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## **The usefulness of melatonin in the field of obstetrics and gynecology**

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## **Abstract**

Disorders of the female reproductive system, including those associated with hormone regulation, fertility rate and fetal health, are issues of great concern worldwide. More recently, melatonin supplementation has been suggested as a therapeutic approach in gynecological practice. In both animal models and in women, melatonin supplementation suggests a therapeutic and preventative potential, effects attributed mainly to its antioxidant properties and action as hormone modulator. The aim of this literature review is to further investigate the evidence available on the effects of melatonin supplementation in animal and human studies, focusing on its potential application to gynecology. Melatonin-containing supplements are easily found in online and high street retailers, and despite its supplementation deemed to be relatively safe, no consensus has been reached on effective dosage and supplementation period. Short term supplementation studies, of up to six months, suggest that a daily posology of 2 to 18 mg of melatonin may have the potential to improve fertility rate, oocyte quality, maturation and number of embryos. However, the evidence available so far on the effects of melatonin supplementation covering gestational age and gestational outcomes is very scarce. Clinical trials and longer-term supplementation studies are required to assess any clinical outcome associated with melatonin supplementation in the field of gynecology.

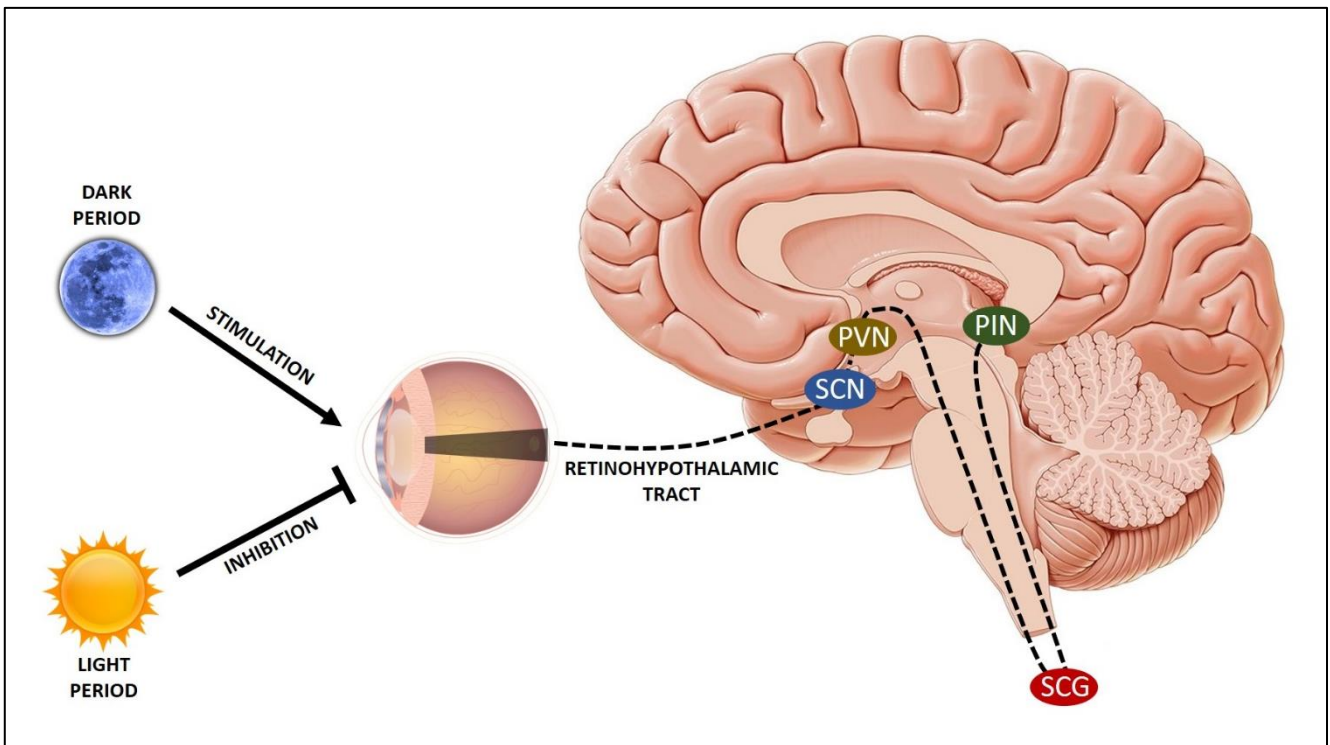
**Keywords:** gynecology; melatonin; reproductive system; antioxidant; fertility.

## 1. Introduction

The mammalian circadian clock covers a wide range of physiological processes and plays pivotal role in reproduction [1, 2]. Such physiological processes are coordinated by a robust genetic machinery known in lay terms as the "clock genes" [3-6]. Clock genes modulate the activity of regulatory nuclei in the hypothalamus-pituitary-gonad axis (HPG) and are abundantly expressed in the reproductive tract [3, 4, 7]. It is currently accepted that dysregulation of the circadian rhythm caused by night shifts, jet lag and sleep deprivation has a detrimental effect on the reproductive system [7-10].

A range of blood-borne stimuli and biomolecules contribute to the regulation of the circadian system. Melatonin is a hormone and an indolamine synthesized mostly at night, as summarized in figure 1. Melatonin is produced not only by the pineal gland, but also in glial cells, meningeal cells, and in other peripheral tissues, and its cyclical pattern of secretion is responsive to zeitgebers [11].

(Insert figure 1 here)



**Figure 1:** Brain circuits involved in melatonin biosynthesis through circadian regulation. Adapted from Tan et al. [11]. PIN, pineal gland; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SCG, superior cervical ganglion.

Melatonin permeability into the central nervous system was described decades ago [12] and its efficient transport through the blood-brain-barrier promotes accumulation in the brain at levels higher than the ones existing in the blood. Melatonin also possesses neuroprotective and antioxidant properties [13-16]. Modulation of redox signaling systems influence the reproductive system in both animals and humans [17, 18], and it is known that insufficient endogenous production of melatonin has been associated with disturbances in the reproductive system due to increased levels of reactive oxygen species (ROS), which are harmful to the male and female gametes [19-21].

As gametogenesis and gamete viability are negatively associated with increased age, successful reproductive strategies are directly related to the life stage of the individual [22]. Additionally, unhealthy lifestyles and psychosocial stress are aspects of modern life that have a negative impact on gynecological health and reproduction [23-25]. Epidemiological studies show that night shifts may negatively influence fetal development and may exacerbate gynecological and metabolic disorders, including endometriosis, diabetes and obesity [26, 27].

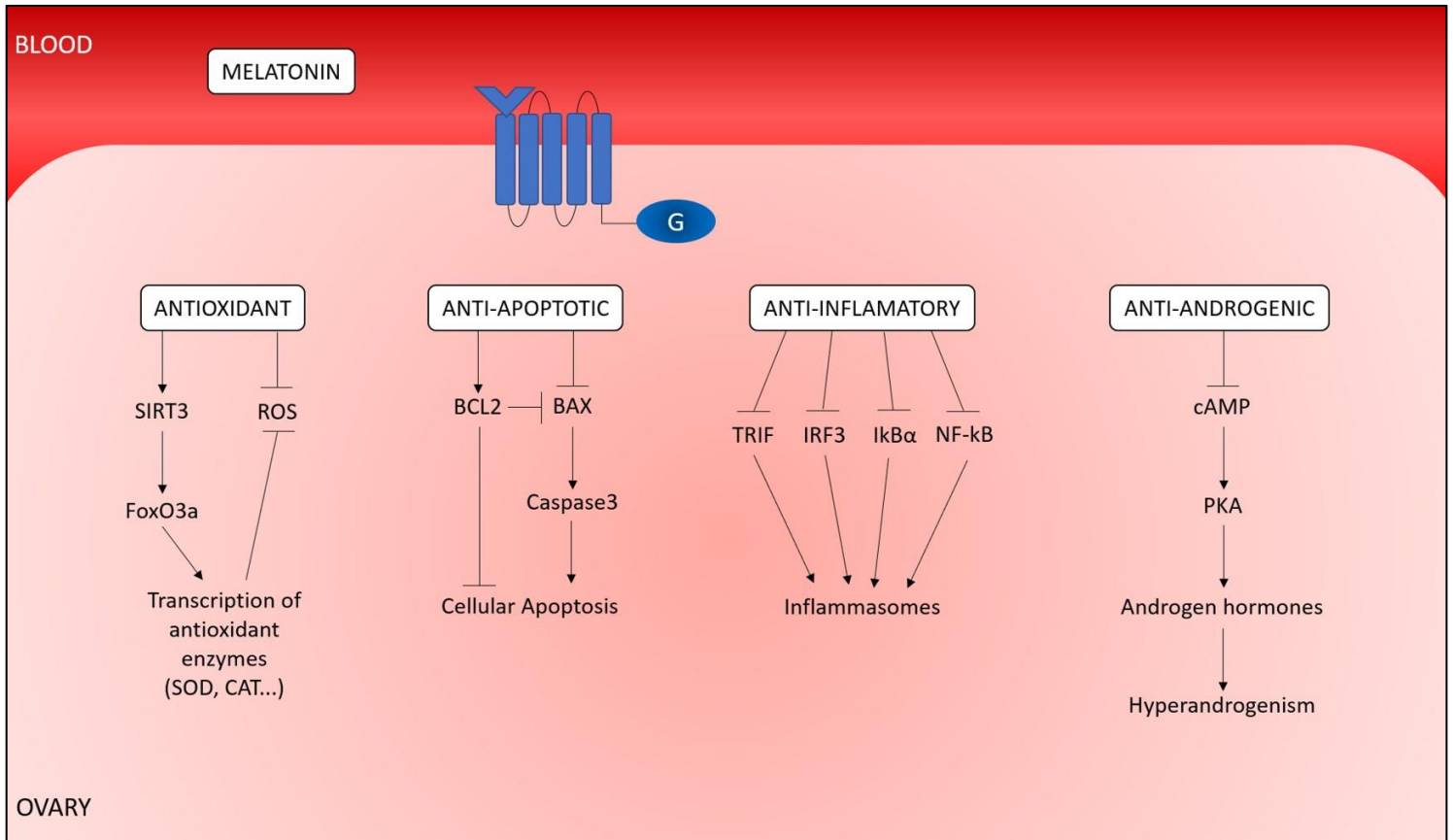
Safe and feasible therapeutic strategies are still required to improve gynecological and reproductive health. Melatonin-containing supplements are easily found in online and high street retailers, with beneficial claims on jet lag [28], as well as occasional sleepiness, sleep problems caused by stress, overall mood and overall health.

