The role of low concentrations of some sulfuric antioxidants on the semen characteristics of rams

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Abstract
This study aims to investigate the effect of low concentrations of sulfuric antioxidants on sperm of rams. This study was conducted in the field of sheep and goats of the Department of Livestock in the college of Agricultural Engineering Sciences, University of Baghdad. The semen was collected using the artificial vagina at weekly rate (Pooled semen) and was subjected to different tests and then was divided into seven treatments but different concentrations of cysteine (0.5, 1mM) and glutathione (0.5, 1mM) and taurine (0.5, 1mM). The sample of the semen was diluted with Tris dilution (10:1). The results showed that the low concentrations of glutathione, cysteine and taurine resulted in an improvement in the motility and integrity of the plasma membrane, dead sperm, DNA damage and survival sperm during the conservation period (0, 24, 48 hours).

1. Introduction
Improving the reproductive performance of Iraqi sheep is important in raising their productivity and increasing their numbers in Iraq[1]. Therefore, attention has been focused on expanding the use of Artificial Insemination (AI) technology for the purpose of disseminating distinct genotypes, as well as the importance of this technology in reproductive technology programs[2]. Cysteine is an essential non-essential amino acid that can be synthesized within living cells through the amino acids L-methionine and L-serine. The amino acid cysteine is determined to produce glutathione tripeptide composed of Kama - Glutamic acid, cysteine and glycine. The sulfur group (SH) in the cysteine is the active group in this peptide, which is an antioxidant and antitoxin[2; 3]. Glutathione isolates the active oxygen molecules before they cause damage to the cells[5]. This ability is due to the group SH (group) in the cysteine, which has the ability and ability to bind to heavy metals such as mercury, cadmium, copper and cobalt, and to make it a desired cellular level as well as to reduce its toxicity[6]. Taurine (2-aminoethanesulfonic acid) is an organic compound that is widely distributed in animal tissues, found in the retina, heart, brain, skeletal muscle, leukocytes and semen of bull, pig and human[7; 8].
The aim of this study is to affect the use of low concentrations of glutathione, cysteine, and taurine concentrations in the extender of the semen of the rams during the cryopreservation of the individual motility and integrity plasma membrane and dead sperm and damage DNA and survival rate of sperm.

2. Materials and Methods

This study was conducted in the field of sheep and goats of the Department of Livestock in the College of Agricultural Engineering Sciences, University of Baghdad - Jadriya for the period from August 2018 to mid-October 2019. All rams were healthy, disease-free, and subject to veterinary supervision on an ongoing basis. The semen was collected using the artificial vagina at weekly rate (Pooled semen) and was subjected to different tests and then was divided into seven treatments but different concentrations of cysteine (0.5, 1mM) and glutathione (0.5, 1mM) and taurine (0.5, 1mM). The sample of the semen was diluted with Tris dilution (10:1) and the diluate was prepared according to the method described by [9]. The individual motility, dead sperm, and plasma membrane integrity, and the damage DNA. [13] Sperm survival after 48 h storage was estimated by dividing the percentage of motility at 48 h by the percentage of initial motility (90%). The Statistical Analysis System, was used for data analysis, and the differences between the averages were compared with the [15].

3. Results and Discussion

The results were showed significant effect (P<0.05) in motility of sperm between treatments at (0 hrs) of cooling preservation. (1 Mm of GSH) achieved the highest sperm motility it was (85.50 ± 0.86%), while the lowest percentage in (0.5 Mm Taurine) compared with the other treatments, There were no significant differences between the treatments at 48 hrs of cooling storage period in sperm motility. The sperm motility was significantly decreased (P<0.01) at (0 and 48 hrs) for all treatments of cooling storage period, the addition of glutathione to rams sperm diluents has improved the movement of sperm, vitality and plasma membrane characteristics and protected the sperm from free radical damage [16]. [17] pointed out that the addition of glutathione (GSH) with concentrations (0.5, 1 and 2 mM) to the 5°C dilution of refrigerated semen for 96 hours had significant effect (P <0.05), significantly improved the ratio of progressive motility and the ratio of live sperm and reduced the percentage of abnormal sperm.

