Relationship between Follicular Volume, Oocyte Competence, and Blastocyst Development in ART

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ABSTRACT

Aim: To analyze oocyte competence in assisted reproductive technology (ART) using gonadotrophin releasing hormone (GnRH) antagonist stimulation protocol with regard to maturity, fertilization, and blastocyst development in relation to follicular volume (FV), measured by three-dimensional (3D) ultrasonography (USG) using SonoAVC software.

Materials and methods: This was a prospective observational single-center study conducted at our center from April 2019 to September 2019 in which 30 cases of ovum pickup were evaluated. A single cycle of *in vitro* fertilization (IVF) per patient was considered. A controlled ovarian stimulation was done by GnRH antagonist induction protocol. Gonadotropin dose calculation was based on the scoring system designed on the basis of parameters which included age, body mass index (BMI), and USG parameters on baseline scan which included antral follicle count, ovarian volume, stromal resistance index (RI), and stromal peak systolic velocity (PSV). Stimulation was started on day 2 of the cycle. Scans at regular interval commencing from day 5 or day 6 of the stimulation were done to track follicular and endometrial development. Multiple follicular development due to controlled ovarian hyperstimulation in ART causes growth of follicles of different sizes and functional activity that will contain oocyte at different maturation status. So, this multiple follicular development results in polygonal shape of the follicles where two-dimensional (2D) measurements are not accurate of its size. Total number of follicles and volume of each follicle were evaluated on the day of trigger using SonoAVC software by 3D USG. Oocyte retrieval was done 35 hours after trigger, and metaphase 2 (M2) oocytes, fertilization, and blastocyst development were tracked according to 3D follicular volume (FV) with the help of our embryologist. Follicles were grouped according to FV, into three arbitrary groups, which included 313 small (0.3–0.9 mL, 8–12 mm), 414 medium (1–6 mL, 13–23 mm), and 11 large (>6 mL, ≥24 mm) follicles, all of which were aspirated and evaluated. The cumulus oophorus complex (COC) recovery rate was statistically significant ($p < 0.0001$) in small follicles (63%) compared with medium (86.4%) and large (63.6%) follicles. However, fertilization rate did not differ when calculating the 2PN/M2 between the three follicle groups (2 pronuclear (PN)/M2: 76.9% in small, 81.9% in medium, 60% in large, $p > 0.2$). Additionally, blastocyst rate per retrieved M2 oocyte was observed in all three groups (36.5% vs 46.2% vs 40%), respectively, but the difference was not statistically significant.

Results: Our data indicate that the optimal follicular size for a high yield of good quality blastocysts is 13–23 mm/1–6 mL. However, oocytes derived from small follicles (8–12 mm/0.3–0.9 mL) still have the capacity for normal development and fertilization.

Conclusion: Earlier clinical practice suggests aspiration of follicles of only 1–6 mL volume, but based on our results, aspiration of small follicles (0.3–0.9 mL/8–12 mm) should be a routine procedure, which would help for better oocyte yield and blastocyst rate.

Keywords: 2 pronuclear, 3D Ultrasonography, ART, Blastocyst, Cumulus oophorus complex, Follicular volume, ICSI, Medium and large follicles, Oocytes, Small, SonoAVC.

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INTRODUCTION

Follicle development during controlled ovarian stimulation, is monitored by transvaginal sonography (TVS). The decision of the follicle size at which final oocyte maturation should be triggered is a critical step in ovarian stimulation. It is well known that oocyte maturity is linked with follicular size.¹ One of the most widely applied protocols is administering the trigger when several follicles have reached a diameter of ≥18 mm.^{1–7} When there is multifollicular development, shape of the follicle changes from round to polygonal, due to pressure effect from adjacent follicles, as for these follicles, the FV assessed by 3D ultrasonography (USG) may be a more reliable parameter for the assessment of its size.

SonoAVC (Automatic Volume Calculation; GE Medical System) is a software program designed to provide automatic estimation of X, Y, Z dimensions, mean diameter, and volume of fluid filled areas. This technique has been proven to be more reproducible and valid in measuring follicular diameter and volume than conventional 2D USG method and may have implication for improving the workflow within an IVF center⁷ in terms of deciding timing of trigger and oocyte retrieval.

