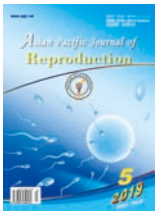

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# Role of melatonin in male reproduction

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ABSTRACT

Melatonin, conventionally accepted as a pineal gland secretion, is a neuromodulator whose physiological concentrations are regulated by circadian rhythms. Alteration in melatonin levels owing to circadian influences is a major regulator of reproductive functions in animal species that are seasonal breeders. Attributing to its antioxidant properties and capability to cross physiological barriers, such as the blood-brain barrier, the blood-testis barrier as well as having almost no toxicity, melatonin finds high relevance in amelioration of male fertility parameters. Melatonin may affect male reproductive functions by influencing the release of hypothalamic gonadotropin-releasing hormone and pituitary luteinizing hormone, which are among the key hormones in regulation of male reproduction. It may directly act on testicular cells to influence testicular functions. The property of melatonin most essential for testicular functions is its ability to scavenge free radicals, thereby preventing testicular oxidative damage. This article summarizes the updated data on the versatility of melatonin as an endogenous rhythm setter, as an antioxidant molecule and its possible physiological impacts in male reproductive functions.

## 1. Introduction

Melatonin or 5-methoxy-*N*-acetyltryptamine was first described from bovine pineal in the year of 1958 by Aaron Lerner[1]. Melatonin is the principal secretion of the pineal gland and it is regulated by the circadian rhythm. Initially, it was thought that it is unique to the pineal gland, but now it is known to be produced in many other tissues all over the body[2]. The rhythmic release of melatonin acts as an endogenous synchronizer, modulating several physiological events like sleep-awake-cycle, blood pressure, body temperature, glucocorticoid or cortisol rhythm, and body defense mechanism, *etc*. Human melatonin has diurnal variations and its secretion gets elevated with the onset of darkness, reaches its peak concentrations in the mid-night followed by gradual decrease in the last part of the night. The gradual rise in melatonin level is seen in individuals from the birth and likewise, become peak during 2 to 4 years of age[3] relatively lesser during the puberty[4] and decreases gradually from the middle age[5]. As the consequence of decline in levels of this natural ubiquitous molecule in old age or in pathological conditions, melatonin may modulate associated physiological functions[6]. Melatonin is applied in combination therapie due to its negligible side-effects together with its ability to reduce the side effects and increase in the functional efficacy of large number of drugs, it finds applications[7]. Melatonin is also well known for being a molecule with antioxidant properties and it also triggers the brain antioxidant defense mechanisms[8].

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Melatonin has been associated with improved male reproductive functions. It has been shown that melatonin exposure is essential for reproductive functions in seasonal breeders[9]. In humans, melatonin has been demonstrated to influence the anterior pituitary gonadotropins, gonadal steroids and testicular functions *via* specific receptors which are profusely expressed in the central nervous system as well as in the reproductive tissues[10]. Moreover, the role of melatonin in protection against testicular pathogenesis and in the sustenance of normal spermatogenesis have been documented. But, the exact mechanism of its action other than its antioxidant properties in testicular cells, remains largely unknown[11]. Attributing to its capability to cross physiological barriers, such as the blood-brain barrier, the blood-testis barrier without obvious toxic impact, melatonin finds high relevance as a therapeutic molecule in male reproductive disorders[12]. In the present global scenario, where male fertility in terms of semen quality is showing a gradual decline[13–17], molecules like melatonin with ameliorative effects on male reproductive functions should be explored further for therapeutic interventions. This article thus aims to review and present an updated report on the properties of melatonin and its receptors along with its versatile roles in the regulation of male reproductive functions.

## 2. Melatonin and melatonin receptors

### 2.1. Synthesis

Melatonin (5-methoxy-*N*-acetyltryptamine) is an endogenously produced indoleamine, also a nocturnal peptide, primarily synthesized from the essential amino acid tryptophan followed by serotonin. It is secreted from the pinealocytes of pineal gland mainly to maintain the circadian rhythms and light is the principal external cue for its secretion[18]. It also involved in various other physiological processes[19] such as blood pressure and body temperature regulation, oncogenesis, and immune functions. During prolong photoperiod melatonin activity is lesser (*e.g.* summer season) and during shorter photoperiod the melatonin activity is higher (*e.g.* winter season)[20]. The rhythmic activity of melatonin may be entrained gradually after shifting to a new short photoperiod. However, compression of the rhythm on long photoperiodic conditions is spontaneous[21]. This photoperiodic regulation of melatonin rhythm acts as an endocrine calendar, as its target organs are informed about the changes in light-dark cycle or seasons of year. Circadian production of pineal melatonin is controlled by endogenous oscillators within the suprachiasmatic nucleus and entrained by daily and seasonal changes in the environmental light-dark cycle[22]. Shift work, travel, and ubiquitous artificial lighting can disrupt natural circadian rhythms of melatonin.

### 2.2. Melatonin receptors

For classical functions, melatonin has two different types of receptors in human called $MT_1$ and $MT_2$ receptors. These two receptors are G-protein-coupled receptors[23]. Melatonin receptor type 1 or $MT_1$ is a cell membrane receptor, also known as Mel1a or $ML_{1A}$ receptor. It is encoded by 4q35.1 and is having 350 amino acid residues[24] in its structure and coupled to $G_i$, specifically $Gi_{\alpha 2}$, $Gi_{\alpha 3}$, and $G_{q/11}$[25]. This $MT_1$ receptor is expressed in the brain including the hypothalamus, cerebellum, hippocampus, substantia nigra and ventral tegmental area; cardiovascular system including peripheral blood vessels, aorta and ventricular wall, coronary and cerebral arteries; on the cells of immune system, skin, liver, kidney, adrenal cortex, retina, pancreas, spleen, placenta, breast tissues, ovary and testes[26].

Whereas, another cell surface receptor MT2, formerly known as Mel1b or $ML_{1B}$ is encoded by 11q21–q22[24] with 363 amino acids with near about 60% homology to $MT_1$ receptor[24] in its structure and couples to the activation of $G_i$. This receptor is located in the brain and various other organs including the testes[27].

For classical functions, melatonin binds to $MT_1/MT_2$ heterodimers that interact with downstream regulating the second messenger systems such as adenylyl cyclase (cAMP), phospholipase A2 and phospholipase C. They act to decrease the production of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate cGMP and/or increase the production of diacylglycerol and inositol triphosphate[28].

