Preparation of Human Spermatozoa Using Glass Wool Filtration Technique Versus Centrifugation Swim-Up Technique for Asthenozoospermia

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Abstract:

Background:

Semen preparation techniques were developed to separate motile sperm that are morphologically normal from seminal plasma to optimize successful assisted reproductive technology cycles which seem to be the most effective options in cases of a male factor infertility.

Objective:

The objective of the present study was to compare between outcomes of two sperm preparation techniques for asthenozoospermic patients includes:1-Centrifugation swim up technique.2-Glass wool filtration technique.

Subjects, Materials and Methods:

Fifty three infertile males were participated in this study during their attendance to the Infertility Clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies; Al- Nahrain University. Semen samples were collected and SFA was done according to WHO (2010 and 1999). Each semen sample was divided into two aliquots. The first one prepared using centrifugation swim up technique, while the other one prepared using glass wool filtration technique then sperm parameters were assessed for both techniques and the results were statistically analyzed.

Results:

After *in vitro* sperm activation using both techniques, there was significant reduction ($P<0.05$) in the sperm concentration, significant improvement ($P<0.05$) in the percentages of sperm motility and morphologically normal sperm when compared to pre-activation. Present study appeared that the glass wool filtration technique resulted in significantly ($P<0.05$) better results for sperm concentration and total number of progressive motile sperm than the swim-up technique.

The present study proved that there was significant ($P<0.05$) improvement in sperm parameters (increment for sperm motility (%), progressive sperm motility (%), total number of progressive motile sperm and normal sperm morphology (%), while reduction for round cell count and sperm agglutination percent) for all cases using glass wool filtration techniques. In contrast, there was failure of sperm activation for 10 cases using centrifugation swim up technique.

Conclusions:

From results of the present study, the sperm parameters outcomes using glass wool filtration technique was superior to the outcomes of centrifugation swim up technique when prepare semen of asthenozoospermic patients.

Key words: Glass Wool Filtration Technique, Centrifugation Swim up Technique, *In vitro* sperm activation.

Introduction:

Infertility affects 15% of couples worldwide, and about 50% of affected couples have male factor infertility (MFI) [1,2]. Despite the identification of many congenital and acquired factors in the etiology of male infertility, the frequency of unexplained cases has increased steadily [3]. Semen analysis (SA) is the initial and most essential step of the infertility evaluation. It is also considered a cornerstone of the laboratory evaluation of the infertile male [4]. Asthenozoospermia, a disorder of sperm motility, is a cause of human male infertility and is implicated in 19% of infertile cases [5]. Isolated asthenozoospermia is found in 24% of infertile men [6], which may be caused by sperm dysfunction, prolonged periods of sexual abstinence, partial blockage of seminal tract, varicocele, infection or genetic factors[7,8]. However, some cases of asthenozoospermia could be idiopathic; namely, no definitive etiology is identifiable by using routine medical tests [9]. Selection of highly motile sperm is the key step to optimize successful ART cycle, thereby determining fertilization rates for ongoing pregnancies [10]. As a result, semen preparation techniques were developed to separate motile sperm that are morphologically normal from seminal plasma [11].

Moreover, many sperm preparation techniques were created such as density gradient centrifugation, centrifugation swim up and glass wool filtration [12]. Therefore, the objective of the present study was the evaluation of two sperm preparation techniques for asthenozoospermic patients including:

1. Centrifugation swim up technique.
2. Glass wool filtration technique.

Subjects, Materials and Methods:

Patients:

Fifty three asthenozoospermic infertile males were participated in this study during their attendance to the Infertility Clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies; Al-Nahrain University.

Seminal fluid analysis

The sample of seminal fluid was collected after 3-5 days of sexual abstinence directly into a clean, dry and sterile disposable Petri-dish by masturbation in a private and quiet room adjacent to the semen analysis laboratory. The container must be labeled with the following information, name, age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37 ºC for 30 minutes to allow liquefaction. The liquefied semen is then carefully mixed for few seconds, and then the specimen was examined by macroscopic and microscopic examinations. The standard form of (WHO 2010 and 1999) [13,14] is used to record the results of seminal fluid analysis . Seminal fluid analysis involving macroscopic and microscopic examinations. Macroscopic parameters were semen volume, liquefaction time, viscosity and acidity (pH). Microscopic parameters of spermatozoa were sperm concentration, sperm motility (%), progressive sperm motility (%), normal sperm morphology (%), sperm agglutination (%), round cells count.

In vitro sperm activation techniques:

Two methods of *In vitro* sperm activation have been used in this study.

Centrifugation swim-up technique

This method was used for the patients by mixing 1mL of liquefied semen gently with 1mL of SMART (simple media for assisted reproductive technology) culture

medium in falcon tube, and then put the mixture in centrifuge at 2300 rpm for 6 minutes. Then, the supernatant was discarded and 1mL of culture medium was added to the pellet carefully and put in the incubator again for 30 minutes period. A drop of 10µL was taken and put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters.

