



SHORT COMMUNICATION

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Effect of melatonin on in vitro maturation of bovine oocyteR. Beheshti^{1*}*Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran***Key words:** Bovine, melatonin, IVM, oocyte.<http://dx.doi.org/10.12692/ijb/4.10.31-35>

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Abstract

This study was aimed at determining the effect of melatonin supplement during in vitro maturation of bovine oocytes. Ovaries were obtained from local abattoir shortly after slaughter and transported to the laboratory in phosphate buffer solution plus 100 IU/ml potassium penicillin G and 100 µg/ml streptomycin sulfate at 35 °C, within 2 hours from slaughter. Cumulus-oocyte complexes (COCs) from abattoir ovaries were collected in were matured in vitro in HEPES- TCM199 supplemented o (control), 50 or 100 µmol/ml of melatonin for 24 hours. When COCs matured in TCM 199 media with 50 µmol/ml melatonin, the rate of maturation were increased none significantly as compared with control group ($P>0.05$). The expansion rate of cumulus oophorus in TCM199 with 50 and 100 µmol/ml melatonin were not increased significantly. Also, the rates of degenerated oocytes in treatment groups lower than control group that it is not significant. In conclusion, melatonin can not increased the bovine oocyte maturation in the TCM199 medium.

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Introduction

Various factors in the medium Such as nutritional and hormonal balance as well as various stresses, such as oxidative stress, heat etc. can influence the *in vitro* maturation of oocytes (Biabani *et al.*, 2012). Oocytes cultured under in-vitro condition affected by various factors such as light, oxygen, high concentrations of metabolites that this causes increased production of free radicals and oxidative stress are increased as a result (Biabani *et al.*, 2012).

There is evidence that the ROS in in-vitro oocyte maturation affect IVP of bovine embryos (Geshi *et al.*, 2000). Free radicals cause damage to RNA and DNA, proteins, carbohydrates, lipids and cause the failure of mitochondria. Decreasing ROS molecules is necessary for oocyte maturation *in vitro* (Morado *et al.*, 2009).

One of the methods to reduce oxidative stress on *in vitro* maturation of oocytes is use of the antioxidants. Melatonin (N-acetyl-5-methoxytryptamine) acts as an antioxidant in living organisms. Melatonin has many physiological functions include its reproductive regulation in seasonal breeders, free radical scavenging and antioxidant capacity, oncostatic actions, neuroimmunological, anti-inflammation and strengthening of circadian rhythms (Srinivasan *et al.*, 2005; Tan *et al.*, 2007). So far, a very important function of melatonin is free radical scavenging, activity (Romero *et al.*, 2010). This hormone directly destroys free radicals and indirectly by stimulating the antioxidant enzymes and inhibition of peroxidation enzymes such as nitric oxide synthetase, acts as antioxidants (Galano *et al.*, 2011). In in-vitro conditions, melatonin in elimination of the hydroxyl free radicals was very effective and in removing peroxy is stronger than vitamin E (Reiter *et al.*, 2004). Melatonin reduces hydroperoxides levels of mitochondria and improves mitochondrial function during oxidative stress. This hormone stimulates gamma-glutamyl-cysteine-synthetase enzyme and then intracellular glutathione increases (Escames *et al.*, 2006). Therefore, addition of melatonin to the culture medium may increase the COCs expansion

and *in vitro* maturation of bovine oocyte. The present work investigated whether melatonin added to culture medium affects the in-vitro maturation of bovine oocytes.

Material and methods

Materials

Chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA).

Oocyte collection

Ovaries were collected at local abattoir (Tabriz abattoir, East Azerbaijan, Iran), shortly after slaughter and transported to the laboratory in 0.9% NaCl solution plus 100 IU/ml potassium penicillin G and 100 µg/ml streptomycin sulfate at 35 °C, within 2 to 4 hours from slaughter (Ball, 1983). Immature bovine cumulus oocyte complex's (CoCs) were aspirated from small antral follicles (2-8 mm) using a 18-gauge needle connected to a 10 ml sterile syringe that contain 1 ml oocyte collection medium (HEPES-TCM 199 (M7528) supplemented with 10% FBS (F6178), and 2 IU/ml of heparin), and the contents recovered into a 15 ml conical tube and allowed to settle for 10 minute. CoCs were washed three times with maturation medium.

