

Retrospective Evaluation of Ovarian Stromal Blood Flow and Ovarian Volume Using Three-Dimensional Power Doppler Ultrasonography in Relation to Serum Anti-Müllerian Hormone Levels in Fertile and Infertile Populations

Üç Boyutlu Power Doppler Ultrasonografi ile Değerlendirilen Over Stromal Kan Akımı ve Over Hacminin Serum Anti-Müllerian Hormon Düzeyleri ile İlişkisi: Fertil ve İnfertil Popülasyonda Retrospektif Bir Değerlendirme

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ABSTRACT

Objective: Ovarian reserve assessment is crucial for fertility management. Serum Anti-Müllerian hormone (AMH) is widely accepted as a reliable biomarker. This study aimed to evaluate the relationship between ovarian stromal blood flow, ovarian volume (measured by three-dimensional power Doppler ultrasonography), and serum AMH levels in fertile and infertile women. **Material and Methods:** This retrospective study included 80 women (40 fertile, 40 infertile) aged between 30 and 40 years, categorized into two age subgroups (30–35 and 36–40 years). Fertile participants had regular menstrual cycles, spontaneous conception within the last year, and no polycystic ovarian appearance. Infertile participants were non-polycystic and had infertility unrelated to male factors. Ovarian volumes, stromal blood flow indices (vascularization index [VI], flow index [FI], and vascularization-flow index [VFI]), antral follicle count (AFC), and serum AMH levels were measured during the early follicular phase using three-dimensional power Doppler ultrasound with VOCAL software. **Results:** AMH levels significantly decreased with age in both fertile ($p<0.01$) and infertile groups ($p<0.01$). AFC values were significantly lower in infertile compared to fertile groups within the same age categories ($p<0.01$). AMH correlated positively with AFC in fertile ($r=0.476$, $p=0.002$) and infertile populations ($r=0.375$, $p=0.01$). Ovarian volume showed a positive correlation with AMH only in the infertile group ($r=0.358$, $p=0.015$). There were no significant correlations between AMH and Doppler indices (VI, FI, VFI) in either group. **Conclusions:** AMH levels and AFC remain the most reliable markers for assessing ovarian reserve. Three-dimensional ovarian volume may be useful particularly in infertile populations, but ovarian stromal Doppler indices seem less predictive. Prospective studies with larger samples are recommended to confirm these observations.

Keywords: AMH; ovarian reserve; infertility; three-dimensional power Doppler; ovarian volume

ÖZET

Amaç: Over rezervinin değerlendirilmesi, infertilite yönetimi açısından büyük önem taşımaktadır. Bu çalışmada, üç boyutlu power Doppler ultrasonografi ile ölçülen over stromal kan akımı, over hacmi ve serum Anti-Müllerian Hormon (AMH) düzeyleri arasındaki ilişkinin fertil ve infertil kadınlarda değerlendirilmesi amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmaya, yaşları 30-40 arasında olan, 40 fertil ve 40 infertil kadın dahil edilmiştir. Fertil grup, düzenli adet döngüsü ve son bir yıl içinde spontan gebelik öyküsüne sahip kadınlardan; infertil grup ise erkek faktörü dışlanarak infertilite tanısı alan, polikistik over görünümü olmayan kadınlardan oluşmaktadır. Katılımcıların tümünde erken foliküler fazda over hacmi, stromal kan akımı (Vaskülarizasyon İndeksi [VI], Akım İndeksi [AI], Vaskülarizasyon-Akım İndeksi [VAI]), antral folikül sayısı (AFS) ve serum AMH düzeyleri ölçülmüştür. **Bulgular:** Her iki grupta da yaş ile birlikte AMH düzeylerinde anlamlı azalma saptandı ($p<0.01$). Aynı yaş grubunda infertil kadınların AFC değerleri fertil kadınlara göre anlamlı olarak düşüktü ($p<0.01$). Fertil ve infertil gruplarda AMH ile AFC arasında pozitif korelasyon gözlemlendi (fertil: $r=0.476$, infertil: $r=0.375$). Sadece infertil grupta AMH ile over hacmi arasında pozitif korelasyon saptandı ($r=0.358$, $p=0.015$). AMH ile Doppler parametreleri arasında anlamlı ilişki bulunmadı. **Sonuç:** AMH ve AFS, over rezerv değerlendirmesinde en güvenilir göstergelerdir. Üç boyutlu ultrasonografi ile ölçülen over hacmi infertil popülasyonda ek bilgi sağlayabilir; ancak stromal Doppler parametrelerinin prediktif değeri sınırlıdır. Daha büyük örneklem içeren prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: AMH; over rezervi; infertilite; üç boyutlu power Doppler; over hacmi