Melatonin may perform its functions also through the putative retinoid orphan receptor (ROR)/retinoid Z receptor (RZR) nuclear receptor[29] and its possible subfamilies that bind melatonin including RZRα, RORα, RORα2, and RZRβ. Among these, the RZRα has been detected in testis[25].

## 3. Physiological functions of melatonin

Role of melatonin in the sleep-wakefulness cycle may be considered as its classical function[30]. Melanopsin is the receptor for light that keeps the body in tune with external time. It contains illumination detecting retinal ganglion cells to signal for the absence or presence of light and it is mediated through the retinohypothalamic tract to the suprachiasmatic nucleus[31]. Melatonin secretion increases near about 10 folds during darkness and falls towards low levels during appearance of the light of day[31]; these fluctuations in melatonin secretion help to entrain the body's biological rhythms with the external light dark cues. It appears to enhance immunity and has been shown to reverse some of the age-related shrinkages of the thymus[32] which is the source of T-lymphocytes. For some other mammals, it controls the seasonal breeding by targeting the hypothalamus[33].

## 4. Melatonin: As free radical scavenger

Free oxygen radicals are produced normally as byproducts during cellular metabolism that utilize oxygen. These radicals of oxygen contain unpaired free valence electrons. Oxygen is particularly susceptible to this conversion, as its outer pair of electrons spin is in the same direction, thus tending to accept electrons one at a time. This free radical form of oxygen is more reactive than the non-radical oxygen, such as the reactive oxygen species (ROS). These include superoxide anion, perhydroxyl radical, singlet oxygen, hydroxyl radical, hypochlorous acid, peroxyl, lipid peroxide, *etc*[34]. Sometimes non-radical molecules are also termed as 'ROS' because of their oxidation nature like the hydrogen peroxide[35]. Generally, ROS are essential for some normal physiological processes like spermatogenesis, sperm capacitation, hyperactivation and acrosome reaction, but an increased ROS production causes oxidative damage to the cells by inducing 'oxidative stress'[36].

Endogenous anti-oxidative agents scavenge the reactive oxygen radicals and inhibit the oxidation of other molecules with ROS to prevent the cellular oxidative damage[36]. Several endogenous antioxidant molecules like catalase, glutathione peroxidases, superoxide dismutase, thioredoxin, vitamin C, vitamin E, *etc* play vital role in maintaining the seminal redox homeostasis[37]. Melatonin has also been considered as an important classical endogenous antioxidant molecule[38] due to its several distinguishable qualities. These include its amphiphilic nature enabling it to easily cross the blood-brain barrier or blood placental barrier[39], and its interactions with nuclear receptor[40]. Melatonin can mediate its antioxidant actions either in a receptor-dependent pathway[41] as well as by directly scavenging the free radicals[42]. Moreover, the ability of melatonin in upregulating antioxidant enzymes and downregulating prooxidant enzymes makes it a unique oxygen scavenger[43]. Melatonin may interact with ROS indirectly by participating in redox cycling unlike vitamin C, glutathione, *etc*, and form several stable metabolites or end products through some add-on reactions in a deluge manner which can act as antioxidant too and also can easily be excreted *via* urine[44]. For example, 3-hydroxymelatonin is more potent than its primary form in reducing hypervalent hemoglobin, on the other hand, $N^1$-acetyl-5-methoxykynuramine may show powerful ROS scavenging capacity than its precursor and thus may prevent protein oxidation[45]. Melatonin can also enhance the activities of some traditional antioxidants including alpha-tocopherol, glutathione peroxidase, ascorbate and superoxide dismutase, *etc*[46]. It also may stimulate the mRNA levels of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and catalase[8].

## 5. Melatonin in male reproduction

### *5.1. In puberty and sexual maturation*

During fetal life (midgestational fetus) the hypothalamic-pituitary-gonadal (HPG) axis is active but becomes quiescent towards the term due to the inhibitory feedback of placental hormones and regains at the birth time[47]. The gonadotropin-releasing hormone (GnRH) and subsequently the gonadotropin levels gradually attain their peaks during the first three months of the life and again declines towards the sixth months remaining quiescent until puberty is attained[47]. Its further reactivation depends on the gradual and continuous release of GnRH resulting in successive secretion of gonadotropins[48]. From one experimental model it has been shown that melatonin secretion may inhibit the hypothalamic pulsatile release of GnRH in male Djungarian hamster[49]. The melatonin levels attain their peak from two to four years of age, and on the other hand the reductions in nocturnal plasma melatonin occur around or prior to pubescence[3]. Thus, it can be said that melatonin may play a role in inhibiting the hypothalamic GnRH activation. During puberty, body mass increases and this is inversely related to melatonin secretion leading to falling in its levels below the threshold concentrations, and so the GnRH pulse generator is re-activated and male reproductive functions are potentiated[50]. Therefore, it is concluded that the decline of melatonin levels trigger puberty and high nocturnal level of melatonin secretion may be associated with delayed puberty in children[51]. Relatively low levels of melatonin than the normal may be associated with precocious puberty in some clinical conditions like destructive pineal tumors in young boys[13].

In the year of 2005, kisspeptin was discovered as a most powerful activator of GnRH neuron located in the hypothalamus[52]. Being a peptide, kisspeptin is expressed in the arcuate nucleus and in the anteroventral periventricular nucleus of the forebrain abundantly and it is encoded by *Kiss1* gene and both estradiol and testosterone regulate the expression of the *Kiss1* gene[53]. It is well known to all that the mammalian reproductive system is controlled under the pulsatile release of GnRH and it is mediated by the effect of that kisspeptin. It is also noticed that, for the process of human puberty, all the GnRH neurons do not always get input from the kisspeptin neurons[54] and some other neurons are also responsible like RFamide-related peptide (RFRP) neurons which secrete RFRP-1 and RFRP-3, a member of the RFamide peptide group, known to inhibit GnRH, and also it may play a role in between melatonin and GnRH release due to the effect of melatonin on RFRP neurons[55]. Some animal studies reveal that followed by pinealectomy (a major source of endogenous melatonin), reduction in kisspeptin expression was observed under exogenous melatonin supplementation along with prolonged photoperiodic exposure[33]. But depending on duration of exogenous melatonin, supplement kisspeptin is regulated; its acute supplementation may reduce the expression of kisspeptin gene at first but when it was used for longer time, it enhanced the kisspeptin gene expression which may again interfere in HPG axis to regulate the release of gonadotropins[56]. Thus, different effects of melatonin on the reproductive system may be modulated by kisspeptin and which is also dependent on the duration of supplementation of the melatonin.

