THE EFFECT OF MELATONIN ON THE OVARIES

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ABSTRACT

Melatonin is a very small molecule whose effects can be both detrimental and beneficial to the ovaries depending on its concentration. Too much of anything is usually not good and so is the case with melatonin. Very high doses can be damaging, but in the right amount melatonin may be able to combat various diseases and increase the chances for fertility in women.

THE PRODUCTION OF MELATONIN

Melatonin is an indoleamine which is produced in the pineal gland. It was first isolated from bovine pineal gland in 1958 and is currently classified as a hormone. This seemingly ambiguous hormone is found in many places in the human body and has many functions. As more research is done on melatonin, more of its importance is being uncovered.

Melatonin is synthesized and released in accordance with how much light reaches the eye. When light enters the eye, the ganglion cells, which contain the photopigment melanopsin, send retinal photic signals to the suprachiasmatic nucleus (SCN) through the monosynaptic retinohypothalamic tract. The ganglion cells which contain melanopsin are sensitive to light with relatively short wavelengths (484 nm) (Macchi and Bruce 2004).

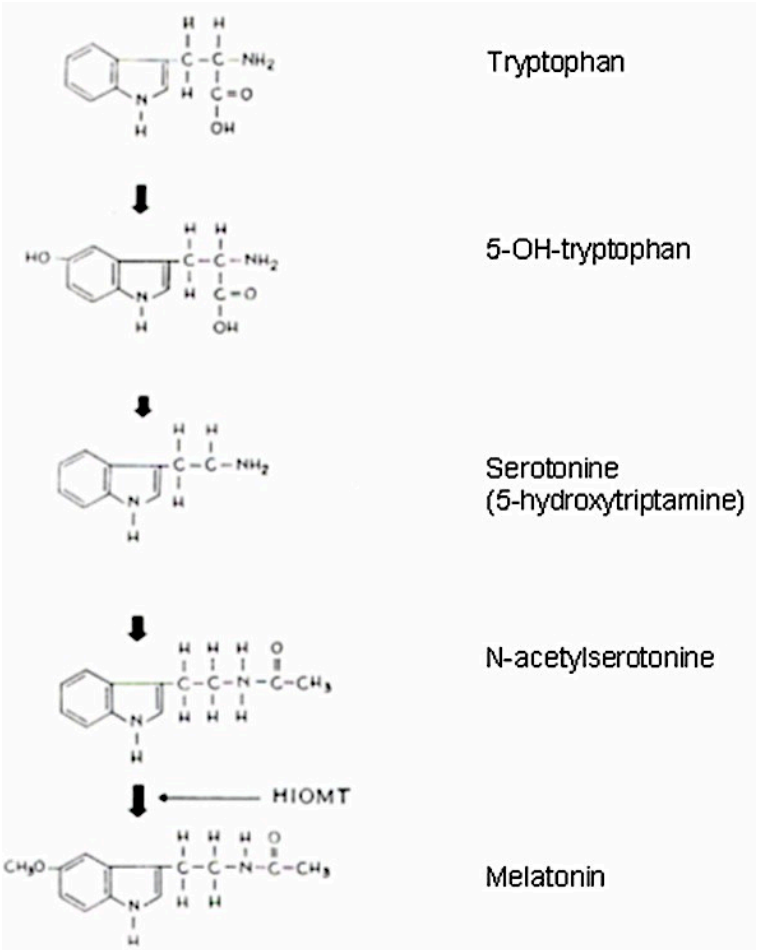

Figure 1: Tryptophan to Melatonin. Source: Macchi and Bruce 2004

There are constant rates of production of melatonin at night, but during daylight hours synthesis is suppressed by light (Macchi and Bruce 2004). The mechanism which causes the light induced suppression and dark induced release of melatonin is due to norepinephrine. Norepinephrine is the input to the pineal gland, and melatonin is the output. During the day, the retinal photoreceptors are hyperpolarized, inhibiting the release of norepinephrine and thus inhibiting the release of melatonin. Conversely, the lack of light at night causes the photoreceptors to release norepinephrine, thereby activating the enzyme N-acetyltransferase which regulates melatonin synthesis (Brzezinski 1997). In addition, melatonin concentrations are affected by exercise and changing posture. During night hours, concentrations of melatonin in the

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plasma and saliva decrease when moving from supine to standing position and vice versa (Macchi and Bruce 2004).

