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Effects of melatonin in polycystic ovary syndrome: is there Hippo pathway crosstalk?

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Abstract

Objective Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder among reproductive women, characterized by hyperandrogenism, oligo-ovulation and polycystic ovarian morphology. Incorporating complementary medicine alongside traditional lifestyle therapies for PCOS may offer additional benefits for affected women. Melatonin (MT), a hormone secreted by the pineal gland, has emerged as a potential treatment for regulating ovarian function in PCOS. However, the specific effects and underlying mechanisms of MT on PCOS need to be elucidated.

Methods This review consolidates evidence from randomized controlled trials, original research articles, systematic reviews, and meta-analyses regarding MT supplementation in PCOS, with a particular focus on its interaction with the Hippo pathway, to provide a comprehensive overview of current knowledge.

Results Current evidence suggests that MT plays a role in modulating PCOS through various mechanisms and is associated with the Hippo pathway. However, several uncertainties and key limitations in the existing literature must be addressed before these treatments can be integrated into standard clinical practice.

Clinical trial number Not applicable.

Keywords Hippo, Melatonin, Ovary, PCOS, YAP

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting approximately 1 in 5 women of reproductive age by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM) [1]. Women with PCOS may experience symptoms such as infertility, metabolic dysfunction, and insulin resistance, with an increased risk of cardiovascular disease, mood disorders, and endometrial cancer in post-menopause [2]. Although clomiphene citrate, metformin, and tamoxifen are widely used in the treatment of PCOS, these conventional therapies often prove insufficient and can cause side effects due to the multifaceted nature of the disorder. Thus, identifying more effective treatment options and alternative therapies to reduce adverse effects is critical. Recent research has increasingly focused on whether

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supplementation with vitamins, vitamin-like nutrients, minerals, and complementary medicines (CM) can contribute to favorable health outcomes in PCOS [3].

Melatonin (MT), an endogenous indoleamine hormone primarily produced by pinealocytes, regulates circadian rhythms, behavior, immune responses, and reproductive functions [4]. Additionally, MT influences body temperature and reproductive hormone secretion and is recognized for its potent antioxidant and anti-inflammatory properties [5]. Evidence suggests that MT directly affects ovarian function and may help ameliorate the pathophysiology of PCOS [6]. Further studies indicate that MT could regulate ovarian function via the Hippo pathway [7]. The Hippo pathway has been implicated in primordial follicle development, oocyte maturation, and granulosa cell (GC) proliferation [8]. Recent findings highlight the role of YAP1 in ovarian GC proliferation, suggesting a link between YAP1 dysregulation and PCOS pathogenesis [9]. These insights deepen our understanding of the Hippo pathway's involvement in PCOS. Notably, emerging evidence points to a potential interaction between MT and the Hippo pathway across various cell types [10].

This review summarizes recent findings from original research articles, systematic reviews, and meta-analyses regarding the efficacy of MT supplementation in managing PCOS, alongside the critical role of the Hippo pathway in treatment strategies. It also addresses the limitations and knowledge gaps that must be overcome before these therapies can be effectively integrated into clinical practice.

PCOS: an overview

PCOS was first described by Stein and Leventhal in 1935 [11]. According to the Rotterdam criteria for diagnosing PCOS, established in 2003, the condition is characterized by at least two of the following three features: oligo- or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology (PCOM) detected *via* ultrasound. Additionally, certain exclusion criteria must be considered, including follicular membrane cell hyperplasia, hyperprolactinemia, adrenal cortical hyperplasia, ovarian masculinized tumors and acanthosis nigricans, etc [12, 13].

The complex pathophysiology of PCOS remains an area of active investigation, with hyperandrogenism (HA) and insulin resistance (IR) playing key roles in initiating and exacerbating the disorder. Insulin dysregulation impacts ovarian function; excessive insulin levels reduce the hepatic production of sex hormone-binding globulin (SHBG) while increasing luteinizing hormone (LH) secretion in the ovaries. This results in ovarian androgen production, which contributes to anovulation [14, 15]. Clinically, PCOS is characterized by dysfunction in the hypothalamus-hypophysis-ovary axis (HHOA), with an elevated frequency of gonadotropin-releasing hormone (GnRH) pulses and increased LH levels, while follicle-stimulating hormone (FSH) remains relatively unchanged. Elevated GnRH levels stimulate ovarian theca cells (TC) to secrete higher amounts of androgens [12]. Furthermore, HA diminishes the sensitivity of hypothalamic gonadotropin cells to estradiol (E) and progesterone (P), which exacerbates GnRH and LH hypersecretion [16]. Ovarian hyperandrogenism, IR, and disrupted intraovarian paracrine signaling, particularly in the context of hyperinsulinemia, impair follicular development in PCOS. This leads to follicular arrest, anovulation, irregular menstrual cycles, and the accumulation of small antral follicles, giving the ovaries a polycystic appearance [11] (Fig. 1). Recent studies have suggested that inflammation may also contribute to the pathogenesis of PCOS [17, 18]. Elevated levels of inflammatory markers, including C-reactive protein (CRP), high-sensitivity CRP (hs-CRP), interleukin-18 (IL-18), and interleukin-6 (IL-6), have been observed in patients with PCOS [19, 20]. Targeting these inflammatory mediators could provide a promising therapeutic strategy, as chronic inflammation may drive metabolic complications associated with the disorder [21].

Mounting evidence suggests that PCOS cannot be attributed to a single genetic factor or cause [22, 23]. Rather, it is associated with a complex interplay of intrinsic mechanisms, including genetic predispositions, environmental factors, and in utero influences [24]. Given that PCOS manifests with reproductive, metabolic, and psychological features, a range of complications can arise, including obesity, gynecological and obstetrical issues (such as infertility, subfertility, endometrial cancer, preeclampsia, and preterm birth), metabolic dysfunction, and mood disorders [25, 26] (Fig. 1).

Due to the multifactorial nature of PCOS, a universally effective treatment plan remains elusive, despite considerable progress in understanding its mechanisms and therapeutic options in recent decades. International evidence-based guidelines emphasize lifestyle modifications-such as diet and exercise-as a primary approach to achieving various health outcomes [27]. When lifestyle changes prove insufficient, pharmacological interventions or ovarian surgery may be considered. Several treatment options have been explored, with selected therapies proposed to improve reproductive outcomes in patients with PCOS. These include metformin, orlistat (a lipase inhibitor), spironolactone (androgen receptor blocker), clomiphene or letrozole (selective estrogen receptor modulators), hormone replacement therapy (HRT), and ovarian surgery (such as ovarian wedge resection or laparoscopic ovarian drilling). While these strategies effectively alleviate symptoms, they come with significant limitations [28–34]. Therefore, there is a clear need for

