

Original Article

Size Of Follicle and Oocyte Maturation Status in an Assisted Reproductive Technology Programme

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Abstract

Background : To assess the correlation between follicular size and oocyte maturation status in assisted reproductive technology programs.

Method : It was a prospective study done from September 2011 to May 2012 in the Department of Reproductive Medicine at a tertiary care hospital. Sixty patients undergoing assisted reproductive cycles either with agonist or antagonist protocol were included in this study. Follicles were subdivided into four arbitrary groups according to their mean two dimension size, >21 mm, 16-20 mm, 12-15 mm and <12 mm. Microscopic examination of the follicular aspirates were performed by the embryologist.

Findings : If follicle size > 21 mm, there was 85% chance of retrieving an MII oocyte; when the size was between 16 – 20 mm, the chance of retrieval was 87%. In 12 – 16 mm group, it was 80% and in the follicles <12mm it was 55%. The level of significance was calculated between each group (with respect to MII oocytes). Between the first three groups, p value was not significant. When the larger sized follicles were compared with < 12 mm group, $p = 0.000$, which was statistically significant ($p < 0.05$).

Conclusion : It is better to trigger with HCG when the lead follicle is between 16 – 20 mm rather than waiting till > 21 mm, as this saves time and money for the patient. Small follicles of size < 12 mm also yielded MII oocytes, hence it is worthwhile aspirating small follicles also.

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Key Words : Follicular size, Oocyte quality, Oocyte maturity, Controlled ovarian hyperstimulation

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Introduction

Follicle size is associated with oocyte development in most species and this may indicate that a specific size is necessary to initiate the molecular cascade of normal nuclear and cytoplasmic maturation.^{1,2}

The development and maturation of a follicle undergo a series of events in the natural menstrual cycle.

- Recruitment of group of follicles – The initial recruitment and growth of primordial follicles are not under the control of any hormone. After certain stage (2 – 5 mm in size) the growth and differentiation are under the control of FSH. Unless the follicles are rescued by FSH at this stage, they undergo atresia.

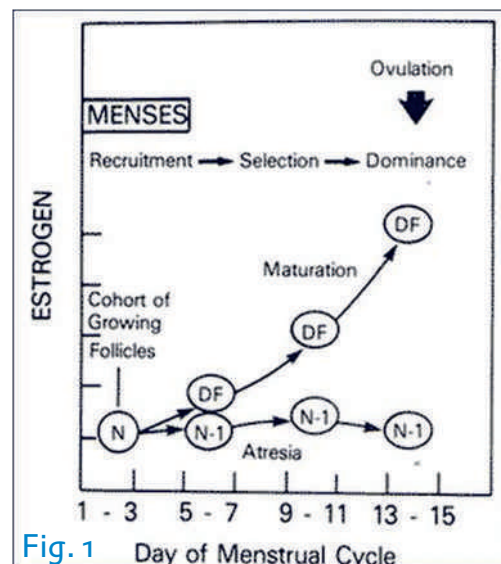


Fig. 1 Day of Menstrual Cycle

- Selection of Dominant follicle and maturation – There is accelerated growth of all the components of the follicles. As early as day 5-7, one of the follicle out of so many in the cohort becomes dominant and undergoes further maturation. The one with maximum receptors for FSH, becomes the dominant follicle (Graafian follicle). The rest become atretic by day 8.
- Ovulation – The cumulus becomes detached from the wall so that the ovum with the surrounding cells float freely in the liquor folliculi. The oocyte completes first meiotic division with extrusion of the first polar body which is pushed to the perivitelline space. The follicular wall near the ovarian surface becomes thinner. The cumulus escapes out of the follicle by a slow oozing process along with varying amount of follicular fluid.
- Corpus Luteum formation – After ovulation, the ruptured Graafian follicle develops into corpus luteum. The colour is yellow due to the presence of lipids.
- Regression – On day 22-23 of cycle, retrogression occurs. The corpus luteum becomes corpus albicans.

