Human Reproduction Update Advance Access published May 12, 2014
Human Reproduction Update, Vol.0, No.0 pp. 1–14, 2014
doi:10.1093/humupd/dmu020

human reproduction update

Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications

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Submitted on October 28, 2013; resubmitted on February 4, 2014; accepted on March 14, 2014

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BACKGROUND: In women, anti-Müllerian hormone (AMH) is exclusively produced by granulosa cells of ovarian follicles during the early stages of follicle development. After an initial increase until early adulthood, AMH concentrations slowly decrease with increasing age until becoming undetectable ~5 years before menopause when the stock of primordial follicles is exhausted. However, major individual variability exists in the pace of follicle pool depletion and the initial size of the follicle pool, reflected by a wide range of age at menopause. Individual AMH serum concentration does accurately reflect the size of the pool of antral follicles, representing the quantity of the remaining primordial follicles. Accordingly, AMH levels may vary significantly in women of the same chronological age, allowing AMH to predict the remaining length of a woman's reproductive lifespan.

METHODS: Following 10 years of intense clinical research in this area (with over 300 papers published in core clinical journals every year), the level of evidence justifying use of AMH in ovarian reserve testing is rapidly increasing. We have conducted a summarizing review regarding all evidence published.

RESULTS: Many studies have convincingly demonstrated that AMH is the best currently available measure of ovarian reserve under a variety of clinical situations, such as infertility treatment (especially IVF), the forecasting of reproductive lifespan, ovarian dysfunction (especially polycystic ovary syndrome) and gonadotoxic cancer treatment or ovarian surgery. Moreover, AMH may help to individualize dosing for ovarian stimulation thereby improving the efficiency and safety of IVF. However, there are concerns about the performance of the AMH assay under different

conditions regarding storage of samples and handling techniques. Therefore an international guideline for laboratories and a reference preparation are needed to make test results between laboratories truly comparable.

CONCLUSIONS: AMH is the best current available measure of ovarian reserve for different clinical conditions. However, prospective well powered studies comparing different infertility treatment strategies based on initial AMH levels using appropriate end-points, such as live birth and cost-effectiveness, are urgently awaited. Such studies could represent a true step forward in rendering counseling and infertility care more patient tailored.

Key words: anti-Müllerian hormone / anti-Müllerian hormone assay / ovarian reserve testing / menopause / polycystic ovaries

Background

The gonadal hormone anti-Müllerian hormone (AMH) is a 140 kDa disulphide-linked homodimeric glycoprotein and a member of the transforming growth factor-β (TGF-β) superfamily of growth and differentiation factors, just like inhibins and activins. AMH, initially referred to as Müllerian-inhibiting substance (MIS), has been known since the 1940s for its role in male sexual differentiation during early embryonic development due to the pioneering work of Alfred Jost. AMH, produced by fetal Sertoli cells, induces regression of the Müllerian duct, allowing Wolffian ducts to develop into the male reproductive tract under the influence of testosterone (Wilson *et al.*, 1981). Testicular production of AMH has been described as early as 8 weeks gestation (Lee *et al.*, 1997).

In the absence of AMH, the embryo develops into a female, allowing the Müllerian duct to differentiate into the upper vagina, uterus and oviduct. However, in later fetal life AMH is synthesized by granulosa cells residing within ovarian follicles, as first described in the adult chicken ovary (Hutson *et al.*, 1981). In the human fetus, ovarian AMH production starts around birth (Rajpert-De Meyts *et al.*, 1999). In antral follicles AMH is predominantly secreted into the intrafollicular compartment giving rise to high follicular fluid concentrations. The secreted quantities are large enough to permit the detection of AMH in the circulation (Hudson *et al.*, 1990; Josso *et al.*, 1990; Lee *et al.*, 1997; Jeppesen *et al.*, 2013).

Molecular mechanisms involved in AMH action exhibit many similarities with those of TGF-β. Ligand binding specifically to the extracellular domain of the AMH type II transmembrane receptor causes phosphorylation of the type I receptor and subsequent downstream signaling via intracellular Smad proteins (Teixeira *et al.*, 2001; Salhi *et al.*, 2004). In the human, mutations have been described in genes encoding AMH itself (located on chromosome 19) or its type II receptor (chromosome 12). Such mutations (affecting ligand binding, signal transduction or intracellular transport) often exhibit autosomal recessive segregation, causing the persistent Müllerian duct syndrome in men (Josso *et al.*, 2005; Belville *et al.*, 2009). Interestingly, several family studies have shown normal fertility of affected sisters (Abduljabbar *et al.*, 2012). AMH type II receptors have also been demonstrated in other tissues (such as the brain, breast and endometrium) although their functional role remains elusive (Segev *et al.*, 2000; Lebeurrier *et al.*, 2008; Wang *et al.*, 2009).

Animal experiments using AMH knock-out mice disclosed important intra-ovarian roles for AMH in inhibiting growth of resting primordial follicles. Augmented primordial follicle recruitment was observed in AMH null mice compared with the wild type. At 4 months, the number preantral and small antral follicles was observed to be increased. In contrast, both at 4 and 13 months of age, the number of primordial follicles was significantly reduced. Collectively, these observations suggest that in mice 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, 2001). Since AMH null mice exhibit low levels of FSH along with increased numbers of growing follicles, it has been hypothesized that follicles are more sensitive to FSH in the absence of AMH. The presumed inhibitory effect of AMH on follicular sensitivity to FSH could play a role in the process of dominant follicle selection (Durlinger *et al.*, 1999; McGee and Hsueh, 2000). As antral follicles become larger, the AMH expression diminishes and this could reduce the threshold level for FSH, allowing follicles to continue growing and to ovulate in the next estrous cycle (Durlinger *et al.*, 2001; Al Qahtani *et al.*, 2005; Visser *et al.*, 2007). It remains to be shown, however, whether such intra-ovarian paracrine roles for AMH are also operative in the human.

Related human ovarian physiology

Immunohistochemistry in human ovarian tissue confirmed the absence of AMH staining in primordial follicles, along with high AMH expression in primary, secondary, pre-antral and early antral follicles <4 mm diameter. AMH staining gradually disappeared in follicles between 4 and 8 mm diameter (Weenen *et al.*, 2004) (Fig. 1). A recent study confirmed this finding, demonstrating that AMH gene expression and total AMH protein in follicular fluid increased until a follicular diameter of 8 mm, after which a sharp decline occurred (Jeppesen *et al.*, 2013). It was demonstrated that 5–8 mm follicles contribute ~60% of the circulating AMH (Jeppesen *et al.*, 2013). In contrast, granulosa cells of larger pre-ovulatory follicles beyond 10 mm fail to produce AMH.

In ovarian hyperstimulation protocols (when the majority of antral follicles develop into large dominant follicles), a significant decline in serum AMH levels is observed (Fanchin *et al.*, 2003). Moreover, with increasing age the prevalence of large follicles increases, which may also have a negative association with AMH (Bentzen *et al.*, 2013). In conclusion, AMH in the peripheral circulation is mainly derived from antral follicles. Since this number is proportionally related to the primordial follicle pool (Gougeon, 1984), AMH may indirectly represent the ovarian reserve.

