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AMH in PCOS: Controlling the ovary, placenta, or brain?

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Abstract

Polycystic ovary syndrome (PCOS) is a very heterogeneous disease of which the exact pathophysiological mechanisms remain unknown. In PCOS, serum anti-Müllerian hormone (AMH) levels are significantly increased. AMH is a member of the transforming growth factor β family and is expressed by growing follicles in the ovaries. In PCOS, the transcriptional regulation of AMH and AMHR2 is altered, increasing and prolonging its temporal expression pattern. Moreover, the recently discovered extragonadal effects of AMH suggest that there might be a crosstalk between the ovary–placenta–brain. This review summarizes the recent findings concerning AMH and its role in the etiology of PCOS.

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Polycystic ovary syndrome, Anti-Müllerian hormone, Ovary, Placenta, Hypothalamus.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in females of reproductive age with a prevalence of 10–15% worldwide [1,2]. It is diagnosed by the presence of at least two of the following three criteria: (1) oligo/amenorrhea, (2) hyperandrogenism, and (3) polycystic ovary morphology (PCOM) [3]. Consequently, four different phenotypes can be recognized, making PCOS a very heterogeneous disease. Genetic predisposition and environmental exposure are thought to play a major role in the pathophysiology of PCOS [4,5]. Yet, the exact pathophysiological mechanisms remain to be unraveled.

In the past years, several studies have implicated anti-Müllerian hormone (AMH) in the pathophysiology of PCOS. AMH is a member of the transforming growth factor β family [6] and is expressed by preantral and small antral follicles in the ovaries [7]. AMH levels strongly correlate with the number of antral follicles, and in PCOS, this is reflected by significantly increased serum AMH levels compared to normoovulatory women [8–10]. It has therefore been suggested that AMH levels may replace the diagnostic criteria (PCOM) in the diagnosis of PCOS. However, a recent paper by Teede et al. [11] reviewing this suggestion, concluded that there is currently a lack of well-defined PCOS and control populations and a gap in assay standardization preventing establishment of clear cut off values. Although it is currently premature to use serum AMH levels as a diagnostic criterion for PCOS, accumulating data implicate a causal role for ovarian AMH function in all three PCOS diagnostic criteria. Moreover, the recently discovered extragonadal effects of AMH suggest that the contribution of AMH to the pathophysiology of PCOS might be even more elaborate than once thought. In this review, we will discuss these recent insights.

AMH and its role in ovarian function in PCOS

The ovary is the most well-studied tissue regarding *AMH* expression and function. The ovarian *AMH* expression is detected in granulosa cells of activated primordial follicles and is highest in preantral and small antral follicles. *AMH* expression is absent in follicular stages following follicle-stimulating hormone (FSH)–dependent selection, although some expression remains in cumulus cells of preovulatory follicles [12]. Expression of the AMH-specific type II receptor (*AMHR2*) coincides with *AMH* expression, albeit that *AMHR2* expression is also detected in theca cells [12]. Thus, AMH may affect both granulosa and theca cell function. Studies using AMH knockout (AMHKO) mouse models revealed that AMH inhibits the primordial follicle recruitment and selection of follicles for dominance, two major steps in folliculogenesis. In the absence of AMH, more primordial follicles are recruited and FSH sensitivity was increased [12,13]. Furthermore, studies in the AMHKO mice suggest that AMH may act as an intraovarian inhibitor of follicular atresia [14]. The effect of AMH on selection of follicles for dominance seems consistent across species. However, species differences

may exist with regard to preantral follicular growth. In nonhuman primates, Xu et al. [15] showed that *in vitro* treatment of macaque secondary follicles with AMH during the first 3 weeks of culture advanced follicle antrum formation with a week, whereas treatment with an AMH neutralizing antibody delayed this. Consistent with the increased growth, estradiol (E2) production of these secondary follicles was also increased [16]. In contrast, in mice, AMH mostly acts as a survival factor for small preantral follicles [14]. Importantly, blocking AMH action *in vivo* through intraovarian infusion of an AMH neutralizing antibody for 4 days resulted in the growth of multiple antral follicles in most animals [15]. In both the *in vitro* and *in vivo* experiments, blocking AMH action in antral follicles increased E2 levels [15]. These findings suggest that AMH may have a follicle stage-dependent effect on E2 production. Several studies have shown that AMH reduces FSH-induced aromatase (*Cyp19a1*) expression and E2 production in human antral follicles, and that in follicular fluid, an inverse relationship between AMH and E2 concentrations exists [17–19]. Hence, it is suggested that AMH in humans acts as a gatekeeper of follicle growth by preventing premature selection and E2 production of small antral follicles. Species differences in FSH-dependency of preantral follicles may explain the observed differences in AMH effects on follicular growth. Although cultured macaque preantral follicles require FSH for survival [15,20], cultured mouse follicles are FSH-independent at this stage [21]. These species differences should be taken into consideration when translating results to human, particularly when implicating AMH in the pathophysiology of PCOS.

