Anti-Müllerian hormone (AMH): Regulator and marker of ovarian function

Hormone anti-müllérienne (AMH) : régulateur et marqueur de la fonction ovarienne

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Résumé
Cette revue consacrée à l’hormone anti-müllérienne (AMH) a pour but de décrire ses fonctions de régulation et de marqueur de la fonction ovarienne. Des études chez la souris ont montré que l’AMH est l’un des facteurs de croissance intra-ovarien qui régule le recrutement du follicule primitif et la sensibilité des follicules en croissance à la FSH sur un mode inhibiteur. Les études d’association des variants communs des gènes de l’AMH et du récepteur de type 2 de l’AMH suggèrent que cette hormone pourrait avoir un rôle similaire dans l’ovaire humain. Quand il a été découvert que les niveaux sériques d’AMH étaient corrélés au nombre de follicules en croissance, l’intérêt clinique pour l’AMH s’est accru quant à son rôle en tant que marqueur des aspects quantitatifs de la réserve ovarienne et comme marqueur diagnostique du syndrome des ovaires polykystiques (OPK).

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Abstract
In this review, the role of anti-Müllerian hormone (AMH) as a regulator and marker of ovarian function is described. Studies in mice showed that AMH is one of the intra-ovarian growth factors regulating primordial follicle recruitment and follicle-stimulating hormone (FSH) sensitivity of growing follicles in an inhibitory manner. Association studies of common variants of the AMH and AMHRII gene suggest that AMH may have a similar role in the human ovary. When it was discovered that serum levels AMH are correlated with the number of growing follicles, AMH gained further clinical interest as a marker for the quantitative aspect of ovarian reserve and as a diagnostic marker for polycystic ovary syndrome (PCOS).

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Keywords: Anti-Müllerian hormone (AMH); AMHRII gene; Polycystic ovary syndrome (PCOS)

1. Introduction
Anti-Müllerian hormone (AMH), or Müllerian inhibiting substance (MIS), has long been known for its involvement in the sexual differentiation of the male embryo. AMH is secreted by the Sertoli cells of the fetal testis and induces regression of the Müllerian duct, the anlagen of the female reproductive tract [1,2]. Although initially not expressed in the ovary, ovarian AMH expression starts after birth in mice and from the 36th week of gestation onwards in the human [3,4]. In the human ovary, AMH is expressed in granulosa cells as soon as follicles are recruited from the primordial follicle pool. Expression is highest in follicles smaller than 4 mm (preantral and small antral follicles) and is nearly lost in follicles larger than 8 mm [5]. Also in rodents, maximum expression is reached during the preantral and small antral stage. AMH expression decreases once follicle-stimulating hormone (FSH)-dependent follicular growth has been initiated [3]. Thus, AMH is expressed by those growing follicles in between initial recruitment and cyclic selection. The similarity of expression pattern in human and mice suggests that AMH may have a comparable role in both species.
AMH is a member of the transforming growth factor β (TGFβ) superfamily, which includes among others TGFβ, activin and bone morphogenetic proteins (BMPs). TGFβ family members signal through a characteristic combination of type I and type II serine/threonine kinase receptors. Upon ligand binding, the type II receptor activates the type I receptor through phosphorylation which in turn phosphorylates and activates the downstream Smad proteins. Based upon the downstream signaling pathway, two main signaling pathways can be identified: the TGFβ/activin-like signaling pathway, signaling through Smad2 and 3; and the BMP-like signaling pathway, signaling through Smad1, 5 and 8 [6].

AMH, signaling through its specific type II receptor (AMH), was shown to activate a BMP-like pathway [7]. Furthermore, in studies focusing initially on Müllerian duct regression, the BMP type I receptors ALK2 and ALK3 were identified as AMH type I receptors. Antisense ALK2 treatment of urogenital ridge organ cultures resulted in inhibition of AMH-induced Müllerian duct regression [8]. Targeted disruption of ALK3 signaling in the Müllerian duct also abolished regression [9]. Interestingly, this phenotype could be rescued in the presence of increased AMH levels [10], suggesting the involvement of an additional type I receptor, such as ALK2. Finally, based on the AMH-induced interaction between AMHRII and ALK6, also ALK6 has been implicated as an AMH type I receptor [11]. In rats, the AMHRII has a similar expression pattern to AMH [12]. The three AMH/BMP type I receptors are also expressed in the ovary [13]. However, their contribution to ovarian AMH signaling, perhaps follicle-stage specific, remains to be investigated.