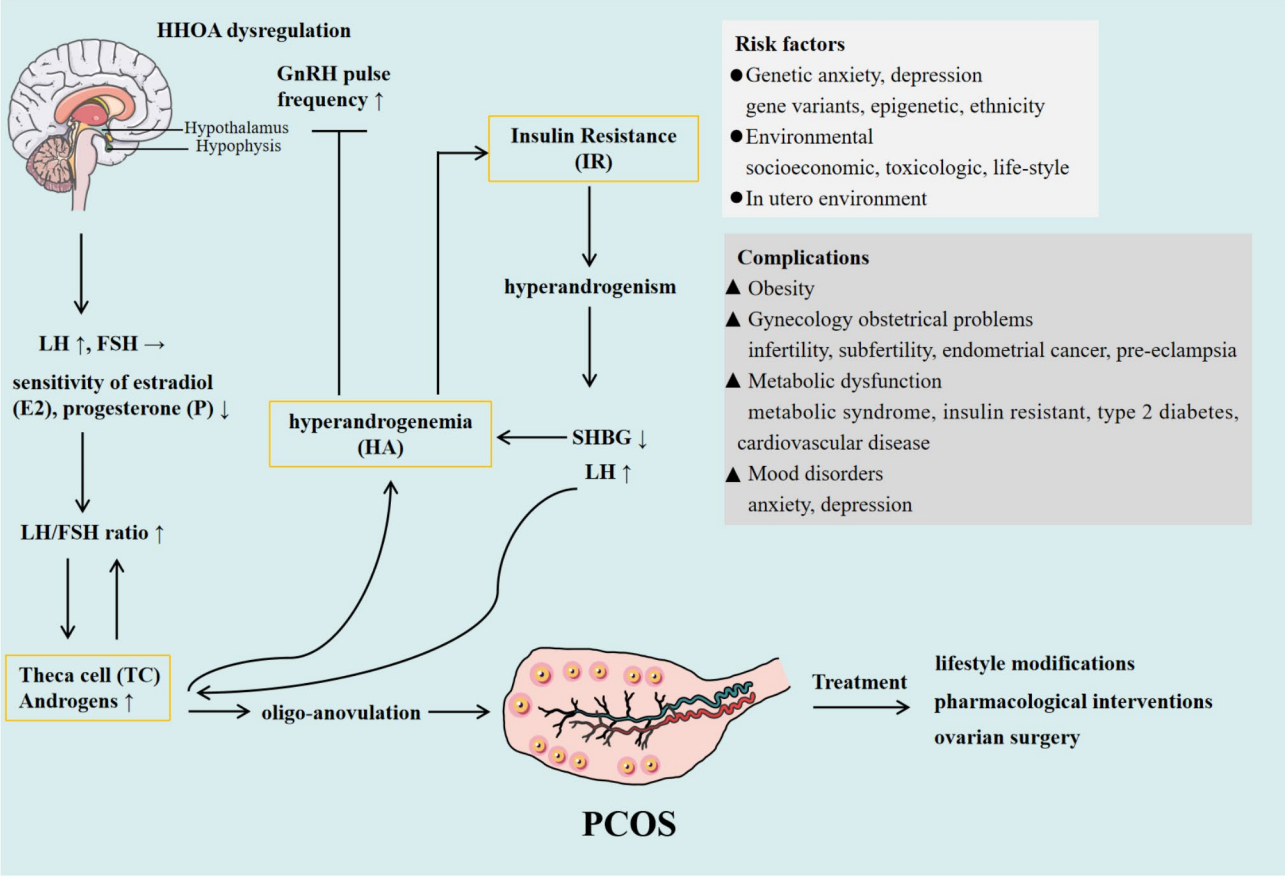

Fig. 1 Schematic representation of the pathophysiological mechanisms, risk factors, and complications associated with PCOS. Insulin resistance (IR) and the hyperinsulinemia (HA) are central mechanisms that perpetuate anovulation. Abbreviations: HHOA: hypothalamus-hypophysis-ovary axis; GnRH: gonadotropin-releasing hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone-binding globulin; ↑: indicates increase in protein level or activity; ↓: indicates decrease in protein level or activity; →: indicates unchanged in protein level or activity

the development of novel pharmacological therapies that can offer additional benefits to patients with PCOS.

MT therapies in PCOS: review of the evidence

MT participates in the normal physiological function of the ovary

MT (N-acetyl-5-methoxytryptamine), is an indoleamine, primarily released at night by the pineal gland, located in the posterior wall of the third ventricle, which is known as the "sleep hormone" [35]. MT exhibits a rhythmic synthesis and release pattern, regulated by a central circadian pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus. This rhythm aligns with the environmental light-dark cycle, with retinal receptors transmitting light/dark signals to the SCN, thereby influencing sexual activity and reproductive function in response to changes in environmental conditions [36]. MT receptors are classified into nuclear and membrane-bound types. Nuclear receptors belong to the RZR/ROR superfamily, while membrane receptors are further divided into three subtypes: MT1, MT2, and MT3 [36]. In humans and other mammals, only two high-affinity, 7-transmembrane G protein-coupled receptors (GPCRs) -MT1 and MT2- are present. Upon agonist binding, the cytoplasmic heterotrimeric G proteins, comprising Gα, β, and γ subunits, dissociate from the GPCRs [37]. The Gα subunits then sequentially activate specific effectors, such as adenylate cyclase (AC), phospholipase C (PLC), or ion channels, influencing second messenger levels like cyclic adenosine monophosphate (cAMP) and inositol trisphosphate (IP3) [38, 39]. Numerous studies have shown that both MT1 and MT2 receptors are co-expressed in various components of the female reproductive system, including the ovary, uterus, and placenta, making these cells potential targets for MT action [40, 41]. Furthermore, recent research has revealed that mRNAs for MT1 and MT2 receptors are detectable on the membranes of human GCs and luteal cells, whose activity is synchronized with the MT cycle [42].

Follicles serve as the functional units of the ovary. As follicles progress from the primary to secondary stage, the surrounding GCs continue to proliferate and expand

[43, 44]. Recent studies have demonstrated that MT is produced in peripheral reproductive tissues, including the whole ovary, GCs, cumulus cells, and oocytes. These cells contribute MT to follicular fluid (FF), alongside blood-derived MT [45, 46]. Interestingly, the concentration of MT in the fluid collected from human follicles exceeds that found in blood samples, suggesting a preferential uptake of circulating MT by the ovary [47]. Moreover, MT appears to play distinct roles at various stages of follicular development [48]. For example, it regulates steroidogenesis, LH mRNA expression, as well as the expression of Bcl-2 and Caspase-3, and modulates the activity of insulin-like growth factor (IGF) and TGF-β in antral follicles [49]. Additionally, MT administration has been shown to delay ovarian aging *via* the MT1 receptor and the Mice/AMP-AMPK pathway [50]. Studies indicate that MT preserves ovarian function and increases oocyte number and quality, resulting in a larger litter size compared to naturally aging ovaries [50]. Other research has reported that MT delays ovarian aging, regulates ovarian biorhythms, promotes follicle formation, and improves oocyte quality and fertilization rates [51].

On the other hand, MT functions as a potent free radical scavenger, protecting oocytes from oxidative damage, particularly during ovulation [52, 53]. Recent studies suggest that mitochondria, rather than just pineal cells, serve as the site of MT synthesis in all cells [54]. Since every cell contains mitochondria, it is hypothesized that all cells may produce MT locally for self-protection against oxidative stress [55]. Ovarian cells, in particular, do not release MT into the systemic circulation but rather utilize it as an antioxidant to benefit themselves and neighboring cells, similar to other extragonadal organs that synthesize MT [56]. Given its antioxidant effects on the hypothalamic-pituitary-gonadal (HPG) axis, MT can mitigate oxidative damage within the follicle, enhancing luteal phase progesterone production and oocyte maturation [57]. Tong et al. found a positive correlation between follicular MT levels and markers of ovarian reserve, including baseline FSH levels, in 61 women undergoing assisted reproductive therapy (ART) [58]. Similarly, there was a significant positive correlation was observed between FF MT concentrations and antral follicle counts in women undergoing in *vitro* fertilization (IVF) [59], supporting the notion that "MT has a protective effect on ovarian cycle progression" (Fig. 2).

Experimental data on MT's therapeutic potential in PCOS

The levels of MT in the serum and saliva of women with PCOS are higher than in healthy controls [60]. A controlled prospective study further revealed that urinary levels of 6-hydroxysulfate melatonin (aMT6s), a key MT metabolite, are significantly elevated in patients with PCOS, while MT levels in FF are lower [61]. Additionally, women with PCOS exhibit altered MT secretion patterns, with diminished nocturnal variation compared to healthy women, which may influence body weight and metabolism [62]. Although the exact etiology of PCOS remains unclear, numerous studies suggest that MT supplementation could offer therapeutic benefits [63]. By modulating key pathways implicated in PCOS, such as insulin signaling, IR, and lipid metabolism, MT supplementation may alleviate the symptoms and severity of the disorder (Fig. 2).

As outlined in Table 1 and detailed below, this study synthesized recent research from molecular studies, animal models, and randomized controlled trials (RCTs) (Materials and Methods are available in Supplementary File 1) to summarize the current evidence on the efficacy of MT supplementation in improving health outcomes in PCOS (Table 1). Due to ethical limitations in human trials, in vitro experiments and animal models of PCOS have been developed. Five studies over the past decade that examine the effects of MT on GCs in vitro were reviewed (Table 1, No.1–5). These studies show that MT mediates various physiological effects through various signaling pathways, such as AC-cAMP, phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B), mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase (ERK), and Akt. MT also regulates hormone levels, exerts anti-inflammatory and anti-apoptotic effects, reduces oxidative stress, and improves mitochondrial dysfunction in patients with PCOS [64–68].

Additionally, reactive oxygen species (ROS) play a significant role in reproductive health, but excessive ROS due to oxidative stress can impair oocyte function, leading to infertility [84, 85]. Studies have demonstrated that MT protects oocytes by reducing free radical levels, including nitric oxide (NO), ROS, and other markers like iNOS, NOX2, and MDA in the follicles [68]. MT's antioxidative actions-both receptor-mediated and direct scavenging of ROS and reactive nitrogen species (RNS)-suggest its potential for improving oocyte development in PCOS [86, 87]. Furthermore, MT is detected in both FF and oocytes, and its administration has shown promising results in mitigating oxidative damage, enhancing oocyte maturation, follicular development, and embryonic progression in PCOS.

