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EFFECT OF REDUCED GLUTATHIONE SUPPLEMENTATION ON MOTILITY, LIVABILITY AND ABNORMALITY OF HOLSTEIN SPERMATOZOA IN: 2- FROZEN SEMEN.

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ABSTRACT

To evaluate the effect of different concentrations of reduced glutathione (GSH) supplemented to the freezing extender on motility, livability and abnormality of Friesian bull spermatozoa during cooling, freezing and thawing phases of the cryopreservation process, a total of five Holstein bulls with average age of 3.5 years were used in this investigation. Semen ejaculates were collected twice weekly by means of artificial vagina. Only ejaculates having mass motility of 70% or more was pooled for each collection day for 5 weeks. The pooled semen was divided into 4 parts including control, and three concentrations of GSH (0.4, 0.8 and 1.2 mM). Percentages of motility, livability and abnormality were determined during different following phases of cryopreservation process: initial percentage in pooled semen pre-dilution; post-dilution; post-equilibration period and post-thawing. Results show insignificant effect of GSH concentration on sperm motility post dilution. During equilibrium period, sperm motility was higher ($P<0.05$) with 0.4 GSH than 0 and 1.2 mM GSH (68.0 vs. 58.5 and 60.0%, respectively), but did not differ significantly than semen with 0.8 mM GSH. However, the differences between semen with 0.8 mM GSH and the other concentrations were not significant. In frozen-thawed semen, sperm motility was higher ($P<0.05$) with 0.04 and 0.8 mM GSH than 0 and 1.2 mM GSH (55.5 and 51.0% vs. 33.0 and 38.1%, respectively). The lowest ($P<0.05$) reduction in sperm motility during all semen processes was obtained with 0.4 or 0.8 mM GSH, which resulted in the highest recovery rate (74%) of motile spermatozoa in thawed semen. The effect of GSH concentration on live sperm percentage post dilution was not significant. Post 4 h at 4°C as an equilibrium period, live sperm percentage was higher ($P<0.05$) with 0.4 mM GSH (68.4%) than 0, 0.8 and 1.2 mM GSH (60.7, 63.4 and 62.2%, respectively). Post freezing and thawing, sperm livability in semen diluted with 0.4 and 0.8 mM GSH was higher ($P<0.05$) than 0 and 1.2 mM GSH (56.8 and 51.3 vs. 35.2 and 40.6%, respectively). The lowest reduction in sperm livability during all semen processes was obtained with 0.4 or 0.8 mM GSH, which resulted in the highest recovery rate (71.4%) of live spermatozoa. The effect of GSH concentrations on sperm abnormality, post dilution, during equilibrium period, and post freezing and thawing was not significant. The lowest ($P<0.05$) increase in sperm abnormality during all semen processes was obtained with 0.4 mM GSH.

In conclusion, the addition of GSH with a concentration of 0.4 mM to the freezing Tris-extender improved percentages of motility, livability and abnormality of bull spermatozoa.

Keywards: *Bull semen, glutathione, equilibrium, motility, livability, abnormality.*

INTRODUCTION

Sperm cells have a high content of unsaturated fatty acids in their membranes and they are exposed to aerobic conditions during processing before freezing, and they have little endogenous antioxidant to protect them

against reactive oxygen species (ROS) that may be present (Foote *et al.*, 2002).

The processes of cooling, freezing, and thawing produce physical and chemical stress on the sperm membrane that reduce sperm viability and their fertilizing ability. The cold shock of spermatozoa is associated with oxidative stress induced by reactive oxygen species (ROS) generation (Chatterjee *et al.*, 2001).

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).

The process of freezing has been resulted in a significant reduction in GSH content in porcine (Gadea *et al.*, 2004) and bovine semen (Bilodeau *et al.*, 2000), which was associated with changes in sperm function, lipid composition and organization of the sperm membrane, and in turn a marked reduction in sperm viability (Buhr *et al.*, 1994).

In relation to sperm membrane functionally, Watson (1995) suggested that cryopreservation induced modifications in sperm membranes make them more prone to capacitation, so that cryopreserved sperm have been thought to be in a partially capacitated state (Bailey *et al.*, 2000).

