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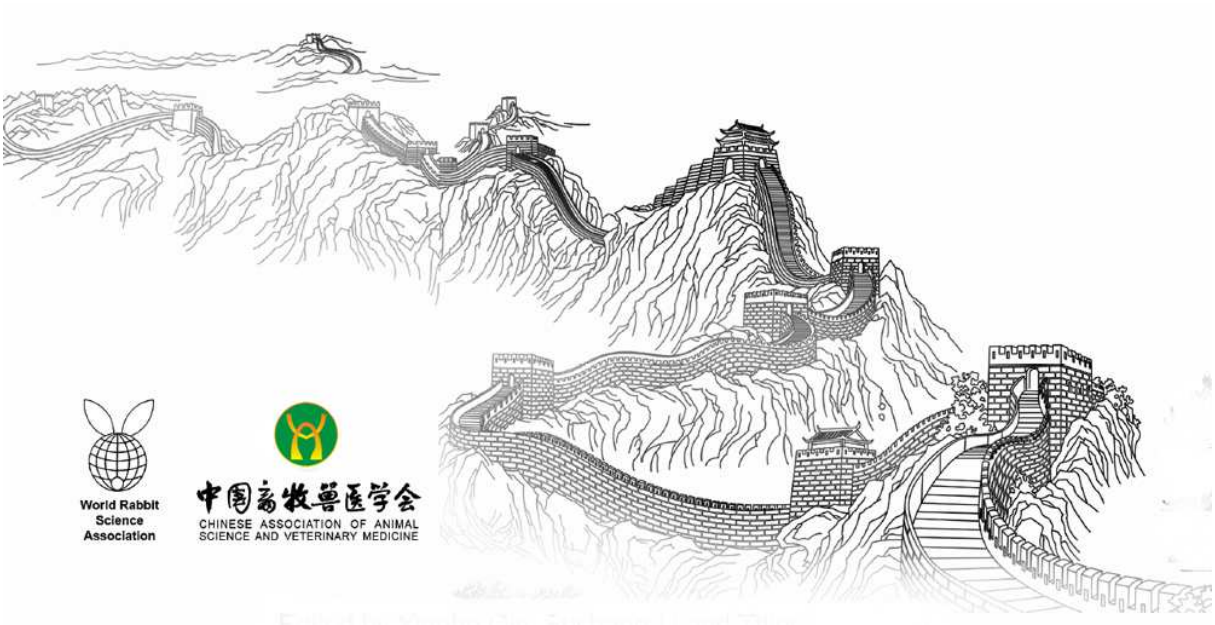

SPERM MOTILITY TRAITS OF COOLED RABBIT SEMEN WITH DIFFERENT LEVELS OF MELATONIN

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ABSTRACT

The present work aimed at investigating the effect of melatonin supplementation at different levels in a semen extender on motility traits of cooled rabbit semen. Semen was collected from ten rabbit bucks twice weekly using artificial vagina. Semen was diluted (1:1 v/v) with Tris-Citric-Glucose extender supplemented with Me2SO and sucrose as cryoprotectants. Extended semen was supplemented with different levels of melatonin (10^{-3}M, 10^{-6}M, 10^{-9}M and control) and cooled at 5 °C for 2h. Motion characteristics of spermatozoa were assessed using computer-assisted sperm analysis (CASA). Total and progressive sperm motility percentages (78.8 and 67.8, respectively) were higher ($P<0.05$) in group supplemented with 10^{-6}M melatonin. Distance straight line (DSL) and velocity curved line (VSL) values (25.5 µm and 56.0 µm/s, respectively) were also high in this group in comparison to the other melatonin groups or control ($P<0.05$). Higher concentration of melatonin (10^{-3} M) *vs.* control had a significant decrease in straightness STR (79.7 *vs.* 81.4%), and a significant increase in distance curved line DCL (62.6 *vs.* 58.4 µm), velocity curved line VCL (135.4 *vs.* 126.4 µm/s) and lateral head displacement ALH (3.8 *vs.* 3.6µm), respectively. The results demonstrate that adding melatonin at 10^{-6}M to rabbit semen extenders enhances sperm motility characteristics after cooling. Higher concentration at 10^{-3} M melatonin decreased the straightness of spermatozoa and increased the undesired parameters like lateral head displacement. Further studies on sperm preservation/cryoprotection are warranted to confirm the present findings.

