Reproductive BioMedicine Online (2013) xxx, xxx–xxx

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ARTICLE

Anti-Müllerian hormone versus antral follicle count for defining the starting dose of FSH

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Abstract This pilot study compared the efficacy and safety of two simple dosing algorithms, one based on anti-Müllerian Hormone (AMH) and the other on the antral follicle count (AFC), to determine the starting dose of recombinant FSH (rFSH) for ovarian stimulation in 348 women. Patients were randomized to a predefined AMH- or AFC-based algorithm. The proportion of cycles with the desired response was similar when rFSH dose was determined using AMH or AFC (35.2% versus 28.4%). There was a significant difference between the groups in the proportion of cycles with a hyperresponse (8.6% and 17.4%, but the incidence of ovarian hyperstimulation syndrome was similar (1.1% and 4.6%). There were no significant differences between two groups in outcomes, including implantation (19.3% versus 19.0%), clinical pregnancy (38.0% versus 46.9%), multiple pregnancy (16.5% versus 15.2%) and miscarriage (7.0% versus 8.3%). However, statistically significant differences in ovarian response were evident among the AMH and AFC subgroups: for AMH, Desired and Hypo; for AFC, Hypo and Hyper. This pilot study provides information for developing protocols to further validate the use of either AMH or AFC to guide the starting dose of rFSH in ovarian stimulation.

KEYWORDS: anti-Müllerian hormone, antral follicle count, assisted reproduction technology, FSH, ovarian stimulation, randomized study

Introduction

It is well established that successful IVF and embryo transfer requires both stimulation of the ovary and suppression of the pituitary. Thus, exogenous gonadotrophins and gonadotrophin-releasing hormone (GnRH) analogues are considered the hormones required to maximize IVF success (Barbieri and Hornstein, 1999). In fact, according to a recent IVF survey including as many as 151,000 cycles/year performed in 273 centres worldwide, the use of GnRH-

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.07.008

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agonists was confirmed in 134,494 (89.1%) cycles (Tur-Kaspa and Fauser, 2010).

The daily dose of gonadotrophin administered in assisted reproduction technology may be fixed but usually it is progressively increased or tapered according to the given patient's response. A key issue in the management of cycles is defining the optimal starting dose of FSH for each patient in order to obtain the optimization of response and outcomes whilst minimizing the risks. This is because it has been shown that for successful induction of multiple folliculogenesis in normally ovulating women there is a critical period during the early follicular phase of the cycle when FSH values should remain above the physiological concentration to stimulate follicle recruitment maximally in the primary cohort (Lolis et al., 1995; Messinis and Templeton, 1990). On the other hand, follicles recruited by exogenous FSH require an FSH threshold concentration that is higher than that in the natural cycle (Lolis et al., 1995) and marked interindividual variation exists in FSH thresholds, as well as in FSH metabolic clearance and ovarian sensitivity to FSH (Ben-Rafael et al., 1995; Porchet et al., 1994; van Santbrink et al., 1995).

The absolute number and functional capacity of follicles and germ cells comprise what is termed ovarian reserve or ovarian age, which affects a given patient's response to stimulation with gonadotrophins and her chance for success. The most important aspect of ovarian reserve is that it declines with age but it is a biological and not just a chronological function (Scott and Hofmann, 1995). Therefore, a major challenge is the assessment of ovarian reserve for prediction of oocyte retrieval.

Serum anti-Müllerian hormone (AMH) and antral follicle count (AFC) both seem to be the most reliable predictors of ovarian ageing and they are equivalent in terms of their accuracy in predicting ovarian response but none of the currently employed tests of ovarian reserve can reliably predict pregnancy success (Broer et al., 2009, 2010, 2013; Domingues et al., 2010; La Marca et al., 2010, 2012; Sills et al., 2009; Younis, 2011). Recently, however, interest has been focused on the evaluation of ovarian reserve in order to personalize the treatment protocol with the aim to predict both a poor response (diminished chance of conception) and a hyperresponse (increased risk of ovarian hyperstimulation syndrome; OHSS; Broer et al., 2011; La Marca et al., 2012; Nardo et al., 2011).

Therefore, this study was aimed to develop a simple, effective and clinically useful approach to individualize the starting dose of recombinant FSH (rFSH) in an assisted reproduction cycle. Thus, the efficacy and safety of two simple dosing algorithms, one based on AMH and the other based on the AFC, to guide the starting dose of rFSH for ovarian stimulation in women undergoing assisted reproduction treatment were compared.

