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EFFECT OF REDUCED GLUTATHIONE SUPPLEMENTATION ON MOTILITY, LIVABILITY AND ABNORMALITY OF HOLSTEIN SPERMATOZOA IN:
1. SEMEN STORED AT ROOM AND COOL TEMPERATURE

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ABSTRACT

Semen was collected twice weekly from five sexually mature Holstein bulls raised at International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, to evaluate the effect of different concentrations of reduced glutathione (GSH) on sperm motility, livability and abnormality in semen stored at 25°C and 5°C for different storage periods. Only ejaculates with mass motility of 70% or more were pooled for each collection day for 5 weeks. The collected semen was diluted with Tris-extender and held in a water bath at 37°C, then divided into four portions. Different concentrations of GSH were added to the four diluted portions of semen (0, 0.4, 0.8 and 1.2 mM/ml Tris extender). Thereafter, each portion of semen was divided into two sub-portions, 10 replicates in each. Replicates of the 1st sub-portion were stored at 25°C (room temperature), while those of the 2nd portion were stored at 5°C (refrigerator temperature) for 0, 6, 24, 48, 72 and 96 h periods. Percentages of progressive motility, livability and abnormality of spermatozoa were determined at various storage times. Results revealed that adding GSH at concentration of 0.4 mM at 25°C showed the highest (P<0.05) sperm motility and livability percentages (56.8 and 57.1%, respectively), and the lowest (P<0.05) sperm abnormality (14.3%) as compared to the control without adding GSH (39.3, 40.8 and 17.3%, respectively). At different storage periods at 25oC, concentration of 0.4 mM GSH showed the highest motility and livability percentages, followed by 0.8 and 1.2 mM levels, respectively, while the control semen showed the lowest percentages. The opposite trend was observed for sperm abnormality. In semen stored at 25°C with 0.4 GSH, sperm cells maintained (P<0.05) their motility and livability only for 12 h; however, the differences in sperm abnormality were not significant between 0 and 48 h. In semen stored at 5°C, semen supplemented with 0.4 mM had the highest (P<0.05) sperm motility and livability and the lowest sperm abnormality. Semen supplemented with 1.2 mM, which was stored at 5°C showed the lowest sperm motility and livability. Sperm motility and livability decreased (P<0.05) by increasing storage period. However, sperm abnormality was not affected by storage period, ranging between 13.1-14.3%. By increasing storage period, sperm motility and livability gradually reduced. Semen supplemented with 0.4 mM showed almost the highest motility and livability percentages and the lowest sperm abnormality percentage The present results suggested the possibility of storage bull semen at 25°C or 5°C for 48 h with maintenance of sperm motility and livability percentages above 50%, and sperm abnormality percentage at a level of 14% in semen supplemented with 0.4 mM glutathione.

Keywords: *Bull sperm, glutathione, storage, motility, livability, abnormality.*

INTRODUCTION

The cytoplasm of somatic cells contains several antioxidant enzyme systems, catalase, glutathione (GSH) and superoxide dismutase (SOD). However, sperm cells are devoid of most of this cytoplasm, so the antioxidant system in sperm cells of different species is weak (Li, 1975). Sperm cells have a high content of unsaturated fatty acids in their membrane. Therefore, they are susceptible to lipid peroxidation by H_2 and H_2O_2 (Alvarez and Storey, 1989 and Storey, 1997) and can readily undergo lipoperoxidation, particularly in the presence of oxygen (Foote *et al.*, 2002).

One of the antioxidant substances that lacked in sperm cells is GSH, where one of its functions is to protect cells against the destructive effect of reactive oxygen species (ROS). Mammalian sperm vary, but tends to be low in GSH, glutathione peroxidase (GSP) and glutethione reductase (GPD), the key components in this protective system. The other antioxidants enzymes active in scavenging ROS are catalase and superoxide dismutase (SOD).

Under natural system of mating, sperm are exposed primarily to anaerobic conditions thus reducing potential damage by ROS. Also, fluids of oviduct contain substantial concentration of taurine (Miller and Shultz, 1987), an important protector of cells against accumulation of ROS when they are exposed to aerobic conditions (Alvarez and Storey, 1983a and Holmes *et al.*, 1992).