In a cross-sectional study, Waldhauser *et al* mentioned that as comparing to age group 1 year to 3 years, the mean nighttime serum melatonin level was dropping progressively throughout the adolescence age group of 15 to 20 years[57]. It indicates that during the adolescence, decreased nocturnal melatonin levels are directly proportional to sexual maturation and inversely proportional with Tanner stage[58]. When exogenous melatonin was used to treat animals, it would suppress the GnRH secretion[59] as the emergence of exogenous melatonin administration may alter the sexual maturation[60]. Nowadays, most important concern during the treatment of sleep disturbances is whether the very young or children or adolescents are being affected on their pubertal growth due to the exogenous melatonin supplementation.

### 5.2. Melatonin and HPG axis

Release of melatonin is utterly dependent on light and darkness cycle as well as seasonal variations. The breeding capacity of seasonal animals varies with these variations and this has already been linked with seasonal variations of melatonin[61]. Melatonin, along with hypothalamic and pituitary hormones, plays prime functions in regulation of reproductive functions. The HPG axis is the principal reproduction regulatory axis[62] but it may be modulated by several crosstalks with metabolic hormones[63], growth factors, and other endogenous influencers thereby interfering with its effects on reproductive functions[64-66]. Melatonin treatment to the pups of female rats showed disrupted melatonin profiles in growing pups indicating its maternal inheritance in the regulation of reproductive development[67]. It has also been reported that delayed onset of pubertal changes due to reduced luteinizing hormone (LH) and prolactin profiles can be mitigated by melatonin treatment to the mother[68]. Exogenous melatonin has been reported to show inhibitory effects on gonadotrophins secretions affecting the male reproductive development by interfering with follicle-stimulating hormone actions on Sertoli cells[69]. It has been shown that in isolated pituitary cells melatonin administration decreases the effects of LH-releasing hormone[70]. These inhibitory actions of melatonin are mediated by alterations in intracellular second messenger concentrations, specifically by increasing $Ca^{2+}$ influx and cAMP accumulation inside the cells[71]. These second messengers potentiate the effects of GnRH on LH secretion, but melatonin administrations affect their concentrations[72]. Melatonin treatment also increases testosterone secretion that negatively regulates gonadotrophin secretions and thus sexual maturation[72].

Melatonin directly affects the neurons in suprachiasmatic nuclei and that secrete GnRH to mediate its effects[73]. Its receptors have also been reported in pars tuberalis and pars distalis of the anterior pituitary[74]. But, the actions of melatonin on sexual functions by hypothalamic and pituitary neurons are reported to be distinct. Inactivation of melatonin receptors has been also reported to disrupt diurnal rhythms and reproductive functions[75]. Expression of melatonin receptors in hypothalamic and pituitary neurons potentiates the hypothesis of its roles in reproductive functions *via* GnRH pulse. Implantation of melatonin containing pellets in hypothalamus has been reported to reduce testicular weights in rats by 60% compared to control rats[76]. Melatonin treatment to male mile over 10 days have shown a decrease in testicular and accessory organ weights along with reduced spermatogenesis[77]. Successive administration of gonadotrophins has been reported to reverse these effects[78]. Inhibitory actions of melatonin on GnRH secretions have been evidenced in isolated GnRH cells following melatonin administration[79].

Melatonin has also been reported to down-regulate the expression of LH-β and follicle-stimulating hormone-β as mediated by gonadotropin-inhibitory hormone (GnIH) to inhibit GnRH synthesis and secretion[80]. In photoperiodic animals, pinealectomy has been found to disrupt reproductive functions due to the decreased melatonin secretion and altered GnIH production which have been reversed with exogenous melatonin administration[81]. Under short-day conditions Siberian hamsters have exhibited higher GnIH expressions, indicating GnIH expression controls actions of melatonin[81]. However, all these data postulate that GnRH alters the actions of melatonin and thereby its regulation over reproductive functions.

### 5.3. Melatonin and testicular steroidogenesis

Melatonin is also a key regulator of steroidogenesis and testicular and accessory sex organ development. Chronic exposure of light to hamsters have been found be related with altered tubular and interstitial structures in testis[82]. Melatonin administration has also been reported to reduce mitochondrial and smooth endoplasmic reticular volume and surface area which are the key locations for testicular steroidogenesis[83]. Moreover, testosterone biosynthesis is dependent on LH-mediated cAMP actions on Leydig cells[84]. Rats treated with exogenous melatonin decreased Leydig cell cAMP concentrations and thus reduced LH signaling. Administration of luzindole, a melatonin receptor antagonist, has been reported to restore the cAMP concentrations in Leydig cells and thus stimulates LH functioning for testicular steroidogenesis[85]. It is also found to reduce the LH-induced expression of steroidogenic acute regulatory protein by decreasing cAMP concentrations in Leydig cells[85]. Other than cAMP-dependent actions of melatonin, it also decreases GnRH-dependent $Ca^{2+}$ release and protein kinases activation which are associated with testosterone biosynthesis and secretion[86].

Along with reduction of LH- and GnRH-induced steroidogenesis, melatonin also affects testosterone production *via* acting through hypothalamic-pituitary-adrenal axis[87] and also through the actions of corticotropin-releasing hormone, cortisol or corticosterone. The corticotropin-releasing hormone produced by Leydig cells

acts as a prime autocrine regulator of GnRH-induced testicular steroidogenesis[88]. Isolated Leydig cells from hamster testis, exposed to different photoperiods and melatonin concentrations showed decreased cAMP and androstane-3α, 17β-diol levels[27], with reduced expressions of steroidogenic acute regulatory protein (StAR), P450scc (side-chain cleavage), 3β- and 17β-hydroxysteroid dehydrogenase[87]. Although it has been reported that melatonin upregulates mRNA expression of corticotropin-releasing hormone, use of corticotropin-releasing hormone antagonists can eliminate the effects of corticotropin-releasing hormone, or melatonin indicating melatonin pathway is independent of corticotropin-releasing hormone regulation[27].

