Melatonin in Physiological/Biological Ovarian Aging

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Melatonin, a highly functionally diverse, low-molecular-weight antioxidant and anti-inflammatory agent has been sparingly studied relative to its ability to reduce patho-physiological processes common to the peripheral female reproductive system. Currently it is known that melatonin reduction is associated with multiple processes underlying changes in the ovary, especially late in the cessation of the reproductive life span. In this sense, it is essential deepen the knowledge about the physiological and molecular actions of melatonin in the maintenance of normal ovaries and in the aging ovaries, and integrate the acquired information for considering melatonin in the treatment of age-related infertility. Additional research is needed to fully understand the effects of melatonin supplementation on potentially enhancing fertility, however, studies published to date suggest it may be a promising option for those struggling with infertility.

oxidative stress oocyte granulosa cells sirtuins melatonin

1. Introduction

Reproductive infertility in both females and males is becoming progressively more common throughout the world [1]. Infertility is a growing public health concern that is acute since most epidemiological studies report a 50% increase in this problem in the last 60 years [2]. It is estimated that 35% of cases in which a couple is unable to conceive are a consequence of ovarian failure with an equal percentage being a result of male infertility. The causes of the failure to produce/shed healthy oocytes are numerous, e.g., hormonal imbalance, poor nutrition, excessive physical or psychological stress, genetic background, ovarian disease, anti-cancer therapies, respiratory pollutants, chemical or heavy metal exposure, advancing age, etc. [3,4,5]. Some of these fall into the category of “endocrine disruptors” (EDCs) [6] and many are the result of industrialization and the introduction of products that contain reproductive toxins which are subsequently inhaled or ingested [7,8]. In addition to significantly reducing fertilization of healthy oocytes and successful pregnancy, there are complex psychological and social consequences associated with failure of conception [9]. In many cases, the basis of female infertility is molecular damage of subcellular molecules at the level of the ovary and related organs that result from the excessive production of reactive oxygen species (ROS); the resulting damage is typically characterized as oxidative stress which functionally interferes with the normal physiology of the oocyte or other mammalian cells.

The loss of the ability to reproduce obviously has serious implications when it occurs prematurely; however, in females, it is also a natural consequence of aging when infertility develops during middle age, well before human females are considered old. Attempts to delay the functional involution of the female reproductive system has attracted significant interest in recent years since in current societies many human females delay having a child to late in their reproductive life, a time when conception may be difficult and the chances of having a child with a specific pathology are increased. One factor related to the shutdown of the reproductive system seems to be the gradual accumulation of molecular damage which depletes the ovary of viable oocytes. Given that melatonin is a ubiquitous and universal antioxidant and the fact that its production has

dropped significantly by the time the reproductive system collapses, herein we consider the possibility that the loss of this critical antioxidant may be a contributor to the onset of infertility and that the shutdown may be a means to protect against the development of preeclampsia, miscarriages, fetal pathologies, and other issues.

2. Regulation of Redox Homeostasis for Delaying Ovarian Aging

Melatonin's protective effects on the ovary, as in other organs, is mediated by both receptor-mediated processes as well as receptor-independent means [36]. The latter occurs when melatonin and its metabolites function as direct free radical scavengers while the former involves receptor-mediated stimulation of antioxidative enzymes or inhibition of pro-oxidative enzymes. It is likely that both pineal-derived melatonin, which is extracted from the blood, and well as locally produced melatonin by granulosa cells and oocytes normally participate in protecting ovarian tissues from age-related deterioration. A significant portion of this protection is lost as age progresses, however, since the concentrations of melatonin in the blood and in the ovarian follicular fluid fall substantially leaving the surrounding tissues increasingly vulnerable to oxidative damage which contributes to the cessation of reproductive capability [52,151]. In addition to the depletion of available melatonin to protect against oxidative stress, the number of melatonin receptors diminishes in the aged ovary thereby limiting the upregulation of antioxidant enzymes [40]. The drop in melatonin also makes the ovary more likely to become inflamed [152]. Both persistent low-grade inflammation and accumulated oxidative damage eventually interfere with oocyte maturation and ovulation leading to the onset of menopause and the termination of fertility. Elevated oxidative stress and prolonged inflammation contribute to an increased frequency of genetic mutations which accelerate ovarian aging [153]. Concurrently, the hormonal patterns that govern ovarian cyclicity and successful ovulation are also altered [154].

