

## Anti-Müllerian hormone: a new marker for ovarian function

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### Abstract

**Anti-Müllerian hormone (AMH) is a member of the transforming growth factor  $\beta$  family of growth and differentiation factors. In the ovary, AMH has an inhibitory effect on primordial follicle recruitment as well as on the responsiveness of growing follicles to follicle-stimulating hormone (FSH). The ovary-specific expression pattern in granulosa cells of growing nonselected follicles makes AMH an ideal marker for the size of the ovarian follicle pool. This review summarizes recent findings concerning AMH and its role as a marker for the quantitative aspect of ovarian reserve as well as ovarian dysfunction.**

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### Introduction

The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of the oocytes therein, declines with increasing age, resulting in the decrease of a woman's reproductive function (te Velde *et al.* 1998a). The size of the follicle pool is established at an early point in life. During fetal life, germ cells populate the ovary and become surrounded by somatic cells, forming the so-called primordial follicles. At birth, about 1 million oocytes are present. This number decreases during childhood, resulting in a primordial follicle pool of 300 000–500 000 follicles at menarche (Faddy *et al.* 1992). Throughout life, follicles leave the primordial follicle pool to enter the growing pool. The majority of these growing follicles will be lost as a result of atresia, unless they are rescued by follicle-stimulating hormone (FSH). This rescue by FSH starts after puberty when the pituitary-gonadal endocrine axis has been activated. Among the cohort of rescued follicles, only one follicle is selected to become the dominant follicle, which will ovulate under the influence of luteinizing hormone (LH) (McGee & Hsueh 2000). This process continues throughout life until the primordial follicle pool is exhausted and, as a consequence, growing follicles are no longer present in the ovary, resulting in menopause. This classical view of a finite primordial follicle pool has been challenged recently by Johnson *et al.* (2004, 2005), who showed that germline stem cells can repopulate a germ cell-depleted postnatal ovary and renew the primordial follicle pool. However, it remains unknown to what extent this process delays the onset of menopause.

In the years preceding menopause, fertility already decreases and the menstrual cycle becomes irregular. This menopausal transition period precedes menopause by a fixed time interval (den Tonkelaar *et al.* 1998, te Velde *et al.* 1998b, te Velde & Pearson 2002). In the Western world, menopause is reached at a median age of 51 years. However, there is considerable individual variation in the age of menopause and, subsequently, also in the age of subfertility (te Velde *et al.* 1998b, te Velde & Pearson 2002). Hence, chronological age is a poor indicator of reproductive aging, and thus of the ovarian reserve.

To assess an individual's ovarian reserve, early follicular phase serum levels of FSH, inhibin B and estradiol ( $E_2$ ) have been measured. Inhibin B and  $E_2$  are produced by early antral follicles in response to FSH, and contribute to the classical feedback loop of the pituitary-gonadal axis to suppress FSH secretion. With the decline of the follicle pool, serum levels of inhibin B and  $E_2$  decrease and subsequently serum FSH levels rise (Burger *et al.* 1995). Because these factors are part of a feedback system, their serum levels are not independent of each other. Furthermore, changes in serum levels of FSH, inhibin B and  $E_2$  occur relatively late in the reproductive aging process (Burger *et al.* 1999). So far, assessment of the number of antral follicles by ultrasonography, the antral follicle count (AFC), best predicts the quantitative aspect of ovarian reserve (Scheffer *et al.* 2003). However, measurement of the AFC requires an additional transvaginal ultrasound examination during the early follicular phase. Therefore, a serum marker that reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, and that is not controlled by gonadotropins, would benefit both patients and clinicians.

In recent years, accumulated data indicate that anti-Müllerian hormone (AMH) may fulfill this role.

