Comparing antral follicle count and serum anti-Mullerian hormone level for determination of gonadotrophin dosing in *in-vitro* fertilisation: a randomized trial

Hang Wun Raymond Li*[1,2], Jennifer Ka Yee Ko[1], Vivian Chi Yan Lee[1], Sofie Shuk Fei Yung[1,2], Estella Yee Lan Lau[1], William Shu Biu Yeung[1,2], Pak Chung Ho[1,2], Ernest Hung Yu Ng[1,2]

[1]Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, Hong Kong

[2]Shenzhen Key Laboratory on Fertility Regulation, Department of Obstetrics and Gynaecology, The University of Hong Kong – Shenzhen Hospital, Shenzhen, People's Republic of China

*Correspondence to:

Professor Ernest H.Y. Ng

Department of Obstetrics and Gynaecology, The University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong.

Email: nghye@hku.hk

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Contribution

What does this work add to what is already known?

This randomised trial compared the proportion of women having a desired ovarian response following ovarian stimulation with gonadotrophin dosing determined based on AFC versus serum AMH level in women undergoing IVF using the GnRH antagonist protocol. A similar study on patients treated on the long GnRH agonist protocol has been reported before.

What are the clinical implications of this work?

Our results confirmed that gonadotrophin dosing algorithms based on either AFC or serum AMH level result in no significant difference in the proportion achieving desired ovarian response, and clinicians can choose to use either of the biomarkers for the said purpose.

Abstract

Objectives: To compare the proportion of women having a desired ovarian response following ovarian stimulation with gonadotrophin dosing determined based on AFC versus serum AMH level in women undergoing IVF using the GnRH antagonist protocol.

Methods: This was a randomised double-blinded trial carried out in a university-affiliated assisted reproduction unit. A total of 200 women undergoing the first IVF cycle between April 2016 and February 2018 were randomised to algorithms for gonadotrophin dosing based on either AFC or AMH in the pre-treatment cycle. They underwent IVF as per standard protocol. The proportion of subjects achieving desired ovarian response, defined as having 6 to 14 oocytes retrieved, was compared between arms.

Results: There was no significant difference in the proportion achieving desired ovarian response between the AFC (56.3%) and AMH (51.6%) groups (p=0.523). The median number of oocytes retrieved were 9 vs 7 (p=0.064), and the median follicular output rate (FORT) was 0.56 vs 0.55 (p=0.759) in the AFC and AMH groups respectively. The same conclusion applied to subgroup analyses on subjects with AFC<=5 and AFC>5 at commencement of ovarian stimulation (P>0.05 for all comparisons). There was moderate concordance between AFC and AMH measured in the pre-treatment cycle and the stimulation cycle (κ = 0.460 and 0.573 respectively).

Conclusions: Gonadotrophin dosing algorithms based on either AFC or serum AMH result in no significant difference in the proportion achieving desired ovarian response.

Introduction

Ovarian stimulation is an integral part of in-vitro fertilization (IVF). The responsiveness to gonadotrophin stimulation has important implications on treatment success[1]. Both antral follicle count (AFC) and serum anti-Mullerian hormone (AMH) level are good predictors of ovarian responses during IVF, although they are not good predictors of pregnancy[2].

AMH is a dimeric glycoprotein that belongs to the transforming growth factor-beta family. In the adult female, AMH is exclusively produced by the granulosa cells of preantral and small antral follicles, and regulates ovarian activity and follicular steroidogenesis in a paracrine manner. Serum AMH level is increasingly used as an ovarian reserve marker[3,4,5]. In IVF cycles, basal serum AMH level was significantly correlated with AFC, the number of follicles obtained after stimulation and the number of retrieved oocytes, and are useful in prediction of suboptimal and excessive ovarian responses upon ovarian stimulation[1,3,6,7,8]. It has been suggested that serum AMH level can be used for patient-tailored individualisation of the dosage of gonadotrophin for ovarian stimulation during IVF[9,10,11].

Compared to AFC, AMH has the advantage of having less intra- and inter-cycle variations[12] and is not operator-dependent, despite its additional cost. A randomised trial suggested that the proportion of cycles attaining a desired response was not different when the gonadotrophin dosing was based on either AMH or AFC (35.2%

versus 28.4%), though the incidence of hyper-response was significantly lower in the AMH group (8.6%) compared to the AFC group (17.4%)[13]. The women in the study were exclusively treated on a long gonadotrophin releasing hormone (GnRH) agonist protocol.

