REVIEW ARTICLE

Melatonin: Adjunct therapeutics in ovarian endometrioma

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ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine) hormone is a methoxyindole primarily released by the pineal gland. Melatonin has dynamic properties of being a free radical scavenger, inflammatory suppressor, anti-apoptotic agent, reproductive hormone modulator and mediator of several signaling pathways in female reproductive system. The presence of differential localization of melatonin receptors in the ovary, influence on the ovarian hormones and ovarian cycle has implied the association between ovarian system and melatonin both at the pathological and physiological levels. On the other hand, the pathophysiology of ovarian endometrioma is remarked by various anomalies likely: generation of various reactive oxygen species, followed by follicular damage, oocyte and embryonic defects, inflammation and apoptosis, which the involvement of melatonin can plausibly counteract. The present review evaluated melatonin's probable protective role against ovarian endometrioma via diverse signaling pathways.

Keywords: Melatonin receptors, ovarian cycle, ovarian endometrioma, oocyte-embryo quality, signaling pathways

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INTRODUCTION

Melatonin is a pleiotropic hormone that acts as a central mediator of the sleep-wake cycle, circadian/seasonal rhythm[1] and reproductive health.[2] Melatonin has antioxidative properties that can shield the female reproductive system from an imbalance in pro-oxidative and antioxidative defense mechanisms.[3] Melatonin indirectly affects gonadotropins,(4) which can modify various processes related to ovarian functions. Recent research is inclined towards melatonin's active role in follicular development, genesis and development of pre-ovulatory follicles into oocytes via various signalling pathways.[5–9]

Conversely, endometriosis is a chronic gynecological disorder affecting around 10% of premenopausal women.[10] This common disease is characterized by the development of ectopic endometrial tissue located outside the uterine walls, mostly in and around the ovaries, fallopian tubes, tissues lining the pelvis, ureters, lungs etc.[11] Endometriosis is marked by a milieu of abnormal conditions, notably oxidative stress,[12,13] inflammatory processes,[14,15] including endometrial angiogenesis and hormonal imbalance.[16] It is responsible for causing chronic pain with dysmenorrhoea and dyspareunia and might even lead to infertility.[17] Endometriosis is broadly classified into three different types i) deep infiltrating endometriosis ii) peritoneal superficial endometriosis and iii) ovarian endometriomas,[18] out of which ovarian endometrioma (OE) is considered to be the most common, affecting around 17 to 44% of women diagnosed with endometriosis.[19] Although the underlying pathogenesis of OE is still under speculation, the origin of endometriomas can be delineated on the basis of three theories. Accordingly, Hughesdon (1957) defined endometriomas as pseudocysts formed from menstrual debris upon bleeding from active implants located at the site of inversion.[20] Nisolle and Donnez (1997)[21] have proposed that the OE originates from metaplasia of the invaginated ovarian coelomic epithelium. And then Jain and Dalton (1999)[22] reported that OE originates from ovarian follicles. Similarly, on the basis of histological appearance, endometriomas are classified into two types: protopathic endometrioma and secondary endometrioma.[23] Collectively, this review delineates the pleiotropic functions of melatonin and summarizes its potential for providing new paradigm in future alternative therapeutics for OE. Additionally, the pharmacological and therapeutic benefits derived from the exogenous administration of melatonin, specifically on the overall ovarian functioning, are also discussed to support the notion of melatonin supplements toward the development of endometriosis. An exponential number of future clinical trials are required to confirm its remedial treatment and safety.

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Melatonin and Its Receptors in Ovary

Melatonin can affect ovarian functions through its receptors (MTs) found in humans[24] and rats.[25] MT1 and MT2 receptors are G-protein coupled receptors[26] expressed throughout the body, mediating its function depending on the cellular microenvironment. Elevated expression of MT1 (or Mel_{1a}) receptor protein was detected in the membrane fraction by western blot analysis and immunocytochemistry in a

study with carp ovary,[27] which was in parallel with reports from rat[25,28] and human[29] studies. This study has opened new avenues in research related to melatonin with other hormones and photoperiodicity. Evidence of numerous forms of melatonin receptor genes[30] were found in the human granulosa luteal cells, which further implied the fact that melatonin has some role in controlling ovarian functions via its receptors. Another study with chicken ovary has reported differential expression of melatonin receptors; Mel_{1a} (MT1), Mel_{1b} (MT2) and Mel_{1c} (MT3) either in the thecal/granulosa layer of different types of follicles.[31] Additionally, both granulosa and thecal layer have showed expression of Mel_{1b} and Mel_{1c} but Mel_{1a} receptor was seen to be expressed only in the thecal layer. Conclusively, differential distribution pattern might influence its downstream regulatory functions related to the ovaries.[32]