### Anti-Müllerian hormone (AMH)

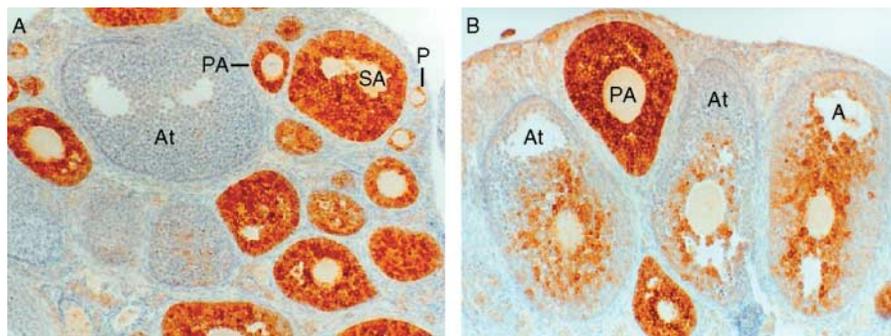
AMH, also known as Müllerian inhibiting substance (MIS), has been mainly studied for its regulatory role in male sex differentiation. AMH, produced by the Sertoli cells of the fetal testis, induces the regression of the Müllerian ducts, the anlagen of the female reproductive tract (Josso *et al.* 1993, Lee & Donahoe 1993). However, after birth, this sex-dimorphic expression pattern is lost and AMH is also expressed in granulosa cells of growing follicles in the ovary.

Detailed studies in rodents have shown that AMH expression starts in the columnar granulosa cells of primary follicles immediately after differentiation from the flattened pregranulosa cells of primordial follicles. Expression is highest in granulosa cells of preantral and small antral follicles, and gradually diminishes in the subsequent stages of follicle development. AMH is no longer expressed during the FSH-dependent final stages of follicle growth (Fig. 1). In addition, AMH expression disappears when follicles become atretic. The level of expression in follicles of the same class does not seem to change during the estrous cycle in rat, although some heterogeneity was observed in AMH expression in preantral and small antral follicles at estrous and diestrous (review by Durlinger *et al.* 2002a).

Interestingly, two major regulatory steps of folliculogenesis, initial follicle recruitment and cyclic selection for dominance (McGee & Hsueh 2000), flank this window of expression. Analysis of the follicle dynamics in AMH null mice provided more insight into the intraovarian role of AMH and revealed that AMH specifically affects these two regulatory steps. Ovaries of 4-month-old AMH null mice contained almost threefold more small nonatretic growing follicles than their wild-type littermates, accompanied by a decrease in the number of primordial follicles (Durlinger *et al.* 1999). This increased recruitment had already started before the initiation of the estrous cycle, since a higher number of growing follicles was already evident in AMH null mice at day 25. These results indicate that, in the

absence of AMH, primordial follicles are recruited at a faster rate. Consequently, the primordial follicle pool is prematurely exhausted and estrous cycling stops at an earlier age in AMH null mice (Durlinger *et al.* 1999). *In vitro* culture of neonatal ovaries in the presence of AMH confirmed the inhibitory effect of AMH on primordial follicle recruitment (Durlinger *et al.* 2002b). Based on the low FSH levels measured in AMH null mice in the presence of an increased number of growing follicles, it was hypothesized that, in the absence of AMH, follicles are more sensitive to FSH. Indeed, AMH inhibited FSH-dependent follicle growth of cultured mouse preantral follicles (Durlinger *et al.* 2001). Similarly, in granulosa cell cultures, AMH attenuates the FSH-dependent increase in aromatase activity and LH receptor expression (di Clemente *et al.* 1994). Furthermore, an *in vivo* study in which FSH levels were modulated showed that in the presence of both low and high serum FSH concentrations more growing follicles are found in AMH null mice than in wild-type mice (Durlinger *et al.* 2001). This inhibitory effect of AMH on FSH sensitivity of follicles could play a role in the process of selection. It is thought that each follicle exerts its own threshold FSH concentration that has to be exceeded to allow selection. A role for AMH in this process is indirectly supported by the differential expression level of AMH in nonatretic, large preantral and small antral follicles in the rat ovary (Baarends *et al.* 1995). A low expression of AMH within the follicle would diminish the threshold level for FSH, allowing these follicles to continue growth and to ovulate in the next estrous cycle.

Although they are mostly based on rodent studies, these results also appear to apply to the human ovary. In women, AMH expression can first be observed in granulosa cells of primary follicles, and expression is strongest in preantral and small antral follicles ( $\leq 4$  mm). AMH expression disappears in follicles of increasing size and is almost lost in follicles larger than 8 mm, where only very weak staining remains, restricted to the granulosa cells of the cumulus (Weenen *et al.* 2004). This expression pattern suggests that, also in man, AMH may play a role in initial recruitment and in the selection of the dominant follicle



**Figure 1** AMH expression in mouse ovaries. (A) AMH is expressed in granulosa cells of primary (P), preantral (PA) and small antral (SA) follicles. (B) AMH expression disappears in antral (A) and atretic (At) follicles. Expression is lost last in the granulosa cells surrounding the oocytes. AMH expression was detected using a monoclonal antibody that recognises rat, mouse and human AMH. Magnification  $\times 200$ .

