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REVIEW ARTICLE

Ke et al: AMH regulation and role in PCOS

Anti-Müllerian hormone in PCOS: Molecular regulation and emerging therapeutic strategies

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ABSTRACT

Anti-Müllerian hormone (AMH), a glycoprotein belonging to the transforming growth factor-beta (TGF-β) superfamily, is a key regulator of ovarian folliculogenesis. Dysregulated AMH expression is a hallmark of polycystic ovary syndrome (PCOS), a common endocrine and metabolic disorder characterized by hyperandrogenism, anovulation, and polycystic ovarian morphology. Elevated AMH levels in PCOS impair follicle-stimulating hormone (FSH) sensitivity, disrupt follicular maturation, and contribute to androgen excess—creating a feedback loop that exacerbates ovarian dysfunction. This review explores the complex regulatory mechanisms governing AMH expression, including transcriptional, post-transcriptional, and post-translational processes. It highlights the interplay between AMH, FSH, and androgen signaling pathways, emphasizing their roles in the pathophysiology of PCOS. Particular attention is given to the downstream SMAD-dependent signaling cascade, which mediates many of AMH's biological effects. Additionally, we summarize emerging therapeutic strategies targeting AMH signaling, such as AMHR2 (anti-Müllerian hormone receptor type 2) antagonists, GnRH (gonadotropin-releasing hormone) antagonists, and aromatase inhibitors. A deeper understanding of AMH regulation and signaling provides critical insights into its role in PCOS progression and supports the development of novel, targeted treatments aimed at alleviating both reproductive and metabolic symptoms.

Keywords: Polycystic ovary syndrome; PCOS; Anti-Müllerian hormone; AMH; regulation; SMAD; therapy.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (PCOM). Its etiology is multifactorial, involving genetic, neuroendocrine, ovarian, and metabolic factors [1-4]. A central feature of PCOS pathogenesis is gonadotropin dysregulation, driven by an increased gonadotropin-releasing hormone (GnRH) pulse frequency, which preferentially stimulates luteinizing hormone (LH) over follicle-stimulating hormone (FSH) secretion (Figure 1) [5,6]. The resulting elevated LH/FSH ratio promotes androgen overproduction by theca cells, contributing to hyperandrogenism, a hallmark of PCOS (Figure 1) [5,6].

Insulin resistance (IR) and hyperinsulinemia also exacerbate PCOS pathophysiology by amplifying hyperandrogenism and metabolic dysfunction [7,8]. Insulin acts synergistically with LH to stimulate theca cell androgen synthesis while also reducing sex hormone-binding globulin (SHBG) levels, thereby increasing the bioavailability of circulating androgens (Figure 1) [7,8]. Moreover, insulin resistance is strongly linked to metabolic disturbances, including dyslipidemia, impaired glucose tolerance, and obesity, all of which further impair ovarian function [9,10].

Another critical contributor to PCOS is anti-Müllerian hormone (AMH), a member of the transforming growth factor β (TGF-β) superfamily, secreted by granulosa cells of pre-antral and small antral follicles [11-13]. In PCOS, AMH levels are abnormally elevated—typically two to three times higher than in healthy individuals—further suppressing FSH sensitivity and exacerbating follicular arrest and anovulation (Figure 1) [11-13]. Additionally, AMH is implicated in neuroendocrine dysregulation, as it can enhance GnRH neuron activity, further increasing LH hypersecretion and perpetuating hormonal imbalances [11-13]. Elevated AMH levels also inhibit aromatase (CYP19A1, Cytochrome P450 family 19 subfamily A member 1) activity, thereby leading to androgen accumulation (Figure 1) [14]. This excess androgen production, driven by elevated LH stimulation of theca cells, further disrupts follicular development and ovulation [11-13].

Beyond its local effects on ovarian function, AMH may also exert systemic influences on the metabolic complications associated with PCOS [11-13]. Emerging evidence indicates that AMH modulates insulin sensitivity, inflammatory signaling, lipid metabolism, and other metabolic processes [11-13,15]. As the primary focus of this review is on the regulatory mechanisms

governing AMH expression in the pathogenesis of PCOS and its potential as a therapeutic target, we will not address the role of AMH in these other biological processes.

REGULATORY MECHANISMS UNDERLYING AMH OVEREXPRESSION IN PCOS

Elevated AMH expression is a defining characteristic of PCOS, and extensive research has explored its underlying regulatory mechanisms at multiple levels, including transcriptional, post-transcriptional, post-translational level, and crosstalk with other signaling pathways.

AMH regulation at the transcriptional level

The transcriptional regulation of *AMH* is orchestrated by a complex interplay of transcription factors and signaling pathways. These include GATA4 (GATA-binding factor 4) [16,17], SF1 (Steroidogenic factor 1) [18], FOXL2 (Forkhead box L2) [19], and WT1 (Wilms Tumor 1) [20], which collectively ensure the precise control of *AMH* expression in granulosa cells (Figure 2A). In addition to the transcription factors identified, our analysis revealed that the AMH promoter region contains binding sites for NF-κB (Nuclear factor-kappa B), TCF4 (Transcription factor 4), and Runx2 (Runt-related transcription factor 2) (Figure 2A). However, no studies to date have reported evidence supporting their roles in the regulation of *AMH* gene expression.

The role of GATA4 in AMH gene expression

GATA4 is a transcription factor belonging to the GATA family, characterized by two zinc finger domains crucial for its function [21]. The C-terminal zinc finger is responsible for recognizing and binding to specific DNA sequences, while the N-terminal zinc finger stabilizes this interaction and facilitates protein-protein interactions with cofactors [21]. These structural features enable GATA4 to regulate the expression of key genes involved in gonadal development, sex determination, and steroidogenesis [16,17].

During gonadal development in mice and humans, GATA4 is prominently expressed in the somatic cells of the developing gonads, playing a pivotal role in the regulation of sex-determining genes such as SRY (Sex determining region Y) and SOX9 (SRY-box transcription factor 9), as well as genes involved in hormone production, including AMH, STAR (Steroidogenic acute regulatory protein), and CYP19A1 [22]. GATA4 cooperatively interacts with transcriptional cofactors like SF1 and FOG2 (Friend of GATA protein 2) to ensure precise regulation of these

genes [22]. In particular, GATA4 is essential for activating *AMH* during male embryonic development [23].

Experimental studies using CRISPR/Cas9-mediated inactivation of the GATA-binding motif in the *AMH* promoter provide direct evidence of GATA4's role in regulating *AMH* expression [16]. In male fetal and neonatal testes, loss of GATA binding significantly reduced *AMH* mRNA and protein levels, although basal transcription was not entirely abolished [16]. This reduction impaired the expected upregulation of AMH during critical developmental windows. Despite markedly lower AMH levels, these reductions were sufficient to allow normal male sexual differentiation, indicating that GATA4 works alongside other transcription factors to ensure adequate *AMH* expression during testis development [16,17]. In contrast, in the adult ovary, GATA4 binding was found to be non-essential for maintaining AMH expression, suggesting that its regulatory role is tissue- and stage-specific [16,17].