The objective of this randomised trial was to compare the proportion of women having a desired ovarian response following ovarian stimulation with gonadotrophin dosing determined based on AFC versus serum AMH level in women undergoing IVF using the GnRH antagonist protocol.

Methods

Subjects

Women undergoing the first-time IVF cycle at Queen Mary Hospital, Hong Kong, between April 2016 and February 2018 were invited to participate in this study. Those with body mass index >=30 kg/m^2 at enrolment (the Centre's limit for offering IVF), undergoing repeated IVF cycles, using donor oocytes or undergoing pre-implantation genetic testing were excluded from this study. Ethics approval was obtained from the Institutional Review Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster (reference number: UW12-358). The study was registered on ClinicalTrials.gov (NCT02739269).

Measurement of AFC and AMH

Women who gave written informed consent attended the clinic on day 2-5 of the menstrual cycle preceding their scheduled IVF. They had transvaginal two-dimensional real-time ultrasound examination to determine the AFC measuring 2-9 mm in mean diameter using a 7-9 MHz probe on the Voluson V730 PRO (GE Medical, Zipf, Austria) machine. The ultrasound scans were performed by one of the five gynaecologists who were all working and had been trained in the same centre and with at least five years of experience in gynaecological ultrasonography. Blood was taken for measurement of serum AMH level using a commercial automated method (Access AMH Assay, Beckman-Coulter, Texas, USA), which had a limit of

detection of 0.02 ng/ml. These measurements were repeated at the commencement of ovarian stimulation, though randomisation was based on the pre-treatment cycle values.

Randomisation

Women were randomised into either the AFC or AMH group, where the gonadotrophin dosing was based on the baseline AFC or serum AMH level assessed one month before the scheduled IVF cycle. Randomisation was performed according to a computer-generated list in blocks of 10, which were read by an independent research nurse who then assigned the initial gonadotrophin dose according to the study protocol. Both the clinicians and women were blinded to the randomization throughout the course of treatment. The pre-treatment AFC and AMH data were kept, separate from the patients' clinical record, by a dedicated research nurse who assigned the gonadotrophin dose based on the regimen detailed below. These data were concealed from the clinical team who managed and monitored the women during the ovarian stimulation cycle.

Ovarian stimulation, oocyte retrieval and embryo transfer

All women received priming with combined oral contraceptive pills for 12 – 21 days in the cycle preceding ovarian stimulation after pre-treatment AFC and AMH were measured. In their stimulation cycle, those in the AFC group were stimulated with the initial gonadotrophin dose of 300, 225 or 150 IU/day if their AFC at first

assessment was <=5, between 6 and 15 inclusive, or >15 respectively. Those in the AMH group were stimulated with the initial gonadotrophin dose of 300, 225 or 150 IU/day if their AMH level at first assessment was <=1.0 ng/ml, >1.0 but <=3.3 ng/ml, or >3.3 ng/ml respectively. The AMH cut-off values were determined based on an in-house correlation in 214 women previously treated in our centre who had AMH and AFC concurrently determined before ovarian stimulation. By both linear and quadratic regression analyses, an AMH value of 1.0 ng/ml and 3.3 ng/ml corresponded to AFC of 5 and 15 respectively. All subjects had ultrasound tracking on the eighth day of ovarian stimulation, with the dose of gonadotrophin adjusted accordingly. Those with five or less follicles beyond 10 mm in size had the gonadotrophin dose stepped up by 75 IU/day with ceiling at 300 IU/day. Those with more than 15 follicles >10 mm in size had the gonadotrophin dose stepped down from 300 to 225 IU/day, from 225 to 150 IU/day, or from 150 to 112.5 IU/day respectively. The gonadotrophin dosage remained unchanged if there were 6 to 15 follicles beyond 10 mm in size. Adjustment of gonadotrophin dosage was allowed only once throughout the whole cycle.