(Fig. 2). The possible mechanisms of action of AMH are reviewed elsewhere (Durlinger *et al.* 2002a, Visser 2003).

### AMH as a marker for ovarian aging

The specific expression pattern of AMH in growing non-selected follicles has prompted us and others to investigate whether serum AMH levels are indicative for the size of the growing follicle pool. As discussed above, the quantitative aspect of ovarian aging is reflected by a decline in the size of the primordial follicle pool. Direct measurement of the primordial follicle pool is impossible. However, the number of primordial follicles is indirectly reflected by the number of growing follicles (Scheffer *et al.* 1999). Hence, a factor primarily secreted by growing follicles will reflect the size of the primordial follicle pool. Since AMH is expressed by growing follicles up to selection (Durlinger *et al.* 2002a), and can be detected in serum (Hudson *et al.* 1990, Lee *et al.* 1996), it is a promising candidate.

In young normal ovulatory women, early follicular phase hormone measurements at 3-year intervals revealed that serum AMH levels decline significantly whereas serum levels of FSH and inhibin B and the number of antral follicles do not change during this interval (de Vet *et al.* 2002). Stratification for age revealed that both serum AMH levels and numbers of antral follicles decline with age (Fig. 3A). Importantly, a strong correlation of serum AMH levels with AFC was observed (Fig. 3B). This positive correlation was later confirmed by Fanchin *et al.* (2003b), who showed a stronger correlation between serum AMH levels and follicle count than between AMH and serum levels of inhibin B, FSH, and  $E_2$  on cycle day 3.

The results of de Vet *et al.* (2002) also suggest that changes in serum AMH levels occur relatively early in the sequence of events associated with ovarian aging. Substantially elevated serum levels of FSH are not found until cycles have already become irregular (Burger *et al.* 1999). Therefore, a marker that already shows a considerable change when cyclicity is still normal would better identify women with declining fertility. Indeed, in studies by van

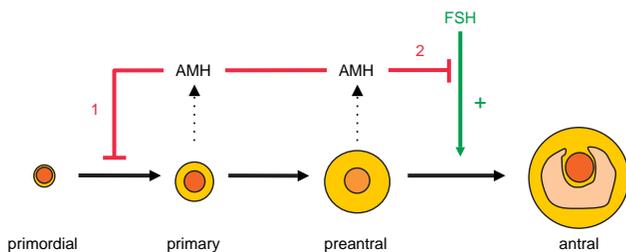
Rooij *et al.* (2004, 2005), in which several markers of aging were measured in normal women at 4-year intervals, serum levels of AMH gave the highest accuracy ( $ROC_{AUC}$  0.87) to predict occurrence of menopausal transition within 4 years. When serum inhibin B levels and age were included in a multivariate model, the  $ROC_{AUC}$  improved to 0.92. Furthermore, compared to other ovarian reserve markers, only serum AMH level showed a mean longitudinal decline over time. Taken together, these data strongly suggest that serum levels of AMH can be used as a marker of ovarian aging.

The usefulness of serum AMH levels as a measure of the ovarian reserve was recently shown in young women after treatment for childhood cancer. Chemotherapy and radiotherapy treatment have adverse effects on the ovary in particular, resulting in loss of primordial follicles. Indeed, in cancer survivors, the partial loss of the ovarian reserve is reflected by increased FSH levels and decreased ovarian volume. Unexpectedly, the number of small antral follicles is unchanged (Bath *et al.* 2003), a finding that may reflect the low accuracy and observer dependency of AFC measurements. Nevertheless, serum AMH levels were decreased in these patients, supporting the use of serum AMH levels as an early predictor of the ovarian reserve.

