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Evaluation of Anti-Mullerian Hormone in Predicting In Vitro Fertilization Cycle Outcomes

Anti-Mülleryan Hormonun İn Vitro Fertilizasyon Siklus Sonuçlarına Etkisinin Araştırılması

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ABSTRACT

Aim: This study was conducted to explore the effect of serum anti-Mullerian hormone (AMH) level on in vitro fertilization (IVF) cycle outcomes.
Material and Methods: A total of 142 patients included in this study, were divided into three groups according to their serum AMH levels as Group 1: AMH level 5-10 ng/ml (n=108), Group 2: AMH level 10-15 ng/ml (n=20), and Group 3: AMH level >15 ng/ml (n=14). Demographic characteristics were recorded. The duration of infertility and stimulation, the number of cycles, initial, final, and total doses of gonadotropins, and estradiol (E2) and progesterone levels on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the total number of oocytes retrieved, the number of mature oocytes, the number and quality of the embryo, and also endometrial thickness on the day of trigger, OPU and ET, the distance of embryo-fundus, the day of ET, and pregnancy outcomes were all recorded.
Results: While the IVF treatment indications and pregnancy outcomes were similar between the groups, body mass index (BMI) was significantly higher in Group 2 and Group 3 than in Group 1 ($p<0.001$). The total doses of gonadotropin were significantly higher in Group 2 than in Group 1 and Group 3, and the total oocyte count was also significantly higher in Group 3 than in Group 1 ($p=0.006$, and $p=0.015$, respectively)
Conclusion: AMH levels were associated with BMI and total oocyte count, but not with mature oocyte count, oocyte quality, and pregnancy outcomes.
Keywords: AMH levels; IVF cycle outcomes; BMI; oocyte count and quality; pregnancy outcomes.

ÖZ

Amaç: Bu çalışma, serum anti-Mülleryan hormon (AMH) seviyesinin in vitro fertilizasyon (IVF) siklus sonuçları üzerindeki etkisini araştırmak için yürütülmüştür.
Gereç ve Yöntemler: Bu çalışmaya dahil edilen toplam 142 hasta, serum AMH düzeylerine göre, Grup 1: AMH düzeyi 5-10 ng/mL (n=108), Grup 2: AMH düzeyi 10-15 ng/mL (n=20) ve Grup 3: AMH düzeyi >15 ng/mL (n=14) olmak üzere üç gruba ayrıldı. Demografik özellikler kaydedildi. İnfertilite ve stimülasyon süresi, siklus sayısı, gonadotropinlerin başlangıç, son ve toplam dozları ile tetikleme, oosit toplama (oocyte pick up, OPU) ve embriyo transferi (ET) gününde östradiol (E2) ve progesteron düzeyleri, toplanan toplam oosit sayısı, matur oosit sayısı, embriyo sayısı ve kalitesi ve ayrıca tetikleme, OPU ve ET gününde endometrial kalınlık, embriyo-fundus mesafesi, ET günü ve gebelik sonuçlarının tamamı kaydedildi.
Bulgular: IVF tedavi endikasyonları ve gebelik sonuçları gruplar arasında benzer iken, vücut kitle indeksi (VKİ) Grup 2 ve Grup 3'te Grup 1'e göre anlamlı olarak daha yüksekti ($p<0,001$). Toplam gonadotropin dozları Grup 2'de Grup 1 ve Grup 3'e göre anlamlı olarak daha yüksek ve toplam oosit sayısı da Grup 3'te Grup 1'e göre anlamlı olarak daha yüksek idi (sırasıyla, $p=0,006$ ve $p=0,015$).
Sonuç: AMH seviyesi VKİ ve toplam oosit sayısı ile ilişkilidir, fakat matur oosit sayısı, oosit kalitesi ve gebelik sonuçları ile ilişkili değildir.
Anahtar kelimeler: AMH seviyeleri; IVF siklus sonuçları; VKİ; oosit sayısı ve kalitesi; gebelik sonuçları.

