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REVIEW ARTICLE

The influence and role of melatonin on *in vitro* oocyte maturation and embryonic development in pig and cattle

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Abstract

Melatonin (N-acetyl-5-methoxytryptamine) is an indole synthesized from tryptophan by the pineal gland in animal. The major function of melatonin is to modulate circadian and circannual rhythms in photoperiodic mammals. Importantly, however, melatonin is also a free radical scavenger, anti-oxidant, and anti-apoptotic agent. Recently, the beneficial effects of melatonin on oocyte maturation and embryonic development *in vitro* have been reported in many species such as pig, cattle, sheep, mouse, and human. In this review, we will discuss recent studies about the role of melatonin in the production of porcine and bovine oocytes and embryos *in vitro* in order to provide useful information of melatonin in oocyte maturation and embryo culture *in vitro*.

Keywords: apoptosis, embryo development, ER stress, melatonin, oocyte maturation, ROS

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Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indole mainly produced in the pineal gland, which plays numerous important physiological functions in animal, such as circadian rhythm regulation, seasonal reproduction, and temperature regulation (Hoffman and Reiter, 1965; Arendt, 1998; Zarazaga et al., 2010; Barrett and Bolborea, 2012; Tamura et al., 2013). Melatonin also functions as a free radical scavenger, anti-oxidant, and anti-apoptotic agent. It can directly scavenge toxic oxygen derivatives (Cruz et al., 2014), reduce ROS formation (Zhao et al., 2016), and stimulate anti-oxidative enzymes (Li et al., 2015) including GSH (glutathione peroxidase) and SOD (superoxide dismutase) (Rodriguez et al., 2004).

The beneficial effects of melatonin on mammalian oocyte maturation and embryo development have been reported in several species. Melatonin has been successfully used to improve oocyte maturation and/or embryo developmental potential in mice (Tian et al., 2010), sheep (Abecia et al., 2002), buffalo (Manjunatha et al., 2009), cattle (Tian et al., 2014; Wang et al., 2014), pig

(Kang et al., 2009; Do et al., 2015), and rabbit (Mehaisen and Saeed 2015). In addition, porcine (Shi et al., 2009) or bovine (Tian et al., 2014) follicular fluid contains high levels of melatonin, suggesting that melatonin may influence oocyte maturation *in vivo*. In pig, supplementation of melatonin during *in vitro* maturation (IVM) and/or *in vitro* culture (IVC) media significantly increased the maturation rate (Kang et al., 2009), reduced reactive oxygen species (ROS) formation (Kang et al., 2009; Nakano et al., 2012), and improved embryonic development (Shi et al., 2009; Do et al., 2015). Melatonin could also protect porcine oocyte during IVM from heat stress by reducing ROS generation and inhibiting apoptosis (Li et al., 2015). In bovine species, melatonin supplementation in oocyte maturation medium significantly increased the expressions of oocyte maturation-related genes and cumulus cell expansion-associated genes (Tian et al., 2014). Melatonin not only enhanced blastocyst formation rates, but also improved the quality of embryos which was indexed by an elevated cryotolerance and the up-regulated expression levels of development-related genes (Wang et al., 2014). Supplementation of melatonin in maturation medium and vitrification solution could significantly inhibit apoptosis and ROS generation of vitrified bovine oocytes and result in improving their developmental capacity (Zhao et al., 2016).

The role and beneficial effects of melatonin on porcine and bovine oocyte maturation and/or embryo culture *in vitro* are summarized in this review.

The effect of melatonin on oocyte maturation and embryo development *in vitro*

Melatonin is known to exist in porcine (Shi et al., 2009) or bovine (Tian et al., 2014) follicular fluid at an approximate concentration of 10^{-11} M, suggesting that melatonin plays an important role in oocyte maturation. Melatonin may affect oocyte maturation through its membrane receptors (Cruz et al., 2014). In bovine oocytes, the expression of melatonin receptors MT1 and MT2 was found in cumulus cells, granulosa cells, and oocytes using RT-PCR, western blotting, and immunofluorescence analysis (Tian et al., 2014). In porcine oocytes, the expression of the MT1 receptor was identified in cumulus and granulosa cells, but not in oocpooytes (Kang et al., 2009). Recently, we obtained a result that could help distinguish good quality COCs from bad quality COCs in pig using RT-PCR analysis. MT1 transcripts derived from good quality porcine COCs (oocytes with more than 3 layers cumulus cells) produced weak signal transduction, whereas MT1 transcripts derived from poor quality COCs (oocytes with less than 3 layers cumulus cells) produced strong MT1 signal transduction (unpublished data). Thus, melatonin probably plays a key role in cumulus cells rather than in oocytes during oocyte maturation.

