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The relationship between oocyte maturation and follicle size: A comparative analysis of 2D and 3D ultrasound

Oosit olgunlaşması ve folikül boyutu arasındaki ilişki: 2D ve 3D ultrasonun karşılaştırmalı analizi

Özge Karaosmanoğlu, Ayşen Yücetürk, Nuri Peker, Ömür Albayrak, Burak Elmas, İlke Özer Aslan, Bülent Tıraş

Acıbadem Maslak Hospital, In Vitro Fertilization Center, İstanbul, Türkiye

Abstract

Objective: To compare the ability of three-dimensional (3D) and conventional two-dimensional (2D) ultrasound to predict oocyte maturity during in vitro fertilization cycles, and to evaluate their contribution to trigger timing and to the determination of optimal cut-off values for maximizing metaphase II (MII) oocyte yield using automated volume-calculation software.

Materials and Methods: Forty-three infertile women who had ≤5 follicles, were younger than 40 years, had a body mass index <30, and had no previous oocyte maturation problems were included in this retrospective study. Follicle diameter was measured using 2D ultrasound, while follicle volume was measured using 3D ultrasound with SonoAVC software. The obtained values were compared with those from MII oocytes, and receiver operating characteristic (ROC) analysis and logistic regression were performed.

Results: A total of 203 oocytes were analyzed; 70% of them were in the MII stage. In the ROC analysis, the optimal cut-off for 2D measurement was determined to be 17.05 mm [area under curve (AUC)=0.737], and for 3D measurement, it was 1.83 cm³ (AUC=0.709). 2D measurements showed specificity, while 3D measurements showed sensitivity. In logistic regression analysis, both 2D diameter and 3D volume were found to be independent predictors of MII oocyte development.

Conclusion: Our findings suggest that 3D ultrasound measurements may provide greater sensitivity for predicting oocyte maturity. However, false-positive results may occur in the presence of multiple or nested follicles, and observer dependence cannot be completely eliminated. Therefore, optimization and large-scale validation studies are needed to improve the accuracy of the method.

Keywords: Oocyte maturation, in vitro fertilization, ultrasonography, imaging, three-dimensional

Öz

Amaç: Bu çalışmada, in vitro fertilizasyon sikluslarında oosit olgunluğunu öngörmede üç boyutlu (3D) ultrason ile ölçülen folikül hacmi ile geleneksel iki boyutlu (2D) ultrason ölçümlerinin karşılaştırılması ve triger zamanlamasında klinik katkılarının araştırılması amaçlanmıştır. Ayrıca, yapay zeka tabanlı otomatik hacim hesaplama yazılım desteğiyle ovaryen hiperstimülasyon döngülerinde maksimum metafaz II (MII) oosit elde edilebilmesi için en uygun cut-off değerinin belirlenmesi hedeflenmiştir.

Gereç ve Yöntemler: Retrospektif olarak planlanan çalışmaya, ≤5 folikülü bulunan, 40 yaş altı, vücut kitle indeksi <30 olan ve daha önce oosit maturasyon problemi bulunmayan 43 infertil kadın dahil edilmiştir. Folikül çapı 2D ultrason ile, folikül hacmi ise SonoAVC yazılımı kullanılarak 3D

PRECIS: Two-dimensional (2D) follicle diameter and three-dimensional follicular volume provide complementary clinical information for predicting oocyte maturity and guiding trigger timing in IVF cycles.

Corresponding Author/Sorumlu Yazar: Özge Karaosmanoğlu MD,
Acıbadem Maslak Hospital, In Vitro Fertilization Center, İstanbul, Türkiye
E-mail: ozge61@gmail.com **ORCID ID:** orcid.org/0000-0001-8184-4747

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ultrason ile ölçülmüştür. Elde edilen değerler MII oositler ile karşılaştırılmış, alıcı işletim karakteristiği (ROC) analizi ve lojistik regresyon testleri uygulanmıştır.

