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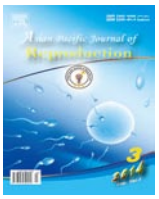

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Basal serum anti-müllerian hormone and antral follicle count are predictors of ovarian response for Asian women in Singapore

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ABSTRACT

Objective: To determine the basal ovarian reserve markers for prediction of poor and high responses and clinical pregnancy outcome in subfertile Asian women requiring controlled ovarian stimulation (COS) treatment in Singapore. **Methods:** Subfertile Asian women, aged ≤40 with basal serum follicle stimulating hormone (FSH) level of ≤12 IU/L, were enrolled prospectively during routine preliminary endocrine ovarian reserve assessment prior to COS regime for intracytoplasmic sperm insemination (ICSI) cycles. Basal serum levels of the endocrine ovarian reserve markers (anti-Müllerian hormone (AMH), estradiol, FSH, luteinizing hormone), antral follicle count (AFC) and ovarian response parameters were compared between the Poor (retrieved oocytes ≤4), Normal (retrieved oocytes, 5 to 19) and High Responder (retrieved oocytes ≥20) groups of women. **Results:** Basal serum AMH and AFC were significantly correlated to age (r =-0.213 and -0.243, respectively) and to the number of retrieved oocytes (r=0.570 and 0.523, respectively) (P<0.05). Both basal serum AMH and AFC were significant discriminators of poor response (cut-off levels of ≤2.0 μg/L and ≤12, respectively) and for high response (AMH ≥ 3.2 μg/L and AFC of ≥20, respectively) to COS. Basal AMH was the only significant predictor for clinical pregnancy outcome, ROCAUC =0.71, cut-off level of ≥3.0 μg/L and odds ratio of 1.42. **Conclusions:** Both basal serum AMH and AFC were reliable ovarian reserve markers for predicting poor and high ovarian response to COS in Asian women. Basal AMH was the only significant predictor for clinical pregnancy outcome.

1. Introduction

Determination of ovarian reserve status prior to commencement of ovulation induction could provide useful information in predicting ovarian response and potential risks to controlled ovarian stimulation (COS). Couples could be counseled and could make informed decision on their treatment options.

Conventional endocrine ovarian reserve markers, such as estradiol (E_2), follicle stimulating hormone (FSH), luteinizing hormone (LH) and inhibin B, have been widely used. However, these markers are cycle dependent, with day to day fluctuations and show significant variations throughout the menstrual cycle[1,2]. Antral follicle count (AFC) is the measurement of "selectable" follicle pool for dominance in the ovaries and is significantly correlated to the number of retrieved oocytes[3].

Recently, anti-Müllerian hormone (AMH) has been proposed as a promising ovarian reserve marker[4-6]. In human ovaries AMH is continuously secreted by the granulosa cells from the pre-antral and antral follicles 4 to 6 mm in diameter into the blood circulation and

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hence measurable in serum[7]. AMH indirectly represents the quantity and quality of the ovarian follicle pool[8]. Furthermore, serum AMH levels has an added advantage in clinical practice as it demonstrates stability and reproducibility with relatively low intra- and inter-cycle variations, flexibility in timing of measurements and at any day of the menstrual cycle[1,9,10].

Most studies on ovarian reserve and response to COS involved Caucasian women or were evaluated independent of ethnic or racial differences. There are limited studies in other races/ethnicity such as Chinese women (who have significantly lower IVF pregnancy rates) and Asian women (reported to have reduced clinical pregnancy and live birth rates in IVF treatment cycles) compared to Caucasian women[11,12].

This study was designed to determine the basal endocrine ovarian reserve markers (AMH, E_2, FSH and LH) and AFC for prediction of poor and high responses to COS, as well as, clinical pregnancy outcome for subfertile Asian women requiring COS in Singapore.

2. Materials and methods

2.1. Subjects

Subjects of Asian origin, aged ≤40 years, either undergoing their first or have had previous intracytoplasmic sperm insemination (ICSI) cycles, with at least one intact ovary were enrolled prospectively between August 2008 and November 2011, at the Centre for Assisted Reproduction, Singapore General Hospital, Singapore. Subjects were excluded if they were oocyte recipients or oocyte donors, have had ovarian surgery, have follicles measuring ≥20 mm in diameter or basal serum FSH level of ≥12 IU/L. Written informed consent was obtained from all subjects during routine preliminary basal endocrine ovarian reserve assessment a few months prior to the commencement of their COS programme. The investigation protocol for this study was approved by the Singhealth Central Institutional Review Board, Singapore.

