The physiology and clinical utility of anti-Mullerian hormone in women

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Introduction

The physiology and clinical utility of anti-Müllerian hormone (AMH) are not completely established. However, because of the tremendous amount of data collected in recent years, it appeared timely for this group of experts to bring together the current knowledge. These experts met in Lille, France, in May 2012 for an ESHRE Campus workshop. This review offers a structured proceeding of this workshop that has been updated with the most recent data published in the literature since then. Its aim is to provide an extensive overview of the current knowledge and position of AMH as a tool in female health and fertility care. While covering most aspects of the physiology and utility of AMH, some aspects (e.g., use in diagnosis of granulosa cell tumours) were not covered, but are discussed in excellent reviews (e.g., La Marca et al., 2007).

Historical perspective and state of the art

AMH is a dimeric glycoprotein and a member of the transforming growth factor β (TGF-β) family of growth and differentiation factors (Cate et al., 1986). AMH has been predominantly known for its role in male sexual differentiation. From castration experiments in the fetal rabbit, Jost demonstrated that a testicular factor distinct from testosterone was responsible for the regression of the Müllerian ducts during male fetal sex differentiation (Jost, 1947). In later years, it was demonstrated that this factor is produced by Sertoli cells in the testis (Josso et al., 1993).

The ovary is also able to produce AMH. In the chicken, this occurs from early embryonic development to adulthood (Hutson et al., 1981) but in human, AMH production by granulosa cells was detected only at the end of gestation (Rajpert-De Meyts et al., 1999). Interest into the role of AMH in the female was principally evoked through studies of AMH-deficient mice. Although female mice appeared fertile in the absence of AMH (Behringer et al., 1994), more detailed analysis of the ovarian follicle pool revealed that AMH acted as an inhibitor of primordial follicle recruitment. Also, later studies demonstrated a potential role for AMH in dominant follicle selection in the follicular phase of the menstrual cycle (Visser and Themmen, 2005). The development of sensitive assays soon enabled measuring AMH in serum (Hudson et al., 1990; Josso et al., 1990; Baker et al., 1990). Release of AMH from the granulosa cells of antral follicles leads to measurable serum levels, and these concentrations have shown to be proportional to the number of developing follicles in the ovaries. Therefore, AMH was considered to be a marker for the process of ovarian ageing (Kevenaar et al., 2006).

To date, AMH has developed into a factor with a wide array of clinical applications, mainly based on its ability to express the number of antral and pre-antral follicles present in the ovaries (Hansen et al., 2011). Predicting ovarian response to hyperstimulation of the ovaries for IVF, with the possibility of individualized counseling and adjustments of the stimulation regimen, is the most appealing application under development so far. Assessment of damage to the ovarian follicle reserve inflicted by iatrogenic

sources such as pelvic irradiation, chemotherapy, uterine artery embolization or ovarian surgery using AMH may open avenues of choosing strategies to prevent this damage in selected cases by applying fertility preservation strategies. The emerging data on the relation between AMH level at a certain age and the timing of menopause has set a scene for an individualized prediction of the reproductive lifespan, and from there potential prevention of infertility based on early ovarian ageing. Finally, marking the excess of antral follicles in women with Polycystic Ovarian Syndrome (PCOS), AMH may soon replace the ultrasound ovarian morphology criterion in the diagnosis of this syndrome, as well as become an additional tool for diagnosing premature ovarian insufficiency (POI).

Physiology

The roles of AMH in ovarian physiology

AMH is specifically expressed in granulosa cells of small growing follicles. In rodents, expression is initiated as soon as primordial follicles are recruited to grow, and highest expression is observed in preantral and small antral follicles. AMH is no longer expressed by mural granulosa cells during the FSH-dependent stages of follicular growth, nor is it expressed in atretic follicles (reviewed in Durlinger et al., 2002). However, expression persists in the cumulus cells of preovulatory follicles (Salmon et al., 2004). In the human ovary, AMH shows a very similar expression pattern (Rey et al., 2000; Weenen et al., 2004; Grondahl et al., 2011; Jeppesen et al., 2013).

Functional roles of AMH in ovarian folliculogenesis were revealed by analysis of the follicle pool in ovaries of AMH-deficient mice at various ages. In the absence of AMH, primordial follicles are recruited at a faster rate, resulting in an exhausted primordial follicle pool at a younger age (Durlinger et al., 1999). The inhibitory effect of AMH on primordial to primary follicle transition was confirmed by *in vitro* studies of neonatal ovaries and ovarian cortical strips of various species, including human (Durlinger et al., 2002; Nilsson et al., 2007; Gigli et al., 2005; Carlsson et al., 2006). However, contradictory results using human ovarian cortical tissue have also been reported (Schmidt et al., 2005). In the mouse AMH inhibited the effect of several growth factors known to have a stimulatory action on primordial follicle recruitment, such as KitL and bFGF (Nilsson, et al., 2007). In the absence of AMH, ovaries contain more growing follicles, yet AMH-deficient mice have a normal ovulation rate. Increased oocyte degeneration and follicular atresia suggests that AMH may also be a survival factor for small growing follicles (Visser et al., 2007). AMH also reduces follicle sensitivity to FSH *in vivo*, and *in vitro* AMH inhibited FSH-induced preantral follicle growth (Durlinger et al., 2001). Thus, there is clear evidence that AMH is involved in the regulation of follicle growth initiation and the threshold for FSH sensitivity (Figure 1).

AMH has also been suggested to exert a physiological effect on antral follicles in the human ovary before final selection. There exists a fine-tuned and delicate balance between estradiol (and inhibin) output by the preovulatory follicle and gonadotrophin secretion by the pituitary to ensure that ovulation

is triggered exactly at the right time (Baird and Smith, 1993). Recently, it has been suggested that AMH may exert a physiological role in down regulating the aromatizing capacity of granulosa cells until the time of follicular selection (Figure 1). Several studies have shown that AMH expression remains high until a follicle reaches a diameter of around 8mm (Weenen, et al., 2004, Andersen et al., 2010, Jeppesen, et al., 2013). The intrafollicular concentrations of AMH in normal human antral follicles show a gradual reduction as the diameter of the follicle increases, and a sharp decline is observed around 8mm (Andersen, et al., 2010). The rapid decline in AMH expression corresponds with the selection of follicles for dominance, which is characterized by a transition from a low-estrogen producing state to one of rapidly increasing estrogen production. Estradiol is instrumental in this decline through estradiol receptor β, which interacts with the AMH promoter region (Grynberg et al., 2012) (Figure 1).