Despite melatonin supplementation believed to be relatively safe, there is no consensus on any potentially effective dosage, treatment period or whether or not it may positively influence gynecological and reproductive health. Melatonin is a key neuroendocrine controller in the modulation of the HPG axis, influencing the synthesis and secretion of gonadotrophins [29-31]. However, little attention has been paid to its potential effects on female health. In this review we aim to address the evidence available on pharmacological aspects of melatonin supplementation and further discuss the clinical relevance of its supplementation in the field of gynecology and reproduction.

## **2. Reduction-Oxidation modulation in the ovary**

The antioxidant potential of melatonin has already been investigated, with the suggestion that it has protective properties upon several physiological systems [32, 33]. Melatonin contributes to the scavenging of free radicals, including hydroxyl cations, at the same time stimulating the expression of antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase, ultimately modulating the redox balance [34, 35]. In the ovary, melatonin shows important actions on mitochondrial protection due to enzymatic activation of sirtuin 3 (SIRT3), superoxide dismutase 2 (SOD2) and catalase (CAT), also positively modulating the forkhead box O3A (FoxO3a) protein [36]. Moreover, melatonin reduces markers of apoptosis (e.g. BAX and caspase 3) and increases the expression of the anti-apoptotic B-cell lymphoma-2 (BCL-2) gene [37, 38]. Viewed collectively, the linkage of these pathways is shown in figure 2.

(Insert figure 2 here)



**Figure 2:** Therapeutic potential of melatonin actions in the ovary: Antioxidant, anti-apoptotic, anti-inflammatory and anti-androgenic regulation. BAX, BCL2 Associated X; BCL2, B-cell lymphoma-2; cAMP, Cyclic adenosine monophosphate; FoxO3a, Forkhead box O3A; IκBα, Inhibitor of NFκB alpha; IRF3, Interferon regulatory factor 3; NF-κB, Nuclear factor kappa B; PKA, Protein kinase A; SIRT3, Sirtuin 3; SOD, Superoxide Dismutase; CAT, Catalase; TRIF, Toll-like receptor-associated activator of interferon.

Given the antioxidant properties of melatonin upon the HPG axis [32, 39, 40], melatonin may reduce intra-follicular oxidative damage, also improving progesterone production in the luteal phase as well as oocyte maturation [40, 41]. Circulating melatonin can be absorbed by the ovary, but the ovarian follicle has the capacity to synthesize and secrete its own melatonin, therefore demonstrating an important paracrine role for melatonin on the female reproductive system [42, 43]. In addition to its antioxidant potential, melatonin has also been employed as a useful clinical tool in ovarian cancer due to its anti-proliferative, anti-inflammatory, anti-angiogenic and immunomodulatory properties [44]. Melatonin is able to reduce the expression of tumor markers related to tumor growth and ovarian metastasis of rats, such as Epidermal growth factor receptors 2 (Her-2), p38 mitogen-activated protein kinases (p38 MAPK), protein kinase B (phospho-AKT) and mammalian target of rapamycin (mTOR) [45]. Melatonin therapy is also related to lower expression of other markers, such as ATP synthase subunit β, fatty acid-binding protein, and 10-kDa heat shock protein [46]. Similarly, the modulatory

capacity of melatonin on Toll-like receptors (TLR4-mediated) results in inflammatory reduction in the ovary of rats, reducing inflammatory markers such as inhibitor of NF $\kappa$ B alpha (I $\kappa$ B $\alpha$ ), nuclear factor kappa B (NF- $\kappa$ B), toll-like receptor-associated activator of interferon (TRIF) and interferon regulatory factor 3 (IRF3) [47].

### **3. The effects of melatonin on reproduction – animal models**

The activity of the reproductive system in mammals is directly linked to food availability, environment cues such as temperature and luminosity, and other zeitgebers [48]. Retinal receptors transmit environmental cues to the suprachiasmatic nucleus, whose activity is inhibited by light and activated by darkness [49, 50]. Consequently, changes in the duration of light-dark periods influence sexual activity and reproduction [51]. However, such influence appears to be species specific. Longer photoperiods appear to show improvement over reproductive parameters in rats [52], whereas shorter photoperiods appear to have a detrimental effect [53]. A different response is observed in other species; a shorter photoperiod appears to show reproductive advantages in the mare, for example [54].

Melatonin significantly downregulates gonadotropin-releasing hormone (GnRH) mRNA levels in hypothalamic GnRH neurons [55], suggesting a direct effect of melatonin over the HPG system. Additionally, an indirect effect also appears to exist; melatonin appears to stimulate the gene expression of cyclo-oxygenase 2A (Cox2A), influencing the synthesis and secretion of prostaglandins, consequently stimulating follicular growth and oocyte maturation [56]. Likewise, melatonin increases the expression of kisspeptin proteins, which are important regulatory molecules for the secretion of luteinizing hormone [57] and follicle-stimulating hormone [37] [58]. It has been shown that the effects of melatonin on improvement of fertility, oocyte development and embryo maintenance are attributed to its capacity to reduce oxidative damage in the ovarian follicles [59, 60]. At the same time, higher concentrations of melatonin observed during pregnancy appear to support fetal maturation and placental homeostasis [59].

Pinealectomized rats show impaired ovulation [61, 62]. Upon pinealectomy, the decline in serum melatonin is followed by a proliferative response of the endometrium; however, melatonin replacement therapy mitigates the morphological dysregulation of its epithelium [63]. The presence of melatonin receptors in the endometrium evidences its importance in reproduction [64]. Song *et al* observed that female mice supplemented with melatonin added to drinking water for 12 months showed reduced ovary ageing parameters, improved quantity and quality of oocytes, and increased litter size [36].

The effects of melatonin in reproduction and follicular maturation has been investigated in non-mammal species, including the zebrafish [56, 65, 66]. Yumnamcha *et al* found that Zebrafish exposed to continuous darkness show higher levels of melatonin in the brain and in the ovary than those exposed to continuous light [67]. The authors also found that the higher melatonin levels were associated with increased GnRH, decreased gonadotropin-inhibiting hormone (GnIH) and improved reproductive rate [67]. Furthermore, melatonin appears to play a role in the zebrafish reproductive cycle by balancing the synthesis of LH and FSH [56].

Melatonin is involved in the modulation of Kiss-1 and Kiss-2 gene activities. Previously observed in other species, including rat [68] and sheep [69], Kiss-1 and Kiss-2 enhance reproductive rate and sexual maturation by promoting the secretion of

gonadotrophins. Clinically, these genes appear to display modulatory roles in the reproductive system from puberty to the hormonal cycle phase; mechanistically, Kiss-1 and Kiss-2 appear to influence the activity of GnRH neurons and mediate metabolic factors, such as adipose tissue hormones and energy balance [70].

#### **4. The effects of melatonin on reproduction – human studies**

Metabolic disarrangement, menstrual cycle abnormalities and increased risk of miscarriage are manifestations reported in women suffering with polycystic ovary syndrome (PCOS) [71, 72]. In a prospective cohort study including 40 women diagnosed with PCOS, melatonin supplementation for six months was effective in restoring their menstrual cyclicity and in normalizing androgenic parameters [73]. The antiandrogenic potential of melatonin is supported by its capacity to decrease the levels of cyclic AMP in granulosa cells (GCs), ultimately reducing the production of androgenic hormones [74]. Endometriosis is another condition that results in reproductive dysregulation, and can lead to infertility in up to 50% of sufferers [75, 76]. Yang *et al* observed that melatonin supplementation appears to be a feasible adjuvant therapy for endometriosis and recurrent spontaneous miscarriage [77], as well as assisting in reduction of pain scores and normalization of menstrual disorders such as dysmenorrhea [78]. As melatonin crosses the placental barrier, it may have the potential to protect the embryo by reducing oxidative damage, possibly hindering the progression of oxidative stress-related fetal abnormalities [79].

The therapeutic properties of exogenous melatonin may be effective in improving fertility rates [80]. As summarized in table 1, melatonin supplementation in disorders of the female reproductive system appears to be clinically relevant, improving fertility rate, oocyte quality, maturation and number of embryos [15, 81-84]. A randomized double-blind placebo-controlled study showed that perimenopausal and menopausal women had lower salivary melatonin levels, a finding that was positively associated with metabolic impairments including lower levels of thyroid hormones [74]. The same study showed that after six months of melatonin supplementation, the investigated biomarkers had shown signs of improvement, as well as significant improvement in mood and depression scores. Another study [85] investigating the effects of melatonin daily supplementation for 3 months in postmenopausal women found that the melatonin-receiving group showed significant improvement in various climacteric symptoms, including physical, psychological and sexual, in comparison to the placebo-receiving group.



(Insert table 1 here)

**Table 1:** Clinical findings on gynecological parameters after melatonin supplementation.

<b>Gynecological Disorder</b>	<b>N</b>	<b>Age mean or range</b>	<b>Daily dose of melatonin</b>	<b>Duration</b>	<b>Outcomes</b>	<b>References</b>
Polycystic ovary syndrome	40	23	2mg	6 Months	Improvement of menstrual irregularities and reduction of hyperandrogenism	[73]
Endometriosis	40	37	10mg	8 Weeks	Reduced pain scores and dysmenorrhea Increased sleep quality	[78]
Primary infertility	85	30	3mg	Acute	Increased mature oocytes and class 1 embryos	[83]
Low fertility rate	18	35	3mg	Acute	Increased fertility rate	[60]
Low fertility rate	60	30	3mg	Acute	Increased number of mature oocytes and class 1 embryos Increased embryo quality	[82]
Infertility	160	18 to 45	4mg 8mg 16mg	3 Days	Increased markers of follicle health and clinical pregnancy rates	[84]
Low fertility rate	72	34	1mg 3mg	Acute	Increased oocyte quality and number of fertilized embryos	[81]

Fetal growth restriction	12	Uninformed	8mg	47 Days	Reduction of the placental oxidative marker malondialdehyde	[86]
Pre-eclampsia	68	33	30mg	Until at birth	Prolonged gestation and reduced the pharmacological need for antihypertensives	[87]
Climacteric Symptoms	240	53	3mg	3 Months	Reduced climacteric symptoms	[85]

Taken together, these findings corroborate the substantial role of melatonin in the reproductive axis, whose reduced production upon aging has been associated with the onset of menopause [88], thus hindering FSH secretion [57].

