SPERM ACTIVATION OF OLIGOSPERMIC PATIENTS AT USING SWIM-DOWN TECHNIQUE AND SWIM-UP TECHNIQUE AFTER CENTRIFUGATION BY FERTICULT FLUSHING MEDIUM WITH GLUTATHIONE

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ABSTRACT

This study was aimed to know effects of activation sperm for Oligospermic infertile patients parameters by activated at using Swim-down technique, Swim-up after centrifugation technique and using FertiCult Flushing Medium with glutathione. This study was performed on 44 samples, belongs to 30 patients suffering oligospermia. In this study divided one samples into two parts. The first part activated by FertiCult Flushing Medium with glutathione and Swim-down technique with incubation period 45 min in 37°C, the second part activated by FertiCult Flushing Medium with glutathione and Swim-up after centrifugation technique with incubation period 45min in 37°C. The results showed that there was a significant improvement (P<0.05) in the sperm motility percent and sperm grade activity to that activation in Swim-down technique, Swim-up after centrifugation technique comparison to the results before activation and the results showed that there was a significant improvement (P>0.05) in the sperm concentration and round cells concentration to that activation Swim-down technique, Swim-up after centrifugation technique comparison to the results before activation and the results showed that there was a significant improvement (P<0.05) in the normal sperm morphology percent to that activation in Swim-up after centrifugation technique and Swim-down technique comparison to the results before activation. Conclusion at using Swim-down technique, a human sperm activation can give a better results on sperm concentration, sperm motility percent and sperm grade activity in Oligospermia infertile patients parameters and using FertiCult Flushing Medium with glutathione.
KEYWORD: Oligospermia, glutathione, swim-up and swim-down activation techniques.

INTRODUCTION

Fertility and Infertility

Man consider fertile if he possessed the ability to proceeding pregnancy in his wife when he have sex with her but if he could not after a year or more and if the wife didn’t appear any clear reason for not getting pregnancy then he deemed sterile.\(^{[1]}\)

Sterility have two types :primary type its mean not getting pregnancy for both couple and secondary type refer to having pregnancy for once time at least however couples cannot make it again.\(^{[2]}\) sterility happen at percentage 2-10 % in couples that recently married a year after of their marriage and be about 40 % be due to the wife and about 40 % be due to the husband while both couple contribute together in 10% of the cases of infertility\(^{[3]}\) as well as there is unexplained infertility form a percentage between 10-15%.\(^{[4]}\)

Man's semen consist of sperms and seminal plasma that carried them and feeds them and provides them protection and transfer them in its currents the sperms swim to reach the uterus.\(^{[5]}\) seminal plasma form more than (90%) of the seminal projectile volume and characterized by chemical character that use to indicate the status of function of accessory glands\(^{[3]}\), and arise changes may be happen on some of the chemical character of the semen result from exposure to the environmental toxic compounds or living active substance, or drugs, or other than of harmful factors that’s lead to deterioration in the quality of sperm and affect the function of sperm generally.\(^{[6]}\)

Fresh normal sperm have gray appearance –homogeneous foggy have high viscosity consider as coagulum liquefy through 60 minute at room temperature spontaneously under the influence of enzymes of prostatic origin to be viscous transparent liquid with slight alkalinity pH between 7,4-8,0.\(^{[3]}\)

Sperm produce small quantity of Reactive Oxygen Species (ROS) in specific physiological conditions which are essential to complete the capacitation process and Acrosome reaction and fertilization. while large quantity consider from active oxygen species that produced by immature sperm and white blood cells have damage to normal sperm result from increase the effectiveness fat oxidation.\(^{[7]}\) The semen contain molecules with high molecular weights and low molecular weight known as Antioxidants or Scavengers system provide methods for
protecting sperm in semen.\textsuperscript{[8]} Glutathione (GSH) consider one of the Antioxidants in the semen and have a great rule in maintaining the sperm motion in normal condition ·this is due to the mention vitamin it works to install plasma membranes chains of sperm cells.\textsuperscript{[9,10]}

**Male Infertility Types**

**Aspermia** which means lack of ejaculation.\textsuperscript{[3]}

**Azoospermia** which means lack of sperm in the semen.\textsuperscript{[11]}

**Oligospermia** when the fall in the number of sperm every one millimeter of semen to less than 20 million sperm, a person likely to become sterile, where counting the latest issuesperm threshold in the field of male fertility (Threshold Limit).\textsuperscript{[3]}

**Asthenospermia** Sperm movement and is one of the most semen parameters in the evaluation of a possibility of fertilization, Active movement and motion progressive are necessary for normal sperm to penetrate the cervical mucus and migrate through the genital tract of female to reach the fallopian tubes to fertilize an egg, sperm animated cannot fertilize an egg whatever there number .Patient consider with asthenospermia if the percentage of sperm animated less than 50% within one hour of ejaculation.\textsuperscript{[3]}

