

EFFECT OF GLUTATHIONE ON SELECTED MOTION PARAMETERS OF RAM COOLING-STORED SPERMATOZOA

EFEKT GLUTATIÓNU NA VYBRANÉ PARAMETRE POHYBLIVOSTI BARANÍCH SPERMÍÍ UCHOVÁVANÝCH V SCHLADENOM STAVE

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ABSTRACT

The goal of this study was to examine the effect of glutathione (GSH) on selected motion parameters of cooling-stored ram sperm. Fresh ram ejaculates were diluted in Triladyl extender, pooled and glutathione was added at concentrations 0; 0.5; 1.5 and 5mmol.l⁻¹. Samples were incubated at 4°C for three days. Sperm motility parameters were measured by CASA system. Glutathione improved average value of total motility and progressive movement (PM) after incubation in cooling condition. Glutathione maintained sperm motility and PM at high level after 24h of cooling-storage, the best results were observed at 1.5 and 5 mmol.l⁻¹ of glutathione. Significant increase in curvilinear velocity (VLC), straight line velocity (VCL), average path velocity (VAP) and distance average path (DAP) were confirmed after 24h of cooling-storage at 1.5 mmol.l⁻¹. After 48h storage GSH maintained sperm motility and PM at higher level in compared with control group. These data indicate that glutathione can maintain good quality of movement of ram sperm during long-term cooling-storage.

Key words: ram sperm, glutathione, motion parameters

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INTRODUCTION

Glutathione (GSH), antioxidant, has elicited beneficial effects in many facets of in vitro production: increasing the IVF success rate (Abeydeera, 2002; Jiang et al., 2007), acting as a cryoprotectant during semen freezing (Munsiet al., 2007), and assisting in antioxidant defense mechanisms during semen thawing (Gadea et al., 2005). Sperm DNA is vulnerable to damage induced by reactive oxygen species (ROS) (Steenken, 1989). Reduced glutathione is present in many organs and tissues of body and has many antioxidant properties (Meister, 1992). High levels of GSH were found in testis of rat (Vina et al., 1992) and in reproductive tract and epididymal sperm of bulls (Agrawal and Vahna-Perttula, 1988). In the semen chilling process, the problem commonly encountered is the damage of spermatozoa plasma membranes due to the formation of lipid peroxidation. This condition occurs as the spermatozoa membranes contain a lot of unsaturated fatty acids which very susceptible to peroxidation damage (Maxwell and Watson 1996). Furthermore, Sikka (1996) stated that mammalian spermatozoa are rich of unsaturated fatty acids and very easily subjected to reactive oxygen species (ROS) that can decrease spermatozoa mortality and increase the morphological damage influencing the sperm capacity and acrosome reaction.

The aim of this study is to examine the effect of glutathione on selected sperm motion parameters of cooling-stored ram sperm.

MATERIAL AND METHODS

All the experiments were carried out with fresh ram spermatozoa, collected from one East-Friesian (EF) and three Lacaune (Lc) rams using artificial vagina. The rams were kept at the Institute of Sheep Breeding (Trenčianska Teplá) under uniform nutritional conditions. Volume, concentration and activity were assessed shortly after collection. The ejaculates from all rams (n=4) were pooled together to make a heterospermia in order to avoid individual variability of ram and were used for the experiment. The ejaculates were diluted in a Triladyl extender (Minitüb, Tiefenbach, Germany). Semen was cooled down to 4 °C and transported to the laboratory, where the samples were divided into four groups, at 1 ml of ejaculate per tube. Then glutathione (Sigma – Aldrich, Germany) was added to marked tubes at concentrations of 0.5; 1.5 or 5 mmol.l⁻¹, control group did not contain glutathione (0 mmol.l⁻¹). The semen samples were kept in a fridge for three days. Analyses of motility parameters were done at 0, 24 and 48 hours of sperm cooling-storage, using computer assisted semen analysis (CASA) - Hamilton Thorn motility analyser (version 7). Each sample was analysed at the time intervals of 0, 0.5h or 2h. Between these time points the sample were incubated at 37 °C. We analysed effect of various concentrations of glutathione on ram sperm total motility

and progressive movement (PM) and on selected movement parameters – curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), amplitude of lateral head displacement (ALH), distance average path (DAP).

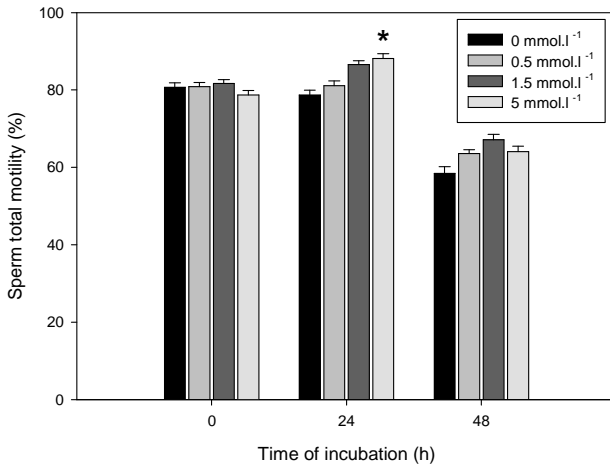
The experiments have been done in two replications. In each experimental group 7 view fields were evaluated, so that at least 350 sperm cells per one experiment were counted. Average values were calculated from three measurements during the day. The results were statistically evaluated by t-test and graphically processed using SigmaPlot graphic software (version 9.0 for windows).

