RBMO

ARTICLE

The association between follicle size and oocyte development as a function of final follicular maturation triggering

BIOGRAPHY

Aya Mohr-Sasson completed her residency at the Obstetric and Gynecology Department at Sheba Medical Center. Since then, she has been practising as a senior physician, leading several investigating teams and promoting academic achievements among young investigators.

Aya Mohr-Sasson[1,2,*], Raoul Orvieto[1,2,3], Shlomit Blumenfeld[1,2], Michal Axelrod[1,2], Danielle Mor-Hadar[1,2], Leonti Grin[1,2], Adva Aizer[1], Jigal Haas[1,2]

KEY MESSAGE

Follicle maximum diameter above 13 mm is associated with higher oocyte recovery rate compared with smaller follicles. Once oocytes are mature, similar fertilization and top quality embryo rates are expected regardless of follicular diameter. Triggering mode does not influence the oocyte recovery rate.

ABSTRACT

Research question: To study the association between follicle size and oocyte/embryo quality, as a function of different triggering modes for final follicular maturation.

Study design: Cohort study conducted in a single tertiary medical centre between July 2018 and May 2019. All women undergoing ovarian stimulation with triggering using human chorionic gonadotrophin (HCG), gonadotrophin-releasing hormone (GnRH) agonist or dual trigger (GnRHa + HCG) were included. Before ultrasound-guided follicular aspiration, follicles were measured and divided into three groups according to maximum dimensions: large ≥16 mm, medium 13–15 mm and small <13 mm. Microscopic examination of the follicular aspirates was performed by an embryologist. Each follicle aspirated was evaluated for oocyte maturation, oocyte fertilization and embryo quality.

Results: A total of 640 follicles were measured, including 402 (62.8%) in the large, 148 (23.1%) in the medium and 90 (14.1%) in the small groups. Oocytes were obtained during aspiration from 76.3%, 70.3% and 55.6% of the large, medium and small follicle groups, respectively ($P = 0.001$). The mature oocyte (metaphase II) rate was significantly higher in the large ($P = 0.001$) and medium ($P = 0.01$) compared with the small follicle group. Nevertheless, no between-group differences were observed in fertilization or top quality embryo rates among mature oocytes regardless of the size of the follicle from which they originated. Triggering mode did not influence oocyte recovery rate in the different follicle size groups.

Conclusion: A higher oocyte recovery rate was observed from follicles >13 mm, however, mature oocytes achieved similar fertilization and top quality embryo rates regardless of follicle size. Triggering mode did not influence oocyte recovery rate.

[1] Department of Obstetrics and Gynecology, Sheba Medical Center, Tel-Hashomer, Israel
[2] Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[3] Tarnesby-Tarnowski Chair for Family Planning and Fertility Regulation, Sackler Faculty of Medicine, Tel-Aviv University, Israel

*Corresponding author. E-mail address: mohraya@gmail.com (A Mohr-Sasson). https://doi.org/10.1016/j.rbmo.2020.02.005
1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest.

KEYWORDS
Dual triggering
Follicle size
Oocyte maturation

INTRODUCTION

Ovarian stimulation is critical to assisted reproduction because it increases the number of oocytes undergoing development. The medications designed to override the selection of a single dominant follicle drive multiple antral follicles into the growth phase. These follicles grow at different rates, and management is guided by their size rather than their competence (*Miller et al., 1996*). Studies have shown that follicles with greater diameter are most likely to yield a mature oocyte that is capable of fertilization and best suited for development into a high-quality embryo. Smaller follicles showed lower rates (60%) (*Bergh et al., 1998*; *Rosen et al., 2008*; *Wittmaack et al., 1994*).

Human chorionic gonadotrophin (HCG) is usually used as a surrogate to LH surge, aiming to induce luteinization of the granulosa cells, final oocyte maturation and resumption of meiosis (*Orvieto, 2015*). This treatment is based on an assumption that follicular size predicts the developmental competence of the oocyte (*Andersen, 1993*). The outcome is that only a portion of the oocytes will be mature and competent for fertilization and further development into viable embryos (*Rosen et al., 2008*). More recently, a new mode of triggering final follicular maturation has been used, aiming to improve the proportion of mature oocytes during retrieval (*Orvieto, 2015*). Following the observations demonstrating comparable or even better oocyte/embryo quality following gonadotrophin-releasing hormone agonist (GnRHa) trigger compared with HCG trigger, GnRHa is now given concomitant to the standard HCG trigger, in order to improve oocyte/embryo yield and quality (*Berg et al., 1998*; *Orvieto, 2015*).

