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***Review Article** (Open Access)*

ROLE OF MELATONIN AS AN ANTIOXIDANT IN FEMALE REPRODUCTION, SPECIAL EMPHASIS ON INFERTILITY

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ABSTRACT
The pineal gland, which is principally responsible for producing and secreting the neurohormone melatonin. Its production is reliant on ambient lighting, and light inhibits its release. Recently, researchers have begun exploring potential mechanisms to stop these effects with the use of novel oxygen scavengers like melatonin. This is because it has been found that the effectiveness of infertility therapies might be adversely impacted by an imbalance of reactive oxygen species. These drugs could increase the success rates of pregnancies following IVF. With a focus on the reproductive system and infertility therapy, we provide an overview of the most recent research on the effect of melatonin on oxidative stress. Melatonin acts as a direct free radical scavenger as well as having anti-oxidative actions through its receptors. How melatonin affects the female reproductive system has been described. The pineal gland secretes melatonin, which is then absorbed from the blood into the follicular fluid. Melatonin is known to scavenge ROS produced within follicles, particularly during the ovulation process, and it may also play a role in oocyte maturation, embryo development, and luteinization of granulosa cells. Here, a clinical trial showed that giving melatonin to infertile women increased the likelihood of fertilization and pregnancy. Good evidence supporting the use of melatonin in the treatment of a number of medical disorders has come from various fields. Reactive oxygen species have the ability to impair the quality of oocytes and embryos and are significantly connected with infertility treatments. The anti-oxidant effect of melatonin holds promise as a complementary therapy for the treatment of infertility.

Keywords: *Melatonin, Antioxidant, Reproduction, Pregnancy, Infertility, ROS*

INTRODUCTION
The neurohormone melatonin (N-acetyl-5-methoxytryptamine), which is primarily produced and secreted by the pineal gland, was initially discovered in 1958 (Chakravarty and Rizvi, 2008). Further research after its discovery has shown that it is also produced by a number of other organs. The digestive system (Madalinski , 2011), brain (Biran *et al.*, 2014), eye (Bai *et al.*, 2013), lungs (Matos *et al.*, 2012), kidney (Russcher *et al.*, 2012), liver (Carbajo *et al.*, 2013), thyroid, thymus, and pancreas (Nowrot *et al.*, 2013), immune system (Csaba ,2013) and reproductive system (Acuna *et al.*, 2014). have all been reported to contain it. Melatonin is an indoleamine, which is synthesised from the essential amino acid, tryptophan (Reiter *et al.*, 2013). Its production is dependent on ambient illumination, with release being suppressed by light. More recently, it has been discovered that an imbalance of reactive oxygen species, or 'oxidative stress', can have a negative impact on the success of infertility treatments, and furthermore, investigators have begun addressing potential mechanisms of preventing these effects with the use of novel oxygen scavengers such as melatonin. These medications may improve the success rates of pregnancies after IVF treatment. We offer an overview of the most recent research on melatonin's impact on oxidative stress, with an emphasis on the reproductive system and infertility treatment. Melatonin has a short half-life and both melatonin and its metabolites can be measured in serum, urine and saliva (Shreeve *et al.*, 2013, Almeida *et al.*, 2011).
Infertility therapy has gained acceptance over the past few decades, and as technology has advanced, pressure has increased for higher success rates. This trend is being supported by the perception that assisted reproductive technologies (ART) can be used to delay pregnancy while still achieving it successfully

(Lundsberg *et al.*, 2014). As a result, in-vitro fertilisation (IVF) success rates must be continuously improved to meet social and technological expectations, which motivates research on innovative adjuvant medicines.

A. 1. General role of melatonin

Melatonin has been identified as a key factor in the regulation of circadian rhythms and the sleep-wake cycle (Reiter *et al.*, 2014) (Fig.1). Long exposure to artificial lighting leads to a reduction in endogenous melatonin exposure (Zhu *et al.*, 2013). Melatoninis thus associated with sleep disturbances including insomnia and much of the literature are focused in this area (Wilhelmsen *et al.*, 2013). It also appears to regulate reproductive seasonal variation in many animal species (Clarke *et al.*, 2013; Zaidi *et al.*, 1995). Nevertheless, a daily circadian rhythm is raising concerns about what other functions it might play in people.