The research articles reviewed highlight the progress of MT in treating PCOS using animal models (Table 1, No.6–13). A total of eight studies were analyzed, including seven PCOS models and one T2DM model [69]. The PCOS models consisted of three using androgen-induced PCOS-like syndrome (T/DHEA) [70–72], three employing aromatase inhibitors (letrozole) [73–75], and one involving continuous light exposure to induce the PCOS-like phenotype [76]. The inclusion of a T2DM model

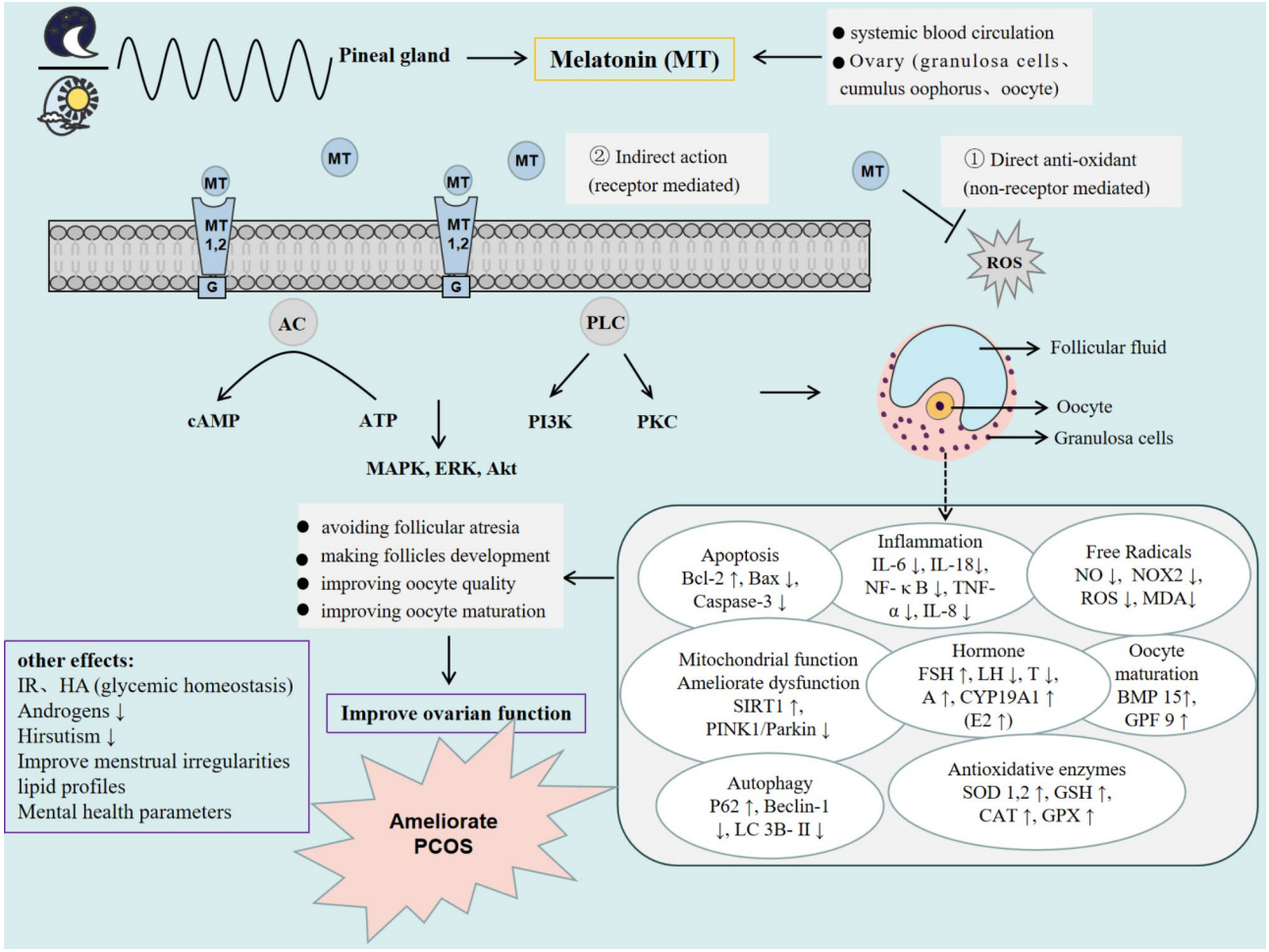

Fig. 2 Schematic representation of Melatonin ameliorates ovarian dysfunction in PCOS. Abbreviations: MT: melatonin; MT 1, 2: melatonin receptor 1, 2; G: G-protein coupled receptor; ROS: reactive oxygen species; AC: adenylyl cyclase; PLC: phospholipase C; ATP: adenosine triphosphate; cAMP: cyclic Adenosine Monophosphate; PI3K: phosphatidylinositol-3 kinase; PKC: protein kinase C; Akt: protein kinase B; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; Bcl-2: B-cell lymphoma-2; Bax: Bcl-2-accociated X protein; IL-18: interleukin-18; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor kappa B; *iNOS: inducible nitric oxide synthetase;* NO: nitric oxide; NOX2: the oxidant-encoding gene (NADPH oxidase 2); MDA: malondialdehyde; SIRT1: NAD-dependent deacetylase sirtuin-1; PINK1: PTEN-induced kinase-1; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; A: Aromatase activity; CYP19A1: cytochrome P450 family 19 subfamily A member 1; E_2: 17β-estradiol; BMP: bone morphogenic protein; GDF: growth differentiation factor; LC 3B- II: light chain 3B-II; SOD: superoxide dismutase; GSH: glutathione; CAT: catalase; GPX: glutathione peroxidase; IR: Insulin resistance; HA: hyperinsulinemia;↑: indicates increase in protein level or activity; ↓: indicates decrease in protein level or activity

aimed to further elucidate MT's role, underscoring the need to differentiate between IR in PCOS and IR as a distinct condition. These studies, all conducted on rodents, examined MT's effects on pubertal animals, showing its ability to regulate hormone levels, reduce inflammation, prevent apoptosis, combat oxidative stress, and improve mitochondrial dysfunction. Notably, MT was found to protect oocytes from apoptosis, increasing Bcl-2 expression while reducing Bax levels [71]. Additionally, MT influenced hypertension, IR, and oocyte maturation in PCOS animal models. Pai SA's findings indicated that MT's impact on reducing intraabdominal fat (IAF), insulin, and CRP was comparable to that of metformin in PCOS treatment [70]. Other studies revealed that MT decreased the expression of IGF-1R/IGF-1, Bcl-2, and PCNA, restoring ovarian morphology. MT also regulated the increased expression of IGF-1R/IGF-1/Bcl-2, and PCNA pathways in the endometrium of PCOS models [75]. Furthermore, Nikmard F observed that MT upregulated GDF-9 and BMP-15, enhancing oocyte maturation, while also boosting antioxidant activity through the upregulation of GPX and SOD1 in PCOS [71, 72].

Collectively, these studies suggest that MT can aid in repairing PCOS in animal models. However, while rodents offer advantages, such as a stable genetic background, short reproductive lifespan, and a brief estrous cycle, their use in reproductive studies has limitations. While rodents and humans share a similar hypothalamic-pituitary-ovarian (HPO) axis, rodents are monoovulatory, resulting in different follicle selection processes driven by FSH [88].

Table 1 Experimental data on the therapeutic potential of melatonin in PCOS. The recent research primarily from molecular studies (No.1–5), animal studies (No.6–13) and RCTs (No.14–20), to summarize current evidence pertaining to the efficacy of MT supplements in improving various health outcomes in PCOS