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In recent years, evaluating ovarian reserve has gained prominence in reproductive medicine, largely due to the increasing tendency of women to postpone childbearing.¹ It is well-established that maternal age advancement correlates with diminished ovarian reserve, encompassing both the quantity and functional competence of ovarian follicles, which adversely affects fertility outcomes and responsiveness to assisted reproductive techniques (ART).² Accurate assessment of ovarian reserve equips clinicians with critical prognostic insights, enabling earlier reproductive counseling and optimizing the management of infertility interventions.^{3,4}

The evaluation of ovarian reserve today involves both biochemical and ultrasonographic approaches. Biochemical indicators include serum levels of follicle-stimulating hormone (FSH), estradiol, inhibin-B, and most notably, anti-Müllerian hormone (AMH).⁵⁻⁷ In particular, AMH has emerged as a highly valuable marker owing to its low variability throughout and between menstrual cycles, as well as its strong association with the size of the ovarian follicular reserve. These attributes position AMH as a dependable tool for predicting ovarian responsiveness during controlled ovarian stimulation.⁸⁻¹⁰

Three-dimensional (3D) ultrasonography combined with power Doppler imaging presents an advanced, non-invasive method for assessing ovarian reserve. This technique enables precise evaluation of ovarian volume along with stromal blood flow parameters, including the vascularization index (VI), flow index (FI), and vascularization-flow index (VFI).¹¹ Existing research indicates that a reduction in ovarian blood supply may negatively affect follicular development by limiting the delivery of gonadotropins to the ovarian tissues.¹²⁻¹⁴ While the clinical utility of AMH and AFC as reliable ovarian reserve indicators is well-established, there remains considerable interest in exploring whether advanced ultrasonographic modalities, such as three-dimensional power Doppler imaging, can enhance diagnostic accuracy or potentially serve as an alternative to serum-based biomarkers in specific clinical contexts.¹⁵

In this retrospective study, we aimed to explore the association between serum anti-Müllerian hor-

mone (AMH) concentrations and ovarian reserve indicators obtained through three-dimensional power Doppler ultrasonography, specifically focusing on ovarian stromal vascular indices and ovarian volume, in both fertile and infertile cohorts. The results may provide further insight into whether these advanced ultrasonographic measurements consistently reflect serum AMH levels and support improved strategies for infertility evaluation and treatment.

MATERIALS AND METHODS

STUDY DESIGN AND ETHICAL APPROVAL

This retrospective study was carried out at the Department of Obstetrics and Gynecology, Gazi University Faculty of Medicine, Ankara, Turkey, between June 2019 and May 2021. The study protocol received approval from the Gazi University Clinical Research Ethics Committee (Approval Number: 44/2021) and conducted in accordance with the Declaration of Helsinki.

STUDY POPULATION

A total of 80 women were enrolled, comprising 40 fertile and 40 infertile patients, and divided into two age-based subgroups (30-35 and 36-40 years).

■ *Inclusion Criteria For Fertile Women:*

- Age below 40 years
- Regular menstrual cycles (25-35 days, cycle-to-cycle variation ≤ 4 days)
- History of spontaneous conception within the previous year
- No ultrasonographic evidence of polycystic ovarian syndrome (PCOS)

■ *Inclusion Criteria For Infertile Women:*

- Age below 40 years
- Diagnosed infertility not attributable to male factor infertility

- No ultrasonographic evidence of PCOS

■ *Exclusion Criteria For All Participants:*

- History of ovarian or tubal surgery
- Smoking
- Use of hormonal contraceptives within the previous 3 months

- Presence of ovarian cysts or masses detected ultrasonographically

Participants were categorized as follows:

- **Group 1:** Fertile women aged 30-35 years (n=20)

- **Group 2:** Fertile women aged 36-40 years (n=20)

- **Group 3:** Infertile women aged 30-35 years (n=20)

- **Group 4:** Infertile women aged 36-40 years (n=20)

Hormonal Measurements

Venous blood samples for serum AMH analysis were collected from all participants during the early follicular phase, specifically on the third day of the menstrual cycle. Serum AMH concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Beckman Coulter, California, USA) at the Biochemistry Laboratory of Gazi University. The assay demonstrated a detection threshold of 0.02 ng/mL, with intra-assay and inter-assay coefficients of variation calculated at 1.5% and 2.8%, respectively.

