Serum AMH levels are not associated with adverse perinatal outcomes in women undergoing IVF treatment due to diminished ovarian reserve

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Abstract

Objective: Anti-Mullerian hormone (AMH) is used as a biomarker for the estimation of fertility related parameters such as quality and quantity of oocytes in in vitro fertilization (IVF) procedures. High oocyte quality may also be associated with healthy trophoblastic invasion and lower complication rates during pregnancy. The aim of this study is to evaluate the relationship between AMH values and perinatal complications in infertile women with poor ovarian reserve (POR).

Material and Methods: A total of 68 women undergoing IVF treatment were included in the study. Thirty six of them constituted the study group (POR) and 32 pregnant women constituted the control group (Tubal factor). All women in the study were chosen from patients who have undergone to their first IVF cycle. Serum AMH levels were analyzed with an ELISA kit in all patients.

Results: AMH level was 5.4 times higher in the control group compared to that of the study group (p<0.05). No significant differences were observed between the groups with regard to preterm birth rate, gestational age at birth, birth weight, 1st and 5th minute Apgar scores, and neonatal intensive care unit admission rates (all, p>0.05).

Conclusion: We found that AMH did not predict adverse perinatal outcomes in women with POR.

Keywords: Anti-Mullerian hormone, IVF, biological marker, diminished ovarian reserve, perinatal outcomes

Introduction

Given the high cost and possible complications of assisted reproduction technologies (ART), investigation of some parameters that can be used to predict the outcomes of ART pregnancies is of great importance. Therefore, such a marker should be able to predict both the response to in-vitro fertilization (IVF) therapy and correlate well with pregnancy rates and as well as perinatal outcomes. Although some evidence indicates that age is the main determinant of IVF success, it is known that the relationship between a woman's chronological age and reproductive capacity is highly variable (1, 2).

Anti-Mullerian hormone (AMH), a dimeric glycoprotein belong to transforming growth factor-β (TGF-β) family, is primarily produced by the fetal Sertoli cells at the time of testicular differentiation and allows the Müllerian channel to regress.

In women, it is secreted by the granulosa cells in the preantral and early antral follicles (3). Therefore, it has been suggested as a marker of ovarian reserve in women, which may predict the number of ovarian follicles and reproductive age (4, 5). In the vast majority of the studies, AMH has been shown to be a better marker than antral follicle count (AFC), baseline FSH, estradiol (E2) and inhibin B in estimating ovarian reserve and ovarian response to IVF treatment (6). Success of IVF procedure is related with different factors including serum AMH (7-9). On the other hand, pregnancy and live birth in women with low/extremely low AMH levels have also been reported over the 40 years of age (10-12). The contradictory observations may be due to the different analytical assays and population characteristics.

The ovarian response to stimulation with medication in IVF is an important step of outcomes, especially live birth rates and adverse effects of the treatment (13-15). Therefore, there is a need for individualization of the gonadotropin-starting dose by using predictive markers to provide a better oocyte yield and minimize the side effects. Even though serum AMH levels may be a useful tool for the prediction of IVF outcomes in low ovarian response patients, it does not seem to be meaningful in IVF patients with normal ovarian response (16). On the other hand, there is still no conclusive data about the optimal cut-off level of blood AMH to use as a marker of IVF prognosis.

At the follicular level, granulosa cells surrounding the oocyte have been shown to express more AMH than mural granulosa cells suggesting that oocytes may play a role in regulation of AMH production (17). In accordance with this hypothesis, granulosa cells placed in the culture medium produced more AMH in the presence of oocytes (18). Moreover, it has been shown that patients with high preovulatory follicular fluid AMH levels, who underwent modified natural cycle IVF, produced reproductively more capable oocytes (19). Thus, based on this scientific basis, it is thought that there may be a relationship between oocyte activity and ovarian AMH production.

Accordingly, there may be a relationship between AMH and oocyte quality. Similarly, high oocyte quality may also be associated with healthy trophoblastic invasion and lower complication rates during pregnancy. However, the controversial results of studies that explain the relationship between AMH, conception and live birth rates have led us to investigate whether there is a relationship between AMH values and perinatal complications.