INTRODUCTION

Anti-Mullerian hormone (AMH) is a homodimeric glycoprotein secreted by the granulosa cells of small antral follicles. It belongs to the transforming growth factor β (TGF-β) family and is located on chromosome 19p13.3 (1). In addition to being an important predictor of ovarian reserve, AMH is also significant in the detection of ovarian response to hormonal stimulation (2). One line of the literature showed that the number of retrieved oocytes was correlated with serum AMH levels in in vitro fertilization (IVF). We know that a high AMH level does not always mean that the number of fertilized oocytes will be high (3,4). Although there is a strong positive correlation between serum AMH level and oocyte quantity, the oocyte and embryo quality are controversial (5-7). In addition, previous studies have demonstrated that a high serum AMH level was correlated with follicular fluid AMH concentration that affects fertilization conditions negatively by changing the ratios of oestradiol-testosterone (8,9).

Therefore, the present study was conducted to explore the effect of serum AMH level on IVF cycle outcomes, especially on the conflicting results observed in the literature.

MATERIAL AND METHODS

This was a retrospective study conducted at the IVF clinic of Etlik Zübeyde Hanım Women's Health Training and Research Hospital of Ankara, Turkey, and was carried out with a total of 142 women. The study protocol was approved by the local ethics committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.07.2022, 2022/143). The women were divided into three groups according to the serum AMH levels, Group 1: AMH level 5-10 ng/mL (n=108), Group 2: AMH level 10-15 ng/mL (n=20), Group 3: AMH level >15 ng/mL (n=14), respectively.

The exclusion criteria included having a history of chronic disease, undergoing preimplantation genetic diagnosis, freeze-thaw, and mild or natural cycle protocols, multiple embryo transfer, severe male factor infertility defined as azoospermia or total progressive motile sperm count being less than 1 million, and the study also excluded the patients with moderate to severe ovarian hyperstimulation syndrome (OHSS).

Demographic characteristics viz. maternal age, gravidity, abortion, live birth, body mass index (BMI), IVF treatment indications (unexplained infertility, male factor, tubal factor), duration of infertility and stimulation, the number of cycles, initial, final, and total doses of gonadotropins (recombinant follicle-stimulating hormone (FSH), Gonal-F® Merck, Germany, human menopausal gonadotropin (hMG), Menopur®, Ferring Pharmaceuticals, Germany), estradiol (E2) and progesterone levels on the day of trigger, oocyte pick up (OPU) and embryo transfer (ET), the total number of oocytes retrieved, the number of mature oocytes, the number and quality of embryo (10), endometrial thickness on the day of trigger, OPU and ET, the distance of embryo-fundus, the day of ET and pregnancy outcomes were all recorded for reference. The gonadotropin releasing hormone (GnRH) antagonist protocol (recombinant FSH, Gonal-F® Merck, Germany, hMG, Menopur®, Ferring Pharmaceuticals, Germany, GnRH antagonist (141 Cetrotide®, Merck, Germany) and agonist trigger (Gonapeptyl® Ferring Pharmaceuticals, Germany) were administrated in all groups (11). The same luteal phase support was provided for all three groups.

Serum AMH level was determined using the IMMULITE 2000 Immunoassay System (Siemens, Berlin, Germany). The detection limit was 0.02-24 ng/mL and the inter-assay and intra-assay coefficients of variation were 3.77%~3.99% for AMH.

Statistical Analysis

Statistical analysis was performed by IBM SPSS Statistics Version 26.0. Descriptive statistics and frequency tables were used to examine the obtained results. Parametric tests were performed for the data which was normally distributed and the analysis of variance (ANOVA) test was used in the analysis of three or more independent groups. Non-normally distributed data were analyzed by the Kruskal Wallis-H test as a non-parametric test. Bonferroni correction test was applied for pairwise comparisons. The relationships between the two qualitative variables were analyzed with the Pearson chi-square test. A p value of <0.05 was considered to be statistically significant.

RESULTS

A total of 142 women were recruited in the study. No significant difference was found in IVF treatment indications and pregnancy outcomes between the three groups according to the AMH value (Table 1).