Additionally, studies in ovarian granulosa cells highlight GATA4's critical role in AMH transcription [24]. By binding to conserved promoter sequences, GATA4 enhances *AMH* gene expression. It operates synergistically with other factors such as FOXL2 and SF1, forming a complex transcriptional regulatory network [24]. These interactions ensure robust control of *AMH* expression, which is modulated further by signaling pathways like those mediated by gonadotropins [24]. This dynamic regulation reflects the hormonal and developmental contexts in which GATA4 functions, emphasizing its importance in both testicular and ovarian physiology [24].

cAMP and SF1-associated transcriptional networks

The classical cyclic AMP (cAMP)-protein kinase A (PKA) signaling is known to increase *AMH* expression, studies have shown that the *AMH* promoter lacks a classical cAMP response element (CRE), suggesting the involvement of alternative pathways and transcription factors [25]. The *AMH* promoter contains binding sites for SOX9 (SRY-box transcription factor 9), SF1, GATA4, and AP1 (Activating protein 1), all of which are implicated in cAMP-responsive gene regulation [25]. Experimental studies using Sertoli cells have demonstrated that these factors mediate cAMP-induced *AMH* transcription [26,27]. Beyond the classical PKA pathway, additional cAMP-regulated cascades, including the cAMP-GEF-PI3K-Akt (Guanine nucleotide exchange factor-Phosphatidylinositol 3-kinase-Protein kinase B) pathway and MAPK (Mitogen-activated protein kinase 1) signaling, further enhance *AMH* promoter activity [28].

Among the transcription factors, SOX9 and SF1 play pivotal roles in *AMH* regulation [29]. SF1 binds to a key element in the *AMH* promoter and cooperates with SOX9, a critical regulator of Sertoli cell differentiation, to amplify *AMH* transcription [29]. Protein-binding studies reveal that SOX9 and SF1 form a functional complex through interactions between their DNA-binding and C-terminal regions, respectively [29]. This combinatorial mechanism ensures cell- and stage-specific AMH expression during embryogenesis, highlighting the intricate transcriptional and signaling network required for proper male sexual differentiation [29].

FBXL12's roles in the regulation of AMH expression

FOXL2 is a transcription factor crucial for ovarian development and function. It directly binds to the *AMH* promoter and interacts with other transcription factors, such as SF1, to regulate *AMH* expression [30]. This interaction forms a transcriptional complex that enhances *AMH* promoter activity, ensuring proper ovarian function. FOXL2 also protects granulosa cells from apoptosis, thereby supporting AMH production by maintaining cell viability [30]. Mutations in FOXL2, observed in conditions like blepharophimosis, ptosis, epicanthus inversus syndrome (BPES), and certain granulosa cell tumors, are associated with dysregulated AMH expression, highlighting FOXL2's significance in ovarian health [31].

Recent studies indicate that AMH can upregulate both the gene and protein expression of FOXL2 [32], suggesting a positive feedback loop that preserves the ovarian follicle reserve. In vivo experiments have shown that knocking down AMH accelerates follicle growth, an effect that can be mitigated by ectopic expression of FOXL2 [19]. This underscores the coordinated interplay between FOXL2 and AMH in controlling ovarian follicle development [19]. Functional FOXL2 is essential for SF1-induced AMH regulation, as it facilitates the association between SF1 and the *AMH* promoter [19]. Mutations in FOXL2 disrupt this interaction, leading to impaired *AMH* expression and subsequent ovarian dysfunction [19].

WT1's influence on AMH transcription

The WT1 transcription factor is essential for mammalian urogenital development, playing a central role in gonadal differentiation and Müllerian duct regression [33]. Mutations in the WT1 gene are associated with several disorders, including Wilms' tumor, a pediatric kidney cancer, and syndromes such as Denys-Drash and Frasier [33]. In severe cases of Denys-Drash syndrome, individuals may experience pseudohermaphroditism or complete sex reversal. During sexual development, WT1 regulates key genes, including *AMHR2* (anti-Müllerian hormone receptor 2)

[33]. AMHR2 is critical for Müllerian duct regression in males, and mutations in this gene result in persistent Müllerian duct syndrome, a rare condition causing male pseudohermaphroditism [33]. WT1 and AMHR2 are coexpressed during urogenital development, with WT1 directly binding to the *AMHR2* promoter to regulate its transcription [33]. Changes in WT1 expression in experimental models lead to immediate alterations in *AMHR2* levels, revealing WT1 as a critical regulator of Müllerian duct regression [33].

In addition to its role in regulating AMHR2, WT1 also influences *AMH* expression. In fetal Sertoli cells, WT1 binds directly to the *AMH* promoter and interacts with transcription factors such as SF1 and GATA4 to regulate basal *AMH* transcription. This ensures sufficient *AMH* expression for Müllerian duct regression [34]. WT1's function, however, is not limited to male development. In females, WT1 is expressed in granulosa cells, where it modulates *AMH* levels within the ovarian microenvironment [35]. This suggests WT1's regulatory role is both context- and tissue-specific, influenced by the presence of co-regulatory proteins and the cellular environment. While its role in male sexual differentiation is well-established, further research is required to elucidate WT1's precise mechanisms in ovarian granulosa cells and its broader implications in ovarian physiology [34,35].

DNA methylation in the regulation of AMH

DNA methylation is a critical epigenetic mechanism regulating gene expression by adding methyl groups to cytosine residues within CpG dinucleotides, particularly in promoter regions [36]. Catalyzed by DNA methyltransferases (DNMTs), this modification alters chromatin structure, inhibiting the binding of transcription factors and RNA polymerase, ultimately silencing gene transcription [36].

We analyzed the GC content and CpG islands in the promoter regions (3,000 bp upstream of the transcription start site) of the human and mouse AMH genes. The analysis revealed that the GC content of the mouse AMH promoter is 60.9%, with no CpG islands identified. In contrast, the human AMH promoter has a GC content of 66.47% and contains a CpG island (Figure 2B). Several studies have highlighted the role of DNA methylation in regulating the expression of the *AMH* gene. For instance, research on multiple sclerosis (MS) patients demonstrated that increased methylation in the *AMH* gene promoter correlates with reduced gene expression, linking methylation to disease activity [37]. Similarly, studies on children born to women with PCOS revealed altered methylation patterns in genes associated with reproductive function, including

AMH, suggesting an impact on ovarian follicle development [38]. This study also reveals that ovarian granulosa cells in PCOS patients found that aberrant methylation of the *AMH* promoter disrupts gene expression, contributing to hormonal imbalances characteristic of the condition [38]. These findings collectively demonstrate that DNA methylation in the *AMH* promoter region is a pivotal regulatory mechanism influencing gene expression.

AMH regulation at the post-transcriptional level

Currently, two major mechanisms of post-transcriptional regulation, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play critical roles in regulating *AMH* expression.

miRNAs involved in the regulation of AMH expression

miRNAs are small, non-coding RNA molecules approximately 22 nucleotides in length that play a pivotal role in post-transcriptional gene regulation [39]. They function by binding to complementary sequences on target messenger RNAs (mRNAs), leading to mRNA degradation or inhibition of translation, thereby modulating gene expression [39].

