

Evaluation of AMH as a Predictor of Clinical Pregnancy and Live Birth Rates and Prognostic Value of AMH Levels in Patients with Poor IVF Outcomes

Running title: AMH Predictor of Clinical Pregnancy and Live Birth Rates

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Abstract: Background: Although Anti-mullerian hormone (AMH) is known to predict ovarian reserve, there is conflicting evidence regarding the association between AMH and clinical pregnancy rate (CPR) or live birth rate (LBR). In an attempt to resolve conflicting findings, the current study was undertaken to analyze CPR and LBR utilizing only AMH as predictor and to evaluate that AMH levels can be used to prognosticate patients of poor IVF outcomes. Materials and Methods: The present study was a retrospective cohort study conducted on 172 women between the age group of 20 -40 yrs. AMH was assayed on any day of the menstrual cycle at patient's convenience using the Generation II AMH (Gen-II AMH assay) enzyme-linked immunosorbent assay (ELISA) kit. All AMH testing was done within 3 months of commencing index IVF cycle. When there were at least 3 leading follicles measuring ≥ 17 mm, recombinant human chorionic gonadotrophin was administered subcutaneously. Progesterone injections were started on the day of oocyte retrieval. If the test was positive, an early pregnancy ultrasound scan was carried out at 6 - 7 weeks gestation to confirm the status of the pregnancy. CPR rate was calculated only when ultrasound appearance of gestational sac is seen. Results: No case of ovarian hyper-stimulation syndrome (OHSS) was reported in this non-high risk population for OHSS. The mean FSH was significantly high in low AMH group ($p=0.005$). The average number of oocytes retrieved was significantly higher in normal AMH group ($p=0.002$). A strong positive correlation was established between oocytes with AMH ($r=0.343$, $p<0.001$). Non-significant increase in pregnancy rate (6%) was observed with AMH > 3.19 ng/ml. Conclusion: The present study concluded that AMH should not be used to counsel patients of occurrence pregnancy especially in good prognosis patients (<35 years). Instead it is a very good marker for oocyte yield and it should be used to make patients aware about their chances of cancellation of cycle. It can be safely said that if a patient with low AMH proceeds with oocyte retrieval-embryo transfer with her own oocytes, her chances of pregnancy are as good as patients with normal AMH.

Keywords: In-vitro fertilization (IVF), Pregnancy, Anti-Mullerian hormone (AMH), Clinical pregnancy rate

1. Introduction

There is an exponential rise in the number of *in vitro* fertilization (IVF) cycles worldwide owing to many factors of infertility (combined male and female) including ovulation dysfunction, endometriosis, sperm abnormalities, reduced ovarian reserves and tubal pathology (1). The fertility potential of women falls beyond 35 years of age, with a further decline after 40 years of age. The underlying reasons are the decreased quantity as well as quality of oocytes (2, 3).

Predicting pregnancy outcome during assisted reproductive technology (ART) procedures is a desire for both care-providers and couples undergoing the treatment to reduce the economical and psychological burden(4). In-vitro fertilization (IVF) procedure outcomes cannot be predicted directly as it involves quantity and quality of gametes, maternal conditions, IVF lab conditions and the uterus. Pregnancy outcomes can be measured indirectly by assessing quantitative ovarian reserve. There are numerous markers of ovarian reserve testing (ORT), i.e. follicle stimulating hormone (FSH), oestradiol, inhibin B, anti-Mullerian hormone (AMH) and antral follicle count

(AFC). However, these markers have some limitations in predicting pregnancy outcomes. FSH is poor marker to predict ovarian response(4). AFC measurement is subjective in nature and is limited by the availability of ultrasonography machine and skilled sonographer. There is no standard cut-off for AFC to predict low response and the ever changing technology had led us to measure AFC upto 2mm in diameter. These limitations of AFC along with inter-observer variability make AMH a feasible marker to predict oocyte yield.

AMH, also called Müllerian inhibiting substance or factor, a member of the transforming growth factor- β (TGF- β) family, is essentially involved in the regression of Müllerian ducts in the male fetus, the initial step of organogenesis of the male genital tract. It appears to be the most robust clinical measure of the number of small antral follicles ready for ovarian stimulation recruitment during IVF (5). AMH and AFC have comparable performance in predicting ovarian response (6, 7) in majority of observational studies but in some AMH was demonstrated better than AFC(8). Few studies with small sample size reported that AMH concentration changes during menstrual cycle (9) but it is a generalized consensus that there is a non-significant

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intracycle and intercycle variation in the values of AMH and thus can be measured at any stage of the menstrual cycle. This makes AMH a favorable marker for ovarian reserve (10, 11)

AMH stands out as a predictor of ovarian response beyond doubt; however, studies on correlation between AMH and live birth rate (LBR) in IVF cycles have shown conflicting results. While some studies show a positive correlation between AMH and LBR, (12, 13) others show a limited predictive value for serum AMH in relation to clinical pregnancy rate (CPR) (11, 14).

