

# Correlation of Lead Follicle Size on Day of Trigger with the Outcome of *In vitro* Fertilization (IVF) – A Retrospective Study

Lakshmanan Saravanan, Mahalakshmi Saravanan, Blessy John\* and Nidhi Sharma

ARC International Fertility Center, Saveetha University, Kuthambaakam, Chennai 600124, Tamil Nadu, India

**Abstract:** *Introduction:* IVF treatment involves the administration of supra-physiological doses of follicle stimulating hormone (FSH) to induce the growth of multiple ovarian follicles. Once ovarian follicles grow to an appropriate size, a trigger is administered to mature the oocytes in preparation for oocyte retrieval. However, no definite data exist to establish which follicle size on the day of trigger is most likely to yield a mature oocyte and successful IVF outcome. Knowledge of the size of follicles on day of trigger from which one could reasonably expect to retrieve a mature oocyte could enable the accurate determination of trigger efficacy. Therefore we sought to determine the size of the follicles on the day of trigger that would be most likely to yield a mature oocyte with increased blastocyst conversion rate after invitro fertilization thereby increasing the clinical pregnancy rate.

*Methodology:* This retrospective study analysed 371 records of infertile women who underwent In-vitro fertilization in ARC International Fertility Center, Saveetha Medical College and Hospital, Chennai from March 2017 to March 2019.

*Results:* In our study, highest oocyte maturation was observed when trigger was given when the lead follicle size was 22.1 to 24mm. a larger number of transferable good quality embryos are harvested from the group with lead follicle size of 22.1 to 24mm (74 %). The 18 to 20mm group had 21% and 20.1 to 22mm group had 40%. The more than 24mm group had 50 % of good embryos. Finally in this study we did not have a statistically significant increase in the clinical viable pregnancy rate among the four groups. Though not statistically significant, we observed a slight increase in the clinical viable pregnancy rate among 22.1 to 24mm group 79% compared to other groups.

*Conclusion:* In this study we observed that an early trigger (less than 22mm lead follicle size) or a very late trigger (>24mm) decreased the oocyte maturation rate thereby reducing the blastocyst conversion rate and clinical pregnancy rate. In this study we observed the ideal size of the lead follicle at the time of trigger to be 22 to 24 mm.

**Keywords:** *In-vitro* fertilization, lead follicle size, trigger.

## INTRODUCTION

IVF treatment involves the administration of supra-physiological doses of follicle stimulating hormone (FSH) to induce the growth of multiple ovarian follicles. Once ovarian follicles grow to an appropriate size, a trigger is administered to mature the oocytes in preparation for oocyte retrieval. It is widely accepted that ovarian follicles that are “too small” are less likely to respond suitably to trigger administration and yield a mature oocyte [1]. Furthermore, once ovarian follicles grow too large, follicles may contain oocytes that are “post-mature” and also not competent for fertilization [2].

While some studies in the literature suggest that oocytes from larger follicles yield superior oocytes with improved fertilization and implantation potential, other studies report that delaying oocyte retrieval neither increases the number of mature oocytes retrieved nor the pregnancy rate and may, in fact, impair embryo quality. Most IVF centers will therefore, monitor follicular size and administer the trigger of oocyte

India; Tel: (+)919445560392; E-mail: drbonuramkumar@yahoo.co.in

maturation once follicles are deemed to have grown to an appropriate size. However, limited data exist to establish which follicle size on the day of trigger is most likely to yield a mature oocyte.

Data on follicle size on day of trigger with greatest propensity to yield oocytes are suggested by Hu and colleagues [3]. The investigators determined that the number of oocytes retrieved was greatest in those with a low proportion of follicles  $\geq 17$  mm. Kyrou *et al.* compared administering the trigger once three follicles were  $\geq 16$  mm in diameter (early), or 24 h later (late), and found that delaying triggering increased the number of mature oocytes retrieved (early 6.1, late 9.2,  $P = 0.009$  [4]. Mochtar and colleagues randomized women to receive trigger once the lead follicle was either 18 or 22 mm, and observed that those with a lead follicle of 22 mm had a greater number of follicles of 20–22 mm on day of trigger (3.95 vs 0.02) and an increase in two oocytes retrieved [5]. Conversely, Tan and colleagues randomized patients to trigger either once the lead follicle was 18 mm, or 1 day later, or 2 days later and observed no differences in the number of oocytes retrieved [6]. Similarly, Tremellen and Lane found that patients with “ideal” timing of the hCG trigger (defined as  $\geq 2$  follicles of  $\geq 17$  mm, with the majority of follicles  $\geq 14$  mm) had similar outcomes to patients

