Reproductive BioMedicine Online (2015) 31, 486–496

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REVIEW

Assessing ovarian response: antral follicle count versus anti-Müllerian hormone

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Richard Fleming has worked as a scientist in research and clinical service in reproductive medicine for more than 30 years. He is currently Scientific Director at GCRM and Honorary Professor of Reproductive Medicine at the University of Glasgow. He has been responsible for a number of innovative developments, including the first use of ultrasound of the ovary (published 1979), and has pioneered the use of GnRH agonists to control pituitary activity during controlled ovarian stimulation (published 1982). Recent work on the assessment of ovarian reserve prior to assisted reproduction has led to debates on how best to use this critically important information.

Abstract Oocyte number and quality decline with age; however, fertility varies significantly even among women of the same age. Various measures have been developed to predict response to ovarian stimulation and reproductive potential. Evaluation of ovarian reserve can identify patients who may experience poor response or hyper-response to exogenous gonadotrophins and can aid in the personalization of treatment to achieve good response and minimize risks. In recent years, two key methods, antral follicle count (AFC), an ultrasound biomarker of follicle number, and the concentration of serum anti-Müllerian hormone (AMH), a hormone biomarker of follicle number, have emerged as preferred methods for assessing ovarian reserve. In this review, a live debate held at the American Society for Reproductive Medicine 2013 Annual Meeting is expanded upon to compare the predictive values, merits, and disadvantages of AFC and AMH level. An ovarian reserve measure without limitations has not yet been discovered, although both AFC and AMH have good predictive value. Published evidence, however, as well as the objectivity and potential standardization of AMH level and the convenience of testing any time throughout the menstrual cycle, leans towards AMH level becoming the gold-standard biomarker to evaluate ovarian reserve and predict ovarian response to stimulation.

KEYWORDS: anti-Müllerian hormone, antral follicle count, hyper-response, in-vitro fertilization, ovarian reserve, poor response

http://dx.doi.org/10.1016/j.rbmo.2015.06.015
1472-6483/

Introduction

The fecundity of women begins to decrease after the age of 30 years, primarily as a result of a decrease in the proportion of normal eggs available, which in turn is a consequence of a continuous process of oocyte atresia (Nelson et al., 2013). Although all women experience this decrease in fecundity, it is difficult to predict the pace of reproductive decline in an individual woman (Broekmans et al., 2006; Faddy et al., 1992; Nelson et al., 2013).

The maximum number of oocytes is 6–7 million, occurring at a gestational age of about 20 weeks in the female fetus. This number decreases to about 1–2 million oocytes at birth, 300,000–500,000 at puberty, 25,000 at an age of 37 years, and 1000 at an age of 51 years, which coincides with the average menopausal age in the USA (Baker, 1963; Block, 1952; Faddy et al., 1992). Women's fecundity decreases gradually, but significantly, beginning after an age of 30 years and then more rapidly in the mid- to late-30s, and is effectively negligible almost a decade before menopause. This age-related decline in fecundity is characterized by a decrease in both egg quality and number, and a population-based change in the expression of markers of ovarian activity, such as a gradual increase in circulating FSH and decreases in circulating anti-Müllerian hormone (AMH) and inhibin B concentrations (Broekmans et al., 2006; Faddy et al., 1992; American College of Obstetricians et al., 2014). It should be noted that a wide variation exists in the number of eggs between women of any age, and that a 30-year-old woman with high ovarian reserve typically demonstrates a total follicle count as much as 100-fold higher than that of a 30-year-old woman with low ovarian reserve (Wallace and Kelsey, 2010).

The follicle maturation process (folliculogenesis) (**Figure 1**) is lengthy and complex (Baerwald et al., 2012; Vegetti and Alagna, 2006). The ovarian reserve is principally composed of 'resting' primordial follicles, which may remain at the arrested stage of development for more than 40 years before developing into primary follicles (Baerwald et al., 2012; Gougeon, 1996; Gougeon et al., 1994). Most (>99%) primordial follicles that undergo further development will be lost to atresia during the maturation process (Baerwald et al., 2012; Baker and Spears, 1999). Once a primordial follicle has been selected to enter active follicular growth, granulosa cells of the now primary follicle begin to express AMH (Visser and Themmen, 2005; Weenen et al., 2004). This expression continues up to the antral stages of development and is discontinued as the follicle becomes dependent on FSH for continued growth (Weenen et al., 2004). Correspondingly, circulating concentrations of AMH are generally considered to be non-cyclic throughout normal menstrual cycles (Hehenkamp et al., 2006; La Marca et al., 2013; Tsepelidis et al., 2007). However, a recent study found that serum AMH levels were significantly lower in the late luteal phase compared with the early follicular phase, with a pattern similar to pituitary FSH (Hadlow et al., 2013). A small, but significant, variation in serum AMH level throughout the menstrual cycle was also reported in a separate study, although the authors indicated that this variation may not have any clinical significance (Deb et al., 2013).