Controlled ovarian hyperstimulation is not critical to assisted reproduction though soft and mild stimulation regimes have been advocated to closely mimic physiological events. Still many prefer the controlled ovarian hyperstimulation to augment the number of oocytes retrieved and embryos generated. Of these, only a small portion will be competent for fertilization and development into viable embryos. Understanding the process of selection, follicular growth and ovulation has guided the development of this important component of treatment. 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. The administration of Human Chorionic Gonadotrophin, in mimicking the endogenous luteinizing hormone [LH] surge, is the final event that determines the follicular maturity and developmental competence. The timing of its administration is typically guided by the size of the lead follicle or lead follicular cohort³.

The treatment is therefore based on an assumption that follicular size predicts the maturity of the oocyte. The assumption is based on limited studies using different models in animals. Yet the available data is conflicting, and although several human studies have suggested oocytes derived from larger follicles outperform [in terms of fertilization and embryo quality] oocytes originating from smaller follicles, the correlation of oocyte competence with follicular size, after controlled ovarian stimulation, has not been well characterized. For instance, while some have suggested the decreasing fertilization rate and embryo quality observed with oocytes originating from smaller follicles can be overcome with intracytoplasmic sperm injection [ICSI]⁴. Others have suggested normal fertilization of

an oocyte is independent of its follicular size origin⁵.

It should be noted that embryo competence is most likely due to the quality of the originating gametes. Therefore, the morphological appearance of the oocyte is likely to contribute to the development potential of the subsequent embryo.

Classification of oocyte maturity⁶

Normal oocyte

1. Metaphase II

A mature or a good metaphase II oocyte is defined as an oocyte with clear, moderately granular cytoplasm, small perivitelline space and clear to colourless zona pellicuda⁷. First polar body is round or ovoid with smooth surface^{8,9,10}. Cumulus cells are fully radiating and easily stretchable.

2. GV (Germinal Vesicle):

It is an immature oocyte where the cumulus cells are tightly packed. The nucleus is large. It is preincubated before insemination.

3. Metaphase – I

It is an immature oocyte which has tightly packed cumulus cells. There is no nucleus or polar body.

4. Very mature :

This oocyte is pale. It has little corona cell.

5. Luteinized:

This oocyte is very pale and difficult to find. Cumulus cell is broken down and becomes a gelatinous mass. There is low probability of fertilization. It is inseminated with little delay.

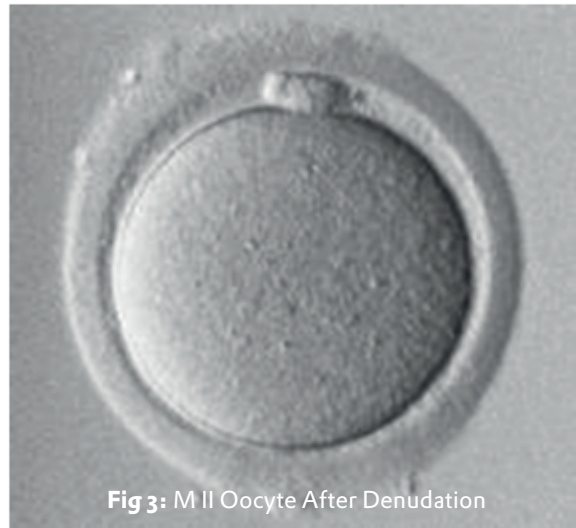
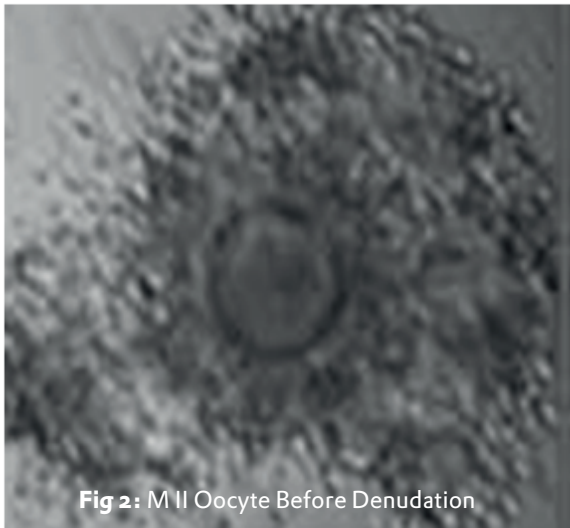
6. Atretic:

It is very dark oocyte with fragmented cumulus cells. It has a lace like appearance and is difficult to identify.

