Review Article

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The Role of Melatonin in Ewes Reproduction: A Review

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Abstract

This review was assigned to illustrate the melatonin (MLT) effect on reproduction in sheep (ewes). However, the pathway by which MLT charge the seasonal reproduction are imperfectly understood in sheep, the researchers consents that MLT regulates reproduction under influence of day length (photoperiod) to guarantee that birth happen in appropriate date of the year, it's called neuro-endocrine process. This mechanism mediates by the pineal hormone (MLT). The major role of MLT in ewes is to translate the photo signals into endocrine pulses (gonadotropin-releasing hormone (GnRH) secretion) at the end of the retino-pineal pathway. In sheep, the MLT receptors distributed in premammillary hypothalamus (PMH), pituitary gland and Suprachiasmatic nucleus (SCN), therefore, many brain loci are participating for MLT pathways to modify the seasonal reproduction. Melatonin stimulates GnRH secretion through effect in different regions and neurons in hypothalamus such as a pre-mammillary nucleus, Arcuate and several factors like kisspeptin, RF-amide related peptide-3 (RFRP-3) and Tyrosine Hydroxylase (TH). In addition, its indirectly control prolactin (PRL) output via an effect on Tuberalin release, which is mediate the mechanism of MLT activity on pituitary PRL secretion and regulate his seasonal cyclicity. The alter in day length is the principle ecological factor that control the breeding in seasonal domestic animals. Several reproductive activities are related to short days and begin during autumn when the day becomes short and a decline in temperature (short-day breeder). While expanding in the duration of light lead to a cessation in reproduction activities during late winter and early spring. In conclusion, according to the major physiological role of MLT, it can be used in different aspects in ewes reproduction industry such as induce oestrus, increment the ovulation rate and In vitro embryo production.

Keywards: Melatonin, Reproduction, Ewe.

دور الميلاتونين في تكاثر النعاج

الخلاصة

تم اجراء هذه المراجعة لتوضيح تأثير الميلاتونين على التكاثر في الأغنام (النعاج). على الرغم من إن المسار الذي يتحكم هرمون الميلاتونين من خلاله بالتناسل الموسمي غير مفهوم بشكل كامل في الأغنام ، يتوافق الباحثون على أن الميلاتونين ينظم التكاثر تحت تأثير طول اليوم (الفترة الضوئية) لضمان حدوث الولادة في التوقيت المناسب من السنة ، وتسمى هذه بعملية الترابط العصبية-الغدد الصماء. تتوسط هذه الآلية هرمون الصنوبرية (الميلاتونين). ان الدور الرئيسي للميلاتونين في النعاج هو ترجمة الإشارات الضوئية إلى دفقات افرازية من الغدد الصماء (إفراز الهرمون المحفز لغشاء القند) في نهاية المسار الشبكي الصنوبري. في الاغنام, تتوزع مستقبلات الميلاتونين في المنطقة المهادية قبل الحلمية, الغدة النخامية والنواة والنواة فوق التصالبية, لذلك، العديد من مواقع الدماغ تشارك في مسارات الميلاتونين لتحفيز التناسل الموسمي. يحفز الميلاتونين إفراز الهرمون المحفز لغشاء القند من خلال التأثير في مناطق وخلايا عصبية مختلفة في منطقة ما تحت المهاد مثل نواة ما قبل الحلمية، النواة الوطائية المقوسة وعدد من العوامل المحفزة مثل القُبل او الكيسبيتين), اميد الببتيد RF والانزيم المختزل للتايورسين. بالإضافة إلى ذلك ، فانه يتحكم بشكل غير مباشر في إنتاج البرولاكتين من خلال التأثير على إطلاق توبيرالين، والذي يتوسط آلية نشاط الميلاتونين على إفراز البرولاكتين من الغدة النخامية وينظم دورته الموسمية. التغيير في طول اليوم هو العامل البيئي الأساسي الذي يتحكم في تربية الحيوانات الحقلية الموسمية. ترتبط العديد من الفعاليات التناسلية مع الأيام القصيرة وتبدأ خلال فصل الخريف عندما يصبح النهار قصيرًا تبدأ درجات الحرارة في الانخفاض. بينما تؤدي الزيادة في مدة الضوء إلى التوقف في أنشطة التكاثر خلال أواخر الشتاء وأوائل الربيع. وفقًا لدورالميلاتونين الفسيولوجي الرئيسي المتعدد الأشكال ، يمكن استخدامه في جوانب مختلفة في الصناعة التكاثرية للنعاج كاستحداث الشبق، زيادة نسبة الخصب وإنتاج الأجنة في المختبر.

