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Anti-Müllerian hormone (AMH): what do we still need to know?

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In the ovary, Anti-Müllerian hormone (AMH) is produced by the granulosa cells of early developing follicles and inhibits the transition from the primordial to the primary follicular stage. AMH levels can be measured in serum and have been shown to be proportional to the number of small antral follicles. In women serum AMH levels decrease with age and are undetectable in the post-menopausal period. In patients with premature ovarian failure AMH is undetectable or greatly reduced depending of the number of antral follicles in the ovaries. In contrast, AMH levels have been shown to be increased in women with polycystic ovary syndrome (PCOS). AMH levels appear to represent the quantity of the ovarian follicle pool and may become a useful marker of ovarian reserve. AMH measurement could also be useful in the prediction of the extremes of ovarian response to gonadotrophin stimulation for *in vitro* fertilization, namely poor- and hyper-response. Although AMH has the potential to increase our understanding of ovarian pathophysiology, and to guide clinical management in a broad range of conditions, a number of important questions relating to both the basic physiology of AMH and its clinical implications need to be answered.

Key words: AMH / PCOS / ovarian reserve / infertility / ART

Introduction

In April 2008, an ESHRE Campus Workshop entitled 'Ovarian reserve: new insights for clinical management' was organized in Modena (Italy) under the auspices of the Special Interest Group in Reproductive Endocrinology. During this meeting a round table on 'Anti-Müllerian hormone (AMH): what do we still need to know?' provided the opportunity for many of the research groups working on AMH to assess our current knowledge, and to discuss the clinical research questions which still need to be addressed.

The issues discussed included: (i) current views on the role of AMH in folliculogenesis, (ii) assays for serum AMH measurement, (iii) AMH and ovarian dysfunction, (iv) the role of AMH in ovarian reserve testing. In this article, the experts' understanding of these topics is summarized and those issues considered to require further research are highlighted.

The role of AMH in ovarian folliculogenesis

AMH is a dimeric glycoprotein member of the transforming growth factor-beta superfamily. Its most clearly defined role is in male sex differentiation. AMH is produced by fetal Sertoli cells at the time of testicular differentiation, and induces regression of the Müllerian ducts. In the absence of AMH, the Müllerian ducts develop into the uterus, fallopian tubes and the upper part of the vagina (Munsterberg and Lovell-Badge, 1991).

In the ovaries of female fetuses, AMH expression has been observed as early as 32 weeks gestation in humans (Rajpert-De Meyts *et al.*, 1999). In primordial follicles, AMH expression seems to be absent. AMH immunostaining can first be observed in granulosa cells of follicles at the primary stage of development. In one study,

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~75% of secondary follicles were positive for AMH immunostaining. The strongest staining was observed in pre-antral and small antral follicles (Weenen *et al*., 2004).

AMH continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which they may be selected for dominance. In the mouse this occurs at the early antral stage in small growing follicles (Durlinger *et al*., 2002), whereas in the human it is evident in antral follicles 4–6 mm in diameter (Weenen *et al*., 2004). Thus, AMH is expressed in follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance. AMH is not expressed in atretic follicles or theca cells (Ueno *et al*., 1989; Munsterberg and Lovell-Badge, 1991; Hirobe *et al*., 1994).

It has recently been demonstrated that oocytes from early pre-antral, late pre-antral and pre-ovulatory follicles up-regulate AMH mRNA levels in granulosa cells, in a fashion that is dependent upon the developmental stage of the oocyte. These findings therefore suggest that oocyte regulation of granulosa cell gene expression occurs during extended periods of follicle development and that oocyte regulation of AMH expression may play a role in intra- and inter-follicular coordination of follicle development (Salmon *et al*., 2004).

The main physiological role of AMH in the mouse ovary seems to be limited to the inhibition of the early stages of follicular development (Themmen, 2005; Visser and Themmen, 2005), since both *in vivo* and *in vitro* experiments have indicated that the transition from primordial into growing follicles becomes enhanced in the absence of AMH, leading to early exhaustion of the primordial follicle pool (Durlinger *et al*., 2001) (Fig. 1).

In one study, the ovaries of 4-month-old AMH knockout mice contained three times as many small non-atretic growing follicles, and a reduced number of primordial follicles compared with their wild-type littermates (Durlinger *et al*., 1999). The increased rate of recruitment from the primordial pool observed in the AMH null mice was already evident before the initiation of the oestrous cycle. These studies confirmed the concept that in the absence of AMH, primordial follicles are recruited at a faster rate, resulting in premature exhaustion of the primordial follicle pool (Durlinger *et al*., 1999). Since AMH null mice have low levels of FSH, and yet increased numbers of growing follicles, it has been hypothesized that follicles are more sensitive to FSH in absence of AMH. The possible inhibitory effect of AMH on follicular sensitivity to FSH could play a role in the process of follicular selection (Durlinger *et al*., 1999; McGee and Hsueh, 2000). Diminished expression of AMH within the follicles would reduce the threshold level for FSH, allowing follicles to continue growing and to ovulate in the next estrous cycle (Durlinger *et al*., 2001; Visser *et al*., 2007). At present however, these views remain largely speculative as few *in vitro* or *in vivo* studies have been conducted which address the physiological role of AMH in the human ovary.