2.2. Hormonal assays

Peripheral blood samples were obtained by venipuncture into two plain tubes from each subject on day 2 or 3 of their spontaneous menstrual cycle. One tube was sent to the central laboratory for the assessment of basal levels of E_2, FSH and LH, using an automated electrochemiluminescence immunoassay system (Roche Cobas e601, Roche Diagnostics Basel). For E_2, the lowest limit of detection (LOD) was 18.4 pmol/L, and intra- and inter-assay co-efficient variations (CVs) were <5% and <6% respectively. For FSH, the LOD was 0.1 IU/L, and intra- and inter-assay CVs were <2% and <3% respectively. For LH, the LOD was 0.1 IU/L, and intra- and inter-assay CVs were <2% and <3% respectively.

The other plain tube was centrifuged to separate the serum. The serum was then frozen in aliquots at −80 ℃ and sent to the central laboratory at the Changi General Hospital, Singapore, for basal serum AMH levels determination in batches, using the commercial MIS/AMH Gen II enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics System Lab-Beckman Coulter, USA) assessed on an automated ELISA system (Evolis, Bio-Rad, USA). AMH results were the mean of duplicate readings with an LOD of 0.08 μg/L, and intra- and inter-assay CVs of <5% and <8% respectively.

2.3. Follicular measurements

On the same day of obtaining peripheral blood for basal endocrine assessment, subjects also had ovarian ultrasound scan using a multi-frequency transvaginal probe (Voluson 730 PRO; GE Medical Systems, Kretz, Austria). The sizes of AFC were measured in mean diameter (mean of two orthogonal diameters). Total number of early follicular phase of sizes 2 to 10 mm were counted for AFC.

2.4. Controlled ovarian stimulation protocol

Three COS protocols were used in our Centre: gonadotrophin-releasing hormone (GnRH) agonist long and short protocols and GnRH antagonist, following decisions made by the team of clinicians prior to the start of the stimulation, based on multiple parameters including age, FSH, BMI and medical history. Recombinant FSH (rFSH) starting dose was based on the Centre's standard operating protocol. Daily doses and duration of stimulation were adjusted according to follicular growth with the intention to harvest maximum number of oocytes.

In the GnRH long agonist protocol, subjects received down-regulation of the pituitary through administration of leuprorelin acetate (1 mg/day, s.c.) (Lucrin; Abbott Australasia, Kurnell, New South Wales), either on day 2 to 4 or day 21 of their spontaneous menstrual cycle. Complete pituitary desensitization was confirmed approximately two weeks later, following the detection of low levels of serum E_2, <200 pmol/L and endometrium thickness of <5 mm. Ovarian stimulation was commenced and administered using rFSH, either Puregon® (Organon, Dublin, Ireland) or Gonal-F (Merck-Serono Pharmaceutical, Nordic, Denmark), starting dose ranged from 100 to 450 IU/L. Both lucrin and rFSH were continued until the day of human chorionic gonadotrophin (hCG) administration.

In the GnRH agonist short protocol, lucrin was administered together with either Puregon® or Gonal-F, starting dose of 450 IU/L, from cycle day 2 to 4 onwards for about two weeks until the day of hCG administration.

In the GnRH antagonist protocol, either Puregon® (100 IU/L) or Gonal-F, (150 IU/L), was started on cycle day 2 to 4 until the day

of hCG administration. In addition, GnRH antagonist Cetrotide® (0.25 mg/day, s.c.) (cetrorelix acetate; Merck-Serono ASTA Medica, Frankfurt, Germany) was administered in a flexible starting date, approximately on cycle day 6 to 8 depending on the presence of a 12 mm follicle.

Ovarian response was monitored in all subjects by serial transvaginal ultrasound and serum E_2 level measurements when necessary. Final oocyte maturation was induced with either 5 000 or 10 000 IU of hCG (Pregnyl®, Organon), when two or more follicles reached the diameter of 18 mm.

Oocyte retrieval was carried out transvaginally under ultrasound guidance 34 to 36 hours after hCG administration. After 2 hours of incubation, oocytes were removed from their cumulus complex. Insemination was performed by standard ICSI procedure on the denuded mature, Metaphase II oocytes. Fertilization was characterized by the presence of two pronuclei when assessed 18 to 20 hours after ICSI. Fertilization rate was defined as number of oocytes fertilized divided by number of mature oocytes and is expressed in percentage. Embryo transfer (ET) was performed 2 to 3 days after oocyte retrieval with ultrasound guidance. Up to two or three cleavage-stage embryos were transferred according to the criteria set by the Ministry of Health, Singapore.