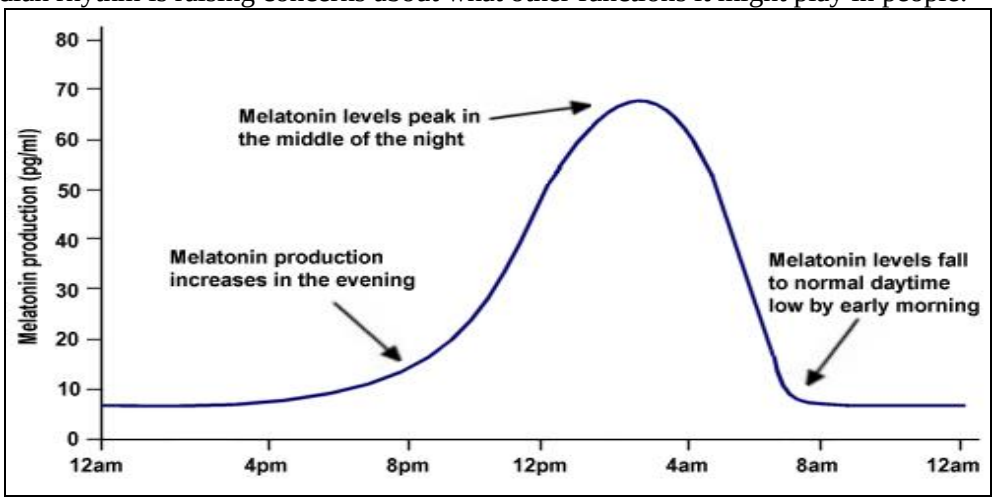

Figure 1: Circadian rhythm of melatonin hormone.

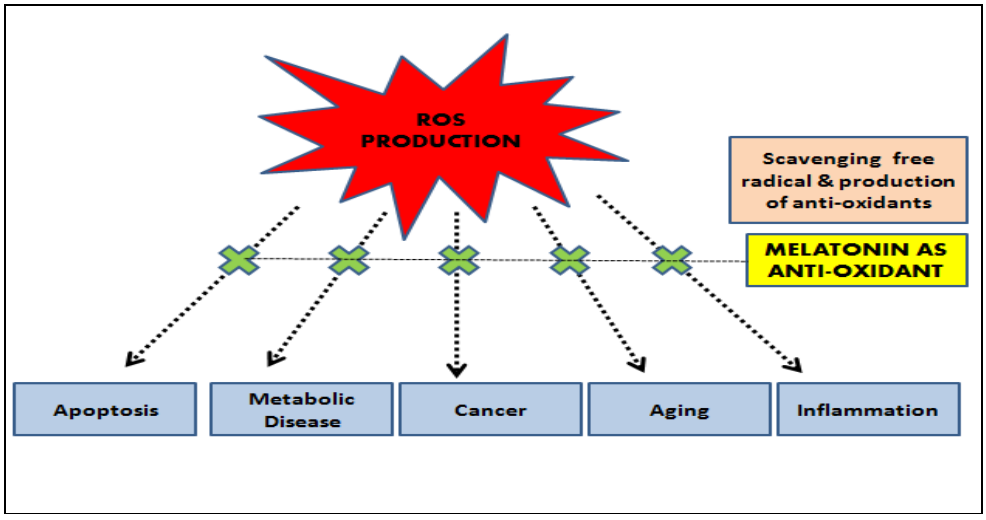

Figure 2: Melatonin act as antioxidant on ROS generation and potential applications in some human diseases.

A. 2. Melatonin as an antioxidant

When oxygen is used in metabolic activities, free oxygen radicals are produced. Since these radicals have 'free' valence electrons, they are extremely reactive and can harm cells (kojo , 2004). According to (Bouayed *et al.*, 2010), the term "reactive oxygen species" (ROS) encompasses not just free radicals but also stable

non-radical molecules that can cause oxidation, such as hydrogen peroxide (H2O2). Compared with other oxygen scavengers, melatonin is of particular interest because it has several qualities distinguishing and rendering it superior to classical anti-oxidative agents. Melatonin has anti-oxidative effects through its receptors, MT1 and MT2 (Agarwal *et al.*, 2005)], but also as a direct free radical scavenger (Srinivasan *et al.*, 2009)). It has binding sites within the nucleus (Tamura *et al.*, 2012), and is amphiphilic, allowing it to cross cell membranes freely (Benitez *et al.*, 1993; Tang *et al.*, 1998). The fact that melatonin is a suicidal terminal anti-oxidant, in contrast to conventional anti-oxidants, is one of its most distinctive qualities. The activity of other endogenous antioxidants like glutathione peroxidase and superoxide dismutase is also improved by melatonin, which is significant (Fig. 2).

Molecular mechanism of melatonin action

Melatonin has both receptor-dependent and receptor-independent actions. The indole binds to well known membrane receptors (MT1 and MT2) and, via several signal transduction pathways, influences a host of physiological effects. MT1 and MT2 may homo- and/or heterodimerize in some cases, and they may interact with nuclear receptors (binding sites). There is considerable debate regarding the existence/function of the orphan nuclear melatonin receptors ROR and RZR. The cytosolic receptor, MT3, is a detoxifying enzyme, quinone reductase 2. Receptor-independent actions are mediated by the ability of melatonin and its metabolites to scavenge reactive oxygen (ROS) and reactive nitrogen species (RNS). These actions allow melatonin to protect against a wide variety of toxins and processes that generate highly toxic reactants. Any cell can simultaneously respond to melatonin by both its receptor-mediated and receptor-independent actions. Many of the documented physiological and molecular actions of melatonin are not listed in this figure. Additionally, the figure does not include melatonin functions in non vertebrates or in plants (Sarti *et al.*, 2013).