Current theories also suggest a role for AMH as a co-regulator of steroidogenesis in granulosa cells, as AMH levels appear to be related to estradiol levels in follicular fluid from small antral follicles (Andersen and Byskov, 2006). This was confirmed by a recent study which showed that polymorphisms in the gene for AMH or AMH receptor type II seem to be related to follicular phase estradiol levels, suggesting a role for AMH in the FSH-induced steroidogenesis in the human ovary (Kevenaar *et al*., 2007).

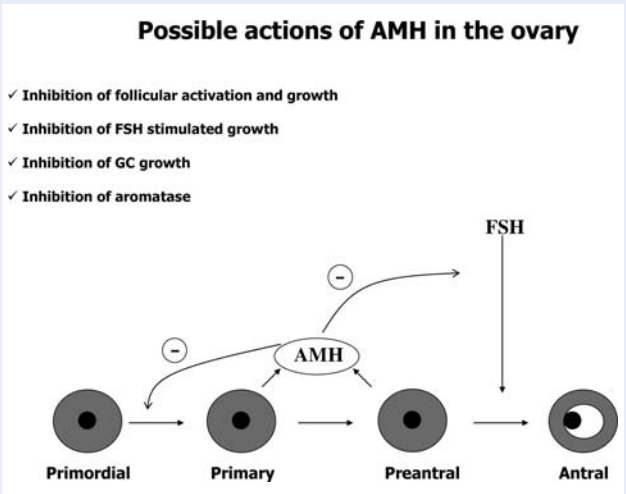

Figure 1 In women, AMH expression can first be observed in primary follicles, and is strongest in pre-antral and small antral follicles.

AMH may play an inhibiting role in initial recruitment and in the selection of the dominant follicle. Other possible autocrine and paracrine effects of AMH have been described and reviewed elsewhere (Visser, 2005; Themmen, 2005). AMH: Anti-Müllerian hormone; GC: Granulosa cell.

Issues requiring further investigation

The vast majority of *in vitro* studies on AMH have been conducted in rodents. Although the main findings also appear to apply to the human ovary, this still needs to be demonstrated. Important aspects which require further clarification include (a) precisely which follicles secrete AMH, (b) the role of AMH present in the follicular fluid, (c) the role of AMH in ovarian folliculogenesis and (d) the possible extra-ovarian effects of AMH.

Although AMH has been shown to have mainly autocrine and paracrine actions in follicle development, the protein is also measurable in serum. Antral follicles are considered to be the primary source of circulating AMH as they contain a large number of granulosa cells. A body of clinical data suggests that AMH is preferentially and constantly secreted by small rather than large antral follicles. The amount and the rate of AMH production by a single antral follicle should be investigated and in particular its modification in relation to the follicular hormonal milieu and to ageing.

Granulosa cells secrete AMH into both the bloodstream and follicular fluid, although concentrations are very much higher in the latter. However, the exact role of AMH in this compartment has not been elucidated. In addition to paracrine and autocrine actions on the granulosa cells, a possible direct role in modulating oocyte physiology may be identified.

A greater knowledge of the physiological role of AMH on human ovarian folliculogenesis is urgently required. Once this is elucidated, then the way will be open to designing experiments in the manipulation of folliculogenesis. As AMH seems to be a powerful brake on follicular transition, supraphysiological exposure to exogenous recombinant AMH may create a novel strategy for hormonal contraception,

and perhaps more importantly, delaying the process of reproductive ageing.

A further new field of research will focus on the possible extra-ovarian effects of AMH. AMH type II receptors have been identified in tissues other than the ovary, such as human endometrium (Wang *et al*., 2008), and several human cancer cell lines, including those originating from the cervix (Barbie *et al*., 2003), endometrium (Renaud *et al*., 2005), ovarian epithelium (Masiakos *et al*., 1999; Ha *et al*., 2000) and breast (Segev *et al*., 2000).

Measuring AMH

Current assays

Until recently, AMH assays were only available in a few laboratories around the world. The lack of access to a single reliable and standardized commercial assay has hindered the development of AMH as a clinical marker of ovarian reserve. A sensitive ELISA assay capable of detecting levels as low as 2 ng/ml was developed 9 years ago (Long *et al*., 2000). However, recently new commercial ultrasensitive sandwich ELISA assays has been developed capable of detecting concentrations <0.1 ng/ml. The increased sensitivity and availability of different assays has highlighted the urgent need to agree on the standard preparations used, in order to avoid confusion in reported levels and interpretation.

At present there are two highly sensitive sandwich ELISA assays available: the Diagnostic Systems Laboratories (DSL) and the Immunotech-Beckman assay. The sensitivity of the DSL is reported to be 0.025 ng/ml compared with 0.07 ng/ml for the Immunotech-Beckman assay, although this difference was not confirmed in a recent clinical study (Taieb *et al*., 2008). The intra- and inter-assay variations of the two assays are similar (<7 and $<5\%$, respectively). The DSL assay is not species-specific, a feature which could be advantageous for research laboratories using rodent models.

Initial studies comparing the two assays have shown that AMH levels appear to be 4–5-fold lower with the DSL assay compared with the Immunotech-Beckman assay (Bersinger *et al*., 2007; Fréour *et al*., 2007). In their report, Bersinger *et al*. (2007) alluded to problems inherent to AMH measurements that stem from residual matrix effects and instabilities of certain antigenic determinants. However, although developed independently, these assays are now both produced by a single company (Beckman-Coulter), and cross-referencing has shown that the correlation between the two assays is >0.9 (personal communication from Beckman-Coulter representative). This is confirmed by recent studies that found similar AMH values with both the assays (Taieb *et al*., 2008; Streuli *et al*., 2009), therefore suggesting that the methodological problems mentioned by Bersinger *et al*. (2007) should have been addressed and solved by the assay manufacturer.