Recent studies have identified specific miRNAs that directly target the *AMH* gene, thereby influencing its expression. For example, the miR-200 family, including miR-200a, miR-200b, miR-200c, miR-141, and miR-429, has been shown to regulate *AMH* expression in Japanese flounder (*Paralichthys olivaceus*), affecting gonadal development [40]. This finding suggests that these miRNAs may modulate *AMH* expression, thereby playing a role in reproductive processes. Similarly, miR-140-3p has been demonstrated to promote the proliferation of follicular granulosa cells and steroid hormone synthesis by directly targeting the *AMH* gene in chickens [41]. This interaction leads to downregulation of *AMH* expression, enhancing granulosa cell activity and increasing hormone production, which are critical for follicular development and ovulation [41]. Another example is miR-155, which has been identified as a potential marker of subfertility in men with chronic kidney disease. While a direct interaction between miR-155 and the *AMH* gene has not been established, the observed correlation suggests that miR-155 may indirectly influence *AMH* expression, impacting male fertility [42].

These findings underscore the intricate regulatory networks involving miRNAs that control *AMH* expression. By binding to specific sites on *AMH* mRNA, miRNAs such as the miR-200 family, miR-140-3p, and miR-155 can modulate its stability and translation efficiency, influencing reproductive development and function (Figure 3A). Understanding these mechanisms provides

valuable insights into the post-transcriptional regulation of AMH and its implications for reproductive health.

H19 in the regulation of AMH expression

Long non-coding RNAs (lncRNAs) are RNA molecules longer than 200 nucleotides that do not encode proteins but are essential regulators of gene expression [43]. At the post-transcriptional level, lncRNAs can interact with mRNAs to influence their splicing, stability, and translation efficiency [43]. Additionally, lncRNAs can act as molecular sponges for microRNAs, sequestering them and preventing their interaction with target mRNAs, thereby indirectly regulating gene expression [43]. One such lncRNA, H19, has been identified as a key player in reproductive biology, particularly in the regulation of ovarian function [44]. The ovarian reserve, comprising follicles and oocytes, declines with age, leading to reduced fertility. Women with diminished ovarian reserve (DOR) exhibit lower levels of circulating and ovarian H19, which are associated with reduced serum AMH levels [44]. Studies on H19 knockout ($H19^{KO}$) mice reveal phenotypes resembling those of AMH knockout (AMH^{KO}) mice, including accelerated follicular recruitment, subfertility, and reduced *AMH* mRNA and protein expression [45]. Notably, *AMH* mRNA contains a functional Let7 microRNA binding site, suggesting that H19 regulates *AMH* expression through the Let7 pathway. In $H19^{KO}$ mice, superovulation increases estradiol production and oocyte yield, indicating that H19 acts to limit the number of ovulating follicles [44,45]. Together, these findings underscore H19's critical role in modulating *AMH* and maintaining ovarian reserves, likely mediated by its interaction with Let7 (Figure 3B).

AMH expression regulation at the post-translational level

The AMH protein in both humans and mice contains two conserved functional domains: the AMH domain and the TGF-β family domain (Figure 4A) [46]. Moreover, three-dimensional structural predictions using AlphaFold demonstrate a high degree of similarity between the mouse and human AMH proteins (Figure 4B), underscoring the evolutionary conservation of their functional architecture. The post-translational mechanisms, such as proteolytic cleavage, glycosylation, dimerization, and interaction with the prodomain, also play important roles in the regulation of AMH activity. Each modification ensures that AMH is properly processed, stable, and active, allowing it to fulfill its crucial roles in reproductive development and function.

Proteolytic cleavage

AMH is initially synthesized as an inactive precursor, a 140 kDa homodimer consisting of a 72 kDa N-terminal pro-region and a 25 kDa C-terminal mature region [10]. To become biologically active, the precursor requires proteolytic cleavage at specific sites, primarily between arginine and serine residues at positions 427-428 [47]. This cleavage separates the pro-region from the mature domain, which is necessary for AMH to exert its biological functions [47]. The proteolytic process is mediated by specific proteases, including subtilisin-like proprotein convertases such as furin [48]. Mutations that disrupt these cleavage sites can result in reduced or absent AMH activity, impairing its role in Müllerian duct regression during male embryogenesis and follicular regulation in females [48]. Studies have demonstrated that the efficiency of this cleavage process directly affects the levels of active AMH available, emphasizing its critical role in AMH functionality [49,50]. For example, mice with mutations impairing cleavage exhibit phenotypes of AMH deficiency, such as retained Müllerian structures in males or altered ovarian folliculogenesis in females [49,50].

Glycosylation

AMH is a glycoprotein, and its glycosylation is essential for proper folding, stability, and secretion. Glycosylation occurs in the endoplasmic reticulum and Golgi apparatus during protein synthesis, where specific carbohydrate moieties are added to asparagine residues in the AMH molecule [51,52]. This modification improves the solubility of AMH, enhances its stability in the extracellular environment, and protects it from proteolytic degradation. Glycosylation also ensures efficient secretion of AMH from Sertoli cells in males and granulosa cells in females [51,52]. Furthermore, variations in glycosylation patterns can alter AMH's receptor-binding properties, affecting its ability to interact with AMHR2 and initiate downstream signaling [51,52]. Aberrant glycosylation has been associated with decreased AMH bioactivity, potentially contributing to reproductive disorders such as PCOS or DOR [51,52]. Understanding the specific glycosylation patterns of AMH could offer new insights into its regulation and functionality.

Dimerization

AMH functions as a disulfide-linked homodimer, a configuration critical for its structural stability and receptor-binding affinity [47,53]. The formation of this dimer occurs in the endoplasmic reticulum during protein synthesis, where disulfide bonds are established between the two identical monomers [47,53]. Dimerization ensures the proper spatial arrangement of AMH,

which is necessary for its interaction with the AMH receptor, AMHR2 [47,53]. The dimeric structure allows the hormone to bind AMHR2 with high specificity and initiate intracellular signaling cascades, such as the SMAD-dependent pathway, which regulates gene expression involved in reproductive processes [47,53]. Defects in dimerization can lead to misfolded or inactive AMH, resulting in reduced biological activity. Studies using recombinant AMH mutants with disrupted dimerization have shown diminished receptor binding and signaling, highlighting the importance of this structural modification in AMH function [47,53].

Interaction with Prodomain

Even after proteolytic cleavage, the N-terminal Prodomain remains non-covalently associated with the C-terminal mature domain of AMH [54,55]. This interaction is crucial for maintaining the structural stability and activity of AMH in the extracellular environment. The Prodomain acts as a chaperone, stabilizing the mature domain and protecting it from premature degradation or denaturation [54,55]. The association between the Prodomain and the mature domain also prevents AMH from interacting with AMHR2 until the Prodomain dissociates [54,55]. This ensures that AMH activity is spatially and temporally controlled, allowing precise regulation of its biological effects. Upon receptor engagement, the Prodomain dissociates, leaving the mature domain free to bind AMHR2 and activate downstream signaling pathways [54,55]. This regulatory mechanism ensures that AMH's activity is tightly controlled, preventing excessive or untimely activation that could disrupt reproductive processes.

Regulation of AMH by signaling pathways

AMH plays a pivotal role in reproductive signaling pathways. Through its interaction with AMHR2, AMH activates the SMAD-dependent signaling cascade, modulating gene expression critical for ovarian folliculogenesis and maintaining the ovarian reserve [46,55]. By inhibiting primordial follicle activation and reducing FSH sensitivity in developing follicles, AMH ensures controlled follicular growth. Dysregulation of AMH signaling, particularly its involvement in the SMAD pathway, is implicated in disorders such as PCOS, where elevated AMH levels disrupt follicular dynamics and contribute to hormonal imbalances [46,55].

