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STUDY OF THE EFFECT OF MELATONIN TO *IN VITRO* OOCYTE MATURATION IN LOCAL IRAQI EWE

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Abstract

Antioxidants and nutrients in the oocyte maturation media have a deep effect on maturation, fertilization and embryo development. In the present study investigate effects to variable dose of melatonin as an antioxidant agent on maturation ability were evaluated in immature oocytes of local Iraqi ewe. Ovaries were collected from local ewe after slaughtered at abattoirs of AL-shulla abattoir/ West of Baghdad. Oocytes were harvested from collected ovaries in Veterinary College Laboratory/Baghdad university after two hours by using slicing techniques. The cumulus oocyte complexes (COCs) was separated under inverted microscope to detect healthy oocyte (oocytes have many cumulus layers add to uniform cytoplasm), healthy oocytes existed randomly divided three groups, group I oocytes culture in maturation media (control group), groups II and group III oocytes culture in maturation media supplement variable doses of melatonin (10^{-5}, 10^{-6}) M respectively than culture 24 hours in a 5 percent CO_2 incubator in 39°C and 95 percent humidity. Oocyte maturation rate was identified using an inverted microscope by found the first polar body in the previtelline space. The result melatonin in concentration 10^{-6} M was maximum effect on *in vitro* maturation rate when improve to maturation media in local Iraqi ewe ($P < 0.05$) than control group and group II. This study concluded melatonin acting effective title role in achieving the maximum maturity rate in local Iraqi ewe, at a concentration of 10^{-6} M supplement to maturation media.

Key words: *in vitro* maturation, melatonin, ewe oocytes.

Introduction

In vitro embryos production (IVP) one of the important significant features in science modern. IVP made up two primary subdivisions: *In vitro* maturation (IVM) plus *in vitro* fertilization (IVF) (Davach *et al*., 2011). *In vitro* maturation is the main and greatest important stage towards successful *in vitro* embryo production by the effects to oocyte efficiency, growth embryo, development of fetal and also off spring safety (Abd El-Aziz *et al*., 2016). The property of the oocytes generated *in vitro* remains low compered oocytes matured *in vivo* (Anckaert *et al*., 2004). Lower quality of matured *in vitro* oocytes was related to *in vitro* environment insufficiency (Rizos *et al*., 2002). The important factor influencing the IVM procedure in mammals the culture medium for used to oocyte maturation, also IVM medium structure influences oocyte and embryo developmental capability (Nabiuni *et al*., 2013). Reactive oxygen species (ROS) released through *in vitro* oocyte maturation can cause severe oxidative damage, apoptosis and stop the embryo development. Naturally under *in vivo* conditions, follicular fluid and oviductal fluid have some free radical antioxidants that can protect oocytes from oxidative stress (Wang *et al*., 2002). However, under conditions of *in vitro* culture this antioxidant state is inferior than under *in vivo* (Nagina *et al*., 2016). Free radicals which have weakening effect to DNA repair, spindle mitotic assembly and maturation oocyte, were the most important hurtful factors affecting oocyte maturation and embryo development (Agarwal *et al*., 2006). The free radicals also effect injury to DNA, RNA, protein, lipids, carbohydrate also cause mitochondrial breakdown so reducing the ROS particles essential for maturation oocyte *in vitro* (Morado *et al*., 2009).

Melatonin (N-acetyl-5-methoxytryptamine) is indole generated in pineal gland beginning tryptophan in farm animals besides other organs such as ovaries (Reiter *et al*., 2009; Sakaguchi *et al*., 2013). Melatonin key role to modulate circadian and circumnial rhythms in photoperiod animals (Lin *et al*., 2017). Also Melatonin is a free radical scavenger, antioxidant and antiapoptotic component for

embryos development (Chen *et al*., 2006). Free radical scavenging function even applies to metabolites, regulate antioxidant enzymes and regulate the prooxidant and proinflammatory enzymes that make melatonin extremely with effect in defensive product from stress oxidative in low concentrations to protective organism from stress oxidative (Galano *et al*., 2011). It is considered a universal antioxidant because its amphiphilic nature enables it to move through the cellular membrane, enabling it to scavenge free radicals directly, bind to different DNA sequences to control antioxidant genes and interrelate with cytosolic molecules (Korkmaz *et al*., 2012). In addition, its presence in fluid follicular with a in height concentration compered in the blood and an increase with the growth of follicles, so suggests play major role in ovarian function (Tamura *et al*., 2016). In present study we assess the optimal concentration melatonin to support *in vitro* maturation of oocytes in local Iraqi ewes.

