THE INFLUENCE OF ADDING GLUTATHIONE ON SEMEN CHARACTERISTICS OF SOHAGI RAMS

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ABSTRACT

The present study was conducted to investigate the influence of adding glutathione (GSH) at levels of 0.4, 0.8 and 1.2 mM to egg yolk-tris extender (EYTE) used for preservation of semen at a temperature of 25^{o}C for 0, 1, 2, 3, 4, 12 and 24 hours. A total of 10 Sohagi rams having 60-65 kg live body weight and aged 3-4 years were used for semen collection in this study. Semen was collected by artificial vagina set up at optimal conditions to induce a good ejaculatory thrust. Semen was collected twice a week in the morning (8 am) for 10 weeks. Each time semen was collected, all ejaculates (10 ejaculates) were pooled and divided into 4 parts and extended on egg yolk-Tris buffered extenders containing three levels of glutathione, 0.4, 0.8, 1.2 and control group of 0.0 mM.

Semen of each treatment was diluted at a rate of one part of semen to 10 parts of egg yolk-Tris buffered extender at 30^{o}C. Semen was evaluated after 0, 1, 2, 3, 4, 12 and 24 h at 25^{o}C using the following *in vitro* fertility tests; sperm motility (%); livability (%); sperm abnormality (%) and spermatozoa with intact acrosome (%).

The addition of GSH to egg yolk-Tris buffer extender at levels 0.4, 0.8 and 1.2 mM improved semen quality. All GSH levels significantly (P<0.05) increased the percentages of sperm motility, sperm livability and intact acrosome while significantly (P<0.05) decrease sperm cells abnormalities. All sperm characteristics were significantly (P<0.05) affected by storage time, as motility, livability and intact acrosome showed significant (P<0.05) gradual reduction, while sperm abnormality showed significant (P<0.05) increase by progress of storage time up to 24 hours. The highest reduction rate was after 4 hours storage period.

In conclusion, this study demonstrate that supplementation of GSH, at various concentrations, to diluted semen kept at 25^{o}C exert beneficial effects on semen quality in term of sperm motility, livability and membrane stabilization of sperm cells. The best result was obtained with 0.4 mM GSH level in egg yolk extender when stored up to 12 h.

INTRODUCTION

Artificial insemination (AI) is one of the major reproductive biotechnologies through which rapid genetic improvement in livestock has been achieved in developed countries. To raise genetic potential of livestock, AI with fresh semen spermatozoa was introduced in most developing countries. It is reported that viability or fertility of fresh semen is somewhat low. Previous studies on ram spermatozoa showed that addition of antioxidants to the semen extender may also be a step closer to improve the quality of ram spermatozoa. Glutathione prevents the damage of important cellular components developed by reactive oxygen species such as free radicals and peroxides **(**Pompella et al., 2003**).** It is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms **(**Scholz et al., 1989**).** It is implicated in metabolic and biochemical reactions such as DNA synthesis and repair, protein synthesis, prostaglandin synthesis, amino acid transport, and enzyme activation **(**Halliwell and Gutteridge, 1989 and Pastore et al., 2003**).**

Glutathione (L-glutamyl-L-cysteinylglycine; GSH) is a tripeptide ubiquitously distributed in living cells, and it plays an important role as an intracellular defense mechanism against oxidative stress (Irvine, 1996).

During various processing procedures of freezing semen, sperm cells used in A.I. are exposed to oxygen and visible light radiation, which could lead to lipid per oxidation and formation of ROS, which negatively affect

sperm cell motility and genomic integrity (Bilodeau *et al*., 2000). The endogenous antioxidative capacity of semen may be insufficient during storage or dilution (Maxwell and Salamon, 1993). A significant reduction in the level of spermatozoa antioxidant has been reported as one of the causes of the enhanced susceptibility of these cells to per oxidative injuries after cryopreservation (Bilodeau, *et al*. 2000). So, adding several types of antioxidant enzymes activates scavenging ROS, which could help to maintain survival and motility of spermatozoa (Foote et al., 2002). The lipid per oxidation cascade is initiated when spermatozoa are attacked by ROS, which results in a loss of polyunsaturated fatty acids from the plasma membrane and a corresponding decline in the survival and fertilizing ability of these spermatozoa (Aitken, 1995). Sperm oxidative damage is the result of improper balance between ROS generation and scavenging activities.

