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An overview of significant role played by melatonin in animal reproduction

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Abstract
Melatonin is a hormone produced by the pineal gland and it is a potent antioxidant with a wide range of uses in both people and animals, including poultry and cattle. Melatonin also has a significant impact on how animals' biological cycles are managed. Melatonin and scientific breeding management have a lot of promise to improve animal physiological processes, but there are still a lot of obstacles to overcome.
We outlined in this article the antioxidant properties of melatonin, its reproductive physiology, and its effect on ovarian function, Granulosa cell, oocytes, testicular function, spermatogenesis, and cryopreservation of semen. Melatonin can have a significant impact on cattle health and reproduction. It is reasonable to state that in the future, we hope to successfully and safely improve animal husbandry with the use of melatonin.

Keywords: Melatonin, granulosa cell, ovary, spermatozoa, oocytes

1. Introduction
Aaron Lerner found melatonin in the pineal gland of bovine in 1958. It was later discovered that melatonin was vital regulator of the circadian rhythm (Yasmin *et al*., 2021) [81]. Melatonin was thus utilized to control the circadian clock, sleep quality, and bodily rhythms, and it was regarded as the "gold standard" medicine in this field. Melatonin has been connected to several tissues and cells in many species during the last 60 years (Olcese, 2020) [46], including the testicles (Li, *et al*., 2020) [34], ovaries (Sundaresan *et al*., 2009) [65], placenta (Reiter, *et al*., 2014) [53], Granulosa cell (Shen, *et al*., 2018) [60], and oocytes (Tian, *et al*. 2014) [71]. In different tissues and cells, melatonin performs several functions, including removing antioxidants and reactive oxygen species (ROS), preventing apoptosis, minimizing inflammation, and delaying aging.
Melatonin, secreted in direct proportion to the period of darkness, is an important candidate in the follicular fluid drawing the attention of various scientists, as it was discovered to prevent oxidative damage to oocytes and increase fertilization rate. (Tamura *et al.,* 2008) [68]. Moreover, higher melatonin concentration was seen in preovulatory follicles of humans as well and the presence of melatonin receptors in the ovary is indicative of melatonin regulation in ovarian function (Nakamura *et al*., 2003) [42]. Heat stress causes DNA oxidation of oocytes, producing 8-hydroxy-2'deoxyguanosine (8- OHdG, Valko *et al*., 2006) [73]. Hydroxyl radical (OH^-) reacts with guanine (the most sensitive DNA base) to form 8-OHdG and is used as a sensitive indicator of oocyte DNA damage (Tamura *et al*., 2008) [68]. The quality and prognosis of oocytes can be assessed by measuring the expression of 8-OHdG as its higher expression adversely affects the cell functions and thus degrades oocyte quality (Seino *et al*., 2002) [57]. The effectiveness of melatonin as an antioxidant and in protecting the cells against stress caused by free radicals (Hardeland *et al*., 2011) [23] is further proven by the fact that melatonin implants were effectively used to counter the summer anestrous with the resumption of ovarian cyclicity in buffalo (Kumar *et al*., 2016) [29] and buffalo heifers (Ghuman *et al*., 2010) [20].
The usefulness of melatonin in the modulation of steroidogenesis has been demonstrated in various *in vitro* experiments (Baraita and Tamanin, 1982) [8] however, scanty information is available in dairy animals emphasizing that further investigation is warranted. Both 17β estradiol and progesterone are principal steroid hormones related to the reproductive tract and alteration in the concentration of 17β estradiol and progesterone may lead to severe reproductive disorders. Moreover, 17β estradiol and progesterone were shown to be associated with good-quality oocytes in buffaloes (Lone *et al*., 2013) [36]. Further, the utility of melatonin in the regulation of reproductive activity in seasonal breeders (sheep and mares) is well recognized.