Both kits are likely to remain in production over the next few years as approximately half of researchers are using the DSL assay and the other half the Immunotech-Beckman product.

AMH levels in women

In females, AMH levels are almost undetectable at birth. After an initially slight increase in the weeks after birth, AMH levels increase, peaking during late puberty (Lee *et al*., 1996) and then show a progressive decline throughout reproductive life as the follicular reserve becomes depleted (Lee *et al*., 1996; Guibourdenche *et al*., 2003), and finally becoming undetectable after menopause (Van Rooij *et al*., 2004; La Marca *et al*., 2005b). Further evidence that circulating AMH appears to be solely of ovarian origin comes from a study in which AMH was undetectable 3–5 days following bilateral ovariectomy (La Marca *et al*., 2005b).

AMH may constitute a unique endocrine parameter for the investigation of ovarian function, since several studies have demonstrated that, in contrast to sex steroids, gonadotrophins and peptides such as inhibin B, AMH serum levels do not significantly change throughout the menstrual cycle (Hehenkamp *et al*. 2006; La Marca *et al*., 2006b; Tsepelidis *et al*., 2007; Streuli *et al*., 2008). However, others have reported significant cyclical fluctuations in AMH levels with a rapid decrease in AMH levels in the early luteal phase (Wunder *et al*., 2008; Streuli *et al*., 2009). Excursions from mean levels of $+3$ to -19%, have been reported (Wunder *et al*., 2008; Streuli *et al*., 2009). These variations are similar to reported intercycle fluctuations for AMH (Streuli *et al*., 2008). In the clinical setting the inter- and intracycle variability in serum AMH levels may be considered to be low enough to permit random timing of AMH measurements during the menstrual cycle. Of course, further studies on a large sample of patients, based on daily blood samples are needed to clarify whether AMH levels vary significantly during the menstrual cycle. Up to now, reported fluctuations appear to be of small amplitude, and therefore probably of minor significance when interpreting data for clinical purposes.

Furthermore, AMH levels appear to be unmodified in conditions under which endogenous gonadotrophin release is substantially diminished, such as during pregnancy (La Marca *et al*., 2005a), under GnRH agonist pituitary down-regulation (Mohamed *et al*., 2006) and oral contraceptive administration (Arbo *et al*., 2007; Somunkiran *et al*., 2007; Streuli *et al*., 2008). This indicates that non-cyclic FSH-independent ovarian activity persists even when pituitary FSH secretion is suppressed. These findings are consistent with the concept that AMH levels reflect the continuous FSH-independent non-cyclic growth of small follicles in the ovary.

The role of AMH in investigating ovarian dysfunction

Hypo- and hypergonadotropic conditions

The observed relationship between the follicular ovarian pool and serum AMH levels, indicates that serum levels could provide additional information (linked to the follicle dynamics) during the diagnostic evaluation of hypogonadism. AMH serum levels have been found to be normal in women with hypogonadotropic amenorrhea indicating that initial follicle recruitment is not abolished in hypogonadotropic hypogonadism (La Marca *et al*., 2006a) (Fig. 2). This finding has been recently confirmed in young women with anorexia nervosa-related amenorrhea (Van Elburg *et al*., 2007). In contrast, in women with hypergonadotropic amenorrhea (Premature Ovarian Failure, POF) serum AMH levels are very low or undetectable. In a recent study in POF patients, the number of AMH immunopositive follicles present in ovarian biopsy material was closely correlated with

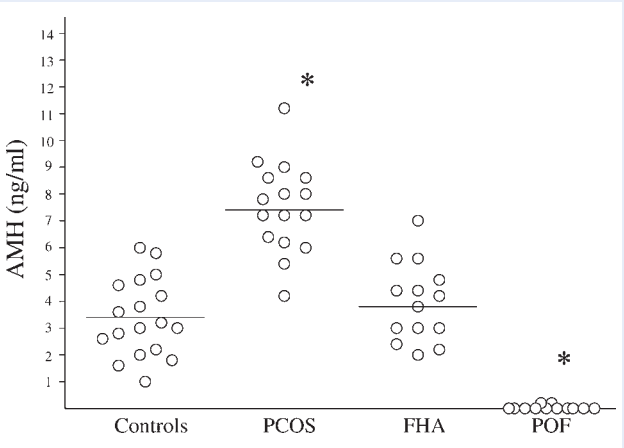

Figure 2 AMH plasma levels in patients and controls.

Mean AMH serum levels were significantly higher in PCOS and lower in POF than in the other groups. Women with functional hypothalamic amenorrhea (FHA) exhibit circulating AMH levels similar to normal cycling women (*$P < 0.05$) (from La Marca *et al*., 2006a, b). AMH: Anti-Müllerian hormone; PCOS: Polycystic ovary syndrome; POF: Premature ovarian failure.

serum AMH levels (Méduri *et al*., 2007), suggesting a diagnostic role for AMH in the evaluation of hypogonadism.