Table 1. Effect of different concentrations of sulfuric antioxidants on sperm motility during cooling(0 and 48 hours)

| Treatment   | Time     | P value |
|-------------|----------|---------|
|             | 0 hour   | 48 hour |         |
| Control     | 82.50 ± 1.04 ABa | 61.00 ± 0.57 b | ** |
| CYC 0.5 mM  | 83.50 ± 1.93 AbAa | 62.75 ± 1.60 b | ** |
| CYS 1 mM    | 83.75 ± 0.62 AAb | 62.25 ± 1.18 b | ** |
| GSH 0.5mM   | 83.25 ± 1.10 AAc | 62.75 ± 2.01 b | ** |
| GSH 1mM     | 85.50 ± 0.86 Aa | 62.75 ± 1.70 b | ** |
| Taurine 0.5mM | 81.25 ± 1.10 Ba | 63.25 ± 0.75 b | ** |
| Taurine 1mM | 83.25 ± 1.10 AAb | 64.00 ± 0.91 b | ** |
| P value     | *        | NS      | -----  |

There were a significant Effect (P<0.05) of (1 Mm of GSH) in plasma membrane integrity it was (88.25±0.94%) at (0 hrs) of cooling storage compared with the control group (82.50±1.40%). No significant differences between the treatments in the same Character at (48 hrs) of cooling storage. As
for the effect of time of cooling storage it was a highly significant (P<0.01) in plasma membrane integrity in all of the treatments in this study (Table2).

**Table 2.** Effect of different concentrations of sulfuric antioxidants on membrane integrity during cooling (0 and 48 hours)

| Treatment           | Time       | P value |
|---------------------|------------|---------|
|                     | 0 hour     | 48 hour |       |
| Control             | 82.50 ± 1.40 Aba | 65.50 ± 0.64 b | **  |
| CYC 0.5 Mm          | 86.75 ± 1.70 Aba | 67.75 ± 1.31 b | **  |
| CYS 1 Mm            | 87.00 ± 0.70 Aba | 66.25 ± 1.18 b | **  |
| GSH 0.5mM           | 86.75 ± 0.85 Aba | 67.25 ± 1.32 b | **  |
| GSH 1Mm             | 88.25 ± 0.94 Aa | 66.75 ± 1.88 b | **  |
| Taurine 0.5mM       | 83.75 ± 1.93 Ba | 67.50 ± 0.64 b | **  |
| Taurine 1mM         | 86.75 ± 1.10 Aba | 67.50 ± 0.64b | **  |

No significant Differences between treatments in dead sperm percentage at (0 hrs) of cooling storage period, 1Mm GSH achieved the lowest percentage it was (21.00 ± 1.22%) while treatment 0.5 mM GSH achieved the highest percentage (23.50 ± 0.64%). There were no significant Differences between the treatments at 48 hrs for cooling storage period in dead sperm percentage. The effect of (0 and 48 hrs) of cooling storage was highly significant (P<0.01) in dead sperm for all treatments (Table3).

**Table 3.** Effect of different concentrations of sulfuric antioxidants on dead sperm during cooling (0 and 48 hours)

| Treatment        | Time       | P value |
|------------------|------------|---------|
|                  | 0 hour     | 48 hour |       |
| Control          | 22.25 ± 0.47 b | 37.25 ± 0.47 a | **  |
| CYC 0.5 mM       | 21.25 ± 0.85 b | 33.25 ± 3.38 a | **  |
| CYS 1 mM         | 21.25 ± 0.94 b | 37.75 ± 0.62 a | **  |
| GSH 0.5mM        | 23.50 ± 0.64 b | 33.75 ± 3.72 a | **  |
| GSH 1Mm          | 21.00 ± 1.22 b | 34.00 ± 4.35 a | **  |
| Taurine 0.5mM    | 21.75 ± 0.25 b | 36.75 ± 1.10 a | **  |
| Taurine 1mM      | 22.25 ± 1.10 b | 32.25 ± 2.86 a | **  |

The results showed (Table4) there were no significant Differences in DNA damage between the treatments at (0 hrs) of cooling period, 1 Mm Taurine was achieved the lowest DNA damage percentage it was (6.75 ± 0.47%). No significant Differences at 48 hrs for cooling period between the treatments on DNA damage, 1 mM Cys achieved the lowest DNA damage percentage it was (11.25±0.94%) compared with the other treatments, While the effect of (0 and 48 hrs) of cooling was highly significant (P<0.01) in DNA damage percentage for all the treatments. The addition of cysteine a few concentrations improved the individual motility of the sperm, this is due to the role of the sulfur group in the cysteine, which has the ability to interact with different free radicals, The role of cysteine in the synthesis of glutathione, and the action of cysteine as an antitoxic agent through its role in the release of the sperm from Singlet oxygen produced by metabolic processes[18]. [19] indicated that the concentration of 10 mM / ml for cysteine may exceed 5mM / ml during the dissolution of frozen ureter rats, while [20]. did not notice any significant differences between the concentration (10, 5 mM / ml). In
our study, no significant differences were observed between the concentration of 0.5 and 1 mM for the studied traits.