Melatonin and Ovarian Functionality

Melatonin controls the female reproductive system by acting upon the ovaries both directly and indirectly. Directly, melatonin has an impact on the ovaries via its anti-apoptotic and antioxidative[33] features, whereas indirectly it might affect the release of gonadotropins from the pituitary gland(4). The presence of MT1 and MT2 receptors in human luteal, granulosa and ovarian cells emphasize its regulatory role on the secretion of gonadal hormones.[24] Ovary has two major functions: driving the female reproductive system by producing hormones and orchestrating the process of ovarian folliculogenesis.

Impact of Melatonin on Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH)

Role of exogenous administration of melatonin at three different time points during the follicular and the mid-luteal phase of female menstrual cycle has hinted towards the modulatory impact of melatonin in context to the menstrual endocrine cycle.[34] Notably, in the follicular phase of menstrual cycle, melatonin application has led to elevation in mean LH levels without any significant change in the luteal phase. Contradictorily, in another clinical trial with twenty postmenopausal women, the suppressive effect of melatonin on LH was confirmed,[35] supporting the preliminary findings[24] of Woo et al. Melatonin receptors (MT1 and MT2) were detected in the ovary's granulosa cells, implying its probable physiological role at the level of ovary.[29] Next, control over the production of progesterone, LH receptor, Gonadotropin-releasing hormone (GnRH) and GnRH receptor gene expression via melatonin receptors in human granulosa-luteal cells was observed, confirming its influence on ovary.[24] Another study with Wistar rats inferred that melatonin to some extent was responsible for suppressing the hypothalamus-pituitary-ovarian (HPO) axis along with divergent regulation of steroid receptors found in the reproductive organs of rats during ovulation.[36]

Presence of melatonin receptors in bovine and porcine ovarian cells by the reverse transcription-polymerase chain reaction (RT-PCR) has highlighted its role in regulating follicular development.[37,38] In an in vitro study with goat preantral follicles, the addition of 1000 pM melatonin along with FSH resulted in a significant increase in follicular and oocyte diameters after culturing for 7 days.[39] Interestingly, another study on mouse granulosa cells (mGCs) has shown that upon silencing MT1, apoptosis of mGCs was increased which was partially reversed by FSH treatment.[40]

Conclusively, effect of melatonin on LH is influenced by different phases of menstrual cycle whereas FSH and melatonin function cooperatively to facilitate follicular development. These findings provide intricate information on the mechanism of action of melatonin in animal reproduction-related hormones.

Effects of Melatonin on Progesterone and Estradiol levels

Role of melatonin in steroidogenesis in the human ovary was tested in a study with granulosa lutein cells obtained from the follicular fluid of women undergoing In Vitro Fertilization (IVF).[41] In this study, the stimulatory effect of human chorionic gonadotropin (hCG) on progesterone (P) secretion was significantly elevated in the presence of melatonin, specifically after 144 and 196 hours of incubation without any notable change in the estradiol production. In bovine granulosa cells, though melatonin administration resonated an increase in progesterone production but had a marked inhibitory action on estradiol biosynthesis in a time-dependent manner.[42] The anti-estrogenic effect (only those ERα that are bound to CaM) of melatonin as a specific inhibitor of E(2)-induced ERalpha-mediated transcription was deduced via calmodulin interaction complex.[43] In a study with pinealectomized female rats, exogenous melatonin treatment for three months has resulted in a significant increase in progesterone receptors along with lower expression of proliferating cell nuclear antigen (PCNA) and vascular endothelial growth factor in their ovaries (VEGF).[44] These findings signify the probable role of melatonin in modulating ovarian structures with effects on the level of progesterone receptors. Melatonin treatment has also shown protection against ROS generation in luteinized granulosa cells with improvement in serum P concentrations in human studies, specifically during the mid-luteal phase.[45] This study points towards the positive effect of melatonin on progesterone production during ovulation. In a female mice model representing perimenopause condition, melatonin treatment interestingly resulted in inhibition of ovarian aromatase expression[46] which is of clinical relevance in menopause-related problems.