### **5. 6-sulfatoxymelatonin concentration: a reliable biomarker on gynecology?**

A surrogate biomarker of melatonin metabolism is 6-sulfatoxymelatonin, a melatonin metabolite whose urinary excretion has already been employed in the assessment of gynecological disorders [89, 90]. Reduced urinary 6-sulfatoxymelatonin levels, associated with higher estradiol and progesterone levels, have been detected in women who work rotating night shifts, in comparison to women who work day shifts [91, 92]. Additionally, decreased circulating levels of 6-sulfatoxymelatonin were reported in girls affected by early onset puberty, which evidences the important relationship between this biomarker and the female reproductive axis [93].

In the opposite direction, Luboshitzky *et al* detected increased urinary levels of 6-sulfatoxymelatonin in a cohort of women diagnosed with PCOS, in comparison with non-PCOS aged-matched women [94], a finding which the authors have associated with the androgenic aspects of the syndrome. However, the urinary excretion of 6-sulfatoxymelatonin does not appear to be a reliable biomarker in patients with endometriosis [95, 96].

### **6. Melatonin in Pregnancy**

A potential application for melatonin supplementation as adjuvant therapy has been proposed to aid positive outcomes in pregnancy. Research on melatonin administration during the gestational period in animal models appears to suggest a protective mechanism upon neurogenesis, placental protection, reduction of oxidative stress, increased reproductive rate and improved fetal development [97-99]. The well described crossing of melatonin through the placental barrier, and its binding to MT1 and MT2 receptors in placental tissue, further evidences its effects upon fetal development; it confirms the role of the mother's own circadian rhythm as the first zeitgeber the fetus is exposed to, which in turn modulates fetal neuroendocrine and immune development, and confers antioxidant protection [100-105].

The antioxidant properties of melatonin upon placental tissue are attributed to its capacity to increase the expression of catalase and superoxide dismutase, whilst its autocrine and paracrine activities in placental endothelial cells appears to influence the development of the fetus' own circadian system [97-99]. Melatonin receptors expressed in the fetal brain are influenced by the mother's hormone variation, and such role is paramount to protect the fetus' developing brain against oxidative stress and variations in oxygen levels [100].

Potentially beneficial effects of melatonin have been proposed as an appealing therapeutic tool in gestational disorders. Fetal growth restriction (FGR) affects 5 to 10% of pregnancies, being the second most common cause of perinatal mortality. It is associated with fetal hypoxia, nutrient deprivation and imbalanced oxidative stress [106, 107]. Taking this concern into account, Miller *et al.* found no safety issues in melatonin supplementation for both mother and fetus, and also found reduced levels of malondialdehyde, which is a placental oxidative biomarker [86].

Pre-eclampsia is another expressive gestational disorder that affects 3 to 10% of pregnancies, classically characterized by high blood pressure and proteinuria [108-110]. Placental reduction of melatonin receptor (MT1 and MT2) expression, alongside inhibition of aralkylamine N-acetyltransferase (AANAT), a key enzyme in melatonin synthesis, have been observed in pre-eclampsia [111]. Animal and *in vitro* studies have identified an important protective effect of melatonin administration against gestational dysregulations, protecting fetal mitochondria and placental DNA, at the same time reducing inflammatory and oxidative biomarkers [87, 112]. In humans, Hobson et al. observed that melatonin supplementation prolonged gestation and reduced the dosage of antihypertensive drugs [87]; further corroborating the safety of melatonin supplementation in pregnancy.

## **7. Approaches to posology**

The evidence so far available supports the suggestion that melatonin supplementation confers positive outcomes in the routine gynecological care, not only improving general well-being but also ameliorating reproductive parameters for women of child-bearing age who wish to become mothers [101-103]. Further evidence suggests that melatonin confers positive outcomes for the musculoskeletal system, improving strength and bone health, alongside positive outcomes of psychological nature [104, 113-116].

The safety of melatonin supplementation as sleep aid is relatively well documented [117]. Its applications in the field of gynecology appear to be not only similarly safe, but also promising. Despite the relatively small number of relevant studies available, most of them investigating short-term effects, a daily posology ranging from 2 to 18 mg appears to be the most commonly employed, as summarized in Table 1.

Notwithstanding the apparent safety and allegedly positive health outcomes, robust long-term clinical trials that investigate the likelihood and severity of risks associated with any melatonin-drug interaction are required before any recommendation can be made for expecting mothers. Very long-term studies which investigate the health of individuals whose mothers received melatonin supplementation during pregnancy are also required to ensure safe recommendations. Directions for future work on melatonin supplementation are suggested in Table 2.

(Insert table 2 here)

**Table 2:** Open-ended questions for clinical trials in the area of gynecology.

***Conceptual and Clinical***

- Is chronic melatonin supplementation safe in pregnancy?
- Is melatonin deficit the culprit for gynecological disorders? Or the other way around?
- How does the antioxidant effect of melatonin compare with other antioxidant molecules for gynecology?
- What are the effects of melatonin on embryo development?
- Can melatonin reduce the occurrence of miscarriages?
- Can melatonin modulate phenotypes related to chromosomal disorders?
- Can melatonin urinary metabolites be employed as diagnostic tool in clinical practice?
- Are there adverse effects of melatonin supplementation for the fetus?
- Is there a safe dose range during pregnancy?

***Hormone profile***

- What are the mechanisms involved in the antiandrogenic effect of melatonin?
- Can melatonin modulate the activity of androgenic receptors?
- How does melatonin modulate the secretion of estrogen?
- Can 6-sulfatoxymelatonin metabolite affect the androgenic response?
- Can 6-sulfatoxymelatonin be an early surrogate biomarker of infertility?
- Can hormone replacement therapy change plasma levels of melatonin?
- Can postmenopausal hormone profile affect the melatoninergetic response?
- In what other gynecological disorders can the 6-sulfatoxymelatonin marker be a useful clinical tool?

## **8. Conclusion**

Melatonin supplementation may offer a therapeutic as well as preventative potential in the area of gynecology and obstetrics due to its antioxidant properties and activity as hormone modulator. It has been observed in melatonin supplementation studies ranging from a few days to up to six months of duration that a daily posology of 2 to 18 mg of melatonin appeared to improve fertility rate, oocyte quality, maturation and number of embryos. In gestational disorders, daily doses of 8 to 30 mg appeared safe for the mother and for the fetus, in addition to an important therapeutic outcome. However, no final consensus has yet been reached in respect to effective dose or supplementation period in the field of obstetrics and gynecology. Despite melatonin supplementation being relatively safe and apparently beneficial, long-term clinical trials are imperative to assess further clinical outcomes, with particular attention to the gestational period.

### **Conflict of interests**

The authors have no competing interests to declare.

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## **The usefulness of melatonin in the field of obstetrics and gynecology**

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## **Abstract**

Disorders of the female reproductive system, including those associated with hormone regulation, fertility rate and fetal health, are issues of great concern worldwide. More recently, melatonin supplementation has been suggested as a therapeutic approach in gynecological practice. In both animal models and in women, melatonin supplementation suggests a therapeutic and preventative potential, effects attributed mainly to its antioxidant properties and action as hormone modulator. The aim of this literature review is to further investigate the evidence available on the effects of melatonin supplementation in animal and human studies, focusing on its potential application to gynecology. Melatonin-containing supplements are easily found in online and high street retailers, and despite its supplementation deemed to be relatively safe, no consensus has been reached on effective dosage and supplementation period. Short term supplementation studies, of up to six months, suggest that a daily posology of 2 to 18 mg of melatonin may have the potential to improve fertility rate, oocyte quality, maturation and number of embryos. However, the evidence available so far on the effects of melatonin supplementation covering gestational age and gestational outcomes is very scarce. Clinical trials and longer-term supplementation studies are required to assess any clinical outcome associated with melatonin supplementation in the field of gynecology.

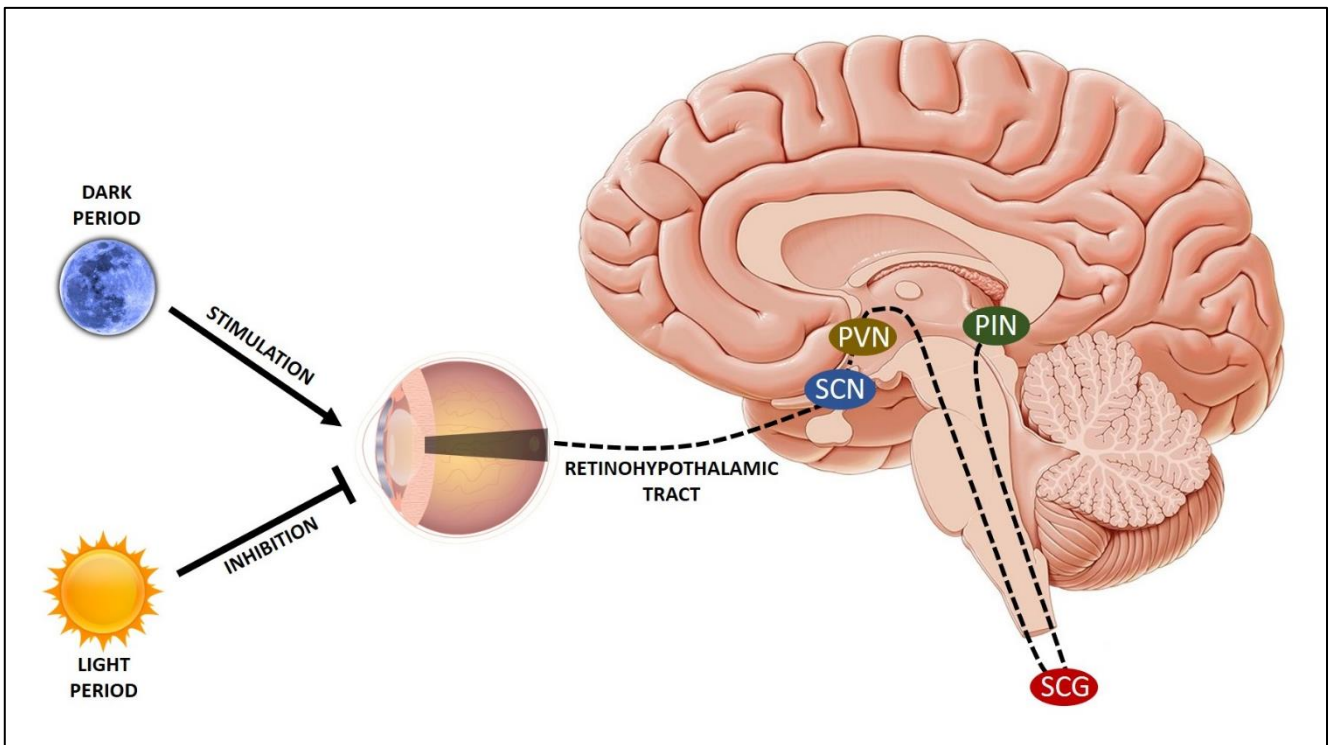
**Keywords:** gynecology; melatonin; reproductive system; antioxidant; fertility.