Early follicle development, before secondary follicle recruitment, is largely gonadotrophin independent (Fauser and Van Heusden, 1997). AMH serum levels are not affected by dominant follicle growth during the late follicular phase of the normal menstrual cycle. This renders AMH easy to use clinically as opposed to other currently available markers of ovarian aging, such as inhibin B, estradiol (E_2) and FSH, which are all menstrual cycle dependent (Fig. 2) and constitute relatively late markers of the ongoing process of primordial follicle pool depletion.

Ovarian aging relates to the decline of the quantity and quality of the ovarian follicle pool with increasing age. Preliminary initial studies

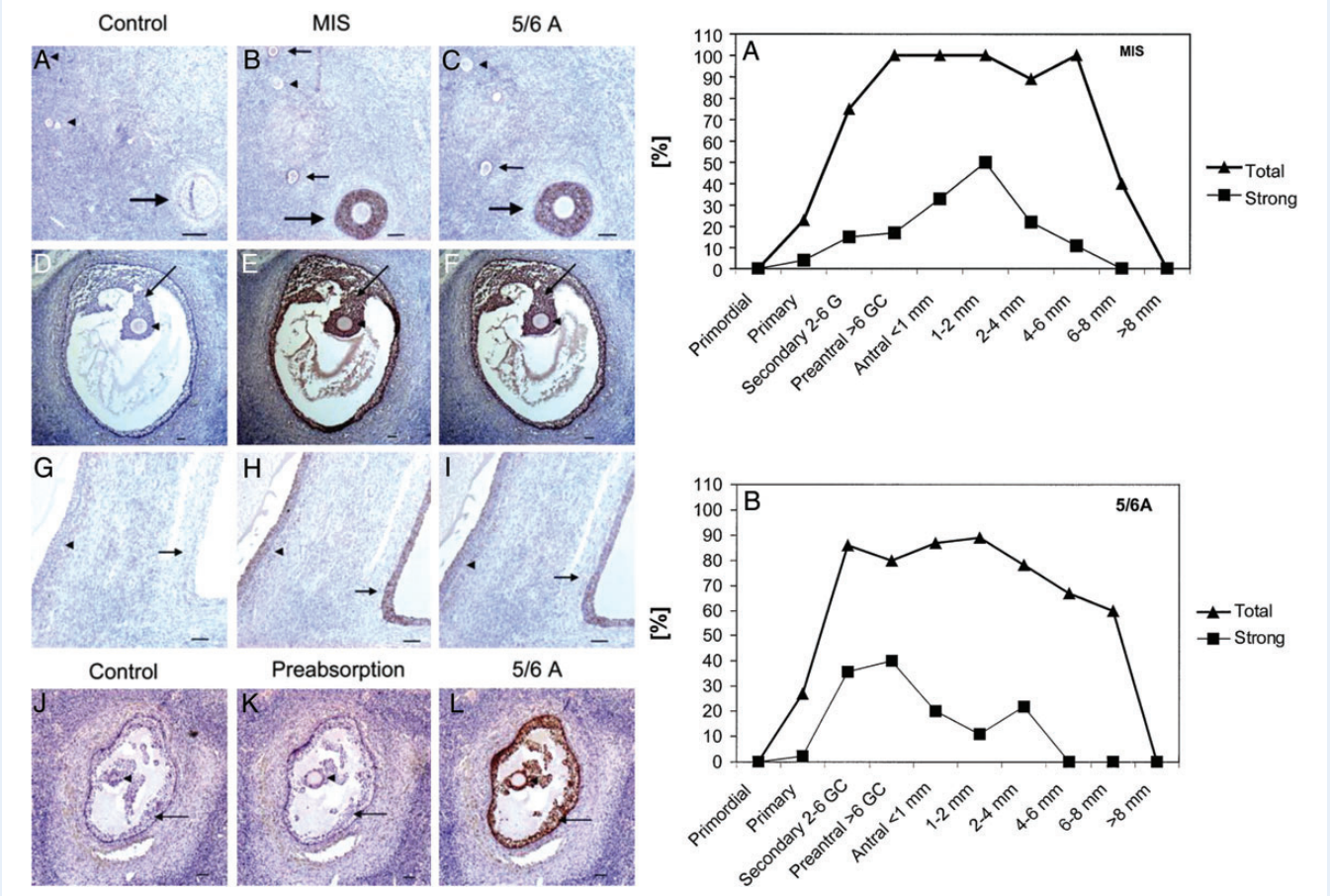

Figure 1 Anti-Müllerian hormone (AMH) immunohistochemical staining in human ovarian tissue sections, along with the graphical summary data, from Weenen *et al.* (2004). Left: Micrographs of AMH immunohistochemistry staining of human ovarian tissue sections. Specific (brown) AMH stain deposition is present in the cytoplasm of granulosa cells (GC). Scale bar = 100 mm. Sections A, D and G are controls; sections B, E and H were stained using the Müllerian-inhibiting substance (MIS) C-20 antibody; sections C, F and I using the 5/6A antibody. (**A**–**C**) 100 magnification with a primordial follicle (arrowhead), a primary follicle (small arrow) and a secondary follicle (large arrow). Primordial follicles show no immunostaining of the cytoplasma of the granulosa cells, primary follicles show normal staining (+) and secondary follicle shows strong staining (++). (**D**–**F**) 340 magnification with a small antral follicle <1 mm. The oocyte shows weak staining (arrowhead), whereas the granulosa cells (arrow) show strong staining (++). (**G**–**I**) 100 magnification showing two larger antral follicles. Left side follicle (6.1 mm diameter) shows weak staining (+/±) of the granulosa cells (arrowhead), smaller follicle (2.5 mm diameter) right side (arrow) shows normal staining (+). (**J**–**L**) 340 magnification with a small antral follicle <1 mm. The oocyte shows weak, non-specific brown staining (arrowhead). The granulosa cells show strong staining (++) with the 5/6A antibody. When the peptide is added to the antibody, no immunohistochemical staining occurs (K). Right: Graphical summary of the immunohistochemical data. Only the percentages of follicles with strong (++) and total staining (+ and ++) are shown. The total staining in the graphical depictions represents an addition of the percentage of follicles within a certain class with normal (+) and strong (++) staining (total staining; triangles). For comparison the percentage of follicles of a certain class that show strong staining is depicted separately (strong staining; squares). Staining increases rapidly with the stage of the follicles and decreases when follicles are >4–6 mm diameter. In the upper graph (A), staining with the MIS antibody is shown. In the lower graph (B), staining with the 5/6A antibody is shown.

proposed AMH as a putative marker of ovarian aging by demonstrating decreasing levels over time in young normo-ovulatory women. A strong correlation between AMH and the antral follicle count (AFC) assessed by transvaginal ultrasound was also observed (de Vet *et al.*, 2002). A similar association between AMH and age, AFC, and FSH was subsequently described in women undergoing IVF (van Rooij *et al.*, 2002). Numerous additional studies confirmed that AMH serum levels decline with increasing chronological age. In women from 21 years of age and onwards the average annual decline has been calculated to be 5.6% (Bentzen *et al.*, 2013). Further proof of the validity of AMH indirectly reflecting the size of the primordial follicle pool comes from an elegant study in 42 women undergoing oophorectomy for benign gynecologic reasons (Fig. 3). After adjusting for age, both AMH and AFC correlated well with the number of primordial follicles present in ovarian tissue (Hansen *et al.*, 2011).

