College of Medicine

Diyala University

Study of effect centrifugal method on sperm motility (Grade A and B) in patients with secondary infertility complaining from (Asthenozoospermia)

Research Presented By:

Ali Bahr Mahdi

Under Supervision Of

Dr. Ahmed Khalid & Dr. Hussein Khalifa

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1-Acknowledgment

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2-Abstract

Objective

Infertility is a inability of a couple of reproductive age to achieve conception after one year of sexual intercourse without contraception. Male infertility means that a man has a problem with his reproductive system.The aim of this study it is compare the results of sperm motility **grade A and B** in patients with secondary infertility pre-activation and post-activation by centrifugal method.

Patient and method

Forty Infertile patients were involved in the present study, the range of ages was between 22-50 years and duration of infertility 1-16 years. From each patient semen sample was taken, most of seminal fluid samples were collected, after 3-5 days of sexual abstinence, directly into clean dry and sterile dish by masturbation in especially room near the laboratory of semen examination.Macroscopic parameters of seminal fluid were evaluated.Then, sperm parameters were assessed microscopically. Progressive motility and total motility were assessed in accordance with WHO 1999 criteria .

Results

Sperm motility grade **(A**) and grade **(B**) were highly Significant **(P<0.01)** increased post-activation by centrifugal method as compared to pre-activation. The average of sperm rapid progressive motility grade **(A)** were significantly increased **(17.87%)** post-activation by centrifugal method as compared to pre-activation. And an Average of sperm slow progressive motility grade **(B)** was decreased **(1.62%)** as compared to pre-activation.

total sperm motility **(grade A+B)** for infertile patients complaining from asthenozoospermia were highly Significant increased **(P<0.01)** post-activation by centrifugal method.

Conclusion

The centrifugal method was suitable for activation sperm motility in patients with secondary infertility complaining from (Asthenozoospermia).

3-Introduction

Infertility is a inability of a couple of reproductive age to achieve conception after one year of sexual intercourse without contraception,it can affect a man, a woman, or both. Male infertility means that a man has a problem with his reproductive system. It means you cannot start a pregnancy with your female partner (1,2). **Primary infertility**: infertility that occurs without any prior pregnancy. **Secondary infertility**: infertility that occurs after a previous pregnancy.

Motility of sperm refers to its ability to swim in a forward direction. Normal sperm motility plays a crucial role in men's reproductive health since the sperm have to be able to reach the egg in the uterus in order to fertilise it. If a sperm is unable to move properly, then getting pregnant and conceiving naturally can become an issue (3). A detailed semen analysis categorises the sperm motility in one of the following grades, the **Grade A** represents the rapid progressive sperms that swim quickly in a straightforward direction and the **Grade B** represents the slow progressive sperms that move forward but in a haphazard line or maybe a little slower.

The treatment of infertile couples has made substantial progress . The rapid increase of assisted reproductive techniques (ART) as treatment

modalities for infertility has led to the development of a wide range of different sperm preparation methods (1,2). With the advancement in the techniques of ART in humans, the need to improve sperm processing and provision of actively motile spermatozoa has increased tremendously by centrifugal method . these technique are capable of effectively increased sperm motility, but to varying degree (4,5). Centrifugation is routinely employed in handling the ejaculates of some specie , this is the preferred technique to select the greater number of motile spermatozoa in cases of asthenozoospermia , the development and implementation of new assisted reproductive technologies, alternative preservation models based on washing sperm from a cellular ageing-accelerating substance such as the seminal plasma, and basic studies in spermatology is associated with the use of centrifugation. This requires a specific evaluation of the centrifugation protocols considering the species-specific relationship with the potential damage produced by this procedure.