Materials and methods

This randomized, parallel, open-label study was conducted from 1 October 2011 to 31 August 2012 (NCT01783301). All patients had baseline FSH, AFC and AMH concentrations determined within 3 months of enrolment in the study. The end-of-study period was defined as a negative pregnancy test according to routine clinical practice or clinical pregnancy confirmed by ultrasound scan performed 6–7 weeks after embryo transfer.

Study population

All patients undergoing routine assisted cycles during the trial period were invited to participate. To be eligible for enrolment, subjects had to be starting treatment with rFSH according to the decision of the investigator and in accordance with the indication and the recommendations of the summary of product's characteristics, aged <40 years at the time of rFSH dosing, have a body mass index <28 kg/m^2), have early follicular phase (day 2–4) basal FSH serum concentrations ≤12 IU/l, receiving a long GnRH-agonist protocol (starting on day 21 of the preceding cycle until day of human chorionic gonadotrophin, HCG) and willing and able to comply with the protocol requirements for the duration of the study.

Patients were excluded from the study if they were already participating in another interventional clinical trial or had concomitant use of either LH or human menopausal gonadotrophin/urinary FSH preparations in the study cycle. The latter was done because considerable debate exists in the literature as to whether the administration of exogenous LH activity could make a difference with regard to the outcome of assisted cycles in GnRH-agonist down-regulated women (Hill et al., 2012).

The Institutional Review Board and Ethics Committee approved the study protocol on 22 September 2011 (IRB reference number: 01/QD-CGRH-NCKH and DT TP.HCM). All patients provided written informed consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

IVF protocol and rFSH treatment

As per the inclusion criterion, all subjects received a long GnRH agonist protocol (starting on day 21 of the preceding cycle until the day of HCG administration) which, according to recent studies on clinical significance of ovarian reserve testing, should still be considered as the standard protocol for ovarian stimulation in patients with normal ovarian reserve and may be used for pituitary suppression in association with increased doses of gonadotrophins in patients with reduced ovarian reserve (La Marca et al., 2012). Down-regulation was monitored according to routine clinical practice. Once down-regulation was achieved (usually after at least 14 days of GnRH agonist and confirmed by serum oestradiol <60 pmol/l), patients were randomized by means of sealed envelopes generated by a computer randomization list to either the AMH or AFC arm for determination of the rFSH starting dose (Gonal-F; Merck Serono, Germany), according to a predefined algorithm based upon a consensus between clinical investigators. As AMH and AFC have the same level of accuracy and clinical value for the prediction of poor response and both markers are highly correlated, using both markers in a prediction model does not improve its performance (Broekmans et al., 2006; Broer et al., 2009; Jayaprakasan et al., 2010). Thus, a third study group including the use of both AFC and AMH was not considered in the current investigation.

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Minimum and maximum starting doses of rFSH were 150 and 375 IU/day, respectively. In the AMH arm, starting dosing of rFSH was 375, 225 or 150 IU/day in patients having basal serum AMH concentrations of <0.7, 0.7–2.1 or >2.1 ng/ml, respectively. In the AFC arm, patients having basal AFC <6, 6–15 or >15 received 375, 225 or 150 IU/day rFSH, respectively. The starting dose of rFSH was given for 5 days, after that the investigator could titrate the dose based on their clinical judgment. Follicular development was monitored using ultrasound and oestradiol concentration but also LH (because a recent report nicely showed that LH is directly related to ovarian response, and preovulatory progesterone concentration is significantly associated with LH concentration but unrelated to pregnancy rates in assisted reproduction cycles; Yding Andersen et al., 2011), starting on day 5 of stimulation, then every 2–3 days depending on the size of the follicles. The criteria for rHCG (Ovitrelle 250 μg; Merck Serono) administration was at least two lead follicles of 17 mm. Ovum retrieval was performed 36 h after HCG administration. Insemination was performed by using intracytoplasmic sperm injection. Embryo transfer was performed 2 days after ovum retrieval. This day was chosen because previous studies have reported that the use of blastocysts in assisted reproduction is not more effective than the use of day-2 or day-3 embryos and resulted in a decreased number of cryopreserved embryos, thus influencing the overall efficacy of treatment (Bennett, 2001; Kolibianakis et al., 2004; Pantos et al., 2004).

The main outcome measure was established on the basis of this study clinic's previous experience where patients had the likelihood of having at least one embryo cryopreserved. Up to four embryos per patient (depending on the age of the patient, the indication for IVF, the number of cycle attempts, the number and quality of embryos available per transfer and the couple's decision) were transferred and luteal-phase support was provided using progesterone gel (Crinone 8% 90 mg, twice a day; Merck Serono). This is in keeping with Vietnam's and also other countries' current legal issues and recent guidelines stressing that 'individual programs are encouraged to generate and use their own data regarding patient characteristics and the number of embryos to be transferred' (Practice Committee of American Society for Reproductive Medicine; Practice Committee of Society for Assisted Reproductive Technology, 2013).