The supplementation of melatonin in oocyte maturation and/or embryo culture media could improve oocyte maturation rate and subsequently embryonic development. Details on melatonin application and evaluation of embryonic parameters are shown in Table 1. Melatonin could improve embryo development and implantation in rats (Dair et al., 2008). In bovine *in vitro* maturation systems, melatonin supplementation significantly increased the levels of maturation-related genes (*GDF9* and *MARF*), cumulus cell expansion-associated genes (*PTX3* and *HAS1/2*), and epigenetic modification gene (*DNMT1a*) (Tian et al., 2014). Supplementation of melatonin in the maturation medium could improve oocyte maturation rate and cumulus cell expansion in bovines (El-Raey et al., 2011). Aged immature bovine oocytes treated with exogenous melatonin showed improved mature oocyte quality, maintained maturation promoting factor (MPF) levels, and had enhanced subsequent embryonic developmental capacity (Liang et al., 2016). Rodrigues-Cunha et al. (2016) reported that melatonin supplementation during IVM in a defined medium could not improve nuclear maturation of bovine oocyte, probably because serum, follicular fluid, or other hormones in the

Table 1. Melatonin tested *in vitro* in pig and cattle.

Species	Exposure		Oocyte maturation		Embryo	Development		Embryo quality					References
	IVM	IVC	Cum	MII		Cle	Bla	TCN	Apo	ROS	MMP	DSBs	
Porcine	+				PA	∨	∨	∨					(Shi et al., 2009)
		+			PA	∨	∨	∨					(Shi et al., 2009)
	+	+			PA	∨	∨	∨					(Shi et al., 2009)
	+			∨	PA	∨	∨	∨		∨			(Kang et al., 2009)
	+			∨	PA	∨	∨	∨					(Nakano et al., 2012)
		+			PA	∨	∨	∨	∨	∨			(Nakano et al., 2012)
		+			SCNT	∨	∨	∨	∨	∨			(Nakano et al., 2012)
	+		∨	∨	PA	∨	∨	∨	∨	∨			(Li et al., 2015)
	+				IVF	∨	∨		∨				(Do et al., 2015)
		+			IVF	∨	∨		∨				(Do et al., 2015)
Bovine	+				IVF	∨	∨	∨					(Tian et al., 2014)
		+			IVF	∨	∨						(Wang et al., 2014)
	+			∨	PA	∨	∨		∨	∨			(Zhao et al., 2015)
	+				IVF	∨	∨	∨		∨	∨	∨	(Zhao et al., 2016)
	+			∨	IVF	∨	∨			∨			(Rodrigues et al., 2016)
	+				IVF		∨	∨	∨	∨	∨	∨	(Liang et al., 2016)
	+		∨	∨									(Nagina et al., 2016)

Abbreviations: +, Melatonin supplementation; ∨, Parameters investigated; IVM, *In vitro* maturation; IVC, *In vitro* culture; Cum, Cumulus cells expansion; MII, Metaphase II stage; Cle, Cleavage; Bla, Blastocyst; TCN, Total cell numbers in blastocyst; Apo, Apoptosis; ROS, Reactive oxygen species; MMP, Mitochondrial membrane potential; DSBs, DNA double-stranded breaks; PA, Parthenogenetic activation; IVF, In vitro fertilization; SCNT, Somatic cell nuclear transfer.

maturation medium could interfere with the melatonin function.

In pig, oocyte maturation medium with melatonin has beneficial effects on nuclear and cytoplasmic maturation of porcine oocytes and subsequent embryonic development (Kang et al., 2009; Shi et al., 2009). Melatonin could affect histone acetylation and autophagy in porcine oocytes resulting in improved porcine oocyte maturation and subsequent embryonic development rates (Chen et al., 2017). Do et al. (2015) reported that the positive influence of melatonin on embryo culture could differ depending on the period of supplementation. For instance, 1-cell stage embryo culture with melatonin could enhance blastocyst formation rate, but oocytes matured with melatonin during *in vitro* maturation could not improve subsequent embryo development (Do et al., 2015). Nakano et al. (2012) reported that even though melatonin supplementation during culture did not improve the developmental rate of the porcine somatic cell nuclear transfer (SCNT) embryos, melatonin treatment significantly reduced the ROS generation in SCNT embryos, suggesting that the beneficial effect of melatonin on embryo culture might be enhancing the quality of embryo conditions.