The comparison of demographic and obstetric characteristics, laboratory data, and duration of infertility between the groups was shown in Table 2. The comparison of IVF cycle characteristics and embryo outcomes between the AMH groups was shown in Table 3.

Table 1. The comparison of IVF treatment indications and pregnancy outcomes according to the AMH value

	Group 1 (n=108) (AMH 5.0-10.0 ng/mL)	Group 2 (n=20) (AMH 10.1-15.0 ng/mL)	Group 3 (n=14) (AMH >15.0 ng/mL)	p
Tubal factor, n (%)	8 (7.4)	2 (10.0)	0 (0.0)	0.509
Male factor, n (%)	32 (29.6)	6 (30.0)	1 (7.1)	0.200
Unexplained infertility, n (%)	68 (63.0)	12 (60.0)	11 (87.6)	0.477
Pregnancy outcomes, n (%)				
No pregnancy	73(67.6)	10 (50.0)	10 (71.4)	
Biochemical	5 (4.6)	2 (10.0)	0 (0.0)	0.479
Clinical	30 (27.8)	8 (40.0)	4 (28.6)	

IVF: in vitro fertilization, AMH: anti-Mullerian hormone (AMH)

Table 2. The comparison of demographic and obstetric characteristics, laboratory data, and duration of infertility according to the AMH value

	Group 1: AMH 5.0-10.0 ng/mL (n=108)		Group 2: AMH 10.1-15.0 ng/mL (n=20)		Group 3: AMH >15.0 ng/mL (n=14)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Maternal age (years)	28.78±3.02	28 [23-35]	29.00±3.33	29.5 [24-35]	28.93±2.67	28 [26-33]	0.955
Gravidity	0.60±0.86	0 [0-4]	0.70±0.66	1 [0-2]	0.29±0.46	0 [0-1]	0.188
Abortion	0.36±0.73	0 [0-4]	0.55±0.61	0.5 [0-2]	0.14±0.36	0 [0-1]	0.061
Live birth	0.15±0.47	0 [0-3]	0.10±0.31	0 [0-1]	0.14±0.36	0 [0-1]	0.929
BMI (kg/m^2)	25.98±4.45^a	25.1 [17.0-40.0]	30.28±4.59^b	30.8 [19.0-37.6]	28.91±3.04^b	29.5 [23.0-33.2]	**<0.001**
E2 (pg/mL), day of trigger	3246.96±1981.83	2946 [437-9438]	4741.87±3179.94	3000 [1400-12115]	4795.81±3520.88	3308 [506-11041]	0.132
Progesterone (ng/mL), day of trigger	1.04±1.43	0.8 [0.1-13.1]	1.28±0.79	1.3 [0.3-2.9]	0.94±0.59	0.8 [0.1-2.1]	0.160
E2 (pg/mL), day of OPU	2241.69±1357.84^a	2061.4 [253.4-7061.7]	3443.42±1761.84^b	3000 [1048.8-7731.9]	3577.18±2125.50^b	3000 [514.0-8128.0]	**0.001**
Progesterone (ng/mL), day of OPU	8.16±4.96	7.3 [0.7-26.8]	9.54±5.27	9.6 [2.5-25.9]	6.01±3.64	5.6 [0.8-16.3]	0.063
E2 (pg/mL), day of ET	1649.45±1417.02	1346.4 [137-6480]	1678.09±1046.43	1677.0 [125-3756]	998.07±820.23	556.5 [325-2275.6]	0.305
Progesterone (ng/mL), day of ET	69.24±69.07	60.0 [1.0-326.6]	49.00±38.24	51.2 [3.1-127.8]	25.98±35.21	15.9 [4.5-112.0]	0.106
Number of cycle	1.71±1.07^a	1 [1-5]	2.45±1.05^b	3 [1-4]	2.00±0.88^{ab}	2 [1-3]	**0.004**
Duration of infertility (month)	68.76±37.49^a	60 [5-180]	95.40±47.80^b	96 [24-168]	89.14±47.47^{ab}	84 [24-216]	**0.022**

