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Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART)

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BACKGROUND: In women, anti-Müllerian hormone (AMH) levels may represent the ovarian follicular pool and could be a useful marker of ovarian reserve. The clinical application of AMH measurement has been proposed in the prediction of quantitative and qualitative aspects in assisted reproductive technologies (ART). In men AMH is secreted in both the serum and seminal fluid. Its measurement may be useful in clinical evaluation of the infertile male.

METHODS: The PubMed database was systematically searched for studies published until the end of January 2009, search criteria relevant to AMH, ovarian reserve, ovarian response to gonadotrophin stimulation, spermatogenesis and azoospermia were used.

RESULTS: AMH seems to be a better marker in predicting ovarian response to controlled ovarian stimulation than age of the patient, FSH, estradiol and inhibin B. A similar performance for AMH and antral follicular count has been reported. In clinical practice, AMH measurement may be useful in the prediction of poor response and cycle cancellation and also of hyper-response and ovarian hyperstimulation syndrome. In the male, the wide overlap of AMH values between controls and infertile men precludes this hormone from being a useful marker of spermatogenesis.

CONCLUSIONS: As AMH may permit the identification of both the extremes of ovarian stimulation, a possible role for its measurement may be in the individualization of treatment strategies in order to reduce the clinical risk of ART along with optimized treatment burden. It is fundamental to clarify the cost/benefit of its use in ovarian reserve testing. Regarding the role of AMH in the evaluation of infertile men, AMH as single marker of spermatogenesis does not seem to reach a satisfactory clinical utility.

Key words: AMH / ART / COS / poor response / OHSS / azoospermia

Introduction

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein, a member of the transforming growth factor-beta superfamily (Jost, 1946; Cate *et al*., 1986), which acts on tissue growth and differentiation. AMH was originally identified because of its fundamental role in male sex differentiation. Indeed, expressed in the Sertoli cells of fetal testis, AMH induces the regression of the Mullerian ducts. In the absence of AMH, Mullerian ducts evolved into uterus, fallopian tubes and the upper part of the vagina (Munsterberg and Lovell-Badge, 1991; Lee and Donahoe, 1993; Josso *et al*., 2001).

In women AMH is produced by granulosa cells, from pre-antral and antral follicles (Weenen *et al*., 2004) and the main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development (Themmen, 2005; Visser and Themmen, 2005).

AMH is secreted by the ovary into circulation, hence AMH is measurable in serum. As serum AMH levels essentially reflect the ovarian follicular pool, reduction in the number of small growing follicles may be followed by a reduction in circulating AMH. Recently, AMH has been evaluated by several groups as a potential novel clinical marker of ovarian reserve and of response to gonadotrophins (Seifer *et al*., 2002; Van Rooij *et al*., 2002; Fanchin *et al*., 2003a, b; Muttukrishna *et al*., 2004; Eldar-Geva *et al*., 2005; Hazout *et al*., 2004; Peñarrubia *et al*., 2005; Tremellen *et al*., 2005; Fiçicioglu *et al*., 2006; La Marca *et al*., 2007). In particular in the last few years several large prospective studies have been published reporting extremely interesting new data on the possible clinical application of AMH measurement in the prediction of quantitative and qualitative ovarian response in assisted reproductive technologies (ART).

In the male, AMH is the earliest Sertoli cell specific protein expressed by the gonad (Tran *et al*., 1977). It is secreted by the testis from the eighth week of pregnancy and remains secreted at high-level until puberty, when Sertoli cell maturation is characterized by decreased AMH production (Rajpert-De Meyts *et al*., 1999). Paralleling the situation in women, the main physiological role of AMH in the adult male seems to be limited to the paracrine control of testicular function.

In the adult man, AMH is secreted both in serum and in seminal fluid (Fénichel *et al*., 1999) and, being a specific marker of Sertoli cell function, its measurement may be useful to obtain information on spermatogenesis in infertile men. In the last few years several studies have been published on the possible clinical use of AMH assay in the diagnostic work-up of patients with oligoasthenoteratozoospermia (OAT) and azoospermia (Fénichel *et al*., 1999; Fujisawa *et al*., 2002; Al-Qahtani *et al*., 2005; Appasamy *et al*., 2007; Muttukrishna *et al*., 2007) and in particular on the predictive value of AMH for the successful sperm retrieval in azoospermic patients (Isikoglu *et al*., 2006; Mostafa *et al*., 2007; Duvilla *et al*., 2008; Goulis *et al*., 2009).

In this review the main findings of published studies have been summarized and some conclusions on the clinical application of AMH measurement in both the infertile male and female have been drawn.

Methods

PubMed database was systematically searched for studies published until the end of January 2009, using search criteria relevant to AMH, ovarian reserve, ovarian response to gonadotrophin stimulation, spermatogenesis and azoospermia. Specifically the following search terms were used: AMH, Müllerian Inhibiting Substance, ovarian reserve, ovarian ageing, poor response, poor responder, hyper-response, hyper-responder, ovarian hyperstimulation syndrome (OHSS), ART, IVF, ICSI, sperm, spermatogenesis, seminal fluid, azoospermia, oligozoospermia, OAT, TESE and TESA. Cross-references picked up during the review search were also selected if they were not included initially. Both prospective and retrospective articles were considered. Methods for selecting and synthesizing the data were based on personal experience.

AMH in female fertility

AMH in ovarian physiology

AMH is produced by granulosa cells from pre-antral and antral follicles, restricting expression to growing follicles, until they have reached the size and differentiation state at which they are selected for dominance by the action of pituitary FSH (Weenen *et al*., 2004) (Fig. 1). In the human this occurs in antral follicles of size 4–6 mm. AMH is not expressed in atretic follicles and theca cells. Ovarian AMH expression has been observed as early as 36 weeks' gestation in the humans' fetus (Raypert-De Meyts *et al*., 1999). Recent studies show that in adult rat ovaries FSH and estradiol may down-regulate AMH expression (Baarends *et al*., 1995).

AMH exerts its biological effects through a transmembrane serine/threonine kinase typeII receptor (AMHRII), which is specifically expressed in the gonads and in the mesenchymal cells adjacent to the Müllerian ducts (Di Clemente *et al*., 2003). In adult female rats, AMH and AMHRII mRNAs are mainly expressed in granulosa cells from pre-antral and smaller antral follicles (Baarends *et al*., 1995). In addition, AMHRII mRNA expression was observed in theca cells of pre-antral and small antral follicles. Besides the exclusive AMHRII, three candidate AMH type I receptors have been identified to be involved in AMH-induced Müllerian duct regression (Visser, 2003). These type I receptors, ALK2, ALK3 and ALK 6, are shared with the bone morphogenetic proteins (BMPs). Subsequently, similar to BMPs, AMH signalling is mediated through the downstream signalling molecules Smad1, Smad5 and Smad8 (Visser, 2003). However, the relative contribution of these three type I receptors to AMH signalling in the ovary remains to be determined.

The main physiological role of AMH in the ovary seems to be limited to the inhibition of the early stages of follicular development (Themmen, 2005; Visser and Themmen, 2005), since both *in vivo* and *in vitro* experiments have indicated that the transition from primordial into growing follicles becomes enhanced in absence of AMH, leading to early exhaustion of the primordial follicle pool (Durlinger *et al*., 2001; Visser and Themmen, 2005). *In vitro* culture of mouse neonatal ovaries and human cortical strips has confirmed the inhibitory role of AMH in primordial follicle recruitment (Carlsson *et al*., 2006). Moreover it has been suggested that follicles are more sensitive to FSH in the absence of AMH. The effects of AMH on FSH sensitivity of follicles was tested in a *in vivo* model in which the follicle dynamics were compared with wild-type and AMH null mice in the presence of low and high FSH serum concentrations (Durlinger *et al*., 2001). The study shows that more growing follicles were found in AMH null mice than in wild-type mice, both in term of numbers and in terms of developmental stage (Durlinger *et al*., 2001).

