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Assessment Quality of Selected Sperm During Glass Wool and Sephadex Filtration Techniques in Infertile Men

Reem Q. M. Wafeeq, Hayder A. L. Mossa*

High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.
Corresponding Author Email: *haydermossa@googlemail.com*

Abstract

Infertility has been defined as the disability to fulfill pregnancy after twelve months of regular sex coition, with no contraceptive taken. Male factor infertility was explained in terms of low spermatozoa concentration (oligozoospermia), or morphology (teratozoospermia), impaired motility (asthenozoospermia), or in some cases, total lack of spermatozoa in the ejaculate (azoospermia). In several cases, a collection of one, or more of these sperm variables defects may be noticed, evaluation these factors by the initial and most important step is seminal analysis. Our study aims to study some sperm characteristics in asthenozoospermia men in comparison with normozoospermia men before and after glass wool and Sephadex activation. This study involved 60 semen samples collected from male patients that came to male infertility clinic at Al-Nahrain University; the recruited semen samples were divided into 2 groups, (40 asthenozoospermic and 20 normozoospermic subjects). After collected semen samples, and assessed analysis of seminal fluid. Each semen samples were divided into 3 aliquots. The first aliquot prepared was *In-Vitro* to assessed sperm characterization before activation, the second part using glass wool filtration (GWF) technique, while the last part was prepared using Sephadex. Both techniques resulted equally in reducing sperm concentration, were equally effective in upgrading sperm motility and in minimizing round cell count; however, Sephadex was superior to glass wool method in upgrading the percentage of morphologically normal sperm. Both Sephadex and glass wool techniques has been proved effective to improve semen quality.

Keywords: Sephadex, Asthenozoospermia, Sperm Preparation Technique

1. Introduction

Infertility has been classified as one of the master issues in medical science, it has been known as the disability to fulfill pregnancy after twelve months of steady sex copulation with no contraceptive taken, which affects 15% of reproductive-aged couples (Choy and Eisenberg [1]). It was about twenty percent of infertility cases were mainly caused by male-factor, and also (30-40)% of status including female and male factors; subsequently, male infertility is present in 50% of infertile couples (PCASRM [2]). Conventionally, male factor infertility was explained in terms of low spermatozoa concentration (oligozoospermia), or morphology (teratozoospermia), impaired motility (asthenozoospermia), or in some cases, total lack of spermatozoa in the ejaculate (azoospermia) (Who [3]). In several cases, a collection of one, two or more of these sperm variables defects may be noticed, evaluation of these factors by the initial and the most important step is seminal analysis (Barrat, et al. [4]). Many of sperm preparation techniques have been developed in ART's, these techniques can be classified into 3 groups: migration of sperm method (swim-up), density gradient centrifugation method and Adherence column method (Sephadex column filtration and glass wool column filtration) (ESHRE Guideline Group on Good Practice in IVF Labs, et al. [5]). These groups have been developed to detach motile sperm from seminal fluid and to achieve the largest number of morphologically normal and free from for seminal plasma, bacteria, leukocytes, agglutination and aggregation (Kiratli, et al. [6]). The principle of

glass wool filtration (GWF) technique is rested on the self-pushed the spermatozoa movement and effect of the glass wool fibers (Kadhim, et al. [7], Kadhim, et al. [8]). The main feature of this filtration is the selection of normal spermatozoa with good quality, considered as a parameter that predictive for fertilization ability in vitro. The GWF technique is very simple, but it is a more expensive procedure (Henkel, et al. [9]). Some debris was usually still present in the sample after the GWF (Beydola, et al. [10]). While, filtration through Sephadex has depended on the capacity of the spermatozoa motion and also the ability that interaction with the filter substrates, Sephadex beads or membrane pores (Henkel and Schill [11]). It has been well thought out that non-motile sperm stickier to the matrix than motile cells. Sephadex permits non-motile and lifeless sperm to agglomerate because of surface charges were changed, or a specific protein found on lifeless sperm that sticky to the particles of Sephadex (Husna, et al. [12]).

