ROLE OF ANTIOXIDANTS ON LIQUID AND FROZEN BUFFALO-BULL SEMEN AND THEIR EFFECT ON PLASMA MEMBRANE INTEGRITY

BY

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SUMMARY

The effects of antioxidants upon the sperm morphology of buffalo liquid and frozen semen and on the membrane of sperm integrity during storage at 4°C and thawing process were studied. Ascorbic acid and glutathione were added to extended semen at concentrations of 0.0, 0.1, 0.5 and 1.0 mg as well and 0.0, 0.1, 0.5 and 1.0 mmol/120×10^6 sperm cell respectively. The samples were stored at 4°C for 4 days and examined daily while, the frozen samples were examined post thawing.

The results in this study demonstrated that, addition of antioxidants improved buffalo-semen quality during storage. Antioxidants, especially high concentration (1.0 mg ascorbic acid or 1.0 mmol glutathione), had significant increasing effects on sperm motility, sperm livability, with acrosomal intact percentages and decreasing effects on sperm abnormality percentages. The ultrastructure investigation of liquid and frozen sperm revealed that, high concentrations of antioxidants had a good protective effect for sperm plasma membrane. Moreover, the glutathione is better than the ascorbic acid upon semen quality and protecting effect for plasma membrane of the spermatozoa against damage.

These results arise the possibility of using antioxidants with diluents for improving the liquid and frozen semen quality.

Key words: Antioxidants, buffalo liquid semen, frozen, plasma membrane.

INTRODUCTION

Preservation of livestock semen requires a reduction or arrest of the metabolism of spermatozoa, thereby prolonging their fertile life (Maxwell and Salamon,1993). However, metabolism is not completely arrested during liquid storage at reduced

temperature. The main changes, which occur, include an irreversible reduction in motility, morphological integrity and fertility of spermatozoa (Maxwell and Stojanov, 1996).

The molecular mechanisms responsible for sperm deterioration during in vitro storage are not clear. The ability of the molecular organization of the sperm membrane to respond to cooling may be impaired through an inability to change fluidity. Fluidity is linked to the integrity of the membrane lipids and changes in the lipid composition of the plasma membrane may therefore by associated with the cooling and storage effects (Buhr, et al., 1989 and Cerolini, et al., 2000).

The term antioxidant has been defined as any substance that delays or inhibits oxidative damage to a target molecule (Gutteridge and Halliwell, 1994). It should be emphasized that, many of these compounds and enzymes work in concert to provide protection against radical reactions. During the physiological process of oxidation of organic materials by molecular oxygen to produce energy, a number of reactive oxygen species (ROS) are formed (Woodford and Whitehead, 1998). It is well known that, during storage of sperm, its phospholipids under peroxidation leads to the formation of toxic fatty acid peroxides. Mammalian sperm are highly susceptible to lipid peroxidation, which may be one of the mechanisms responsible for the negative biochemical and physiological changes during storage (Sinha, et al., 1996 and Cerolini, et al., 2000). Spontaneous lipid peroxidation of the sperm membranes damages the structural of the lipid matrix and is associated with loss of the motility (Kessopoulou, et al., 1992 and Maxwell and Stojanov, 1996). These changes may be contributed to the accumulation of the toxic products of metabolism and more importantly of ROS (Maxwell and Stojanov, 1996).

ROS in bovine and ram semen are produced by dead spermatozoa via an aromatic amino acid oxidase (Shannon and Curson, 1982 and Upreti, et al., 1994). It has a bad effect on sperm survival and fertilizing ability (Vishwanath and Shannon, 1997). Glutathione as a substrate of glutathione peroxidase present in the sperm head. It plays an important role in sperm activities including viability, movement and their fertility (Jain, 1997 and Krizanovic, 1997). It protects cellular and subcellular membranes from peroxidative damage (Slaweta, et al., 1988).

From the available literatures, attempts to improve the liquid and frozen buffalo semen quality and fertility by adding glutathione and ascorbic acid are lacking. Therefore, the aim of the present investigation was to study the effect of different levels of glutathione and ascorbic acid on sperm motility, alive, acrosomal intact, abnormalities percentages, lipid peroxide (LPO), lactate dehydrogenase enzyme (LDH) and sperm integrity from liquid and frozen semen after storage semen.

MATERIALS AND METHODS

Semen collection :-

Semen samples were collected from healthy two buffalo-bulls raised in the farm belonging to Faculty of Veterinary Medicine, Assiut University, Egypt. The animals maintained under identical nutritional and managemental conditions. The semen was collected twice weekly for three weeks at early morning using artificial vagina (42-43 °C) and female buffalo used as a teaser. Within 2-3 minutes after collection, the samples were placed in a water bath at 37°C and immediately transferred to the laboratory.

Semen evaluation :-

Semen was evaluated according to Ahmed et al. (1996). Ejaculates having less than 60% motility were discarded and good quality samples were pooled and kept in water bath at 37°C before dilution for the subsequent examination. Evaluation of pooled ejaculates include, mass activity, individual motility and sperm cell concentration. Morphology and alive sperm percentages were assessed by using alkaline methyl violet and eosin-nigrosin stains respectively. Acrosomal intact was determined by using Giemsa staining technique according to Watson (1975).

Extension and treatment for liquid semen :-

The pooled semen, after evaluation, was extended with egg yolk citrate to give a final concentration of 120×10^6 sperm cell/ml. The prepared liquid semen was divided into two portions, the first contained ascorbic acid (L-ascorbic acid, Sigma) and the second one contained glutathione (Sigma). The ascorbic acid was added to the extended semen at concentrations of 0.0 mg (control), 0.1, 0.5 and 1.0 mg / 120×10^6 sperm cell. The glutathione was added to the extended semen at concentrations of 0.0 mmol (control), 0.1, 0.5 and 1.0 mmol/ 120×10^6 sperm cell. Five samples from each of the above mentioned concentrations were prepared. All

samples were stored in refrigerator (4°C) and examined daily for 4 days to evaluate sperm motility, livability, secondary abnormalities (especially free loss head and bent tail) and percentages of intact acrosomal.