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Introduction

The MLT of cattle Pineal gland (PG) is discovered in 1958, the and distinguished as (N-acetyl-5-methoxy-tryptamine) by dermatologist Aaron Lerner and his colleagues, the MLT is came from lightening skin of Lerners coworkers during the work on amphibians, certain fishes and reptiles (1). Melatonin is a small indole molecule that has the following chemical names: '5-Methoxy-N-acetyltryptamine' and 'N-Acetyl-5-methoxytryptamine' with atomic formula: C13H16N2O2 (Hattori *et al*., 1995), MLT half-life in sheep is (16-18) min. (2). Melatonin has a major factor that coordinate the reproduction in seasonal estrous animal model, in ewes, the MLT can prompt estrous and enhance litter size (3), increment the ovulation rate (4), improve luteal activity by a reduction in the antiluteolytic mechanisms, it also increases embryo viability and improve lamb production (5). Furthermore, the MLT treatment have an effect on ovine fecundity, fertility and sex ratio (6). In ram, MLT could improve the spermatozoal progressive motility and the fertile spermatozoa quantity (7).

Melatonin was considered as direct potent and indirect antioxidants (free radical scavengers) that maximized many antioxidant enzymes expression like superoxide dismutase and glutathione peroxidase (8). This review was designed to describe the MLT effect on reproduction in ewe.

1. Biosynthesis of Melatonin

Biosynthesis of MLT occured through the precursor tryptophan in four enzymatic steps hydroxylation, decarboxylation, acetylation and methylation (9). Firstly, 'L-tryptophan' is hydroxylated at indole ring through 'tryptophan hydroxylase' (10). Then, the intermediate '5-hydroxyl-L-tryptophan' is decarboxylated via '5-hydroxy-L-tryptophan decarboxylase' to produce serotonin called '5-hydroxytryptamine' (11). After that, 'serotonin' is transformed into 'N-acetyl-serotonin' via 'serotonin arylalkylamine N-acetyl transferase' (AA-NAT) and by 'acetyl-CoA' (12). At the end, 'N-acetyl-serotonin' changed over into MLT by 'Hydroxyindole O-methyl transferase (HIOMT)' through methylation of the hydroxyl group (13). Whereas levels of HIOMT action stay decently consistent, the diurnally MLT production is controlled by a concurrent rhythm of AA-NAT enzyme action (14). The Pineal is a unique endocrine gland that is affected straightly by outer/external conditions by means of retina and changes over natural environmental signals into neuroendocrine messages (15). The neural information that is produced in the retina passed to hypothalamic SCN via retino-hypothalamic tract (16). The SCN is connected to the PG by many synaptic ways include hypothalamus (paraventricular nucleus) that connects and sends neural signals into 'spinal cord', the spinal neurons (sympathetic spinal neurons) send the neural signals to 'superior cervical ganglion (SCG)' neurons (17). Finally, the noradrenergic sympathetic neurons of the SCG are synapses to the PG via noradrenergic fibers (18).

These sympathetic fibers spur both MLT receptors (α- and β- adrenergic receptors) in the pinealocyte to made intracellular increment of 'cyclic Guanosine Monophosphate (cGMP)' and 'cyclic Adenosine Monophosphate (cAMP)'; these increase in intracellular cAMP improved Nacetyltransferase (NAT) activity (19). The AA-NAT function is controlled by 'retino-pineal path', and that represent crucial component of MLT synthesis (20). (Figure 1).

