Original Article

Normal and Abnormal Oocytes Observed During Assisted Reproductive Technique (ART) Procedures

Dr.P.Savitha*, Dr.D.Ramesh Raja*, Dr.Radha Pandiyan**

Chettinad Health City Medical Journal 2012; 1(1): 4 - 11

Dr.P.Savitha, B.D.S., D.C.E., Clinical Embryologist, Department of Reproductive Medicine. Completed Bachelor of Dental Surgery in the year 2004. Did Post Graduate Diploma in Clinical Embryology in Chettinad University in the year 2008 and joined as Embryologist in Chettinad Super Specialty hospital. Attended hands on work shop at Chennai in 2010 for vitrification and at Delhi in 2011 for IMSI (Intracytoplasmic Morphologically Selected sperm Injection) and micromanipulation techniques.

*Clinical Embryologist, CSSH, **Senior Consultant in Reproductive Medicine, CSSH (Chettinad Super Speciality Hospital).

Abstract

A competent human MII (Metaphase II) oocyte is regarded as crucial for efficiency of assisted reproductive technique (ART). The non invasive morphological appearance of the oocyte helps in evaluation of developmental competency of oocyte. The purpose of this observational study was to document different patterns of oocyte morphology in our IVF laboratory. This study includes 121 patients who underwent ART from January 2011 to December 2011. The results showed that there were a considerable number of normal and abnormal oocytes observed during ART cycles. From the morphological features of 1070 oocytes retrieved from 121 ART patients, 311(29.06%) oocytes were abnormal. Of 121 patients, oocytes retrieved from 90 patients (74.28%) had one of the abnormalities. Most common abnormality observed were empty and broken zona which were found in 57 oocytes (18.3%) and fragmented polar body which were found in 49 oocytes (15.7%). Of the 90 patients who showed oocyte abnormalities, oocytes from 61 patients (67%) showed either of the two abnormalities mentioned above. We find 74.28% of our patients out of 121 patients had one of the abnormalities. This can be due to underlying infertility, effect of ovarian hyper stimulation or advanced maternal age. Further work is required to assess the benefits of oocyte assessment in selecting best embryo for transfer.

Key Words: Assisted Reproduction, normal and abnormal oocytes

Introduction

Human reproduction is the result of union of two highly specialized cells- the oocyte and the spermatozoon. Of these, the oocyte deserves special mention because of its key functions; it receives the spermatozoon during fertilization, contributes the majority of the cytoplasm for early embryo development and provides half of the genome for one the resulting zygote. In lower animals, it provides information to initiate the events of early embryo development.

Important changes take place in the nucleus and cytoplasmic components of the oocyte at maturation, in preparation for fertilization i.e.

1. Unequal cell division by the oocyte, retaining the haploid set of chromosomes and major portion of cytoplasmic organelles.
2. Exclusion of small sized 1st polar body with remaining chromosomes.
3. Cumulus expansion around various layers of cells surrounding the oocyte.

These intracellular and extracellular components are critical for the survival and fertilization of oocyte. Proper evaluation of nuclear and cytoplasmic maturation of human oocytes is extremely important for the result of In Vitro Fertilization. Low quality oocytes are unlikely to fertilize or will not be competent to produce good embryos. One third of the collected oocytes during IVF may show at least one morphologic anomaly that could negatively influence the embryo development. This paper aims to investigate different patterns of oocyte morphology we encountered in our IVF laboratory.

Methodology:

Data collection

In this observational study the data were collected from the records of all sub-fertile couples who attended the IVF centre at the Department of Reproductive Medicine, Chettinad Health City, from January 2011 to December 2011. Data from 121 patients who underwent assisted reproductive technique (ART) were included in the study. All women were less than 40 years. In all patients, controlled ovarian hyperstimulation was carried out with urinary follicle stimulating hormone (FSH, i.m.). The ovarian response was monitored by serial transvaginal ultrasound followed by the injection of 10,000 IU of human chorionic gonadotrophin (hCG, i.m.). Transvaginal ultrasound-guided oocyte retrieval was performed about 35hours post hCG injection.

Oocyte evaluation

The morphological feature of each oocyte was evaluated with the aid of an inverted microscope.