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To date, few studies have analyzed the relationship between blastocyst development and follicular size following one-by-one follicle measurement.^{8–10} In spite of several studies, there is still controversy regarding the optimal follicular size for oocyte retrieval evaluated by conventional 2D TVS, we aimed to perform a prospective study using 3D TVS using SonoAVC software, which is considered to be a more accurate method for calculation of spherical and nonspherical follicles.¹¹

MATERIALS AND METHODS

Patient and Study Design

This prospective study was conducted from April 2019 to September 2019 at our center.

Methods

Inclusion Criteria

Women of ≤ 45 years undergoing antagonist downregulation with gonadotropin stimulation protocol. Only one cycle per patient was included.

Patient data including maternal age, height, weight, BMI, cause of infertility, preconditions and previous H/o IVF attempts, and pregnancies were collected. Data from 30 IVF cycles and 738 aspirated follicles with individual 3D ultrasound follicle measurement and single embryo cultures were included.

On day 2, the patient was asked to empty her bladder and was undressed and placed in lithotomy-like position on a gynecology couch after counseling, and a verbal consent was obtained from the patient. Transvaginal probe of frequency 5–9 mHz of Voluson E10 (GE Medical System, Kretz) was used for all scans. After assessment of the uterus, the scanning was continued for assessment of the ovaries. B mode assessment of the ovaries for measurement of ovarian diameters, volume, and counting of the antral follicles (diameter up to 9 mm) was done. Once ovaries were located, the probe was rotated to find out the longest diameter of the ovary and was stored as one frame on a dual screen. The probe was then rotated 90° anticlockwise to get a transverse section of the ovary (Fig. 1).

The longitudinal diameter (X) is the longest diameter on the longest section of the ovary. Anteroposterior diameter (Y) is the longest diameter perpendicular to longitudinal diameter. Transverse diameter (Z) is the maximum width on the transverse section of the ovary. The ovarian volume can be calculated by the formula $X \times Y \times Z \times 0.523$ in milliliter. The antral follicles were counted in the whole ovary by taking a 2D sweep across the whole ovary and eyeballing. SonoAVC is a specific volume calculation software that identifies several small echolucent areas and color codes them in order to prevent overcounting of follicles. If AFC was >15 , we used 3D SonoAVC software for more accurate assessment of AFC. For assessment of ovarian stromal flow and to calculate resistance index (RI) and peak systolic velocity (PSV), the color Doppler PRF was set at 0.3 and wall motion filters (WMFs) at the lowest with optimal gains and balance setting. For pulse wave, the WMF was set at 30 Hz. The brightest vessel was selected for interrogation. The gonadotropin dose was calculated based on the baseline scoring system devised by Panchal et al.¹² (Table 1).

Figs 1A and B: Measurement of ovarian diameters in longitudinal and transverse section in 2D ultrasound

The doses according to the patient's score were decided (Table 2) based on the study by Panchal et al.¹²

Gonadotropin was started on day 2 or day 3 of menstrual cycle. Here, we have used recombinant follicle stimulating hormone (FSH) or highly purified urinary FSH. Human menopausal gonadotrophin (HMG) was used only in poor responders. Either fixed or flexible protocol of antagonist was followed. In the fixed protocol, GnRH antagonist was started on day 6 of stimulation till the date of ovulation trigger.¹³ In the flexible protocol, GnRH antagonist was started once the leading follicle is more than 14 mm.¹⁴ Antagonist used was cetrorelix, 0.25 mg/day.

Follicular growth together with endometrial thickness was monitored by transvaginal scans commencing on day 5 to day 6 of the treatment cycle. The gonadotropin dosage was adjusted according to ovarian response. When three or more follicles greater than 18 mm in diameter were documented, Doppler was done to assess the flow to confirm functional maturity. Perifollicular vessels (vessels overlying the follicular wall) covering at least 2/3rd of the circumference of the follicle preferably 3/4th, with RI of 0.4–0.48¹⁵ and PSV >10 cm/second, were the desired parameters. When the criteria for trigger were fulfilled, follicular diameter and volume were measured using SonoAVC software. The volume for the specific color coded, identified areas may be calculated automatically. For using SonoAVC follicle, we obtained a 3D volume of the ovary by placing the 3D box over the ovary with an angle of acquisition so that it covers the entire ovary. An automatic measurement in each of the three planes, together with the volume of the follicle, gets generated in a table form (Figs 2 and 3).