Glass wool filtration (GWF) technique

Glass wool column were prepared by gently inserting glass wool into the barrel of a one mL syringe, and compressed to a final thickness of 3 mm. The column was then rinsed with 1mL of medium. Prior to GWF, 1mL semen was diluted with one mL SMART medium and mixed gently. Following dilution, the semen suspension was centrifuged for 6 min at 2300 rpm. Supernatant was removed and 1mL of SMART medium was added then left for 8-10 min after that the solution was aspirated. The washed sperm suspension was placed gently over the wet glass wool and allowed to filter by gravity. After the first three drops were discarded, the remaining filtrate was collected and analyzed for sperm parameters.

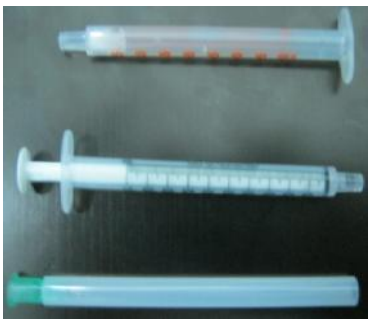

Figure 1: Glass wool tools.
(A= Glass wool syringe, B= insulin syringe, C= Glass wool needle)

Statistical analysis:

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago). Sperm parameters, pre and post activation assay were analyzed using complete randomized design (CRD) (one way ANOVA). Differences among means were compared using the Duncan multiple ranges test [15].

Results:

Table (1) shows sperm parameters of pre- and post-*in vitro* sperm activation for 43 asthenozoospermic infertile males using centrifugation swim up and glass wool filtration techniques in which centrifugation swim up was succeed. There was a significant decrease ($P<0.05$) in sperm concentration, agglutination (%) and round cell count. In contrast, there was a significant increase ($P<0.05$) in the percentages of sperm motility, progressive sperm motility, total number of progressive motile sperm and normal sperm morphology following *in vitro* sperm activation by both techniques. From same table can noticed that there was significant increase ($P<0.05$) in sperm concentration and total number of progressive motile sperm for glass wool filtration technique as compared to swim up technique. In contrast, a significant increase ($P<0.05$) in sperm motility (%) and progressive sperm motility (%) for centrifugation swim up technique as compared to glass wool filtration technique.

For normal sperm morphology (%), there was significant increment ($P<0.05$) for swim up technique in comparison to glass wool filtration technique. Significant decrease ($p<0.05$) in round cell count for swim up technique compared to glass wool filtration technique was recorded. However, there was no significant difference ($P<0.05$) between two techniques for the sperm agglutination (%) in the present study.

Table (2) showed that *in vitro* activation of human sperms for 10 asthenozoospermic infertile males using

glass wool filtration technique, whereas swim up technique was failed. Significant decline (P<0.05) in the sperm concentration compared to pre-activation. The percentages of sperm motility, progressive sperm motility, total number of progressive motile sperm and normal sperm morphology were significantly increased (P<0.05) than that pre- activation. The number of round cells (cell/HPF) and sperm agglutination (%) were significantly reduced (P<0.05) after *in vitro* sperm activation using glass wool filtration technique.

Table 1: Sperm parameters for asthenozoospermic infertile males pre- and post- *in vitro* sperm activation using glass wool filtration and centrifugation swim up techniques with successful outcome post-activation.

Sperm Parameters		Pre-activation	Post-activation	
			Glass wool	Swim up
Sperm concentration(millions/mL)		25.900 a ±2.88	13.600 b ±1.42	–
Sperm motility (%)		32.700 b ±4.23	79.500 a ±1.74	–
Sperm grade activity (%)	Progressive sperm motility (%)	8.100 b ±2.88	61.000 a ±4.00	–
	Non Progressive sperm motility (%)	24.600 a ±2.23	18.500 b ±2.89	
	Immotile sperm (%)	67.300 a ±4.23	20.500 b ±1.74	–
Total Progressive sperm (millions/ejaculate)		1.879 b ±0.58	8.200 a ±0.88	–
Normal sperm morphology (%)		40.400 b ±1.78	64.600 a ±2.31	–
Sperm agglutination (%)		6.200 a ±4.91	0.000 b ±0.00	–
Round cells count (HPF)		8.400 a ±1.35	0.000 b ±0.33	–

- Means with different superscripts within each row are significantly different (P<0.05)
- Means with similar superscipts within each row are nga significantly different (P>0.05).
- Data are mean ± S.E
- Number = 43

Table 2: Sperm parameters for asthenozoospermic infertile males pre- and post- *in vitro* sperm activation using glass wool filtration and centrifugation swim up techniques with failed outcome post-activation for centrifugation swim up technique.