Sperm cells used in AI are exposed to oxygen and visible light radiation during various processing procedure or in semen stored by cooling or at room temperature, which could lead to formation of ROS, and negatively affect sperm cell motility and genomic integrity (Aitken and Clarkson, 1988; Storey, 1997; Aitken *et al.*, 1998 and Bilodeau *et al.*, 2001). Under these conditions, adding several types of antioxidants could help to maintain survival and motility of spermatozoa (Alvarez and Storey 1983a, b, 1989; Lindemann *et al.*; 1988; Beconi *et al.*, 1993; Lapointe *et al.*, 1998; Bilodeau *et al.*, 2001 and Foote *et al.*, 2002), when storage has been for several hours.

The objective of the current study was to testing the effect of adding different levels (0, 0.4, 0.8 and 1.2 mM) of reduced glutathione to Tris extender on motility, livability and abnormality of spermatozoa of Holstein bull semen stored at both room (25°C) and cool (5°C) temperatures for different storage times (0, 6, 12, 24, 48, 72 and 96 h).

MATERIALS AND METHODS

This study was carried out at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture. Egypt, during the period from 15 December 2005 to 30 January 2006.

Animals and management:

Five sexually mature Holstein bulls (3-5 years old and average LBW of 532±12.4 kg) raised at the ILMTC were used in this study. All bulls were free from any diseases with healthy appearance and were used for semen

collection as a routine work in ILMTC. Each bull was fed on daily ration composed of 8 kg concentrate feed mixture (CFM), 10 kg berseem (*Trifolium alexandrinum*) and 5 kg rice straw. Bulls were housed individually under semi-open sheds.

Semen collection:

Semen was collected twice weekly from each of the experimental bulls as a common practice in ILMTC using the conventional artificial vaginal method. One ejaculate from each bull (5 ejaculates for each run) was taken immediately to the laboratory and the ejaculates were pooled. Semen was collected before feeding at 8.00 a.m. A bull was used as teaser animal for sexual preparation.

Only ejaculates with mass motility of 70% or more were pooled for each collection day for 5 weeks. The collected semen was diluted with Tris-extender and held in a water bath at 37°C, then divided into four portions. Different concentrations of GSH were added to the four diluted portions of semen (0, 0.4, 0.8 and 1.2 mM/ml Tris extender). Thereafter, each portion of semen was divided into two sub-portions, 10 replicates in each. Replicates of the 1st sub-portion were stored at 25°C (room temperature), while those of the 2nd portion were stored at 5°C (refrigerator temperature) for 0, 6, 24, 48, 72 and 96 h periods.

Semen was diluted in at a rate of 1:20 in heated (37°C) Tris extender. The Tris extender was prepared with Tris (0.05 g), citric acid (3.35 g), streptomycin (0.5 g), lincomycin (0.01 g) and distilled water (100 ml) then 16 ml egg yolk, 12 ml glycerol and 72 ml distilled water were added to obtain 200 ml of the Tris extender, then it was mixed and kept at 37°C.

Assessment and evaluation of semen:

Semen of each GSH concentration was stored for 0, 6, 12, 24, 48, 72 and 96 h at room temperature (25°C) and refrigerator temperature (5°C). After each storage time at 25 and 5 °C, percentage of progressive motility, livability and abnormality of spermatozoa in 10 replicates of each concentration were determined.

The percentage of motile spermatozoa (progressive motility) in each replicate was assessed using research microscope with warmed stage (37°C) under the high power magnification (x400) according to Amman and Hammerstedt (1980). Sperm livability percentage was determined using eosin and nigrosin mixture stain according to Hackett and Macpherson (1965). Live spermatozoa (unstained ones) and dead spermatozoa (stained ones) were counted in field of a total of 200 spermatozoa. Then percentage of live spermatozoa was calculated. Sperm abnormalities percentage was determined during the examination of live/dead sperm percentage at a high power magnification (400x), according to the classification adopted by Blom (1983).

Statistical analysis:

Results were statistically analyzed according to Snedecor and Cochran (1982) using SAS system (1985). The differences among means

were tested using new multiple range test (Duncan, 1955). The percentage values of sperm progressive motility, livability, and abnormality were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed

RESULTS AND DISCUSSION

Semen stored at room temperature (25°C):

Results presented in table (1) showed significant (P<0.05) effect of GSH supplementation on all sperm characteristics studied. Supplementation of 0.4 mM GSH showed the highest (P<0.05) sperm motility and livability percentages. However, all GSH supplements reduced (P<0.05) sperm abnormality as compared to the control (without additives), being the best in semen supplemented with 0.4 mM GSH.