Production of melatonin begins when pinealocytes take up the amino acid tryptophan from the blood. Tryptophan is then hydoxylated and decarboxylated, thus converting it into serotonin. The enzyme N-acetyl transferase (NAT) then converts the serotonin into N-acetyl serotonin. Hydroxyindole-O-methyl transferase (HIOMT) methylates the N-acetyl serotonin, converting it into indoleamine N-acetyl-5-methoxytryptamine, which is melatonin (Figure 1). (Macchi and Bruce 2004). Additionally, studies suggest that melatonin may be synthesized from serotonin in the ovaries. (Itoh et al 1999).

After production, melatonin is released into the capillaries where 70% of it is bound to albumin. Melatonin is metabolized primarily in the liver where it undergoes hydroxylation and conjugation into sulfate and glucuronide (Macchi and Bruce 2004).

A highly diffusible hormone, melatonin is found in the blood, saliva, urine, cerebrospinal fluid (CSF), anterior chamber of eye, retina, gut, and bone marrow, as well as in many reproductive fluids such as breast milk, preovulatory follicles, semen, and amniotic fluid. Three melatonin receptors, MT1, MT2, and MT3, have been identified (Macchi and Bruce 2004; Adriaens et al. 2006). These receptors are localized in lymphocytes, prostate epithelial cells, granulosa cells, preovulatory follicles, spermatozoa, blood, platelets, and the mucosa and submucosa layers of the colon.

Melatonin and its receptors are found in many parts of the human body, indicating that the hormone may have many functions rather than only one main one. The fact that melatonin and melatonin receptors are found in many reproductive fluids and in preovulatory follicles suggests that melatonin plays a role in reproduction.

THE FUNCTION OF MELATONIN

Melatonin has various functions which are mediated either through specific membrane receptors or nuclear binding sites (Tamura et al. 2009). However, unlike other hormones, melatonin is unique in its ability to act as a free radical scavenger. It is an even better antioxidant than glutathione, vitamin E, and mannitol, which are known as effective free radical scavengers. It is one of the more effective antioxidants because it permeates and disperses through cells easily. Therefore it can prevent oxidative damage at any site of a cell (Pandey et al. 2003). Since melatonin acts forcefully upon free radicals, it is a very important hormone (Macchi and Bruce 2004).

Besides melatonin's role as an antioxidant, it plays a part in the immune system as well. Maestroni (1993) showed that suppression of melatonin (which is found in lymphocytes) caused a decrease in thymus and spleen activity. It also caused a decrease in the primary response of antibodies to T-dependent antigens. These results were reversed with administration of melatonin. In addition, constant administration of melatonin caused an increase in helper T cell activity and in IL-2 production (Macchi and Bruce 2004). High estrogen levels cause suppression of cell mediated immune response. Melatonin acts as an anti-estrogen, improving immune response (Sanchez-Barcelo et al. 2005).

Another important function of melatonin is its role in cardiac activity. When humans sleep at night, heart rate and blood pressure are lowered. It is also during the night that melatonin is produced. This concurrence seems to demonstrate a link between melatonin and a decrease in heart rate and blood pressure. Studies have shown that pineal extracts lower blood pressure in humans and in rats. But when exogenous melatonin was administered, heart rate and blood pressure only went down when given during the day. When melatonin

was administered at the time of endogenous melatonin production, only systolic blood pressure was lowered. Since melatonin has a beneficial effect on our cardiac system, it is more common for a stroke to occur in the morning when melatonin levels are low. Coronary disease patients have low melatonin levels, making melatonin's role in cardiac function even more plausible (Macchi and Bruce 2004).

Melatonin is released in a circadian rhythm. Therefore, it has been used to help regulate sleep in people with insomnia by entraining their circadian rhythm (Dubocovich 1988). In addition to helping regulate sleep, melatonin also regulates seasonal reproduction in photoperiodic animals (Macchi and Bruce 2004). These types of animals only reproduce during specific seasons of the year. Melatonin regulates reproduction in photoperiodic animals because it has a progonadotropic effect on these animals during certain seasons by increasing follicular stimulating hormone (FSH) concentrations and luteinizing hormone (LH) pulses (Dair et al. 2008).