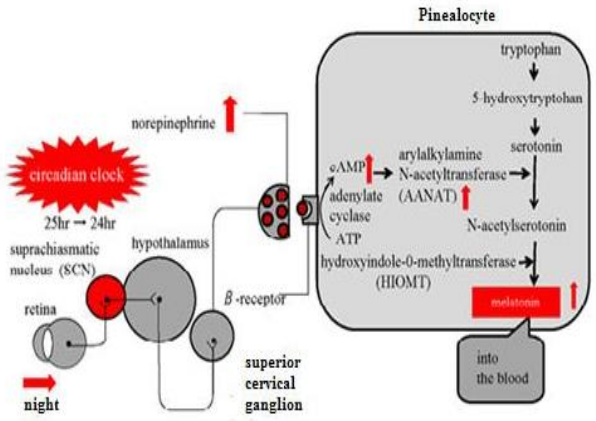

Figure 1: The neural pathway and MLT production (21).

2. Secretion and excretion of Melatonin

The MLT which is synthesized by pinealocyte and diffuses directly into cerebrospinal Fluid (CSF) and capillary blood without store inside the PG (22). The MLT could reach the CSF via two ways, the first deliver during daytime via the little number of distended pinealocytes of the PG which is located at the basal part of pineal recess, so the interstitial fluid discharged into ventricular lumen or through the connection with CSF (23). The rest pinealocyte of PG that secreted the MLT straight forward to blood flow and are taken up from the blood to CSF by the choroid plexus (24). The brain conveyed MLT through the blood capable to elicit the effectiveness of light (photoperiod) on reproduction (23).

The metabolism of MLT occurs via cytochromes P450 (P450s) in liver through changing over it to 6-Hydroxy-MLT as a final product and is cleared through the liver after a single passage, a little amount is discharged into urine and a small amount is found in saliva (25).

3. Melatonin receptors and signaling pathway

Melatonin receptor 1 (MTNR1) is referred to the first type of MLT receptors which is cloned and has characterized (26). It is mediate inhibition of the cAMP through a G protein-coupled receptor (27). According to the same researchers, the the pituitary gland specially pars tuberalis (PT) contains a vast number of MTNR1. It also presents in PMH, that considered as a target structure of MLT for it is reproductive effects (28).

Melatonin receptor 2 (MTNR2) refers to the ovine MLT2 receptor, it is a 'pertussis toxin (PTX)-sensitive (Gi) protein-linked receptor', which is able of inhibit cGMP and cAMP production, it also spurs 'Protein Kinase C (PKC)' action in SCN, additionally, the receptor was expressed in PT, choroid plexus and retina (29). Both MLT receptors have a general motif and which have 7 trans-membrane á-helical sections containing a (20- 25) hydrophobic residues, these á-helical segments span the cell membrane, and it associated with extra and intracellular loops, the structure also bind with the amino acids at the end of the external membrane side and on the 'carboxyl term group' at the internal side (30). About 350 amino acids which encoded by MLT1 receptor gene and 362 amino acid by MLT2 receptor gene, additionally, two consensus locales for 'N-terminal asparagine connected glycosylation' demonstrated by MLT1 receptor and single site in MLT2 receptor (31). The inner receptors (2 n) have consensus sites for regulatory signal enzymes like PKC, casein kinase I and casein kinase II at carboxyl end (32).

The signaling pathway of both MLT receptors are firmly associate to the Gi/cAMP path that inhibits cAMP production via Gi proteins, so the activation of both MLT receptors diminishes cAMP formation by forskolin stimulation (33). The signaling of MTNR1 can couple to both Gi and 'PTX-insensitive (Gq) proteins' (34). The activation of MTNR1 diminishes forskolin-stimulated cAMP formation via Gi protein, therefore, the inhibition includes 'Protein kinase A(PKA)' and 'cAMP responsive

element-binding protein (CREB)' (35). Godson and Reppert (1997) (36) mentioned that the βγ subunit of Gi protein of MTNR1 mediate the phospholipase via prostaglandin F2α (PGF2α) stimulation that prompts to increment in phosphoinositide (PI3) turnover. The intracellular calcium is rised by activation of the endogenous MLT1 receptors in PT cells of sheep, this mechanism happened through Gi proteins/ PI3 of MTNR1 likewise control ion fluxes pathway (37) (Figure 2).

