

Sperm swim-up: a simple and effective technique of semen processing for intrauterine insemination

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Abstract

Objective: To study the impact of sperm swim up technique of seminal processing for the purpose of intrauterine insemination (IUI) in couples presenting with subfertility.

Methods: Hundred and twenty-one couples presenting with subfertility underwent 281 cycles of IUI in Combined Military Hospital Kharian, Lahore and PNS SHIFA Karachi from June 2002 to March 2005. Men had prior semen analysis to assess for parameters like those that total sperm count, morphology and progressive motility. Only those couples were enrolled in whom there was either no obvious cause or the male partner had some problem with the seminal counts/motility.

In standard swim-up technique, after liquefaction, the semen sample was centrifuged and supernatant was discarded. Pellet was suspended in pre-warmed 2.5 ml of Ham's F-10 culture medium and thereafter centrifuged once more. The pellet was gently over-layered with medium in the tube which was sealed, inclined at 45° and kept at 37°C for 60-90 minutes in 5% CO₂. A sterile Pasteur pipette was used to remove the supernatant containing actively motile sperms. The specimen was kept at 37 °C till dispatched to be inseminated. Data was analyzed by using SPSS version-10. Motility and morphology were used to present qualitative parameters and chi-square test was applied to assess the effectiveness of IUI in relation with these variables.

Results: Post swim-up semen parameters including total motile sperm count and motility were observed. There was a trend towards an increased sperm count, motility and pregnancy rate after the swim up procedure.

Conclusion: Our findings suggest that sperm swim-up technique is an easy reliable and effective sperm processing method for insemination purposes (JPMA 58:71;2008).

Introduction

With the advancement in the techniques of assisted reproduction in humans, the need to improve sperm processing and provision of actively motile spermatozoa has increased tremendously.¹ The human ejaculate comprises of a mixture of seminal plasma, mature and immature spermatozoa, non-reproductive cells, various micro organisms and non-specific debris.² In preparation for assisted fertilization procedures such as intrauterine insemination, the motile and hopefully, the most fertilizable population of sperm must be separated from the surrounding milieu.

The demands on sperm separation techniques have increased with our expanding knowledge of sperm physiology and on their genetic contribution to the embryo. Because of this, there has been rising concern over the safety of any sperm separation procedure with respect to not only the viability of the sperm, but to the long-term effects on any resulting pregnancy.³

Several available sperm separation methods include simple washing, sperm migration into culture medium (swim-up), Sephadex and glass wool columns and density gradient centrifugation.⁴ All of these techniques are capable

of effectively separating sperm from the seminal plasma, but to varying degree. Recovery rates, motility, morphology and degree of DNA damage vary greatly between procedures.⁵

The swim-up technique originally described by Mahadevan and Baker⁶ from a washed pellet is the oldest and most commonly used sperm separation method. Swim-up is the standard technique for patients with normozoospermia and female subfertility.⁶

The methodology of this conventional swim-up is based on the active movement of spermatozoa from the pre-washed cell pellet into an overlaying medium. A yield of very high percentage (>90%) of motile morphologically normal spermatozoa can be obtained with the help of this technique.⁷ Keeping in mind that the efficiency of the technique is based on the surface of the cell pellet and the initial sperm motility in the ejaculate. Many layers of cells in the pellet may cause potentially motile spermatozoa in the lower levels of the pellet never to reach the interface with the culture medium layer.⁸

In an attempt to improve the sperm yield in oligospermic males the "swim-up" can be performed directly from the liquefied semen avoiding the centrifugation and multiple washing steps. During this

procedure, several aliquots of liquefied semen are taken from a sample and placed in tubes underneath an overlay of culture medium. Round-bottom tubes or 4-well dishes should be used to optimise the surface area of the interface between the semen layer and the culture medium. The tubes may also be prepared by gently layering culture medium over the liquefied semen. A maximum recovery is obtained by using multiple tubes with small volumes of semen per tube, thus maximizing the combined total interface area between semen and culture medium.⁹

Subjects and Methods

In this multi-centric study, total of 121 couples presenting with the complaints of primary or male factor subfertility completed 281 IUI cycles. All these couples had been trying unsuccessfully to conceive for a minimum of 2 years before being considered for assisted fertility procedure. Complete history and physical examination - both systemic and local of the husband and wife was carried out. Testing for the women included at least: basal temperature chart, post-coital tests, hormone levels and a hystero-gram. If either the hystero-gram or vaginal ultrasonography suggested peritoneal adhesion or endometriosis, a laparoscopy was performed before any treatment.

