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Serum anti-Müllerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles—implications for individualization of therapy

Scott M. Nelson[1,3], Robin W. Yates[2] and Richard Fleming[1]

[1]*Reproductive and Maternal Medicine, Division of Developmental Medicine,University of Glasgow, 10 Alexandra Parade, Glasgow G31 2ER, UK;* [2]*Assisted Conception Unit, Glasgow Royal Infirmary, Glasgow G31 2ER, UK*

[3]Correspondence address. Tel: +44 141 211 4706; Fax: +44 141 552 0873; E-mail: s.nelson@clinmed.gla.ac.uk

BACKGROUND: Serum concentrations of anti-Müllerian hormone (AMH) correlate with oocyte yield in assisted reproduction treatment (ART) cycles, however, performance of AMH for prediction of live birth is unknown. METHODS: A total of 340 first cycle IVF/ICSI patients (median age 34.0 years, inter-quartile range 31.0–37.0 years), had basal plasma AMH and FSH measured and their predictive values for live birth and oocyte yield compared. RESULTS: AMH predicts live birth [contribution to variance (CTV) 3.84%, $P < 0.001$] and oocyte yield ($r = 0.71$, $P < 0.0001$, CTV 7.3%, $P < 0.0001$). Compared with age and FSH, AMH performs better in prediction of live births [area under receiver operating characteristic curve (AUC) 0.62, 95% CI 0.55–0.68; FSH AUC 0.42, 95% CI 0.35–0.49; age AUC 0.48, 95% CI 0.41–0.55, $P = 0.0028$] and excessive response to ovarian stimulation (AMH AUC 0.90, 95% CI 0.83–0.96; FSH AUC 0.32, 95% CI 0.23–0.40; age AUC 0.57, 95% CI 0.43–0.71, $P < 0.001$). AMH prediction of oocyte yield is independent of age ($r = -0.28$, $P < 0.0001$, CTV 1.4%, $P = 0.006$), however, a significant negative interaction (CTV 3.6%, $P < 0.0001$) exists. AMH demonstrates improved differential distributions for non-, poor, normal and excessive ovarian responses relative to FSH and age. CONCLUSIONS: Plasma AMH is a superior predictor of live birth and anticipated oocyte yield compared with FSH and age, facilitating individualization of therapy prior to first ART cycle.

Keywords: anti-Müllerian hormone; live birth; controlled ovarian stimulation

Introduction

There has been increasing interest in identification and the relative performance of tests of ovarian reserve prior to embarking on controlled ovarian stimulation (Broekmans *et al.*, 2006). Although there is a clear relationship between age and declining reproductive capacity (Templeton *et al.*, 1996), this is highly variable (de Bruin *et al.*, 2004) and therefore a variety of endocrine, stimulatory and ultrasound tests have been suggested as functional assessments of potential oocyte yield and pregnancy (Broekmans *et al.*, 2006). The overall aim of these tests is to provide a more accurate estimate of potential success for patients before embarking on therapy and, more importantly, to facilitate optimization and individualization of therapy prior to commencement of the first cycle of assisted reproduction treatment (ART) (Tarlatzis *et al.*, 2003). It is important for a clinic programme to be able to predict both extremes of response, as high responding patients are at risk of ovarian hyperstimulation syndrome, while poor responders can be forewarned, and modified stimulation approaches employed. A reliable indicator of responses to conventional treatment can be used to prospectively test different therapeutic approaches.

Anti-Müllerian hormone (AMH, Müllerian-inhibiting substance), a member of the transforming growth factor-β family, has the primary role of regression of the Müllerian duct in the male fetus during early testis differentiation. However, expression of AMH persists after completion of reproductive duct system development in males, and commences in females in early fetal life, where it is produced by ovarian granulosa cells (Modi *et al.*, 2006). In females, AMH appears to have inhibitory effects upon the recruitment of primordial follicles (Durlinger *et al.*, 2002) and it may decrease the sensitivity of large pre-antral and small antral follicles to FSH (Durlinger *et al.*, 2001). However, recent analyses of human follicles examining AMH receptor expression suggests that inhibitory effects at the earliest stages of follicle development are unlikely (Rice *et al.*, 2007). Although AMH is initially observed in granulosa cells of primary follicles, maximal expression occurs in pre-antral and small antral follicles (Laven *et al.*, 2004; Weenen *et al.*, 2004). AMH expression declines as antral

follicles increase in size, with nominal expression restricted to the granulosa cells of the cumulus (Weenen *et al.*, 2004). This loss of AMH expression during the FSH-dependent final stages of follicular growth, and the lack of expression by atretic follicles (Baarends *et al.*, 1995), suggests that basal levels of AMH may more accurately reflect the total developing follicular cohort and consequently potential ovarian response to FSH. Furthermore, AMH has been shown to not fluctuate across the menstrual cycle (Cook *et al.*, 2000; La Marca *et al.*, 2004,2006), consistent with its role reflecting the continuous, non-cyclic growth of small follicles in the ovary.