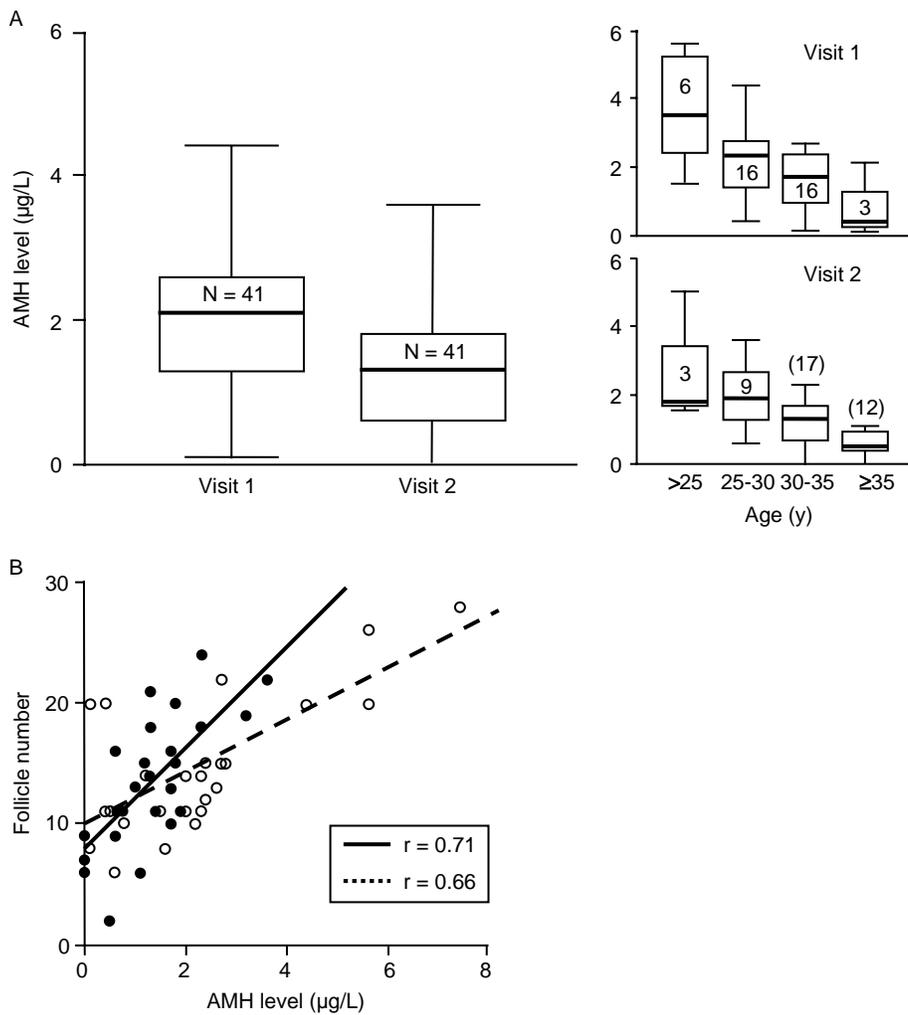
### AMH as a marker of ovarian responsiveness

AMH's role as a peripheral signal of the size of the growing follicle pool may have important clinical benefits. In women undergoing treatment for infertility, ovarian aging is characterized by decreased ovarian responsiveness to exogenous gonadotropin administration and poor pregnancy outcome. On the one hand, correct identification of poor responders by assessment of their ovarian reserve before entering an *in vitro* fertilization (IVF) program is important. On the other hand, assessment of the ovarian reserve may also benefit patients that would generally be excluded from IVF programs because of advanced age.

Several studies have shown that AMH is an excellent marker to determine ovarian responsiveness also in an IVF program. Hormone measurements in the early follicular phase (day 3 of spontaneous cycle), retrospectively or in a group of unselected patients, revealed that AMH levels are lower in patients with poor ovarian response than in women with normal response (Seifer *et al.* 2002, van Rooij *et al.* 2002), ovarian responsiveness being defined as the number of oocytes retrieved, or as cancellation due to impaired or absent follicular growth. In agreement with the studies described above, AMH serum levels were shown to be highly correlated with the number of antral follicles before treatment and number of oocytes retrieved upon ovarian stimulation (van Rooij *et al.* 2002). Logistic regression analysis for prediction of poor response showed that serum AMH levels had a better predictive value than serum levels of FSH, inhibin B and  $E_2$ , and that the



**Figure 2** Model of AMH action in the ovary. Progressing stages of folliculogenesis are depicted. AMH is produced by the small growing (primary and preantral) follicles in the postnatal ovary and has two sites of action. It inhibits initial follicle recruitment (1) and inhibits FSH-dependent growth and selection of preantral and small antral follicles (2).