The luteal phase was supported with oral Duphaston (10 mg, 3 times per day) (dydrogesterone; Solvay Pharmaceuticals, Holland) starting on the evening of embryo transfer day until the pregnancy test day. Serum beta-hCG level was measured 17 days after oocyte retrieval and the level of more than 50 IU/L was defined as a positive pregnancy outcome. An ultrasound scan at 6 to 7 weeks of gestation was performed to confirm viability of the fetus and defined as clinical pregnancy outcome. Live birth was defined as birth of one or more infants and the birth of twins or triplets was counted as one live birth delivery.

Subjects were grouped as Poor Responders (defined as retrieval of $\leqslant$ 4 oocytes). Normal Responders (number of retrieved oocytes of 5 to 19) and High Responders (defined as retrieval of $\geqslant$20 oocytes or no embryo transfer due to risk of ovarian hyper stimulation syndrome (OHSS)).

2.5. Statistical analysis

The Statistical Package for the Social Sciences (version 17.0; SPSS, Chicago, IL) was used for statistical analyses. The distribution of data was checked for normality using the normal probability plot and Kolmogorov-Smirnov test. Basic descriptions of parameters were presented either in mean and standard deviation (± SD) for normally distributed data or in median and ranges for data distribution that were skewed. The relationships between two continuous variables were evaluated using non-parametric Spearman's correlation coefficient, r Comparison between two or more groups were evaluated using non-parametric Kruskal Wallis test, Mann-Whitney U test, Chi-Square or Fisher's exact test, as appropriate.

Receiver operating characteristic (ROC) curve analysis was performed for determination of predictive accuracy (area under curve, AUC) of ovarian reserve marker(s) to discriminate poor ovarian response (normal and high response were combined as one group), high ovarian response (poor and normal response were combined as one group) and clinical pregnancy outcome. The optimum cut-off levels were indicated by the highest sensitivity and specificity levels. Univariate logistic regression analysis was performed to identify significant predictor(s) for poor and high responses to COS and clinical pregnancy outcome. A $P<0.05$ was considered as statistically significant.

3. Results

A total of 106 women of Asian origin seeking assisted reproductive treatment (ART) at Singapore General Hospital, Singapore had enrolled and had given written informed consent to participate in the study. Of these, eighty women underwent COS for ICSI treatment cycles. Four women had basal serum FSH level $\geqslant$12 IU/L and four women from other nationalities were excluded, thus, a total of 72 women were included in the final data analyses. Majority of the women, 74% (n=53) received GnRH agonist long protocol, 14% (n=10) received agonist short protocol treatment and the remaining 12% (n=9) underwent GnRH antagonist treatment. Indications for ART were female factors 36%, male factors 22%, combined factors 31% and unexplained subfertility, 11%.

Baseline characteristics were presented in mean±SD. Subjects were (34.6±2.8) years of age, with BMI of (23.6±4.4) kg/m^2 and duration of subfertility of (5.2±2.9) years. Basal serum levels of AMH was (3.74±3.07) μg/L, E_2 (132.1±49.2) pmol/L, FSH (6.7±1.7) IU/L, LH (4.4±2.1) IU/L and AFC was (18.1±8.7). The women underwent (11.1±1.5) days of gonadotrophin stimulation with starting dosage of (296.4±116.5) IU and total dosage of (3376.4±1282.6) IU. Assessment of ovarian response parameters to COS included number of retrieved oocytes (12.7±7.3), mature oocytes (9.9±6.2), fertilized oocytes (7.2±5.2) and cleaved embryos (6.6±4.9) and number of transferred embryos was (1.8±0.7). Mean fertilization rate was (70.8±26.7) %.

In this study, basal serum levels of AMH and AFC were significantly and negatively correlated to age, the correlation coefficients, r=-0.213, P=0.036 and r=-0.243, P=0.020, respectively. When the women were stratified to 4 age-groups, basal AMH and AFC gradually declined from age-group >35 to 38 years to the lowest levels of 1.6 μg/L and 12 μg/L, respectively, in the oldest age group >38 to 40 years (Table 1). None of the other basal endocrine ovarian reserve markers (E_2, FSH, LH) and ovarian response parameters

Table 1
Comparison of basal levels of ovarian reserve markers and antral follicle count in 4 age-groups.