No	Author, year (Ref)	Sample, species	Induced PCOS/ PCOS patients	Method	MT Duration, Incubation period	Mechanism	Main findings	Effects
1	Xie F, 2021 [64]	Clinical samples from PCOS patients/cell line KGN/Sixty female Sprague-Dawley rats (25 days old)	infertile women with PCOS (n = 20), non-PCOS women undergoing IVF or ICSI (n = 20)/ DHEA (100 μM) -exposed KGN cells/ DHEA (6 mg/100 g/day for 20 days)-induced PCOS rat model;	clinical examination + cell culture + animal models	Cell culture (200 μM)/ animal model: MT (5 mg/100 g for 20 days);	Autophagy was activated in the ovarian tissue of the PCOS rat model, whereas additional MT inhibited autophagy by increasing PI3K-Akt pathway expression.	PCOS group: MT expression ↓, IL-6 ↑, IL-18 ↑, IL-8 ↑, IL-10 ↓; MT-treated group: Serum-free T ↓, inflammatory cytokine ↓, apoptosis indexes ↓;	Protective
2	Yi S, 2020 [65]	the granulosa cells of PCOS patients/ cell line KGN/ Forty-eight female C56BL/6 mice (21 days old)	PCOS group: women with PCOS (n = 60), Control group: patients requiring IVF, IVF-ET or normal ovulation including those with infertility caused by tubal or male factors (n = 30)/ DHT (500 nM)-treated KGN cells/ DHT (6 mg/100 g/ day for 35 days)-induced PCOS-like mice;	clinical examination + cell culture + animal model	Cell culture (100 pM)/ animal model: MT (10 mg/kg for for 14 days);	MT protects against mitochondrial injury in GCs of PCOS by enhancing SIRT1 expression to inhibit excessive PINK1/Parkin mediated mitophagy.	PCOS: P62 ↓, Beclin-1 ↑, LC3B-II ↑, PINK1 and Parkin ↑, SIRT1 ↓; MT-treatment: SIRT1 ↑, PINK1/ Parkin ↓, ameliorate mitochondrial dysfunction, repress the excessive mitophagy;	therapeutic
3	Zheng B, 2021 [66]	Clinical samples from PCOS patients/Twenty-four female C56BL/6 mice (3-week-old)	PCOS group: women with PCOS (n = 12), Control group: Infertile patients who have common ovulation with tubal factors (n = 12)/ DHT (6 mg/100 g/ day for 35 days)-induced PCOS-like mice;	clinical examination + animal models	animal model: MT (10 mg/kg for 14 days);	MT enhances SIRT1 to ameliorate mitochondrial membrane damage by activating PDK1/Akt in granulosa cells of PCOS.	PCOS: mitochondrial swelling and membrane defect mitochondria, cytochrome C level in the cytoplasm ↑, BAX in mitochondria ↑, p-Akt ↓; MT-treatment: mPTP ↓, cytochrome C level in the cytoplasm ↓, BAX ↓, p-PDK 1 and p-Akt ↑, SIRT1 ↑;	not applicable
4	Guo R, 2022 [67]	Clinical samples from PCOS patients/human ovarian GCs (SVOG)	PCOS group: women with PCOS (n = 20), Control group: Infertile patients with tubal factors or normal women (n = 20)/PA-induced IR cell model;	clinical examination + cell culture	Cell culture (100 μM)	MT can reduce IR in GCs and PA-induced SVOG cells via the PI3K/Akt signaling pathway, providing more evidence for treating PCOS.	PCOS: LH ↑, T ↑, LH/FSH ↑, IRS-1 ↓, GLUT4 mRNA ↓, glucose uptake capacity ↓; MT-treatment: IRS-1 ↑, GLUT4 ↑, p-IRS-1 (Ser307) ↓, glucose uptake ↑, p-PI3K and p-Akt ↑;	not applicable
5	Yu K, 2019 [68]	GCs and FF were collected from human	PCOS group: 15 female PCOS patients, Control group (non-PCOS patients: normal menstrual cycles and infertility caused by oviductal dysfunction);	Cell culture	Cell culture (10^{-7} M, 24 h)	MT promoted the expression of CYP19A1 and reduced androgen levels through ERK pathway in GCs.	MT-treated group: Bcl-2 ↑, Bax ↓, the level of T ↓, the level of estrogen ↑, CYP19A1 ↑, Aromatase activity ↑, p-ERK/total ERK ratio ↑, inflammatory factors (IL-6, TNF-α, IL-18) ↓, NF-κB ↓, free Radicals (iNOS, NO, NOX2, ROS, MDA) ↓, oocyte maturation: ↑;	not applicable

Table 1 (continued)

No	Author, year (Ref)	Sample, species	Drug induced PCOS	MT Intervention dose/daily	Duration	Main findings (biochemical)	Ovarian/ uterine histopathological features	Effects
6	Rahma MM, 2017 [69]	Male white Sprague-Dawley rats	fed with a high-fat diet for 45 days (T2DM model)	5 mg/kg (oral gavage)	45 days	Hyperglycemia, IR, hyperlipidemia, hyperleptinemia, increased oxidative stress, inflammatory cytokines, hypertension and fatigue were effectively ameliorated by the combined action of melatonin supplementation and exercise regimentation. (Mitochondrial biogenesis ↑, GLUT4 ↑, antioxidant activities ↑, adiponectin ↑; Oxidative stress ↓, dyslipidemia ↓, inflammation ↓, leptin ↓, visceral obesity ↓;)	N/R	therapeutic
7	Pai SA, 2014 [70]	21-day-old female rats	subcutaneous testosterone (20 mg/kg/day) for 35 consecutive days (PCOS model)	1 mg/kg (intraperitoneally)	14 days	body weight ↓, body mass index ↓, IAF ↓, insulin ↓, CRP ↓, favourable lipid profile, normal glucose tolerance, the percentage of estrus smears ↓;	ovary, uterus and IAF revealed cystic follicles ↓, neoplastic endometrial glands ↓, adipocyte hypertrophy ↓;	protective
8	Nikmard F, 2022 [71]	Females pre-puberal (21–25-day-old) C57BL/6 mice	Dehydroepiandrosterone (DHEA) 6 mg/100 g/day for 20 consecutive days, subcutaneous	10^{-5}, 10^{-6} and 10^{-7} M of MT were added into medium culture for 24 h (in vitro)		oocyte maturation-related genes (GDF 9 ↑, BMP15 ↑), antioxidant-related genes (GPX 1 ↑, SOD1↑), ROS ↓, apoptotic biomarkers (Bcl-2 ↑, Bax ↓);	N/R	therapeutic (*in vivo + in vitro)*
9	Nikmard F, 2017 [72]	Seventy-seven female prepu-bertal (21-25-day-old) C57BL/6 mice	dehydroepiandrosterone (DHEA) 6 mg/100 g/day for 20 consecutive days, subcutaneous.	10^{-5}, 10^{-6} and 10^{-7} M of MT were added into medium culture for 24 h (in vitro)		nuclear maturation of PCOS oocytes ↑, cleavage rate ↑,	N/R	therapeutic (in vivo + in vitro)
10	Basheer M, 2018 [73]	Wistar female Rat	Letrozole (1 mg/kg/day) orally for 2-3 weeks	200 µg/100 g i.p	2-3 weeks	PCOS condition: MT1 and MT2 receptor expression ↓, estrogen (ER-α) ↓, cytokine (IL-2R and IL-6R) receptors ↓; IL-6 and TNF-α↑; Exogenous melatonin: MT1↑, ER-α: reversal, IL-6R ↑;	N/R	protective
11	Hansda SR, 2021 [74]	Adult female golden hamsters	Letrozole (3 mg/kg/day for 40 days, oral gavage)	1 mg/kg (intraperitoneally)	40 days	Letrozole: testosterone ↑, leptin ↑, insulin ↑; uterine InsR/GLUT-4 expression ↓; oxidative load (SOD/catalase/LPO) ↑, inflammatory markers (NF-κB/COX-2) ↑; Melatonin: normalized all the above parameters;	letrozole + melatonin group: ovary with few cystic follicles, atretic follicles and also corpus lutea and several pre-antral and few antral follicles;	therapeutic

Table 1 (continued)

12	Seymen CM, 2021 [75]	6-8-week -old Sprague Dawley rats	Letrozole (1 mg/kg/day, oral gavage) for 21 days;	2 mg/kg (subcutaneous)	21 days	endometrial IGF-1R/IGF-1/Bcl-2 and PCNA pathways ↑	returned to its normal appearance generally (body ↓, ovary weights ↓, uterine weights ↑, lumen structures returned to their normal appearance, Fibrosis was completely eliminated in the stroma and blood vessels were found to be normal;	protective
13	Lombardi LA, 2019 [76]	adult-female rats	at permanent-estrous phase induced by 60 days of continuous illumination	0.4 mg/Kg drinking water	60 days	Ki-67-positive cells ↑, Caspase-3positive cells in granulosa cells ↓;	the presence of corpus luteum, primary and antral follicles ↑, ovarian cysts ↓, in the area occupied by interstitial cells ↓;	therapeutic
No	**Author, year (Ref)**	**Study Design**	**Population, n**	**weight**	**Age mean or range**	**MT Intervention dose/day**	**Duration**	**Main findings**
14	Tagliaferri V, 2018 [77]	prospective cohort study	*n* = 40 women for PCOS	normal-weight (N.S)	23.25	2 mg	6 Months	Improvement of menstrual irregularities, androgens ↓, FSH↑, AMH↓, LDL ↓, hyperandrogenism ↓, glucoinsulinemic N.S, lipid parameters N.S.
15	Mousavi R, 2022 [78]	randomized double-blind, placebo-controlled trial	*n* = 84 women for PCOS (*n* = 20 placebo group, 21 MT group, 22 MT + Mg group, 21 Mg group)	BMI ≤ 35 (N.S)	18-40	6 mg	8 weeks	BMI ↓; WC ↓; TNF-α↓; TAC N.S;
16	Jamilian M, 2019 [79]	randomized, double-blinded, placebo-controlled clinical trial	*n* = 56 women for PCOS (*n* = 28 placebo group, 28 MT group)	N.S	18-40	10 mg (5 mg twice/day)	12 weeks	Hirsutism ↓; TT ↓; hs-CRP ↓; MDA ↓; TAC ↑; GSH ↑; IL-1 ↓; TNF-α ↓;
17	Shabani A, 2019 [80]	randomized, double-blind, placebo-controlled trial	*n* = 58 women for PCOS (*n* = 29 placebo group, 29 MT group)	N.S	18-40	10 mg	12 weeks	Pittsburgh Sleep Quality Index ↓; Beck Depression Inventory index ↓; Beck Anxiety Inventory index ↓; serum insulin ↓; QUICKI ↑; PPAR-γ ↑; LDLR ↑;

Table 1 (continued)

