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Cysteamine Mitigates the Deleterious Impact of Cryopreservation on Sperm Parameters

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Abstract

Background: Sperm cryopreservation can reduce the quality of sperm. Antioxidants can improve sperm parameters by decreasing oxidative stress.

Objectives: This study was performed to determine the effect of supplementing sperm freezing media with cysteamine on the quality of sperm after freezing.

Methods: This descriptive-analytical research was conducted at Omolbanin Center for Infertility Treatment in Dezful, Iran, in 2022. The samples included 60 men with normosperm based on the World Health Organization standards. Each sample was divided into three parts: 1. fresh semen group, 2. semen frozen with 10 mmol cysteamine supplementation, and 3. semen frozen without cysteamine supplementation. Viability, motility, morphology, total antioxidant capacity (TAC), and malondialdehyde (MDA) levels were evaluated. The results were described using SPSS version 22, and the significance level was considered $P \leq 0.05$.

Results: The mean of progressive motility was significantly higher in the fresh semen group (48.3 ± 4.74) compared to the two other groups. The mean progressive motility was significantly lower in the group without cysteamine supplementation (24.4 ± 3.38) than in the group with cysteamine supplementation (35.3 ± 4.9). Moreover, TAC was significantly higher in the group with cysteamine supplementation (816.6 ± 4.36) than in the other groups. We found that the MDA level was significantly higher in the group without cysteamine supplementation than in the other groups (36.5 ± 2.51).

Conclusions: According to the results of the present study, appropriate conditions for preserving the sperm parameters after cryopreservation could be created by adding cysteamine to the sperm cryopreservation media as an antioxidant effective on sperm parameters.

Keywords: Cysteamine, Semen, Sperm, Antioxidant

1. Background

Male fertility relies on the production of many cells during the spermatogenesis process. Oxygen plays a crucial role in the aerobic metabolism of spermatogenetic cells but may induce harmful effects on cells by producing reactive oxygen species (ROS). A strong positive correlation has been reported between various levels of ROS and the percentage of sperm with different types of sperm disorder (1, 2). An increase in ROS levels in semen and abnormal spermatozoa can be among the leading causes of subfertility or infertility (3). Sperm cryopreservation helps fertilization techniques by preserving cells at extremely low temperatures for long periods without any decrease in their viability or function (4). However, sperm cryopreservation reduces sperm motility, chromatin stability, and membrane integrity, causing significant morphological changes in the sperm. This decrease in sperm quality can be quite important, especially in patients with weak sperm parameters (5). Sperm motility is the most affected parameter following freezing (6, 7). The aforementioned damage mechanism can be related to osmotic pressure, cold shock, formation of

intercellular ice crystals, and excessive production of ROS or a combination of these factors (8). Although ROS production plays a vital role in sperm function during capacitation, acrosome reaction, and binding of the zona pellucida, excessive exposure to ROS can harm the mitochondria, cell membrane, and DNA and even decrease the motility and viability of sperms. Antioxidants are the most critical defensive factors against oxidative stress caused by free radicals (9, 10).

Cysteamine is an aminothiol antioxidant with low molecular weight that increases cysteine absorption and glutathione synthesis and acts as a protective mechanism against oxidative stress (11, 12). Cysteamine also plays a role in fetal development and improves the quality of the fetus during laboratory maturity (13).

2. Objectives

Commercial culture media can be expensive, and specific specialized media formulations containing antioxidants may be less accessible or even unavailable in some regions. Therefore, the present study aimed to determine the effectiveness of cysteamine supplementation as a strong antioxidant to human semen freezing media on the freezing of sperms, semen quality parameters, lipid peroxidation, and total antioxidant activity. Although several studies have investigated this effect, it is important to note that most of these studies have focused on animal samples.

3. Methods

After receiving an ethical code (IR.DUMS.REC.1398.052) from the Ethics Committee of Dezful University of Medical Sciences, patients were identified and selected from the Omolbanin Center for Infertility Treatment in Dezful, Iran. All necessary data were obtained and completed from checklists based on the relevant criteria. This experimental study included 60 normospermic samples aged 25 - 45 years divided into three groups. The semen samples were collected in steel containers via patients' self-ejaculation. Patients had not ejaculated semen for 3 - 5 days before sample collection. The samples were incubated at 37°C for 20 - 30 min. All procedures were conducted for three groups, each consisting of 20 samples.

The first, second, and third groups included fresh semen samples, samples frozen with 10 mmol cysteamine supplementation of the freezing media, and samples frozen without cysteamine supplementation, respectively. To freeze a portion of the sample, 0.5 mL was taken from the semen sample and mixed with the freezing solution (SpermFreeze, Vitrolife, Sweden) in a sterilized cryovial with a 1: 1 ratio until it reached 1 mL of volume. Next, it was preserved at room temperature for 10 and 20 min in nitrogen vapor before being placed in -196°C liquid nitrogen.