Outcome measures

It has been previously reported that AMH and AFC have highly significant correlations with the number of oocytes obtained and are comparable predictors in this respect (Ben-Haroush et al., 2012; Majumder et al., 2010). Therefore, in this study the primary endpoint was the proportion of patients with an appropriate ovarian response, defined as between eight and 12 oocytes retrieved. This range was based on clinical data from 2010 during which there were 2818 ovarian retrievals. The lower figure of eight oocytes is based on the observation that this was associated with a high likelihood of having at least one embryo for cryopreservation; if the number of oocytes was less than 8, the percentage of patients who had at least one embryo frozen was only 5.1%, whereas if the number of oocytes was eight or more, the percentage of patients with at least one embryo frozen was 50.8% (odds ratio 9.1, 95% confidence interval 3.7–16.5).

The secondary endpoints were: proportion of patients with hyporesponse (≤3 oocytes); proportion of patients with hyperresponse (>20 oocytes); number of MII oocytes; number of oocytes retrieved; number of fertilized 2PN oocytes; number of mature follicles ≥14 mm on day of HCG administration; LH and oestradiol concentration on day of HCG; rFSH dose (daily dose, treatment duration, total dose); clinical pregnancy rate; multiple pregnancy rate; implantation rate (sacs with heartbeat per total number of embryos transferred); and cycle cancellation prior to HCG due to poor response or hyperresponse.

Safety

The rates of moderate and severe OHSS were recorded. Moderate OHSS was defined as moderate abdominal pain, nausea ± vomiting, ultrasound evidence of ascites and ovarian size usually 8–12 cm. Severe OHSS was defined as clinical ascites (occasionally hydrothorax), oliguria, haemoconcentration (haematocrit > 45%), hypoproteinaemia and ovarian size usually >12 cm.

Statistical analysis

Based on the assumption of at least a 16% difference in the proportion of subjects with 8–12 oocytes between the two treatment groups, with 90% power and a 2-sided *P*-value of 0.05, the number of subjects required was 120 per group (total 240). The recruitment target was 140 subjects per group (total 280) to allow for dropouts. The aim was to have 40/60/40 patients in the upper/middle/lower ranges of AMH or AFC concentrations as defined in the dosing algorithm. However, after 280 patients had been randomized, the target number in the low AMH and the high AFC subgroups had not been reached. Therefore, recruitment initially continued with the aim of achieving the required number of patients in each subgroup. However, subsequently a clinical decision was taken to stop recruitment in the low AMH subgroup prior to reaching the target number of patients. This was because AMH concentration <0.7 ng/ml was an infrequent occurrence and most patients with concentrations in that range elected to have egg donation rather than ovarian stimulation.

The number of patients within the predetermined range of retrieved oocytes in each of the two groups was compared using the chi-squared test. Other assessments include normal clinical parameters for an IVF cycle. Receiver operating characteristic (ROC) analysis was applied to analyse the predictive value of AMH and AFC for predicting the total dose of rFSH and the number of oocytes retrieved. The mean of number oocytes obtained was compared across the three strata of AMH and AFC using ANOVA test to show differences in the number of oocytes and to test sensitivity and specificity of the different cut-offs to predict ovarian response. In addition, the predictive value of a combined AMH/AFC parameter (compared with AMH or AFC alone) for number of oocytes retrieved was determined. Data were analysed for both intention-to-treat (ITT) and per-protocol (PP) populations. Patients who had major deviations (e.g.

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in dose of rFSH) who proceeded to oocyte retrieval were included as part of the ITT population.

Results

A total of 348 subjects undergoing assisted reproduction technology were recruited into this study. The patient characteristics and demographic details are summarized in Table 1. Patients in the two groups were largely comparable at baseline with the exception of significantly lower AMH concentrations and AFC in patients whose rFSH dosage was guided by AMH (all $P \leq 0.01$). There were 10 cycles in both groups that had not reached oocyte retrieval stage. The reasons were inadequate follicular development in five cycles, risk of ovarian hyperstimulation in two cycles (both couples were offered cryopreservation of all embryos but they did not accept) and personal reasons in three cycles.

Ovarian response

The proportion of cycles with the desired response to ovarian stimulation was similar when rFSH dosing was guided using AMH or AFC (Table 1). There were significant differences between the two dosing algorithm groups with respect to the proportion of cycles with a hyperresponse ($P = 0.02$).