Material and Methods

The present study was approved by the ethics committee and informed written consent was obtained from each patient before the study was undertaken. The Helsinki Declaration was followed throughout the study. A total of 68 women undergoing IVF treatment were included in the study. Thirty six of them constituted the study group (POR) and 32 pregnant women constituted the control group (Tubal Factor). Patients in both groups were recruited from infertile women aged 22-38 years, who became pregnant with IVF treatment. All women in this study were chosen from patients who have undergone to their first fresh-embryo transfer cycles, while those with tubal factor infertility were included as the controls. Women with chronic systemic disease, drug users other than folic acid, patients who underwent any pelvic surgery, radiotherapy and chemotherapy were excluded from the study. All patients were followed regularly in our antenatal outpatient clinics throughout pregnancy and received multivitamin supplementation after 4 months and iron supplement after 6 months. Luteal phase support with vaginal progesterone was also given to all patients in the IVF group during the first trimester of pregnancy.

The birth weights, gestation weeks at birth, obstetric characteristics and perinatal outcomes including newborn APGAR scores and neonatal intensive care unit admission (NICU) rates were recorded for analysis. Cesarean

decisions were based on obstetric indications and maternal demand. The number of birth and twins were also recorded for each group.

Statistical analyses

The statistical analysis was done with statistical computer software (IBM SPSS Statistics version 22 software, IBM Corporation, Armonk, NY, USA). The distributions of data were analyzed by Kolmogorov Smirnov test. Normally distributed data were analyzed with parametric test (student's t test) for the comparison of two independent groups. Mann–Whitney U test was used to analyze the comparison of non-normally distributed findings. Continuous variables were shown as mean ± standard deviation (SD) whereas categorical variables were expressed as number (percentage). Differences between categorical data were evaluated using the Chi-square test or Fisher's exact test. Statistical significance was considered for $P<0.05$.

Results

The characteristics of 36 females in the study group and 32 females in the control group are summarized in Table 1. There was no statistically significant age difference between two groups. The gestational weeks of the study and control groups were not significantly different. The mean birth weight of control group was slightly but not statistically significantly higher compared to that of the study group. No significant differences were observed between the groups in terms of route of birth, infant gender, 1st and 5th minute APGAR scores and NICU admission rates. On the other hand, number of infants for each birth in the POR group was 1.19±0.40 (n=7) and significantly higher than the control group (p<0.05). Although preterm birth rate was more frequent in the POR group, the difference did not reach a statistically significant level (16.7% vs. 9.4%, p>0.05). Other obstetric complications such as preeclampsia, gestational diabetes and intrauterine growth restriction were only detected in one patient in both groups which was statistically insignificant when compared between the groups.

The mean serum AMH concentrations were 3.0±1.4 (range 1.1-6.0) and 0.6±0.3 (0.1-1.0) ng/ml in the study and control groups, respectively (p<0.001). The AMH level of the control group was 5.4 times higher than the POR group (Figure 1). There was no correlation between AMH levels and other parameters including age, gestation week, birth weight and number of infants at birth in the groups. Table 2 summarizes the characteristics of POR group according to number of infants in each birth. When IVF patients were divided into low and high oocytes yield groups, the number of oocytes collected and fertilized was statistically significantly lower in the former group (p<0.001) (Table 3).

In POR group, there were negative correlations between gestational weeks and age (r=-0.352, p: 0.035), and between gestational weeks and number of infants (r=-0.401, p: 0.015). There was a strong positive correlation between gestational weeks and infant weight at birth (r=0.856, p<0.001) within the POR group. There was a positive correlation between the duration of stimulation and number

of oocytes collected (r=0.497, p: 0.003) and, number of fertilized oocytes (r=0.522, p: : 0.002) in the POR group. Number of oocytes collected and number of oocytes fertilized were strongly positively correlated (r=0.897, p<0.001).

However, there was a negative correlation in the POR group between number of oocytes collected and fertilization rate (r=-0.489, p: 0.004).

Table 1: Comparison of obstetric and perinatal outcomes between the POR and control groups

	Control	POR	P value
Number of subjects	32	36	NS
Age (year)	30.4±4.3	30.6±4.5	NS
Gestational age (weeks)	38.1±1.8	37.3±2.1	NS
Preterm birth n(%)	3/32(%9.4)	6/36(16.7)	NS
Route of birth n(%)			NS
Abddominal	12(37.5)	11(30.6)	
Vaginal	20(62.5)	25(69.4)	
Infant weight at birth (gr)	3180.2±448.2	2960.6±522.4	NS
Infant gender n(%)			NS
Male	17/33(48.5)	28/43(65.1)	
Female	16/33(51.5)	15/43(34.9)	
Apgar score			NS
1st minute	7(6-7)	7(5-7)	
5th minute	9(8-9)	9(8-10)	
Number of infants	1.03±0.18	1.19±0.40	<0.05
Twin pregnancies n(%)	1/32(3.1)	7/36(19.4)	NS
NICU admission rate n(%)	2/33(6.1)	6/43(14.0)	NS

NICU : neonatal intensive care unit admission. P<0.05 is considered as statistically significant.