MELATONIN AS AN ANTIOXIDANT

The cells in our body undergo a process called apoptosis, which is programmed cell death. This occurs in order to renew the cells in our body, eliminate defective cells, and maintain homeostasis. Apoptosis can be induced by growth factor deprivation, radiation induced DNA damage, activation of death receptors, and oxidative stress. Overall, it is a beneficial process, but only if it occurs when necessary. Excessive apoptosis can lead to an array of diseases such as Alzheimer's, Parkinson's, ischemia, stroke, and AIDS (Pandey et al. 2003). One of melatonin's most important features is its ability to act as a free radical scavenger, thus protecting against oxidative stress. Much research has been done on the use of melatonin as an antioxidant since it is a safe molecule and is therefore an excellent candidate for use in treatments for diseases which are caused by excessive apoptosis, which is due to oxidative stress (Pandey et al. 2003).

Research was done on rat hepatoma cells in order to see if serum deprivation induced oxidative stress, leading to apoptosis. Serum deprivation did induce oxidative stress, but treatment with antioxidants such as vitamin E and melatonin prevented apoptosis. One hundred (100) μM of melatonin were added to the medium six hours before serum deprivation. Oxidative stress caused by serum deprivation induced production of radioactive oxygen species (ROS), but with melatonin pre-treatment, the level of ROS was lowered (Figure 2) as seen by the significant reduction in nuclear condensation and cellular blebbing (features of cellular apoptosis). To ascertain that the pre-treated hepatoma cells not only survived but remained functional, they were re-plated in complete media. Most of the cells divided; they doubled three times in seventy two hours. In contrast, serum-deprived cells (without melatonin treatment) died within ninety six hours (Pandey et al. 2003).

Another antioxidant, glutathione, is a tripeptide which acts as a free radical scavenger by reducing oxidative stress. In the latter experiment, glutathione levels were tested in the serum deprived cells and in the melatonin treated serum deprived cells. In the untreated cells, glutathione levels were decreased, but in the melatonin pre-treated cells, glutathione levels remained the same (Pandey et al. 2003). This study demonstrates that melatonin not only has an anti-apoptotic effect as an antioxidant, but also has a protective effect over other antioxidants.

MELATONIN AND WOMEN

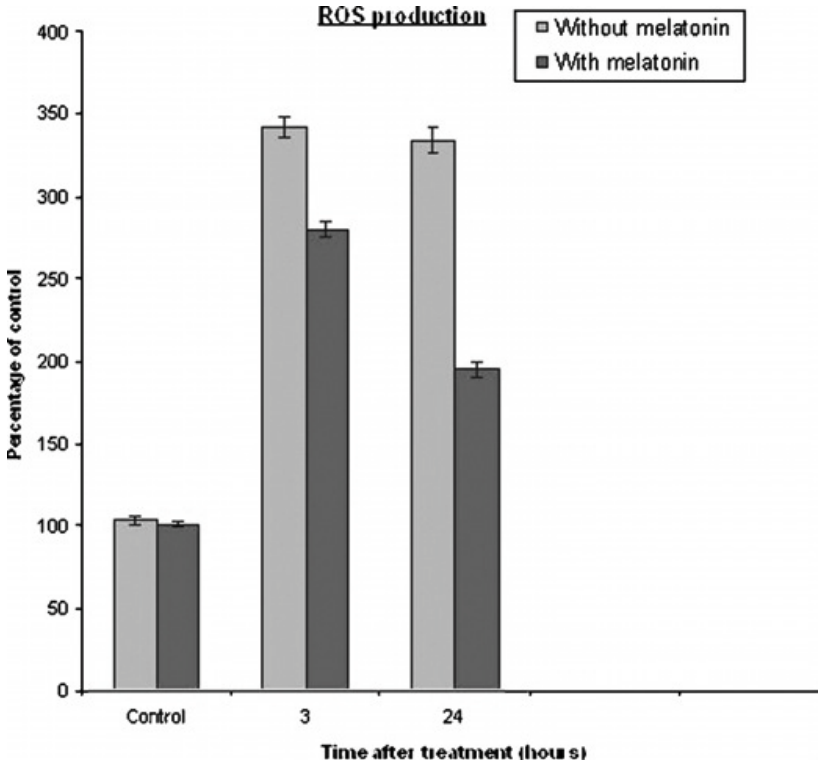

Figure 2: The effect of ROS on cells treated with melatonin versus control cells. Source: Pandey et al. 2003