Table (1):**Effect of GSH supplementation and storage time on percentages of motility, livability and abnormality of Holstein spermatozoa in semen stored at 25 C°.**

Item	Motility (%)	Livability (%)	Abnormality (%)
Effect of GSH supplementation (mM):			
0	39.3±3.06^{d}	40.8±3.08^{c}	17.3±0.28^{a}
0.4	56.8±2.28^{a}	57.1±2.21^{a}	14.3±0.38^{b}
0.8	48.1±2.78^{b}	48.9±2.65^{b}	15.1±0.41^{b}
1.2	42.3±2.92^{c}	43.0±2.80^{c}	15.1±0.37^{b}
Effect of storage time (h):			
0	71.8±0.89^{a}	71.2±1.34^{a}	14.2±0.47^{c}
6	68.1±1.16^{a}	67.7±1.13^{a}	14.6±0.61^{c}
12	61.0±1.56^{b}	61.7±1.49^{b}	14.9±0.53^{c}
24	50.1±2.04^{c}	51.9±1.99^{c}	15.1±0.53^{c}
48	41.2±2.33^{d}	42.0±2.38^{d}	15.4±0.47^{bc}
72	23.5±2.35^{e}	25.5±2.17^{e}	16.6±0.38^{ab}
96	10.6±1.57^{f}	12.6±1.67^{f}	17.4 ±0.37^{a}

a, b and c: Means denoted within the same column with different superscripts are significantly different at (P<0.05).

In semen supplemented with concentrations of 0.5, 1.0, 1.5 and 2.0 mM of GSH and stored at 25°C, Foote *et al.* (2002) found that addition of 1.0 and 1.5 mM of GSH in TALP-extender increased (P<0.05) sperm motility, being 52.0 and 51.0%, respectively as compared to 45.7% in the control semen (0 mM GSH). In the present study, the beneficial effect on sperm motility and livability percentages in semen supplemented with GSH was obtained for all GSH supplements, being the best for 0.4 mM of GSH (56.8 and 57.1%, respectively) as compared the control semen (39.3 and 40.8%, respectively). The different trends of GSH supplements in our study may be due to type of the extender used in semen dilution. The later author found that the corresponding effect of GSH levels (0.5, 1.0, 1.5 and 2.0 mM) in whole milk (WM) was not significant, but sperm motility was significantly

higher with WM than TALP extenders. Accordingly, it was suggested an interaction effect between GSH level and type of extender. In this respect, Raina *et al.* (2002) showed that sperm motility was affected significantly ($P<0.01$) by extender–antioxidant combination.

Effect of storage period on all sperm characteristics studied was significant ($P<0.05$). Sperm cells significantly ($P<0.05$) maintained heir motility and livability percentages only for 6 h, thereafter these percentages significantly ($P<0.05$) reduced with increasing storage time, reaching the minimum values at storage period of 96 h. On the other hand, percentage of sperm abnormality did not differ significantly by increasing storage time from 0 up to 48 h, and then significantly ($P<0.05$) increased after 96 h (Table 1).

Raina *et al.* (2002) found that overall mean percent motility observed after 0, 4, 8, 12 and 24 h of preservation at 37°C were 68.5, 58.9, 45.0, 38.1 and 18.1%, respectively. Type of antioxidant added and storage interval were found to significantly ($P<0.01$) affect sperm motility on room temperature preservation.

The effect of interaction between GSH level and storage period on percentages of motility, livability and abnormality of spermatozoa was not significant. Although, sperm motility and livability percentages in control and all supplemented semen showed gradual reduction with increasing storage time, semen supplemented with 0.4 mM GSH showed the highest values, followed by those supplemented with 0.8 and 1.2 mM, respectively. While, the control semen showed the lowest values (Figs. 1 and 2, respectively). The opposite trend was observed for sperm abnormality percentages (Fig. 3).

Such trends indicated the possibility of maintaining percentage above 50% as motile and live spermatozoa with about 14% sperm abnormality in semen diluted with Tris-extender supplemented with 0.4 mM GSH for 48 h.