The sperm plasma membrane is rich in polyunsaturated fatty acids. Therefore it is susceptible to per oxidative damage with consequent loss of membrane integrity, decreased sperm motility, and eventually loss in fertility, resulting from reactive oxygen species during aerobic incubation (Alvarez et al. 1987). Therefore, supplementation with antioxidants such as GSH during the storage of semen is necessary for elimination of free radicals (Bucak *et al*. 2007).

The present study was conducted to investigate the influence of adding antioxidants, glutathione at levels of 0.4, 0.8 and 1.2 mM to egg yolk-tris extender for semen stored at room temperature (25^{o}C) for 0, 1, 2, 3, 4, 12 and 24 hours.

MATERIALS AND METHODS

Animals and Management:

This study was carried out at Animal Production Department, Faculty of Agriculture, Sohag University.

Ten Sohagi Rams having 60-65 kg live body weight and aged 3-4 years were used for semen collection in the study. Throughout the semen collection period rams were fed formulated diets based on NRC (1985) allowances and water was available all day times.

Experimental designs:

Treatments involved three levels, 0.4, 0.8 and 1.2 mM of GSH; and control level without any additives with one type of egg yolk-Tris buffered extender which stored at room temperature (25^{o}C) according to Foote et al. (2002) and evaluated by *in vitro* fertility test after storing for 0, 1, 2, 3, 4, 12 and 24 h.

Semen collection and evaluation:

Semen was collected by artificial vagina set up at optimal conditions to induce a good ejaculatory thrust. At time of collection, one or more ewes were used. One false mount had been always allowed before collection of the first ejaculates. Two consecutive ejaculates with 4-6 minutes interval were obtained from each rams each day of collection.

Semen was collected twice a week in the morning (8 am) for 10 weeks as collection period. Immediately after collection, the ejaculates were transferred to the laboratory to be placed in a water bath at 30ºC. Only ejaculates with more than 70% initial motility were used in the study.

Semen extension:

Each time semen is collected, all ejaculates (10 ejaculates) were pooled, divided into 4 parts, then extended on egg yolk-Tris buffered extender containing glutathione at three levels of 0.4, 0.8, 1.2 and 0.0 mM as control.

Egg yolk-Tris buffered extender (EYTE) was used (Maxwell and Salamon, 1993). Semen of each treatment was diluted at rate of one part of semen to 10 parts of extender at 30^{o}C with a single stepwise addition of egg yolk-Tris buffered extender.

Semen evaluation:

Semen was evaluated after 0, 1, 2, 3, 4, 12 and 24 h of storage at 25^{o}C using the following *in vitro* fertility tests:

Sperm motility (%):

Percentage of sperm motility in each semen sample was determined.

Live and dead sperm (%):

A smear from diluted semen was made on a glass slide and stained by eosin (1.67%) and nigrosin (10%) stain mixture (Hackett and Macpherson, 1965).

Sperm abnormality (%):

The morphological abnormalities of spermatozoa were determined per 200 spermatozoa according to classification adopted by Bolm (1983). Then percentage of sperm abnormalities was calculated.

Spermatozoa with intact acrosome (%):

To determine the variability and acrosome reaction, under bright field microscope, about 100 spermatozoa were randomly selected per slide and examined for the live sperm with intact acrosome (live reacted, LR). Examination of the acrosome status was carried out by adding one drop of diluted semen incubated at 37^{o}C to one drop of sodium citrate (2.9%) at the same temperature, then the mixture was placed on a slide to make a smear, which dried at 37^{o}C. The dried slides were fixed in 10% formal solution for 15 minutes and washed by tap water for 15 minutes then immersed in Gimsa stain at 37^{o}C for 3 hours. The stained slides were washed by tap water for 15 minutes and dried at 37^{o}C. The prepared slides were examined at high magnification (x 100) for determination of spermatozoa with and without intact acrosome per 200 spermatozoa in each field. The acrosome stained light purple-dark pink, while sperm remains unstained. Percentage of spermatozoa with intact acrosome was calculated.