Extension and treatment for frozen semen :-

Egg yolk citrate extender was prepared (one part egg yolk and four parts sod. citrate), then divided into two equal parts. Glycerol (14%) was added to the first part and the semen was added to the second part. Equal amount of first part of extender was added in 3 lots with 5 minutes apart to obtained the final concentration of 120×10^6 sperm cell/ml and 7% glycerol.

The final extend semen was divided into two parts. Ascorbic acid and glutathione were added at the same levels of concentrations of used for liquid semen. Five samples from each concentrations were prepared. The treated and control extended semen were filled in mini-tub straws (each 0.25 ml capacity) and sealed. The straws were collected in a special container and transported to the refrigerator to equilibration at 5°C for 3 h. The straws were exposed to liquid nitrogen vapour (-100 °C) for 10 minutes, then kept in liquid nitrogen large flask. Evaluation was done after 24 h from complete processing. Ten straws from each concentrations of ascorbic acid and glutathione were thawed in water bath (45°C) for one minute. Each group of straws were collected in the test tube and kept in water bath (30°C) till examination. Evaluation of each sample was done as in case of liquid semen.

Biochemical analysis :-

After sperm evaluation the remainders of samples were centrifuged at 3000 rpm for 20 minutes. The supernatant fluid was collected and kept at -20 °C till used for biochemical analysis. Lactate dehydrogenase (LDH) was determined by using commercial kit (Stanbio Lab. INC., San Antonio, Texas). Lipid peroxide (LPO) concentration was estimated by the malonaldehyde (MDA) level according to Benge and Aust (1978).

Electronic microscopically examination :-

After centrifugation, the sediment was prepared, in the Unit of Electronic Microscope, Assiut University, for examination by transmission electron microscope to evaluate changes in the

plasma membrane of spermatozoa after being stained by uranyl acetate and lead citrate.

Statistical analysis :-

Data were expressed as the mean ± S.D for all treatment. Analysis of Variance (ANOVA) was done and differences between treatments were analyzed by least significant difference (LSD) using PC-stat computer programme. Results were considered significant at $P<0.05$ or less.

RESULTS

The effect of addition of antioxidants upon the liquid and frozen buffalo-semen were represented in Tables (1-4) and Figures (1-5).

Effect of ascorbic acid and glutathione on quality of liquid semen :-

Sperm motility percent (S.M) and livability were affected by the addition of either ascorbic acid or glutathione (Tables 1, 2). S.M and sperm livability increased significantly ($P<0.01$) with all concentrations of ascorbic acid and glutathione. It was observed that, the increase of S.M and livability was noticed with higher concentration of ascorbic acid and glutathione till the 4^{th} day of storage when compared with the control samples. High concentration of glutathione (1. mmol) had a highest improving effect on the S.M and sperm livability than in case of ascorbic acid (1.0 mg).

Sperm abnormalities percent (S.Ab) reduced by the addition of antioxidants to the liquid buffalo semen (Table 3). All concentrations of both ascorbic acid and glutathione had a significant ($P<0.01$) reducible effect when compared with control. In between concentrations, the high concentration of ascorbic acid had a non-significant effect when compared with the second concentration but significantly with the first concentration all over the storage time. However, in case of glutathione, there are significant effects between each concentration through 3^{rd} and 4^{th} day of storage, but there are non-significant effects between high and second concentration at 1^{st} and 2^{nd} day of storage. The results presented at Table (4) show the effect of ascorbic acid and glutathione on the percentages of intact acrosomal sperm stored at 4 °C for 4 days. All concentrations of ascorbic acid had a significant ($P<0.01$) increasing effect on percentages of intact

acrosomes among storage time. Moreover, all concentrations of glutathione had a highest effect especially at the 3rd and 4th day of storage thereby the increasing percent.

The changes in the levels of LDH and LPO were illustrated in Figures (1,2 and 3,4) respectively. The significant decreasing ($P<0.01$) effect was observed for each of LDH and LPO levels in all concentrations of ascorbic acid and glutathione among all days of storage. The high concentration of both ascorbic acid and glutathione (1.0 mg/ml and 1.0 mmol/ml respectively) had a highest significant decreasing ($P<0.01$) effect among the last day of storage. The decreasing percent at last day of storage after adding of high concentration of glutathione (129.13 %) was better than in case of ascorbic acid (89.58 %) at the same concentration and the same day of storage.

Effect of ascorbic acid and glutathione on the quality frozen buffalo-semen after thawing :-

The obtained results in this parts of the present study were illustrated in Table (5). It revealed the effect of ascorbic acid upon the thawing frozen semen, as well as, it was noticed that, S.M had a significantly increasing ($P<0.01$) effect in all concentrations of ascorbic acid when compared with control samples. However, high concentration (1.0 mg/ml) had a significant ($P<0.05$) different when compared with other concentrations of ascorbic acid, but there is no- significant different between first and second concentrations of ascorbic acid upon the S.M after thawing of frozen semen. All concentrations of ascorbic acid had a significantly increasing ($P<0.01$) effect upon the sperm livability and intact acrosomal percent of thawing frozen semen as compare with the control samples. In addition, S.Ab decreased significantly ($P<0.01$) with all concentrations of ascorbic acid. When compared between the effects of all concentrations upon sperm livability, S.Ab and intact acrosomal percent, it was noticed that, the third (high) concentration was considered the more effective and it had a significantly ($P<0.05$) effect as compared with first and second concentrations of ascorbic acid.

