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Do ovarian reserve markers predict the subsequent pregnancy outcomes in women with recurrent pregnancy loss?

Over rezerv belirteçleri tekrarlayan gebelik kaybı olan kadınlarda sonrası gebeliklerin sonuçlarını öngörür mü?

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Abstract

Objective(s): Chromosomal abnormalities are more commonly seen in embryos with decreased oocyte quality. Therefore aneuploidy due to diminished ovarian reserve may be one of the causative reasons of RPL. We investigated the relationship between ovarian reserve markers especially serum antimullerian hormone (AMH) level and antral follicle count (AFC) and recurrent pregnancy loss (RPL).

Materials and methods: This prospective cross-sectional clinical trial including 88 women with RPL and 84 age-matched women without RPL.

Results: There were statistically significant differences in body mass index, live birth number, menstrual cycle length, AFC and serum AMH level between groups. There was no statistically significant difference between groups regarding age, menstrual cycle regularity and serum follicle stimulating hormone (FSH) and estradiol (E₂) levels. The percentage of women with levels of AMH <1 was 21.4% in the RPL group and 11.4% in the control group. AFC <7 in both ovaries was lower in the RPL group when compared with the control group (73.8% vs 44.3%, respectively).

Conclusion(s): Serum AMH levels and AFC can be assessed in patients with RPL as a part of the work up parameters. Lower Serum AMH levels and AFC especially may predict the quantity of oocytes that may be consequently be related with RPL.

Keywords: Recurrent pregnancy loss; Ovarian reserve; Antimullerian hormone; Antral follicle count; Follicle stimulating hormone.

Özet

Amaç: Kromozomal anomaliler sıklıkla azalmış oosit kalitesi ile ilişkili gebeliklerin embryolarında izlenmektedir. Dolayısıyla azalmış overan rezerv ile ilişkili anöploidiler tekrarlayan gebelik kayıplarının bir nedeni (TGK) olabilir. Çalışmamızda over rezerv testleri özellikle de antimüller- yan hormone (AMH) ile antral folikül sayısı (AFS) ile TGK arasındaki ilişkiiyi incelemeyi amaçladık.

Materyal ve Method: Prospektif cross-sectional olan çalış- maz 88 TGK öyküsü olan ve 84 TGK öyküsü olmayan kadın hastayı kapsamaktadır.

Bulgular: İki grup arasında vücut kitle indeksi (VKİ), canlı doğum sayısı, menstrüel siklus uzunluğu, AFS ve serum AMH değeri açısından istatistiksel anlamda farklı bulunuyor. Yaş, menstrüel siklus düzeni, serum folikül stimüle edici hormon ve östradiol değerleri açısından fark tespit edildi. AMH <1 ng/mL, TGK grubunda %21.4 iken, kontrol grubunda %11.4 olarak hesaplandı. AFS<7 olması, TGK grubunda kontrol grubuna göre daha yüksek oranda tespit edildi (%73.8 vs %44.3).

Sonuç: AMH ve AFS, TGK olan hasta grubunda artışa yapılırdı bu parametrelerden biri olabilir. Düşük AMH değeri ve AFS, azalmış oosit kalitesiyle ilişkii olup TGK ile sonuçlanabilir.

Anahtar sözcükler: Tekrarlayan gebelik kaybı; Over rezervi; Antimülleryan hormon; Antral folikül sayısı; Folikül stimül edici hormone.

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Introduction

Recurrent pregnancy loss (RPL), also named as habitual abortus or recurrent miscarriage, is defined as two or more failed clinical pregnancies as demonstrated by ultrasonography or histopathologic examination or three consecutive pregnancy losses, which are not required to be intrauterine [1, 2]. Although there are many causes of RPL including endocrinological, prothrombotic, structural, uterine or autoimmune factors, there is still no clearly identified etiology in 50% of RPL cases [3]. These couples need more understanding and information about future risk of subsequent pregnancy loss. Unfortunately, there is a little data which have inconsistent results in literature to predict the risk of pregnancy loss.