AMH: anti-Mullerian hormone, BMI: body mass index, E2: estradiol, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation, ^{a,b}: different superscripts denote the significant difference according to the post hoc test results

Table 3. The comparison of IVF cycle characteristics and embryo outcomes according to the AMH value

	Group 1: AMH 5.0-10.0 ng/mL (n=108)		Group 2: AMH 10.1-15.0 ng/mL (n=20)		Group 3: AMH >15.0 ng/mL (n=14)		p
	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	Mean±SD	Median [min-max]	
Initial doses of gonadotropins (IU)	158.33±28.60	150 [100-225]	166.25±29.82	175 [100-200]	143.75±29.72	150 [87-200]	0.069
Final doses of gonadotropins (IU)	126.97±48.91^{ab}	150 [25-275]	148.75±46.40^a	150 [25-225]	104.46±34.53^b	100 [50-150]	**0.014**
Total doses of gonadotropins (IU)	1517.62±488.83^a	1400 [800-4350]	1753.75±405.43^b	1718.8 [1013-2575]	1339.29±267.42^a	1337.5 [825-1700]	**0.006**
Duration of stimulation (day)	10.38±1.60^a	10 [7-15]	11.30±1.53^b	11.5 [9-14]	11.00±1.10^{ab}	11 [9-13]	**0.019**
Follicle number (15-17 mm)	5.02±3.45	4.5 [0-22]	4.95±3.15	4 [0-11]	5.79±2.64	6 [2-11]	0.462
Follicle number (≥17 mm)	4.50±3.06	4 [0-13]	4.65±3.70	4 [0-11]	4.14±3.28	3.5 [0-10]	0.904
Total oocyte count	16.93±8.32^a	15 [2-38]	21.15±9.95^{ab}	22 [6-37]	22.71±7.63^b	25 [5-33]	**0.015**
Mature oocyte count	12.64±7.51	11 [0-32]	14.25±7.95	12.5 [4-35]	14.29±7.46	13 [0-26]	0.463
Grade 1 embryo	0.44±0.56	0 [0-2]	0.50±0.51	0.5 [0-1]	0.21±0.42	0 [0-1]	0.261
Grade 2 embryo	0.30±0.48	0 [0-2]	0.65±0.45	1 [0-2]	0.36±0.63	0 [0-2]	0.462
Grade 3 embryo	0.14±0.35	0 [0-1]	0.10±0.31	0 [0-1]	0.07±0.27	0 [0-1]	0.721
Endometrial thickness (mm), day of	10.14±2.14	9.9 [5.6-17.0]	10.18±1.51	10.0 [7.5-12.8]	10.49±2.61	9.8 [6.5-15.0]	0.860
Endometrial thickness (mm), day of OPU	9.53±2.14	9.1 [4.5-16.0]	9.24±1.74	8.8 [7.0-13.0]	9.32±2.79	8.6 [5.0-14.3]	0.773
Endometrial thickness (mm), day of ET	9.94±2.11	9.6 [7.0-17.2]	9.53±1.72	9.5 [7.2-13.3]	10.41±1.85	10.6 [7.4-12.9]	0.464
Distance embryo-fundus	11.11±3.51	11.0 [0.8-19.0]	10.24±4.61	10.4 [1.1-18.0]	12.99±4.31	13.5 [6.4-20.0]	0.238
Day of embryo transfer	3.74±0.97	3 [3-5]	3.94±1.03	3 [3-5]	4.13±0.99	4.5 [3-5]	0.425

AMH: anti-Mullerian hormone, OPU: oocyte pick up, ET: embryo transfer, SD: standard deviation, ^{a,b}: different superscripts denote the significant difference according to the post hoc test results

There was no significant difference in maternal age, gravidity, abortion, live birth, E2, and progesterone level on the trigger day, progesterone level on the OPU day, E2, and progesterone level on the ET day between the groups. As a result of the Bonferroni correction method, BMI was significantly higher in Group 2 and Group 3 than in Group 1, OPU E2 level was significantly higher in Group 2 and Group 3 than in Group 1, the number of cycle was significantly higher in Group 2 than in Group 1, and duration of infertility was significantly longer in Group 2 than in Group 1 ($p<0.001$, $p=0.001$, $p=0.004$, and $p=0.022$, respectively, Table 2).