Major individual variability exists in ovarian aging—believed to predominantly result from differences in the pace of follicle pool depletion—coinciding with a large age range of normal menopause between 40 and 60 years (te Velde and Pearson, 2002; Broekmans *et al.*, 2009). Some women at age 35 years may present with much more advanced ovarian aging than others; i.e. her individual AMH concentration may be similar to mean levels of women at age 45 years (Kelsey *et al.*,

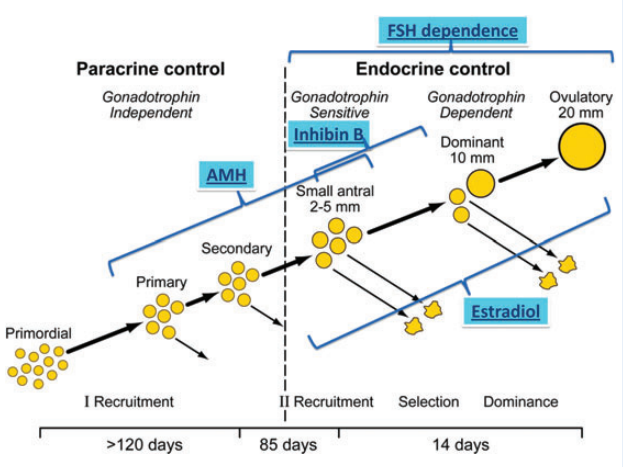

Figure 2 Schematic representation of follicle development emphasizing that AMH is produced in early stages of follicle development (characterized by gonadotrophin-independent growth), as opposed to Inhibin B and estradiol produced by follicles at later stages of development where growth is FSH-dependent. Adapted from McGee and Hsueh (2000).

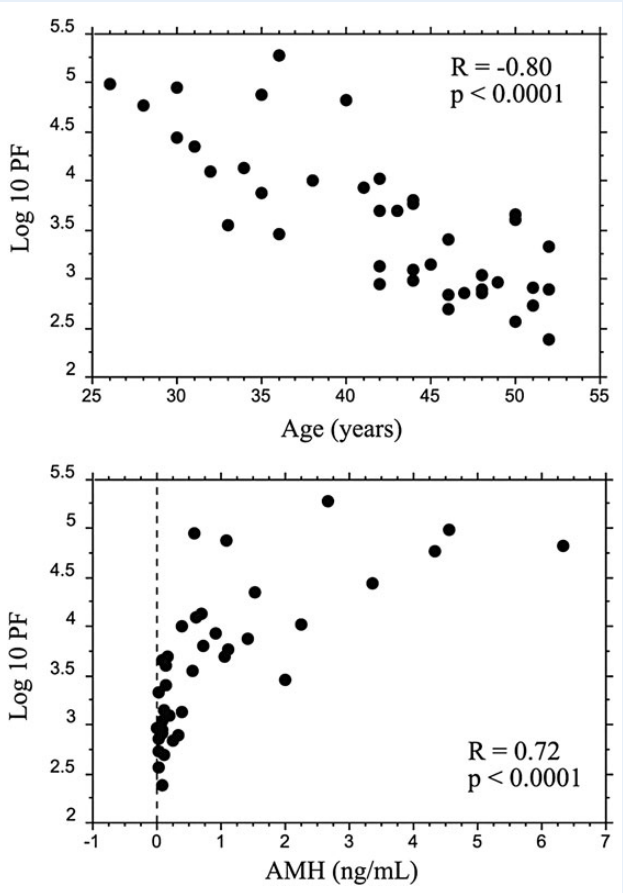

Figure 3 Age (top panel) and AMH serum levels (lower panel) in relation to number of remaining primordial follicles (PF) as assessed in human ovarian tissue, from Hansen et al. (2011).

2011). In recent years several nomograms for normal levels of serum AMH from birth to menopause have been developed (Kelsey et al., 2011; Nelson et al., 2011a, b). Collectively these studies show that AMH levels are low during prepubertal development, rise during early puberty and reach a plateau ~20–25 years of age, followed by a gradual decline thereafter until becoming undetectable around menopause (Fig. 4) (Hagen et al., 2010; Kelsey et al., 2011; Lie Fong et al., 2012).

It is not yet understood why AMH levels rise during childhood and adolescence but changes in the hypothalamic–pituitary–ovarian axis along with differences in the dynamics of early follicle recruitment and growth of the ovary have been proposed as the most likely explanation (Buzi et al., 1998). Hence, it appears that AMH can only be used to assess the extent of ovarian aging in women beyond 25 years of age.

AMH measurement

AMH assays

Between 2002 and 2010 two different AMH assays have been used in human female studies. These assays have been simultaneously developed by Diagnostic Systems Laboratory (DSL) and by Immunotech (IOT), applying different AMH antibodies (Nelson and La Marca, 2011). Until today, various sources for the AMH standard have been used for calibration and no international standard has yet been developed. The IOT assay produced AMH concentrations ~40% higher compared with DSL, rendering the combined analysis of trials employing different assays problematic (Freour et al., 2007).

In 2010 both companies merged under Beckman Coulter and a single new two-step, sandwich-type enzymatic, microplate assay (the AMH gen II assay) was introduced. A more stable antibody is now used to bind to the mature region of AMH along with the IOT calibrator standard curve and AMH levels can be measured in 20 μl serum in <3 h (Fleming and Nelson, 2012). The gen II assay is calibrated to the old IOT standards and AMH levels are thus comparable to the IOT assay and 40% higher than the previous DSL version (Kumar et al., 2010; Wallace et al., 2011; Fleming and Nelson, 2012). The gen II assay has a 2-fold greater sensitivity (0.08 ng/ml) than the IOT assay and the cross-reactivities of inhibin A, activin A, FSH and LH were below the detection limit of the assay (Kumar et al., 2010).

Several studies have been performed to investigate the robustness of the gen II assay for clinical application. These studies have determined several factors that could affect the reproducibility of the test result. Intra/inter-assay differences, between laboratory differences and sample stability in storage all may be influenced by sometimes unknown factors.

Regarding the reproducibility of the new assay, the inter and intra-assay variation was shown to be small (<5%) in several studies, where measurements were performed in the same laboratory (Kumar et al., 2010; Rustamov et al., 2012). However, when 10 laboratories tested 20 serum samples with the same gen II assay from Beckman Coulter, the in-laboratory reproducibility was good but the between laboratory results showed a wide range of average values relative to the consensus value (Zuvela et al., 2013). These differences may represent dissimilarities in storage and shipping conditions, or differences in the work-up of this manual enzyme-linked immunosorbent assay (ELISA) test system.