Statistical analysis:

Data were statistically analyzed by the methods of analysis of variance according to Snedecor and Cochran (1982). Duncan's Multiple Range Test was used to test the differences among means **(**Duncan, 1955**)**.

RESULTS AND DISCUSSION

Effect of GSH levels:

The use of egg yolk-Tris buffered extender supplied with glutathione at concentration of 0.4, 0.8 and 1.2 mM for extension of ram semen has beneficial effects on semen characteristic as indicated on the significant (P<0.05) increase in percentage of sperm motility, semen livability and intact acrosome and significant decrease (P<0.05) in the percentage of sperm abnormalities (Table 1).

With the exception of the percentage of intact acrosome, there were non-significant variations between the effects of different levels of glutathione on the studied semen parameters. The lowest GSH level (0.4 mM) had the best effect (P<0.05) on the percentage of intact acrosome compared with either control or 1.2 mM level of GSH (Table 1).

Comparable results were recorded by Foote et al. (2002) on bull semen extended with TALP (Tyrodes Albumin Lactate Pyruvate medium) supplemented with GSH at concentrations 0.5, 1.0, 1.5 and 2.0 mM. They found that addition of 1.0 and 1.5 mM of GSH to TALP-extender increased (P<0.05) sperm motility compared to the control semen.

Ahmed (2008) observed also beneficial effect of GSH on live sperm percentage in post-diluted buffalo's semen. In accordance with our results, El-Sherbieny *et al.* (2006) found that supplementation of 0.4 mM GSH showed the highest percentages (P<0.05) of sperm motility and livability compared with other GSH levels.

The acrosome reaction test is a stable parameter of sperm function and is useful to predict fertilization **(**Maji *et al*., 2010**)**. In agreement with the results of the current study, individual variations in the proportion of spermatozoa undergoing acrosome reaction were reported by Ucar and Parkinson (2003). Moreover, El-Nagar (2007) and Ahmed (2008) recorded comparable results concerning the variable responses to different GSH levels on the percentage of sperms undergoing acrosome reaction.

Table (1): Effect of glutathione supplementation with different levels on semen characteristics of rams

Treatment		Sperm characteristics (%)			
		Sperm motility	Sperm livability	Abnormal sperm	Intact acrosome
	Control	80.20±0.25^{b}	60.92±0.22^{b}	11.52±0.09^{a}	58.92±0.42^{c}
GSH	0.4 mM	85.40±0.17^{a}	73.24±0.15^{a}	6.62±0.08^{b}	71.73±0.22^{a}
GSH	0.8 mM	84.61±0.13^{a}	68.24±0.17^{a}	8.15±1.05^{b}	66.26±0.17^{ab}
GSH	1.2 mM	83.74±0.15^{a}	66.37±0.16^{a}	8.60±0.05^{b}	64.72±0.12^{b}

a, b, …..d: Means within the same column with different superscripts are significantly different at P<0.05.

In mammalian semen, the antioxidant defense capability consists of enzymatic and non-enzymatic systems, in which the latter is represented mainly by glutathione. The basic function of GSH in mammalian semen is related to its interaction with other systems as a preventive mechanism against ROS. This scavenging function of GSH helps to counteract the effects of oxidative stress in sperm cells, which could result in lipid peroxidation of plasmalemma, irreversible loss of motility, leakage of intracellular enzymes and damage of the chromatin (Aitken, 1999 and Luberda, 2001).

Effect of storage time:

Analysis of variance presented in Tables (2) showed that all sperm characteristics were affected significantly (P<0.001) by storage time. As expected, percentages of motility, livability and intact acrosome showed significant (P<0.05) gradual reduction, while sperm abnormality significantly (P<0.05) increased by progressing storage time up to 24 hours. The highest reduction rate was observed after 4 hours of storing.

