MELATONIN AS AN ADDITIVE TO SHIELD THE HAZARDOUS EFFECTS OF CRYOPRESERVATION ON KANKREJ BULL SEMEN

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ABSTRACT

Melatonin was supplemented at 1, 2 and 3 mM in Tris-Fructose Egg Yolk Citrate Glycerol (TFYG) extender to assess the impact on various seminal and acrosomal characteristics of 24 ejaculates collected from Kankrej bulls (n=3). In brief, melatonin supplementation at 2 mM increased ($p<0.05$) percent sperm motility, sperm viability, HOST reactive spermatozoa, live sperm with intact acrosome and acrosomal integrity. In addition, 2 mM melatonin supplementation reduced ($p<0.05$) percent dead spermatozoa, dead sperm with damaged acrosome, dead sperm with intact acrosome and abnormal spermatozoa at post-dilution, post-equilibration and post-thaw stages of cryopreservation.

Keywords: Cryopreservation, Kankrej bull, Melatonin, Semen, Seminal characteristics

INTRODUCTION

During semen cryopreservation, a lot of damage occurs to sperm characteristics in which the production of reactive oxygen species also plays a role (Agarwal and Said, 2005). The supplementation of antioxidants in the semen extender could decrease the impact of oxidative stress and therefore improve sperm quality following freeze–thawing process (Berra and Rizzo, 2009). Melatonin (N-acetyl-5-methoxy tryptamine) is an indole derivative endogenous compound that is secreted rhythmically by the pineal gland and plays a major role in regulating the circadian clock and seasonal reproduction in mammals. Also, melatonin as well as its metabolites has indirect antioxidant action with powerful direct scavenging ability of free radicals (Pieri *et al.,* 1994). The beneficial impact of melatonin during cryopreservation was observed in Holstein bulls (Ashrafi *et al.,* 2013), Egyptian buffalo bulls (El-Raey *et al.,* 2014) and in human (Karimfar *et al.,* 2015). It was indicated that the protective effect of melatonin on cryopreservation injuries occurs in a dose-dependent manner (Fujinoki, 2008). Thus, the present study for Kankrej bull semen was planned to improve seminal and acrosomal characteristics during cryopreservation after the addition of melatonin in semen extender.

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MATERIALS AND METHODS

Twenty-four semen ejaculates over an eight-week period were collected from Kankrej bulls (n=3, age 6-7 year) maintained under optimum healthy conditions. Semen ejaculate with >70% initial progressive motility was used in the present study. These ejaculates were divided into four equal aliquots, with 80 million sperms/ml each, and received either 1, 2, 3 or 0 mM (control) melatonin as an additive in Tris-Fructose Egg Yolk Citrate Glycerol (TFYG) extender. The extended semen aliquots were filled, sealed and printed in French Mini Straw of 0.25 ml capacity using automatic machine (IS-4, IMV-France) and were stored in liquid nitrogen at -196^{0}C. After 24 h cryopreservation period, straws were thawed at 37^{0}C for 30 seconds in a water bath for evaluation. The semen evaluation was performed at post-dilution, post-equilibration and post-thaw stages to assess the individual motility, sperm viability, sperm abnormality, Hypo osmotic swelling (HOST; Revel and Mrode, 1994) and acrosomal integrity (Kutty *et al.,* 1996). The data obtained for various parameters was analyzed using one-way

ANOVA followed by the Duncan *post hoc* test for all the stages of cryopreservation.

RESULTS AND DISCUSSION

The individual motility, sperm viability, HOST-reactive sperm, live sperm with intact acrosome and acrosomal integrity was higher (P<0.05) and sperm abnormalities, dead sperm with intact acrosome and llve sperm with damaged acrosome were lower (P<0.05) in 2 mM melatonin group as compared to other groups at all the stages of cryopreservation (Table 1 and 2). Cryopreservation reduces fertilizing ability of spermatozoa by causing damage to acrosome membrane and spermatozoal mitochondria (Pena *et al.*, 2009). However, the addition of melatonin tends to maintain sperm motility by stabilizing the integrity of acrosome and spermatozoa plasmalemma (Perumal *et al.,* 2015). In fact, melatonin in sperm cells is able to scavenge many ROS directly for protecting mammalian cells against oxidative stress (lipid peroxidation), and hence maintain sperm motility (Karimfar *et al.,* 2015). Moreover, the additive might have displayed cryoprotective effect on the functional integrity of mitochondria that is responsible for the generation of energy from intracellular stores of ATP, and hence improved post-thaw sperm motility (Reddy *et al.,* 2010). The mechanism implicated in increasing the HOST reactive sperm in the present study may include melatonin-induced maintenance of plasma and mitochondrial membrane integrity and cytoskeleton structure of flagella of sperm by stimulating the activities of antioxidant enzymes (Perumal *et al.,* 2015). Similarly, a lower acrosomal abnormality using 2 mM melatonin at post-thaw stage was recorded in Holstein bulls (Ashrafi *et al.,* 2013). Moreover, an improvement in post-thaw acrosomal integrity was recorded using a lower concentration of Melatonin in Egyptian buffalo bulls (El-Raey *et al.,* 2014). In the present study, the observed difference (p<0.05 - >0.05, Table 1 and 2) in various seminal and acrosomal parameters using