Men had at least two semen analyses and microbiological tests before any treatment. Serological tests for HIV, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) were conducted for both members of the couple. Additional testing depended on any abnormalities observed. Both the partners were informed about the procedure and its implications in detail. A certificate was signed by both that they were the legal husband and wife and they had understood the whole procedure of IUI with no objection to any of its part.

Normal semen analyses were defined by the threshold values of the WHO.¹⁰ (concentration $20 \times 10^6/\text{ml}$, total count 40×10^6 , progressive motility 50%, typical morphology 30%).

Sperm motility was determined by assessing at least five microscopic fields to classify minimum 200 spermatozoa ($\times 400$ magnification). The motility was graded progressive, non-progressive or immotile.

For sperm morphology, smears were prepared and the percentages of morphologically normal spermatozoa and of various sperm abnormalities were evaluated on 100 sperms at a final magnification of $\times 1000$. A male factor was diagnosed if either the sperm concentration was $< 20 \times 10^6/\text{ml}$, total progressive motility $< 50\%$ or sperm morphology showing $\leq 10\%$ normal forms according to the WHO criteria.¹⁰

Semen was collected by masturbation into a sterile container after 2-4 days of sexual abstinence. Immediately after liquefaction, a drop of the well-mixed specimen was placed on a clean and pre-warmed glass slide at 37°C , covered with a cover slip and parameters were noted down to be compared with the values after processing.

The standard swim-up technique was used for collection of motile and active sperms. In the swim-up technique, the sperm sample was centrifuged at 400 g for 15 minutes. The supernatant was discarded; the pellet was suspended in pre-warmed 2.5 ml of Ham's F-10 (Sigma Chemical, St. Louis, MO) culture medium or Earle's balanced salt solution (Sigma), supplemented with human serum albumin thereafter centrifuged once more. After removing the supernatant the pellet was gently over-layered with medium in the tube which was sealed, inclined at 45° and kept at 37°C for 60-90 min in 5% CO_2 . A sterile Pasteur pipette was used to remove the supernatant containing actively motile sperms. Before dispatching the prepared sample, a drop was examined under light microscope. Motility and morphology was recorded and the specimen was kept at 37°C till dispatched to be inseminated.

Urinary excretion of human beta chorionic gonadotrophin was measured 15 days after insemination (luteal - day 15) by latex agglutination test for the diagnosis of pregnancy. Findings were confirmed by ultrasonography at later stages.

Data was analyzed by using SPSS version-10. Frequency and percentage were used to present qualitative parameters and chi-square test was applied to assess the effectiveness of IUI in relation with these variables. Quantitative variables like seminal fluid before and after IUI were presented by mean $\pm$ standard deviation and paired samples t-test was used to compare.

Results

Semen analysis, showed subnormal sperm count in 34 patients (30.9%). The following sperm abnormalities, defined according to the WHO criteria, were observed: isolated oligospermia ($n=3$), isolated asthenospermia ($n=22$), isolated teratospermia ($n=7$), oligoasthenospermia ($n=3$), oligoteratospermia ($n=5$), asthenoteratospermia ($n=6$) and oligoasthenoteratospermia ($n=13$). Twenty one (21) of them had increased leukocytes in the semen. None of the patients or their male partners had detectable antisperm antibodies. In all the semen specimens subjected to swim up procedure the pre and post treatment values were noted. All the three variables i.e. sperm count, motility and sperm concentration showed marked improvement after the swim up procedure. (Table 1)

All the candidates received a total of 281 IUI cycles.

Table 1. Data before and after the swim-up procedure fluid of males:

Semen characters	Values on semen analysis (n=281)	
	Base line	After swim up technique
Semen volume (ml)	3.7	-
Sperm concentration (million /ml)	12 ± 3.02	59 ± 3.11*
% Motile spermatozoa	25 ± 2.56	72 ± 4.48*
Motility (0-4)	1.2 ± 0.45	3.7 ± 0.81*

(All the data is mean ± standard deviation of the findings)*Significant higher p < 0.001

Table 2. Impact of intrauterine insemination according to female characteristics.

