A New Sperm Preparation Technique by Glass Wool Filtration Combined with Pentoxifylline Techniques versus Glass Wool Filtration alone for Infertile and Fertile Men

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Abstract:

Background:

The procedures of assisted reproductive technologies have been developed during recent years and have been revealed the need for new suitable and effective techniques of sperm treatment in the laboratory.

Objectives:

This study is designed to normozoospermic and asthenozoospermic compare the semen outcomes of two *in vitro* sperm activation techniques and evaluate their efficiency, namely; Glass wool filtration technique and a new technique of Glass wool filtration combined with Pentoxifylline.

Subjects, Materials and Methods:

Fifty five semen samples were collected from the patients, fifteen were obtained from normozoospermic men, forty were obtained from asthenozoospermic men who at tend the infertility clinic were involved in this study. Sperm parameters were assessed according to WHO (2010 and 1999). Post- activation of each sample were divided into two aliquots, the first one : using Glass wool filtration technique , the second : using Glass wool filtration technique with Pentoxifylline.

Results:

A significant increase of sperm motility, progressive sperm motility and morphologically normal sperm when using Glass wool filtration technique combined with Pentoxifylline as compared to Glass wool filtration technique. Regarding sperm agglutination and Round cells count the study showed the best results by decreasing their levels when using Glass wool filtration technique combined with Pentoxifylline as compared to Glass wool filtration technique alone.

Conclusions:

From the results of the present study, the sperm function parameters outcome when using glass wool filtration technique combined with Pentoxifylline were superior to other technique since it yields better sperm function parameters rather than the outcome of glass wool filtration ; respectively especially when using a low quality of semen samples such as decreased sperm function parameters (sperm motility) so it was fit in cases of asthenozoospermia which taken as an example of infertile group within this study.

Keywords: Glass Wool Filtration, Pentoxifylline, Sperm preparation techniques.

Introduction:

Infertility is defined as the inability to become pregnant after 12 months of regular, unprotected intercourse. In a survey from 2006 to 2010, more than 1.5 million U.S. women, or 6% of the married population 15 to 44 years of age, reported infertility, and 6.7 million women reported impaired ability to get pregnant or carry a baby to term [1]. Among couples 15 to 44 years of age, nearly 7 million have used infertility services at some point [2] . This encompasses couples with infertility and impaired ability to get pregnant, but it does not capture those who are not married, so actual numbers may be underestimated. These numbers are comparable to those of other industrialized nations [3,4] . Infertility may arise from male factors, female factors, or a combination of these factors [5] .

Assisted reproductive technologies (ART's) consist of develop into the treatment of option in many cases for male and female infertility. The class of semen samples is one of the factors determining the winning assisted reproduction [6], for that reason, the perfect sperm preparation technique is to reach the largest number of motile spermatozoa, morphologically normal, in a little volume of physiological culture media from seminal plasma, leukocytes , bacteria and debris [7]. With the advancement in the techniques of assisted reproduction, the need to improve sperm processing methods and provision of actively motile spermatozoa has increased tremendously [8]. Glass wool filtration (GWF) technique separate motile spermatozoa from other contents of semen by filtration through densely packed glass wool fibers (9). Nonviable spermatozoa adhere to the matrix more than motile functional spermatozoa [10]. Immotile and dead spermatozoa may agglomerate because of changes in surface charges [11].

The principle of GWF technique is rested on the self-propelled movement of the spermatozoa and filtration effect of the glass wool fibers [12]. A major advantage of this approach is the selection of normally chromatin- condensed spermatozoa, a parameter considered as predictive for fertilization ability *in vitro*. The GWF technique is very simple but it is a more expensive procedure [13]. The filtration method is useful for eliminating leukocytes major sources of Reactive Oxygen Species (ROS) and selecting motile and morphologically normal spermatozoa [14].

Pentoxifylline(PX) is a methylxanthine derivative in the same pharmacologic group as caffeine. The PX belongs to a group of vasoactive drugs used in human for treatments of peripheral and cerebral vascular diseases caused by impairment of microcirculation [15]. The PX is also used in treatment of male infertility in human by enhancing sperm motility both *in vivo* [16] and *in vitro* [17]. In previous experimental as well as clinical studies, the role of *in vitro* application of PX on motility of spermatozoa retrieved from different sources of ejaculate, epididymis, and testis were investigated [18]. Pentoxifylline is phosphodiesterase inhibitor that inhibits the breakdown of cyclic adenosine monophosphate (cAMP) [19]. Asthenozoospermia is one of the major causes of infertility or reduced fertility in men [20]. Asthenozoospermia, is defined as ‘total motility’ (progressive + non-progressive) less than 40% or progressive motility less than 32% (WHO, 2010) [21]. Sperm motility is a critical indicator of semen quality and fertility potential because the sperm motility is required for the penetration of cervical mucus, transport

through the female genital tract and penetration through the corona radiate and zona pellucida before oocyte fertilization [22].