2. Anti-Müllerian hormone and primordial follicle recruitment

The formation of the finite primordial follicle pool occurs within a few days after birth in rodents [14]. From this pool, follicles are recruited to develop through primary and secondary stages to become preantral follicles. Once entering the antral stage, only a few will reach the preovulatory stage, while the remaining follicles will become atretic [15].

Insight into the role of AMH in folliculogenesis came from studies performed in the AMHKO mice. In these studies, the complete follicle population was determined in AMHKO and wild-type mice. At 4 months of age, ovaries of AMHKO mice contained more growing follicles and less primordial follicles compared to wild type mice. This increased recruitment in the absence of AMH resulted in a much faster depletion of the primordial follicle pool, evident at 13 months of age when ovaries of AMHKO mice were nearly devoid of primordial follicles whereas wild type ovaries still contained primordial follicles [16]. These results strongly suggested that AMH has an inhibitory role in primordial follicle recruitment (Fig. 1). This conclusion was confirmed in a neonatal ovary culture system in which ovaries of 2-day-old mice were cultured in the presence or absence of AMH. In the presence of AMH, cultured ovaries contained 40–50% less growing follicles compared with control ovaries [17]. Similarly, grafting of mouse neonatal ovaries beneath the chorioallantoic membrane of chick embryos, which results in exposure to high levels of AMH secreted by the chick gonads, suppressed primordial follicle recruitment. Interestingly, follicle recruitment was not suppressed when neonatal ovaries of AMHRII null mice were grafted [18]. Similar results were obtained using bovine ovarian cortical strips. In vitro culture of cortical strips in the presence of AMH inhibited follicle recruitment, whereas recruitment occurred in bovine ovarian cortex grafted in gonadectomized chicks [18]. Likewise, treatment of human ovarian cortical strips with AMH for 7 days in vitro suppressed the initiation of primordial follicle growth [19]. In contrast, Schmidt et al. [20] reported that follicle growth was more advanced in the presence of AMH in cryopreserved human ovarian cortical tissue cultured for 4 weeks. The difference in material (fresh vs. frozen-thawed) and the duration
of culture (7 days vs. 4 weeks) perhaps explain these conflicting results.

3. Anti-Müllerian hormone and cyclic recruitment

The specific window of AMH expression, i.e. in between the two major regulatory steps of folliculogenesis (Fig. 1), suggests that in addition to primordial follicle recruitment, AMH may also regulate FSH-dependent cyclic selection. The increase in FSH during each cycle results in the selection of a limited number of follicles from the cohort of small growing follicles. For this cyclic selection of follicles, FSH levels need to rise to a certain threshold concentration to prevent follicles becoming atretic [15]. Studies in mice and human suggest that AMH is one of the intra-ovarian growth factors contributing to the establishment of this threshold.

In the AMHKO mice, more growing follicles were observed despite lower FSH levels [16]. Detailed analysis throughout the estrous cycle revealed that the FSH surge was blunted in AMHKO mice. Nevertheless, the FSH-dependent selection of small antral follicles was more pronounced in the absence of AMH. In addition, recruitment of large preantral follicles was observed in AMHKO mice at estrous, whereas in wild type mice these follicles are not sensitive to FSH [21]. Thus, in the absence of AMH follicles display an enhanced and premature sensitivity to FSH (Fig. 1).

A role for AMH in the inhibition of FSH sensitivity was confirmed in in vitro cultures of mouse preantral follicles. Follicles cultured in the presence of both FSH and AMH displayed smaller diameters compared to those cultured in the presence of FSH alone [22]. In addition, AMH inhibited the FSH-dependent increase in LH receptor expression and aromatase activity in rodent and porcine granulosa cells [23]. Recently, AMH was also shown to inhibit FSH-induced aromatase expression, and subsequent estradiol production, in granulosa cell cultures of normo-ovulatory women [24]. In follicular fluid of human small antral follicles, a negative correlation between AMH and estradiol levels was observed, suggesting indirectly that AMH inhibits FSH-induced estradiol production [25].