The MTNR2 signaling inhibits both of cGMP and cAMP forming (38). In SCN, MLT activate the MTNR2 signaling throughout PKC, this pathway mediates the MLT at both night and dawn (phase-shifting effects of MLT) (39). The MTNR2 signaling by Gi proteins pathway can shut down the 'PKC-mediated c-fos induction' in the PT cells (40). Additionally, MTNR2 inhibit neurotransmitter liberation in the retina by through intracellular calcium regulation (41) (Figure 2).

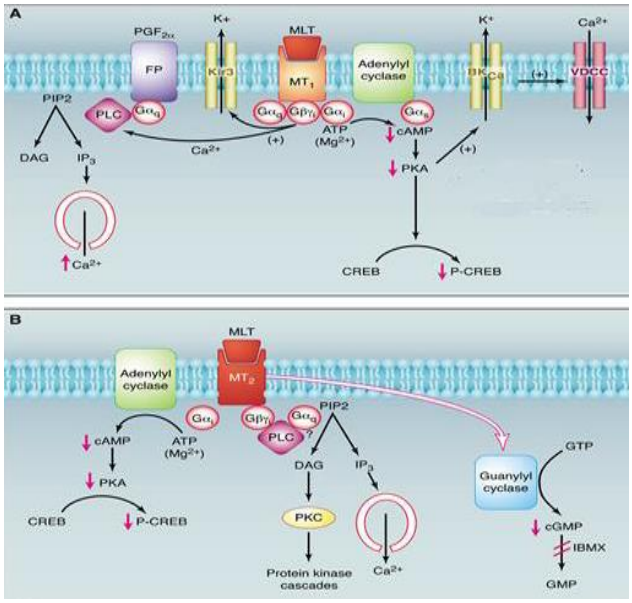

Figure 2: A- Signaling pathways of MLT1A through activation of the MTNR1, B- Signaling pathways of MTNR2 activation (42). PLC: phospholipase C. PKA: protein kinase A, PGF2α: prostaglandin F2α, GTP: guanosine triphosphate, DAG: diacylglycerol, PIP2: phosphatidylinositol bisphosphate, GMP: guanosine monophosphate.

4. Mechanism of action of melatonin

4.1. Effect on GnRH

In ewes, the large environmental factor that controls the breeding is the day length (photoperiod) (43). The day light exposure variance modify the production and releasing of MLT from PG, and in turns that binds to the nuclei of hypothalamus and regulate the pulses releasing of GnRH (44).

Despite the fact that MLT work at several aspects of the reproductive system in ewes, the MLT principle activity in the premammillary region of the caudal hypothalamus within the central nervous system (45).

In the ewes, Malpaux et al (2001) (46) listed various evidences about the target site of MLT is pre-mammillary nucleus of premammillary hypothalamus (PMH) to which acts to modulate GnRH/gonadotropin releasing and regulate reproductive actions. While, the short time MLT administration into central nervous system of ewes does not spur GnRH and LH secretion during seasonal anestrus (47).

The ovine PMH region of the cerebrum engages the caudal district of Arcuate region (ARC) and the ARC containing an intensive population of kisspeptin cells (45). These cells are responsible for Kisspeptin output by the expression of the Kiss1 gene, its a peptide hormone that empower GnRH production (48) (49).

The MLT treatment control the expression of Kiss1 in cell lines (50). The expression of Kiss1 was higher three time in the ARC region in Soay ovary-intact ewes that placed in a photoperiod of 16 dark hrs. and eight hrs. light (16D:8L) than other ewes on longer photoperiods (51). In ewes, a minimize kisspeptin function is related with loss of cyclicity at non-breeding period or season, and the kisspeptin injection in