In harmony with the established relationship between age and declining ovarian reserve, AMH falls linearly with increasing age (de Vet *et al.*, 2002; Fleming *et al.*, 2006). This occurs in conjunction with reductions in the antral follicular count, which is strongly correlated to plasma AMH levels (van Rooij *et al.*, 2002; Fanchin *et al.*, 2003). AMH has consequently been explored as a predictor of ovarian response to FSH in cycles of ART (van Rooij *et al.*, 2002; Fanchin *et al.*, 2003; Hazout *et al.*, 2004; Muttukrishna *et al.*, 2004,2005; Penarrubia *et al.*, 2005) and oocyte quality (Ebner *et al.*, 2006). However, most of these studies have included relatively small series of patients ($n = 56–141$). In combination with its ability to be measured throughout the menstrual cycle, AMH has been proposed as an optimal measure of 'ovarian reserve' and an accurate predictor of cycle cancellation (Muttukrishna *et al.*, 2004; Penarrubia *et al.*, 2005) or poor responder status (van Rooij *et al.*, 2002; Eldar-Geva *et al.*, 2005; Muttukrishna *et al.*, 2005; Tremellen *et al.*, 2005). Although these studies collectively demonstrate the potential value of AMH, its merits with respect to the prediction of live birth are unknown. Furthermore, clarification of its merits across the whole spectrum of ovarian response, including an excessive response, relative to the established markers of age and early follicular FSH is required. The current study, a large prospective cohort study which examines the value of AMH to predict live birth and ovarian response to controlled ovarian stimulation in a first cycle of ART in an unselected population, provides a comprehensive analysis as well as the opportunities for considering different therapeutic strategies based on basal AMH values.

Materials and Methods

Patients and treatment

Successive patients undergoing their first ART cycle ($n = 340$) were down-regulated with a depot GnRH agonist (Prostap SR 3.75 mg, Wyeth, Maidenhead, UK) initiated on cycle day 21. Stimulation was commenced 2 weeks later, when the circulating estradiol (E_2) was <100 pg/ml (350 pmol/l), combined with a thin endometrium, and no ovarian cysts on transvaginal ultrasound scan. Ovarian stimulation was effected with exogenous gonadotrophins in the form of either Menogon (Ferring Pharmaceuticals, Langley, UK) or Gonal-F (Serono, Feltham, UK). The starting daily dose of FSH was determined by age, whereby women of <36 years received 225 IU and those >36 years received 300 IU each day. Ovarian follicular responses were monitored with serum E_2 concentrations and transvaginal ultrasound assessment of follicular growth. The first response scan was performed on S8 (stimulation day 8), providing the 'FolsS8' value, and subsequent scans were performed according to the S8 response. Ovulation was induced with 6500 IU HCG (Ovitrelle, Serono, Feltham, UK), provided that three follicles were ≥ 17 mm in diameter and serum E_2 was ≥ 200 pg/ml. Transvaginal oocyte retrieval was performed under ultrasound guidance 38 h after HCG administration and the number of oocytes retrieved recorded. Frozen embryo transfers were performed 3 days after an LH surge.

Assays

One month before treatment, an early follicular blood sample (cycle day 2–5) was taken for assay of FSH and AMH. The FSH concentrations in peripheral plasma were estimated using the Immulite semi-automated assay system (DPC, Los Angeles, CA, USA). Inter and intra-assay coefficients of variations were 6.5% and 6.1% respectively. Early follicular FSH concentrations were only considered valid if circulating E_2 levels were ≤ 150 pmol/l. The AMH assay was performed in batches using the enzyme-linked immunosorbent assay provided by DSL (Webster, Texas, USA), with values presented as pmol/l (conversion factor to pmol/l = ng/ml $\times$ 7.143). Inter and intra-assay coefficients of variation were 5.3 and 5.4% respectively.

Definitions

'FolsS8'—the stockpile of follicles responding to exogenous FSH was estimated by counting the number of follicles ≥ 12 mm, determined on on the basis of previous observations that the minimum size of lead follicle at S8 was 12 mm in $>85\%$ of cycles (Fleming *et al.*, 2006).

'No response' to FSH in ART cycles was defined as discontinuation of treatment because of negligible follicular development after 12 days of stimulation.

'Poor response' to FSH in ART cycles was defined as ≤ 2 oocytes obtained at retrieval, as this represented -2 SDs from the mean number of oocytes collected and allowed for a 66% yield per follicle at oocyte retrieval, given the HCG criteria of three follicles ≥ 17 mm.