Ovarian reserve describes the number and quality of the follicles in the ovaries at any given time which can be evaluated by endocrine and ultrasound markers. Although there is a no ideal test to measure ovarian reserve, age, antral follicle count (AFC), ovarian volume, stromal blood flow, serum level of follicle stimulating hormone (FSH), estradiol (E2), inhibin B and antimullerian hormone (AMH) are used as markers for ovarian reserve. Among these markers, age, AMH and AFC are accepted to be the most reliable markers [4]. AMH, also known as müllerian inhibiting substance (MIS), belongs to the transforming growth factor-β (TGF-β) family and is secreted by the granulosa cells of the recruited follicles until they become sensitive to FSH [5]. The antral follicles measuring 2–10 mm in diameter within the ovary were defined as AFC.

Although the ovarian reserve could be understood with these markers, they are inadequate for the prediction of the pregnancy outcome. However, to our knowledge, one of the reasons underlying RPL is chromosomal abnormalities which are seen more commonly in embryos with decreased oocyte quality. So it seems reasonable to determine the likelihood of pregnancy outcome in women with RPL with these markers evaluating ovarian reserve. The associations between serum AMH level and oocyte quality, pregnancy outcome and live birth rate have been demonstrated in some studies, but this condition has not been confirmed in other studies [6].

We aimed to compare the patients with RPL and without a history of RPL according to their clinical characteristics and ovarian reserve markers. We also investigated whether there is a relationship between ovarian reserve markers especially serum AMH level and pregnancy loss.

Methods

This study was conducted as a prospective cross-sectional clinical trial including 88 women with RPL and 84 age-matched women without RPL at a tertiary referral center between January 2016 and December 2016. The reason for age-matching instead of another parameter was that age is known to be the most important parameter determining the pregnancy potential [7]. RPL was defined based on a documented history of at least three spontaneous, consecutive pregnancy losses before 20 weeks of gestation, with the same partner. These women with RPL in whom routine blood work up including maternal and paternal chromosomal analyses, serum levels of thyroid stimulating hormone (TSH), lupus anticoagulant and anticardiolipin antibody, HbA1c assessment and pelvic ultrasonography were within reference limits were assigned to the RPL group. The control group consists of healthy women with no history of RPL who were given birth and seeking contraception in the family planning center. We excluded the women with polycystic ovary syndrome because of their elevated AMH and luteinizing hormone (LH) levels. Exclusion criteria were also as follows: (1) presence of endometriosis, (2) history of ovarian surgery, (3) history of pelvic radiation and/or systemic chemotherapy, (4) familial history of premature menopause, (5) existence of ovarian follicles >10 mm, (6) use of any hormone therapy during past 3 months, (7) genetic abnormalities, (8) tobacco and/or alcohol use, (9) evidence of postmenopausal FSH levels, (10) any suspicion of malignant ovarian disease. Because women with these conditions have already poor ovarian reserve and related complications.

All clinical and demographic characteristics including age, body mass index (BMI), live birth number, abortus number, menstrual cycle length and ovarian reserve markers were recorded for both groups and these two groups were compared regarding all of these factors.

Serum FSH, LH, E2 and AMH levels were measured on the 3rd day of the menstrual cycle and the FSH:LH ratio was calculated. The AFC in both ovaries were assessed by transvaginal ultrasonography. All ultrasonographic examinations were done by the same gynecologists using a Voluson 730 Expert with transvaginal probe. On the day of ultrasonography, blood samples were obtained from antecubital vein. The patients’ sera were obtained from blood samples by centrifuge at 3000×g for 10 min. The serum was stored at −80°C until assayed. Serum FSH, LH and E2 levels were measured by chemiluminescence method with original Abbott assays. Serum AMH levels were measured by ELISA (Diagnostic Systems Laboratories; Heidelberg, Germany).
Germany). The normal range for this assay is 0.03–1.5 μg/L. The coefficients of intra- and interassay variations are <10% and <12%, respectively. The cut-off values of diminished ovarian reserve markers were defined as a serum FSH level ≥11 IU/L, a serum E2 level ≥60 nmol/L, a FSH:LH ratio of ≥3, an AMH level ≤1 μg/L and an AFC count ≤7 [8].

Our hospital’s Ethics Committee (Istanbul, Turkey) approved our study which was in accordance with the Declaration of Helsinki (diary number 2016.22.31). All participants were informed about the study and gave written informed consent. The trial was also registered with ClinicalTrials.gov, number NCT03009370.