\*Address correspondence to this author at the ARC International Fertility Center, Saveetha University, Kuthambaakam, Chennai 600124, Tamil Nadu,

triggered either a day earlier or later [7], whereas Vandekerckhove *et al.* observed that a 24 h delay in trigger administration of patients with  $\geq 3$  follicles of  $\geq 18$  mm (and 30–50% of follicles  $\geq 10$  mm were also  $\geq 18$  mm) increased the number of mature oocytes retrieved by 2.4 [8].

However, no definite data exist to establish which follicle size on the day of trigger is most likely to yield a mature oocyte and successful IVF outcome. Knowledge of the size of follicles on day of trigger from which one could reasonably expect to retrieve a mature oocyte could enable the accurate determination of trigger efficacy. Therefore we sought to determine the size of the follicles on the day of trigger that would be most likely to yield a mature oocyte with increased blastocyst conversion rate after invitro fertilization thereby increasing the clinical pregnancy rate.

#### Aim of the Study

1. To identify the ideal follicle size on the day of trigger which most likely yields a mature oocyte
2. To correlate the lead follicle size on day of trigger on the outcomes of an IVF cycle
  - a) To correlate the lead follicle size on day of trigger on the M2 conversion rates in an IVF cycle
  - b) To correlate the lead follicle size on day of trigger on Blastocyst formation rates in an IVF cycle
  - c) To correlate the lead follicle size on day of trigger on clinical viable pregnancy rates in an IVF cycle

#### Type of the Study

A retrospective analysis of data of women who underwent In-Vitro Fertilization for a period of two years – from March 2017 to March 2019 in ARC International Fertility Center, Saveetha Medical College and Hospital, Chennai

#### MATERIALS AND METHODS

This retrospective study analysed 371 records of infertile women who underwent In-vitro fertilization in ARC International Fertility Center, Saveetha Medical College and Hospital, Chennai from March 2017 to March 2019.

#### Inclusion Criteria

1. Age – 21 to 37 years
2. Patients who were given Recombinant FSH for ovarian stimulation
3. Patients who had Recombinant HCG for trigger
4. Patients having normal ovarian reserve – Antimullerian hormone (AMH) = 1.5ng/ml to 4ng/ml
5. Patients who were transferred with good quality embryos.

#### Exclusion Criteria

1. Age –more than 37 years
2. Patients with AMH < 1.5ng/ml or > 4ng/ml
3. Patients who had ovarian stimulation with drugs other than recombinant FSH
4. Patients who had GnRH agonist or urinary HCG or Dual trigger
5. Patients with uterine and adnexal pathology eg., hydrosalpinx, submucous polyps or myomas, or uterine septum.

#### Procedure

This study was approved by the Institutional Review Board at the Saveetha University, Chennai.

IVF Protocol: In-vitro fertilization (IVF) is a type of assistive reproductive technology (ART) which involves retrieving eggs from a woman's ovaries and fertilizing them with sperm to form an embryo.

#### Ovarian Stimulation

When the period starts, a baseline ultrasound and blood estradiol and progesterone test was scheduled on day 2. The purpose of these tests was to confirm that the ovaries are suppressed to a baseline state. After ovarian suppression was confirmed, ovarian stimulation using gonadotropin fertility drugs commenced. These gonadotropin fertility drugs were continued throughout the stimulation phase of the cycle, that is, until hCG is administered. In this study we analysed patients who were stimulated with only recombinant FSH and recombinant HCG for trigger. The dose of gonadotropins

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were based upon age, weight, number of antral follicles and day 2 estradiol levels. Sonograms were conducted starting on day 6 or 7 of the stimulation. In general, follow-up sonograms and estradiol blood tests occurred every one to three days to monitor the growth of the follicles. Patients were asked to return more frequently toward the end of their ovarian stimulation. Most people required 10 to 12 days of ovarian stimulation.