**Figure 3** Serum AMH levels in normo-ovulatory women. (A) Box and whiskers plots show declining AMH levels with increasing age. AMH levels were measured at two time points with a  $2.6 \pm 1.7$ -year interval. (B) AMH levels positively correlate with numbers of antral follicles at visit 1 (closed circles, solid line) and visit 2 (open circles, dotted line). Reproduced from de Vet *et al.* (2002), with permission from American Society for Reproductive Medicine.

**Table 1** Logistic regression for prediction of poor response after ovarian hyperstimulation.

Univariate analysis	Odds ratio (95% CI)	P	ROC <sub>AUC</sub>
AFC (per follicle)	0.70 (0.61–0.81)	<0.001	0.86
AMH (per 0.1 µg/l)	0.82 (0.75–0.90)	<0.001	0.85
FSH (per IU/l)	1.41 (1.22–1.63)	<0.001	0.83
Inhibin B (per ng/l)	0.98 (0.97–0.99)	<0.001	0.76
Age (per year)	1.07 (0.99–1.16)	NS	0.60
E <sub>2</sub> (per pmol/l)	1.003 (1.000–1.006)	NS	0.52
Multivariate analysis	OR (95% CI)		ROC <sub>AUC</sub> (final model)
<i>All variables</i>			
AFC (per follicle) and	0.77 (0.65–0.90)	0.001	
Inhibin B (per pg/ml) and	0.98 (0.97–0.99)	0.006	
FSH (per IU/l)	1.27 (1.07–1.50)	0.006	0.92
<i>AFC excluded from analysis</i>			
AMH (per 0.1 µg/l) and	0.90 (0.82–0.98)	0.018	
Inhibin B (per pg/ml) and	0.98 (0.97–0.99)	0.005	
FSH (per IU/l)	1.26 (1.07–1.50)	0.006	0.90

Data taken from van Rooij *et al.* (2002), © European Society of Human Reproduction and Embryology. Reproduced by permission of Oxford University Press/Human Reproduction.

predictive values for AMH and AFC were almost identical ( $ROC_{AUC}$  0.85 vs 0.86) (Table 1). Inclusion of FSH and inhibin B together with AMH in a multivariate model improved this predictive value to 0.90 (van Rooij *et al.* 2002) (Table 1). Similarly, cycle day-5 AMH levels are a better marker of ovarian responsiveness than inhibin B levels (Muttukrishna *et al.* 2004). Measurement of serum AMH levels has several advantages over other serum markers such as FSH, inhibin B and  $E_2$ . To achieve a reliable predictive outcome, one single hormone measurement for AMH seems sufficient (Fanchin *et al.* 2005a). Furthermore, in contrast to FSH, inhibin B and  $E_2$ , AMH levels remain relatively constant during the follicular phase and entire menstrual cycle (Cook *et al.* 2000, La Marca *et al.* 2004a, and our own unpublished results (F H J, J L, A T and Dr F Broekmans)), consistent with the continuous, noncyclic growth of small follicles.

The absence of regulation of AMH by gonadotropins was shown in both rodents and man. Heterozygous AMH null mice present with an ovarian phenotype between that of wild-type and homozygous AMH null mice (Durlinger *et al.* 1999), suggesting that AMH acts as a paracrine rather than a systemic factor, and thus is not part of a negative feedback loop with involvement of gonadotropins. In agreement, treatment of IVF patients with a single, high dose of gonadotropin-releasing hormone (GnRH) agonist, resulting in a rise of endogenous FSH and LH, does not affect AMH serum levels (van Rooij *et al.* 2002). Similarly, in conditions where FSH levels are suppressed, such as pregnancy, AMH levels remain constant (La Marca *et al.* 2005). Thus, AMH is not influenced by the gonadotropic status and reflects only the follicle population. The latter conclusion was confirmed in a more detailed study by Fanchin *et al.* (2003a), who treated women with FSH and human chorionic gonadotropin (hCG) after complete pituitary desensitization with a GnRH agonist. In a normal menstrual cycle, the early antral follicle pool remains intact throughout the follicular phase. However, upon ovarian hyperstimulation, all small antral follicles are stimulated to the preovulatory stage, thus providing a model to determine the relationship between AMH levels and follicle dynamics. Serum AMH levels, determined at three days during FSH treatment and at the day of hCG administration, decline significantly at each consecutive measurement (Fanchin *et al.* 2003a), reflecting the reduction in number of small antral follicles. A decline in serum AMH was also observed after FSH administration immediately following a spontaneous cycle (La Marca *et al.* 2004a). Moreover, on day 5 of gonadotropin therapy, levels of serum AMH and estradiol constitute an even better prediction of the ovarian response than cycle day 3 AMH levels (Penarrubia *et al.* 2005). However, from a clinical point of view, poor responders should be identified before treatment; therefore, it is more useful to determine serum AMH levels during a spontaneous cycle.