Key words: Rabbit, Sperm Motility, Cooling, Melatonin.

INTRODUCTION

It would be highly beneficial in routine artificial insemination (AI) programs on commercial rabbit farms if semen could be cryopreserved without affecting fertility. Most of the protocols need long periods of cooling (between 2 and 4 h) before they are frozen or transported to distant farms. Although several authors have developed different extenders and protocols for cooling or freezing the rabbit sperm, a multitude of factors affect the results of fertility and prolificacy, including the initial quality of the semen samples, cryopreservation protocol, freezing extenders and environmental factors (Lavara *et al.*, 2013). Rabbit spermatozoa are relatively sensitive to cryoprotectants containing hydroxyl, amide or methyl groups (Castellini *et al.*, 1992). In this context, Viudes de Castro *et al.* (2014) developed a rapid protocol for freezing rabbit sperm using an extender with Tris, citric acid and glucose supplemented with sucrose and dimethyl sulphoxide (Me2SO) as cryoprotectants; the results obtained both in motility and fertility were comparable to those obtained with fresh sperm.

Cold storage of semen is used to reduce metabolism and to maintain sperm viability over an extended period of time. During this extended storage period, spermatozoa face the risk of exposure to high levels of reactive oxygen species (ROS) and free radicals due to the relative high content of unsaturated fatty acids in the phospholipids of the sperm membrane. These conditions adversely affect sperm motility

(Alvarez and Storey, 1982), inactivate glycolytic enzymes, damage the acrosomal membranes (Alvarez and Storey, 1984) and oxidize DNA, which would render the sperm cell unable to fertilize the oocyte (Ashrafi *et al.*, 2013).

Melatonin (N-aceyl-5-methoxytryptamine) is secreted by the pineal gland in the brain (Awad *et al.*, 2006) and is well known as an antioxidant because of its high efficacy as a free radical scavenger (Reiter, 1998; El-Sokkary *et al.*, 2003). The free radical scavenging activity of melatonin also extends to its metabolites, which up-regulate antioxidant enzymes and down-regulate the pro-oxidative and proinflammatory enzymes making melatonin highly effective, even at low concentrations, in protecting organisms from oxidative stress (Tan *et al.*, 2007). Reports about the beneficial effects of melatonin in protecting spermatozoa from different kind of injuries have also been emerged in mouse (Sarabia *et al.*, 2009), sheep (Casao *et al.*, 2010) and human (Du Plessis *et al.*, 2010).

To our knowledge, few previous studies have reported the effects of melatonin on sperm motility traits of rabbit semen during the cooling phase of cryopreservation protocols. Therefore, this study was carried out to investigate the effect of melatonin supplementation at different levels in a semen extender on motility traits of cooled rabbit semen.

MATERIALS AND METHODS

Animals

A total of 10 rabbit bucks belonging to the Baladi breed (Khalil, 2002) were used in this experiment. All bucks aged 6 months old and the average weight was ranged from 3.5 to 4 kg at the beginning of the study. All bucks were housed during the study period in a semi-closed rabbitry housing system (Faculty of Agriculture, Cairo University). They were maintained under the same standard environmental conditions with light alternating on a cycle of 16 light hours and 8 dark hours, fed with the same pellets commercial diet (18.4% CP, 3.1% ether extract, 12.7% crude fibre and 2.600 kcal DE/kg) and had free access to water. All the experimental protocols were approved by the Research Ethics Committee at the Faculty of Agriculture, Cairo University.