### 5.4. Melatonin and oxidative stress in spermatozoa

In the sperm, melatonin reduces oxidative damage in the mitochondria, DNA fragmentation, lipid peroxidation of plasma membrane, and apoptotic markers by improving antioxidant activity of enzymatic systems and reducing ROS levels[11]. In addition to the antioxidative properties of melatonin in seminal plasma[89], it has been also been demonstrated to have a direct action in sperm capacitation[90,91]. Thus, high levels of seminal melatonin may protect sperm from oxidative damage, and prevent capacitation at the same time[92]. Though there is less amount of melatonin found in ejaculated sperm in the female reproductive tract, the melatonin present in follicular fluid helps in the process of capacitation[42]. It has been reported that melatonin regulates seminal calmodulin level and thus controls several reproductive functions, like hyperactivation, capacitation, and acrosome reaction[93]. Melatonin, through its receptors on sperm membrane, $MT_1$ and $MT_2$, exert direct actions on spermatozoa[92]. $MT_2$ has been reported to be directly concerned with the process of capacitation[89]. Melatonin has been reported to regulate secretion of bicarbonates and mobilization of calcium, and thus it may control the process of sperm capacitation by regulating the effects of calcium and bicarbonates on sperm[94].

### 5.5. Melatonin and semen quality

Spermatogenic disruption can be observed in testes exposed to endocrine disruptors or genital infections[13,95–98]. Melatonin is well-known to protect testis from these reproductive disruptions[44,62]. Lower seminal levels of melatonin have also been associated with male infertility[99]. Declined seminal melatonin has also been reported to cause decreased sperm motility[100]. These data point toward the role of melatonin in the regulation of semen quality. The receptor of the sperm membrane also indicated its role in sperm production and maturation[101]. It has been reported that in rams reproductive functions are under the influence of photoperiod and semen quality decreases after the breeding season[102]. Melatonin has been proven to improve semen quality in rams and male Damascus goats in non-breeding seasons[92]. Isolated ram spermatozoa exposed to melatonin showed improved capacitation and phosphatidylserine translocation. These changes decline at lower dosage of melatonin[92]. Melatonin due to its potential antioxidative properties has been reported to protect sperm cells from ischemia and improves sperm abnormalities[103]. Sperm maturation medium with high melatonin has been found to increase sperm progressive motility, raise the number of motile sperms, improve sperm mitochondrial activity, along with decreasing endogenous nitric oxide levels[104]. Cryopreserved sperms for insemination has shown an improvement in motility, straight-linear velocity and average path velocity when exposed to high levels of melatonin, even stored at 5 ℃ and 17 ℃[105]. The role of melatonin in amelioration of sperm damage is associated with various signaling cascades. It reduces sperm DNA fragmentation and apoptosis induced by hydrogen peroxide through $MT_1$ and extracellular signal-regulated kinases[11].

### 5.6. Melatonin on sperm preservation

As discussed above, melatonin due to its antioxidative and antiapoptotic functions improves semen quality[106]. It has been reported that boar spermatozoa treated with 100 nM melatonin improved sperm motility, sperm membrane integrity, sperm mitochondrial functions along with increased numbers of embryos produced through *in vitro* fertilization[107]. Succu *et al* with different doses of melatonin showed increased sperm motility, sperm chromatin integrity and intracellular adenosine triphosphate (ATP) concentration in ram spermatozoa[108]. Souza *et al*[109] with various concentrations of melatonin also supported these observations in ram spermatozoa and reported higher sperm motility, sperm membrane and acrosome integrity, and mitochondrial activity in melatonin treated sperms. It has been proposed that improvement of sperm motility in melatonin-treated spermatozoa is attributed to its effects of sperm mitochondrial functions[110]. Ashrafi *et al*[105] with different concentrations of melatonin to a freezing extender for bull spermatozoa reported better sperms functions with improved antioxidant profile in seminal plasma. El-Raey *et al*[111] with two different concentrations of melatonin (0.10 and 0.25 mM) to a freezing extender for buffalo spermatozoa have reported improved semen quality, plasma membrane and acrosome integrity, with higher conception rate after freeze-thawing. Lanconi *et al*[112] with 1 mM melatonin added to a freezing extender for horse spermatozoa reported same observations. For human spermatozoa, Karimfar *et al*[113] have reported the same with multiple doses of melatonin. Altogether, these reports reveal the semen quality-improving potential of melatonin and its beneficial effects on sperm cryo survival with fertility-enhancing actions in different species. However, in dogs, semen quality-improving and cryo survival-enhancing functions are not very much evident. Epididymal spermatozoa of German and Belgian Shepherd dogs incubated with

1 mM melatonin showed similar sperm motility, plasma membrane and acrosome integrity, capacitation status, and membrane fluidity after freeze-thawing compared to untreated sperms[114].

In some cases, the negative effects of the dissolving medium have been noted on semen quality. Gwayi and Bernard have reported negative effects of ethanol in rat spermatozoa[115]. But, Martín-Hidalgo *et al*[116] did not find any detrimental effects of ethanol as a melatonin-dissolving medium on boar spermatozoa.

Studies have also reported negative effects of high levels of melatonin on cryopreserved sperms on its fertilizing potential. It has been hypothesized that excessive neutralization of ROS generation and inhibition of oxidative phosphorylation may reduce sperm viability as well as motility[109]. Melatonin, which has been evident to facilitate sperm functions including capacitation, hyperactivation, and acrosome reaction, is a universal antioxidant, but its excessive dosage may hinder the normal functioning of ROS needed for these sperm functions. These reports indicate the importance of identification of optimal dosage of melatonin for cryopreservation in different species.

## 6. Conclusions

The present review article has discussed the properties of melatonin, its receptors and the possible mechanisms by which it influences male reproductive functions. It appears to be clear that melatonin affects both the HPG axis as well as testicular hormone actions and prevents testicular oxidative damage *via* its antioxidative properties thereby improving sperm quality. Further studies should be undertaken to unveil considering melatonin as a therapeutic target to combat oxidative stress-induced male subfertility or infertility.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

## References

[1] Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *J Am Chem Soc* 1958; **80**(10): 2587-2587.

[2] Singh M, Jadhav HR. Melatonin: Functions and ligands. *Drug Dis Today* 2014; **19**(9): 1410-1418.