Table 2: Means (±SD) of AMH levels, ages, gestational weeks and birth weight at birth according to number of twins in the POR group

IVF group	Single	Twin	P value
Number of subjects	29	7	NS
AMH (ng/ml)	0.552±0.334	0.600±0.374	NS
Age (year)	30.03±4.65	33.14±2.8	NS
Gestational week	37.7±2.0	35.6±1.6	<0.05
Infant weight at birth (gr)	3087.6±500.4	2434.3±148.1	<0.05

IVF; in vitro fertilization, AMH; anti mullerian hormone. P<0.05 is considered as statistically significant.

Table 3. Clinical data of the POR patients by oocyte yield. The POR group was divided into two groups according to the fertilized oocytes after the oocyte collection: "low" represented a yield of 1–3 oocytes and "high" a yield of 4 or more oocytes

Clinical characteristics	1-3 Oocytes (n=20)	≥ 4 oocytes (n=12)	*P* value
Serum AMH level, ng/ml	0.470±0.285	0.642±0.355	NS
Age, years	30.0±4.4	32.2±4.6	NS
Gestational week	37.4±2.0	36.8±2.5	NS
Infant weight at birth, gr	2941.5±508.7	2905.0±585.5	NS
Number of infants	1.20±0.41	1.25±0.45	NS
Duration of stimulation, days	11.3±1.0	12.17±1.3	NS
Number of oocytes collected	3.30±2.16	9.25±3.36	<0.001
Number of oocytes fertilized	1.95±0.89	5.83±2.73	<0.001
Low oocyte yield	1.35±1.69	3.42±1.73	0.002
Fertilization rate	0.74±0.28	0.62±0.12	NS

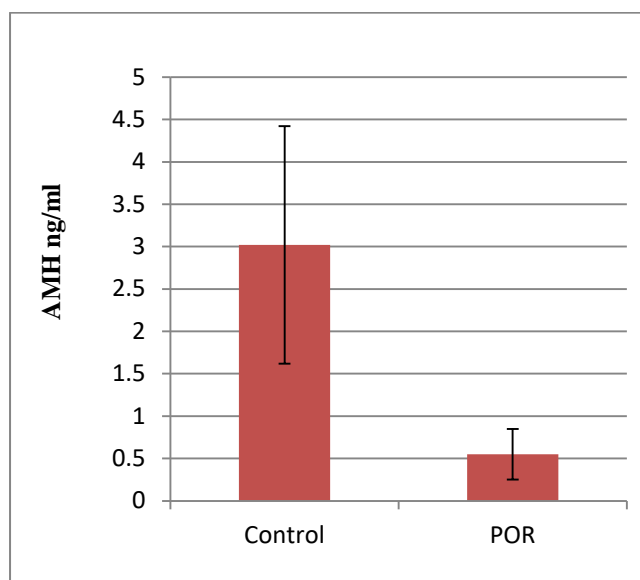*P<0.05 is considered as statistically significant.*

Figure 1: The serum levels of Anti-Mullerian hormone (AMH) in the control (n=32) and perinatal outcome (POR) (n=36) groups. (p value: NS)

Discussion

There are some reports that infertility caused by female factor may be associated with increased perinatal risks (20). The quality of the oocyte determines embryo quality, and embryo quality may also affect pregnancy outcomes and result in increased perinatal risks. In this study, we hypothesized that pre-pregnancy serum AMH concentrations may predict adverse perinatal complications. However, we observed that pre-pregnancy low AMH values which are indicative of ovarian reserve and oocyte quality did not worsen perinatal outcomes. Therefore, poor ovarian reserve is not associated with increased risk for negative perinatal results. We also investigated the cycle characteristics and multiple pregnancy rates of POR patients conceived by IVF in relation to preconceptional serum AMH levels. Our secondary outcome is that AMH has some value for the prediction of fertility parameters, ovarian reserve, IVF procedure, etc., but there is still a need for more information in different populations.

It is very well known that AMH is closely related to ovarian reserve. The decrease in serum AMH due to aging in the ovaries is accompanied by a decrease in the size of the primordial follicle pool, as well as increased apoptosis in the granulosa cells per follicle, which indicates reduced oocyte quality. Although low AMH levels in IVF cycles indicate that oocyte counts to be collected may be low and oocyte quality may be poor, studies have shown that even a poor quality embryo, which can form from such an oocyte, may result in a live birth (21).