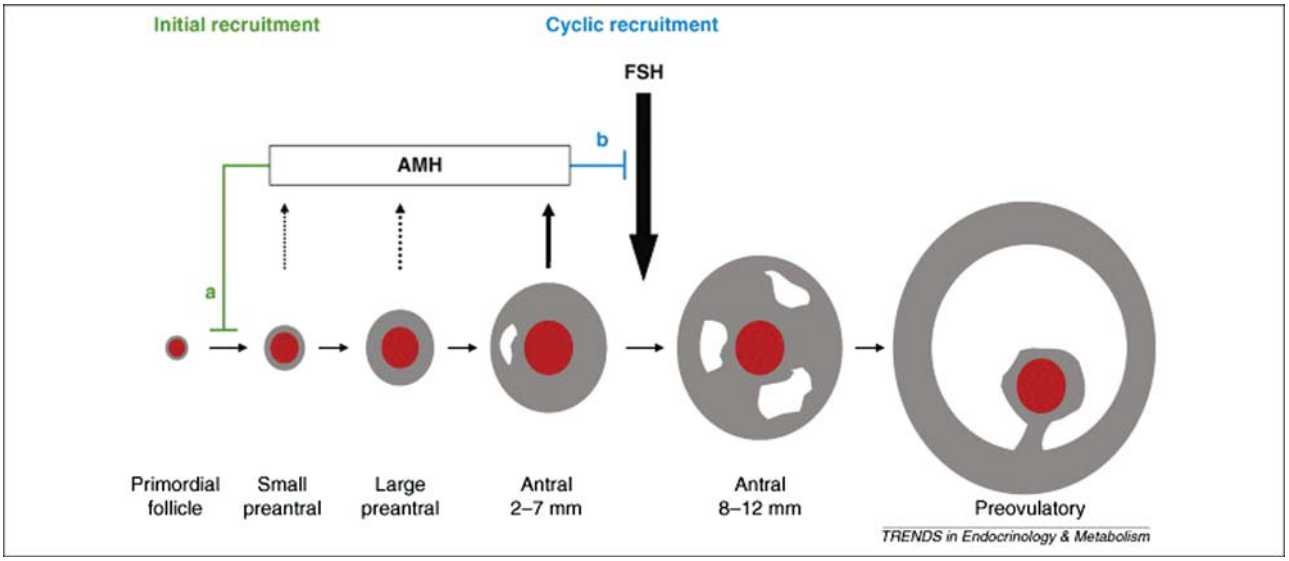

Figure 1 AMH is secreted by pre-antral and antral follicles.

It seems to inhibit initial follicle recruitment and FSH-stimulated follicle growth. The role of AMH in the two main compartments of normal ovarian follicle development (the red centre represents the oocyte, the grey area represents the granulosa cell layer and the white area represents follicle fluid in the antrum). AMH is expressed in small and large pre-antral follicles (broken arrows) and in small antral follicles (whole arrow), and the latter mainly contributes to serum levels. Initial recruitment takes place as a continuous process, whereas cyclic recruitment is driven by a rise in FSH serum levels at the end of a previous menstrual cycle. The inhibitory effects of AMH are shown (a) on the initial recruitment of primary follicles from the resting primordial follicle pool and (b) on the sensitivity of antral follicles for FSH (reproduced with permission from Broekmans *et al*., 2008).

Recently, ovaries from rats placed in organ culture and incubated in the absence and presence of AMH, show that AMH alters the expression of several hundred genes (Nilsson *et al*., 2007). The overall effects of AMH exposure was to decrease the expression of stimulatory factors, increase the expression of inhibitory factors and regulate cellular pathways that result in the inhibition of primordial follicle development (Nilsson *et al*., 2007).

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

Factors modulating AMH levels in women

AMH is produced and secreted by the gonads into the circulation, and AMH is measurable in serum from both men and women. Serum AMH levels from women are lower than those in men throughout life. In women AMH levels are almost undetectable at birth with a subtle increase within the first 2 or 4 years of age, after that AMH appears to be stable until adulthood but found to decrease as a sign of follicular reserve exhaustion becoming undetectable at menopause (Fig. 2) (Lee *et al*., 1996; Guibourdenche *et al*., 2003; La Marca *et al*., 2005a; Van Rooij *et al*., 2005; Bergada *et al*., 2006; Shin *et al*., 2008; Robertson *et al*., 2008; La Marca 2009a). Interestingly, in women circulating AMH appears to be solely of ovarian origin since AMH is undetectable 3–5 days following bilateral ovariectomy (La Marca *et al*., 2005a). As AMH levels essentially reflect the follicular ovarian pool, reduction in the number of small growing follicles may be followed by a reduction in circulating AMH. The reduction in ovarian reserve is a physiological process occurring in the late reproductive period and consistently associated with a decrease in AMH levels (Van Rooij *et al*., 2005; Robertson *et al*., 2008). The strong correlation existing between AMH levels and the resting pool of follicles has recently been highlighted by some papers showing that AMH measurement may be used to predict the occurrence of menopause (Sowers *et al*., 2008; Van Disseldorp *et al*., 2008).

Non-significant variations of AMH throughout the menstrual cycle have been reported by our group (La Marca *et al*., 2006a) and confirmed by a number of independent studies (Hehenkamp *et al*., 2006; Tsepelidis *et al*., 2007; Streuli *et al*., 2008) (Fig. 2). Others have reported significant cyclical fluctuations in AMH levels with a rapid decrease in the early luteal phase (Wunder *et al*., 2008; Streuli *et al*., 2009). However, excursions from mean levels of +3% to −19% have been calculated (Wunder *et al*., 2008; Streuli *et al*., 2009). These variations are similar to reported inter-cycle variability for AMH (Fanchin *et al*., 2005a, b; Streuli *et al*., 2008). Hence in the clinical setting the inter- and intra-cycle variability in serum AMH levels may be considered to be low enough to permit random timing of AMH measurement during the menstrual cycle.

In women, AMH levels seem to be unmodified under conditions in which endogenous gonadotrophin release is substantially diminished, such as during pregnancy (La Marca *et al*., 2005b), GnRH agonist treatment (Mohamed *et al*., 2006) and short-term oral contraceptive administration (Arbo *et al*., 2007; Somunkiran *et al*., 2007; Streuli *et al*., 2008), indicating that non-cyclic FSH-independent

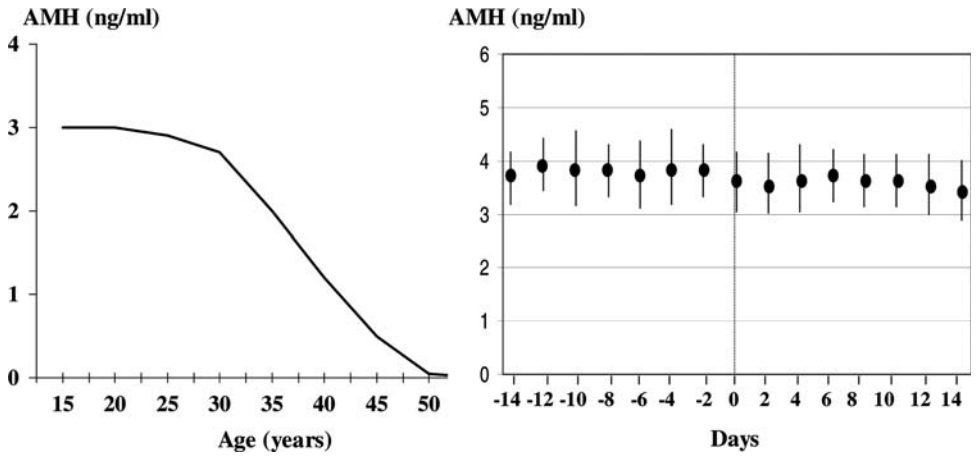

Figure 2 *Left*: Mean serum AMH levels show a reduction throughout reproductive life. Undetectable AMH levels after spontaneous menopause have been reported (constructed graphic). *Right*: Circulatory pattern of AMH during the menstrual cycle of young healthy women aged 18–24 years. Serum AMH levels have been shown to be stable throughout the menstrual cycle. Day 0 = day of LH surge (reproduced with permission from La Marca *et al*., 2006a).

ovarian activity persists even when pituitary FSH secretion is suppressed.