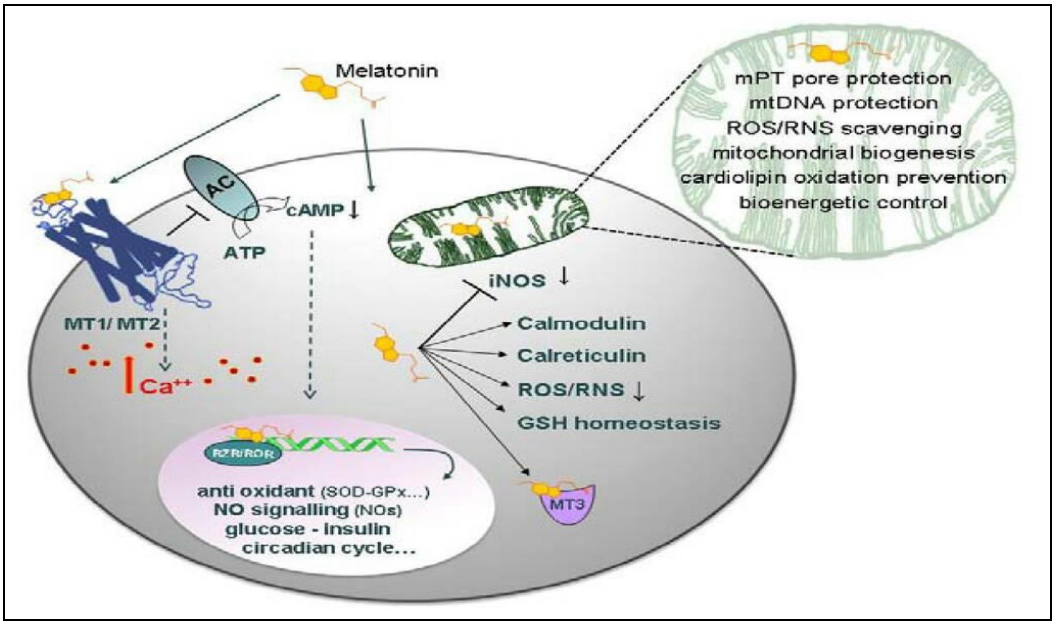

Figure 3: Action of melatonin at cellular level (Sarti *et al.*, 2013).

Melatonin on the cell stage. Melatonin interacts with cells in a receptor-dependent or -independent manner. The receptors on the cell membrane, MT1 (*Mel 1a*) and MT2 (*Mel 1b*), consist of seven transmembrane helices, G protein-coupled. Activating G protein signalling, the receptors mediate a wide variety of effects; among others, inhibition of the adenylate cyclase (AC), with a consequent cyclic AMP (cAMP) decrease, regulation of gene transcription, activation of protein kinase C subtypes and changes of intracellular Ca^{++} levels. Independently of receptors, melatonin permeates cell membranes and, owing to its low redox potential, $E_o = -980$ mV, scavenges the ROS in the cell cytoplasm, mitochondria and nucleus. In the

cytoplasm, melatonin maintains GSH homeostasis and interacts with proteins, such as calmodulin (CaCaM), calreticulin and the cytosolic quinone reductase 2 enzyme, (MT3). Melatonin is also a ligand for a nuclear retinoid related orphan nuclear hormone receptor (RZR/RORa) regulating the expression of anti-oxidant enzymes, such as glutathione peroxidase (GPx), glutathione reductase (GRd) and superoxide dismutase (SOD), and down regulating pro-oxidant enzymes, such as the NOSs, particularly the iNOS . Melatonin is accumulated in mitochondria at high concentrations, where it scavenges ROS and RNS. Melatonin also protects cardiolipin from oxidation and prevents respiratory chain complexes, as well as mtDNA from free radical attack, thus ultimately protecting the membrane permeability transition (mPT) pore, thus preventing cell apoptosis (Sarti *et al.*, 2013) (Fig. 3).

B. Role of melatonin in female reproductive organ and reproduction process

B. 1. In ovulation and luteinization

In humans, the only data on cyclical melatonin changes comes from women undergoing ovarian stimulation. Levels of melatonin reach a nadir in the preovulatory phase and peak in the luteal phase (Webley *et al.*, 1987)(Fig. 4). This suggests that melatonin has variable effects dependent on the menstrual phase. It is also well known that shift-workers are more likely than daytime workers to experience circadian disruption and longer menstrual cycles, more menorrhagia and dysmenorrhoea (Attarchi *et al.*, 2013; Lawson *et al.*, 2011). These results are corroborated by a very large cohort study, which also found that duration of shift work was modestly associated with menstrual cycle irregularity (Beczek *et al.*, 2007). In fact, in very high doses, when combined with progesterone, melatonin has the ability to suppress ovulation in humans, possibly by interfering with LH release (Itoh *et al.*, 1999).Interestingly, melatonin receptors have been found on granulosa cells, indicating that this may be an additional site of melatonin activity (Carlomagno *et al.*, 2011).Melatonin appears to be more important for reproduction during pregnancy (Tamura *et al.*, 2008), and studies have begun to look at its potential as a treatment for pre-eclampsia and neonatal neurological morbidity (Maria *et al.*, 2013).