Postprocessing like addition of the uncounted follicles, deletion of areas overcounted as follicles, merging, and cutting of the follicles was done as required for both the ovaries. Trigger used was GnRH agonist, triptorelin 0.2 mg.

Oocyte retrieval was done 35 hours posttrigger under i.v. sedoanalgesia using propofol. Oocyte retrieval was done by a well-experienced single operator, under ultrasound guidance using standard protocol with Cook's no. 16 gauge, single-lumen aspiration needle. Follicles were aspirated one by one in falling order of size, under aspiration pressure of 100 to 105 mm Hg. For each follicle, one separate prewarmed test tube (37°C) was used and a track was kept on the number of follicles and laterality of the ovary and marked and numbered according to the identification on the SonoAVC result sheet. All follicles up to 10 mm diameter were aspirated.

The aspirated follicular fluid was examined one by one under a stereozoom microscope mounted in a class 2 cabinet laminar air flow. On identification of the cumulus oocyte complex (Fig. 4), it was segregated from the follicular fluid and gently agitated in a buffered media, HEPES' media, to remove any blood cells. The cumulus oocyte complexes (COCs) from right and left ovaries were incubated separately in one-step culture media for 1.5–2 hours in carbon dioxide incubator.

Aspirated follicles were grouped according to the FV, into the following arbitrary groups: small follicles with a volume of 0.3–0.9 mL corresponding to 8–12 mm, medium size follicles with a volume of 1–6 mL corresponding to 12.1–23 mm, and large size follicles with a volume of >6 mL corresponding to >23 mm. The oocyte recovery rate was determined by the number of COC retrieved in relation to the number of follicles aspirated according to FV.

For ICSI, denudation of COC was done 30 minutes before the injection or normally 38–40 hours posttrigger. Denudation of COCs was done in 4-well Petri dish (NUNC's petri dish) containing 1 mL

Table 1: Scoring system for deciding the stimulation protocol¹²

Score	1	2	3	4	5
Age	>40	35.1–40	30.1–35	25.1–30	≤25
BMI	>30	30–28.1	28–25.1	25–22.1	≤22
AFC	<5	5–10	10.1–15	15.1–20	>20
Ovarian volume	<3	3–5	5.1–7	7.1–10	>10
Stromal RI	>0.75	0.75–0.65	0.65–0.55	0.55–0.45	<0.45
Stromal PSV	<3	3–5	5.1–7	7.1–10	>10

Table 2: Starting dose of recombinant follicle stimulating hormone in IVF cycle, based on the scoring system

Score	Dose (IU)
≥23	75
21–22	150
16–20	225
11–15	300
6–10	375

Fig. 2: SonoAVC for the counting of number of follicles

of hyaluronidase solution and three drops (1 mL each) of HEPES-buffered media. The COCs were washed in well 1 which contained hyalase for 30 seconds using 3-mL Pasteur pipette followed by washing with HEPES media in well 2 with 175-μL denuding pipette attached to stripper. It was again washed in HEPES media in well 3 with 135-μL pipette attached on stripper, and finally, M2 oocytes were placed in well 4 and metaphase 1 (M1) and germinal vesicle (GV) in well 3.

After denudation, the quality and maturity of oocytes were assessed under an inverted microscope. Once denuded, mature (M2) oocytes (Fig. 5) with first polar body expelled were placed in a 30-μL droplet of culture media after gently washing. Those oocytes which are immature either M1 (Fig. 6) or GV (Fig. 7) were placed in separate droplets.

M2 oocyte rate was calculated per follicle aspirated and per COC retrieved in correlation with 3D FV.

