The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach

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ABSTRACT

CONTEXT: Reduced ovarian response to stimulation represents one of the most intractable problems in infertility treatment. As failed cycle can cause considerable amount of emotional and economical loss, there are various attempts made to predict ovarian response. **AIMS:** To evaluate different factors influencing outcome of assisted reproduction in women with predicted reduced response (antimullerian hormone between 1 and 5 pmol/L) and to develop a model using of AMH and age to predict the number of oocytes in poor responders. **SETTINGS AND DESIGN:** Retrospective study in a teaching hospital. **MATERIALS AND METHODS:** We analyzed 85 cycles (57 women) with predicted reduced response with serum AMH value between 1 and 5 pmol/L. Standard ovarian stimulation protocol was used. Primary outcome measures were clinical pregnancy rates and oocytes retrieved. **STATISTICAL ANALYSIS USED:** Data were analyzed using Microsoft excel and MetlabR software. **RESULTS:** Clinical pregnancy rate/ET was 20.33%, in this group. AMH and age was analyzed using linear regression model which produced an equation to give predicted oocyte count if AMH and age are known. (Oocytes = age × (-ß) + Serum AMH × α) (Constant ß=0.0102 and α = 1.0407). **CONCLUSIONS:** Combined use of serum AMH and age to predict ovarian response within reduced responder group should be further evaluated. For first time, we suggested combining both factors to predict ovarian response using a simple equation which allow developing tailored strategy.

KEY WORDS: Ovarian hyperstimulation, poor responders, serum antimullerian hormone

INTRODUCTION

Failed-assisted conception cycle causes considerable amount of emotional and economical loss. There are various attempts made to predict reduced response. Evidence has shown convincingly that poor ovarian response is a first sign of ovarian ageing.[1] The Bologna criteria[2] define the poor response as presence of two or more features (i) advanced maternal age or any other risk factor for poor ovarian response; (ii) a previous poor ovarian response; and (iii) an abnormal ovarian reserve test. Two episodes of poor ovarian response after maximal stimulation deemed sufficient to define a patient as poor responder in the absence of other criteria. Ovarian ageing can occur independently of chronological age.[3] AMH suggested being a better marker of ovarian responsiveness, as it reflects the size of the larger resting pool of prefollicle-stimulating hormone (FSH)-dependent follicles. AMH is a more direct and independent measure of the growing preantral and antral follicular pool.[4-6] Thus age and serum AMH are the most successful predictors of reduced ovarian response. Our aim was to evaluate the role of age and AMH influencing outcome of assisted reproduction in women with predicted reduced response (AMH between 1 and 5 pmol/L) and to develop a model using of AMH and age together to predict the number of oocytes in poor responders. Such model will help clinicians to predict the response and design appropriate protocol. It will also help in patient counselling and advice on alternative management options.

MATERIALS AND METHODS

We have analyzed 85 cycles undergoing controlled ovarian stimulation for IVF or ICSI cycle. These women were treated for either primary or secondary infertility at teaching hospital. All women included were predicted reduced responders with serum AMH value between 1 and 5 pmol/L. We defined reduced responders based on AMH level between 1 and f5 pmol/L based on previous study by.[7] There was no age cut off. Population was from different ethnic background. We have excluded all cases with AMH 5 pmol/L and above as they proved to have normal response and different strategies were used in these cases. We have excluded women with AMH of less than 1 pmol/L, as they demonstrated to have maximum cycle cancellation and no pregnancy occurred in this group. Ovarian stimulation was performed with exogenous gonadotropins initiated on the third or forth cycle day in the form of either Menogon (Ferring Pharmaceuticals, Langley, UK) or Gonal-F (Serono, Feltham, UK). The starting daily dose of FSH was either 225 or 300 IU each day. Ovarian follicular responses were monitored with serum E2 concentrations and transvaginal ultrasound assessment of follicular growth. The GnRH antagonist Cetrotide 0.25 mg/day s.c. (Merck Serono Feltham, U.K.) or Ganirelix was commenced on days 4-7 if serum E2 exceeded 200 pg/mL. Follicular responses were monitored with serum E2 and transvaginal ultrasound assessment of follicular growth. Ovulation was induced with 6500 IU HCG (Ovitrelle, Serono, Feltham, UK), provided that three follicles were 17 mm in diameter and serum E2 was 200 pg/ml. Trans-vaginal oocyte retrieval was performed under ultrasound guidance 38 h after HCG administration and the number of oocytes retrieved were recorded. Women were either offered IVF-ET or ICSI-ET. The study analysis includes only fresh cycles.

Primary outcome studied was clinical pregnancy rates and secondary outcomes were positive pregnancy rate, oocytes retrieved, and cancellation rate, duration of stimulation, and FSH drug consumption.