Several lines of evidence suggest that AMH acts as gatekeeper of follicular estrogen production:

1) Early studies on fetal ovine ovaries showed that AMH repressed aromatase biosynthesis (Vigier et al., 1989). A quantitative bioassay for AMH was subsequently developed based on inhibition of cAMP-induced aromatase activity in fetal rat ovaries (di Clemente et al., 1992).
2) In granulosa-lutein cells from IVF patients AMH reduces the expression of CYP19a1 at both gene and protein level and FSH-induced E2 production was significantly reduced in the presence of AMH (Grossman et al., 2008).
3) In human small antral follicles there is a distinct inverse association between intrafollicular concentrations of AMH and estradiol concentrations and *CYP19a1* gene expression in the corresponding granulosa cells (Andersen and Byskov, 2006, Andersen and Lossl, 2008, Nielsen et al., 2011).
4) Using adjacent ovarian sections of preovulatory sheep follicles it was observed that the oocyte cumulus complex showed an almost complete inverse expression pattern of AMH and aromatase (Campbell et al., 2012). AMH continues to be expressed in cumulus cells of preovulatory follicles in the human (Grondahl, et al., 2011).
5) Association analysis of genetic variants of the AMH signaling pathway showed that the AMH Ile^{49}Ser and AMH type 2 receptor (AMHR2) -482A>G variants were related to follicular-phase estradiol levels in normo-ovulatory women. Women carrying the minor allele of the AMH or the AMHR2 polymorphism had higher estradiol levels compared to non-carriers, with carriers of both minor alleles having the highest levels (Kevenaar et al., 2007). *In vitro,* the AMH ^{49}Ser variant yields a less active AMH protein that could result in weaker inhibition of FSH-induced aromatase activity and follicle growth (Kevenaar et al., 2008).

Thus, AMH may act as a follicular gatekeeper and ensure that each small antral follicle produces little estradiol prior to selection (i.e. up to a follicular diameter of approximately 8mm) allowing a direct ovarian/pituitary dialogue regulating the development of the selected follicle that will undergo ovulation (Jeppesen, et al., 2013) (Figure 1).

Assessment of AMH in serum: assay development

AMH is produced as a precursor protein, consisting of 70 kDa disulphide-linked monomers (Picard and Josso, 1984). Proteolytic processing yields a 55 kDa N-terminal proregion and a 12.5 kDa C-terminal mature region (Pepinsky et al., 1988, Nachtigal and Ingraham, 1996). The pro- and mature homodimers remain non-covalently associated, resulting in a 140 kDa complex in circulation (Lee and Donahoe, 1993) (Figure 2). The mature region of AMH holds the biological activity of the protein, but in contrast to other TGFβ family members, requires the N-terminal proregion to obtain its full activity (Wilson, 1993). It has been suggested that the proregion is involved in protein stability and folding (Belville et al., 2004).

Measurement of serum AMH was first reported in the 1990s, with the development of three AMH enzyme-linked immunosorbent assays (ELISAs) (Baker et al., 1990; Hudson, et al., 1990; Josso et al., 1990). The AMH ELISAs were initially developed to measure AMH as a marker for testicular function during childhood, when serum concentrations are much higher than in females. Using a monoclonal and a polyclonal antibody that were both raised against recombinant human AMH (rhAMH), and which both recognize epitopes in the proregion of AMH, a sensitivity of 0.5 ng/ml was reached (Hudson, et al., 1990). Baker *et al* developed an assay with antibodies raised against bovine AMH and rhAMH, but this assay was unable to detect AMH in female serum samples because of the relatively high detection limit of 6.25 ng/ml and the presence of inhibitory effects of serum. The assay developed by Josso and colleagues used a single polyclonal antibody raised against purified bovine AMH with rhAMH as the standard (Josso, et al., 1990). In this assay, the minimal detectable dose of AMH was 0.02 ng. This assay was subsequently modified to a sandwich ELISA using a monoclonal and polyclonal antibody raised against rhAMH. These antibodies recognize epitopes in the pro- and mature region of AMH (Figure 2), and increased the sensitivity of the assay to 14 pmol/L (1 ng/ml = 7.14 pmol/L) (Carre-Eusebe et al., 1992). A further improvement in sensitivity to 0.7 pmol/L was reached by the use of two different monoclonal antibodies (Long et al., 2000). This ultrasensitive assay, known as the IOT assay, became commercially available through Beckman-Coulter (originally Immunotech-Coulter).

The importance of assessment of serum AMH levels in females followed the insight (based on the expression pattern) that serum AMH might be a proxy for the size of the primordial follicle pool (reviewed in Visser et al., 2006). This led to the development of an additional sensitive AMH ELISA. Highly specific monoclonal antibodies to the proregion of AMH were generated by immunization of female AMH-deficient mice with rhAMH (Al-Qahtani et al., 2005). These antibodies had different epitope specificities and, with rhAMH as the standard, the detection limit improved to 0.078 ng/ml (Al-Qahtani, et al., 2005). This assay was subsequently improved with another pair of highly specific monoclonal antibodies, which recognize epitopes in both the proregion (F2B/7A) and mature regions (F2B/12H) (Kevenaar, et al., 2006) (Figure 2). This assay is therefore expected to measure total AMH, and was commercially available through Diagnostic Systems Lab (DSL), has a detection limit of 6.3 pg/ml (Kevenaar, et al., 2006).

With the availability of two commercial assays, research on the clinical application of serum AMH increased tremendously. However, because these assays use different antibody pairs, and even more importantly different AMH calibrators, values of serum AMH differed significantly between the assays being 3- to 4- fold lower in the DSL assay (Freour et al., 2007). In later publications, similar AMH values were reported for both assays (Streuli et al., 2009, Lee et al., 2011), indicating that the assays continued to evolve. This may, in part, explain the different conversion factors that have been reported in various studies (Hehenkamp et al., 2006, Freour, et al., 2007). As a consequence, values obtained by one assay may not be directly translated to results obtained with the other assay.