A variety of events conspire to reduce oocyte availability and maturation as menopause approaches. The age-associated reduction in healthy oocytes is in part a consequence of atresia, a process that is common but not well understood [155]. A second cause of the persistent loss of available oocytes is apoptosis; these two processes eventually lead to a diminished ovarian reserve (DOR). Women with DOR may still have a menstrual cycle but they are typically infertile. By the time of menopause, the primordial follicle pool containing immature oocytes is reduced to several hundred from the many thousands that were present at menarche. This reduced number of follicles signals an elevation in the rate of loss of the few remaining follicles; as a result, at the end of the menopause period, follicle number is nearly totally depleted [156].

Whereas the molecular processes related to menopause are well studied, the events that cause ovarian aging have been less thoroughly investigated. They have, however, many common features. Since it is not uncommon in today's societies for a woman to delay having a child until late in her reproductive life and because of the number of serious diseases that often occur during the post-menopause period, there is intense interest in procedures/treatments that delay ovarian aging and prolong female reproductive life [157].

Proposed pathophysiological processes that have been implicated in ovarian aging are multiple, such as the accumulation of disfigured molecules resulting from oxidative stress including damaged DNA, increased number of mutations, molecular errors that accompany meiosis, telomere shortening, mitochondrial malfunction which reduces ATP generation while concurrently increasing ROS formation, and disordered cytosolic protein metabolism [103,158,159]. Identifying ways to delay or reduce the degenerative changes in the ovarian reserve of women nearing menopause may safely extend their reproductive life span, prevent the likelihood of giving birth to offspring with genetic deficits, improve the success rate of women who undergo in vitro fertilization–embryo transfer (IVF-ET), and delay POI, also known as premature ovarian failure

(POF) or premature menopause. POI is identified in woman under the age of 40 years and is likewise associated with an earlier than normal reduction in oocyte number as well as early menopause [160]. While DOR and POI are described as distinctly different conditions, there is significant overlap in their clinical presentations and in the underlying causes [161], so any potential treatments could have benefits for both conditions.

Considering the major role that oxidative stress plays in the cessation of successful gametogenesis and in ovarian decline [113], the administration of a multifunctional, mitochondria-targeted antioxidant in an attempt to maintain the ovarian reserve and preserve healthy oocytes may be a good choice [78,79,80]. Melatonin meets these specifications. Because of their high energy requirements, oocytes are especially enriched with mitochondria where their number is greater than in any other mammalian cell [162]. The shunting of electrons between the complexes of the electron transport chain (ETC), which is required for eventual ATP synthesis, also leads to the formation of an abundance of ROS when electrons are leaked from the ETC and reduce neighboring oxygen molecules to the superoxide anion radical ($O_2^{\bullet -}$) [163]. Considering the high reactivity of free radicals and, in many cases, their very short half-life, it is imperative that oocyte mitochondria are equipped with adequate endogenous antioxidative defense mechanisms to prevent damage to this critically important cell. Melatonin, a highly efficient inhibitor of oxidative stress, is synthesized in the oocyte [46], particularly in the mitochondria [45]. As a result, melatonin is in the optimal position to prevent oxidative mutilation of the oocyte. Additionally, pineal-derived melatonin circulating in the blood gains access to cells via several routes [164,165]. It is likely, however, that the continually synthesized and potentially inducible mitochondrial melatonin has a much greater role in protecting the oocyte from ROS-mediated damage considering that melatonin in the blood is only available during darkness when it is being produced by the pineal gland [166]. Unfortunately, aging is associated with a gradual loss of melatonin in the blood and its likely diminished production in the oocytes (and in granulosa cells) leaving the follicles in a highly vulnerable situation at a time when ETC efficiency is waning and free radical generation is increasing [167]. We speculate that the reduction in melatonin and the simultaneously elevated ROS generation in the ovary accounts, in part, for reproductive failure and menopause onset .