The oocytes were classified as follows

A. normal oocytes

B. oocytes with intracytoplasmic abnormalities

C. oocytes with extracytoplasmic abnormalities

D. oocytes with abnormal shape

Results:

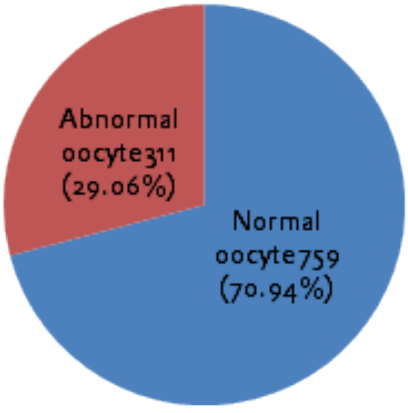
The results showed that there were a considerable number of normal and abnormal oocytes observed during ART cycles. Table 1 demonstrates the morphological features of 1070 oocytes retrieved from 121 ART patients, of which 311(29.06%) oocytes were abnormal. Of 121 patients, oocytes retrieved from 90 patients (74.28%) had one of the abnormalities mentioned in Table 1. Pie chart 1 shows the percentage of abnormal oocytes retrieved and the pie chart 2 shows the percentage of different types of oocyte abnormalities from retrieved oocytes.

Table -1 Different forms of oocyte abnormalities observed during our ART procedure.

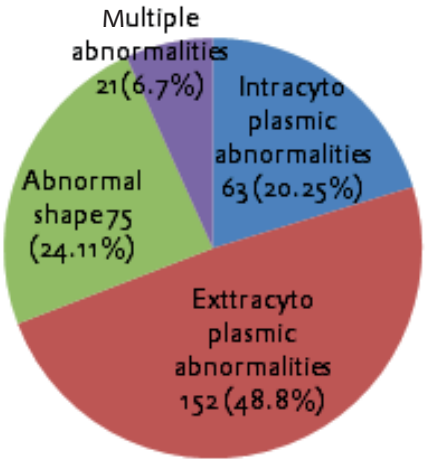
s.no	Forms of Oocyte abnormalities	Total number of oocytes with abnormalities	Total number of patients with oocyte abnormalities
	Intracytoplasmic abnormalities		
1.	Vacuolated	23(7.3%)	17(18.8%)
2.	Abnormal texture	31(9.9%)	11(12.2%)
3.	Degenerated	8(2.5%)	7(7.7%)
4.	Parthenogenetic oocyte	1(0.3%)	1(1.1%)
	Extracytoplasmic abnormalities		
5.	Debris in PVS	39(12.5%)	8(8.8%)
6.	Large PVS	19(6.1)%	12(13.3%)
7.	Fragmented polar body	49(15.7%)	26(28.8%)
8.	Big polar body	1(0.3%)	1(1.1%)
9.	Thick zona	33(10.6%)	5(5.5%)
10.	Thin zona	11(3.5%)	3(3.3%)
	Abnormal shaped oocyte		
11.	Empty zona	57(18.3%)	35(38.8%)
12.	Zona free	5(1.6%)	5(5.5%)
13.	Irregular shape	11(3.5%)	8(8.8%)
14.	Giant oocyte	1(0.3%)	1(1.1%)
15.	Small oocyte	1(0.3%)	1(1.1%)
16.	**Multiple abnormalities**	**21(6.7%)**	**9(10.0%)**
	Total	**311(29.06%)**	**90(74.28%)**

PVS - perivitilline space, **Abnormal texture** - dark and granular, **Parthenogenetic oocyte** - a form of asexual reproduction means development of an embryo from an unfertilized egg cell, **thin zona** - ≤10 µm thickness, **thick zona** - ≥ 22 µm thickness.

Serial no 1-4 from the table shows total of 63 oocytes with Intracytoplasmic abnormalities (20.25%) from 36 patients (40.0%). Serial no 5-10 from the table shows total of 152 oocytes with Extracytoplasmic abnormalities (48.8%) from 55 patients (61.1%). Serial no 11-15 from the table shows total of 75 oocytes with abnormal shape (24.11%) from 50 patients (55.5%). Serial no 16 from the table shows 21 oocytes with multiple abnormalities (6.7%) from 9 patients (10.0%)



Pie chart 1. Percentage of abnormal oocytes retrieved



Pie chart 2. Percentage of different types of oocyte abnormalities from retrieved oocytes

Most common abnormalies observed were empty and broken zona which were found in 57 oocytes (18.3%) and fragmented polar body which were found in 49 oocytes (15.7%). Of the 90 patients who showed oocyte abnormalities, oocytes from 61 patients (67%) showed either of the two abnormalities mentioned above. This means 67% of patient in our ART programme have oocytes with an empty and broken zona or fragmented polar body. All types of abnormal oocytes we observed are shown in the following figures. Figures 1 & 2 show normal oocytes. Figures 3- 8 show oocytes observed with extracytoplasmic abnormalities. oocytes with intracytoplasmic abnormalities are shown in figures 9- 18. Abnormal shaped oocytes are shown in figures 19- 26.