Serum AMH measurement may also have a role in identifying incipient ovarian failure in young eumenorrheic women with moderate hypergonadotropism. Incipient ovarian failure (Soules *et al*., 2001) may precede the onset of cycle irregularity (transitional ovarian failure) and hence the menopausal transition by 3–10 years. In a recent study, serum levels of AMH were able to discriminate between incipient and transitional ovarian failure, defined as elevated follicular phase FSH levels along with a regular menstrual cycle or cycle disturbances, respectively. In women with incipient failure, levels were below the 5th percentile of normo-ovulatory women in 25% and undetectable in 7%, compared with 66 and 52% in women with transitional ovarian failure (Knauff *et al*., 2008). This finding indicates that AMH provide an accurate assessment of ovarian follicle pool in young hypergonadotropic patients especially in the clinically challenging subgroups of patients with elevated FSH who do not fulfil the strict definition of POF (Knauff *et al*., 2008).

Moreover, young sisters or daughters of women affected by POF, patients undergoing ovarian surgery, and patients affected by Turner Syndrome may all benefit from the information which AMH levels might provide in this context. This is illustrated in a recent report on the fertility preservation in girls with Turner Syndrome (Borgström *et al*., 2008). Forty-seven young girls with Turner Syndrome underwent laparoscopy for ovarian tissue cryopreservation and 15 of them had follicles in the tissue piece analyzed. When investigating which factors had the highest predictive value for finding follicles, the most powerful were the presence of 46XX/XO chromosomal mosaicism, serum FSH levels below 11 IU/l and serum AMH levels >0.28 ng/ml (Borgström *et al*., 2008). AMH may therefore have a role in the diagnostic work-up and fertility counselling of patients with Turner Syndrome.

A particularly promising field for further research is assessing the value of AMH as a potential marker of ovarian function in women who have undergone chemotherapy or radiotherapy for malignant disease. Longitudinal studies have demonstrated that AMH measurement can be used as a reliable and early marker of ovarian damage, and that a decrease in AMH precedes alterations in other markers (Anderson *et al*., 2006; Loverro *et al*., 2007; Lutchman Singh *et al*., 2007, Van Beek *et al*., 2007; Lie Fong *et al*., 2008b). The ability to predict the impact of these treatments on the ovarian follicular pool (and consequently future fertility) in individual cases may be of considerable value in guiding clinicians and patients when considering whether or not fertility preservation strategies should be employed, and if so, which strategy is most appropriate. At present, care is guided largely by the nature of the intervention and the age of the woman.

Polycystic ovary syndrome

A number of studies have shown serum AMH levels to be increased in women with polycystic ovary syndrome (PCOS) compared with controls (Cook *et al*., 2002; Pigny *et al*., 2003; La Marca *et al*., 2004b; Laven *et al*., 2004; Mulders *et al*., 2004; Eldar-Geva *et al*., 2005b; Piltonen *et al*., 2005; La Marca *et al*., 2006a; Wachs *et al*., 2007) (Table I). This is thought to be the result of increased synthesis by

Table I Serum AMH levels have been found to be significantly increased in PCOS compared with healthy women.

Author	Year	Patients (*n*)	AMH levels (ng/ml)		
			Controls	PCOS	Mean % increase
Cook *et al*.	2002	47	2.4 ± 0.2	5.3 ± 0.7	+120
Pigny *et al*.	2003	104	2.9 ± 0.3	6.6 ± 0.4	+127
La Marca *et al*.	2004b	29	1.3 ± 0.5	5 ± 1.8	+284
Laven *et al*.	2004	109	2.1 ± 0.6	7.6 ± 1.8	+261
Piltonen *et al*.	2005	170	2.4 ± 0.2	8.1 ± 0.8	+237
Eldar-Geva *et al*.	2005b	52	1.6 ± 0.8	5.1 ± 1.3	+218
La Marca *et al*.	2006a	34	3.5 ± 1.5	7.4 ± 1.7	+111
Wachs *et al*.	2007	27	2.1 ± 0.4	7.2 ± 0.5	+242

AMH: Anti-Müllerian hormone; PCOS: Polycystic ovary syndrome.

granulosa cells and secretion of AMH in the polycystic ovaries (Mulders *et al*., 2004). Indeed levels of AMH are on average 75 times higher in granulosa cells from polycystic ovaries, compared with levels in normal ovaries (Pellatt *et al*., 2007). In addition, increased AMH levels in PCOS may be due to the disruption in folliculogenesis leading to an excess accumulation of pre-antral and small antral follicles (Wang *et al*., 2007).

Increased AMH levels have also been found in prepubertal (Sir-Petermann *et al*., 2007) and peripubertal (Crisosto *et al*., 2007) daughters of PCOS women as well as in adolescent PCOS girls with normal menstrual cycles (Siow *et al*., 2005), suggesting that altered follicle development is already present during infancy and early adulthood before the clinical phenotype of ovarian dysfunction is present.

AMH levels appear to be related to the severity of the syndrome since levels have been observed to be higher in insulin-resistant PCOS women than in patients with normal insulin sensitivity (Fleming *et al*., 2006). Similarly AMH is higher in amenorrheic compared with oligomenorrheic women with PCOS (La Marca *et al*., 2004b), which could indicate a role for AMH in the pathogenesis of PCOS-related anovulation. Alternatively, high AMH values could reflect more impaired disruption in folliculogenesis and granulosa cell function in the ovary of amenorrheic compared with oligomenorrheic PCOS women (La Marca *et al*., 2004b).