Women with polycystic ovary syndrome (PCOS) show increased development of antral follicles compared with normal women (Pigny *et al*., 2006). On histological examination, polycystic ovaries (PCO) exhibit a normal number of primordial follicles, whereas the number of developing follicles is double compared with normal ovaries (Webber *et al*., 2003). Accordingly circulating AMH levels in women with PCOS are two to three times higher than healthy controls (Fallat *et al*., 1997; Cook *et al*., 2002; Pigny *et al*., 2003; La Marca *et al*., 2004a, b; Laven *et al*., 2004; Mulders *et al*., 2004; Eldar-Geva *et al*., 2005; Piltonen *et al*., 2005; Wachs *et al*., 2007). In women with PCOS, increased AMH levels may not only be due to excessive accumulation of antral follicles (Wang *et al*., 2007) but also to increased granulosa cell AMH secretion (Mulders *et al*., 2004). Indeed, levels of AMH are on average 75 times higher in granulosa cells from PCO, compared with levels in granulosa cells from normal ovaries (Pellatt *et al*., 2007).

AMH levels appear to be related to the severity of the syndrome, since levels have been observed to be higher in insulin-resistant PCOS women than in patients with normal insulin sensitivity (Fleming *et al*., 2006). Similarly AMH is higher in amenorrheic compared with oligomenorrheic women with PCOS (La Marca *et al*., 2004a, b), which could indicate a role for AMH in the pathogenesis of PCOS-related anovulation. The relationship between AMH levels and the severity of the syndrome seems to be confirmed by studies demonstrating that PCOS patients ovulating during a weight loss-programme had AMH levels lower than women remaining anovulatory (Moran *et al*., 2007; Thomson *et al*., 2009). Interestingly, in one study no significant changes in AMH levels were observed in either responders or non-responders during the weight loss-programme (Thomson *et al*., 2009). In order to clarify the complex relationship existing between insulin resistance, androgen excess and high levels of AMH, a prospective, randomized, double-blind 26 week-long study was undertaken in women with PCOS (Carlsen *et al*., 2009). All patients received diet and lifestyle counselling, and metformin. Concomitantly, they were randomized to either dexamethasone or placebo. The study clearly demonstrated that circulating AMH concentrations were unaffected by 6 months of lifestyle counselling with metformin and placebo treatment. AMH levels were also unaffected by 6 months of androgen suppression with dexamethasone in addition. These results may indicate that high serum AMH levels in PCOS may be more strongly related to the presence of PCO than to the full spectrum of the syndrome (PCOS) as modifications in androgens and insulin sensitivity are not followed by changes in ovarian AMH output (Carlsen *et al*., 2009).

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

Obesity has been associated with reduced fertility, even in the presence of ovulatory menstrual cycles, and to increased probability of miscarriage compared with normal weight women (Rich-Edwards *et al*., 2002; Fedorcsák *et al*., 2004). Non-PCOS obese women show reduced levels of inhibin B and AMH (Gracia *et al*., 2005; Freeman *et al*., 2007) suggesting that obesity may be associated with impaired ovarian reserve. However, a recent study (Su *et al*., 2008) examined the correlation of obesity with hormonal and ultrasound-derived markers of ovarian reserve and found that serum AMH levels are lower in obese women compared with age-matched women of normal weight, despite similar antral follicular count. This suggests that AMH levels in obese women may be lower for physiological reasons related to obesity itself and may not be necessarily indicative of impaired ovarian reserve (Su *et al*., 2008). Other factors related to reduced AMH levels are smoking (Freour *et al*., 2008), alcohol use (Nardo *et al*., 2007) and race or ethnicity (Seifer *et al*., 2008).

Table I Studies on AMH as marker of ovarian response to controlled ovarian stimulation (COS)

Author	*n*	R with oocytes*	AMH better than					
			AFC	Ov. Vol	d3 FSH	d3 E2	d3 inhB	Age
Seifer *et al.* (2002)	107	0.48			√	√		
Van Rooij *et al.* (2002)	130	0.57	=		√	√	√	√
Fanchin *et al.* (2003a, b)	93	0.43						
Muttukrishna *et al.* (2004)	69	0.69			√		√	
Hazout *et al.* (2004)	109	0.38			√	√	√	√
Muttukrishna *et al.* (2005)	108	0.5	=		√			
Eldar-Geva (2005)	56	0.64	X		√		√	
Silberstein *et al.* (2006)	257	0.33			√			
Fiçicioglu *et al.* (2006)	50	0.56	√		√	√		√
Lekamge *et al.* (2007)	126	0.34	=					
La Marca *et al.* (2007)	48	0.7						
Kwee *et al.* (2007)	110	0.63	X	√	√			√
Nakhuda *et al.* (2007)	77	0.63			√			
McIlveen *et al.* (2007)	84	0.78	√	√	√		=	√
Nelson *et al.* (2007)	340	0.71			√			√
Elgindy *et al.* (2008)	33	0.88	=	√	√			
Lie Fong *et al.* (2008)	125	0.47						
Jee *et al.* (2008)	59	0.53					X	
Jayaprakasan *et al.* (2008)	135	0.47	=	√	√	√		√
Wunder *et al.* (2008)	276	0.35			√		X	

Comparison with other predictors.
*R with oocytes: correlation between serum AMH levels and the number of retrieved oocytes; √, better than; X, worse than; =, equal to.

Prediction of quantitative ovarian response in ART

AMH levels seem to decline gradually during gonadotrophin administration as a part of controlled ovarian stimulation (COS) (Fanchin *et al.*, 2003a, b; La Marca *et al.*, 2004a, b). The reduction of AMH levels during COS could be due to a negative direct or indirect effect of FSH on ovarian AMH secretion. During exogenous administration of FSH there is an increase in estradiol levels, which could be a reason for decreased AMH. Indeed estradiol has been implicated in the down-regulation of AMH and AMHII mRNA in the ovary (Baarends *et al.*, 1995). Stimulation with FSH induces growth of follicles that enlarge and lose their AMH expression, and this is probably the main reason for AMH reduction. Hence, due to the reduction of AMH levels during FSH administration, AMH measurement to predict the ovarian response to FSH should not be performed during gonadotrophin treatment, but some months to some days prior commencing FSH treatment.

Much data show a strong and positive correlation between basal AMH serum levels and the number of retrieved oocytes in women undergoing ovarian stimulation (Table I). In the evaluation of AMH as a marker of ovarian response to FSH, the first article to report an association between circulating AMH and ovarian response to gonadotrophin was by Seifer and colleague segues (Seifer *et al.*, 2002). The authors observed that higher AMH on Day 3 of the stimulation protocol was associated with a greater number of retrieved oocytes. In particular, AMH levels were 2.5-fold higher in patients with at least 11 oocytes compared with those with six oocytes or fewer retrieved. Results from this study were successively confirmed by several retrospective and prospective studies by different independent groups.

In Table I all retrospective and prospective studies that have found a correlation between the number of retrieved oocytes and AMH levels have been summarized. The majority of authors compared AMH with age and other hormonal markers (FSH, estradiol and Inhibin B), but only a few studies also compared AMH levels with ultrasound markers of ovarian reserve. The balance of the published studies seems to indicate that AMH is a better marker in predicting ovarian response to COS than age of the patient, Day 3 FSH, estradiol and inhibin B.

Almost all of these studies found a significant correlation between AMH and antral follicular count, but very few studies have compared the performance of the two markers in the prediction of the number of retrieved oocytes. Only Fiçicioglu *et al.* (2006) and McIlveen *et al.* (2007) concluded that AMH is better than AFC, whereas two studies found AFC to be superior to AMH (Eldar-Geva *et al.*, 2005; Kwee *et al.*, 2007) and five studies reported a similar performance of the two markers (Van Rooij *et al.*, 2002; Muttukrishna *et al.*, 2005; Elgindy *et al.*, 2007; Lekamge *et al.*, 2007; Jayaprakasan *et al.*, 2008).

Hence, it may be concluded that AFC and AMH perform with similar power in the prediction of the number of retrieved oocytes.

This was confirmed by a recent meta-analysis in which the value of serum AMH levels as a test to predict ovarian response in IVF in comparison to the performance of the AFC was been assessed (Broer *et al*., 2008). A total of 13 studies were analyzed reporting on AMH and 17 on AFC. The ROC curves for the prediction of ovarian response indicated no significant difference between the performances of AMH and AFC. Hence it may be concluded that at present AMH appears to offer at least the same level of accuracy and clinical value for the prediction of ovarian response as AFC (Broer *et al*., 2008).