Bulgular: Toplam 203 oosit analiz edilmiştir; bunların %70'i MII evresinde bulunmuştur. ROC analizinde 2D ölçüm için en uygun cut-off 17,05 mm [eğrinin altındaki alan (AUC)=0,737], 3D ölçüm için ise 1,83 cm³ (AUC=0,709) olarak belirlenmiştir. 2D ölçümler özgüllük açısından, 3D ölçümler ise duyarlılık açısından daha yüksek performans göstermiştir. Lojistik regresyon analizinde hem 2D çap hem de 3D hacim MII oosit gelişimi için bağımsız prediktör bulunmuştur.

Sonuç: Bulgularımız, literatür ile uyumlu olarak 3D ultrason ölçümlerinin oosit olgunluğunu öngörmede duyarlılık avantajı sağlayabileceğini göstermektedir. Ancak çok sayıda veya iç içe folikül varlığında yanlış pozitif sonuçlar oluşabilmekte, ayrıca gözlemci bağımlılığı tamamen ortadan kalkmamaktadır. Bu nedenle yöntemin doğruluğunun artırılmasına yönelik optimizasyon ve geniş ölçekli doğrulama çalışmalarına ihtiyaç vardır.

Anahtar Kelimeler: Oosit maturasyonu, in vitro fertilizasyon, ultrasonografi, görüntüleme, üç boyutlu

Introduction

Infertility is a significant health issue affecting millions of individuals worldwide. In vitro fertilization (IVF) procedures, which are part of assisted reproductive technologies, are among the most commonly used and promising treatment methods for infertile couples. IVF success has many components; however, oocyte maturity, particularly the number of metaphase II (MII) oocytes, is a critical factor directly affecting embryo quality, implantation rates, and live birth rates[1-3].

Three-dimensional ultrasound (3D-US), a significant milestone in gynecological and obstetric diagnostic imaging, has been increasingly adopted clinical practice in recent years. In obstetrics, it is widely used to examine fetal malformations and to perform facial assessments[4,5]. In gynecology, its primary applications include the evaluation of uterine anomalies using sonohysterography and the detection of intracavitary pathologies. However, antral follicle counting and follicle monitoring are still routinely performed using two-dimensional ultrasound (2D-US)[5,6]. This raises the question of whether 3D ultrasound can replace 2D ultrasound for assessing oocyte maturity via follicular volume measurements and for determining trigger timing.

Transvaginal 2D ultrasound has been widely used in recent years for the evaluation, treatment planning, and follow-up of infertile women. This method has become the preferred approach for determining the number, size, and volume of follicles in real time. However, follicle monitoring with 2D-US has limitations such as manual measurements, interobserver variability, increased time requirements, and errors related to follicle shape[7,8]. In contrast, 3D-US allows imaging of the entire ovarian tissue in a single sonographic section. Follicle count, diameter, and volume measurements are performed using automated volume calculation software. This software automatically identifies fluid-filled structures in the area of interest and reports the size and volume information for each follicle. Although 3D-US has been shown to provide reliable estimates of follicle size, it is still used to a limited extent to monitor ovarian response to stimulation[9-11]. Thus, current studies indicate that the contribution of 3D-US to predicting oocyte maturity and trigger timing remains incompletely clarified in clinical terms[12,13].

This study aimed to compare follicle volume measured by 3D-US with 2D diameter measurements in predicting oocyte maturity during IVF cycles and to investigate their contributions to more accurate trigger timing. Additionally, the study aimed to determine the optimal cut-off value to achieve the maximum number of MII oocytes during ovarian hyperstimulation cycles using artificial intelligence-based software.

Materials and Methods

Study Design and Participants

This retrospective study included 43 infertile women who presented to the IVF center at Acıbadem Hospital and underwent IVF with controlled ovarian stimulation.

Inclusion criteria were defined as patients who started stimulation for IVF with an antagonist protocol, were under 40 years of age, and had ≤5 follicles in both ovaries. Patients with a history of oocyte maturation problems, endometrioma, or smoking, or who had a body mass index (BMI) ≥30, were excluded from the study.

