A PHYSIOLOGICAL STUDY TO SHOW THE EFFECT OF SEMEN FREEZING ON SPERM PARAMETERS AND ITS RELATIONSHIP TO THE SUCCESS RATES OF IVF OPERATIONS

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Abstract: The study aimed to try to identify the effect of rapid freezing and thawing processes on the parameters of semen and sperm, interleukin-10 and the concentration of Almalone Dialdehyde (MDA), in addition to studying the effect of adding types of antioxidants, which included vitamin C to the sperm freezing medium, in reducing the negative effects resulting from the freezing processes. Rapid and dissolving in the sperm. The study included the use of Normozoospermic sperm, and the number of samples was 16 samples of semen samples. The mean age of both sperms was (29.2 ± 1.11 years). The study of the freezing time by calculating the parameters of the sperm in the sixth month after freezing and thawing compared to before freezing and thawing for samples of the subjects included in the study showed a significant decrease (P<0.05) in the percentage of sperms with progressive movement and the percentage of sperm viability and a significant increase (P<0.05) in the averages of MDA concentration and no significant differences (P<0.05) in sperm concentration, percentage of normal sperm, circulating cells concentration and interleukin-10 concentration in the sixth month after freezing and thawing compared to before freezing and thawing. The results of the study showed that the use of sperm freezing medium (Sperm Freeze) added to the antioxidants of the type C vitamin led to significant differences (P<0.05) in the studied parameters of the sperm of the subjects included in the study, which led to a significant (P<0.05) increase in The percentage of sperms with progressive motility and the percentage of sperms viability and a significant decrease (P<0.05) in the rates of each of the concentrations of Malone Dialdehyde and the absence of significant differences (P<0.05) in each of the sperm concentration and the percentage of normal sperms and The concentration of circulating cells and the concentration of interleukin-10 when comparing their rates with the use of sperm freezing medium alone or before freezing. It is concluded from this study that the processes of rapid freezing and thawing of sperm have negative effects on the parameters of semen and sperm and the concentration of Malone Dialdehyde (MDA) Which leads to lower pregnancy rates and IVF technical success, and that the addition of antioxidants of vitamin C type to the medium of freezing sperm (Sperm Freeze) showed resistance to these negative effects reflected in a significant improvement in studied landmarks.

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INTRODUCTION

Infertility is a pathological condition that affects about 15% of all couples who want to have children, and it is a widespread medical problem and has great psychological effects. Male infertility factors constitute about 50% of this case, and about 25% of them are caused by Diagnosed Idiopathic (1).

Assisted reproduction techniques have become a successful treatment option for many cases of infertility, and in general, success rates are still below the required level (2).

Assisted reproduction techniques include a number of different methods of in vitro fertilization (IVF), which require the presence of both the male gamete (sperm) and the female gamete (the egg) outside the vivo to cause in vitro fertilization. Outside the body, this necessitated the provision of methods to try to preserve the life of these gametes until the time when fertilization is required (3), and the best way to achieve this goal is to preserve these gametes using cryopreservation) And in which biological materials are preserved at a temperature less than zero, such as 80 - or 196 - C 0, in which the cellular biochemical reactions responsible for cell death are effectively curbed, and the freezing process aims mainly "to try to preserve the survival of cells and functional activity after a period of Preservation at a temperature less than zero degrees Celsius, as cells kept at a temperature equal to –196 degrees Celsius have a halt in the phenomenon of cellular diffusion at this point, as well as a reduction in the thermal energy required to complete chemical reactions (4).

The freezing of sperm and testicular tissue helped preserve fertility regardless of the cause that leads to infertility, and the

process of preserving sperm and testicular tissue using freezing has become of greater importance as a result of research requirements and the development of clinical applications in the means of assisted reproduction and semen banks, and for the purpose of preserving Fertility after exposure to chemotherapy, radiotherapy or surgical procedures with potential effects on fertility (3).

There are two types of freezing processes: Slow Freezing and Rapid Freezing, and all methods aim to achieve one goal, which is to try to preserve the cell from the effects of cooling, the formation of cellular ice, dehydration and toxic effects at low temperature, and one of the science techniques Modern freezing, within this field of reproductive science, is a method called "vitrification", which means "transformation into a glass-like state", and includes a number of methods that have been modified from the slow method, which aims to achieve the same goal, which is to try to preserve the cell from the effects of cooling, the formation of cellular ice. , drought and toxic effects at low temperature, and the technology does not need special requirements to achieve this (5)

The process of freezing sperm includes conducting a number of operations, such as the process of diluting the medium to be frozen by adding chemicals with low partial weights known as preservatives from Cryoprotectants Substances, as well as the process of immersing the mixture in liquid nitrogen with a temperature of - 196 ° C and the process of heating and thawing the samples, which in turn includes A number of processes, from heating using the water bath, and conducting the process of separating the freezing medium by conducting the centrifugal process and adding activation media and others (6). It is believed that each step of these processes induces negative effects that may lead to the generation of damage in both the structure and function of the sperm (7).