The main objective of this study was to evaluate the effect of different concentrations of GSH supplemented to the freezing extender on motility, livability and abnormality of Friesian bull spermatozoa during cooling, freezing and thawing phases of the cryopreservation process.

MATERALS AND METHODS

The current work was carried out at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal production Research Institute, Ministry of Agriculture, during the period from 15 December 2005 to 30 January 2006.

Animals:

A total of five Holstein bulls with average age of 3.5 years were used in this investigation. They were housed individually under semi-open sheds, allowed to drink water twice daily. All bulls were healthy and clinically free of external and internal parasites. All bulls were fed according to live body weight on the basis of requirements recommended by the Animal Production Research Institute.

Semen collection:

Semen ejaculates were collected twice weekly at 8.0 a.m. by means of artificial vagina, where ejaculate of each bull (5 ejaculates for each run) was taken immediately to the laboratory and pooled. Only ejaculates having mass motility of 70% or more was pooled for each collection day for 5 weeks. On each collection day, the pooled semen was divided into 4 parts. The 1st was served as a control without treatment and the other three parts were supplemented with 0.4, 0.8 and 1.2 mM GSH.

Tris-based extender was consisted of 3.61 g Tris (hydroxymethyl) amino methane, 1.89 g citric acid, 20 ml egg yolk, 5 ml glycerol, 0.005g streptomycin, 0.25 g lincomycin and completed with bi-distilled up to 100 ml.

Semen processing and freezing procedure:

Pooled ejaculates were further processed for freezing using 0.25 ml French straws containing 20 x 10^6 motile sperm before freezing, whereas semen of each treatment was extended at rate 1: 17-20 in heated (37°C) Tris extender.

The Tris-egg yolk extender was gently mixed and warmed up to 37°C in a water bath during processing of semen extension. The vial containing the extended semen were placed in a water bath at 37°C, and then placed into a refrigerator at 5°C for 4 hours for gradual cooling as an equilibration period of spermatozoa.

Filling, freezing and thawing processes:

Extended semen before filling in straw was always kept in iced water bath to keep its temperature at 5°C while semen packed in straws was placed in a cooled ice chest.

At the end of the equilibration period, the extended semen in each tube was filled by semen automatic filling machine in French straw of 0.25 ml capacity. The semen-filling machine connected with computer and printer, which print all the data on the straws such as bull number, breed and freezing date. The filled straws were sealing by heat.

The extended packed semen was transferred into processing container and located horizontally in static nitrogen vapor 4 cm above the surface of liquid nitrogen for 10 minutes, then the straws were placed vertically in a metal canister and immersed completely in liquid nitrogen container for storage at -196°C for 24 h, thereafter the frozen semen was thawed by dipping the frozen straws into a water bath at 38°C for 30 seconds.

Semen evaluation:

Percentages of motility, livability and abnormality were determined by placing 2 sample aliquots on warm glass slides (37°C) and examined under light microscopy. The percentage of motile sperm was estimated to the nearest 5%. Percentage of progressive motility, livability and abnormality was estimated during different following phases of cryopreservation process:

- Initial percentage in pooled semen pre-dilution
- Post-dilution (pre-cooling for equilibration period)
- Post- equilibration period (pre-freezing) for 6 h
- Post-thawing after 24 h freezing period

The reduction in each characteristic post each process was calculated and the total reduction of each parameter was computed. Also, recovery rate of motility, livability and abnormality was calculated as:

Recovery rate = percent in thawed semen/initial percent

Statistical analysis:

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

Sperm motility:

Results presented in table (1) show insignificant effect of GSH concentration on sperm motility post dilution, although semen supplemented with 0.4 mM GSH showed the highest percentage (71.5%), followed by 0 and 0.8 mM (70.0 and 69.5%, respectively). While, semen supplemented with 1.2 mM GSH showed the lowest values (69.0%). However, the differences between the highest and lowest sperm motility post-dilution were not significant.