Semen stored at cool temperature (5°C):

Results presented in table (2) showed significant ($P<0.05$) effect of GSH supplementation on all sperm characteristics studied. Supplementation of 0.4 mM GSH at 0.4 mM significantly ($P<0.05$) showed the highest percentage of motility and livability of sperm cells and the lowest sperm abnormality percentage. Adding 1.2 mM of GSH to semen stored at 5°C showed the lowest motility and livability percentages, being lower than those in the control semen. This may suggest the toxic effect of this concentration in semen stored at the cooler temperature than at 25°C.

During cooling to 4 °C increasing production of ROS was measured in human semen (Wang *et al.*, 1997). Hydrogen peroxide (H_2O_2) as ROS has been shown to decrease motility in mouse, human, bull, and rabbit spermatozoa (Alvarez and Storey, 1989). Under these conditions, adding several types of antioxidants could help to maintain survival and motility of spermatozoa (Alvarez and storey 1983a, b, 1989; Lindemann *et al.*; 1988; Beconi *et al.*, 1993; Lapointe and Sirard, 1998; Bilodeau *et al.*, 2001 and Foote *et al.*, 2002). These findings were indicated in our study when of 0.4 mM GSH was supplemented to semen, whereas percentages of sperm motility and livability were 52.8 and 53.3%, respectively as compared to 41.6 and 43.0% in semen without supplementation.

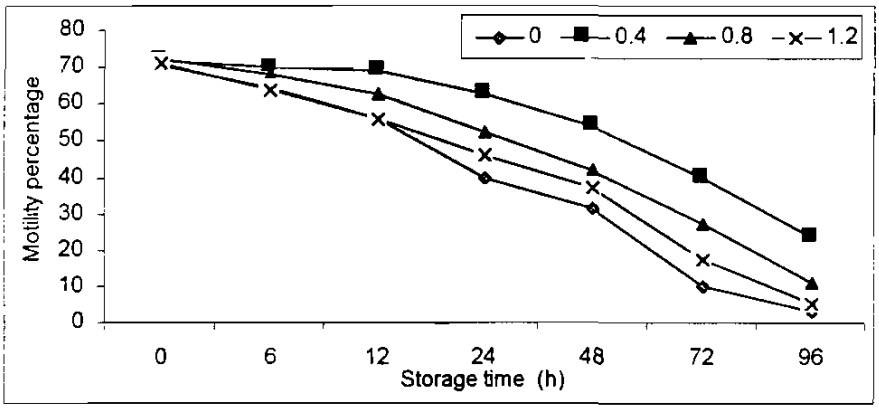

Fig. (1): Effect of GSH supplements on sperm motility percentage in semen stored for different times at 25°C.

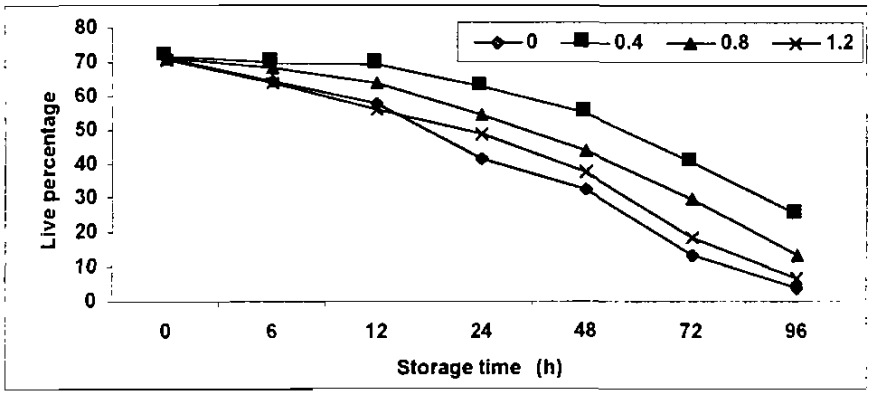

Fig. (2): Effect of GSH levels on live sperm percentage in semen stored for different times at 25°C.

