

# Melatonin effects on ovarian follicular cells: a systematic review

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## SUMMARY

Melatonin is known for its effects on both the sleep and reproductive system of mammals. The latter has melatonin receptors type 1 and 2, which act to regulate, among other things, cyclic AMP. Notwithstanding all the literature data, there is still no sound knowledge or a clear understanding of the hormone's action on the physiology of ovarian follicular cells.

**OBJECTIVE:** To review and evaluate studies about melatonin action on the ovarian granulosa/theca interna cells from the literature.

**METHODS:** The systematic review was carried out according to the PRISMA recommendations. The MEDLINE and Cochrane primary databases were consulted with the use of specific terms. There was no limitation on language or publication year.

**RESULTS:** Seven papers about melatonin action on granulosa cells were selected. The following can be attributed to the hormone's effects: a) progesterone increase in culture medium; b) increased estrogen production; c) antagonistic action on estrogen; d) improvement in cell quality resulting in improved embryo and higher pregnancy rates; e) improved cell proliferation via MAPK; f) reduction of free radicals. Nevertheless, there are contrarian papers reporting a reduction in progesterone production.

**CONCLUSION:** Melatonin interferes in sex steroid production, boosting progesterone output. Such action may help improve oocyte quality.

KEYWORDS: Melatonin. Ovary. Granulosa cells.

## INTRODUCTION

Melatonin is the main hormone produced by the pineal gland. Of all its functions, the most studied and with the best-structured data is the regulation of the circadian cycle and seasonal rhythms of the body<sup>12</sup>. Only recently have other functions been

studied more intensively. Some of these are control of glucose metabolism, modulation of humoral immune activity and vascular tone, and regulation of human reproductive function<sup>3-6</sup>.

Melatonin is an indolamine resulting from sero-

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tonin acetylation and methylation<sup>7</sup>. As a powerful antioxidant<sup>6,8,9</sup>, more so than glutathione<sup>7</sup>, it has an important role in controlling free radicals. Using endocrine, paracrine, and autocrine signaling, mediated or not by membrane receptors like MT1 and MT2<sup>10,11</sup>, melatonin plays a still unclear role in human ovarian physiology<sup>7,10</sup>. The human follicular fluid contains high melatonin concentrations – higher than those in serum concentrations – mainly in pre-ovulatory follicles<sup>2</sup>. There are studies correlating melatonin concentrations in follicular fluid with those of progesterone, estradiol, and even oxytocin<sup>12-14</sup>. Thus, the scientific investigation into melatonin's role and action mechanisms in the ovary may open up new horizons for the treatment of several diseases of the female reproductive system, including endometriosis, ovarian neoplasms, and polycystic ovary syndrome<sup>6,15</sup>. In short, the key question to address, which has been the source of much discussion, is melatonin's influence on ovarian follicle cells<sup>7</sup>.

This systematic review aimed at gathering and analyzing research work about melatonin effects on human ovarian follicle cells published in the literature up to the present. The ultimate objective was to consolidate knowledge in this field.

## METHODS

The systematic review followed the procedures established by PRISMA<sup>16</sup>. The search strategy and the databases that were consulted are shown in Fig-

ure 1. An option was made not to exclude papers with respect to publication time to allow an analysis of the relevance of the topic throughout the decades. The search, thereby, yielded articles ranging from 1986 to 2017. The PICO was defined as follows: P (Patients) patients with infertility; I (intervention) melatonin in the granulosa cells; C (control) women with normal menstrual cycle and a male factor; and O (outcome) melatonin's effect.

The initial selection was carried out based on the title; studies unavailable in English, Portuguese, Spanish, Italian, or French or those not addressing the central issue of this review were excluded. Only original papers reporting on research conducted with humans and reviews on the subject were included. Work using animal models was excluded. This phase was followed by the reading of abstracts and the screening out of articles unrelated to the topic.

Study selection was carried out by two researchers (I.P.M. and R.S.S.) who worked independently, following the eligibility criteria. When there was disagreement, a third reviewer was consulted (J.M.S.J.).

The phase above yielded 15 studies to be read entirely (Fig. 2). In addition, reviews, as well as references, were examined to enhance our research (evaluation of the literature in the "gray area").

A table was used to organize the following data from the articles: title, authors, year, study design, number of participants (N), general objectives, methods, results, and study limitations.