Table 1 Baseline patient characteristics, gonadotrophin treatment, ovarian response, ovum retrieval and outcome in the AMH and AFC arms.

Parameter	*AMH*	*AFC*	*P-value*
Patients	*n* = 174	*n* = 174	
Age (years)	32.3 ± 4.0	33.1 ± 4.1	NS
Body mass index (kg/m^2)	20.8 ± 2.1	20.8 ± 1.9	NS
FSH (IU/l)	5.7 ± 2.5	5.8 ± 2.4	NS
Free testosterone index	0.5 ± 0.4	0.5 ± 0.3	NS
AMH (ng/ml)	2.6 ± 1.7	3.1 ± 1.9[a]	≤0.01
AFC	8.9 ± 4.8	11.2 ± 6.4[a]	≤0.01
Ovarian stimulation	*n* = 169	*n* = 169	
Response (*n* = 345)[b]	*n* = 173	(*n* = 172	
Desired	61 (35.3)	49 (28.5)	NS
Hypo	17 (9.8)	8 (4.7)	NS
Hyper	15 (8.7)	30 (17.4)	0.02
Cycles cancelled (*n* = 348)	*n* = 174	*n* = 174	
Due to hyporesponse	3 (1.7)	2 (1.1)	NS
Due to hyperresponse	1 (0.6)	1 (0.6)	NS
Duration of stimulation (days)	11.8 ± 1.6	11.6 ± 1.3	NS
FSH dose			
Total (IU)	2694 ± 1053	2872 ± 1188	NS
Daily (IU/day)	224 ± 71	243 ± 84	0.03
No. of follicles ≥14 mm on HCG day	8.7 ± 4.3	10.9 ± 5.1	<0.01
Oestradiol on HCG day (pmol/l)	4726 ± 4142	7769 ± 5674	<0.01
LH on HCG day (IU/l)	1.1 ± 0.3	1.2 ± 0.3	NS
Oocytes retrieved	10.8 ± 6.3	13.6 ± 7.3	<0.01
MII	8.9 ± 5.5	11.1 ± 6.4	<0.01
Embryos	6.3 ± 4.1	8.1 ± 4.7	<0.01
Embryos transferred	3.1 ± 0.9	3.0 ± 0.8	NS
Frozen embryos	1.7 ± 2.5	2.7 ± 3.3	<0.01
Outcome per embryo transfer	*n* = 158	*n* = 145	
Beta-HCG positive	72 (45.6)	80 (55.2)	NS
Clinical pregnancy rate	60 (38.0)	68 (46.9)	NS
Multiple pregnancy rate	26 (16.5)	22 (15.2)	NS
Miscarriage rate	11 (7.0)	12 (8.3)	NS
Implantation rate	101/523 (19.3)	97/510 (19.0)	NS

Values are mean ± standard deviation, *n* (%) or *n*/total (%).
[b]Three cycles were cancelled for personal reasons.AFC = antral follicle count; AMH = anti-Müllerian hormone; HCG = human chorionic gonadotrophin; NS = not significant.

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Data on ovarian stimulation for the different dosing algorithm groups are reported in Table 1. Overall, duration of stimulation was similar in the AMH and AFC arms but ovarian response in terms of follicle development and oocyte and embryo yield was significantly higher in the AFC arm (all $P < 0.01$). The mean number of embryos transferred was 3.1 in the AMH group and 3.0 in the AFC group. There were no significant differences between the groups in outcomes per embryo transfer, including implantation, clinical pregnancy, multiple pregnancy and miscarriage (Table 1).

In the AMH algorithm group, there were significant decreases in the total and daily dose of rFSH, and significant increases in the number of follicles ≥14 mm per HCG day, oocytes, MII oocytes and embryos, as the AMH concentration increased (all $P < 0.01$) (Table 2). The proportion of cycles with a desired response was 5.9% in the AMH <0.7 ng/ml group, 38.7% in the 0.7–2.1 ng/ml group and 38.3% in the >2.1 ng/ml group ($P = 0.02$). The proportion of cycles with a poor response was significantly higher in the lowest AMH group (Table 2) ($P < 0.01$). Although there were no statistically significant differences across the subgroups in pregnancy, multiple pregnancy, miscarriage or implantation rates, these showed increasing numerical trends across increasing AMH concentration groups, particularly the clinical pregnancy rate (Table 2).