When looking into the inter-sample stability and reproducibility, conflicting results were obtained regarding the variation caused by various

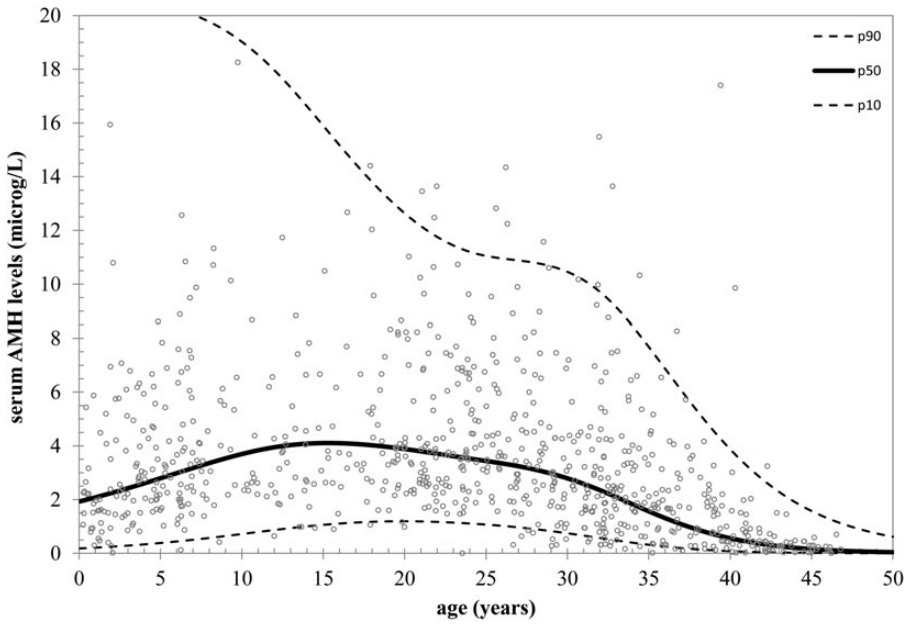

Figure 4 AMH nomogram from birth to menopause in 804 healthy females from Lie Fong *et al.* (2012). Reference lines of serum AMH for the 10th, 50th and 90th percentiles of predicted AMH values versus age.

storage conditions. A first study demonstrated a good sample stability and reproducibility of the gen II assay, also after freezing and thawing (Kumar *et al.*, 2010). This could not be confirmed by one other study demonstrating a very high between sample variation, amounting to a variation coefficient of up to 60% (Rustamov *et al.*, 2012). The explanation for this may lie in several factors. Regarding storage and handling of samples, it was demonstrated that in samples stored at room temperature for 7 days the AMH serum levels increase. Samples stored at −20°C yielded on average 23% higher values, while the same samples stored at −80°C showed no change (Rustamov *et al.*, 2012). Another explanation for sample instability may be the effects of complement binding. Due to complement interference the test result may be lower than expected. This risk is highest in freshly drawn samples. It has been suggested that this can be avoided, or minimized, by adding a buffer and recent studies have already shown promising results by the pre-mixing of samples with assay buffer to potentiate AMH stability at all temperatures (ESHRE meeting London 2013). Also, automatic pipetting or centrifugation of samples within 5 h seems to reduce the influence of complement binding (Fleming and Nelson, 2012).

Data for the new Anhs Labs ultra-sensitive AMH and picoAMH ELISA assay were published recently (Welsh *et al.*, 2014). The study demonstrates that this assay has a different calibration than the Gen II assay, but that its performance is suitable for clinical use. Enhanced sensitivity of the Ansh Labs picoAMH assay enables measurement of low AMH concentrations; however, further studies in different centers regarding the stability of this new assay still have to be performed (Welsh *et al.*, 2014).

In conclusion, uncertainty remains concerning the stability of the AMH assay, specifically regarding optimal storage and handling conditions as well as the role for complement interference causing sample instability. Until all these factors have been studied thoroughly or an automated assay is available, we are in need of international guidelines for all laboratories on how to store samples and perform AMH assays under the same condition, so that the influence of handling technique in causing different AMH results will not alter clinical management. Moreover, there is an urgent need to establish an international reference preparation to make test results comparable. Another possibility would be to use a harmonization sample against which every laboratory can gauge its own measurements. Until that time, we have to be careful in translating AMH cut-off levels from studies into our clinical practice, since it remains unclear if we can directly translate the AMH values from research projects into daily practice (Schipper *et al.*, 2012).

Factors influencing AMH levels

Variation in AMH levels could also be explained by biological variance. Contradictory results have been described regarding intra- and inter-cycle variability of AMH levels. Some studies show these to be limited (Van Disseldorp *et al.*, 2010) and merely represent fluctuations by chance, possibly related to gradual changes in the number of antral follicles present in both ovaries (Hehenkamp *et al.*, 2006). However, other studies have demonstrated substantial fluctuations in the menstrual cycle (Wunder *et al.*, 2007), which would argue in favor of measuring AMH levels at the early follicular phase only. Especially in young women, this fluctuation in AMH over a time period of several weeks may be quite extensive and needs to be taken into consideration if applied in clinical conditions (Overbeek *et al.*, 2012).

Furthermore, we have to take into account the clinical conditions under which the samples were drawn. It has been suggested that AMH levels remain constant under the influence of exogenous sex steroids used for contraception (Somunkiran *et al.*, 2007; Streuli *et al.*, 2008; Steiner *et al.*, 2010; Li *et al.*, 2011; Deb *et al.*, 2012). In a recent large

cohort study in >2000 women it was demonstrated that AMH levels decrease under current use of oral contraceptives (Dolleman et al., 2013b). Such an effect was also demonstrated in other studies (Arbo et al., 2007; Shaw et al., 2011; Kristensen et al., 2012). Previous use of oral contraception was not associated with lower AMH levels (Dolleman et al., 2013b) and AMH levels may even be increased after discontinuation of oral contraceptives (van den Berg et al., 2010). Both findings support the notion of a reversible suppressive subtle effect of oral contraceptives on AMH.

It was also demonstrated that under mid-luteal GnRH agonist administration AMH levels changed significantly across the initial 4 weeks (Hagen et al., 2012a; Su et al., 2013). Such observations suggest that if a patient is receiving GnRH agonist medication, for example in cancer treatment, AMH may not be a reliable marker of ovarian reserve.

Finally, various other factors were recently described to influence absolute AMH concentrations, including overweight (Freeman et al., 2007; Su et al., 2008; Piouka et al., 2009b; Buyuk et al., 2011), ethnicity (Seifer et al., 2009), Vitamin D status (Dennis et al., 2012; Merhi et al., 2012), polymorphisms of AMH and its receptor (Kevenaar et al., 2007), and genetic variants across the genome (Schuh-Huerta et al., 2012). Current smoking has also been associated with lower AMH levels (Dolleman et al., 2013b). The clinical relevance of these observations remains to be determined.