All women received cetrorelix (Cetrotide®, Merck, Germany) or ganirelix (Orgalutran®, NV Organon, The Netherlands) 250 microgram subcutaneous injection starting from the sixth day of ovarian stimulation until the day of ovulatory trigger by either human chorionic gonadotrophin 10,000 IU (Pregnyl®, N.V. Organon, Oss, The Netherlands or Ovidrel®, EMD Serono, Inc. MA, USA) or triptorelin 0.25

mg (Decapeptyl®, Ferring, Kiel, Germany) when the leading follicle reached 18 mm, and preferably two or more other follicles reached 16 mm in mean diameter. The latter was used for ovulatory trigger if there were more than 15 dominant follicles, serum estradiol level was higher than 25,000 pmol/L or there were signs of ovarian hyperstimulation syndrome, in which case no fresh embryos transfer would be carried out. Oocyte retrieval was carried out 34-36 hours post-trigger, where all follicles >10 mm were aspirated. All women were allowed to proceed to ovulatory trigger and oocyte retrieval except for those with no follicle developing beyond 10 mm after 12 days of stimulation, or when there was other medical contraindication to continue treatment.

Embryo transfer was carried out two days post-fertilisation by default, except when there were six or more embryos of transferrable quality on the second day post-fertilisation where extended culture to blastocyst stage would be discussed and considered. A maximum of two embryos were transferred on any occasion. Elective freezing of all embryos would be considered when clinically indicated, such as when the patient was at risk of ovarian hyperstimulation syndrome, having premature progesterone elevation, having untreated hydrosalpinx or endometrial polyp, or other personal circumstances where fresh embryo transfer was not preferred.

Outcome measures and statistical analyses

The primary outcome was the proportion of women having a desired ovarian response, defined as the number of oocytes retrieved being 6 to 14 inclusive[14]. Requirement for gonadotrophin dose adjustment (step-up or step-down), the number of oocytes retrieved, as well as the follicular output rate (FORT), defined as the ratio of follicles reaching 16 mm or beyond on the ovulatory trigger date / AFC at baseline[15,16], were also compared between the two groups. Subgroup analyses on those with AFC >5 and <=5 were also performed. Pregnancy was defined by a positive urine or serum hCG test, whereas ongoing pregnancy was defined as the presence of at least one intrauterine pregnancy with foetal heart pulsations detected sonographically at 8 weeks of gestation. Between-group comparisons were performed using χ^2 test or Mann-Whitney U test for categorical and continuous variables respectively. Statistical analyses on both intention-to-treat and per protocol basis were performed using the IBM SPSS Statistics 24 software.

Sample size estimation

According to our Centre's record (unpublished data), about 50% of our patients had the desired ovarian response when using AFC to determine gonadotrophin dosing as in the current practice. Assuming that an increase to 70% achieving desired ovarian response in the AMH group was clinically meaningful, a minimum of 93 women per group was required to demonstrate a significant difference between the AMH and AFC groups with 80% power and type I error of 0.05. To allow for drop-outs, a total of 200 women (i.e. 100 per group) was recruited.

Results

A total of 200 women were initially recruited. Of them, 5 deviated from the study protocol, 2 did not proceed with IVF as scheduled, and 4 had cycle cancellation (2 for poor ovarian response upon patient's request and 2 for premature ovulation) (Figure 1).

Background characteristics and clinical outcomes

No differences were found in the background demographic and clinical characteristics between the AFC and AMH groups (Table 1). The age of women ranged from 26 to 40 years, and their body mass index on day 1 of the stimulation cycle ranged from 15.2 to 30.4 kg/m^2. Those in the AFC group had significantly lower total gonadotrophin dose, shorter duration of stimulation, higher number of follicles reaching 16mm and higher peak serum oestradiol level compared to those in the AMH group. There was no significant difference in pregnancy and ongoing pregnancy rates per women. Out of those with fresh embryo transfer, the AFC and AMH groups did not differ significantly in pregnancy rate (48.4% versus 40.9%; p=0.395) and ongoing pregnancy rate (37.1% vs 34.8%; p=0.791) per transfer either (Table 2).