With the acquisition of DSL by Beckman-Coulter, the two existing assays were replaced by a new ELISA. This Beckman-Coulter AMH Gen II assay continues to use the antibodies of the previous DSL assay but uses native AMH in heat-inactivated bovine calf serum as a standard. The Gen II assay was calibrated to the IOT AMH ELISA, yielding a sensitivity of 0.08 ng/ml (Kumar et al., 2010). Comparison of the AMH Gen II assay with the previous assays showed that AMH values obtained with the AMH Gen II assay had a good correlation with those of the DSL assay but higher values (22-40%) were obtained with the Gen II assay (Wallace et al., 2011, Li et al., 2012). Because the AMH Gen II assay was calibrated to the IOT assay, this difference could potentially be accounted for by the previously observed difference between the DSL and IOT assays. However, Li et al also observed a 35% increase in sample value in the Gen II assay compared to the IOT assay (Li, et al., 2012). This finding is unexpected given that the AMH Gen II assay was calibrated to the IOT assay. Furthermore, there have been studies questioning the stability of AMH upon storage, sample handling and sample diluting, either prior to or by sequential addition to the microtitre plate, which all might affect serum AMH values (Rustamov et al., 2012). In contrast, stable serum AMH values were reported upon long term storage at -20°C with the previous DSL assay (Kevenaar, et al., 2006). Also with the AMH Gen II assay fairly stable values were reported for serum AMH but not for whole blood (Kumar, et al., 2010, Fleming and Nelson, 2012, Fleming, et al., 2013). Concerns about the robustness of the AMH Gen II assay have been fuelled by recent safety notices and technical update letters from Beckman-Coulter, indicating that undiluted samples may give falsely low values due to interference from complement, but also that some samples diluted prior to addition to the plate may give falsely elevated values. Therefore, results published so far with the AMH Gen II assay have to be taken with caution and will probably need to be revisited once the technical issues are resolved. Furthermore, it is recommended that these changes are validated in independent research before clinical application of the assay. Adapting clinical cut-off values from the IOT assay to the Gen II assay is not recommended, because a different antibody pair is used. Likewise, a simple conversion factor to recalculate values from the DSL assay to AMH Gen II is also not recommended, given the issues raised above. Therefore, although the clinical application of serum AMH, as discussed in this review, is not in question, it is also not recommended to compare absolute values from clinical studies that use different assays. To maximise the clinical utility of AMH measurement it is also critical to develop an

international standard for AMH that is safeguarded and distributed by a competent authority such as the National Institute for Biological Standards and Control. This would allow harmonization of current and potential new AMH assays, thereby eliminating the need to establish assay-specific normative and cut-off values.

Variability of serum AMH in normal women

Inter-individual variability of AMH is high, mainly due to the very high variability in the number of antral follicles within groups of subjects of similar age (Gougeon, 1998; La Marca et al., 2011; Almog et al., 2011). There also seems to be ethnic variation, with African-American (Seifer et al., 2009; Schuh-Huerta et al., 2012) and Hispanic (Seifer, et al., 2009) women having lower serum AMH levels than those found in Caucasian women which may indicate a discrepancy between ovarian follicle number and AMH production. Some studies have indicated a negative relationship between BMI and AMH (Freeman et al., 2007; Steiner et al., 2010) but this has not been consistent (Halawaty et al., 2010; Skalba et al., 2011, La Marca et al., 2012; Overbeek et al., 2012). In a recent study, AMH was negatively related to BMI but the relationship was age-dependent (La Marca, et al., 2012) suggesting that the relationship is secondary to the stronger relationship of the two variables with age. Contradictory result have also been reported on the relationship between smoking and AMH, with some studies reporting reduced AMH levels in smokers (Freeman, et al., 2007, Plante et al., 2010, Freour et al., 2012) and others reporting similar values (Nardo et al., 2007; Dafopoulos et al., 2010; Waylen et al., 2010; La Marca, et al., 2012).

Analysis of intra-individual variability may be secondary to true biological variations in AMH levels in the circulation. The inter-menstrual cycle variability has been appropriately analyzed in two prospective studies (Fanchin et al., 2005, van Disseldorp et al., 2010), both of which concluded that 89% of the variation in AMH was due to between-subject variation, while only 11% of variability was secondary to individual fluctuation in AMH levels. Both studies found a similar intra-class coefficient (ICC) of 0.89, which is the ratio of the inter-individual variability over the total variability thus the higher the ICC, the lower the intra-individual variability. The majority of studies indicate that AMH is relatively stable through the menstrual cycle, as would be expected since the dominant follicle and corpus luteum do not secrete AMH (Hehenkamp, 2006; La Marca et al., 2006; Tsepelidis et al., 2007) (Figure 3). Van Disseldorp, et al. (2010) calculated the intra-individual CV of AMH to be 13 %, with intra-individual fluctuations within the same quintile in 72% of women and to cross two quintiles in only 1%. In contrast, a recent but small study found a reduction in circulating AMH in the luteal phase and intra-individual variance of AMH to be as high as 80% (Hadlow et al., 2013). In a prospective study based on 20 women, the authors described two different patterns for AMH dyanmics throughout the menstrual cycle. The "younger ovary" pattern had higher mean AMH and significant variations in AMH levels throughout the cycle. This was in contrast with an "aging ovary" pattern with low mean AMH, shorter menstrual cycle

lengths, and very low variation in AMH levels, suggesting diminished ovarian reserve. Fluctuations were randomly distributed during the cycle indicating that measuring on a fixed day would not be advantageous.

The literature also contains contradictory reports regarding the influence of conditions associated with gonadotrophin suppression, particularly hormonal oral contraception use and pregnancy, with serum AMH level. It seems likely that weak study size and design underlies this confusion. Recently a cohort study based on 863 women (228 OC-users and 504 non-users) reported that AMH serum levels were 29.8% lower in oral contraception users than controls (Bentzen et al., 2012). This has been recently confirmed by Dolleman et al. (2013). In a small but randomized trial of 42 healthy women administered oral, transdermal or vaginal ring hormonal contraception for 9 weeks, AMH levels decreased by almost 50% in all treatment groups (Kallio et al., 2013). Conversely, serum AMH level increases in subsequent natural cycles after stopping with hormonal contraception (van den Berg et al., 2010). Similarly in relation to pregnancy, in the only longitudinal study available (n=60) a significant decrease in AMH levels was found in the 2^{nd} and 3^{rd} trimesters compared to the 1^{st} trimester, with a mean reduction at the end of pregnancy of about 50% (Nelson et al., 2010). Such a decline in AMH levels during pregnancy has been recently confirmed by Köninger et al. (2013) in a cross-sectional study. While this no doubt reflects reduced follicular maturation, there may also be a contribution of pregnancy-associated haemodilution and increased plasma-protein binding.