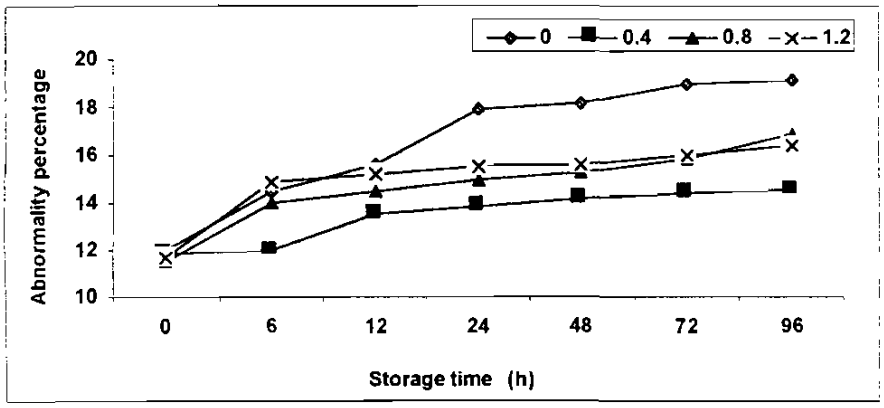

Fig. (3): Effect of GSH supplements on sperm abnormality percentage in semen stored for different times at 25°C.

The effect of addition of natural antioxidants into semen diluents on the preservability of buffalo semen was studied by Raina *et al.* (2002). At 4–7°C, overall mean of percent motility for semen extended in MYE, MYC, MYO, and TYE, TYC and TYO were 44.8, 42.7, 38.7, 36.0, 35.2 and 33.0%, respectively. The results showed that motility was significantly (P<0.01) affected by extender–antioxidant combination

Also, the effect of storage time on all sperm characteristics studied was significant (P<0.05). Sperm motility and livability percentages significantly (P<0.05) decreased by increasing storage time. However, sperm abnormality percentage was not affected by storage period, ranging between 13.1-14.3% (Table 2).

Foote *et al.* (2002) found no effect of GSH addition (0.5 and 1.0 mM) in WM on sperm motility immediately after cooling to 5°C and then after 24 h storage at 25°C, a beneficial effect was detected for 0.5 of GSH level on sperm motility.

Table (2): Effect of GSH supplementation and storage time on percentages of sperm motility, livability and abnormality of Holstein spermatozoa in semen stored at 5 C°.

Item	Motility (%)	Livability (%)	Abnormality (%)
Effect of GSH supplementation (mM):			
0	41.6±2.7^{bc}	43.0±2.7^{b}	16.0±0.35^{a}
0.4	52.8±2.2^{a}	53.3±2.2^{a}	11.8±0.41^{c}
0.8	44.3 ±2.7^{b}	44.6±2.7^{b}	13.9±0.45^{b}
1.2	40.6±2.8^{c}	42.6±2.7^{b}	13.8±0.36^{b}
Effect of storage time (h):			
0	70.6±0.74^{a}	70.9±0.83^{a}	13.1±0.49
6	62.5±0.95^{b}	63.7±0.85^{b}	13.7±0.56
24	52.8±1.3^{c}	53.8±1.3^{c}	13.1±0.58
48	39.4±1.8^{d}	40.7±1.8^{d}	14.3±0.51
72	27.8±1.7^{e}	28.8±1.7^{e}	14.3±0.55
96	16.5±1.7^{f}	17.1±1.7^{f}	14.0±0.43

A, b and c: Means denoted within the same column with different superscripts are significantly different at (P<0.05).

The effect of interaction between GSH level and storage period on percentages of motility, livability and abnormality of spermatozoa was not significant. A gradual reduction in sperm motility and livability percentages was observed in control and semen supplemented with increasing storage time. At all storage times, semen supplemented with 0.4 mM of GSH showed the highest values, followed by those supplemented with 0.8 and 0 mM of GSH, respectively. While, semen supplemented with 1.2 mM showed the lowest values (Figs. 4 and 5, respectively). However, sperm abnormality percentages in supplemented and control semen showed gradual increase by increasing storage time, being the lowest in semen supplemented with 0.4 mM of GSH (Fig. 6).