Prediction of poor response and cycle cancellation

A proportion of women (2–30%) undergoing COS experience poor response (Hendriks *et al*., 2005) for which there is no universally accepted definition. Numerous criteria have been used to characterize poor response. The number of developed follicles and the number of retrieved oocytes are two of the most important criteria for defining poor response. The proposed number varies among different authors and ranges from less than three to less than five dominant follicles on the day of hCG, and from less than three to less than five retrieved oocytes (reviewed in Tarlatzis *et al*., 2003). More logically, poor response is generally considered to have occurred if the cycle is cancelled due to an inadequate ovarian response to stimulation. Whatever definition is used, poor responders have definitely lower pregnancy rates compared with normal responders of similar age (El-Toukhy *et al*., 2002; Ulug *et al*., 2003; Kailasam *et al*., 2004; Galey-Fontaine *et al*., 2005; Klinkert *et al*., 2005; Saldeen *et al*., 2007).

In the clinical setting it may be useful to correctly predict the occurrence of poor response as this may lead to avoiding treatment in women destined not to respond to COS, thus contributing to reducing the cycle cancellation rate, the treatment costs and psychological stress for the couple. Finally improved counselling for the prediction of poor response may ameliorate disappointment and distress.

A large number of clinical parameters have been shown to predict the poor ovarian response to stimulation with exogenous gonadotrophins and have been introduced in the clinical practice. These include age, basal serum FSH and inhibin B levels, antral follicle count, ovarian volume, a number of dynamic tests and more recently AMH (Navot *et al*., 1987; Fanchin *et al*., 1994; Faddy and Gosden, 1996; Lass *et al*., 1997; Tomas *et al*., 1997; Hall *et al*., 1999; Ravhin *et al*., 2000; Bancsi *et al*., 2002; Broekmans *et al*., 2006).

Several authors investigated the utility of AMH in the prediction of poor response to FSH. Reported sensitivity and specificity ranged between 44–97% and 41–100%, respectively (Table II). Sensitivity–specificity points for all studies reporting on the performance of AMH in the prediction of poor response are reported in Fig. 3. It is clear that not all studies found an optimal sensitivity (>0.75) and specificity (>0.85) for AMH predicting poor response (Fig. 3). However, as will be discussed later, if AMH is measured with the aim of refraining bad prognosis couples from IVF, then in order to have a low number of false positive results, specificity more than sensitivity should be taken into consideration. On this basis it should be highlighted that more than half of the studies on AMH have reported a specificity higher than 0.85 (Fig. 3).

One of the main advantages of AMH with respect to the other hormonal markers of ovarian reserve is the possibility to be used as a menstrual cycle-independent marker since AMH seems to be stable and to have very low inter- and intra-cycle variability. In the first published study based on a single random measurement of AMH, it has been calculated a sensitivity of 80% and specificity of 93% for the prediction of poor response (La Marca *et al*., 2007).

Variable predictive performance for AMH was reported in the various studies and this has been considered by some authors (Seifer and Maclaughlin, 2007; Nakhuda, 2008) to be partly due to the use of different variants of AMH assay. Two different kits have been developed for AMH measurement [Immunotech–Beckman Coulter and Diagnostic System Laboratories (DSL)]. The main difference between the two assays is in the antibodies which have been obtained by using different standard proteins, thus leading to differences in the assay sensitivities. Initial studies comparing the two assays have shown that AMH levels appear to be 4–5-fold lower with the DSL assay compared with the Immunotech–Beckman assay (Bersinger *et al*., 2007; Fréour *et al*., 2007). In their report, Bersinger and colleagues (2007) alluded to problems inherent to AMH measurements that stem from residual matrix effects and instabilities of certain antigenic determinants. However, although developed independently, these assays are now both produced by a single company (Beckman–Coulter), and cross-referencing has shown that the correlation between the two assays is very high as confirmed by recent studies that found similar AMH values with both assays (Taieb *et al*., 2008; Streuli *et al*., 2009), therefore suggesting that the methodological problems mentioned by Bersinger and colleagues (2007) should have been addressed and solved by the assay manufacturer. Both kits are likely to remain in production over the next few years as approximately half of researchers are using the DSL assay and the other half the Immunotech–Beckman product. However, it is anticipated that within 2 years, an automated system for AMH measurement will become available, and industry sources indicate that it is likely that this will be calibrated to the Immunotech–Beckman kit.

Most importantly, the performance of any test of ovarian reserve, including AMH, is strictly dependent on the prevalence of the disease (poor response) we want to identify. Throughout the published studies the prevalence of poor response may vary on the basis of the percentage of older (high incidence of poor response) and younger (low incidence of poor response) patients included in the study and, of course, on the basis of the adopted definition for poor response. As a consequence, the same test, measured at the same laboratory, will have different predictive performance if the proportion of older patients and the definition of poor response will change.

In conclusion the balance of all the clinical studies on AMH seems to suggest that AMH measurement, prior to gonadotrophin secretion, may be useful in the prediction of women at risk for poor-response or no response to gonadotrophins. Moreover the absence of modifications in serum AMH levels throughout the menstrual cycle permits clinicians to have a reliable serum marker of ovarian reserve that can be measured independently of the day of the cycle.

Prediction of hyper-response and OHSS

Ovarian hyper-response is the opposite end of the spectrum of ovarian reserve and might lead to a potentially life threatening condition, the OHSS.

OHSS refers to an exaggerated ovarian response to gonadotrophin treatment. The syndrome has a broad spectrum of clinical manifestations, from mild illness needing only careful observation to severe

Table II Sensitivity and specificity of AMH for the prediction of poor response to gonadotrophin stimulation

Author	*n*	Study design	Cut-off value	Sens (%)	Spec (%)	Definition of poor response	AMH assay
Van Rooij *et al.* (2002)	119	Prosp	0.3 μg/l	60	89	<4 oocytes	Immunotech–Beckman–Coulter
Muttukrishna *et al.* (2004)	69	Prosp	0.1 ng/ml	87.5*	72.2*	<4 oocytes or cancellation	Immunotech–Beckman–Coulter
Muttukrishna *et al.* (2005)	108	Retro	0.2 ng/ml	87	64	≤4 oocytes	Immunotech–Beckman–Coulter
Tremellen *et al.* (2005)	75	Prosp	8.1 pmol/l	80	85	≤4 oocytes	Immunotech–Beckman–Coulter
Peñarrubia *et al.* (2005)	80	Prosp	4.9 pmol/l	53*	96*	cancellation	Immunotech–Beckman–Coulter
Ebner *et al.* (2006)	141	Prosp	1.66 ng/ml	69	86	<4 oocytes	Immunotech–Beckman–Coulter
Fiçicioglu *et al.* (2006)	50	Prosp	0.25 pg/ml	90.9	90.9	<5 oocytes	Diagnostic System Laboratories
La Marca *et al.* (2007)	48	Prosp	0.75 ng/ml	80	93	<4 oocytes or cancellation	Immunotech–Beckman–Coulter
Fréour *et al.* (2007)	69	Prosp	1.3 μg/l	44	100	<6 oocytes	Immunotech–Beckman–Coulter
Smeenk *et al.* (2007)	80	Prosp	1.4 μg/l	62	73	≤4 oocytes	Immunotech–Beckman–Coulter
McIlveen *et al.* (2007)	84	Prosp	1.25 ng/ml	58	75	≤4 oocytes	Immunotech–Beckman–Coulter
Kwee *et al.* (2007)	110	Prosp	1.4 μg/l	76	86	<6 oocytes	Diagnostic System Laboratories
Nakhuda *et al.* (2007)	77	Prosp	0.35 ng/ml	90.1*	81.8*	cancellation	Diagnostic System Laboratories
Lekamge *et al.* (2007)	126	Retro	14 pmol/l	73	73	≤4 oocytes	Immunotech–Beckman–Coulter
Nelson *et al.* (2007)	340	Prosp	5 pmol/l	75†		≤2 oocytes	Diagnostic System Laboratories
Gnoth *et al.* (2008)	132	Prosp	1.26 ng/ml	97	41	≤4 oocytes	Diagnostic System Laboratories
Nardo *et al.* (2008)	165	Prosp	1.0 ng/ml	87	67	≤4 follicles on day 8 of COH	Diagnostic System Laboratories
Jayaprakasan *et al.* (2008)	135	Prosp	0.99 ng/ml	100	73	<4 oocytes or cancellation	Diagnostic System Laboratories

*For cycle cancellation identification; †percentage of correctly classified poor responder patients; Retro, retrospective study; Prosp, Prospective study.

illness requiring hospitalization and intensive care, being a potentially life-threatening condition. Mild and moderate forms of OHSS may occur in 15–20% of all ovarian stimulation cycles, however, the severe form of the syndrome has been reported as frequently as 1–3% (Practice Committee of ASRM, 2008).