Age-groups (years)	≤32 (16)	>32 to 35 (24)	>35 to 38 (23)	>38 to 40 (9)
Anti- müllerian hormone (μg/L)	3.16* (1.53-15.37)	2.97* (0.85-13.86)	3.43 (0.12-9.29)	1.60 (0.88-3.55)
Estradiol (pmol/L)	119.0 (72.7-316.0)	133.0 (18.4-239.0)	126.0 (64.2-190.0)	119.0 (74.9-200.0)
Follicle stimulating hormone (IU/L)	6.4 (4.6-9.7)	6.2 (2.1-10.3)	7.1 (3.4-11.9)	6.7 (4.7-9.8)
Luteinising hormone (IU/L)	4.2 (1.3-7.9)	4.0 (0.2-11.9)	4.3 (1.5-9.1)	3.7 (1.8-9.3)
Antral follicle count	18* (9-44)	21.5 (5-37)	16 (3-29)	12 (7-31)

Note: Values are median (range). Mann-Whitney *U* Test, (2-tailed): * = Compared to age-group, >38 to 40 years, $P<0.05$.

showed any correlations to age.

Basal serum AMH was highly and significantly correlated to AFC, $r=0.778$, $P<0.001$, followed by FSH, $r=-0.340$ and LH, $r=0.250$, $P<0.05$. Among the ovarian reserve markers, only basal serum levels of AMH and AFC showed significant correlations to ovarian response parameters (number of retrieved, mature and fertilized oocytes and cleaved embryos) (Table 2).

Table 2
Correlations of basal serum anti-Müllerian hormone and antral follicle count to ovarian response parameters.

Parameters	Retrieved Oocytes	Mature Oocytes	Fertilised Oocytes	Cleaved Embryos
Anti-Müllerian hormone(μg/L)	0.570[a]	0.496[a]	0.427[a]	0.375[b]
Antral follicle count	0.523[a]	0.448[a]	0.383[a]	0.329[b]

Spearman's correlation coefficient, *r*. Significant value, [a]$P<0.01$, [b]$P<0.05$

Overall, 11% ($n=8$) of subjects demonstrated poor ovarian response to COS, normal response was 71% ($n=51$), and high response was 18% ($n=13$) (Table 3). The Poor responder group of women had significantly the lowest median basal serum AMH level of 1.25 μg/L and AFC of 7. The median fertilization rate, 41.7%, was not significantly different compared to the other responder groups. High responders had significantly the highest median basal level of AMH, 4.65 μg/L and AFC of 26. Figure 1, illustrates the box and whisker plots of AFC and ovarian response parameters among the various responder groups. The conventional basal endocrine ovarian reserve markers were not significantly different among the different responder groups.

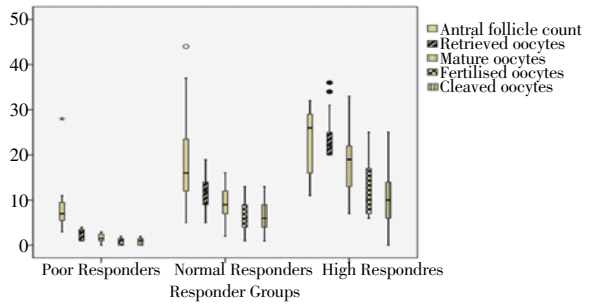

Figure 1. Comparison of antral follicle count and ovarian response parameters among the responder groups.
Box and whisker plot indicating the median (bold line), interquartile range (box) and 95% limit (whiskers).

Embryo transfer (ET) was performed on 66 subjects as 6 cycle cancellations occurred, due to fertilization failure in the Poor Responders ($n=3$) and mild OHSS in the High Responder group ($n=3$). The total positive pregnancy rate/ET cycle was 45.4% (30/66), clinical pregnancy/ET cycle was 37.9% (25/66) and live birth/ET cycle was 33.3% (22/66) (Table 3). Eighteen singletons, three sets of twins and a set of triplets were born, resulting in a total of 27 live births.

Both AMH and AFC showed significant discriminative abilities for poor ovarian response (optimal cut-off levels, AMH ≤2.0 μg/L and AFC ≤12) and for high ovarian response (optimal cut-off levels, AMH ≥3.2 μg/L and AFC ≥20) to COS treatment, as determined using ROC curve analysis and univariate logistic regression analysis (Table 4). Basal serum AMH was the only significant predictor for clinical pregnancy outcome, ROCAUC =0.71, at optimal cut-off level of ≥3.0 μg/L and odds ratio of 1.42. The other endocrine reserve markers (E_2, FSH and LH) were not predictive for poor or high response or clinical pregnancy outcome to COS.