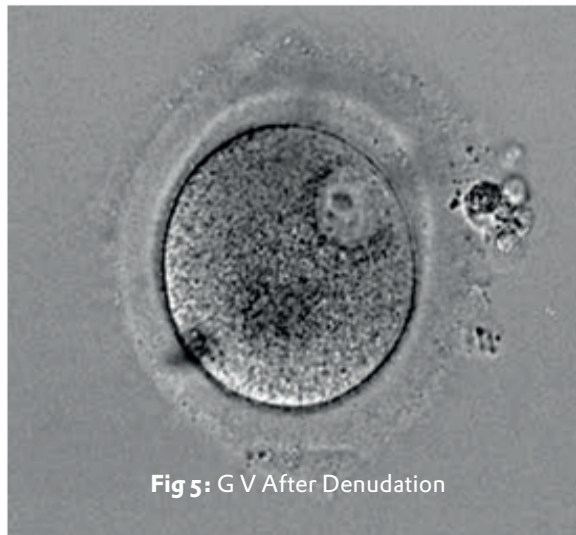
Materials and Methods

The present study is a prospective study conducted in the Department of Reproductive Medicine at a tertiary care hospital from September 2011 to May 2012. 60 patients undergoing assisted reproductive cycles either with agonist or antagonist protocol were included in this study. Individuals were serially monitored with transvaginal ultrasound from day 5 onwards of stimulation and all follicles were measured in two dimensions. The decision to administer HCG was based on the lead follicular cohort, usually with at least 3 follicles measuring 20mm in diameter and also an endometrial thickness of 8 mm onwards. Follicular size measurements were made serially and on the day of HCG trigger. A transvaginal ultrasound guided follicular aspiration was conducted 35 hours after hCG administration.

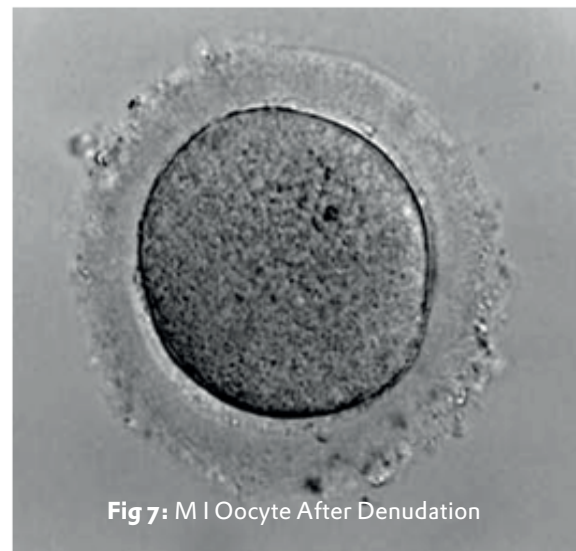
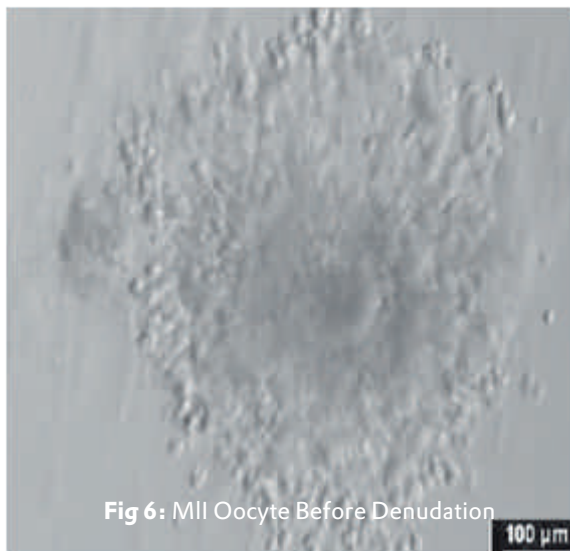
Normal Oocytes Metaphase II