So far, AMH has not been found to be an independent predictor of LBR. In an attempt to resolve these conflicting findings, the current study was undertaken to analyze CPR and LBR utilizing only AMH as predictor. Further, we evaluated the role of AMH levels for prognosis of patients with poor IVF outcomes.

2. Materials and Methods

Study Design

This was a retrospective cohort study conducted at IVF and Reproductive biology Centre, INTERNATIONAL FERTILITY CENTRE, New Delhi.

Inclusion and Exclusion Criteria

Only women (20-40 years) who underwent first IVF/ICSI non-donor cycle with regular menstrual pattern were included in the study. Some of these women had subsequent cycles during the study period. Subsequent cycles were not included during the study period. Only women with complete data records were included in the study. All the fresh IVF/Intracytoplasmic sperm injection (ICSI) cycles from May 2015 to May 2017 were retrospectively analyzed and recorded from the electronic database and patient records. Women with polycystic ovarian syndrome (PCOS) were excluded; as it is known that PCOS is associated with significantly elevated basal AMH concentrations and sometimes need to freeze all embryos in PCOS. Some non-donor 200 fresh IVF/ICSI cycles, with own oocytes were performed during the study period.

Hormone Measurements

AMH was assayed on any day of the menstrual cycle at patient's convenience using the Generation II AMH (Gen-II AMH assay) enzyme-linked immunosorbent assay (ELISA) kit (2015 Beckman Coulter, Inc. USA). AMH values are reported in ng/ml. All AMH testing was done within 3 months of commencing index IVF cycle.

Stimulation Regimen

Women were treated using the standard operating procedure of the unit and either of the antagonist or agonist protocol was used. AMH tailored step up protocol was followed with standard dose of 225 IU FSH (highly purified FSH) for AMH values 2.0 g/ml and above. When there were at least 3 leading follicles measuring ≥ 17 mm, recombinant human chorionic gonadotrophin (rHCG; Ovitrelle, Merck-Serono, UK) 6500 IU was administered subcutaneously. Cycle cancellation was discussed if fewer than three follicles

developed after at least two weeks of COH with maximum dose of FSH.

Ovarian Retrieval and Embryo Transfer

Transvaginal ultrasound guided oocyte retrieval was performed under general anesthesia, 34 - 36 hours after the hCG injection using single lumen 17 G ovum pick up (OPU) needle. Routine insemination of oocytes using direct swim up or density gradient centrifugation or intracytoplasmic sperm injection (ICSI) was done depending upon semen parameters. Progesterone injections intramuscular or subcutaneous were started the day after oocyte retrieval. As per our local policy, a single or double embryo on Day 2 - 3 or a single blastocyst on Day 5 - 6 is transferred by soft embryo transfer catheter depending upon the woman's age and number of available embryos. Urinary pregnancy test and serum β hCG is performed two weeks after embryo transfer, if no menstrual period followed. If the test is positive, an early pregnancy ultrasound scan is carried out at 6 - 7 weeks gestation to confirm the status of the pregnancy. CPR is calculated only when ultrasound appearance of gestational sac is seen. Negative pregnancy test, biochemical pregnancy and ectopic pregnancy were counted as negative result.

Measurement of outcomes

A total of 172 subjects with complete data records were identified. This data also had subjects whose cycles were cancelled due to no response and rescued IUI cycles. Outcome of these was not available. CPR, LBR and cancellation rate (CR) we recalculated initially for overall data including cancelled cycles. As every laboratory has its own AMH reference values for low, normal and high levels; AMH < 2 ng/ml is taken as low in our unit and AMH > 7 ng/ml is taken as high. Further, whole data was divided in two groups of low AMH < 2 ng/ml and normal AMH ≥ 2 ng/ml. Across each AMH group, number of oocytes retrieved, number of days of stimulation, CPR and LBR per embryo transfer we recalculated. Later, data was divided in 3 age groups of < 30 years, 30-34 years and 35 years and above to access effect of age on pregnancy and LBR.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 15.0 (SPSS, USA). Categorical data were expressed as number and percentage, and the numerical data as mean \pm SD. Independent sample t-test, Fisher's exact test, χ^2 test and Mann-Whitney U tests were used where appropriate. A *p* value of less than 0.05 was considered significant.