'Excess response' to FSH in ART cycles was defined as a yield of ≥ 21 oocytes obtained at retrieval, as this was used by our unit to dictate freezing of all embryos and no immediate replacement.

'Live birth' incorporated all births arising from the study cycle of controlled ovarian stimulation and included all live births derived from fresh and subsequent frozen embryo transfers. Embryos were only frozen if there were two embryos with a quality score of ≥ 7 out of 10 using a standardised scoring system (Association of Clinical Embryologists).

Statistics

The distribution of groups of variable data was assessed, and Gaussian or non-Gaussian distributions were treated appropriately. Analysis across groups was by analysis of variance or Kruskal–Wallis as appropriate. Spearman correlation coefficients were used to assess the relationships between the parameters. FSH and AMH were logarithmically transformed for assessment in general linear models. Stepwise logistic regression was performed using an alpha of $P \leq 0.15$ for adding or removing predictors from the model. Non-parametric receiver operating characteristic (ROC) curves were generated for FSH and AMH to compare ability of parameters to predict ovarian response and the area c (AUC) tested for equality (DeLong *et al.*, 1988). The sensitivity, specificity, percentage correctly classified and the positive and negative likelihood ratio were calculated for derived AMH cut-off values (Choi, 1998). AMH and FSH quintiles were calculated for the whole population and mean oocyte yield and live birth calculated for each quintile. The statistics packages used were Minitab v14 (Minitab®, State College, Pennsylvania, USA) and Stata v8 (StataCorp LP, College Station, Texas, USA). Significance was determined when $P \leq 0.05$.

Table 1: Patient characteristics relative to ovarian response

Variables	Normal	No HCG given	≤2 oocytes	≥21 oocytes	*P*-value
ART cases (*n*)	262	34	19	25	
Age (years)	34 (31.0–37.0)	37.2 (33.8–39.8)	34 (33.0–38.0)	30.0 (25.5–35.0)	<0.001
Duration of infertility (years)	3 (3–4)	4 (3–4)	4 (3–4)	4 (3–4)	0.29
Treatment type: ICSI/IVF (% ICSI)	111/151 (42.3)	11/23 (32.4)	11/8 (57.8)	9/16 (36.0)	0.30
BMI	24.4 ± 3.2	25.1 ± 3.1	23.7 ± 3.0	25.2 ± 3.6	0.36
Follicles on S8	3 (1–5)	0 (0–1)	1 (0–3)	5 (3–8)	0.001
Oocytes collected	8.5 (5.0–12.0)	0 (0–0)	2 (2–2)	23 (22–27.5)	<0.001
Live birth (%)	30.5	0	10.5	44	<0.001

Values are presented as median (inter-quartile range) or mean ± SD. *P*-value reflects analysis of variance or Kruskal–Wallis as appropriate. Follicles on S8, number of follicles ≥12 mm on stimulation day 8. ART, assisted reproduction treatment.

Results

Baseline patient information

Median age was 34.0 years (inter-quartile range 31.0–37.0 years), and the BMI was 24.5 ± 3.2 (mean ± SD). There was a 4 year history of infertility (inter-quartile range 3–4 years), with 142 cases (41.7%) having a male component requiring ICSI. Concentrations of FSH showed a non-Gaussian distribution with median value of 7.5 IU/l (inter-quartile range 5.9–10.0 IU/l) and a mean of 12.6 IU/l (SEM 0.28 IU/l). AMH showed a similar distribution with median 9.3 pmol/l (inter-quartile range 5.7–16.4 pmol/l) and a mean of 12.6 pmol/l (0.72 pmol/l). The overall live birth rate for the cohort was 27.4%.

Patient characteristics relative to ovarian response (Table 1) revealed the known relationship between age and response to exogenous gonadotrophins. Non-responders were older than women who had a normal ($P < 0.01$) or excessive response ($P < 0.01$) (Table 1). Women with an excessive response were characterized as being significantly younger ($P < 0.01$). This association between age and ovarian response was reflected on stimulation day 8 with the excessive response group having a higher number of follicles ≥12 mm ($P < 0.05$). Live birth rates reflected oocyte yields with the highest rates in those women with an excess response ($P < 0.001$).

AMH and FSH relative to live birth

Analysis of live birth rate relative to AMH and FSH quintiles (Fig. 1) demonstrated that although there was a significant relationship between increasing AMH and escalating live birth rate, above an AMH concentration of >7.8 pmol/l there was no discrimination in live birth rates. Similarly, for women with FSH <10.7 IU/l there was no significant differences in live birth rates. ROC analysis demonstrated that AMH [AUC 0.62, 95% confidence interval (CI) 0.55–0.68] was superior to FSH (AUC 0.42, 95% CI 0.35–0.49; $P < 0.001$) or age (AUC 0.48, 95% CI 0.41–0.55) in predicting live birth.