Previous studies showed different results for effect centrifugal method on sperm motility, like study on (Effects of Shaking and Centrifugation on Human Sperm Motility) that published on Journal of Reproductive Systems which demonstrate that the significant drop in sperm motility by use centrifugal method (6). and onther studie witch demonstrate that the total progressive sperm motility (grades A and B) were significantly increased ($P<0.05$) post-activation in vitro as compared to pre-activation using direct swim-up technique (7).

The aim of this study it is compare the results of sperm motility **grade A and B** in patients with secondary infertility pre-activation and post-activation by centrifugal method.

4-patient and Method

Infertile males

This study was conducted in the Specialized Clinic for General Surgery, Infertility and in-vitro-fertilization for Dr. Hussein Khalifa Al-Dulaimi from November-2021 to february - 2022, and forty Infertile patients were included. The range of ages was between 22-50 years and duration of infertility 1-16 year.

Seminal fluid analysis (SFA)

Most of seminal fluid samples were collected, after 3-5 days of sexual abstinence, directly into clean dry and sterile dish by masturbation in especially room near the laboratory of semen examination. The normal semen specimen was liquefied within 60minutes at 37 ºC. Macroscopic parameters of seminal fluid were evaluated (10). Then, sperm parameters were assessed microscopically (11). Progressive motility and total motility were assessed in accordance with WHO 1999 criteria.

Semen preparation techniques by centrifugal method

This is the preferred technique to select the greater number of motile spermatozoa in cases of asthenozoospermia. In this method, good quality sperms can be separated from dead sperms, leukocytes and the other components of the seminal plasma by a density discontinuous gradient. Cells with different density and motility can be selected during the centrifugation by the colloidal silica coated with silane of the gradient; the sperms with high motility and good morphology are at the bottom of the tube, finally free from dead spermatozoa, leukocytes, bacteria and debris.

The most applied discontinuous density-gradient is a two layers density-gradient, formed by a top layer of 40% (v/v) and a lower layer of 80% (v/v). Density gradient media are available in commerce ready to use or ready to make the different density layers; the top layer phase (40%) is prepared by adding 4 ml of density gradient medium to 6 ml isotonic sterile medium (BWW, Earle, Ham F-10 or HTF) supplemented with HAS (Human Serum Albumin); the lower layer phase (80%) is prepared by adding 8 ml of density gradient medium to 2 ml of isotonic sterile medium. The density gradient is prepared by layering 1 ml of 40% medium over the 80% medium, or by layering the 80% medium under the 40% medium in a conical centrifuge tube . The number of the tubes depends on the volume of the semen sample, but the total volume could be divided in not more of 1 ml of semen per tube (12).

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means in this study (13).

*($P \leq 0.05$) was considered statistically signification.

*($P \leq 0.01$) considered highly signification.

*NS- mean not signification.

5-Results:

Results of the present study appeared that the sperm motility grade **(A**) and grade **(B**) were highly Significant **(P<0.01)** increased post-activation by centrifugal method as compared to pre-activation (Table 1).

Total sperm motility **(grade A+B)** for infertile patients complaining from asthenozoospermia were highly Significant increased **(P<0.01)** post-activation by centrifugal method (Table 2).

Table 1: Comparison between Before and After activation by centrifugal method in Sperm motility of Grade A and Grade B.

Activation	No	Mean ± SE of Sperm motility	
		Grade A	Grade B
Before activation	40	15.25 ± 1.53	25.87 ± 1.34
After activation	40	33.12 ± 2.39	24.25 ± 1.25
T-test	---	5.653 **	3.6432 NS
P-value	---	0.0001	0.3772
** (P≤0.01).			

Table 2: total sperm motility (grade A+B) for infertile patients complaining from asthenozoospermia pre- and post-activation in vitro by centrifugal method.

Activation	No	Mean ± SE of Sperm motility
Before activation	22	28.86 ± 2.56
After activation	22	45.00 ± 3.43
T-test	---	8.654 **
P-value	---	0.0005
** (P≤0.01).		