The specific risk factors for OHSS include young age, low BMI, signs of PCOS, previous history of OHSS and high estradiol on the day of hCG (Macklon *et al.*, 2006; Fauser *et al.*, 2008; Practice Committee of ASRM, 2008). The key to preventing OHSS is the recognition of risk factors for OHSS leading to an individualization of gonadotrophin starting dose which should be the minimum dose necessary to achieve the therapeutical goal. However, the accurate prediction of OHSS in an individual IVF cycle remains a difficult task. Indeed, PCOS (the main risk factor used in the prediction of OHSS) is present only in 20% of women undergoing COH and in <20% of patients developing symptoms of impending OHSS (Bellver *et al.*, 2003; Tummon *et al.*, 2005).

The recognition of a dose–response relationship between AMH and ovarian response to FSH leads to the hypothesis that hyper-response to ovulation induction might result from high AMH. In this context high basal AMH may be associated with an increased risk of developing OHSS.

At present few studies have been published reporting on this issue (Table III). However, it seems that hyper-response and OHSS may be associated with significantly higher mean basal AMH levels (Eldar-Geva 2005; Tremellen *et al.*, 2005; Nakhuda *et al.*, 2006; La Marca *et al.*, 2007; Nelson *et al.*, 2007; Lee *et al.*, 2008; Nardo *et al.*, 2008). Recently, four prospective studies performed on large number of

subjects have been published (Kwee et al., 2007; Nelson et al., 2007; Lee et al., 2008; Nardo et al., 2008) reporting relevant value for AMH for the prediction of hyper response and OHSS (Table IV).

Particularly the studies by Lee et al. (2008) and Nardo et al. (2008) have independently calculated a similar performance of AMH for the prediction of hyper response and OHSS. The reported cut-off value is of about 3.5 ng/ml, above which hyper-response/OHSS may be anticipated. In the study by Lee et al. (2008), a cohort of 262 IVF cycles was investigated, in order to evaluate the predictive value for OHSS by means of age, BMI, estradiol and AMH levels. Authors found that the ROC of the basal AMH was larger than age and BMI, and works equally well as the number of follicles and estradiol levels on the day of hCG. Basal AMH levels predicted OHSS with a sensitivity of 90.5% and specificity of 81.3%. Interestingly the cut-off value calculated (3.36 ng/ml) corresponded to the highest quartile of the AMH values in their population, suggesting that hyper-response and OHSS may be caused by gonadotrophin administration to women with 'enhanced ovarian reserve' (Lee et al., 2008). This was also evident in a previous study by our group (La Marca et al., 2007) in which all cases with ovarian hyper-response to COS where in the group of patients with basal AMH levels in the highest AMH quartile. Considering that PCOS has been associated with high AMH levels, it is logical to conclude that the prevalence of PCOS patients among women with AMH levels in the highest AMH quartile may be increased thus in part explaining the observed high rate of OHSS in this group of women.

In conclusion, AMH measurement prior to gonadotrophin stimulation could provide useful information to direct the application of mild patient-friendly stimulation protocols in order to avoid OHSS.

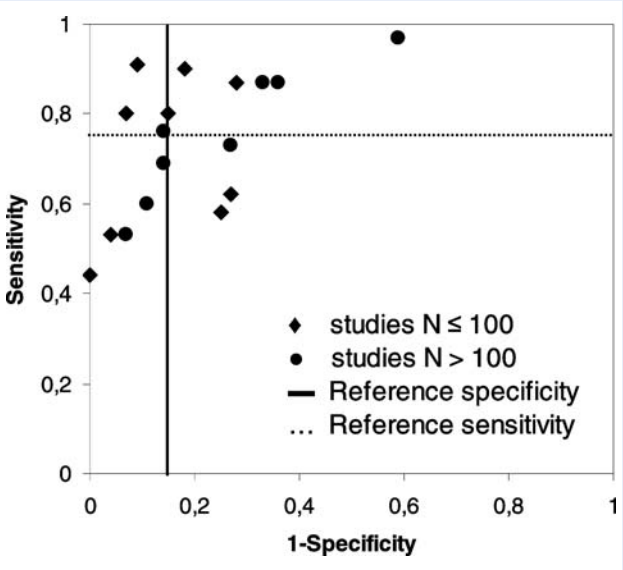

Figure 3 Sensitivity–specificity points for all studies reporting on the performance for AMH in the prediction of poor response.

Reference lines indicate a desired level for sensitivity (0.75) and specificity (0.85).

Prediction of qualitative ovarian response in ART

It is extensively recognized that pregnancy in ART is mostly related to the qualitative than quantitative aspects of IVF. As the status of the ovarian reserve includes both the quantity and quality of ovarian follicle pool, AMH may reflect not only quantitative but also qualitative ovarian responsiveness. Indeed several authors have found a significant positive correlation between AMH levels, oocyte quality (Hazout et al., 2004; Ebner et al., 2006; Silberstein et al., 2006; Cupisti et al., 2007; Fanchin et al., 2007, Lekamge et al., 2007) and embryo morphology (Silberstein et al., 2006). However, this relationship has not been confirmed by others (Smeenk et al., 2007; Lie Fong et al., 2008). In order clarify the complex relationship between AMH and oocyte quality, embryo quality and implantation and pregnancy rate, we should separately comment on studies of AMH in the follicular fluid and in serum.

Studies on AMH in the follicular fluid

In an elegant study AMH was measured in the follicular fluid obtained from both small and large follicles on the day of oocyte retrieval

Table III Basal AMH levels in women with normal response, hyper-response to controlled ovarian stimulation (COS) and ovarian hyperstimulation syndrome (OHSS)

Author	Design	*n*	Mean AMH levels		
			Normal response	Excessive response	OHSS
Tremellen et al. (2005)	Prosp	75	15.47 pmol/l	21.53 pmol/l[a]	
Eldar-Geva et al. (2005)	Prosp	56	14.1 pmol/l	37.8 pmol/l[b]	
Nakhuda et al. (2006)	Retro	30	0.63 ng/ml		3.6 ng/ml
La Marca et al. (2007)	Prosp	48	5.98 ng/ml	10.13 ng/ml[c]	
Nelson et al. (2007)	Prosp	340	10 pmol/l	27 pmol/l[d]	
Nardo et al. (2008)	Prosp	165	3.04 ng/ml	5.56 ng/ml[b]	

Retro: retrospective study; Prosp: prospective study.
[a]Excessive response if ≥18 oocytes retrieved.
[b]Excessive response if ≥20 oocytes retrieved.
[c]Excessive response if ≥16 oocytes retrieved.
[d]Excessive response if ≥21 oocytes retrieved.

Table IV AMH cut-off values for the prediction of hyper-response to COS and OHSS

Author	*n*	Study design	Cut-off value	Sensitivity (%)	Specificity (%)	Prediction of hyper-response	Prediction of OHSS
Kwee *et al.* (2007)	110	Prosp	5 mcg/l	53	91	√[a]	
Nelson *et al.* (2007)	340	Prosp	25 pmol/l	60	94.9	√[b]	
Lee *et al.* (2008)	262	Prosp	3.36 ng/ml	90.5	81.3		√
Nardo *et al.* (2008)	165	Prosp	3.5 ng/ml	88	70	√[a]	

Prosp: prospective study.
[a]Excessive response if >20 oocytes retrieved.
[b]Excessive response if ≥21 oocytes retrieve.

(Fanchin *et al*., 2005a, b). AMH levels in follicular fluid were found to be roughly three times higher in small than in large follicles confirming the hypothesis that AMH production by granulosa cells probably declines during final follicular maturation. Moreover in both small and large follicles, follicular fluid AMH levels correlated positively with the number of early antral follicles on cycle Day 3 before COS, growing follicles on the day of hCG administration and oocytes retrieved. This interesting finding may indicate that peripheral AMH levels are not exclusively dependent on the number of follicles; they are also modulated by individual follicular ability to produce AMH. Hence, elevated peripheral AMH levels indicate not only that the number of antral follicles is increased, but also that each follicle probably produces more AMH individually. This offers us a new understanding of the reported association between peripheral AMH levels and the ovarian fertility potential, and leads the authors to speculate that serum AMH measurement could reflect not only quantitative but also qualitative ovarian responsiveness to COS (Fanchin *et al*., 2005a, b).