Within the ovary, AMH is involved in the regulation of the number of primordial follicles that begin maturation, preventing premature exhaustion of the ovarian reserve (La Marca et al., 2009; Visser and Themmen, 2005). In granulosa cells, AMH is also involved in the regulation of steroidogenesis (La Marca et al., 2009; Visser and Themmen, 2005). Maturation into small antral (fluid-filled) follicles is characterized by maximal expression and concentrations of AMH within the follicle, followed rapidly by diminishing expression of AMH and growing dependence on FSH, which promotes further development into large antral and then pre-ovulatory follicles (Baerwald et al., 2012). The concentration of FSH rises above a critical threshold to drive this follicle development, and then lowers again during the late follicular phase owing to ostrogen-derived negative feedback (Baerwald et al., 2012). Therefore, FSH deprivation leads to atresia, whereas sustained elevation of FSH leads to follicular growth and maturation.

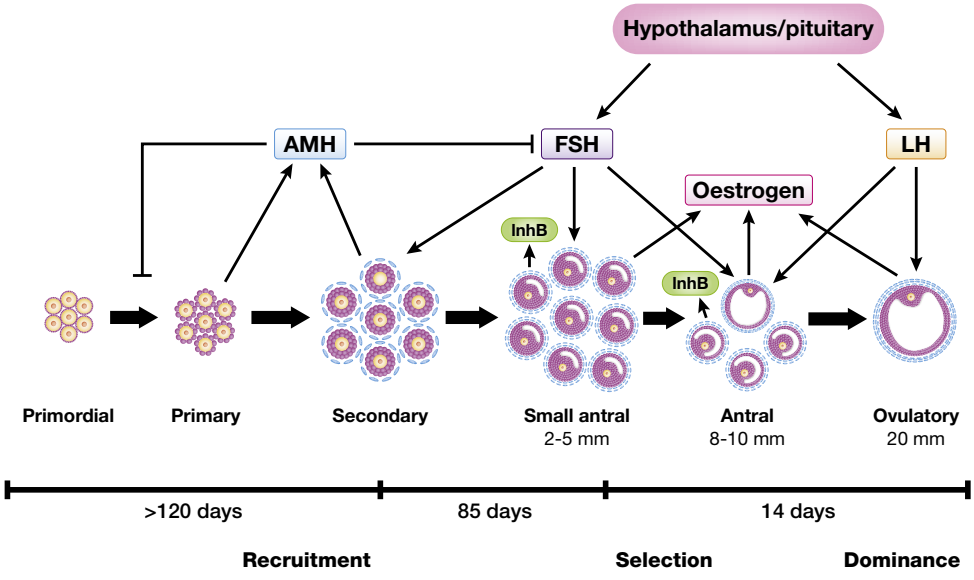

Figure 1 Folliculogenesis. AMH = anti-Müllerian hormone; InhB = inhibin B.

The basis of ovarian stimulation for assisted reproduction treatment is the administration of exogenous gonadotrophins. To permit retrieval of multiple oocytes during a single cycle, it is necessary to maintain FSH and LH concentrations above a critical threshold, so that multiple competent follicles are selected for growth and maturation (Mochtar et al., 2007; Vegetti and Alagna, 2006). A gonadotrophin-releasing hormone (GnRH) agonist or antagonist is given concurrently to ensure prevention of a premature spike of LH that would induce ovulation (Mochtar et al., 2007; Shoham et al., 1993). Final oocyte maturation is then typically triggered by administration of a bolus of human chorionic gonadotropin (HCG), which is structurally and biologically similar to LH and binds to the same LH/HCG receptor (Humaidan et al., 2012). Alternatively, a bolus dose of GnRH agonist to induce a short endogenous surge of pituitary gonadotropins with or without concomitant HCG can be used for final oocyte maturation (Humaidan et al., 2012).

Some women respond poorly to ovarian stimulation, e.g. development of less than four retrieved oocytes (Hendriks et al., 2005), and others may experience a hyper-response, e.g. development of over 15 retrieved oocytes (Steward et al., 2014; Sunkara et al., 2011). Although oocyte reserve and the number of developing follicles decline generally with increasing age, it is a weak predictor of either poor or hyper-response (Alviggi et al., 2012; La Marca et al., 2012). Therefore, if adaptations to the ovarian stimulation protocol are to be made, more reliable indicators of response are required.

Why measure ovarian reserve?