Germinal vesicle



METAPHASE I



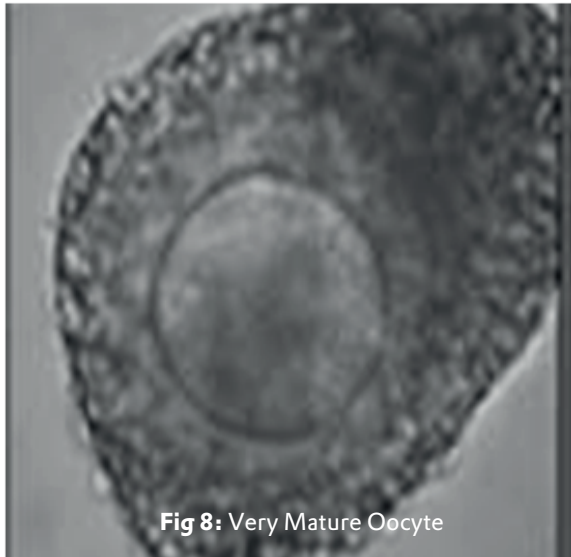


Fig 8: Very Mature Oocyte

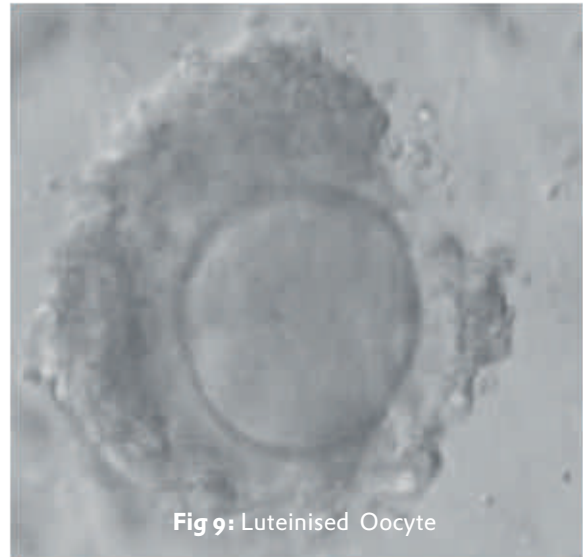


Fig 9: Luteinised Oocyte

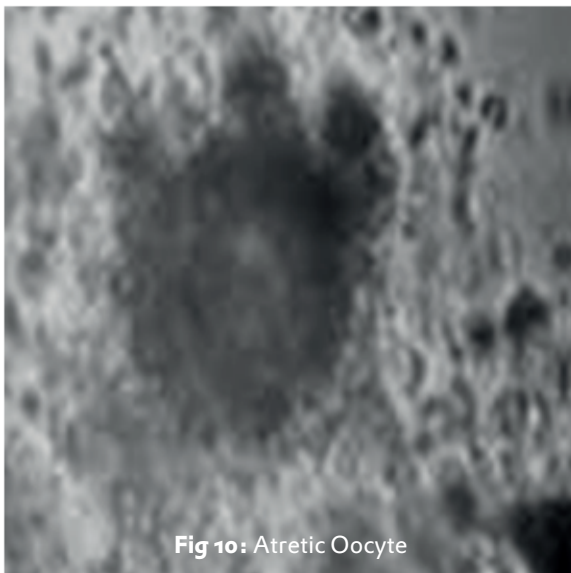


Fig 10: Atretic Oocyte

Follicles were subdivided into four groups according to their mean two dimension size, >21 mm, 16-20 mm, 12-15 mm and <12 mm. All follicles were aspirated under

transvaginal ultrasound guidance with a double lumen 17G oocyte pickup needle (Swemed) at 100 mmHg pressure. Flushing of aspirated follicles was performed with flushing medium phosphate buffered saline (PBS-SAGE) once or twice if oocyte was not obtained in the initial aspirate. Volume of the aspirate was found to be >3 ml in larger follicles, around 2 ml in the intermediate follicles and < 1 ml in small follicles