Melatonin affects the female reproductive system by acting upon the ovaries both directly and indirectly. It acts indirectly on the ovaries by acting on gonadotropin releasing hormone (GnRH), thereby effecting the release of gonadotropins (GnRH is responsible for the release of LH and FSH from the anterior pituitary gland) (Romeu et al. 2010). Melatonin acts on the ovaries directly through its antioxidant and anti-apoptotic properties, as well as its regulation of LH mRNA in the ovaries (Tamura et al. 2009).

One indication that melatonin is involved in reproduction is that melatonin levels decrease at the start of puberty. This may hint to melatonin's role in reproduction because high levels of melatonin are known to inhibit ovulation (Macchi and Bruce 2004). Secondly, the concentration of melatonin in human preovulatory follicular fluid is three times more than in the plasma and melatonin and its precursors are found in human ovary extracts. For example, NAT and HIOMT are found in human ovarian homogenates (Itoh et al. 1999). In addition to melatonin itself, MT1 and MT2 receptors are also found in ovarian extracts such as ovarian granulosa, luteal cells of humans, and rat ovaries. The binding site for the receptors has been detected in the granulosa cell membrane.

There are two possibilities for the source of the melatonin found in the female reproductive system. It is possible that it is synthesized in the ovary and then released directly into the follicular fluid, or it may be derived from the circulation. The latter seems more likely because when infertile women were given a 3 mg tablet of melatonin (taken orally), there were higher levels of melatonin in the follicular fluid (Tamura et al. 2009).

High or low melatonin levels in rats can affect their ovaries. In an experiment done to determine the effects of melatonin on rat endometrial morphology and embryo implantation, results indicated that melatonin had a positive effect on the endometrium by allowing for better implantation of embryos (probably by raising progesterone levels) (Soares et al. 2003).

Rats are non-photoperiodic, just like humans. Melatonin was administered to the rats in large doses in the afternoon of their proestrus phase (the phase proceeding ovulation). As a result, the surge in LH which normally causes ovulation was prevented, not allowing for ovulation. In another experiment, when rats were given exogenous melatonin, ovarian weight

was decreased. But, when the rats were subjected to prolonged light, decreasing the high melatonin levels, an increase in ovarian weight was observed (Soares et al. 2003).

This antigonadotropic effect was seen not only when melatonin levels were too high, but also when melatonin levels were too low. When melatonin production was suppressed in rats, either by pinealectomy or light, precocious puberty, ovarian atrophy, chronic anovulatory state, permanent estrous condition, and hyperprolactinemia were observed. Pinealectomy induced a prolonged estrous stage in the rats, possibly by raising estrogen levels and lowering progesterone levels (Dair et al. 2008). Moreover, when rats underwent a pinealectomy, there was an increase in atretic follicles in the ovaries (Tamura et al. 2009). Atresia is hormone controlled apoptosis; approximately ninety nine percent of ovarian follicles undergo this degenerative process; the rest proceed to the preovulatory stage (Manabe et al. 2008). Pinealectomy of rats in another experiment resulted in overgrowth of the ovarian stroma (Romeu et al. 2010).

From these experiments on rats, it seems that high levels of melatonin have antigonadotropic effects (indirectly affecting the ovaries) resulting in a lack of ovulation, and low levels of melatonin cause an increase in atretic follicles and decrease in ovarian weight. But it should be noted that high levels appear to improve the endometrium for implantation by having an effect on hormones such as estrogen and progesterone. It is also possible that in humans, high melatonin levels are detrimental to fertility because infertility may be a result of low estrogen levels and high melatonin levels (Macchi and Bruce 2004).