Ovarian reserve testing

Ovarian reserve testing aims to assess the reproductive potential of a given individual as a function of the quantity and quality of remaining oocytes. Evidence is accumulating suggesting that AMH is the best currently available test in terms of sensitivity and specificity as opposed to AFC, FSH, E_2 and inhibin B concentrations or various ovarian challenge tests (Practic Committee ASRM, 2012). Different areas of reproductive medicine exist where ovarian reserve testing by AMH may prove to be of distinct clinical benefit.

Fecundity and menopause prediction in the general population

In many Western countries the average age of women giving birth to their first child is approaching 30 years. Due to the ongoing trend of delayed childbearing, a significant proportion of women aiming to have a child beyond 30 years will already exhibit a reduced probability of spontaneous pregnancy. Accurate ovarian reserve testing may motivate some women to start a family at an earlier age (or alternatively apply fertility preservation by means of oocyte freezing) or alternatively reassure others that postponing childbearing will not interfere with her chances to achieve a pregnancy later on.

A first study in 100 unselected women aiming to conceive spontaneously aged 30–44 years disclosed a good correlation between initial AMH concentrations and natural fertility during a 6-month follow-up (Steiner et al., 2011). Such an association could not be confirmed in a comparable number of younger women between 20 and 35 years of age (Hagen et al., 2012b). More data are needed to draw meaningful conclusions regarding the ability of AMH to predict the chance of spontaneous pregnancy, especially at younger age.

It has been hypothesized that a fixed interval exists between age at natural sterility and age at menopause (te Velde and Pearson, 2002). Lately, several studies have been undertaken regarding the prediction of age at natural menopause. In a long-term follow-up study, 257 women were followed for 11 years. It was demonstrated that using age and AMH the age range in which menopause will subsequently occur can be individually calculated (Fig. 5) (Broer et al., 2011). Other studies have confirmed these findings (Tehrani et al., 2011; Freeman et al., 2012). These results have identified AMH as a promising marker for menopause prediction, although wide confidence intervals exist especially in women sampled at a younger age. Larger cohort studies are now starting to emerge, where the predictive role for AMH in conjunction with female age and other potential factors, such as BMI and smoking, will be defined in greater detail (Dolleman et al., 2013a; La Marca et al., 2013).

Prediction of pregnancy chances in couples presenting with infertility

Advanced female age is becoming increasingly important in the infertility clinic. Ovarian reserve testing may allow for a better assessment of the fertility potential of a given woman. This could permit to tailor the treatment plan accordingly, which may include expectant management in women with a good prognosis. Multiple attempts have been described in recent years to develop age-specific nomograms for AMH concentrations involving tens of thousands of infertile women of reproductive age (Nelson et al., 2011a; Seifer et al., 2011; Leader et al., 2012). Although age-specific AMH levels may have the potential to be of much clinical benefit, the disturbing implications of discordant findings between AMH and FSH (especially abnormal FSH coinciding with reassuring AMH levels occurring in 1 out of 18 women) remain to be addressed, but most likely emphasize the different nature of the two tests (Leader et al., 2012; Schipper et al., 2012). Ultimately, clinical studies should be undertaken in infertile couples to correlate individual AMH levels independent of chronological age with live birth rates, either after natural conception or following various infertility treatments.

Patient management in IVF

Many studies have been published regarding the prediction of IVF outcome by using ovarian reserve tests, such as AMH. Most studies published so far concerning AMH and subsequent IVF outcomes have been carried out in heterogeneous patient cohorts. Accordingly, conflicting results were obtained with regard to the capacity of AMH to predict treatment outcome. One important outcome measure is the response to ovarian hyperstimulation. Ovarian hyperstimulation is an integral part of IVF and is usually applied in a uniform standardized fashion regardless of individual patient characteristics. The majority of applied stimulation regimens are very complex, time consuming and costly. A distinct individual variability in ovarian response to stimulation is usually observed, varying from low or virtually no response (resulting in treatment cancelation or very poor IVF outcomes) all the way to an exaggerated response (associated with the potentially hazardous ovarian hyperstimulation syndrome (OHSS)). Furthermore, a relationship between the number of oocytes retrieved and pregnancy exists. Several studies have demonstrated that a response of between 9–13 or 6–15 oocytes is associated with the highest pregnancy or live birth rate (van der Gaast et al., 2006; Sunkara et al., 2011; Ji et al., 2013).

Clinicians are interested in predicting the response to ovarian hyperstimulation. Recently two individual patient data meta-analyses have been published regarding poor and excessive response prediction. The