### Statistical analysis

Statistical analysis were performed with the Statistical Package for the Social Sciences (SPSS Inc; Chicago, IL, USA) statistics 22.0 version for Windows. Difference in mean values and characteristics between groups were analyzed with independent samples t-test and χ² test. Means were presented with standard deviation (SD). Threshold for the association of serum AMH level and RPL was determined by initially using receiver operating characteristics (ROC) curves. While evaluating the area under the curve (AUC), a 5% type-I error level was used to accept a statistically significant predictive value of the test variables. p < 0.05 was considered statistically significant.

### Results

Between January 2016 and December 2016, a total of 88 women for the RPL group and a total of 84 women for the control group met the criteria. The mean age of the patients in the study was 28.4 ± 5.4 years, and the mean BMI was 25.6 ± 4.4 kg/m². The clinical and laboratory characteristics of the patients were summarized in Table 1. Most of the subjects in the study (122 patients, 70.9%) were multiparous. The patients in the RPL group had abortus number ranging from 3 to 7. A total of 158 patients (91.9%) had regular menses.

The RPL group and control group were compared regarding all of these factors showing in Table 2. There was no statistically significant difference between the groups regarding age, menstrual cycle regularity and serum FSH and E2 levels. Women within the RPL group had higher BMI, less live birth number, longer menstrual cycle length as statistically significant (p < 0.001 and <0.001, respectively). There was also statistically significant difference between groups according to AFC and serum AMH level. Women within the RPL group had lower AFC and serum AMH level (p = 0.030 and <0.001, respectively). The percentage of women with AFC ≤7 in both ovaries was 73.8% in the RPL group and 44.3% in the control group (p = 0.013). The comparison of women according to levels of FSH ≥11 IU/L, E2 ≥60 nmol/L, AMH ≤1 μg/L and FSH:LH ratio ≥3 was found to be statistically insignificant. 21.4% of the patients in the RPL group had levels of AMH ≤1 μg/L, while 11.4% of the patients in control group did (Figure 1). We also drawn ROC curves to evaluate the association between serum AMH level and RPL. Area under curves for the prediction of RPL was 62% [95% confidence interval (CI), 54–70%] for serum AMH level (p = 0.007) (Figure 2).

We also divided the patients according to their AMH levels with cut-off value of 1 μg/L: group 1 with AMH level <1 μg/L and group 2 with AMH level ≥1 μg/L. They were compared based on clinical and demographic characteristics and laboratory parameters (Table 3). Women within the group 1 were older and had lower AFC and higher FSH levels as expected (p = 0.001, p = 0.001 and

### Table 1: Clinical characteristics and laboratory values of the patients.

| Characteristics | Mean ± SD or number (%) |
|-----------------|--------------------------|
| Age (years)     | 28.4 ± 5.4               |
| BMI (kg/m²)     | 25.6 ± 4.4               |
| Menstrual cycle length (days) | 28.6 ± 3.9           |
| Live birth number | 1.3 ± 1.1              |
| Serum FSH level (IU/L) | 6.9 ± 3.1              |
| Serum E2 level (nmol/L) | 57.7 ± 51.3          |
| Serum AMH level (μg/L) | 4.6 ± 3.2             |
| AFC in both ovaries | 9.5 ± 4.2             |
| Serum FSH level  |                          |
| <11 IU/L        | 161 (93.6)               |
| ≥11 IU/L        | 11 (6.4)                 |
| Serum E2 level  |                          |
| <60 nmol/L      | 126 (73.3)               |
| ≥60 nmol/L      | 46 (26.7)                |
| Serum AMH level |                          |
| ≤1 μg/L         | 28 (16.3)                |
| >1 μg/L         | 144 (83.7)               |
| AFC in both varies |                    |
| ≤7              | 61 (35.5)                |
| >7              | 111 (64.5)               |
| FSH:LH ratio    |                          |
| <3              | 169 (98.3)               |
| ≥3              | 3 (1.7)                  |

BMI, Body mass index; FSH, follicle stimulating hormone; E2, estradiol; AMH, antimullerian hormone; AFC, antral follicle count.
Table 2: Comparison of the clinical characteristics and laboratory parameters of the patients between the RPL and control group.