Prompted by the aforementioned observations, this study sought to evaluate the association between follicle size and oocyte development and quality, as a function of the different final follicular maturation triggering modes.

MATERIALS AND METHODS

This was a prospective cohort study conducted in a single university-affiliated tertiary medical centre, between July 2018 and May 2019. Women undergoing ovarian stimulation using the multiple-dose GnRH antagonist protocol with final follicular maturation triggering using either HCG (Ovitrelle 250 μg), GnRH agonist (GnRHa) (Decapeptyl 0.2 mg), dual trigger using concomitant administration of GnRHa and HCG (Ovitrelle 250 μg + Decapeptyl 0.2 mg) 36 h before retrieval or double triggering using the same treatment 40 h and 36 h before retrieval, were included. Women ≥43 years old, those with a history of endometriosis or fragile X gene mutation were excluded. Data concerning women's demographic, medical history, gynaecological and obstetrical history, fertility investigation, past fertility treatments and current treatment protocol were collected from their medical files.

The decision about final follicular maturation triggering was based on physician judgement, with the double triggering usually offered to patients with previous abnormal final follicular maturation or poor embryo quality (*Haas et al., 2014*; *Zilberberg et al., 2015*). The timing was based on the lead follicular cohort, usually with at least two leading follicles measuring ≥17 mm in maximum diameter. A transvaginal, ultrasound-guided follicular aspiration was conducted 36 h after triggering administration.

At retrieval, up to four leading follicles were measured before aspiration from each woman. Follicles were divided into three follicular groups according to their maximum dimensional size: large ≥16 mm, medium 13–15 mm and small <13 mm. Retrieval was done separately for each follicle measured. Microscopic examination of the follicular aspirates was performed by the embryologist. In cases where no oocyte was detected, flushing of the system was performed using 0.5–1 ml of medium with HEPES (Quinn's Advantage®, Sage, USA).

Oocytes were fertilized using conventional insemination or intracytoplasmic sperm injection (ICSI) as indicated. Fertilization was determined by the presence of two pronuclei (2PN) and two polar bodies. Each embryo was cultured separately and evaluated after 72 h.

Day 3 embryo grading, based on cellular cleavage and fragmentation, was recorded separately. Fragmentation was scored by the degree of fragmentation proportional to the whole embryo volume: (i) no fragmentation; (ii) <10%; (iii) 10% to 25%; (iv) 25% to 50%; (v) >50%. A top quality embryo (TQE) was defined as a day 3 embryo with 7–8 cells and ≤10% fragmentation rate. The information for each oocyte, starting from the follicular size, was followed through all laboratory procedures including insemination, oocyte stripping for ICSI, ICSI, pronuclear assessment and embryo culture.

The primary outcome was defined as the number of oocytes retrieved; from each of the follicular groups (oocyte recovery rate); and using the different final follicular maturation triggering modes. Secondary outcomes included the number of oocytes which had undergone nuclear maturation–metaphase II oocytes (MII); fertilization rate; and TQE rate.

Statistical analysis

Normality of the data was tested using the Shapiro–Wilk or Kolmogorov–Smirnov tests. Data are presented as median and interquartile range (IQR). Comparison between unrelated variables was conducted with Student's *t*-test, Mann–Whitney *U*-test or ANOVA test as appropriate. Chi-squared and Fisher's exact tests were used to compare categorical variables. Significance was accepted at $P < 0.05$. Statistical analyses were conducted using SPSS Statistics for Windows, Version 19 (IBM Corp., Armonk, NY, USA).

Ethical approval

The study protocol was approved by the Institutional Review Board (ID 4689-17-SMC) on 21 December 2017, and was supported by the National Institutes of Health (NCT02821702).

RESULTS

During the study period 204 women met the inclusion criteria, from whom 640 follicles were measured, including 90 (14.1%) in the small [median 11.2 (IQR 10.5–11.2)], 148 (23.1%) in the medium [median 14.5 (IQR 13.8–15.3)] and 402 (62.8%) in the large [median 19.0 (IQR 17.2–21.2)] follicle groups (FIGURE 1).