The significant decreasing ($P<0.01$) effect was noticed for each of LDH and LPO levels from control in all concentrations of ascorbic acid as well as, there was a significant effect ($P<0.01$) between all concentrations upon LDH and LPO levels of thawing frozen semen. The variations in the effect of glutathione upon the

thawing/ frozen semen were presented at Table (5b). It had a significant increasing ($P<0.01$) effect on S.M, sperm livability, and intact acrosomal percent when compared with the control samples. It also, had a significant decreasing ($P<0.01$) effect on S.Ab (except first concentration), LDH and LPO levels as compared with the control samples.

When compared between all concentrations, it was observed that, there was a non-significant effect between first and second concentrations of glutathione in case of S.M, livability, S.Ab and intact acrosomal percent but the third concentration had a significant effect ($P<0.05$) with 1st and 2nd concentrations in case of S.M but it was non-significant effect with 2nd concentration in case of sperm livability, S.Ab and intact acrosomal percent. However, All concentrations of glutathione had a significant effect ($P<0.05$) between them in case of LDH and LPO levels.

Effect of antioxidants upon the ultrastructure of treated and control liquid and frozen buffalo-semen :-

The ultrastructure examination of treated and control samples (Figure 6) revealed that, the high concentrations of treatment either ascorbic acid or glutathione had a protective effect for sperm against plasma membrane integrity when compared with control. The other concentrations had a bad effect (especially lower concentration), which observed in the form of sever swelling and sever disintegration of the plasma membrane of sperm head. The protective effect of glutathione (high concentration) against plasma membrane disintegration was better than that of ascorbic acid treatment. For this reason, the treated of liquid and frozen samples with high concentration of ascorbic acid and glutathione were used in insemination to calculate the fertility.

DISCUSSION

The dilution of semen in appropriate media not only provides an environment suitable for extended storage of the spermatozoa, but also dilutes the sperm concentration to a level appropriate for making multiple insemination (Garner, 1991). Artificial insemination (AI) has resulted in the control of venereal diseases and the reduction in frequency of undesirable recessive genes (Maxwell and Salamon, 1993).

This study is a trial to investigate the effect of different concentrations of antioxidants (ascorbic acid and glutathione) on buffalo-liquid and frozen semen. The obtained results showed that, the addition of antioxidants (ascorbic acid and glutathione) improved the semen quality especially sperm motility along the storage time and after thawing. This improvement increased with increasing of the adding concentration of antioxidants. These results are in agreement with that reported by Slaweta and Laskowska (1987), Maxwell and Stojanov (1996), Sinha et al. (1996), Bilodeau et al. (2002) and Foote et al. (2002) who observed that the addition of antioxidants was beneficial improvement in progressive motility of diluted and frozen bovine semen. However, Donnelly et al. (1999) and Ball et al. (2001) concluded that adding of water-soluble antioxidants (ascorbic acid and glutathione) did not significantly improve the maintenance of motility during liquid semen storage.

The improvement of sperm motility during the present study is attributed to the addition of antioxidants to semen increase the intracellular sperm ATP level and prevented sperm ATP loss (Bilodeau, et al., 2002). Moreover, Slaweta and Laskowska (1987) and Sinha et al. (1996) suggested that antioxidants (e.g. glutathione) play an active role in sperm fructolysis. It is a coenzyme of 1,3- diphosphoglyceric aldehyde dehydrogenase which leads to oxidation of triose phosphate to phosphoglyceric acid which is later reduced to pyruvic acid then lactic acid. This may be the reason for improved metabolic activity and increased motility of the sperm.

The values of sperm viability percentages along the storage time take the same trend of motility with different doses of the antioxidants (Cerolini, et al., 2000). Moreover, there are non-significant decreasing effects of antioxidants on the sperm abnormalities. This may be attributed to the protective action of the antioxidants against lipid peroxidation which similar to egg yolk that may also provide lipid substrates for peroxidation (Erochin and Derjasencev, 1992).

A complex antioxidant system present in spermatozoa and seminal plasma to scavenge the oxygen radicals and prevent their damage action under normal physiological conditions (Surai, et al., 1998). Nevertheless, the antioxidant system of the cell did not potent enough to prevent lipid peroxidation completely, especially

during in vitro storage when the production of free radicals could be significantly enhanced as a result of metabolic changes (Hammerstedt, 1993 and Halliwell, 1994). However, the present results show that, in the buffalo-semen, the addition of antioxidant into the storage diluents improve any deterioration in the parameters of sperm quality and decrease the acrosomal integrity. The findings in this study seem to agree with those of Vishwanath et al. (1994), Maxwell and stojanov (1996), Baumber et al. (2000) and Ball et al. (2001). They concluded that, antioxidants had an additive effect on the acrosomal integrity of spermatozoa and significantly increase their resistance to lipid peroxidation (Griveau, et al., 1995). In our investigation, the finding revealed a significant decreasing of the release of LDH and LPO in the treated samples than in control samples which agree with the reports of Slaweta and Laskowska (1987), Cotran et al. (1989) and Sinha et al., (1996). Bell et al. (1993) reported that, there was a negative correlation between sperm motility and LPO in the cryopreserved Samples.

Concerning the protection of sperm cell and plasma membrane by addition of antioxidants, the obtained results revealed that, the high concentration of the uses antioxidants protected the sperm cell membrane (as observed on ultrasturcture of sperm). It is clear that, oxidative damage is only one of a number of stresses the cryopreserved sperm cells (Alvarez and Storey. 1993). The Sudden increase in oxygen utilization by spermatozoa during storage and thawing, following the dormant metabolic stage, might be responsible for increased production of free radicals, leading to spermatozoal membrane damage (Sinha, et al. 1996 and Bilodeau, et al., 2000). This stress found to be counteracted in presence of glutathione and ascorbic acid (Slaweta and Laskowska, 1987). The obtained findings agrees with that reported by Maxwell and Watson (1996), Sinha et al. (1996), O'Flaherty et al. (1997), Watson (2000) and Yousef et al. (2003). They concluded that antioxidants play an important role in scavenging reactive oxygen and other free radicals and may be delay the membrane destabilization associated with sperm storage and thawing.