The Average of sperm rapid progressive motility **(Grade A)** was significantly increased **(17.87%)** post-activation in vitro by using centrifugal method as compared to pre-activation. And a Average of sperm slow progressive motility **(Grade B)** was decreased **(1.62%)** as compared to pre- activation .

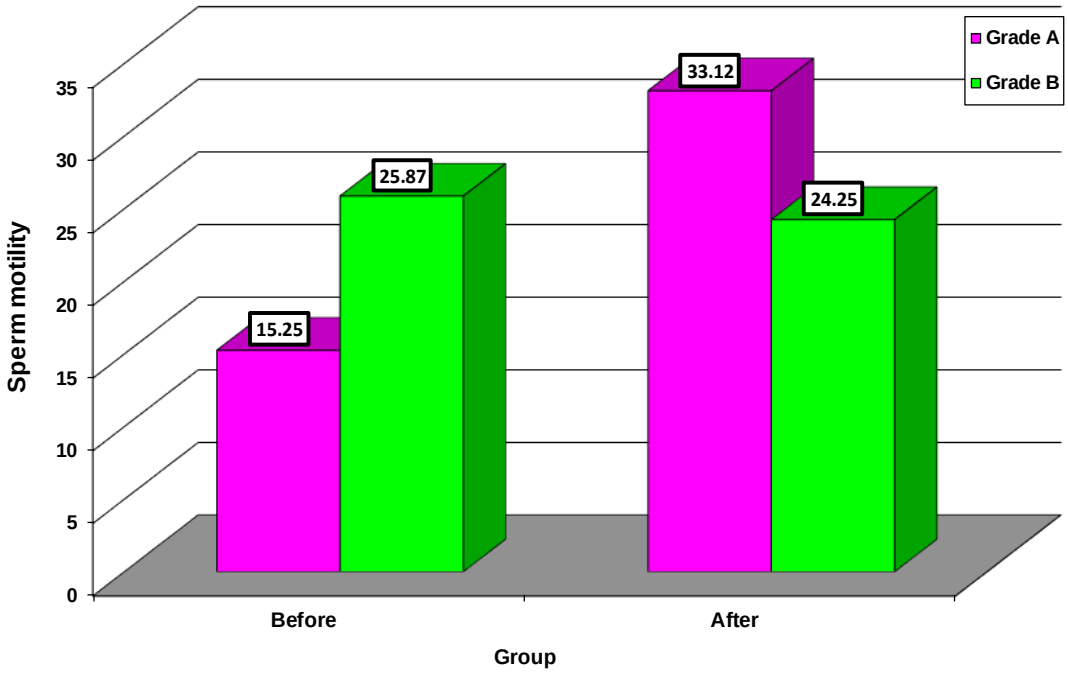

Figure 1. Comparison between Before and After activation in Sperm motility of Grade A and Grade B

The Average of total sperm motility **(grade A+B)** In infertile patients complaining from asthenozoospermia, significantly increased **(16,14%)** post-activation in vitro by using centrifugal method.