In a successive study by the same group (Fanchin *et al*., 2007), 118 monodominant follicle cycles were prospectively studied. AMH was measured in the follicular fluid and the fate of oocytes and embryos generated was observed. It was found that embryo implantation, clinical pregnancy and ongoing pregnancy rate increase dramatically from the low to the high follicular fluid AMH groups. The embryo morphology was similar within the groups, indicating that AMH in follicular fluid may be an additional factor in the selection of the oocyte (Fanchin *et al*., 2007). This is particularly relevant in countries with restrictive laws limiting the number of oocytes that may be inseminated. A recent study on a large number of subjects ($n = 276$) confirmed the previous finding that levels of AMH in follicular fluid were significantly increased in women who became pregnant in the respective IVF /ICSI treatment cycle (Wunder *et al*., 2008).

Studies on circulating AMH

Although studies on follicular fluid seem to indicate that AMH may be useful in the prediction of oocyte and embryo quality and finally pregnancy, the same could not be said for circulating AMH. At present only few studies concluded that serum AMH measurement may be able to give relevant information on gametes and embryo quality and on the outcome of the treatment cycle.

Silberstein and colleagues (2006) found that serum AMH measured on the day of hCG correlated with the quality of embryos obtained thus allowing discrimination between embryos with high- and low-implantation potential. Consequently implantation rate, but not pregnancy rate, was higher in the group with high basal AMH levels (Silberstein *et al*., 2006). However, the lack of a consistent correlation between serum AMH and embryo morphology and embryo aneuploidy rate, which is not in favour of a direct relationship between oocyte quantity and embryo quality, has been clearly demonstrated (Lie Fong *et al*., 2008). Hence serum AMH seems not to be an adequate marker for embryo quality.

The vast majority of the studies investigating the performance of serum AMH in the prediction of pregnancy occurrence following IVF reported that AMH measurement is not useful in the prediction of success. Only few studies reported a significant cut-off for AMH levels able to distinguish between pregnancy and non-pregnancy. It should be noted that the only two positive prospective studies (Eldar-Geva *et al*., 2005; Elgindy *et al*., 2008) were limited by very small numbers of subjects ($n = 56$ and 33, respectively). Conversely the largest study ($n = 109$) concluding that serum AMH may be predictive of pregnancy had a retrospective design, hence limiting the scientific soundness of the finding (Lekamge *et al*., 2007). However, the study by Lekamge and colleagues (2007) analyzed for the first time the cumulative pregnancy rate from both fresh and freezed/thawed embryos. As a consequence of the relationship between serum AMH and the quantitative ovarian response to COS, women with low AMH levels yielded fewer oocytes and generated fewer embryos, culminating in halving of the cumulative pregnancy rate compared with the high AMH group (Lekamge *et al*., 2007). Hence the higher pregnancy rate observed in the group of patients with high basal AMH levels, when compared with those with low AMH levels, may be explained on the basis of an increased availability of oocytes.

Until now only one study has been published relating serum AMH levels to the live birth rate following IVF (Nelson *et al*., 2007). In this large prospective study of 340 patients it was demonstrated that the live birth rate dramatically increased with increasing basal AMH values (Fig. 4). However, this was valid only for women with basal levels <7.8 pmol/l. Above this value there was no discrimination for the live birth. Basal AMH does not seem to predict pregnancy or non-pregnancy, but simply enables patients to be identified as being at a low or high probability of pregnancy after IVF. As concluded by the same authors, this finding may, at least in part, be explained by the very good correlation existing between basal AMH and the number of retrieved oocytes (Nelson *et al*., 2007).

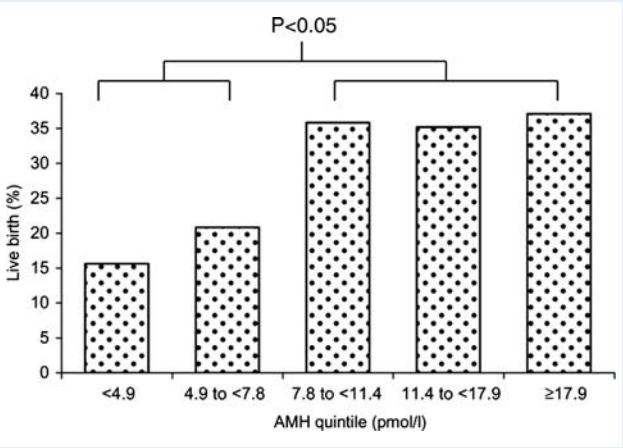

Figure 4 The mean live birth rate following IVF according to basal serum AMH levels.

In this prospective study of 340 patients it was demonstrated that the live birth rate dramatically increased with increasing basal AMH value. However this was valid only for women with basal levels <7.8 pmol/l. Above this value there was no discrimination for the live birth (reproduced with permission from Nelson *et al.*, 2007).

In conclusion the possible prediction of qualitative aspects of ART programmes by serum AMH measurement remains largely controversial. Evidence suggests that this relationship may only be indirect and related to the strong correlation existing between serum AMH and the quantitative ovarian response to COS.

AMH in ovarian reserve testing

The ideal ovarian reserve test should permit identification of women who have a chance of pregnancy after IVF close to zero as a consequence of an extremely reduced ovarian reserve. The exclusion of these couples from ART could effectively reduce costs for the health system. Moreover useless medical treatments, surgical risks, stress and disappointment could be avoided. On the other hand, as we have previously seen, the predictive value of AMH for poor response is not absolute, with consequent false positive and negative results. Especially false positive results may have negative consequences on the couple's life since this result might incorrectly prohibit these women from undergoing IVF. Furthermore, it has been widely demonstrated that many poor responders achieve pregnancy and live birth (Klinkert *et al.*, 2004; van der Gaast *et al.*, 2006). In particular young poor responders have a different prognosis compared with older poor responders (Lashen *et al.*, 1999; Ulug *et al.*, 2003).

Hence, before proposing AMH measurement in the ovarian reserve testing, we should define what is the aim of the testing itself. The possible aim of ovarian reserve testing in the IVF setting is: (i) to counsel the patients about the risk/benefit of the treatment; (ii) to reduce the cost by denying treatment to bad prognosis couples and (iii) to individualize treatment strategy.

Significance of low AMH levels before IVF

For women with low AMH levels either cycle cancellation or poor response may be anticipated. Hence, couples would need to accept protracted treatment programmes and should be informed that not every cycle may result in embryo transfer and that it is highly probable that the chance of success may be reduced.

Counselling and management of this group of patients is difficult for several reasons. First of all, accuracy of testing for poor response appears to be better than for the prediction of pregnancy, but is not fully reliable since a false positive rate of 10–20% can be expected. This indicates that AMH measurement, similarly to the other ovarian reserve markers, should not be used to exclude couples from IVF (Broer *et al.*, 2008). Cut-off values for AMH of 0.7–0.75 ng/ml have been proposed for the identification of poor responders by several groups (La Marca *et al.*, 2007; Nelson *et al.*, 2007). Although it seems to have a good sensitivity and specificity by which 75% of poor responders are correctly classified, one should note that the prevalence of young women with AMH levels less than 0.7 ng/ml is estimated to be rather low. In a large population from our clinic ($n = 381$), 97 patients (25%) had AMH values <0.7 ng/ml and among these only 53 women (13.9%) were younger than 38 years, indicating that the added value of AMH to chronological age in the identification of poor responders may be lower than expected (personal data, unpublished). Most importantly, the live birth rate for women with basal AMH <0.7 ng/ml is estimated to be 15% (Nelson *et al.*, 2007) which may be considered highly acceptable for patients anticipated to be poor responders.