Subjects, Materials and Methods:

Fifty five semen samples were collected from the patients, fifteen were obtained by masturbation from normozoospermic men, forty were obtained by masturbation from asthenozoospermic men who at tend the infertility clinic were involved participated in this study during their attendance to the infertility clinics at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University . Semen samples were collected and semen analysis was done according to WHO (2010) and (1999). Each semen sample was divided into two aliquots. The first one using the Glass wool filtration(GWF) technique, the second using, Glass wool filtration technique with Pentoxifylline(GWF+PX) (combination technique), then sperm parameters were assessed for these technique and the results were statistically analyzed.

Glass wool filtration technique with Pentoxifylline (combination technique):

As a new sperm preparation technique was performed as the following, adding 1mL of Add 1mL of FertiCult Flushing medium + PX to1mL liquefied semen sample then Centrifugation at 2000 rpm for 5 minute and remove supernatant and add 1mL of FertiCult Flushing medium + PX to pellet. Shaking the sample then left for 8-10 minutes in an incubator, after that the semen suspension placed gently over the wet glass wool syringe and allowed to filter by gravity. A drop of 10µL was aspirated, put on a slide with cover slip and examined under the microscope at 400X objective to assess the sperm parameters as recommended by WHO (2010) and (1999).

Statistical Analysis:

The Statistical Analysis System-SAS (2016) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) or T-Test was used to significant compare between means. Estimate of Correlation coefficient between parameters in this study**.**

Results:

The present study showed a significant decrease in the certain sperm parameters (sperm concentration, sperm agglutination and round cells count) during post-*in vitro* sperm activation when using these four techniques as compared to pre-activation. While, a significant increase in the other certain sperm parameters (sperm motility, progressive sperm motility and morphologically normal sperm) during post –*in vitro* sperm activation when using these four activation techniques as compared to pre-activation. Table 1 showed the sperm function parameters for infertile men with asthenozoospermia (n=40), during pre- and post-*in vitro* sperm activation techniques. The same table showed a significant decrease for the concentration of sperm when using Glass wool filtration combined with Pentoxifylline technique as compared with pre-activation. In contrast, table (1) showed significant increase in the certain sperm parameters (sperm motility, progressive sperm motility and

morphologically normal sperm) when using Glass wool filtration combined with Pentoxifylline as compared with the Glass wool filtration techniques only. Table 1 also showed non- significant difference for the sperm agglutination and round cells count among these activation techniques.

Table (1): Sperm Function Parameters for asthenozoospermic infertile men pre- and post- *in vitro* sperm activation.

Certain sperm function parameters		Before activation	After *in vitro* activation		LSD value	P-value
			Glass wool M ± S.E.	Glass wool+px M ± S.E.		
Sperm concentration value (m/ml)		44.40 ± 1.79 a	39.25 ± 2.11 ab	39.78 ± 1.74 ab	6.073 **	0.001
Progressive sperm motility (%)	Grade A	10.78 ± 0.52 d	26.87 ± 0.94 b	35.37 ± 1.06 a	6.158 **	0.001
	Grade B	29.95 ± 0.87 b	45.27 ± 2.28 a	41.32 ± 1.74 a	6.733 **	0.001
	Grade A+B	40.73 ± 1.53 c	72.27 ± 3.41 a	76.70 ± 3.76 a	7.165 **	0.001
Non progres sperm motility (%)	Grade C	38.18 ± 2.38 a	18.50 ± 0.89 c	16.67 ± 0.63 c	6.738 **	0.001
Immotile sperm (%)	Grade D	21.10 ± 0.82 a	9.23 ± 0.43 c	6.50 ± 0.18 c	4.064 **	0.001
Morphologically Normal Sperm (%)		36.98 ± 1.57 d	58.62 ± 2.26 b	66.63 ± 2.77 a	5.983 **	0.001
Sperm agglutination (%)		6.30 ± 0.17 a	0.125 ± 0.05 c	0.050 ± 0.03 c	1.419 **	0.001
Round cell count (HPF)		8.275 ± 0.16 a	0.625 ± 0.07 c	0.625 ± 0.07 c	2.194 **	0.001

- Data are Mean(M) ± Standard Error (S.E.)
- Number = 40

Table (2) can clarify sperm parameters for normozoospermic males (No.15:15%) pre- and post-*in vitro* sperm activation techniques. It was noticed that significant increase post-activation for certain sperm parameters (progressive sperm motility grade A, progressive motility grade B, sperm motility Grade A+ Grade B (GA+GB),and morphologically normal sperm) On the other hand, significant reduction (Sperm concentration, non-progressive sperm motility Grade C (GC), immotile sperm Grade D(GD), sperm agglutination and round cells count) when using these two techniques as compared with pre-activation as shown in table [(2)].