Oocyte number and quality are known to decline with age; however, large variations in oocyte reserve exist between individual patients, as do ovarian responses to gonadotrophin stimulation, even among women of the same age (Fleming et al., 2013; La Marca et al., 2012). Women with a low ovarian reserve are more likely to respond to ovarian stimulation with a modest degree of follicular development ('poor responders') and may require greater management of their expectations for outcome success (Fleming et al., 2013; La Marca et al., 2012). At the other end of the spectrum, women with a high ovarian reserve are at increased risk for excessive ovarian response that can lead to ovarian hyperstimulation syndrome (OHSS) (Broer et al., 2011; Fleming et al., 2013; La Marca et al., 2012), which is a common (occurring in up to 30% of IVF cycles) and potentially severe (even rarely fatal) iatrogenic adverse outcome associated with gonadotrophin preparations (Broer et al., 2011; Humaidan et al., 2010).

Additionally, it has been suggested that some patients may benefit from one protocol over another, i.e. antagonist versus long agonist protocol, or from other protocol and FSH dose adjustments. Correspondingly, women predicted to have excessive responses may benefit from a GnRH antagonist protocol, as comparative trials have shown they are associated with fewer developing follicles when using milder stimulation and allow for a GnRH agonist trigger instead of an HCG trigger in cases with a risk of OHSS (Fleming et al., 2013). Women predicted to have poor response may benefit from a higher gonadotrophin dose for maximal stimulation in a GnRH antagonist protocol, long GnRH agonist protocol, or GnRH flare-agonist protocol, which adds a burst of endogenous FSH and LH stimulation at the start of the protocol to enhance follicular recruitment (Fleming et al., 2013).

No convincing data that increasing the dose of FSH above a standard level leads to increased egg yields have been published (Haas et al., 2015; Karande and Gleicher, 1999; Land et al., 1996; van Hooff et al., 1993). It is likely the best result that can be achieved in this group of women is avoidance of a submaximal response. Personalization of stimulation protocols for assisted reproduction techniques may thus improve both outcomes and patient safety and reduce the incidence of cycle cancellations (Fleming et al., 2013; La Marca et al., 2012). Cycle cancellations owing to poor or hyper-response contribute to the psychological burden of assisted reproduction techniques by adding significant emotional and financial costs (Fiedler and Ezcurra, 2012; Humaidan et al., 2010; Kee et al., 2000), as well as further delaying time to pregnancy by eliminating the opportunity to proceed with a fresh embryo transfer.

Therefore, accurate and reliable predictors of ovarian reserve are needed to identify patients likely to have poor response or hyper-response to treatment and to guide physicians in selecting the optimal dose of gonadotrophins for ovarian stimulation. To predict ovarian reserve and reproductive potential, several different measures of ovarian reserve have been identified over time, including biochemical measures and ovarian imaging, with varying degrees of success. To discuss the need for consistently useful measures of ovarian reserve, a debate was held at the American Society for Reproductive Medicine (ASRM) 2013 Annual Meeting to compare and contrast two key methods (antral follicle count [AFC] and AMH level) for determining ovarian reserve. Presentations of contrasting views on the predictive value, additional advantages, and disadvantages of AFC versus AMH level were given based on available published literature.

Predictors of ovarian reserve

A number of predictors of ovarian reserve have been identified, including patient age; concentration of hormonal markers, such as basal (perimenstrual) FSH, LH, oestradiol, inhibin B, and, more recently, AMH; and dynamic tests, such as the clomiphene citrate challenge test. Other predictors of ovarian reserve include ultrasound measures, such as pre-treatment ovarian volume and AFC, which is the sum of small antral follicles in both ovaries. Most of these measures, however, have limited predictive value, often because they are indirect measures of ovarian reserve, e.g. FSH, clomiphene citrate challenge test, or have substantial inter-patient variability, e.g. age, or intra-cycle fluctuation, e.g. basal FSH. (Biasoni et al., 2011; Broekmans et al., 2006; Broer et al., 2010; Fleming et al., 2013; La Marca et al., 2012). In recent years, data have emerged to support AFC and AMH level as preferred methods for predicting ovarian reserve with a varied degree of precision (Hendriks et al., 2005; Iliodromiti et al., 2014a; La Marca et al., 2010; Lukaszuk et al., 2013; Polyzos et al., 2013).

Receiver operating characteristic (ROC) curve studies within single centres and meta-analyses have demonstrated that both AFC and AMH level can identify patients likely to respond to exogenous gonadotrophins with poor, normal, or hyper-response (Hendriks et al., 2005; La Marca et al., 2010; Lukaszuk et al., 2013; Polyzos et al., 2013). Notable differences, however, exist: AMH natural values show large inter-individual variability by

age, indicating a wide range of ovarian reserve among the healthy population (La Marca et al., 2013), whereas the range of values described by AFC do not show the same degree of sensitivity owing to technical limitations, restriction to antral follicles of measurable size, and differences in methodology for counting antral follicles (Arce et al., 2013b; Broekmans et al., 2010).

