up method. This study was conducted to evaluate the efficiency of swim-up technique on the yield of motile spermatozoa in the different types of semen samples.

MATERIALS AND METHODS

Semen Samples

Semen samples were obtained from the patients enrolled for the IUI program in our centre. Semen was collected by masturbation after three days of sexual abstinence. After liquefaction at room temperature for 30 min, a standard semen analysis was performed according to the guidelines of WHO (13). Semen samples were evaluated for volume, sperm concentration, motility, and morphology. Sperm count and motility was assessed using Makler's chamber (Sefi Scientific, Israel). A total of 100 squares were analyzed for sperm count and at least 200 spermatozoa were analyzed for the evaluation of motility. Sperm morphology was assessed in 200 spermatozoa by Eosin-Nigrosin method. Based on the semen analysis, the samples were classified into six groups (Table I). A total of 100 samples were analyzed from each group out of the 600 samples included for this study.

Swim-Up Technique

The culture medium used for all preparations was Earls Balanced Salt Solution (EBSS, Cat No. E 2888, Sigma Chemical Co, USA) supplemented with 10% heat inactivated maternal serum, prescreened for antisperm antibodies and assayed for sperm survival. The medium was equilibrated at 37°C in a carbon dioxide incubator (Nuaire, USA). After liquefaction, the semen was diluted 1:1 with medium and centrifuged at 300 × g for 10 min after which the supernatant was discarded. The pellets were resuspended in 5-ml medium and centrifuged at the same speed for 10 min. The supernatant was removed, and the pellets were overlayered with fresh medium (0.5–1.0 ml) and incubated at 37°C for 1 h. The upper layer rich in motile sperm was collected carefully, sperm count and motility were assessed, and percent enhancement was calculated for every group.

Calculation of Relative Yield (RY)

The relative yield (RY) is the proportion of progressively motile spermatozoa that are present in the final preparation before insemination, and was calculated according to the method described by Mortimer (14).

$$\text{Yield}\ (\%) = \frac{v \times c \times \text{pm}\% \times 100}{V \times C \times \text{PM}\%}$$

where v is volume of sperm preparation (ml), V is volume of semen used for the procedure (ml), c is sperm concentration in the prepared sample (10^6/ml), C is sperm concentration in the semen sample (10^6/ml), pm% is progressive motility of the prepared sperm population (decimal), and PM% is progressive motility in the semen sample (decimal).

The RY of each sample was calculated and comparisons were made between each group.

The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Statistical significance was defined as $p < .05$.

RESULTS

Influence of Swim Up on Sperm Concentration

A total of 600 samples were used for the comparison of sperm recovery, motility enhancement, and relative sperm yield between six different groups of semen samples of different qualities. The average sperm density in oligospermic group was 17.86 ± 0.93 million, which resulted in the recovery of 2.67 ± 0.2 million spermatozoa. A maximum enhancement in sperm density after swim up is observed in this group, whereas percent enhancement was lowest in

Table II. Comparison of Initial and Final Sperm Count and Motility in Various Types of Semen Samples

Group	Initial count ($\times 10^6$)	Final count ($\times 10^6$)	Percent recovery (% ± SEM)	Initial motility (% ± SEM)	Final motility (% ± SEM)	Percent enhancement in motility
I Normal (20–60 million/ml)	74.5 ± 4	8.54 ± 0.74	11.79 ± 0.8	65.04 ± 0.8	81.89 ± 0.7	127.42 ± 1.67
II Normal (>60 million/ml)	204.92 ± 9.41	18.59 ± 1.11	9.63 ± 0.54†	67.74 ± 0.8	82.92 ± 0.7	124.3 ± 1.78
III Oligospermia	17.86 ± 0.93	2.67 ± 0.2	16.52 ± 1.2	64.72 ± 0.9	78.45 ± 1.1	122.56 ± 2
IV Asthenospermia	91.63 ± 6.98	6.55 ± 0.8	7.6 ± 0.64†	38.28 ± 1.5	60.6 ± 2.87	157.69 ± 4.71*
V Teratospermia	110.05 ± 11.43	12.74 ± 1.22	14.1 ± 1.5	64.85 ± 1.5	77.3 ± 1.39	120.48 ± 2.28
VI Oligo Astheno Teratospermia	22.48 ± 4.56	2.37 ± 0.32	13.05 ± 1.1	49.53 ± 2.7	61.3 ± 4.16	122.71 ± 5.03

Note. All values are expressed as means ± SEM.
*$p < 0.05$ compared with all other groups.
†$p < 0.05$ vs. group III.

asthenospermic group (Group IV). In normal (>60 million/ml) samples (Group II), average sperm density before swim up was 204.92 ± 9.41 million, which produced 18.59 ± 1.11 million spermatozoa after swim up and a significant decline in the percent recovery was observed in the normal (>60 million/ml) samples (9.63 ± 0.54) than in the normal (20–40 million/ml) group, which had an initial count of 74.5 ± 4 and a percent recovery of 11.79 ± 0.8.