Table 1: The impact of melatonin (MT) **as an additive in semen extender on seminal characteristics** (%, Mean±SE) **of Kankrej bull semen**

	MT	Post-Dilution	Post-Equilibration	Post-Thaw
Individual motility	1 mM	79.38±0.50^{p}	70.17±0.42^{p}	54.58±0.31^{p}
	2 mM	82.33±0.47^{q}	74.42±0.50^{q}	58.08±0.47^{q}
	3 mM	78.00±0.44^{r}	69.00±0.38^{r}	53.13±0.29^{r}
	Control	76.83±0.40^{s}	67.67±0.36^{s}	52.33±0.33^{r}
Sperm viability	1 mM	82.00±0.41^{p}	74.08±0.35^{p}	62.13±0.26^{p}
	2 mM	85.17±0.41^{q}	78.42±0.44^{q}	65.67±0.31^{q}
	3 mM	80.96±0.37^{r}	72.54±0.36^{r}	60.38±0.30^{r}
	Control	79.83±0.38^{s}	71.29±0.33^{s}	59.0±0.31^{s}
Sperm abnormality	1 mM	5.04±0.25^{p}	6.71±0.19^{p}	8.33±0.10^{p}
	2 mM	3.71±0.22^{q}	5.29±0.14^{q}	6.92±0.10^{q}
	3 mM	5.50±0.22^{p}	7.54±0.20^{r}	8.88±0.20^{p}
	Control	6.54±0.19^{r}	8.54±0.18^{s}	10.21±0.17^{r}
HOST-reactive sperm	1 mM	78.83±0.47^{p}	70.88±0.40^{p}	58.42±0.59^{p}
	2 mM	82.46±0.42^{q}	74.58±0.97^{q}	62.46±0.59^{q}
	3 mM	77.13±0.49^{r}	69.29±0.33^{r}	56.50±0.37^{r}
	Control	75.83±0.50^{r}	67.79±0.29^{s}	55.04±0.34^{s}

Mean Means with different (p, q, r, s) superscripts for each parameter within column differ at P<0.05

Table 2: The impact of melatonin (MT) **as an additive in semen extender on acrosomal characteristics** (%, Mean±SE) **of Kankrej bull semen**

	MT	Post-Dilution	Post-Equilibration	Post-Thaw
Live sperm with intact acrosome	1 mM	81.67 ± 0.38^{p}	73.67 ± 0.35^{p}	61.50 ± 0.30^{p}
	2 mM	85.00 ± 0.38^{q}	77.79 ± 0.52^{q}	65.29 ± 0.31^{q}
	3 mM	80.83 ± 0.39^{p}	72.75 ± 0.48^{p}	59.88 ± 0.30^{r}
	Control	79.42 ± 0.39^{r}	70.67 ± 0.29^{r}	58.67 ± 0.54^{s}
Dead sperm with intact acrosome	1 mM	6.46 ± 0.25^{p}	9.88 ± 0.40^{pr}	13.79 ± 0.50^{pr}
	2 mM	5.04 ± 0.24^{q}	8.29 ± 0.32^{q}	12.13 ± 0.46^{q}
	3 mM	6.67 ± 0.29^{p}	10.42 ± 0.43^{r}	14.21 ± 0.49^{r}
	Control	7.46 ± 0.26^{p}	11.38 ± 0.40^{sr}	15.21 ± 0.51^{sr}
Live sperm with damaged acrosome	1 mM	0.33 ± 0.10^{p}	0.45 ± 0.10^{p}	0.63 ± 0.10^{p}
	2 mM	0.17 ± 0.08^{p}	0.28 ± 0.09^{p}	0.42 ± 0.10^{p}
	3 mM	0.37 ± 0.08^{p}	0.50 ± 0.10^{p}	0.67 ± 0.10^{p}
	Control	0.41 ± 0.10^{p}	0.67 ± 0.10^{p}	0.71 ± 0.10^{p}
Dead sperm with damaged acrosome	1 mM	11.54 ± 0.29^{pr}	16.00 ± 0.30^{p}	24.08 ± 0.44^{p}
	2 mM	9.81 ± 0.24^{q}	13.64 ± 0.36^{q}	22.16 ± 0.45^{q}
	3 mM	12.13 ± 0.34^{r}	16.33 ± 0.37^{p}	25.24 ± 0.48^{r}
	Control	12.71 ± 0.22^{sr}	17.37 ± 0.32^{s}	25.41 ± 0.45^{r}
Acrosomal integrity	1 mM	88.13 ± 0.27^{pr}	83.54 ± 0.24^{p}	75.29 ± 0.46^{p}
	2 mM	90.04 ± 0.22^{q}	86.08 ± 0.38^{q}	77.42 ± 0.45^{q}
	3 mM	87.50 ± 0.24^{r}	83.17 ± 0.38^{p}	74.08 ± 0.48^{r}
	Control	86.88 ± 0.24^{sr}	82.04 ± 0.34^{r}	73.88 ± 0.50^{r}

Means with different (p, q, r, s) superscripts for each parameter within column differ at P<0.05

various dosage of melatonin supplementation seems to be due to its potent antioxidant activity. Conclusively, 2 mM melatonin was most effective in shielding seminal and acrosomal characteristics of Kankrej bull by keeping a check on hazardous effects encountered during cryopreservation.

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