In women with stress induced, exercise induced, or functional hypothalamic hypogonadism, melatonin levels are higher than normally present. Additionally, women with functional amenorrhea, have high melatonin levels which are inversely related to their estrogen levels, suggesting a relationship between high melatonin concentrations and hypothathalamic-pituitary-gonadal hypofunction which can cause temporary infertility by preventing menstruation in women. In one study, exogenous melatonin and progestagen were given to women; as a result LH secretion decreased, not allowing for ovulation. Although the luteal phase increase in progesterone was blocked, FSH and estradiol were not affected (Macchi and Bruce 2004).

The relationship between melatonin and estrogen was also studied in breast cancer patients. High melatonin levels decreased the amount of estrogen, and low melatonin caused an increase in estrogen. In breast cancer patients, tumors are sometimes estrogen-dependent. These women were treated with estrogen inhibitors such as tamoxifen, which is a drug that acts as an estrogen receptor antagonist and agonist. Melatonin acts as an anti-estrogen as well, but in a different manner; it interacts with the estrogen receptor signaling pathway. Melatonin binds to its own receptors and causes a decrease in expression of estrogen receptors, thereby blocking estradiol from binding to the estrogen receptors (Sanchez-Barcelo et al. 2005). Therefore, infertile women may have high levels of estrogen which could possibly be treated with administration of melatonin.

Low levels of melatonin in human ovaries may also be unfavorable by affecting the ovaries directly. Many studies provide evidence that follicular cell death during atresia occurs through apoptosis. Many women who are having ovarian failure such as premature ovarian failure, polycystic ovary syndrome, oophoritis, or unexplained infertility, may have these disorders due to a large number of follicular cell deaths via apoptosis. In order for apoptosis to occur, the cell's mitochondrial membrane must become more permeable, so as to release pro-apoptotic factors. Melatonin has been shown to directly inhibit the permeability of transition pores (a protein pore), causing anti-apoptotic effects.

An experiment was done in which mice were injected with either xenogenic anti-ovarian antibodies or allogenic anti-ovarian antibodies. One hour before antibody injection, melatonin was administered (5 mg/kg). In the control mice, exogenous melatonin had no effect on the ovaries, but in the mice which had been injected with xenogenic anti-ovarian antibodies, melatonin reduced the negative effects caused by the antibodies; it improved oocyte quality and caused a decrease in the number of follicular cell deaths by apoptosis. Melatonin reversed the antagonistic effects of anti-ovarian antibodies on the mouse ovaries, but it did not protect against the larger immune reaction of the xenogenic antibodies on a systemic level. On the other hand, melatonin administration in mice injected with allogenic anti-ovarian antibodies had no effect (Voznesenskaya et al. 2007).

In addition to providing anti-apoptotic factors, melatonin in the follicles can scavenge free radicals such as radioactive nitrogen species (RNS) and ROS, as mentioned above. The capability of melatonin to act as a free radical scavenger is especially important because ovulation stimulates a local inflamatory response, causing inflammatory cells such as macrophages and neutrophils to produce free radicals such as ROS and RNS, which induce apoptosis in ovarian cells. Higher melatonin levels in ovarian follicles offers the ovarian cells a greater chance of maturing and developing (Tamura et al. 2009). Therefore, if a woman's melatonin levels are too low, there may be an increase in the percentage of follicular deaths which would result in a lower number of follicles that have the potential for ovulation. Clearly, melatonin levels must be maintained within a certain range, since very high and very low levels can prevent ovulation, leading to infertility.

Very few follicles mature and become capable of releasing their ovum for fertilization purposes. The maturing follicle is filled with follicular fluid which contains water, electrolytes, serum proteins, and steroid hormones secreted by granulosa cells. Melatonin affects sex steroid production at different phases of follicular growth by regulating the steroidogenic enzyme, which activates gene expression in thecal and granulosa cells. Melatonin has been proven to alter granulosa cell steroidogenesis and follicular function. As follicles mature, they shift from dependency on FSH to LH. The process by which follicles are selected to continue maturing may be linked to the timing of mRNA expression encoding LH receptors in granulosa cells. Melatonin may directly affect the ovaries by regulating LH mRNA expression; melatonin treatment (10 pM-100 nM) increased the LH receptor mRNA expression in granulosa cells (Tamura et al. 2009). This confirms that higher levels of melatonin in the ovaries can be beneficial, but what concentrations are too high?