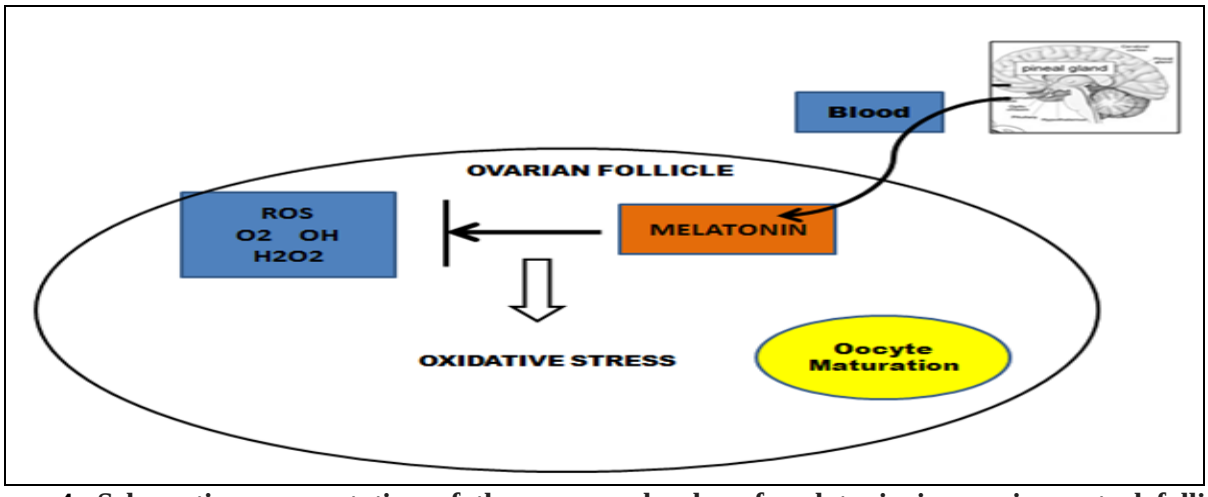

Figure 4: Schematic representation of the presumed roles of melatonin in ovarian antral follicle. Melatonin, secreted by pineal gland, is taken up into the follicular fluid from the blood. Melatonin scavenges ROS produced within the follicles, particularly during the ovulation process, and reduced oxidative stress may have a role in oocyte maturation and embryo development.

Melatonin also appears to have a role in the prevention of postmenopausal bone loss, with effects being exerted via inhibition of oxidative stress, induction of osteoblastogenesis and inhibition of osteoclast genesis (Meliska *et al.*, 2011). These findings and evidence from a small randomised controlled trial suggests

that melatonin may be useful in the treatment of perimenopausal and menopausal symptoms and sequelae (Barron, 2007)).The positive implications of higher melatonin levels on the human menstrual cycle, fertility and pregnancy are therefore well documented, with varying levels of evidence (Hemadi *et al.*, 2012).

B. 2. In placenta

The placenta is a critical component of the life support system of the foetus. For the foetus to normally develop, the placenta must function optimally. Considering melatonin's protective actions for its antioxidant and anti-inflammatory functions, it would be advantageous for the foetal/placental unit to produce melatonin for its local use.

In 2005, a study reported the presence of mRNA transcripts for alkylamine *N*-acetyltransferase (AANAT), sometimes considered the rate-limiting enzyme for melatonin synthesis (Reiter *et al.*, 2013), and acetylserotonin methyltransferase (ASMT), the melatonin-forming enzyme , in placental tissue obtained during the first trimester of human pregnancy. DNA sequencing of the RT-PCR products were found to be identical for the genes for AANAT and ASMT. The authors surmised that melatonin produced locally works in a paracrine manner to enhance the function of placental tissue. One action that they showed melatonin to mediate at the level of the trophoblast cells was the augmented release of human chorionic gonadotropin (hCG). Since they also documented the presence of RNA transcripts for the MT1 and MT2 melatonin membrane receptors in placental tissue, the action of melatonin on hCG release was assumed to be mediated by these receptors although no direct proof for this was provided (Iwasaki *et al.*, 2005)

The proliferating villous cytotrophoblasts (stem cells) differentiate and fuse into the syncytiotrophoblast, which is non-proliferative and undergoes rapid apoptosis (Lanoix *et al.*, 2012)as diagrammatically represented in Figure 5. Thus, during a normal pregnancy, the syncytiotrophoblast turns over and is continually renewed. A precise balance between the fusions of the villous cytotrophoblasts into the syncytiotrophoblast is needed to prevent placental pathology. Given the actions of melatonin in regulating apoptosis in normal cells (in this case, the cytotrophoblasts) and causing apoptosis in cancer-type cells (in this case, the tumour-like syncytiotrophoblast), the indole could have a major influence in creating a stable villous cytotrophoblast/syncytiotrophoblast homeostasis (Fig. 5).

It is well recognised that a placenta that is not working regularly can lead to a variety of diseases that affect both the mother and the foetus. Melatonin has been demonstrated to be helpful or is anticipated to be helpful in the treatment of certain ailments.