AFC: the evidence as a predictive method

Transvaginal ultrasound is required to determine AFC by imaging and manually measuring the diameter of all small antral follicles and counting those between 2 and 10 mm in diameter (**Figure 1**) (Broekmans et al., 2010; Chang et al., 1998a, 1998b). Beginning with the early antral follicle stage, folliculogenesis becomes cyclic, with maturation dependent on waves of FSH and other factors (Baerwald et al., 2012; Vegetti and Alagna, 2006); therefore, AFC is typically carried out at the beginning of a cycle (Chang et al., 1998a, 1998b). Recent evidence, however, suggests that AFC can be obtained at any point in the cycle without compromising accuracy (Deb et al., 2013). With the recent development of three-dimensional ultrasound and other improvements in ultrasound resolution, antral follicles as small as 2 mm in diameter can now be reliably counted (Broekmans et al., 2010).

For several important IVF outcomes, AFC has been associated with good predictive value, showing a linear relationship with the number of retrieved oocytes (Chang et al., 1998a, 1998b; Himabindu et al., 2013; Hsu et al., 2011; Tsakos et al., 2014) and correlation with measures of ovarian response to gonadotrophins, including cycle cancellations as a result of poor response (Frattarelli et al., 2000; Tomas et al., 1997). Reports examining the correlation with clinical pregnancy rate and live birth rate, however, are more variable (Chang et al., 1998a, 1998b; Hsu et al., 2011; Jayaprakasan et al., 2012; Lukaszuk et al., 2014), and AFC has not been shown to be predictive of embryo quality (Chang et al., 1998a, 1998b; Hsu et al., 2011; Jayaprakasan et al., 2012). It has also been suggested that determination of AFC may be helpful in identifying an appropriate stimulation protocol (Hsu et al., 2011).

AMH: the evidence as a predictive method

Circulating levels of AMH derive from the total cohort of granulosa cells in developing small follicles (Visser and Themmen, 2005). The overwhelming majority of these cells are found in the later stages of pre-antral and early antral follicles (**Figure 1**) (La Marca et al., 2009; Weenen et al., 2004). Correspondingly, AMH levels are correlated with the number of early stage antral follicles (Amer et al., 2013; La Marca et al., 2010; Laven et al., 2004). Just as the distribution of the total number of follicles present in the ovary shows a large log-scale variation in the healthy population at any age (Faddy et al., 1992), so does the circulating AMH level (Nelson et al., 2007; Seifer et al., 2011). This may have a practical advantage in defining specific cut-off values for predicting ovarian response to stimulation. Unlike basal FSH levels, only mild intra- and inter-cycle fluctuations are observed (La Marca et al., 2013). The decrease in AMH levels that occurs with increased age may be noted before changes in other age-related variables (La Marca et al., 2009), suggesting serum AMH levels may be the best marker of ovarian ageing; the next generation of AMH assays is being developed to demonstrate greater sensitivity, and they are likely to show greater value in this regard.

Similar to AFC, AMH level has also demonstrated good predictive value for a number of IVF outcomes. When a single stimulation protocol is used, a linear relationship develops between oocyte yield and AMH level (Blazar et al., 2011; Nardo et al., 2009; Tsakos et al., 2014). Although AMH level has been shown to decrease during stimulation with exogenous FSH (Anckaert et al., 2012; Blazar et al., 2011; La Marca et al., 2004), AMH levels during and on the last day of stimulation are still positively correlated with the number of retrieved oocytes (Anckaert et al., 2012; Blazar et al., 2011). In addition to oocyte yield, one report indirectly suggested AMH level may also predict embryo quality (Irez et al., 2011); however, direct confirmation of any potential predictive value for embryo quality is needed.

Given that AMH is associated with oocyte yield and oocyte yield has been shown to be a strong predictor of live births (Sunkara et al., 2011), it is plausible that AMH level could be used to predict pregnancy outcomes. Findings from several large-scale retrospective analyses of women undergoing IVF found a positive association between AMH level and live birth rates (Arce et al., 2013b; Khader et al., 2013; Li et al., 2013; Lukaszuk et al., 2014). A recent prospective study in nearly 900 women undergoing 1230 IVF cycles confirmed these findings, reporting that AMH level is strongly associated with both pregnancy and live birth rates, independent of age and oocyte yield (Brodin et al., 2013). However, other studies have not shown an association between AMH level and pregnancy rates or live births (Kedem et al., 2013; Lin et al., 2013; Mutlu et al., 2013; Reichman et al., 2014), including two separate meta-analyses (Broer et al., 2013; Iliodromiti et al., 2014b). A third meta-analysis found a weak association between AMH level and implantation and clinical pregnancy rates (Tal et al., 2015). Thus, AMH may be useful in predicting pregnancy and live birth rates, but further prospective analyses are still needed.