Microscopic examination of the follicular aspirates were performed by the same embryologist. Once the oocytes were identified, they were collected and kept in the four well dishes with G-IVF media. Aspirates were screened individually and the maturity of oocytes were noted from each size of the follicle. Further study of the development of individual oocyte was not possible due to group culture.

Table 1 shows the association between the size of the follicles and maturity of the oocytes obtained in the

Results

Table 1 Oocyte Maturity and Size

SIZE(mm)	OOCYTE MATURITY				TOTAL (N=489)
	MII	M ₁	GV	ABNORMAL	
>21	110(85%)	5(3.8%)	9(6.9%)	6(4.3%)	130
16-20	179(87%)	8(3.9%)	6(2.9%)	13(6.2%)	206
12-15	71(80%)	10(11.2%)	6(6.7%)	2(2.1%)	89
<12	35(55%)	13(20%)	11(17.2%)	5(7.8%)	64

study. In follicle size > 21 mm, there was an 85% chance of retrieving MII oocyte. When the size was between 16 – 20 mm, the chance of retrieval was 87%. In 12 – 16 mm group, it was 80% and in the follicles < 12 mm it was 55%. The level of significance was calculated between each group (with respect to MII oocytes). Between the first 3 groups, p value was not significant. When the larger size follicles were compared with < 12 mm group, $p = 0.000$, which was statistically significant ($p < 0.05$).

Discussion

In the study by Mitchell et al³, they grouped the size of follicles into arbitrary five groups, > 18 mm, 16-18 mm, 13-15 mm, 10-12 mm and < 10 mm. The percentage of MII oocytes from each group were 90%, 79%, 73%, 53% and 47.6% respectively. The effect was a monotonic decrease in the odds of obtaining a mature oocyte with decrease in the follicle size group.

In a study done by Ectors et al¹¹, they grouped the aspirated follicular fluid volume into three groups, < 2ml as small (size < 16 mm), 2-6 ml as medium (16 – 23 mm) and >6 ml as large (>23 mm). Higher percentage of oocytes were collected from the medium size follicles. 50.8% were MII oocytes, which were collected from medium sized follicles as compared to 24.7% and 24.5% from small and large follicles respectively.

They further concluded that good embryos were found in medium sized group. In the present study, a good yield of follicles were achieved from large follicles but small follicles also yielded mature oocytes.

In the present study. the chance of retrieving a MII oocyte from follicle size > 21 mm was 85%. When the size was between 16 – 20 mm, the chance of retrieval was 87%. In 12 – 16 mm group, it was 80% and in the follicle < 12mm it was 55%. The level of significance was calculated between each group (with respect to MII oocytes). Between the first 3 groups, p value was not significant. When the larger size follicles were compared with < 12 mm group, $p = 0.000$, which was statistically significant ($p < 0.05$).

In view of the above findings, it is impossible to lay down a treatment scheme. We conclude that it is advisable to aspirate all ultrasonically visible follicles (regardless of size) at the time of oocyte retrieval in order to achieve the maximum benefit from each cycle. Since each patient in every cycle responds differently to ovarian stimulation, it is impossible to lay down a uniform treatment scheme applicable to everyone. It is necessary to arrange the ovarian stimulation as individually as possible, according to patient's age, cause and the way they respond to stimulation protocol, aiming at a continuous multifollicular oocyte development. The degree of this maturation is indicated by follicular growth and development and is assessed most readily by transvaginal ultrasonography. Traditionally, oocyte retrieval is based on ultrasonographic measurement of the leading follicle, but if smaller follicles have oocytes of equal developmental potential, HCG could be administered earlier at a follicular size of about 16-18 mm to save time and expense for the patient.

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