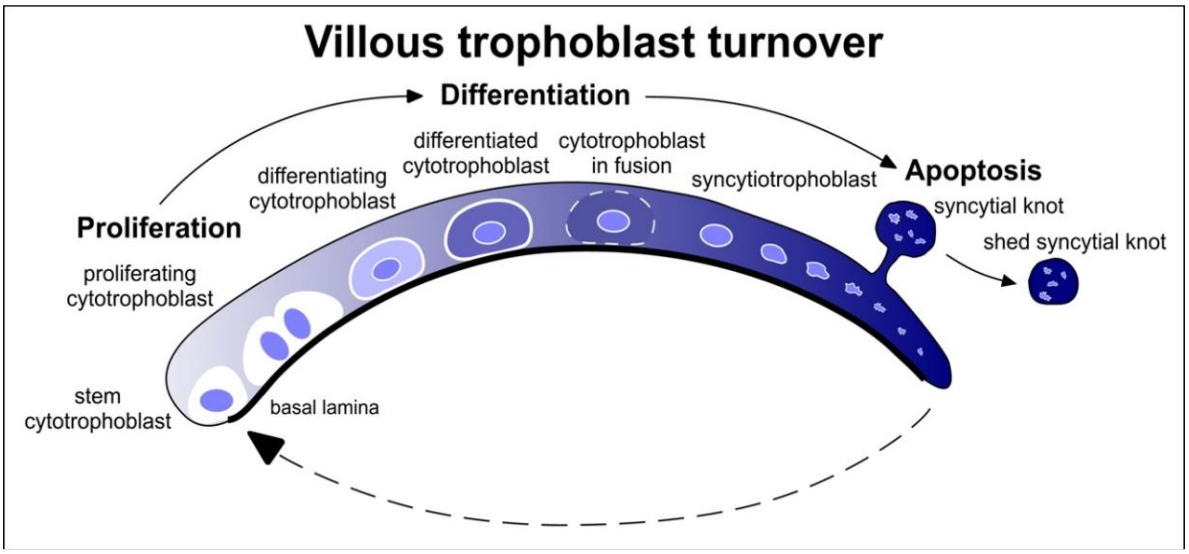

Figure 5: Turnover of cells that make up the villous trophoblast is essential for proper functioning of the placenta. Proliferative cytotrophoblast stem cells differentiate and eventually exit

the cell cycle. These cells then fuse to form a multinucleated syncytium, the syncytiotrophoblast. Within several days cells of the syncytiotrophoblast undergo apoptosis. The additions of differentiated cytotrophoblasts replace the syncytiotrophoblast cells that are lost via apoptosis. The apoptotic loss of the syncytiotrophoblast is balanced by the fusion of differentiated cytotrophoblast cells. Locally produced melatonin seems to be involved in maintenance of homeostasis by limiting apoptosis of the differentiated cytotrophoblasts while enhancing apoptosis of the syncytiotrophoblast; these latter cells have characteristics of cancer cells in which melatonin also causes apoptosis (Lanoix *et al.*, 2012).

B.3. In pregnancy and delivery

Serum melatonin levels in humans are known to be substantially greater while pregnant and labour than they are throughout the postpartum period. It has previously studied the blood melatonin concentrations in normal pregnant women at daytime (15 00 h) and night time (02 00 h) (Nakamura *et al.*, 2001) (Fig. 5). In typical singleton pregnancies, the daytime serum melatonin levels did not significantly increase as the pregnancy progressed. Serum melatonin levels, on the other hand, were consistently and considerably higher at night than during the day throughout pregnancy, progressively rising after 24 weeks of gestation and demonstrating significantly higher levels after 32 weeks. Thereafter, they declined to the non-pregnant levels on the 2nd day of puerperium in normal singleton pregnancies. It also recently measured the maternal serum melatonin levels throughout pregnancy in rats (Nakamura *et al.*, 2001) (Fig. 6).