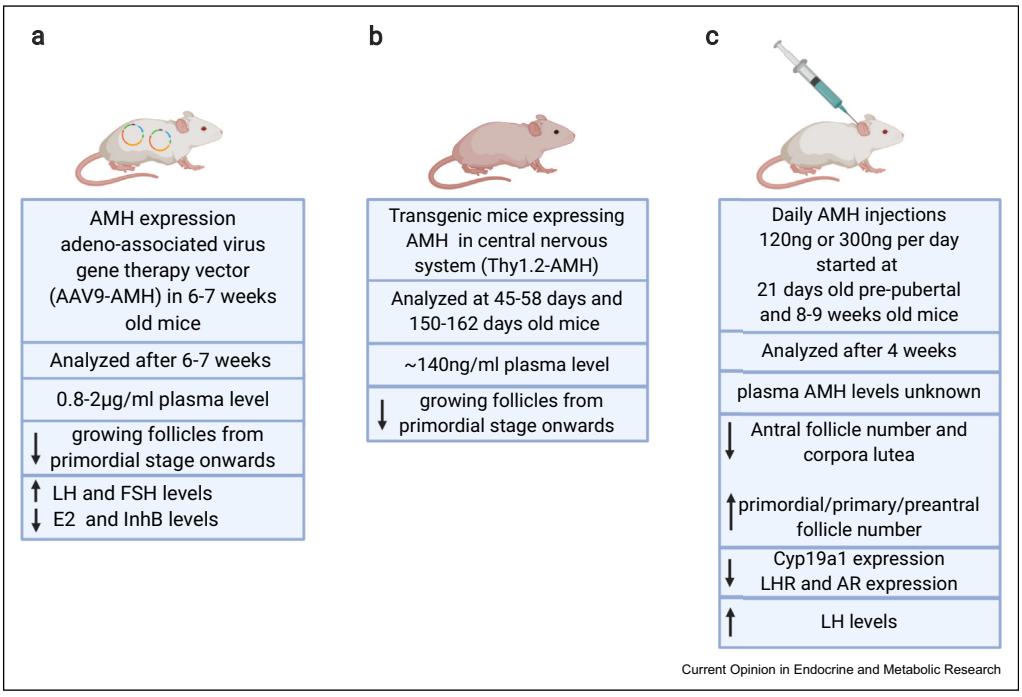
In PCOS, where AMH levels are increased, the AMH effects on follicular growth/survival and FSH sensitivity may be exacerbated leading to increased follicle numbers combined with follicular arrest. Several studies showed that the increased serum AMH level in PCOS is not only explained by the increased follicle number but also by increased production per follicle compared to normal ovaries [22,23]. In both follicular fluid and isolated granulosa cells obtained after controlled ovarian hyperstimulation for *in vitro* fertilization, AMH levels as well as *AMH* and *AMHR2* expression were increased in samples from PCOS women compared to control women [24,25]. Two additional studies in which the switch from a gonadotropin-independent to gonadotropin-dependent follicular stage was taken into account confirmed these findings [26,27]. *AMH* expression and follicular fluid AMH levels decline in gonadotropin-dependent follicles in normoovulatory women, whereas this did not occur in PCOS patients [26,27]. Likewise, the coincided increase in E2 levels was absent in PCOS patients [27]. This altered *AMH* expression may be the result of intrinsic granulosa cell dysregulation in PCOS. Both theca and granulosa cells of small antral follicles express higher levels of the luteinizing hormone (LH) receptor in PCOS women compared to normoovulatory women [28]. Combined with the elevated LH levels in PCOS, this leads to hyperstimulation of the theca cells and premature luteinization of granulosa cells. Interestingly, LH stimulation increased *AMH* expression in granulosa cells of PCOS women but not in normoovulatory women [24,29,30]. Treatment with 5-α-dihydrotestosterone yielded similar results [30]. Furthermore, although estrogens suppress *AMH* expression, mediated via estrogen receptor β, in normoovulatory women, this suppression was not observed in granulosa cells of anovulatory PCOS women [30,31]. Combined, these results suggest a failure in the downregulation of *AMH* expression in gonadotropin-dependent follicular stages in PCOS, which may contribute to a failure in follicular growth.