In the AFC algorithm group, there were significant decreases in the total and daily dose of rFSH and significant increases in the number of follicles ≥14 mm per HCG day, oocytes, MII oocytes and embryos, as the AFC concentration increased (all $P < 0.01$) (Table 3). In contrast with AMH, the proportion of cycles with a desired response did not differ significantly between the three AFC subgroups (28.9%, 35.5% and 17.6% in the <6, 6–15 and >15 groups, respectively). Significantly more women in the <6 group had a poor ovarian response ($P < 0.01$) and significantly more in the >15 group had a hyperresponse ($P < 0.01$)(Table 3). There were no statistically significant differences across the AFC subgroups in clinical pregnancy, multiple pregnancy, miscarriage or implantation rates (Table 3).

Usefulness of the different algorithms

Area under the curve (AUC) values and 95% confidence intervals (CI) for AMH, AFC and the AMH/AFC ratio for predicting hyporesponse to ovarian stimulation (≤3 oocytes retrieved) were 0.88 (0.81–0.95), 0.80 (0.73–0.89) and 0.71 (0.61–0.8), respectively (all $P < 0.0001$). The cut-off values to predict hyporesponse are shown in Figures 1A and 2A.

For the prediction of hyperresponse (>20 oocytes retrieved), AUC values and 95% CI were statistically

Table 2 Main characteristics and outcomes in the three strata of the AMH algorithm group.

Parameter	*AMH concentration (ng/ml)*			*P-value*
	<0.7	*0.7–2.1*	*>2.1*	
Characteristics ($n = 174$)	$n = 17$	$n = 62$	$n = 95$	
AMH (ng/ml)	0.4 ± 0.1	1.4 ± 0.4	3.8 ± 1.3	<0.01
AFC	3.4 ± 2	7.0 ± 2.8	11.1 ± 4.8	<0.01
Ovarian response ($n = 173$)[a]	$n = 17$	$n = 62$	$n = 94$	
Desired	1 (5.9)	24 (38.7)	36 (38.3)	0.02
Hypo	12 (70.6)	6 (9.7)	2 (2.1)	<0.01
Hyper	0 (0)	3 (4.8)	13 (13.8)	NS
Ovarian stimulation ($n = 169$)	$n = 14$	$n = 62$	$n = 93$	
FSH dose				
Total (IU)	4607 ± 696	3147 ± 826	2104 ± 678	<0.01
Daily (IU/day)	375 ± 0	261 ± 42	177 ± 37	<0.01
Follicles ≥14 mm on HCG day	3.3 ± 1.7	7.5 ± 3.3	10.3 ± 4.3	<0.01
Oocytes retrieved	3.5 ± 2.3	8.9 ± 5.3	13.1 ± 6.2	<0.01
MII	2.8 ± 2.3	7.3 ± 4.7	10.9 ± 5.5	<0.01
Embryos	1.9 ± 1.4	5.2 ± 3.5	7.8 ± 4.1	<0.01
Outcome per embryo transfer ($n = 158$)	$n = 14$	$n = 60$	$n = 84$	
Clinical pregnancy rate	2 (14.3)	20 (33.3)	39 (46.4)	NS
Multiple pregnancy rate	1 (7.1)	9 (15.0)	16 (19.0)	NS
Miscarriage rate	1 (7.1)	4 (6.7)	6 (7.1)	NS
Implantation rate	4/23 (17.4)	33/198 (16.7)	64/302 (21.2)	NS

Values are mean ± standard deviation, *n* (%) or *n*/total (%).
AFC = antral follicle count; AMH = anti-Müllerian hormone; HCG = human chorionic gonadotrophin; NS = not significant.
[a]One cycle was cancelled for personal reasons.

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Table 3 Main characteristics and outcomes in the three strata of the AFC algorithm group.