4. Polymorphisms and anti-Müllerian hormone function

The studies performed in the AMHKO mice revealed that AMH inhibits initial recruitment and cyclic selection. The few studies available suggest that AMH may have a similar role in the human ovary. To study the role of AMH in the human ovary in more detail, our group took a genetic approach to determine whether genetic variants of the AMH signaling pathway influence the rate of primordial follicle usage and FSH sensitivity.

Analysis of the AMH and AMHR2 genes identified two common polymorphisms, AMH Ile49Ser (MAF = 0.20) and AMHR2 -482 A>G (MAF = 0.19), which capture the genetic variation within each gene. Association of these two polymorphisms with natural age at menopause was not associated with natural age at menopause. In contrast, an association was observed for the AMHR2 -482 A>G polymorphism, albeit in interaction with parity (p < 0.001) (Fig. 2). Nulliparous women homozygous for the minor allele had a nearly three years earlier onset of menopause compared with nulliparous women homozygous for the major allele [26]. These results suggest that AMH signaling plays a role in the process of ovarian aging. However, little is known about the underlying mechanism of the relation between age at menopause and parity, and the influence of the AMHR2 polymorphism on this mechanism.

Association analysis of the AMH Ile49Ser and AMHR2 -482 A>G polymorphisms with menstrual cycle characteristics in two Caucasian cohorts of normo-ovulatory women revealed that both polymorphisms were associated with estradiol levels during the follicular phase of the menstrual cycle. Women carrying the AMH 49Ser allele or the AMHR2 -482 G allele had higher estradiol levels compared to non-carriers (p = 0.03 and p = 0.012 respectively). Interestingly, carriers of both the AMH and AMHR2 minor alleles had the highest estradiol levels (p = 0.001) [27]. These findings further support a role for AMH in the regulation of intra-ovarian FSH sensitivity.

Aberrant FSH sensitivity of follicles has been suggested as a cause for the disturbed selection of the dominant follicle in polycystic ovary syndrome (PCOS) [28]. Since AMH suppresses FSH sensitivity, the high AMH levels in women with PCOS (discussed in more detail below) may contribute to the disturbed follicle development in PCOS women. Furthermore, since AMH inhibits FSH-induced aromatase activity in human granulosa cells, AMH may contribute to the characteristic increased androgen levels in PCOS women. Analysis of the AMH and AMHR2 polymorphisms in a large cohort of Caucasian PCOS women suggests that these polymorphisms do not contribute to the risk of developing PCOS. However, the AMH Ile49Ser polymorphism contributes to the severity of the PCOS phenotype. Carriers of the 49Ser allele had less frequently polycystic ovaries (92.7% vs. 99.5%, p = 0.0004), reflected also by a 12% lower total follicle number. In addition, nearly 10% lower testosterone and androstenedione levels were observed in PCOS women carrying the 49Ser allele. The milder PCOS phenotype in carriers of the 49Ser polymorphism suggests that the AMH 49Ser variant is less effective in suppressing FSH-sensitivity [29]. Functional analysis confirmed that the AMH 49Ser protein induced a lower maximal response of an AMH-responsive luciferase reporter compared to the AMH 49Ile protein, suggesting a lower bioactivity of the AMH 49Ser protein [29].

In addition to the AMH and AMHR2 polymorphisms, the contribution of genetic variants of one of the AMH type I receptors to PCOS susceptibility and phenotype was investigated. Seven, so-called, tagging polymorphisms capturing the common genetic variation across the ACVR1 gene, encoding ALK2, were selected. In addition to AMH, ALK2 also mediates effects of BMP ligands [6]. Interestingly, also BMP ligands have been shown to suppress FSH sensitivity [30]. Therefore, observed associations for ACVR1 variants may reflect subtle alteration in both AMH and BMP signaling. The seven tagging polymor-
Fig. 2. Interaction between AMHR2 -482 A>G genotypes and parity in the Rotterdam cohort.
Age at menopause for women with zero, one or two, and more than two children, by AMHR2 -482 A/A, A/G and G/G genotype groups adjusted for age, BMI, smoking, socioeconomic status, age at menarche and hormone use. Data are presented as mean ± SEM. **: G/G genotype significantly different from A/A genotype, $p = 0.005$. (Figure reprinted from [26], with permission from Oxford University Press/Human Reproduction).