In conclusion, fluctuations in AMH levels have been reported for a number of conditions and this has to be taken into account when interpreting values in clinical practice. While fluctuations in the menstrual cycle appear to be random and minor hence permitting the measurement of AMH independently of the cycle phase, ovarian suppression as induced by physiological or pharmacological interventions may reduce AMH levels. Thus, serum AMH may not retain its accuracy as a predictor of the ovarian reserve in women using long-term hormonal contraception.

Derivation of a normative model for AMH from conception to menopause

The emerging value of AMH measurement requires understanding of its pattern across the whole female life-course. Most published studies that report AMH in normal girls and women include only a relatively small age range, thus a 'data-driven' approach has been used (Kelsey et al., 2012). This involved extracting data using a semi-automated procedure, and combined it with other unpublished data. The resulting combined dataset (n = 3,260; age range -0.3 years to 54 years)(Kelsey et al., 2011) forms a representative sample of AMH levels in the population of healthy female humans, and can therefore be used as a basis for a predictive model of serum AMH level with changing age and was used to generate and validate the model.

Analysis of the model shows that the dynamics of circulating AMH levels throughout life can be split into several distinct phases (Figure 4). A peak shortly after birth confirms that girls also undergo a 'mini puberty' of the neonate, following which there is a sustained rise to about 9 years of age. There is an inflection with even a slight decline during the pubertal ages (9–15 years), followed by a second growth phase to a peak at an age of about 25 years. After this, there is a steady decline to undetectable levels at an average age of 50–51 years, corresponding to the menopause.

When non-growing follicle (NGF) recruitment dynamics are considered and compared to AMH levels (Figure 4) there is a strong and positive correlation ($r = 0.96$) between declining AMH and declining numbers of recruited NGFs after age 25 (the average age of peak AMH). This observation underpins the use of serum AMH level as an indirect indicator of human ovarian reserve for ages after the mid-twenties. Before the age of 25, the relationships between AMH and ovarian reserve are more complex with overall a positive relationship between rising AMH and increasing follicle growth activation, and thus we would recommend caution in the interpretation of AMH concentrations in girls and young women as an indirect indicator of ovarian reserve.

Ovarian Reserve assessment

Assessment of Ovarian Reserve in normal women

From the ART literature, it is clear that AMH can predict the ovarian response to hyperstimulation (Broer et al., 2013). AMH is superior to female age in assessing the quantitative aspects of the ovarian reserve but its value is much more limited in the prediction of ongoing pregnancy. Indeed no combination of ovarian reserve tests (ORTs) has been able to improve the accuracy of female age in identifying those with a close to zero prognosis (Hendriks et al., 2008, Broer, et al., 2013). Qualitative aspects of the ovarian reserve are much more difficult to capture.

The role for AMH as a predictor of natural fertility has been studied in a limited number of papers. In a prospective study of women mostly in their 30's, those with low AMH had significantly reduced fecundability, after adjustment for age (Steiner, et al., 2010). In contrast, fecundability in healthy young women with no prior knowledge of their fecundity, appeared not to be compromised if very low AMH levels were present (Hagen et al., 2012). However, it must be stressed that these results were obtained with the Gen II assay that provided at that time lower measurement than it was believed (see "assay" section). Conversely, the probability of conceiving was reduced in women with high AMH levels, suggesting that this represented women with overt or mitigated conditions of anovulation. Being a quantity marker, the true value for AMH may therefore be found in predicting the timelines in the ovarian ageing process that are dictated by quantity alone.

To study the value of the ORTs in the assessment of the future ovarian reserve status, long term follow up studies are required, where several factors assessed at initiation of the follow up are linked to the final outcome age at menopause. As menopause has a fixed time relation to earlier events such as onset of cycle irregularity (average age 46 years) and the loss of natural fertility (average age 41 years), a woman's reproductive lifespan can be predicted from forecasting age at menopause. To date, a total of four datasets are available addressing this issue. In two small studies, it has been demonstrated that across a period of 9 and 12 years, AMH level will adjust the predictions that can be based on female age at the moment of AMH sampling, so that women with low age-specific AMH will have menopause earlier and vice versa (Tehrani et al., 2009, Broer et al., 2011). A larger analysis is now available from the Iranian study (Tehrani et al., 2013). A third study confirmed these findings in a group of women of late reproductive age, but with still detectable levels of AMH (Freeman, et al., 2007). All these datasets however have very wide confidence intervals in the predictive value of a single AMH measurement. The rate of change over time may also affect the time to menopause, and be susceptible to extrinsic as well as intrinsic factors.

Genetic factors have proven to play a major role in determining the variation in menopausal age, as demonstrated in several mother-daughter, twin and sib-pair studies. Next to genetic factors, several environmental and life-style factors like smoking, body mass index, use of alcohol and parity have claimed to influence menopausal timing as well. Thus, menopausal age is considered a complex genetic trait. From a recent review (Voorhuis et al., 2010), it became apparent that a number of genetic regions and variants involved in several possible pathways underlying timing of age at menopause could be identified. Regarding a potential role for AMH or its receptor in modulating the rate of follicle loss form the primordial follicle pool, it has been demonstrated in two separate studies that common variation in the AMHR2 gene modifies the relationship between parity and age at natural menopause (Kevenaar et al., 2007; Voorhuis et al., 2010). Moreover, interactions between common variation in the AMH and AMH receptor II gene in their effect on menopause have further supported a potential role for factors that steer initial follicle recruitment (Braem et al., 2013).

The value of predicting age at menopause serves multiple targets. First of all, the ability to assess the future ovarian reserve status, and thereby the reproductive lifespan of an individual women, will have implications for female infertility. Because of the fixed time interval that is believed to be present, prediction of age at menopause will predict the age of natural end of fertility. If such predictions could be made early in life, with sufficient accuracy, this could have a great influence on individual women making decisions regarding career and a wish to have children. It is at present unclear whether AMH measurement meets those criteria.

AMH in the assessment of ovarian damage from chemotherapy, radiotherapy and surgery.

The relationship between serum AMH and the number of small growing and indeed primordial follicles has made it a prime potential tool for the investigation of gonadotoxicity of cancer therapy and of loss of the ovarian reserve from ovarian surgery. AMH offers the possibility of a more accurate assessment, revealing partial loss of the ovarian reserve, as well as ovarian failure. It may also be of value in children where FSH and inhibin B are not useful, and in individualising the degree of damage when measured prospectively.