The AMH assay was performed in batches one month before treatment cycle using the enzyme-linked immunosorbent assay provided by DSL in study period 2011 (Webster, Texas, USA), with values presented as pmol/L (conversion factor to pmol/L¼ng/mL_7.143). The Reduced responders are defined as predicted with low serum AMH (serum AMH between 1 and 5 pmol/L) and cycle cancellation is defined as less than two matured sized follicles after 2 weeks of stimulation with maximum dose of gonadotropins. Embryo cryopreservation was done with more than one high grade embryo remained after fresh embryo transfer. Data were analyzed using Microsoft excel and MetlabR software.[8,9] Data were presented in descriptive statistic. The factors influencing outcome of assisted conception were analysed using linear regression model. A simple matrix formula was derived to calculate predictive response when these factors are known.

RESULTS

The baseline demographic characteristics and outcome are shown in Table 1. The AMH level was ranging from 1.1 to 4.9 as per inclusion criteria. Among 44 eggs retrieved, two failed to fertilize in IVF cycle. A total of 29 (34.1%) cycles were treated by ICSI. Thus, fertilization rate was 91.6% in ICSI cycles. Duration of stimulation was from 4-14 days and from 675 IU to 5100 IU FSH was used. Out of 85 cycles started, eight (9.41%) were cancelled due to poor response. A total of 59 embryos were transferred; two embryos were frozen along with ET in those women. All cancelled cycles are shown in Table 2. Clinical pregnancy was achieved in 12 cases. Characteristics are discussed in Table 3.

Thus, there was no significant difference between these groups. Lower AMH value showed trend toward cancellation and higher AMH value showed trend toward clinical pregnancy in this group. We have analyzed age and AMH, using linear regression model.

The factors Age and AMH were put in linear regression model to produce a solution.

Table 1: Demographic characters

Number of patients	
cycles	85
Age at stimulation (years)	39 (31-46)
BMI (kg/m^2)	24.7±4.6
AMH (pmol/L)	2.9 (1.1-4.9)
Procedure	
IVF (%)	56 (65.9)
ICSI (%)	29 (34.1)
Length of stimulation (days)	10 (4-14)
Total dose (IU)	2100 (675-5100)
Cancelled cycles **	8 (9.41%)
Number of oocytes	3 (0-15)
Number of embryos transferred	59
Normal fertilization rate %	
IVF (%)	37 (94.8)
ICSI (%)	22 (91.6)
Cohort outcomes	
No transfer[ß]	26 (30.5%)
Not pregnant	42 (49.4%)
Positive pregnancy test[δ]	17 (20%)
Clinical pregnancy	12 (14.1%)
Clinical pregnancy per OR	16%
Clinical pregnancy per ET	20.33%
Clinical pregnancy per cycle	14.11%

Values are presented as median (interquartile range) or mean±standard deviation
**Percentage per cycle started, (*n*=85). [ß]Includes women with cancelled cycle; no eggs or failed fertilisation; [δ]Includes clinical and biochemical pregnancy. AMH=Antimullerian hormone; BMI=Body mass index; ET= Embryo transfer; IU=International units; ICSI=Intra-cytoplasmic sperm injection; IVF= *In vitro* fertilization; OR=Odds ratio

Table 2: Cancelled cycle and all cycles characters

Characteristics	All	Cancelled cycles
Total	85	8
Age	39 (31-46)	39.9 (35-45)
BMI	24.7±4.6	25.9±4.6
Amh	2.9 (1.1-4.9)	2.2 (1.1-3.3)
Total dose of FSH	2100 (675-5100)	1162.5 (675-4200)
Duration of FSH	10 (4-14)	7.5 (7-10)

BMI=Body mass index; FSH=Follicle-stimulating hormone

Table 3: Clinical pregnancy and all cycle characteristics

Characteristics	All	Clinical pregnancy
Total	85	12
Age	39 (31-46)	37 (35-39)
BMI	24.7±4.6	24.5±6
AMH	2.9 (1.0-4.9)	3.15 (1.2-4.7)
Total dose of FSH	2100 (675-5100)	2287.5 (1350-3600)
Duration of FSH	10 (4-14)	10 (8-14)
Number of oocyte retrieved	3 (0-15)	4 (2-8)

AMH=Antimullerian hormone; BMI=Body mass index; FSH=Follicle-stimulating hormone

$$\text{⚜} = \begin{pmatrix} age \\ AMH \end{pmatrix}$$

$$y = oocytes$$

$$y = \beta^T X$$

Where, $\beta = \begin{pmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{pmatrix}$ we used the dataset to estimate β.

The solution is $\hat{\beta} = YX^T\left(XX^T\right)^{-1}$ where X is a 2 × 85 matrix and Y is a 1 × 85 matrix.

Finally we got $\hat{\beta} = \begin{pmatrix} -0.0102 \\ 1.0407 \end{pmatrix}$

Thus with this model when age and AMH is known (⚜), oocytes yield can be predicted by formula: $y^* = \hat{\beta}^T \mathrm{x}^*$.