Table (1): Effect of GSH concentrations on sperm motility percentages during dilution, equilibrium period and freezing processes.

Semen process	Reduced glutathione concentration (mM)			
	0	0.4	0.8	1.2
Initial motility (%)	75.0	75.0	75.0	75.0
Dilution (37°C):				
Post dilution	70.0±1.8	71.5±1.5	69.5±1.0	69.0±1.0
Reduction	5.0±1.54	3.5±1.28	5.5±1.10	6.0±1.24
Equilibration period (for 6 h at 5°C):				
Post 6 hours	58.5±1.5^{b}	68.0±1.4^{a}	63.5±1.8^{ab}	60.0±1.6^{b}
Reduction	11.5±1.06^{a}	3.5±1.06^{c}	6.0±1.25^{bc}	9.0±1.25^{ab}
Reduction from initial	16.5±1.7^{a}	7.0±0.89^{c}	11.5±1.2^{bc}	15.0±1.4^{ab}
Freezing (24 h at –196°C):				
Post-thawing	33.0±1.53^{b}	55.5±1.89^{a}	51.0±3.12^{a}	38.1±1.67^{b}
Reduction	25.5±2.03^{a}	12.5±1.54^{b}	12.5±2.50^{b}	21.9±2.17^{a}
Reduction from dilution	37.0±2.26^{a}	16.0±1.79^{b}	18.5±2.5^{b}	30.9±3.17^{a}
Total reduction	42.0±2.62^{a}	19.5±2.01^{b}	24.0±1.84^{b}	36.9±2.42^{a}
Recovery rate (%)	44	74	68	50.8

$^{a, b \text{ and } c}$: Means denoted within the same row with different superscripts are significantly different at P<0.05.

During equilibrium period, effect of GSH concentration was significant (P<0.05) on sperm motility, being higher with 0.4 mM GSH than each of 0 and 1.2 mM GSH (68.0 vs. 58.5 and 60.0%, respectively), but did not differ significantly than semen supplemented with 0.8 mM GSH. However, the differences between semen with 0.8 mM GSH and the other concentrations were not significant. Such trend in sperm motility post equilibrium period reflected the same trend of significance among GSH concentrations in percentage of reduction in sperm motility during equilibrium period and reduction from the initial motility (Table 1).

In frozen-thawed semen, the differences in sperm motility in semen diluted with different concentration of GSH was more pronounced, being significantly (P<0.05) higher with 0.04 and 0.8 mM GSH than 0 and 1.2 mM GSH (55.5 and 51.0% vs. 33.0 and 38.1%, respectively). The reduction in sperm motility during freezing and thawing was significantly (P<0.05) lower in semen with 0.4 and 0.8 mM GSH than 0 and 1.2 mM GSH, being the lowest with 0.4 mM GSH (Table 1).

Generally, the lowest reduction in sperm motility during all semen processes was obtained significantly (P<0.05) with semen diluted with Tris-extender containing 0.4 or 0.8 mM GSH, which resulted in the highest recovery rate of motile spermatozoa (74%) as compared to 0.8 mM (68%)

and 1.2 mM (50.8%). Meanwhile, unsupllemente semen showed the lowest recovery rate (44.0%, Table 1).

Such results indicated the benefits of all GSH supplementations on motility of cryopreserved spermatozoa, in particular for 0.4 mM GSH supplementation.

Sperm livability:

Table (2) shows insignificant effect of GSH concentrations on live sperm percentage post dilution, although semen supplemented with 0.4 mM GSH showed the higher percentage (73.9%) than the other supplementations (70.7, 69.8 and 69.0% for 0, 0.8 and 1.2 mM GSH, respectively). This trend led to significant ($P<0.05$) reduction in live sperm percentage post dilution, being lower with 0.4 mM GSH (5.6%) than the other supplementation (8.8, 9.7 and 10.5% for 0, 0.8 and 1.2 mM GSH, respectively (Table 2).