Finally, it may be concluded that, the higher concentrations of antioxidants used in our investigation were necessary to protect spermatozoa from the potentially toxic conditions created in the unnatural environment of liquid storage semen and during

frozen/thawing processes. As well as, antioxidants might be protect the spermatozoa from membrane damage by inhibiting the lipid peroxidation process, which leads to improve the liquid and frozen semen quality.

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Table (1) : Effect of antioxidants upon sperm motilitypercentages of liquied semen stored at 4 ^{O}C for 4 days.

Storage time (days)	Control samples (n=20)	Ascorbic acid (n=20)			Glutathione (n=20)		
		0.1 mg (n = 20)	0.5 mg (n = 20)	1.0 mg (n = 20)	0.1 mmol (n = 10)	0.5 mmol (n = 10)	1.0 mmol (n = 10)
1	58.33 ± 2.89^{3}	$63.33 \pm 2.88^{2,a}$	$68.33 \pm 2.17^{1,2,a}$	$73.35 \pm 2.89^{1,a}$	$66.67 \pm 2.88^{2,a}$	$69.33 \pm 1.16^{2,3,a}$	$74.45 \pm 2.19^{3,a}$
2	51.67 ± 1.88^{3}	$61.67 \pm 2.19^{2,b}$	$66.67 \pm 2.89^{1,2,a}$	$71.66 \pm 2.1^{1,a,b}$	$61.67 \pm 2.16^{2,b}$	$67.35 \pm 2.34^{3,a}$	$72.33 \pm 2.92^{4,a}$
3	48.33 ± 1.39^{3}	$53.33 \pm 2.67^{4,c}$	$63.33 \pm 2.15^{1,a}$	$68.33 \pm 1.92^{1,b,c}$	$56.67 \pm 1.65^{2,c}$	$64.67 \pm 2.54^{3,a}$	$69.54 \pm 2.21^{4,b}$
4	41.67 ± 2.88^{3}	$48.33 \pm 2.82^{4,c}$	$56.76 \pm 5.77^{1,b}$	$63.52 \pm 2.88^{2,c}$	$50.76 \pm 1.67^{2,d}$	$58.33 \pm 1.17^{3,b}$	$66.67 \pm 1.35^{4,b}$

-Means with different superscript numbers in the same row are significantly different (P< 0.01).
-Means with different superscript letters in the same column are significantly different (P< 0.01).

Table (2) : Effect of antioxidants upon sperm viability percentage of liquid semen stored at 4 ^{O}C for 4 days.

Storage time (days)	Control samples (n=20)	Ascorbic acid (n=20)			Glutathione (n=20)		
		0.1 mg (n = 20)	0.5 mg (n = 20)	1.0 mg (n = 20)	0.1 mmol (n = 10)	0.5 mmol (n = 10)	1.0 mmol (n = 10)
1	77.82 ± 0.98^{1}	$84.76 \pm 0.69^{2,a}$	$85.97 \pm 1.08^{3,a}$	$87.28 \pm 0.61^{4,a}$	$85.97 \pm 0.64^{2,a}$	$86.57 \pm 0.46^{3,a}$	$87.46 \pm 0.68^{4,a}$
2	70.78 ± 1.05^{1}	$83.86 \pm 0.76^{2,a}$	$84.37 \pm 1.15^{3,b}$	$85.56 \pm 1.27^{3,b}$	$84.16 \pm 0.36^{2,a}$	$85.06 \pm 1.05^{2,3,b}$	$86.86 \pm 065^{3,b}$
3	67.78 ± 0.75^{1}	$78.09 \pm 0.79^{2,b}$	$81.75 \pm 0.73^{3,c}$	$84.35 \pm 1.12^{4,b}$	$80.59 \pm 0.56^{2,b}$	$83.36 \pm 1.03^{3,c}$	$84.86 \pm 1.76^{4,c}$
4	51.44 ± 0.48^{1}	$65.69 \pm 0.61^{2,c}$	$78.08 \pm 0.65^{3,d}$	$81.62 \pm 0.89^{4,c}$	$75.35 \pm 0.60^{2.}$	$80.16 \pm 0.63^{3,d}$	$83.03 \pm 0.47^{4,d}$

-Means with different superscript numbers in the same row are significantly different (P< 0.01).
-Means with different superscript letters in the same column are significantly different (P< 0.01).

Table (3) : Effect of antioxidants upon sperm abnormalities percentage of liquid semen stored at 4 °C for 4 days.

Storage time (days)	Control samples (n=20)	Ascorbic acid (n=20)			Glutathione (n=20)		
		0.1 mg (n = 20)	0.5 mg (n = 20)	1.0 mg (n = 20)	0.1 mmol (n = 10)	0.5 mmol (n = 10)	1.0 mmol (n = 10)
1	25.06 ± 1.19^{1}	$19.41 \pm 0.64^{2,a}$	$17.38 \pm 1.09^{3,a}$	$16.49 \pm 1.26^{3,a}$	$19.27 \pm 0.73^{2,a}$	$17.09 \pm 0.24^{3,a}$	$15.52 \pm 0.55^{3,a}$
2	28.55 ± 0.76^{1}	$21.89 \pm 0.75^{2,b}$	$19.24 \pm 1.04^{3,b}$	$17.58 \pm 0.90^{3,a,b}$	$20.73 \pm 0.57^{2,a,b}$	$18.04 \pm 0.77^{3,a,b}$	$16.57 \pm 051^{3,a}$
3	32.45 ± 2.36^{1}	$23.82 \pm 0.92^{2,c}$	$20.04 \pm 0.28^{3,b}$	$17.63 \pm 1.27^{4,a,b}$	$21.65 \pm 0.73^{2,b,c}$	$19.43 \pm 1.04^{3,b,c}$	$17.09 \pm 1.07^{4,a}$
4	37.36 ± 0.69^{1}	$25.34 \pm 0.95^{2,c}$	$20.78 \pm 0.93^{3,b}$	$19.23 \pm 0.82^{3,b}$	$22.85 \pm 0.62^{2,c}$	$20.47 \pm 0.86^{3,c}$	$18.79 \pm 0.43^{4,b}$

-Means with different superscript numbers in the same row are significantly different (P< 0.01).
-Means with different superscript letters in the same column are significantly different (P< 0.01).