## 1. Introduction

The mammalian circadian clock covers a wide range of physiological processes and plays pivotal role in reproduction [1, 2]. Such physiological processes are coordinated by a robust genetic machinery known in lay terms as the "clock genes" [3-6]. Clock genes modulate the activity of regulatory nuclei in the hypothalamus-pituitary-gonad axis (HPG) and are abundantly expressed in the reproductive tract [3, 4, 7]. It is currently accepted that dysregulation of the circadian rhythm caused by night shifts, jet lag and sleep deprivation has a detrimental effect on the reproductive system [7-10].

A range of blood-borne stimuli and biomolecules contribute to the regulation of the circadian system. Melatonin is a hormone and an indolamine synthesized mostly at night, as summarized in figure 1. Melatonin is produced not only by the pineal gland, but also in glial cells, meningeal cells, and in other peripheral tissues, and its cyclical pattern of secretion is responsive to zeitgebers [11].

(Insert figure 1 here)



**Figure 1:** Brain circuits involved in melatonin biosynthesis through circadian regulation. Adapted from Tan et al. [11]. PIN, pineal gland; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SCG, superior cervical ganglion.

Melatonin permeability into the central nervous system was described decades ago [12] and its efficient transport through the blood-brain-barrier promotes accumulation in the brain at levels higher than the ones existing in the blood. Melatonin also possesses neuroprotective and antioxidant properties [13-16]. Modulation of redox signaling systems influence the reproductive system in both animals and humans [17, 18], and it is known that insufficient endogenous production of melatonin has been associated with disturbances in the reproductive system due to increased levels of reactive oxygen species (ROS), which are harmful to the male and female gametes [19-21].

As gametogenesis and gamete viability are negatively associated with increased age, successful reproductive strategies are directly related to the life stage of the individual [22]. Additionally, unhealthy lifestyles and psychosocial stress are aspects of modern life that have a negative impact on gynecological health and reproduction [23-25]. Epidemiological studies show that night shifts may negatively influence fetal development and may exacerbate gynecological and metabolic disorders, including endometriosis, diabetes and obesity [26, 27].

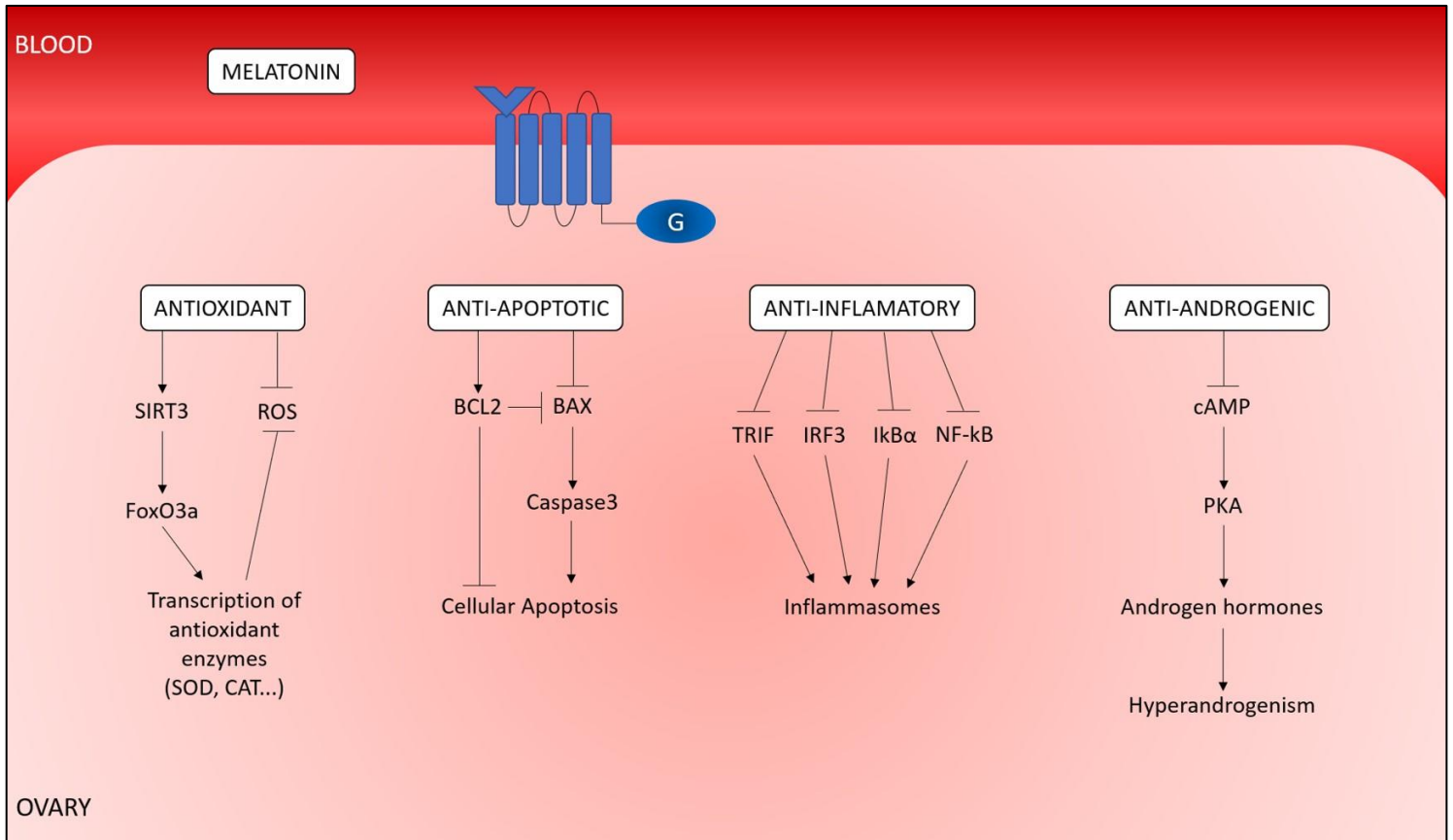
Safe and feasible therapeutic strategies are still required to improve gynecological and reproductive health. Melatonin-containing supplements are easily found in online and high street retailers, with beneficial claims on jet lag [28], as well as occasional sleepiness, sleep problems caused by stress, overall mood and overall health.

Despite melatonin supplementation believed to be relatively safe, there is no consensus on any potentially effective dosage, treatment period or whether or not it may positively influence gynecological and reproductive health. Melatonin is a key neuroendocrine controller in the modulation of the HPG axis, influencing the synthesis and secretion of gonadotrophins [29-31]. However, little attention has been paid to its potential effects on female health. In this review we aim to address the evidence available on pharmacological aspects of melatonin supplementation and further discuss the clinical relevance of its supplementation in the field of gynecology and reproduction.

## **2. Reduction-Oxidation modulation in the ovary**

The antioxidant potential of melatonin has already been investigated, with the suggestion that it has protective properties upon several physiological systems [32, 33]. Melatonin contributes to the scavenging of free radicals, including hydroxyl cations, at the same time stimulating the expression of antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase, ultimately modulating the redox balance [34, 35]. In the ovary, melatonin shows important actions on mitochondrial protection due to enzymatic activation of sirtuin 3 (SIRT3), superoxide dismutase 2 (SOD2) and catalase (CAT), also positively modulating the forkhead box O3A (FoxO3a) protein [36]. Moreover, melatonin reduces markers of apoptosis (e.g. BAX and caspase 3) and increases the expression of the anti-apoptotic B-cell lymphoma-2 (BCL-2) gene [37, 38]. Viewed collectively, the linkage of these pathways is shown in figure 2.

(Insert figure 2 here)



**Figure 2:** Therapeutic potential of melatonin actions in the ovary: Antioxidant, anti-apoptotic, anti-inflammatory and anti-androgenic regulation. BAX, BCL2 Associated X; BCL2, B-cell lymphoma-2; cAMP, Cyclic adenosine monophosphate; FoxO3a, Forkhead box O3A; IκBα, Inhibitor of NFκB alpha; IRF3, Interferon regulatory factor 3; NF-κB, Nuclear factor kappa B; PKA, Protein kinase A; SIRT3, Sirtuin 3; SOD, Superoxide Dismutase; CAT, Catalase; TRIF, Toll-like receptor-associated activator of interferon.

Given the antioxidant properties of melatonin upon the HPG axis [32, 39, 40], melatonin may reduce intra-follicular oxidative damage, also improving progesterone production in the luteal phase as well as oocyte maturation [40, 41]. Circulating melatonin can be absorbed by the ovary, but the ovarian follicle has the capacity to synthesize and secrete its own melatonin, therefore demonstrating an important paracrine role for melatonin on the female reproductive system [42, 43]. In addition to its antioxidant potential, melatonin has also been employed as a useful clinical tool in ovarian cancer due to its anti-proliferative, anti-inflammatory, anti-angiogenic and immunomodulatory properties [44]. Melatonin is able to reduce the expression of tumor markers related to tumor growth and ovarian metastasis of rats, such as Epidermal growth factor receptors 2 (Her-2), p38 mitogen-activated protein kinases (p38 MAPK), protein kinase B (phospho-AKT) and mammalian target of rapamycin (mTOR) [45]. Melatonin therapy is also related to lower expression of other markers, such as ATP synthase subunit β, fatty acid-binding protein, and 10-kDa heat shock protein [46]. Similarly, the modulatory

capacity of melatonin on Toll-like receptors (TLR4-mediated) results in inflammatory reduction in the ovary of rats, reducing inflammatory markers such as inhibitor of NF $\kappa$ B alpha (I $\kappa$ B $\alpha$ ), nuclear factor kappa B (NF- $\kappa$ B), toll-like receptor-associated activator of interferon (TRIF) and interferon regulatory factor 3 (IRF3) [47].