Throughout the controlled ovarian hyperstimulation protocol, serum AMH levels correlated well with the decrease in number of small antral follicles ( $\leq 12$  mm) (Fanchin *et al.* 2003a), reflecting the complete conversion of small antral follicles into large antral follicles in response to FSH stimulation. Indeed, no correlation with the number of growing follicles ( $> 12$  mm) was observed (Fanchin *et al.* 2003a), in line with the low expression of AMH in these follicles (Weenen *et al.* 2004). In the days following hCG treatment, AMH serum levels initially declined, possibly as a result of the luteinization of granulosa cells upon hCG treatment that also causes a decline in  $E_2$  levels. During the midluteal phase, AMH serum levels slightly increased, probably as a result of the presence of newly developed, small antral follicles (Fanchin *et al.* 2005b). Thus, these changes in serum AMH levels seem to reflect follicle dynamics rather than regulation by gonadotropins.

All combined these studies strongly support a role of serum AMH level as a marker for ovarian responsiveness. However, the application of AMH to predict ongoing pregnancy seems limited, although day 3 serum AMH levels are higher in patients that become pregnant after IVF treatment than in those who do not (Hazout *et al.* 2004). However, data on pregnancy outcome were not stratified for the number of retrieved oocytes, which also in this study showed a positive correlation with AMH levels. Therefore, it is likely that the quantitative aspect of AMH as a marker of the ovarian reserve has contributed predominantly to the association with pregnancy outcome. Indeed, other studies did not observe a predictive value of AMH serum levels for ongoing pregnancy after IVF treatment (van Rooij *et al.* 2002, Penarrubia *et al.* 2005).

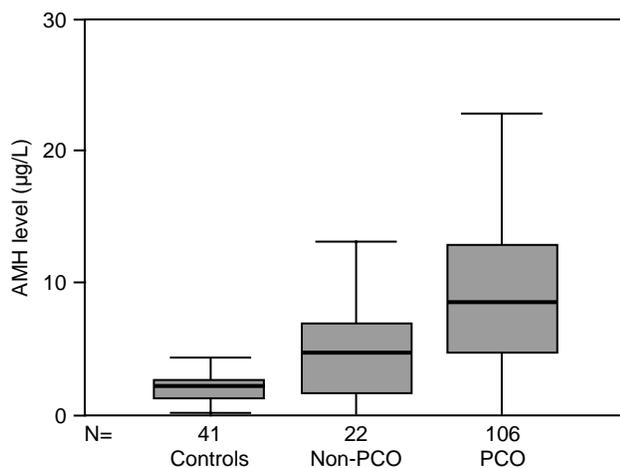
### AMH as a marker for ovarian pathophysiology

The results of the studies described above indicate that serum AMH level can be used as a marker for the number of growing follicles. Besides being a marker for a diminishing follicle pool, serum AMH level can also serve as a marker in ovarian pathophysiology, such as polycystic ovary syndrome (PCOS), in which the antral follicle pool is enlarged. PCOS is one of the most common endocrine disorders in women of reproductive age (Franks 1995). It is characterized by anovulation manifested as oligo- or amenorrhea, elevated levels of circulating androgens, and polycystic ovaries as visualized by ultrasound. The diagnosis is based on the presence of at least two of the described characteristics, as defined by the Rotterdam Consensus (2004). PCOS encompasses a broad spectrum of clinical and biochemical characteristics, and, although the mechanisms leading to PCOS are still poorly understood, the common denominator is a disturbance in the selection of the dominant follicle resulting in anovulation. The defective selection mechanism results in an accumulation of small antral follicles, which contribute

significantly to the production of AMH. As discussed above, studies in mice showed that AMH lowers the sensitivity of follicles to FSH (Durlinger *et al.* 2001), possibly contributing to deranged follicle selection. It has been suggested that aromatase activity in PCOS patients might be decreased because follicles from PCOS women do not produce large amounts of  $E_2$  (Agarwal *et al.* 1996). AMH also inhibits aromatase activity, as discussed above, suggesting that AMH contributes to the severity of PCOS.