18	Mokhtari F, 2019 [81]	double-blinded RCT	*n* = 198 IUI PCOS (*n* = 100 control group, 94 MT group)	N.S	28.9 ± 5.5	3 mg	From the third day of menstruation until HCG administration	chemical pregnancy rates ↑; ET ↑;
19	Pacchiarotti A, 2016 [82]	randomized, controlled, double-blind trial	*n* = 526 ICSI PCOS (*n* = 195 folic acid group, 165 myo-inositol + folic acid + MT group, 166 myo-inositol + folic acid group)	BMI: 20 to 26 kg/m^2 (N.S)	27-38	3 mg	From the first day of the cycle until 14 days after embryo transfer;	Myo-inositol and melatonin can enhance oocyte and embryo quality;
20	Alizadeh M, 2021 [83]	randomized, double-blind, placebo-controlled trial	84 subjects with PCOS (*n* = 20 placebo group, 21 MT group, 21 Mg group, 22 MT + Mg group)	28.40 ± 3.86 (N.S)	18-40	6 mg	8 weeks	mean PSQI score ↓ (sleep improvement), testosterone concentrations ↓, serum insulin levels ↓, HOMA-IR ↓, serum cholesterol ↓, LDL-C↓, TT ↓, HDL-C ↑;

Abbreviations symbol is a sign of decreasing variables in the intervention group;↑This symbol is a sign of increasing variables in the intervention group;↔This sign indicates that there is no difference between the two groups;N/R: not reported; N.S: not significant; MT: melatonin; MT 1,2: melatonin receptor 1, 2; PCOS: Polycystic Ovary Syndrome; IVF: in vitro fertilization; ICSI: Intra-Cytoplasmic Sperm Injection; DHEA: dehydroepiandrosterone; PI3K: phosphatidylinositol-3 kinase; Akt: protein kinase B; IL-18: interleukin-18; T: testosterone; IVF-ET: In Vitro Fertilization-Embryo Transfer; DHT: dihydrotestosterone; SIRT1: NAD-dependent deacetylase sirtuin-1; PINK1: PTEN-induced kinase-1; LC 3B-II: light chain 3B-II; PDK1/Akt: phosphoinositide dependent proteinkinase-1/Protein Kinase B; Bax: Bcl-2-accociated X protein; mPTP: mitochondrial permeability transition pore; GCs: granulosa cells; PA: palmitic acid; IR: Insulin resistance; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; IRS-1: insulin receptor substrate-1; GLUT4: glucose transporter-4; CYP19A1: cytochrome P450 family 19 subfamily A member 1; ERK: extracellular signal-regulated kinase; Bcl-2: B-cell lymphoma-2; A: aromatase activity; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor kappa B; iNOS: inducible nitric oxide synthetase; NO: nitric oxide; NOX2: the oxidant-encoding gene (NADPH oxidase 2); ROS: reactive oxygen species; MDA: malondialdehyde; T2DM: Type 2 diabetes mellitus; IAF: intraabdominal fat; CRP: C reactive protein; DHEA: Dehydroepiandrosterone; GDF: growth differentiation factor; BMP: bone morphogenic protein; GPX: glutathione peroxidase; SOD: superoxide dismutase; InsR: insulin receptor; LPO: lipid peroxidation; COX-2: cyclooxygenase-2; PCNA: proliferation cell nuclear antigen; IGF-1: insulin-like growth factor-1; IGF-1R: IGF-1 receptor; Casp-3: caspase-3; AMH: anti-müllerian hormone; LDL: low-density lipoprotein cholesterol; BMI: body mass index; WC: waist circumference; TAC: total antioxidant capacity; TT: serum total testosterone; hs-CRP: high-sensitivity C-reactive protein; GSH: glutathione; QUICKI: quantitative insulin sensitivity check index; PPAR-γ: peroxisome proliferator-activated receptor gamma; LDLR: low-density lipoprotein receptor; RCT: randomized clinical trial; IUI: intrauterine insemination; HCG: human chorionic gonadotropin; ET: endometrial thickness; ICSI: Intracytoplasmic sperm injection; PSQI: Pittsburg Sleep Quality Index; HOMA-IR: homeostasis model of assessment-insulin resistance; LDL-C: low-density lipoprotein cholesterol; TT: total testosterone; HDL-C: high-density lipoprotein cholesterol; IVM: in-vitro maturation; COC: cumulus-oocyte-complexes; G: G-protein linked receptor; ROS: reactive oxygen species; AC: adenylyl cyclase; PLC: phospholipase C; ATP: adenosine triphosphate; cAMP: cyclic Adenosine Monophosphate; PKC: protein kinase C; MAPK: mitogen-activated protein kinase; E2: 17β-estradiol; CAT: catalase; HA: hyperinsulinemia

Additionally, differences in follicle growth regulation and timing between rodents and humans must be considered, limiting the direct applicability of rodent-based findings to human conditions [89, 90].

To further explore MT's regulatory effect on PCOS, we reviewed human RCT studies [77–83] (Table 1, No.14–20). A prospective cohort study showed a significant reduction in serum AMH levels and an increase in FSH in patients with PCOS, with six months of MT supplementation restoring menstrual cycles and normalizing androgen levels [77]. A recent clinical trial involving 56 patients with PCOS demonstrated significant reductions in hirsutism, serum TT, hs-CRP, TNF-α, and plasma MDA, alongside increases in plasma TAC and total GSH following 12 weeks of MT supplementation [79]. Another study reported improvements in the Pittsburgh Sleep Quality Index, Beck Depression and Anxiety Inventories, serum insulin, and the quantitative insulin sensitivity test index (QUICKI) after 12 weeks of MT [80]. Similarly, Alizadeh M's study confirmed that MT supplementation improved sleep quality, reduced testosterone, insulin levels, homeostasis model of insulin resistance (HOMA-IR), and serum total cholesterol and LDL levels [83]. Overall, MT administration showed beneficial effects on menstrual disorders, ovarian function, glycemic control, lipid profiles, mental health, and related gene expression in women with PCOS.

PCOS often leads to anovulation, contributing to IVF failure. To improve oocyte quality, a double-blind RCT with 198 patients with PCOS undergoing intrauterine insemination (IUI) found that MT (3 mg/day) significantly improved follicle quality, endometrial thickness (ET), and the chemical pregnancy rate [81]. Another RCT involving 526 patients with PCOS undergoing Intra-Cytoplasmic Sperm Injection (ICSI) demonstrated that MT supplementation was a strong predictor of positive IVF outcomes, correlating with higher oocyte and embryo quality [82]. Additional evidence supports the benefit of MT supplementation, with higher implantation rates observed in MT-supplemented groups compared to non-supplemented controls when using a human chorionic gonadotropin initiation regimen. This suggests that MT may improve cytoplasmic maturation and subsequent clinical outcomes in human immature oocytes [91, 92].

In summary, current clinical trials suggest that MT improves oocyte maturation, quality, and chemical pregnancy rates in women with PCOS. Despite its limited applications, MT presents a promising therapeutic option and warrants further investigation.

Relationship between Hippo pathway and PCOS

The Hippo pathway and ovary

The Hippo pathway, initially discovered in Drosophila, regulates organ size during development and is evolutionarily conserved [93]. In mammals, the core components of the Hippo pathway include mammalian sterile 20-like (MST) 1/2, large tumor suppressor (LATS) 1/2, transcriptional activator Yes-associated protein (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ) [94]. The pathway is primarily governed by a kinase cascade: MST1/2 phosphorylate the Salvador homolog (Sav) 1, which activates LATS1/2. LATS1/2, in turn, phosphorylate and inactivate YAP. Phosphorylated YAP binds to the cytoplasmic scaffold protein 14-3-3, preventing its nuclear translocation. When unphosphorylated, YAP enters the nucleus and interacts with transcriptional enhanced associate domain (TEAD) transcription factors to regulate downstream target genes such as cystein-rich 61 (CYR61) and connective tissue growth factor (CTGF) [95, 96]. Activation of YAP and TAZ in the nucleus promotes gene expression linked to cell proliferation, tissue growth, and organ size, particularly when the Hippo pathway is inactivated.

Components of the Hippo pathway, including MST1/2, LATS1/2, YAP1, and phosphorylated YAP1, are expressed throughout all stages of folliculogenesis in oocytes, GCs, and TCs, and in atretic follicles and corpus luteum [9, 97–100]. Studies have shown that disruption of the Hippo pathway in ovarian cells leads to increased nuclear YAP, stimulating the expression of downstream growth factors and promoting follicle growth, resulting in the generation of mature oocytes [20, 101]. Inhibition of YAP1 using verteporfin, a specific Hippo pathway inhibitor, further confirms the role of this pathway in regulating follicular development [101]. Moreover, disruption of the Hippo pathway enhances the secretion of CCN (cellular communication network) growth factors, which also promote follicle growth [21].

Comprehensive studies of drosophila ovaries have shown that the Hippo pathway interacts with several pathways, including PI3K-Akt and Notch, to regulate ovarian development and function [102, 103]. Disruption of the Hippo pathway, along with Akt activation, promotes follicle growth and oocyte maturation, offering potential applications in infertility treatments [97, 101, 104]. Recent research suggests that PI3K pathway activation is essential for follicular growth, particularly during the primordial and primary stages. While the Hippo and PI3K/Akt/mTOR pathways have opposing effects during the gonadotropin-independent phase of follicular development, they work synergistically during the gonadotropin-dependent phase to support follicle maturation, particularly the development of GCs and oocytes in preovulatory follicles. This coordination ensures proper follicle activation and oocyte quality [105].