Human sperm is highly sensitive to a state of lowering the temperature beyond the freezing point of water (0°C), which is due to the peculiarity in the composition of plasma membranes and intracellular membranes, which are major sites for cellular wounding when frozen (8).

Studies have also shown that the freezing process leads to a significant decrease in the levels of both enzymatic and non-enzymatic antioxidants after freezing and thawing procedures, and an increase in the levels of Reactive Oxygen Species (ROS) within the medium (9). Increasing levels of ROS at the expense of antioxidants leads to oxidative stress, a condition that has significant negative effects. All cellular components of sperm such as lipids, proteins, nucleic acids and sugars are potential targets for the toxic effects of oxidative stress (10).

A high increase in the levels of active oxygen species (ROS) and a decrease in antioxidants also leads to an increase in the rates of programmed cell death in the sperm cell population present in the sample (11).

Seminal fluid Analysis

After completion of the urine whose time was fixed, each sample was examined microscopically and microscopically and recorded Information and results of semen tests based on the report of the World Health Organization (12), according to the following:

Macroscopic examination

volume of semen: The volume of semen was measured by inserted test tubes, the normal volume of a man's ejaculate ranged between 2-6 ml and the sample was considered undersized.

Color: The semen appears homogeneous, milky in color.

Liquefaction Time: The duration of the semen was measured from the time of taking the sample until the completion of its liquefaction by changing the consistency of the semen from semi-liquid to liquid consistency, where the complete fluidization of the semen sample takes place within 15 minutes at room temperature and is rarely completed within 60 minutes.

Viscosity: After washing, the liquefied semen was estimated by observing the mucous suture, by flowing the sample from the pipette drop by drop.

Microscopic examination: The sample was placed on a warm glass slide and covered with a cover slide, then the sample was examined under the objective lens first under 10X and then 40X. The following parameters of sperm were measured:

Sperm Concentration: The sperm concentration was estimated in 1 milliliter of average sperm count in ten random microscopic fields, and the average number was multiplied by a factor of 10^6.

Sperm motility: the movement percent of sperms was measured according to sperm grade activity (A, B, C, and D) in ten fields, and estimate the mean of the number was. Grade A means the high speed of motility of the sperms, Grade B means active sperms, Grade C means sluggish motility, and the movement is circularity, and grade D for immobile sperms .

Sperm Morphology: Determination of sperm morphology according to the following formula:

Abnormal sperm morphology = No. of abnormal sperm / total sperm conc. ×100

Round cell concentration: The pus cell concentration is measured by ten random fields and multiplied by 10^6, and its normal value is less than 1 million per ml.

Sperm viability percent: Eosin alone was used in the Vitality Test Using Eosin alone, where 5 µl of eosin and 5 µl of semen were taken and the two drops were placed on the glass slide and mixed well and placed on the cover slid and left for 30 seconds and then examined under the microscope. Observe the live (alive) sperms whose outer membrane is dyed only with the dye without entering the inside of the cell, considering that its cell membrane prevents the passage of the dye, while the dead (dead) sperm will be dyed with the dye because the damage to its membrane loses control of its permeability and allows the dye to enter and becomes red in color. and then the percentage of sperm viability was calculated according to the following equation: -

Percentage of sperm viability = (number of live sperms) / (total number of sperms) x 100

malondialdehyde concentration

The concentration of MDA in the seminal plasma of the semen was calculated according to the method described in (13), where the semen samples were separated by placing them in a centrifuge at a speed of 3000 rpm for 15 minutes to obtain the seminal plasma. according to the procedure (See appendix 1) .

Human Interleukin-10

According to the results of measurement of interleukin-10 concentration by using enzyme-linked immunosorbent assay according to procedure (See appendix 2) .

Medium Sperm Freeze

It contains in its composition glycerol, human serum albumin HAS and HEPES, and the medium is supplied by the company Fertipro . N.V Belgium, and it is used for the purpose of freezing sperm, and it is preferable to leave it after mixing with

the sample at room temperature for 10 minutes before placing it in liquid nitrogen.

preparation of vitamin C

Vitamin C (commercially called Citrate C), manufactured by the Syrian pharmaceutical company Aleppo-Syria, was used in the form of capsules with a concentration of 500 mg, and it was prepared as follows: -

1- Add vitamin C to 20 ml of distilled water.

2- Mix well until it forms an emulsion, then filter to get a clear solution.

3- Concentration of vitamin were used a 0.04 mg/ml..