The fresh semen sample, including 1 mL of semen liquid, was evaluated by a person under an optical microscope with $\times$ 400 magnification. The sperm parameters of number, motility (rate of sperms with progressive and non-progressive motility and immotile sperms), and morphology (form and size) were examined using a Neubauer hemocytometer and a counter. Sperms were randomly examined in some microscopic fields, and the mean of the obtained numbers was calculated. Papanicolaou staining techniques were used to examine the morphology of the sperm. In contrast, eosin staining (0.5% in saline) was used to examine and distinguish between motile and immotile sperms and dead sperms.

In order to separate the seminal plasma from the part containing sperm, the samples were centrifuged at 2000 rpm for 10 min, and the supernatant was separated. Afterward, the MDA and TAC levels were measured in the separated seminal plasma of the fresh semen sample. The samples frozen with and without cysteamine were thawed at laboratory temperature after two weeks, and MDA and TAC levels in their seminal plasma were measured. In addition, all sperm quality parameters were evaluated.

Data analysis was performed by SPSS version 16 using the one-way analysis of variance to assess the variance of the groups and Fisher's least significant difference to evaluate significant differences between the groups. In all statistical analyses, $P \leq 0.05$ was considered significant.

4. Results

According to the results presented in Table 1, in the fresh semen group, the mean of progressive motility was 48.3 ± 4.74, which was significantly higher than the frozen group with cysteamine supplementation (35.3 ± 3.99) and the frozen group without cysteamine supplementation (24.4 ± 3.38) (P = 0.004). The mean of non-progressive motility was 21.4 ± 2.52 in the group frozen-thawed without cysteamine supplementation, which was significantly higher than the group with cysteamine supplementation (15.5 ± 4.23) and the fresh semen group (13.4 ± 2.78) (P = 0.001).

The mean of immotile sperms was 59.5 ± 3.12 in the group frozen-thawed without cysteamine supplementation, which was significantly higher than the group with cysteamine supplementation (49.4 ± 4.15) (P = 0.041). The mean of sperms with normal morphology was 12.3 ± 3.91 in the group thawed without cysteamine

Table 1. Sperm Analysis in Different Groups [a]

Variables	Fresh	Freeze with Cisteamin	Freeze Without Cisteamin	P-Value
Count (10^6)	68.5 ± 2.11 [A]	70.5 ± 1.27 [A]	70 ± 2.51 [A]	0.211
Progressive motility	48.3 ± 4.74 [A]	35.3 ± 4.9 [B]	24.4 ± 3.38 [C]	0.004
Non-progressive motility	13.4 ± 2.78 [B]	15.5 ± 2.23 [B]	21.4 ± 2.2 [A]	0.011
Immotile	37.4 ± 2.3 [C]	49.4 ± 4.15 [B]	59.5 ± 3.12 [A]	0.041
Normal morphology	14.3 ± 3.75 [A]	13.6 ± 4.11 [A]	12.3 ± 3.91 [A]	0.061

[a] Different superscript letters in each row indicate significant differences at $P \leq 0.05$.

supplementation and 13.6 ± 4.11 in the group thawed with cysteamine supplementation. In the group of fresh semen samples, the mean number of sperms with normal morphology was 14.3 ± 3.75.

Based on the results in Table 2, the mean of TAC was 721.4 ± 3.36 in the group frozen-thawed without cysteamine supplementation, which was significantly lower than the group with cysteamine supplementation (816.6 ± 4.36). Furthermore, the TAC level was significantly lower in the groups thawed with and without cysteamine supplementation compared to the fresh semen group (P = 0.033).

The mean MDA was 36.5 ± 2.51 (nmol/mL) in the group without cysteamine supplementation, significantly higher than in the group with cysteamine supplementation (25.4 ± 4.35). The MDA level was significantly higher in the groups thawed with and without cysteamine supplementation compared to the fresh semen group (P = 0.002).

5. Discussion

Human sperm is very susceptible to the harm caused by oxidative stress due to its many lipid groups. Therefore, this study aimed to assess the effect of adding cysteamine supplementation, as an antioxidant compound, to the sperm freezing media on the sperm quality parameters and oxidant-antioxidant status.

According to Agarwal et al., freezing the sperm can trigger lipid peroxidation in the sperm cell membrane and increase the number of free radicals in the sperm cytoplasm. In addition, freeze-thawing the sperm increases the production of superoxide radicals harmful to DNA. This study demonstrated that freezing and the following thawing process raised the number of peroxide free radicals and MDA levels compared to the fresh sperm, which was in line with the findings of Agarwal et al. (14).

Grossfeld et al. considered free radicals as cryopathogenic species and explained that, based on several experiments, antioxidant supplementation to the sperm-freezing media seems necessary to preserve sperm quality to some extent after thawing (15). However, according to Li et al., there is limited information regarding the different types of effective antioxidants and how they protect the human sperm (16). This study also demonstrated that the addition of antioxidant compounds to the sperm media after the thawing process influences the TAC level positively.