Semen Collection and Processing

Semen was collected twice a week from the bucks by using an artificial vagina. The ejaculates were transferred immediately to the laboratory and maintained in a water bath (38.5 ºC), until semen evaluation. Only those ejaculates with more than 70% progressive motility were pooled. The pooled semen was divided equally into four groups and diluted (1:1 v/v) with rabbit semen extender supplemented with different levels of melatonin (10^{-3}M, 10^{-6}M and 10^{-9}M) or without melatonin as control. The composition of semen extender was 250 mMtris-hydroxymethylaminomethane, 83 mM citric acid and 50 mM glucose; supplemented with 3 M Me2SO and 0.1M sucrose as cryoprotectants. All groups were cooled at 5 °C for 2h. After cooling all groups were evaluated for motion characteristics by using Computer Assisted Sperm Analysis (CASA; instrument SpermVision™ software minitube Hauptstraße 41. 84184 Tiefenbach, Germany). The motion characterization was recorded including: distance curved line (DCL, µm), distance average path (DAP, µm), distance straight line (DSL, µm), velocity curved line (VCL, µm/sec), velocity average path (VAP, µm/sec), velocity straight line (VSL, µm/sec), linearity (LIN=VSL/VCL), straightness (STR=VSL/VAP), wobble (WOB=VAP/VCL), beat cross frequency (BCF, H_z) and amplitude of lateral head displacement (ALH, µm).

Statistical Analysis

The data of motion characteristics were analyzed using the general linear model procedure (SAS, 2001). Duncan's range test was performed to test the significance.

RESULTS AND DISCUSSION

The physiological concentrations of melatonin in plasma of rabbits were previously estimated by radioimmunoassay (RIA) to be 22.7 pg/ml (equivalent to 10^{-10} M) (Noguchi *et al.*, 2003). In the current study, we used a concentration of melatonin close to physiological levels (10^{-9}M) and higher concentrations (10^{-6}M and 10^{-3}M) to study its effect on sperm motility traits of cooled rabbit semen. As shown in Table 1, our results indicated that the treatment of cooled semen samples with 10^{-9} M melatonin did not provide any significant effect on all motility parameters analyzed by CASA when compared to the control group; maybe due to that, this level is very close to the physiological concentrations of melatonin in plasma of rabbits (Noguchi *et al.*, 2003).

Table 1. Sperm motility traits (mean ± SE) of cooled rabbit semen as affected by different levels of melatonin.

Parameters	control	10^{-3}	10^{-6}	10^{-9}
Total motility (%)	74.0 ± 0.9 [b]	74.3 ± 0.9 [b]	78.8 ± 1.0 [a]	75.1 ± 1.0 [b]
Progressive motility (%)	57.4 ± 0.6 [c]	59.7 ± 1.0 [c]	67.8 ± 1.2 [a]	62.9 ± 1.1 [b]
DAP (µm)	28.6 ± 0.5 [b]	29.9 ± 0.5 [ab]	31.0 ± 0.6 [a]	29.3 ± 0.6 [b]
DCL (µm)	58.4 ± 0.9 [b]	62.6 ± 0.9 [a]	62.3 ± 1.0 [a]	60.2 ± 1.0 [ab]
DSL (µm)	23.5 ± 0.5 [b]	24.0 ± 0.5 [b]	25.5 ± 0.5 [a]	23.9 ± 0.5 [b]
VAP (µm/s)	61.9 ± 1.1 [b]	64.9 ± 1.1 [ab]	67.9 ± 1.3 [a]	63.3 ± 1.2 [b]
VCL (µm/s)	126.4 ± 2.0 [c]	135.4 ± 2.0 [ab]	136.1 ± 2.2 [a]	129.9 ± 2.2 [bc]
VSL (µm/s)	51.0 ± 1.0 [b]	52.3 ± 1.0 [b]	56.0 ± 1.2 [a]	51.9 ± 1.1 [b]
STR (%)	81.4 ± 0.0 [a]	79.7 ± 0.0 [b]	81.8 ± 0.0 [a]	81.3 ± 0.0 [ab]
LIN (%)	39.7 ± 0.0 [ab]	38.1 ± 0.0 [b]	40.7 ± 0.0 [a]	39.6 ± 0.0 [ab]
WOB (VAP/VCL)	0.485 ± 0.0 [ab]	0.474 ± 0.0 [b]	0.494 ± 0.0 [a]	0.485 ± 0.0 [ab]
ALH (µm)	3.6 ± 0.1 [bc]	3.8 ± 0.1 [a]	3.6 ± 0.1 [b]	3.4 ± 0.1 [c]
BCF (H_z)	34.2 ± 0.5 [a]	34.0 ± 0.5 [a]	34.9 ± 0.5 [a]	34.2 ± 0.5 [a]