Proportion achieving the desired ovarian response, FORT and gonadotrophin dose adjustment

The AFC and AMH groups had a similar proportion achieving the desired ovarian response (54% vs 49% respectively, p=0.479), with an odds ratio of 1.222 (95% confidence interval 0.701 – 2.129). There was no significant difference in the number of oocytes retrieved as well as FORT between the two groups. Significantly fewer women required no gonadotrophin dose adjustment and more women required stepping up in the AMH group compared to the AFC group. The findings by intention-to-treat analyses are detailed in Table 2. Per-protocol analysis (including only those 189 women who underwent ovarian stimulation according to study protocol and proceeded to oocyte retrieval) resulted in similar findings (data not shown).

Sub-group analysis based on baseline AFC in the treatment cycle

In the sub-groups of women with baseline AFC >5 and <=5 in the treatment cycle, there was no significant difference in the proportion attaining the desired ovarian response (p>0.05). In the sub-group with baseline AFC <=5, the median FORT (25-75th percentile) in the AFC and AMH groups were 1.00 (0.75 – 1.33) and 1.00 (0.75 – 1.40) respectively (p=0.969).

Concordance in AFC and AMH between pre-treatment and ovarian stimulation cycle

There were strong correlations of AFC (r=0.758, p<0.001) and AMH (r=0.888, p<0.001), values in the pre-treatment cycle with those in the treatment cycle as shown in Supplementary Figure 1.

In the pre-treatment cycle and at commencement of the ovarian stimulation, the median (25-75th percentile) of AFC were 11.0 (7.0 – 15.3) and 10.0 (6.0 – 14.0) respectively (p=0.006), while that of AMH was 2.59 (1.40 – 4.20) and 2.10 (1.35 – 3.55) respectively (p<0.001, Wilcoxon signed rank test). Both AFC and AMH were significantly higher in the pre-treatment cycle than at commencement of the stimulation cycle.

Classifying AFC into low (<=5), normal (6 to 15) and high (>15) categories, there was moderate concordance between AFC categorization measured in the pre-treatment versus the stimulation cycle (κ = 0.460). Likewise, by classifying AMH into low (<=1.0 ng/ml), normal (>1.0 but <=3.3 ng/ml) and high (>3.3 ng/ml) categories, there was moderate concordance between AMH categorization measured in the pre-treatment versus the stimulation cycle (κ = 0.573) (Table 3).

Concordance in categorisation by AFC and AMH in the pre-treatment cycle

Among all 200 women enrolled, 147 (73.5%) of them showed concordance between categorisation based on either AFC and AMH in the pre-treatment cycle, whereas

the remaining 53 (26.5%) had mismatch if their dosing regimen were based on the alternative unassigned parameter (κ=0.560) (Table 4).

Discussion

Main findings

Our results showed that there was no significant difference in the proportion achieving desired ovarian response defined by the number of oocytes retrieved when either AFC or serum AMH level was used for determination of gonadotrophin dosing in IVF.

Strengths and limitations

The main strength of our study is that it is the first randomised double-blinded trial to compare the use of AFC versus serum AMH for determination of gonadotrophin dosing in IVF under the GnRH antagonist protocol. Ovarian response is often subjected to many confounding factors, which can only be controlled for by having a randomised study design. One limitation is that in our study, oral contraceptive priming was used to programme the treatment cycle in our centre, which could have altered the ovarian response or the biomarkers used for predicting it. For this reason, randomisation was based on AFC and AMH measured in the month preceding the stimulation cycle. As shown in previous studies as well as our results, considerable cycle-to-cycle variation in both AFC and AMH do exist[2], and the status measured in the pre-treatment cycle is not precisely equivalent to that in the stimulation cycle, although the two are moderately concordant as shown by our results. Nonetheless, in real clinical practice, assessment in the pre-treatment cycle is often necessary, especially for AMH measurement which usually does not have

results immediately available in most centres to guide gonadotrophin dosing on the same day. Although the baseline ultrasound scans were performed by the same team of clinicians that managed the women's IVF cycle, they were all blinded from the initial AFC and AMH results during the ovarian stimulation cycle, and the gonadotrophin dosing was assigned by the research nurse according to the study protocol to avoid bias. Furthermore, this study was carried out in women undergoing IVF in a single centre using a unified protocol. Other ovarian stimulation regimens used in anticipated good and poor responders may be adopted in other centres, and whether the same findings can be extrapolated to women treated under other regimens is yet to be confirmed in further studies.