Material and Method

Local ewes ovaries were collected in thermal flasks containing phosphate-buffered solution (PBS) in 37°C and 100 IU Penicillin to ml and streptomycin 100 mg to ml immediately after slaughter on the AL-shulla abattoir / west of Baghdad send to the Veterinary College Laboratory / University of Baghdad. In laboratory ovaries washed in phosphate-buffered solution with antibiotics three times. Oocytes were collected using the slicing technique described by Wang *et al*., (2007) when the ovaries sliced to small parts in surgical scalpel in a graded plastic Petri dish containing a collection medium (TCM199 with an antibiotic). The cumulus oocyte complexes (COCs) were isolated under inverted microscope and transferred to 35 mm Petri dish containing oocyte collection medium and wash three times to isolated oocytes from debris, only oocytes with multiple coats of cumulus cell and constant cytoplasm carefully chosen to *in vitro* maturation (AL-Maeeni *et al*., 2012). These oocytes were randomly separated to the three groups, the first group Petri dish contained oocyte with 1ml maturation medium (TCM-199 supplement with 10 percent FCS, 10 IU / mL FSH, 10 IU / mL LH, 100 IU / ml penicillin and 100 mg / ml streptomycin) alone (control group). Other group II and group III Petri dish oocyte culture in maturation media supplemented with melatonin at two concentrations (10^{-5}, 10^{-6})M respectively, in three investigational groups coated by mineral oil. All oocyte in maturation media in the three dishes were put in incubator instrument with 5 percent CO_2 for 24 hours in 39°C and about 95% humidity. The oocytes maturation was assessed by finding the one polar body to be extruded in the prevetlin space after maturation of the oocytes when gently denuded by micropipette.

Melatonin was liquefied in dimethyl sulfoxide (DMSO) and PBS to create 10^{-5} or 10^{-6} M concentrations that were administered to the group II and group III oocytes, respectively. DMSO's final media concentration was 0.01% v/v, with no effect on oocyte maturation, as Ishizuka *et al*., (2000).

Statistical Analysis

The Statistical Analysis System- SAS, (2012) program was used to perceive the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 probability) in this study.

Result and Discussion

Oocyte maturation consists of important events which require completion of the oocytes to profitable fertilization and prompt fetal development. Suitable maturity is the base to attaching, introduction of gestation and progress of fetuses (Sirard *et al*., 2006).

In present study three different groups of oocytes harvest culture from ovaries by slicing method, group I (control group) oocytes culture with maturation media alone, group II oocytes culture in maturation media supplement with 10^{-5} M Melatonin and group III oocyte culture in maturation media supplement with 10^{-6} M Melatonin. After incubator 24 hours with 5 percent CO_2, in 39°C and 95 percent humidity, the oocyte maturation is observed in an inverted microscope by extruding the one polar body. We show high significant effect ($P < 0.05$) in maturation rate (70.34%) in group III (when concentration 10^{-6} than group II (concentration of melatonin 10^{-5}) and control group (64.13%, 61.07%) respectively. Also the maturation rate in group II high significant ($P < 0.05$) than control group (Table 1). This result agreement with Casao *et al.,* (2010)

Table 1: Effect addition of melatonin at different concentration on of Oocytes maturation media in local Iraqi ewe.