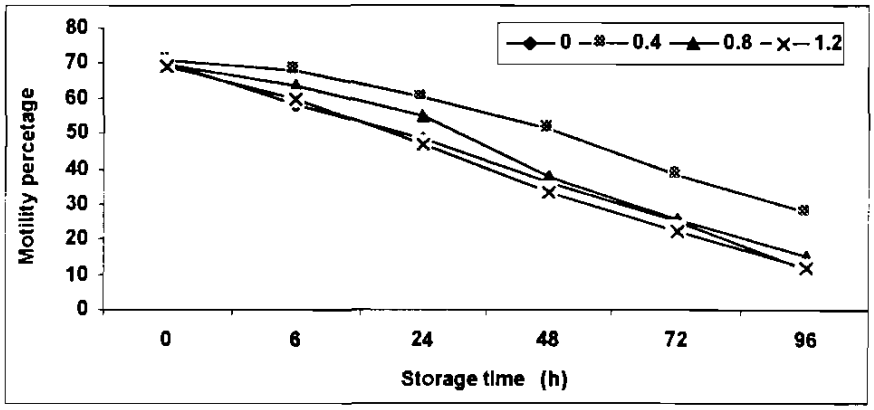

Fig. (4): Effect of GSH supplements on sperm motility percentage in semen stored for different times at 5°C.

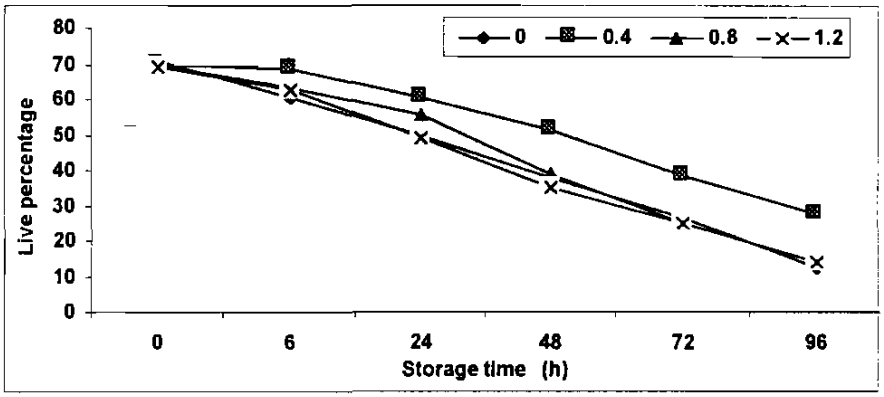

Fig. (5): Effect of GSH supplements on live sperm in semen stored for different times at 5°C.

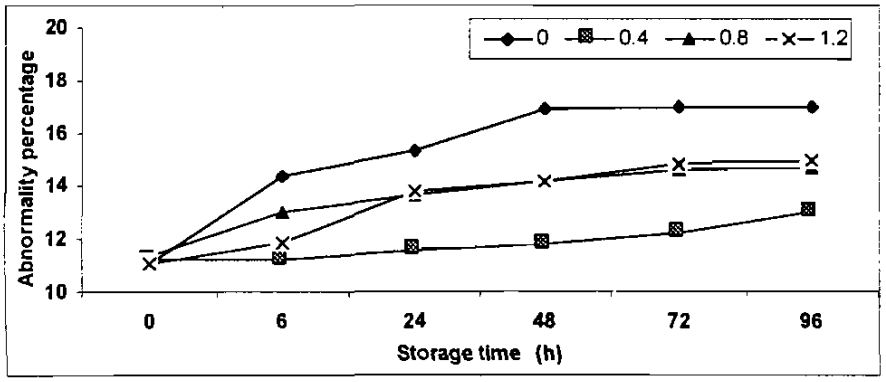

Fig. (6): Effect of GSH supplements on sperm abnormality in semen stored for different times at 5°C.

Such trends indicated the possibility of cooling bull semen to 5 °C for 48 h with appropriate percentages of motility, livability (>50%) and abnormality (12%).

In conclusion, supplementation of semen diluted with Tris-extender with 0.4 mM of GSH resulted in bull spermatozoa to maintain their motility and livability above 50% and abnormality percentage less than 15% at room temperature (25°C) or at cooling temperature (5°C) for 48 hours.

REFERENCES

Aitken, R.J. and Clarkson, J.S. (1988). Significance of reactive oxygen species and antioxidants in defining the efficacy sperm preparation techniques. J. Androl. 9,367-376.

Aitken, R.J., Gordon, E., Harkiss. D., Twigg, J. P., Milne, P., Jennings, Z., Irvine, D.S., (1998). Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol. Reprod. 59, 1037-1046.