A decrease in serum AMH was first described in women who had had childhood cancer but who still had regular menses, compared to an age matched control group (Bath et al., 2003). In contrast there was no difference in serum FSH or inhibin B between groups. Similar findings have been shown in breast cancer survivors (Partridge et al., 2010). AMH was decreased in a study of ovarian function in young adults following treatment for childhood Hodgkin lymphoma with a clear dose response demonstrated between the number of chemotherapy cycles and the serum AMH (van Beek et al., 2007). FSH also rose with increasing treatment, but AMH appeared to have greater sensitivity to detect ovarian damage at lower doses of chemotherapy. The gonadotoxicity of alkylating agent based protocols has been shown in a range of childhood and adult malignancies (Rosendahl et al., 2008; Lie Fong et al., 2009; Gracia et al., 2012) but is most clearly demonstrated in a prospective study in young women with lymphoma (Decanter et al., 2010): AMH concentrations fell in all women during therapy but in the non-alkylating agent group there was then recovery to concentrations similar to pre-treatment whereas there was no evidence of recovery in women treated with alkylating agent based therapies.

Radiotherapy is also widely recognised to cause ovarian damage even at low doses and women treated with radiotherapy that includes the pelvis (including abdominal pelvic therapy in children or total body irradiation) generally have very low or undetectable AMH concentrations (Gracia, et al., 2012) (Lie Fong, et al., 2009).

Most of these studies were retrospective in nature, with no pre-treatment samples taken. There is also a dearth of data linking post treatment AMH to other clinical variables, most importantly fertility and subsequent reproductive lifespan, although a recent analysis shows a high prevalence of successful pregnancy in childhood lymphoma survivors despite low AMH concentrations (Hamre et al., 2012). A prospective study in women with newly diagnosed breast cancer linked pre-treatment AMH with long term ovarian function at 5 years (Anderson and Cameron, 2011), pre-treatment serum AMH being markedly higher in women who continued to have menses. The predictive value of AMH for post-chemotherapy ovarian function has subsequently been confirmed (Anderson et al., 2013) allowing the development of prediction tools combining age and AMH (Figure 5). It therefore appears that in addition to reflecting post-chemotherapy (or radiotherapy) damage, AMH is also able to predict on-going ovarian activity after such treatment, and the existing data suggest it is likely to be more robust than either FSH or inhibin B in this regard. Consistent with this, a study in younger women has demonstrated that pretreatment AMH predicts post-chemotherapy recovery, with a more rapid recovery in women with

higher pretreatment AMH (Dillon et al., 2013). Older women with cancer may have lowered pretreatment AMH concentrations; this was not observed in younger women (Su et al., 2013). Substantial prospective studies are required to develop a clearer analysis of the predictive value of AMH in different circumstances and it may be of value in information provision for example regarding the need for fertility preservation strategies.

AMH is detectible in girls of all ages, unlike other reproductive hormones, and rises steadily through childhood thus may be of value in the assessment of ovarian function in pre-pubertal girls. In a prospective analysis of girls with varied diagnosis (and therefore undergoing differed therapies) at different ages, AMH declined during repeated chemotherapy cycles (Brougham et al., 2012). Strikingly, in girls judged to be at medium or low risk of long-term ovarian damage, AMH recovered to concentrations similar to pre-treatment, whereas in girls judged to be at high risk, serum AMH at the end of treatment was undetectable and showed no evidence of recovery. Post-treatment AMH therefore appeared to identify even very young girls who are very likely to require pubertal induction, distinct from others who may be able to be reassured as to the likelihood of satisfactory ovarian function later in life. Long term follow up of these different groups is required to ascertain fully the value of post childhood cancer AMH in predicting long term ovarian function whether reflected in achieving spontaneous puberty, fertility or reproductive lifespan.

The impact of ovarian surgery on the ovarian reserve as measured by AMH has also been investigated, and two systematic reviews of the impact of ovarian surgery for endometriosis have been published (Raffi et al., 2012; Somigliana et al., 2012). Both analyses highlight the heterogeneity of study design and the difficulty in pooling data. However both conclude that ovarian endometrioma surgery is associated with a decline in serum AMH, indicating the removal of a significant part of the ovarian reserve. A subsequent large retrospective analysis has confirmed the impact of endometrioma surgery on the ovarian reserve as detected by serum AMH (Streuli et al., 2012), and these findings should be taken in to account in the planning and decision making process relating to ovarian surgery in women desirous of future pregnancy.

Assessment of Ovarian Reserve in infertility and ART patients

Age and ovarian reserve are potentially the most important patient characteristics determining the success of assisted conception, with interpretation of AMH in an age-specific manner now feasible (Nelson et al., 2011a; Nelson et al., 2011c; Almog, et al., 2011). Recognition of the linear relationship of AMH with oocyte yield was a critical step forward (Nelson et al., 2007; La Marca et al., 2010). That AMH can predict ovarian response accurately (Broer et al., 2011)(Broer et al., 2009) enables clinicians to avoid iatrogenic complications and to choose the optimal stimulation strategy. This also ensures that patients are counselled appropriately with realistic expectations of the outcome of their ovarian stimulation.

At one extreme of the response spectrum we can identify women who are at risk of OHSS (Al-Inany et al., 2011, Broer, et al., 2011). We can adjust our stimulation strategy to incorporate GnRH antagonists (Al-Inany, et al., 2011) reducing the risk of this potentially fatal complication (Acolet et al., 2005, Braat et al., 2010). Choosing a GnRH antagonist prtocol and adjusting the FSH dose according to a high serum AMH level should preclude OHSS but at present, however, only locally-derived thresholds can be used since there is no consensus on an universal threshold (Broer, et al., 2011). This approach has particular benefits for women undergoing altruistic oocyte donation, removing much of the integral risk of IVF (Bodri et al., 2009). Conversely maximising follicular recruitment would seem appropriate if a poor response was anticipated, although the optimal strategy for the poor responder remains debated (Ferraretti et al., 2011). At present the value of a mixed strategy in an ART programme has yet to be fully elucidated, but for centres where agonist strategies still dominate the advantage of an AMH-based approach over conventional dose adjustment and long course agonist for all has been demonstrated (Nelson et al., 2009).

The ability to predict a very poor response has resulted in some centres withholding the first treatment cycle if a very low AMH is detected, with an overall improvement in results of the programme and substantial cost savings (Yates et al., 2011). However even women with AMH concentrations at the limit of assay sensitivity have a significant chance of conception through IVF, thus this approach appears unjustified (Anderson et al., 2012). Inevitably this chance will be lower than for a woman of the same age with a higher ovarian reserve (La Marca, et al., 2010) but to withhold treatment and not actually confirm a predicted poor response at present purely based on an AMH would seem inappropriate. This is particularly the case as this approach has not been incorporated into cost-effectiveness models with other more accurate population level models available (Lawlor and Nelson, 2012; Nelson and Lawlor, 2011).

