Original Article

Anti- Mullerian Hormone as A Potential Predictive Marker of Estradiol Concentration In Controlled Ovarian Stimulation

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Abstract

Anti-Mullerian hormone (AMH) plays an important role in outcomes of controlled ovarian hyperstimulation (COS) and live birth rate following intracytoplasmic sperm injection. this study aims to analyze the effect of baseline AMH serum concentration on estradiol levels at the HCG administration day in infertile women enduring intracytoplasmic sperm injection.

Material and method :In this retrospective study, 39 women received a daily dose of 200–225 IU of recombinant FSH and cetrorelix at a daily dose of 0.25 mg before going through oocyte recovery. Levels of AMH were analyzed on cycle day two before the ovarian hyper-stimulation, their ovarian response was observed by conducting transvaginal ultrasonography and serum estradiol measurements on day 6 of the treatment cycle. Blood samples were collected on the morning of the second day of the cycle to determine the baseline serum AMH, FSH, LH, and estradiol levels. Additionally, a blood sample was collected on the morning of the HCG injection to determine the estradiol plasma levels.

Result :there was a significant elevation of serum estradiol concentration in women with a higher level of AMH. The basal serum AMH and estradiol levels on the day of HCG injection showed a significant moderate positive correlation. Estradiol levels on the day of HCG injection significantly correlated negatively with patient age and, BMI.

Conclusion :The baseline AMH level is a much more reliable predictor of ovarian responses in COS than basal serum estradiol, BMI and age. Therefore, the AMH level can be used as a marker of ovarian reserve.

Keywords: Anti-Mullerian hormone (AMH), estradiol, controlled ovarian hyperstimulation.

1. INTRODUCTION

Gonadotrophin-releasing hormone agonists (GnRH-a), which were developed in the mid-1980s, effectively eliminated the disadvantage of an early luteinizing hormone (LH) surge. Human gonadotrophin (HMG), which has equal amounts of follicle-stimulating hormone (FSH) and luteinizing (LH), has gradually been replaced by pure urine-derived FSH preparations and finally by recombinant human FSH (rFSH), which has no LH activity [1].

Most stimulation procedures now begin with daily doses of 150–300 IU of gonadotropin, with 225 IU being the average starting dosage for most infertile females [2]. The possible gonadotropin dosage for most women who undergo Assisted reproductive technology (ART) is 150–300 IU per day, with a daily maximum of 450 IU. The drug dosage is frequently determined by the patient's age and some indicators of ovarian reserve, with 150 IU assigned to young females who are projected to have vigorous reactions and higher doses assigned to older females who are likely to respond poorly. Aside from a limited range of daily gonadotropin dosage in controlled ovarian stimulation (COS), the approach to COS will likely remain patient-centered, influenced by clinical experts, and based on a continuous evaluation of published research [3].

Clinically, it is important to distinguish between young women with a diminished ovarian reserve and older women who have had their conception ability gradually impaired by aging but still have an adequate ovarian potential. If this is achievable, care can be personalized and optimized using modified stimulation techniques, which will decrease the risk of extreme responses and fall rates while increasing the likelihood of conception [4].
Many clinicians determine this initial dose based on the patient's age and ovarian reserve markers, such as anti-Mullerian hormone (AMH) levels, antral follicle counts (AFCs), early estradiol levels, and follicle stimulating hormone (FSH) levels [5,6].
Recently, it has been discovered that there is a strong link between the AMH levels in the blood and the ovarian reserve or ovarian response during IVF cycles [7–9].
AMH is a glycoprotein of the transforming growth factor (TGF) superfamily, and it is released by granulosa cells at the primary follicle stage (pre-antral and small antral follicles). The release of AMH by the granulosa cells decreases as the follicles begin their FSH-dependent cyclic recruiting process [10,11].
The highest AMH expression levels have been observed in human antral follicles smaller than 6 mm, with the levels decreasing as the antral follicles grow larger [11].
The aim of this study, is to investigate the changes in AMH concentrations in infertile women with different causes of infertility on the serum concentration of estradiol on the day of HCG administration. Estradiol concentration on the day of HCG administration correlation with AMH, age and BMI will be evaluated.