Interestingly, there may be another side of the AMH-PCOS coin. Two recent studies from Gorsic et al. [46,47] describe several PCOS-specific heterozygous variants in the *AMH* and *AMHR2* genes. *In vitro* studies revealed that these variants significantly reduce AMH signaling activity, through dominant negative effects (*AMH* variants) and through splicing defects, reduced expression, or reduced signaling (*AMHR2* variants) [46,47]. Based on the role of AMH in testicular Leydig cells, the authors hypothesized that reduced AMH signaling might lead to increased theca cell testosterone (T) production because of loss of CYP17 inhibition. However, reduced AMH bioactivity may also lead to less inhibition of aromatase activity, thereby increasing the conversion of T into E2. Thus, it remains to be determined how reduced AMH action affects follicular growth and function.

Recently, studies have been performed in adult mice, which may provide insight into the contribution of elevated bioactive AMH in the PCOS pathophysiology (summarized in Figure 1). Kano et al. and Pankhurst et al. [32,33] both showed that treatment with supraphysiological levels of AMH resulted in a phenotype that resembles ovarian insufficiency rather than PCOS, because a severe reduction in the number of growing follicles from the primary follicle stage onwards was observed. In the model of Hayes et al., daily AMH treatment seemed to affect FSH sensitivity more strongly than primordial follicle recruitment. In this model, a significant reduction in antral follicle number and number of corpora lutea was observed, along with an increase in primordial, primary, and preantral follicle number. However, in contrast to what is observed in PCOS, LH receptor (*LHCGR*) and androgen receptor (*AR*) expression were decreased. Unfortunately, androgen levels were not reported for this model [34].

Overall, these studies suggest that exposure to strongly elevated AMH levels during adulthood do not or only partly induce PCOS characteristics, at least in a

Figure 1



Summary of mouse models exposed to elevated AMH levels. (a) Refers to the model of Kano et al. (2017). **(b)** Refers to the model of Pankhurst et al. (2018). **(c)** Refers to the model of Hayes et al. (2016). AMH levels in control fertile mice range between 28.34 ± 7.12 ng/ml measured with the DSL AMH ELISA (DSL-10-14400). The figure was created with BioRender.com. AMH, anti-Müllerian hormone; *AR*, androgen receptor; FSH, follicle-stimulating hormone; LH, luteinizing hormone; *LHR*, luteinizing hormone receptor; E2, estradiol; InhB, inhibin B.

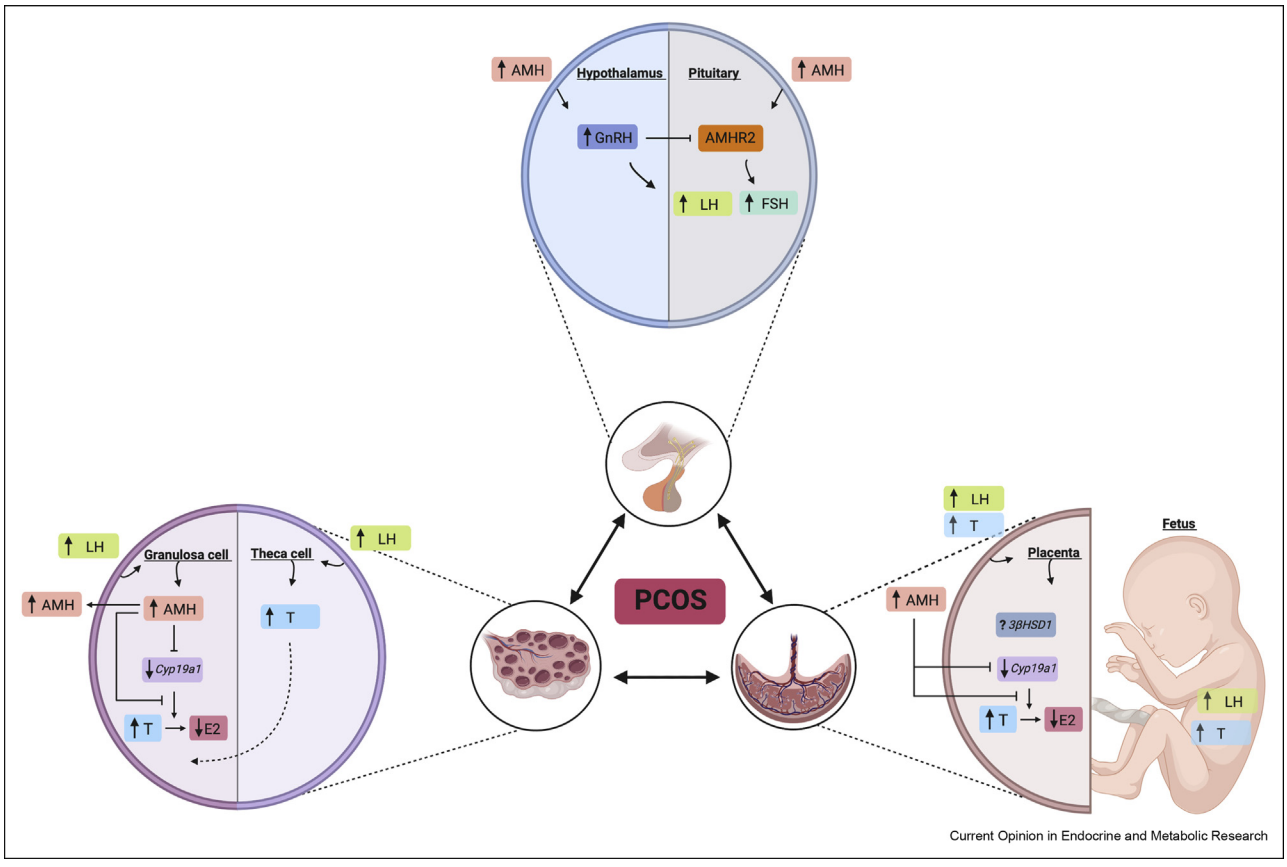
polyovulatory species. The very high AMH levels used in these studies seem to override the effects on FSH sensitivity as it already affects primordial follicle recruitment. Recent studies, highlighting extragonadal effects of AMH, strongly suggest that additional mechanisms should be taken into account when addressing a role of AMH in PCOS.