The study was approved by the Acıbadem Medical Research Ethics Committee (Acıbadem) under decision number 2025-08/61, dated: 22.05.2025. Informed consent was obtained from all participants.

Ovarian Stimulation Protocol

During controlled ovarian hyperstimulation, the gonadotropin-releasing hormone antagonist (Cetrorelix, Merck Serono, Darmstadt, Germany) was initiated at 250 µg/day when the leading follicle diameter reached ≥14 mm and was continued until the trigger day.

The trigger was administered when at least half of the follicles were ≥17 mm in diameter as determined by ultrasound. For this purpose, recombinant human chorionic gonadotropin (1000-2000 IU, Ovitrelle, Merck, Lyon, France) and triptorelin acetate, 0.1 mg/mL (two doses; Gonapeptyl, Ferring Pharmaceuticals) were used. Oocyte retrieval (OPU) was performed under sedation 36 hours after the trigger.

Ultrasound Evaluations

Patients' ultrasonographic examinations were performed by a single specialist with at least 10 years of experience in IVF. All

evaluations were performed using a Voluson S8 (GE Medical Systems, Zipf, Austria) equipped with a 5-9 MHz transvaginal volume probe.

First, follicles were measured using 2D ultrasound, and their number and size in both ovaries were recorded. Then the ovary's maximum diameter was measured, and the image was fixed for 3D volume scanning. Follicle volumes were calculated semi-automatically using SonoAVC software.

In cases where the software mistakenly evaluated vascular cross-sections or free fluid areas as follicles, the cross-sections were reacquired and volume measurements were repeated (Figure 1).

Since the primary goal was to track each oocyte individually and match it to the MII stage, cases with a large number (>20) of follicles were excluded because reliable follicle-oocyte matching could not be performed (Figure 2).

Oocyte Retrieval and Evaluation

Follicles marked on ultrasound were aspirated individually during OPU. Oocytes were evaluated by embryologists after denudation, and their maturity status was recorded.

Statistical Analysis

Descriptive statistics were calculated for all variables. Continuous variables [2D follicle measurements (mm) and 3D follicle volumes (cm^3)] were summarized as mean ± standard deviation when normally distributed and as median (minimum-maximum) when not normally distributed. Normality was assessed using the Shapiro-Wilk test. Categorical variables were presented as counts and percentages. Receiver operating characteristic (ROC) curve analyses were performed separately for 2D and 3D measurements to evaluate the discriminatory power of follicle size to predict MII oocytes. The area under the curve (AUC) and 95% confidence intervals (CIs) were reported. The optimal cut-off points were determined using the Youden index, and diagnostic performance was summarized by sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Additionally, logistic regression analyses were performed to predict MII outcome from follicle size. Logistic regression analyses were performed using univariate models. In model 1, 3D follicular volume was used as a continuous variable; in model 2, volume was categorized according to a cut-off value of 1.83 cm^3 (<1.83 vs. ≥1.83 cm^3). The same analyses were performed in parallel for 2D measurements. All analyses were performed using R software (version 4.4.2). P<0.05 was considered statistically significant.

Results

Among the analyzed oocytes, the mean 2D follicle measurement was 16.54±3.66 mm (range: 6.4-24.0 mm), and the mean 3D follicle volume was 2.37±1.26 cm^3 (range: 0.20-5.41 cm^3). Of the 203 oocytes evaluated, 142 (70.0%) were in the MII stage, 14 (6.9%) were in the metaphase I (MI) stage, and 15 (7.4%) were in the germinal vesicle stage. Additionally, 13 (6.0%) of the 202 oocytes examined were at the MA stage (Table 1).