In similar trend with results of the present study, El-Sherbieny et al. (2006) found significant (P<0.05) gradual reduction in sperm motility and livability percentages by increasing storage time of Friesian semen diluted with Tris-extender supplemented with different GSH levels and stored for 96 h at 25^{o}C.

The marked gradual reduction in percentage of individual motility of spermatozoa recorded for the control semen may be attributed to gradual increase in ROS (Iwasaki and Gagnon, 1992). The increase in ROS (superoxide anion, hydrogen peroxide, and hydroxyl radicals), as well as the fatty acid peroxide generated by ROS attack cell membrane phospholipids, reduce mammalian sperm motility and decrease capacity for sperm-oocyte fusion (Aitken and Clarkson, 1987). In addition, the degradation products of these lipid peroxides such as hydroxyalkenals and malonaldehyde are also highly toxic to spermatozoa and cause an irreversible loss in motility. However, these bad effects on sperm motility were combated by addition of GSH at levels 0.4, 0.8 and 1.2 mM until 4 h post dilution, while 0.4 level kept the same motility percentage more than 75% until 12 h (Table 2).

Semen fertility is closely related to percentage of motility (>75%, Swanson and Herma, 1944). The acceptable sperm motility for using semen for A.I. is achieved with 0.4, 0.8 and 1.2 mM at 4 hr post-dilution. In contrast control semen cannot be used beyond 2 hrs of storage at 25^{o}C. However, the acceptable sperm motility (>75%) was maintained until 12 hr with 0.4 GSH level.

Data presented in Table (2) indicated that the recorded percentage of intact acrosome in control group were the lowest in all post-diluation hours compared with all GSH levels. Statistical analysis showed that there were significant (P<0.05) differences in percentage of intact acrosome between 0.4 compared with either 0.8 or 1.2 mM. Also, it can be observed that, the treatment 0.4 mM GSH had high percentage of intact acrosome with all times. GSH treatment improved the percentage of intact acrosome stored at 25^{o}C. According to the present results, percentage of intact acrosome could be preserved for 4 hours at room temperature with acceptable percentage of intact acrosome when it diluted by EYTE at rate of 1:10 with supplemented with 0.4 mM of GSH.

Table (2): Percentage of sperm motility, sperm livability, abnormal sperm and intact acrosome in ram semen as affected by GSH levels and storage time at 25°C.