2. Melatonin: A free radical scavenger and powerful antioxidant

Free radicals are byproducts of regular cellular metabolism and are defined as molecules with a number of electrons that are unpaired in their molecular or atomic orbitals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are terms used to describe radicals and the non-radical species that they are connected to. A range of processes, both physiological and pathological, including cell survival, proliferation, and division at the level of physiological function, as well as cell death via necrosis or apoptosis at the higher level, are impacted by reactive oxygen species. Free radicals have a role in the sperm, oocyte, and follicular fluid microenvironments (Agarwal *et al*., 2005) [3] and are regarded as essential signaling molecules for a variety of ovarian processes (Sugino *et al*., 2002) [64]. The follicular fluid contains many antioxidant enzymes that guard the oocyte from ROS-induced oxidative damage. Superoxide dismutase (SOD), catalase, GSH-Px, Cu, Zn-SOD, and Mn-SOD dismutate are some of these enzymes. Oxidative stress is a result of a contradiction among ROS and antioxidants that can cause DNA damage, protein oxidation, or lipid peroxidation. Follicular cells suffer significant oxidative damage due to the imbalance in the oxidant-antioxidant system, demonstrating the importance of oxidative damage in the pathophysiology of reproduction. By modulating the gene transcription for the antioxidant enzymes, melatonin, and its byproducts are effective immediate scavengers of free radicals and indirect antioxidants. The principal function of melatonin in defending organisms against the constant threat of oxidative stress was as a form of antioxidant. Together with other well-known antioxidants including the enzyme glutathione peroxidase, superoxide dismutase, the antioxidant vitamin E, and selenium, melatonin has a synergistic effect (Reiter *et al*., 1997) [51]. Melatonin is thought to be more efficient at neutralizing free radicals than mannitol and glutathione (GSH). Melatonin was shown to have the power to reduce DNA damage, lipid peroxide concentrations, and harmful reactive oxygen species (ROS), all of which improve the survival of embryonic cells. Inhibiting the activity of several pro-oxidant enzymes including lipoxygenase and NO synthase allows melatonin to reduce the production of reactive oxygen species (Reiter *et al*., 2001) [53]. Additionally, both granulosa and cumulus cells have melatonin-binding sites that have been discovered (Tian *et al*., 2017) [72] and the ovary's Granulosa cell may directly bind to melatonin (Yie *et al*., 1995) [82]. Additionally, melatonin increases the activity of a number of enzymes involved in the antioxidative defense system. This effect is probably mediated by membrane receptors, nuclear, or cytosol-binding sites, or both. Melatonin works by forming a complex with calmodulin and inhibiting the gene transcription that produces the rate-limiting enzyme, inducible NO synthetase. Following a single subcutaneous administration of melatonin for 28 days, it was shown that postpartum summer anestrous buffaloes had higher serum total antioxidant capacity and lower serum levels of nitric oxide and malondialdehyde when compared to controls (Kumar *et al*., 2015) [30]. It is possible that melatonin plays a protective effect by suppressing the generation of lipid peroxidation and lowering the concentration of malondialdehyde, as indicated by a lower level of oxidative biomarkers after melatonin administration.

One of the main byproducts of DNA oxidation is 8-hydroxy-2'deoxyguanosine (8-OHdG), which is also a highly reactive genotoxic biomarker of oxidatively deteriorated DNA (Valko *et al*., 2006) [73]. It is a biomarker for oxidative stress related to DNA. Guanine, the most sensitive DNA nucleotide, interacts with the hydroxyl radical (OH-) to produce 8-hydroxy-2' deoxyguanosine (8-OHdG). Melatonin inhibits DNA, protein, and lipid peroxidation. Tamura *et al*. (2008) [68] investigated the relationship between the quality of the oocytes, the proportion of degenerate oocytes, and the amount of 8-OHdG in the follicular fluid of females going through IVF-ETT. They found that the intrafollicular 8-OHdG levels in women alongside a significant amount of deteriorated oocytes were significantly greater compared to those in women with a small number of degenerate oocytes. Melatonin and 8-OHdG concentrations in the intrafollicular area were significantly and negatively correlated. Accordingly, it may be hypothesized that melatonin in the follicle dissipates into the cumulus and oocyte to shield them from free radical damage and that as a result, melatonin's primary function in the follicle is as a free radical scavenger that lessens oocyte damage. Immature rats given human chorionic gonadotropin treatment had greater levels of 8-OHdG, and those levels dramatically rose nine hours after injection, immediately preceding ovulation (Tamura *et al*., 2008) [68]. In women undergoing IVF, there was an increase in intrafollicular melatonin levels and a decrease in the concentration of 8-OHdG adhering to therapy with oral melatonin @ 3 mg from the fifth day of the menstrual cycle to the day of oocyte retrieval (Tamura *et al*., 2008) [68]. This suggests that melatonin reduces oxidative damage within the follicles. Due to its proximity to the inner mitochondrial membrane, where oxidants are produced and DNA repair activity is deficient, mitochondrial DNA is the main target for ROS. The mitochondria are the target organ for melatonin activity because it lessens DNA and protein damage to the mitochondria and enhances the electron transport chain (Tamura *et al*., 2008) [68].