### **3. The effects of melatonin on reproduction – animal models**

The activity of the reproductive system in mammals is directly linked to food availability, environment cues such as temperature and luminosity, and other zeitgebers [48]. Retinal receptors transmit environmental cues to the suprachiasmatic nucleus, whose activity is inhibited by light and activated by darkness [49, 50]. Consequently, changes in the duration of light-dark periods influence sexual activity and reproduction [51]. However, such influence appears to be species specific. Longer photoperiods appear to show improvement over reproductive parameters in rats [52], whereas shorter photoperiods appear to have a detrimental effect [53]. A different response is observed in other species; a shorter photoperiod appears to show reproductive advantages in the mare, for example [54].

Melatonin significantly downregulates gonadotropin-releasing hormone (GnRH) mRNA levels in hypothalamic GnRH neurons [55], suggesting a direct effect of melatonin over the HPG system. Additionally, an indirect effect also appears to exist; melatonin appears to stimulate the gene expression of cyclo-oxygenase 2A (Cox2A), influencing the synthesis and secretion of prostaglandins, consequently stimulating follicular growth and oocyte maturation [56]. Likewise, melatonin increases the expression of kisspeptin proteins, which are important regulatory molecules for the secretion of luteinizing hormone [57] and follicle-stimulating hormone [37] [58]. It has been shown that the effects of melatonin on improvement of fertility, oocyte development and embryo maintenance are attributed to its capacity to reduce oxidative damage in the ovarian follicles [59, 60]. At the same time, higher concentrations of melatonin observed during pregnancy appear to support fetal maturation and placental homeostasis [59].

Pinealectomized rats show impaired ovulation [61, 62]. Upon pinealectomy, the decline in serum melatonin is followed by a proliferative response of the endometrium; however, melatonin replacement therapy mitigates the morphological dysregulation of its epithelium [63]. The presence of melatonin receptors in the endometrium evidences its importance in reproduction [64]. Song *et al* observed that female mice supplemented with melatonin added to drinking water for 12 months showed reduced ovary ageing parameters, improved quantity and quality of oocytes, and increased litter size [36].

The effects of melatonin in reproduction and follicular maturation has been investigated in non-mammal species, including the zebrafish [56, 65, 66]. Yumnamcha *et al* found that Zebrafish exposed to continuous darkness show higher levels of melatonin in the brain and in the ovary than those exposed to continuous light [67]. The authors also found that the higher melatonin levels were associated with increased GnRH, decreased gonadotropin-inhibiting hormone (GnIH) and improved reproductive rate [67]. Furthermore, melatonin appears to play a role in the zebrafish reproductive cycle by balancing the synthesis of LH and FSH [56].

Melatonin is involved in the modulation of Kiss-1 and Kiss-2 gene activities. Previously observed in other species, including rat [68] and sheep [69], Kiss-1 and Kiss-2 enhance reproductive rate and sexual maturation by promoting the secretion of

gonadotrophins. Clinically, these genes appear to display modulatory roles in the reproductive system from puberty to the hormonal cycle phase; mechanistically, Kiss-1 and Kiss-2 appear to influence the activity of GnRH neurons and mediate metabolic factors, such as adipose tissue hormones and energy balance [70].

#### **4. The effects of melatonin on reproduction – human studies**

Metabolic disarrangement, menstrual cycle abnormalities and increased risk of miscarriage are manifestations reported in women suffering with polycystic ovary syndrome (PCOS) [71, 72]. In a prospective cohort study including 40 women diagnosed with PCOS, melatonin supplementation for six months was effective in restoring their menstrual cyclicity and in normalizing androgenic parameters [73]. The antiandrogenic potential of melatonin is supported by its capacity to decrease the levels of cyclic AMP in granulosa cells (GCs), ultimately reducing the production of androgenic hormones [74]. Endometriosis is another condition that results in reproductive dysregulation, and can lead to infertility in up to 50% of sufferers [75, 76]. Yang *et al* observed that melatonin supplementation appears to be a feasible adjuvant therapy for endometriosis and recurrent spontaneous miscarriage [77], as well as assisting in reduction of pain scores and normalization of menstrual disorders such as dysmenorrhea [78]. As melatonin crosses the placental barrier, it may have the potential to protect the embryo by reducing oxidative damage, possibly hindering the progression of oxidative stress-related fetal abnormalities [79].

The therapeutic properties of exogenous melatonin may be effective in improving fertility rates [80]. As summarized in table 1, melatonin supplementation in disorders of the female reproductive system appears to be clinically relevant, improving fertility rate, oocyte quality, maturation and number of embryos [15, 81-84]. A randomized double-blind placebo-controlled study showed that perimenopausal and menopausal women had lower salivary melatonin levels, a finding that was positively associated with metabolic impairments including lower levels of thyroid hormones [74]. The same study showed that after six months of melatonin supplementation, the investigated biomarkers had shown signs of improvement, as well as significant improvement in mood and depression scores. Another study [85] investigating the effects of melatonin daily supplementation for 3 months in postmenopausal women found that the melatonin-receiving group showed significant improvement in various climacteric symptoms, including physical, psychological and sexual, in comparison to the placebo-receiving group.

(Insert table 1 here)

**Table 1:** Clinical findings on gynecological parameters after melatonin supplementation.

<b>Gynecological Disorder</b>	<b>N</b>	<b>Age mean or range</b>	<b>Daily dose of melatonin</b>	<b>Duration</b>	<b>Outcomes</b>	<b>References</b>
Polycystic ovary syndrome	40	23	2mg	6 Months	Improvement of menstrual irregularities and reduction of hyperandrogenism	[73]
Endometriosis	40	37	10mg	8 Weeks	Reduced pain scores and dysmenorrhea Increased sleep quality	[78]
Primary infertility	85	30	3mg	Acute	Increased mature oocytes and class 1 embryos	[83]
Low fertility rate	18	35	3mg	Acute	Increased fertility rate	[60]
Low fertility rate	60	30	3mg	Acute	Increased number of mature oocytes and class 1 embryos Increased embryo quality	[82]
Infertility	160	18 to 45	4mg 8mg 16mg	3 Days	Increased markers of follicle health and clinical pregnancy rates	[84]
Low fertility rate	72	34	1mg 3mg	Acute	Increased oocyte quality and number of fertilized embryos	[81]

Fetal growth restriction	12	Uninformed	8mg	47 Days	Reduction of the placental oxidative marker malondialdehyde	[86]
Pre-eclampsia	68	33	30mg	Until at birth	Prolonged gestation and reduced the pharmacological need for antihypertensives	[87]
Climacteric Symptoms	240	53	3mg	3 Months	Reduced climacteric symptoms	[85]



Taken together, these findings corroborate the substantial role of melatonin in the reproductive axis, whose reduced production upon aging has been associated with the onset of menopause [88], thus hindering FSH secretion [57].

### **5. 6-sulfatoxymelatonin concentration: a reliable biomarker on gynecology?**

A surrogate biomarker of melatonin metabolism is 6-sulfatoxymelatonin, a melatonin metabolite whose urinary excretion has already been employed in the assessment of gynecological disorders [89, 90]. Reduced urinary 6-sulfatoxymelatonin levels, associated with higher estradiol and progesterone levels, have been detected in women who work rotating night shifts, in comparison to women who work day shifts [91, 92]. Additionally, decreased circulating levels of 6-sulfatoxymelatonin were reported in girls affected by early onset puberty, which evidences the important relationship between this biomarker and the female reproductive axis [93].

In the opposite direction, Luboshitzky *et al* detected increased urinary levels of 6-sulfatoxymelatonin in a cohort of women diagnosed with PCOS, in comparison with non-PCOS aged-matched women [94], a finding which the authors have associated with the androgenic aspects of the syndrome. However, the urinary excretion of 6-sulfatoxymelatonin does not appear to be a reliable biomarker in patients with endometriosis [95, 96].

### **6. Melatonin in Pregnancy**

A potential application for melatonin supplementation as adjuvant therapy has been proposed to aid positive outcomes in pregnancy. Research on melatonin administration during the gestational period in animal models appears to suggest a protective mechanism upon neurogenesis, placental protection, reduction of oxidative stress, increased reproductive rate and improved fetal development [97-99]. The well described crossing of melatonin through the placental barrier, and its binding to MT1 and MT2 receptors in placental tissue, further evidences its effects upon fetal development; it confirms the role of the mother's own circadian rhythm as the first zeitgeber the fetus is exposed to, which in turn modulates fetal neuroendocrine and immune development, and confers antioxidant protection [100-105].

The antioxidant properties of melatonin upon placental tissue are attributed to its capacity to increase the expression of catalase and superoxide dismutase, whilst its autocrine and paracrine activities in placental endothelial cells appears to influence the development of the fetus' own circadian system [97-99]. Melatonin receptors expressed in the fetal brain are influenced by the mother's hormone variation, and such role is paramount to protect the fetus' developing brain against oxidative stress and variations in oxygen levels [100].

Potentially beneficial effects of melatonin have been proposed as an appealing therapeutic tool in gestational disorders. Fetal growth restriction (FGR) affects 5 to 10% of pregnancies, being the second most common cause of perinatal mortality. It is associated with fetal hypoxia, nutrient deprivation and imbalanced oxidative stress [106, 107]. Taking this concern into account, Miller *et al.* found no safety issues in melatonin supplementation for both mother and fetus, and also found reduced levels of malondialdehyde, which is a placental oxidative biomarker [86].

Pre-eclampsia is another expressive gestational disorder that affects 3 to 10% of pregnancies, classically characterized by high blood pressure and proteinuria [108-110]. Placental reduction of melatonin receptor (MT1 and MT2) expression, alongside inhibition of aralkylamine N-acetyltransferase (AANAT), a key enzyme in melatonin synthesis, have been observed in pre-eclampsia [111]. Animal and *in vitro* studies have identified an important protective effect of melatonin administration against gestational dysregulations, protecting fetal mitochondria and placental DNA, at the same time reducing inflammatory and oxidative biomarkers [87, 112]. In humans, Hobson et al. observed that melatonin supplementation prolonged gestation and reduced the dosage of antihypertensive drugs [87]; further corroborating the safety of melatonin supplementation in pregnancy.