2. STUDY DESIGN

2.1. Materials and Methods

This study was carried out at the YAD IVF Center in Erbil, the Kurdistan Region of Iraq. Data collection for this retrospective study began on March 1, 2018, and ended on February 1, 2019. The participants of this study were thirty-nine infertile women, twelve of whom had polycystic ovarian syndrome (PCOS) based on the Rotterdam criteria, six had unexplained infertility, six had endometriosis, seven had a tubal factor, and eight had a male factor. The control group was normal women with infertility due to male factors.
The inclusion criteria were females aged 24 to 44, women with primary or secondary infertility, and females who were attending the IVF center for their first, second, or third therapy session.
The exclusion criteria were infertile women with endocrine disorders, such as hyperprolactinemia, hypothyroidism, hyperthyroidism, hyperadrenocorticism, and congenital adrenal hyperplasia (CAH).
The College of Medicine/Hawler Medical University's Research Ethics Committee approved this study on February 20th, 2018, meeting code 3 and paper code 4. All participants signed informed consent forms.

The GnRH antagonist protocol of COH was used in this study. The infertile women were administered intradermally a daily dose of 200–225 IU of recombinant FSH (follitropin alfa, Gonal-F, Merck Serono), starting from day two or three of their menstrual cycle. Their ovarian response was observed by conducting transvaginal ultrasonography and serum estradiol measurements on day 6 of the treatment cycle. A GnRH antagonist, cetrorelix acetate (Cetrotide; Serono, Switzerland), was administered intramuscularly to the women at a daily dose of 0.25 mg from the day the dominant follicle reached a mean diameter of ≤14 mm to the day of human chorionic gonadotropin (HCG) administration. The daily rFSH dose was adjusted on an individual basis during the treatment period.

Transvaginal oocyte retrieval was performed 36 h after HCG administration.
Venous blood samples were collected on the morning of the second day of the cycle to determine the baseline serum AMH, FSH, LH, and estradiol levels. Additionally, a venous blood sample was collected on the morning of the HCG injection to determine the estradiol plasma levels. The estradiol serum levels were determined by performing an electrochemiluminescence immunoassay with an E-170 autoanalyzer (Roche Diagnostics, USA).

2.2. Statistical Analysis

The analysis of data was done by SPSS V 27.0. Data analysis was made using a one-way analysis of variance (ANOVA). The comparison between groups was done using the Tukey test. Pearson's correlation was used to examine the associations between the parameters. A P-value of 0.05 or less was considered statistically significant.

3. RESULTS

Table 1 lists demographic information on the women, including their age, body mass index (BMI), FSH, LH, basal AMH, progesterone and estradiol levels.

Table 1: Demographic information on the 39 patients.

Number of patients	39
Female age (year)	30.13 ± 5.15
Female BMI (kg/m^2))	30.24 ± 5.17
Baseline plasma FSH (IU/L)	5.69 ± 2.20
Baseline plasma LH (IU/L)	6.90 ± 4.18

AMH (ng/mL)	5.32 ± 3.98
Baseline plasma progesterone (ng/mL)	0.22 ± 0.14
Baseline plasma estrogen (pg/mL)	33.63 ± 32.79

The infertility causes identified in the infertile female include PCOS, endometriosis, male infertility factor, tubal ligation, and unexplained infertility.

3.1. Blood AMH Level

There was no noticeable difference in the basal AMH levels between the control group and the tubal abnormality and unexplained infertility groups. Nonetheless, the infertile PCOS group had significantly higher basal AMH levels than the control group, while the endometriosis group had significantly lower basal AMH levels than the control group (i.e., the male factor group [$p < 0.5$]; Table 2).

Table 2: Basal AMH levels in women with various infertility causes. There is a significant difference in values ($p < 0.5$).

Infertility causes	Polycystic ovary (n = 12)	Endometriosis (n = 6)	Male factor (n = 8)	Tubal blockage (n = 7)	Unexplained (n = 6)
Basal AMH levels (ng/mL)	8.47 ± 1.45 c	0.86 ± 0.24 a	4.50 ± 1.39 b	5.66 ± 2.39 b	4.3 ± 2.09 b

Different letter indicates a significant difference

3.2. Plasma Estradiol Level

The baseline serum estradiol level was measured on day 2 of the cycle before the treatment began, and there was no significant difference between the groups.