Whether knowing the anticipated oocyte response has a beneficial psychological effect for the couple and thereby reduces cycle drop out has not been formally evaluated. Discussion of the ovarian assessment report may set patient's expectations appropriately particularly at the bottom end of the spectrum where only a few oocytes may be retrieved. Given that many women do not fully appreciate the detrimental effect of age on oocyte number, the ability to guide them on overall success using a combination of their age as a surrogate for oocyte quality, and AMH for oocyte yield is a powerful tool (La Marca et al., 2011).

It is likely in the future that with standardisation of AMH measurement and stimulation strategies, multivariate prediction models with tight confidence intervals will be able to be created and individualised reports generated. Steps on this path have already been made with optimal prediction of excessive response achieved by combining age, AMH and antral follicle count (Broer et al., 2011a) and refinement of gonadotropin dosing by combining AMH with FSH and age (La Marca et al., 2012). The

future is therefore likely to harness the collective power of biomarkers including AMH to ensure true personalisation of ovarian stimulation.

Factors influencing the relationship between and the predictability of AMH and antral follicle count (AFC)

The follicular pool that influences serum AMH levels the most probably is that of 1-2 mm follicles, although some analyses have suggested a slightly larger size (Jeppesen, et al., 2013) (see section 3-1). This notion assumes a particular importance not only when we analyze the strength of the relationship between the ultrasonographic counting of antral follicles (AFC) and serum AMH levels but also when we compare the clinical predictability of both parameters.

Although the positive relationship between AFC and serum AMH levels has been recognized for over ten years (Fanchin et al., 2003), cases of discrepancy are sporadically observed (Schipper et al., 2012). These cases may result, at least in part, from technical difficulties but other physiological contingencies may influence this expected relationship. According to recent guidelines (Broekmans et al., 2010) and current clinical practice worldwide, ultrasonographic counting considers antral follicles whose diameter varies considerably, from 2 to 10 mm. It is also noteworthy that ultrasound technology cannot distinguish healthy from atretic follicles. Therefore, the strength of the correlation between AFC and serum AMH is influenced by at least 2 additional factors. The first is antral follicle sizes. It is likely that a patient whose AFC is mostly represented by small follicles (1-2mm) will display higher serum AMH levels than a patient who has a majority of large antral follicles (>6 mm). The second factor is follicle “health“ as granulosa cell atresia may hinder AMH production. Further clinical studies are needed to confirm these hypotheses.

In line with this, both AMH and AFC have been shown to be useful markers of the ovarian response to controlled ovarian hyperstimulation (Broer, et al., 2013). Again here, two other refinements should be brought to this clinical observation. On the one hand, it is probable that, in the beginning of the follicular phase, it is the large antral follicles that will respond first to gonadotropin treatment. As these follicles are already losing their ability to produce AMH, AFC might better predict ovarian response than AMH (Mutlu et al., 2013). On the other hand, if we consider that atretic antral follicles will not properly respond to exogenous FSH, AMH should be the most reliable marker as it is not produced by atretic follicles that still are counted by ultrasound. Another pertinent issue regarding both biomarkers is that, contrary to AFC, AMH is also an important regulator of ovarian function, as discussed above. In the ovary, AMH exerts an inhibiting role on many follicular functions, including granulosa cell sensitivity to FSH. In support of this, antral follicle responsiveness to exogenous gonadotropins, clinically assessed by the Follicle Output RaTe (FORT), is inversely correlated with serum AMH (Genro et al., 2011).

Therefore, from a clinical standpoint, both AMH and AFC provide the physician with useful information regarding ovarian follicular status and responsiveness to controlled ovarian hyperstimulation. While AMH provides information essentially on the number of very small, non-atretic follicles, AFC is contributive to detect follicle sizes and evaluate size discrepancies, with both analyses being complementary to the proper adaptation of the type of stimulation required by the patient.

Polycystic Ovary Syndrome (PCOS)

AMH and its putative role in PCOS pathophysiology

PCOS, a heterogeneous condition, is the most prevalent endocrine disorder in women, affecting 5 to 10% of the female population (Franks, 2008). Women with PCOS present with a range of symptoms such as acne, hirsutism and/or menstrual irregularities and have an increased risk of type II diabetes. The condition imposes a considerable economic burden on health systems internationally (Azziz et al., 2005). Polycystic ovaries (PCOs) are characterised by an increase in the number of follicles at all growing stages (Hughesdon, 1982; Webber et al., 2003; Maciel et al., 2004). PCOS is almost certainly a genetic condition (Kosova and Urbanek, 2013), but the cause of the change in ovarian and the cause of anovulation which affects a subgroup of these women remains unknown.

The ability of AMH to alter early follicle growth was demonstrated by the AMH knock-out mouse model (Durlinger, et al., 1999, Durlinger, et al., 2002) in which there is an increase in the initiation of primordial follicles into the growing pool (see section 3). This morphology appeared similar to that seen in polycystic ovaries (PCOs) and so an assessment of the production of AMH by PCOs was carried out. Stubbs et al., 2005 found fewer primordial and transitional follicles positively stained for AMH from anovulatory PCO than in normal ovaries. Reduced AMH in anovulatory PCO might enhance the transition of follicles to the growing phases, or might be a marker of abnormal early follicle growth in PCOS.

Serum AMH is two to four-fold higher in women with PCOS than in normal women (Pigny et al., 2003; Laven et al., 2004; Park et al., 2010; Lie Fong et al., 2011). This increase in serum AMH was thought to reflect the increased number of small antral follicles in which AMH production is highest. However, when production of AMH per granulosa cell was compared between normal ovaries, ovulatory and anovulatory PCOs (Pellatt et al., 2007), AMH production was on average 75 times higher per granulosa cell from anovulatory PCOs and 20 times higher from ovulatory PCOs. This indicates that the increase in AMH is due to an intrinsic property of granulosa cells in PCOs, a property that persists even after stimulation for IVF (Catteau-Jonard et al., 2008). These increased concentrations are also found in follicular fluid (Das et al., 2008).