Microinjection dishes for ICSI were prepared with 4–8 droplets of 2–5 μL of HEPES-buffered culture media for each individual oocyte and a river-like flat and long spread of polyvinylpyrrolidone (PVP) in the center. A small volume of media evaporate very quickly, and they were therefore covered with a layer of oil. Equilibration of dishes was done in CO₂ incubator for at least 20–30 minutes.

Name										Pat. ID E6665-17-06-08-1									
SonoAVC™ (Semi-) Automatic										Total#: 40									
Left Ovary	Nr.	d(V) mm	dx mm	dy mm	dz mm	mean d mm	V cm ³			Right Ovary	Nr.	d(V) mm	dx mm	dy mm	dz mm	mean d mm	V cm ³		
1	12.6	16.9	14.6	8.7	13.4	10.6				1	14.9	21.7	15.7	12.0	16.5	1.74			
2	11.2	14.4	11.8	8.7	11.6	0.73				2	12.4	13.8	12.3	11.7	12.6	0.99			
3	10.2	15.8	10.6	9.5	12.0	0.56				3	10.7	13.1	11.4	9.4	11.3	0.63			
4	9.7	17.5	12.9	6.8	12.4	0.47				4	10.6	13.5	10.6	9.8	11.3	0.62			
5	9.5	11.5	10.8	7.3	9.9	0.45				5	9.4	17.6	10.4	6.2	11.4	0.44			
6	9.5	16.6	10.6	6.5	11.2	0.44				6	9.3	11.9	9.7	8.0	9.9	0.43			
7	8.8	13.3	12.5	8.0	11.2	0.35				7	9.1	13.2	9.3	6.9	9.8	0.40			
8	8.7	19.2	9.8	4.9	11.3	0.35				8	9.0	12.3	9.1	7.5	9.6	0.38			
9	8.4	10.9	9.4	6.1	8.8	0.31				9	9.0	12.8	11.2	5.5	9.8	0.38			
10	7.4	11.2	7.8	5.5	8.1	0.22				10	8.7	13.4	10.3	6.1	9.9	0.34			
11	7.3	9.4	7.8	5.6	7.6	0.21				11	8.5	11.4	8.7	6.6	8.9	0.32			
12	7.3	13.5	8.0	4.4	8.6	0.20				12	7.7	12.7	7.8	6.5	9.0	0.24			
13	7.0	8.3	7.3	5.8	7.1	0.18				13	6.7	12.5	6.5	4.9	8.0	0.16			
14	7.0	10.6	8.0	5.4	8.0	0.18				14	6.4	11.6	8.0	3.9	7.8	0.14			
15	6.8	12.4	6.5	4.9	7.9	0.17				15	5.7	8.4	5.4	4.3	6.0	0.10			
16	6.8	12.6	8.1	4.1	8.3	0.17				16	5.4	8.3	5.5	4.1	6.0	0.08			
17	6.8	12.3	7.4	4.6	8.1	0.16				17	5.3	8.8	4.7	3.7	5.7	0.08			
18	6.2	12.0	7.1	3.2	7.4	0.13				18	4.9	6.7	4.6	4.4	5.2	0.06			

Fig. 3: Calculation of follicular diameters and volume in a tabular form by SonoAVC software **Fig. 4:** Oocyte cumulus complex

A small aliquot of sperm suspension (3–4 μL) was added to the central PVP line, and oocytes were transferred into the droplets, one in each droplet. After appropriate setting of the micromanipulator, sperm selection and immobilization of the sperm was done and was injected into oocyte one by one following oocyte activation. Only M2 oocytes were injected with single morphologically normal sperm after ensuring that both nuclear and cytoplasmic maturity was synchronized and normal fertilization could occur.

After completion of microinjection, the oocytes were transferred in 40-μL droplets of one-step culture media in a closed culture system and kept in CO₂ incubator.

Pronuclear stage (Fig. 8) embryos were evaluated 16–18 hours after fertilization. The key parameters monitored at this included pronuclei (size and symmetry), nucleoli (size, number, and

Fig. 5: Metaphase 2 oocyte showing polar body

Fig. 6: Metaphase 1 oocyte

Fig. 7: Germinal vesicle

Fig. 8: Two pronuclei—two polar body stage seen indicating fertilization

distribution), and cytoplasmic appearance. The presence of two fully formed pronuclei, two polar body, and an intact zona pellucida indicated proper fertilization. Fertilization rate was calculated per follicle aspirated, per COC retrieved, and per M2 oocyte obtained in correlation with FV.