Interpretations

Our main results are compatible with previous meta-analyses using ROC curve prediction models that both AFC and serum AMH level had comparable performance in predicting suboptimal ovarian response[17] and excessive ovarian response[18]. Despite the respectable sample size included, these meta-analyses were based on different studies with possible heterogeneity and possible biases incurred. To our knowledge, there has been only one randomised trial comparing the AFC-based and AMH-based gonadotrophin dosing algorithms[13], which showed no difference in the proportion of women having the desired number of oocytes retrieved in either group. In that study, it was not known if the subjects and clinicians were blinded, and women were treated exclusively on the long GnRH agonist protocol. Yet, the GnRH

antagonist protocol has been increasingly adopted in recent years for its simplicity and lower risk of ovarian hyperstimulation syndrome associated with its use. Therefore, findings from the present study, which basically concurred with those of Lan et al (2013)[13], will reinforce our knowledge on this issue as applied to IVF cycles treated on the GnRH antagonist protocol.

We observed a higher total gonadotrophin dose required, longer duration of ovarian stimulation, fewer number of follicles reaching 16 mm or beyond, as well as lower peak serum oestradiol level in the AMH group compared to the AFC group, which may be a difference by chance since the recruited women were randomised. Although women in the AMH group had apparently older age, lower baseline AFC and AMH level than the AFC group, statistical comparison in these parameters were inappropriate as per general principle of randomised trials and such differences could just happen by chance. Less women in the AFC group required dose adjustment, which may be considered as a merit. Yet there was no significant difference in the number of oocytes retrieved, which was the primary outcome measure and the more important outcome measure regarding ovarian stimulation.

We defined the desired ovarian response as 6 to 14 oocytes being retrieved[14]. We performed subgroup analysis by further stratifying subjects according to their baseline AFC at commencement of ovarian stimulation (<=5 versus >5), in view of the fact that collection of 6 to 14 oocytes was unrealistic among those with low AFC.

In the subgroup with low AFC, a higher FORT may be the more realistic goal for "optimal" ovarian stimulation, and indeed our results showed no significant difference in FORT between the AFC and AMH groups.

Both AFC and AMH measurements have their merits and limitations. Sonographic measurement of AFC is immediately available in most IVF centres, and is not laboratory-dependent. However, the counting of antral follicles is operator-dependent and is also influenced by the resolution of the ultrasound probe and machine. Moreover, visualization of AFC may be limited by patient's factors like obesity. Comparatively, AMH measurement is more objective and less subjected to the latter limitations. However, there has been no standardization in AMH values measured by the different available assay methods[19]. Keeping these in mind, our findings suggest that clinicians can choose to use AFC or AMH interchangeably to determine the gonadotrophin dosing in IVF. This was supported by the moderate concordance in the categorisation by AFC and AMH in the same women (κ=0.560).

Conclusion

Gonadotrophin dosing algorithms based on either AFC or serum AMH level result in no significant difference in the proportion achieving desired ovarian response.

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Details of Ethics Approval

Ethics approval was obtained from the Institutional Review Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster (reference number: UW12-358; approval date: 21 August 2012).

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References

1. Li HWR, Lee VCY, Lau EYL, Yeung WSB, Ho PC, Ng EHY. Role of baseline antral follicle count and anti-Mullerian hormone in prediction of cumulative live birth in the first in vitro fertilisation cycle: a retrospective cohort analysis. PLoS One 2013; 8(4):e61095.
2. Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update 2015; 21(6):698-710.
3. Ledger WL. Clinical utility of measurement of anti-Mullierian hormone in reproductive endocrinology. J Clin Endocrinol Metab 2010; 95: 5144-5154.
4. Anderson RA, Nelson SM, Wallace WHB. Measuring anti-Mullerian hormone for the assessment of ovarian reserve: when and for whom is it indicated? Maturitas 2012; 71(1): 28-33.
5. Nelson SM, Anderson RA, Broekmans FJ, Raine-Fenning N, Fleming R, La Marca A. Anti-Mullerian hormone: clairvoyance or crystal clear? Hum Reprod 2012; 27(3): 631-636.
6. Lekamge DN, Barry M, Kolo M, Lane M, Cilchrist RB, Tremellen KP. Anti-Mullerian hormone as a predictor of IVF outcome. Reprod BioMed Online 2007: 14: 602-610.
7. Broer SL, Mol BWJ, Hendriks D, Broekmans FJM. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril 2009; 91(3):705-714.

8. Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 2011;17(1):46-54.
9. Nelson SM, Yates RW, Lyall H, Jamieson M, Traynor I, Gaudoin M, Mitchell P, Ambrose P, Fleming R. Anti-Mullerian hormone-based approach to controlled ovarian stimulation for assisted conception. Hum Reprod 2009; 24:867–875.
10. Yates AP, Rustamov O, Roberts SA, Lim HY, Pemberton PW, Smith A, Nardo LG. Anti-Müllerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011; 26:2353–2362.
11. Andersen AN, Nelson SM, Fauser BC, García-Velasco JA, Klein BM, Arce JC; ESTHER-1 study group. Individualized versus conventional ovarian stimulation for in vitro fertilization: a multicenter, randomized, controlled, assessor-blinded, phase 3 noninferiority trial. Fertil Steril 2017;107(2):387-396.e4.
12. van Disseldorp J, Lambalk CB, Kwee J, Looman CWN, Eijkemans MJC, Fauser FC, Froekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod 2010; 25(1):221-227.
13. Lan VT, Linh NK, Tuong HM, Wong PC, Howles CM. Anti-Müllerian hormone versus antral follicle count for defining the starting dose of FSH. Reprod Biomed Online 2013; 27(4): 390-399.

14. Broer SL, Mol B, Dolleman M, Fauser BC, Broekmans FJM. The role of anti-Mullerian hormone assessment in assisted reproductive technology outcome. Curr Opin Obstet Gynecol 2010; 22: 193-201.
15. Genro VK, Grynberg M, Scheffer JB, Roux I, Frydman R, Fanchin R. Serum anti-Müllerian hormone levels are negatively related to Follicular Output RaTe (FORT) in normo-cycling women undergoing controlled ovarian hyperstimulation. Hum Reprod 2011;26(3):671-677.
16. Gallot V, Berwanger da Silva AL, Genro V, Grynberg M, Frydman N, Fanchin R. Antral follicle responsiveness to follicle-stimulating hormone administration assessed by the Follicular Output RaTe (FORT) may predict in vitro fertilization-embryo transfer outcome. Hum Reprod 2012;27(4):1066-1072.
17. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, Eijkemans MJ, Mol BW, Broekmans FJ; IMPORT study group. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013a;19(1):26-36.
18. Broer SL, Dólleman M, van Disseldorp J, Broeze KA, Opmeer BC, Bossuyt PM, Eijkemans MJ, Mol BW, Broekmans FJ; IPD-EXPORT Study Group. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis. Fertil Steril 2013b;100(2):420-429.e7.

19. Li HWR, Wong BPC, Ip WK, Yeung WSB, Ho PC, Ng EHY. Comparative evaluation of three new commercial immunoassays for anti-Müllerian hormone measurement. Hum Reprod 2016;31(12):2796-2802.

Figure legends

Figure 1. Flow diagram of study recruitment.

Table 1. Background demographic and clinical characteristics of subjects. The medians (25-75th percentile) are shown for continuous variables, and number (percentage) are shown for categorical variables.

	AFC group (n=100)	AMH group (n=100)
Age of women (years)	35 (33 – 37)	36 (34 – 38)
Body mass index (kg/m^2) on day 1 of ovarian stimulation	21.4 (19.5 – 23.1)	20.8 (19.6 – 23.2)
Type of infertility		
Primary	75 (75%)	74 (74%)
Secondary	25 (25%)	36 (36%)
Duration of infertility (years)	4 (3 – 6)	4.5 (3 – 6)
Cause of infertility		
Tubal factor	16 (16%)	15 (15%)
Endometriosis	5 (5%)	7 (7%)
Male factor	46 (46%)	42 (42%)
Mixed factors	16 (16%)	14 (14%)
Unexplained	17 (17%)	22 (22%)
AFC in the pre-treatment cycle	12.5 (8.0 – 15.0)	10.0 (6.5 – 16.0)
AFC in the treatment cycle	11 (7 – 14)	9 (5 – 14)
AMH in pre-treatment cycle (ng/ml)	2.9 (1.8 – 4.5)	2.5 (1.3 – 3.9)
AMH in the treatment cycle (ng/ml)	2.4 (1.4 – 3.5)	1.9 (1.3 – 3.8)

Table 2. Comparison of ovarian stimulation parameters and pregnancy outcome between the AFC and AMH-based algorithms for gonadotrophin dosing by intention-to-treat analysis.