TABLE 1 displays the demographic and clinical characteristics of the women divided by the three follicular size groups. No between-group differences were demonstrated in the total dose

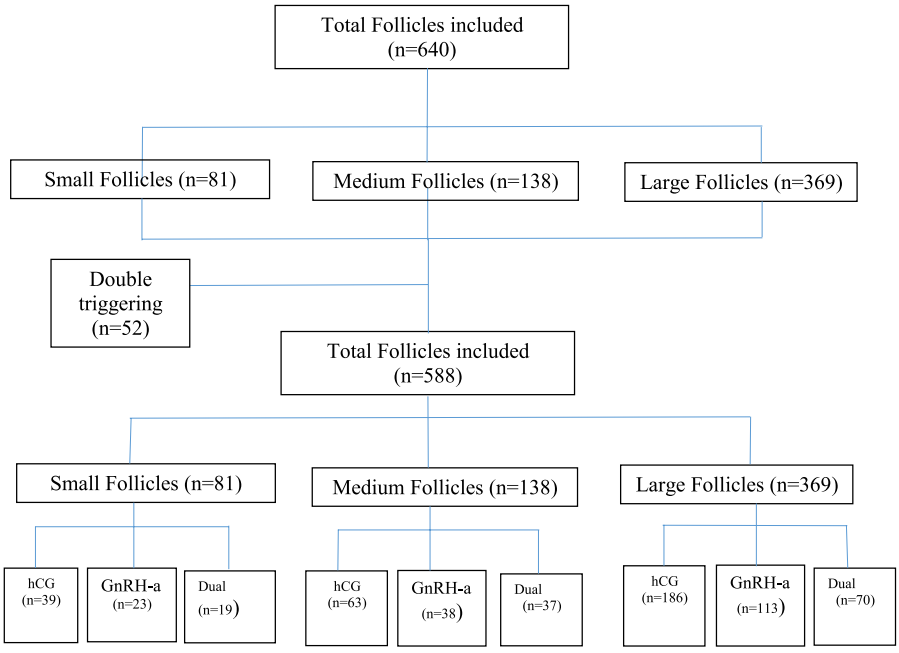

FIGURE 1 Study population.

of gonadotrophins used, duration of stimulation or days of antagonist administration.

Oocytes were obtained during aspiration from 55.6%, 70.3% and 76.3% of the small, medium and large follicle groups, respectively (TABLE 2). This difference was found to be statistically significant when comparing the medium and the large follicle groups to the small group ($P = 0.02$, $P = 0.001$, respectively), but no statistically significant difference was observed when comparing the medium and large groups ($P = 0.15$). The probability of retrieving mature oocytes (MII) was significantly higher in the medium and large compared with the small follicle size groups ($P = 0.01$, $P = 0.001$, respectively), with no difference when comparing the medium and large groups ($P = 0.10$). Nevertheless, after achieving mature oocytes (MII), no difference was observed in fertilization or in TQE rates between the three groups ($P = 0.14$) (TABLE 2). Similarly, in a sub-analysis comparing fertilization rate between insemination and ICSI, no differences were observed between all three follicle size groups ($P = 0.55$) (data not shown).

Further analysis according to the different final follicular maturation triggering modes, including HCG, GnRHa and the dual triggering, was performed. Demographic and clinical characteristics of the women by triggering mode is presented in TABLE 3.

Differences were observed between the groups in women's age ($P = 0.001$),

TABLE 1 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BY FOLLICLE SIZE

	Follicle size			
	Small (*n* = 90)	**Medium (*n* = 148)**	**Large (*n* = 402)**	***P*-value**
Age (years)	37 (32–41)	35 (32–40)	36 (32–40)	0.38
BMI (kg/m^2)	24 (21–25)	23 (21–28)	23 (20–27)	0.42
Gravidity	0 (0–1)	1 (0–1)	1 (0–2)	0.03
Parity	0 (0–1)	0 (0–1)	0 (0–1)	0.04
IVF cycle number	2 (1–4)	2 (1–4)	2 (1–4)	0.65
Duration of stimulation (days)	9 (8–11)	10 (9–11)	10 (9–11)	0.59
GnRH antagonist (days)	5 (4–6)	5 (4–6)	5 (4–6)	0.81
HMG dose (mIU/ml)	1575 (912–2250)	1200 (825–2325)	1500 (900–2400)	0.57
FSH dose (mIU/ml)	2700 (1800–3750)	2212 (1575–3525)	2400 (1625–3750)	0.57
Total oocytes	7 (4–11)	10 (5–15)	8 (4–12)	0.05
Endometrial thickness (mm)	9.5 (8–10.5)	9.5 (8–11)	9.7 (8–11)	0.27

Data are presented as median and interquartile range.

BMI = body mass index; GnRH = gonadotrophin-releasing hormone; HMG = human menopausal gonadotrophin.