1. 3. Reproductive physiology of melatonin

The "hormone of darkness," also known as melatonin (N-acetyl-5-Methoxytryptamine), is an indole derivative produced systematically via the pineal gland with pleiotropic operations that include circadian rhythm in vertebrates to seasonal procreation in many mammals (Altun and Ugur-Altun, 2007) [5]. It was first identified in cattle pineal extracts due to its ability to construct melanin granules. When it was initially discovered, it was believed to have the power to lighten the skin of amphibians by causing the melanin granules in frog dermal melanosomes to assemble around the nuclei of skin cells (Lerner, 1959) [33]. The preovulatory follicular fluid contains melatonin, which is a ubiquitous chemical that can be found practically everywhere in the body. Its concentration there is substantially higher than in peripheral serum. The circadian cycle of melatonin in vertebrate animals, with its highest level at night and low concentrations throughout the day, is one of its most distinctive characteristics. Melatonin is an excellent signaling molecule to inform internal organs of ambient photoperiodic variations as a result of this feature. Since the dark portion of the light/dark cycle usually corresponds with the melatonin peak in vertebrates' blood, melatonin is sometimes referred to as the chemical manifestation of the darkness (Reiter, 1991) [55]. In photoperiodic animals, the circadian melatonin rhythm, which is characterized by high levels at night, is crucial for coordinating the reproductive response to suitable

environmental circumstances (Reiter, 1998) [54].

Many mammalian species rely on the nocturnal release of melatonin, which serves as a transducer of the photoperiod signals to the neuroendocrine reproductive axis, to determine their seasonal patterns of reproductive activity (Arendt, 1985) [7]. As a result of changing steroid feedback, the length of melatonin's nocturnal release regulates the pulsatile production of the GnRH hormone. The GnRH system is affected by melatonin, however, it is thought that melatonin does not modify GnRH neurons directly. Instead, it works by way of an interneuronal pathway that finally forms synapses with GnRH neurons. Direct steroid-dependent regulation of the secretion of GnRH and an alteration in the steroid adverse effect on the GnRH secretion are two complementary mechanisms regulated by melatonin that together account for the change in GnRH release (Malpaux *et al*., 2001) [38]. In seasonal breeders, melatonin also serves as a neural signal to the regions in charge of regulating the gonadotropins released by the pituitary gland, causing their activity to correspond to the season of the year. When pregnant sheep were exposed to constant illumination for 138 days (146 gestation days), according to Okatani and Sagara (1993) [45], there was a drop in levels of melatonin and a spike in the rate of metabolism in the reproductive organs. Melatonin also modifies the release of the hormones FSH and LH, which control gonadal activity and function. The fact that pinealectomy in combination with melatonin therapy changed the daily pattern of fetal respiration in pregnant sheep provides more proof that photoperiodic information provides the fetus with circadian fluctuations through maternal melatonin.