Ultrasonographic Assessment

Transvaginal ultrasonography was performed on the third day of the menstrual cycle using a General Electric Healthcare Voluson E6 system equipped with a 3D transvaginal probe (RIC 5-9-D, General Electric, USA). All ultrasound assessments were carried out by the same experienced clinician to ensure consistency.

Ovarian volume measurements and stromal vascular assessments were performed using three-dimensional power Doppler ultrasonography. Data were analyzed with the Virtual Organ Computer-Aided Analysis (VOCAL) software, which is incorporated into the ultrasound platform. The specific ovarian stromal blood flow indices evaluated included:

- **Vascularization Index (VI)**-indicates the percentage of ovarian volume occupied by vessels.

- **Flow Index (FI)**-represents the average blood flow intensity.

- **Vascularization Flow Index (VFI)**-a composite measure calculated by multiplying VI and FI, representing overall ovarian vascularization and flow.

Ovarian volumes were determined using the VOCAL software, which analyzed 12 sequential contours obtained at 30-degree intervals, encompassing a complete 180-degree rotation of the ovary. In addition, antral follicle counts (AFC) were documented during the same ultrasonographic session.

STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS Statistics version 22.0 (SPSS Inc., Chicago, IL, USA). The distribution of continuous variables was evaluated through the Kolmogorov-Smirnov test and visual inspection of histograms. Variables that did not exhibit a normal distribution were expressed as median with corresponding minimum and maximum values. Group comparisons were conducted using the Mann-Whitney U test. Associations between serum AMH concentrations and ultrasonographic parameters were assessed using Spearman's rank correlation coefficient. Correlation strength was classified as weak (0.10-0.39), moderate (0.40-0.69), or strong (≥ 0.70). Statistical significance was defined as a p-value below 0.05.

RESULTS

PATIENT DEMOGRAPHICS AND CLINICAL CHARACTERISTICS

A total of 80 women, categorized equally into fertile (n=40) and infertile (n=40) groups, were evaluated. Each group was further subdivided according to age ranges: 30–35 years (Groups 1 and 3) and 36-40 years (Groups 2 and 4). The clinical characteristics and ovarian reserve markers for all groups are summarized in [Table 1](#).

COMPARISON OF OVARIAN RESERVE PARAMETERS

AFC

AFC was significantly higher in fertile women compared to infertile women within both age groups:

- **30-35 years:** median AFC 11.5 (range: 7-19) in fertile vs. 7 (range: 3-15) in infertile group ($p < 0.01$).

TABLE 1: Clinical and ovarian reserve characteristics according to study groups

Parameter	Fertile (30-35 y) (n=20)	Fertile (36-40 y) (n=20)	Infertile (30-35 y) (n=20)	Infertile (36-40 y) (n=20)	p-value
Age (years)	32.5 (30-35)	38.5 (36-40)	32.5 (30-35)	38.5 (36-40)	-
BMI (kg/m ²)	26 (18-36)	26 (19-39)	23.6 (16-31)	24.5 (17-33)	NS
Total AFC	11.5 (7-19)*	9 (4-15)*	7 (3-15)*	4 (2-14)*	<0.01
Total ovarian volume (cm ³)	12.7 (7.7-36.9)	10.5 (5.9-30.5)	9.8 (2.9-16.7)	7.9 (2.3-16.6)	NS
VI (%)	8.2 (0.05-68.3)	8.7 (0.7-40.9)	7.5 (0-28)	5.9 (0-15.7)	NS
FI	61.4 (35.1-86.7)	60.2 (43.7-76.3)	58.1 (0-81)	56.6 (0-75.9)	NS
VFI	2.8 (0-44.4)	2.4 (0.2-16.1)	2.2 (0-11.8)	2.0 (0-6.1)	NS
Echogenicity (MGV)	82.2 (50.4-97.0)	77.7 (59.9-99.6)	80.5 (51.5-102.9)	80.7 (53.5-93.0)	NS
AMH (ng/mL)	3.1 (0.8-6.7)**	1.1 (0.1-3.8)**	2.4 (0.4-6.4)**	0.4 (0.02-5.8)**	<0.01 (age groups)

*Significant difference between fertile and infertile groups within age categories ($p < 0.01$).