At low concentrations of melatonin may not improve embryonic development, quality and/or scavenge sufficient ROS, while at higher concentrations, melatonin may cause embryo damage and reduce embryo development (Cruz et al., 2014). The different *in vitro* culture conditions and/or potential difference in sensitivity to melatonin between porcine and bovine embryos, the concentrations used is various. The optimal concentrations of melatonin used *in vitro* in pig and cattle are shown in Table 2.

Table 2. The optimal concentrations of melatonin used *in vitro* in pig and cattle.

Species	Concentrations		References
	IVM	IVC	
Porcine	10^{-9} M	10^{-9} M	(Shi et al., 2009)
	10 ng/mL		(Kang et al., 2009)
		10^{-7} M	(Nakano et al., 2012)
	10^{-7} M		(Li et al., 2015)
	25 ng/mL	25 ng/mL	(Do et al., 2015)
Bovine	10^{-9} M to 10^{-7} M		(Tian et al., 2014)
		10^{-7} M	(Wang et al., 2014)
	10^{-9} M		(Zhao et al., 2015)
	10^{-9} M		(Zhao et al., 2016)
	10^{-6} M		(Rodrigues et al., 2016)
	1 μM		(Liang et al., 2016)
	250 μM		(Nagina et al., 2016)

Melatonin is a powerful anti-oxidant

The ROS produced during *in vitro* oocyte maturation can induce serious oxidative damage, apoptosis, and cease embryo development. Naturally, under *in vivo* conditions, the follicular fluid and oviductal fluid contain some free radical scavenging antioxidants and they can protect the oocytes against oxidative stress (Wang et al., 2002). However, this antioxidative environment becomes weaker under *in vitro* culture conditions than under *in vivo* (Nagina et al., 2016). Thus, oocytes or embryos are exposed to serious oxidative stress under *in vitro* culture conditions. The most effective way to overcome this problem is to supplement the culture media with antioxidant agents. Compared to other free radical scavengers, melatonin is universal due to its solubility in water and lipids (Hardeland, 2005; Do et al., 2015).

It has been reported that melatonin directly protects mammalian oocytes from oxidative stress. In pigs, melatonin supplementation during *in vitro* culture significantly reduced ROS generation (Kang et al., 2009; El-Raey et al., 2011; Zhao et al., 2015) and increased GSH generation (Li et al., 2015). The presence of melatonin during porcine oocyte maturation *in vitro* improved oocyte maturation rate and subsequent embryo development by reducing ROS and apoptosis levels (Kang et al., 2009; Li et al., 2015). Also, addition of melatonin to embryo culture medium improved the embryonic development of parthenogenetic embryos, and significantly reduced the ROS levels in 4-cell embryos (Nakano et al., 2012). In bovine species, melatonin supplementation in IVM medium protects cumulus cells from nuclear fragmentation, increases the expression levels of antioxidant enzymes, and decreases ROS formation in oocytes (Rodrigues-Cunha et al., 2016). Recently, it has been reported that melatonin supplementation in the vitrification solution for oocyte storage significantly decreased ROS levels, inhibited apoptosis, and increased subsequent embryonic development after thawing (Zhao et al., 2016). In buffalo, melatonin supplementation during *in vitro* maturation could also improve oocyte maturation rate by reducing oxidative stress and DNA damage (Manjunatha et al., 2009).