Sperm Parameters		Pre-activation	Post-activation	
			Swim up	Glass wool
Sperm concentration (millions/mL)		63.209 a ±3.39	17.572 c ±0.81	40.326 b ±2.54
Sperm motility (%)		42.721 c ±1.87	93.023 a ±0.49	81.535 b ±0.80
Sperm grade activity (%)	Progressive sperm motility (%)	14.953 c ±1.33	79.791 a ±1.17	68.651 b ±1.20
	Non Progressive sperm motility (%)	27.767 a ±1.13	13.233 c ±0.85	15.884 b ±0.82
	Immotile sperm (%)	57.279 a ±1.87	6.977 c ±0.49	15.349 b ±0.81
Total Progressive sperm (millions/ejaculate)		9.134 c ±0.94	13.841 b ±0.66	27.815 a ±1.85
Normal sperm morphology (%)		39.512 c ±1.14	78.209 a ±0.91	68.279 b ±1.14
Sperm agglutination (%)		4.419 a ±0.89	0.000 b ±0.00	0.000 b ±0.00
Round cells count (HPF)		8.349 a ±0.97	0.000 c ±0.00	0.930 b ±0.21

- Means with different superscripts within each row are significantly different (P<0.05)
- Means with similar superscipts within each row are nga significantly different (P>0.05).
- Data are mean ± S.E
- Number = 10

Discussion:

The present study showed that *in vitro* sperm activation causes significant (P<0.05) reduction in sperm concentration as compared to pre-activation for both methods, this is due to inability of dead and abnormal sperm morphology with poor motility to swim up and migrate into upper layer of culture media, these results were in agreement with Anderson [16], Kouty [17] and Rasheed [18], this is for swim up technique. On the other hand, similar situation in the cervix abnormal or dead spermatozoa are held back by adhesion to glass wool fibers [19].

Furthermore, post activation using different techniques resulted in a significant (P< 0.05) reduction in the count of round cells and sperm agglutination (%) while a significant increment in the percentage of normal sperm morphology, same result achieved from study of Shaaban [20], this is because the sperm preparation techniques for ART have been developed to remove the undesired sperm, round cells, debris, and thereby increase the overall sperm quality [21,22].

The present study proved that there is significant increase (P<0.05) in the percentages of sperm motility and progressive sperm motility using both techniques, these results in agreement with studies of Shajer [23] and

Mohammed-Ali [24]. This is regarded as normal response for sperm activity after removal of seminal plasma since it contain dead sperm, leukocytes, epithelial cells, particulate debris and microbial contamination that produce many oxygen radicals that can negatively influence the sperm functions [25].

Another factor increase sperm motility is the effects of culture medium (CM) composition, that may explain these results including protein source and buffers to promote sperm capacitation and hyperactivation [26]. In this study, SMART medium was used and it was concluded that the SMART medium was suitable for enhancement of sperm parameters of asthenozoospermic patients as certified by Fakhrildin and Flayyih [27] in their study.

The present study proved that the sperm concentration is significantly higher for glass wool filtration technique as compared to centrifugation swim up technique. Also, there was significantly higher ($P<0.05$) results in total number of progressive motile sperm for glass wool filtration technique compared to centrifugation swim up technique.

The present study proved that there was significant ($P<0.05$) improvement in sperm parameters(increment for sperm motility, progressive sperm motility, total number of progressive motile sperm and normal sperm morphology, while reduction for round cell count and sperm agglutination percentage) for all ejaculates studied using glass wool filtration technique. In contrast, there was failure of activation of 10 cases by centrifugation swim up technique.

Furthermore, the present study also proved that the glass wool filtration technique resulted in significantly ($P<0.05$) higher results for sperm concentration and total number of progressive motile sperm than the swim up technique from all types of ejaculates.

The SUP technique relies on the ability of the motile spermatozoa to “swim up” into the culture medium, while slow and immotile sperm remain behind, along with other components in the semen pellet [28]. This technique is distinguished by a very high percentage (>90%) of progressive motile sperm, as the presence of 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 [29].Only a small fraction of total motile sperm is recovered by the SUP methodology, therefore its use is mostly restricted to ejaculates with high sperm counts and good motility [30].

The results of present study are in agreement with the results vander Ven [31] which concluded that the number and viability of spermatozoa recovered by glass wool column filtration and a swim-up procedure were compared using different types of ejaculates, such as normal, asthenozoospermic and very viscous oligozoospermic semen**,** the filtration procedure resulted in significantly higher ($P< 0.01$) recovery of viable spermatozoa than the swim up procedure from all types of ejaculates studied so that glass wool column filtration is superior to the swim-up procedure since it yields a higher recovery of viable spermatozoa. These results also agreed with results done by Coetzee [32] and Arzondo [33] which concluded that the glass wool filter procedure consistently produced significantly ($P < 0.05$) higher viable sperm concentrations and progressively motile sperms than swim up procedure.

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