**Significant difference between younger and older groups within fertile or infertile categories ($p < 0.01$).

BMI: Body mass index; AFC: Antral follicle count; VI: Vascularization index; FI: Flow index; VFI: Vascularization-flow index; MGV: Mean gray value; AMH: Anti-Müllerian hormone; NS: Not significant.

■ **36-40 years:** median AFC 9 (range: 4-15) in fertile vs. 4 (range: 2-14) in infertile group ($p < 0.01$).

Serum AMH Levels

AMH levels significantly decreased with increasing age within both fertile and infertile populations:

■ Fertile women: AMH median 3.1 ng/mL (range: 0.81-6.71) in younger group (30-35 years) vs. 1.1 ng/mL (range: 0.05-3.77) in older group (36-40 years), ($p < 0.01$).

■ Infertile women: AMH median 2.4 ng/mL (range: 0.43-6.42) in younger group vs. 0.4 ng/mL (range: 0.02-5.76) in older group, ($p < 0.01$).

However, AMH values were comparable between fertile and infertile women within the same groups ($p > 0.05$).

CORRELATIONS BETWEEN AMH AND ULTRASONOGRAPHIC PARAMETERS

Fertile Population

■ AMH positively correlated with AFC ($r = 0.476$, $p = 0.002$).

■ No significant correlations were observed between AMH and ovarian volume, VI, FI, VFI, or echogenicity in the overall fertile population.

When stratified by age groups:

■ In younger fertile women (30-35 years), AMH positively correlated with AFC ($r = 0.467$, $p = 0.038$)

(Figure 1). No correlations were observed with ovarian volume or Doppler parameters.

■ In older fertile women (36-40 years), no significant correlations were found between AMH and AFC or other ultrasonographic parameters.

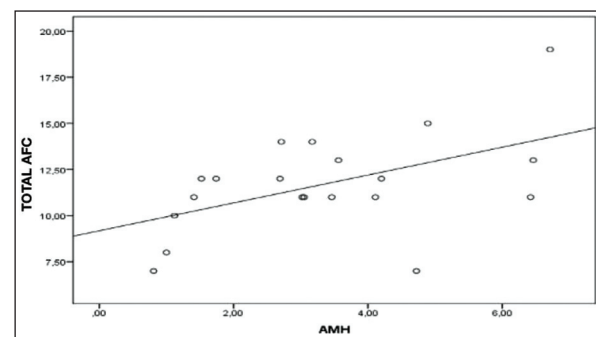


FIGURE 1: Correlation between Serum AMH Levels and AFC in Fertile Women (30-35 years).

TABLE 2: This table presents the statistically significant positive correlations between serum AMH levels and both AFC ($r = 0.375$, $p = 0.01$) and ovarian volume ($r = 0.358$, $p = 0.015$) in infertile women, indicating that higher AMH levels are associated with increased AFC and larger ovarian volumes.

	AMH (ng/mL)	
	r value	p value
Total AFC	0,375	0,01
Total Ovarian Volume (cm ³)	0,358	0,015
Total Ovarian VI (%)	-0,104	0,494
Total Ovarian FI	-0,077	0,610
Total Ovarian VFI	-0,087	0,563
Total Ovarian Echogenicity, MGV	0,106	0,484

AMH: Anti-Müllerian hormone; AFC: Antral Follicle Count; VI: Vascularization Index; FI: Flow Index; VFI: Vascularization Flow Index; MGV: Mean Gray Value.

Infertile Population

■ AMH positively correlated with AFC ($r=0.375$, $p=0.01$) and ovarian volume ($r=0.358$, $p=0.015$) (Table 2).

■ No significant correlations between AMH and Doppler indices (VI, FI, VFI) were observed.