On day 3 (64–67 hours) post insemination, the embryos were evaluated on the size of blastomers and fragmentation. From the morula, the embryos develop into a blastocyst (Fig. 9) and undergo the first cell division forming two layers, the trophoblast and inner cell mass on day 5. Blastocysts were graded according to the Gardner blastocyst grading scale.^{16,17} Blastocyst with expansion 2–6 and grade A for inner mass cell and trophectoderm or a combination of grades A and B were scored as top blastocyst. Blastocyst rates were determined per follicle aspirated per COC retrieved and per M2 and per 2 pronuclear (PN) obtained in correlation with the FV.

Statistical Analysis

The sample size was calculated based on both of the main outcome parameters namely oocyte recovery rate and blastocyst rate, applying a 15% minimum clinically relevant difference, a type I error of $\alpha = 0.05$ and $1 - \beta = 0.8$, resulting in a minimum of 30 samples. A statistical difference in oocyte retrieval, oocyte maturation, fertilization rate, and blastocyst rate was calculated by Pearson's

Fig. 9: Expanded blastocyst (day 5 embryo)

Chi-square. A 95% confidence interval of the relative risk and risk difference was applied. An error of $\alpha < 0.05$ was considered statistically significant. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 21.0 for Windows.

RESULTS

Subjects

Follicles were individually aspirated in 30 patients. The demographic data of these patients are shown in Table 3. The mean dose of gonadotropin used was 2,552 IU (1,200–4,200 IU) with mean duration of 10.6 days. The overall oocyte recovery rate was 76% out of 738 follicles aspirated.

Follicular growth under antagonist downregulated protocol.

Automated 3D ultrasound investigation of follicular growth at the time of ovum pickup showed that out of all detected follicles, 14.9% had a volume of >6 mL (≥ 24 mm spherical diameter), 56.1% had a volume of 1–6 mL (13–23 mm spherical diameter), and 42.4% had a volume of 0.3–0.9 mL (8–12 mm spherical diameter).

COC recovery, M2 oocyte, 2PN, and blastocyst development.

Follicles were grouped according to FV, into three arbitrary groups, which included 313 small (0.3–0.9 mL), 414 medium (1–6 mL), 11 large (>6 mL) follicles. The COC recovery rate was statistically significant ($p < 0.0001$) in small follicles (63%) compared with medium (86.4%) and large (63.6%) follicles. The area under the curve (AUC) as depicted in receiver operating characteristic (ROC) Figure 10 quantifies the overall ability of the test to discriminate between the small, medium, and large follicles in COC retrieval. Our study shows that medium-sized follicles are correctly predicted with an accuracy of 0.824 (good), followed by small follicles with an accuracy of 0.779 (fair) whereas failed to predict in large follicles.

The proportion of M2 oocytes per aspirated follicles was significantly reduced in small follicles, compared with medium and large follicles (33.2% vs 77.2% vs 45.41%) respectively; p value < 0.0001, Table 4.

The same trend was observed when the rate of M2 oocytes was calculated per retrieved COC (M2/COC, 53.1% in small follicles vs 89.4% in medium vs 71.4% in large follicles, respectively). The AUC as depicted in ROC Figure 11 quantifies the overall ability of the test to discriminate the ability of small, medium, and large follicles in retrieval of M2 oocyte. Our study shows that retrieval of M2 oocyte is correctly predicted in medium follicles with an accuracy of 0.837 (good), followed by small follicles with an accuracy of 0.790 (fair) whereas failed to predict in large follicles.