Interaction entre les génotypes AMHR2 -482 A>G et la parité dans la cohorte de Rotterdam.

polymorphisms did not contribute to PCOS susceptibility. However, three of the seven polymorphisms were associated with serum AMH levels in PCOS patients. Women homozygous for the minor allele had 30, 70, and 34% higher AMH levels (rs1220134 A/A, rs1049189 C/C, and rs2033962 T/T respectively) compared to women homozygous for the major allele. Similarly, these polymorphisms were or tended to be associated with follicle number. After adjustment for follicle number, the associations with AMH levels remained significant, and are thus, in part, independent of follicle number [31]. Interestingly, Pellatt et al. [32] showed that AMH production per granulosa cell is increased in PCOS patients. Our findings suggest that ACVR1 variants may contribute to this increased AMH production. Furthermore, our findings suggest that, besides AMH, other TGF/B family members may also contribute to the pathophysiology of PCOS.

5. Anti-Müllerian hormone as a marker for ovarian reserve and responsiveness

The reproductive capacity of women declines with increasing age. This decline in reproductive function reflects the decrease in ovarian reserve, constituted by both the attenuated size and quality of the primordial follicle pool. Particularly, the decline in the number of primordial follicles determines the onset of menopause. Once the primordial follicle pool is exhausted, growing follicles can no longer be recruited, resulting in menopause. In the years preceding menopause, the number of growing follicles already decline, leading to subfertility [33]. Due to the variation in age of menopause, chronological age appears a poor indicator of ovarian reserve.

Currently, direct measurement of the primordial follicle pool is not possible. However, the number of growing follicles is proportionally related to the size of the primordial follicle pool [34]. The finding that AMH is expressed in growing follicles prior to FSH-dependent selection, suggested that AMH constitutes an intra-ovarian marker of the number of growing follicles, and thereby indirectly for the quantitative aspect of the ovarian reserve. Indeed, in mice serum AMH levels decline gradually with increasing age, whereas the expression of AMH in individual growing follicles did not change. This decline in serum AMH correlated strongly with the decreasing number of growing follicles, and more importantly, with the decline in number of primordial follicles [35] (Fig. 3). Similarly, in adult women serum AMH levels decline with increasing age to undetectable levels after menopause [36,37]. AMH levels correlated strongly with antral follicle count (AFC), but also with other markers for ovarian aging such as FSH and inhibin B on cycle day 3 [36]. Interestingly, AMH levels remain relatively stable during the menstrual cycle and also do not vary significantly between cycles [38,39], suggesting that AMH expression is not regulated by gonadotropins. This may explain why AMH is considered one of the earliest markers for ovarian reserve. In young normoovulatory women AMH levels decreased over a 3-years interval, whereas serum levels of FSH and inhibin B and AFC did not change. In agreement, analysis of normal women at an on average 4-years interval revealed that of these markers, serum AMH level is the best predictor for the occurrence of menopausal transition [40]. Furthermore, in a study by van Disseldorp et al. [41], in which AMH levels of a cohort of normal women were related to the observed age at menopause distribution of a prospective cohort, it was observed that AMH is a much better marker to predict a women’s reproductive age than chronological age.

With the increase in number of women that postpone childbearing, thereby risking sub-fertility, AMH levels may provide a good test to determine the status of the ovarian reserve. Assessment of the ovarian reserve may be particularly important in childhood cancer survivors. Gonado-toxicity after cancer treatment is a well-known side effect. The damage to the ovaries causes accelerated primordial follicle loss and can eventually result in premature ovarian failure (POF) [42].
Fig. 3. Serum anti-Müllerian hormone (AMH) levels and follicle numbers in aging mice. The relative numbers of primordial (open bars, \(n=8–10\) mice per age group) and growing follicles (closed bars, \(n=4\) mice per age group) declined in mice of increasing age (\(r=−0.89, p<0.0001\), and \(r=−0.94, p<0.0001\), respectively). Likewise, serum AMH levels declined in aging mice (\(n=8–10\) per age group, \(r=−0.84, p<0.0001\), grey line). a, b, and c: indicate age-groups of mice with statistically significant numbers of primordial follicles, number of growing follicles, and AMH levels, respectively, \(p<0.05\). (Figure modified from [35], Copyright 2006, The Endocrine Society).

Analysis of AMH levels in childhood cancer survivors showed that AMH levels were indicative of a limited ovarian reserve [43–45]. Furthermore, based on AMH levels subgroups of childhood cancer survivors at risk for POF could be identified. Particularly survivors treated with abdominal irradiation, total body irradiation, or three or more procarbazine containing chemotherapy cycles had AMH levels below the 10th percentile and are highly at risk for POF [44].