such animals can prompt ovulation (52). Additionally, Kisspeptin treatment stimulate the hypothalamic–pituitary– gonadal axis during non-breeding season in goat, the removal of inactivity is associated with rising of plasma testosterone concentration (53).Otherwise, Median Eminence (ME) is also the target of MLT in ewes, the MLT caused block the TH activity (is a crucial enzyme in dopamine synthesis), so the MLT induce alteration (decline) in TH function on the dopamine secretory ME neurons can regulate GnRH pulsatile secretion because an rise in dopamine inhibits of GnRH and therefore LH secretion is diminish, these opinion was confirmed by the experiment of Viguie et al (1998) (54), they find that LH secretion was stimulated by suppress of TH in the ME long day inhibited ewes. In addition, Goodman et al (2012) (55) support the speculation that dopamine prevents synthesis and release of GnRH and LH at anestrum phase in sheep by exert a repressing role on the 'ARC kisspeptin neurons' because these neurons are critical for reproductive function and seasonal changes.

A new observation is suggested that MLT control of season through its effect on Gonadotropin-inhibitory hormone termed RFRP-3 that influence the GnRH neurons (negative correlation) (56). In sheep, RFRP3 expressing cells are found transcendently in the specific part of hypothalamus (dorso-medial nuclei) and these cells project to the ME, that region of GnRH cells (57). In ewes model, the increases in RFRP-3 gene expression happen at long daylight hrs. (20L: 4D) (58). In addition, the impacts of season on RFRP-3 hormone seemed to be based on on seasonal MLT fluctuations and the response to photoperiod is anticipated by pinealectomy and neutralized by MLT treatment (59).Morever, the melatonin implantation give a good results for improving seminal quality in Holstein bulls, conception rate and reproductive performance in cows (60)(61).

4.2. Effect on Prolactin

In seasonal mammals, PT plays a direct role in regulating the annual PRL cycle (62). The researchers reported a PRL releasing factor called Tuberalin, which is released by the ovine PT specific thyrotrophs that impacts on increment of c-fos gene expression and to stimulate PRL promoter activity in a subpopulation of lactotrophs to prompt Messenger Ribonucleic acid (mRNA) expression and PRL secretion (63) (Figure 3). Over 90% of ovine PT cells are chromophobe cells that produce Tuberalin (64).

In ewes, the PT has a high concentration of MLT receptors (65). The hypothalamo-pituitary-disconnected rams showed well-defined seasonal cycles in PRL release with low PRL blood concentration under short day conditions (66). On the other hand, the MLT implants in post-partum ewes caused a decline in PRL secretion under long photoperiod occurred in spite of the high stimulation of suckling (67).

The release of Tuberalin is enhanced by forskolin and the cAMP that activated Tuberalin secretion is inhibited by MLT that lead to decrease in PRL production (63). Because of the absence of MLT receptors on lactotrophs, it is reasonable to propose that PT may mediate the monitored effect of MLT by secretes Tuberalin and proposed that endocrine effect of Tuberalin which is necessary in the pituitary mechanism of MLT activity specially in regards to the regulation of the seasonal cycle of PRL (68).

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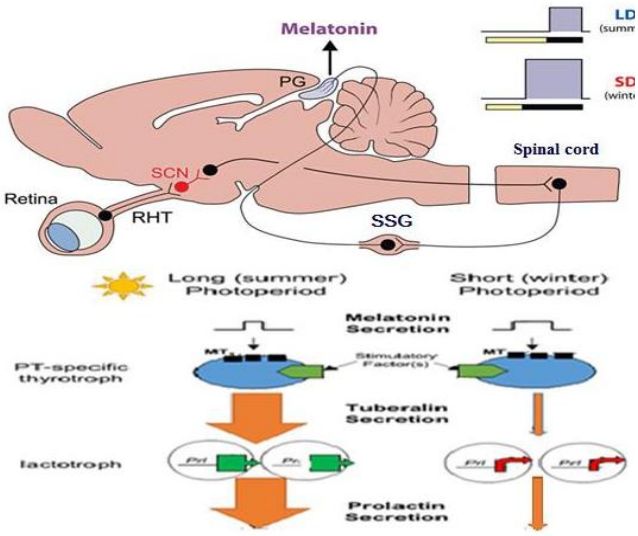

Figure 3: Regulation of PG the secretion of MLT by photoperiod and Model of intra-pituitary mechanisms driving photoperiodic PRL secretion (69)(70).