RESULTS

Average values of ram sperm total motility and progressive movement following 3 days of cooling-storage were evaluated. It was not visible effect of glutathione on sperm motility parameters at day 0 of storage. Effect of glutathione after 24 h was more expressed. Higher concentrations 1.5 and 5 mmol.l⁻¹ had considerable effect on sperm total motility. Significant increase in total motility was noted at the highest concentration of GSH (5 mmol.l⁻¹), where total motility increased from 78.73% (control group) to 88.14% (Fig 1). Similar tendencies were observed for sperm progressive movement. Glutathione at highest concentration significantly improved proportion of progressively moved sperm from 71.61% (control group) to 82.24%. Furthermore, positive effect of GSH at 1.5 mmol.l⁻¹ was visible, where proportion of progressive moved sperm was 80.32%, but this increase was not significant (Fig 2). Glutathione at 0.5 mmol.l⁻¹ did not affect sperm total motility and PM, what can demonstrate dose-depend manner of glutathione effect.

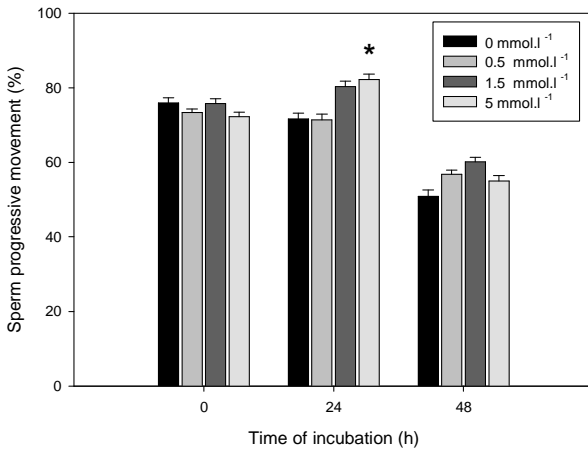
This positive effect of GSH persisted following 48 h of cooling-storage, when all tested concentrations increased sperm total motility and PM. Similarly, the highest effect was visible at concentration 1.5 and 5 mmol.l⁻¹.

Figure 1: Effect of glutathione on sperm total motility after long-term cooling-storage



* Significant difference compared to control: P < 0.05

Figure 2: Effect of glutathione on sperm progressive movement after long-term cooling-storage



* Significant difference compared to control: P < 0.05

Determination of movement characteristics is important for good prediction of sperm fertilization ability. Glutathione at all tested concentration positively improved motion parameters of ram sperm after 24h of cooling storage (Tab 1). Glutathione positively affected sperm velocity parameters - curvilinear velocity, straight line velocity and average path velocity. Furthermore distance average path and amplitude of lateral head displacement were increased by all tested concentration in compare with control group. The most marked effect on velocity parameters was at 1.5 mmol.l⁻¹, where VAP, VCL and VSL were significantly increased (P< 0.05). These results indicate that glutathione may improve quality of movement of cooling storage sperm.

Table 1: Effect of different glutathione concentrations on selected motility characteristics after 24 h of cooling storage

Parameter	Mean ± SE			
	0mmol.l ⁻¹	0.5mmol.l ⁻¹	1.5mmol.l ⁻¹	5mmol.l ⁻¹
Motility (%)	78.73± 1.20	81.07 ± 1.28	86.51 ± 1.03	88.14 ± 1.21*
PM (%)	71.61 ± 1.58	71.38 ± 1.51	80.32 ± 1.46	82.24 ± 1.44*
ALH (µm)	4.53 ± 0.18	5.19 ± 0.46	5.25 ± 0.47	5.14 ± 0.37
DAP(µm)	34.94 ± 0.97	39.44 ± 2.49	42.69 ± 1.35*	39.47 ± 3.28
VCL (µm/s)	145.41 ± 5.35	165.93 ± 13.67	171.97 ± 9.47*	156.13 ± 11.83
VSL (µm/s)	63.71± 2.32	74.09 ± 5.00	81.97 ± 3.45*	74.27 ± 7.09
VAP (µm/s)	77.71± 2.19	90.00 ± 5.98	97.68 ± 3.89*	90.15 ± 7.37

* Significant difference compared to control: P < 0.05

VCL – curvilinear velocity – velocity over the total distance moved, i.e. including all deviations of sperm head movement (µm.sec⁻¹)