Table (4) : Effect of antioxidants upon sperm acrosomal intact percentage of liquid semen stored at 4 °C for 4 days.

Storage time (days)	Control samples (n=20)	Ascorbic acid (n=20)			Glutathione (n=20)		
		0.1 mg (n = 20)	0.5 mg (n = 20)	1.0 mg (n = 20)	0.1 mmol (n = 10)	0.5 mmol (n = 10)	1.0 mmol (n = 10)
1	77.32 ± 1.49^{1}	$79.25 \pm 0.24^{2,a}$	$81.54 \pm 1.23^{3,a}$	$85.48 \pm 0.81^{4,a}$	$84.38 \pm 0.79^{2,a}$	$86.09 \pm 0.39^{3,a}$	$88.15 \pm 0.48^{4,a}$
2	71.46 ± 0.79^{1}	$78.05 \pm 0.64^{2,a}$	$79.47 \pm 0.74^{3,b}$	$83.62 \pm 0.87^{4,b}$	$83.31 \pm 0.35^{2,a,b}$	$85.32 \pm 0.47^{3,a}$	$87.10 \pm 0.47^{4,a}$
3	69.45 ± 0.61^{1}	$76.54 \pm 0.59^{2,b}$	$78.83 \pm 0.19^{3,b}$	$81.18 \pm 0.78^{4,c}$	$82.55 \pm 0.66^{2,b,c}$	$83.67 \pm 0.43^{3,b}$	$85.23 \pm 0.39^{3,b}$
4	66.02 ± 1.25^{1}	$74.98 \pm 0.76^{2,c}$	$76.98 \pm 0.13^{3,c}$	$79.96 \pm 0.32^{4,c}$	$81.68 \pm 0.73^{2,c}$	$82.35 \pm 0.85^{3,c}$	$84.06 \pm 0.76^{3,d}$

-Means with different superscript numbers in the same row are significantly different (P< 0.01).
-Means with different superscript letters in the same column are significantly different (P< 0.01).

Table (5) : Effect of antioxidants upon the frozen semen quality after thawing.

Storage time (days)	Control samples (n=20)	Ascorbic acid (n=20)			Glutathione (n=20)		
		0.1 mg (n = 20)	0.5 mg (n = 20)	1.0 mg (n = 20)	0.1 mmol (n = 10)	0.5 mmol (n = 10)	1.0 mmol (n = 10)
S.M	44.00 ± 4.18^{1}	56.00 ± 6.52^{2}	60.00 ± 5.00^{2}	62.00 ± 4.18^{3}	56.00 ± 6.52^{2}	60.00 ± 5.00^{2}	62.00 ± 4.18^{3}
S.V	57.66 ± 5.48^{1}	67.89 ± 6.43^{2}	$73.93 \pm 4.61^{2,3}$	78.76 ± 3.30^{3}	67.89 ± 6.43^{2}	$73.93 \pm 4.61^{2,3}$	78.76 ± 3.30^{3}
S.Ab	24.29 ± 2.79^{1}	19.60 ± 1.52^{2}	18.99 ± 0.85^{2}	17.59 ± 0.79^{2}	19.60 ± 1.52^{2}	18.99 ± 0.85^{2}	17.59 ± 0.79^{2}
S.A.I	69.74 ± 4.51^{1}	74.69 ± 4.21^{2}	79.74 ± 1.78^{2}	80.51 ± 1.75^{2}	74.69 ± 4.21^{2}	79.74 ± 1.78^{2}	80.51 ± 1.75^{2}
LDH	52.81 ± 0.42^{1}	42.97 ± 0.38^{2}	39.14 ± 0.13^{3}	31.83 ± 0.41^{4}	42.97 ± 0.38^{2}	39.14 ± 0.13^{3}	31.83 ± 0.41^{4}
LPO	02.17 ± 0.11^{1}	01.77 ± 0.07^{2}	01.67 ± 0.06^{3}	01.43 ± 0.06^{4}	01.77 ± 0.07^{2}	01.67 ± 0.06^{3}	01.43 ± 0.06^{4}

-S.M = Sperm motility percentages.
-S.V = Sperm viability percentages.
-S.Ab = Sperm abnormalities percentages.
-S.A.I = Sperm acrosomal intact percentages.
-LDH (U/L) = Lactate dihydrogenease enzyme
-LPO (nmol/L) = Lipid peroxide.
-Means with different superscript numbers in the same row are significantly different (P< 0.01).

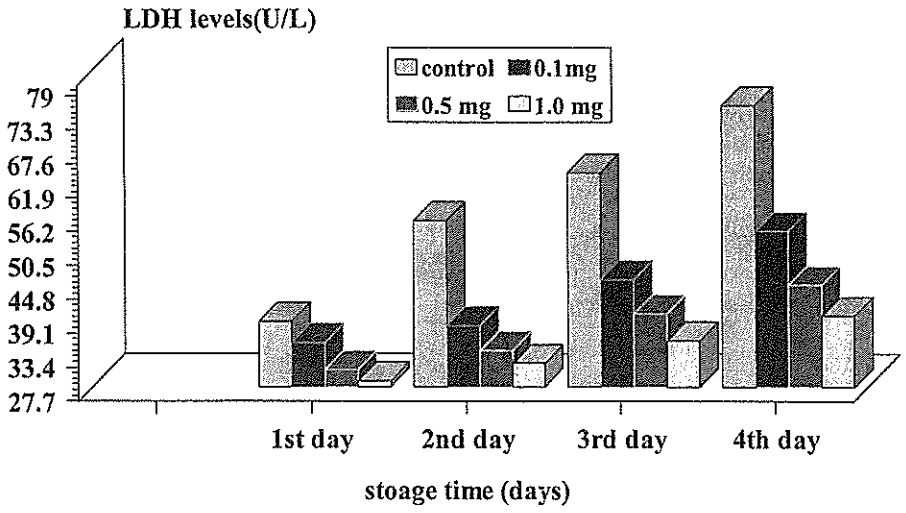


Figure (1) : LDH levels (U/L) of treated liquid semen with ascorbic acid stored at 4 C for 4 days.

