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Short communication

Upstream; a novel method for separating human motile sperms

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Abstract

Background: Sperm preparation is a fundamental step in ART and attempt for developing new methods to be continued.

Objective: To evaluate the efficacy of upstream method for separation of human spermatozoa and its effects on sperm parameters including: motility, morphology, and concentration.

Materials and Methods: Semen samples from 17 men who referred to infertility clinic for evaluating their infertility problems were collected. Semen was placed in floor of the upper container of device, and then media was gently placed on the upper semen portion. During the procedure, semen flowed from upper container and dropped in lower container. When semen flowed, the non-motile sperms, cells, debris and seminal plasma enter the lower container, while only motile sperms separated by swimming upstream and go to the upper part of the medium. Finally, all of the semen flowed from the upper container to the lower one, and merely the medium remained, which contained motile normal sperms.

Results: With application of upstream method, the recovered spermatozoa showed significant improvement with motility and normal morphology. The data showed that mean sperm motility increased from 39% to 90% ($p<0.0001$) and normal morphology increased from 25% to 32 % ($p<0.0001$) after processing using new method. In addition, the concentration of round cells decreased to zero in all samples.

Conclusion: The results indicate that upstream method can enrich the sperm population of normal motile sperms. Also, the new method is simple and does not require centrifugation step or any chemicals.

Key words: *Sperm preparation, Swim up, Seminal plasma, Sperm separation, Upstream.*

Introduction

To increase the chance of fertilization, several kinds of treatment including IUI, IVF, GIFT and ICSI are performed with sperm preparations composed of viable and motile sperms, free of seminal plasma and debris. Potentially the separation of fertile spermatozoa from immotile ones is done under in vivo conditions. Another step is debris and seminal plasma in the female genital tract by active migration through the cervical mucus (1). During this process, not only progressively the selection of motile sperms is done, but male germ cells also undergo physiological changes called capacitation. The sperm's functional competence needs these changes as fundamental prerequisites with regard to acrosome reaction (2, 3).

Probably, first attempt for sperm separation was done by Lopata *et al* in Australia. He reported that spermatozoa can migrate into a diluent, contained in Pasteur pipettes that were dipped into semen for 1 hour (4).

The development of a wide range of different sperms separation methods is the result of the introduction of assisted reproduction, especially

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IVF, during the 1980's. More complicated techniques were developed to increase the number of motile spermatozoa even in severe cases after the development of the classical swim-up technique by Mahadevan and Baker (5). On principle, these techniques can be differentiated in migration, density gradient centrifugation and filtration techniques (6, 7).

Two currently used standard preparation techniques are double density gradient centrifugation and the swim-up procedure. Moreover, for providing sperm samples with comparable recovery rates, motility, morphology, and fertilizing capacity another technique is used which is glass wool filtration (8). For separation of sperm in all of these methods, the centrifugation step is mandatory.

Aitken and Clarkson discovered that the centrifugal pelleting of unselected human sperm populations often resulted in the generation of free radical or reactive oxygen species (ROS) within the sperm pellet that could adversely affect sperm function in vitro (9,10).

Moreover, Mortimer prepared a detailed review of the literature of in vitro tests of human sperm function and IVF that clearly demonstrated that sperm preparation methods that included such a centrifugation step could impair sperm function to such an extent as to cause a decrease in fertilization rates and even cause fertilization failure in more extreme cases (11).

It has been reported that when sperms are put into a fluid flow, the motile sperms rapidly align themselves and swim upstream (12, 13). Non-motile and sluggish sperms, along with other cellular components, are washed downstream away from the motile sperms. Cilia have been shown to be present in the endometrial cells of many mammals. Ciliary's currents in both the fallopian tubes and the uterus move in the same direction and extend towards the external os. One may expect that this flow act as a guide for sperms, leading sperm with the correct motility parameters towards the site of fertilization at the ampoule of the fallopian tubes. Secondly, this flow acts as a natural selection mechanism to optimize the quality of sperm able to reach the fertilization site. Based on swimming upstream phenomenon of sperm, we developed new methods for separation of normal sperm name as upstream that use a new patented device. This method and relative device dose not need centrifugation step or any chemicals. In this study we evaluate the efficacy of this new method for separating normal motile sperms.