[3] Wurtman RJ. Age-related decreases in melatonin secretion—clinical consequences. *J Clin Endocrinol Metab* 2000; **85**(6): 2135-2136.

[4] Payne JK. The trajectory of biomarkers in symptom management for older adults with cancer. *Semin Oncol Nurs* 2006; **22**(1): 31-35.

[5] Karasek M. Melatonin, human aging, and age-related diseases. *Exp Gerontol* 2004; **39**(11-12): 1723-1729.

[6] Hardeland R. Melatonin in aging and disease—multiple consequences of reduced secretion, options and limits of treatment. *Aging Dis* 2012; **3**(2): 194.

[7] Reiter RJ, Tan Dx, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: Reducing the toxicity and increasing the efficacy of drugs. *J Pharm Pharmacol* 2002; **54**(10): 1299-1321.

[8] Rodriguez C, Mayo JC, Sainz RM, Antolín I, Herrera F, Martín V, et al. Regulation of antioxidant enzymes: A significant role for melatonin. *J Pineal Res* 2004; **36**(1): 1-9.

[9] Malpaux B, Viguie C, Skinner D, Thiery J, Pelletier J, Chemineau P. Seasonal breeding in sheep: Mechanism of action of melatonin. *Anim Reprod Sci* 1996; **42**(1-4): 109-117.

[10]Shiu SY, Law IC, Lau KW, Tam PC, Yip AW, Ng WT. Melatonin slowed the early biochemical progression of hormone-refractory prostate cancer in a patient whose prostate tumor tissue expressed $MT_1$ receptor subtype. *J Pineal Res* 2003; **35**(3): 177-182.

[11]Espino J, Ortiz Á, Bejarano I, Lozano GM, Monllor F, García JF, et al. Melatonin protects human spermatozoa from apoptosis *via* melatonin receptor–and extracellular signal–regulated kinase-mediated pathways. *Fertil Steril* 2011; **95**(7): 2290-2296.

[12]Rocha C, Rato L, Martins A, Alves M, Oliveira P. Melatonin and male reproductive health: Relevance of darkness and antioxidant properties. *Curr Mol Med* 2015; **15**(4): 299-311.

[13]Sengupta P, Dutta S, Krajewska-Kulak E. The disappearing sperms: Analysis of reports published between 1980 and 2015. *Am J Men's Health* 2017; **11**(4): 1279-1304.

[14]Sengupta P, Borges Jr E, Dutta S, Krajewska-Kulak E. Decline in sperm count in European men during the past 50 years. *Hum Exp Toxicol* 2018; **37**(3): 247-255.

[15]Sengupta P, Nwagha U, Dutta S, Krajewska-Kulak E, Izuka E. Evidence for decreasing sperm count in African population from 1965 to 2015. *Afr Health Sci* 2017; **17**(2): 418-427.

[16]Sengupta P, Dutta S, Tusimin MB, Irez T, Krajewska-Kulak E. Sperm counts in Asian men: Reviewing the trend of past 50 years. *Asian Pac J Reprod* 2018; **7**(2): 87-92.

[17]Sengupta P. Reviewing reports of semen volume and male aging of last 33 years: From 1980 through 2013. *Asian Pac J Reprod* 2015; **4**(3): 242-246.

[18]Pévet P. Melatonin receptors as therapeutic targets in the suprachiasmatic nucleus. *Exp Opinion Ther Targ* 2016; **20**(10): 1209-1218.

[19]Reiter RJ, Mayo JC, Tan DX, Sainz RM, Alatorre-Jimenez M, Qin L. Melatonin as an antioxidant: Under promises but over delivers. *J Pineal Res* 2016; **61**(3): 253-278.

[20]Xu X, Liu X, Ma S, Xu Y, Xu Y, Guo X, et al. Association of melatonin production with seasonal changes, low temperature, and immuno-responses in hamsters. *Molcules* 2018; **23**(3): 703.

[21]Walton JC, Weil ZM, Nelson RJ. Influence of photoperiod on hormones, behavior, and immune function. *Front Neuroendocrinol* 2011; **32**(3): 303-319.

[22]Foulkes NS, Whitmore D, Sassone-Corsi P. Rhythmic transcription: The molecular basis of circadian melatonin synthesis. *Biol Cell* 1997; **89**(8): 487-494.

[23]Johansson LC, Stauch B, McCorvy JD, Han GW, Patel N, Huang XP, et al. XFEL structures of the human MT2 melatonin receptor reveal the basis of subtype selectivity. *Nature* 2019: **569**: 289-292.

[24]Liu J, Clough SJ, Hutchinson AJ, Adamah-Biassi EB, Popovska-Gorevski M, Dubocovich ML. MT1 and MT2 melatonin receptors: A therapeutic perspective. *Ann Rev Pharmacol Toxicol* 2016; **56**: 361-383.

[25]Slominski RM, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: Distribution and functions. *Mol Cell Endocrinol* 2012; **351**(2): 152-166.

[26]Nosjean O, Nicolas JP, Klupsch F, Delagrange P, Canet E, Boutin JA. Comparative pharmacological studies of melatonin receptors: MT1, MT2 and MT3/QR2. Tissue distribution of MT3/QR2. *Biochem Pharmacol* 2001; **61**(11): 1369-1379.

[27]Rossi S, Windschuettl S, Matzkin M, Terradas C, Ponzio R, Puigdomenech E, et al. Melatonin in testes of infertile men: Evidence for anti-proliferative and anti-oxidant effects on local macrophage and mast cell populations. *Andrology* 2014; **2**(3): 436-449.

[28]Amaral FGd, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metab* 2018; **62**(4): 472-479.

[29]Jockers R, Delagrange P, Dubocovich ML, Markus RP, Renault N, Tosini G, et al. Update on melatonin receptors: IUPHAR Review 20. *Brit J Pharmacol* 2016; **173**(18): 2702-2725.

[30]Emet M, Ozcan H, Ozel L, Yayla M, Halici Z, Hacimuftuoglu A. A review of melatonin, its receptors and drugs. *Eur J Med* 2016; **48**(2): 135.

[31]Hankins MW, Peirson SN, Foster RG. Melanopsin: An exciting photopigment. *Trends Neurosci* 2008; **31**(1): 27-36.

[32]Tian YM, Li PP, Jiang XF, Zhang GY, Dai YR. Rejuvenation of degenerative thymus by oral melatonin administration and the antagonistic action of melatonin against hydroxyl radical-induced apoptosis of cultured thymocytes in mice. *J Pineal Res* 2001; **31**(3): 214-221.