Initial studies showed that follicular fluid and serum of PCOS women contained increased AMH levels (Fallat *et al.* 1997, Cook *et al.* 2002). In agreement with previous results obtained in normal cycling women, also in PCOS women serum AMH levels were correlated with antral follicle number. The two- to threefold increase in the number of growing follicles is reflected by a two- to threefold increase in serum AMH level (Pigny *et al.* 2003, Laven *et al.* 2004) (Fig. 4). In PCOS, the follicular excess is mainly caused by an increase of small antral follicles upto 2–5 mm in size (Hughesdon 1982, Jonard *et al.* 2003). Interestingly, in follicles beyond this stage, AMH expression diminishes (Weenen *et al.* 2004). Therefore, it is not surprising that serum AMH levels positively correlate with the number of 2–5 mm, but not 6–9 mm, follicles in PCOS women (Pigny *et al.* 2003). The finding that AMH levels are also increased in the follicular fluid of PCOS women (Fallat *et al.* 1997) suggests that the increase in serum AMH levels is not only due to an increase in the number of growing follicles, but may also result from increased AMH production per follicle. So far little is known about the factors that regulate AMH expression in the ovary.

Categorization of anovulatory women into groups with or without polycystic ovaries (PCO), by number of antral follicles ( $\geq 12$  follicles per ovary measuring 2–9 mm) or



**Figure 4** Serum AMH levels are increased in normogonadotropic anovulatory infertile women. Box and whisker plots show AMH levels in infertile women with PCO and those without (non-PCO) compared with control women. Reproduced from Laven *et al.* (2004) (©2004, Endocrine Society).

ovarian volume ( $> 10$  ml), revealed that serum AMH levels were significantly higher in the PCO group than in non-PCO patients (Laven *et al.* 2004). Nevertheless, serum levels in non-PCO women were still significantly elevated compared to those in control women (Fig. 4). This suggests that the pool of smaller follicles, which is not detected on ultrasound, may be increased and contributes significantly to serum AMH levels in anovulatory women. In a study by Eldar-Geva *et al.* (2005), categorization of PCOS women by presence or absence of hyperandrogenism showed that AMH levels were significantly different between groups. Both groups had increased AMH levels compared to control women, but levels in women with PCO and hyperandrogenism were even further elevated. Interestingly, the numbers of small antral follicles did not differ between the two PCO groups, and multiple regression analysis showed that follicle number and testosterone levels independently correlated with AMH serum levels (Eldar-Geva *et al.* 2005). These results suggest that the nonvisible pool of follicles may be further increased in the presence of increased androgen levels. Indeed, in the rhesus monkey, androgens stimulate the initiation of primordial follicle growth (Vendola *et al.* 1999). In addition, androgens stimulate the proliferation of granulosa and theca cells of growing follicles in rhesus monkeys (Vendola *et al.* 1998). It is possible that under these conditions follicles may produce more AMH. Since AMH inhibits aromatase activity, local androgen concentrations may be increased, possibly resulting in a positive feedback mechanism between AMH and androgens. However, the mechanism behind the positive association between androgens and AMH in PCOS women requires further studies, in particular, since in males during puberty an inverse relationship between AMH and testosterone levels was found (Rey *et al.* 1993). In this respect, it would be interesting to analyze the expression level and pattern of AMH by immunohistochemical analysis in polycystic ovaries of women with or without hyperandrogenism.

In PCOS women, levels of serum AMH are also correlated with other clinical features, such as cycle duration, mean ovarian volume, testosterone and androstenedione levels, and free androgen index, whereas no correlations with inhibin B and  $E_2$  levels were observed (Pigny *et al.* 2003, Laven *et al.* 2004).