Correlation between Hippo pathway and PCOS

In patients with PCOS, the ovaries are enlarged, with a thickened sclerotic capsule containing numerous small

antral follicles but no pre-ovulatory follicles. PCOS affects 5–10% of women of childbearing age and is a common cause of infertility [106]. Although the factors influencing ovarian response to invasive procedures like wedge resection or laparoscopic ovarian drilling (LOD) remain unclear, changes in actin polymerization and the Hippo pathway may play a pivotal role [107]. The mechanism of the Hippo pathway in PCOS has been briefly described. Normally, as follicles enlarge and enter the softer cortical regions, the Hippo pathway is reactivated, slowing follicular growth, and maintaining physiological ovarian androgen secretion and normal LH/FSH ratios. However, in the classic PCOS phenotype described by Stein and Leventhal, increased cortical collagen and stromal hypertrophy result in an abnormally rigid, sclerotic cortex [108]. This may be linked to defects in actin polymerization, leading to the increased F-actin content and/or the abnormal extracellular matrix protein biosynthesis. The hardened cortical layer exhibits increased polymerized actin, leading to an enhancement in the tensile strength of the matrix tissue (elevated F-actin levels), potentially disrupting the local Hippo pathway [12]. This disruption causes excessive YAP activity in stromal and follicular cells, which in turn increases CCN growth factor secretion, promoting the proliferation of stromal cells, follicular membrane cells, and GCs, leading to the growth of multiple early antral follicles. The proliferation of TCs and increased androgen production may elevate LH secretion from the pituitary, contributing to a higher LH/FSH ratio. Stromal and thecal hyperplasia leads to excessive androgen biosynthesis, follicular arrest, and ovarian enlargement, characteristic of PCOS [12, 107]. In summary, while the Hippo pathway supports proper follicular development, its dysregulation—through increased YAP1 expression—can result in ovarian enlargement and cyst formation, hallmark features of PCOS [109].

As discussed earlier, IR, hyperinsulinemia, and hormonal imbalances, particularly elevated serum testosterone (T) levels, are key characteristics of PCOS. Hyperinsulinemia contributes to ovarian hyperandrogenism by stimulating follicular membrane cells to produce androgens. Recent evidence in murine GCs suggests that YAP1 is critical for GC proliferation, and ovarian androgens can increase YAP1 expression in human GCs. Specifically, T has been shown to enhance both the expression and activity of YAP1. Acute hyperandrogenism disrupts LH actions and induces oligo-ovulation by activating YAP1, linking YAP1 dysregulation to the pathogenesis of PCOS [9]. Genomic and genetic studies further highlight the pivotal role of YAP1 in PCOS phenotype and metabolic disorders. Genome-wide association studies (GWAS) have identified three SNPs (rs11225138, rs11225161, and rs11225166) in the YAP gene, associated with increased susceptibility to PCOS [110]. Additionally, low methylation of the YAP promoter and elevated YAP expression have been observed in GCs of patients with PCOS, which may influence follicular growth [111].

Defects in Hippo pathway genes are linked to PCOS, ovarian reserve, and infertility in mice. GWAS in diverse populations have also shown that YAP, as a Hippo effector, is associated with PCOS [112, 113]. Moreover, the specific loss of YAP in mice results in increased GC apoptosis, reduced corpora lutea, and subfertility [114]. Overall, understanding the intraovarian mechanisms through which the Hippo pathway operates could pave the way for novel therapeutic strategies for patients with PCOS (Fig. 3).

Relationship between Hippo pathway, MT, and PCOS

The potential cross-talk between MT and Hippo pathway through the gas molecular (based on theoretical basis)

MT exerts its effects through the MT1 and MT2 GPCRs, which interact primarily with the Gαi protein, and to a lesser extent with Gαq/11 and Gαs proteins [115]. The signaling pathways activated by MT receptors are cell type- and tissue-specific, leading to distinct cellular responses and suggesting potential crosstalk with other signaling networks [116]. As previously discussed, the Hippo pathway regulates organ development and growth, and its dysregulation in patients with PCOS may contribute to the disease's multifactorial etiology. YAP and TAZ are key targets of the Hippo pathway, and GPCRs have been shown to act as upstream regulators of this pathway [117]. Evidence indicates that MT inhibits hepatocellular carcinoma (HCC) cell proliferation and glucose metabolism by suppressing YAP expression and its downstream targets Bcl-2 and GLUT-3, thereby promoting cisplatin-induced apoptosis [118]. Further studies have demonstrated that MT can prevent bleomycin(BLM)-induced pulmonary fibrosis by inhibiting YAP translocation from the cytoplasm to the nucleus, both in vivo and in vitro [119]. These findings suggest potential crosstalk between MT and the Hippo pathway, revealing possible mechanisms of action.

Shiu SY demonstrated that the dual activation of Gαs and Gαq proteins is involved in the signal transduction of the anti-proliferative effect of MT1 (MTNR1A) recepto on human prostate cancer cells [120]. This activation leads to an increase in intracellular cAMP, which in turn activates protein kinase A (PKA) and protein kinase C (PKC) in parallel. GPCR-mediated signals can either positively or negatively regulate YAP/TAZ activity, depending on the signal properties, receptor type, and associated adaptor proteins. For instance, serum-borne lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) inhibit the kinase Lats1/2 kinase through Gα12/13, Gαq/11 or Gαi/o coupled

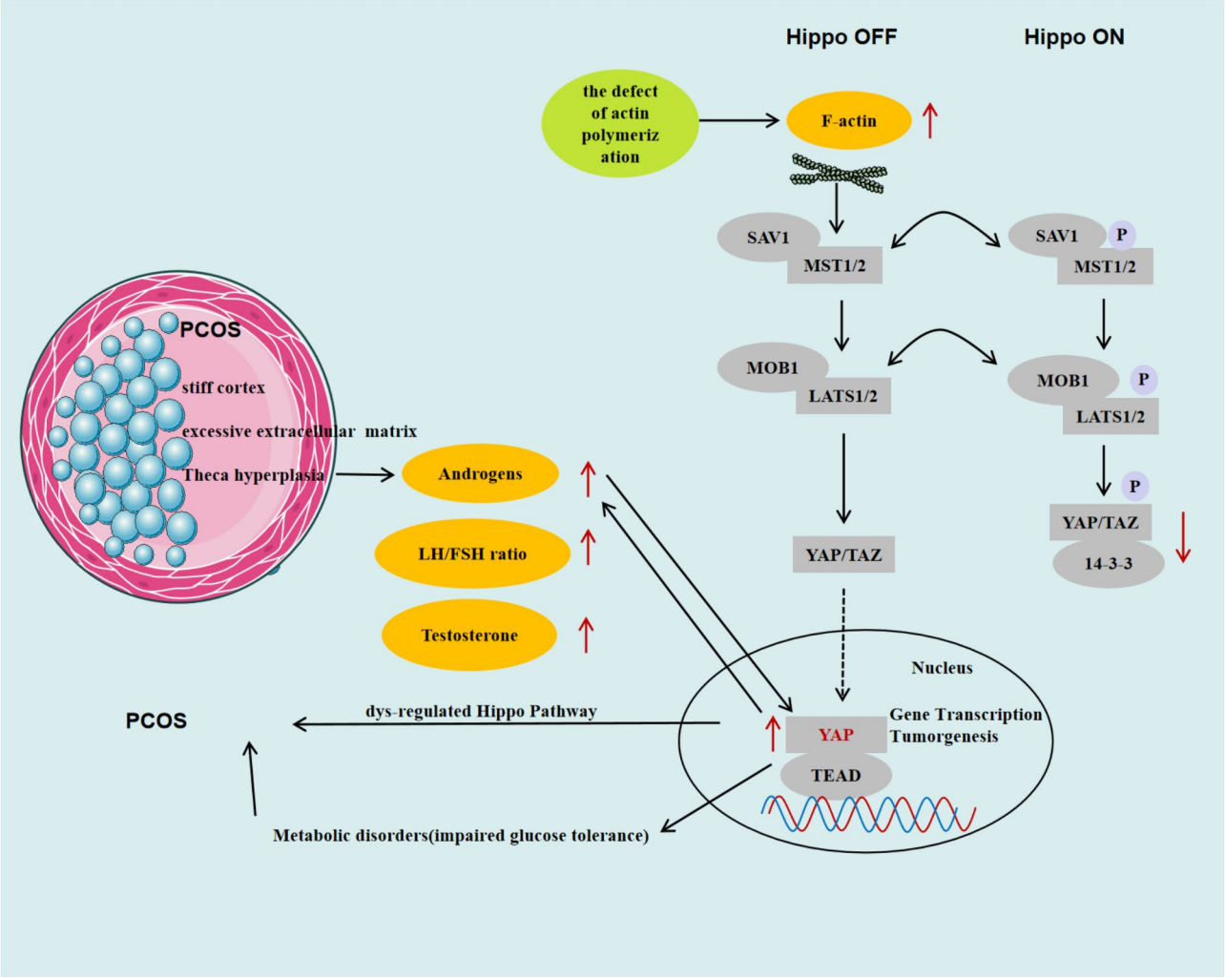

Fig. 3 Disruption of the ovarian Hippo pathway through actin polymerization, resulting in increased nuclear YAP expression and contributing to PCOS formation. Abbreviations:↑ indicates increase in protein level or activity; ↓ indicates decrease in protein level or activity; YAP: Yes-associated protein; TAZ: PDZ-binding motif; MST 1/2: mammalian STE20-like protein kinase 1/2; SAV1: salvador homologue 1; LATS1/2: large tumor suppressor 1 and 2; MOB1A: MOB kinase activator 1