Thus, we proposed that only women with a very poor prognosis should be refused treatment. These patients are those at very high risk for cycle cancellation and might be identified by serum AMH levels lower than 0.1–0.35 ng/ml (Muttukrishna *et al.*, 2004; Lekamge *et al.*, 2007). Of course the use of these very low cut-off values would implicate that only a small percentage of abnormal tests will be found and that many poor responders will pass unrecognized. In our infertile population ($n = 381$), only 34 women (8.9%) had AMH values lower than 0.35 ng/ml and only half of these women (4.5%) were younger than 38 years. Only 9 of 381 patients had AMH values less than 0.1 ng/ml and of these patients only two were younger than 38 years (personal data, unpublished). This clearly indicates that age alone would identify the majority of women who will have a cycle cancelled for absent ovarian response.

In conclusion AMH measurement in ovarian reserve testing should be used with very low cut-off values in order to minimize the occurrence of false positive tests. As a consequence the added value of AMH assay to chronological age is expected to be minimal. If the ovarian reserve test should be used to reduce costs by denying treatment to bad prognosis couples, an AMH assay may only permit meagre cost reductions.

Regarding treatment, it is still not clear whether the individualization of the therapy may improve outcome. Indeed, although high doses of gonadotrophins are widely administered to poor responders and to patients with an anticipated poor response, results reported in literature have been controversial. Published trials have shown little or no benefit (Popovic-Todorovic *et al.*, 2003; Klinkert *et al.*, 2005). Similarly it is not clear what kind of GnRH analog, either agonist or antagonist, may be more suitable for the pituitary suppression in these patients. Natural IVF and the use of adjuvant therapies seem to give results similar to the standard IVF and still need to be studied in large randomized controlled trials (Tarlatzis *et al.*, 2003; Ubaldi *et al.*, 2005; Shanbhag *et al.*, 2007; Loutradis *et al.*, 2008).

In conclusion AMH measurement, when low AMH values are found, may have an added value to chronological age only in the counselling of the patients. Doubts remain regarding both the possible reduction of costs (consequent to IVF refusing) and the possible improvement of the outcome (consequent to the individualization of the treatment).

Significance of normal AMH levels before IVF

Women with normal AMH levels are most probably normal responders and a good prognosis may be anticipated. Currently, there is no evidence to modify the normal strategy based on the standard long protocol (Daya, 2000). In recent years, mild ovarian stimulation protocols for predicted good responder women have been proposed as a valid and alternative standard treatment in order to achieve cost-effective, patient-friendly regimens which optimize the balance between outcomes and risks of treatment (Hohmann *et al*., 2001, 2003; Heijnen *et al*., 2007; Verberg *et al*., 2009). However, further, sufficiently-powered prospective studies applying novel mild treatment regimens are required.

Significance of high AMH levels before IVF

Women with high AMH levels are considered to be at risk for hyper-response and OHSS. Hence these women should be informed about this risk. Women with high AMH levels are those who may really benefit from the individualization of the treatment. Indeed a low FSH starting dose followed by the use of GnRH antagonists (Al Inany *et al*., 2006; Doldi *et al*., 2006; Griesinger *et al*., 2006) have been shown to reduce the incidence of OHSS and may be proposed as a first line treatment for patients with high serum AMH levels. Moreover the use of GnRH antagonist permits the triggering of ovulation by means of GnRH agonist instead of hCG and this practice has been recognized as useful in the prevention of OHSS (Olivennes *et al*., 2002; Engmann *et al*., 2006; Griesinger *et al*., 2006; Orvieto *et al*., 2006; Kol and Solt, 2008).

In conclusion it seems that AMH measurement, when high AMH values are found, may have relevant clinical value for the specialist. Indeed such information may improve the counselling of the patients (about the increased risk for OHSS), and permit an individualization of the treatment with the aim of reducing the incidence of OHSS, and finally, AMH measurement may reduce the costs linked to hospitalization.

AMH in male fertility

AMH in testicular physiology

AMH is the earliest Sertoli cell specific protein expressed by the male gonad (Tran *et al*., 1977). It is secreted by the testis from the eighth week of pregnancy and remains secreted at high levels until puberty, when Sertoli cell maturation is characterized by a decreasing AMH production (Rajpert-De Meyts *et al*., 1999). During puberal development AMH expression falls, coinciding with the increase in androgen secretion by Leydig cells (Rey *et al*., 1993). The reduction in AMH levels at puberty is considered a clear marker of the elevation of intratesticular androgen concentration which inhibits Sertoli cell AMH production at puberty (Fig. 5). Paralleling the situation in women, the main physiological role of AMH in the adult male seems to be limited to the paracrine control of testicular function. AMH inhibits aromatase activity in Sertoli cells (Rouiller-Fabre *et al*., 1998) and testosterone production by Leydig cells (Behringer *et al*., 1990). Indeed, male mice that over-express AMH have lower levels of testosterone and Leydig cell hypoplasia (Lyet *et al*., 1995), and conversely, mice with null mutations in AMH or AMH RII have Leydig cell hyperplasia (Behringer *et al*., 1990).

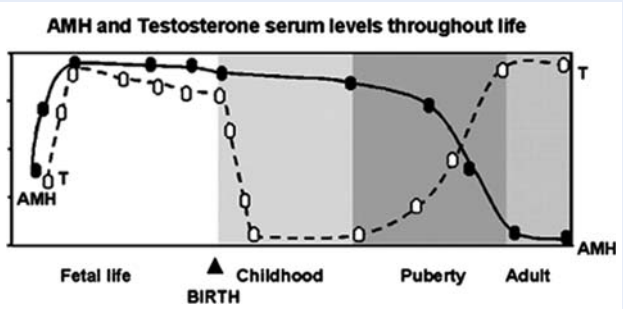

Figure 5 Profiles of serum AMH and testosterone (T) in the male. AMH is not down-regulated by testosterone during fetal life and in the first months after birth due to the lack of expression of the androgen receptor in Sertoli cells. The physiological androgen insensitivity of fetal and neonatal Sertoli cells explains, thus, the transient coexistence of high concentrations of androgens and AMH. During puberal development AMH expression falls coincidently with the increase in androgen secretion by Leydig cells (reproduced with permission from Rey, 2005).

As AMH is produced at high level before puberty its measurement can serve as a reliable marker for the presence of testicular tissue in childhood when levels of testosterone are very low. On this basis AMH is useful in the differential diagnosis of intersex conditions and disorders associated with androgen insensitivity (Gustafson and Donahoe, 1994; Rey *et al*., 1994). AMH measurement is particularly helpful in patients with bilateral non-palpable gonads (Lee *et al*., 1997). In these patients normal AMH levels provide reassurance that the testis can be present but not descended.

In the adult man, AMH is also present in seminal fluid at concentrations that may be significantly higher than those observed in serum (Fénichel *et al*., 1999). The data comparing seminal and serum AMH concentrations in adults suggests that after puberty AMH is secreted preferentially by the apical pole of the Sertoli cells toward the lumen of the seminiferous tubules resulting in higher concentrations of AMH in the seminal plasma than in the serum (Fénichel *et al*., 1999).

Value of AMH measurement in infertile men

As AMH is a specific marker of Sertoli cell function and is secreted in the serum and seminal fluid, its measurement in both the compartments may be useful in obtaining information on spermatogenesis, particularly in infertile men.

Studies on serum AMH

One study found significantly reduced serum AMH levels in men with oligozoospermia compared with controls (Al-Qahtani *et al*., 2005). The difference in serum AMH between men with normal and reduced sperm concentration was not confirmed by a second study

by the same group (Appasamy et al., 2007), however, a correlation of serum AMH levels with sperm count and serum FSH levels has been reported (Appasamy et al., 2007).

In the largest study to date, performed on 199 men, no significant differences were found in serum AMH levels between controls and men with oligozoospermia (Tüttelmann et al., 2009), confirming that serum AMH is not of diagnostic significance in men with impaired spermatogenesis. Serums AMH levels have been found to be significantly lower in non-obstructive azoospermic (NOA) than in obstructive azoospermic (OA) patients and normal fertile men (Muttukrishna et al., 2007). However, the wide overlapping of values between controls and infertile men prevents this hormone from being a useful diagnostic marker.

Other studies have investigated whether serum AMH levels may be predictive of the presence of sperm in testis from NOA patients (Isikoglu et al., 2006; Goulis et al., 2009). It has been clearly demonstrated that serum AMH could not predict the presence of sperm in fine-needle aspiration (Goulis et al., 2009) or in testicular sperm extraction (TESE) (Isikoglu et al., 2006) performed in men with NOA.