Influence of Swim Up on Sperm Motility

No significant difference in the enhancement of sperm motility after swim up was seen among different groups except asthenospermia (Group IV). The average initial motility in this group was 38.38 ± 1.5, which is significantly lower than all other groups, and resulted in 78.45% motile spermatozoa after swim up and a percent enhancement of 157.69 ± 4.71. Table II shows the sperm count and motility before and after swim up and also the enhancement of these parameters in various types of semen samples.

Relative Sperm Yield (RY)

The relative sperm yield was maximum in the oligospermic group (Group III) (RY = 20.41 ± 1.5) and it was significantly higher than in all the other groups ($p < .05$) except Teratospermia and Oligo Astheno Teratospermia (Group V and VI). However, RY was lowest in normal (>60 million/ml) samples (Group II) and asthenospermic samples (Group IV) where it was 11.6 ± 0.6 and 11.97 ± 1.1, respectively. Teratospermia (Group V) and OATS (Group VI) also had RY values of 16.98 ± 1.68 and 16.59 ± 1.74, respectively, which were higher than that of the normal (20–40 million/ml) group where RY was 15.03 ± 1.04 (Fig. 1).

DISCUSSION

The present study showed the effectiveness of swim-up method in the variety of semen samples, which are commonly encountered in the IUI program. Recovery of sperm after swim up varies according to the quality of the semen sample. We observed a good outcome in oligospermic samples followed by teratospermic and oligo astheno teratospermia samples as evidenced by an increase in RY. However, semen samples with normal (20–60 million/ml) and normal

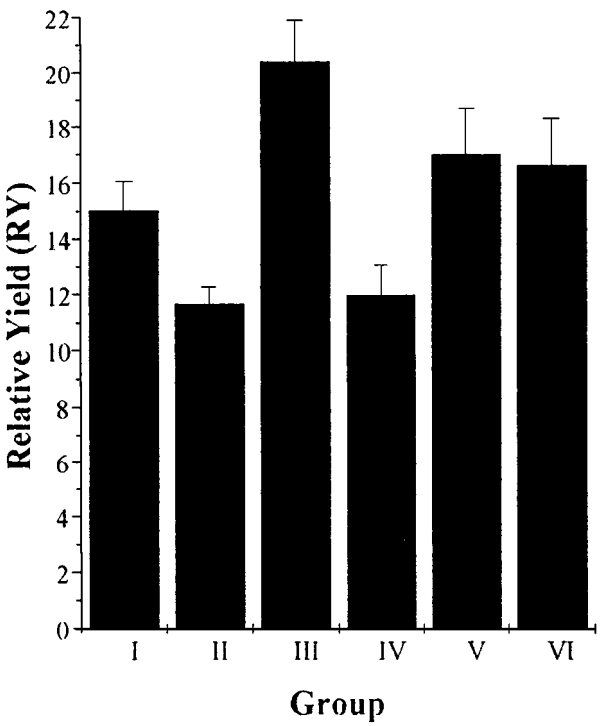

Fig. 1. Relative yield of spermatozoa after swim up in different types of semen parameters. Data are expressed as means ±SEM.

(>20 million/ml) parameters (described in Table I) did not produce a significant increase in the sperm density after swim up when compared with their initial parameters. Similarly in asthenospermic samples, swim-up method significantly enhanced the sperm motility. However, when the overall outcome was calculated for RY, no significant improvement was seen in this group because of recovery of less number of spermatozoa. In oligospermic group, after swim up, even though there is no significant difference in the sperm motility with other groups, except asthenospermia, RY was significantly higher than in all the other groups, and swim-up method was found to be very effective. RY was also higher in the teratospermia and oligo astheno teratospermia samples.

The purpose of swim-up technique is to separate spermatozoa from the seminal plasma and to improve the general sperm quality prior to assisted conception. Purvis and Egdetveit (15) observed sperm yield from normozoospermic men and men with borderline asthenoteratozoospermia represented 40 and 15% of the total motile cells in the ejaculated aliquots, respectively. In general, in both groups, spermatozoa in the swim-up fraction exhibited superior motility, vitality, and morphology compared to the original ejaculates.