ROC analysis of 2D follicular measurements determined an optimal cut-off value of 17.05 mm (sensitivity=0.67, specificity=0.79, PPV=0.88, NPV=0.51). Below this threshold, the MII rate was 49.5% (47/95), while above the threshold, this rate increased to 88.0% (95/108). This finding indicates that larger 2D follicular diameters are significantly associated with the likelihood of reaching MII (Figure 3; Table 2).

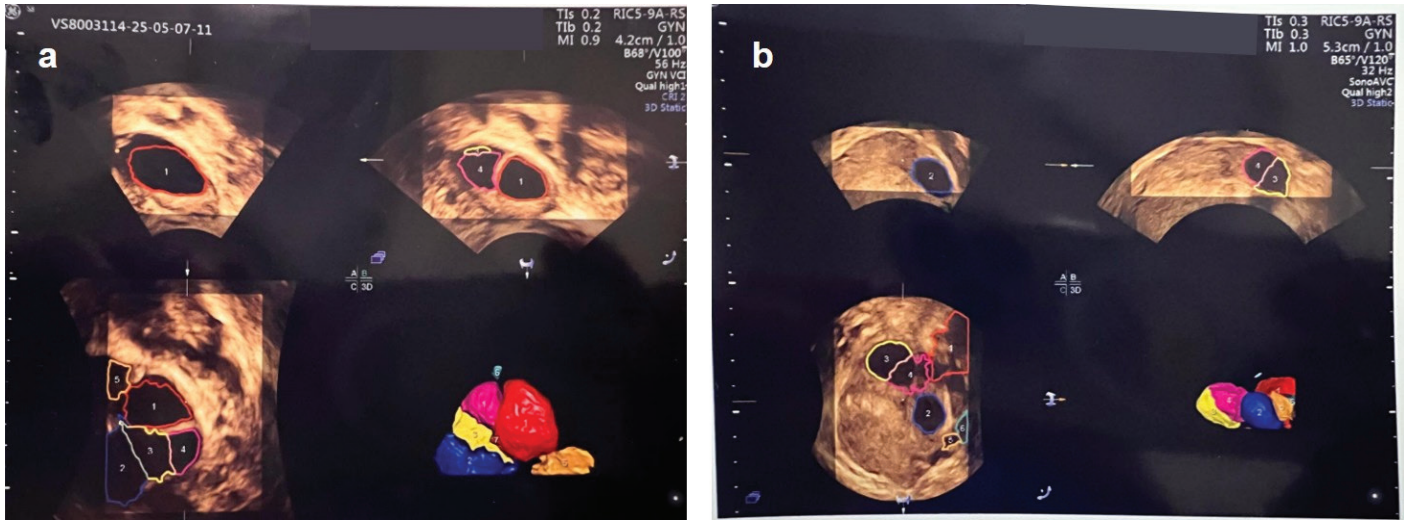

Figure 1. Examples of semi-automatic measurement of follicle volumes using three-dimensional ultrasound and SonoAVC software. Each follicle is marked with a different color and numbered. (a) Number "2" was incorrectly identified as a follicle by the software and required manual correction as it was a blood vessel section. (b) Number "1" is an example of free fluid around the ovary being detected as a follicle by the software