### **Ovulation Triggering**

When the follicles had grown to a certain size, the recombinant HCG trigger injection was administered. The purpose of this trigger is to induce the final stages of oocyte maturation

### **Oocyte Retrieval**

This is a daycare procedure done under anaesthesia. During the procedure, fluid containing egg cells were aspirated with a thin, long needle through vaginal fornix from the follicles in ovaries, under ultrasound guidance. The ultrasound guidance enabled the visualization of the ovaries and follicles which contain eggs, as well as the tip of the needle which was introduced precisely into every follicle, and the follicular fluid was successfully collected.

### **Oocyte and Sperm Preparation**

In the laboratory, the identified eggs were stripped of surrounding cells and prepared for fertilisation. An oocyte selection was performed prior to fertilisation to select eggs with optimal chances of successful pregnancy. In the meantime, semen was prepared for fertilisation by removing inactive cells and seminal fluid in a process called sperm washing.

### **Fertilization**

*In-vitro* fertilization involves the sperm and the egg incubated together at a ratio of about 75,000:1 in a culture media in order for the actual fertilisation to take place. In certain situations, such as low sperm count or motility, a single sperm was injected directly into the egg using intracytoplasmic sperm injection (ICSI). The fertilised egg was passed to a special growth medium and left for about 48 hours until the egg consists of six to eight cells.

### **Embryo Culture**

The main durations of embryo culture are until cleavage stage (day two to four after co-incubation) or the blastocyst stage (day five or six after co-incubation).

Embryo culture until the blastocyst stage confers a significant increase in live birth rate per embryo transfer, but also confers a decreased number of embryos available for transfer and embryo cryopreservation. In this study we considered 5<sup>th</sup> day or 6<sup>th</sup> day embryo in the blastocyst stage.

### **Embryo Transfer**

The number to be transferred depends on the number available, the age of the woman and other health and diagnostic factors. Maximum of two embryos are transferred at a time. In our study, all patients had two embryo transferred.

### **Luteal Support**

In this study luteal support was administered by progesterone, progestins, hCG, or GnRH agonists, and often accompanied by estradiol, to increase the success rate of implantation and early embryogenesis. Beta HCG level was tested after 2 weeks of embryo transfer.

All details were collected from patients' data file and the patients were categorized based on the diameter of the largest follicle recorded on the day of ovulation trigger into 4 groups: 18 to 20 mm, 20.1 to 22mm, 22.1 – 24mm and > 24mm. The data was tabulated and statistically analysed.

## **RESULTS AND ANALYSIS**

A total of 371 patients who met the inclusion and exclusion criteria were included in this study. The results were analysed using Pearson Chi Square Test.

## **DISCUSSION**

In this study, we quantified the relationship between follicle size and oocyte maturation, blastocyst formation and achievement of clinical viable pregnancy relative to the lead follicle group. This enabled us to predict potential outcomes based on the egg origin and to explain some of the outcomes of the *in vitro* fertilization (IVF) process.

In this study, the data of total of 371 patients were analysed. Based on the size of the lead follicle on the day of trigger, the patients were grouped into 4 groups. 47 patients (13%) fell into the 18mm to 20mm group. 102 patients (27%) was from 20.1 to 22mm, 164 patients (44%) was from 22.1 to 24mm and 58 patients (16%) had lead follicle size >24mm.

**Correlation of Lead Follicle Size on Day of Trigger International Journal of Gynecological and Obstetrical Research, 2019, Vol. 7 27 Table 1:**

LEAD FOLLICLE SIZE	M2 CONVERSION RATE			TOTAL
	< 50%	50 – 75%	>75%	
18 – 20mm	8 (17%)	19 (40%)	20 (43%)	47 (100%)
20.1 – 22mm	6 (6%)	42 (41%)	54 (53%)	102 (100%)
22.1 – 24mm	10 (6%)	35 (21%)	119 (73%)	164 (100%)
>24mm	3 (5%)	15 (26%)	40 (69%)	58 (100%)

Pearson's Chi-squared test = 24.963, p-value = 0.0003469 (significant).