Assessment of the independence of AMH, FSH and age to predict live birth rate was undertaken by a stepwise regression model. This revealed that AMH was the only independent predictor [β positive, contribution to variance (CTV) 3.84%, $P < 0.001$]. Analysis of whether AMH predicted live birth independent of a correlation with oocyte yield was performed by inclusion of oocyte yield in a second model. In this model oocyte yield (β positive, CTV 5.8%, $P < 0.001$) was the only predictor of live birth.

AMH and FSH relative to category of ovarian response

The relationship between oocyte yield at oocyte retrieval and baseline parameters was examined (Table 2). Consistent with previous studies, age was positively correlated with FSH, and was negatively related to AMH and oocyte yield. Increasing FSH was associated with a reduction in AMH and oocyte yield. AMH demonstrated a remarkably strong correlation to oocyte yield and appeared to be the best predictor of oocyte yield ($r = 0.71$, $P < 0.0001$). Analysis of the independent predictive ability of age, BMI, log FSH and log AMH in a general

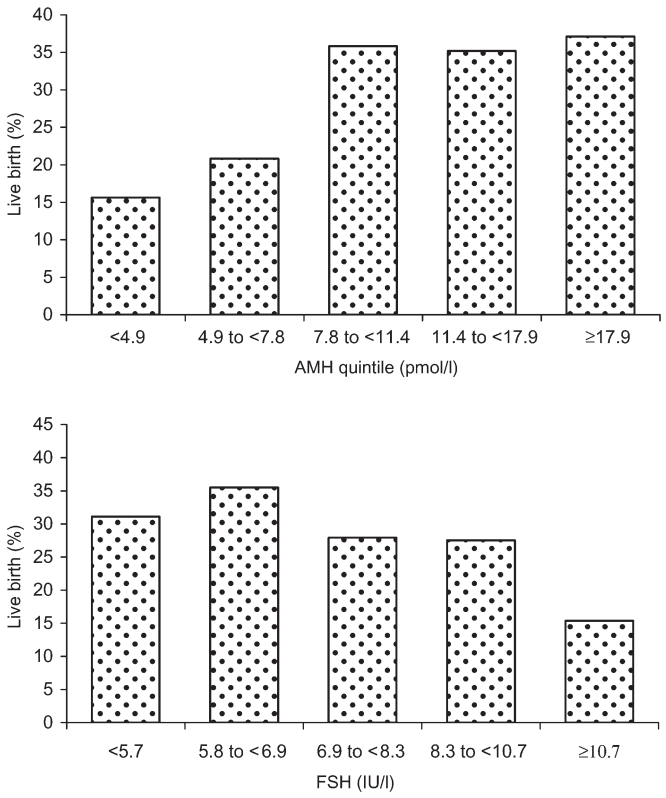

Figure 1: Live birth rate per AMH and FSH quintile Values are live birth rates for all embryos derived from a single cycle, including fresh and frozen embryo transfers

Table 2: Spearman correlation coefficients of phenotype and endocrine markers to oocyte yield

	Age	BMI	FSH	AMH	Oocytes
Age	–	−0.03	0.23	−0.38	−0.28
BMI	0.61	–	0.04	0.06	0.02
FSH	<0.0001	0.52	–	−0.47	−0.46
AMH	<0.0001	0.28	<0.0001	–	0.71
Oocytes	<0.0001	0.75	<0.0001	<0.0001	–

AMH, anti-Müllerian hormone.

linear model revealed that age (CTV 1.4%, $P = 0.006$) and AMH (CTV 7.3%, $P < 0.0001$) were the only independent predictors of oocyte yield, however, a significant negative interaction between age and AMH was present (CTV 3.6%, $P < 0.0001$).

The baseline concentrations of FSH and AMH were examined in the four categories of responders. FSH was significantly higher in non- ($P < 0.001$) and poor ($P < 0.001$) responders compared with normal responders (Fig. 2). Women with an excessive response had similar FSH levels to normal responders. AMH was significantly lower in non- ($P < 0.001$) and poor ($P < 0.001$) responders and was higher in women who had an excessive response ($P < 0.001$) (Fig. 2). AMH appeared to be a better discriminator of the four groups; however, there appeared to be significant overlay of low values of AMH in women who either had a poor, non- or normal response (Fig. 3). Consistent with the strong correlation between AMH and oocyte yield there was little overlay in women who had an excessive response (Fig. 3). A comparison of the frequency distributions of log AMH and log FSH demonstrated that each category of AMH response demonstrated greater discrimination compared with that of FSH (Fig. 3).