Melatonin directly affects ovarian function in hens (Murayama *et al*., 1997) [41], hamsters (Tamura *et al*., 1998) [67], and people (Woo *et al*., 2001) [77], altering granulosa cell steroid production and follicular function. Progesterone and androstenedione are produced more often when melatonin is incubated with mice's preantral follicles for 12 days (Adriaens *et al*., 2006) [1]. Melatonin results in higher blastocyst total cell counts and embryo cleavage rates in the porcine model. Additionally, melatonin affects seasonal variations in sperm concentration, chromatin condensation, embryo quality, and fertilization rates. Melatonin clearly plays a significant role in blastocyst attachment and reproductive activity, as demonstrated by research in a variety of animal species, including sheep, horses, and ferrets. Maternal transmission of melatonin in animals also suggests that the fetus receives the daily photoperiod experienced by the mother's body during pregnancy or breastfeeding via the placenta or milk respectively. It is apparent that melatonin acts through its receptors, which have been found in the Pars tuberalis of the pituitary and the suprachiasmatic nuclei of the hypothalamus (Dupre *et al*., 2008) [13]. The Pars tuberalis part of the pituitary has been discovered to have the greatest number of melatonin receptors in all the species investigated, with the exception of humans (Morgan and Williams, 1996) [40]. Melatonin receptors were additionally found in the ovary, the mammary glands, the testis, the epididymis, and the prostate gland utilizing a radioreceptor binding assay. The membrane portion of human Granulosa cell, luteal cells (Woo *et al*., 2001) [77], and rodent ovaries (Soares *et al*., 2003) [62] have all been found to have melatonin binding sites. Thus, it may be hypothesized that melatonin affects a variety of levels, including those in the hypothalamus, pituitary, gonads, and reproductive tissue, producing a strong photoperiodic regulatory mechanism in animal reproduction. The high levels of melatonin activity are crucial for a healthy pregnancy and birth of offspring at a period when the surroundings are most favorable and there is an abundance of food for the species' survival (Pang *et al*., 1998) [47].

3.1 Melatonin and ovarian functions

There is evidence to support that melatonin has a direct impact on ovarian function, in addition to its well-established involvement in the control of reproduction activity among seasonal breeders (Nakamura *et al*., 2003) [42]. According to research done on humans, melatonin has a role in the pathophysiology of polycystic ovary syndrome, endometriosis, and precocious ovarian follicles, as well as in the development of follicles, the ovulation oocyte maturation, luteal function, and embryo development (Tamura *et al*., 2009) [66]. It serves as both an immediate free radical scavenger and an indirect antioxidant. Melatonin has been found in preovulatory follicles in human investigations before (Nakamura *et al*., 2003) [42], and intrafollicular melatonin level has been found to be three times greater than blood serum (Yie *et al*., 1995) [82]. Additionally, studies on humans have shown that larger follicles have greater melatonin concentrations than smaller follicles. Anovulation and poor oocyte quality may be caused by low follicular melatonin concentrations, whereas high levels of melatonin in the follicular fluid (FF) are necessary for follicle development, ovulation, and oocyte quality. Melatonin therapy for infertile human females enhances the likelihood of conception and pregnancy while decreasing intrafollicular oxidative damage (Tamura *et al*., 2008) [68]. Melatonin helps to luteinize Granulosa cell, which in turn helps to stimulate the synthesis of progesterone (Tamura *et al*., 2009) [66]. It also shields Granulosa cell from harm during ovulation. Melatonin therapy increases corpus luteum progesterone production in infertile women with luteal phase insufficiency. Initial mouse embryo development was considerably supported by melatonin supplementation at rates of $^{10\text{-}6}$ and 10^{-4} M (Ishizuka *et al*., 2000) [27]. This may be explained by melatonin's ability to neutralize free radicals and function as a robust antioxidant. Human Granulosa cell treated with melatonin (10 pM-100 nM) had significantly higher LH (as opposed to FSH) receptor mRNA expression.

Some earlier studies claimed that melatonin has no effect on the production of 17 estradiol (Fiske *et al*., 1984; Webley and Luck, 1986; and Tanavde and Maitra, 2003) [18, 76, 69], while others claimed that it stimulates the production of 17 estradiol in Granulosa cell in culture (Sirotkin, 1994; Yie *et al*., 1995) [61, 82]. According to other studies (Sirotkin, 1994; Murayama *et al*., 1997; Tamura *et al*., 1998; and Nakamura *et al*., 2003) [61, 41, 67, 42], melatonin has little impact or even a negative effect on the generation of progesterone in granulosa cell culture. In contrast, it was also suggested that melatonin and progesterone synthesis in Granulosa cell from ovine, rats (Fiske *et al*., 1984) [18], cattle (Webley and Luck, 1986) [76], and humans (Yie *et al*., 1995) [82] were positively correlated. Progesterone and melatonin levels in females undergoing IVF after hCG injection appear to be positively correlated in several human investigations (Webley and Luck 1986) [76]. According to Nakamura *et al*. (2003) [42], after hCG injection, Granulosa cell transform into luteal cells, and steroidogenesis shifts from 17 estradiol predominance to progesterone dominance, showing that melatonin indirectly stimulates progesterone synthesis. Furthermore, through reducing LH