When ovarian aging is considered, it is usual that the viable primordial follicle stockpile and the number of oocytes that are biologically capable of maturing to undergo ovulation, fertilization and embryo development are the major concern [159,168]. Very closely physiologically related to these processes is the functional state of the granulosa cells [169]. During the reproductively capable period, the granulosa cells undergo morphophysiological changes typical of functional decay accompanied by a reduction in superoxide dismutase 1 and 2 and in catalase making them less capable of resisting free radical damage [170]. This increases the frequency of mitochondrial DNA mutations and is accompanied by the upregulation of glutathione S-transferase theta 1. This enzyme also exaggerates oxidative stress since it catalyzes the conjugation of chemically-reduced glutathione to a variety of compounds; glutathione is an important intracellular antioxidant that is typically in high concentrations in granulosa cells [171]. As with the oocyte itself, the granulosa cells also synthesize the antioxidant melatonin, but as these cells age, they lose that capability just as other tissues do. Thus, not only does the oocyte decay, but the supporting cells do as well, which leads to a lower reproductive capability.

The ovarian stroma surrounds each follicle during its development and plays an essential role in the maintenance of a healthy follicle/oocyte unit [172]. During its maturation, a follicle moves from the collagen-rich cortex to the less dense medulla and eventually back to the cortex for ovulation. The mechanics of this movement and the cellular composition of the stroma during these movements significantly determines the functional state of the follicle and its oocyte [173]. This was proven by in vitro studies where follicles were grown in different stromal compositions which generated different extracellular matrices (ECM) that differentially impacted the rate of

follicle maturation [174]. During aging, the ECM of the stroma changes substantially relative to its viscoelastic properties [175]. In general, the stroma generally becomes more rigid as menopause approaches, which may restrict the movement of follicles to the surface for ovulation. Among a number of other alterations in the stroma, there is also a change in the ratio of M1/M2 macrophages in favor of the M1 proinflammatory cell type contributing to what is referred to as inflammaging [176], an age-associated change related to aging that is not unique to the ovarian stroma since it occurs in many other tissues as well. How precisely this low-grade inflammation alters the rate of reproductive decay remains to be determined, but, as inflammation always does, it increases the amount of oxidatively damaged stromal elements, rendering them less functional.

Sirtuins, a family of highly conserved histone-deacetylating enzymes that are differentially distributed in cells, are frequently described as influencing aging and longevity pathways [69]. The roles of these critically important enzymes, with the exception of SIRT1, have not been extensively investigated relative to senescent processes of the ovary. SIRT1 knockout mice survive but are severely compromised relative to their ability to reproduce. SIRT1 also influences oocyte maturation and quality. For example, pharmacological inhibition of SIRT1 interferes with meiosis and those that continue on to meiosis II are defective in terms of spindle formation and chromosome misalignment [177]. While SIRT2 has been rather extensively investigated in relation to somatic cell division, there is a dearth of information on its actions in gametes [178].

Mitochondrial SIRT3 protein has been identified in human in all components of the developing follicle, but knockout of this deacetylase in mice does not interfere with successful reproduction. Despite the absence of SIRT3, these gametes can be successfully fertilized, at least in vitro. This is somewhat unexpected since oocytes with a deficiency of SIRT3 exhibit elevated levels of oxidative stress [179]. SIRT4 is a weak deacetylase; as a lipoamidase [180], it reduces the activity of the mitochondrial enzyme pyruvate dehydrogenase complex which limits mitochondrial acetyl-coenzyme production. This, in turn, may lower mitochondrial melatonin synthesis [72]. Overexpressed SIRT4 in oocytes causes spindle and chromosomal disorders and altered mitochondrial physiology with elevated ROS generation [181]. How or whether these changes relate to oocyte physiology, however, is undefined. SIRT6 curtails oocyte DNA damage mediated by oxidative stress and also functions in stabilizing their telomeres. With a deficiency of this deacetylase, aged oocytes have shorter telomeres, an indication of aging-related decay [182]. SIRT6 is also involved in maintaining redox homeostasis via its influence on Nrf2 [183]. Even less is known about the functional significance of SIRT5 or SIRT7 to peripheral reproductive physiology. In oocytes, as in somatic cells, advancing age is associated with diminished mitochondrial function which causes increased oxidative stress [184]; this also contributes to potential deficiency in the NAD+-dependent deacetylase activities, thereby preventing healthy oocyte aging.