Normal human oocyte

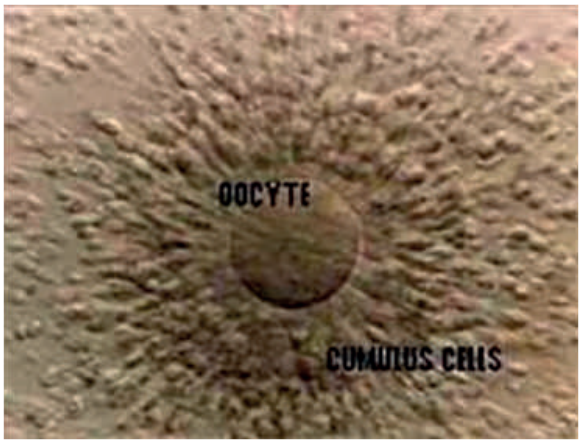


Fig 1. Oocyte with cumulus corona complex

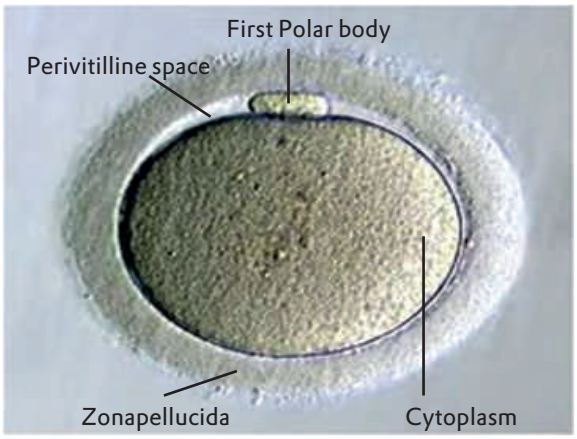


Fig 2. Normal mature Metaphase two human oocyte after cumulus removal

Abnormal human oocytes
Extracytoplasmic abnormalities:

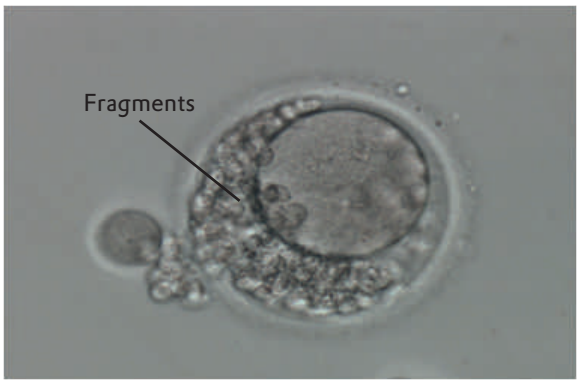


Fig 3. Fragments in PVS.