AMH and its role in placental function in PCOS

Recently, placental *AMHR2* expression has been reported in several species, including human [35–37]. Conflicting results exist regarding *AMH* expression itself, because expression was detected in human but not in bovine placenta [35,37]. Interestingly, although in pregnant normoovulatory women, AMH levels decline after the first trimester, in women with PCOS, AMH levels remain significantly higher during pregnancy, but only in lean PCOS women [36,38]. The presence of elevated maternal AMH levels throughout pregnancy and the existence of an AMH signaling pathway in the placenta raises the question whether AMH affects placental function in PCOS.

Tata et al. [36] showed that daily injection of AMH during E16.5–E18.5 of pregnancy significantly suppressed placental *Cyp19a1* expression. Placental aromatization is important to protect the fetus from virilization by fetal androgens and to prevent the accumulation of high androgen levels in the maternal circulation [39]. Thus, elevated AMH may induce a hyperandrogenic intrauterine environment, which reprograms the reproductive axis of female offspring. Indeed, increased T levels were measured in AMH-treated pregnant mice and masculinization of the brain was observed in the female offspring [36]. In agreement with prenatal androgenized PCOS models, *in utero* AMH-treated females displayed several reproductive PCOS-like phenotypes, including oligoanovulation, altered gonadotropin-releasing hormone (GnRH) pulsatility with elevated LH and T levels, whereas body weight remained normal. In line with the oligoanovulation, the number of large-antral follicles was reduced.

Figure 2



Potential mechanisms of the contribution of AMH in the pathophysiology of PCOS. AMH may intervene at all three levels in the ovary–placenta–brain crosstalk, thereby contributing to the etiology of PCOS. In the ovary, elevated AMH levels decrease *Cyp19a1* expression in granulosa cells, leading to increased T levels. Elevated LH levels increase *AMH* expression in granulosa cells and increase T production in the theca cells (dotted arrow: transfer of T to granulosa cells). In the hypothalamus, exposure to increased AMH levels results in an increase in GnRH/LH pulsatility and concentrations. In the pituitary, exposure to increased AMH levels results in increased FSH levels. Moreover, GnRH treatment may decrease *AMHR2* expression in the pituitary, favoring LH over FSH production. In the placenta, increased maternal AMH decreases *Cyp19a1* expression, preventing aromatization of elevated maternal T levels, which in turn results in a hyperandrogenic intrauterine environment and masculinization of the fetus. The figure was created with BioRender.com. *3βHSD1*, 3-beta-hydroxysteroid-dehydrogenase-1; AMH, anti-Müllerian hormone; AMHR2, AMH-specific type II receptor; E2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; T, testosterone.

However, whether gestational AMH treatment affected the number of smaller growing follicles and AMH levels itself was not reported. Importantly, the authors demonstrated that AMH did not cross the placental barrier, excluding a direct effect of AMH in the offspring [36].