The cause of such high levels of AMH in antral follicles in PCOS is currently unknown. However there is evidence to support a role for androgens as a positive correlation with AMH in serum has been reported

(Carlsen et al., 2009; Pigny, et al., 2003; Laven, et al., 2004; Eldar-Geva et al., 2005), and over-production of androgens is an intrinsic defect of theca from PCOs (Gilling-Smith et al., 1994). It is curious that AMH should be lower in preantral follicles and then higher once the follicle reaches the antral stage, however prenatal testosterone treatment of sheep produced precisely this effect (Veiga-Lopez et al., 2011). In vitro however, androgens have not been shown to do this and indeed androgens have been shown to reduce antral follicle granulosa cell AMH production in a bovine model (Crisosto et al., 2009). In human, serum AMH levels decrease in female to male transsexual women using testosterone as cross-sex therapy (Caanen M et al., 2013). Other groups have demonstrated inhibition of AMH production by gonadotrophins, particularly FSH (Baarends et al., 1995; Panidis et al., 2011). Others found no such inhibitory effect on granulosa cells from normal ovaries; in contrast, FSH did inhibit AMH production in cultured granulosa cells from polycystic ovaries (Pellatt, et al., 2007) whereas LH significantly stimulated production.

Although many aspects of AMH action in the ovary remain to be elucidated, knowledge is emerging. AMH significantly decreases FSH- and LH- induced aromatase expression in granulosa cells as well as reducing the activity of the ovary-specific aromatase promoter II (see section 3). This results in a significant reduction in estradiol production (Pellatt et al., 2011). AMH also inhibits FSH-stimulated FSH receptor mRNA expression (Pellatt, et al., 2011). The fact that AMH is inhibitory of factors required for follicle growth adds considerable significance to the finding of high AMH in PCOS. LH reduces AMHRII expression in granulosa luteal cells collected from women with normal ovaries and ovulatory PCOS, but was unable to do so in women with anovulatory PCOS (Pierre et al., 2013). It can be envisaged that AMH content in antral follicles in these ovaries would be sufficient to inhibit FSH-stimulated aromatase expression and would thus prevent the inhibitory effect of estradiol on AMH production (Figure 1). This effect would be amplified by the loss of LH-induced down-regulation of AMHRII expression in women with anovulatory PCOS. These findings suggest that AMH may contribute to anovulation in PCOS. In agreement, it has been shown that emergence of a dominant follicle in anovulatory women with PCOS under recFSH is preceded by a significant reduction in serum AMH level (Catteau-Jonard et al., 2007).

AMH in diagnosing PCOS: a shift from ultrasound to laboratory

Given its strong involvement in the pathophysiology of PCOS (see section 5.1), serum AMH is a subject of special interest for clinicians involved in this field. There is considerable interest in whether it might become part of the diagnostic criteria for the condition, although this is at present premature. It may also shed light on different subtypes of this diverse condition leading to greater understanding of the disordered follicle growth. Certainly, the serum AMH concentration appears to be greatly increased in most patients with PCOS (Pigny, et al., 2003, Laven, et al., 2004, Li et al., 2011). This elevation is highly pertinent as it has been shown that polycystic ovaries (PCO) exhibit an increased number of AMH-producing pre-antral and small antral follicles, the latter expressing the most AMH (Weenen, et al.,

2004) and contributing the most to the circulating AMH (Jeppesen, et al., 2013). In addition, production of AMH is greatly increased in GC from PCO, especially if the patient is oligo-anovulatory, as discussed above (Pellatt, et al., 2010). Therefore, not surprisingly, many authors have reported a strong correlation between plasma levels of AMH and follicle count on ultrasound in PCOS patients. The strength of this relationship is even greater with newer ultrasound technology allowing the counting of 1-2 mm follicles (Dewailly et al., 2011).

The strong association between AMH and follicle count has led some authors to compare the performance of one against the other for the diagnosis of PCOS. However, the results in the current literature are not homogeneous between studies, as well demonstrated in a recent compilation (Iliodromiti et al., 2013). Part of this heterogeneity is due to the lack of well-defined populations. In particular, it must be stressed that many authors have used the threshold for follicle excess that was established in 2003 at the Rotterdam Consensus Conference to define PCOM (Balen et al., 2003), namely 12 follicles of 2-9 mm diameter per ovary. With the latest generation of ultrasound equipment and using well-defined populations, recent studies have proposed to increase this threshold to 19 or 25 (Dewailly, et al., 2011, Lujan et al., 2013, respectively). This threshold will probably continue to evolve in parallel with the technical improvement of ultrasound equipment.

Beside the flaw in the ultrasound definition of controls and patients, the variability of the results can also be explained by the problem that prevails with serum AMH assays. About half of the previous studies were performed using either the DSL or IOT assays (Iliodromiti, et al., 2013), for which concordance in the values is problematic (see above). More recent studies using the Gen II kit should also be interpreted with caution (see above).

It is therefore impossible to date to propose a consensual and universal diagnostic threshold for serum AMH that is predictive of PCOS. Using the IOT assay, serum AMH was found to be more efficient than the follicle count with excellent sensitivity and specificity for a threshold of 35 pmol/l (4.9 ng/ml) (Dewailly, et al., 2011). Contrary to other studies, specific thresholds for AMH and follicle count were calculated without using pre-determined values. In addition, women with supposedly asymptomatic PCOM were excluded from the control group of regularly menstruating women by cluster analysis. If these results can be replicated with the new AMH assays, serum AMH may become an accurate and reliable marker that may eventually replace the follicle count which itself, in turn, suffers from great controversy in the current literature. It is reasonable to propose that the increased serum AMH is a surrogate to the term "PCOM" in the Rotterdam classification (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004). Further, since we have now at our disposal two different markers, one being morphological (PCOM) and the other being biochemical (increased serum AMH), the terms “PCO-like abnormalities” (PCO-L) may become more accepted as the third item of the Rotterdam classification (Robin et al., 2012).

In addition, the serum AMH correlates with the severity of PCOS and precisely with the severity of both hyperandrogenism (Piouka et al., 2009) and oligo-anovulation (Laven, et al., 2004, Catteau-Jonard et al., 2012). By principal component analysis, it has been shown that a high serum AMH level can be considered a marker of hyperandrogenism and may therefore also be considered as a replacement for this other item in the Rotterdam classification (Dewailly et al., 2010). This would reconcile the different classifications currently available for the diagnosis of PCOS since some of them necessarily require the presence of hyperandrogenism to retain the diagnosis (Azziz et al., 2009). The only exception to this assertion would be the presence of PCOS in women with type 1 diabetes, where serum AMH does not correlate to androgen levels (Codner et al., 2007).