Pregnancy			
Variables	Conceived (n=34)	Not conceived (n=76)	Significance
Age (in years):			
< 35 (49)	19 (55.8%)*	30(44.2%)	p = 0.003*
>35 (61)	15 (24.5%)	46 (75.5%)	
Duration of infertility (in years)			
≤ 5	29 (85.2%)	36 (47.3%)	p = 0.002*
> 5	5 (14.8%)	40 (52.7%)	
Type of infertility			
Primary (84)	23 (64.5%)	61 (83.7%)	p = 0.050*
Secondary (26)	11 (35.5%)	15 (16.3%)	
Aetiology of infertility			
Unexplained (76)	24 (31.5%)	52 (68.5%)	p = 0.002*
Male factor (34)	10 (29.4%)	24 (70.6%)	

Key:

Given measurements is Mean ± Standard deviation (Range)

* Shows significantly greater at p < 0.05

‡Show significantly less at p < 0.05

Out of the total 110 couples, 13 (11.83%) women conceived just after first IUI cycle, 12 (10.9%) after second, 9 (8.18%) after third cycle while 76 (69.09%) could not conceive. (Table 2).

Majority of the women in our study were in age group of >35 years (n=61, 55.50%), followed by 49 (44.5%) in age group < 35 years. Out of those 34 women who conceived, 19 (55.8 %) were aged less than 35 years and pregnancy rate per cycle was also high (23.3%) in this group (Table-2). It shows a significant association (p = 0.003) of conceiving pregnancy after IUI with age less than 35 years.

Eighty four (76.3%) cases in our study had primary infertility and the proportion of women who conceived pregnancy was marginally significant (p = 0.05), but rate of pregnancy per cycle was slightly high in secondary infertile women (Table 2).

IUI was found more effective in case of unexplained

aetiology of infertility as out of such 23 cases, 15(%) conceived pregnancy (p=0.002) while rate of pregnancy per cycle was also higher (16.85% vs. 13.79%) in this group (Table 2).

Discussion

The practice of intrauterine insemination with treated motile and morphologically normal spermatozoa involves bypassing the cervical mucus barrier resulting in an increased gamete density at the site of fertilization.¹¹

The present results show that the swim-up method for recovery of motile sperm is reliable. The washing procedure is necessary to remove prostaglandins, infectious agents and leukocytes.¹² One advantage of the method is the limited number of technical steps that besides being more practical, avoids damage to the spermatozoal cytoplasmic membrane.¹³

These and other techniques are designed to recover morphologically better motile sperm. Some procedures are preferred because of their capacity to maximize motile sperm recovery. Sperm recovery does not necessarily need to be maximized, but adequate for a good pregnancy rate per cycle.¹⁴

The percentage of actively motile sperms recovery obtained in the present study ensures efficacy of the method; indeed, the pregnancy rates obtained in the present study were similar to those reported in the literature.¹¹ The swim-up method can at times be more successful if one omits the initial centrifugation process while dealing with oligoasthenozoospermic samples. In cases of severe oligozoospermia, other methods, involving centrifugation and concentration of sperm, seem advisable.¹⁵

The swim-up method of sperm preparation does not require particular expertise and saves material; it is, therefore, more practical for the operator and less expensive.¹⁶ Regarding the policies and procedures of motile sperm preparation, the present technique reduces the risks without affecting the outcome as our results show remarkable improvement in the yield of motile sperms with the procedure of swim up. The concentration of morphologically normal sperms also increased substantially due to better forward movement of normal spermatozoa.¹⁷ Another substantial advantage of the technique is removal of non-motile spermatozoa, leukocytes and immature germ cells. This might contribute to enhance sperm quality by decreased release of lymphokines and/or cytokines and a reduction in the formation of free oxygen radicals after sperm preparation.¹⁸ This results in better sperm fertilizing ability in vitro and in vivo.¹⁹

A threshold effect for the average total motile sperm count seems significant. With the average count of <10

million/ cmm, pregnancy rates are reported to be very low after IUI and when the average sperm count is above 30 million, a higher pregnancy rates are noted per the IUI cycle.²⁰

Most studies evaluating the effect of semen parameters on outcome of IUI have concluded that couples with a male-factor for infertility have low pregnancy rates after IUI.²¹

Sperm morphology is another factor and the number of motile inseminated sperms may influence the IUI results. Several IVF studies have shown that the fertilization rate decreases with a low level of normal sperm.²²

It is thus logical that sperm morphology modifies the results of IUI. Semen preparation may modify sperm characteristics considerably, and the number of motile spermatozoa and the morphological criteria should logically be assessed after semen preparation.²³ In our study, morphology improved after preparation in nearly three-quarters of the men (83/110) with a rate of normal sperm before preparation <30%: the rate of normal features exceeded the 30% threshold. This result justifies the use of morphology only after preparation for assessing IUI prognosis.²⁴

Conclusion

Swim-up method of sperm preparation for IUI is a step towards optimization of the procedure and reduction of costs, enabling the gynaecologist to work in a safe and effective way.

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