**Table 4. Effect of different concentrations of sulfuric antioxidants on DNA Fragmentation during cooling (0 and 48 hours)**

| Treatment | Time          | P value |
|-----------|---------------|---------|
|           | 0 hour        | 48 hour |         |
| Control   | 6.75 ± 0.47 b | 12.50 ± 0.86 a | ** |
| CYC 0.5 mM| 7.25 ± 0.75 b | 11.25 ± 0.94 a | ** |
| CYS 1 mM  | 7.50 ± 0.28 b | 11.50 ± 0.28 a | ** |
| GSH 0.5mM | 7.00 ± 0.40 b | 11.50 ± 0.50 a | ** |
| GSH 1mM   | 7.50 ± 0.50 b | 11.50 ± 0.50 a | ** |
| Taurine 0.5mM| 7.00 ± 0.40 b | 12.50 ± 1.25 a | ** |
| Taurine 1mM| 6.75 ± 0.47 b | 11.50 ± 0.50 a | ** |

The results of study showed no significant differences between the treatments in sperm survival (Table5) 1Mm Taurine was a highest average it was (7.80 ± 0.84), while the control group was the lowest average for sperm survival (67.70 ± 0.63) compared with the other treatments. Taurine is an intracellular amino acid found in majority of the mammalian tissues and plays its role in cell proliferation, viability, and osmoregulation and prevents injuries induced by oxidants in many tissues[21; 22]. [23; 24] report that the main causes of low sperm effectiveness and DNA damage during (refrigeration, freezing, and thawing) are oxidative stress. The addition of antioxidants to sperm diluents will improve the antioxidant properties of sperm [25; 26].

**Table 5. Effect of different concentrations of sulfuric antioxidants on sperm survival during cooling**

| Treatment  | Sperm Survival |
|------------|----------------|
| Control    | 67.70 ± 0.63   |
| CYC 0.5 Mm | 69.45 ± 1.73   |
| CYS 1 mM   | 69.10 ± 1.31   |
| GSH 0.5mM  | 69.70 ± 2.24   |
| GSH 1mM    | 69.72 ± 1.89   |
| Taurine 0.5mM| 69.67 ± 0.85   |
| Taurine 1mM| 70.80 ± 0.84   |
| P value    | N.S            |

The addition of cysteine a few concentrations improved the individual motility of the sperm, this is due to the role of the sulfur group in the cysteine, which has the ability to interact with different free radicals, The role of cysteine in the synthesis of glutathione, and the action of cysteine as an antitoxic agent through its role in the release of the sperm from Singlet oxygen produced by metabolic processes[18]. [19] indicated that the concentration of 10 mM / ml for cysteine may exceed 5mM / ml during the dissolution of frozen ureter rats, while [20]. did not notice any significant differences between the concentration (10, 5 mM / ml). In our study, no significant differences were observed between the concentration of 0.5 and 1 mM for the studied traits. Taurine is an intracellular amino acid found in majority of the mammalian tissues and plays its role in cell proliferation, viability, and osmeregulation and prevents injuries induced by oxidants in many tissues[21; 22]. [23; 24] report that the main causes of low sperm effectiveness and DNA damage during (refrigeration, freezing, and
thawing) are oxidative stress. The addition of antioxidants to sperm diluents will improve the antioxidant properties of sperm[25; 26].

4. Conclusion
The results of the study showed that the use of low concentrations of sulfur compounds improved the calculation of the characteristics mentioned in the study and there was no significant improvement.

5. Acknowledgments
Authors would like to express their high appreciation to the Higher Education and Scientific Research Ministry/University of Baghdad/col lege of Agricultural Engineering Sciences for providing the facilities utilized in accomplishing of this research project.

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