2. Materials and Methods

A total of 60 semen samples collected from infertile males were involved in this study, they were separated into 2 groups, (40: asthenozoospermia and 20: normozoospermia) during their visits to the male infertility clinic at High Institute for Infertility Diagnosis and Assisted Reproductive Technologies; Al-Nahrain University. The seminal fluid analysis was assessed, and each semen sample was divided into 3 aliquots. The first aliquot was prepared for sperm characterization assessment before activation, the second aliquot using the GWF technique and assessment of sperm characterization after activation,

while the last aliquot was prepared using Sephadex with an evaluation of sperm characterization after activation.

2.1 Glass wool filtration (GWF) technique

Glass wool (GW) gently inserted inside one mL disposable syringe and compacted to a final thickness of 3 mm, was commercially available (Srivastava, et al. [13]). Before the GWF technique, 1mL of seminal fluid was diluted with one mL of FertiCult Flushing medium and mixed gently. Following dilution, the semen suspension was centrifuged for 10 minutes at 2500 rpm. The supernatant was removed and 1mL of FertiCult medium was added then left for 15-20 minutes after that the solution was aspirated. The glass wool was then rinsed with 1mL of the medium, then washed sperm suspension was located quietly over the wet glass wool and allowed to filter by gravity. A drop of 10 µL was aspirated and put on a slide with cover-slip and examined under the microscope at 400X objective to assess the sperm parameters.

2.2 Sephadex G – 25 filtration technique

Glass wool gently inserting inside 5 mL syringe and compressed to adopter of the syringe and then one ml of Sephadex was added to disposable syringe. Prior to Sephadex filtration technique, 1mL semen was diluted with one mL of FertiCult Flushing medium and mixed gently. Following dilution, the semen suspension was centrifuged for 10 minutes at 2500 rpm. The supernatant was removed and 1 mL of FertiCult medium was added then left for 15-20 minutes after that the solution was aspirated. The Sephadex column was rinsed with 1 mL of the medium, then

washed sperm suspension was placed gently over the Sephadex column and allowed to filter by gravity. A drop of 10 µL was aspirated and placed on a slide with a coverslip and scanned under the microscope at 40X or 400X objective to measure the sperm parameters.

3. Results

3.1 Sperm characteristic in normozoospermia group before and after activation

Sperm characteristics of normozoospermia group before and after activation where contrasted statistically and the results were shown in ***Table (1)***. Sperm concentration decreased significantly following treatment with glass wool or Sephadex and there was no significant variance in mean sperm concentration between glass wool and Sephadex treatment ($P > 0.05$), ***Table (1)***. There was no significant difference in mean grade A % ($P > 0.05$), significant rise in grade B % ($P < 0.05$) without significant difference following glass wool and Sephadex activation ($P > 0.05$); in addition to significant reduction in both grade C and D % ($P < 0.05$); however, there was no significant difference following treatment with either glass wool or Sephadex ($P > 0.05$), ***Table (1)***. Mean morphologically normal sperm percentage (%) became significantly higher following treatment with either glass wool or Sephadex ($P < 0.05$); and the effect of Sephadex was more significant ($P < 0.05$), ***Table (1)***. Round cells and sperm agglutination were totally reduced with either glass wool or Sephadex, ***Table (1)***.