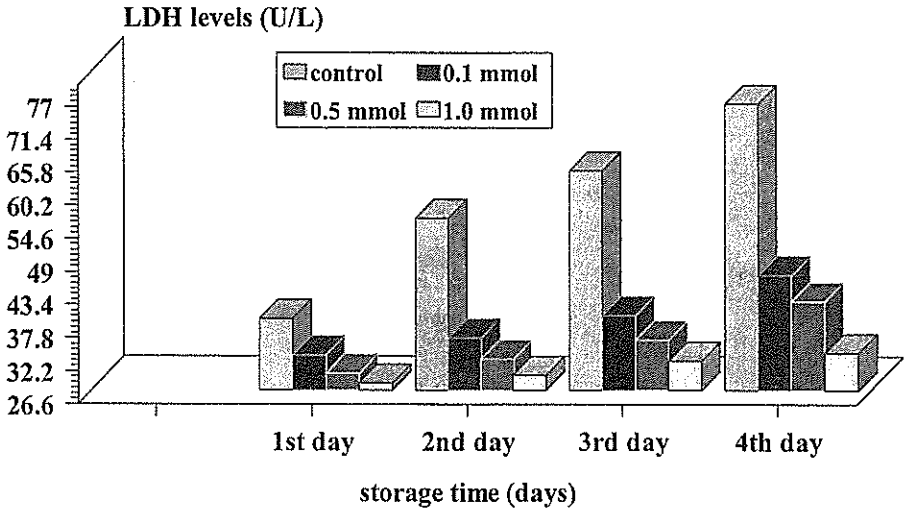


Figure (2) : LDH levels (U/L) of treated liquid semen with glutathione stored at 4 C for 4 days.

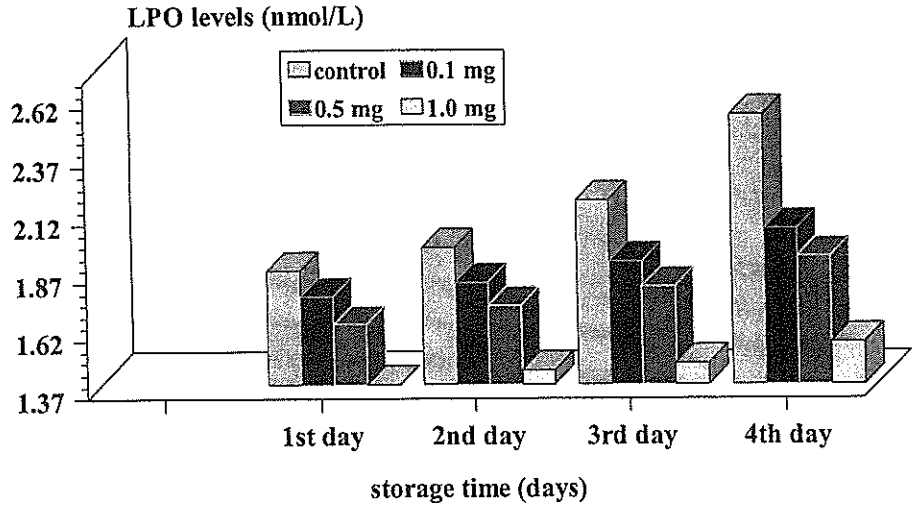


Figure (3) : LPO levels (nmol/L) of treated liquid semen with ascorbic acid stored at 4 C for 4 days.

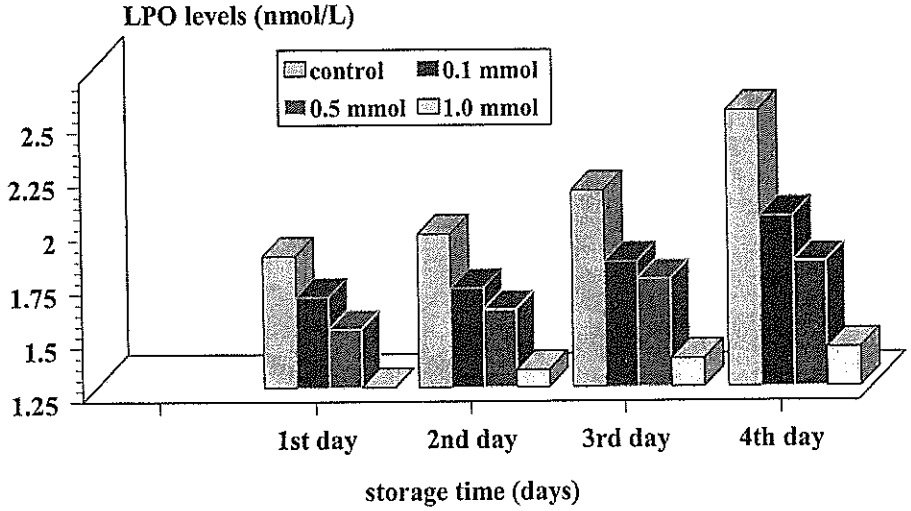


Figure (4) : LPO levels (nmol/L) of treated liquid semen with glutathione stored at 4 C for 4 days.