TABLE 2 OUTCOMES BY FOLLICLE SIZE

	Follicle size, *n* (%)				*P*-value			
	Small (*n* = 90)	Medium (*n* = 148)	Large (*n* = 402)	Total (*n* = 640)	Small/ medium	Small/ large	Medium/ large	Total
Oocytes	50 (55.6)	104 (70.3)	306 (76.1)	460 (71.9)	0.02	0.008	0.15	0.001
Fertilizations[a]	22 (24.4)	70 (47.3)	178 (44.3)	270 (42.2)	0.05	0.11	0.40	0.14
TQE	13 (14.4)	48 (32.4)	123 (30.6)	77 (12.0)	0.03	0.06	0.40	0.09
Mature (MII) oocytes	22 (24.4)	59 (39.9)	192 (47.8)	273 (42.7)	0.01	0.001	0.10	0.001
MI oocytes	7 (7.8)	5 (3.4)	17 (4.2)	29 (4.5)	0.13	0.16	0.65	0.25
GV/AT	6 (6.7)	6 (4.1)	9 (2.2)	21 (3.3)	0.37	0.03	0.24	0.09

[a] Fertilization was determined by the presence of two pronuclei (2PN) and two polar bodies.GV/AT = germinal vesicle/atretic oocyte; MI = metaphase I; MII = metaphase II; TQE = top quality embryo.

TABLE 3 DEMOGRAPHIC AND CLINICAL CHARACTERISTICS BY TRIGGERING MODE

	Triggering			
	HCG (*n* = 288)	Dual (*n* = 126)	GnRHa (*n* = 174)	*P*-value
Age (years)	36 (31–40)	37 (35–42)	35 (31–38)	0.001
BMI (kg/m^2)	23 (31–40)	23 (20–27)	22 (20–28)	0.44
Gravidity	0 (0–2)	1 (0–1)	1 (0–1)	0.30
Parity	0 (0–1)	0 (0–1)	0 (0–1)	0.47
IVF cycle number	2 (1–4)	3 (2–5)	2 (1–3)	0.001
Duration of stimulation (days)	9 (8–11)	9 (9–10)	10 (9–11)	0.51
GnRH antagonist (days)	5 (4–6)	5 (4–6)	5 (5–6)	0.001
HMG (mIU/ml)	1312 (900–2925)	1800 (1312–2700)	1162 (731–2100)	0.001
FSH (mIU/ml)	2700 (1575–3862)	3150 (2212–4200)	2218 (1537–3000)	0.001
Total oocytes	7 (4–10)	7 (3–12)	13 (8–18)	0.001
Endometrial thickness (mm)	9.7 (8.0–10.9)	9.7 (8.0–10.8)	9.7 (8.2–11.1)	0.37

Data are presented as median and interquartile range (IQR).

BMI = body mass index; GnRH = gonadotrophin-releasing hormone; HMG = human menopausal gonadotrophin.

previous IVF cycle attempts (P = 0.001) and total gonadotrophin doses during ovarian stimulation (P = 0.001). Nevertheless, no between-group differences were observed in the rate of oocyte retrieval per triggering mode in the different follicle size groups (TABLE 4).

Furthermore, choice of triggering mode appeared to have no influence on fertilization and TQE rates per oocyte (TABLE 5).

DISCUSSION

The main findings of this study are:

- Higher rates of oocyte recovery were obtained from follicles >13 mm in maximum diameter compared with smaller follicles.
- Mature oocytes reached similar fertilization and TQE rates regardless of the maximum diameter of the follicle from which they were retrieved.
- When comparing HCG, GnRHa and dual triggering, no difference was observed in oocyte recovery rate from the different follicle size groups.
- Oocyte fertilization and TQE rates were not influenced by the different triggering methods.

The association between oocyte maturity and follicle size has been known about for more than three decades and is the basis for the timing of final follicular maturation trigger when several follicles reach a diameter of more than 17–20 mm (*Dubey et al., 1995*; *Ectors et al., 1997*; *Scott et al., 1989*, *Simonettiet et al., 1985*). The results of this study demonstrated higher oocyte recovery rates in the medium (13–15 mm) and large (≥16 mm) compared with the small (<13 mm) follicle groups. This finding is consistent with previous studies (*Haines et al., 1991*, *Scott et al., 1989*, *Triwitayakorn et al., 2003*; *Wittmaack et al., 1994*). Of note, however, although some studies suggested higher oocyte recovery rate as follicle maximal diameter is increased (*Dubey et al., 1995*; *Scott et al., 1989*), in the present study, no difference in recovery rate was observed between medium and large size follicles.

In concordance with oocyte recovery rate, mature oocytes (MII) were more commonly found in the medium and large follicle groups, demonstrating that follicles ≥15 mm provide the highest probability of retrieving mature oocytes. Similar results were reported by *Scott et al. (1989)*. In a study conducted by *Mehri et al. (2014)*, including 360 follicles, 99% of the oocytes recovered from follicles ≥18 mm (n = 147) were in MII.