Post 6 h at 4°C as an equilibrium period, effect of GSH concentration was significant ($P<0.05$) on live sperm percentage, being higher with 0.4 mM GSH (68.4%) than each of 0, 0.8 and 1.2 mM GSH (60.7, 63.4 and 62.2%, respectively). Such trend in sperm livability post equilibrium period reflected lower reduction in percentages of sperm livability during equilibrium period with all GSH supplementations (5.5-6.8%) than the control one (10%). However, the reduction from initial to post-equilibrated sperm livability was significantly ($P<0.05$) lower with 0.4 mM GSH (11.1%) than 0, 0.8 and 1.2 mM GSH (18.8, 16.1 and 17.3%, respectively, Table 2).

Post freezing and thawing, sperm livability percentage in semen diluted with 0.4 and 0.8 mM GSH was significantly ($P<0.05$) higher than 0 and 1.2 mM GSH (56.8 and 51.3 vs. 35.2 and 40.6%, respectively). The reduction in live sperm percentage during freezing and thawing or from equilibrated semen was significantly ($P<0.05$) lower with 0.4 and 0.8 than 0 and 1.2 mM, while semen with 0.4 mM GSH showed the lowest reduction (Table 2).

Table (2): Effect of GSH concentrations on sperm livability percentages during dilution, equilibrium period and freezing processes.

Semen process	Reduced glutathione concentration (mM)			
	0	0.4	0.8	1.2
Initial livability (%)	79.5	79.5	79.5	79.5
Dilution (37°C):				
Post dilution	70.7±2.02	73.9±1.69	69.8±1.24	69.0±1.40
Reduction	8.8±1.71^{a}	5.6±1.12^{b}	9.7±1.05^{a}	10.5±1.22^{a}
Equilibrium period (for 6 h at 5°C):				
Livability post 6 hours	60.7±1.48^{b}	68.4±1.05^{a}	63.4±1.59^{b}	62.2±1.78^{b}
Reduction (%)	10.0±1.7^{a}	5.5±1.51^{b}	6.4±0.99^{ab}	6.8±1.31^{ab}
Reduction from initial	18.8±1.52^{a}	11.1±1.48^{b}	16.1±0.84^{a}	17.3±1.02^{a}
Freezing (24 h at –196°C):				
Post-thawing livability	35.2±2.18^{b}	56.8±1.98^{a}	51.3±3.14^{a}	40.6±3.69^{b}
Reduction	25.5±2.29^{a}	11.60±1.86^{b}	12.1±2.28^{b}	21.6±3.03^{a}
Reduction from dilution	35.5±1.71^{a}	17.10±2.27^{b}	18.5±2.62^{b}	35.5±1.71^{a}
Total reduction	44.3±1.45^{a}	22.7±1.89^{b}	28.2±2.05^{b}	38.9±1.41^{a}
Recovery rate (%)	44.3	71.4	64.5	51.1

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

In general, the lowest reduction in sperm livability during all semen processes was obtained with semen supplemented with 0.4 or 0.8 mM GSH, which resulted in the highest recovery rate of live spermatozoa (71.4%) as compared to 0.8 mM GSH (64.5%) and 1.2 mM GSH (51.1%).

Such results indicated the beneficial effects of all GSH supplementations on livability of cryopreserved spermatozoa, in particular for 0.4 mM GSH supplementation.

Sperm abnormality:

It is of interest to note that the effect of GSH concentrations on abnormal sperm percentage post dilution and during equilibrium period was not significant. Also, the differences in increase of abnormal sperm percentage post dilution and equilibration period were not significant, although, the lowest increase post equilibrium period from the initial percentage was obtained for 0.4 mM GSH as compared to the other supplementations and control one (Table 3).

Also, the effect of GSH supplementation on abnormal sperm percentage post freezing and thawing was not significant. However the increase in abnormal sperm percentage during freezing and thawing or the increase from equilibrated semen was affected significantly ($P<0.05$) by GSH concentration, being lower in semen diluted with 0.4 mM GSH than 0, 0.8 and 1.2 mM GSH (Table 3).

Table (3): Effect of GSH concentrations on sperm abnormality percentages during dilution, equilibrium period and freezing processes.