Reda Elkhawagah et al. reported that sperm penetrability rose significantly after cysteamine supplementation to buffalo sperm. Supplementing sperm with 10 mmol cysteamine had a protective effect on the sperm. It improved the motility of the sperm after the thawing process (17), which is consistent with the results of the present study. Tuncer et al. showed that the activities of superoxide dismutase and glutathione peroxidase in frozen-thawed semen samples supplemented with 2.5 and 7.5 mmol cysteamine supplementation, were higher than the control group (18), which was in line with the current study.

Beheshti and Ghiyasi reported that adding 20 mmol cysteamine and 1.5 mmol vitamin E to a commercial diluent increased the motility and some other sperm quality parameters in buffalo sperm after thawing (19), which was congruent with the results of this study regarding the effect of cysteamine on providing proper conditions for preserving the sperm quality parameters after cryopreservation.

Some researchers believe that cryopreservation is related to sperm DNA fragmentation. It is presumed that mechanisms damaging the human sperm depend on several factors, including cold shock, osmotic pressure, formation of intracellular ice crystals, and oxidative stress. Supplements with antioxidant properties reduce the harm caused by ROS and cold shock. There are many studies on the effects of antioxidants on the cryopreservation process to improve semen quality after the thawing process.

Swami et al., in a study titled “The Influence of Trehalose, Taurine, Cysteamine, and Hyaluronan on Ram Semen: Microscopic and Oxidative Stress Parameters after

Table 2. Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA) in Different Groups [a]

Variables	Fresh	Freeze with Cisteamin	Freeze Without Cisteamin	P-Value
TAC, mmol/L	985 ± 3.37 [A]	816.6 ± 4.36 [B]	721.4 ± 3.36 [C]	0.033
MDA, nmol/mL	15.3 ± 4.18 [C]	25.4 ± 4.35 [B]	36.5 ± 2.51 [A]	0.002

[a] Different superscript letters in each row indicate significant differences at $P \leq 0.05$.

Freeze-thawing Process," explained that cryopreservation is accompanied by the production of ROS, which leads to the peroxidation of sperm membrane lipids and loss of sperm motility (20). In biochemical measurements, antioxidant supplementation results in a significant difference in MDA, glutathione, and glutathione peroxidase after the thawing process compared to the groups without supplementation. Therefore, their results regarding cysteamine's effect on improving sperm parameters aligned with the current study.

Jumintono et al. reported that cysteamine supplementation decreased the total number of antioxidants while increasing the MDA concentration of sperm and explained that cysteamine cannot trigger intracellular glutathione synthesis in the sperm to fight the free radicals because the sperm loses its cytoplasm (which is essential for chemical reactions) at the time of maturity. Therefore, the results of this study demonstrated that cysteamine is not the proper supplement for the sperm freezing media. The findings are not in line with the current study regarding the effect of cysteamine on the total number of antioxidants and increasing sperm MDA content (21).

Bansal and Bilaspuri, in a study titled "Impacts of Oxidative Stress and Antioxidants on Semen Functions," explained that oxidative stress is an important factor in infertility. Oxidative stress results from an imbalance between ROS and the body's antioxidants, which can cause harm to the sperm, lead to its deformation, and finally result in male infertility. Although high concentration of oxidants can cause sperm pathology (decrease of adenosine triphosphate), lipid peroxidation, and the loss of sperm motility and vitality, there is much evidence demonstrating that low and controlled oxidative stress plays a vital role in the physiological processes of sperm (22).

In a study conducted in 2022, the antioxidant, anti-inflammatory, and anti-apoptotic effects of cysteamine on testicular torsion-induced damage were investigated. The results showed that cysteamine protects the testis from the damage of reperfusion injury, inflammatory and oxidative pathways, and apoptosis. These findings strengthen the results of our study (23).

5.1. Limitations

The main limitation of this study was patients' reluctance to participate, and the second limitation was the low availability of normozoospermic samples during our study period. In order to address this issue, larger sample sizes and more extended study periods are needed.

5.2. Conclusions

Although the quality of semen decreases when preserved under freezing conditions, this study shows that cysteamine reduces lipid peroxidation and prevents the structural damage caused by freezing. Adding cysteamine to the freezing media increased TAC levels and reduced MDA levels compared to other groups. Moreover, cysteamine has the potential to improve various sperm parameters after freezing. Therefore, it can be inferred that supplementing the freezing media with cysteamine is likely to enhance the sperm's function after cryopreservation. However, further in vivo studies are required to investigate these impacts.

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Footnotes

Authors' Contribution: M. A. B. conceived and designed the research and drafted the manuscript. S. M. P., S. J., and B. Gh. designed the research, performed parts of the statistical analysis, and helped draft the manuscript. M. A. B. and A. B. re-evaluated the clinical data, revised the manuscript, performed the statistical analysis, and revised the manuscript. S. M. P. and M. A. B. collected the clinical data, interpreted them, and revised the manuscript. M. A. analyzed the clinical and statistical data and revised the manuscript. All authors read and approved the final manuscript.

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