Explanation of Plates

Plate (I): Liquid semen after 4 days storage.

Fig. 1 : Sagital section through the head of non-treated buffalo-bull spermatozoa.
Fig. 2 : Sagital section through the head of treated buffalo-bull spermatozoa with 1st conc. of ascorbic acid.
Fig. 3 : Sagital section through the head of treated buffalo-bull spermatozoa with 2nd conc. of ascorbic acid.
Fig. 4 : Sagital section through the head of non-treated buffalo-bull spermatozoa with high conc. of ascorbic acid.
Fig. 5 : Sagital section through the head of treated buffalo-bull spermatozoa with 1st conc. of glutathione.
Fig. 6 : Sagital section through the head of treated buffalo-bull spermatozoa with 2nd conc. of glutathione.
Fig. 7 : Sagital section through the head of non-treated buffalo-bull spermatozoa with high conc. of glutathione.

Plate (II): Frozen semen after thawing.

Fig. 1 : Sagital section through the head of non-treated buffalo-bull spermatozoa.
Fig. 2 : Sagital section through the head of treated buffalo-bull spermatozoa with 1st conc. of ascorbic acid.
Fig. 3 : Sagital section through the head of treated buffalo-bull spermatozoa with 2nd conc. of ascorbic acid.
Fig. 4 : Sagital section through the head of non-treated buffalo-bull spermatozoa with high conc. of ascorbic acid.
Fig. 5 : Sagital section through the head of treated buffalo-bull spermatozoa with 1st conc. of glutathione.
Fig. 6 : Sagital section through the head of treated buffalo-bull spermatozoa with 2nd conc. of glutathione.
Fig. 7 : Sagital section through the head of non-treated buffalo-bull spermatozoa with high conc. of glutathione.

Plate I

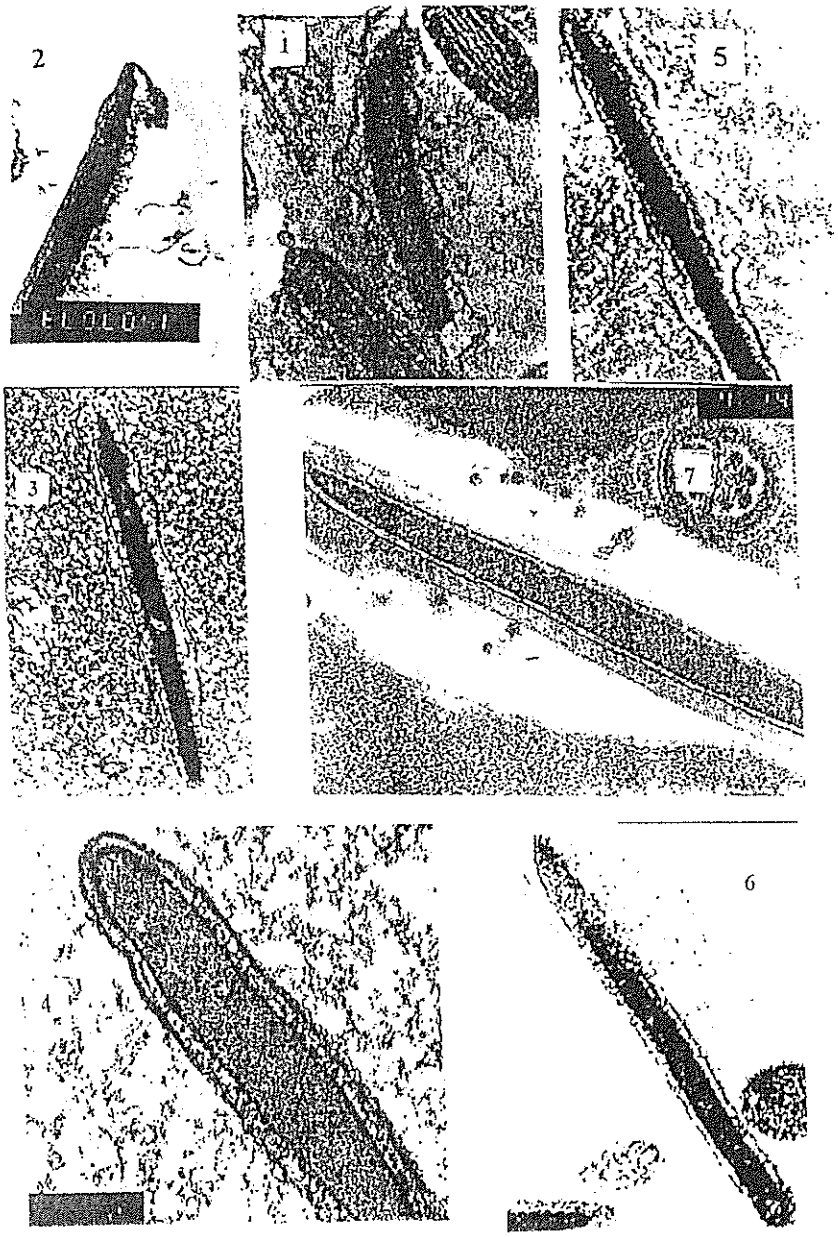
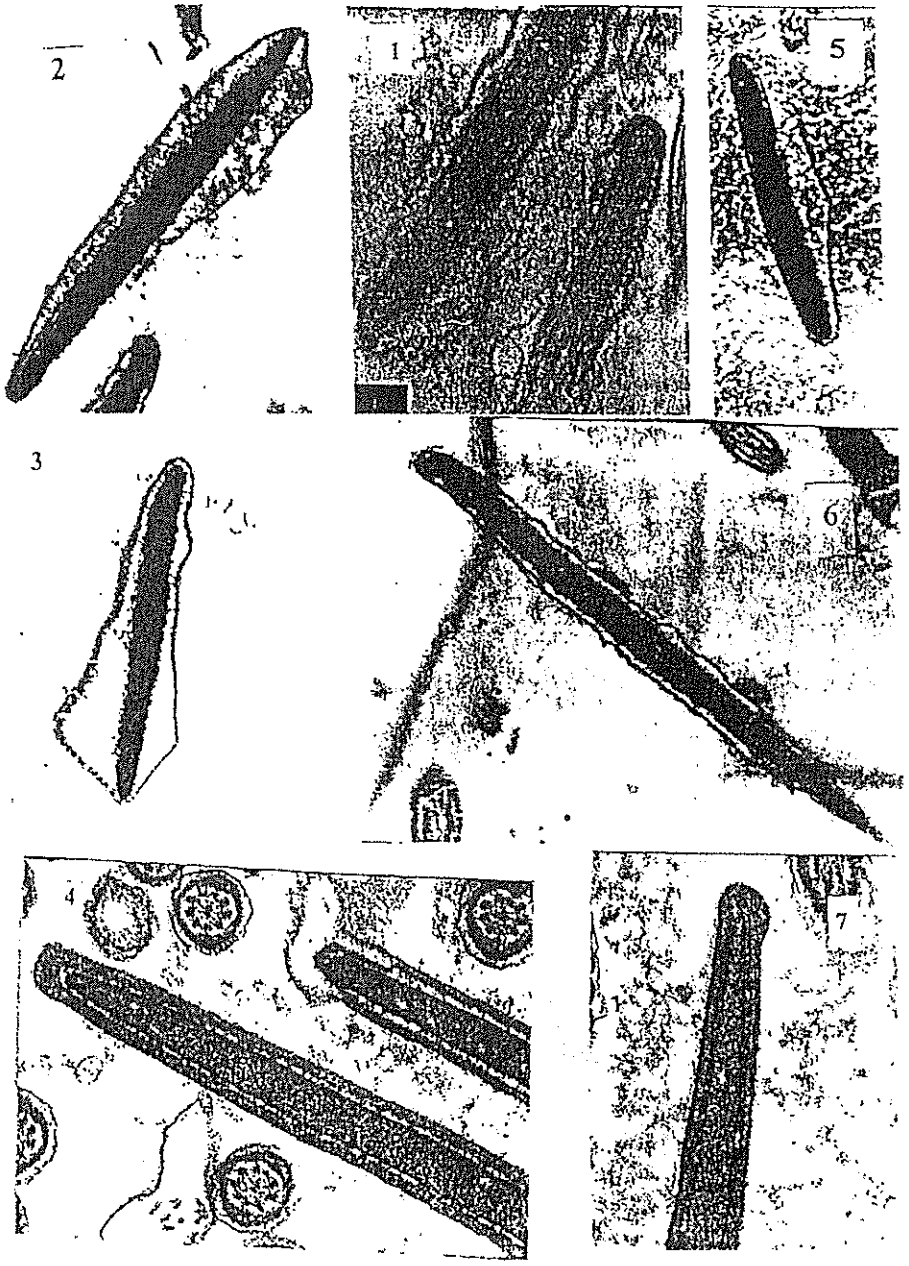