The recovery of sperm after swim up varies according to the various modifications of the techniques used. Harris *et al.* (16) reported a recovery of 58% for normozoospermic men. Purvis and Egdetveit (15) reported a reduction in sperm recovery in normozoospermic men. We also observed poor sperm recovery in the samples of men having normal semen parameters. This may be due to reduced possibility of sperm movement from the lower portion of the centrifuged pellet, when the sperm are more densely packed. It may also reflect damage to the sperm by free oxygen radicals associated with centrifugation (17). In normozoospermic men, the proportion of motile sperm entering the overlaying medium remained relatively constant at about 40% with increasing sperm concentration in the seminal plasma (15). However, the proportion increased with temperature, the area of the interphase between the two liquids, and incubation time. In such situations where sperm density is very high in the semen, swim up may be performed in more than one tubes depending on the sperm count in the semen or the size of the pellet after first centrifugation.

The time of incubation during swim up is only 1 h in our study, which is kept same for all kind of samples. Vijayakumar *et al.* (6) reported that a maximum sperm yield was reached 2–6 h after the start of incubation, which may be a reason for low sperm recovery in the samples with normal semen parameters having thick sperm pellet in the present study.

Percoll does not appear to be suitable for human use as reported by many studies. In this situation, swim-up technique may still represent more appropriate alternative for sperm preparation. However, in the present study, the reason for poor sperm recovery from the higher sperm density samples could be technical difficulty rather than related to function of the sperm. To avoid such problems, modifications (multiple tube swim up) in technique and selecting appropriate incubation time based on the initial semen parameter will enhance the sperm yield after swim up to achieve good success in assisted conception procedures.

REFERENCES

1. Taylor PJ, Kredentser JV: Washed intrauterine insemination—indications and success. Int J Fertil 1989;34:378–384
2. Russell LD, Rogers BJ: Improvement in the quality and fertilization potential of a human sperm population using the rise technique. J Androl 1987;8:25–33
3. Gorus FK, Pipeleers DG: A rapid method for the fractionation of human spermatozoa according to their progressive motility. Fertil Steril 1981;35(6):662–665
4. Carreras A, Palma A, Mendoza C: Hypoosmotic swelling test in normo-, oligo-, astheno- and oligoasthenozoospermic men before and after swim-up separation of spermatozoa. Andrologia 1990;22(4):313–317
5. Oehninger S, Acosta R, Morshedi M, Philput C, Swanson RJ, Acosta AA: Relationship between morphology and motion characteristics of human spermatozoa in semen and in the swim-up sperm fractions. J Androl 1990;11(5):446–452
6. Vijayakumar R, Ndubisi B, De Leon F, Heine W: Sperm wash in three culture media: Maximization of motile sperm recovery during swim-up incubation. Andrologia 1987;19(5):579–584
7. Carson SA, Reiher J, Scommegna A, Prins GS: Antibody binding patterns in infertile males and females as detected by immunobead test, gel-agglutination test, and sperm immobilization test. Fertil Steril 1988;49(3):487–492
8. Berger T, Marrs RP, Moyer DL: Comparison of techniques for selection of motile spermatozoa. Fertil Steril 1985;43(2):268–273.
9. Ord T, Patrizio P, Marello E, Balmaceda JP, Asch RH: Mini-Percoll: A new method of semen preparation for IVF in severe male factor infertility. Hum Reprod 1990;5(8):987–989
10. Van der Zwalmen P, Bertin-Segal G, Geerts L, Debauche C, Schoysman R: Sperm morphology and IVF pregnancy rate: Comparison between Percoll gradient centrifugation and swim-up procedures. Hum Reprod 199;6(4):581–588

11. Claassens OE, Menkveld R, Harrison KL: Evaluation of three substitutes for Percoll in sperm isolation by density gradient centrifugation. Hum Reprod 1998;13(11):3139–3143
12. World Health Organization: WHO Laboratory Manual for Examination of Human Semen and Semen-Cervical Mucus Interaction, 2nd Ed. Cambridge, The Press Syndicate of the University of Cambridge, 1987, p. 28
13. World Health Organization: WHO Laboratory Manual for Examination of Human Semen and Semen-Cervical Mucus Interaction, 3rd Ed. Cambridge, The Press Syndicate of the University of Cambridge, 1992, p. 44
14. Mortimer D: Practical Laboratory Andrology. Oxford University Press, 1994
15. Purvis K, Egdetveit I: Factors affecting sperm yield during swim-up. J Assist Reprod Genet 1993;10(2):145–150
16. Harris SJ, Milligan MP, Masson GM, Dennis KJ: Improved separation of motile sperm in asthenospermia and its application to artificial insemination homologous (AIH). Fertil Steril 1981;36(2):219–221
17. Aitken RJ, Clarkson JS: Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm preparation techniques. J Androl 1988;9(6):367–376