3. Results

Study Population

Overall, 200 women underwent fresh donor IVF cycles. After excluding subjects with PCOS and patients with AMH > 7 ng/ml, a total of 172 subjects were identified where AMH records were available. In all, 15.7% cycles got cancelled during stimulation period. A total of 145 subjects underwent ovum pick up and embryo transfer procedure in first IVF cycle. Baseline characteristics of the subjects are as shown in **Table 1**. The mean age of study subjects was 29.7 ± 10 years. No woman above 40 years was included in the study.

AMH Assay

Main causes of infertility in the study population were tubal and peritoneal factor infertility (33%), male infertility (30%), unexplained (20%), diminished ovarian reserve (8%), severe endometriosis (7%) and hypogonadotropic hypogonadism (2%). Overall, 60% subjects had primary infertility and 40% had secondary infertility. No cases of ovarian hyperstimulation syndrome (OHSS) were reported in this non-high risk population for OHSS. The data was analyzed in two groups on the basis of AMH levels, i.e., low AMH and normal AMH as shown in **Table 2**. The mean FSH was significantly high in low AMH group ($p=0.005$). The average number of oocytes retrieved was significantly higher in normal AMH group ($p=0.002$). A strong positive correlation was established between oocytes with AMH ($r=0.343$, $p<.001$) **Figure 1**.

Evaluation of Pregnancy Outcomes:

On calculating clinical pregnancy, no statistical significance difference was observed between low and normal AMH group. CPR per embryo transfer was high in low AMH group as shown in **Table 3**. LBR being final end point of IVF cycle was again not statistically different in the two groups. Overall, 25% cycles got cancelled in low AMH group as compared with 12% cancelled in normal AMH group with a difference of 13% which was statistically significant ($p=0.011$). Miscarriage rate was non-significantly lower in normal AMH group. LBR and CPR did not vary significantly in various age groups as the study population had a lower proportion (11.3%) of women of advance age group (>35). Predictive value of AMH was observed as 3.10 ng/ml. Non-significant increase in pregnancy rate (6%) was observed with AMH > 3.19 ng/ml.

4. Discussion

AMH concentration and AFC are two increasingly popular static measures used to predict ovarian response prior to IVF treatment. Both can reliably predict poor and hyper response (15). Response prediction helps patients to decide the reliable estimate of most important aspect of IVF-ET i.e. Oocyte yield. A significant positive relationship exists between IVF CPR and the quantitative ovarian reserve as measured by the serum AMH. However, the strength of the association is modulated by patient's age (16).

Elgindy *et al* showed that AMH levels varied only marginally across menstrual cycle, which was not significant and early follicular, ovulatory and mid luteal AMH levels correlated well with clinical pregnancy (17). According to the American Society of Reproductive Medicine (ASRM) committee opinion, it was concluded that AMH is a useful tool to predict ovarian reserve in the general IVF population, as well as for women at a risk of diminished ovarian reserve (18).

The cut off levels of AMH for poor response vary as per the available literature. The value $<0.5-1.1$ ng/ml is considered as a predictor of poor response as per the Bologna criteria (19).

A Bologna criterion is in fact very strict in terms of definition of poor responders. Many new studies including

ESTHER study used higher cut off for AMH to define poor response (20). Our study too utilized AMH value as 2ng/ml as the women below .5ng/ml were very few to bring about meaningful conclusions. Our study could not identify any association between age and live birth in contrast to many studies including the most recent one by Goswami (21). This could be the younger age at marriage and early presentation of infertility in this part of world. Ovarian ageing of Indian women is few years early compared to European women as pointed by Iglesias *et.al.* and the majority of population in our study was in age group <35 years. Several studies have shown a positive correlation between basal AMH concentrations and oocyte yield following ovarian stimulation, so is in our study it was significantly correlated with oocyte yield (22).

The earlier assumption was that AMH does not predict LBR (23, 24).

In the present study, the average number of oocytes retrieved was significantly higher in normal AMH group and a statistically significant difference for pregnancy outcomes between low and normal AMH group was observed. FSH was negatively correlated with AMH. Also, LBR being final end point of IVF cycle was not statistically different in two groups. There was no significance in LBR and CBR in advance age group greater than 35 years. AMH levels also negatively correlated with female age, as already known (25). Researchers have reported that age-dependent predictability of AMH is possibly related to the age-related decline in oocyte quality and quantity (26).

Previous studies reported that AMH had a weak association with CPRs in ART, but had considerable predictive accuracy in poor ovarian reserve cases (25). In our study, AMH levels did not correlated with LBR which is in contrast with the recent meta-analysis which concluded that AMH correlates well with the cumulative LBR in fresh and frozen cycles in the antagonist protocol (25). The probable reason for this is we took only fresh cycles in account, if may be cumulative outcome of both fresh and frozen cycles were taken into account the live birth rate increases in normal AMH vs low AMH group. Non-significant increase in pregnancy rate (by 6%) with AMH > 3.19 ng/ml for predicting pregnancy was observed in the present study. It may be possible that the AMH threshold level of 3.19ng/ml predicting pregnancy is related to the larger cohort of antral follicles in these women or higher AMH level being associated with better quality oocytes in comparison to other women in the same age group.