When stratified by age groups:

■ In both younger (30-35 years) and older infertile groups (36-40 years), no significant correlations were found between AMH and ultrasonographic parameters individually.

AMH AND OOCYTE YIELD IN INFERTILE WOMEN UNDERGOING ART

A subgroup analysis in infertile women undergoing ART treatments showed that serum AMH positively correlated with the number of retrieved oocytes ($r=0.566$, $p=0.004$) (Figure 2). However, other ultrasonographic parameters, including AFC, ovarian volume, and Doppler indices, did not significantly correlate with oocyte yield.

Correlations of AMH and AFC with Age

Negative correlations with age were found for both AMH and AFC:

■ Fertile group: AMH ($r=-0.424$, $p=0.006$), AFC ($r=-0.364$, $p=0.021$) (Table 3).

■ Infertile group: AMH ($r=-0.590$, $p<0.001$), AFC ($r=-0.493$, $p=0.021$) (Table 4).

No significant correlations were observed between age and ovarian Doppler parameters (VI, FI,

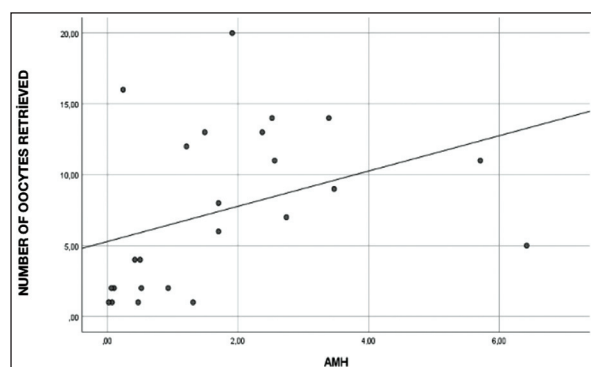


FIGURE 2: Correlation between Serum AMH levels and the number of oocytes retrieved in infertile women undergoing ART

TABLE 3: Table showing a statistically significant negative correlation between age and serum AMH levels in fertile women (Spearman's $r=-0.424$, $p=0.006$), indicating that AMH levels decrease with advancing age.

	Age r value	p value
AMH (ng/ml)	-0,424	0,006
Total AFC	-0,364	0,021
Total Ovarian Volume (cm ³)	-0,070	0,667
Total Ovarian VI (%)	0,168	0,301
Total Ovarian FI	-0,190	0,400
Total Ovarian VFI	0,065	0,400
Total Ovarian Echogenicity, MGv	0,033	0,021

TABLE 4: Table showing a statistically significant negative correlation between age and serum AMH levels in infertile women (Spearman's $r=-0.590$, $p<0.001$), indicating that AMH levels decline with increasing age.

	Age r value	p value
AMH (ng/ml)	-0,590	<0,001
Total AFC	-0,493	0,021
Total Ovarian Volume (cm ³)	-0,194	0,460
Total Ovarian VI (%)	-0,209	0,163
Total Ovarian FI	0,368	0,460
Total Ovarian VFI	0,130	0,460
Total Ovarian Echogenicity, MGv	0,225	0,460

VFI) or ovarian volume in either fertile or infertile populations.

DISCUSSION

The primary aim of this study was to retrospectively evaluate ovarian stromal blood flow and ovarian volume, assessed by three-dimensional (3D) power Doppler ultrasonography, and to examine their relationship with serum AMH levels in fertile and infertile populations. Our findings indicate that serum AMH and AFC remain the most reliable markers for assessing ovarian reserve, whereas ovarian volume may serve as an additional predictor, particularly in infertile populations. In contrast, the Doppler-derived ovarian stromal blood flow indices (VI, FI, VFI) showed limited predictive value in the context of ovarian reserve evaluation.

CORRELATION OF AMH AND AFC IN FERTILE AND INFERTILE POPULATIONS

Consistent with previous literature, our results confirmed a significant positive correlation between serum AMH levels and AFC, reinforcing their established clinical roles in evaluating ovarian reserve.^{16,17} Numerous studies have demonstrated the predictive accuracy of AMH and AFC for ovarian response during assisted reproductive technology (ART) treatments.^{7-9,18} Our findings align with these data, suggesting the continued value of these two markers in routine clinical practice.