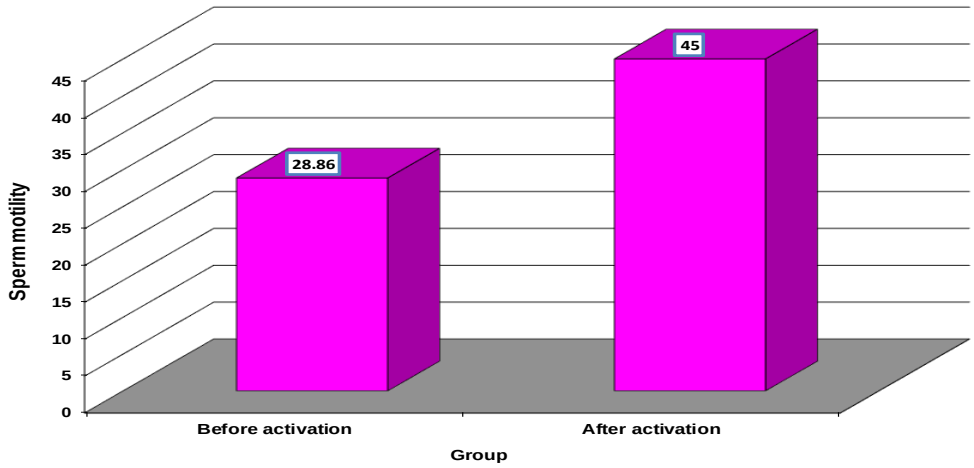

Figure 2. Comparison between Before and After activation in Sperm motility of Grade A + Grade B

6-Discussion:

Assisted reproductive techniques (ART) have become the treatment of choice in many cases of male infertility. The quality of semen samples is one of the factors determining successful assisted reproduction. A variety of sperm preparation techniques are available to select motile spermatozoa (14) . It is noticed that the use of in vitro centrifugal method increases sperm motility. The reason is that the seminal fluid with high viscosity obstructs sperm progressive motility so that the uses of in vitro centrifugal method with aqueous nature lead to decrease the viscosity of the seminal fluid and as a result sperms move more freely. This is why sperm motility increases with the use of centrifugal method (15).

The ultimate function of a sperm cell is to fertilize an ovum. The oviduct of a female selects only those fittest sperm in order to ensure fertilization of the ovum.studys show Reduced fertility is associated with poor quality of sperm that includ decrease sperm motility (16).

Demonstrated a gentle , non - invasive technique of separating low- , medium- and high - quality sperm with increase sperm motility
In use centrifugal method(17). Results of the present study appeared that the sperm motility grade **(A)** and grade **(B)** were highly Significant **(P<0.01)** increased post-activation by centrifugal method as compared to pre-activation. Sperm rapid progressive motility **(Grade A)** was significantly increased **(17.87%)**, sperm slow progressive motility **(Grade B)** was decreased **(1.62%)**, Average of total sperm motility **(grade A+B)** In infertile patients complaining from asthenozoospermia, significantly increased **(16,14%)**. total sperm motility **(grade A+B)** for infertile patients complaining from asthenozoospermia were highly Significant increased **(P<0.01)** post-activation by centrifugal method.

Our study demonstrate that the total sperm motility highly Significant increased **(P<0.01)** post-activation by centrifugal method. as a compared

with study that published at 2011 done by Assist. Prof. Dr. Muhammad-Baqir and Nisreen Khazal , Institute of Embryo Research and Infertility Treatment, Al-Nahrain University, Baghdad, IRAQ , Which demonstrate that there total progressive sperm motility **(grades A and B)** were significantly increased (P<0.05) post-activation *in vitro* as compared to pre-activation using SMART medium by direct swim-up technique(7).

Our findings are better than findings on study (Effects of Shaking and Centrifugation on Human Sperm Motility) that published on Journal of Reproductive Systems Which demonstrate that the Shaking for 15 sec by mechanical vibrator did not induce any drop in sperm percentage of motility, while shaking for 30 to 180 sec was followed by immediate drop of this parameter. Sperm velocity increased for a short duration in all shaken specimens, after which it dropped considerably in most cases. Following centrifugation below 330 g for 10-20 min, sperm motility was almost unaffected. However, there was a significant drop in sperm motility after specimens were subjected to rates above 580 g for the same time duration (6).

From the results of the present study, it was concluded that the centrifugal method was suitable for enhancement of sperm parameters of asthenozoospermic patients . Further studies are needed to evaluate effects of centrifugal method on successful rate of intrauterine insemination.

7-Conclusion:

Centrifugal method was suitable for activation sperm motility in patients with secondary infertility complaining from (Asthenozoospermia). Our findings suggest highly Significant increased total sperm motility after activation by centrifugal method. Further studies are needed to evaluate effects of centrifugal method on successful rate of intrauterine insemination.

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