VAP – average path velocity – the average velocity of sperm movement (µm.sec⁻¹)

VSL – straight line velocity - velocity calculated using the straight line distance between the beginning and end of the sperm track (µm.sec⁻¹)

ALH – amplitude of lateral head displacement – the mean width of sperm head oscillation (µm)

DAP - distance average path - distance that the sperm head travelled along its average trajectory (µm)

DISCUSSION

The processing and storage of ram semen reduce the motility and may cause disruption the membrane integrity of spermatozoa. It is generally assumed that these changes are detrimental and are associated with a loss of fertilising capacity. Fertility of stored sperm is generally lower after cervical insemination than fertility of fresh ram semen (Maxwell and Watson, 1996). Glutathione is a significant component of the collective antioxidant defences, and a highly potent antioxidant and antitoxin in its own right. GSH is essential both to the functionality and to the structural integrity of cells, the tissues and organ systems. Superoxide, peroxide, hydroloxy radical and other free radicals derived from oxygen are highly reactive and therefore threatening to the integrity of essential biomolecules such as DNA and RNA, enzymes and other proteins, and the phospholipids responsible for membrane integrity (Kidd, 1997). Oxidative damage to ram sperm resulting from reactive oxygen species generated by the cellular components of semen during liquid storage is possibly one of the main causes for the decline in motility and fertility during storage (Bucak and Tekin, 2007).

There are reports about positive effects of glutathione on sperm. Addition of 0.5 mmol.l⁻¹ of GSH to diluents is sufficiently effective to protect the plasma membranes and maintain the percentage of spermatozoa motility stored at 5 °C (Triwulanningsih et al., 2008). Sinha et al. (1996) reported that addition of 2 or 5 mmol.l⁻¹ of GSH to goat semen diluents increased the motility and reduced proportion of acrosome abnormality of goat spermatozoa after being chilled. The anti-oxidant treatments GSH 5–10 mmol.l⁻¹ and taurineat 50 mmol.l⁻¹ provided a significant improvement in ram sperm survival during the 6 h of liquid storage at 5 °C (Bucak and Tekin, 2007). Our results indicate the highest effect of GSH after 24h of cooling-storage where GSH at 1.5 mmol.l⁻¹ and at 5 mmol.l⁻¹ (P<0.05) increased average value of total sperm motility and sperm progressive movement. Positive effect of GSH was visible after 48h too, where all concentrations increased sperm total motility and PM. Similar results were obtained with bull's semen. Sperm motility of chilled bull semen was significantly higher in semen treated with 0.5 mmol.l⁻¹ of glutathione. The optimum sperm motility (≥50%) for AI was retained for 3 days at 0.5 – 2.0 mmol.l⁻¹ of glutathione (Munsi et al., 2007).

The addition of 0.5 mmol.l⁻¹ GSH to diluents is sufficiently effective in improving the condition of plasma membranes of chilled semen stored at 5 °C, thus it can increase percentage of bull sperm motility (Triwulanningsih et al., 2008). Munsi et al. (2007) reported that GSH at 3.0 mmol.l⁻¹ did not retain bull sperm motility over 50% for 3 days. Similarly, El-kon and Darwish (2011) reported that the decline rate in the motility percentage was higher in buffalo semen samples treated with 2.00 or 3.00 mmol.l⁻¹ of GSH. Our results indicate, that higher concentration of GSH (1.5 and 5 mmol.l⁻¹) had more beneficial effect on ram sperm movement parameters than lower (0.5 mmol.l⁻¹). Glutathione addition positively affected sperm velocity parameters (VCL, VSL, VAP). Significantly increase was noted at 1.5 mmol.l⁻¹. These results can indicate that GSH at 1.5 mmol.l⁻¹

may have positive impact on quality of movement of cooling-storage ram sperm. Results of El-kon and Darwish (2011) indicate, that addition of 0.50 or 1.00 mmol.l⁻¹ of GSH to semen diluent improves quality of liquid semen up to 120 hours, reduces DNA damage and improves the fertility of frozen-thawed buffalo semen. Different effect of GSH on another species was reported. No effect of glutathione at 5 mmol.l⁻¹ on progressive movement of post-thawed boars sperm (Whitaker et al., 2008) and on human sperm PM and other CASA movement parameters (VSL, VAP, VCL, ALH) over a 4h period was reported (Donnelly et al., 2000). Similarly, no significant effect on motion parameters (VCL, VSL, VAP, ALH) of boar sperm was found after addition of GSH to the thawing medium (Gadea et al., 2005).

CONCLUSION

Our results indicate that addition of glutathione can maintain the quality of cooling storage ram sperm. Glutathione at 1.5 and 5 mmol.l⁻¹ had beneficial impact of total sperm motility and progressive movement and significantly increased sperm movement characteristics (VAP, VCL, VSL and DAP).

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