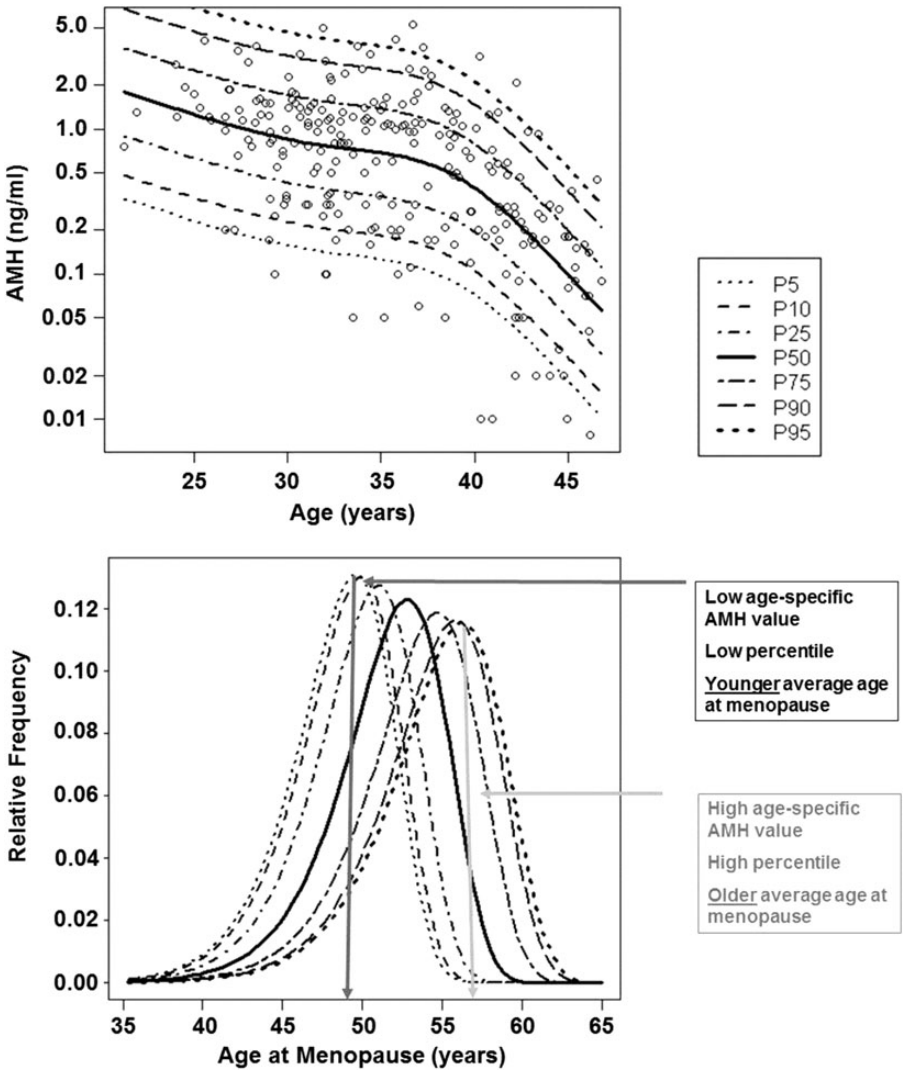

Figure 5 Nomogram for the relation between age-specific AMH concentrations and the distribution of age at menopause. AMH levels measured at entry to the study ($n = 257$, average age 35 years) (upper panel) and age at menopause assessed 11 years later (bottom panel), from Broer *et al.* (2011). (Lines represent the upper margins of the different percentiles of AMH.)

first IVF treatment cycle of 5705 women was analyzed, showing that AMH adds to age in predicting poor response. More importantly, a single test of AMH fully covers the prediction of poor response with an acceptable area under the receiver operating characteristic (ROC) curve of 0.78 (Broer *et al.*, 2013b). Results of the first IVF treatment cycle of >4700 women were available for analysis of excessive response prediction and the same level of accuracy was demonstrated (Broer *et al.*, 2013a). It can therefore be concluded that AMH is a useful predictor of ovarian response to ovarian hyperstimulation.

Changing gonadotrophin doses in the course of the ovarian stimulation treatment, often applied in poor responders, has failed to show a clinical benefit (van Hooff *et al.*, 1993). The ability to assess ovarian responsiveness to FSH before the actual start of stimulation allowing adjustment of the initial doses for ovarian stimulation, is expected to improve the efficacy and safety of IVF treatment (Fauser *et al.*, 2008b). Initial studies aiming to develop algorithms for individualized dosing applied patient characteristics such as FSH, the AFC, ovarian volume and age. It could be demonstrated that daily doses of exogenous FSH varying between 37 and 225 IU may be used in individual cases to achieve a desired ovarian response (Fauser *et al.*, 2008a). Others studies provided contradictory results. Two studies showed no effect of increasing the FSH dosage for expected poor responders (Klinkert *et al.*, 2005; Lekamge *et al.*, 2008), whereas decreasing the FSH dosage for expected excessive responders did show promising results (Olivennes *et al.*, 2009). Only a single RCT has been performed, demonstrating that in the individual dosing group the incidence of a poor or excessive response was reduced (Popovic-Todorovic *et al.*, 2003). A large RCT is necessary to validate these findings. Moreover, the cost-effectiveness of an individual approach to ovarian hyperstimulation has not been studied to date. Currently the multi-center OPTIMIST trial (registration nr: NTR2657) is being conducted in the Netherlands, investigating the effect of individualization of ovarian hyperstimulation and its cost-effectiveness (van Tilborg *et al.*, 2012).

Only recently have algorithms been developed to individualize dosing for ovarian hyperstimulation based on initial AMH concentrations. These treatment strategies resulted in a reduction of both an excessive response and canceled cycles, reduced risk of OHSS, increased pregnancy and live birth rates, in addition to a reduction in costs (Nelson et al., 2009; Yates et al., 2011; La Marca et al., 2012). A recent meta-analysis has summarized the current status of the available evidence supporting the use of individualized ovarian hyperstimulation (Fleming et al., 2013; La Marca and Sunkara, 2013).

The ability of AMH to predict pregnancy chances is less promising. In an individual patient data meta-analysis it was clearly demonstrated that AMH does not add to the prediction of ongoing pregnancy in IVF (Broer et al., 2013b). However, two recent studies, both in many hundreds of women undergoing IVF, did establish an association between AMH and cumulative live birth rates. It was demonstrated that women in the higher AMH categories have a higher ongoing pregnancy rate as well as a higher live birth rate (Arce et al., 2013). This finding was confirmed in a larger prospective study of almost 900 women, where increasing AMH levels were associated with increasing live birth rates, which remained significant even after adjustments were made for age and oocyte yield (Brodin et al., 2013). It has also been demonstrated that when female age and AMH are combined it is possible to make a distinction between couples with a good and poor prognosis (La Marca et al., 2011).

Management of women with cancer

Childhood cancer treatment has improved dramatically resulting in current overall survival rates of over 90%. Therefore, the long-term implications of treatment, such as gonadal damage, and related infertility are gaining increasing attention. AMH could play a role in several aspects of cancer treatment and outcome. First, AMH appears to facilitate establishing which chemotherapeutic agents are particularly toxic to the ovaries (van Beek et al., 2007; Brougham et al., 2012).

Second, AMH may also be able to identify diminished ovarian reserve when ovulatory cycles are restored following cessation of cancer treatment. Typically, AMH levels drop during chemotherapy with some recovery 3–6 months thereafter. Radiation cancer therapy is known to be particularly toxic to ovaries. AMH, both before and after treatment, may be useful in the management of young women diagnosed with cancer, since many women are concerned about their future fertility potential (Anderson and Cameron, 2011; Dillon et al., 2013) and fertility preservation may be considered. Recently a small study emerged showing that also in adolescents (age <18 years) treated for cancer AMH is a marker of ovarian function (Krawczuk-Rybak et al., 2013).

Moreover, the real value of measuring AMH levels in young women surviving cancer would be to forecast the long-term reproductive outcome. A first study looking into this matter has emerged. A 10-year re-follow-up study of childhood cancer survivors now in their mid-thirties showed a decrease in AMH level according to the gonadotoxic effect of the treatment in their childhood. In general the percentage of childless women in this group was higher than in the normal Danish population and, especially in the group of women who received the maximum gonadotoxic treatment, the pregnancy rate and outcome was very poor (Nielsen et al., 2013). However, whether AMH could play a role in forecasting reproductive outcome in these women has yet to be established.

In the context of gonadotoxic cancer treatment ovarian tissue can be cryopreserved. Although resumption of ovarian function and subsequent pregnancies have been reported following the orthotopic transplantation of ovarian tissue, AMH levels are undetectable in the great majority of women (Janse et al., 2011; Andersen et al., 2012). It remains to be elucidated whether undetectable AMH under those circumstances is due to accelerated follicle loss during thawing of cryopreserved ovarian tissue, poor vascularization of transplanted ovarian material or other causes.

In women with breast cancer it was demonstrated recently that pretreatment AMH levels are a useful predictor of the long-term post-chemotherapy loss of ovarian function, adding significantly to the only other established individual predictor, which is age. The area under the curve for predicting amenorrhea at 2 years post-chemotherapy was 0.90. Therefore pretreatment AMH measurements may aid in decision-making regarding treatment options and the need for applying fertility preservation procedures (Anderson and Wallace, 2013; Anderson et al., 2013).