In a recent longitudinal study, serum AMH levels were measured in 98 women with PCOS and 41 controls at two time points (interval between the visits: 0.3–9 years). Although serum AMH levels declined over time in both groups, the reduction observed in PCOS patients was less than that in controls. The authors of this study postulated that this may indicate a longer reproductive life span in PCOS patients (Mulders *et al*., 2004). On histological examination, polycystic ovaries exhibit a median density of small pre-antral follicles, including those at primordial and primary stages, 6-fold greater than in normal ovaries (Hughesdon, 1982; Webber *et al*., 2003). Hence it may be proposed that the process of ovarian ageing is delayed in women with PCOS since high AMH levels may inhibit the primordial follicle pool depletion (Mulders *et al*., 2004).

AMH measurement has been found to offer a relatively high specificity and sensitivity (92 and 67%, respectively) as a diagnostic marker for PCOS (Pigny *et al*., 2006). On this basis it has been proposed that in situations where accurate ultrasound data are not available, AMH could be used instead of the follicle count as a diagnostic criterion for PCOS (Pigny *et al*., 2006).

Other than for diagnostic evaluation, AMH measurement may also be useful in the therapeutic approach of PCOS patients. Indeed overweight women with PCOS who respond to weight loss with menstrual improvements have significantly reduced preweight-loss AMH levels, indicating that baseline AMH may provide a potential clinical predictor of menstrual improvements with weight loss in PCOS (Moran *et al*., 2007). Similarly, basal AMH level evaluation may be useful in the prediction of ovarian response to clomiphene citrate (El-Halawaty *et al*., 2007). Finally it has been shown that metformin administration in women affected by PCOS is associated with a reduction in both AMH serum levels and antral follicles, suggesting that the measurement of AMH could be used to evaluate the treatment efficacy with insulin sensitizers (Piltonen *et al*., 2005).

Issues requiring further investigation

Studies to date indicate that AMH may be very informative in defining conditions associated with hypogonadism. In patients with hypergonadotropic hypogonadism AMH appears helpful in characterizing the degree of ovarian failure and for identifying women who may still have some residual ovarian activity. It remains to be clarified whether differences in individual AMH levels in women with incipient, transitional or POF may indicate a different pathophysiology of the hypogonadism itself. The idea that AMH measurement may be predictive of the duration of reproductive life span in sisters and daughters of patients with POF is very attractive but still needs to be investigated.

As an early marker capable of identifying subtle damage to the ovaries, AMH may become the test of choice in studies on ovarian damage due to any kind of agent, such as chemotherapy, ovarian surgery and even disease processes like endometriosis. Studies on longitudinal changes in AMH levels during and after chemotherapy may also be very informative in establishing the gonadotoxicity of different chemotherapeutics and the extent of possible protective effect of oral contraceptive and GnRH analog co-treatment. Similarly studies are needed on the possible negative effect of endometriosis on the ovarian reserve and in particular on the consequences of different therapeutic strategies (medical versus surgical therapy) for endometrioma on the residual ovarian reserve.

Regarding PCOS, the collective data clearly demonstrate that increased AMH levels are consistently associated with this syndrome. Concerning the pathophysiology of PCOS, it is fundamental to know whether this exaggerated AMH production might be the consequence of the disrupted folliculogenesis (accumulation of small antral follicles) or whether it may also be the cause (AMH inhibits the follicular transition). This may also have clinical implications. If AMH is shown to cause the PCOS-associated follicular disruption, the development of an AMH antagonist may provide the basis for new therapy.

The use of AMH measurement as a substitute of follicle count as a diagnostic criterion in the definition of PCOS should be tested in well-designed large trial. Moreover, additional studies are needed to evaluate the utility of AMH in the early identification of adolscents at risk for the development of PCOS. An interesting field of clinical research is the AMH-based possibility to predict patients who will respond to treatments such as weight loss and clomiphene citrate and to monitoring treatment with insulin sensitizers.

The role of AMH as a marker for ovarian reserve

AMH as longitudinal marker for ovarian reserve

The age-related decline in female reproductive function due to the reduction of the ovarian follicle pool and the quality of oocytes has been well established (Macklon and Fauser, 2005). A reliable marker for the age at which subfertility will occur would have great potential value as a predictor of future reproductive lifespan. The ideal marker would show a significant change in levels from adolescence to the late reproductive period. Increased basal levels of FSH, and a decrease in inhibin B and in the antral follicle count (AFC) on ultrasound

examination are widely taken to indicate a reduced ovarian reserve (Broekmans *et al*., 2006).

Recent studies have indicated that AMH may constitute an important novel measure of ovarian reserve. Evidence for this comes from studies demonstrating that serum AMH levels fall throughout reproductive life (de Vet *et al*., 2002), with levels becoming undetectable after spontaneous menopause (Lee *et al*., 1996; Van Rooij *et al*., 2004; La Marca *et al*., 2005b).

Serum AMH levels on day 3 of the menstrual cycle show a progressive decrease with age, and appear to correlate well with AFCs age, and FSH (de Vet *et al*., 2002). In an interesting prospective study, a group of women was followed longitudinally, with an interval between the two visits ranging from 1.1 years to 7.3 years. Although the number of antral follicles and the levels of FSH and inhibin B did not change, a reduction in mean AMH levels of about 38% was observed (de Vet *et al*., 2002).

The same group prospectively studied 81 women for 4 years (mean age 39.6 and 43.6 at the beginning and at the end of the study, respectively). Although the AFCs did not change over this time period, AMH, FSH and inhibin B all demonstrated significant changes. However, AMH was the only marker of ovarian reserve showing a mean longitudinal decline over time both in younger women (<35 years) and in women over 40 years (Van Rooij *et al*., 2004).