receptors, thus activating YAP and TAZ, which play a role in LPA-induced gene expression, cell migration, and proliferation. In contrast, signals from glucagon, epinephrine, and dobutamine, through Gαs, lead to increased intracellular cAMP and activation of PKA. This subsequently inhibits RhoA GTPase activity (thereby suppressing F-actin) and activates LATS1/2, resulting in YAP/TAZ phosphorylation. Phosphorylated YAP/TAZ are retained in the cytoplasm by 14-3-3 proteins, reducing their nuclear activity and inhibiting the expression of genes involved in cell proliferation, migration, and anti-apoptotic processes [121, 122]. This suggests that MT may inhibit the oncogenic functions of YAP/TAZ by enhancing LATS1/2 activity following PKA and PKC activation [123]. Other studies indicate that MT may also suppress YAP/TAZ's carcinogenic activity by reducing TAZ transcription through NF-κB inhibition, as the TAZ promoter is directly activated by NF-κB. This inhibition may reduce cell proliferation and aggressiveness in AR-positive cells [7, 124–126] (Fig. 4).

Genetic and experimental studies have highlighted the critical role of the Hippo pathway in PCOS development. A defect in actin polymerization (increased F-actin) disrupts the Hippo pathway, leading to reduced YAP phosphorylation and elevated nuclear YAP levels. Nuclear YAP, in conjunction with TEAD, activates the transcription of downstream CCN growth factors, contributing to PCOS pathogenesis [101, 107]. Increased nuclear YAP is closely associated with excessive androgen production, exacerbating PCOS. MT has the potential to reduce nuclear YAP expression by inhibiting F-actin polymerization or through GPCR signaling, offering a mechanism through which MT may mitigate the symptoms of PCOS. This suggests that MT could improve PCOS outcomes by modulating the Gαs-mediated Hippo pathway.

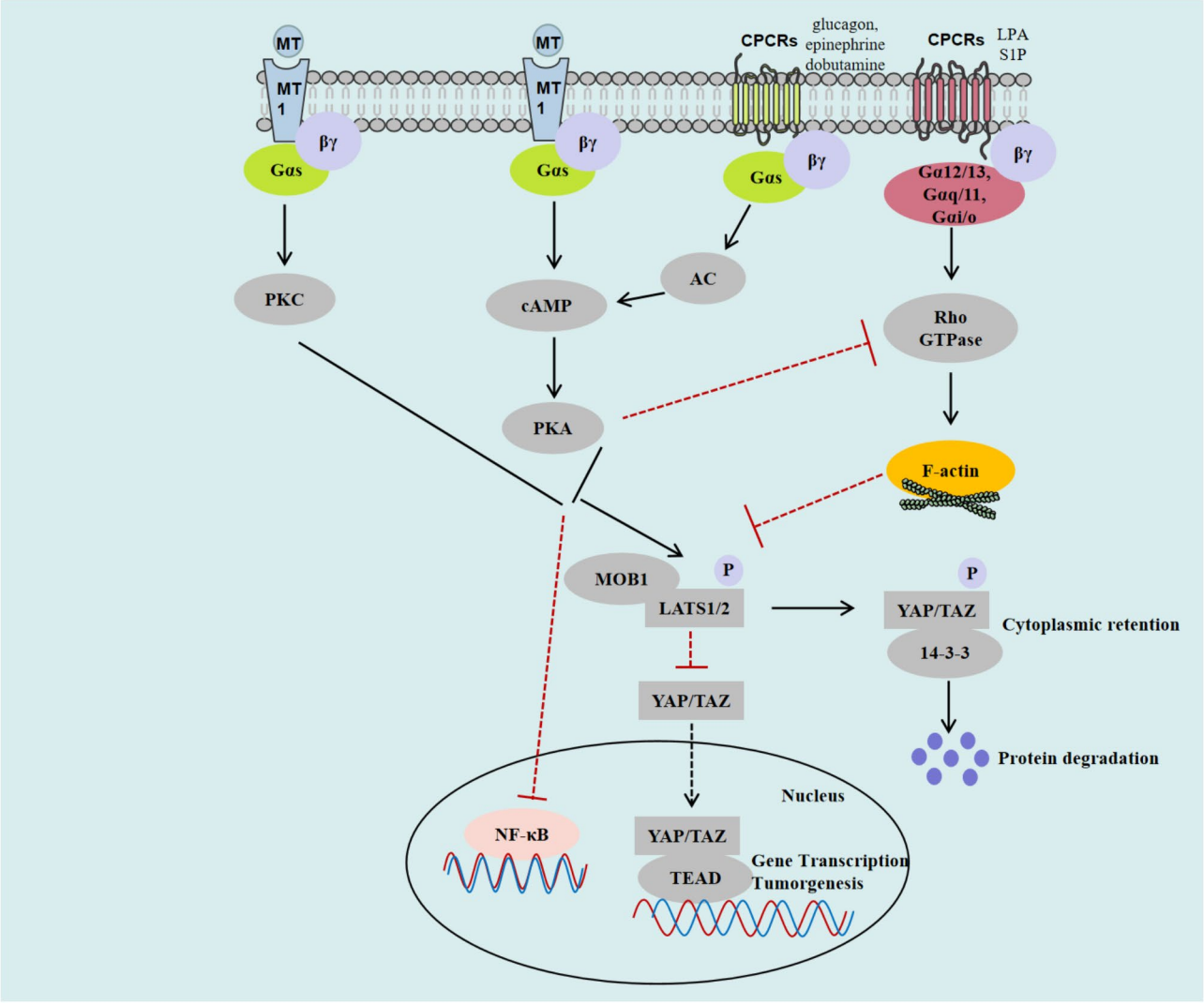

Fig. 4 Melatonin-regulated GPCR signaling interacts with GPCR signal-regulated Hippo pathway. Abbreviations:↑ indicates increase in protein level or activity; ↓ indicates decrease in protein level or activity; ⊣ : indicates inhibition in protein level or activity; MT: melatonin; MT 1: melatonin receptor 1; GPCR: G-protein coupled receptor; Gαi: inhibitory guanine triphosphate-binding protein α-subunit; Gαs: stimulatory guanine triphosphate-binding protein α-subunit; cAMP: cyclic adenosine monophosphate; AC: adenylyl cyclase; PKC: protein kinase C; PKA: Protein Kinase A; LATS1/2: large tumor suppressor 1 and 2; MOB1A: MOB kinase activator 1; TEAD: TEA domain family member; YAP: Yes-associated protein; TAZ: PDZ-binding motif (also known as WW domain-containing transcription regulator protein 1, WWTR1); NF-κB: nuclear factor-κB

Theoretical levels of MT antagonize the metabolic pathway of the Hippo pathway in PCOS

PCOS presents with a range of phenotypes, including reproductive, endocrine, and metabolic disturbances. Beyond the reproductive system, its extra-reproductive manifestations encompass IR, metabolic syndrome (MS), low-grade chronic inflammation, anovulatory infertility, and type 2 diabetes (T2D) [127]. IR, along with glucose metabolism disorders, plays a pivotal role in the pathogenesis of PCOS. It induces compensatory hyperinsulinemia, which not only directly enhances ovarian androgen synthesis but also stimulates LH secretion [128].