For example, woman with age 33 years and AMH value 2, the predicted response (oocyte yield) can be calculated as

$$\mathrm{x}^* = \begin{pmatrix} 33 \\ 2 \end{pmatrix} \text{ and } \hat{\beta} = \begin{pmatrix} -0.0102 \\ 1.0407 \end{pmatrix}$$

$$\text{oocytes} = y^* = \hat{\beta}^T \mathrm{x}^*$$

so oocytes = 33× (- 0.0102) + 2 × 1.0407

Thus this model, with use of simple calculator, gives approximate numbers of oocytes number two or three.

DISCUSSION

The evaluation and tailored treatment strategy for poor responder group is important to improve overall pregnancy rates. Unfortunately, lack of uniform criteria to define this group, lack of evidence to predict outcome in this particular group, and debated treatment strategy are major influencing factors on outcome. This group of women are usually with older age and more anxious to get pregnant than younger age group. Previous failed treatment cycles add in frustration of couple and clinician. Predicting response allows tailoring individual treatment strategy. This approach was suggested by.[7] We have further extended it for poor responder group. Our results show increasing AMH value is associated with increasing oocyte yield in this reduced responders group which is consistent with previous study.[7] Our results show that combining two strong predictors allow clinicians to develop a model for predicting ovarian response. This will help clinicians to counsel patient, design protocol for optimal outcome. Reduced responders with predicted poor response using various factors including age and AMH will allow opting for natural cycle IVF. Other options like oocyte donation, adoption, could be discussed with couple without subjecting ovarian stimulation. At same time predicted reasonable response will allow to go ahead with antagonist and stimulation protocols. Systematic review supported antagonist protocol in this group Pandian and Marci *et al*.[10,11] Hence, prediction of response is significant.

Age is always labelled as significant factor affecting fertility[12,13] but biological aging and ovarian aging are not always same.[3] Various attempts to develop ideal predictive test for ovarian reserve were failed.[14] At same time, AMH emerged as a promising option for predicting both oocyte number and quality.[15] AMH showed definitive advantages over other bio markers.[5,6] It has linear relationship with age.[4] Previous studies clearly established linear relationship between AMH and oocyte yield using AMH and age to develop a linear regression model for oocyte yield is a justified approach based on past evidence. The accuracy of multivariate models for the prediction of ovarian reserve and pregnancy in women undergoing IVF compared with the antral follicle count (AFC) as single test was reviewed by Verhagen *et al*.[16] He reviewed age + FSH, age + inhibin B, age + AFC and different combinations for predictive model. He concluded the use of more than one single test for the assessment of ovarian reserve cannot be supported. Thus, the models incorporating inhibin B, FSH, ovarian volume, clomiphene challenge test, and so on failed to deliver successful prediction. Further evidence is required to support the multivariate model of AMH and age to predict ovarian response.

Other factors like body mass index, procedure, previous pregnancy, and duration of pregnancy were analyzed but failed to establish any significant relationship. There are various factors suggested to be significant predictors of clinical pregnancy such as age, serum E (2) concentration on the day of hCG administration, embryo quality, and number of embryos transferred sperm motility and ICSI operator.[17] It is practically impossible to establish relationship between all factors at a time. Hence, we considered most significant

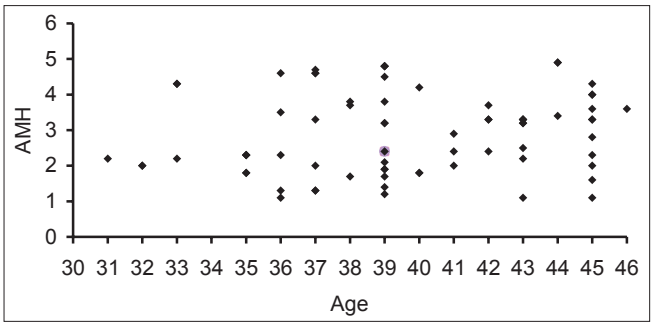

Figure 1: Age and AMH relationship

factors particularly affecting subgroup of women labelled as reduced responders and analysed to get a user friendly predictive model.

This study evaluated the relationship between two important factors, age, and AMH, influencing outcome of assisted reproduction in reduced responder group. The relationship between age and AMH is shown in Figure 1. As figure shows the value of AMH is not same within same age group. Hence it is crucial to have both predictors taken into the consideration whilst predicting ovarian response. This figure illustrates the importance of having both predictors that is age and AMH calculated together to predict more accurate response. All previous studies analyzing predictive potential were done in normal, reduced, and high responders with many variables at same time. In present study, we analyzed combined predictive potential of AMH and age affecting outcomes. This study has relatively small population size and it is a major limitation. There is a need to focus on this particular group of women to improve outcome without adding any financial burden or emotional stress. This can be achieved only with good prediction of response and individualizing therapy accordingly. Current evidence favors antagonist approach to treat reduced responder group. Current evidence supports AMH and age as good predictors of ovarian response over any other single or multiple available predictors. We have tried to introduce new approach of combining both factors using a simple equation. We accept the limitation of having small number of study subjects to draw any conclusions based on this study alone. Hence, further studies are required to establish strong evidence before recommending combination of age and AMH prediction model and consequent individualized treatment strategy in practice.

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