

## Detection of sperm-motivating factor in cervical mucus of ovulatory women

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**Abstract:** *Objective:* The objective of the present study was to detect the presence of any sperm selecting and / or motivating factor in the cervical mucus (CM) of ovulatory women, which, in turn, might influence pregnancy rate. *Materials & Methods:* The study was conducted with 190 infertile couples, having indications for moderate seminopathy, enlisted for intra-uterine insemination (IUI), following routine ultrasound and laparoscopic evaluation at our centre, between April 2006 to August 2010. The female partners were in the age group 25-30 years and were randomly divided into 3 groups to receive sperm processed in 3 different methods, with or without CM. Pre and peri-ovulatory CM was subjected to paper electrophoresis on cellulose acetate membrane (CAM) strip, to study the protein band pattern. The different procedures of sperm processing were compared primarily on the basis of sperm motility and quality in the swim-up layer and also the pregnancy outcome following IUI, with due emphasis on improvisation of current laboratory methodologies used in IUI. *Results:* The use of CM layer alongwith medium for swim-up proved to be the best regarding selection of sperm with maximum motility and normal morphology, as well as the pregnancy outcome. The CM of ovulatory women around ovulation showed the presence of a specific band in electrophoresis. *Conclusion:* The use of CM in sperm preparation technique proves to be beneficial to patients undergoing IUI.

**Keywords:** cervical mucus, fetal cord serum, intra-uterine insemination, ovulatory women, swim-up technique, cellulose acetate membrane.

### Introduction

The overall pregnancy rate of IUI, though a frequently indicted therapeutic modality in infertility, and generally considered to be an intermediate step of low to moderate complexity before the application of more sophisticated assisted reproductive technologies (ART), is not at all satisfactory (less than 20% per cycle) [1-6]. An increase in the success rate is being attempted all over the world, without much effect. In normal pregnancy, the sperm cells pass through the cervical canal where there is a natural barrier called CM, that constitutes the natural defense system of uterine cervix and acts as a strainer for sperm before entry to the uterine cavity [7-8].

In IUI program, these cells are separated from seminal plasma by some technical procedures, mostly in usual cell culture medium. Sometimes, additives like human serum albumin or fetal cord serum are added to the medium for potentiating sperm, and isolated and processed spermatozoa are released in the uterine cavity by-passing cervix so that they can reach the site of

fertilization quickly. This does not allow the treated sperm to be strained through CM, the multifactorially determined filtering system [9]. This man-made sperm selection might affect the pregnancy rate in IUI program. With this idea in mind, a study was designed to investigate the role of CM as a whole or any of its components on quality (motility as well as morphology) of selected sperm cells, which might influence fertilization and hence, success rate of IUI.

### Material and Methods

Between April 2006 and August 2010, 190 couples undergoing IUI at our centre for moderate male factor problem (seminopathy) were included in the study. For this purpose, formal approval was taken from the Institute's Ethics Committee and written consent from the patients. The selection criteria for the patients were as follows – the ovulatory as well as the tubal status of female partners were evaluated, and those with patent tubes of adequate length and clear Pouch of Douglas

(POD) observed in laparoscopic examination, were enlisted for the study. Either these female partners ovulated normally or had minor ovulatory dysfunctions, which were corrected by oral ovulation-inducing (OI) agents. The strict seminal criteria for inclusion into the study were as follows – total sperm count in between 10-15 millions with initial motility (0 hour) 15-20%, pus cell count 2-4/HPF and total seminal volume 1-1.5 ml. The viscosity was normal. For ovarian stimulation, the female partners received Clomiphene Citrate 100 mg. (Ovofar from Organon India, now MSD) daily from 3<sup>rd</sup> to 7<sup>th</sup> day of menstrual cycle (D3-D7), and injection Human Menopausal Gonadotrophin or hMG 75 IU (IM) (GMH from Sun Pharma) on D4, D6 and D8. Those who developed at least one 18 mm. follicle by D16 were subjected to IUI. The age group of patients, that is, female partners was between 25-35 years, and they were randomly distributed into 3 groups (viz. Groups A, B and C), to receive sperm processed in 3 different ways - (1) using Ham's F10 medium in Group-A, (2) same medium supplemented with fetal cord serum in Group-B; and (3) same medium, where CM was added in Group-C. CM was collected from wife of the couple concerned, between D12 and 13 and at the time of insler cervical score of 8-10 cms [10]. In every case, to exclude any infection, the mucus was subjected to culture once on previous cycle before commencing IUI, and again on the cycle of IUI. Ovulation monitoring was performed through trans-vaginal ultrasonography (TV-USG) from D8/9 onwards, on daily or alternate day basis.

In all the 3 groups, semen samples were prepared by single wash technique [11]. The sperm pelette was layered with 0.5 ml of medium in each case. In Group-C, the sperm pelette was first layered with CM and then medium on top and subjected to swim-up. Incubation was performed in 5% CO<sub>2</sub> environment. The swim-up layer was used for IUI after 1 hour of incubation, leaving aside a small volume for observation of sperm motility for 24 hours, at 6 hourly interval following initial assessment after 1<sup>st</sup> hour, to ascertain the period of retention of active motility (PRAM). Only progressive motility was taken into consideration. In each case, the mucus sample from the respective female patient was mixed with saline and 2.0–3.0 µl of it and was placed on CAM strip and subjected to electrophoresis in barbitone

buffer, pH 8.6 [12-13]. After electrophoretic run, the strips were fixed in 5% Trichloroacetic Acid (TCA), stained with 0.5% Ponceau S stain and de-stained in 3-4 washes of 5% acetic acid. The CM samples collected between D9 and D13 of period were subjected to the above procedure at a daily collection basis. Lastly, the pregnancy outcomes from different protocols were compared.