TABLE 4 OUTCOMES BY TRIGGERING MODE

		Triggering mode, *n* (%)			*P*-value			
	All	**HCG**	**Dual**	**GnRHa**	**HCG / dual**	**HCG/GnRHa**	**Dual/GnRHa**	**Total**
Follicles <13 mm	81 (100)	39 (100)	19 (100)	23 (100)				
Oocytes	45 (55.6)	24 (61.5)	8 (42.10)	13 (56.5)	0.26	0.79	0.54	0.37
MII oocytes	20 (24.7)	12 (30.8)	3 (15.79)	5 (21.7)	0.34	0.56	0.71	0.43
MI oocytes	5 (6.2)	3 (7.7)	2 (10.52)	0 (0)	1	0.29	0.2	0.32
GV/AT	5 (6.2)	1 (2.6)	2 (10.52)	2 (8.7)	0.25	0.55	1	0.42
TQE	11 (13.6)	7 (17.9)	2 (10.52)	2 (8.7)	1	1	1	0.93
Fertilizations[a]	20 (24.7)	13 (33.3)	4 (21.05)	3 (13.0)	0.7	0.42	1	0.57
Follicles 13–15 mm	138 (100)	63 (100)	37 (100)	38 (100)				
Oocytes	98 (71.0)	46 (73.0)	27 (72.97)	25 (65.8)	1	0.5	0.62	0.71
MII oocytes	57 (41.3)	24 (38.1)	18 (48.6)	15 (39.5)	0.4	1	0.5	0.56
MI oocytes	5 (3.6)	2 (3.2)	2 (5.40)	1 (2.6)	0.62	1	0.61	0.79
GV/AT	4 (2.9)	1 (1.6)	0 (0)	3 (7.9)	0.29	1	1	0.39
TQE	47 (34.1)	23 (36.5)	14 (37.83)	10 (26.3)	1	0.61	0.56	0.75
Fertilizations	68 (49.3)	31 (49.2)	21 (56.76)	16 (42.1)	0.45	1	0.76	0.71
Follicles ≥16 mm	369 (100)	186 (100)	70 (100)	113 (100)				
Oocytes	286 (77.5)	145 (78.0)	55 (78.57)	86 (76.1)	1	0.67	0.72	0.88
MII oocytes	178 (48.2)	94 (50.5)	33 (47.14)	51 (45.1)	0.67	0.4	0.88	0.65
MI oocytes	15 (4.1)	8 (4.3)	5 (26.3)	2 (1.8)	0.35	0.33	0.11	0.2
GV/AT	8 (2.2)	3 (1.6)	3 (4.28)	2 (1.8)	0.35	1	0.37	0.4
Fertilizations	115 (31.2)	58 (31.2)	22 (31.41)	35 (30.9)	1	0.77	1	0.93

[a] Fertilization was determined by the presence of two pronuclei (2PN) and two polar bodies.GV/AT = germinal vesicle/atretic oocyte; GnRHa = gonadotrophin-releasing hormone agonist; MI = metaphase I; MII = metaphase II; TQE = top quality embryo.

TABLE 5 EMBRYOS BY TRIGGERING

		Triggering mode, *n* (%)			*P*-value			
	Total	**HCG**	**Dual**	**Decapeptyl**	**HCG / dual**	**HCG / Deca-peptyl**	**Dual / Deca-peptyl**	**Total**
Small	37 (100)	22 (100)	8 (100)	7(100)				
Fertilizations[a]	20 (54.1)	13 (59.1)	4 (50)	3 (42.9)	0.7	0.67	1	0.73
TQE	11 (29.7)	7 (31.8)	2 (25)	2 (28.6)	1	1	1	0.98
Medium	87 (100)	42 (100)	24 (100)	21 (100)				
Fertilizations	68 (78.2)	31 (73.8)	21 (87.5)	16 (76.2)	0.59	1	0.75	0.72
TQE	47 (54.0)	23 (54.8)	14 (58.3)	10 (47.6)	0.8	0.61	0.56	0.76
Large	241 (100)	125 (100)	46 (100)	70 (100)				
Fertilizations	167 (69.3)	90 (72.0)	26 (56.5)	51 (72.9)	0.13	0.28	0.28	0.08
TQE	115 (47.7)	58 (46.4)	22 (47.8)	35 (50.0)	1	0.65	0.76	0.89

[a] Fertilization was determined by the presence of two pronuclei (2PN) and two polar bodies.TQE = top quality embryo.