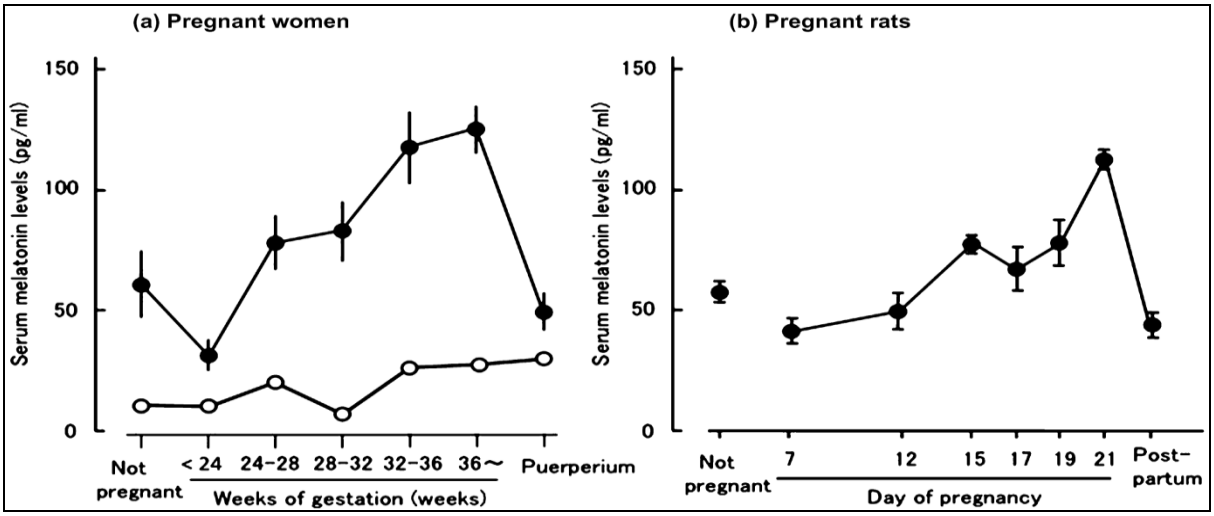

Figure 6: Changes of maternal serum melatonin in pregnancy. (a) Levels of maternal serum melatonin during the night (solid line) and day (dotted line) in normal singleton pregnancy. Values are means standard error of the mean. (b) Changes in maternal serum melatonin levels at night-time during pregnancy in rats. Maternal circulating night-time melatonin concentrations were measured on days 7, 12, 15, 17, 19 and 21 of pregnancy, and on day 2 of postpartum. Data are shown as the mean standard error of the mean for 7–10 rats (Nakamura *et al.*, 2001).

Human foetuses exhibit circadian rhythms in hormones, behavior, heart rate and sleep. The photoperiodic information perceived by the mother is thought to play an important role in synchronizing foetal physiology. Presumably, the information about day length and circadian phase, mediated by the maternal melatonin rhythm, is transferred to the foetus.

B. 4 . An contraceptives

The effect of melatonin on the hypothalamus pituitary axis and therefore the reproductive system have initiated studied aimed at the use of melatonin as an oral contraceptive. When using high doses of melatonin (75 and 300 mg daily) in combination with norethisteron, no pick in LH hormone secretion was observed

during the menstrual cycle. Furthermore plasma FSH hormone levels remained constant. These circumstances prevented ovulation and the increase in progesterone during luteal phase.
Melatonin drugs are normally use in combination with progesterone analogue hormones. High level of melatonin acts on hypothalamic pituitary axis and prevents LH surge during menstrual cycle, Hence ovulation does not occur due to the absence of LH surge. On the other hand progesterone analogue hormone(progestin) increases the thickness of cervical mucus, blocking passage of sperm into uterus, thus acting as another way of contraception.

C. Melatonin use in infertility treatment

C. 1. The importance of oxidative stress in assisted reproductive technology (ART)

The relevance of oxidative stress in ART has gained increasing attention in recent literature, in particular with regards to IVF. IVF can result in exposure of oocyte sand embryos to high levels of superoxide free radicals, which begins prior to oocyte retrieval (Guerin *et al.,* 2001). Ovarian stimulation protocols are associated with significant changes to the in-vivo follicular environment, altering endogenous levels of oxygen scavengers (Palini *et al.,* 2014). Furthermore, in-vitro, these oocytes are no longer protected by antioxidant-rich follicular fluid, leaving them more susceptible to oxidative damage (Yoshoda *et al.,* 1993; Fatechi *et al.,* 2005; Huang *et al.,* 2014). They may also be exposed to high oxygen concentrations in incubators and during handling throughout the IVF process, with higher concentrations of oxygen being associated with more ROS, and a positive effect of melatonin being more marked in oocytes exposed to higher oxygen tensions (Papis *et al.,* 2007). This oxidative stress modifies the quality of oocytes and embryos, decreasing the fertilisation rate and the success of the infertility treatment (Takahashi *et al.,* 2003; Plessis *et al.,* 2008).
While reactive oxygen species are required for\sperm capacitation events (O'Flaherty *et al.,* 2006; Dona *et al.,* 2011), an imbalance of ROS has been implicated as a factor in reducing the quality and function of sperm (Dona *et al.,* 2011), with most protection from these effects being afforded by the enzymatic antioxidant, superoxide dismutase (Aitken *et al.,* 1987; Alvarez *et al.,* 1987 ; Guz *et al.,* 2013)).The recognition of the association between exposure of gametes and embryos to oxidative stress and a reduction in the success rates of IVF has led investigators to assess whether these adverse effects can either be prevented or reversed, with emphasis being placed on the adjuvant use of oxygen scavengers including melatonin.

C. 2. The role of melatonin in assisted reproductive technology

In the course of treating infertility, oxidative stress manifests itself in a variety of ways (Koppers *et al.,* 2011). Recent interventional studies have focused on the impact of oral melatonin supplementation on the quality of gametes and embryos during the ovarian stimulation phase of IVF cycles.