When lower cut-off levels of AMH ≤1.0 μg/L, and AFC ≤8 were selected, the positive predictive values (PPV) and positive likelihood ratios (+LR) increased to the highest values for poor responders (Table 4). Likewise, selecting higher cut-off levels for AMH ≥5.0 μg/L and AFC ≥27, the PPV and +LR increased to the highest values for high responders. At AMH levels of ≥4.5 μg/L, the PPV for clinical pregnancy increased from 51.4% to 64.7% and the +LR increased from 1.7 to 3.0.

Table 3
Comparison of basal characteristics, ovarian parameters among the responder groups.

Parameters	Poor responder(n=8)	Normal responder (n=51)	High responder(n=13)
Age (years)	35.5(29.1-38.1)	34.6(28.5-39.7)	34.9(29.3-38.6)
Body mass index (kg/m^2)	21.0(19.0-37.0)	22.4(17.0-36.2)	23.0(20.0-35.0)
Duration of subfertility (years)	4.0(1-8)	5.0(1-13)	4.0(1-10)
Anti-müllerian hormone (μg/L)	1.3(0.12-3.74)*	3.0(0.85-15.37)**	4.7(1.95-13.86)***
Estradiol (pmol/L)	116.0(18.4-190.0)	125.0(57.6-316.0)	132.0(103.0-252.0)
Follicle stimulating hormone(IU/L)	8.5(2.1-11.9)	6.3(3.4-10.3)	7.7(4.8-9.3)
Luteinising hormone(IU/L)	4.8(0.2-9.3)	3.9(1.3-11.9)**	4.9(2.4-8.1)
Antral follicle count	7(3-28)*	16.0(5-44)	26.0(11-32)***
Duration of stimulation (days)	10.0(7-14)	11.0(8-16)	12.0(9-13)
Starting dose (IU)	450.0(150-450)*	300.0(100-450)	225.0(150-450)***
Total dose (IU)	4500.0(2625-5850)*	3300.0(1000-5850)	2350.0(1350-5850)
Number of retrieved oocytes	3.0(1-4)*	12.0(5-19)**	22.0(20-36)***
Number of mature oocytes	1.5(0-3)*	9.0(2-6)**	19.0(7-33)***
Number of fertilized oocytes	1.0(0-2)*	6.0(1-13)**	10.0(6-25)***
Number of cleaved embryos	1.0(0-2)*	6.0(1-13)**	10.0(0-25)***
Fertilization rate (%)	41.7(0-100)	80.0(18.2-100)	75.0(30-100)
Positive pregnancy/ET (%)	1.0/66(1.5)	23.0/66(34.8)	6.0/66(9.1)
Clinical pregnancy/ET (%)	0.0/66(0)	20.0/66(30.3)	5.0/66(7.6)
Live birth/ET (%)	0.0/66(0)	18.0/66(27.3)	4.0/66(6.0)

All values are in median (range). ET = embryo transfer; Significant difference (P<0.05) for comparison of Poor versus normal responder (*);Normal versus High responder (**), Poor versus High responder (***).

Table 4
Significant predictors and diagnostic characteristics of poor response, high response and clinical pregnancy outcome to control ovarian stimulation in Asian women.

Parameters	Odds ratio P value (95% CI)	ROCAUC P value (95% CI)	Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR
Poor Response								
Anti-Müllerian hormone (μg/L)	4.60 P=0.009 (1.47-14.42)	0.86 P=0.001 (0.68-1.00)	≤2.0	87.5	73.4	29.2	97.9	3.3
			≤1.0	37.5	98.4	75.0	92.6	23.4
Antral follicle count	1.28 P=0.008 (1.07-1.53)	0.86 P=0.001 (0.00-1.00)	≤12.0	87.5	75.0	30.4	98.0	3.5
			≤8.0	75.0	95.3	66.7	96.8	16.0
High Response								
Anti-Müllerian hormone (μg/L)	1.26 P=0.010 (1.06-1.51)	0.75 P=0.005 (0.62-0.89)	≥3.2	69.2	59.3	27.3	89.7	1.7
			≥5.0	46.2	89.8	50.0	88.3	4.5
Antral follicle count	1.08 P=0.039 (1.00-1.16)	0.71 P=0.022 (0.56-0.85)	≥27.0	61.5	69.5	30.8	89.1	2.0
			≥20.0	46.2	84.7	40.0	87.7	3.0
Clinical pregnancy								
Anti-Müllerian hormone (μg/L)	1.42 P=0.008 (1.09-1.84)	0.71 P=0.004 (0.59-0.84)	≥3.0	72.0	58.5	51.4	77.4	1.7
			≥4.5	44.0	85.4	64.7	71.4	3.0

ROC = Receiver operating characteristic, AUC = Area under curve, (CI) = Confidence Interval; PPV =Positive predictive value, NPV = Negative predictive value; +LR: Likelihood ratio of a positive test = Sensitivity/(1-Specificity).