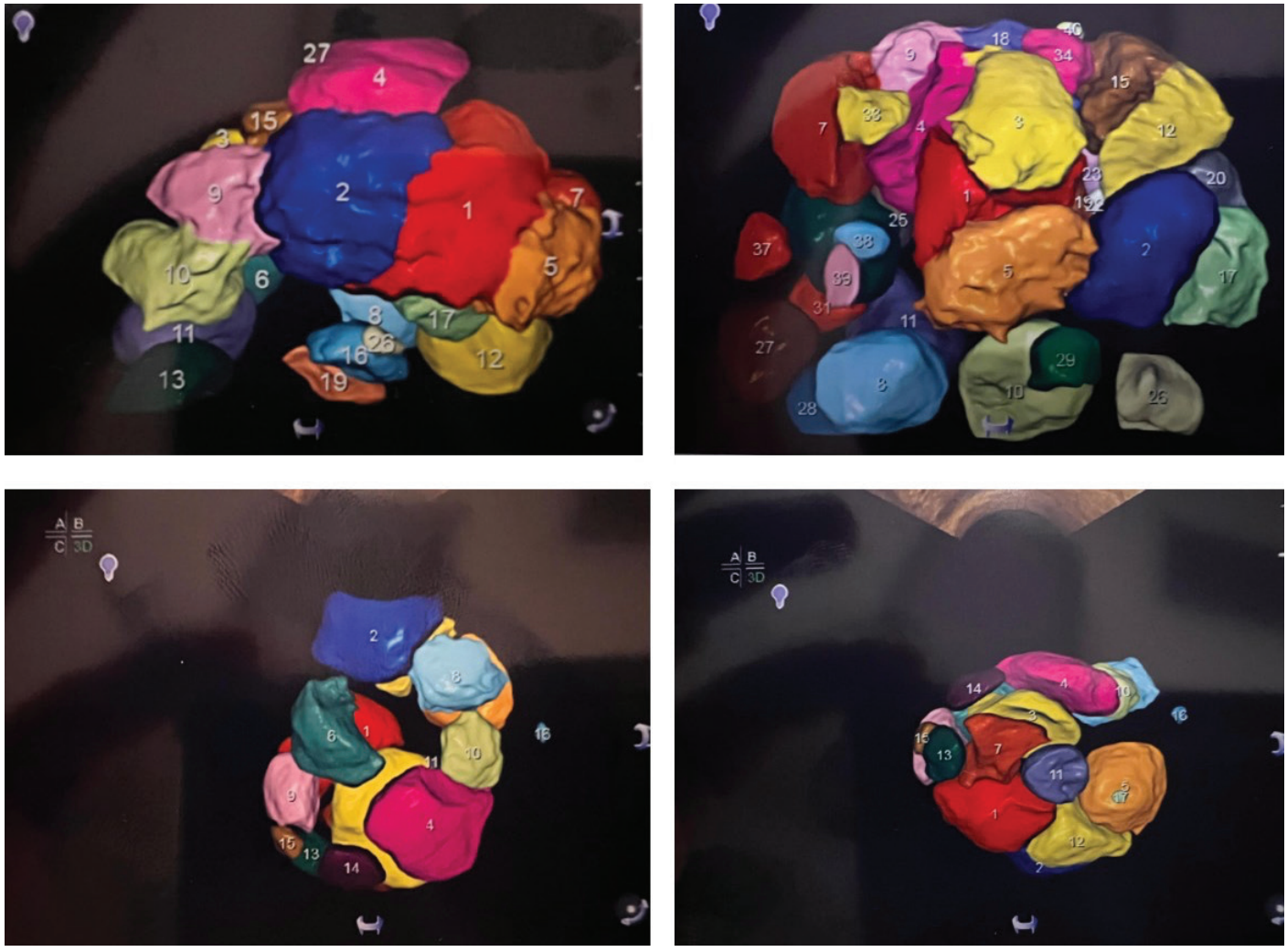

Figure 2. Examples of volume reconstruction using three-dimensional ultrasound and SonoAVC software in different cases with multiple follicles. Each follicle is marked with a different color and numbered. Since follicle-oocyte matching cannot be reliably performed in such cases, they were not included in the study to maintain methodological consistency

Table 1. Distribution of oocytes according to 2D follicle measurements, 3D follicle volumes, and maturation stages

Variable	Mean	SD	Min	Max
2D measurement	16.54	3.66	6.4	24.00
3D volume	2.37	1.26	0.2	5.41
	N	Count (1)	Count (0)	% (1)
MII	203	142	61	70
MI	203	14	189	6.9
GV	203	15	188	7.4
MA	202	13	189	6.0

SD: Standard deviation, MII: Metaphase II, MI: Metaphase I, GV: Germinal vesicle, MA: Metaphase arrest, Min: Minimum, Max: Maximum, 3D: Three-dimensional, 2D: Two-dimensional

The cut-off value of 17.05 mm, determined for 2D measurement, provided a meaningful distinction for predicting MII oocytes. The MII rate in follicles below this threshold value was approximately half that in follicles above the threshold, where a significantly higher rate of 88.0% was observed. This finding supports the utility of 2D measurements in clinical decision-making.