Determination of functional ovarian reserve as indicated by a woman's AMH level is likely to be helpful in selecting an appropriate stimulation protocol, starting dose of exogenous gonadotropins, or both. The risk for OHSS may be reduced in women with high AMH levels who receive lower doses of gonadotropins for shorter periods (a mild stimulation protocol) (Anckaert et al., 2012; Nelson et al., 2007). Thus, the choice of an antagonist-controlled protocol (with mild stimulation doses) may minimize the frequency of cycle cancellations caused by OHSS in women with high AMH levels (Arce et al., 2014; Casano et al., 2012; Fauser et al., 2010; Hamdine et al., 2014; Nelson et al., 2007). Additionally, a recent analysis of two prospective, randomized trials comprising over 1400 assisted reproduction technique cycles indicated that selection of gonadotrophin, i.e. purified menotropins (human menopausal gonadotropins compared with recombinant FSH) may also help to reduce the risk of high response and also improve outcomes among women with high AMH levels, regardless of agonist or antagonist protocol (Arce et al., 2014). These findings are supported by a retrospective study that evaluated outcomes for women assigned to a stimulation protocol based on basal FSH and age (conventional determination) compared with AMH levels (Yates et al., 2011). Patients

in the conventional group received either a long GnRH agonist downregulation protocol (low FSH) or a co-flare GnRH agonist protocol (high FSH) with human menotropins of 150 or 300 IU depending on patient age; those in the AMH-tailored group received a GnRH antagonist protocol with 300 IU of menotropins (low AMH), a long GnRH agonist downregulation protocol with 200 IU of recombinant FSH or 225 IU of menotropins (moderate AMH), or a GnRH antagonist protocol with 150 IU of menotropins. The study found that the rates of embryo transfer, pregnancy, and live births were higher in the AMH-tailored group, whereas the incidence of OHSS was reduced (Yates et al., 2011). In addition to the introduction of an antagonist protocol (which did not affect live birth rate in this study), the AMH-tailored group is notable for assigning the starting dose of FSH or menotropins based on predicted response to stimulation. A separate study found AMH level positively predicted the need for FSH or menotropin dose adjustments after a fixed starting dose, leading the investigators to suggest that tailoring of the initial starting dose based on AMH level might improve outcomes while eliminating the need for later dose adjustments (Anckaert et al., 2012).

The debate: selection of AFC versus AMH level for prediction of ovarian reserve

Both AFC and AMH level are good predictors of ovarian response during assisted reproduction techniques compared with other traditional measures, e.g. age and basal FSH level (Hendriks et al., 2005; La Marca et al., 2010; Lukaszuk et al., 2013; Polyzos et al., 2013). Direct comparisons of AFC and AMH level have generally shown similar predictive value for ovarian response and outcome (Amer et al., 2013; Broekmans et al., 2006; Broer et al., 2009, 2011; Li et al., 2013; Lukaszuk et al., 2013; Panchal and Nagori, 2012; Sunkara et al., 2011), with one prospective, multicentre study that indicated a significantly stronger predictive value for AMH (Arce et al., 2013b; Brodin et al., 2013), and three others that demonstrated a stronger predictive value for AFC (Himabindu et al., 2013; Rosen et al., 2012; Tsakos et al., 2014), depending upon the specific outcome and patient subpopulation evaluated. Beyond general predictive value, AFC and AMH level each have specific advantages and disadvantages (**Table 1**).

Advantages and disadvantages of AFC

In specialist IVF centres, AFC is easy to carry out and provides immediate results (Chang et al., 1998a, 1998b; Frattarelli et al., 2000; Hendriks et al., 2005; Hsu et al., 2011; Iliodromiti et al., 2014a; Tomas et al., 1997). According to the American Society for Reproductive Medicine, the use of AFC is recommended to predict poor response to ovarian stimulation and pregnancy outcome, but should not be the sole criterion for the application of assisted reproduction techniques (2012).

Key disadvantages of AFC mainly derive from hardware and operator variability and the failure to establish category-defining criteria. Significant variation between, as well as

Table 1 Advantages and disadvantages for antral follicle count and anti-Müllerian hormone level.