Time (h)	Control	GSH supplementation		
		0.4 (mM)	0.8 (mM)	1.2 (mM)
Sperm motility				
0	80.20±0.25^{AB}	85.40±0.17^{aA}	84.61±0.13^{aA}	83.74±0.15^{aA}
2	74.18±0.62^{bB}	81.12±0.25^{bA}	80.12±0.15^{bA}	79.22±0.51^{bA}
4	70.26±0.14^{cB}	79.45±0.14^{bcA}	77.25±0.22^{bcA}	75.12±0.41^{cA}
6	66.18±0.47^{dB}	77.41±0.74^{cA}	74.69±0.24^{cA}	71.18±0.08^{dA}
12	58.12±0.12^{eB}	75.02±0.85^{dA}	70.18±0.28^{dA}	68.15±0.29^{dA}
24	49.21±0.47^{fB}	69.15±0.29^{eA}	62.13±0.74^{eA}	60.74±0.47^{eA}
Sperm livability				
0	60.92±0.22^{aB}	73.24±0.15^{aA}	68.24±0.17^{aA}	66.37±0.16^{aA}
2	57.12±0.11^{bD}	71.18±0.25^{abA}	65.23±0.69^{bB}	61.49±0.25^{bC}
4	54.26±0.17^{cC}	68.25±0.78^{bA}	61.48±0.26^{dcB}	57.26±0.15^{bcBC}
6	50.14±0.28^{dD}	65.47±0.14^{cA}	58.26±0.23^{cB}	53.26±0.36^{cC}
12	45.17±0.26^{eC}	60.48±0.95^{dA}	52.15±0.58^{dB}	48.25±0.06^{dBC}
24	38.25±0.74^{fD}	52.47±0.36^{eA}	47.26±0.14^{eB}	42.16±0.48^{eC}
Abnormal sperm				
0	11.52±0.09^{fA}	6.62±0.08^{fB}	8.15±1.05^{eB}	8.60±0.05^{eB}
2	13.27±0.15^{eA}	8.47±0.11^{eB}	11.21±0.22^{deAB}	12.48±0.21^{dAB}
4	16.48±0.14^{dA}	11.26±0.23^{dC}	13.47±0.41^{dB}	15.85±0.11^{cAB}
6	18.24±0.25^{cA}	13.25±0.47^{cdB}	16.48±0.63^{cAB}	17.36±0.23^{bcAB}
12	23.56±0.63^{bA}	17.22±0.51^{bC}	20.26±0.29^{bB}	22.25±0.60^{bAB}
24	35.26±0.41^{aA}	26.45±0.26^{aD}	29.12±0.15^{aC}	32.78±0.41^{aB}
Intact acrosome				
0	58.92±0.42^{aC}	71.73±0.22^{aA}	66.26±0.17^{aB}	64.72±0.12^{aB}
2	56.25±0.14^{bC}	69.25±0.13^{abA}	64.23±0.48^{abB}	63.14±0.12^{abB}
4	53.47±0.25^{cC}	67.21±0.48^{bA}	62.15±0.74^{bB}	60.45±0.58^{bBC}
6	50.13±0.74^{dD}	63.58±0.25^{cA}	59.25±0.12^{bcB}	54.13±0.02^{cC}
12	48.15±0.85^{eD}	60.14±0.06^{dA}	55.78±0.32^{cB}	51.26±0.14^{dC}
24	44.42±0.63f^{C}	55.48±0.17^{eA}	50.23±0.56^{dB}	48.13±0.21^{eBC}

A and B: Means within the same row with different superscripts are significantly different at P<0.05.
a, b, …..e: Means within the same column with different superscripts are significantly different at P<0.05.

Depending on the fact that percentage of sperm abnormalities should not exceed 15% (Terill, 1962), the quality of diluted semen in the current study is considered good until 6 , 4 h and 4 h post dilution by egg yolk extender containing GSH at levels 0.4, 0.8 and 1.2 mM. However, the coincidence between percentages of motility and abnormal sperms revealed more or less parallelism in the trend of reduction of these two parameters. Where the lowest accepted motility vs. abnormal sperm cells percentages were (75.02 vs. 17.22), (77.25 vs. 13.47) and (75.12 vs. 15.85) in cases of GSH levels of 0.4, 0.8 and 1.2 mM at 12 , 4 h and 4 h post dilution, respectively. These finding may indicate intact relationship between the two parameters. Such parallelism may be detected to some extent between either of these two parameters and percentage of intact acrosome. However, previous studies (Terrill, 1962) recorded 20% of dead sperms in most normal bovine semen samples and that there is little correlation with fertility.

In conclusion, this study demonstrate that supplementation of GSH at various concentrations to diluted semen kept at 25°C

exert beneficial effects on semen quality in term of sperm motility, livability and membrane stabilization of sperm cells. The best result was obtained with 0.4 mM GSH level with egg yolk extender when stored up to 12 h..

REFERENCES

Ahmed, I. A. (2008). Effect of adding ascorbic acid to semen of Egyptian buffalo bulls. M. Sc. Fac. Agric. Mansoura University.

Aitken, R.J. and Clarkson, J. S. (1987) Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. J. Reprod. and Fert., 81: 459–469.

Aitken, R. J. (1995). Free radicals, lipid peroxidation and sperm function. Reprod. Fert. and Develop., 7: 659-668.

Aitken R.J. (1999). The amoroso lecture. The human spermatozoon – a cell in crisis? Journal of Reproduction and Fertility 115 1-7.

Alvarez, J.G.; Touchstone, J.C.; Blasco, L. and Storey, B.T. (1987). Spontaneous lipid per oxidation and production of hydrogen peroxide and superoxide in human spermatozoa. J. Androl., 8:338–348.