Semen process	Reduced glutathione concentration (mM)			
	0	0.4	0.8	1.2
Initial abnormality (%)	12.5	12.5	12.5	12.5
Dilution (37°C):				
Post dilution	17.8±1.11	17.2±0.76	18.4±1.05	17.1±0.71
Increase	5.3±1.10	4.7±0.84	5.9±0.91	4.6±1.02
Equilibrium period (for 6 h at 5°C):				
Post 6 hours	23.9±0.99	21.2±1.20	24.4±0.86	22.1±0.91
Increase	6.1±0.87	4.0±1.09	6.0±1.01	5.0±1.25
Increase from initial	11.4±1.01	8.7±0.95	11.9±0.93	9.6±1.13
Freezing (24 h at –196°C):				
Post-thawing	30.7±1.09	24.6±0.83	31.9±1.24	31.2±0.81
Increase	6.8±0.73^a	3.4±1.21^b	7.5±0.84^a	9.1±0.82^a
Increase from dilution	12.9±0.74^a	7.4±0.71^b	12.9±0.94^a	14.1±1.02^a
Total reduction	18.2±1.13^a	12.1±0.94^b	19.4±0.98^a	18.7±1.20^a

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

It is worthy noting that the lowest increase in sperm abnormality during all semen processes was obtained significantly ($P<0.05$) with semen diluted with Tris- extender containing 0.4 mM GSH, which resulted in the lowest increase in sperm abnormality percentage (12.1%) as compared to the other supplementations and unsupplemented one (Table 3).

Such results indicated the benefits of all GSH supplementations on motility of cryopreserved spermatozoa, in particular for 0.4 mM GSH supplementation.

DISCUSSION

Freezing is associated with damage of sperm function affecting those processes required for the successful *in vivo* fertilization of an oocyte (Bailey *et al.*, 2000). During freezing, the production of ROS (Bilodeau *et al.*, 2000 and Ball *et al.*, 2001) can induce changes in membrane function and structure of spermatozoa. The detrimental effects of freezing could be blocked, at least in part, by the addition of exogenous GSH, since the cell employs GSH and thioredoxin systems to reverse oxidative stress. In addition an alteration in antioxidant defense systems may occur (Bilodeau *et al.*, 2000), including a decrease in intracellular GSH content (Bilodeau *et al.*, 2000 and Gadea *et al.*, 2004). So, one obvious way to improve the viability, livability and subsequent fertilizing capacity of frozen-thawed spermatozoa would be the addition of antioxidants to the freezing media.

An attempt with relative success was carried out to improve motility, livability and abnormality of spermatozoa in bull semen stored at room temperature (25°C) and refrigerated at 5°C by supplementation of different GSH concentration (El-Sherbieny *et al.*, 2006). Almost, supplementation of Tris-extender with 0.4 mM GSH resulted in the best results.

The present results indicated that the addition of 0.4 mM GSH to the freezing extender resulted in an increasing motility of thawed spermatozoa and a higher number of total live and abnormal spermatozoa. This may indicate the protective effects of exogenous GSH on sperm function, which was dose dependent, and it was more pronounced in semen supplemented with 0.4 mM GSH than those supplemented with 0.8 and 1.2 mM GSH.

During the equilibrium period in the present study, the addition of GSH to the Tris-extender had significant effects on sperm motility and livability. But, the changes in sperm abnormality were not significant. In this respect, Gadea and Matas (2000) and Gadea *et al.* (2004) found that the addition of GSH to the media did not have any significant effects on sperm function during the cooling phase (previous to the freezing phase). They suggested a decrease in intracellular GSH content only during the freezing step. However, the decrease of intracellular GSH content was not observed during the cooling step. Therefore, the addition of exogenous GSH in the current study, which was preserved sperm function during equilibration period, may indicate that endogenous GSH concentration decreased during this step.