Ours study could find significant increase in cancelled cycles in low AMH group ($p<.05$) in accordance with study by Wang *et al.* (27) The notion that low AMH predicts very well poor response and it could never reach the stage of oocyte retrieval but once oocyte retrieval and embryo transfer is reached in low AMH group pregnancy and live birth rate did not differ in these patients in comparison to women with normal AMH as explained in recent study that low AMH is not a predictor of embryo quality (28)

Thus, AMH may have a role in predicting chance of conception after IVF along with other factors like age,

embryo quality, transfer technique, luteal support and endometrial receptivity which can independently affect the cycle outcomes.

Present study demonstrated that AMH alone cannot be a strong independent predictor of CPR and live birth rate. Young patients with lower AMH concentrations may be reassured about favourable pregnancy outcomes. A combination of low AMH and increasing age on the other hand may be looked upon less favourably.

5. Conclusion

The present study concluded that AMH is not a predictor of CPR and LBR. However it can very well predict oocyte response to gonadotropins. It can be useful to counsel women about their chances of cancelled cycles. In the present study, we observed that AMH value; ≥ 3.19 ng/ml signifies positive pregnancy but with very poor sensitivity and specificity. We support that preferential usage of AMH to counsel the patients regarding the probability of pregnancy should not be done; instead young women can be reassured of good pregnancy outcome following ART.

6. Acknowledgement

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7. Conflict of Interest

The authors do not have any conflict of interest.

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Table and Figures

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Table 3: Pregnancy outcome from fresh embryo transfer in the low AMH and high AMH
Figure 1: Correlation between number of oocytes and AMH levels in study subjects

Table 1: Baseline characteristics of the patients

Number of patients	Cancelled Cycles	Continued cycles	Clinical pregnancy rate	Live birth rate	Mean age (years)	Mean AMH(ng/ml)
172	15%(n=27)	145	44.14%(n=64)	33.1%(n=48)	29.7±3.5	3.2±1.8

The values are expressed as mean ±SD unless otherwise specified.

Table 2: IVF treatment parameters in the low AMH (<2pmol/l) and normal AMH (≥2pmol/l) in study groups

Parameter	Low AMH	Normal AMH	p value
Age (years)	30.78 ±3.44	29.30±3.48	0.011
AMH	1.17±0.58	4.08±1.51	<0.001
FSH	8.07±2.7	6.94±2.21	0.005
Days	10.67±2.27	11.57±2.64	0.034
No. of Oocytes retrieved	7.16±5.8	10.97±6.74	0.002

The values are expressed as mean ±SD unless otherwise specified.

Table 3: Pregnancy outcome from fresh embryo transfer in the low AMH and high AMH

Parameter	Low AMH	Normal AMH	P value
CPR per embryo transfer	18/38 (47.37%)	46/107 (42.99%)	0.320
LBR per embryo transfer	13/38 (34.2%)	35/107 (32.7%)	1.000
Cancellation rate from initiated cycles	13/51 (25.49%)	14/121 (11.57%)	0.011
Miscarriage rate	5/38 (13%)	11/107 (10.2%)	0.763

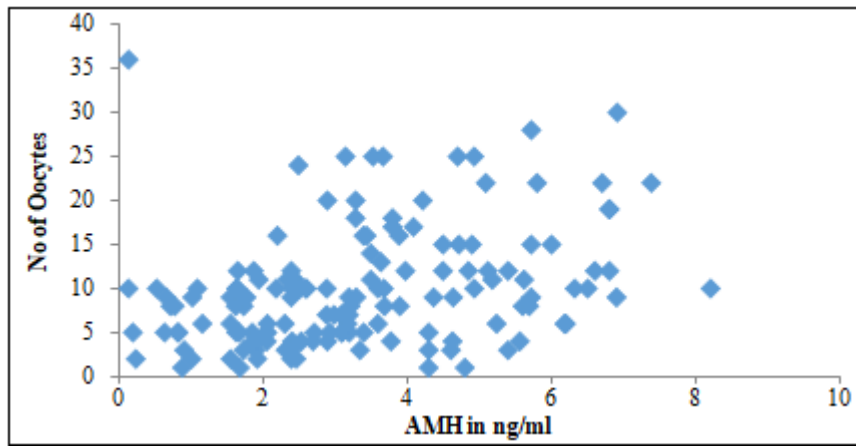


Figure 1: Correlation between number of oocytes and AMH levels in study subjects