The transmembrane variants MT1 and MT2 are expressed in Langerhans cells, where they regulate insulin secretion from β-cells and glucagon secretion from α-cells. Research has consistently highlighted the critical role of MT-insulin interactions in glucose metabolism [129]. Additionally, extensive studies have emphasized the importance of crosstalk between insulin/IGF-1 receptors and GPCR signaling in regulating both normal and pathological functions in patients with T2DM [130]. Guo R demonstrated that MT significantly upregulates IRS-1 and GLUT4 expression, downregulates p-IRS-1 (Ser307), and enhances glucose uptake in patients with PCOS [67]. Moreover, MT influences the expression of endometrial IGF-1R/IGF-1/Bcl-2 and PCNA pathways in individuals with PCOS [79]. PI3K/Akt serves as a central mediator of insulin/IGF-1 signaling. Guo R further found that MT mitigates IR in PCOS cells through

the PI3K/Akt pathway [67]. Similarly, Xie F reported that MT protects ovarian function by modulating autophagy in PCOS, likely *via* the PI3K-Akt pathway [64]. YAP and TAZ have emerged as novel insulin sensors, with YAP playing a pivotal role in insulin/IGF signaling. The Hippo pathway, a key downstream effector of both PI3K and GPCR signaling, is integral to this process [131]. Wang C identified the most significant insulin resistance-related (IRR) gene as a major contributor to IR by comparing IRS1/2 expression levels between obese and non-obese patients. In patients with endometrial cancer (EC), IRS1/2 expression was positively correlated with YAP/TAZ [132]. Hao F explored the positive crosstalk between insulin/IGF-I receptors and GPCR signaling, noting that GPCR signaling synergizes with the IRS-1 pathway to promote YAP nuclear translocation [133]. Additionally, MT helps maintain glucose homeostasis by modulating glucagon activity in pancreatic α cells [134]. As mentioned earlier, glucagon activates the Gαs protein, leading to increased cAMP levels, which in turn activates LATS1/2 kinase activity, resulting in YAP/TAZ phosphorylation and subsequent inhibition of YAP function [121].

Xu H proposed a potential mechanism by which MT activates the Hippo pathway in POF rats, showing that MT inhibits LATS1, MOB1 and YAP phosphorylation, thereby activating the Hippo pathway and promoting its downstream targets, CYR61 and CTGF [135]. This suggests that MT and the Hippo pathway may interact at various levels. Taken together, this body of evidence indicates a possible interplay between MT, the Hippo pathway, and insulin signaling, aligning with prior studies. It is proposed that MT could ameliorate PCOS through metabolic pathways that antagonize the Hippo pathway (Fig. 5).

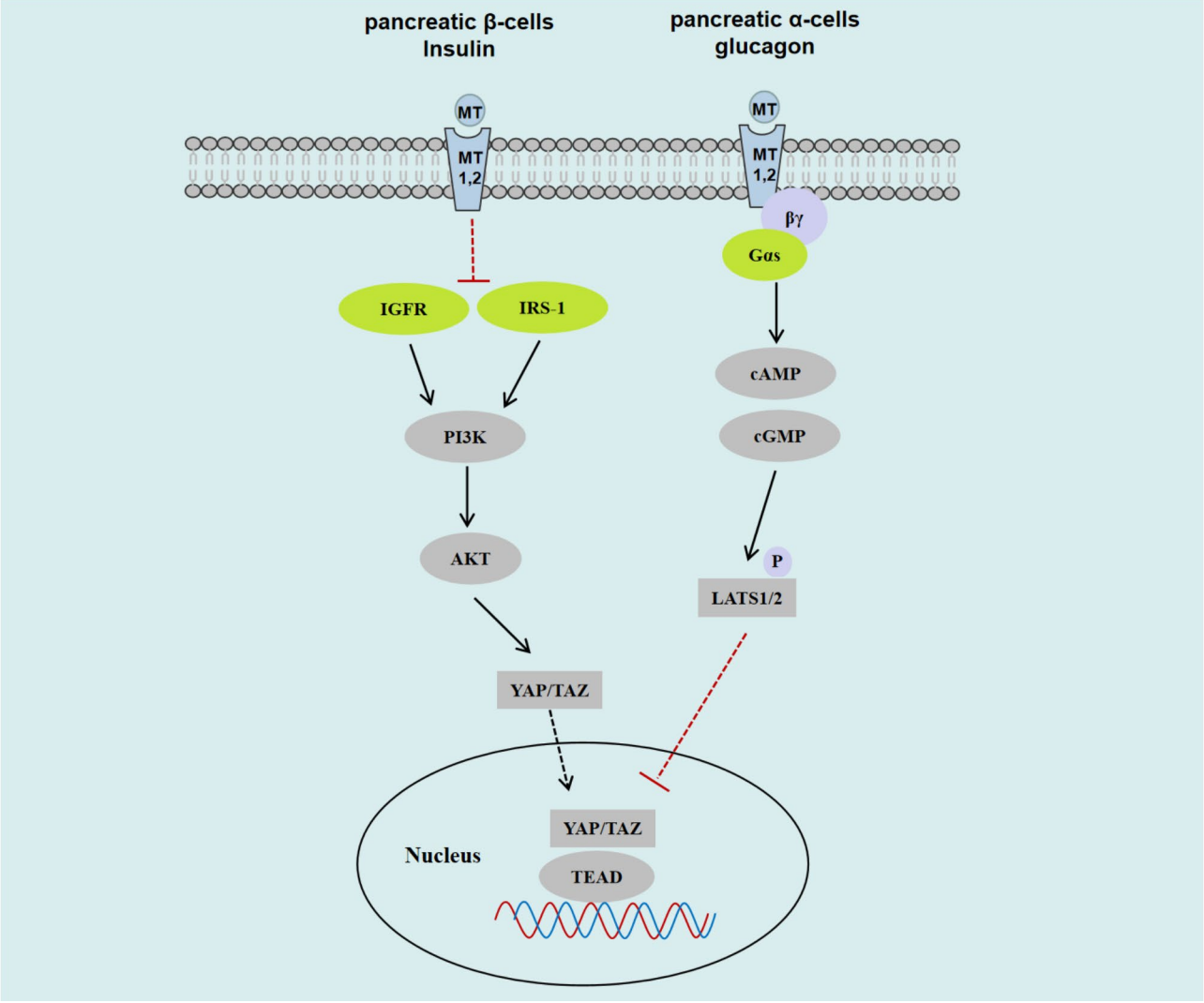

Fig. 5 Interaction between melatonin, insulin, and the Hippo pathway. Abbreviations: Gαs: stimulatory guanine triphosphate-binding protein α-subunit; cAMP: cyclic adenosine monophosphate; cAMP: cyclic guanosine monophosphate; LATS1/2: large tumor suppressor 1 and 2; YAP: Yes-associated protein; IGF-I: insulin-like growth factor; IRS-1: insulin receptor substrate-1

Conclusions and perspectives

Currently, MT supplementation shows promise in mitigating some of the adverse health outcomes associated with PCOS. However, the underlying etiology and mechanisms of PCOS remain incompletely understood. This review summarizes current evidence on the etiology of PCOS and explores the effects of MT, which has been investigated in recent studies involving experimental models, animal trials, and human subjects. Various methods, such as androgen administration, non-steroidal aromatase inhibitors, and continuous light exposure, have been employed to induce PCOS-like phenotypes in these studies. Serum hormone levels, hyperandrogenism, IR, ovarian histopathology, and PCOS symptoms were assessed before and after MT treatment. Most studies reported positive outcomes, suggesting that MT may be effective in treating PCOS. Given its antioxidant properties and role as a hormone modulator, MT supplementation holds therapeutic and preventive potential in obstetrics and gynecology. Additionally, ovarian stiffness and fibrosis, potentially mediated through the Hippo signaling pathway, may contribute to PCOS pathogenesis, and approaches to alleviate fibrosis could improve fertility. Consequently, targeting the Hippo pathway with pharmacological agents may offer non-surgical therapeutic options for PCOS.

Despite the promising preliminary data on MT's potential to alleviate PCOS-related complications, several limitations need to be addressed before these therapies can be widely implemented in clinical practice. First, there is significant variability in the MT dosages and intervention durations studied, making it difficult to draw definitive conclusions (Table 1). With varying dosages and treatment timelines, it remains unclear what the optimal dose for PCOS is, leading to possible overestimation or underestimation of the therapeutic benefits. Second, while this review highlights MT's potential therapeutic effects, the quality of the supporting evidence varies, ranging from robust RCTs to single retrospective observational studies. Some studies are limited by small sample sizes, with participant numbers under 100, reducing the strength of the conclusions (Table 1). Third, discrepancies in receptor functions between humans and rodents complicate the translation of findings. For instance, MT has been shown to increase insulin secretion in isolated human pancreatic islets, whereas other studies found a decrease in rodent pancreatic islet insulin secretion [136, 137]. This difference may be attributed to the circadian differences in MT release, with nocturnal species like rats peaking in MT during active hours, while in humans, peak MT release coincides with rest and sleep periods.

This review also discusses the potential role of MT in promoting follicle recovery in PCOS, the involvement of the Hippo pathway in follicle growth, and the possibility of an interaction between MT and the Hippo pathway. Evidence supporting MT's regulation of PCOS through the Hippo pathway is also examined. Future research should focus on developing preventive strategies to minimize the onset of metabolic dysfunction in adolescent girls. Although MT's application remains limited, it lays a strong foundation for future investigation.

Supplementary Information

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Supplementary Material 1

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Author contributions
Lijun Wang and Yuanyuan Jin were in charge of the conception, study design, and literature research. Zhenzhen Li and Meili Wang wrote the scripts manuscript and designed figures. Boda Wang, Lijun Wang, Yuanyuan Jin, Xinbo Wang and Yuanyuan Zhi contributed to editing the manuscript and designing Table 1. Xinbo Wang and Yuanyuan Zhi reviewed the manuscript and supervised the work. The authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
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Competing interests
The authors declare no competing interests.

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