Notably, melatonin and progesterone might function synergistically and/or additively in reversing the excess estrogen state as evident in many female reproductive diseases.

Influence of Melatonin on Ovarian Cycle

Melatonin supplementation in *in-vitro* cultured mouse ovarian follicles can have direct effect on its folliculogenesis, oogenesis and theca cell steroidogenesis depending on the dosage being used.[47] In a randomized controlled trial, melatonin application to culture media of GC or cumulus-oocyte-complexes (COC) after *in-vitro* maturation (IVM) media has resulted in a positive effect on cytoplasmic maturation of immature human oocytes collected from patients with the polycystic ovarian syndrome (PCOS).[48] The protective effect of melatonin on primordial follicle might have some linkage with the mammalian target of the rapamycin (mTOR) pathway.[49] This study implies melatonin's role in fertility-related processes. In comparison to early follicular phase, melatonin did not have much impact on the mid-cycle LH surge during ovulation in women[50] as confirmed b^{125}I-radioimmunoassay. This study has suggested towards an inverse relationship between melatonin and LH levels. Studies with either a combination of synthetic progestin norethisterone and melatonin or melatonin alone has resulted in inhibition of ovulation:[51,52] suggesting its role in oral contraceptives. Similar results were obtained in ewes with melatonin treatment for 60 days.[53] Contradictorily, in some reports no changes/increase in ovulation rate upon melatonin treatment were observed.[54-56] Interestingly, in female pinealectomized Wistar rats upon melatonin supplementation, the ovarian polycystic condition was reversed,[57] implying its role in anovulation. In a clinical trial, melatonin administration has shown the protection of human GC cells undergoing luteinization against ROS(45) which was in line with another study in mice model.[58] Additionally, melatonin increased the production of progesterone,[59] further strengthening its role in the luteal phase of the cycle. During IVM rescue, dosage of 1nM concentration of melatonin had improved the nuclear maturation of human oocyte[60] which was supported by another study with controlled ovarian hyperstimulation[61] suggesting its role in oocyte development.

Conclusively, melatonin alone or with progesterone[62] administration has shown improvement at various ovarian cycle stages, implying its probable role in future combinatorial medication.

Ovarian Endometrioma and Ovarian Pathophysiology

Ovarian endometrioma (OE) is an estrogen dependent, inflammatory disease with ability to mimic metastasis and recurrence.[63] Disruption in estrogen-progesterone receptor expression was observed in patients diagnosed with OE.[64] In a retrospective case-control study including women diagnosed with OE undergoing IVF has raised concern over ovarian responsiveness to gonadotropins.[65] A study with 7-8 week old mouse preantral follicles was cultured in the presence of human endometriotic fluid, which adversely affected folliculogenesis and oocyte retrieval rate.[66] In a different experiment with ovarian endometriotic patients it was observed that follicular density obtained from the cortex of endometriomas ≤ 4cm was significantly lower as compared to the cortex of control ovaries, providing new insights into the loss caused at a very early stage of the disease.[67] The follicular loss can be correlated with oxidative stress which might pave way for studying early necrosis in follicles.[68] High concentration of ROS was reported in cases with endometrioma as compared to normal cysts,[69] which might adversely affect the Mitogen-activated protein kinase (MAPK) pathways, the Activator protein 1 (AP-1) transcription factor, the Nuclear factor kappa light chain enhancer of activated B cells (NF-kB) pathway and hypoxia-inducible transcription factors.[70,71] In patients undergoing IVF therapy, cumulus cell apoptosis was observed to be elevated with mitochondrial impairment in GC cells[72] Elevated expression of Matrix metalloproteinase-1 (MMP-1)[73] and MMP-9[74] has been reported in ovarian endometriotic tissues with lower levels of Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) in serum or peritoneal fluid of endometriotic patients.[75] Higher levels of interleukin (IL)-8 (IL-8) and IL-6[76] has been reported with some contradictory reports[77] in the endometrioma cyst. A prospective cohort study with unilateral and bilateral endometrioma patients observed significantly lower oocyte retrieval number without any significant change in the fertilization rate compared to the control patients.[78] This study can indicate that low success rate in these patients undergoing IVF is not due to a low receptive endometrium but due to poor quality of oocytes and embryos retrieved in due course.