AMH and SMAD-dependent signaling pathway

The SMAD-dependent signaling pathway is a critical mechanism through which members of the TGF-β superfamily, including AMH, regulate cellular processes such as growth, differentiation, and development [46,55]. Upon binding to AMHR2, AMH initiates the formation

of a receptor complex that recruits and phosphorylates receptor-regulated SMADs (R-SMADs), specifically SMAD1, SMAD5, and SMAD8 [46,55]. These phosphorylated R-SMADs form a complex with the common mediator SMAD4 and translocate into the nucleus. In the nucleus, the SMAD complex interacts with transcription factors and co-regulators to modulate the expression of target genes (Figure 5) [46,55]. These genes are involved in ovarian follicular development, including primordial follicle recruitment and granulosa cell differentiation, underscoring the role of SMAD signaling in reproductive health.

AMH plays a key role in folliculogenesis by modulating SMAD-dependent signaling to regulate the recruitment and growth of ovarian follicles [56]. Specifically, AMH inhibits the initial activation of primordial follicles, preserving the ovarian reserve and ensuring the availability of oocytes throughout reproductive life [57]. AMH also reduces FSH sensitivity in growing follicles, thus controlling the pace of follicular development. Dysregulation of AMH expression or SMAD signaling can lead to disruptions in ovarian function, contributing to the pathogenesis of reproductive disorders [57].

In the context of PCOS, elevated levels of AMH are a characteristic feature and play a significant role in disease development. Excessive AMH enhances SMAD1/5/8 activation, which amplifies the inhibitory effects on follicular recruitment and sensitivity to FSH [58]. This results in an accumulation of small antral follicles and the characteristic follicular arrest seen in PCOS. Furthermore, high AMH levels contribute to hyperandrogenism by influencing granulosa cell function, creating a feedback loop that exacerbates the hormonal imbalance in PCOS [58]. This dysregulated AMH-SMAD pathway not only disrupts normal follicular development but also alters endocrine signaling, worsening the reproductive and metabolic features of PCOS [58].

Interplay between AMH and FSH

The interaction between AMH and FSH is crucial in regulating ovarian folliculogenesis and maintaining reproductive health [59,60]. AMH inhibits the recruitment of primordial follicles into the growing follicle pool, thereby preserving the ovarian reserve and ensuring a steady supply of oocytes throughout a woman's reproductive lifespan [59,60]. FSH promotes the growth and maturation of follicles, acting as the primary driver for the selection of a dominant follicle during each menstrual cycle. The balance between these two hormones is critical for normal ovarian function [59,60].

AMH modulates FSH activity by reducing the sensitivity of granulosa cells to FSH in developing follicles [61]. This action prevents premature follicular recruitment and overactivation, ensuring that only follicles with sufficient FSH receptors progress to dominance [61]. Studies have shown that AMH decreases FSH-induced aromatase expression, which reduces estradiol production in granulosa cells. By controlling FSH sensitivity, AMH regulates the transition of follicles through key developmental stages and contributes to the fine-tuning of ovarian function [61].

In PCOS, the interplay between AMH and FSH is significantly altered. Women with PCOS typically exhibit elevated AMH levels, which exacerbate FSH insensitivity in granulosa cells, leading to follicular arrest where small antral follicles accumulate but fail to progress to the dominant stage, resulting in anovulation [58,59]. Additionally, elevated AMH levels in PCOS are thought to suppress FSH-mediated granulosa cell differentiation, contributing to the hormonal imbalance characteristic of the disorder [58,59].

Androgens and their role in AMH regulation

Androgens, such as testosterone and dihydrotestosterone (DHT), play a significant role in the regulation of AMH expression within the ovary [59,62]. Granulosa cells, which produce AMH, express androgen receptors (AR), enabling them to respond directly to androgenic signals. This interaction is crucial for normal folliculogenesis and ovarian function. Studies have shown that androgens can modulate AMH levels in granulosa cells [59,62]. For instance, exposure to DHT has been associated with increased AMH production, suggesting a stimulatory effect of androgens on AMH expression [63]. This upregulation is mediated through the AR pathway, highlighting the direct influence of androgens on granulosa cell function [63]. In the context of PCOS, a condition characterized by hyperandrogenism, elevated androgen levels contribute to increased AMH expression [64]. This elevation in AMH is linked to disrupted follicular development and anovulation, key features of PCOS. The heightened AMH levels may inhibit FSH sensitivity, leading to impaired follicle maturation and ovulatory dysfunction [64].

THERAPEUTIC POTENTIALS TARGETING AMH IN PCOS

Given the critical role of AMH in the pathophysiology of PCOS, particularly in disrupting folliculogenesis and contributing to anovulation, targeting AMH may represent a promising

therapeutic avenue. Multiple strategies are currently being developed to modulate AMH signaling or reduce its overexpression, aiming to restore normal ovarian function and improve fertility outcomes [65].

AMH receptor antagonists

Developing antagonists that block AMH binding to AMHR2 is a direct approach to mitigating the excessive AMH signaling. AMHR2 antagonists are gaining attention as promising therapeutic agents across multiple fields, particularly in oncology. By inhibiting the AMHR2 signaling pathway, these antagonists can impede tumor growth in cancers expressing this receptor [66]. For example, murlentamab, a humanized monoclonal antibody developed by LFB Biotechnologies, targets AMHR2 and is being investigated for its efficacy against various tumors [66]. Four AMHR2 agonists, including SP600125, CYC-116, gandotinib, and ruxolitinib, can repress folliculogenesis in mice and rats [67]. Gandotinib has undergone evaluation in a Phase II clinical study (NCT01594723) targeting various myeloproliferative disorders, such as myelofibrosis, polycythemia vera, and essential thrombocythemia [67,68]. Ruxolitinib has received regulatory approval for treating intermediate to high-risk myelofibrosis, polycythemia vera in patients who are unresponsive or intolerant to hydroxyurea, and graft-versus-host disease [67,69]. GM102 is a humanized, glyco-engineered monoclonal antibody that targets AMHRII [70]. After comprehensive pharmacological characterization and toxicity assessments conducted in cynomolgus monkeys, a Phase I clinical trial involving GM102 was launched in patients diagnosed with gynecologic malignancies (NCT02978755) [70]. In the context of PCOS, AMHR2 antagonists have been proposed as a novel therapeutic approach to restore normal folliculogenesis and ovulation by counteracting the effects of excessive AMH. While this concept holds promise, current research is largely theoretical, and clinical trials are needed to evaluate the efficacy and safety of AMHR2 antagonists in managing PCOS [71]. Moreover, a monoclonal antibody targeting AMH, referred to as B10, has been shown to reduce cell viability and induce apoptosis in four ovarian cancer cell lines, as well as in ascitic cells derived from ovarian cancer patients [72], suggesting its potential as a therapeutic agent for PCOS.