affinity to its receptor, melatonin regulates theca cell steroidogenesis (Tamura *et al*., 1998) [67]. When LH stimulates steroidogenic enzymes, CAMP-protein kinase catalyzes the transformation of cholesterol to C21 and C19 steroids. Additionally, healthy follicles and oocytes of high quality are linked to high estradiol levels. According to Alberto *et al*., (2009) [4], a high estrogenic milieu in the follicular fluid exerts anti-atresia actions and is associated with oocyte competency and follicular expansion. It is debatable how intrafollicular progesterone affects oocyte quality. A high progesterone content, according to some writers, is a reliable indicator of egg maturity, follicle luteinization, implantation, and pregnancy (Enien *et al*., 1995) [16]. Others claimed that postmature oocytes were linked to elevated follicular fluid progesterone levels, which resulted in aberrant fertilization and produced multipronuclear embryos (Ben-Rafael *et al*., 1987) [9]. Greater levels of 17-estradiol or a greater 17-estradiol to progesterone concentration ratio in the follicular fluid have been linked to earlier stages of oocyte development and higher pregnancy rates, according to earlier research (Tarlatzis *et al*., 1993) [70]. In contrast, others reported no such relationship (Costa *et al*., 2004) [12].

3.2 Melatonin in Bovine Granulosa cell

Granulosa cell play a critical part in the development of ovarian follicles, and follicle atresia significantly contributes to the decline in the function of bovine reproduction. Any alteration in the state of these cells, including apoptosis, autophagy, a cell cycle arrest, or an accumulation of ROS, might result in follicular atresia (Wang *et al*., 2021) [75]. Furthermore, alterations in the synthesis of steroid hormones have an effect on the physiological conditions of Granulosa cell (Wang *et al*., 2018) [74].

Melatonin, which is well recognized for its ability to remove ROS and influence cellular physiology, can decrease ROS and avoid apoptosis in Granulosa cell through a number of mechanisms (Yang *et al*., 2019) [79]. While the cumulus-oocyte and outer Granulosa cell do not undergo apoptosis in the early phases of follicular atresia, the interior layer of Granulosa cell does (Nakayama *et al*., 2000) [43]. Therefore, a crucial initial step in the treatment of atresia of the follicle may be identifying the reason for granulosa cell variation. Granulosa cell play important functions in sustaining and encouraging the *in vivo* formation of follicles, with various physiological conditions dictating follicle destiny (Yuan *et al*., 2018). Important consequences include ROS release, which results in apoptosis and autophagy when crucial processes in cells, especially those of the mitochondria, are modified. One of the primary producers of ROS is mitochondria. Although some O2 is utilized in the synthesis of ATP, some will additionally up ROS, which is essential for mitochondria to function. Extracellular Ca2+ may enter the cells at high concentrations of this, which might result in swelling or possibly mitochondrial rupture. Superoxide dismutase (SOD) and catalase (CAT) activity can be increased by melatonin, which can also maintain antioxidant enzymes and remove active oxygen via altering ER oxidoreductin1 (ERO1) (Fernández *et al*., 2015) [17]. Melatonin may also inhibit -zearalenol-induced oxidative damage and apoptosis, and SOD and CAT levels of proteins can be significantly raised (Yang *et al*., 2019) [79]. Melatonin effects can be reduced and cell cycle changes can be prevented by inhibiting the MT1 and MT2 receptors for melatonin (Wang *et al*., 2021) [75]. Melatonin has a range of actions that rely on temperatures, oxygen content, and scavenging ROS. Low melatonin concentrations boosted cell proliferation at 37.5 °C and 5% O2, which replicates *in vivo* circumstances, whereas high melatonin concentrations stimulated cell multiplication at 40 °C (Zeebaree *et al*., 2018) [84]. Melatonin may therefore be able to lessen the stress brought on by high temperatures due to its dependent on dosage interactions with temperature. Exogenous melatonin consumption is difficult due to changes in the body's physiological state and melatonin release, highlighting the necessity for trustworthy data to support evidence-based decisions.