Parameter	*AFC*			*P-value*
	<6	*6–15*	*>15*	
Characteristics (*n* = 174)	*n* = 46	*n* = 77	*n* = 51	
AMH (ng/ml)	1.9 ± 1.8	3.2 ± 1.9	4.1 ± 1.6	<0.01
AFC	4.0 ± 1.0	10.3 ± 2.8	19.3 ± 3.9	<0.01
Ovarian response (*n* = 172)[a]	*n* = 45	*n* = 76	*n* = 51	
Desired	13 (28.9)	27 (35.5)	9 (17.6)	NS
Hypo	8 (17.8)	2 (2.6)	0 (0)	<0.01
Hyper	2 (4.4)	10 (13.2)	19 (37.3)	<0.01
Ovarian stimulation (*n* = 169)	*n* = 44	*n* = 75	*n* = 50	
FSH dose				
Total (IU)	4479 ± 829	2717 ± 528	1690 ± 186	<0.01
Daily (IU/day)	360 ± 44	236 ± 29	149 ± 6	<0.01
Follicles ≥14 mm on HCG day	7.2 ± 4.1	10.9 ± 4.4	14.1 ± 5.1	<0.01
Oocytes retrieved	8.2 ± 5.5	13.2 ± 5.7	19.1 ± 7.3	<0.01
MII	6.8 ± 5.1	10.6 ± 5	15.7 ± 6.3	<0.01
Embryos	4.7 ± 3.3	8.0 ± 4.2	11.2 ± 4.7	<0.01
Outcome per embryo transfer (*n* = 145)	*n* = 42	*n* = 68	*n* = 35	
Clinical pregnancy rate	17 (40.5)	34 (50.0)	17 (48.6)	NS
Multiple pregnancy rate	6 (14.3)	10 (14.7)	6 (17.1)	NS
Miscarriage rate	1 (2.4)	5 (7.4)	6 (17.1)	NS
Implantation rate	25/130 (19.2)	47/250 (18.8)	25/130 (19.2)	NS

Values are mean ± standard deviation, *n* (%) or *n*/total (%).
AFC = antral follicle count; AMH = anti-Müllerian hormone; HCG = human chorionic gonadotrophin; NS = not significant.
[a]Two cycles were cancelled for personal reasons.

significant ($P < 0.0001$) for AMH (0.76, 0.69–0.83) and AFC (0.81, 0.74–0.88), but not for AMH/AFC (0.47, 0.39–0.857). The associated cut-off values are shown in **Figures 1**B and **2**B.

Safety

The incidence of OHSS was not significantly different between the two study groups: 2/174 (1.1%) in the AMH group versus 8/174 (4.6%) in the AFC group. In the AMH group, there was one event each of mild and moderate intensity. In the AFC group, there were two mild cases and six moderate cases.

Discussion

This study has shown that algorithms based on AMH and AFC have similar effectiveness for guiding the starting dose of rFSH in ovarian stimulation, with no significant difference between groups in proportion of cycles with the desired response (8–12 oocytes) (35.2% and 28.4%, respectively). This is in keeping with a meta-analysis on the subject (Broer et al., 2009). In the current study, the overall proportion of cycles with a desired response was about 30%. The study was quite strict in the definition based on the clinic's experience of trying to achieve a relatively high percentage of cycles with embryos cryopreserved without increasing the risk of OHSS. However, it is widely accepted that it is difficult to predict ovarian response, making it challenging to prospectively define the optimal response range (Broer et al., 2009; Popovic-Todorovic et al., 2003), particularly across different patient populations such as the Asian patients included in this trial.

The proportion of cycles with hyperresponse was significantly higher in the AFC algorithm group compared with AMH (17.4% versus 8.6%; $P = 0.02$). This may be the result of baseline differences between the two algorithm groups, despite the random allocation to treatment. In particular, mean AMH concentration and AFC were significantly higher in the AFC versus AMH group. The differences in AMH and AFC between the two dosing algorithm groups at baseline are also the likely explanation for the greater number of follicles ≥14 mm, oocytes retrieved, MII, embryos and frozen embryos in the AFC compared with AMH group. Algorithm-driven adjustments in the rFSH dose did not appear to be sufficient to negate the effects of significantly different AMH and AFC at baseline. Furthermore, physician-initiated rFSH dose adjustments as part of standard clinical practice did not appear to have any impact on ovarian response.

Another factor that could have affected the findings of this study is that the cut-off values chosen as part of the