| Characteristics               | RPL group | Control group | p-Value   |
|-------------------------------|-----------|---------------|-----------|
| Age (years)                   | 29.4 ± 5.4| 28.0 ± 5.3    | NS        |
| BMI (kg/m²)                   | 26.9 ± 4.9| 24.4 ± 3.5    | <0.001    |
| Live birth number             |           |               |           |
| 0                             | 48 (57.1) | 2 (2.3)       | <0.001    |
| ≥1                            | 36 (42.9) | 86 (97.7)     |           |
| Menstrual cycle regularity    |           |               |           |
| Irregular                     | 4 (4.8)   | 10 (11.4)     | NS        |
| Regular                       | 80 (95.2) | 78 (88.6)     |           |
| Menstrual cycle length (days) |           |               |           |
| Serum FSH level (IU/L)        | 7.3 ± 2.7 | 6.5 ± 3.4     | NS        |
| Serum E₂ level (nmol/L)       | 60.8 ± 53.8| 54.8 ± 35.6  | NS        |
| Serum AMH level (μg/L)        | 3.7 ± 2.9 | 5.4 ± 4.1     | 0.030     |
| AFC in both ovaries           | 7.9 ± 1.8 | 11.2 ± 5.3    | <0.001    |
| Serum FSH level <11 IU/L      | 79 (94)   | 82 (93.2)     | NS        |
| ≥11 IU/L                      | 5 (6)     | 6 (6.8)       |           |
| Serum E₂ level <60 nmol/L     | 64 (76.2) | 62 (70.5)     | NS        |
| ≥60 nmol/L                    | 20 (23.8) | 26 (29.5)     |           |
| Serum AMH level ≤1 μg/L       | 18 (21.4) | 10 (11.4)     | NS        |
| >1 μg/L                       | 66 (78.6) | 78 (88.6)     |           |
| AFC in both ovaries ≤7        | 62 (73.8) | 39 (44.3)     | 0.013     |
| >7                            | 22 (26.2) | 49 (55.7)     |           |
| FSH:LH ratio                  |           |               |           |
| ≤3                            | 84 (100)  | 85 (96.6)     | NS        |
| ≥3                            | 0         | 3 (3.4)       |           |

RPL, Recurrent pregnancy loss; BMI, body mass index; FSH, follicle stimulating hormone; E₂, estradiol; AMH, antimullerian hormone; AFC, antral follicle count; NS, non-significant. Italic values indicates p-values <0.005 as statistically significant.

Figure 1: The AMH levels in control group and in recurrent pregnancy loss group.

Figure 2: The ROC curve between serum AMH level and RPL.

Table 3: Comparison of the clinical characteristics and laboratory parameters of the patients according to serum AMH levels.

| Characteristics               | AMH (μg/L) | p-Value   |
|-------------------------------|------------|-----------|
| Age (years)                   | 31.1 ± 4.6 | 28.9 ± 5.3 | <0.001    |
| BMI (kg/m²)                   | 26.0 ± 4.0 | 25.6 ± 4.5 | NS        |
| Live birth number             |            |            |           |
| 0                             | 7 (25)     | 43 (29.9)  | NS        |
| ≥1                            | 21 (75)    | 101 (70.1) |           |
| Menstrual cycle regularity    |            |            |           |
| Irregular                     | 1 (3.6)    | 13 (9)     | NS        |
| Regular                       | 27 (96.4)  | 131 (91)   |           |
| Menstrual cycle length (days) |            |            |           |
| Serum FSH level (IU/L)        | 9.4 ± 4.3  | 6.4 ± 2.6  | 0.001     |
| Serum E₂ level (nmol/L)       | 56.5 ± 46.0| 58.0 ± 52.4| NS        |
| AFC in both ovaries ≤7        | 6.8 ± 2.3  | 10.1 ± 4.3 | <0.001    |
| >7                            |            |            |           |
| Serum FSH level <11 IU/L      | 22 (78.6)  | 139 (96.5) | <0.001    |
| ≥11 IU/L                      | 6 (21.4)   | 5 (3.5)    |           |
| Serum E₂ level <60 nmol/L     | 22 (78.6)  | 104 (72.2) | NS        |
| ≥60 nmol/L                    | 6 (21.4)   | 42 (27.8)  |           |
| AFC in both ovaries ≤7        | 18 (46.3)  | 43 (29.9)  | <0.001    |
| >7                            | 10 (25.7)  | 101 (70.1) |           |
| FSH:LH ratio                  |            |            |           |
| ≤3                            | 26 (92.9)  | 143 (99.3) | 0.017     |
| ≥3                            | 2 (7.1)    | 1 (0.7)    |           |