**Teratospermia** Sometimes the person is sterile despite he has the normal number of sperms, was found in such cases that half the number of sperm have abnormal physically as sperms have two heads or their heads abnormal.\textsuperscript{[11]}

**Necrospermia** Semen may contain the normal number of sperm with natural forms, but these sperms are immobile, this case known as the death of sperm. Therefore it is not expected to reach the sperm animated not penetrate the cervical mucus.\textsuperscript{[3]}

**Leukospermia** There are white blood cells, especially neutrophil in most semen projectile but high concentrations of these cells are index to infect bacterial in reproductive tract and glands, the presence of more than a million cells per milliliter of semen on the leukospermia.\textsuperscript{[12]}

**Cryptospermia** It means the presence of a small number of sperm in ejaculation and cannot be discerned in the initial screening only through the use of Centrifugation of the sample and the sample of semen and examination of the pellet.\textsuperscript{[13]}
**Sperm Activation Techniques**

Is the treatment of infertility in the present time in the developed centers for the treatment of infertility through an activated sperm operations outside the body by using different techniques. It is important to choose the necessary to prepare the sperm technology to suit the samples of semen characteristics and should be available the following conditions to become activation technique used more ideal

Be easy, fast and with an appropriate cost.

Insulated as much as possible high percentage of moving sperm.

Production them not to crash sperm and do not cause physiological changes in sperm isolated.

Fact dead sperm and white blood cells and bacteria).

And found several techniques that using in sperm activation such as:

Washing and Swim-up.

Glass Wool-Column Filtration technique.

Migration-Gravity sedimentation technique.

Density gradient technique.[14,15]

**Sperm activation media**

There are a lot of media used in the activation sperm in vitro the body and that various types of assisted reproduction techniques, have proven their quality in this area ,and found several types of media which using in sperm activation techniques such as: Modified Earle’ s medium, Ham’ s F-10, Whittingham’ s -T6, BWW, Menez’ s -B2 and TC-199.

The media are used, in general, to the activation of sperm operations and fertilization in vitro the body and implant embryos are as modifications to the solutions Physiological balanced inorganic ions contain such as sodium, potassium, calcium, magnesium, chlorine and phosphate as well as to organic substances and vitamins that contribute to liberalization of energy suitable to improve the movement of sperm, As added lactate or sodium pyruvate or both together with glucose as a source of energy, and to protect the medium of bacterial growth added antibiotics, often used penicillin and streptomycin and organize osmosis for these crops including mimics those found in serum. And maintain the pH of the medium by the buffer System of bicarbonate and carbon dioxide that is working to reduce the agglutination of cultured cells and reduce the viscosity of the semen that helps to increase sperm movement which plays an important role in maintaining the vitality of the sperm thus
facilitating the processes of empowerment and interaction of the particle terminal then facilitate the processes of fertilization and normal fetal growth.[16]

Aim the Study
Activation by using swimming towards the bottom technique (Swim-down technique) and swimming towards the top after centrifuge (Swim-up technique after centerfucation) to oligospermic patient by using FertiCult Flushing Medium with glutathione and clarify effect of glutathion in the activation.

Sample Collection
Few sperm semen samples collect on container clean, sterile and dry its size (40) ml and wrote the name of husband on container after taking samples by masturbation after Sexual abstinence period not less than three days and not more than seven days for patient who were reviewing the fertility center of Sader city medical in the province of Najaf, then put semen samples before examined in the incubator in 37C° to allow natural liquefaction (less than 60 minute) then examined by eyes and microscope depending on the type of sample[3] the samples were then examined before activation the samples of patients with infertility have oligospermia has been activated with two technicality swimming to the down and swim-up after centerfucation and incubation period 45 minute in 37C° and use a FertiCult Flushing Medium with glutathione.

Semen analysis
After liquefaction is complete that was that are installed every sample examine by eyes and microscopic and information record and the result of sperm tests came as the following:

Macroscopic Examination
Volume
The measurement of sample size by test tube and the range of normal size of man ejaculation 1.5-6 ml.[3]

Color
Normal sperm have specular color gray and homogeneous appearance. As for brown red color it may be due to red blood cell existence also the presence of threaded mucous and yellowing color of mucus may indicate the presence of inflammation.[3]
Liquefaction
Features of recently gusher sperm being liquid and soon turns to semisolid or coagulate and natural liquefaction happen through 15-20 minute.[3]

Viscosity
It was estimated the sperm viscosity through noticing mucous thread by paying sample from Pasteur pipette, sperm consistency consider normal when flow drop by drop from the pipette, while consistency be anomalous when the sample thread more than 2 cm.[3]

pH
It has been measure the pH after liquefaction by special strips for this purpose (Lackmuspaper rot – Merck), the pH for semen sort of mild alkaline between 7.2- 8.0.[3]

Microscopic examination
Take 10 Ml drops from semen sample for people with oligospermia and binding well after liquefaction, then put the drops on the slide and examine under (10x) then (40x) by this way we can study the characteristics of semen and sperms.