Bilodeau, J. F. ; Chatterjee, S. ; Sirard, M. A. and Gagnon, C. (2000). Levels of antioxidant defenses are decreased in bovine spermatozoa after cycle of freezing and thawing. Mol. Reprod. Dev., 55: 282-288.

Blom, E. (1983). The spermiogram of the bull. Nordisk Veterinear Medicin, 35: 105.

Bucak, M. N. ; Ateşşahin, A. ; Varışlı, O. ; Yüce, A. ; Tekin, N. and Akçay, E. (2007). The influence of trehalose, taurine, cyst amine and hyaluronan on ram semen: Microscopic and oxidative stress parameters after freeze-thawing process. Theriogenology, 67: 1060-1067.

Duncan, D. B. (1955). Multiple Range and Multiple F-Test. Biometrics, 11: 1-42.

El-Nagar, H. A. A. (2007). Studies on frozen buffalo bull semen. Ph. D. Thesis Agric. Sci., Fac. Agric., Al-Azhar Univ., Egypt.

El-Sherbieny, M.A. ; El-Nenaey, M. H. ; El-Siefy, E. M. E. and Abdel-Khalek, A. E. (2006). Effect of reduced glutathione supplementation on motility, livability and abnormality of Holstein spermatozoa in: 1. Semen stored at room and cool temperature. J. Agric. Sci. Mansoura Univ., 31 (4): 1913-1931.

Foote, R. H. ; Brockette, C. C. and Kaproth, M. T. (2002). Motility and fertility of bull sperm in whole milk extender containing antioxidants. Anim. Prod. Sci., 71:13 – 23.

Hackett, A. J. and Macpherson, J.W. (1965). A method for differential staining of bovine spermatozoa after extension in sterile milk. Can. Vet. J., 6: 117-120.

Halliwell, B. and Gutteridge J. M. C. (1998). Antioxidant defences. In: HALLIWELL B, GUTTERIDGE JMC (Eds), Free radicals in biology and medicine. Oxford, University Press (London), pp. 155-158.

Halliwell, B. and Gutteridge J. M. C. (1989). Free radicals in biology and medicine. Oxford: Clarendon Press.

Irvine S.; Cawood E.; Richardson D.; MacDonald E. and Aitken J. (1996). Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. BMJ. 312: 467–471.

Iwasaki, A. and Gagnon, C. (1992) Formation of reactive oxygen species in spermatozoa of infertile patients. Fertil. Steril., 57: 409–416.

Luberda Z. (2001). Present conception regarding the effect on reactive oxygen species on functions of mammalian spermatozoa. In Polish with English summary. Postępy Biologii Komórki 28 309-316.

Maji, S. ; Datta, U. and Hembram, M.L. (2010). Cell surface changes associated with in vitro capacitation and acrosome reaction of goat epididymal sperm by a marine bio-active compound from the snail Telescopium telescopium. Veterinarski Arhiv, 80(5): 561-570.

Maxwell, W. M. C. and Salamon, S. (1993). Liquid storage of ram semen. Reprod. Fertil. Dev., 5: 613-638.

NRC (1985). Nutrient Requirements of sheep. Nat. Acad. Press, Washington, DC.

Pastore, A.; Federici, G.; Bertini, E. and Piemonte F. (2003). Analysis of glutathione:

implication in redox and detoxification. Clin. Chim. Acta., 333(1):19-39.

Pompella, A., Visvikis, A., Paolicchi, A., (2003). The changing faces of glutathione, a cellular protagonist. Biochem. Pharmacol., 66:1499-1503.

Scholz, R. W., Graham, K. S., Gumpricht, E., and Reddy, C. C. (1989). Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. Ann. N. Y. Acad. Sci. 570: 514–517.

Snedecor, G.W. and Cochran, W. G. (1982). Statistical Methods. 7th Ed. Iowa Univ. Press, Ames. Iowa, USA.