Conversely, OE can adversely affect the follicles, oocyte maturation, oocyte reserve, cause dysregulation in MMPs-TIMPs balance, imbalance in cytokine levels and modulation of gonadotropin levels along with changes in expression of progesterone and estrogen receptors in women diagnosed with OE (Figure 1).

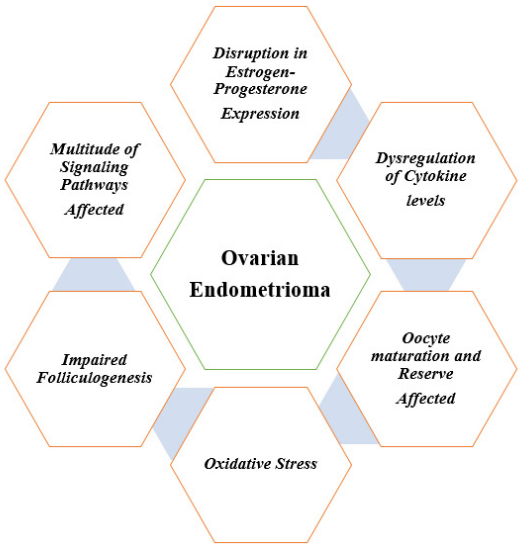

Figure 1: Pathophysiological anomalies in ovarian endometrioma

Ovarian endometrioma affects various signalling pathways, modulates the hormonal regulation of ovary, affects various stages of ovarian cycle with impairment of oocyte and embryo quality.

Protective Role of Melatonin

Oocyte and Embryo quality

In an IVM- IVF-embryo transfer programme for PCOS patients, melatonin supplementation has been reported to have an indirect role in improving poor cytoplasmic maturation and positively affectson implantation rates.[79] In another pilot study with unexplained infertility, irrespective of melatonin dosage concentration, intra-follicular oxidative stress was mitigated with betterment in oocyte quality.[80] In a mouse model, melatonin supplementation ameliorated Fenoxaprop-ethyl (FE, which is used as a herbicide) induced meiotic defects in oocytes, strengthening its protective impact on oocytes.[81] In a study with bovine oocytes, melatonin via MT1 receptor has led to increase in concentrations of Cyclic adenosine monophosphate (cAMP) and Cyclic Guanosine Monophosphate (cGMP) in oocytes and cumulus cells, respectively in promoting oocyte development.[9] Interestingly, mice oocytes with mitochondrial dysfunction and meiotic errors were prevented upon treatment with 10^{-3} M melatonin in culture medium with significant upregulation of sirtuin (SIRT1) expression.[82] This study's findings imply future perspectives on investigating SIRT1 dependent pathway in endometrioma patients undergoing IVF. A similar effect of melatonin was deduced in autophagy via expression of SIRT1[83] with a decrease in ROS generation in the oocytes of aged mice.

Cytokines and Inflammation

Melatonin administration in the Lupus mouse model has shown decreased production of IL-6 and IL-13;[84] which are an integral part of cytokine network in the development of endometriosis. Similarly, with human pulmonary fibroblasts, melatonin administration was able to counter the acrolein-induced IL-8 secretion.[85] The inhibitory effect of melatonin against lipopolysaccharide (LPS) induced inflammation and ROS damage in case of bovine mammary epithelial cells[86] was observed; implying the anti-inflammatory action of melatonin in diseased conditions. Another study with mice ovary after autograft transplantation along with melatonin supplementation has also shown improvement against oxidative stress and inflammatory conditions.[87]