3.3 Application of Melatonin in Bovine Oocyte Cells

Oocyte quality is a significant barrier to the transfer of embryos from cattle and a reliable indicator of the reproductive success of female animals. When fresh embryos were implanted compared to those cryopreserved, live birth rates were much higher (Insogna *et al*., 2021) [25]. Oocyte cryogenic preservation has traditionally been difficult because of things like their survival, the process of fertilization and development rates (Chen *et al*., 2003) [11]. Oocytes developed *in vitro* prior to freezing have plenty of potential to be of higher quality thanks to melatonin.

Numerous studies have demonstrated that melatonin enhances the ability of oocytes to mature in both vitro as well as *in vivo* (Sananmuang *et al*., 2020) [56]. For example, by reducing ROS, it improves the effectiveness of oocyte production and the growth of embryos in prepubertal and mature cows. Melatonin boosts the transcription of genes that regulate mitochondrial function, which speeds up oocyte maturation, reduces the amount of ROS in heat-shocked oocytes, and enhances the number of embryos that mature into blastocysts (Yaacobi *et al*., 2020) [78]. Melatonin's preservation of bovine oocytes also prevents paraquat-induced oocyte damage and preserves the embryo's capacity for development (Pang *et al*., 2019) [48]. Melatonin has been found in several studies to help bovine oocytes grow (Lan *et al*., 2018) [31]. Melatonin encourages the development of antioxidant enzymes to eliminate ROS through certain membrane or nuclear receptors. Cumulus-oocyte complexes (COCs) may produce melatonin as a result of the activity of the enzyme of acetylserotonin O-methyltransferase (ASMT) (El-Raey *et al*., 2011) [14]. By turning on the MT1 transmembrane receptor, melatonin lessens the oxidative stress that oocytes experience, preserving the spindle body's ability to support oocyte growth. On days 190–262 of pregnancy, Holstein cows received 20 mg of melatonin, which enhanced uterine blood flow because it may affect steroid metabolism (Brockus *et al*., 2016) [10]. In heifers, melatonin changed the way estradiol was broken down to encourage uteroplacental development (Lemley *et al*., 2017) [32]. Exogenous melatonin significantly raised progesterone levels, SOD, CAT, and glutathione peroxidase (GSH-Px) activity, decreased the amount of MDA in bovine blood, and substantially increased pregnancy rate in studies for estrus and artificial insemination in cattle (Guo *et al*., 2021) [21]. Increased *in vivo* fertility and the growth of embryos and oocytes in culture may be achieved with the help of melatonin.

3.4 Melatonin and Testicular Function, Spermatogenesis, and Semen Cryopreservation in Bulls

For the long-term conservation of sperm in ruminant species like sheep and cattle, cryopreservation is a vital assisted

reproductive technique (Ofosu *et al*., 2021) [44]. High-quality semen is crucial for protecting endangered species and expediting genetic development in addition to the significance of oocytes. Semen the preservation process technology is being developed for this reason. The reactive oxygen species, that are required for sperm capacitation, are present at a certain concentration in semen (Ofosu *et al*., 2021) [44]. However, when levels of ROS are high, oxidative stress can impair sperm physiological functions, including structure and DNA integrity (Medrano *et al*., 2017) [39]. Due to its capacity to scavenge ROS, melatonin lowers ROS-induced harm caused by oxidative stress during sperm preservation and enhances the purity of frozen-thawed sperm (Shahat *et al*., 2022) [59].