Research was done in order to find a melatonin concentration that would protect female oocytes without being toxic. This experiment was specifically performed to find a way for young women who are undergoing treatments such as chemotherapy and radiation to

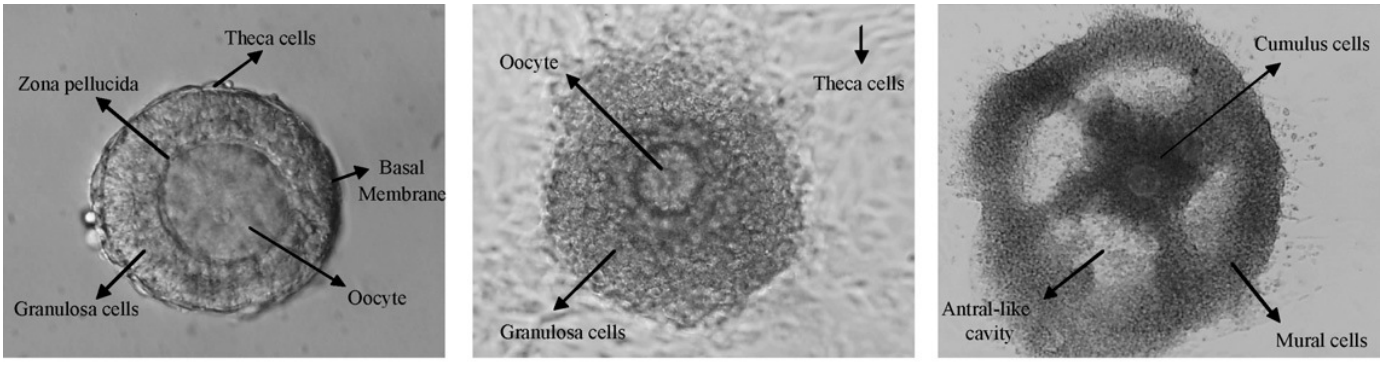

Figure 3: The granulosa cells of the ovaries, which each house an oocyte, go through three major phases: the follicular, diffuse, and antral stages. Source: Adriaens et al. 2006

retain fertility. Mouse follicles were cultured in vitro and different concentrations of melatonin were added to each. Ovulation was then stimulated. Two (2) mM of melatonin was found to be toxic (this is about four thousand times more than physiological melatonin concentrations in the follicular fluid); it affected follicular differentiation leading to a decreased amount of antral follicles (Adriaens et al. 2006).

One (1) mM negatively influenced oocyte development, and 100 µM influenced steroidogenesis. Ten (10) µM was the highest concentrations that had no negative effect on the follicular system. This dosage could possibly be used to protect female ovaries. Additionally, progesterone levels increased significantly in follicles with 100 µM and 1 mM of melatonin. After ovulation was stimulated, progesterone levels increased in all cases except for with 2 mM melatonin. In the control groups, estradiol increased exponentially, but in follicles with 1 or 2 mM of melatonin, estradiol amounts were much lower. As seen by the results in Figure 4, large doses of melatonin caused a reduction in the number of granulosa