3.2 Sperm characteristics in asthenozoospermia group before and after activation

Sperm characteristics of asthenozoospermia group before and after activation where contrasted statistically and the results were shown in ***Table (2)***. Sperm concentration decreased significantly following treatment with glass wool or Sephadex and there was no significant difference in mean sperm concentration between glass wool and Sephadex treatment ($P > 0.05$), ***Table (2)***. Grade A sperms were obtained following treatment with either glass wool or Sephadex and the difference between both methods was insignificant ($P > 0.05$), a significant rise in grade B ($P < 0.05$) without significant difference following glass wool and Sephadex activation ($P > 0.05$). In addition to a significant reduction in both grade C ($P < 0.05$); however, without significant difference following treatment with either glass wool or Sephadex ($P > 0.05$). Moreover, there was a significant reduction in grade D% ($P < 0.05$); Sephadex was significantly better than glass wool with this regard ($P > 0.05$) ***Table (2)***. Mean morphologically normal sperm % became significantly higher following treatment with either glass wool or Sephadex ($P < 0.05$); and the effect of Sephadex was more significant ($P < 0.05$), ***Table (2)***. Round cells and sperm agglutination were totally reduced with either glass wool or Sephadex, ***Table (2)***.

4. Discussion

In the present study, no significant difference in mean sperm concentration between normozo-ospermia and asthenozoospermia before activation, respectively; however, in terms of sperm motility,

grade A% and B% sperm were significantly lower and grade C% and D% sperm were significantly higher in patients with asthenozoospermia in comparison with normozoospermia. In addition, there was no significant difference in morphologically normal sperm % and in round cell count between both groups. Indeed, the lack of difference in sperm concentration between both groups is self-explanatory because the main defect in patients with ashenozoospermia is in the sperm motility rather than in sperm concentration (Liu, et al. [14], Guo, et al. [15]). Some cases of asthenozoopermia may be associated with oligozoospermia (Curi, et al. [16]). However, in the present study, only patients complained of pure asthenozoospermia were included. On the other hand, it has been documented that the coexistence of morphologically abnormal sperm in combination with abnormal motility (asthenoteratozoospermia) is frequent in infertile men (Hristova, et al. [17], Jenkins, et al. [18]). However, in the present study, infertile men with pure asthenozoospermia were recruited. Regarding the comparative study of the mentioned parameters in relevance to treatment with glass wool and Sephadex there are no previous researches discussed the mentioned parameters. Sperm concentration decreased signifi-cantly following treatment with glass wool or Sephadex and there was no significant difference in mean sperm concentration between glass wool and Sephadex treatment. Grade A sperms were obtained following treatment with either glass wool or Sephadex and the difference between both methods was insignificant, significant rise

Table (1): *Sperm characteristics of normozoospermia group before and after activation*

Characteristics	Before	Glass wool	Sephadex
Sperm concentration (m/ml)	58.25 ±10.79 A	30.20 ±7.20 B	30.40 ±6.49 B
Sperm Motility Grade A%	25.00 ±0.00 B	34.25 ±6.34 B	35.35 ±5.36 B
Sperm Motility Grade B%	38.75 ±4.55 B	48.75 ±7.76 A	48.90 ±9.12 A
Sperm Motility Grade C%	18.50 ±4.06 A	13.75 ±6.26 B	13.25 ±6.13 B
Sperm Motility Grade D%	17.75 ±4.18 A	3.50 ±2.86 B	2.75 ±3.02 B
Morphologically Normal Sperm %	37.65 ±5.71 C	61.05 ±6.24 B	70.00 ±5.13 A
Round cells	5.55 ±4.44 A	---	---
Sperm agglutination%	0.0 (0.0)	---	---

Data were expressed as mean standard deviation or median (inter-quartile range); Comparison was carried out using one-way ANOVA followed by post hoc LSD test; Capital letters were used to indicate level of significance; similar letters indicate no significant difference at $P \leq 0.05$ whereas, different letters indicate significant difference at $P \leq 0.05$; letter A is the highest value.