Parameter	AFC group (n=100)	AMH group (n=100)	P value#
Number of oocytes retrieved			0.460
<=5	26 (26.0%)	34 (34.0%)	
6 - 14	54 (54.0%)	49 (49.0%)	
>=15	20 (20.0%)	17 (17.0%)	
Desired ovarian response^			0.479
Yes	54 (54.0%)	49 (49.0%)	
No	46 (46.0%)	51 (51.0%)	
Gonadotrophin dosage adjustment			0.002*
None	64 (64.0%)	56 (56.0%)	
Stepping-up	28 (28.0%)	44 (44.0%)	
Stepping down	8 (8.0%)	0 (0.0%)	
Number of oocytes retrieved	9 (6 – 14)	7 (4 – 12)	0.070
Follicular output rate (FORT)	0.56 (0.36 – 0.86)	0.55 (0.31 – 0.80)	0.702
Total gonadotrophin dose (IU)^^	2250.0 (1837.5 – 2737.5)	2475.0 (2025.0 – 3300.0)	0.018*
Duration of stimulation (days)^^	10 (9 – 11)	11 (10 – 13)	0.009*
Number of follicles reaching 16 mm^^	6.0 (4.0 – 8.5)	5.0 (3.0 – 7.0)	0.037*
Peak serum oestradiol (pmol/L)^^	7463 (4556 – 10606)	5810 (3253 – 8539)	0.016*
Ovulatory trigger method^^			0.004*
hCG	88 (91.7%)	93 (100.0%)	
GnRH agonist	8 (8.3%)	0 (0.0%)	
Number of embryos transferred			0.558
0	38 (38.0%)	34 (34.0%)	
1	45 (45.0%)	43 (43.0%)	
2	17 (17.0%)	23 (23.0%)	
Pregnancy rate per woman	30/100 (30.0%)	27/100 (27.0%)	0.639

Ongoing pregnancy rate per woman	23/100 (23.0%)	23/100 (23.0%)	1.000
Ovarian hyperstimulation syndrome			0.512
No	97 (97.0%)	96 (96.0%)	
Mild	2 (2.0%)	1 (1.0%)	
Moderate to severe	1 (1.0%)	3 (3.0%)	

#Mann-Whitney U test for continuous variables and χ^2 test for categorical variables

*Statistically significant (P<0.05)

^Two cases not proceeding with treatment after randomisation were regarded as having zero oocyte obtained and not requiring gonadotrophin dose adjustment (intention-to-treat analysis)

^^Only applying to subjects who received treatment per-protocol and proceeded to oocyte retrieval

Table 3. Concordance in antral follicle count (Panal A) and serum anti-Mullerian hormone (Panel B) categorisation between the pre-treatment cycle and the stimulation cycle. Those showing concordance are in bold. Only women who started ovarian stimulation per-protocol (n=193) were analysed.

(A) AFC categorisation:

Stimulation cycle / Pre-treatment cycle	AFC <=5	AFC 6 – 15	AFC >15
AFC <=5	**23**	6	0
AFC 6 – 15	19	**84**	13
AFC >15	1	19	**28**

$\kappa = 0.477$

(B) AMH categorisation:

Stimulation cycle / Pre-treatment cycle	AMH <=1.0 ng/ml	AMH >1.0 and <=3.3 ng/ml	AMH >3.3 ng/ml
AMH <=1.0 ng/ml	**21**	9	0
AMH >1.0 and <=3.3 ng/ml	8	**77**	7
AMH >3.3 ng/ml	0	24	**47**

$\kappa = 0.587$

Table 4. Concordance in AFC and AMH categorization in the pre-treatment cycle in all women enrolled. Those showing concordance are in bold.

Categorisation by AMH / Categorisation by AFC	AMH <=1.0 ng/ml	AMH >1.0 and <=3.3 ng/ml	AMH >3.3 ng/ml
AFC <=5	**24**	6	1
AFC 6 – 15	6	**80**	32
AFC >15	0	8	**43**

$\kappa = 0.560$