Interestingly, in women with PCOS, also a lower placental *Cyp19a1* expression has observed, accompanied by increased placental 3-beta-hydroxysteroid-dehydrogenase-1 (*3βHSD1*) expression [40], which potentially could contribute to a hyperandrogenic intrauterine environment. However, in the model of Tata et al. [36], AMH treatment suppressed placental *3βHSD1* expression. It remains questionable whether the placenta itself is solely responsible for the hyperandrogenic intrauterine environment, because AMH treatment also resulted in elevated maternal LH levels, which in turn could increase maternal T levels in these mice. Indeed, combined treatment with a GnRH antagonist prevented AMH-induced suppression of *Cyp19a1* and *3βHSD1* expression in the placenta and the development of a PCOS-like phenotype in the female offspring. These results suggest that AMH may also have a central action.

AMH and its role in brain function in PCOS

In addition to ovarian dysfunction, PCOS is also characterized by neuroendocrine abnormalities, with increased GnRH pulse frequency favoring LH over FSH production. Recent studies suggest that AMH may also play a role in the neuroendocrine dysregulation in PCOS [36,41,42]. This hypothesis builds on the discovery of *AMHR2* expression in hypothalamic GnRH neurons in

both rodents and human [42]. These neurons are AMH responsive because AMH stimulated the excitability and release of GnRH in neuronal explants of rats [42]. Furthermore, AMH injection directly into the lateral ventricle of female mice induced a rapid increase in GnRH-mediated LH pulsatility and secretion [42]. More support for a central action of AMH is provided by additional studies from the same group [36,41]. In the previously mentioned prenatal AMH-treated mouse model, Tata et al. [36] observed increased LH concentration and pulsation via induced GnRH neuronal activity in the pregnant dams. Unfortunately, these studies did not report whether AMH-stimulated GnRH pulsatility also affected FSH levels. In PCOS patients, AMH levels are positively correlated with LH concentrations [10]. High LH levels are known to stimulate the release of ovarian androgen production by theca cells, and as discussed earlier, LH enhances *AMH* expression in granulosa cells of PCOS women. This suggests the existence of a positive feedback loop between AMH, GnRH, and LH in PCOS.

In addition to hypothalamic function, AMH may also regulate pituitary function given the presence of *AMHR2* expression, predominantly at prepubertal ages [43–45]. In the LβT2 pituitary cell line, AMH stimulated FSH secretion and *FSH*β expression, which was confirmed *in vivo* in immature female rats 18 hours post-AMH injection [44]. In contrast, in these studies, no effect was observed on LH levels. In addition, it was observed that pituitary *AMHR2* expression was downregulated on continuous GnRH agonist treatment in both mice and humans [44]. This may suggest that increased GnRH release desensitizes the pituitary for AMH, thereby contributing to LH over FSH production. However, effects on *AMHR2* expression may differ depending on the rate of GnRH pulsatile release [43].

Given the stimulatory central actions of AMH, particularly in the hypothalamus, it is remarkable that *AMH* and *AMHR2* heterozygous mutations leading to reduced signaling (as discussed above) have been identified in a subset of PCOS patients [46,47]. At the same time, *AMH* and *AMHR2* heterozygous loss-of-function mutations were identified in patients with congenital hypogonadotropic hypogonadism. Loss of AMHR2 signaling in mice impairs GnRH neuronal migration resulting in reduced LH levels at adult age [41]. The involvement of AMH mutation in both PCOS and congenital hypogonadotropic hypogonadism raises the question whether these mutations display a different penetrance and expressivity.

Conclusion

AMH research in the scope of PCOS remains a very intriguing topic. The recent studies highlighting extragonadal functions of AMH indicate that the contribution of AMH to the pathophysiology of PCOS is more complex than once thought. They suggest that AMH may intervene at multiple levels of the brain–ovary–placenta crosstalk (Figure 2). Perhaps all three tissues can contribute to the pathophysiology of PCOS by establishing a vicious circle that reinforces itself through an androgen–gonadotrophin feedback loop. However, it remains unclear whether ovarian *AMH* expression is a driving force or an accomplice in this crosstalk. As it has been shown now that AMH also has extragonadal functions, this requires the need for tissue-specific AMHR2 knockout models. Furthermore, the identification of genetic AMH and AMHR2 mutations suggests that AMH action not necessarily needs to be increased to contribute to the PCOS pathophysiology, implying that there may be different etiologies in PCOS. Further studies into the mechanisms by which AMH contributes to PCOS will help to understand this heterogeneous disease.

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* of special interest
** of outstanding interest

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