Low oocyte quality and hence quality of embryo may be associated with impaired energy production in oocyte cytoplasm, although they result in a live birth (22). This energy impairment may have different effects in a wide range from implantation to nutrition, from birth to postnatal period, and can also determine the degree of quick response and adaptation of the newborn after birth. The number of

studies examining the relationship between ovarian reserve, oocyte quality and perinatal complications is not very high. Even as far as we know, there is no study comparing IVF pregnancies with non-infertile and spontaneously conceived women in terms of serum AMH levels. The researchers generally investigated the relationship with preeclampsia and found conflicting results (23).

Woldringh et al. (24) pointed out that if the pregnancy develops, the risk of developing preeclampsia will be higher in patients with decreased ovarian reserve which is characterized by decreased response to FSH in the IVF cycle. It is thought that the inadequate vascular reserve in patients with low ovarian reserve may lead to pregnancy-related vascular complications. In another study by Van Disseldorp et al. (25), the incidence of hypertensive disorders of pregnancy did not differ between poor responders and normal responders for ovarian stimulation.

In their study, Levron et al. (26) have shown that women who become pregnant by receiving oocyte from younger donors have a higher risk of developing preeclampsia and that this may be due to immunologic interactions rather than quality of oocytes. The perinatal period also includes the neonatal period, which is defined as the first 7 postnatal day. Low Apgar score, NICU admission rates for newborns, necrotizing enterocolitis and low birth weight, preterm delivery, respiratory or gastrointestinal complications and poor neonatal complications are all perinatal complications. The only study in the literature that also encompassing neonatal period was done by Oron et al (27). In this study, pregnancies resulting from single fresh poor-quality embryo transfer did not constitute any risk of adverse obstetric or perinatal outcome when compared with transfer of good quality single fresh embryos.

As compared with fresh embryo transfer, having less perinatal complication rates of frozen embryo transfer have been explained by less asynchronization between the endometrium and the embryo (28, 29). We have seen in our own patient group that we cannot support the idea of asynchronization, because of none of the patients with low AMH values conceived by the freeze-thaw IVF cycles. There is also some evidence that laboratory or medical procedures may be responsible for controversial perinatal outcomes in IVF pregnancies (30). Specific laboratory procedures, such as embryo culture media, culture duration, intracytoplasmic sperm injection (ICSI), and cryopreservation method can disturb implantation by creating stress in the developing embryo despite high AMH levels and subsequently increase complication rates by affecting following intrapartum and perinatal processes.

Recently, Nelson et al. report AMH-based approach for a controlled ovarian stimulation in IVF cycles (11). AMH level was associated with oocyte yield following to the ovarian stimulation. The levels between 1 and 5 pmol/l have been associated with a reduced clinical pregnancy rate. The previous studies evaluating that relationship mostly report a positive correlation between AMH levels and better IVF outcomes, however few of them note a poor association as well (31-33).

The result of our study supports the literature data presented in most of the studies and demonstrates AMH as a good predictor of IVF outcomes.

In our study, we did not observe a significant difference in AMH levels of the IVF patients when they were classified to the number of infants delivered as single or twin. In the literate there is limited data on number of infants and AMH levels (34, 35). Tal et al. report an age dependent association between AMH levels and twin births. AMH level seems to predict twin pregnancy over 34 years old of age but not in the patients with lower age group (34). This finding is consistent with our result because the mean age of the IVF patients in our study was 30.6 years.

Different analytical methods have been described for the determination of AMH levels in serum (36-38). In the literature, it has been stated that type of the analytical method may be one of the factors as a possible reason for the contradictory results, especially in the patients with low AMH levels. In the present study, we used a conventional ELISA kit which provided the analysis of all the samples accurately within the detection range. All the values measured in the groups were within the detection limit. Therefore, we do not expect analytical procedure-dependent error as a confounder in the present study.

The major drawback of this study was small number of samples in the groups and evaluation of only AMH as a predictive marker. Therefore, perinatal complication rates may be low in our study. In addition, we could not perform logistic regression analysis which would be more meaningful to reveal real effect of serum AMH levels on adverse perinatal outcomes due to the limited number of patients.

Conclusion

As a result, we found that AMH did not predict adverse perinatal outcomes. The present study also supports the literature data that suggest a positive association between AMH and ovarian response in IVF cycles. Since the number of studies evaluating the relationship between AMH and perinatal outcome is limited, further extensive studies including multicenters with more patients should be designed in order to make the results more meaningful.

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