Addition of GSH to the freezing extender during the freezing phase in this study improved the quality and fertilizing ability of frozen-thawed spermatozoa. This is in agreement with the addition of GSH to the freezing extender of boar semen (Nishimura and Morii, 1993). This was attributed to that the addition of GSH has been help to maintain bull sperm motility (Lindemann *et al.*, 1988; Bilodeau *et al.*, 2001 and Foote *et al.*, 2002), to protect sperm against oxidative damage (Alvarez and Storey, 1989), to preserve sperm viability by protecting membrane structure and function (Gadea 2005). Such effects resulted in fewer changes in sperm function in frozen bull spermatozoa (Chatterjee *et al.*, 2001), and subsequently better sperm motility.

Recently, Gadea (2005) reported a significant correlation between GSH supplementation to the thawing extender and *in vitro* fertilization. In contrast, Gadea *et al.* (2004) did not find any improvement in either standard semen parameters or sperm fertilizing ability *in vitro* as affected by GSH addition to the freezing and thawing extender.

In the current study, addition of 0.4 GSH led to improvement in bull sperm quality. However, Gadea (2005) found that the concentration of 1 mM of GSH tends to be more adequate in preserving boar sperm function. Therefore, species differences must be taken in consideration to determine the optimal concentration of GSH for each species. Also, Foote *et al.* (2002) found individual differences in the response of sperm to exogenous GSH concentration. The lower results obtained from high GSH concentration (1.2 mM) as compared to low GSH concentration (0.4 mM) could be attributed to the lower membrane stability caused by a high concentration of exogenous GSH when endogenous GSH concentration are within normal limits, as has been previously reported in studies of bull spermatozoa (Foote *et al.*, 2002).

In conclusion, the addition of GSH with a concentration of 0.4 mM to the freezing Tris-extender improved percentages of motility, livability and abnormality of bull spermatozoa.

Further studies are needed to understand changes that occur in bull spermatozoa during freezing and the potentially beneficial effects of antioxidants in improving the fertility outcome in artificial insemination using bull frozen-thawed spermatozoa and the mechanism(s) by which GSH exerts its effect(s).

REFERENCES

Alvarez, J.G. and 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.

Bailey, J.L.; Bilodeau, J.F. and Cormier, N. (2000). Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. J. Androl., 21:1–7.

Ball, B.A., Vo, A.T. and Baumber, J. (2001). Generation of reactive oxygen species by equine spermatozoa. Am. J. Vet. Res., 62:508–515.

Bilodeau, J.F.; Blanchette, S., Gagnon, C. and Sirard, M.A. (2001). Thiols prevent H_2O_2-mediated loss of sperm motility in cryopreserved bull semen. Theriogenology, 56:275–286.

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

Buhr, M.M.; Curtis, E.F. and Kakuda, N.S. (1994). Composition and behavior of head membrane lipids of fresh and cryopreserved boar sperm. Cryobiology, 31:224–238.

Chatterjee, S.; de Lamirande, E. and Gagnon, C. (2001). Cryopreservation alters membrane sulfhydryl status of bull spermatozoa: protection by oxidized glutathione. *Mol. Reprod. Dev.*, 60:498–506.

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

El-Sherbieny, M.A.; M.H. El-Nenaey; E.M.E. El-Siefy and A.E. Abdel-Khalek (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 University, 31 (4): 1913- 1931.

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

Gadea, J. and Matas, C. (2000). Sperm factors related to in vitro penetration of porcine oocytes. Theriogenology, 54:1343–1357.

Gadea, J.; Selles, E.; Marco, M.A.; Coy, P.; Matas, C.; Romar, R. and Ruiz, S. (2004). Decrease in glutathione content in boar sperm after cryopreservation: effect of the addition of reduced glutathione to the freezing and thawing extenders. Theriogenology, 62:690–701.

Gadea, J. (2005). Sperm factors related to in vitro and *in vivo* porcine fertility. Theriogenology, 63:431–444.

Irvine, D.S. (1996). Glutathione as a treatment for male infertility. Rev Reprod., 1:6–12.

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

Nishimura, K. and Morii, H. (1993). Effects of glutathione on the motility of frozenthawed boar spermatozoa. Anim. Sci. Technol. (Jpn), 64:433–439.

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

Watson, P.F. (1995). Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. Reprod. Fertil. Dev., 7:871–891.