C. 3. Effects of melatonin on oocyte quality

Recent research on mice revealed that oxidative stress in oocytes may start as soon as 8 hours after ovulation and increase rapidly from there. It was also discovered in this study that in-vitro addition of 1 mM of melatonin to oocyte culture medium greatly ameliorated these time-dependent effects, leading to 54% of fertilised oocytes reaching the blastocyst stage in the presence of melatonin compared to 29% in the controls (Lord , 2013). This study not only showed that an imbalance of ROS is an important cause of impaired oocyte quality in-vitro, but also that the addition of melatonin could reverse these effects (Fig. 7).
Melatonin is an effective mitigator of mitochondrial DNA damage (Ei-Raey *et al.,* 2011), likely as a result of an increase in electron transport efficiency, within mitochondria, thus preventing the formation of ROS (Hardeland *et al.,* 2005). In some situations melatonin may be even more effective at performing this function than specific mitochondrial antioxidants (Lowes *et al.,* 2013), and this particular characteristic may have relevance to its use in the treatment of infertility and the improvement of oocyte quality and maturity (Fig. 8).
A recent review concluded that oral administration of melatonin reduces intra follicular oxidative damage and increases fertilisation rates (Tamura *et al.,* 2008). Unfortunately, most studies addressing the use of melatonin in infertility treatment have been conducted with patients as their own controls (‘before and after’

comparison) (Nishihara *et al.,* 2014; Barnett *et al.,* 2005). In the absence of proper control or placebo groups, it must be assumed that any beneficial effects thus observed are explained by the phenomenon of regression toward the mean (Rombauts, 2007).

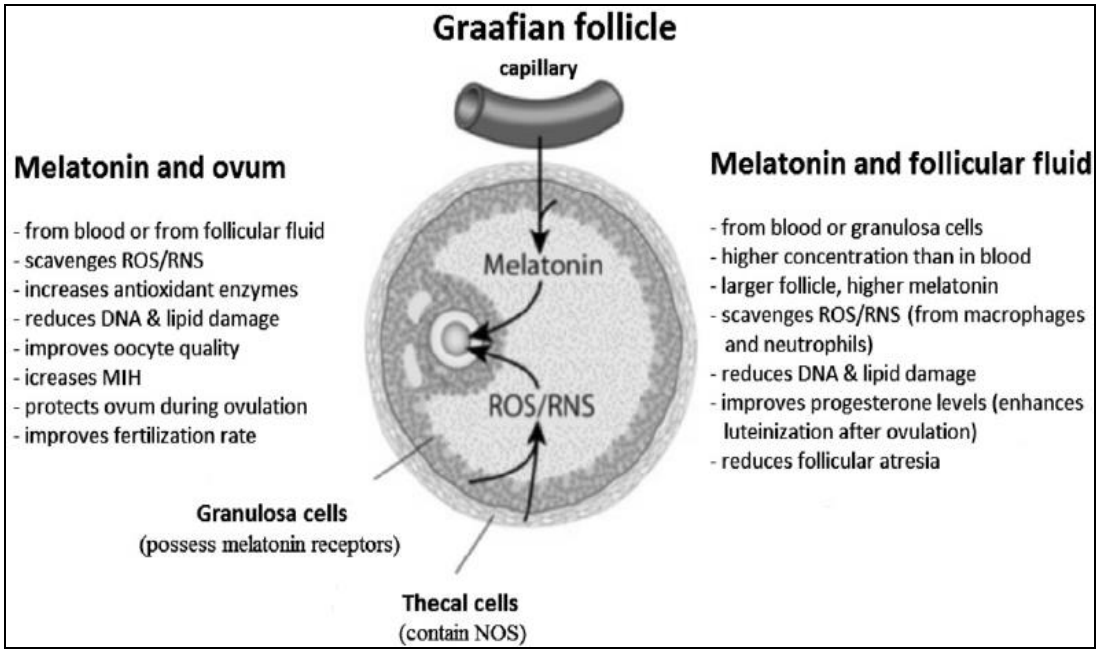

Figure 7: Some of the proposed function of melatonin in the Graafian follicle. ROS reactive oxygen speies, RNS reactive nitrogen species, MIH maturation-inducing hormone, NOS nitric oxide synthase **(Dikic *et al.*, 2014).**

Despite its limitations, these findings were in keeping with another larger unblinded randomised trial looking specifically at the effect of melatonin on IVF outcomes. Eighty women were randomised to receiving melatonin 3 mg/day or no treatment from the commencement of GnRH agonist administration. The percentage of mature oocytes was higher in the melatonin group ($p < 0.05$) as was the proportion of high quality embryos, however, an increase in clinical pregnancy rate did not reach statistical significance (Espino *et al.,* 2011). Additionally, patients with cancelled cycles were not included in the analysis making these findings susceptible to attrition bias.

In general, it is accepted that a higher percentage of motile sperm is associated with improved fertilisation rates and Ortiz et al. has shown that the addition of melatonin to seminal samples can improve the overall motility and the percentage of progressively motile spermatozoa (Espino *et al.,* 2010). Melatonin also appears to inhibit apoptosis in spermatozoa, with a reduction in early apoptotic events being demonstrated in human sperm thus prolonging sperm survival (Moghadam *et al.,* 2014). These effects would serve to improve sperm quality , therefore increasing the probability of successful fertilisation.