Antral follicle count	*Anti-Müllerian hormone level*
Advantages	*Advantages*
•Good predictive value for the number of oocytes retrieved and stimulation response. •May help guide protocol and other treatment decisions. •Easy to perform and personalize. •Fairly non-invasive. •Provides immediate results.	•Good predictive value for the number of oocytes retrieved and stimulation response. •May help guide protocol and other treatment decisions. •Well-characterized across adolescent and reproductive ages. •Can be performed at any point during a cycle (low intra-cycle variability). •Good inter-cycle consistency. •Good inter-operator and inter-centre consistency. •Relatively low cost (depending upon the specific anti-Müllerian hormone assay).
Disadvantages	*Disadvantages*
•Must be carried out at the beginning of a cycle because of intra-cycle variation. •Inter-centre variation because of subjective determination and differences in technology, training, and methodology. •May be overestimated owing to inclusion of atretic follicles. •Inappropriate for many juvenile and adolescent individuals. •Greater inter-cycle variation observed with overweight and obese women. •Requires cost of ultrasound technician and availability of ultrasound machine.	•Labour intensive, requiring several hours (note: a new, fully automated assay will take minutes and thus eliminate this disadvantage). •Requires careful sample preparation and storage. •No standardization across assays.

within, centres has been observed with AFC, and is consistently greater with AFC versus AMH (Arce et al., 2013a, 2013b; Broekmans et al., 2006; Broer et al., 2009; Fleming et al., 2013; Iliodromiti et al., 2014a; La Marca et al., 2010). This variation may be caused by differences in training, specific methodology, technological resources, i.e. resolution of ultrasound for visualization of antral follicles, or both. Poor-quality images may affect the reliability of AFC, particularly in women with ovarian cysts, fibroids, or scars from previous surgeries. The choice of ultrasound machine also has an impact on image quality (Broekmans et al., 2010; Vandekerckhove et al., 2014). In addition, AFC tends to overestimate the true number of FSH-sensitive follicles and oocytes retrieved, perhaps because it also includes non-viable atretic follicles of the same size. Some estimates indicate that more than one-half of follicles detected in young women by transvaginal ultrasound could be undergoing atresia (Broekmans et al., 2010).

Standardization of AFC determination could improve the usefulness of AFC for determination of ovarian reserve, similar to what has been seen for the nuchal translucency assay for determining a fetus's risk of certain chromosomal abnormalities. Factors for consideration in the standardization of AFC include the timing of AFC, e.g. days 2–4 of a spontaneous menstrual cycle or oral contraceptive cycle; size of follicles to be included, e.g. 2–10 mm in diameter; ultrasound technology used (to ensure adequate resolution); a systematic process for counting antral follicles; and consistent training for personnel (Broekmans et al., 2010; Iliodromiti et al., 2014a).

The use of three-dimensional ultrasound, automated identification and quantification of follicles, and post-hoc image analysis have the potential to further reduce variability in AFC (Broekmans et al., 2010; Iliodromiti et al., 2014a). Although studies are limited, automated three-dimensional ultrasound imaging has been shown to reduce intra- and inter-observer variability, thus providing more reliable AFC measurements in a shorter amount of time (Broekmans et al., 2010; Deb et al., 2009, 2010, 2011; Jayaprakasan et al., 2010). Follicular measurement data can also be stored for future review and training of technicians and investigators (Vandekerckhove et al., 2014). Further evaluation of this technology and associated costs, however, are needed before regular use in the clinic (Broekmans et al., 2010; Iliodromiti et al., 2014a). Obtaining an AFC is already associated with costs for the ultrasound machine and ultrasound technician; increased costs associated with three-dimensional ultrasound may be prohibitive. Furthermore, three-dimensional imaging has not been shown to improve clinical results, and, as with standard two-dimensional imaging, image quality can vary depending on the patient (Vandekerckhove et al., 2014).

Another disadvantage of AFC is the lack of standardization in some patient populations. Although transvaginal ultrasound is easy to carry out and relatively non-invasive in women, it is generally considered inappropriate for juvenile and adolescent patients (Bauman, 2012). Therefore, the predictive value of AFC has not been well characterized for these age groups owing to limited data.

Recent reports have also suggested that AFC can be affected by certain environmental and biological factors, such as the decrease in AFC observed with continuous use of certain contraceptives, although this may apply to other measures of ovarian reserve as well (Bentzen et al., 2012; Deb et al., 2012; Iliodromiti et al., 2014a; Peterson et al., 2014). Smoking has also been associated with decreased age-specific AFC (Iliodromiti et al., 2014a). Additionally, greater intra- and inter-cycle variation has been noted in overweight and obese women, limiting its predictive usefulness in these populations (Broekmans et al., 2006; Broer et al., 2009; La Marca et al., 2010). Additional studies are required to confirm many of these findings, as well as provide an accurate estimate of their associated effects. In many instances, such as in overweight and obese women (characteristics that currently apply to 69% of adults in the USA) (National Center for Health Statistics. Health, United States, 2014), it may be necessary to combine AFC with other ovarian reserve measures.