Gonadotropin-releasing hormone (GnRH) antagonists

GnRH antagonists, such as cetrorelix and ganirelix (Figure 6), indirectly reduce AMH levels by suppressing gonadotropin secretion and lowering ovarian androgen production [73]. Androgens are known stimulators of AMH production in granulosa cells, creating a feedback loop

that exacerbates the condition in PCOS [4]. By disrupting this loop, GnRH antagonists may lower serum AMH levels, thus enhancing FSH efficacy in follicular recruitment and maturation [73]. Clinical trials have indicated that GnRH antagonists are effective in restoring ovulation in women with PCOS, making them a valuable adjunct to AMH-targeted therapy [74].

Aromatase inhibitors

Aromatase inhibitors, such as letrozole (Figure 6), are widely used in ovulation induction protocols for PCOS and indirectly counteract the effects of elevated AMH [75]. By inhibiting the conversion of androgens to estrogens, these inhibitors reduce estrogen-mediated negative feedback on the hypothalamic-pituitary axis, leading to increased FSH secretion [75]. Higher FSH levels can override the inhibitory effects of AMH on follicular recruitment, promoting the growth and maturation of dominant follicles. Letrozole has shown superior efficacy compared to clomiphene citrate in inducing ovulation in PCOS patients, suggesting its potential role in overcoming AMH-mediated follicular arrest [75].

Androgen-targeting therapies

Since androgens can stimulate AMH production in granulosa cells, targeting androgen levels may indirectly reduce AMH overexpression. Anti-androgens, such as spironolactone and flutamide (Figure 6), or androgen-lowering agents like oral contraceptives, can be used to modulate this axis [76]. Lower androgen levels not only reduce AMH production but also improve follicular dynamics, offering a two-fold benefit in managing PCOS [76].

FUTURE DIRECTION

AMH is a critical regulator of ovarian function and plays a central role in the pathophysiology of PCOS. Elevated AMH levels are a hallmark of PCOS and contribute to the disrupted follicular dynamics, anovulation, and hormonal imbalances characteristic of the condition [8,10]. By inhibiting FSH sensitivity and primordial follicle activation, AMH perpetuates the follicular arrest observed in PCOS. Moreover, AMH interacts with androgen signaling, exacerbating hyperandrogenism and creating a feedback loop that further impairs ovarian function [8,10].

Despite significant advances, many aspects of AMH regulation and its role in PCOS remain insufficiently understood. AMH expression is controlled by transcriptional, post-transcriptional, and post-translational mechanisms. At the transcriptional level, key transcription factors such as

GATA4, FOXL2, SF1, and WT1 have been identified as regulators of AMH gene expression [16,17,18,19,20]. However, the precise mechanisms by which these factors interact with various co-regulators and chromatin-modifying complexes to fine-tune *AMH* transcription remain largely elusive. Deciphering these complex interactions is crucial, as they may reveal novel regulatory nodes that contribute to the aberrant AMH levels observed in PCOS. Non-coding RNAs, such as miRNAs and lncRNAs, play essential roles in post-transcriptional regulation, yet the mechanisms driving their dysregulation in PCOS are unclear [40,41,42]. Furthermore, post-translational modifications are critical for the proper maturation and function of AMH. Processes such as proteolytic cleavage, glycosylation, and other covalent modifications not only activate AMH but also influence its stability, bioactivity, and interactions with its receptor, AMHR2. The variability of these modifications and their subsequent impact on AMH activity in the context of PCOS remain underexplored. Understanding these mechanisms is essential for decoding AMH's contributions to PCOS pathophysiology.

The SMAD-dependent signaling pathway activated by AMH has been extensively studied for its role in follicular development [51]. Elevated AMH levels in PCOS enhance SMAD1/5/8 signaling, which suppresses follicular recruitment and growth. This pathway, along with its interplay with FSH and androgen signaling, disrupts the hormonal balance and follicular maturation [51]. However, the full spectrum of genes and proteins regulated by AMH in these pathways has yet to be fully characterized. Additionally, AMH's potential roles beyond the ovary, including systemic metabolic effects, require further investigation.

Given the promising preclinical findings, targeting AMH may offer an effective strategy for managing PCOS. Various approaches, such as developing AMHR2 antagonists, GnRH antagonists, and aromatase inhibitors, have been proposed to modulate AMH activity, restore FSH sensitivity, and promote normal folliculogenesis [69,70,71,72]. Moreover, combination therapies targeting both AMH and androgen signaling could potentially yield synergistic improvements in reproductive and metabolic outcomes. However, the direct therapeutic targeting of AMH remains a nascent concept or is still in the preclinical stage. To fully establish AMH as a viable target in PCOS treatment, further research is needed to develop more specific AMH inhibitors and to conduct comprehensive clinical trials that rigorously assess their efficacy and safety. In addition, PCOS presents with considerable phenotypic variability, encompassing differences in hyperandrogenism, ovulatory function, and polycystic ovarian morphology [1-4]. These diverse

presentations are shaped by genetic, hormonal, and metabolic factors, leading to variable responses to treatment. AMH levels are often disproportionately elevated in specific PCOS phenotypes, particularly in those with severe anovulation and polycystic ovarian features [1-4]. This suggests that AMH-targeted therapies may not be universally applicable, but rather most effective in selected subgroups. A personalized medicine approach—stratifying patients based on AMH levels, ovarian function, and clinical phenotype—may enhance therapeutic outcomes. For example, individuals with high AMH expression and anovulatory cycles may derive greater benefit from AMH or AMHR2 blockade. Future studies should aim to define these subpopulations more precisely and evaluate targeted interventions within well-characterized phenotype clusters.

Future research should prioritize the upstream and downstream signaling networks of AMH. Investigating how transcriptional regulators and epigenetic modifiers control AMH expression, as well as profiling miRNAs and lncRNAs in PCOS, could uncover novel therapeutic targets. miRNAs and lncRNAs have demonstrated significant potential in disease diagnosis and therapy [77]. Although members of the miR-200 family, miR-140-3p, miR-155, and the lncRNA H19 have been shown to regulate AMH expression, their specific roles in the diagnosis and treatment of PCOS have not yet been established [40-44]. Moreover, exploring AMH's systemic roles and its interactions with metabolic pathways may reveal broader implications for PCOS management. Stratifying patients based on AMH levels and hormonal profiles could enable personalized treatment strategies, improving outcomes for individuals with diverse PCOS phenotypes.

CONCLUSION

In conclusion, AMH is a pivotal factor in PCOS pathogenesis, serving as both a biomarker and a regulator of ovarian dysfunction. While significant progress has been made, many gaps remain in our understanding of its regulatory mechanisms and signaling pathways. Addressing these knowledge gaps will enhance our understanding of PCOS and support the development of targeted therapies to improve reproductive and metabolic health. Through continued research, the potential of AMH-targeted interventions can be fully realized, offering hope for more effective and individualized treatments for PCOS.