The estradiol plasma level was measured on the day of HCG administration, and there was a significant increase in the estradiol plasma levels in the control, unexplained infertility, and PCOS groups. However, the endometriosis group showed no significant increase in plasma estradiol when compared to the control group, (Table 3).

Table 3: Estradiol plasma levels on the day of HCG administration in women with various infertility causes. There is a significant difference in variables ($p < 0.5$).

Infertility causes	Polycystic ovary (n = 12)	Endometriosis (n = 6)	Male factor (n = 8)	Tubal blockage (n = 7)	Unexplained (n = 6)
Basal plasma estradiol (pg/mL)	26.06 ± 12.46 a	32.74 ± 9.88 a	26.49 ± 10.99 a	29.70 ± 14.61 a	36.50 ± 12.38 a
Plasma estradiol on the day of HCG administration (pg/mL)	1659.0 ± 534.73 bc	721.50 ± 137.26 a	1765.63 ± 815.7 bc	2052.57 ± 1138.82 c	1070.00 ± 420.88 ab

Different letter indicates significant difference

3.3. Correlation of AMH with HCG Day Administration Serum Estradiol

As shown in Figure 1, there was a significant correlation between the estradiol plasma levels on the day of HCG administration and the serum AMH levels before treatment ($r = 0.61$ and $p = 0.0001$).

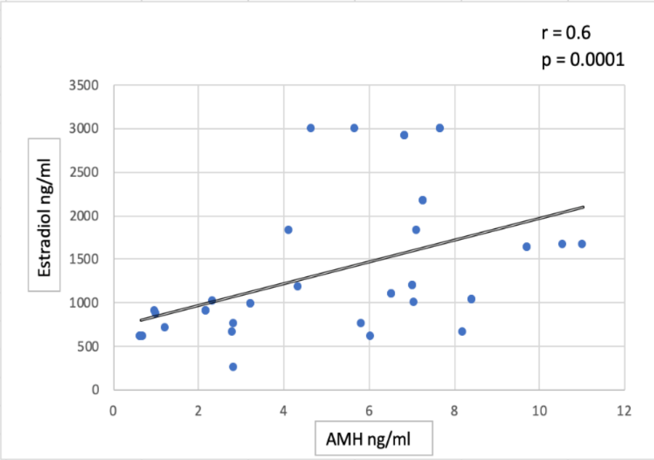

Figure 1: Correlation analysis of the AMH and estradiol plasma levels in the patients on the day of HCG administration

3.4. Correlation of Age and BMI with HCG Day Administration Serum Estradiol

Figures 2 and 3 demonstrate that there was a significant negative correlation between the serum estradiol levels on the day of HCG administration and patients' age ($r = -0.362$ and $p = 0.02$). Significant negative correlation between the estradiol plasma levels on the day of HCG administration and patients' BMI ($r = -0.318$ and $p = 0.04$).

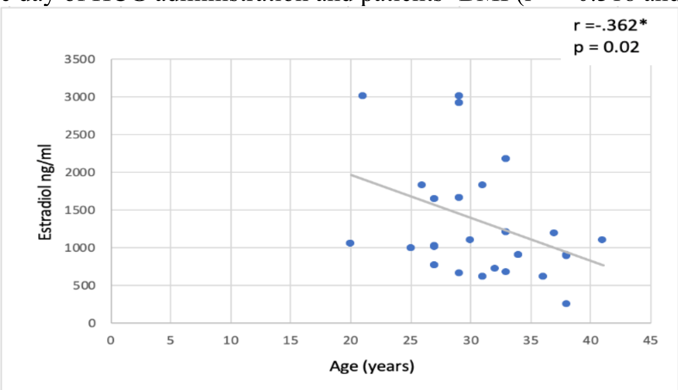

Figure 2: Correlation analysis of patients' age in years and serum estradiol levels on the day of HCG injection.

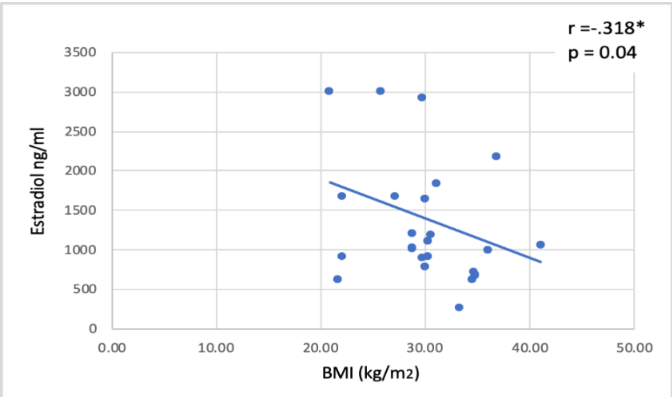

Figure 3: Correlation analysis of patient BMI in kg/m^2 and plasma estradiol levels on the day of HCG injection.