Melatonin attenuates endoplasmic reticulum stress

Endoplasmic reticulum (ER) plays a key role in protein synthesis, lipid biosynthesis, calcium regulation, and cell homeostasis maintenance (Sharma et al., 2014), as well as in mammalian embryonic development (Latham, 2015; Lin et al., 2016a). A short-term ER stress can protect cells, but long-term ER stress will lead to the induction of ROS, apoptosis, or autophagy (Yorimitsu et al., 2006; Ciechomska et al., 2013; Lin et al., 2016b; Utaipan et al., 2017), and finally trigger cell death. Melatonin is widely used to attenuate ER stress in cells or organs. For instance, melatonin can reduce tunicamycin-induced ER stress in human hepatocellular carcinoma cells (Fan et al., 2013) or skeletal muscle cells (Quan et al., 2015). Hadj Ayed Tka et al. (2015) reported that melatonin improved the recovery of renal function by inhibiting ER stress and activing Akt pathway in renal ischemia/reperfusion injury. However, there are a limited number of studies focusing on the possible mechanisms of melatonin on the aspects ER stress in oocyte maturation and/or embryo development. Recently, one study suggested that melatonin could enhance porcine oocyte maturation rate and cumulus cells expansion by regulating of UPR signal genes against the ER stress during *in vitro* maturation (Park et al., 2017). It has been reported that the *in vitro* culture environment could lead to increased ER stresses, which damage embryo development (Zhang et al., 2012; Lin et al., 2016a), suggesting that mammalian embryos are always exposed to ER stress under *in vitro* culture conditions. Thus, melatonin could neutralize ER stress by regulating the ER stress pathway which would result in an improved embryonic developmental capacity. This possible mechanism of melatonin on ER stress during embryo development needs to be further investigated.

Melatonin protects oocyte maturation *in vitro* from heat stress

For normal physiological functions of organisms, a constant body temperature is very important (Li et al., 2015). Under heat stress conditions, an animal's physiological functions could be damaged (Jardine 2007; Li et al., 2015). It was reported that heat stress in porcine COCs at germinal vesicle breakdown stage induced apoptosis in cumulus cells and reduced oocyte maturation rate (Yuan et al., 2008). Also, heat stress induced oxidative damage and led to cell apoptosis during *in vitro* maturation of oocytes (Wang et al., 2009). However, melatonin is known to have the capacity to protect cells from heat stress. Recently, Li et al. (2015) reported that heat stress significantly reduced the maturation rate of porcine oocytes and blastocyst formation rate, but melatonin supplementation not only enhanced the maturation rate and developmental capacity of embryos, but also helped maintain normal steroid hormone levels which are disrupted under heat stress. In addition, under heat stress, melatonin could also reduce ROS and apoptosis, and increase GSH production and the expression levels of *Polg2*, *SIRT1*and *AKT2* genes (Li et al., 2015). In a recent study that examined the effect of resveratrol and melatonin on protecting porcine oocytes from heat stress, melatonin exhibited a more powerful protective activity than resveratrol alone during oocyte maturation under heat stress (Li et al., 2016).

Melatonin protects oocytes during cryopreservation

Cryopreservation of oocytes and embryos is an important assisted reproductive technology procedure along with *in vitro* fertilization, somatic cell nuclear transfer, intracytoplasmic sperm injection, etc. One of the major adverse factors to the cryopreservation technique is the damage caused by oxidative stress (Mehaisen et al., 2015). Oxidative stress-induced DNA damage may accelerate the process of cell apoptosis (Agarwal et al., 2003). Several studies have

reported that oocytes cryopreservation significantly increased the apoptosis rate of oocytes, resulting in a reduction of their developmental potential (Hwang et al., 2013; Morato et al., 2010; Vallorani et al., 2012; Zhao et al., 2016). Melatonin can protect oocyte and embryos from damage during the freezing process probably due to its known antioxidant activity and the multi-faceted ways it counteracts oxidative stress and apoptosis (Zhao et al., 2016). The observed beneficial effects of melatonin on vitrified oocytes are attributed to its ability to inhibit apoptosis, ROS level, and DNA fragmentation (Zhao et al., 2016). In addition, melatonin supplemented to *in vitro* culture medium had a dramatic beneficial effect on bovine IVF blastocyst cryotolerance, significantly enhancing hatched blastocyst rate (Wang et al., 2014).

Concluding remarks

Melatonin supplementation can not only improve quality of oocytes and their maturation rate , but can also enhance subsequent embryo developmental capacity by reducing oxidative stress and apoptosis during *in vitro* culture. Porcine and bovine oocytes and embryos are hypersensitive to oxidative stress during *in vitro* culture due to the presence of high lipid content. The beneficial effects of melatonin on oocyte maturation, embryonic development, endoplasmic reticulum stress, and heat stress, as well as cryopreservation in pig and cattle have been distinctly reported in the literature, suggesting that melatonin has an important role in the production of mammalian gametes and embryos. However, the mechanisms of melatonin in oocytes and embryos needs to be further investigated.

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