Formal assessment of the predictive ability of age, AMH and FSH to correctly identify women at risk of non-, poor or excessive responses was undertaken by calculating ROC curves and the AUC, 95% CI. In the 'non-responder' category, AMH concentrations were significantly lower than other categories, but AMH was a poorer predictor (AUC 0.073, 95% CI 0.02–0.12; $P < 0.001$) than FSH (AUC 0.782, 95% CI 0.68–0.88) or age (AUC 0.717, 95% CI 0.62–0.82), which were of equivalent efficacy ($P = 0.32$). In women who had an FSH >25 ($n = 5$) there was only one woman who responded and had ≤2 oocytes, and once FSH was ≥30 all women ($n = 4$) were non-responders. Similar cut-offs could not be derived for age, as even at 44 years old, the oldest age within our cohort, there were cases of normal responders.

In the poor responder category, AMH appeared to be inferior (AUC 0.227, 95% CI 0.14–0.31; $P < 0.001$) to FSH (AUC 0.762, 95% CI 0.64–0.88) or age (AUC 0.569, 95% CI 0.43–0.71), which again showed equivalent efficacy ($P = 0.06$).

AMH performed significantly better at identifying women at risk of an excessive response to controlled ovarian stimulation (AUC 0.90, 95% CI 0.83–0.96; $P < 0.001$) than FSH (AUC 0.321, 95% CI 0.23–0.40) or age (AUC 0.569, 95% CI 0.43–0.71), which were of equivalent efficacy ($P = 0.44$).

ROC performance can be driven by extreme values, as evident by inclusion of women in this prospective cohort with FSH >30. Therefore, analysis of oocyte yield relative to AMH and FSH quintiles (Fig. 4) demonstrated that although there was a relationship between increasing FSH and declining oocyte yield, this compared poorly to the predictive ability of AMH.

Further analysis of AMH with determination of pragmatic clinical cut-offs; <1.0, 1 to <5.0, 5.0 to <15, 15 to <25 and ≥25 pmol/l, demonstrated that in the 23 women who had an AMH of <1.0 pmol/l, 19 women did not receive HCG, 1 woman had a poor response and 3 women responded normally as per our criteria, although only 3 oocytes were retrieved in each case. When AMH was 1.0 to <5.0 pmol/l, 17 women out of 41 (41%) were either non- or poor responders, with a median of 4 oocyte (inter-quartile range 3.0–7.75) in normal responders. For identification of women at risk of an excessive response, an AMH ≥15 pmol/l had 88.0% sensitivity and 76.9% specificity, 77.8% women were identified correctly, positive likelihood ratio 3.8 and a negative likelihood ratio of 0.15. If AMH was ≥25 pmol/l this had a lower sensitivity of 60%, 94.9% specificity, 92.2% women were correctly identified, positive likelihood ratio 11.8 and a negative

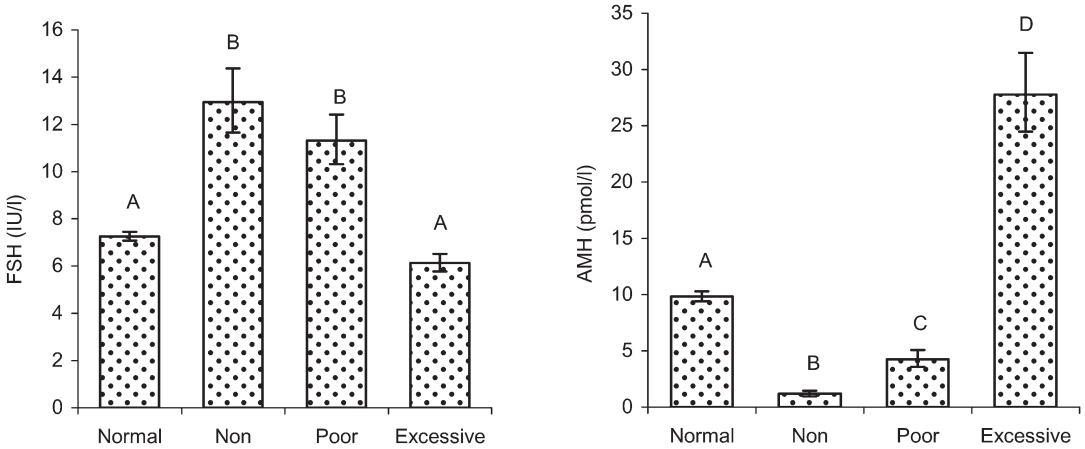

Figure 2: FSH and AMH concentrations relative to category of ovarian response
Values are geometric mean ± SEM of geometric mean. Groups with a letter in common do not differ significantly at $P < 0.01$