Total count and sperm concentration
We can estimate the sperm content in full ejaculation from the average of sperm number in 10 microscopic fields then multiply the average by 10, divided the result on semen volume of the sample to calculate sperm concentration in 1ml.[3]

Sperm motility percent
We can calculate the percentage ratio for sperm motility according to this equation:

\[
Sperm \ motility \ percent = \frac{Number \ of \ Sperm \ motility}{Total \ sperm \ count} \times 100\%
\]

Normal sperm morphology percent
Calculate the percentage ratio for normal sperm according to this equation:

\[
Normal \ sperm \ morphology \ percent = \frac{Number \ of \ normal \ sperm \ morphology}{Total \ sperm \ count} \times 100\%
\]

Round cell concentration
To Calculate the concentration of round cell by calculate the number of these cells in 10 microscopic fields and multiply the average by 10, The round cells in human semen sample
are white cells and immature sperm cell such as spermatid, The normal number of white cells in semen sample are less than one million per milliliter, while the normal number of phagocytic cells are less than half million per milliliter in semen sample.

**Activation media in vitro sperm**

Used (Ferticult Flushing Medium) which is balanced and ready to activate sperms in a glass equipped by the company (Fertipro.N.V.Belgium), This media contain bicarbonate, salts, glucose, pyruvate, lactate, insuline and protein, that not need carbon dioxide and preferably before use it, put media at a temperature of 37°C for a period of 12 hours.

**Preparation reduced glutathione**

Use of glutathione manufactured by the Syrian Company (Aleppo Syria) for the drug and it has been prepared as follows

Glutathione 0.1 gm added to 10 ml of distilled water.

Mixing well in order to be nominated for an emulsion and then we get a clear solution.

Prepare different concentrations of glutathione a 0.02 mg/ml and 0.04 mg/ml and 0.06 mg/ml

Add glutathione to FertiCult Flushing Medium.

The culture medium (FertiCult Flushing Medium) user in activating sperm in vitro by using glutathione three different concentrations of a 0.02 mg/ml and 0.04 mg / ml and 0.06 mg / ml, and then was selected the concentration 0.04 mg/ml in the activation process for being the best in activation when conducting the experiment.

Add the glutathione concentration of 20% to media User in the activation of sperm and by mixing 20 ml of the glutathione solution with 80 ml of the previously prepared cultured solution for each concentration of the solution concentrations used.

**Experimental Design**

**This study was designed according to the two major pathways**

**The first pathway:** sperm activation by using (Swim-down technique) was done by incubation a test tube containing 1ml (FertiCult Flushing Medium) with glutathione and 1 ml of semen at 37°C for 45 minutes, after which the sample was examined by taking a drop from the second half (the lower part) to the culture medium and examination of sperm parameters after activation.
The second pathway: sperm activation by using (Swim-up technique) was done after centrifugation of a mixture of 0.5 ml of (FertiCult Flushing Medium) with glutathione with 2 ml of semen in 2000 rpm, and the supernatant was removed after the centrifugation process and the sperm pellet covered with 1 ml of cultured sample was incubated at 37°C degree for 45 minutes, after that the sample was examination by taking a drop from the second half (the higher part) to the culture media and examination of sperm parameters after activation.

Statistical analysis
Significant Difference (LSD) has also been used to compare between the results in addition to the standard methods to determine the average and the Standard error.[17]

RESULT AND DISCUSSION
In the present study, we use (FertiCult Flushing Medium) with glutathione in the activation techniques. Several studies have suggested the using of activated culture for the purpose of reducing the agglutination of cells and subsequently the viscosity of semen to help increase the movement of sperm. these cultures contain many ions such as sodium, potassium, calcium, magnesium, phosphate, pyruvate and lactate as is the case of the Culture media (FertiCult Flushing Medium), where these ions work to activate the sperms that increase its movement[18] that use (FertiCult Flushing Medium) that show a significant impact to increase the sperm motility percent and sperm grad by adding of glutathione to the culture media to increase reactive oxygen species production that result from the oxidation of fats and damaged the cells as well as the white blood cells where glutathione as a non-enzymatic antioxidants that works to curb reactive oxygen species and protect sperm from the effect of increased oxidative stress. The following table was illustrating the sperm activation results.