Recently, AMH levels were measured in 144 fertile normal volunteers and used to determine an estimate of mean AMH as a function of age (Van Disseldorp *et al*., 2008). There was good conformity between the observed distribution of age at menopause and that predicted from declining AMH levels, further supporting the hypothesis that AMH levels may predict the age of onset of menopause. Other studies have recently confirmed that a single AMH measurement may be a good predictor for the onset of menopause in ageing women (Sowers *et al*., 2008; Tehrani *et al*., 2009).

In conclusion, compared with other known markers, AMH seems to better reflect the continuous decline of the oocyte/follicle pool with age (Van Rooij *et al*., 2004). The decrease in AMH with advancing age may be present before changes in currently-known ageing-related variables, suggesting that serum AMH levels may be the best marker of ovarian ageing and menopausal transition (Fig. 3).

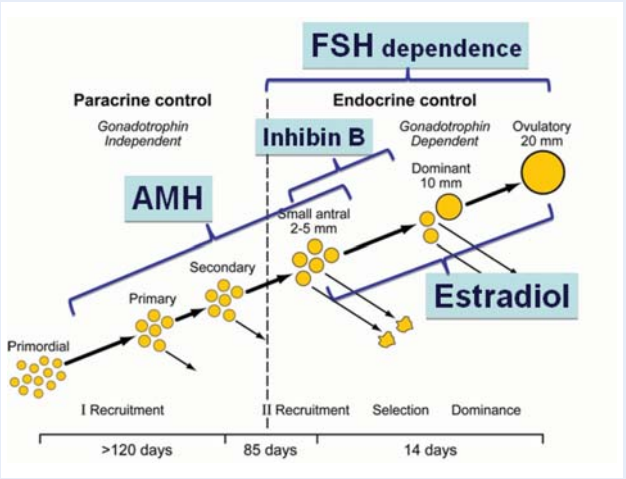

Figure 3 In contrast to the other known ovarian reserve markers, AMH may not only reflect the number of early and developing antral follicles, but also earlier stages of follicle development.

The FSH, estradiol and inhibin secretion are mutually connected by negative feedback. Therefore, their circulating levels are only an indirect reflection of the number of antral follicles. The E2 levels are less a reflection of the number of antral follicles, but rather of their growth activity during the follicular phase. On this basis, the highest biological plausibility as marker of ovarian reserve is to be attributed to AMH, followed by inhibin B, FSH and E2. AMH: Anti-Müllerian hormone; E2: estradiol; FSH: follicle stimulating hormone.

AMH as predictor of outcome in infertile women

During ovarian stimulation, serum AMH levels correlate positively with the number of small (but not large) antral follicles, and with inhibin B serum levels (Fanchin *et al*., 2003a, b). Serum AMH levels have been shown to decline gradually during multiple follicular development, probably reflecting the dramatic reduction in the number of small antral follicles, and confirming the reported low levels of AMH expression by larger follicles (Bézard *et al*., 1988; Fanchin *et al*., 2003a, b; La Marca *et al*., 2004a).

In the evaluation of AMH as a marker of ovarian response, the first paper reporting an association between circulating AMH and ovarian response to gonadotrophin stimulation was published by Seifer *et al*. (2002) who reported that higher day 3 serum AMH levels were associated with a greater number of retrieved oocytes. This finding was confirmed by several retrospective and prospective studies by a number of independent groups (Van Rooij *et al*., 2002; Fanchin *et al*., 2003a, b; Hazout *et al*., 2004; Muttukrishna *et al*., 2004, 2005; Eldar-Geva *et al*., 2005a; Tremellen *et al*., 2005; Peñarrubia *et al*., 2005; Fiçicioglu *et al*., 2006; La Marca *et al*., 2007; Kwee *et al*., 2007; Elgindy *et al*., 2008; McIlveen *et al*., 2007; Nelson *et al*., 2007; Wunder *et al*., 2008; Gnoth *et al*., 2008; Nardo *et al*., 2008; Jayaprakasan *et al*., 2008; Nelson *et al*., 2009).

The currently available literature indicates that AMH may be a superior marker for predicting ovarian response over either age of the patient, day 3 FSH, estradiol or inhibin B, whereas the vast majority of studies have found AMH and AFC to have similar predictive value for the poor response. Recently, a meta-analysis compared the value of serum AMH levels with the performance of the AFC as a test to predict poor ovarian response and pregnancy occurrence after IVF. A total of 13 studies were found reporting on AMH and 17 on AFC. The receiver operator curves for the prediction of poor response revealed no significant difference between the predictive value of AMH and AFC. For the prediction of non-pregnancy, both serum AMH levels and AFC were shown be similarly poor performers. In conclusion, at present AMH appears to offer at least the same level of accuracy and clinical value for the prediction of poor response and non-pregnancy as AFC (Broer *et al*., 2008) (Fig. 4).

Serum AMH levels may also be predictive of a hyper-response to FSH, and consequently may be useful in the prediction of women at risk of ovarian hyperstimulation syndrome (OHSS) (Nakhuda *et al*., 2006; Tremellen *et al*., 2005; Lee *et al*., 2008). Lee *et al.* (2008) have clearly demonstrated that basal AMH measurement works as well as the number of follicles and estradiol levels on the day of hCG in the identification of women who will develop OHSS.