[a,b,c] Means having different superscripts within the same row differ significantly (P<0.05) DCL: Distance Curved Line (µm); DAP: Distance Average Path (µm); DSL: Distance Straight Line (µm); VCL: Velocity Curved Line (µm/s); VAP: Velocity Average Line (µm/s); VSL: Velocity Straight Line (µm/s); LIN: Linearity (VSL/VCL); STR: Straightness (VSL/VAP); WOB: Wobble (VAP/VCL); BCF: Beat Cross Frequency (H_z); ALH: Amplitude of Lateral Head Displacement (µm).

The cooling extender supplemented with 10^{-6}M melatonin led to higher percentages of total and progressive motility (78.8 and 67.8%, respectively) and higher values of DSL and VSL (25.5 µm and 56.0 µm/s, respectively), in comparison to the other melatonin groups or control (P<0.05). Moreover, the addition of 10^{-6} M melatonin significantly (P<0.05) increased the sperm DAP, DCL, VAP and VCL traits when compared to the control, while the other motion characteristics (STR, LIN, WOB, ALH and BCF) did not express any significant differences to the control (Table 1). These results are consistent with previous investigators who found higher percentages of motile spermatozoa in the extender supplemented with melatonin (Succu *et al.*, 2011; Ashrafi *et al.*, 2013). Motility may increase as a consequence of the protective effect of melatonin on sperm mitochondria after the freeze–thawing process (Paradies *et al.*, 2010).

On the other hand, increasing melatonin concentration at 10^{-3} M in semen extender did not have significant effect on almost motility parameters when compared to the control following the cooling process, except a marked decline in STR (79.7 *vs.* 81.4%) and a marked increase in DCL (62.6 *vs.* 58.4 µm), VCL (135.4 *vs.* 126.4 µm/s) & ALH (3.8 *vs.* 3.6 µm); in 10^{-3} M group *vs.* control group, respectively (P<0.05; Table 1). These results suggest that there may be a negative effect of high concentrations of melatonin on sperm quality (Succu *et al.*, 2011) since the high lateral head displacement (ALH) is undesired movement and may interfere with cell cycle progression (Arruda *et al.*, 2003). Furthermore, extreme doses of antioxidants in the freezing medium can counteract the ROS-induced oxidative stress and so impede the ROS-associated functions of spermatozoa (Roca *et al.*, 2004). In this study, the VCL of sperm in control group recorded the lowest value after cooling; it may be due to cryoinjuries to the mitochondrial apparatus (Jones and Stewart, 1979) and axoneme (Courtens *et al.*, 1989) of spermatozoa.

CONCLUSIONS

The present study demonstrates that adding melatonin at 10^{-6} M to rabbit semen extenders enhances sperm motility characteristics after cooling. This effect strongly suggests an antioxidant protection of melatonin against injuries after cooling process. Higher concentration at 10^{-3} M melatonin decreased the straightness of spermatozoa and increased the undesired parameters like lateral head displacement. In the future, sperm preservation/cryoprotective studies are warranted to confirm the present findings.

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