5. Factors effecting melatonin biosynthesis and secretion

The biosynthesis and secretion of MLT follow a circadian rhythm with high level at night and low levels during the day in both blood and CSF (71). The exposure to light quickly inhibits MLT synthesis and its secretion into the blood (72). Hence, because of changes in the time of night and day, the rhythm of MLT secretion is effected by the cycle of the seasons (73).

The concentration of MLT secretion varies highly between species, the sheep and Siberian hamsters considered type C animals; that mean the MLT levels reach a peak slightly after the onset of the dark night (10-30 min.) and stay elevated along the entire night and decline at the time of the light onset (74). In sheep which kept in the same conditions, the night-time concentration in circulating blood varies between individuals of similar age, which is originated from the variances in MLT production but not from catabolism (2). This changeability is under hereditary control because of the heritability coefficient which was observed to be 0.53 in humans and 0.45 in sheep (75).

There are several factors that effect on MLT like location of sampling, pregnancy status, flock, ewe age interaction (76). The MLT concentration in old age ewes lower than ewes in 12-18 months old and in three years old ewes (77). Furthermore, Redondo et al (2003) (78) point out that the plasma MLT levels are higher in pubertal sheep than in infants or matured sheep. On the other hand, a decline in reproductive activity is observed correlated in the aging sheep with decease in MLT concentration that is restored by administration of MLT (79). The daily MLT concentration is different between in Seasonal and Aseasonal ewes. Hatif and Laith (2018a) (80) clarified that the relative MLT level was significant higher in non-seasonal as a compare with seasonal Awassi ewes under same circumference.

The MLT secretion may be affected by extra factors, since the precursor tryptophan is provided to the PG by the circulating blood, dietary intake of tryptophan may impact MLT fluctuation (81). In spite of the fact that the AA-NAT is a rate limiting enzyme, serotonin availability is one of the major factor that play an important regulatory role in MLT synthesis (82) and some evidences supposed that MLT synthesis is a part under serotoninergic control (83). In addition, MLT is inhibited by benzodiazepines via benzodiazepine receptors in the PG (84). Moreover, many reports pointed out that the polymorphism (genetic effect) in AA-NAT and MLTR genes influenced the sheep seasonal reproduction via effect on MLT output and affinity of its own receptor (85) (86) (87).

6. The use of MLT in Reproductive Techniques

The earlier induce reproductive activity in ewes showed by persistent MLT implant treatment (88), therefore, it been used in vivo to induce oestrus.

Melatonin was utilized to to enhance embryo production in Ovine multiple ovulation and embryo transfer (MOET) technique (89). The MLT implant was used for 3 months to promote the collected embryos viability and the ratio of oocyte reaching blastocysts for Rasa Aragonesa breed (90). In another way, the MLT has been demonstrated to keep most favorable conditions for homeostasis and mitochondrial function (91). This happen by reducing mitochondrial oxidative stress and consequently restrict subsequent apoptotic events and cell death (92), therefore, the MLT uses to improving oocyte quality in sheep superovulation (93). Both ovulation rate and the retrieve embryos number from ewes were significantly improved after MLT used (93).

Melatonin implicated in in vivo oocyte maturation, this suggestion came because existence of MLTR in granulosa cells (94). Its stimulate ovarian steroidogenic gene expression (95) and luteinisation of graffian follicle (96). During the anoestrous period, MLT regulate a follicular growth (have a strong role in regulation and development) and oocyte efficiency (97). Exogenous MLT conserve cumulus cells from DNA damage during In *vitro* maturation (IVM) (98) (99), and the MLT supplementation to the IVM and culture medium can reduced Reactive oxygen species (ROS) (antioxidant effect) and get better competence of oocytes, which led to rise the quality and quantity blastocyst proportions and improve the embryos quality.

Conclusions

Based on this review, there were a vast benefit for MLT uses. The induce estrus during breeding season by effect on GnRH is the major role for MLT. Its a part of IVM medium, because its preserve the oocyte competence and improve the embryo quality. The litter size is important in sheep industry, so the using of MLT showed a partial advantage.

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