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FIGURES WITH LEGENDS

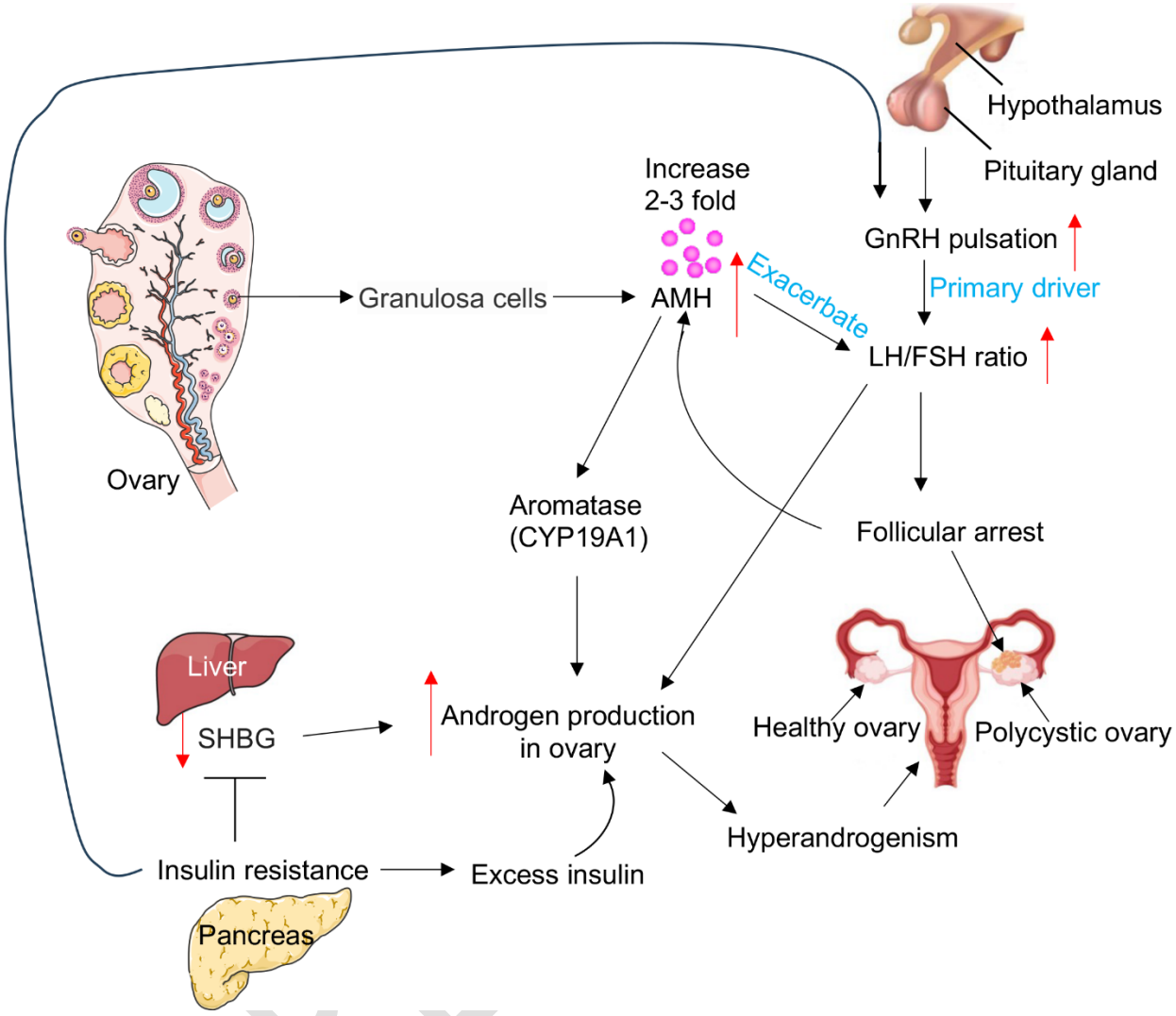

Figure 1. Involvement of AMH, GnRH, LH/FSH, androgen, and insulin resistance in PCOS pathogenesis

The pulsatile release of GnRH from the hypothalamus is often disturbed in PCOS, leading to an increased LH/FSH ratio. Elevated AMH further exacerbates this imbalance. Abnormal secretion of FSH and LH leads to follicular arrest, which in turn contributes to elevated AMH levels. Moreover, high AMH inhibits aromatase (CYP19A1) activity, leading to increased androgen production. The altered LH and FSH ratios impair ovulation: elevated LH promotes

hyperandrogenemia by stimulating androgen secretion from follicular theca cells, while reduced FSH levels contribute to anovulation. Additionally, insulin acts synergistically with LH to enhance androgen synthesis and decreases SHBG levels, thereby increasing the bioavailability of circulating androgens. Insulin resistance can also exacerbate PCOS by altering GnRH pulse frequency, affecting ovarian function, and increasing androgen levels.

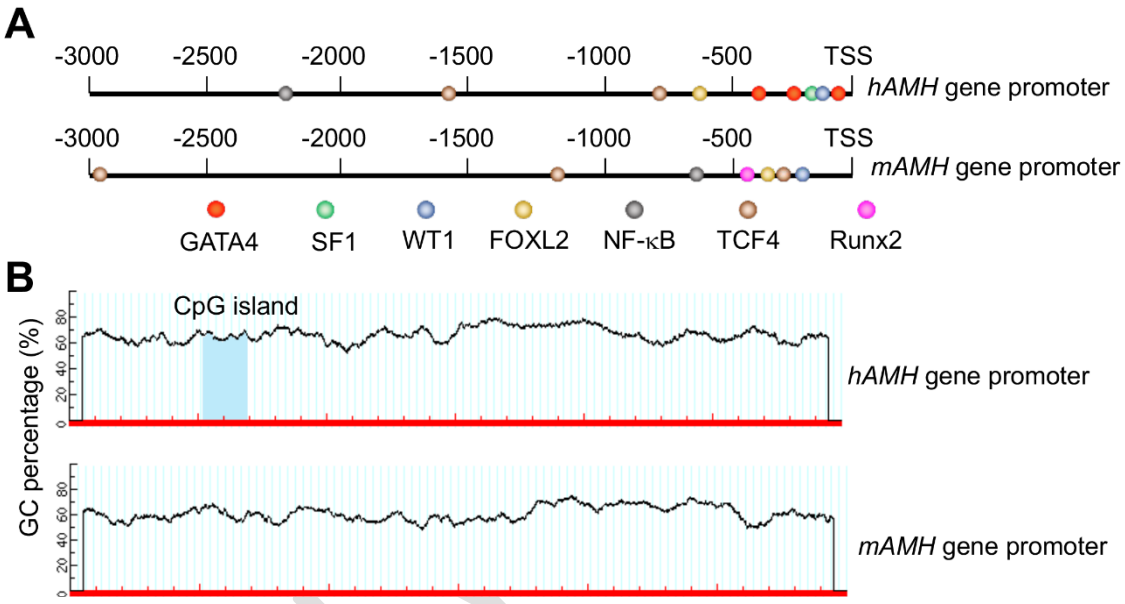

Figure 2. Transcription factors and DNA methylation are involved in the transcriptional regulation of *AMH* gene

(A) Transcription factors binding to the human *AMH* (*hAMH*) gene promoter (3000 bp upstream of the transcription start site, TSS) and the mouse *AMH* (*mAMH*) gene promoter. **(B)** GC content percentages in the *hAMH* and *mAMH* gene promoters, with the CpG island in the *hAMH* promoter highlighted.