## **7. Approaches to posology**

The evidence so far available supports the suggestion that melatonin supplementation confers positive outcomes in the routine gynecological care, not only improving general well-being but also ameliorating reproductive parameters for women of child-bearing age who wish to become mothers [101-103]. Further evidence suggests that melatonin confers positive outcomes for the musculoskeletal system, improving strength and bone health, alongside positive outcomes of psychological nature [104, 113-116].

The safety of melatonin supplementation as sleep aid is relatively well documented [117]. Its applications in the field of gynecology appear to be not only similarly safe, but also promising. Despite the relatively small number of relevant studies available, most of them investigating short-term effects, a daily posology ranging from 2 to 18 mg appears to be the most commonly employed, as summarized in Table 1.

Notwithstanding the apparent safety and allegedly positive health outcomes, robust long-term clinical trials that investigate the likelihood and severity of risks associated with any melatonin-drug interaction are required before any recommendation can be made for expecting mothers. Very long-term studies which investigate the health of individuals whose mothers received melatonin supplementation during pregnancy are also required to ensure safe recommendations. Directions for future work on melatonin supplementation are suggested in Table 2.

(Insert table 2 here)

**Table 2:** Open-ended questions for clinical trials in the area of gynecology.

***Conceptual and Clinical***

- Is chronic melatonin supplementation safe in pregnancy?
- Is melatonin deficit the culprit for gynecological disorders? Or the other way around?
- How does the antioxidant effect of melatonin compare with other antioxidant molecules for gynecology?
- What are the effects of melatonin on embryo development?
- Can melatonin reduce the occurrence of miscarriages?
- Can melatonin modulate phenotypes related to chromosomal disorders?
- Can melatonin urinary metabolites be employed as diagnostic tool in clinical practice?
- Are there adverse effects of melatonin supplementation for the fetus?
- Is there a safe dose range during pregnancy?

***Hormone profile***

- What are the mechanisms involved in the antiandrogenic effect of melatonin?
- Can melatonin modulate the activity of androgenic receptors?
- How does melatonin modulate the secretion of estrogen?
- Can 6-sulfatoxymelatonin metabolite affect the androgenic response?
- Can 6-sulfatoxymelatonin be an early surrogate biomarker of infertility?
- Can hormone replacement therapy change plasma levels of melatonin?
- Can postmenopausal hormone profile affect the melatoninergetic response?
- In what other gynecological disorders can the 6-sulfatoxymelatonin marker be a useful clinical tool?

## **8. Conclusion**

Melatonin supplementation may offer a therapeutic as well as preventative potential in the area of gynecology and obstetrics due to its antioxidant properties and activity as hormone modulator. It has been observed in melatonin supplementation studies ranging from a few days to up to six months of duration that a daily posology of 2 to 18 mg of melatonin appeared to improve fertility rate, oocyte quality, maturation and number of embryos. In gestational disorders, daily doses of 8 to 30 mg appeared safe for the mother and for the fetus, in addition to an important therapeutic outcome. However, no final consensus has yet been reached in respect to effective dose or supplementation period in the field of obstetrics and gynecology. Despite melatonin supplementation being relatively safe and apparently beneficial, long-term clinical trials are imperative to assess further clinical outcomes, with particular attention to the gestational period.

### **Conflict of interests**

The authors have no competing interests to declare.

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## **The usefulness of melatonin in the field of obstetrics and gynecology**

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## **Abstract**

Disorders of the female reproductive system, including those associated with hormone regulation, fertility rate and fetal health, are issues of great concern worldwide. More recently, melatonin supplementation has been suggested as a therapeutic approach in gynecological practice. In both animal models and in women, melatonin supplementation suggests a therapeutic and preventative potential, effects attributed mainly to its antioxidant properties and action as hormone modulator. The aim of this literature review is to further investigate the evidence available on the effects of melatonin supplementation in animal and human studies, focusing on its potential application to gynecology. Melatonin-containing supplements are easily found in online and high street retailers, and despite its supplementation deemed to be relatively safe, no consensus has been reached on effective dosage and supplementation period. Short term supplementation studies, of up to six months, suggest that a daily posology of 2 to 18 mg of melatonin may have the potential to improve fertility rate, oocyte quality, maturation and number of embryos. However, the evidence available so far on the effects of melatonin supplementation covering gestational age and gestational outcomes is very scarce. Clinical trials and longer-term supplementation studies are required to assess any clinical outcome associated with melatonin supplementation in the field of gynecology.

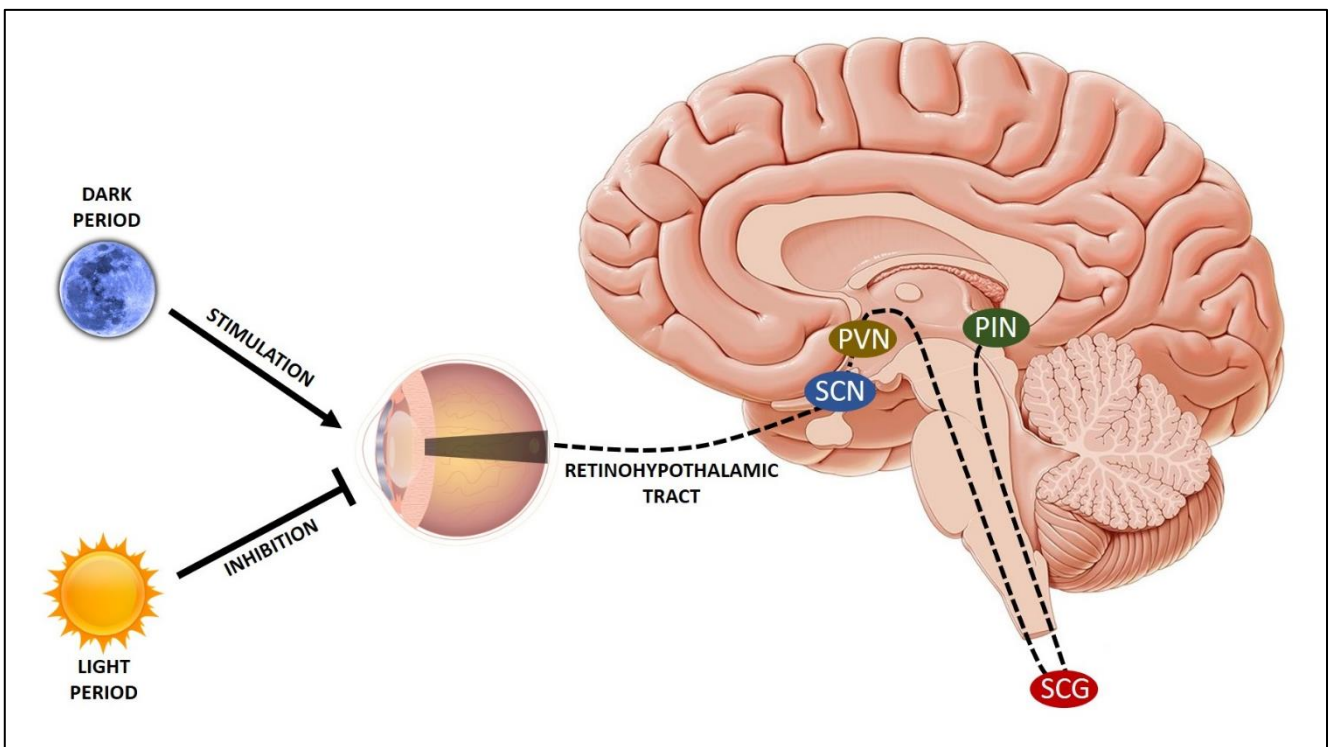
**Keywords:** gynecology; melatonin; reproductive system; antioxidant; fertility.

## 1. Introduction

The mammalian circadian clock covers a wide range of physiological processes and plays pivotal role in reproduction [1, 2]. Such physiological processes are coordinated by a robust genetic machinery known in lay terms as the "clock genes" [3-6]. Clock genes modulate the activity of regulatory nuclei in the hypothalamus-pituitary-gonad axis (HPG) and are abundantly expressed in the reproductive tract [3, 4, 7]. It is currently accepted that dysregulation of the circadian rhythm caused by night shifts, jet lag and sleep deprivation has a detrimental effect on the reproductive system [7-10].

A range of blood-borne stimuli and biomolecules contribute to the regulation of the circadian system. Melatonin is a hormone and an indolamine synthesized mostly at night, as summarized in figure 1. Melatonin is produced not only by the pineal gland, but also in glial cells, meningeal cells, and in other peripheral tissues, and its cyclical pattern of secretion is responsive to zeitgebers [11].

(Insert figure 1 here)



**Figure 1:** Brain circuits involved in melatonin biosynthesis through circadian regulation. Adapted from Tan et al. [11]. PIN, pineal gland; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SCG, superior cervical ganglion.

Melatonin permeability into the central nervous system was described decades ago [12] and its efficient transport through the blood-brain-barrier promotes accumulation in the brain at levels higher than the ones existing in the blood. Melatonin also possesses neuroprotective and antioxidant properties [13-16]. Modulation of redox signaling systems influence the reproductive system in both animals and humans [17, 18], and it is known that insufficient endogenous production of melatonin has been associated with disturbances in the reproductive system due to increased levels of reactive oxygen species (ROS), which are harmful to the male and female gametes [19-21].

As gametogenesis and gamete viability are negatively associated with increased age, successful reproductive strategies are directly related to the life stage of the individual [22]. Additionally, unhealthy lifestyles and psychosocial stress are aspects of modern life that have a negative impact on gynecological health and reproduction [23-25]. Epidemiological studies show that night shifts may negatively influence fetal development and may exacerbate gynecological and metabolic disorders, including endometriosis, diabetes and obesity [26, 27].

Safe and feasible therapeutic strategies are still required to improve gynecological and reproductive health. Melatonin-containing supplements are easily found in online and high street retailers, with beneficial claims on jet lag [28], as well as occasional sleepiness, sleep problems caused by stress, overall mood and overall health.