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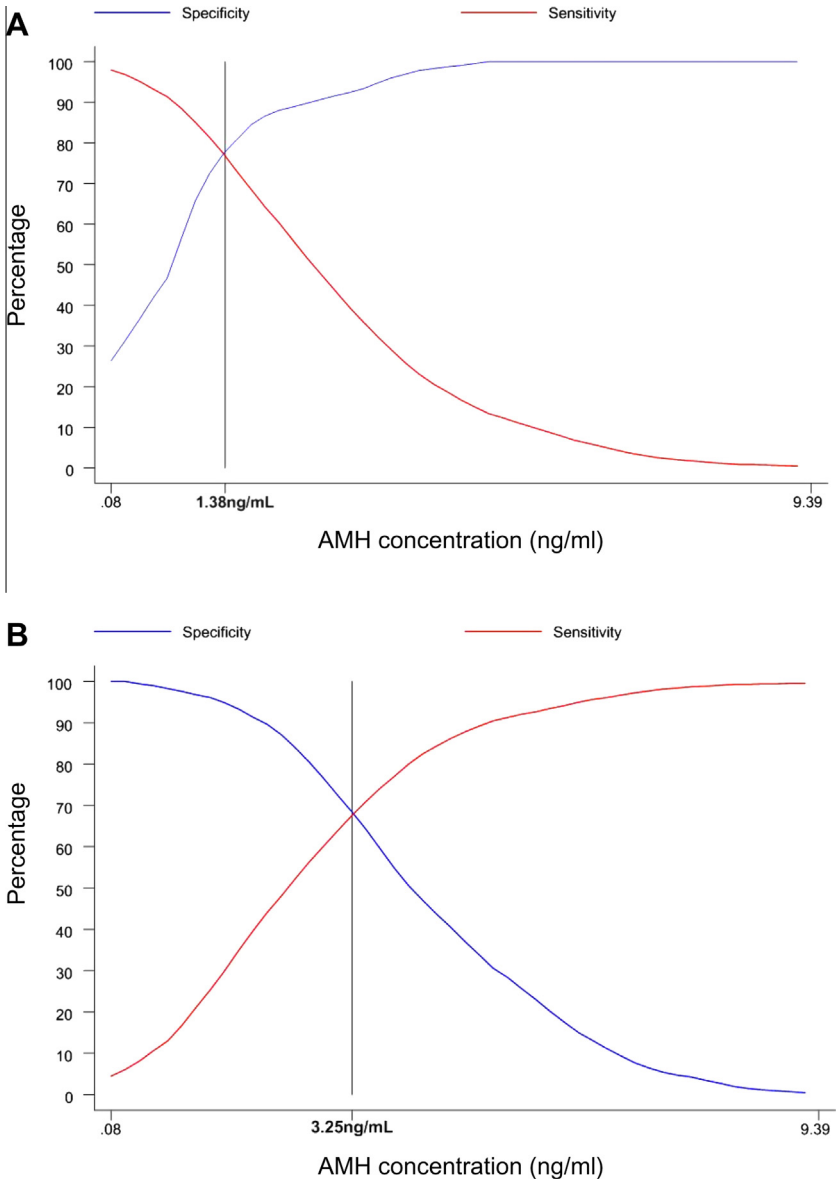

Figure 1 Anti-Müllerian hormone (AMH) cut-off values to predict hyporesponse (A) or hyperresponse (B) to ovarian stimulation.

algorithms. When comparing outcomes across the three subgroups of AMH concentrations, more than 70% of those in the lowest category (<0.7 ng/ml) were classified as having a poor ovarian response. Therefore, even increasing the dose of rFSH to 375 IU was insufficient to compensate for the low level of response. These findings are in line with a previous study (Nelson et al., 2009).

AMH cut-off values for this study were based on those used in a previous study conducted in the UK (Nelson et al., 2009). However, there is some evidence that AMH concentrations vary with races (Seifer et al., 2009), although there are no specific data for Asians. As previously reported, ovarian response in women of Asian descent may be lower than that in Caucasian women (Purcell et al., 2007).

Comparing the proportion of cycles with desired response across the three AFC subgroups did not reveal any significant differences, indicating that the cut-off values chosen for this dosing algorithm were more appropriate than those in the AMH arm. The different starting doses of rFSH defined by the AFC-based algorithm ensured that the response to ovarian stimulation was more consistent across patients with different initial ovarian reserve. Additional evidence that the AFC dosing algorithm cut-off values were more appropriate comes from the level that predicted hyporesponse which, at 6, was equivalent to the definition of the lowest AFC group. This is very similar to AFC cut-off values used in previous studies to predict poor ovarian response (AFC 5–7; Broer et al., 2009). In contrast, the rate of hyperresponse in the AFC >15 subgroup was relatively high (37.3%), suggesting that these patients still had a particularly high response even though the starting dose of rFSH was 150 IU. Perhaps even lower dosages could be considered in these patients. In addition, the AFC cut-off to predict hyperresponse in this study was 12.25. Therefore, setting >15 as the cut-off to receive an initial rFSH dose of 150 IU means that a proportion of patients in the middle-dose