Table 2: Sperm Function Parametersfor normozoospermic fertile men pre- and post- *in vitro* sperm activation.

Certain sperm function parameters		Before activation	After in vitro activation		LSD value	P-value
			Glass wool M ± S.E	Glass wool+px M ± S.E		
Sperm concentration value (m/ml)		56.00 ± 2.13 a	45.67 ± 2.07 bc	47.33 ± 1.95 b	6.314 **	0.001
Progressive sperm motility (%)	Grade A	20.33 ± 0.78 c	36.67 ± 1.66 b	43.00 ± 2.19 a	6.309 **	0.001
	Grade B	37.00 ± 1.16 c	43.67 ± 1.52 ab	45.67 ± 2.09 a	5.875 **	0.001
	Grade A+B	57.33 ± 2.68 e	80.33 ± 3.96 b	88.67 ± 3.62 a	7.042 **	0.001
Non progres sperm motility (%)	Grade C	26.67 ± 1.03 a	9.67 ± 0.38 c	7.33 ± 0.25 c	5.311 **	0.001
Immotile sperm (%)	Grade D	16.00 ± 0.74 a	7.67 ± 0.50 c	4.00 ± 0.07 d	3.258 **	0.001
Morphologically Normal Sperm (%)		43.33 ± 2.06 d	70.73 ± 3.09 b	78.00 ± 2.82 a	6.302 **	0.001
Sperm agglutination (%)		4.067 ± 0.33 a	0.00 ± 0.00 c	0.00 ± 0.00 c	1.073 **	0.001
Round cell count (HPF)		8.067 ± 0.26 a	1.333 ± 0.16 c	1.067 ± 0.15 c	2.0671 **	0.001

- Data are Mean(M) ± Standard Error(S.E.)
- Number = 15

Discussion:

Sperm preparation techniques are a very important component of assisted reproductive technologies [23] in the meantime, enhancement in the sperm parameters improved the sperm fertilizing capacity and outcomes of ART's [24-25]significantly thought of sperm separation techniques is to the treat spermatozoa *in vitro* in order to improve their functionality i.e. motility and provide a protective environment with the purpose to maintain or get better their functional capacity for winning fertilization. An advance in the percentages of sperm motility and progressive sperm motility is regarded as normal reply for sperm activity after exclusion of seminal plasma since it contain dead sperm, leukocytes, epithelial cells, debris and microbial contamination that produce many oxygen radicals so as to can negatively influence the sperm functions[26].

In the glass wool filtration technique and glass wool filtration (GWF) combined with Pentoxifylline there are lower decrease in the sperm concentration compared with before activation. These results were in agreement with other studies using culture medium for the separation and activation of sperm *in vitro* which based on the existence of the higher percentage of good motile sperm in these two methods compared with other methods The current study clarified that significant increase for the progressive sperm motility after when using GWF+PX technique as compared to the GWF techniques. Also, this study clarified that a significant increase for the same parameter post-activation when using the GWF technique as compared to the pre-activation [27-30]. The sperm motility parameters within the techniques of Glass wool filtration alone or with combination with Pentoxifylline showed that the sperm motility Grade A+B were significantly improved in comparison to other techniques, while there were a decrease in the poor sperm motility Grades(C,D). These results were similar to the findings that reported the main improving role of Glass wool filtration which based mainly on the reduction process of dead immotile spermatozoa that are held back by adhesion to glass wool fibers[31]. Sperm morphology is considered as a sensitive indicator of overall testicular health, because the sperm morphological characteristics are determined during spermatogenesis The present study clarified that a significant increase in the normal sperm morphology when using GWF+PX technique as compared to the GWF techniques. Significant increase for the same parameter when using GWF technique as compared to the pre-activation [32]. Also plays a crucial role in the diagnosis of male fertility potential and it has demonstrated a predictive value for fertilization and pregnancy outcomes in IVF [33].

Conclusions:

From the results of the present study, the sperm function parameters outcome when using glass wool filtration technique combined with Pentoxifylline were superior to other technique since it yields better sperm function parameters rather than the outcome of glass wool filtration ; respectively especially when using a low quality of semen samples such as decreased sperm function parameters (sperm motility) so it was fit in cases of asthenozoospermia which taken as an example of infertile group within this study.

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