A substantial proportion of PCOS women are obese and exhibit insulin resistance and compensatory hyperinsulinemia (Dunaif 1997). The increased insulin levels in some PCOS women can, in part, account for the hyperandrogenism, because insulin acts synergistically with LH to enhance androgen production by theca cells (Franks *et al.* 1999). However, serum AMH levels do not seem to correlate with BMI and insulin levels (Pigny *et al.* 2003, Laven *et al.* 2004, Fleming *et al.* 2005). In contrast, in a small study, La Marca *et al.* (2004b) observed a positive correlation between serum AMH levels and the HOMA index, an insulin resistance index calculated from fasting insulin and fasting glucose levels.

Improvement of insulin levels by insulin-lowering drugs, such as metformin and thiazolidinediones, which also indirectly affect androgen production, have been shown to be beneficial in PCOS women (Franks *et al.* 1999). In a study of obese PCOS women, metformin treatment suppressed androstenedione levels and ovulation rate (Fleming *et al.* 2005), although androgen levels were still above the upper limit of the normal range. Metformin administration also resulted in a small but significant reduction of serum AMH levels after 8 months of treatment, whereas the follicle number did not change significantly (Fleming *et al.* 2005). In a smaller study, metformin treatment for 6 months also decreased serum AMH levels only slightly (Piltonen *et al.* 2005), and levels remained strongly elevated compared to controls. Although AMH and androgen levels are positively correlated in PCOS women, the decrease in AMH levels may be secondary to the decrease in androgens upon metformin treatment. Furthermore, since the number of follicles is not likely to change in a short period of time, more detailed studies with longer follow-up are required to determine the long-term effect of insulin-lowering agents on AMH levels.

As in cycling women, AMH levels decline with increasing age in PCOS. However, the decrease in serum levels is significantly different from that in controls (Laven *et al.* 2004). A follow-up study investigated this phenomenon in more detail by measuring serum AMH levels in control and PCOS women on two occasions with a median time interval of 2.6 years. Although AMH levels had declined over time in both groups, the decline was less pronounced in PCOS women (Mulders *et al.* 2004). These results were confirmed by Piltonen *et al.* (2005), who showed that, in contrast to older control women with low to undetectable AMH levels, women with PCOS of the same age still had high AMH levels. This suggests that the ovarian aging process in PCOS women may have been slowed down, possibly due to suppressed primordial follicle outgrowth by the high levels of AMH observed in these women. However, it has also been suggested that exhaustion of the primordial follicle pool occurs later in PCOS women because their intrinsic primordial follicle pool may be increased (Webber *et al.* 2003). Data regarding the menopausal age in PCOS women are scarce. However, smaller studies seem to indicate that women with PCOS reach menopause at an older age (Dahlgren *et al.* 1992).

## Conclusions and future directions

The studies described here indicate that serum AMH levels decrease with age in premenopausal women. In addition, serum levels of AMH correlate strongly with the number of antral follicles, suggesting that AMH levels by extension reflect the size of the primordial follicle pool. Assessment of the ovarian reserve is particularly important in the IVF clinic, where AMH may be useful as a predictor of poor response. Since a considerable proportion of

subfertility is due to postponement of childbearing, measurement of AMH levels to assess the ovarian reserve may also be of interest in women in general. Assessment of the ovarian reserve, at least of the size of the ovarian follicle pool, may provide insight into the number of fertile years a woman has left. However, in order to determine whether serum AMH level has prognostic value, additional prospective studies in a normal population are necessary to provide definite proof for this concept.

The positive correlation between serum AMH levels and number of antral follicles is also observed in women with PCOS. The elevated levels of AMH in these women strongly suggest that serum AMH levels may also be used in the diagnosis of PCOS. The difference in serum levels of AMH between subgroups of PCOS women suggests that AMH might also be used to establish a subclassification of this heterogeneous syndrome. However, more studies, preferably prospective, with thoroughly analyzed patient cohorts are necessary to define cutoff values. In addition, studies are necessary to determine whether serum AMH levels are also indicative of improved ovarian function upon treatment of PCOS women.

In conclusion, recent studies have validated the use of serum AMH levels as a marker for the quantitative aspect of ovarian reserve. Because AMH levels are strongly correlated with the size of the follicle pool, and because of the lack of cycle variations, serum levels of AMH are a good candidate for inclusion in standard diagnostic procedures to assess other ovarian dysfunctions, such as premature ovarian failure. Knowledge of the serum AMH levels in such conditions might provide more insight into the possible cause or effect of altered AMH levels. Genetic studies of well-defined population cohorts would also provide more knowledge about the role of AMH in ovarian physiology.

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