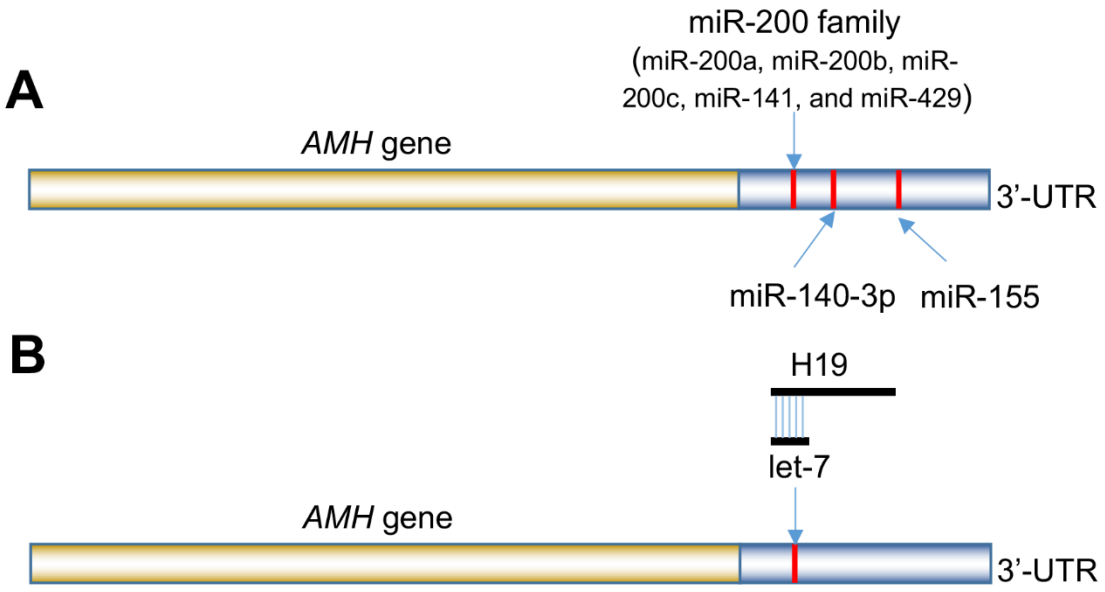

Figure 3. miRNAs and the HT19 lncRNA are involved in the regulation of the *AMH* gene at the post-transcriptional level

(A) The miR-200 family, miR-140-3p, and miR-155 target the 3'-UTR of *AMH* genes across various species. **(B)** The H19 lncRNA indirectly regulates *AMH* gene expression by acting as a molecular "sponge" for let-7, which targets the 3'-UTR of *AMH*.

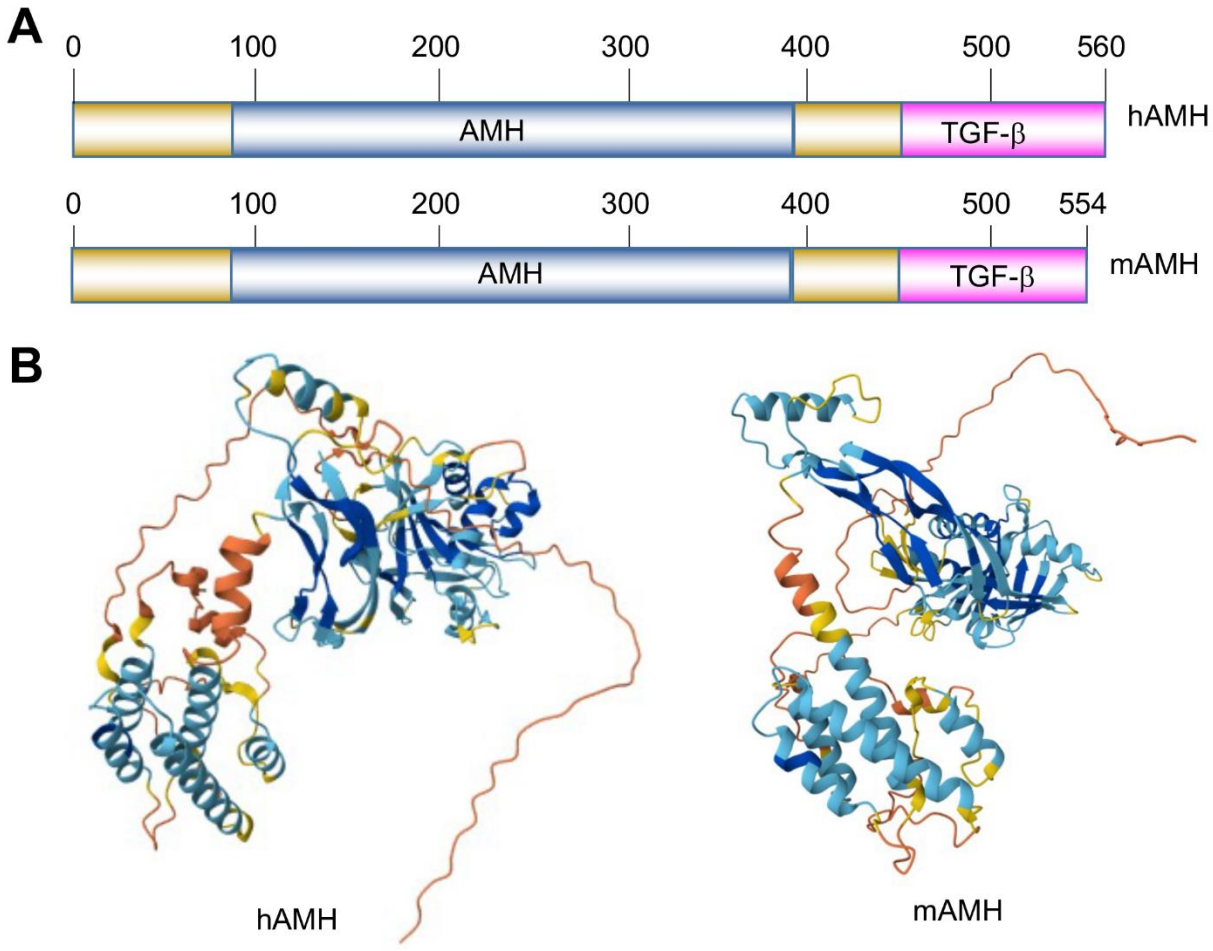

Figure 4. Functional domains and structures of AMH proteins

(A) Conserved domains of hAMH and mAMH proteins, highlighting two main functional domains: the AMH-specific domain and the conserved TGF-β family domain. **(B)** Predicted three-dimensional structures of hAMH and mAMH generated by AlphaFold.