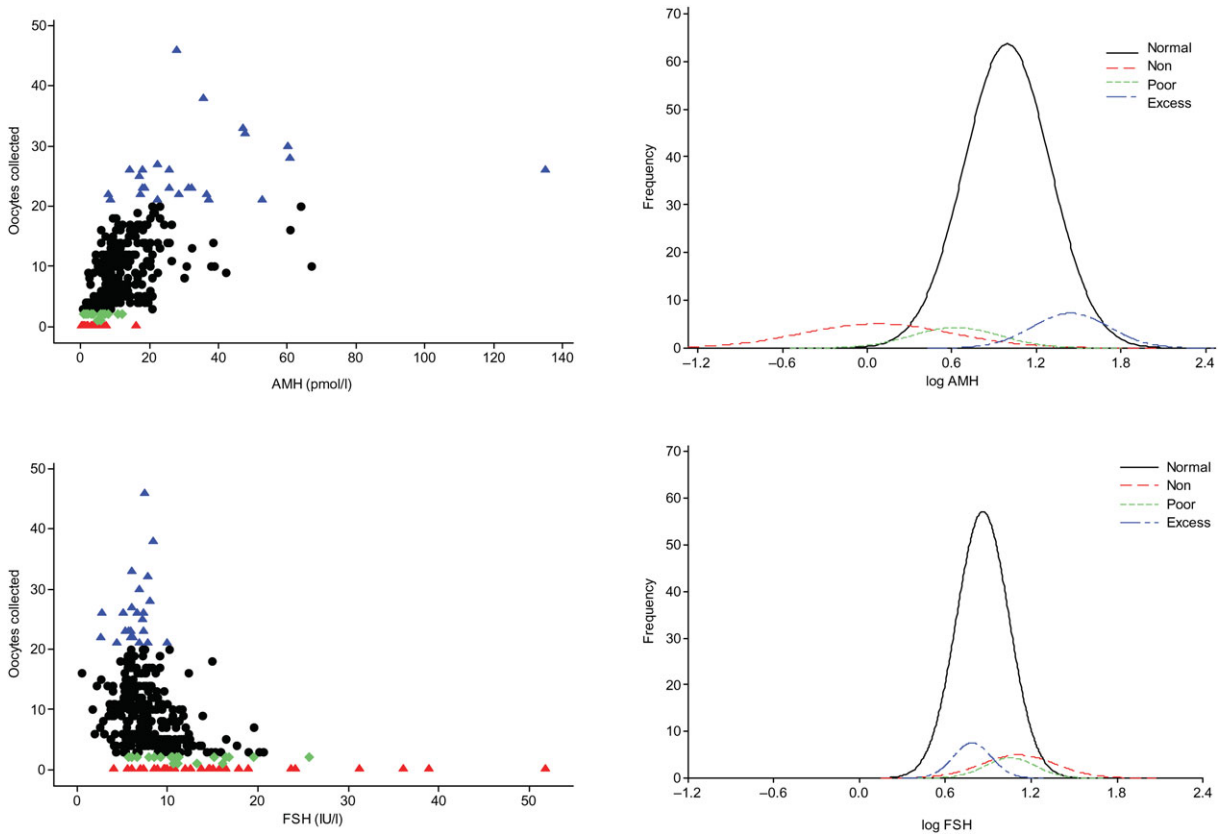

Figure 3: AMH and FSH related to oocyte yield and log distributions for each category of ovarian response
Key: red triangles, cycle cancelled; green diamonds, ≤2 oocytes retrieved; black circles, ≥3 but ≤20 oocytes and blue triangles, ≥21 oocytes.

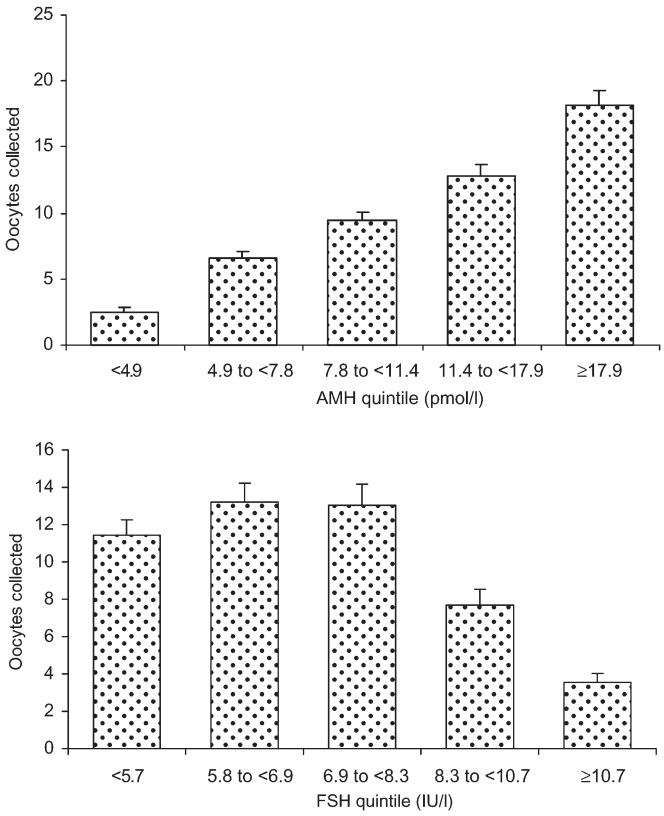

Figure 4: Mean oocyte yield per AMH and FSH quintile
Values are mean ± SEM

likelihood ratio of 0.42. Using this upper limit of 25 pmol/l AMH, even in women who were not classed as having an excessive response by our definition, a median of 13 oocytes (inter-quartile range 10–14) were collected.