Alvarez, J.G., Storey, B.T. (1989). Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. Gamete Res., 23:77-90.

Alvarez, J.G., Storey, B.T. (1983a). Role of superoxide dismutase in protecting rabbit spermatozoa from O2 toxicity due to lipid peroxidation. Biol. Reprod. 28, 1129-1136.

Alvarez, J.G., Storey, B.T. (1983b). Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. Biol. Reprod. 548-555.

Amman,R.P. and Hammerstedt, R.H. (1980). Validation of a system for computerized. Measurements of spermatozoal velocity and percentage of motile sperm. Biol. Reprod., 23:647-656.

Beconi, M.T., Francia, C.R. and Affranchino, M.A. (1993). Effect of natural antioxidants on frozen bovine semen preservation. Theriogenology; 40:841-51.

Bilodeau, J.F.; Blanchette, S.; Sirard, M.A. and Gagnon, C. (2001). Thiols prevent H_2O_2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology. 56:275-86.

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

Duncan, D.B. (1955). Multiple Range and Multiple F. test Biometrics, 11-10.

Foote, R. H., (1967). Influence of light and agitation on bovine spermatozoa stored with protective agents. J. Dairy Sci., 50, 1468-1474.

Foote, R. H., Brockett, C.C., and Kaproth, M. T. (2002). Motility and fertity of bull sperm in whole milk extender contaning antioxidants. Anim. Prod. Sci., 71: 13-23.

Hackett, A.J. and Macpherson, J.W. (1965). Some staining procedures for spermatozoa: A review. Canadian. Vet. J. 5-55.

Holmes, R.P.; Goodman, H.O.; Shihabi, Z.K.; Jarow, J.P. (1992). The taurine and hypotaurine content of human semen. J. Androl. 13, 289-292.

Lapointe, S.; Sulivan, R. and Sirard, M.A. (1998). Binding of a bovine oviductal fluid catalase to mammalian spermatozoa. Biol. Reprod. 58:747-53.

Li, T.K. (1975). The glutathione and thiol content of mammalian spermatozoa and seminal plasma. Biol Reprod., 12:641-646.

Lindemann, C.B; O,Brien, J.A. and Giblin, F.J. (1988). An investigation of the effectiveness of certain antioxdants preserving the motility of reactivated bull sperm models. Biol. Reprod. 38, 114-120.

Miller, J.G.O. and Shultz, G.A. (1987). Amino acids content of preimplantation embryos and fluids of the reproductive tract. Biol. Reprod. 36, 125-129.

Raina, V. S., Gupta, A. R. and Kiran, S. (2002).Effect of antification on preserability of buffalo semen. Asian – aust.G.A.nm. Sci.,15(1)16 - 18

S.A.S. (1985). SAS User's guide. Statistical Analysis System Institute, Inc., Cary. NC. USA.

Snedecor, G.W. and Cochran, W.G. (1982). Statistical methods. 7th ed. Iowa state Univ. press, USA.

Storey, B.T. (1997). Biochemistry of the induction and prevention of lipoperoxidative damage in human spermatozoa. Mol. Hum. Reprod. 3, 203-214.

Wang, A.W.; Zhang, H.; Jkemoto, I.; Anderson, D.J. and loughlin, K.R. (1997). Reactive oxygen species generation by seminal cells during cryopreservation. Urology; 49: 921-5.

تأثير إضافة الجلوتاثيون المخفض على حيوية، حياتية وشواذ الحيوانات المنوية لطلائق الهولستين في: السائل المنوي المحزن على درجة حرارة الغرفة (٢٥درجة مؤوية) والبرد على ٥ درجات مؤوية

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**معهد بحوث الإنتاج الحيواني- مركز البحوث الزراعية- وزارة الزراعة.