The quality of swamp buffalo sperm has improved with the inclusion of 1 mM melatonin in the semen extender (Inyawilert *et al*., 2021) [26]. The quality of the sperm from heat-stressed rams which had been frozen and thawed was unaffected by the addition of melatonin to the semen extender (Shahat *et al*., 2023) [58]. 2-month melatonin implantation dramatically decreased morphologically aberrant sperm and enhanced both the curve and linear velocity of sperm in Murrah buffalo bulls. In seminal plasma from Murrah buffalo males during the absence of breeding under tropical circumstances, melatonin also enhanced the levels of overall protein and cholesterol (Ramadan, *et al*., 2019) [50].

The impact of melatonin on semen freezing, oocyte development, and embryonic development was thoroughly examined by Su *et al*. in 2021. Depending on the approach, different melatonin concentrations were ideal. *In vitro* fertilization (IVF) with 10^7 M greatly increased the rate of oocyte maturation and the overall quantity of blastocysts, although semen freezing was optimum with 10^3 M. In earlier research, we discovered that various melatonin concentrations had variable impacts on antioxidant markers and sperm motility. Melatonin decreased the ROS concentration by 0.125 mg/mL and 0.5 mg/mL, respectively. Melatonin, at a modest dosage (0.25 mg/mL), also decreased the MDA levels. The medium dosage of melatonin provided the most beneficial benefits, even though all three levels increased antioxidant markers (Appiah *et al*., 2019) [6]. The quality of frozen-thawed sperm, embryo development, and *in vitro* fertilization efficiency can all be enhanced by adding the proper quantity of melatonin to the semen extender and sperm preparation.

Melatonin has a crucial role in spermatogenesis, which controls the function of the testes via the hypothalamic-pituitary-gonadal axis, in addition to increasing semen quality when administered exogenously (Shahat *et al*., 2022) [59]. The HPG axis has a significant impact on the control of reproductive hormones. Melatonin treatment delayed puberty in the offspring of expectant female rats by lowering prolactin and LH levels. Additionally, by lowering GnRH-induced LH release, which in turn decreases testosterone synthesis, melatonin receptor MT1 inhibitor luzidole effectively counteracts the effects of melatonin (Li *et al*., 2015) [34]. Melatonin is required for the development and growth of multiple kinds of testicular cells as well as the process of hormone release assert that melatonin shields the testicles against localized swelling and reactive oxygen species. In order for the HPG axis to function correctly in the reproductive system of male, melatonin can influence the production of hormones by, for example, controlling the development and growth of several types of cells in the testicles through its receptor (Heidarizadi *et al*., 2021) [24]. Melatonin produced by the pineal gland and consumed by the testes through circulation of blood may alter testicular function (Frungieri *et al*., 2017) [19]. The production of the hormone testosterone, apoptosis, and phagocytosis may all be controlled by melatonin via its many receptors (Qingyu, 2022) [49]. Bovine Sertoli cells were incubated with melatonin *in vitro*, which elevated the expression of spermatogenesis-related genes such Pdgfa, Cyclin D1, Occludin, Cyclin E, Dhh, and Claudin (Yang *et al*., 2014) [80]. After using it for six months, several characteristics of healthy men's semen altered, which may be related to melatonin's potential ability to suppress aromatase (Luboshitzky *et al*., 2002) [37]. In a testicular ischemia-reperfusion scenario, melatonin significantly reduced morphologically abnormal sperm (Koksal *et al*., 2012) [28]. Via raising the fraction of motile sperm and elevating mitochondrial activity, melatonin is said to have changed spermatogenesis and development via crossing the blood-testis barrier *in vitro*. These findings showed that melatonin regulated the development of testicular cells, which regulated spermatogenesis.

Conclusion

Increasing the efficiency of animal reproduction is necessary to increase the long-term viability of livestock reproduction. However, a variety of factors affect domestic cow breeding. Domestic animals' physiological circumstances will have an effect on how they reproduce. Melatonin has a substantial impact on the development, growth, and metabolism of several cells. Melatonin has been found to promote embryonic and placental development, stimulate ovulation, and increase pregnancy rates in female cattle. Male calves with elevated levels of melatonin had improved testicular function, sperm shape and motility, sperm content of proteins, and mitochondrial activity. Melatonin may work best when combined with modern breeding techniques, which would improve domestic animals' reproductive capacities.

Conflict of interest

Authors declare that there is no conflict of interest

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