The rate of 2PN (fertilization rate) was significantly lower in small follicles than in medium and large follicles, when calculated per number of aspirated FV (25.6% vs 63.3% vs 27.3% respectively, $p < 0.05$) or per COC retrieved (40.8% vs 73.2% vs 42.9%, respectively, although it was statistically significant between small and medium

Table 3: Demographics of 30 patients undergoing ovarian stimulation using gonadotropins with antagonist downregulation

Characteristics	Value
Female age (years)	32.57 $\pm$ 3.98 (25–40)
BMI (kg/m ²)	26.85 $\pm$ 4.32 (19.6–35.8)
Total dose FSH (IU)	2552.5 $\pm$ 812.68 (1,200–4,200)
Stimulation (days)	10.57 $\pm$ 1.85 (8–14)
Aspirated follicles (n)	24.6 $\pm$ 10.30 (12–62)
COC retrieved (n)	18.7 $\pm$ 10.10 (8–55)
Oocyte retrieval rate (%)	76.01
M2 rate (%)	76.8

Values are given as mean $\pm$ SD (range) or percentage; SD, standard deviation; BMI, body mass index; COC, cumulus oophorus complex; FSH, follicle stimulating hormone; M2, metaphase 2 oocyte

Fig. 10: ROC curve for CR (COC retrieved) was obtained from small, medium, and large follicles. The AUC for CR from small follicles is 0.779, from medium follicles is 0.824, and from large follicles is 0.585. Test variables are CR_SMALL FOLLICLE, CR_MEDIUM FOLLICLE, and CR_LARGE FOLLICLE

follicles only, $p < 0.0001$). However, fertilization rate did not differ when calculating the 2PN/M2 between the three follicle group (2PN/M2: 76.9% in small, 81.9% in medium, 60% in large, though the p value was not significant). The AUC as depicted in ROC Figure 12 quantifies the overall ability of the test to discriminate the ability of small, medium, and large follicles to fertilize and reach 2PN stage. Our study shows that medium-sized follicles are correctly predicted in reaching 2PN stage with an accuracy of 0.806 (good), followed by small follicles with an accuracy of 0.783 (fair), and failed to predict large follicles.

Finally, a lower blastocyst rate was observed in small compared with medium and large follicles per aspirated follicles (12.1% vs 35.7% vs 28.6%, respectively) or per COC (19.4% vs 41.3% vs 28.6%, respectively) although the difference was statistically significant only between small and medium follicles ($p < 0.0001$). Additionally, blastocyst rate per retrieved M2 oocyte was observed in all three groups (36.5% vs 46.2% vs 40%) respectively, but the difference was not statistically significant. The AUC as depicted in ROC Figure 13 quantifies the overall ability of the test to discriminate between small, medium, and large follicles in progression to blastocyst stage. Our study shows that blastocyst development in small-sized follicles is predicted with an accuracy of 0.759 (fair), followed by medium follicles with an accuracy of 0.737 (fair), and failed to predict in large follicles.

DISCUSSION

This study indicates that though the number of M2 oocytes derived out of smaller follicles were less, they still have the capacity for normal development, fertilization, and blastocyst formation, indicating the usefulness of puncturing this cohort of follicles.

Table 4: Oocyte maturity and blastocyst development in 738 follicles aspirated individually, grouped according to follicular volume

Variable	Small follicles (8–12 mm/0.3–0.9 mL)	Medium follicles (13–23 mm/1–6 mL)	Large follicles (≥24 mm/≥6 mL)	p value*
Follicles analyzed (n)	313	414	11	
COCs retrieved (n)	196	358	7	
COCs/follicle (%)	63	86.4	63.6	<0.00001 ⁺ , <0.05 ^ε , NS [§]
M2 (n)	104	320	5	
M2/follicle (%)	33.2	77.2	45.4	<0.00001 ⁺ , NS ^{ε§}
M2/COC (%)	53.06	89.4	71.4	<0.00001 ⁺ , NS ^{ε§}
2PN (n)	80	262	3	
PN/follicle (%)	25.6	63.3	27.3	<0.00001 ⁺ , <0.05 ^ε NS [§]
2PN/COCs (%)	40.8	73.2	42.9	<0.00001 ⁺ , NS ^{ε§}
2PN/M2 (%)	76.9	81.9	60	NS ^{+ε§}
Blastocyst (n)	38	148	2	
Blastocyst/follicle (%)	12.1	35.7	18.2	<0.00001 ⁺ , NS ^{ε§}
Blastocyst/COC (%)	19.4	41.3	28.6	<0.00001 ⁺ , NS ^{ε§}
Blastocyst/M2 (%)	36.5	46.25	40	NS ^{+ε§}