[33]Revel FG, Masson-Pévet M, Pévet P, Mikkelsen JD, Simonneaux V. Melatonin controls seasonal breeding by a network of hypothalamic targets. *Neuroendocrinology* 2009; **90**(1): 1-14.

[34]Silva JP, Coutinho OP. Free radicals in the regulation of damage and cell death--basic mechanisms and prevention. *Drug Disc Ther* 2010; **4**(3): 144-167.

[35]Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 2015; **30**(1): 11-26.

[36]Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. *BioMed Res Int* 2014; 2014: 761264. Doi: 10.1155/2014/761264.

[37]He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol Biochem* 2017; **44**(2): 532-553.

[38]Dragojevic Dikic S, Jovanovic AM, Dikic S, Jovanovic T, Jurisic A, Dobrosavljevic A. Melatonin: A "Higgs boson" in human reproduction. *Gynecol Endocrinol* 2015; **31**(2): 92-101.

[39]Tarocco A, Caroccia N, Morciano G, Wieckowski MR, Ancora G, Garani G, et al. Melatonin as a master regulator of cell death and inflammation: Molecular mechanisms and clinical implications for newborn care. *Cell Death Dis* 2019; **10**(4): 317.

[40]Dai J, Ram PT, Yuan L, Spriggs LL, Hill SM. Transcriptional repression of ROR α activity in human breast cancer cells by melatonin. *Mol Cell Endocrinol* 2001; **176**(1-2): 111-120.

[41]Dubocovich ML, Markowska M. Functional MT 1 and MT 2 melatonin receptors in mammals. *Endocrine* 2005; **27**(2): 101-110.

[42]Tamura H, Takasaki A, Taketani T, Tanabe M, Kizuka F, Lee L, et al. The role of melatonin as an antioxidant in the follicle. *J Ovarian Res* 2012; **5**(1): 5.

[43]Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. *Clin Inter Aging* 2018; **13**: 757.

[44]Darbandi M, Darbandi S, Agarwal A, Sengupta P, Durairajanayagam D, Henkel R, et al. Reactive oxygen species and male reproductive hormones. *Reprod Biol Endocrinol* 2018; **16**(1): 87.

[45]Tan DX, Manchester L, Esteban-Zubero E, Zhou Z, Reiter R. Melatonin as a potent and inducible endogenous antioxidant: Synthesis and metabolism. *Molecules* 2015; **20**(10): 18886-18906.

[46]Milczarek R, Hallmann A, Sokołowska E, Kaletha K, Klimek J. Melatonin enhances antioxidant action of α-tocopherol and ascorbate against NADPH-and iron-dependent lipid peroxidation in human placental mitochondria. *J Pineal Res* 2010; **49**(2): 149-155.

[47]Kuiri-Hänninen T, Sankilampi U, Dunkel L. Activation of the hypothalamic-pituitary-gonadal axis in infancy: Minipuberty. *Horm Res Paediatr* 2014; **82**(2): 73-80.

[48]Rey RA, Campo SM, Ropelato MG, Bergadá I. Hormonal changes in childhood and puberty. In: Kumanov P, Agarwal A. (eds.) *Puberty*. Switzerland: Springer, Cham; 2016, p. 23-37.

[49]Buchanan KL, Yellon SM. Delayed puberty in the male Djungarian hamster: Effect of short photoperiod or melatonin treatment on the GnRH neuronal system. *Neuroendocrinology* 1991; **54**(2): 96-102.

[50]Silman R. Melatonin and the human gonadotrophin-releasing hormone pulse generator. *J Endocrinol* 1991; **128**(1): 7-11.

[51]Hay I, Cohen H, Annesley T, Wallace A, Spooner R, Thomson J, et al. Serum immunoreactive melatonin in boys with idiopathic delayed puberty. *Clin Endocrinol* 1982; **17**(5): 517-521.

[52]Han SK, Gottsch ML, Lee KJ, Popa SM, Smith JT, Jakawich SK, et al. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. *J Neurosci* 2005; **25**(49): 11349-11356.

[53]Dungan HM, Clifton DK, Steiner RA. Minireview: Kisspeptin neurons as central processors in the regulation of gonadotropin-releasing hormone secretion. *Endocrinology* 2006; **147**(3): 1154-1158.

[54]Critchlow V, Bar-Sela ME. Control of the onset of puberty. *Neuroendocrinology* 1967; **2**: 101-162.

[55]Simonneaux V, Ancel C, Gauer F, Poirel VJ. Kisspeptins and RFRP-3

act in concert to synchronize rodent reproduction with seasons. *Front Neurosci* 2013; **7**: 22.

[56]Gingerich S, Wang X, Lee P, Dhillon S, Chalmers J, Koletar M, et al. The generation of an array of clonal, immortalized cell models from the rat hypothalamus: Analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. *Neuroscience* 2009; **162**(4): 1134-1140.

[57]Waldhauser F, Weiszenbacher G, Tatzer E, Gisinger B, Waldhauser M, Schemper M, et al. Alterations in nocturnal serum melatonin levels in humans with growth and aging. *J Clin Endocrinol Metab* 1988; **66**(3): 648-652.

[58]Crowley SJ, Acebo C, Carskadon MA. Human puberty: Salivary melatonin profiles in constant conditions. *Dev Psychobiol* 2012; **54**(4): 468-473.

[59]Roy D, Belsham DD. Melatonin receptor activation regulates *GnRH* gene expression and secretion in GT1–7 GnRH neurons signal transduction mechanisms. *J Biol Chem* 2002; **277**(1): 251-258.

[60]Andersen LPH, Gögenur I, Rosenberg J, Reiter RJ. The safety of melatonin in humans. *Clin Drug Invest* 2016; **36**(3): 169-175.

[61]Tabecka-Lonczynska A, Mytych J, Solek P, Kulpa M, Koziorowski M. New insight on the role of melatonin receptors in reproductive processes of seasonal breeders on the example of mature male European bison (*Bison bonasus*, Linnaeus 1758). *J Photochem Photobiol B: Biol* 2017; **173**: 84-91.

[62]Dutta S, Sengupta P, Muhamad S. Male reproductive hormones and semen quality. *Asian Pac J Reprod* 2019; **8**(5): 189-194.