ROC analysis revealed an AUC of 0.709 for 3D follicular volume. This value indicates that 3D volume measurement has moderate discriminatory power for distinguishing oocytes that reach the MII stage. In the literature, AUC values between 0.70 and 0.80 are considered acceptable, between 0.80 and 0.90 are considered good, and values >0.90 are considered to indicate excellent discriminatory power. The ROC curve lying above the diagonal reference line indicates that the model performed better than random guessing. Furthermore,

the curve approaching the upper left corner indicates that certain cutoff points (e.g., 1.83 cm³) provide an appropriate balance between sensitivity and specificity. Clinically, follicles with larger 3D volumes were more likely to reach the MII stage (Figure 4).

Table 2. MII rates according to 2D follicle diameter cut-off values

2D cut-off group	Follicle count	MII number	Ratio (%)
<17.05 mm	95	47	49.5%
≥17.05 mm	108	95	88.0%

MII: Metaphase, 2D: Two-dimensional

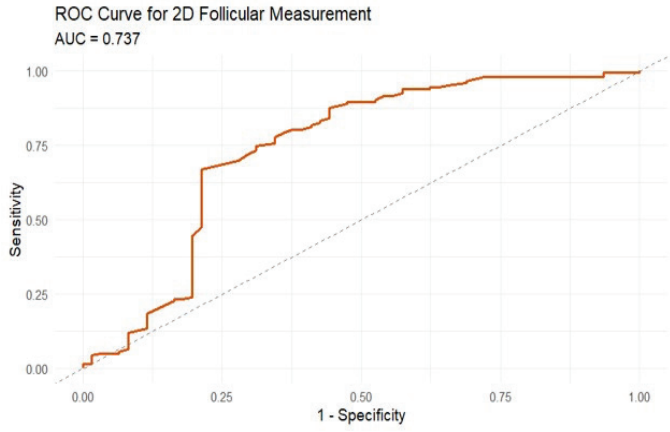

Figure 3. ROC curve for 2D follicle measurement. The analysis determined a cutoff value of 17.05 mm as the optimal threshold (AUC=0.737). This value has moderate discriminatory power in predicting oocytes that reach the metaphase II stage. The curve indicates an appropriate balance between sensitivity and specificity

ROC: Receiver operating characteristic, AUC: Area under the curve, 2D: Two-dimensional

Table 3. MII rates according to the 3D follicular volume cut-off value

3D cut-off group	Follicle count	MII number	Ratio (%)
<1.83 cm³	74	34	45.9%
≥1.83 cm³	129	108	83.7%

3D: Three-dimensional, MII: Metaphase

This analysis contributes to the objective selection of a specific cut-off value and to the decision-making process in clinical practice (Table 3).

ROC analysis for 3D follicular volume determined an optimal cut-off value of 1.83 cm³. Below this threshold, the MII rate was 45.9% (34/74), while above the threshold, this rate increased to 83.7% (108/129). At this cut-off value, sensitivity was 0.76, specificity was 0.66, PPV was 0.84, and NPV was 0.54. These findings indicate that 3D follicular volume has significant discriminatory ability to predict the likelihood of reaching MII.

Logistic regression analysis showed that both 2D follicular diameter and 3D follicular volume are meaningful predictors of MII oocyte development. Larger follicle size was associated with a significantly increased probability of reaching MII, both when analyzed as a continuous variable and when categorized using cutoff values from ROC analysis (Table 4). Both 2D follicle diameter and 3D follicle volume were found to be significant predictors of reaching the MII stage. For continuous variables, each one-unit increase significantly increased the likelihood of achieving MII. When ROC-derived cut-off values were applied, larger follicle sizes also were associated with a marked increase in the likelihood of achieving MII.