The delay in childbearing has as a result that significantly more women rely on assisted reproduction. However, advanced ovarian aging is characterized by a decreased ovarian response to ovulation induction treatment. Several studies have investigated the usefulness of AMH as a marker for ovarian response. AMH levels proved to be highly correlated with AFC prior to treatment and number of oocytes retrieved upon treatment. As may be expected, AMH levels were significantly lower in patients responding poorly than in those with a normal response [46,47]. Studies also showed that AMH and AFC have a similar predictive value for poor response, and are superior to FSH and inhibin B levels at cycle day 3 [47]. This suggests that not only the increased number of follicles contribute to the elevated serum AMH levels, but also that the production per granulosa cell is increased. Indeed, Pellatt et al. [32] observed that AMH expression in granulosa cells of PCOS patients was nearly 75-times higher than in those of control women.

6. Anti-Müllerian hormone as a marker for polycystic ovary syndrome

In addition to ovarian reserve tests, AMH levels have also been assessed in ovarian pathological conditions, such as PCOS. Based on the Rotterdam criteria, PCOS is characterized by two of the three following criteria: clinical or biochemical hyperandrogeism, oligo- or amenorrhea, and polycystic ovaries (PCO) [49]. PCOS is one of the most frequent forms of infertility and affects about 6–8% of women worldwide [28]. Although its etiology is unknown, the failure in dominant follicle selection leading to an accumulation of small antral follicles suggests that FSH sensitivity is altered in PCOS ovaries.

A large proportion of PCOS patients are obese and present with abdominal adiposity. Abdominal adiposity is considered a risk factor for metabolic disease. Indeed, many PCOS patients are insulin resistant resembling type 2 diabetes [50,51]. The high insulin levels due to insulin resistance also affect ovarian function. Insulin was shown to stimulate androgen production by theca cells in synergy with LH [52]. Thus the increased androgen and insulin levels form a vicious circle, which may lead to an exaggerated PCOS phenotype.

Several studies showed that serum AMH levels are 2–3 fold increased in PCOS women, and correlate with the increased follicle number [53–56]. In addition, serum AMH levels are positively correlated with androgen levels. Increased AMH levels were also observed in follicular fluid of PCOS women [32,53]. This suggests that not only the increased number of follicles contribute to the elevated serum AMH levels, but also that the production per granulosa cell is increased. Indeed, Pellatt et al. [32] observed that AMH expression in granulosa cells of PCOS patients was nearly 75-times higher than in those of control women. Interestingly, AMH levels are highest in those PCOS patients with the more severe phenotype. PCOS women with PCO had higher AMH levels than those without [55]. Likewise, subdividing PCOS women in ovulatory and anovulatory women, showed that anovulatory PCOS women had increased AMH levels [57]. Women with both PCO and hyperandrogeism had higher AMH levels than women with PCO only, although the number of small antral follicles was not different between the two groups [58]. A relationship with insulin resistance is less clear. A lack of correlation between serum AMH levels and insulin levels or BMI has been reported [55,56], whereas other
studies did observe a positive correlation with the HOMA index and higher AMH levels in insulin-resistant PCOS women than in PCOS women with normal insulin sensitivity [59,60]. However, treatment with insulin-lowering drugs, such as metformin, only weakly lowers AMH levels and only after a prolonged treatment period [61,62], suggesting that insulin does not directly regulate AMH production. Since AMH levels appear to reflect the severity of PCOS, AMH may not only be used as a diagnostic marker but also as a marker to monitor the normalization of ovarian function in response to treatment.

7. Conclusion

AMH is an important regulator of the rate of primordial follicle usage and FSH-dependent follicle selection. Association studies of genetic variants of the AMH signaling pathway suggest that AMH may also fulfill these roles in the human ovary. Based on the strong correlation of serum AMH with the number of growing follicles, a role for AMH as a marker of ovarian function has emerged. AMH levels reflect the quantitative aspect of the ovarian reserve and are predicative for poor response to ovulation induction treatment. Currently, data on normal levels in a wide age range and data on cut-off values for a clinical setting are still missing. Such data are necessary before AMH can be included as a marker in routine diagnostic screening.

Conflict of interest statement

None.

References


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