Although mature oocyte recovery rate was higher among follicles >13 mm, it was found that once a mature oocyte was recovered, no difference was observed in fertilization rate or in embryo quality, regardless of follicle size.

Data regarding fertilization and embryo quality derived from small follicles are inconsistent (*Mehri et al., 2014*; *Salha et al., 1998*; *Triwitayakorn et al., 2003*). *Dubey et al. (1995)* reported that fertilization rate of all oocytes, regardless of morphological type, had a positive linear correlation as follicle diameter increased. *Nogueira et al. (2006)* found that matured oocytes retrieved from small follicles generated embryos of lower developmental potential than oocytes derived from larger follicles. In a prospective study conducted by *Triwitayakorn et al. (2003*; *Mehri et al., 2014*), including 991 follicles, the fertilization rate of mature oocytes, as well as the rate of good-quality embryos, increased from the small follicle group to the large follicle group, but this finding

was not statistically significant. The results of this study are in concordance with *Wirleitner et al. (2018)*, who found a significantly lower MII oocyte recovery rate for small follicles compared with larger ones, but similar fertilization rates and blastocyst rates per mature MII. They concluded that oocytes derived from small follicles still have the capacity for normal development and subsequent delivery of healthy children, suggesting that aspiration of these follicles should be encouraged, as this would increase the total number of blastocysts retrieved per stimulation, and consequently give rise to a higher potential cumulative live birth rate (*Drakopoulos et al., 2016*).

Final oocyte maturation is commonly triggered by the injection of HCG 36 h before oocyte retrieval. Recently, alternative triggering modes have been practised in order to improve treatment outcomes (*Orvieto, 2015*).

The use of GnRHa was first introduced in 1990 by *Gonen et al. (1990)*, who demonstrated that ovulation may also be triggered by GnRHa, causing the release of both endogenous LH and FSH. This mimics the natural cycle surge and is therefore considered to be more physiological. Moreover, today it is commonly used as a rescue treatment in order to eliminate ovarian hyperstimulation syndrome for women at risk treated by the GnRH antagonist ovarian stimulation protocols. Numerous studies have emerged comparing the effect of HCG versus GnRHa trigger following an IVF treatment cycle. In these studies, GnRHa triggering was found to be comparable with or superior to HCG when measuring the number of oocytes retrieved, percentage of mature oocytes and the number of resultant top quality embryos (*Acevedo et al., 2006*; *Erb et al., 2010*; *Fauser et al., 2002*; *Kolibianakis et al., 2005*).

Due to improved results reported after GnRHa triggering, the concomitant administration of both GnRHa and a standard bolus of HCG (5000–10,000 units) prior to oocyte retrieval (dual triggering) was given to further improve oocyte and embryo quality. Dual triggering has been specifically used to treat suboptimal responders and those with abnormal final follicular maturation (*Griffin et al., 2014*; *Haas et al., 2014*; *Lu et al., 2016*; *Orvieto, 2015*; *Zilberberg et al., 2015*). Recent studies report mixed results. *Eser et al. (2018)* reported that dual triggering did not improve oocyte maturation, clinical pregnancy and ongoing pregnancy rates. In a study comparing 224 women who underwent dual triggering to 101 women triggered with HCG alone, oocyte retrieval rate was comparable, with no difference in live delivery rate between the groups (*Zhou et al., 2018*). *Decleer et al. (2014)* reported no between-group differences in the mean number of oocytes retrieved, mature oocytes or pregnancy rates, between women treated with dual triggering and those treated with HCG alone. However, the number of patients whose treatment yielded at least one embryo of excellent quality and the number of cryopreserved embryos were significantly higher following dual triggering.

As far as is known, no studies have been published comparing the influence of all three triggering modes on oocyte recovery and maturation as a function of follicle size. This study revealed no differences between the triggering modes in oocyte recovery rate, mature oocytes retrieved, fertilization rate and TQE in all follicle size groups. These findings were observed despite the significantly less favourable demographics and infertility background of the dual triggering group. The dual trigger group were older women, underwent more IVF cycle attempts and were offered the dual trigger in an attempt to overcome their suboptimal/poor prognosis and/or previous abnormal final follicular maturation (*Haas et al., 2014*; *Orvieto, 2015*; *Zilberberg et al., 2015*). Indeed, the dual trigger resulted in comparable embryological outcome, which means that it effectively 'normalized' the prognosis of this group of patients.