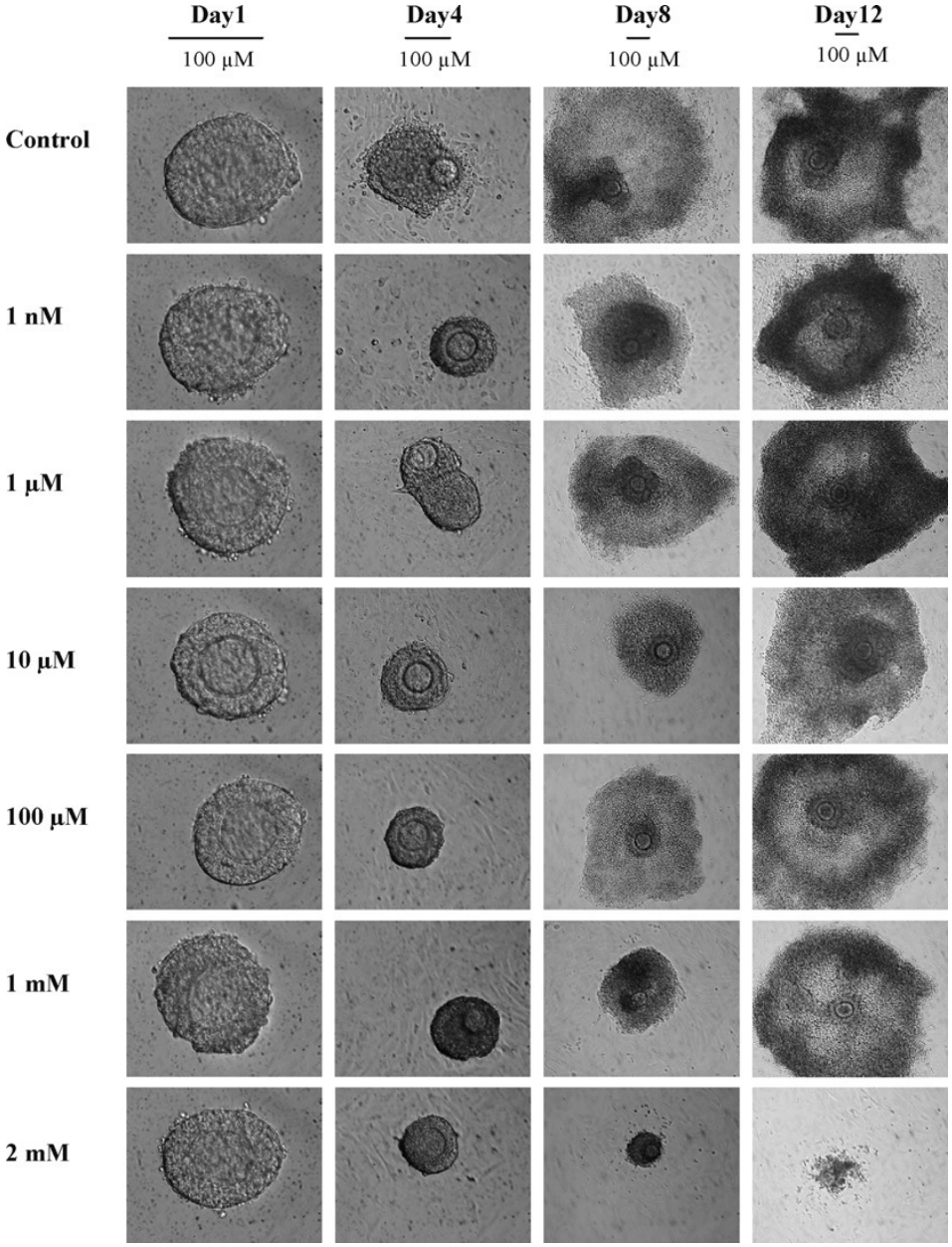

Figure 4 : Morphological changes of follicles over a 12 day period under different melatonin concentrations Source: Adriaens et al. 2006

cells. But by day twelve, most of the follicles reached the antral stage, with concentrations of up to 1 mM melatonin (Adriaens et al. 2006).

This experiment only used three mice (six ovaries) for evaluation; this is a very small control group making its validity questionable. Everyone has varying levels of hormones, such as estrogen, progesterone and melatonin, in circulation so a larger number of ovaries should have been used in this study. On the other hand, this study was done well because they used concentrations of melatonin spanning physiological and pharmacological follicular fluid concentrations (1nM-2mM). According to this study, there would be no health hazard (in regards to fertility) for women to take 3 mg tablets of melatonin (the normal dosage found over-the-counter).