Materials and methods

Device

Device is compromised of a cylindrical shape tube which is made from polystyrene. At the middle part of the tube there is a nylon mesh that divides tube to lower and upper container. By using this nylon mesh upper container completely distinct from the lower one. The dimension of nylon mesh pores are around 80 µm. For regulating the flow velocity of semen and exiting air there are some fine passage between upper and lower container just below the nylon mesh (Figure 1).

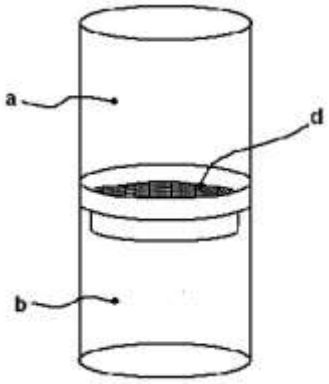

Figure 1. Side view of device. a, upper container. b, lower container. d, nylon mesh.

Procedure

Semen samples were obtained from 17 men attending the infertility clinic for evaluating their infertility problems. After 3 to 4 days of abstinence semen samples were collected in a room close to the laboratory in sterile polystyrene cups. All samples were allowed to liquefy for at least 30 minutes. Before preparation, semen samples were evaluated according to WHO (1999) which are described in detail in the joint European Society of Human Reproduction and Embryology–Nordic Association for Andrology (ESHRE-NAFA) manual (14).

Initial sperm concentration and motility were determined under magnification using 400X. Assessment of motility was made according to four group's classification including rapid progressive, slow progressive, non-progressive and immotile sperm count. Finally percentages for the two categories (rapid progressive + slow progressive) were written on the sample report form. The morphology of spermatozoa was subsequently determined. Each spermatozoon without morphological"defects" was defined as ideal. All deviations from the ideal morphology were classified as defects. The presence of defects in each region of the spermatozoon was expressed as

"defects per 100 spermatozoa" for that region. A spermatozoon with a head defect, a neck/mid-piece defect and a tail defect was registered as having defects in all three sperm regions (i.e. three defects), but was still only one defective cell. Only intact spermatozoa, i.e. those with both head and tail, were counted. All borderline cases classified as defective.

For performing procedure, about 2 ml semen was placed in floor of the upper container just on the mesh, via using a Pasteur pipette, then using a different Pasteur pipette 1 ml Ham's F-10 is gently placed on the upper semen portion.

At last, the device was put in a 37ºC incubator with 5% CO2 in air. During the procedure, semen flowed from upper container, because of the gravity and dropped in lower container via mesh pores. The air in lower part exited, simultaneously, via the fine passage which was the upper part of wall of the lower container. When semen flowed in mesh pores, the non-motile sperms, cells, debris, and seminal plasma entered the lower part, while only motile sperms separated by swimming to upstream and went to the upper parts of the medium.Finally, all of the semen flowed from the upper container of the device to the lower one, and merely the media remained, which contains motile normal sperms. After 30 min, the media with active sperms was aspirated via a Pasteur pipette.

Statistical analysis

Statistical differences among percentage of sperm motility, percentage of normal morphology and sperm concentration in two groups (unprocessed and upstream specimens) were analyzed by paired test.

Results

When using upstream method, the recovered sperms showed significant improvement in motility and normal morphology. Our data showed that mean sperm motility increased from 39%to 90% ($p<0.0001$) (Figure 2) and normal morphology increased from 25% to 32 % ($p<0.0001$) (Figure 3) after processing with upstream method. However, there was some decrease in sperm concentration, 42×10^6 to 23×10^6 (Figure 4) that is predictable because abnormal sperms as well as non-motile sperms were eliminated from samples. In addition, the concentration of round cells decreased to zero in all processed samples.