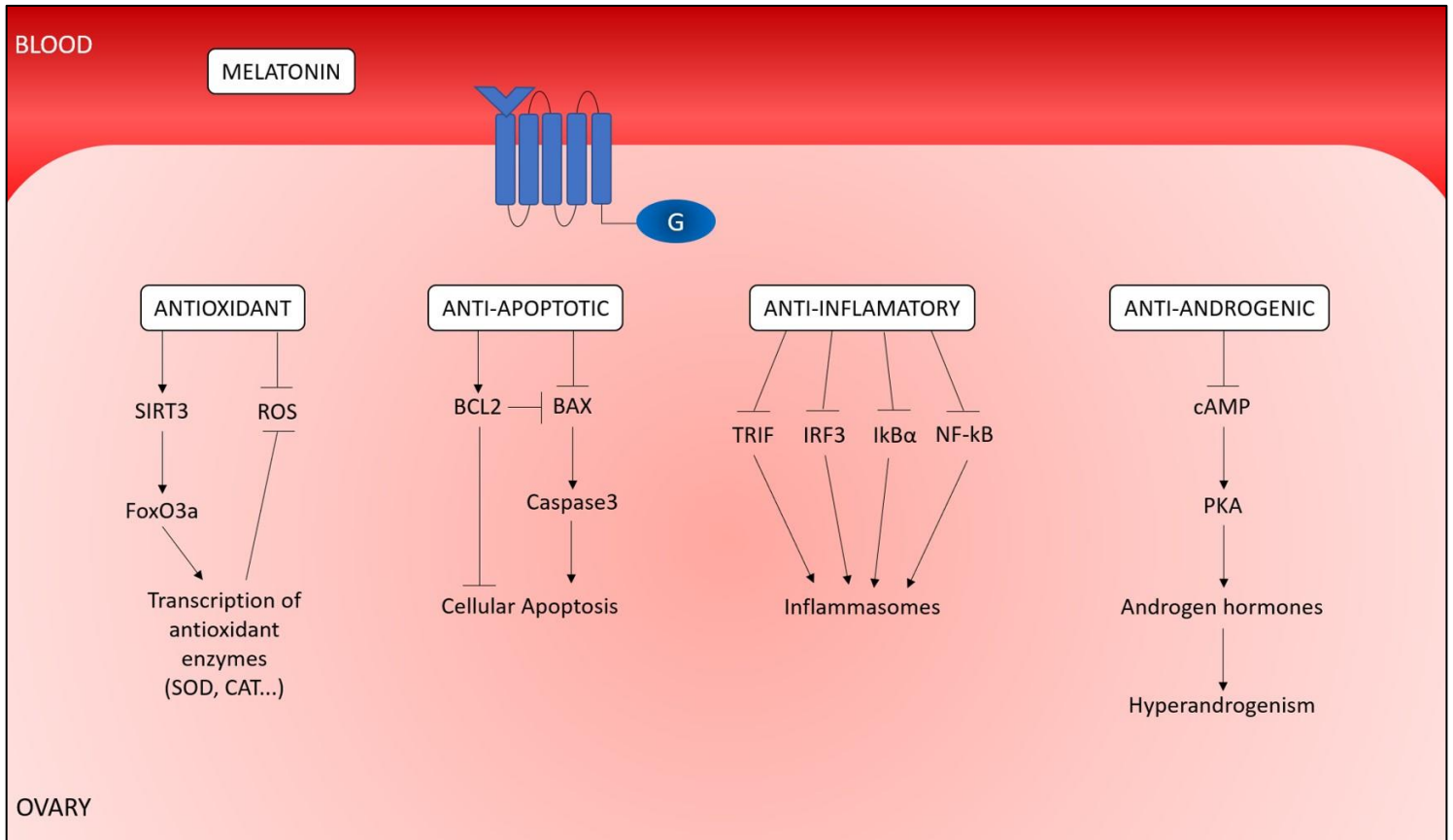
Despite melatonin supplementation believed to be relatively safe, there is no consensus on any potentially effective dosage, treatment period or whether or not it may positively influence gynecological and reproductive health. Melatonin is a key neuroendocrine controller in the modulation of the HPG axis, influencing the synthesis and secretion of gonadotrophins [29-31]. However, little attention has been paid to its potential effects on female health. In this review we aim to address the evidence available on pharmacological aspects of melatonin supplementation and further discuss the clinical relevance of its supplementation in the field of gynecology and reproduction.

## **2. Reduction-Oxidation modulation in the ovary**

The antioxidant potential of melatonin has already been investigated, with the suggestion that it has protective properties upon several physiological systems [32, 33]. Melatonin contributes to the scavenging of free radicals, including hydroxyl cations, at the same time stimulating the expression of antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase, ultimately modulating the redox balance [34, 35]. In the ovary, melatonin shows important actions on mitochondrial protection due to enzymatic activation of sirtuin 3 (SIRT3), superoxide dismutase 2 (SOD2) and catalase (CAT), also positively modulating the forkhead box O3A (FoxO3a) protein [36]. Moreover, melatonin reduces markers of apoptosis (e.g. BAX and caspase 3) and increases the expression of the anti-apoptotic B-cell lymphoma-2 (BCL-2) gene [37, 38]. Viewed collectively, the linkage of these pathways is shown in figure 2.

(Insert figure 2 here)





**Figure 2:** Therapeutic potential of melatonin actions in the ovary: Antioxidant, anti-apoptotic, anti-inflammatory and anti-androgenic regulation. BAX, BCL2 Associated X; BCL2, B-cell lymphoma-2; cAMP, Cyclic adenosine monophosphate; FoxO3a, Forkhead box O3A; IκBα, Inhibitor of NFκB alpha; IRF3, Interferon regulatory factor 3; NF-κB, Nuclear factor kappa B; PKA, Protein kinase A; SIRT3, Sirtuin 3; SOD, Superoxide Dismutase; CAT, Catalase; TRIF, Toll-like receptor-associated activator of interferon.

Given the antioxidant properties of melatonin upon the HPG axis [32, 39, 40], melatonin may reduce intra-follicular oxidative damage, also improving progesterone production in the luteal phase as well as oocyte maturation [40, 41]. Circulating melatonin can be absorbed by the ovary, but the ovarian follicle has the capacity to synthesize and secrete its own melatonin, therefore demonstrating an important paracrine role for melatonin on the female reproductive system [42, 43]. In addition to its antioxidant potential, melatonin has also been employed as a useful clinical tool in ovarian cancer due to its anti-proliferative, anti-inflammatory, anti-angiogenic and immunomodulatory properties [44]. Melatonin is able to reduce the expression of tumor markers related to tumor growth and ovarian metastasis of rats, such as Epidermal growth factor receptors 2 (Her-2), p38 mitogen-activated protein kinases (p38 MAPK), protein kinase B (phospho-AKT) and mammalian target of rapamycin (mTOR) [45]. Melatonin therapy is also related to lower expression of other markers, such as ATP synthase subunit β, fatty acid-binding protein, and 10-kDa heat shock protein [46]. Similarly, the modulatory

capacity of melatonin on Toll-like receptors (TLR4-mediated) results in inflammatory reduction in the ovary of rats, reducing inflammatory markers such as inhibitor of NF $\kappa$ B alpha (I $\kappa$ B $\alpha$ ), nuclear factor kappa B (NF- $\kappa$ B), toll-like receptor-associated activator of interferon (TRIF) and interferon regulatory factor 3 (IRF3) [47].

### **3. The effects of melatonin on reproduction – animal models**

The activity of the reproductive system in mammals is directly linked to food availability, environment cues such as temperature and luminosity, and other zeitgebers [48]. Retinal receptors transmit environmental cues to the suprachiasmatic nucleus, whose activity is inhibited by light and activated by darkness [49, 50]. Consequently, changes in the duration of light-dark periods influence sexual activity and reproduction [51]. However, such influence appears to be species specific. Longer photoperiods appear to show improvement over reproductive parameters in rats [52], whereas shorter photoperiods appear to have a detrimental effect [53]. A different response is observed in other species; a shorter photoperiod appears to show reproductive advantages in the mare, for example [54].

Melatonin significantly downregulates gonadotropin-releasing hormone (GnRH) mRNA levels in hypothalamic GnRH neurons [55], suggesting a direct effect of melatonin over the HPG system. Additionally, an indirect effect also appears to exist; melatonin appears to stimulate the gene expression of cyclo-oxygenase 2A (Cox2A), influencing the synthesis and secretion of prostaglandins, consequently stimulating follicular growth and oocyte maturation [56]. Likewise, melatonin increases the expression of kisspeptin proteins, which are important regulatory molecules for the secretion of luteinizing hormone [57] and follicle-stimulating hormone [37] [58]. It has been shown that the effects of melatonin on improvement of fertility, oocyte development and embryo maintenance are attributed to its capacity to reduce oxidative damage in the ovarian follicles [59, 60]. At the same time, higher concentrations of melatonin observed during pregnancy appear to support fetal maturation and placental homeostasis [59].

Pinealectomized rats show impaired ovulation [61, 62]. Upon pinealectomy, the decline in serum melatonin is followed by a proliferative response of the endometrium; however, melatonin replacement therapy mitigates the morphological dysregulation of its epithelium [63]. The presence of melatonin receptors in the endometrium evidences its importance in reproduction [64]. Song *et al* observed that female mice supplemented with melatonin added to drinking water for 12 months showed reduced ovary ageing parameters, improved quantity and quality of oocytes, and increased litter size [36].

The effects of melatonin in reproduction and follicular maturation has been investigated in non-mammal species, including the zebrafish [56, 65, 66]. Yumnamcha *et al* found that Zebrafish exposed to continuous darkness show higher levels of melatonin in the brain and in the ovary than those exposed to continuous light [67]. The authors also found that the higher melatonin levels were associated with increased GnRH, decreased gonadotropin-inhibiting hormone (GnIH) and improved reproductive rate [67]. Furthermore, melatonin appears to play a role in the zebrafish reproductive cycle by balancing the synthesis of LH and FSH [56].

Melatonin is involved in the modulation of Kiss-1 and Kiss-2 gene activities. Previously observed in other species, including rat [68] and sheep [69], Kiss-1 and Kiss-2 enhance reproductive rate and sexual maturation by promoting the secretion of

gonadotrophins. Clinically, these genes appear to display modulatory roles in the reproductive system from puberty to the hormonal cycle phase; mechanistically, Kiss-1 and Kiss-2 appear to influence the activity of GnRH neurons and mediate metabolic factors, such as adipose tissue hormones and energy balance [70].