This study has several limitations. Women included in the study were treated for infertility caused by a variety of factors. Furthermore, treatment protocols were not homogeneous across the study population, so follicles exposed to different gonadotrophins were included. This may have influenced the rate of oocyte retrieval. Another limitation is the lack of randomization for the triggering mode, exposing outcomes to potential selection bias. For example, those offered dual triggering were often patients with previous abnormal final follicular maturation or poor embryo quality.

Although various studies have examined the association between follicular size and oocyte recovery rate at retrieval, data are inconsistent. This study's strength is in its being conducted in a single centre by a consistent professional team on a large study group. Moreover, this is thought to be the first study to assess the association of follicular size with oocyte retrieval rate as a function of the different final follicular maturation triggering modes.

In summary, the results of this study indicate that follicles with a maximum diameter ≥16 mm are comparable in oocyte recovery rate to those with diameters between 13 mm and 15 mm, and both are associated with a higher oocyte recovery rate compared with follicles smaller than 13 mm. Once oocytes are mature (MII), similar fertilization and TQE rates are observed, and no correlation is found with the original follicular diameter. Triggering mode did not influence oocyte recovery rate across the different follicle size groups. This information should be of value to physicians and patients alike. Further investigation is required to strengthen these findings.

ACKNOWLEDGEMENTS

We acknowledge the Embryological Laboratory Team of Sheba Medical Center for their cooperation.

REFERENCES

Acevedo, B., Gomez-Palomares, J.L., Ricciarelli, E., Hernández, E.R. **Triggering ovulation with gonadotropin-releasing hormone agonists does not compromise embryo implantation rates.** Fertil. Steril. 2006; 86: 1682–1687

Andersen, C.Y. **Characteristics of human follicular fluid associated with successful conception after in vitro fertilization.** J. Clin. Endocrinol. Metab. 1993; 77: 1227–1234

Bergh, C., Broden, H., Lundin, K., Hamberger, L. **Comparison of fertilization, cleavage and pregnancy rates of oocytes from large and small follicles.** Hum. Reprod. 1998; 13: 1912–1915

Decleer, W., Osmanagaoglu, K., Seynhave, B., Kolibianakis, S., Tarlatzis, B., Devroey, P. **Comparison of hCG triggering versus hCG in combination with a GnRH agonist: a prospective randomized controlled trial.** Facts Views Vis. Obgyn. 2014; 6: 203–209

Drakopoulos, P., Blockeel, C., Stoop, D., Camus, M., de Vos, M., Tournaye, H., Polyzos, N.P. **Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos?** Hum. Reprod. 2016; 31: 370–376

Dubey, A.K., Wang, H.A., Duffy, P., Penzias, A.S. **The correlation between follicular measurements, oocyte morphology, and fertilization rates in an in vitro fertilization program.** Fertil. Steril. 1995; 64: 787–790

Ectors, F.J., Vanderzwalmen, P., Van Hoeck, J., Nijs, M., Verhaegen, G., Delvigne, A., Schoysman, R., Leroy, F. **Relationship of human follicular diameter with oocyte fertilization and development after in-vitro fertilization or intracytoplasmic sperm injection.** Hum. Reprod. 1997; 12: 2002–2005

Erb, T.M., Vitek, W., Wakim, A.N. **Gonadotropin-releasing hormone agonist or human chorionic gonadotropin for final oocyte maturation in an oocyte donor program.** Fertil. Steril. 2010; 93: 374–378

Eser, A., Devranoğlu, B., Bostancı Ergen, E., Abide, Ç.Yayla **Dual trigger with gonadotropin-releasing hormone and human chorionic gonadotropin for poor responders.** J. Turk. Ger. Gynecol. Assoc. 2018; 19: 98–103

Fauser, B.C., de Jong, D., Olivennes, F., Wramsby, H., Tay, C., Itskovitz-Eldor, J., van Hooren, H.G. **Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization.** J. Clin. Endocrinol. Metab. 2002; 87: 709–715

Gonen, Y., Balakier, H., Powell, W., Casper, R.F. **Use of gonadotropin-releasing hormone agonist to trigger follicular maturation for in vitro fertilization.** J. Clin. Endocrinol. Metab. 1990; 71: 918–922

Griffin, D., Feinn, R., Engmann, L., Nulsen, J., Budinetz, T., Benadiva, C. **Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates.** Fertil. Steril. 2014; 102: 405–409

Haas, J., Zilberberg, E., Dar, S., Kedem, A., Machtinger, R., Orvieto, R. **Co-administration of GnRH-agonist and hCG for final oocyte maturation (double trigger) in patients with low number of oocytes retrieved per number of preovulatory follicles–a preliminary report.** J. Ovarian. Res. 2014; 7: 77