Studies on AMH in the seminal fluid

After puberty, AMH is preferentially secreted by the apical side of Sertoli cells, into the seminiferous tubules, explaining the higher concentration of AMH in the seminal fluid when compared with the serum in adult men (Fénichel et al., 1999). This observation suggests a closer link between spermatogenesis and seminal AMH than serum AMH. Seminal AMH correlated with sperm concentration and, as a consequence, seminal AMH levels in controls were significantly higher than in oligozoospermic men (Fujisawa et al., 2002; Duville et al., 2008; Mostafa et al., 2007). As expected, seminal AMH levels have been reported to be significantly lower in azoospermic men than in oligozoospermic and healthy men (Duville et al., 2008; Mostafa et al., 2007). In particular AMH was not detectable in semen from OA patients (Fénichel et al., 1999; Mostafa et al., 2007) whereas it was detectable in 39–57.5% of NOA patients (Fénichel et al., 1999; Mostafa et al., 2007).

When evaluating the predictive value of seminal AMH on TESE outcome in NOA patients, all studies confirmed that AMH measurement in the seminal fluid is not useful in distinguishing between cases with positive and negative outcome (Fénichel et al., 1999; Isikoglu et al., 2006; Mostafa et al., 2007; Duvilla et al., 2008). This is not surprising as, similarly to other endocrinological markers of testicular function (FSH and inhibin B), variations in AMH levels can occur for reasons unrelated to spermatogenesis.

Conclusions

Recent studies have indicated that AMH may constitute an important novel measure of ovarian reserve. Serum AMH levels show a reduction throughout reproductive life and are undetectable after menopause (Van Rooij et al., 2004; Van Rooij et al., 2005; La Marca et al., 2005a, b; Robertson et al., 2008; Shin et al., 2008; Van Disseldorp et al., 2008). Similarly, early ovarian ageing and premature ovarian failure have been associated with very low or undetectable serum levels, respectively (La Marca et al., 2006b; De Koning et al., 2008; Knauff et al., 2008). Furthermore AMH levels do not significantly change during the menstrual cycle (Hehenkamp et al., 2006; La Marca et al., 2006a; Streuli et al., 2008), whereas all other hormones secreted by the ovary show significant variations throughout the cycle. The stability and consistency of its levels indicate that AMH could be used as the most reliable single marker of ovarian ageing and ovarian response to COS.

For women who want to become pregnant by means of ART, it is important to offer counselling about the optimal balance between benefit and risk. Since these outcomes are highly dependent on ovarian reserve, much effort has been put into identifying good clinical markers of ovarian reserve regarding individual prognosis for success and to design appropriate stimulation protocols.

Although AMH measurement is of course more expensive than age evaluation as a single marker of ovarian reserve, it clearly performs better in the prediction of both poor and hyper-response to COS (Table V). Furthermore, AMH ease of measurement confers a relevant advantage to FSH which is cycle-dependent and less powerful. AMH may also be informative on ovarian reserve in women during GnRH agonist treatment or hormonal contraception that consequently exhibit suppressed FSH levels. Finally, it seems that poor response may be predicted by AMH with a performance which is similar to the AFC. Conversely AMH seems superior to AFC in the prediction of hyper response (Nardo et al., 2008). Although AFC is a very common and useful measurement it may be sometimes technically challenging and operator-dependent. Considering all these peculiar characteristics, it may be concluded that AMH is a candidate proposed as the ideal test for the ovarian reserve evaluation (La Marca et al., 2006a, b, c; 2009b) (Table V).

One new interesting field of application for AMH measurement, may be its use in the individualization of ovarian stimulation regimens. In many centres, the starting FSH dose for the first IVF is often selected on the basis of age and possibly also BMI of the patient. Some authors have recently proposed adjusting the treatment strategy on the basis of AMH levels (Nelson et al., 2007, 2009; Gnoth et al., 2008).

As low and high AMH values are predictive of poor- and high-response to gonadotrophins, respectively, it has been proposed that the daily dose of FSH is tailored according to the pre-IVF AMH levels, and independently of the age and BMI of the patient (Nelson et al., 2007, 2009; Gnoth et al., 2008).

Table V Comparison of characteristics of the most widely used markers of ovarian reserve

Characteristics for a good marker	Age	AMH	FSH	AFC
Prediction of poor response	+	+++	++	+++
Prediction of hyper response	+	+++	−	++
Low inter-cycle variability	+++	++	−	++
Low intra-cycle variability	+++	++	−	++
Blinded to the operator	+++	+++	+++	−
Applicable to all patients (a)	+++	+++	+	+
Cheapness	+++	−	−	−

(a) FSH and antral follicle count (AFC) are not informative in patients on hormonal contraception or GnRH agonist treatment. Moreover the count of antral follicles may be difficult in women with ovarian cysts or with previous pelvic surgery.

If AMH measurement is proposed to all women before entering an IVF programme, a clear definition of cut-off values for the prediction of poor- and hyper-response is required. Similarly the treatment strategies for the various groups of patients should be elaborated. Finally an analysis of cost and benefit of this programme is mandatory. Most of these aspects have been addressed in a recent prospective study in which the COS strategies have been based only on serum AMH levels (Nelson *et al*., 2009). More than five hundred patients were divided into four groups on the basis of AMH levels: the predicted negligible response category (AMH $<$ 1 pmol/l), the predicted reduced response category (AMH $\geq$ 1, $<$5 pmol/l), the predicted normal response category (AMH $\geq$ 5, $<$15 pmol/l) and the predicted high response category (AMH $\geq$ 15 pmol/l). Different stimulation protocols were then applied only on the basis of this stratification, independently of the age of the patients. In particular, women with low AMH received a high starting FSH dose followed by the GnRH antagonist, women with normal AMH levels received the standard long protocol and women with high AMH received a low FSH dose followed by the GnRH antagonist. The authors found that this AMH-based strategy of COS was associated with a significant reduction of excessive response to stimulation and in reduced treatment burden, reduced cycle cancellation and a trend towards increased clinical efficacy. Even if the study by Nelson and colleagues (2009) has several limitations such as the non-randomized design and the non-random use of different gonadotrophin formulations, it clearly demonstrates that a single AMH assay may be used to individualize treatment strategies for IVF, potentially resulting in reduced clinical risks, along with optimized treatment burden and clinical pregnancy rate (Nelson *et al*., 2009).

Finally AMH may be incorporated into a more complex predictive calculation of response like the CONSORT formula (Olivennes *et al*., 2009). The CONSORT dosing algorithm individualizes recombinant FSH doses for ART according to certain patient characteristics: basal FSH, body mass index, age and antral follicle count. The use of the CONSORT algorithm seems to achieve an adequate oocyte yield and good pregnancy rates (Olivennes *et al*., 2009). Adjustment of the algorithm by incorporating further powerful markers such as AMH may, in turn, increase the clinical efficacy of the formula.

In summary, published studies indicate a relevant role for AMH measurement in the identification of both the extremes of ovarian response to stimulation, and probably in the consequent individualization of treatment strategies in order to possibly reduce the incidence of cycle cancellation and OHSS. It still remains to clarify the cost/benefit of its use as a single assay before beginning an IVF cycle and whether the AMH-determined strategy of COS for assisted conception may be associated with improved live birth rate.

Concerning the role of AMH in the evaluation of infertile men, it should be highlighted that much research has been focused in the last years on the identification of serum and seminal markers able to improve the understanding of germinal deficiency and to allow discrimination between absent, incomplete or reduced spermatogenesis. AMH seems to be a good candidate marker since it is of testicular origin, it is specifically secreted by Sertoli cells, it is correlated with spermatogenesis and is present in both serum and seminal fluid in detectable concentrations. Serum AMH seems to be significantly lower in men with NOA than OA and controls, however, the wide overlapping of values between subjects prevents this hormone from being of clinical utility. On the contrary AMH is undetectable in seminal fluid for men with obstructive azoospermia, thus being useful to formulate the, not always easy, diagnosis of obstructive azoospermia. Unfortunately in the studies to date, the seminal AMH predictive value on TESE outcome in case of NOA is not optimal in the identification of men with successful sperm retrieval. Inclusion of AMH in an equation obtained by multivariate logistic regression analysis and including other preoperative factors may be a strategy to obtain a satisfactory clinical use of AMH determination in men.

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