Discussion

In this study, we demonstrate that plasma AMH is strongly correlated to oocyte yield and is an accurate predictor of live birth and risk of an excessive response to controlled ovarian stimulation. This, the largest cohort to date of an unselected population of patients undergoing their first treatment cycle, extends previous observations regarding pregnancy rates (Eldar-Geva *et al.*, 2005) to actual live births and supports previous observations that AMH is a reliable marker of the number of follicles attaining FSH sensitivity (Seifer *et al.*, 2002; van Rooij *et al.*, 2002; Laven *et al.*, 2004). AMH is thereby a strong predictor of the number of viable antral follicles and oocyte yield in ART cycles stimulated with FSH in controlled ovarian stimulation.

The relationship between increasing AMH and live birth probably reflects the strong correlation between AMH and oocyte yield, as AMH was not an independent predictor of live birth after incorporation of oocyte yield into our models. High AMH has been associated with improved pregnancy rates (Eldar-Geva *et al.*, 2005), consistent with a correlation with numbers of mature oocytes and subsequently embryos (Hazout *et al.*, 2004) available for transfer. The observation

that AMH does not predict live birth independent of oocyte yield indicates that AMH does not predict oocyte or embryo quality (Smeenk *et al.*, 2007). Increasing FSH (Frazier *et al.*, 2004; Abdalla *et al.*, 2006) and age (Frazier *et al.*, 2004) have previously been shown to be negative predictors of live birth, however, our ROC analysis suggests that these are inferior predictors relative to AMH, reflecting the relative correlations to oocyte yield—the principal determinant of live birth.

The ability of AMH to identify women at risk of an excessive response may reflect the relative contribution from the different follicle classes to final plasma levels and their potential recruitment. Ovarian AMH expression is minimal in primordial follicles but increases in association with follicular development and expression is maximal in pre-antral follicles (Weenen *et al.*, 2004). In conditions where pre-antral follicle numbers are increased, such as polycystic ovary syndrome (PCOS) (Hughesdon, 1982), AMH plasma levels are elevated (Laven *et al.*, 2004; Fleming *et al.*, 2005; Piltonen *et al.*, 2005). Consistent with our described relationship between increased AMH and excessive response to controlled ovarian stimulation, women with PCOS are at substantial risk of excess ovarian responses and development of ovarian hyperstimulation syndrome (OHSS) (Delvigne *et al.*, 2002). Improved discrimination of women at risk of OHSS should be possible using AMH prior to controlled ovarian stimulation.

Although there is a clear association between AMH and oocyte yield, we demonstrate that AMH performs relatively poorly as a screening test for either potential cycle cancellation or poor response to controlled ovarian stimulation according to the ROC analyses. This is due to the overlap in distribution of plasma levels of AMH in women who have a potentially normal response. In women with premature ovarian failure, AMH levels are low compared with normal (Meduri *et al.*, 2007), however, plasma AMH levels are still within the detectable range in 40% of such women and within the normal range in a few patients, despite minimal to nil follicles on ovarian biopsy (Meduri *et al.*, 2007). This overlap hinders AMH as an absolute predictor of non-responder status to controlled ovarian stimulation and therefore it is not feasible to suggest that a woman should not undergo controlled ovarian stimulation based on a low plasma AMH value. This contrasts with extremely elevated FSH, where we clearly demonstrate that the risk of cancellation is high and even when undergoing oocyte retrieval, the number of oocytes obtained is very low. Consequently, adjustment of a patients expectations is required and consideration given to individualization of a therapeutic strategy.