Haines, C.J., Emes, A.L. **The relationship between follicle diameter, fertilization rate, and microscopic embryo quality.** Fertil. Steril. 1991; 55: 205–207

Kolibianakis, E.M., Schultze-Mosgau, A., Schroer, A., van Steirteghem, A., Devroey, P., Diedrich, K., Griesinger, G. **A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists.** Hum. Reprod. 2005; 20: 2887–2892

Lu, X., Hong, Q., Sun, L., Chen, Q., Fu, Y., Ai, A., Lyu, Q., Kuang, Y. **Dual trigger for final oocyte maturation improves the oocyte retrieval rate of suboptimal responders to gonadotropin-releasing hormone agonist.** Fertil. Steril. 2016; 106: 1356–1362

Mehri, S., Levi Setti, P.E., Greco, K., Sakkas, D., Martinez, G., Patrizio, P. **Correlation between follicular diameters and flushing versus no flushing on oocyte maturity, fertilization rate and embryo quality.** J. Assist. Reprod. Genet. 2014; 31: 73–77

Miller, K.F., Goldberg, J.M., Falcone, T. **Follicle size and implantation of embryos from in vitro fertilization.** Obstet. Gynecol. 1996; 88: 583–586

Nogueira, D., Friedler, S., Schachter, M., Raziel, A., Ron-El, R., Smitz, J. **Oocyte maturity and preimplantation development in relation to follicle diameter in gonadotropin-releasing hormone agonist or antagonist treatments.** Fertil. Steril. 2006; 85: 578–583

Orvieto, R **Triggering final follicular maturation–hCG, GnRH-agonist or both, when and to whom?** J. Ovarian. Res. 2015; 8: 60

Rosen, M.P., Shen, S., Dobson, A.T., Rinaudo, P.F., McCulloch, C.E., Cedars, M.I. **A quantitative assessment of follicle size on oocyte developmental competence.** Fertil. Steril. 2008; 90: 684–690

Salha, O., Nugent, D., Dada, T., Kaufmann, S., Levett, S., Jenner, L., Lui, S., Sharma, V. **The relationship between follicular fluid aspirate volume and oocyte maturity in in-vitro fertilization cycles.** Hum. Reprod. 1998; 13: 1901–1906

Scott, R.T., Hofmann, G.E., Muasher, S.J., Acosta, A.A., Kreiner, D.K., Rosenwaks, Z. **Correlation of follicular diameter with oocyte recovery and maturity at the time of transvaginal follicular aspiration.** J. In. Vitro. Fert. Embryo. Transf. 1989; 6: 73–75

Simonetti, S., Veeck, L.L., Jones, H.W. **Correlation of follicular fluid volume with oocyte morphology from follicles stimulated by human menopausal gonadotropin.** Fertil. Steril. 1985; 44: 177–180

Triwitayakorn, A., Suwajanakorn, S., Pruksananonda, K., Sereepapong, W., Ahnonkitpanit, V. **Correlation between human follicular diameter and oocyte outcomes in an ICSI program.** J. Assist. Reprod. Genet. 2003; 20: 143–147

Wirleitner, B., Okhowat, J., Vištejnová, L., Králíčková, M., Karlíková, M., Vanderzwalmen, P., Ectors, F., Hradecký, L., Schuff, M., Murtinger, M. **Relationship between follicular volume and oocyte competence, blastocyst development and live-birth rate: optimal follicle size for oocyte retrieval.** Ultrasound Obstet. Gynecol. 2018; 51: 118–125

Wittmaack, F.M., Kreger, D.O., Blasco, L., Tureck, R.W., Mastroianni, L., Lessey, B.A. **Effect of follicular size on oocyte retrieval, fertilization, cleavage, and embryo quality in in vitro fertilization cycles: a 6-year data collection.** Fertil. Steril. 1994; 62: 1205–1210

Zhou, X., Guo, P., Chen, X., Ye, D., Liu, Y., Chen, S. **Comparison of dual trigger with combination GnRH agonist and hCG versus hCG alone trigger of oocyte maturation for normal ovarian responders.** Int. J. Gynaecol. Obstet. 2018; 141: 327–331

Zilberberg, E., Haas, J., Dar, S., Kedem, A., Machtinger, R., Orvieto, R. **Co-administration of GnRH-agonist and hCG, for final oocyte maturation (double trigger), in patients with low proportion of mature oocytes.** Gynecol. Endocrinol. 2015; 31: 145–147

Received 4 January 2020; received in revised form 5 February 2020; accepted 11 February 2020.