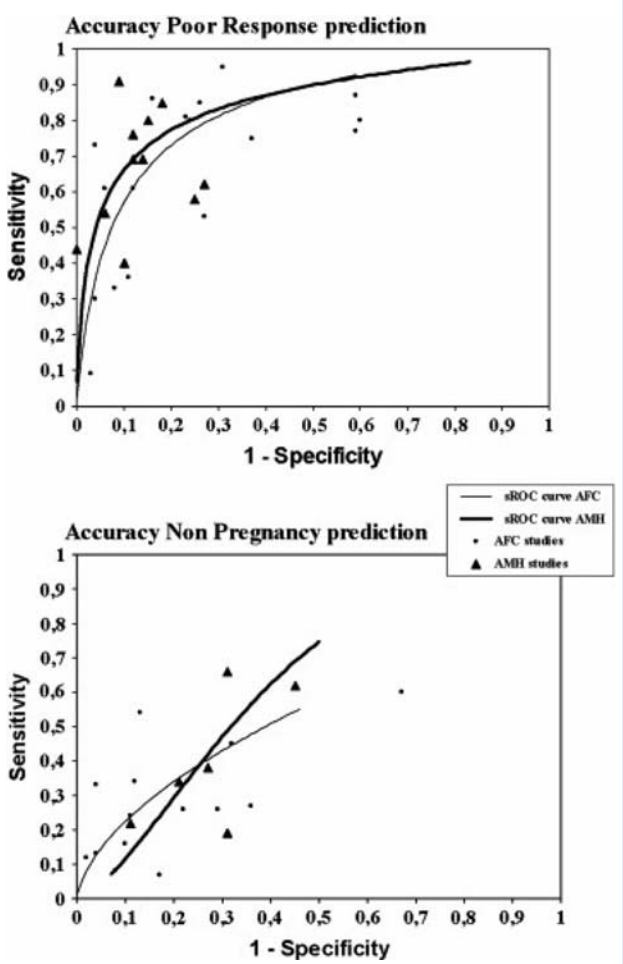

Figure 4 Accuracy of poor response and non-pregnancy predictions.

AMH is, next to the AFC, the currently best predictor of the occurrence of poor response to hyperstimulation for IVF. Pregnancy prediction remains inadequate (from Broer *et al*., 2008). AFC: Antral follicle count; AMH: Anti-Müllerian hormone; ROC: Receiver operating characteristic.

One of the main advantages of AMH measurement in ART compared with the other markers of ovarian reserve may derive from its low inter- and intra-cycle variability. The relative stability and consistency of AMH serum levels indicate that AMH could be used as a menstrual cycle-independent marker of ovarian response to controlled ovarian stimulation. The first study to address this demonstrated that AMH measurement on any cycle day has a predictive performance for response to ovarian stimulation in ART (La Marca *et al*., 2007). This finding has recently been confirmed in a large prospective study on 538 patients undergoing ART (Nelson *et al*., 2009), hence strengthening the concept that blood sampling at any point of the menstrual cycle will be the preferred method for AMH measurement, because of its undisputed advantages for both the patient and the clinician.

In addition to reflecting the quantitative ovarian response, several authors have found a significant positive correlation between AMH levels and oocyte quality (Hazout *et al*., 2004; Ebner *et al*. 2006; Silberstein *et al*., 2006; Cupisti *et al*., 2007), and embryo morphology (Silberstein *et al*., 2006). However, this relationship has not been confirmed by others (Smeenk *et al*., 2007; Lie Fong *et al*., 2008a). Moreover, in a recent study, no consistent correlation between AMH and embryo morphology, and most importantly embryo aneuploidy rate, was demonstrable (Lie Fong *et al*., 2008a). Hence the possible prediction of qualitative aspects of ART programs by AMH measurement remains largely controversial. This is also evident from studies reporting on the pregnancy rate following IVF. A number of authors have tried to identify cut-off levels for AMH that are able to distinguish between pregnancy and non-pregnancy (Hazout *et al*., 2004; Eldar-Geva *et al*., 2005a; Kwee *et al*., 2007; Elgindy *et al*., 2008). However, the majority of them indicated that AMH measurement is not useful for predicting this end-point (Van Rooij *et al*., 2002; Fanchin *et al*., 2003b; Penarrubia *et al*., 2005; Ebner *et al*., 2006; Fiçicioglu *et al*., 2006; Kwee *et al*., 2007; Smeenk *et al*., 2007). Up to the present, only one study has been published relating serum AMH levels to the live birth rate following IVF (Nelson *et al*., 2009). In this prospective study of 340 patients it was demonstrated that the live birth rate dramatically increases with increasing basal AMH value. However, as concluded by the same author, this finding may at least in part be explained by the very good correlation existing between basal AMH and the number of retrieved oocytes (Nelson *et al*., 2009), indicating that circulating AMH may definitely be considered a better marker for quantitative than for qualitative aspects of ART.

Issues requiring further investigation

Since a considerable proportion of female subfertility arises due to postponement of childbearing, any reliable measurement of ovarian reserve may also be of interest to women in general. On this basis, commercial tests have become available to the general public which, by combining measurements of AMH and other markers, claims to indicate individual fertility chances and the state of advancement of ovarian ageing. However, at present no longitudinal data on AMH values during the reproductive period are available, and the normal ranges according to the age of patients remain to be clarified. Most importantly it is still not known whether AMH levels may be predictive, independent of age, of spontaneous pregnancy in the general population. Taking into account these current uncertainties, the application of AMH measurement for fertility assessment in the general population is premature (Broekmans *et al*., 2008).