An interaction between melatonin and SIRT3 has clear implications for ovarian function as uncovered in several recent studies. In humans, obesity is a negative factor for the successful fertilization of the oocyte by the sperm with the mechanisms for this inhibition probably being multifactorial but only ill-defined [185,186]; excessive body weight is believed to contribute to the decline in female fertility. NAD+ (nicotinamide adenine dinucleotide) is a necessary co-factor for SIRT3 and for its regulation of mitochondrial redox homeostasis [187]. Feeding female mice a high-fat diet that promotes obesity reduces their fertility, which is accompanied by a significant drop in NAD+ levels in whole ovaries and meiosis II oocytes [188]; as anticipated, MitoSOX immunocytochemistry documented the elevated levels of ROS in the oocyte mitochondria, along with a reduced membrane polarization and a depression of genes related to oxidative phosphorylation. Supplementing the obese mice with nicotinamide riboside, a precursor of NAD+, elevated the transcription of SIRT3 and protected the oocyte from oxidative stress.

Using the same obese mouse model, we had previously reported that feeding a high-fat diet caused the acetylation of SIRT3 in oocytes with extensive oxidative damage to these organelles

[189]. Conversely, treating these animals orally with melatonin caused the deacetylation of SIRT3, which allowed for the deacetylation and activation of SOD2 [96], leading to a significant reduction in the exaggerated oxidative stress measured in the oocytes of the obese animals. Finally, the use of morpholino knockdown of SIRT3 expression further confirmed the essential role of the SIRT3/SOD2 pathway in maintaining viable oocytes in obese animals and re-emphasized the importance of melatonin in regulating this axis.

3. Conclusions

The data accumulated to date suggest that melatonin may be a molecule capable of delaying normal ovarian aging and ensuring successful reproduction in humans to a later age than usual. Significant advances have been achieved in defining the molecular and cellular events associated with reproductive decline in recent years as well as the potential role of melatonin in deferring the aging of the ovary and also of the oocyte quality. For many organs and for organisms as a whole, melatonin has been frequently advanced as an age-delaying agent [223–225]. As animals including humans age, melatonin production typically declines markedly, which is believed to contribute to the general aging phenotype [113,226]. Given that melatonin levels gradually wane throughout life, reproductive cessation in females coincides with a time when melatonin perhaps drops to a level that is no longer compatible with ovulation and a high oocyte quality. While other cells can tolerate some accumulated oxidative damage throughout life, in the case of the oocyte, molecular damage is more serious since it would be disastrous in terms of successful implantation and the delivery of normal offspring [227]. This seems to be the case in late reproductive life pregnancies in humans where the frequency of functionally compromised offspring increases as menopause approaches [149] with these abnormalities being prevented by melatonin administration in experimental animals [228]. A major feature of melatonin that likely contributes to its ability to reduce molecular damage to tissues is its multifaceted function in reference to maintaining redox homeostasis [83,229,230]. Melatonin and its metabolites are all highly efficient scavengers of ROS [87,231,232]. This is particularly important at the mitochondrial level, since partially reduced oxygen derivatives are abundantly produced in these organelles and provide the basis for massive free radical damage that occurs when electrons leak from the electron transport chain [233]. To compensate for the large number of oxidizing agents produced in these organelles, mitochondria, as recently discovered, also generate their own melatonin [33,45] to aid in combatting the massive molecular destruction that would otherwise occur. Moreover, recent evidence suggests that mitochondrial melatonin production is upregulated under conditions of elevated free radical generation [234]. It is presumed that local melatonin synthesis in mitochondria of ovarian cells also diminishes simultaneous with the drop in pineal-derived blood melatonin concentrations which would mean by middle-aged, these values may only be half of what they are in younger individuals at their peak reproductive capability.