[63]Irez T, Karkada IR, Dutta S, Sengupta P. Obestatin in male reproduction and infertility. A*sian Pac J Reprod* 2019; **8**(5): 239-243.

[64]Krajewska-Kulak E, Sengupta P. Thyroid function in male infertility. *Front Endocrinol* 2013; **4**: 174.

[65]Sengupta P, Dutta S. Thyroid disorders and semen quality. *Biomed Pharmacol J* 2018; **11**(1): 1-10.

[66]Sengupta P, Dutta S, Tusimin M, Karkada IR. Orexins and male reproduction. *Asian Pac J Reprod* 2019; **8**(5): 233-238.

[67] Tenorio FD, Simões MD, Teixeira VW, Teixeira ÁA. Effects of melatonin and prolactin in reproduction: Review of literature. *Rev Assoc Med Bras* 2015; **61**(3): 269-274.

[68]Esouifino A, Villanua M, Agrasal C. Effect of neonatal melatonin administration on sexual development in the rat. *J Steroid Biochem* 1987; **27**(4-6): 1089-1093.

[69]Lang U, Aubert M, Rivest R, Vinas-Bradtke J, Sizonenko P. Daily afternoon administration of melatonin does not irreversibly inhibit sexual maturation in the male rat. *Endocrinology* 1984; **115**(6): 2303-2310.

[70]Hattori A, Herbert DC, Vaughan MK, Yaga K, Reiter RJ. Melatonin inhibits luteinizing hormone releasing hormone (LHRH) induction of LH release from fetal rat pituitary cells. *Neuroscience letters* 1995; **184**(2): 109-112.

[71]Vanecek J. Melatonin inhibits release of luteinizing hormone (LH) *via* decrease of [$Ca^{2+}$] i and cyclic AMP. *Physiol Res* 1998; **47**(5): 329-335.

[72]Damassa DA, Kobashigawa D, SmitH ER, Davidson JM. Negative feedback control of LH by testosterone: A quantitative study in male rats. *Endocrinology* 1976; **99**(3): 736-742.

[73]Wierman ME, Bruder JM, Kepa JK. Regulation of gonadotropin-releasing hormone (*GnRH*) gene expression in hypothalamic neuronal cells. *Cell Mol Neurobiol* 1995; **15**(1): 79-88.

[74]Weaver D, Stehle J, Stopa E, Reppert S. Melatonin receptors in human hypothalamus and pituitary: Implications for circadian and reproductive responses to melatonin. *J Clin Endocrinol Metab* 1993; **76**(2): 295-301.

[75]Comai S, Ochoa-Sanchez R, Gobbi G. Sleep–wake characterization of double MT1/MT2 receptor knockout mice and comparison with MT1 and MT2 receptor knockout mice. *Behav Brain Res* 2013; **243**: 231-238.

[76]Glass D, Knotts LK. A brain site for the antigonadal ation of melatonin in the white-footed mouse *(Peromyscus leucopus*): Involvement of the immunoreactive GnRH neuronal system. *Neuroendocrinology* 1987; **46**(1): 48-55.

[77]Forger NG, Zucker I. Photoperiodic regulation of reproductive development in male white-footed mice (*Peromyscus leucopus*) born at different phases of the breeding season. *Reproduction* 1985; **73**(1): 271-278.

[78]Ghosh PK, Biswas R, Mandal H, Biswas N. Effect of continual light deprivation and alpha-2u-globulin replacement therapy on serum concentration of gonadotropins and testicular activity in rats. *Andrologia* 1996; **28**(6): 309-313.

[79]Kelestimur H, Ozcan M, Kacar E, Alcin E, Yılmaz B, Ayar A. Melatonin elicits protein kinase C-mediated calcium response in immortalized GT1–7 GnRH neurons. *Brain Res* 2012; **1435**: 24-28.

[80]Tsutsui K, Ubuka T, Bentley GE, Kriegsfeld L. regulatory mechanisms of gonadotropin-inhibitory hormone (GnIH) synthesis and release in photoperiodic animals. *Front Neurosci* 2013; **7**: 60.

[81]Ubuka T, Son YL, Bentley GE, Millar RP, Tsutsui K. Gonadotropin-inhibitory hormone (GnIH), GnIH receptor and cell signaling. *Gen Comp Endocrinol* 2013; **190**: 10-17.

[82]Mason AO, Duffy S, Zhao S, Ubuka T, Bentley GE, Tsutsui K, et al. Photoperiod and reproductive condition are associated with changes in RFamide-related peptide (RFRP) expression in Syrian hamsters (*Mesocricetus auratus*). *J Biol Rhythm* 2010; **25**(3): 176-185.

[83]Redins C, Redins G, Novaes J. The effects of treatment with melatonin on the ultrastructure of mouse Leydig cells: A quantitative study. *Braz J Biol* 2002; **62**(3): 517-523.

[84]Stojilković SS, Chang JP, Ngo D, Keiichi T, Shun-Ichiro I, Catt KJ. Mechanism of action of GnRH: The participation of calcium mobilization and activation of protein kinase C in gonadotropin secretion. *J Steroid Biochem* 1989; **33**(4): 693-703.

[85] Wu CS, Leu SF, Yang HY, Huang BM. Melatonin inhibits the expression of steroidogenic acute regulatory protein and steroidogenesis in MA-10 cells. *J Androl* 2001; **22**(2): 245-254.

[86]Valenti S, Thellung S, Florio T, Giusti M, Schettini G, Giordano G. A novel mechanism for the melatonin inhibition of testosterone secretion by rat Leydig cells: Reduction of GnRH-induced increase in cytosolic $Ca^{2+}$. *J Mol Endocrinol* 1999; **23**(3): 299-306.

[87]Frungieri MB, Mayerhofer A, Zitta K, Pignataro OP, Calandra RS, Gonzalez-Calvar SI. Direct effect of melatonin on Syrian hamster testes: Melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. *Endocrinology* 2005; **146**(3): 1541-1552.

[88]Dufau ML, Tinajero JC, Fabbri A. Corticotropin-releasing factor: Aan antireproductive hormone of the testis. *FASEB J* 1993; **7**(2): 299-307.

[89]Gonzalez-Arto M, Hamilton TRdS, Gallego M, Gaspar-Torrubia E, Aguilar D, Serrano-Blesa E, et al. Evidence of melatonin synthesis in the ram reproductive tract. *Andrology* 2016; **4**(1): 163-171.