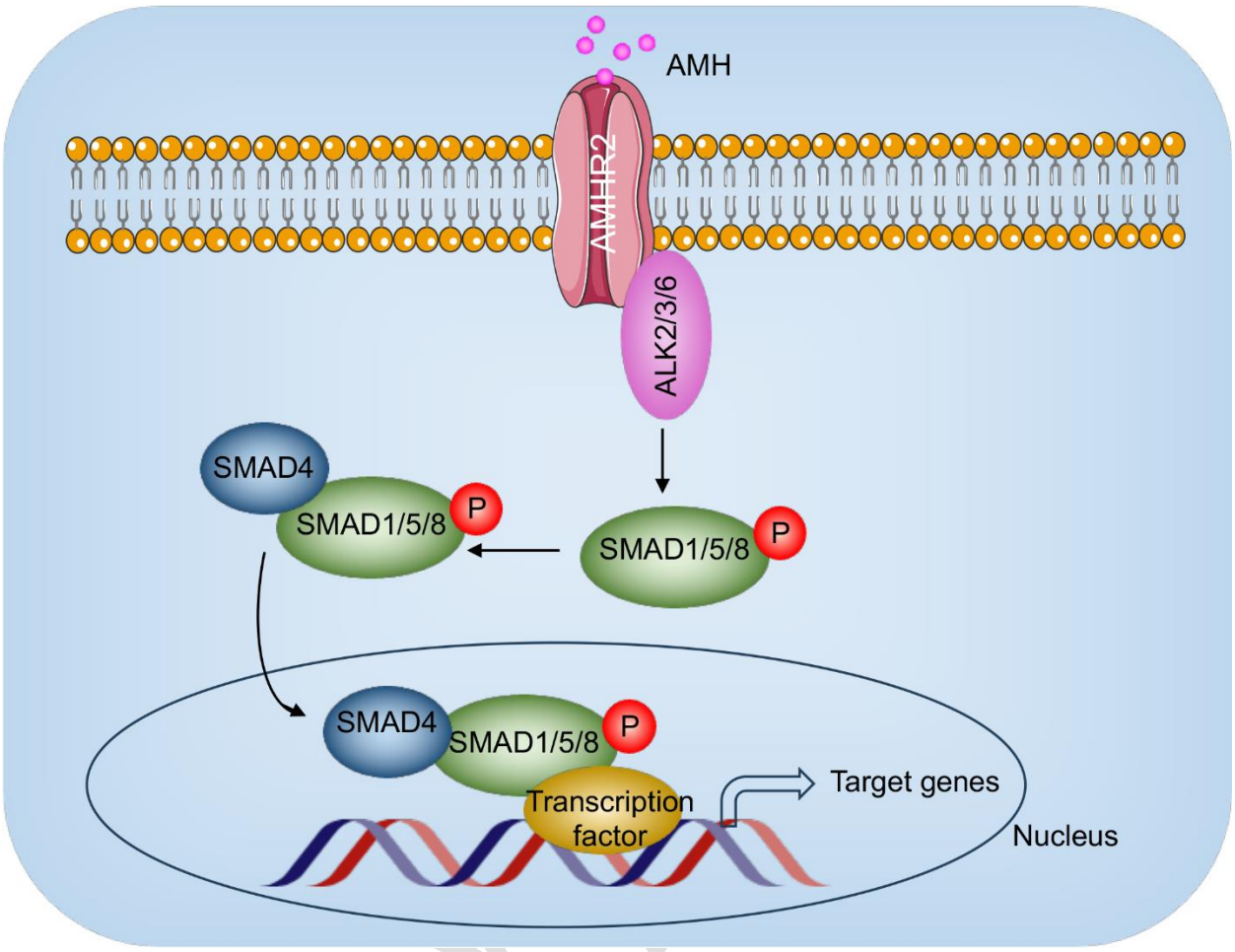

Figure 5. AMH/AMHR2 signaling pathway in the pathogenesis of PCOS

AMH binds to its receptor AMHR2, which interacts with ALK2/3/6 to activate SMAD proteins 1/5/8. Phosphorylated SMAD1/5/8 translocate to the nucleus alongside SMAD4, where they cooperate with transcription factors (TFs) to regulate the expression of AMH target genes.

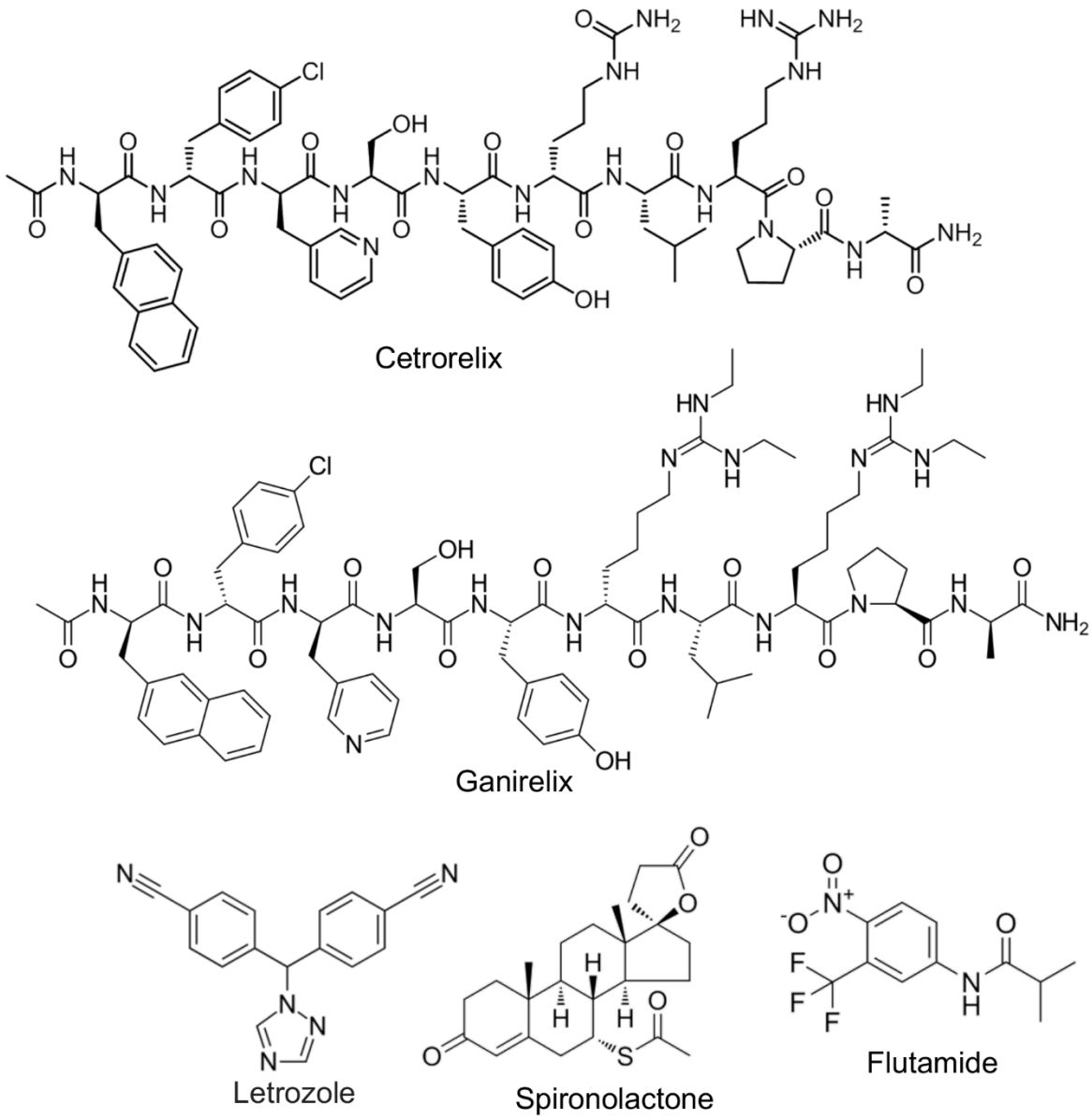

Figure 6. Chemical structures of inhibitors of GnRH and aromatase and anti-androgens

Chemical structures of cetrorelix and ganirelix (GnRH antagonists), letrozole (Aromatase inhibitors), and spironolactone and flutamide (anti-androgens) are indicated.