*Statistical significance was evaluated using Chi-square, between ⁺: small and medium follicles; ^ε: small and large follicles, and [§]: medium and large follicles; NS, not statistically significant; COC, cumulus oophorus complex; M2, metaphase 2 oocyte;

Fig. 11: ROC curve for M2 oocyte obtained from small, medium, and large follicles. AUC for M2 oocyte from small follicles is 0.790, from medium follicles is 0.837, and from large follicles is 0.545. Test variables are M_2_SMALL FOLLICLE, M_2_MEDIUM FOLLICLE, M_2_LARGE FOLLICLE

Other studies have reported lower oocyte recovery rate and decreased M2 oocyte rate in small follicles (<2 mL);^{2,5,18–20} however, as per our study, we found that the oocyte recovery rate and M2 rate (M2/follicle, M2/COC) in small follicles are statistically significant to that of the medium follicles, and the finding is in line with some previous publications^{5,9,18,21} although not others.^{6,19}

The fertilization rate (2PN/M2) was comparable in small follicles and medium follicles which is in accordance with the finding of Wirleitner et al.²² indicating that M2 derived from small oocytes are capable of normal fertilization.

Fig. 12: ROC curve for 2PN stage obtained from small, medium, and large follicles. The AUC for 2PN from small follicles is 0.783, for medium follicles is 0.806, and for large follicles is 0.540. Test variables are 2PN_SMALL FOLLICLE, 2PN_MEDIUM FOLLICLE, and 2PN_LARGE FOLLICLE

Similar blastocyst rate (blastocyst/follicle, blastocyst/M2) was seen for small follicles (0.3–0.9 mL) compared with large follicles (1–6 mL, >6 mL) though the finding was not significant and is in line with Wirleitner et al.²²

A slight decrease in blastocyst rate was noted for follicles >6 mL, which may indicate that a delay in trigger might decrease the chances of blastocyst formation.

So the main strength of this study is that it has analyzed oocyte competence and blastocyst development in relation to individual 3D ultrasound FV measurement. Limitation of the study was

Fig. 13: ROC curve for blastocyst obtained from small, medium, and large follicles. The AUC for blastocyst (BC) from small follicles is 0.759, from medium follicles is 0.737, from large follicles is 0.571. Test variables are BC_SMALL FOLLICLE, BC_MEDIUM FOLLICLE, and BC_LARGE FOLLICLE

that the study group was quite small. Regarding the inevitable heterogeneity of IVF patients, and thus the confounding factor, our findings may not be fully representative of the general IVF patients.

In general, premature administration of hCG can result in retention of the oocyte within the follicle or delivering of oocytes with impaired developmental potential.²³ In 1973, Edwards showed a strong relationship between oocyte recovery and follicle size.²⁴ In a natural menstrual cycle, preovulatory follicle reaches 17–25 mm in diameter.²⁵ Data indicate that in ovulation stimulation, the relationship between follicular size and oocyte maturity might be different, although a relationship is undoubtedly present.^{2,6}

The estimation of FV in ovulation induction cycle is a difficult task as the shape is often not spherical but rather ellipsoid or even complex. Calculation of the FV ($V_f = 4/3 \times r^3 \times \pi$) with conventional 2D TVS bears the risk of false estimation, possibly contributing to the controversial publications on this topic in the past.²⁶ In contrast, the 3D ultrasound allows a more accurate calculation.^{27,28}

CONCLUSION

Our data indicate that the optimal FV for a high yield of good quality blastocysts is 13–23 mm/1–6 mL. However, oocytes derived from small follicles (8–12 mm/0.3–0.9 mL) still have the capacity for normal development and fertilization. Earlier clinical practice suggests aspiration of follicles of only 1 to 6 mL volume, but based on our results aspiration of small follicles (0.3–0.9 mL/8–12 mm) should be done, which would help in increase in number of oocyte retrieval and blastocyst rate especially in low reserve patients and would give better chances of pregnancy.

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