Signalling pathways

Depending upon the concentration and timing of melatonin administration, fluctuation in MAPK activity was observed for the first time[24] with a probable role in steroidogenesis. In this study, a significant increase in LH receptor mRNA levels without any notable changes in the expression of the FSH receptor gene was also observed, implying a direct role of melatonin in modulating ovarian functions. Similarly, in bovine *in-vitro* study, it was found that melatonin via activation of phosphoinositide-3 kinase (PI3K)/AKT pathway can trigger expression of the steroidogenic acute regulatory protein (STAR) with the production of progesterone especially in small follicles through MT1 and MT2 receptors in ovarian theca cells.[5] This study further supported the role of melatonin in ovarian steroidogenesis. Additionally, the potential role of AKT signaling in bovine oocytes had shown a significant decrease in oocyte development in the presence of AKT inhibitor rescued by melatonin treatment.[6] Notably, this study has also accounted for the down-regulation of Bcl-2 associated X apoptosis regulator (BAX), caspase3 and p21 involvement in apoptosis of blastocysts, reporting the positive effect of both melatonin and AKT in embryo development. Another study with pregnant mice showed similar protective effect of melatonin via PI3K/AKT/GSK3β signalling pathway with reduced apoptosis in ovarian cells.[7] Melatonin has shown promising effect in improving cellular oxidative stress in human umbilical vein endothelial cells (HUVECs), possibly by inhibiting Janus kinase/ signal transducers and activators of transcription (JAK2/STAT3) pathway.[8] Here partial reversal effect of melatonin on increased levels of p-JAK2, p-STAT3, Cytochrome c, Bax and Caspase 3 was observed; suggesting its antioxidative property. Additionally, melatonin can be used to increase Epidermal growth factor receptor (EGFR) expression causing improvement in cumulus-oocyte complex maturation[9] which plays a pivotal role in mediating various factors involved in oocyte development (Figure 2).

Melatonin plays a protective role at various stages of the ovarian cycle by alleviating the generation of reactive oxygen species via JAK2/STAT3 pathway by an unknown mechanism. A decrease in the production of cytokines upon supplementation of melatonin can improve the inflamed condition. Improvement of oocyte development can be manifested by the increase in cAMP and cGMP upon stimulation by melatonin.

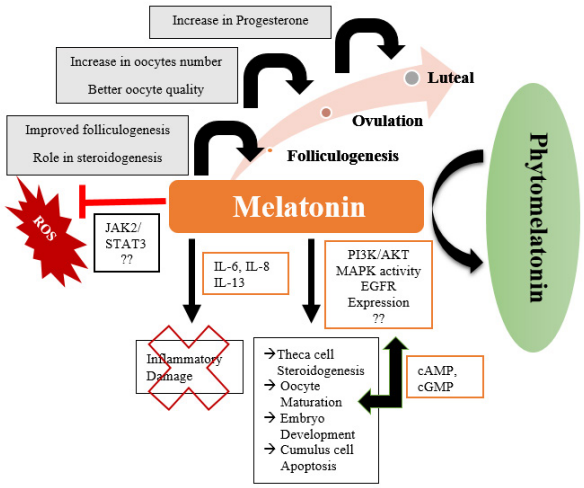

Figure 2: Hypothetical schematic for the protective effect of melatonin on ovarian functions via different pathways

CONCLUSION

From the review, it can be inferred that ovarian endometrioma is a dynamic disease that damages growing follicles, oocytes and embryo quality. The abnormal ovary is influenced by alterations at various phases of ovarian cycle, notably changes in gonadotropin levels, inflammatory cytokines, and apoptosis via various signaling pathways. Generally, melatonin functionality is thought to be proactive, specifically at the level of the pituitary gland for various reproductive processes. But in the recent literature, its presence is detected in follicular fluid along with binding sites in the granulosa-luteal cells; implying direct action of melatonin via receptor-mediated pathways at the level of the ovary. In particular, melatonin administration can have variable effects depending upon a plethora of other factors likely the cell type in the ovarian tissue, dosage, experimental setup, treatment timing, genetic variabilities, gender differences, stages of the disease and with nutritional status. Further clinical research with melatonin and/or novel analogs of melatonin as an adjunct might be effective in studying various downstream signaling pathways in OE patients probably in ways we cannot even imagine yet.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.