In certain number of patients who had good CM (23 cases), another observation was made. CM was collected around 11<sup>th</sup> - 13<sup>th</sup> day, when the Insler Score was nearly 8-10cms and that mucus was subjected to electrophoresis. The above couples were asked to have intercourse thereafter and the CM was again collected 4-6 hours following intercourse, and was subjected to electrophoresis again, to find out the difference, if any, in the band pattern of pre and post-coital mucus. Care was taken to collect only a drop of mucus for electrophoresis and not to suck out the whole mucus pre-coitally.

## Results

The difference in morphology and motility of spermatozoa yielded by 3 different procedures following 1 hour of incubation was compared primarily, though the pregnancy rate following IUI was the main outcome measure. In Group-A, the post-preparation motility of sperm cells in swim-up increased upto 50-60% from the initial 15-20%, whereas the same in Group-B was 55-70% and in Group-C was 70-85%. Regarding the quality of spermatozoa in swim-up layer, it was observed that the normal morphology was 60-70% in both Group-A and Group-B but >90% in Group-C. The pregnancy rate in patients of Group-A was 15%, in Group-B was 20% and in Group-C was 33%.

In the present study, it has been observed that sperm motility was enhanced in all cases, i.e., in Group-A, Group-B and Group-C, but maximum induction was achieved with CM, i.e., in Group-C. The rating of motility (viz., fast forward, sluggish, etc.), morphological quality and PRAM were significantly improved with CM compared to HAM F10

alone, or the same supplemented with FCS. The scenario was the same in case of pregnancy outcome where IUI with CM strained sperm cells offered 33% success rate in comparison with 15% success in Group-A and 20% success in Group-B (Table – 1).

**Table-1: Comparison of Sperm Preparation in 3 Different Procedures**

| Group | Normal Morphology | Motility | Pregnancy Rate |
|-------|-------------------|----------|----------------|
| A     | 60-70%            | 50-60%   | 15%            |
| B     | 60-70%            | 55-70%   | 20%            |
| C     | 92-95%            | 70-85%   | 33%            |

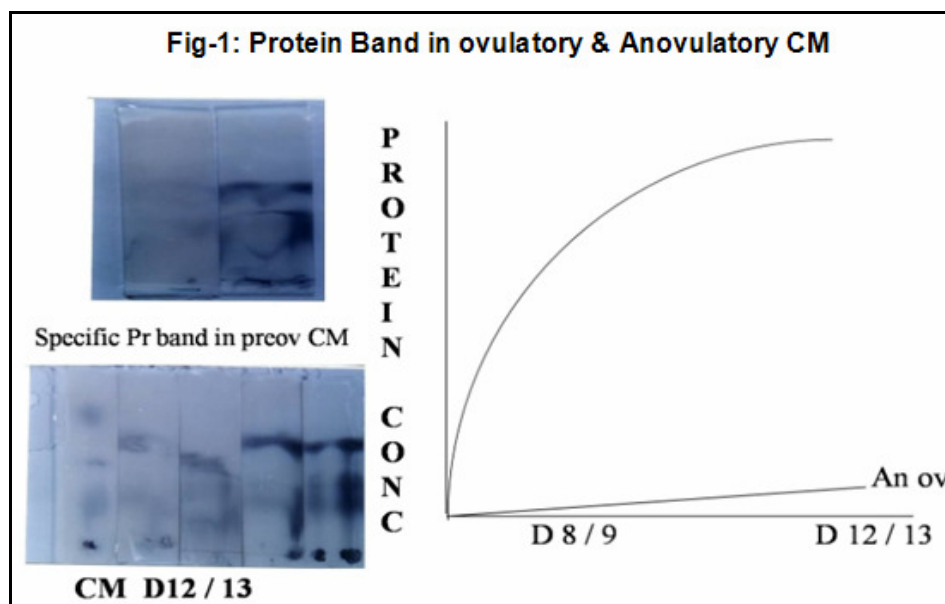
In electrophoresis, the group of patients presenting with anovulation, no protein band could be detected on the CAM strip after fixing and staining. In some cases where folliculogenesis took place but the protein band did not appear or appeared poorly, pregnancy did not occur. When the CM was collected pre and post-coitally, it was found that the specific protein band appearing pre-coitally was either absent or minimal in post-coital mucus sample.

**Discussion**

One of the most important criteria of success in IUI is availability of good number of actively motile and morphologically normal spermatozoa as well as an oocyte at the site of fertilization. For IUI, the problem of Oligozoospermia can be

countered by concentrating the semen sample, but asthenozoospermic samples need proper fortified media for their improvement [14]. The addition of CM in the sperm wash media has been shown to be promising in this respect. It appears that the protein content of CM exerts this effect. The present study has also pointed out a specific protein which appeared as a persistent band on CAM strip following electrophoresis of CM of ovulatory women. The non-occurrence of pregnancy in cases where this protein band was either absent or poorly present therefore indicated the possibility of empty follicles [15-17] (Fig-1).

This concept is similar to that of anembryonic pregnancy, which always ends up in abortion [18-20], though the initial hormone ( $\beta$ -hCG) profile may be normal. Moreover, the absence or minimal presence of this specific band following electrophoresis of post-coital mucus sample pointed to its probable utilization by the sperm cells, when they encountered it. Hence, it appears that the oocyte is the driving force for the above protein synthesis, which probably motivates sperm cells and dictates fertilization. Moreover, isolation of selected sperm populations by new methodology for providing optimum environment for fertilization at the time of insemination, is likely to play a pivoting role in enhancing the success of IUI in the near future.



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