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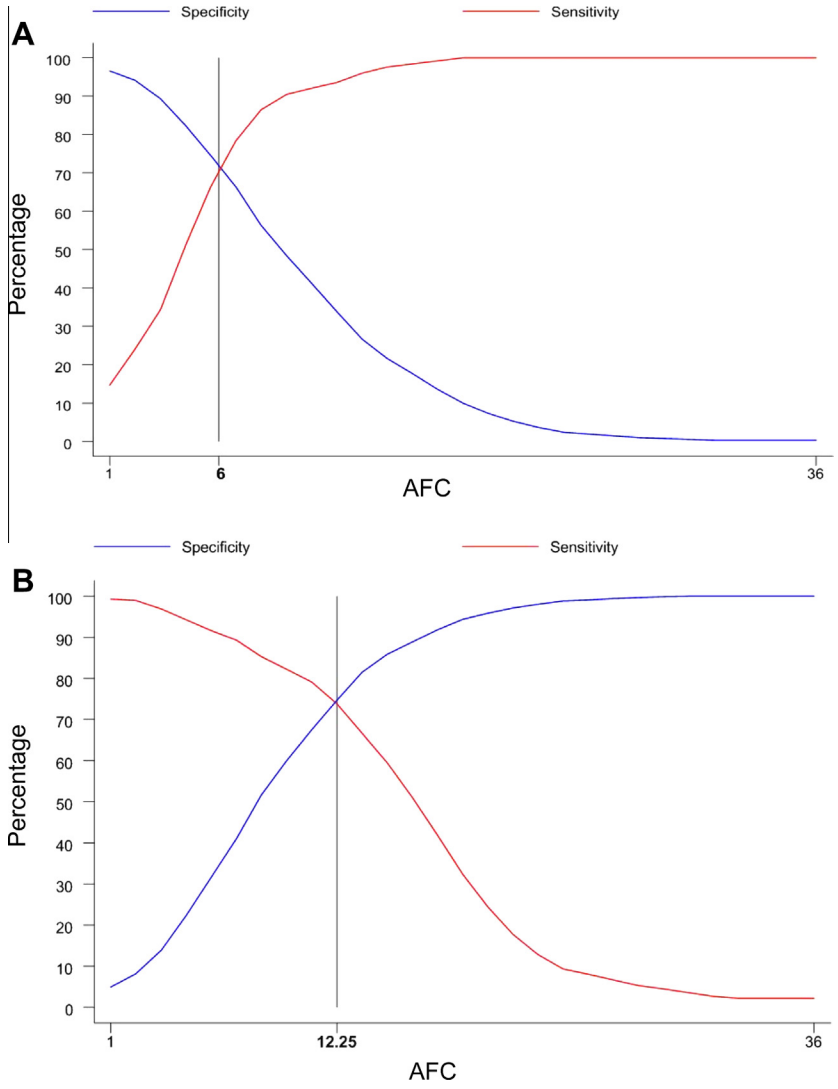

Figure 2 Antral follicle count (AFC) cut-off to predict hyporesponse (A) or hyperresponse (B) to ovarian stimulation.

group were effectively receiving a dose that was too high (225 versus 150 IU). To increase the proportion of cycles with desired response in both dosing algorithm groups, adjustments in the cut-off values used to guide the initial dose would be required.

Data from this trial indicate that AMH is better than AFC for predicting hyporesponse, and it has been suggested that AMH may eventually replace AFC or FSH as a predictor of poor response (Fauser et al., 2008). Conversely, AFC appeared to be a better predictor of hyperresponse than AMH. However, the cut-off values for both AMH and AFC identified in this Vietnamese patient population was different to those previously reported in other groups of women (from <4 to <10) (Broekmans et al., 2006).

The overall multiple pregnancy rate obtained in the current study was lower than that reported in the recent European database registry (Ferraretti et al., 2012) while implantation rate was within an acceptable range of 15–21%.

With subtle differences, both AMH and AFC appear to have the ability to predict poor ovarian response and guide the starting dose of rFSH. Therefore, other factors might influence the choice of test. Advantages of AMH include intracycle stability (Cook et al., 2000; Hehenkamp et al., 2006; La Marca et al., 2007) and the fact that concentrations can be determined from blood obtained during routine IVF testing (Broer et al., 2009). In contrast, AFC needs to be determined early in the follicular phase of the cycle by a skilled ultrasound operator (Broer et al., 2009; Pache et al., 1990) and the measurement requires standardization (Broekmans et al., 2010).

As far as is known, this study is one of the first to compare the use of AMH- or AFC-based algorithms to guide the starting dose of rFSH during ovarian stimulation. Although both appear to have utility in this setting, the appropriate cut-off values remain to be determined. In addition, it appears that population-specific recommendations may be required. Therefore, additional data are needed before the widespread use of either AMH or AFC to determine the rFSH starting dose in the clinic.

Acknowledgement

This study was funded by a grant from Merck Serono.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 12 May 2013; refereed 17 July 2013; accepted 17 July 2013.