Swanson, E. W. and Herman, H. A. (1944). Seasonal variation in semen quality of some Missouri dairy bulls. J. Dairy Sci., 27: 303-307.

Terrill, C. E. (1962). The reproduction of sheep. Ch. 14. in reproduction in farm animals edn. 1. Ed. F. S. E. Hafez, Philadelphia, USA.

Ucar, O. and Parkinson, T. J. (2003). *In vitro* induction of the acrosome reaction in ovine spermatozoa by calcium ionophore A23187. Acta Veterinaria Hungarica, 51(1): 103–109.

THE INFLUENCE OF ADDING GLUTATHIONE ON SEMEN CHARACTERISTICS OF SOHAGI RAMS

تأثير اضافة الجلاتاثيون كمضاد للاكسدة على صفات السائل المنوى فى الكباش السوهاجى

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يهدف هذا البحث الى دارسة تأثير اضافة الجلاتاثيون كمضاد للاكسدة بمستويات مختلفة هى 0.4 و 0.8 و 1.2 مل مول الى السائل المنوى المخفف بمخفف صفار البيض مع الترس وذلك فى درجة حرارة 25 مئوية لمدة 0 و 1و 2 و 3 و 4 و 12و 24 ساعة. أجريت هذة الدراسة على عشرة كباش من الاغنام السوهاجى وزنها من 60 -65 كجم وزن حى وعمرها من 3 -4 سنوات استخدمت لجمع السائل المنوى . استخدم المهبل الصناعى لجمع السائل المنوى . وتم الجمع مرتين فى الاسبوع فى الثامنة صباحا لمدة 10 اسابيع. فى كل مرة يتم فيها الجمع ، يتم جمع القذفات معا ثم تخفف بمخفف الترس المحتوى على الجلاتاثيون بتركيزات 0.4 ، 0.8 ، 1.2 وتركيز صفر للكنترول بدون اى اضافة من الجلاتاثيون. فى كل مرة يتم فيها الجمع (10 قذفات) كانت تقسم بعد التخفيف الى اربعة اجزاء بمخفف الترس المحتوى على ثلاث مستويات بالنسب 0.4 ، 0.8 ، 1.2 ، صفر (كنترول) .

تم تخفيف السائل المنوى لكل معاملة جزء سائل منوى 1: 10 اجزاء مخفف ترس مع صفار البيض على 30 °م . تم حفظ السائل المنوى وتقييمه على صفر ، 1 ، 2 ، 3 ، 4 ، 12 ، 24 ساعة على حرارة 25ْم واجريت اختبارات الخصوبة التالية معمليا : حيوية السائل المنوى ، حياتية السائل المنوى والحيوانات المنوية الشاذة والتغير فى شكل الاكروسوم.

اوضحت النتائج ان اضافة الجلاتاثيون على مخفف الترس – صفار البيض بمستويات 0.4 ، 0.8 ، 1.2 مل مول حسن من صفات وجودة السائل المنوى للكباش. كان تأثير جميع مستويات الجلاتاثيون عالى المعنوية على حيوية السائل المنوى ، وحياتية السائل المنوى والتغير فى شكل الاكروسوم. وخفض معنويا الحيوانات المنوية الشاذة. تأثرت كل صفات السائل المنوى معنويا بوقت التخزين حيث انخفضت كل من حيوية السائل المنوى ، وحياتية السائل المنوى والتغير فى شكل الاكروسوم بينما زادت الحيوانات المنوية الشاذة معنويا بالتقدم فى التخزين . كان اعلى تغير بعد مرور 4 اساعات بعد التخزين.

توصى هذه الدراسة بان اضافة الجلاتاثيون كمضاد للاكسدة بتركيزات مختلفة مع الحفظ على حرارة 25ْم قد حسن صفات السائل المنوى حيث انعكس ذلك على حيوية السائل المنوى ، وحياتية السائل المنوى والتغير فى شكل الاكروسوم وكانت افضلها عند اضافة تركيز 0.4 مل مول الى مخفف ترس – صفار البيض حتى مدة حفظ 12 ساعة..