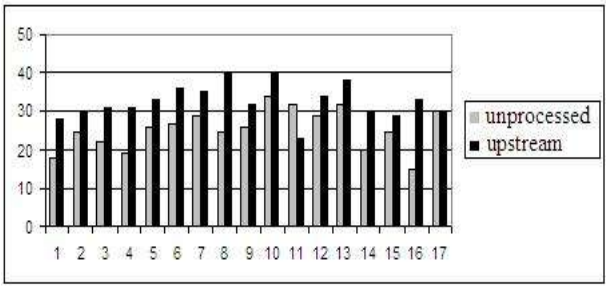

Figure 2. Percentage of sperm motility in unprocessed and upstream specimens; $p<0.0001$.

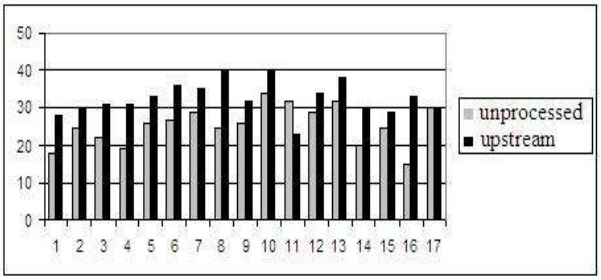

Figure 3. Percentage of normal morphology in unprocessed and upstream specimens; $p<0.0001$.

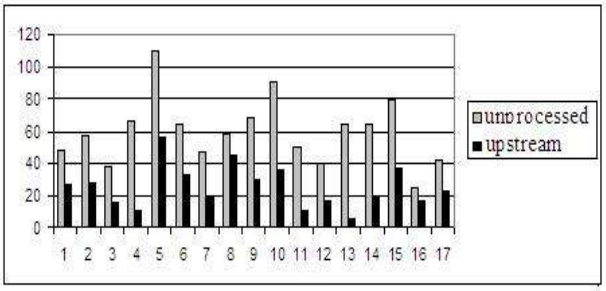

Figure 4. Sperm concentration unprocessed and upstream specimens; $p<0.0001$.

Discussion

Depending on the quality of ejaculates, standard preparation, such as, swim-up and double density gradient methods have different efficiency and applications (6). In these methods, functional spermatozoa can come into close cell-to-cell contact with defective sperms or leukocytes by centrifugation. Thus, causing massive oxidative damages of the sperm plasma membrane by ROS and consequently of sperm functions (9, 10). Therefore, the quality of the ejaculates has direct consequences on the choice of sperm separation method. The deleterious effect of free radicals or ROS upon sperm function and their role in the aetiology of male infertility was originally described (9, 10).

Recently new sperm separation methods such as flow cytometric, magnetic cell separation and electrophoretic methods are introduced (15-18). The ideal sperm separation technique should (i) be quick, easy and cost-effective, (ii) isolate as much

motile spermatozoa as possible, (iii) not cause sperm damage or non-physiological alterations of the separated sperm cells, (iv) eliminate dead spermatozoa and other cells, including leukocytes and bacteria, (v) eliminate toxic or bioactive substances like decapacitation factors or reactive oxygen species (ROS), and (vi) allow processing of larger volumes of ejaculates (6).

Since none of the methods available meets all these requirements, swim-up and density gradient are mandatory in clinical practice to obtain an optimal yield of functionally competent spermatozoa for insemination purposes (6) and attempt for developing new methods to be continued. Our data showed that upstream method can enrich the sperm population by separating normal motile sperm for using in ART and IUI procedures.

This study confirm our previous suggestion that swimming upstream phenomenon of motile sperm acts as a natural selection mechanism for normal motile sperms. In addition upstream is simple, and does not require any centrifugation step or chemicals. However, more studies are needed to measure the levels of ROS and also DNA integrity of isolated sperms.

Acknowledgment

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