Ovarian reserve is currently defined as the number and quality of the follicles left in the ovary at any given time, and the values of tests for 'ovarian reserve' (meaning the number of recruitable follicles) prior to ovarian stimulation, particularly if it is maximal has recently been questioned (Broekmans *et al.*, 2006). Most of the follicles in the ovary will be primordial, and although AMH is not thought to be expressed in human primordial follicles (Weenen *et al.*, 2004), there is a strong correlation between AMH and primordial follicle number in mice (Kevenaar *et al.*, 2006), consistent with a role for AMH as a measure of ovarian reserve. However, this role is dependent upon a close relationship between the stockpile of primordial follicles and the number of developing follicles. In fact, not all primordial follicles will successfully make the transition to primary follicles, and age has profound effects on rates of follicular growth and atresia dynamics (Faddy, 2000). Consequently it is not until this transition has occurred and a potentially FSH recruitable primary follicle has developed that AMH starts to be expressed in significant amounts, thereby potentially explaining why age is a poorer correlate than AMH of the number of oocytes obtained after controlled ovarian stimulation. The observed independence of age and AMH in predicting oocyte response may reflect their individual contributions to the process of follicular growth dynamics, with age influencing the proportion of follicles making the transition from primordial to recruitable follicles. AMH in turn may reflect the number of follicles, which successfully made the transition to a FSH sensitive phenotype. The negative interaction between AMH and age in response to controlled ovarian stimulation, suggests that older women with a low AMH will yield fewer oocytes than a younger woman with the same AMH. This interaction may reflect age dependent increases in apoptotic changes in granulosa cells, which occur independent of oocyte yields (Sadraie *et al.*, 2000). Furthermore, the degree of granulosa cell apoptotic damage is reflected in oocyte quality (Nakahara *et al.*, 1997a,b; Oosterhuis *et al.*, 1998). The reduction in AMH with age may therefore reflect a reduction in number of functional granulosa cells per follicle, with this relationship also underlying the association between AMH and oocyte quality (Ebner *et al.*, 2006).

The clear relationship between plasma AMH and oocyte yield may allow optimization of treatment strategies prior to the first cycle of ovarian stimulation. In women with a low AMH (e.g. <1.0 pmol/l) either cycle cancellation (~80%) or ≤ 2 oocytes are anticipated. For this group of women, management is difficult and perhaps the shorter regimens may result in less stress to the patient than a long course agonist cycle with 3–4 weeks intensive treatment to yield few oocytes. A variety of options have been explored as alternative concepts with at best limited success (Tarlatzis *et al.*, 2003; Garcia-Velasco *et al.*, 2005; Balasch *et al.*, 2006; Massin *et al.*, 2006). An alternative strategy for this group could be natural cycle IVF, although at present results are inconsistent (Bassil *et al.*, 1999; Feldman *et al.*, 2001; Pelinck *et al.*, 2002; Tarlatzis *et al.*, 2003; Kolibianakis *et al.*, 2004; Ziebe *et al.*, 2004; Check, 2005; Elizur *et al.*, 2005; Trokoudes *et al.*, 2005). Furthermore, patients would need to accept that a protracted treatment programme would be required and that not every cycle would result in embryo transfer. However, women who are forewarned of the likelihood of these events can begin to formulate coping strategies prior to treatment. For women with an AMH of 1 to <5 pmol/l, they are still at risk of either cycle cancellation or a poor response and therefore a protracted long course agonist cycle is likely to achieve no benefit over an antagonist regimen, which may be more appropriate. Whether a maximal gonadotrophin dose is beneficial remains to be determined, but with a category based upon AMH concentration these concepts can be explored prospectively and objectively. Although there is no evidence that antagonist regimens

are better than agonist regimens in poor responders (Akman *et al.*, 2000,2001; Copperman, 2003; Malmusi *et al.*, 2005; Marci *et al.*, 2005) and in fact may result in, on average, one fewer oocyte, they have the substantial benefit of enabling early cycle cancellation if an inadequate response is obtained. Women with an AMH of 5 to <15 pmol/l have a high probability of a normal response to a standard long course agonist regime, and we suggest that there is little current evidence to alter that strategy. Again with this categorization, the appropriate FSH dose can be explored—perhaps depending upon age. Women with an AMH of 15 to <25 pmol/l show an excessive response and we would recommend consideration of a protocol mindful of the increased risk of OHSS. This could employ a GnRH agonist regimen with a reduced dose of 150 IU FSH, depending on BMI, or an antagonist regime where the risk of OHSS may be reduced (Al-Inany *et al.*, 2006). Women with an AMH ≥ 25 pmol/l show an excessive response to exogenous gonadotrophins and are at substantial risk of developing OHSS. This cohort of women should be treated with a GnRH antagonist regimen (again dose explored with respect to BMI) which has been shown to reduce the risk of OHSS (Al-Inany *et al.*, 2006).

In summary, this study demonstrates that basal plasma AMH can predict live birth rates in ART cycles stimulated with long course controlled ovarian stimulation, through its relationship with oocyte yield. AMH and age are independently related to oocyte yield and also have a significant negative interaction reflecting their relative contributions to follicular and oocyte ontogeny. We propose that clinicians can prospectively examine the concept of individualized and optimized treatment strategies based on AMH prior to the first treatment cycle. This approach will also allow adjustment of patient expectations accordingly and potentially minimize psychological and physical morbidity due to the extremes of response observed with controlled ovarian stimulation.

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