Plate II



الملخص العربى

دور مضادات الأكسدة على المنى السائل والمجمد للعجول الجاموسي و تأثيرها على سلامة أغشية الحيوانات المنوية

تمت دراسة تأثير مضادات الأكسدة (حمض الاسكوربك و مادة الجلوتاثيون) على المنى السائل والمجمد للعجول الجاموسي وأيضا على سلامة أغشية الحيوانات المنوية أثناء حفظ المني السائل في درجة حرارة 4م لمدة أربعة أيام وكذلك أثناء عملية الإساحة بعد التجميد للمني السائل.

تم تخفيف السائل المنوي باستخدام صفار البيض المذاب فى سترات الصوديوم للحصول على تركيز 120 × 10 ^{6} حيوان منوي لكل واحد ملليمتر ثم أضيفت مواد مضادات الأكسدة بالتركيزات الآتية:-

- 0.1 , 0.5 , 1.0 مليجرام لكل 120 × 10 ^{6} حيوان منوي بالنسبة لحمض الاسكوربك

- 0.1 , 0.5 , 1.0 ملي مول لكل 120 × 10 ^{6} حيوان منوي بالنسبة للجلوتاثيون

وأيضا أضيفت نفس التركيزات السابقة للسائل المنوي اثناء تجميده. وتم فحص عينات المني السائل يوميا لمدة أربعة أيام بعد حفظها عند درجة حرارة 4م ، كما تم فحص السائل المنوي المجمد بعد عملية الإساحة.

وأظهرت هذه الدراسة أن إضافة مضادات الأكسدة لها تأثير معنوي في تحسين الحركة الفردية للحيوانات المنوية وأيضا لها تأثير معنوي على خفض نسبة الحيوانات المنوية الميتة. وكذلك أدت هذه المواد إلى خفض نسبة الحيوانات المنوية الغير طبيعية. وحدث انخفاضا معنوية في LDH وأيضا LPO سواء المنى السائل أو المجمد. و كان إضافة مواد مضادات الأكسدة إلى كل من المنى السائل أو المجمد أدى إلى المحافظة على سلامة أغشية الحيوانات المنوية.

ونستخلص من هذه الدراسة أن إضافة مضادات الأكسدة وعلى وجه التحديد التركيز العالي(واحد مليجرام لحمض الاسكوربك) أو واحد مليمول (لجلوتاثيون) لكل 120×10 ^{6} حيوان منوي يؤدى إلى تحسين جودة السائل المنوي وكذلك المحافظة على سلامة أغشية الحيوان المنوي ومنعه من التحطيم أثناء الحفظ في درجة حرارة 4م لمدة أربعة أيام أو بعد عملية إساحة السائل المنوي المجمد.

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