تأثير إضافة الجلوتاثيون المنخفض علي حيوية وحياتية وشواذ الحيوانات المنوية لطلائق الهولشتين في : ٢- السائل المنوى المجمد

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لتقدير تأثير التركيزات المختلفة من الجلوتاثيون المنخفض المضافة الى مخفف السائل المنوي علي حيوية وحياتية وشواذ الحيوانات المنوية لطلائق الهولشتين أثناء مراحل التبريد والتجميد والإسالة لعمليات الحفظ استخدم في هذا البحث عدد خمس طلائق هولشتين ناضجة جنسيا متوسط عمرها ٣,٥ سنة تم جمع السائل المنوي مرتين أسبوعيا باستخدام المهبل الصناعي وتم خلط القذفات التي حيوتيها ٧٠% فاكثر فقط ثم تخفيفها بمخفف الترس/كل يوم على حدة لمدة خمسة أسابيع. تم تقسيم السائل المنوي المخفف على أربعة أجزاء المقارنة وثلاث تركيزات من الجلوتاثيون المنخفض (٠,٤ -٠,٨ -١,٢ مل مول). وتم تقدير النسبة المئوية للحيوية والشواذ خلال المراحل المختلفة للحفظ (قبل التخفيف - بعد التخفيف - بعد فترة الموازنة - بعد التجميد والإسالة). أظهرت النتائج ان تأثير الجلوتاثيون المنخفض على الحيوية بعد التخفيف أثناء فترة الموازنة كان معنويا عند مستوى ٥% بالنسبة لتركيز ٠,٤ عن صفر ، ١,٢ مل مول (٦٨ مقابل ٥٨,٥ ، ٦٠%) على التوالي لكنها لم تختلف معنويا عن السائل المنوي المخفف المضاف إليه ٠,٨ مل مول. بالنسبة للسائل المنوي بعد التجميد والإسالة كانت الحيوية عالية المعنوية عند مستوى ٠,٤ ، ٠,٨ مل مول عن المقارنة ، ١,٢ مل مول حيث كانت ٥٥,٥ ، ٥١ % مقابل ٣٣ ، ٣٨,١% على التوالي وأقل انخفاض في نسبة الحيوية عند مستوى ٥% في جميع خطوات التصنيع (التخفيف - الموازنة - التجميد والإسالة) كانت عند مستويات ٠,٤ ، ٠,٨ مل مول تأثير تركيز الجلوتاثيون المنخفض على النسبة المئوية للحيوانات المنوية الحية بعد التخفيف كان غير معنوي. بعد فترة الموازنة على درجة ٥م° كانت أعلى نسبة ٦٨,٤% عند مستوى ٠,٤مل مول عن صفر ، ٨ ، ١,٢ مل مول (٦٠,٧ ، ٦٣,٤ ، ٦٢,٢%) على التوالي. بعد التجميد والإسالة كانت النسبة المئوية للحيوانات المنوية الحية عالية المعنوية عند تركيز ٠,٤ ، ٠,٨ مل مول عن صفر ، ١,٢ مل مول (٥٦,٨ ، ٥١,٣% مقابل ٣٥,٢ ، ٤٠,٦% على التوالي). كان أقل انخفاض في النسبة المئوية للحيوانات المنوية الحية في جميع المراحل (التخفيف - الموازنة - التجميد والإسالة) كانت عند مستوى ٠,٤ ، ٠,٨ مل مول تأثير الجلوتاثيون على النسبة المئوية للحيوانات المنوية الطبيعية بعد التخفيف - فترة الموازنة - التجميد والإسالة كانت غير معنوية. أقل انخفاض في النسبة المئوية للحيوانات المنوية الطبيعية في جميع المراحل (التخفيف ، الموازنة ، التجميد والإسالة) كانت عند مستوى ٠,٤ مل مول.

لذلك يجب إضافة الجلوتاثيون المنخفض إلى مخفف السائل المنوي حيث أنه أدى الى تحسين جودة السائل المنوي (الحيوية - النسبة المئوية للحيوانات المنوية الحية وتقليل نسبة الشواذ).