#### **4. The effects of melatonin on reproduction – human studies**

Metabolic disarrangement, menstrual cycle abnormalities and increased risk of miscarriage are manifestations reported in women suffering with polycystic ovary syndrome (PCOS) [71, 72]. In a prospective cohort study including 40 women diagnosed with PCOS, melatonin supplementation for six months was effective in restoring their menstrual cyclicity and in normalizing androgenic parameters [73]. The antiandrogenic potential of melatonin is supported by its capacity to decrease the levels of cyclic AMP in granulosa cells (GCs), ultimately reducing the production of androgenic hormones [74]. Endometriosis is another condition that results in reproductive dysregulation, and can lead to infertility in up to 50% of sufferers [75, 76]. Yang *et al* observed that melatonin supplementation appears to be a feasible adjuvant therapy for endometriosis and recurrent spontaneous miscarriage [77], as well as assisting in reduction of pain scores and normalization of menstrual disorders such as dysmenorrhea [78]. As melatonin crosses the placental barrier, it may have the potential to protect the embryo by reducing oxidative damage, possibly hindering the progression of oxidative stress-related fetal abnormalities [79].

The therapeutic properties of exogenous melatonin may be effective in improving fertility rates [80]. As summarized in table 1, melatonin supplementation in disorders of the female reproductive system appears to be clinically relevant, improving fertility rate, oocyte quality, maturation and number of embryos [15, 81-84]. A randomized double-blind placebo-controlled study showed that perimenopausal and menopausal women had lower salivary melatonin levels, a finding that was positively associated with metabolic impairments including lower levels of thyroid hormones [74]. The same study showed that after six months of melatonin supplementation, the investigated biomarkers had shown signs of improvement, as well as significant improvement in mood and depression scores. Another study [85] investigating the effects of melatonin daily supplementation for 3 months in postmenopausal women found that the melatonin-receiving group showed significant improvement in various climacteric symptoms, including physical, psychological and sexual, in comparison to the placebo-receiving group.

(Insert table 1 here)

**Table 1:** Clinical findings on gynecological parameters after melatonin supplementation.

<b>Gynecological Disorder</b>	<b>N</b>	<b>Age mean or range</b>	<b>Daily dose of melatonin</b>	<b>Duration</b>	<b>Outcomes</b>	<b>References</b>
Polycystic ovary syndrome	40	23	2mg	6 Months	Improvement of menstrual irregularities and reduction of hyperandrogenism	[73]
Endometriosis	40	37	10mg	8 Weeks	Reduced pain scores and dysmenorrhea Increased sleep quality	[78]
Primary infertility	85	30	3mg	Acute	Increased mature oocytes and class 1 embryos	[83]
Low fertility rate	18	35	3mg	Acute	Increased fertility rate	[60]
Low fertility rate	60	30	3mg	Acute	Increased number of mature oocytes and class 1 embryos Increased embryo quality	[82]
Infertility	160	18 to 45	4mg 8mg 16mg	3 Days	Increased markers of follicle health and clinical pregnancy rates	[84]
Low fertility rate	72	34	1mg 3mg	Acute	Increased oocyte quality and number of fertilized embryos	[81]

Fetal growth restriction	12	Uninformed	8mg	47 Days	Reduction of the placental oxidative marker malondialdehyde	[86]
Pre-eclampsia	68	33	30mg	Until at birth	Prolonged gestation and reduced the pharmacological need for antihypertensives	[87]
Climacteric Symptoms	240	53	3mg	3 Months	Reduced climacteric symptoms	[85]

Taken together, these findings corroborate the substantial role of melatonin in the reproductive axis, whose reduced production upon aging has been associated with the onset of menopause [88], thus hindering FSH secretion [57].

### **5. 6-sulfatoxymelatonin concentration: a reliable biomarker on gynecology?**

A surrogate biomarker of melatonin metabolism is 6-sulfatoxymelatonin, a melatonin metabolite whose urinary excretion has already been employed in the assessment of gynecological disorders [89, 90]. Reduced urinary 6-sulfatoxymelatonin levels, associated with higher estradiol and progesterone levels, have been detected in women who work rotating night shifts, in comparison to women who work day shifts [91, 92]. Additionally, decreased circulating levels of 6-sulfatoxymelatonin were reported in girls affected by early onset puberty, which evidences the important relationship between this biomarker and the female reproductive axis [93].

In the opposite direction, Luboshitzky *et al* detected increased urinary levels of 6-sulfatoxymelatonin in a cohort of women diagnosed with PCOS, in comparison with non-PCOS aged-matched women [94], a finding which the authors have associated with the androgenic aspects of the syndrome. However, the urinary excretion of 6-sulfatoxymelatonin does not appear to be a reliable biomarker in patients with endometriosis [95, 96].

### **6. Melatonin in Pregnancy**

A potential application for melatonin supplementation as adjuvant therapy has been proposed to aid positive outcomes in pregnancy. Research on melatonin administration during the gestational period in animal models appears to suggest a protective mechanism upon neurogenesis, placental protection, reduction of oxidative stress, increased reproductive rate and improved fetal development [97-99]. The well described crossing of melatonin through the placental barrier, and its binding to MT1 and MT2 receptors in placental tissue, further evidences its effects upon fetal development; it confirms the role of the mother's own circadian rhythm as the first zeitgeber the fetus is exposed to, which in turn modulates fetal neuroendocrine and immune development, and confers antioxidant protection [100-105].

The antioxidant properties of melatonin upon placental tissue are attributed to its capacity to increase the expression of catalase and superoxide dismutase, whilst its autocrine and paracrine activities in placental endothelial cells appears to influence the development of the fetus' own circadian system [97-99]. Melatonin receptors expressed in the fetal brain are influenced by the mother's hormone variation, and such role is paramount to protect the fetus' developing brain against oxidative stress and variations in oxygen levels [100].

Potentially beneficial effects of melatonin have been proposed as an appealing therapeutic tool in gestational disorders. Fetal growth restriction (FGR) affects 5 to 10% of pregnancies, being the second most common cause of perinatal mortality. It is associated with fetal hypoxia, nutrient deprivation and imbalanced oxidative stress [106, 107]. Taking this concern into account, Miller *et al.* found no safety issues in melatonin supplementation for both mother and fetus, and also found reduced levels of malondialdehyde, which is a placental oxidative biomarker [86].

Pre-eclampsia is another expressive gestational disorder that affects 3 to 10% of pregnancies, classically characterized by high blood pressure and proteinuria [108-110]. Placental reduction of melatonin receptor (MT1 and MT2) expression, alongside inhibition of aralkylamine N-acetyltransferase (AANAT), a key enzyme in melatonin synthesis, have been observed in pre-eclampsia [111]. Animal and *in vitro* studies have identified an important protective effect of melatonin administration against gestational dysregulations, protecting fetal mitochondria and placental DNA, at the same time reducing inflammatory and oxidative biomarkers [87, 112]. In humans, Hobson et al. observed that melatonin supplementation prolonged gestation and reduced the dosage of antihypertensive drugs [87]; further corroborating the safety of melatonin supplementation in pregnancy.

## **7. Approaches to posology**

The evidence so far available supports the suggestion that melatonin supplementation confers positive outcomes in the routine gynecological care, not only improving general well-being but also ameliorating reproductive parameters for women of child-bearing age who wish to become mothers [101-103]. Further evidence suggests that melatonin confers positive outcomes for the musculoskeletal system, improving strength and bone health, alongside positive outcomes of psychological nature [104, 113-116].

The safety of melatonin supplementation as sleep aid is relatively well documented [117]. Its applications in the field of gynecology appear to be not only similarly safe, but also promising. Despite the relatively small number of relevant studies available, most of them investigating short-term effects, a daily posology ranging from of 2 to 18 mg appears to be the most commonly employed, as summarized in Table 1.

Notwithstanding the apparent safety and allegedly positive health outcomes, robust long-term clinical trials that investigate the likelihood and severity of risks associated with any melatonin-drug interaction are required before any recommendation can be made for expecting mothers. Very long-term studies which investigate the health of individuals whose mothers received melatonin supplementation during pregnancy are also required to ensure safe recommendations. Directions for future work on melatonin supplementation are suggested in Table 2.

(Insert table 2 here)

**Table 2:** Open-ended questions for clinical trials in the area of gynecology.

***Conceptual and Clinical***

- Is chronic melatonin supplementation safe in pregnancy?
- Is melatonin deficit the culprit for gynecological disorders? Or the other way around?
- How does the antioxidant effect of melatonin compare with other antioxidant molecules for gynecology?
- What are the effects of melatonin on embryo development?
- Can melatonin reduce the occurrence of miscarriages?
- Can melatonin modulate phenotypes related to chromosomal disorders?
- Can melatonin urinary metabolites be employed as diagnostic tool in clinical practice?
- Are there adverse effects of melatonin supplementation for the fetus?
- Is there a safe dose range during pregnancy?

***Hormone profile***

- What are the mechanisms involved in the antiandrogenic effect of melatonin?
- Can melatonin modulate the activity of androgenic receptors?
- How does melatonin modulate the secretion of estrogen?
- Can 6-sulfatoxymelatonin metabolite affect the androgenic response?
- Can 6-sulfatoxymelatonin be an early surrogate biomarker of infertility?
- Can hormone replacement therapy change plasma levels of melatonin?
- Can postmenopausal hormone profile affect the melatoninergetic response?
- In what other gynecological disorders can the 6-sulfatoxymelatonin marker be a useful clinical tool?



## **8. Conclusion**

Melatonin supplementation may offer a therapeutic as well as preventative potential in the area of gynecology and obstetrics due to its antioxidant properties and activity as hormone modulator. It has been observed in melatonin supplementation studies ranging from a few days to up to six months of duration that a daily posology of 2 to 18 mg of melatonin appeared to improve fertility rate, oocyte quality, maturation and number of embryos. In gestational disorders, daily doses of 8 to 30 mg appeared safe for the mother and for the fetus, in addition to an important therapeutic outcome. However, no final consensus has yet been reached in respect to effective dose or supplementation period in the field of obstetrics and gynecology. Despite melatonin supplementation being relatively safe and apparently beneficial, long-term clinical trials are imperative to assess further clinical outcomes, with particular attention to the gestational period.

### **Conflict of interests**

The authors have no competing interests to declare.

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