Melatonin, through its neutralisation of reactive oxygen and nitrogen species, has been shown in both animal and human studies to improve seminal quality in-vitro. A study investigating the addition of melatonin to semen extender in cryopreserved seminal samples from Holstein bulls resulted in amelioration of the oxidative effects of the freeze-thaw process (Kim *et al.,* 2013). Studies in rats also have shown that melatonin has a positive effect on sperm that have been subjected to oxidative stress, improving sperm number, viability and motility (Liu *et al.,* 2013; Plessis *et al.,* 2010). Similar results have been found in a small human study in which in-vitro melatonin-treated samples showed a higher percentage of sperm motility and a lower proportion of non-viable spermatozoa (Ishizuka *et al.,* 2000) (Fig. 8).

C. 4. Effects of melatonin on embryo culture media

According to numerous studies (Choi *et al.*, 2008; Sugino *et al.*, 1999), adding melatonin to in-vitro culture media has a positive overall impact on the development of pig, murine, and bovine embryos. With lower doses being more advantageous (and less hazardous) than higher ones, it appears that in-vitro supplementation of embryo culture material with melatonin has a substantial impact on the development and quality of embryos (Fig. 8).

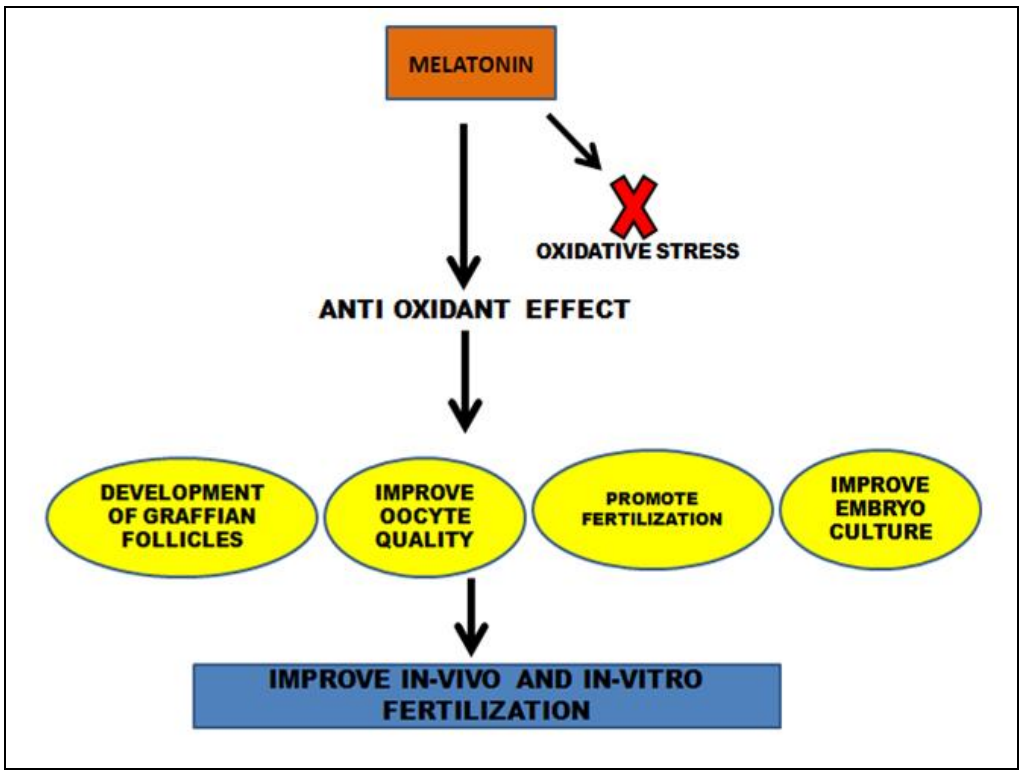

Figure 8: schematic presentation of melatonin role in fertilization

CONCLUSION

Compared with other oxygen scavengers, melatonin is of particular interest because it has several qualities distinguishing and rendering it superior to classical anti-oxidative agents. Melatonin has anti-oxidative effects through its receptors but also as a direct free radical scavenger. It has been outlined how melatonin functions in the female reproductive system. This study showed that placental hormones control the night time melatonin concentrations in pregnant women, which rise towards delivery. Melatonin serum levels that are higher at night may control when a woman gives birth. There was also a correlation between perimenopausal women's lipid profiles and their serum melatonin levels. Additionally, it has been discovered that melatonin therapy may enhance lipid metabolism and has concentrated on the intrafollicular function of melatonin in the ovaries.

Melatonin is secreted from the pineal gland, is taken up into the follicular fluid from the blood. It has been noted that ROS produced within the follicles, especially during the ovulation process, are scavenged by melatonin, while reduced oxidative stress may be involved in oocyte maturation, embryo development and luteinisation of granulosa cells. Clinical study demonstrated here that melatonin treatment in infertile women increases fertilization and pregnancy rates.

Good quality evidence has emerged from other disciplines indicating the utility of melatonin in the treatment of a variety of medical conditions. Despite this, melatonin use in infertility treatment still lacks adequate evidence to recommend routine use. Infertility treatments are associated with significant levels of reactive

oxygen species which have the potential to negatively affect the quality of oocytes and embryos. Melatonin anti oxidant property shows promise as an adjunctive therapy in the treatment of infertility.

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