For patients with hormone-sensitive breast cancer, knowledge of the precise time point by which the ovarian reserve is depleted is of great importance for the decision regarding the optimal adjuvant hormonal treatment. Unfortunately, the currently available measures to determine the post-menopausal status, such as FSH, are of limited value. Recently it has been proposed to use AMH under those circumstances. A practical guideline based on the currently existing scientific evidence using AMH as a marker has been proposed and research to validate this guideline is underway (De Vos et al., 2012).

Finally, as ovarian granulosa cells secrete AMH, serum AMH levels may be used in diagnosis and follow-up of ovarian granulosa cell tumors. AMH performance for diagnosing a granulosa cell tumor seems very good with a sensitivity ranging between 76 and 93%. Post-operatively it may be used as a marker for the efficacy of surgery and for disease recurrence. One study followed 31 patients post-operatively for 7 years and demonstrated AMH as a useful tool in diagnosing recurrence of disease. This was confirmed in a second report on 56 patients, of whom 36 were followed post-operatively (Lane et al., 1999; Long et al., 2000; La Marca and Volpe, 2007).

In conclusion, multiple small single-center studies have been performed regarding AMH in young women surviving childhood cancer or women treated for (breast) cancer later in life. They all show promising results for AMH, but more research is needed to confirm these findings and assess the clinical utility.

Novel indications for ovarian reserve testing

A diminished ovarian reserve has been disclosed in a variety of clinical conditions. Women who were born small for gestational age (Sir-Petermann et al., 2010), women with type 1 diabetes mellitus (Soto et al., 2009), women suffering from the auto-immune disease lupus erythematosus (Lawrenz et al., 2011), women having undergone ovarian surgery (chiefly cystectomy in women with endometriosis) (Raffi et al., 2012) or uterine artery embolization for fibroids (Berkane and Moutafoff-Borie, 2010), all may be at risk of various degrees of reduced ovarian reserve. The recent observation of reduced AMH levels and a significantly earlier age of natural menopause in BRCA1/2 mutation carriers (Titus et al., 2013) further underlines the potential significance of ovarian reserve testing in these specific groups. Finally, both fecundity data along with decreased AMH levels suggest accelerated ovarian aging in tall women who were treated with high-dose estrogens during puberty to reduce adult height (Hendriks et al., 2011, 2012).

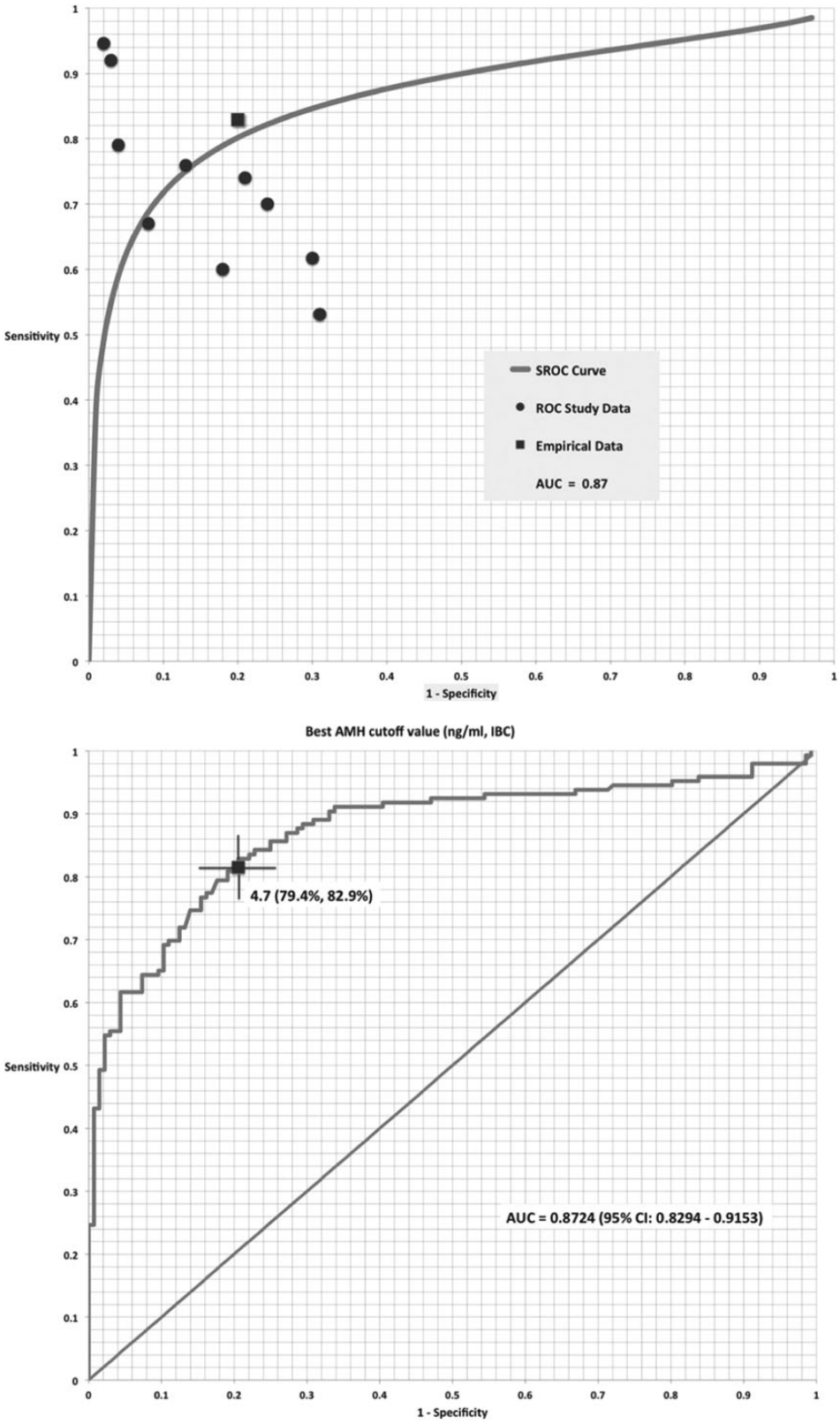

Figure 6 AMH in the diagnosis of polycystic ovary syndrome (PCOS). Top panel: the summary receiver operating characteristic (ROC) curve, reported sensitivity and specificity values of the ten included studies (circles), and the sensitivity and specificity values for the individual patient data aggregation meta-analysis (squares). Bottom panel: ROC curve, optimal cut-off value and area under the curve (AUC) for the individual patient data aggregation meta-analysis. From Iliodromiti *et al.* (2013).

Besides the use of predicting age at menopause for the association with the preceding decrease in natural fecundity, predicting menopausal age may also have implications for female health in general. Age of menopause is known to be related to many general health issues, such as osteoporosis, breast cancer, cognition and Alzheimer disease, cardiovascular disease and stroke (De Vos et al., 2010). The ability to predict age at menopause by assessing AMH at a relatively young age may enable the design of screening and prevention programs according to the risk profile of the individual woman. Indeed, a relationship between AMH and subsequent atherosclerosis risk has recently been described for the first time in a monkey model (Appt et al., 2012).