**Table 2:**

LEAD FOLLICLE SIZE	BLASTOCYST CONVERSION RATE			TOTAL
	< 30%	30 – 50%	>50%	
18 – 20mm	19 (41%)	18 (38%)	10 (21%)	47 (100%)
20.1 – 22mm	8 (8%)	53 (52%)	41 (40%)	102 (100%)
22.1 – 24mm	16 (10%)	27 (16%)	121 (74%)	164 (100%)
>24mm	11 (19%)	18 (31%)	29 (50%)	58 (100%)

Pearson's Chi-squared test = 95.687 p-value = < 0.001 (significant).

**Table 3:**

LEAD FOLLICLE SIZE	CLINICAL PREGNANCY		TOTAL
	YES (CARDIAC ACTIVITY SEEN)	NO (NEGATIVE AND BIOCHEMICAL PREGNANCY)	
18 – 20mm	26 (55%)	21 (45%)	47 (100%)
20.1 – 22mm	72 (71%)	30 (29%)	102 (100%)
22.1 – 24mm	129 (79%)	35 (21%)	164 (100%)
>24mm	39 (67%)	19 (33%)	58 (100%)

Pearson's Chi-squared test = 9.2033, p-value = 0.02671 (non-significant).

It is well known that nuclear maturation, defined as oocytes that have completed their first meiotic division and are in MII, is necessary for normal fertilization. In this study, while correlating the lead follicle size with the M2 conversion rate, p value was less than 0.001 and hence statistically significant proving that the lead follicle size has a statistically significant effect over the nuclear maturation of oocytes. In this study, oocyte maturation rate rose from 43% from follicles 18- 20mm in size to 53% in 20.1 to 22mm group to 73% in 22.1 to 24mm group. In the more than 24mm group, the oocyte maturation fell slightly to 69%. This shows that In our study, highest oocyte maturation was observed when trigger was given when the lead follicle size was 22.1 to

24mm. This was consistent with the work of Dubey and colleagues, whereby 85% of oocytes were collected from follicles of 14–24 mm at the time of oocyte retrieval [9]. Wirleitner B *et al.* observed the same trend in his study, when the rate of MII oocytes was calculated per retrieved COC (MII/COC rate, 59.2% in small follicles vs 83.6% in medium follicles and 86.2% in large follicles;  $P < 0.001$ ), showing that when an oocyte is retrieved from small follicles, it has a lower capability of being mature. Furthermore, Mochtar and colleagues [5] randomized women to receive trigger once the lead follicle was either 18 or 22 mm, and observed that those with a lead follicle of 22 mm had a greater number of follicles of 20–22 mm on day of trigger and an increase in two oocytes retrieved. Conversely, Tan and colleagues randomized

patients to trigger either once the lead follicle was 18 mm, or 1 day later, or 2 days later and observed no differences in the number of oocytes retrieved [6].

This study indicated that a larger number of transferable good quality embryos are harvested from the group with lead follicle size of 22.1 to 24mm (74 %). The 18 to 20mm group had 21% and 20.1 to 22mm group had 40%. The more than 24mm group had 50 % of good embryos. This slight decrease (50%) in the blastocyst conversion rate in the more than 24mm group compared 74% in 22,1 to 22mm group, denotes that a delay in ovulation trigger or early trigger might impair the chances of good embryo development. Finally in this study we did not have a statistically significant increase in the clinical viable pregnancy rate among the four groups. This could be because we transferred only good quality embryos to all patients. Though not statistically significant, we observed a slight increase in the clinical viable pregnancy rate among 22.1 to 24mm group 79% compared to other groups.

## CONCLUSION

Knowledge of the size of follicles on day of trigger from which one could reasonably expect to retrieve a mature oocyte with increased blastocyst conversion rate and establishment of a clinical pregnancy could enable the accurate determination of trigger efficacy. In this study we observed that an early trigger (less than 22mm lead follicle size) or a very late trigger (>24mm) decreased the oocyte maturation rate thereby reducing the blastocyst conversion rate and clinical pregnancy rate. In this study we observed the ideal size of the lead follicle at the time of trigger to be 22 to 24 mm. However more multicentral large size studies are required to universalise this hypothesis.

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