In another experiment, three week-old mice were whole-body irradiated with gamma-radiation. Gamma radiation causes an increase in atresia of ovarian follicles. The purpose of this experiment was to observe if melatonin provided a protective effect against radiation. One group of mice was treated with 10 µg of melatonin before radiation, and another was treated with 100 µg. After radiation, the ovaries were taken out and examined. The number of follicles (including normal and atretic) was about one hundred and twenty five in the largest cross sections; in the irradiated mouse ovaries, the number of follicles was reduced to about one hundred and three. Primordial follicles were the most sensitive to radiation; melatonin treatment seemed to protect these follicles from radiation. Ten (10) µg of melatonin did not have a large protective effect on the primary follicles from radiation, but 100 µg did display a protective effect on them. But lower melatonin concentration did have a protective effect on preantral and antral follicles. Different concentrations of melatonin have protective effects against radiation at different stages of follicular development (Kim and Lee 2000).

As opposed to most of the previous studies mentioned, one study found that melatonin increased follicular atresia. Research was done to study the effects of pineal indoles (including melatonin) on gonadotropin-induced ovulation in mice. To stimulate ovulation, mice were injected with PMSG (pregnant mare serum gonadotropin) and hCG (human chorionic gonadotropin). Then various pineal indoles, at a dose of 0.2 mg/25 g body weight, were administered. After the mice were sacrificed, the ovaries were removed and examined. Although ovarian weight and quality remained the same, the number of atretic follicles appeared to increase in the mouse ovaries which had been injected with melatonin. Plasma levels of estradiol and progesterone were decreased (Chan and Ng 1995).

The two latter experiments discussed (on gamma radiation and on pineal indoles) both used young mice which were prepubertal. Puberty in humans causes a decrease in endogenous melatonin production, as well as other hormonal changes. When examining whether melatonin has protective effects on follicles for the purpose of helping prepubertal females, using prepubertal mice is appropriate. Otherwise, mature mice should be used for experimentation because hormone levels and interactions would be more similar to that in mature women.

Future Research on Melatonin

As seen by the experiments and research done on melatonin, it is apparent that the indoleamine affects the reproductive system. It appears that melatonin is beneficial in high concentrations when it is present in the ovaries because it directly decreases apoptosis and acts as a free radical scavenger. Therefore, low melatonin levels in the ovaries are detrimental to fertility. On the other hand, high melatonin concentrations in the blood can inhibit ovulation by inhibiting the surge in LH. It is necessary to determine the concentrations of

melatonin that will be most beneficial in increasing ovulation by protecting and helping oocyte maturation, without being too high to be antigonadotropic. In addition, research should be done to see if melatonin can be directly administered into the ovaries (in order to decrease apoptosis) without too much of it entering circulation and possibly inhibiting ovulation (by inhibiting the release of GnRH).

Moreover, more research needs to be done on finding a concentration of melatonin that has protective properties on oocytes (by acting as free radical scavengers) without having detrimental effects. This would be beneficial to young women undergoing chemotherapy or radiation treatments whose fertility in the future might be at risk. Ten micromoles of melatonin is a dose that should be experimented with further.

Additional work should be done on the relationship between melatonin and estrogen. A few of the experiments done showed that low levels of melatonin, as a result of pinealectomy, raised estrogen levels and caused an increase in atretic follicles. On the other hand, other research indicated that high levels of melatonin and low estrogen levels were detrimental to fertility.

Melatonin could also be used as a supplement for treatments of various disorders since it is generally safe (Brzezinski 1997). As an antioxidant and mitochondrial membrane permeability transition pore inhibitor, melatonin may be a good candidate to help in the management of cancer, Alzheimer's disease, diabetes, and viral infections (Tamura et al. 2009). Research has shown that melatonin can fight against tumors by reducing vascular endothelial growth factor (Romeu, et al. 2010). It is also permeable enough to disperse throughout cells and can even penetrate the nucleus where it can protect DNA from oxidative damage, reducing the risk of cancer (Meki et al. 2001).

CONCLUSION

Melatonin has a relationship with estrogen and other hormones, has an anti-apoptotic effect, and regulates LH mRNA. With these properties melatonin can be beneficial as it may help increase fertility in women who are infertile, prevent damage in diseases where there is too much apoptosis, and possibly protect the ovaries from radiation. Although more research has to be done on melatonin and the doses which work best in each case, it seems that the hormone will play a role in various therapies in the future.

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