Ovarian dysfunction

Women with presumed ovarian dysfunction presenting with oligomenorrhea or amenorrhea are classified based on serum FSH and E_2 concentrations. This classification was developed more than half a century ago and was subsequently adopted by the World Health Organization (WHO). In brief, a distinction can be made between either a central (hypothalamic-pituitary unit) origin of ovarian dysfunction (WHO group 1, characterized by low FSH and low E_2 levels), abnormalities residing within the ovary itself (WHO group 3; high FSH, low E_2), or a pituitary-ovarian 'imbalance' (WHO group 2; normal FSH and E_2). The latter condition (WHO group 2), mainly involving polycystic ovary syndrome (PCOS), is observed in ~80% of cases of oligo/amenorrhea. As described earlier, AMH is exclusively produced by pre-antral and early antral follicles independent from FSH. Assessing AMH concentrations in anovulatory women may therefore provide useful additional information concerning early follicle dynamics along with ovarian reserve.

PCOS

Women diagnosed with PCOS often present with oligo/anovulation, hyperandrogenism and characteristic ovarian features. Polycystic ovarian morphology (PCOM) as assessed by transvaginal ultrasound is one of the three criteria for PCOS diagnosis. Ovarian dysfunction in women with PCOS is characterized by follicle maturation arrest and disturbed dominant follicle selection. Accordingly, 2–3 fold increased serum AMH concentrations have been reported in PCOS, directly reflecting the increased number of early antral follicles. Increasing evidence suggests that AMH levels may replace PCOM assessment (Dewailly et al., 2011, 2013; Eilertsen et al., 2012), but it has also been suggested that AMH can replace features of hyperandrogenism or anovulation (Casadei et al., 2013). Recently a systematic review and meta-analysis was performed regarding the capacity of AMH to diagnose PCOS. Ten studies could be included in the meta-analysis and a summary ROC curve was constructed. Using a cut-off level of 4.7 ng/ml, AMH has a sensitivity and specificity of 82.8 and 79.4%, respectively. The AUC was 0.87, which was identical to the summary ROC curve of the 10 studies (Fig. 6) (Iliodromiti et al., 2013).

Moreover, the magnitude of AMH elevations in PCOS is associated with the extent of disease (Laven et al., 2004; Piouka et al., 2009a), improved reproductive performance in relation to weight loss (Thomson et al., 2009), and improved ovulatory function with age (Carmina et al., 2012). Moreover, ovarian response to infertility treatment by laparoscopic ovarian diathermy (Elmashad, 2011) may be predicted by initial AMH levels. AMH is also elevated in prepubertal and adolescent girls with PCOS (Villarroel et al., 2011) and in daughters of mothers with PCOS (Crisosto et al., 2007). Hence, AMH testing may allow the early detection of subclinical disease in siblings of women diagnosed with PCOS.

The observed decline of AMH with increasing age in PCOS appears to be significantly less pronounced compared with normal controls (Mulders et al., 2004). Retarded ovarian aging and a delayed age of menopause has therefore been proposed in PCOS. This hypothesis, however, remains to be further substantiated (Mulders et al., 2004; Piltonen et al., 2005; Tehrani et al., 2010).

Other forms of anovulation

Women presenting with functional hypothalamic amenorrhea were shown to have normal AMH levels suggesting a normal size of the cohort of early growing follicles (Luisi et al., 2012). Accordingly, initial AMH levels predict chances for recovery of ovarian function following weight gain in women with anorexia nervosa (van Elburg et al., 2007). However, a case report has been published in which a woman with a hypothalamic amenorrhea initially presented with low AMH levels which increased after stimulation with gonadotrophins (Tran et al., 2011).

In contrast, AMH levels are undetectable in the great majority of women diagnosed with primary ovarian insufficiency (POI) (WHO group 3) suggesting premature follicle pool exhaustion (Knauff et al., 2009). Moreover, AMH may provide useful information regarding the extent of follicle pool depletion in various POI-like conditions, such as incipient ovarian failure (Knauff et al., 2009), ovarian failure due to autoimmunity (La Marca et al., 2009) or FSH receptor loss of function mutation (Kallio et al., 2012). In this context, it should be noted that current criteria used to define POI (such as FSH concentrations >40 IU/L) are not based on sound scientific evidence.

Women with (mosaic) Turner syndrome are destined to develop POI at an early age. AMH has been found to be higher in women with Turner syndrome who do achieve puberty (Visser et al., 2013), and in girls with karyotypes associated with a fair probability of fertility (Purushothaman et al., 2010). Moreover, in the case of fertility preservation in women with Turner syndrome, AMH represents one of the predictors of the presence of follicles in biopsied ovarian tissue (Borgstrom et al., 2009). Further studies have to be undertaken to assess whether AMH measured at a young age could aid in the decision-making regarding future attempts at fertility preservation.

Conclusion

AMH serum concentration accurately reflects the size of the pool of antral follicles, representing the quantity of the remaining primordial follicles. Accordingly, AMH levels may vary significantly in women of the same chronological age. AMH is the best currently available measure of the ovarian reserve in several clinical conditions. In IVF it can be used to predict outcome measures, most importantly the ovarian response, and may also aid in the individualization of dosing for ovarian hyperstimulation. However, prospective well powered studies comparing different infertility treatment strategies based on initial AMH levels using appropriate end-points, such as live birth and cost-effectiveness, are urgently awaited. Moreover, AMH has a role in forecasting reproductive lifespan, ovarian dysfunction (especially PCOS) and the impact

of gonadotoxic cancer treatment or ovarian surgery. However, concerns regarding the performance of AMH assay under different conditions indicate an urgent need for an international guideline regarding the storage of samples and handling techniques for the AMH assay, to allow the comparison of test results between laboratories.

Authors' roles

All authors contributed to writing and critically reviewing the manuscript.

Funding

No specific funding was received for preparing this review.

Conflict of interest

S.L.B. has nothing to declare. F.J.M.B. is a member of the external advisory board for Merck Serono (The Netherlands) the advisory board Roche (Switzerland), performs consultancy work for Gedeon Richter (Belgium), for MSD (The Netherlands), and is involved in educational activities for Ferring BV (The Netherlands) and for MSD (The Netherlands). J.S.E.L. received unrestricted research grants from the following companies (in alphabetical order): Ferring, Genovum, Merck-Serono, Merck Sharp and Dome (MSD), Organon, Shering-Plough, and Serono. B.C.J.M.F. has received fees and grant support from the following companies (in alphabetic order): Andromed, Ardana, COGI, Euroscreen, Ferring, Genovum, Gedeon-Richter, Merck Serono, MSD, Organon, Ova Science, Pantharei Bioscience, PregLem, Roche, Schering, Schering Plough, Serono, Uteron, Watson laboratories and Wyeth.

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