4. Discussion

In this study on Asian women who underwent COS at our Centre in Singapore, we observed basal AMH level and AFC declined to the lowest median levels of 1.60 μg/L and 12, respectively, after the age of 38 years. The AFC of 12 in the women of age-group 38 to 40 years was found to correspond with the optimal cut-off level of the Poor Responders group of women. Thus, both AMH and AFC are reliable markers of ovarian reserve and aging for Asian women. AMH levels have been shown to decline progressively in parallel with follicle pool with advancing age; the decline starts earlier than the conventional

markers of ovarian reserve[13-15]. Asian women requiring ART should be advised to start COS regime at an earlier age preferably before 38 years, as our study has shown the decline in ovarian reserve with advancing age.

Our study suggests that both AMH and AFC were similarly associated with ovarian response outcome to COS, *i.e.* the number of retrieved oocytes, as noted previously by La Marca and colleagues[16]. In their review, AMH was either equal to AFC or was a better marker than AFC, age of patients, E_2, FSH, or inhibin B, with correlation coefficients (r) of AMH to number of retrieved oocytes ranging from 0.33 to 0.88.

Low levels of AMH were predictive of poor response to COS, which frequently lead to cycle cancellation, either due to inadequate response to stimulation or fertilization failure[16,17]. In contrast, high levels of AMH (indicative of excessive response) could lead to cycle cancellation due to risk of life-threatening OHSS[16,18]. In our study population, both basal serum AMH and AFC showed similar predictive accuracies (ROCAUC) for poor and high response to COS[13,16-18].

A recent study involving infertile couples undergoing COS in Taiwan, basal serum AMH was a significant discriminator for OHSS (ROC_{AUC} of 0.902), at a cut-off level of 3.36 μg/L. Age was the only significant predictor for clinical pregnancy outcome (odds ratio 0.9436)[19]. In our Asian study cohort, basal serum AMH was discriminative for high response and OHSS at cut-off level of ≥3.20 μg/L (odds ratio of 1.26) and for clinical pregnancy at a cut-off level of ≥3.0 μg/L (odds ratio of 1.42). Other studies have reported that AMH at cut-off levels of 18 pmol/L (approximately 2.5 μg/L) was predictive for ongoing pregnancy, and 2.7 μg/L for clinical pregnancy[20, 21].

Nelson and colleagues have demonstrated basal AMH as a better predictor of excessive response (ROC_{AUC} =0.90) and live births (ROC_{AUC} =0.62) and demonstrated a significant relationship between increasing basal AMH quintiles to oocyte yield and live birth rates[22]. A follow-up study at two centres using AMH predetermined values (AMH <1.0, 1.0 to <5.0, 5.0 to <15.0 and ≥15.0 pmol/L) to optimize treatment strategies for COS reported reduced clinical risks and improved clinical pregnancy rates[23].

We propose using, either AMH (cut-off level of ≤1.0 μg/L) or AFC (≤8), for categorizing or identifying poor responders in Asian women, as the positive predictive values (PPVs) of these cut-off levels were the highest at 75% or 66.7%, respectively, with corresponding positive likelihood ratios (+LRs) of 23.4 or 16.0, respectively. Cut-off levels of either AMH ≥5.0 μg/L (PPVs were highest at 50% and +LRs were also at highest of 4.5) or AFC ≥27 (PPV were highest at 40% and +LR highest of 3.0) could be used for categorizing or identifying high responders (Table 4). Further study involving a larger cohort is needed in optimizing ART based on these proposed cut-off levels to determine ovarian response outcomes and potential risks to COS.

In conclusion, our current study data has demonstrated both basal AMH and AFC were reliable markers of ovarian aging and was significant predictors of poor and high ovarian response to COS for the Asian women. Basal AMH was the only predictive marker of clinical pregnancy outcome.

Conflict of interest statement

We declare that authors have no potential conflicts of interest to disclose.

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