Advantages and disadvantages of AMH level

Advantages of AMH level as a measure of ovarian reserve include well-characterized reference ranges for adolescent and reproductive-aged patients (Hehenkamp et al., 2006; La Marca et al., 2009, 2013), as well as minimal variability between and within natural cycles (Hehenkamp et al., 2006; Iliodromiti et al., 2014a; La Marca et al., 2013; Tsepelidis et al., 2007). Additionally, AMH level reflects the number of granulosa cells in early growing follicles, all of which are active, and appears sensitive enough to detect changes in ovarian reserve occurring over just a few years (La Marca et al., 2009). The AMH assay is also relatively inexpensive, and the newer, fully automated AMH assays may further reduce costs by enhancing ease of use and minimizing technician handling time (Fleming et al., 2013; Beckman Coulter, 2014). Recommendations from the American Society for Reproductive Medicine describe AMH level as a promising screening test, likely to be most useful in the general IVF population and among women at high risk for diminished ovarian reserve (2012). At the other end of the spectrum, AMH is also being discussed as a potential diagnostic marker for polcystic ovary syndrome (Dewailly et al., 2011; Iliodromiti et al., 2013). Anti-Müllerian hormone allows for the interpretation of a specific value to an individual's lifetime reproductive potential, which is a contextualization that should be a part of the reproductive physician's armory.

One current disadvantage of the measurement of AMH is that assays cannot provide an answer to ovarian reserve and expected ovarian response in real time. Traditional enzyme-linked immunosorbent assays (ELISAs) for AMH are labour intensive and require several hours to obtain results (Gassner and Jung, 2014; Rustamov et al., 2012), thus delaying any treatment decisions that would be guided by determination of ovarian reserve. New automated AMH assays in the final stages of development, however, are described as providing faster results than currently used ELISAs (in a few minutes), which facilitates routine office use, and showing a wider range of sensitivity. Despite their speed, the new assays will demonstrate improved precision and reliability (Gassner and Jung, 2014; Rustamov et al., 2012).

Although AMH level is an objective test, and therefore should not have the inter-operator variability issues associated with subjective determination of AFC (Arce et al., 2013b), values from manual assays have been shown to vary between assays as well as laboratories, owing to differences in site-specific processes (Iliodromiti et al., 2014a; Rustamov et al., 2014). Now, numerous commercial assays are available for AMH, including the original laboratory assays; the first generation

assays introduced by Diagnostic Systems Laboratories and Immunotech; the Beckman Coulter Generation II (Gen II) assay, which uses the Diagnostic Systems Laboratories antibodies and Immunotech standards; a newer ELISA assay from Ansh Labs that uses different antibodies; and several new, fully automated assays, as described previously (Nelson, 2013). Improper storage and handling of samples, e.g. collection in ethylenediaminetetraacetic acid tubes instead of serum separator tubes, delayed centrifugation, storage at room temperature, can drastically affect AMH levels (Nelson, 2013). In addition, interference by the serum complement that binds to the assay antibodies in fresh samples can lead to variability in AMH assays (Craciunas et al., 2014; Rustamov et al., 2012, 2014).

Improper sample handling and storage may have affected the reliability of earlier studies of AMH levels; results from these early studies should thus be considered critically, although together they point to good predictive value and limited inter-cycle variability. Recent modifications to the Gen II assay sample storage recommendations and test protocol have obviated these effects, achieving more consistent results with samples stored in various conditions (Broer et al., 2014; Craciunas et al., 2014; Han et al., 2014; Welsh et al., 2014). Fleming and Nelson reported consistent AMH levels in serum samples assayed before and after a week of refrigerated storage using the Gen II kit, and confirmed that long-term storage of serum samples at −20°C over 3 years did not affect assay performance (Fleming and Nelson, 2012). Thus, the Gen II assay is a reliable method to measure serum AMH, provided samples are prepared and stored appropriately. The new, fully automated AMH assays, which allow for more efficient sample processing, should help to further reduce the influence of procedural errors (Iliodromiti et al., 2014a), and preliminary evidence suggests consistent AMH values are obtained under most storage conditions (Gassner and Jung, 2014). A recent study evaluating the fully automated Elecsys AMH assay from Roche reported consistent AMH values that were correlated with age and AFC across different centres (Anderson et al., 2015). Of note, significant variation in AFC was seen, suggesting that AMH level measured with the Elecsys assay may be a better indicator of ovarian reserve than AFC. A comprehensive evaluation of these new automated assays will be required to establish confidence in their ability to provide consistently accurate results.