Assessment of the ovarian reserve is particularly relevant in the IVF clinic, where AMH may be useful as predictor of the extremes of ovarian response to FSH, namely the poor- and hyper-response. In this context AMH measurement is useful in improving the counselling of patients, enhancing the ability to inform them about the risk and consequences of poor response (cycle cancellation, embryo transfer cancellation, low chance of pregnancy and protracted treatment) and hyper-response (fresh embryo transfer cancellation and OHSS).

A more contentious point is whether AMH measurements should be used to deny IVF treatment to couples shown by such a test to have a poor prognosis. This is an issue which may arise with other predictors of ovarian response to the IVF treatment. Although a number of markers, including AMH, may be predictive of ovarian response, none are 100% reliable. Moreover, AMH, as with other markers of ovarian reserve, is a poor predictor of who will achieve a pregnancy

Table II Changes in markers of ovarian function during women's life and in some pathological conditions (modified from La Marca *et al*., 2006c).

Markers*	Late reproductive period	Post-menopause	Pregnancy	PCOS	Hypogonadotropic hypogonadism	Hypergonadotropic hypogonadism
FSH	↔/↑	↑	↓	↔	↔/↓	↑
Estradiol	↔/↑	↓	↑	↔/↑	↔/↓	↓
Inhibin B	↓	↓	↔	↔/↑	↔	↓
AMH	↓	nd	↔	↑	↔	↓/nd

*Normal values are considered those found in healthy women of reproductive age.
Symbols: ↔: Not modified when compared with healthy women of reproductive age.
↑: Increased when compared with healthy women of reproductive age.
↓: Decreased when compared with healthy women of reproductive age.
nd: Not detectable.
AMH: Anti-Müllerian hormone; PCOS: Polycystic ovary syndrome.

after IVF. Indeed, it has been widely demonstrated that many poor responders, in particular young ones, achieve pregnancy and live birth (Lashen *et al*., 1999; Ulug *et al*., 2003; Klinkert *et al*., 2004; van der Gaast *et al*., 2006). This indicates that AMH measurement, similarly to the other ovarian reserve markers, should not be offered with the aim of withholding IVF (Broer *et al*., 2008), in particular to women undergoing their first IVF.

A new interesting field of application is the individualization of treatment strategy on the basis of the AMH-based ovarian reserve assessment, in order to possibly reduce the incidence of cycle cancellation and OHSS. Since low and high AMH values are predictive of poor- and high-response to gonadotrophins, respectively, it has been proposed that FSH dose may be adjusted according to the pre-IVF AMH levels and independently of the age and BMI of the patient (Nelson *et al*., 2007; Gnoth *et al*., 2008; Nelson *et al*., 2009). In a recent prospective study performed on more than 500 patients undergoing IVF (Nelson *et al*., 2009), the AMH-based strategy of controlled ovarian stimulation (COS) was associated with a significant reduction of excess response to stimulation and in reduced treatment burden, reduced cycle cancellation and a trend towards increased clinical efficacy. Although limited by its non-randomized design, this study indicates that a single AMH assay may be used to individualize treatment strategies for IVF. However, the cost/benefit of its use as a single assay before initiation of an IVF cycle and whether the AMH-determined strategy of COS for assisted conception may be associated with an improved live birth rate still need to be clarified in well-designed prospective studies.

Conclusions

In summary, the currently available data indicate that AMH is produced by growing pre-antral and early antral follicles. It has been demonstrated in mice that AMH inhibits initial follicle recruitment from the resting primordial stage. In addition, AMH may affect FSH-dependent growth of more mature follicles. However, the precise nature of the function of AMH within the human ovary, and in particular the paracrine role of AMH on ovarian folliculogenesis and steroidogenesis in the human remains largely speculative.

As AMH is related to the ovarian follicular status, circulating AMH measurement may provide useful information in women with ovarian dysfunction. The changes in AMH levels in several physiological and pathological conditions are reported in the Table II.

Circulating AMH levels are increased in women with PCOS and its use as a clinical diagnostic marker for the syndrome has been proposed. It is still unclear whether AMH levels may reflect the severity of ovarian function disruption or have a role in predicting the outcome of individual treatment regimens.

AMH could be a valuable marker of ovarian reserve in the general population, which may facilitate reproductive life planning for women. However, longitudinal data on AMH values during the reproductive life span are not available, and it remains unknown whether AMH levels may enable age-independent prediction of an individuals reproductive lifespan and spontaneous pregnancy in the general population. At present, the application of the AMH measurement for fertility assessment in the general population outside the context of research studies is inappropriate.

The relative stability and consistency of AMH serum levels indicate that AMH could be used as a predictor of ovarian response to controlled ovarian stimulation. In this application, the predictive power of AMH for ovarian response to FSH appears to be similar to that demonstrated by the antral follicular count. One main advantage of AMH measurement may be its use as a cycle-independent test. The exact role of AMH measurement in the IVF setting should be clarified also considering a cost-benefit analysis. With high probability the most useful clinical application of AMH measurement may be in the individualization of treatment strategies for COS.

In summary, although AMH has the potential to increase our understanding of ovarian pathophysiology, and to guide the clinical management of a broad range of conditions, a number of important questions relating to both the basic physiology of AMH and its clinical implications still need to be unravelled, particularly in the human.

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