The current search results and there is a significant decrease (p<0.05) in the sperm concentration for samples that have been activation by using swim-up technique after centrifugation compared to the sperm activation by swim-down technique and before activation and found a significant between activation by swim-down technique and before activation due to the impact of the process swim-down, as there will be no effect on the attractiveness of the ground in the movement and direction of the sperm swim sperm normal and abnormal freely on the contrary swim-up technique after centrifugation as the swim-up technique after centrifugation that sperm migrate toward the opposite effect of gravity, and this is consistent with the findings of the[19], where he found a significant decrease in the use
of the swim-up technique compared with mixing technique and confused before activation (Table 1).

Table 1: activated human sperm in vitro of sperm concentration to oligospermic by using Swim-down and Swim-up techniques after centerfucation techniques

<table>
<thead>
<tr>
<th>Semen parameter</th>
<th>Before activation</th>
<th>Activation by swim-down technique</th>
<th>Activation by swim-up technique after centerfucation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration</td>
<td>15±0.5 a</td>
<td>10±0.1 b</td>
<td>7±0 c</td>
</tr>
<tr>
<td>Sperm motility percent (%)</td>
<td>50±3.5 a</td>
<td>71±3.9 b</td>
<td>66±4.6 b</td>
</tr>
<tr>
<td>Sperm grad</td>
<td>3±0.5 a</td>
<td>3.7±0.3 b</td>
<td>3.5±0.2 b</td>
</tr>
<tr>
<td>Sperm normal morphology percent (%)</td>
<td>80±6 a</td>
<td>90±8.1 b</td>
<td>92±6.6 b</td>
</tr>
<tr>
<td>Round cell concentration</td>
<td>1±0.5 a</td>
<td>0.3±0.1 b</td>
<td>0.1±0 b</td>
</tr>
</tbody>
</table>

Disparate characters denote significant differences at the significance level (p>0.05).
The cells inside the columns represent the standard error rate.

44 represent the number of samples
The current research's results also showed that there is a significant rise (P>0.05) in the sperm motility percent and sperm grad for samples that activated by swim–down technique and samples that activated by swim–up technique after centerfucation compared with results before activation while moral differences in the sperm motility percent and sperm grad by using swim-down technique and swim-up technique after centerfucation that agree the findings results of the[20] that indicated a significant improvement in the sperm motility percent and sperm grad by using technical tiered simple technique washing and ostracism in a row for a period of incubation a 30 minutes. At The phenomenon of both technologies rely swimming towards the top that the increase occurring in the sperm motility percent. This simple technique tiered be the result of the removal of white blood cells and phagocytic cells and moving sperm. Also, the ability of sperm to take advantage of food material and inorganic ions that’s found in media layers within a period of incubation, working on mitochondrial processing materials, food necessary to stimulate mitochondria to generate power, which is the source required for the movement of sperm energy by converting compound adenosine triphosphate (ATP) to the compound adenosine diphosphate (ADP), and transmission of energy from the mitochondria to the tail of the sperm to increase the sperms movement.[21] As well as the results showed higher insignificant (P>0.05) in the sperm motility percent and sperm grad for samples that have been activation by swim-down
technique compared with the results of the sperm activation by swim-up technique after centerafucation and perhaps the reason is that swimming technique based on the same principle which is a swimming process, albeit towards the top or the bottom, which depends on the sperm that can swim towards the media is the sperm of the best attractions in the sample where these two technologies can be considered as a form of the sperm in the positive insulation in the sample, leaving the sperm parameters of Rogue from being used in the insemination (Table 1).

Also showed the results of research found height significant (P>0.05) in the sperm normal morphology percent of samples which has been activated by using swim-down and swim-up techniques after centerfucation compared with results before activation while differences be insignificant in activation by swim-down and swim-up techniques after centerfucation. It may be because of the normal shape of sperm that characterized by its higher activity is solely that can boarding up or down from the remainder with abnormal shapes and the movement, which is characterized by weak mostly in the bottom of or in the up\textsuperscript{122} (Table 1).

Also showed the results of search that there is a significant decrease (0.05>P) in a round cells concentration for samples that have been activated by swim-down and swim-up techniques after centerfucation compared with results before activation while the differences were not significant in the round cells concentration when activated by swim-down and swim-up techniques after centerfucation and the reason for this is the survival of cells in the bottom of the tube or in a Supreme being immobile and cannot climb to the top or go down to the bottom where the work force of gravity on survival in the bottom of the tube. And this is consistent with the finding of the\textsuperscript{19}, where the observed decrease significantly (0.05>P) at a concentration of white blood cells using swim-up technique compared with its emphasis in the samples prior to activation (Table 1).

REFERENCES