AMH, Antimullerian hormone; RPL, recurrent pregnancy loss; BMI, body mass index; FSH, follicle stimulating hormone; E₂, estradiol; AFC, antral follicle count; NS, non-significant. Italic values indicates p-values <0.005 as statistically significant.
The percentage of women with levels of FSH <11 IU/L, AFC ≥7 and FSH:LH ratio <3 was higher in women within group 2 as statistically significant (p < 0.001, p < 0.001 and p = 0.017, respectively).

Discussion

The definition of RPL depends on three or more consecutive pregnancy losses before the completed 20th weeks of gestation with the same partner. This condition affects up to 15% of all clinically confirmed pregnancies [9]. RPL is a heterogeneous condition with a number of underlying reasons such as uterine, infectious, genetic, thrombophilic, endocrine, metabolic, immunologic and environmental. Unfortunately, the cause of RPL could be determined in only 50% of patients.

The women with RPL have an emotionally traumatic experience, similar to that associated with stillbirth or neonatal death. So these couples are seeking predictive markers for subsequent pregnancy outcomes. But we have limited data to predict the risk of pregnancy loss in their future pregnancies.

To our knowledge, an important cause of RPL is genetic abnormalities which are closely related to poor oocyte quality and diminished ovarian reserve [10]. So our aim in this study was to determine the relationship between ovarian reserve markers especially serum AMH level which is accepted to be the most reliable ovarian reserve marker and RPL.

There are several publications with inconsistent results on this topic [11, 12]. Serum levels of FSH, LH, E₂, inhibin B and recently AMH and ultrasonographic measurements such as AFC, ovarian volume and stromal blood flow have been used for ovarian reserve testing. Among them, serum AMH level has been evaluated as a potential clinical marker for ovarian reserve and pregnancy outcome [13]. There is still no consensus on the threshold value for AMH suggesting the reduced fertility potential. Interpretation of AMH levels is laboratory assay-dependent and clinicians decide regarding their own laboratory’s reference ranges. The analytical performances of the different AMH reagents from different manufacturers and the cut-off values for AMH could show differences between each other. This may lead to statistically different results from the measurements. Therefore we assessed serum AMH values in a single laboratory and used this laboratory’s cut-off values [14].

We found the statistically significant difference based on serum AMH level between the RPL group and control group. Also women within the RPL group had lower AFC in comparison to women within control group. Similarly, there are reports in literature supporting the relationship between serum AMH level and pregnancy outcomes [8, 15]. On the other hand, Morel et al. showed that the possibility of live birth or miscarriage could not be predicted by the levels of AMH [16, 17]. The reason for this weak predictive value could be explained by the small size and retrospective design of these studies. Also AMH value reflects the size of the cohort of antral follicles, not the quality of the oocytes.

Pils et al. claimed in their study that the presence of idiopathic recurrent miscarriage could be predicted by only lower basal E₂ and AMH levels, in spite of age and basal serum FSH and LH levels. Because they found more reasonable correlation between AMH and oocyte quality. They explained the relationship between lower E₂ and RPL that these women had been accepted in an earlier stage of losing their ovarian function [18].

There is no established biomarker to inform an individual’s probability of becoming pregnant or experiencing pregnancy loss. On the other hand, there are systematic reviews and meta-analyses suggesting that AMH has an association with clinical pregnancy. So it may have some clinical utility in counseling women regarding the pregnancy outcomes.

One limitation of the study may be the absence of cytogenetic testing of the miscarriages. The other limitation of this study is that AFC may have interobserver and intraobserver variability.

In conclusion, all possible potential confounding factors in the etiology of RPL could not be controlled. However, we showed in this study that AMH could have limited predictive value for pregnancy loss, because women within the RPL group had lower serum AMH levels. So effective management of women with RPL may be provided by the measurement of serum AMH level as a part of the work up parameters which protects us from unnecessary tests and costs.

Ethical considerations

Name of the Ethical Committee: S.B.U. Kanuni Sultan Süleyman Eğitim ve Araştırma Hastanesi Baştabipliği Klinik Araştırmalar Etik Kurulu.

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