**TABLE 1. CHARACTERISTICS OF THE STUDIES INCLUDED IN THE SYSTEMATIC REVIEW**

Authors and year of publication	Country	Sample	Kind of study	Outcome
Kim et al.3, 2013	South Korea	Human and oocyte granulosa cells	Cell culture	Improvement in embryo implantation
Taketani et al.9, 2011	Japan	Granulosa cells	Cell culture	Decrease of free radicals
Nakamura et al.7, 2003	Japan	Human granulocyte cells and follicles	Cellular and follicular culture	Increased melatonin is accompanied by increased progesterone
Woo et al.10, 2001	Canada	Human granulosa cells	Cell culture	Activation of MAPK (mitosis) and discrete progesterone increase
Bódis et al.17, 2001	Hungary	Human granulosa cells	Cell culture	Estradiol stimulation and progesterone reduction
Niles et al.11, 1999	Canada	Human granulosa cells	Without cell culture	Presence of the melatonin receptor
Yie et al.18, 1995	Canada	Human granulosa cells	Cell culture	Presence of the melatonin receptor Increased production of progesterone

## RESULTS

The database search produced a total of 116 articles. Selection narrowed this number down to 7 studies about melatonin action on the granulosa cells (Fig. 2). The data are summarized in Table 1. Most papers were from Canada. Only one study did not use cells from a cell culture; measurements were made directly on the cells after collection. The patients were in the 22 to 35 age range. The studies had a transverse design.

What follows can be attributed to melatonin action on the granulosa cells: a) progesterone increase in culture medium; b) stepped up estrogen production; c) antagonistic action on estrogen; d) improvement in cell quality resulting in improved embryo and higher pregnancy rates; e) improved cell proliferation via MAPK; and f) reduction in free radicals. Nevertheless, there are contrarian papers reporting a reduction in progesterone production<sup>8</sup>.

The studies imposed limitations on this review, given the diverse stimulation protocols for the women in the assisted fertility programs and different methods for evaluating melatonin. The varied intracellular signaling pathways further compounded the complexities of the review.

## DISCUSSION

Ovulation is a process involving adequate interaction between follicular cells and substances participating in the inflammatory process, such as prostaglandins and cytokines, as well as the action of proteolytic enzymes and vasoactive substances<sup>9</sup>. Regulating this process is crucial for successful egg release and oocyte quality. It is also known that the macrophages, neutrophils, and vascular endothelium itself in the follicles produce reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) during the ovulation process. The ROS participate in follicle maturation and rupture for oocyte release. However, excessive production of such substances is potentially harmful to the granulosa cells as it may hinder ovulation and corpus luteum formation and

even impair embryo quality due to changes in DNA. It may compromise the lipid peroxidation of the oocyte membrane as well<sup>5,8</sup>. Therefore, melatonin's antioxidant action may aid in the process<sup>5,8,9</sup>. Other melatonin roles reported in the studies were those as hormone receptor regulator and as an aid in adequate follicle growth.

Taketani et al.<sup>9</sup> evaluated ROS melatonin effects in a culture of follicle cells from healthy women undergoing in vitro fertilization (IVF). Melatonin regulated progesterone production in the cells and reduced ROS. These mechanisms may explain the data showing enhanced implant rate under melatonin treatment<sup>17,19</sup>.

Melatonin receptors are located in the ovarian granulosa cells<sup>17,10</sup>. Perhaps the reduction in ROS is related to melatonin's intracellular signaling<sup>7</sup>. However, there is evidence that melatonin has other properties, which are independent of its receptors<sup>11,18</sup>. Nonetheless, during the ovulation process, the melatonin levels in the follicular fluid increase threefold over those in blood circulation<sup>20</sup>. The aim is possibly to regulate follicle growth and decrease reactive oxygen species, as well as to influence sex steroid production and action in the follicle microenvironment.

During follicle growth, melatonin's Gi-mediated intracellular signaling pathway, which influences the second messengers (AMPc and GMPc), and its Gs-mediated pathway, which affects PKC activity<sup>1</sup>, possibly stimulate granulosa cell proliferation through MAPK activation. This potential mechanism was confirmed in vitro with melatonin treatment and ELK-1 phosphorylation, which is a dose-and-time-dependent action. During ovulation with a high melatonin concentration, the reverse occurs, i.e., a reduction in cell proliferation and in MAPK activation<sup>10</sup>. This is a property that may be important for egg release and enables cell apoptosis, acts as a brake on follicle growth, and reduces ROS<sup>9</sup>.