In vitro Oocytes Maturation

The basic medium for IVM was a Hepes-buffered tissue culture medium199 supplemented with 0.2 mM sodium pyruvate, 1 µg/ml 17-β-estradiol, and 10% fetal calf serum (FCS) and 0.5 µg/ml FSH. In treatment groups melatonin was supplemented at two levels (50, 100 µM). The oocytes were cultured in 50 µL droplets (10 oocytes per droplet) of the culture medium under mineral oil at 38.5°C under an atmosphere of 5% CO₂ in air with maximum humidity.

Statistical analysis

Results are expressed as the mean. The proportion of MII oocytes for each treatment was analyzed by chi-squared analysis. A significance level of P<0.05 was used throughout this study.

Results

Cumulus-oocyte complex expansion

After 24 h culture of Cumulus-oocyte complexes in TCM 199 containing 0, 50 and 100 μ M melatonin, there was no significant difference in cumulus

expansion between experimental treatments and control and also among treatments. The degree of cumulus expansion did not changed significantly in all groups (Table 1).

Table 1. Effect of melatonin addition during *in vitro* maturation on COCs expansion degree.

Treatments	Number of COCs	COCs expansion
Control	45	11.33 (75.53%) a
Melatonin	50 μ M	12.66 (84.40%) a
	100 μ M	10.33 (68.86%) a
P value		0.687

^a Within the same column, values with different letters were significantly different ($P < 0.05$).

Bovine oocyte *in vitro* maturation

After assessment of cumulus cells expansion, the cumulus cells were removed and matured oocytes in

control and other groups were clearly visible with presence of first polar body in previtellin space (metaphase-II stage oocytes) (Table 2).

Table 2. Effect of melatonin addition during *in vitro* maturation of bovine oocytes.

Treatments	Oocytes used	Unmatured Oocyte (%)	Matured oocyte (%)	Degenerated Oocyte (%)
Control	45	3.67 (24.46%) ^a	10.00 (66.67%) ^a	1.33 (8.87%) ^a
Melatonin	50 μ M	2.67 (17.80%) ^a	11.33 (75.53%) ^a	1.00 (6.67%) ^a
	100 μ M	1.67 (11.13%) ^a	9.66 (64.40%) ^a	3.67 (24.46%) ^a
P value		0.408	0.508	0.687

^a Within the same column, values with different letters were significantly different ($P < 0.05$).

Discussion

Previous studies of mammalian oocytes IVM has been reported in over 70 years that Pincus and his associates observed, some of oocytes from human and rabbit spontaneously resumed meiosis when released from follicles and cultured in a medium (Beheshti *et al.*, 2011). In this study, the addition of melatonin to the TCM 199 was not improvement in maturation of bovine oocytes. The addition of melatonin increased the COCs expansion and MII oocytes in group with 50 μ M, when compared to the control.

Melatonin has the high lipophilicity and hydrophilicity and therefore it can easily pass through cell membranes. Melatonin and its metabolites such as cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK) are ROS scavengers and

melatonin can increase the production of endogenous antioxidants (Tamura *et al.*, 2012). Wei *et al.*, (2013) observed that addition of melatonin to the culture medium on in-vitro maturation of immature human oocytes, lead to enhancing *in vitro* oocyte maturation, significantly. The researchers suggested that adding melatonin to the culture medium could ameliorate nuclear maturation of human oocyte.

In another study, Tian *et al.*, (2010) assessed the effects of different concentrations of melatonin (3 to 13 moles) on the growth of *in vitro* cultured mouse embryos. In their study, addition of 5 to 13 mol melatonin caused to increasing blastocyst cell number. Farahvar and colleagues (2010) was added various concentrations of melatonin, 0, 0.01, 1 and 100 μ M to the culture medium of bovine oocytes. They found that more than 80% of oocytes and

cumulus cells complexes were fully expanded but the maturation rate significantly decrease to 65.24% when melatonin concentrations were increased to 100 μ M. Consequently melatonin cannot enhance cumulus cells expansion and it can't be used as tools to enhance the efficiency of bovine oocytes *in vitro* maturation.

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