Experimental design

This study was designed by dividing the semen sample into three sections Part One: Study of the parameters included in the study immediately before freezing and considered as a control group.The second part The sample was frozen for 6 months in the freezing medium and it was thawed and the parameters included in the study were studied. The third part A concentration of vitamin C was added to the freezing medium and the parameters of the study were studied after thawing after freezing the sample for 6 months.

Statistical Analysis

In this study, an electronic system for statistics was used, which is accurate and efficient in giving results (Publication Chart Board Version 5), and the ANOVA test was used to compare the results. The results were compiled using the (mean ± standard error) method. Descriptive statistics significance and correlation coefficients were performed using mega stat (v 10.12) for Excel 2010.

The seminal fluid parameters of studying specimens Before freezing (Control group), After freezing (without vitamin C) and After freezing (with vitamin C).

Analysis of seminal fluid samples showed a significant difference (P< 0.05) in some parameters (Table 1).

Table 1. The seminal fluid parameters of studying specimens Before freezing (Control group), After freezing (without vitamin C) and After freezing (with vitamin C).

Semen and Sperms Parameters	Before freezing (Control group) N=13	After freezing (without vitamin C) N=13	After freezing (with vitamin C) N=13
Concentration (Sperm/ml.)×10^6	55.1±1.5 a	52.3±2.5 a	56.00±1.4 a
Sperms motility (%)	60.2±1.75 a	45.8±1.15 b	58.60±1.67 a
Sperm viability(%)	80.1±3.5 a	51.1±5.5 b	70.1±1.5 a
concentration of round cell ×10^6 cells	0.82±0.07 a	0.94±0.16 a	0.9±0.15 a

Table 2. The Interleukin 10 level and malondialdehyde concentration In the studying specimens Before freezing (Control group), After freezing (without vitamin C) and After freezing (with vitamin C).

Analysis of seminal fluid samples showed a significant difference (P< 0.05) in some parameters (Table 2).

Sperms Parameters	Before freezing (Control group) N=13	After freezing (without vitamin C) N=13	After freezing (with vitamin C) N=13
Interleukin 10 pg/ml	6.9±0.5 a	7.0±1.5 a	6.7±0.4 a
malondialdehyde concentration nmol/l	0.56±0.4 a	1.3±0.5 b	0.88±0.2 a

DISCUSSION

In this current study, the process of freezing semen for a period of six months on the parameters of sperm, the concentration of interleukin-10 and aldehyde in semen, as well as the effect of adding vitamin C to a freezing medium in order to reduce this effect was demonstrated. Several studies have shown that the process of freezing sperm has a significant effect on These landmarks.

The results of the current research showed that there was a significant (p>0.05) decrease in both the concentration of sperms, and the concentration of circulating cells in relation to before freezing compared to after freezing without or with the addition of vitamin C. To the dilution process that occurs as a result of mixing semen and culture, if compared to a group before freezing These results agree with the findings (14) where they noted that there was a decrease, but not significant, in the concentration of sperms, the concentration of circulating cells and the percentage of normal sperms for samples of infertile patients with activated oligospermia by mixing technique, while the decrease was significant (p>0.05) regarding the viability of sperm and its progressive movement for samples after freezing compared to before freezing and after freezing to which vitamin C was added. In the middle of freezing, this effect is due to the reason for this to be due to the great role of vitamin in reducing the oxidative stress resulting from freezing. This study agrees with the finding of the (15), as it was shown in a special study that vitamin C reduces the level of active oxygen species and maintains the movement of sperm by scavenging the oxygen species. effective and protect the sperm from oxidative stress.

The results of the current research also showed a significant increase (p>0.05) in the concentration of MDA after freezing compared to its concentration before freezing and after freezing with the addition of vitamin C. Perhaps the reason for this is that this increase in the concentration of MDA may be due to the negative effects of freezing and thawing processes on the body. Sperm parameters and lead to a significant increase in the concentration of malondial aldehyde, because the increase in

concentration is linked to an increase in the active oxygen species. (16) It was noticed when the level of active oxygen species increased as a result of an increase in oxidative stress accompanied by an increase in the concentration of malondialdehyde. This increase when freezing may be due to the freezing and thawing processes leading to a significant increase ($p<0.05$) in the percentage of abnormal or damaged sperm chromatin In the (DNA) also due to the increase in oxidative stress, while this concentration decreased when adding vitamin to the freezing block due to the decrease in oxidative stress (15).

The results of the current research also showed that there were differences, but not significant, in the concentration of interleukin-10 after freezing and after freezing by adding vitamin C compared to its concentration before freezing. In their semen there is an increase in the blood cells, the interleukin level remained normal because the freezing did not affect the level of the white blood cells. of white blood cells in the body(17).

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