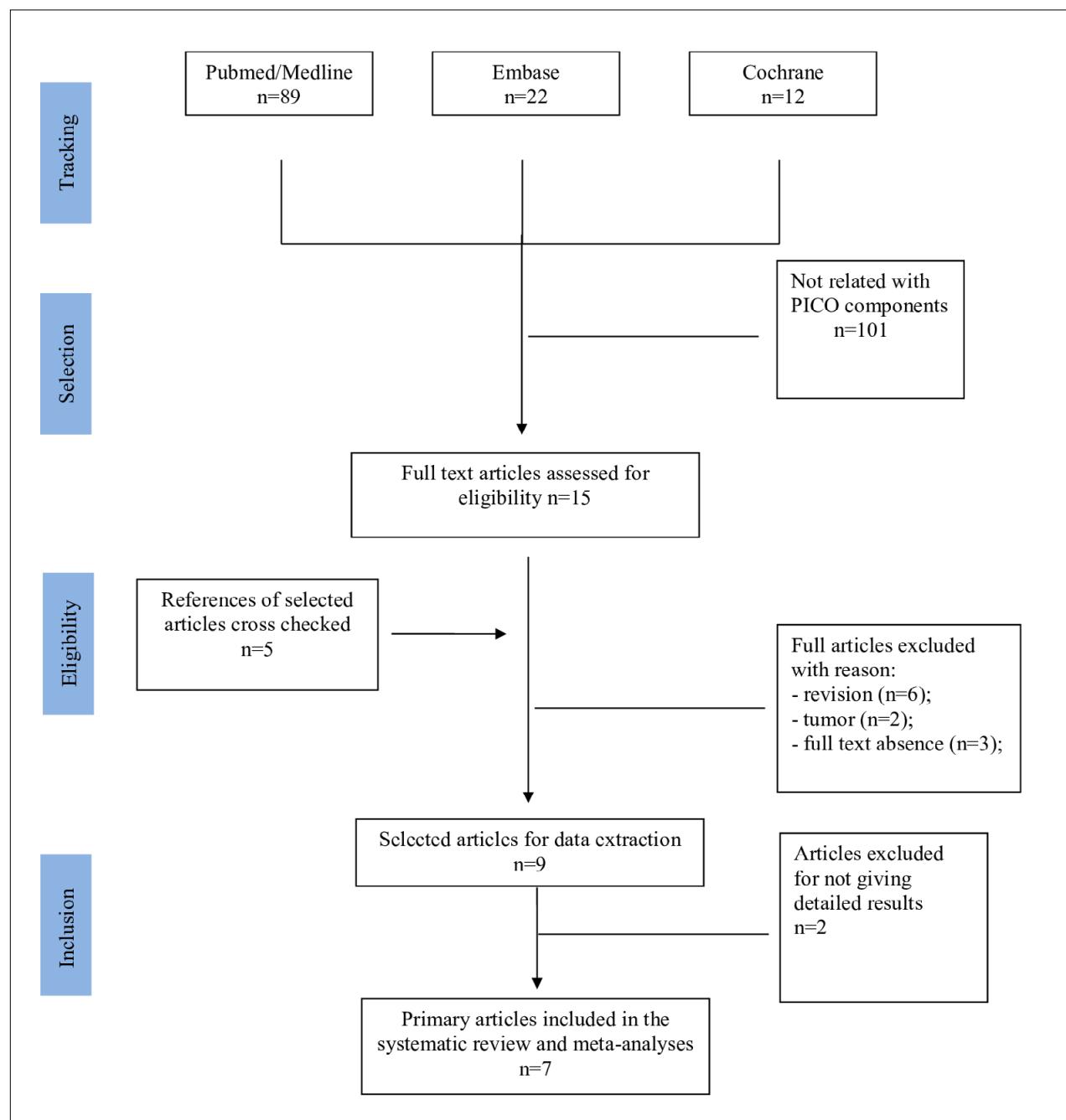
Melatonin's influence on steroidogenesis seems to be dose-dependent, involving both central mechanisms and the ovarian microenvironment. Between 10pM and 100nM of melatonin, there is an increase

**FIGURE 1.** DATABASES USED AND SEARCH STRATEGIES.

**Medline:** ("Ovarian Function Tests" OR "Ovarian Reserve" OR "Ovarian Follicle" OR "Theca Cells" OR "Follicular Fluid" OR "Ovary" OR "Ovarian Tissue" OR "Granulosa Cells") AND ("Melatonin" OR "Melatonin Receptors")