[90]Li C, Zhou X. Melatonin and male reproduction. *Clin Chim Acta* 2015; **446**: 175-180.

[91]Saki G, Mirhoseini M, Hemadi M, Khodadadi A, Amiri FBT. The effect of the melatonin on cryopreserved mouse testicular cells. *Int J Reprod BioMed* 2016; **14**(1): 23.

[92]Casao A, Mendoza N, Pérez-Pé R, Grasa P, Abecia JA, Forcada F, et al. Melatonin prevents capacitation and apoptotic-like changes of ram spermatozoa and increases fertility rate. *J Pineal Res* 2010; **48**(1): 39-46.

[93]Tulsiani DR, Zeng H, Abou-Haila A. Biology of sperm capacitation: Evidence for multiple signalling pathways. *Soc Reprod Fertil Suppl* 2007; **63**: 257.

[94]Cebrián-Pérez J, Casao A, González-Arto M, dos Santos Hamilton T, Pérez-Pé R, Muiño-Blanco T. Melatonin in sperm biology: Breaking paradigms. *Reprod Domest Anim* 2014; **49**: 11-21.

[95]Sengupta P. Current trends of male reproductive health disorders and the changing semen quality. *Int J Prev Med* 2014; **5**(1): 1.

[96]Sengupta P. Recent trends in male reproductive health problems. *Asian J Pharm Clin Res* 2014; **7**(2): 1-5.

[97]Sengupta P. Environmental and occupational exposure of metals and their role in male reproductive functions. *Drug Chem Toxicol* 2013; **36**(3): 353-368.

[98]Dutta S, Biswas A, Sengupta P. Obesity, endocrine disruption and male infertility. *Asian Pac J Reprod* 2019; **8**(5): 195-202.

[99]Awad H, Halawa F, Mostafa T, Atta H. Melatonin hormone profile in infertile males. *Int J Androl* 2006; **29**(3): 409-413.

[100]Yie SM, Daya S, Brown G, Deys L, YoungLai E. Melatonin and aromatase stimulating activity of human seminal plasma. *Andrologia* 1991; **23**(3): 227-231.

[101]Van Vuuren RJ, Pitout MJ, Van Aswegen CH, Theron JJ. Putative melatonin receptor in human spermatozoa. *Clin Biochem* 1992; **25**(2): 125-127.

[102]Azawi O, Al Khashab A, Al Kadoo N. Effect of gonadotropin releasing hormone treatment on semen characteristics and enzymatic activities of Awassi rams in breeding and non breeding seasons. *Iran J Appl Anim Sci* 2012; **2**(1): 13-19.

[103]Koksal M, O uz E, Baba F, Eren MA, Ciftci H, Demir M, et al. Effects of melatonin on testis histology, oxidative stress and spermatogenesis after experimental testis ischemia-reperfusion in rats. *Eur Rev Med Pharmacol Sci* 2012; **16**(5): 582-588.

[104]Du Plessis S, Hagenaar K, Lampiao F. The *in vitro* effects of melatonin on human sperm function and its scavenging activities on NO and ROS. *Andrologia* 2010; **42**(2): 112-116.

[105]Ashrafi I, Kohram H, Ardabili FF. Antioxidative effects of melatonin on kinetics, microscopic and oxidative parameters of cryopreserved bull spermatozoa. *Anim Reprod Sci* 2013; **139**(1-4): 25-30.

[106]Bejarano I, Monllor F, Marchena AM, Ortiz A, Lozano G, Jiménez MI, et al. Exogenous melatonin supplementation prevents oxidative stress-evoked DNA damage in human spermatozoa. *J Pineal Res* 2014; **57**(3): 333-339.

[107]Jang H, Kim Y, Kim B, Park I, Cheong H, Kim J, et al. Ameliorative effects of melatonin against hydrogen peroxide-induced oxidative stress on boar sperm characteristics and subsequent *in vitro* embryo development. *Reprod Domest Anim* 2010; **45**(6): 943-950.

[108]Succu S, Berlinguer F, Pasciu V, Satta V, Leoni GG, Naitana S. Melatonin protects ram spermatozoa from cryopreservation injuries in a dose-dependent manner. *J Pineal Res* 2011; **50**(3): 310-318.

[109]Souza WL, Moraes EA, Costa J, Sousa PH, Lopes Junior ES, Oliveira RP, et al. Effect of different concentrations of melatonin to ram spermatozoa on oxidative stress after cryopreservation. *Pesq Veter Brasil* 2016; **36**(7): 657-664.

[110]Jou MJ, Peng TI, Yu PZ, Jou SB, Reiter RJ, Chen JY, et al. Melatonin protects against common deletion of mitochondrial DNA-augmented mitochondrial oxidative stress and apoptosis. *J Pineal Res* 2007; **43**(4): 389-403.

[111]El-Raey M, Badr M, Assi M, Rawash Z. Effect of melatonin on buffalo bull sperm freezability, ultrastructure changes and fertilizing potentials. *Assiut Vet Med J* 2015; **61**(144): 201-208.

[112]Lançoni R, Celeghini E, Bianchi-Alves M, Santos G, Florez-Rodriguez S, Leite T, et al. Use of melatonin and ferulic acid as promoters of cryopreserved equine sperm. *Anim Reprod* 2015; **12**(3): 559.

[113]Karimfar M, Niazvand F, Haghani K, Ghafourian S, Shirazi R, Bakhtiyari S. The protective effects of melatonin against cryopreservation-induced oxidative stress in human sperm. *Int J Immunopathol Pharmacol* 2015; **28**(1): 69-76.

[114]Varesi S, Vernocchi V, Morselli MG, Luvoni GC. DNA integrity of fresh and frozen canine epididymal spermatozoa. *Reprod Biol* 2014; **14**(4): 257-261.

[115]Gwayi N, Bernard R. The effects of melatonin on sperm motility *in vitro* in Wistar rats. *Andrologia* 2002; **34**(6): 391-396.

[116]Martín-Hidalgo D, Barón F, Bragado M, Carmona P, Robina A, Garcia-Marin L, et al. The effect of melatonin on the quality of extended boar semen after long-term storage at 17 ℃. *Theriogenology* 2011; **75**(8): 1550-1560.