Groups	Treatment	No. of Oocyte culture	No. of Oocytes mature	Maturation rate
Group I	TCM alon	167	102	61.07%
Group II	TCM +10^{-5} Melatonin	184	118	64.13%
Group III	TCM +10^{-6} Melatonin	172	121	70.34%
Chi-Square (χ^2)		-	-	4.371 *
* ($P \leq 0.05$).				

when evaluating the impact of melatonin on oocyte maturation, fertilization and *in vitro* culture of embryo sheep, melatonin concentrations 10^{-6} M appeared to boost the rate of cleavage following *in vitro* fertilization. This explains role melatonin provides protection against the oxidative stress. Increased oxidative stress cause injury the mitochondria and subsequently will impair ATP production and impede meiotic and mitotic spindles formation in growing oocyte (Farahavar and Shahne, 2010). Since of the amphiphilic nature of melatonin it widely diffuses barriers in different cellular partitions. Also it high active antioxidant and antiapoptotic vehicle to prevents oxidative stress injury to entirely macromolecules in cell parts by express scavenging of poisonous oxygen products and its capability to diminish ROS (Ramis *et al*., 2015). Others agree with Tian *et al*., (2017) when exploring a beneficial effect of melatonin, its function in regulating oocyte maturation in sheep is mainly intermediated by ML1 receptors when it has found a higher presence of ML1 in the germ vesicle in the oocyte cumulus. Other researchers Goodarzi *et al*., (2018) practice antioxidant of melatonin to storage ovary medium to benefit influences on the maturation of oocytes in sheep and the production of embryos. Ishizuka *et al*., (2000) also found that the optimum melatonin concentration in the IVM medium was 10^{-6} M and that very small and very high doses had adverse effects. Thus as reactive oxygen species in a precise concentration was vital for oocyte maturation, very high and very low doses could be harmful for oocytes during IVM, therefore the concentration of antioxidants is crucial. So shows that increasing concentrations of melatonin above 250 μM have no major impact on buffalo oocyte maturation levels. Higher-concentration melatonin can be toxic, resulting to cells damage and inferior blastocysts levels through toxicity (Nagina *et al*., 2016). Conclusion beneficial effect of melatonin in local Iraqi ewe oocyte maturation by add to tissue culture media to improves the maturation rate, also we show the concentration 10^{-6} more effect to improve maturation rate.

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Reference

Abd El-Aziz, A.H., U.E. Mahrous, S.Z. Kamel and A.A. Sabek (2016). Factors influencing *in vitro* production of bovine embryos: a review. *Asian Journal of Animal and Veterinary Advances*, **11(12):** 737-756.

Agarwal, A., T.M. Said, M.A. Bedaiwy, J. Banerjee and J.G. Alvarez (2006). Oxidative stress in an assisted reproductive techniques setting. *Fertility and sterility*, **86(3):** 503-512.

AL-Maeeni, I.M. (2012). Study the effect of addition alcoholic extract of Tribulus terrestris on bovine oocyte maturation *in vitro* (IVM). *The Iraqi Journal of Veterinary Medicine*, **36(2):** 199-203.

Anckaert, E., T. Adriaenssens, S. Romero, S. Dremier and J. Smitz (2004). Unaltered imprinting establishment of key imprinted genes in mouse oocytes after *in vitro* follicle culture under variable follicle-stimulating hormone exposure. *International Journal of Developmental Biology*, **53(4):** 541-548.

Casao, A., J.A. Abecia, J.C. Pérez, T.M. Blanco, M.I. Vázquez and F. Forcada (2010). The effects of melatonin on *in vitro* oocyte competence and embryo development in sheep. *Spanish Journal of Agricultural Research*, **8(1):** 35-41.

Chen, H.Y., T.Y. Chen, M.Y. Lee, S.T. Chen, Y.S. Hsu, Y.L. Kuo and E.J. Lee (2006). Melatonin decreases neurovascular oxidative/nitrosative damage and protects against early increases in the blood–brain barrier permeability after transient focal cerebral ischemia in mice. *Journal of pineal research*, **41(2):** 175-182.

Davachi, N.D., H. Kohram and S. Zeinoaldini (2011). Effect of the presence of corpus luteum on the ovary and the new oocyte recovery method on the oocyte recovery rate and meiotic competence of ovine oocytes. *African Journal of Biotechnology*, **10(47):** 9706-9709.

Farahavar, A. and A.Z. Shahne (2010). Effect of melatonin on *in vitro* maturation of bovine oocytes. *African Journal of Biotechnology*, **9(17):** 2579-2583.

Galano, A., D.X. Tan and R.J. Reiter (2011). Melatonin as a natural ally against oxidative stress: a physicochemical examination. *Journal of pineal research*, **51(1):** 1-16.