There was no significant difference in the initial doses of gonadotropins, the number of follicles, mature oocyte counts, grade 1-2-3 embryo, and endometrial thickness on the day of trigger, OPU and ET, embryo-fundus distance, and the day of ET between the groups. As a result of the Bonferroni correction method, the final doses of gonadotropin were significantly higher in Group 2 than in Group 3, the total doses of gonadotropin were significantly higher in Group 2 than in Group 1 and Group 3, the duration of stimulation was significantly longer in Group 2 than in Group 1, and total oocyte count was significantly higher in Group 3 than in Group 1 ($p=0.014$, $p=0.006$, $p=0.019$, and $p=0.015$, respectively, Table 3).

DISCUSSION

In the present study, it was observed that as the AMH level increased BMI and the total oocyte count increased, the mature oocyte count, oocyte quality, and pregnancy outcome did not change. While it was expected that the increase in the total oocyte count would also be reflected on the number of mature oocyte and embryo, this did not take place for the present study. It is worth highlighting that the number of oocyte retrieved did not always mean an increased fertilized quality of oocytes.

In contrast with the current study, it was demonstrated in the literature that there occurred a negative correlation between AMH level and BMI (12). In fact, numerous theories have been put forward for the analysis of the association between BMI and AMH level (13). It has been argued that insulin resistance in obese women may disrupt the function of granulosa cells through lipotoxic effect and alter AMH production (14,15).

When the literature was reviewed, it was understood that the AMH level had a positive correlation with the number of retrieved oocytes and the OPU E2 level in IVF cycles was in agreement with the present study (6,16). The results were controversial regarding embryo quality. Garcia-Velasco et al. (17) reported that high AMH level blocks aromatase expression in granulosa cell that contributes to an intraovarian hyperandrogenic environment, which impairs oocyte development and causes poor quality embryo. In addition, Grossman et al. (18) revealed that the expression of cytochrome P450 aromatase (CYP19) via FSH in granulosa cells is inhibited by AMH that leads to a detrimental effect on the development of oocytes. In accordance with our study, Lie Fong et al. (19) demonstrated that there was no correlation between AMH level and embryo quality.

The mean goal of IVF treatment was to obtain the maximum number of high-quality embryos and increased the live birth rate as much as possible with minimum complications. Therefore, the optimization and individualization of IVF treatment have been accepted as the best practice, especially for patients with high AMH level. Because of the concerns regarding OHSS, cycle cancellation was more common in high AMH levels resulting in an increase in the number of failed IVF cycles as shown in the present study (20). In addition, in the current study, the initial gonadotropin doses were similar in all groups, but as the AMH level increased, we gradually reduced the doses through the end of the ovarian stimulation due to the risk of OHSS. In this way, even if we administered different doses of gonadotropin, the number of mature oocytes obtained was similar, so the controlled low-dose ovarian stimulation seemed more rational in those with high AMH levels.

The literature results are conflicting on the association between AMH level and pregnancy outcome, namely, a number of studies showed a positive relationship in this sense while some others demonstrated an inverse relationship. Wang et al. (21) indicated that endometrial cells contain AMH protein and when it binds its receptor, cellular viability is declined. This could explain the inverse relation between high AMH levels and decreased implantation rate. On the other hand, Kaya et al. (22) demonstrated that high AMH level had a positive correlation with pregnancy rate. In the current study, the pregnancy outcomes do not seem to be affected by AMH values.

CONCLUSION

In conclusion, AMH levels were associated with BMI and total oocyte count but not with mature oocyte count, oocyte quality, and pregnancy outcomes. Extensive studies are required to confirm the results of this study.

Ethics Committee Approval: The study was approved by the Ethics Committee of Etlik Zübeyde Hanım Women's Health Training and Research Hospital (21.07.2022, 143).

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