Table (2): *Sperm characteristics of asthenozoospermia group before and after activation*

Characteristics	Before	Glass wool	Sephadex
Sperm concentration (m/ml)	54.15 ±13.01 A	24.88 ±7.09 B	24.98 ±6.50 B
Sperm Motility Grade A%	---	20.83 ±6.77 B	22.33 ±6.53 A
Sperm Motility Grade B%	35.63 ±4.41 B	54.18 ±6.68 A	56.00 ±5.33 A
Sperm Motility Grade C%	33.43 ±3.75 A	21.75 ±6.46 B	19.80 ±7.31 B
Sperm Motility Grade D%	30.95 ±4.72 A	3.00 ±2.73 B	2.13 ±2.50 C
Morphologically normal sperm %	34.63 ±6.79 C	58.38 ±7.54 B	65.63 ±5.90 A
Round cells	5.78 ±2.66 A	---	---
Sperm agglutination%	1.88 ±4.63 A	---	---

Data were explained as mean standard deviation or median (inter-quartile range); Comparison was carried out using one-way ANOVA followed by post hoc LSD test; Capital letters were used to indicate level of significance; similar letters indicate not at all significant difference at $P \leq 0.05$ whereas, different letters indicate significant difference at $P \leq 0.05$; letter A is the highest value

in grade B without significant difference following glass wool and Sephadex activation; in addition to a significant reduction in grade C; however, there was no significant difference following treatment with either glass wool or Sephadex. Moreover, there was a significant reduction in grade D%; Sephadex was significantly better than glass wool with this regard. Mean morphologically sperm % became significantly higher following treatment with either glass wool or Sephadex, and the effect of Sephadex was more significant. Round cells and sperm agglutination were lacking with either glass wool or Sephadex. Agglutination of sperms is one of the obstacles that reduce natural male fertility due to interference with normal sperm motility and passage of sperm through the cervical mucus, and interference with zonal binding and passage (Vasan, SS [19]). The result of the current study agrees with the findings of some experimental studies that have shown equally effective role for both Sephadex and glass wool sperm preparation techniques in upgrading the proportion of progressively motile sperm with no significant difference between both methods (Lee, et al. [20]). Other experimental studies showed that Sephadex was superior to glass wool technique in obtaining better sperm quality in terms of progressive motility ([21]). Therefore, in the present study, both techniques resulted equally in reducing sperm concentration, were equally effective in upgrading sperm motility and in minimizing round cell count. However, Sephadex was superior to glass wool method in upgrading the percentage of morphologically normal sperm.

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Author Contribution

This research was done by Dr. Reem Q. M. Wafeeq as a part of her Ph.D. thesis under the supervision of Assist. Prof. Dr. Hayder A. L. Mossa (corresponding author).

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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Biography

Reem Qays Mohammed Wafeeq

Born in Baghdad in 1991, received the B.Sc. of Biology - College of Science, University of Baghdad in 2013, and the M.Sc. of Applied Embryology 2019 with excellence. She worked in the field of companies, scientific and medical offices, she participated in many conferences, seminars, training courses, and workshops, especially related to the field of Assisted Reproductive Technologies and Applied Embryology. She is a Member of Organization of Doctors Without Borders and a Member of the Iraqi Fertility Society.

Dr. Hayder A. L. Mossa

He is an assistant professor at Al Nahrain University. He is a member of Iraqi Society of Fertility, Iraqi Society of Zoonotic Diseases (ISZD) & Iraqi Zoonotic Communicable Diseases (ISZCD), Iraqi Academics Syndicate, European Society of Human Reproduction and Embryology (ESHRE). An editorial board member of Merit Research Journal of Medicine and Medical Sciences (MRJMMS), International Journal of Medical Research & Health Sciences

(IJMRHS). A reviewer at global journals Inc. (USA). He was a candidate for award of Arabian Young Researchers in 2007. He granted for many appreciation certificates in the scientific & administrative fields. He participated & supervised in more than 60 scientific & medical conferences, workshops & training course. Has many kinds of research & articles published in Iraqi & national medical journals. Chief & member of nearly 60 scientific & administrative committees from 2006-till now. Has interests in assisted reproductive technologies, reproductive micromanipulation, medical terminology, research methods and seminars, reproductive physiology, advanced embryology.

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