Goodarzi, A., A.Z. Shahneh, H. Kohram, M. Sadeghi, M.H. Moazenizadeh, A. Fouladi-Nashta and N.D. Davachi (2018). Effect of melatonin supplementation in the long-term preservation of the sheep ovaries at different temperatures and subsequent *in vitro* embryo production. *Theriogenology*, **106:** 265-270.

Ishizuka, B., Y. Kuribayashi, K. Murai, A. Amemiya and M.T. Itoh (2000). The effect of melatonin on *in vitro* fertilization and embryo development in mice. *Journal of pineal research*, **28(1):** 48-51.

Korkmaz, A., S. Rosales-Corral and R.J. Reiter (2012). Gene regulation by melatonin linked to epigenetic phenomena. *Gene*, **503(1):** 1-11.

Lin, T., J.E. Lee, J.W. Kang, S.Y. Kim and D.I. Jin (2017). The influence and role of melatonin on *in vitro* oocyte maturation and embryonic development in pig and cattle. *Korean Journal of Agricultural Science*, **44(3):** 309-317.

Morado, S.A., P.D. Cetica, M.T. Beconi and G.C. Dalvit (2009). Reactive oxygen species in bovine oocyte maturation *in vitro*. *Reproduction, Fertility and Development*, **21(4):** 608-614.

Nabiuni, M., R. Ganji and M. Bahadori (2013). Melatonin Impact on *In vitro* Development of Mouse Preantral Follicles and Oocyte Maturation. *Anatomical Sciences Journal*, **10(1):** 7-14.

Nagina, G., A. Asima, U. Nemat and A. Shamim (2016). Effect of melatonin on maturation capacity and fertilization of Nili-Ravi buffalo (Bubalus bubalis) oocytes. *Open veterinary journal*, **6(2):** 128-134.

Ramis, M., S. Esteban, A. Miralles, D.X. Tan and R. J Reiter (2015). Protective effects of melatonin and mitochondria-targeted antioxidants against oxidative stress: A review. *Current medicinal chemistry*, **22(22):** 2690-2711.

Reiter, R.J., D.X. Tan, L.C. Manchester, S.D. Paredes, J.C. Mayo and R.M. Sainz (2009). Melatonin and reproduction revisited. *Biology of reproduction*, **81(3):** 445-456.

Rizos, D., F. Ward, P.A.T. Duffy, M.P. Boland and P. Lonergan (2002). Consequences of bovine oocyte maturation, fertilization or early embryo development *in vitro* versus *in vivo*: implications for blastocyst yield and blastocyst quality. *Molecular reproduction and development*, **61(2):** 234-248.

Sakaguchi, K., M.T. Itoh, N. Takahashi, W. Tarumi and B. Ishizuka (2013). The rat oocyte synthesises melatonin. *Reproduction, Fertility and Development*, **25(4):** 674-682.

Sirard, M.A., F. Richard, P. Blondin and C. Robert (2006). Contribution of the oocyte to embryo quality. *Theriogenology*, **65(1):** 126-136.

SAS (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

Tamura, H., A. Takasaki, T. Taketani, M. Tanabe, F. Kizuka, L. Lee and N. Sugino (2012). The role of melatonin as an antioxidant in the follicle. *Journal of ovarian research*, **5(1):** 5.

Tian, X., F. Wang, L. Zhang, C. He, P. Ji, J. Wang and Z. Lian (2017). Beneficial effects of melatonin on the *in vitro* maturation of sheep oocytes and its relation to melatonin receptors. *International journal of molecular sciences*, **18(4):** 834.

Wang, X., T. Falcone, M. Attaran, J.M. Goldberg, A. Agarwal and R.K. Sharma (2002). Vitamin C and Vitamin E supplementation reduce oxidative stress–induced embryo toxicity and improve the blastocyst development rate. *Fertility and sterility*, **78(6):** 1272-1277.

Wang, Z.G., Z.R. Xu and S.D. Yu (2007). Effects of oocyte collection techniques and maturation media on *in vitro* maturation and subsequent embryo development in Boer goat. *Czech Journal of Animal Science*, **52(1):** 21.