4. DISCUSSION

baseline serum AMH and estradiol levels in infertile women with various infertility causes were investigated in this study. This result demonstrates that infertile women with PCOS had significantly higher baseline AMH levels than women in the control group. This finding is consistent with prior findings that revealed that women with PCOS have higher AMH levels than controls, leading to the theory that an elevated AMH tone is responsible for the follicular stoppage observed in polycystic ovaries by interfering negatively with FSH [12,13].

The women with PCOS had a significantly high serum AMH level [14], which has been linked to PCOS sonographic characteristics, including the presence of many tiny follicles in the periphery of ovaries [15].

Although the endometriosis group had significantly lower AMH levels than the control group, as has been demonstrated in previous studies infertile women due to endometriosis had lower AMH concentration [16], and unmanaged endometriosis patients above the age of 36 experienced a faster decrease in AMH levels with age when compared to the control group [17].

There was no notable change in basal plasma estradiol between all the groups in this study, in addition, other meta-analysis studies have demonstrated that baseline estradiol does not improve the prognostic capacity of other widely used parameters for ovarian pool and is therefore not recommended for routine use in clinical practice [18].

On the day of HCG administration, there was a significant increase in plasma estradiol in the control women, unexplained infertility, tubal blockage, and PCOS groups. However, the endometriosis group did not reveal a significant increase in plasma estradiol when compared to the control group on the HCG injection day.

The principal female sex steroid, estradiol or 17 estradiol, is primarily secreted by the granulosa cells in the Graafian follicles. It is produced during the pre-ovulatory phase of the menstrual cycle, reaches its maximum around ovulation before the LH surge, and then gradually decreases and plateaus [19]. An increase in estradiol/fol (estradiol concentration per Graafian follicle with a diameter of >14 mm) was found to be parallel with an increase in the number of recovered follicles, mature oocytes, and fertilized oocytes. Therefore, an increase in estradiol/fol is associated with an improved oocyte quality. Estradiol/fol doses of 200–299.99 pg/mL have been reported to have the highest pregnancy rate [20,21].

Ozdegirmenci et al., Loutradis et al., and Pena et al. demonstrated that elevated serum estradiol levels on the HCG injection day were associated with a higher number of oocytes and embryos as well as excellent-grade blastocysts for transplantation and cryopreservation. They discovered a positive link between estradiol/follicle and the number of recovered oocytes, mature oocytes, and fertilized oocytes [21–23].

In this study, there was a substantial positive link between the estradiol plasma levels on the day of HCG administration and the AMH levels prior to treatment ($p = 0.0001$); in fact, previous research has demonstrated that AMH levels precisely predict the number of recovered oocytes. [24]. In a study by Malathi et al. demonstrated the number of follicles, oocytes, and mature oocytes exhibited a significant positive correlation with the AMH levels and a substantial positive correlation with the estradiol levels on the HCG administration day [25].

In this study, there was a modest negative correlation between patients' age and plasma estradiol on the HCG administration day ($r = −0.362$ and $p = 0.02$). This finding is consistent with the findings of Malathi et al. and Vaughan et al., who reported a substantial negative association between age and estradiol levels, demonstrating that ovarian reserves decline with age [25,26].

Although there was a noticeable negative correlation between patients' BMI and plasma estradiol on the HCG administration day ($r = −0.318$ and $p = 0.04$), which is in agreement with the findings of Wang et al., who concluded the larger the BMI, the lower the estradiol levels [27].

5. CONCLUSION

A positive link was found between the estradiol plasma concentration on the day of HCG administration and the AMH levels before treatment. The baseline AMH level is a much more reliable predictor of ovarian responses in COS than basal serum estradiol, BMI, and age. Therefore, the baseline serum AMH level could be used as a marker of ovarian reserve.

6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. REFERENCES

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