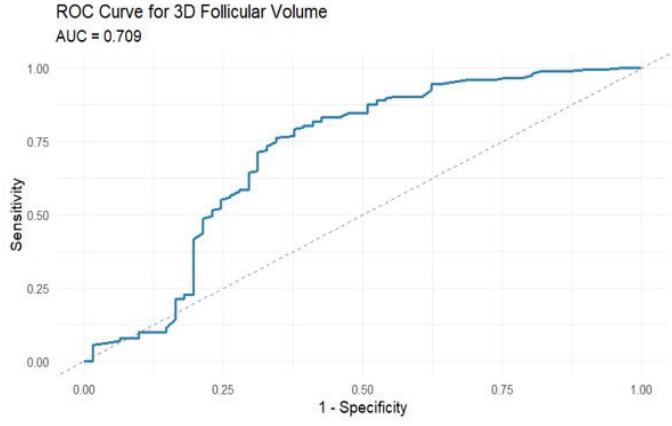

Figure 4. ROC curve for 3D follicular volume. The curve indicates that the model has moderate discriminatory power in predicting oocytes that reach the metaphase II stage (AUC=0.709)

ROC: Receiver operating characteristic, AUC: Area under the curve, 3D: Three-dimensional

Table 4. Logistic regression analysis showing the relationship between follicle size and the probability of obtaining MII oocytes

Variable	OR	95% CI	p-value
3D volume (continuous)	1.85	(1.42; 2.47)	0.0000126000
3D volume (≥1.83 vs. <1.83 cm³)	6.05	(3.18; 11.82)	0.0000000681
2D diameter (continuous)	1.30	(1.19; 1.44)	0.0000000521
2D diameter (≥17.05 vs. <17.05 mm)	7.46	(3.78; 15.62)	0.0000000235

OR: Odds ratio, CI: Confidence interval, MII: Metaphase

3D volume (continuous): For each 1 cm³ increase, the odds of achieving MII increased 1.85-fold [odds ratio (OR) = 1.85, 95% CI: 1.42-2.47].

For 3D volume (cut-off ≥1.83 cm³), follicles above this threshold showed a sixfold higher probability of obtaining MII compared to those with smaller volumes (OR=6.05, 95% CI: 3.18-11.82).

2D diameter (continuous): Each 1-mm increase was associated with a 1.30-fold higher likelihood of achieving MII (OR=1.30, 95% CI: 1.19-1.44).

2D diameter (cut-off ≥17.05 mm): Larger follicles had approximately 7.5-fold higher odds of MII than smaller ones (OR=7.46, 95% CI: 3.78-15.62).

Discussion

Many researchers have recently questioned the accuracy of the aforementioned 2D measurement approach, particularly during controlled ovarian hyperstimulation cycles, due to the irregular shape of follicles.[5,6] Measuring follicle size often yields inaccurate results because follicles, which are actually 3D structures, are assessed as 2D structures, even in ovaries containing few or small follicles. Current literature on the accuracy of 3D and 2D ultrasound in IVF follicular assessment shows that 3D ultrasound is more accurate than real-time 2D ultrasound in measuring follicular size and volume[14,15]. However, software errors and technical limitations have been observed in 3D ultrasound measurements, and the accuracy and clinical utility of the method require further optimization. In this study, both 2D-US and 3D-US measurements were found to be valuable in predicting oocyte maturity. Analyses revealed that 2D measurements reduced false positives, whereas 3D measurements were more sensitive in detecting mature oocytes. These findings indicate that both methods contribute to determining trigger timing from different perspectives and may be complementary. 3D-US provides practical benefits in assessing maturity, especially in patients with few follicles, but 2D examinations remain important in the clinical process to validate the measurements. Additionally, these results suggest that 3D-US may offer a significant advantage in clinical practice, particularly in patients with a low follicle count and a BMI <30.