في هذه الدراسة تم جمع السائل المنوي مرتين أسبوعيا باستخدام المهبل الصناعي من خمسة طلائق هولشتين ناضجة جنسيا (عمرها من ٣ إلي ٥ سنوات ومتوسط الوزن ٥٣٢±١٢,٤ كجم) وذلك لتقييم اضافة مستوبات مختلفة من الجلوتاثيون كأحد مضادات الأكسدة على حيوية وحياتية وشواذ الحيوانات المنوية. وتم خلط السائل المنوي للخمس طلائق فى يوم الجمع لمدة خمسة أسابيع وتم استخدام عينات السائل المنوي التي لا تقل حيويتها عن ٧٠%. وضع السائل المنوي في حمام مائي على درجة حرارة ٣٧م°. بعد ذلك قسمت إلى أربع معاملات بها عشر مكررات لكل معاملة. المعاملة الأولى كانت بدون إضافة (كنترول) بينما الثلاث معاملات الأخرى كانت مضاف إليها الجلوتاثيون بتركيز (٠,٤ ، ٠,٨ ، ١,٢ مل مول/مل مخفف الترس) وكان معدل التخفيف بنسبة ١:٢٠.

تم تخزين السائل المنوي لكل معاملة لمدة صفر ،٦ ،١٢ ، ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ساعة على درجة حرارة ٢٥م° وكذلك على درجة ٥م° ثم قدرت النسبة المئوية للحركة التقدمية و النسبة المئوية لكل من الحيوانات المنوية الحية والشاذة وقد أظهرت النتائج مايلى:

١- كان تأثير اضافة الجلوتاثيون بنسبة ٠,٤ مل مول إلي السائل المنوي المخزن على درجة حرارة ٢٥م° إيجابي ومعنوي على كل من الحيوية ونسبة الحيوانات المنوية الحية بينما حسنت إضافة مستويات الجلوتاثيون الأخرى النسبة المئوية للحيوانات المنوية الشاذة مقارنة بالكنترول وكانت أقل نسبة للحيوانات المنوية الشاذة عند مستوى ٠,٤ مل مول جلوتاثيون.

٢- حافظت الحيوانات المنوية على حيويتها ونسبة الحي منها لمدة ٦ ساعات فقط من التخزين، بعد ذلك انخفضت النسبة المئوية للحيوية والحى مع طول فترة التخزين ووصلت إلى أدنى قيمة بعد ٩٦ ساعة. ومن ناحية أخرى فإن نسبة الحيوانات المنوية الشاذة لم تختلف معنويا بين صفر - ٤٨ ساعة من التخزين وكانت اقل قيمة في المعاملة بتركيز ٠,٤ مل مول يليها مستويات ٠,٨ و ١,٢ مل مول جلوتاثيون، على الترتيب، بينما اعلى قيمة كانت في السائل المنوى المقارن.

٣- كانت أعلى قيمة لنسبة الحيوية والحيوانات المنوية الحية وأقل قيمة للنسبة المئوية للحيوانات المنوية الشاذة في السائل المنوي المخزن على درجة حرارة ٥م° مع مستوى جلوتاثيون ٠,٤ مل مول.

٤- إضافة الجلوتاثيون بمستوى ١,٢ مل مول للسائل المنوي المخزن على درجة ٥م° أدى إلى انخفاض الحيوية والنسبة المئوية للحيوانات المنوية الحية مقارنة بالكنترول. التخزين على درجة ٥م° أدى إلى انخفاض الحيوية ونسبة الحيوانات المنوية الحية بينما نسبة الحيوانات المنوية الشاذة لم تختلف معنويا وتراوحت بين ١٣,١ -١٤,٣ %. وحدث نقص تدريجي في نسبة الحيوية ونسبة الحيوانات المنوية الحية بزيادة فترة التخزين.

٥- أظهر السائل المنوي المعامل بـ ٠,٤ مل مول أعلى القيم للحيوية والحيوانات المنوية الحية تلاها مستوى ٠,٨ مل مول جلوتاثيون ثم السائل المنوى المقارن، على الترتيب. بينما اظهر السائل المنوي المعامل بـ١,٢ مل مول أقل القيم. وزادت النسبة المئوية للحيوانات المنوية الشاذة تدريجيا في السائل المنوي بتركيز ٠,٤ مل مول جلوتاثيونمع زيادة فترة التخزين.

أشارت النتائج إلى إمكانية تخزين السائل المنوي لطلائق الهولشتين على درجة حرارة ٢٥م° أو ٥م° لمدة ٤٨ ساعة بإضافة الجلوتاثيون بتركيز ٠,٤ مل مول، حيث كانت النسبة المئوية للحيوانات المئوية الحية أكبر من ٥٠% والنسبة المئوية للحيوانات المنوية الشاذة ١٢%.