Standardized cut points have been developed for each available commercial AMH assay, however, no standardization exists across assays (Broer et al., 2011; Iliodromiti et al., 2014a; Nelson et al., 2009; Toner and Seifer, 2013; Practice Committee of the American Society for Reproductive Medicine, 2012). Therefore, when using AMH cut points in clinical practice, it is important to use the same assay as that used in the reference study population; results obtained using different assays may show a high degree of correlation, but the specific cut points have rarely been formally tested, making results difficult to compare between assays. Development of an international standard of AMH level is necessary for future clinical use (Broer et al., 2011; Iliodromiti et al., 2014a; Toner and Seifer, 2013).

As with AFC, a number of environmental and biological factors have been suggested to cause changes in AMH levels (**Table 2**). For example, both the continuous use of certain contraceptives (Bentzen et al., 2012; Dolleman et al., 2013; Iliodromiti et al., 2014a; Kallio et al., 2013; Peterson et al., 2014; Shaw et al., 2011) and current cigarette smoking have

Table 2 Effect of patient, reproductive, and lifestyle factors on anti-Müllerian hormone level.

Potential factor	*Effect on anti-Müllerian hormone level*
Patient characteristics	
Ethnicity	Latina, black, Chinese less than white (Bleil et al., 2014; Iliodromiti et al., 2014a; La Marca et al., 2013; Schuh-Huerta et al., 2012; Seifer et al., 2009)
Socio-economic status	No effect (Bleil et al., 2014; Dolleman et al., 2013)
Body mass index/obesity	Inconsistent (Bleil et al., 2014; Dolleman et al., 2013; Iliodromiti et al., 2014a; La Marca et al., 2013; Shaw et al., 2011; Su et al., 2008)
Elevated leptin	Decrease (Merhi et al., 2013)
Low vitamin D	Decrease (Dennis et al., 2012)
Reproductive factors	
Continuous contraceptive use	Decrease (Bentzen et al., 2012; Dolleman et al., 2013; Iliodromiti et al., 2014a; Kallio et al., 2013; Peterson et al., 2014; Shaw et al., 2011)
Current pregnancy	Decrease (Dolleman et al., 2013; Iliodromiti et al., 2014a)
Parity	Increase (Bleil et al., 2014; Dolleman et al., 2013)
Irregular menstrual cycle	Decrease (Dolleman et al., 2013)
Age at menarche	Inconsistent (Bleil et al., 2014; Dolleman et al., 2013; Shaw et al., 2011)
Lifestyle factors	
Current tobacco use	Decrease (Dolleman et al., 2013; Freour et al., 2008; Iliodromiti et al., 2014a; Plante et al., 2010; Waylen et al., 2010)
Past tobacco use	No effect (Dolleman et al., 2013; Plante et al., 2010)
Alcohol use	No effect (Dolleman et al., 2013)
Physical exercise	No effect (Dolleman et al., 2013)

been linked to decreased AMH levels (Dolleman et al., 2013; Freour et al., 2008; Iliodromiti et al., 2014a; Plante et al., 2010; Waylen et al., 2010), although correlation between tobacco dose with effect on AMH level is inconsistent. Greater clinician awareness of factors that may affect patients' AMH levels, and thus misrepresent actual ovarian reserve, is needed; however, the clinical impact of these findings is still unknown and requires additional characterization.

In conclusion, evaluation of ovarian reserve can help to identify patients who will have poor response or hyper-response to ovarian stimulation for assisted reproduction techniques. This information can aid in the personalization of treatment to achieve good response and minimize safety risks. The ideal ovarian reserve test should be reproducible, with limited inter- and intra-cycle variability, and demonstrate high specificity to minimize the risk for incorrectly categorizing women as having decreased ovarian reserve. No measure of ovarian reserve is perfect; however, both AFC and AMH level have good predictive value. Composite measures that incorporate both methods could potentially be used to provide a comprehensive assessment of ovarian reserve, although AMH has been shown to be a better predictor of oocyte yield in patients with discordant AFC and AMH results (Li et al., 2014). The objectivity, convenience of untimed sampling, and potential standardization of AMH level make this a preferred method for the evaluation of ovarian reserve in most women.

Acknowledgements

Medical writing and editorial assistance for the development of this manuscript were provided by Kimberly Brooks, PhD, of SciFluent, based upon the authors' direction, and was financially supported by Ferring Pharmaceuticals, Inc.

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Declaration: David B. Seifer has received royalties from a licensing agreement between Beckman-Coulter and Rutgers Medical School/MGH for the use of AMH in determining ovarian reserve. Jane Ruman is an employee of Ferring Pharmaceuticals, Inc. Richard Fleming and John L. Frattarelli have no conflicts of interest to disclose.

Received 26 February 2015; refereed 27 May 2015; accepted 23 June 2015.