Our findings are consistent with the existing literature. For instance, Shmorgun et al.[15] reported that 3D volume measurements showed a higher, though limited, correlation with oocyte maturation than did 2D diameter measurements. Hernández et al.[16] demonstrated a significant relationship between follicle volume classes and the number of mature oocytes based on automatic volume measurements performed with SonoAVC software. In a more recent study, Rodr´guez-Fuentes et al.[2] showed that a follicular volume >0.56 cm³ on the trigger day could predict the retrieval of mature oocytes, but sensitivity and specificity were moderate. Similarly, in our study, 3D volume measurements showed moderate discriminatory power, and our analyses determined the most appropriate cut-off value to be 1.83 cm³. This finding indicates that threshold values may vary across populations, but 3D-US generally offers greater sensitivity. In addition, a newly published study by Yang et al.[14] reported that follicular sphericity was predictive only in patients with normal ovarian reserve, had limited discriminatory power for MII oocytes, and showed no correlation with clinical outcomes in the low-reserve group. These findings indicate that the clinical use of volume- and shape-based 3D parameters still requires optimization and larger, multicenter validation studies.

These studies commonly conclude that the conventional approach based on follicle diameter has limitations and that volume-based assessment has the potential to guide clinical decisions more accurately. Our study supports these findings, particularly among a homogeneously selected group of patients with few follicles.

Automated 3D ultrasound software, such as SonoAVC, saves clinicians time by enabling rapid evaluation of follicles in the correct sections and significantly reduces observer dependence often seen in manual 2D measurements. However, in this study evaluation was difficult in patients with a large number of follicles, because closely adjacent or intertwined follicles were sometimes perceived as a single follicle. This situation necessitated that the physician performing the ultrasound also conduct a 2D examination to verify the measurements. Furthermore, vascular structures in the ovarian section or surrounding fluids can also be interpreted as follicles by the software, potentially leading to misleading results in volume measurements. Indeed, these limitations reported in early studies such as Coelho Neto et al.[7] and Vandekerckhove et al.[17], these limitations, which have been reported in early studies, are still valid in some other recent studies [Rodr´guez-Fuentes et al.[2]], highlighting the need for optimization and validation in the clinical use of the method.

Our findings indicate that 2D-US follicle diameter remains reliable for routine trigger decisions, whereas 3D follicle volume measurement may provide additional value in specific clinical scenarios, particularly for patients with low follicle counts or borderline follicle growth. The higher sensitivity of 3D-US may help clinicians avoid premature triggering in such cases. However, due to software limitations and the risk of false-positive measurements caused by overlapping follicles, the routine use of 3D ultrasonography should be reserved for complex or uncertain cases, rather than replacing traditional 2D assessment.

Study Limitations

The main limitations of this study include its retrospective nature and its relatively small, homogeneous study population. These conditions may also limit the generalizability of the findings. The exclusion of patients with a high number of follicles raises questions regarding the applicability. Furthermore, when interpreting the results, the technical

limitations of automated 3D volume measurements and the absence of clinical outcomes beyond oocyte maturity should be considered.

Conclusion

Our study demonstrates that follicular volume measurements obtained by 3D ultrasound may be clinically valuable for predicting oocyte maturity and determining trigger timing. However, significant limitations of the method include the possibility of measurement errors, particularly in patients with multiple or overlapping follicles, and the potential for misidentification of blood vessels or areas of free fluid as follicles by the software. Therefore, while 3D measurements offer clinical benefits, further optimization studies are needed for the method's clinical application.

Ethics

Ethics Committee Approval: The study was approved by the Acıbadem Medical Research Ethics Committee (Acıbadem) under decision number 2025-08/61, dated: 22.05.2025.
Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Concept: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Design: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Data Collection or Processing: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Analysis or Interpretation: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Literature Search: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T., Writing: Ö.K., A.Y., N.P., Ö.A., B.E., İ.Ö.A., B.T.
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