AMH and Ovarian Volume

Our results indicated a significant positive correlation between ovarian volume measured by 3D ultrasonography and serum AMH levels in the infertile group. This finding suggests that ovarian volume may reflect ovarian reserve status more reliably in infertile women. Previous meta-analyses have shown that ovarian volume alone is less predictive than AFC and AMH but may complement these markers, particularly in subgroups at higher risk of reduced ovarian reserve.^{14,19,20} Our data support this hypothesis, highlighting that ovarian volume measurement could provide adjunctive clinical information, particularly when AFC measurement or AMH assays are challenging or inconclusive.

LIMITED ROLE OF DOPPLER INDICES (VI, FI, VFI)

Contrary to our initial expectations, ovarian stromal Doppler indices (VI, FI, VFI) did not correlate significantly with serum AMH or other ovarian reserve markers in either fertile or infertile populations. Previous studies examining the role of 3D Doppler parameters have produced inconsistent findings. While some authors reported correlations between ovarian blood flow and ovarian reserve status, others found no significant associations.^{11,21,22} Jokubkiene et al., for example, observed a decline in ovarian stromal blood flow with increasing age but noted limited clinical applicability for predicting ovarian reserve or fertility outcomes.¹²

Our results align with those studies demonstrating limited clinical utility of Doppler indices in routine ovarian reserve assessment. It is plausible that

ovarian stromal blood flow may vary significantly based on physiological and technical factors, reducing the consistency and reliability of Doppler-derived indices. Therefore, we recommend that Doppler parameters should not replace established ovarian reserve markers, such as AMH and AFC, but may be considered complementary tools in select clinical scenarios, warranting further prospective investigation.

Impact of Age on AMH and AFC

Age-related decline in ovarian reserve is well established. Our study demonstrated significant negative correlations between age and both serum AMH levels and AFC, consistent with previous reports.^{23,24} These findings underscore the sensitivity of AMH and AFC as robust biomarkers for reproductive aging. Given the growing number of women delaying childbearing, accurate age-dependent ovarian reserve assessment is increasingly important for effective clinical counseling and fertility management strategies.

AMH as a Predictor of Oocyte Yield in ART

Our subgroup analysis of infertile women undergoing ART confirmed a moderate yet significant correlation between AMH and the number of retrieved oocytes. Several studies have highlighted AMH as the best predictor of oocyte yield in ART cycles, surpassing other markers such as basal FSH, estradiol, and ovarian volume.^{6,23,25,26} Our results reinforce these findings, further validating AMH's clinical utility in predicting ovarian response during controlled ovarian stimulation.

STUDY LIMITATIONS

This study has several limitations, including its retrospective design, relatively small sample size, and single-center nature, potentially limiting the generalizability of findings. Furthermore, the lack of longitudinal follow-up precludes assessment of clinical pregnancy outcomes, thus limiting conclusions regarding the predictive value of Doppler parameters for clinical fertility outcomes. Future prospective multicenter studies with larger sample sizes and comprehensive follow-up are needed to confirm these preliminary findings and clarify the role of Doppler parameters in ovarian reserve assessment.

CONCLUSION OF DISCUSSION

In summary, our study provides additional evidence supporting the use of serum AMH and AFC as primary ovarian reserve markers in clinical practice. While ovarian volume measured by 3D ultrasonography may be useful in specific patient populations, ovarian stromal Doppler indices (VI, FI, VFI) appear to have limited predictive utility. Further research is necessary to determine any potential complementary role of advanced Doppler techniques in reproductive medicine.

CONCLUSION

This retrospective analysis confirms that serum AMH levels and AFC remain the most consistent and reliable markers for evaluating ovarian reserve in both fertile and infertile populations. Ovarian volume assessed via three-dimensional ultrasonography also demonstrates potential utility, particularly within infertile groups, as an adjunct marker of ovarian reserve. In contrast, ovarian stromal Doppler indices (VI, FI, VFI) exhibit limited predictive capacity and currently should not replace established ovarian re-

serve biomarkers such as AMH and AFC.

Further prospective, multicenter studies involving larger patient cohorts and longitudinal follow-up are necessary to comprehensively evaluate the clinical value and potential complementary role of advanced ultrasonographic techniques in ovarian reserve testing and infertility management.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

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