**Cochrane:** Follicular Cells AND melatonin

**Embase:** 'ovarian follicles/exp AND (melatonin/exp OR ovary/exp).

**FIGURE 2.** ALGORITHM OF THE SELECTION OF THE STUDIES

in the luteinizing hormone (LH) receptor messenger RNA with no concomitant changes in the follicle-stimulating hormone (FSH) receptor gene expression, and there is also a reduction superior to 45% in gonadotropin-releasing hormone (GnRH) receptor messenger RNA<sup>3,9,10,16</sup>. This action has immediate repercussions in steroid production<sup>11</sup>. With respect to melatonin's direct action on the granulosa cells, it appears that melatonin is involved in the luteinization of the cells and in a rise in progesterone production<sup>21</sup>. This would be an important effect with a bearing on endometrial preparation and embryo receptivity. On the other

hand, there is a study showing a reverse action<sup>10</sup>. A tentative explanation is that the heterogeneity of the stimulation protocols used in the studies influenced the number of gonadotropin receptors. Another point is the amount of melatonin on the granulosa cells and their time of exposure to it, which may have impacted steroidogenesis<sup>10</sup>. In general, via its receptor, melatonin can negatively influence estrogen production<sup>16</sup>. This could be important for the pituitary release of LH (peak) as well as for egg release<sup>22</sup>.

Our study was hindered by a few limitations capable of affecting our results. For example, the adop-

tion of different stimulation protocols in the ovarian stimulation programs may have influenced both gonadotropin action and steroidogenesis. The time span between the beginning of cell culture and the melatonin treatment varied among the studies. This fact may have biased the analyses, given that gonadotropin receptors may be more abundant in the longer-span cultures and less so in those with a shorter time span. Moreover, melatonin action is known to depend on the interaction with gonadotropins, especially LH. A further limitation was the use of different protocols to evaluate melatonin.

Finally, melatonin seems to interfere in sex steroid production (progesterone increase and estrogen decrease) and in the reduction of free radicals in follicle cells after ovarian stimulation protocols by assisted reproduction techniques. However, it is necessary to confirm this melatonin action on the quality of both the granulosa cells and the oocytes.

## CONCLUSIONS

We conclude that melatonin has actions in the production of sex hormones, in the improvement of antioxidant parameters, in the increase of cellular proliferation and in the oocyte quality. However, further studies are necessary to verify the actions of melatonin.

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### Competing interests

The authors declare that they have no competing interests

### Author Contributions

**IPM** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

**CML** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data anal-

ysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

**MCPB** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published.

**CCM** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published. –

**CON** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published. –

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**ECAV** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis and final approval of the version to be published

**JCP** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

**ECB** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

**JMSJ** - made substantial contributions to the concept, design of the study and definition of intellectual content; was involved in literature search, data analysis, statistical analysis, manuscript preparation, manuscript writing; drafting the article or revising it critically for important intellectual content; and final approval of the version to be published.

## RESUMO

A melatonina é conhecida por seus efeitos no sono e no sistema reprodutivo dos mamíferos. Este último tem receptores de melatonina tipos 1 e 2, que atuam para regular, entre outras coisas, o AMP cíclico. Apesar de todos os dados da literatura, ainda não há um conhecimento sólido ou uma compreensão clara da ação do hormônio na fisiologia das células foliculares ovarianas.

**OBJETIVO:** Revisar e avaliar estudos da ação da melatonina na literatura sobre as células internas da granulosa/teca ovariana.

**MÉTODOS:** A revisão sistemática foi realizada de acordo com as recomendações do Prisma. As bases de dados primárias Medline e Cochrane foram consultadas com o uso de termos específicos. Não houve bar na língua ou ano de publicação.

**RESULTADOS:** Sete artigos sobre a ação da melatonina nas células da granulosa foram selecionados. O que se segue pode ser atribuído aos efeitos do hormônio: a) aumento de progesterona no meio de cultura; b) aumento da produção de estrogênio; c) ação antagônica no estrogênio; d) melhoria na qualidade celular, resultando em melhor embrião e maiores taxas de gravidez; e) melhor proliferação celular via MAPK; f) redução de radicais livres. No entanto, existem artigos controversos relatando redução na produção de progesterona.

**CONCLUSÃO:** A melatonina interfere na produção de esteroides sexuais, aumentando a produção de progesterona. Tal ação pode ajudar a melhorar a qualidade do óvulo.

**PALAVRAS-CHAVE:** Melatonina. Ovário. Células da granulosa.

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