Therefore, to establish the diagnosis of PCOS, after exclusion of other diagnoses, oligo-anovulation and HA should first be required. In the cases where one is missing, then "PCO-L" (i.e., high AFC and/or serum AMH level) could be used as a surrogate for either oligo-anovulation or HA. It must be stressed, however, that the thresholds for an excessive AFC and serum AMH level have to be revisited and validated worldwide in populations of different ethnicity. Meanwhile, local in-house control data can be used. We think this information is important and useful for diagnostic concerns as well as for phenotype/genotype analysis within genetic studies.

The diagnostic value of serum AMH concentrations has also been studied in adolescents since ultrasound is often unreliable in detecting PCOM in this population. A study in Chilean adolescents identified a cut-off serum AMH concentration of 60 pmol/l (with the IOT assay) to diagnose PCOM in regularly menstruating adolescents, with a sensitivity and specificity of 64% and 90% (area under the ROC curve = 0.87) (Villarroel et al., 2011). The results were not as good in Australian adolescents with the same assay (area under the ROC curve = 0.67) leading the authors to conclude that serum AMH was a questionable surrogate for PCOM in adolescents (Hart et al., 2010).

Finally, in addition to its diagnostic role, the determination of AMH could be used in the future to establish treatment protocols, and in particular to define the strategy for the induction of ovulation in infertile oligo-anovulatory PCOS women. To date, there are very few studies that have examined the predictive power of AMH assay for response to clomifene, recombinant FSH or to ovarian drilling. Similarly, AMH is of value as a good predictor of the risk of ovarian hyperstimulation in an IVF setting (Broer, et al., 2011) .

The current technical difficulties with the determination of serum AMH may have dampened the enthusiasm of some clinicians for this marker of PCOM. However there are sufficient data to support the view that this assay may replace (or be an alternative for) AFC in the Rotterdam classification, which will make it even more reliable and more flexible, especially in situations when ultrasound is uninformative or impossible, as in obese women or adolescents.

Future avenues

Recent years have shown multiple ways in which AMH is not only a “male” hormone but is emerging as an invaluable tool offering new insights into ovarian function in childhood, adolescence and through the reproductive years. Although knowledge of its precise roles in ovarian physiology still requires extensive fundamental and clinical studies, it is already clear that AMH is crucial in maintaining the right tempo of folliculogenesis in the ovary (although there are only very limited human data), making it one of the most important ovarian hormones and one of the most crucial factors underpinning female fertility. Whether its action is exclusively intra-ovarian, within and between follicles, is a challenging issue for future research. We should think about possible endocrine effects of this hormone, possibly in ovary-to-ovary interaction or in hypothalamic-pituitary-ovarian integration.

At the current time, the clinical use of serum AMH assay is hampered by technical issues undermining its reliability. It is likely that these issues will be rapidly solved and the advent of more sensitive assays may confirm that serum AMH level is the best biochemical marker of ovarian function in a large array of clinical situations, both in childhood and adulthood. For the first time in female reproductive biology, we have at our disposition an easy measure of the submerged part of the iceberg of follicle growth, i.e., the intrinsic so-called “acyclic” ovarian activity.

Author's Roles

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Legends to figures

Figure 1: Schematic model of anti-Müllerian hormone (AMH) actions in the ovary.

AMH, produced by the granulosa cells of small growing follicles, inhibits initial follicle recruitment and FSH-dependent growth and selection of preantral and small antral follicles. In addition, AMH remains highly expressed in cumulus cells of mature follicles. The inset shows in more detail the inhibitory effect of AMH on FSH-induced *CYP19a1* expression leading to reduced E2 levels, and the inhibitory effect of E2 itself on AMH expression. AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone; T, testosterone; E2, estradiol; Cyp19a1, aromatase. Figure modified from van Houten et al. (2010).

Figure 2: Schematic presentation depicting the processing of AMH.

AMH is produced as a precursor protein consisting of disulphide-linked monomers. Upon cleavage by prohormone convertases the protein is cleaved into pro- and mature homodimers, which remain non-covalently associated. AMH enzyme-linked immunosorbent assays (ELISA) have been developed to detect AMH in circulation. The regions that are recognized by the monoclonal antibodies used in the ultrasensitive IOT assay and the Gen II assay (previously DSL) are indicated. For the Gen II assay, the capture antibody recognized the mature region and the detector antibody recognizes the proregion.

Figure 3: AMH variability throughout the menstrual cycle. Serum AMH appears to be stable.

(*Reproduced with permission from (a) La Marca et al., 2006, (b) Hehenkamp et al., 2006 and (c) Tsepelidis et al., 2007).*

Figure 4: AMH and follicular recruitment profile across the lifespan.

Comparison of serum AMH concentrations with NGF recruitment rates. The red line is the log-unadjusted validated AMH model (Kelsey et al., 2011), peaking at 24.5 years. The blue line denotes the numbers of NGFs recruited per month towards the maturation population (Wallace and Kelsey, 2010), with peak numbers lost at age 14.2 years on average. Correlation coefficients (r) are given for AMH concentrations against follicular recruitment for each developmental phase; from birth to puberty (age 9 years), during puberty (9 – 15 years), post-puberty (15- 25 years) and mature adults (>25 years).

Figure 5: Classification mosaic chart for ongoing menses (M) or chemotherapy-related amenorrhea (A) using pre-chemotherapy serum AMH and chronological age as predictor variables, in women with early breast cancer.

The primary cutoff values are both for AMH, with below 3·8 pmol/L predicting amenorrhea and above 20·3 pmol/L predicting ongoing menses. Between these AMH levels there is an age threshold at 38·6 years, above which amenorrhea is predicted and below which ongoing menses are predicted. The

classification schema has sensitivity 98·2% and specificity 80·0%. Reprinted with permission from Anderson et al 2013, Eur J Cancer.

Figure 6: rationale for the use of serum AMH assay as a probe for PCOM

(A) All growing follicles secrete AMH but serum AMH reflects only the secretion from bigger follicles that are in contact with the vascular bed. As the numbers of follicles in all growth stages are strongly related to each other, serum AMH is considered to reflect the sum of growing follicles but not the number of primordial follicles that do not secrete AMH (see section 3-1).

(B) In PCO, the numbers of all growing follicles is increased, resulting in a marked increase in serum AMH level (see section 5). This marker may be considered as a deeper and more sensitive probe to define follicle excess than the follicle count by ultrasound (U/S) since it appraises more follicle classes (blue arrows).