Fig 4. Thin zona

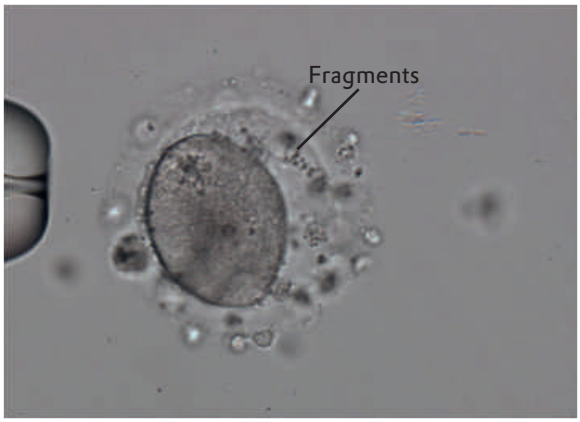


Fig 5. Metaphase one oocyte-Fragments in PVS

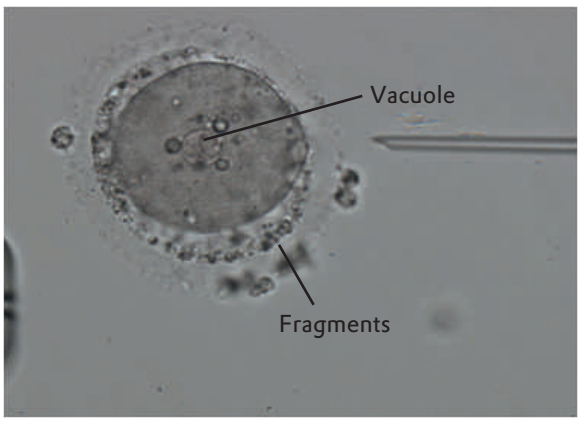


Fig 6. Metaphase one Oocyte- Fragments in PVS with central vacuole

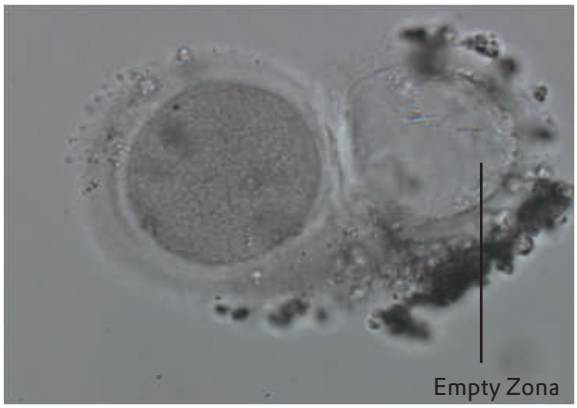


Fig 7. Extra empty zona

Fig 8. Big polar body

Intracytoplasmic abnormalities

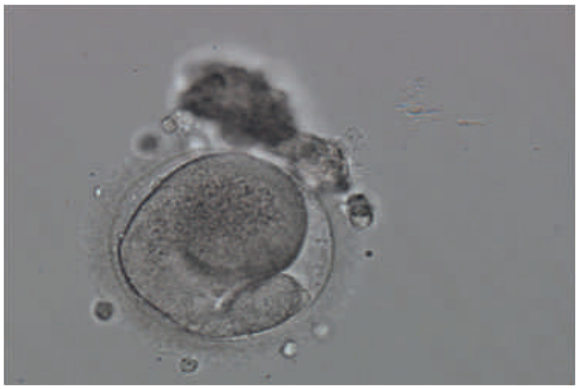


Fig 9. Abnormal shaped cytoplasm

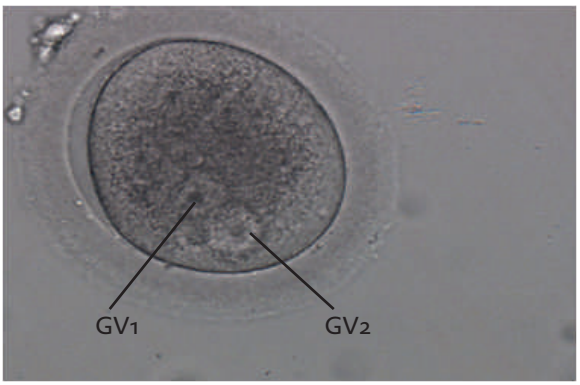


Fig 10. Central granularity with two germinal vesicles

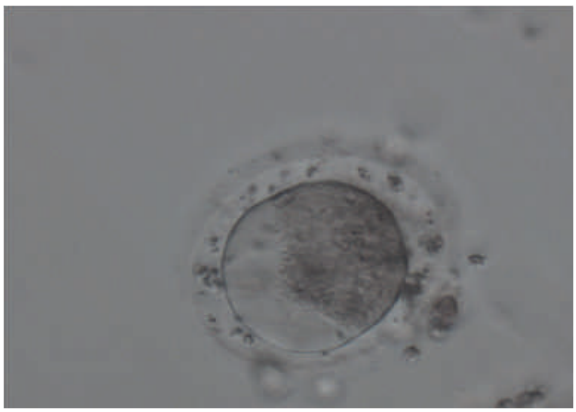


Fig 11. Abnormal textured cytoplasm

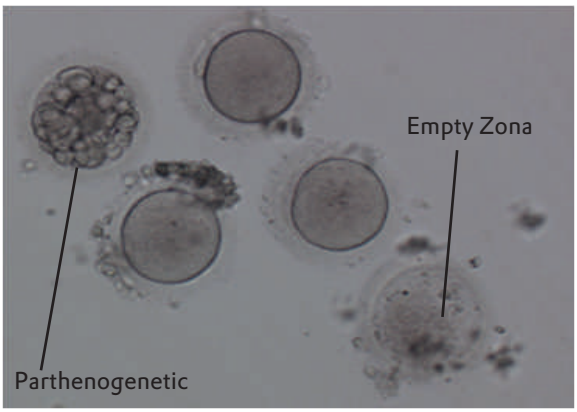


Fig 12. Parthenogenetic oocyte and empty zona

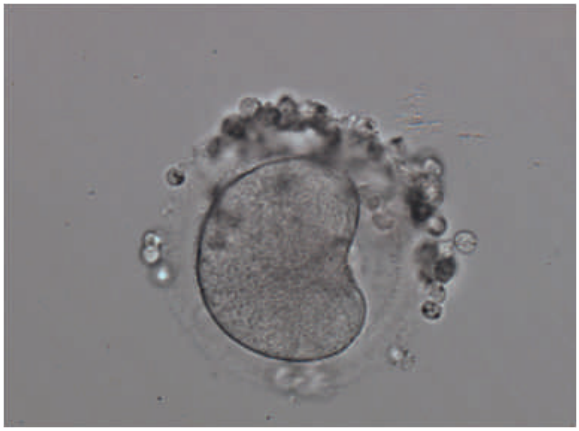


Fig 13. Abnormal shaped oocyte cytoplasm

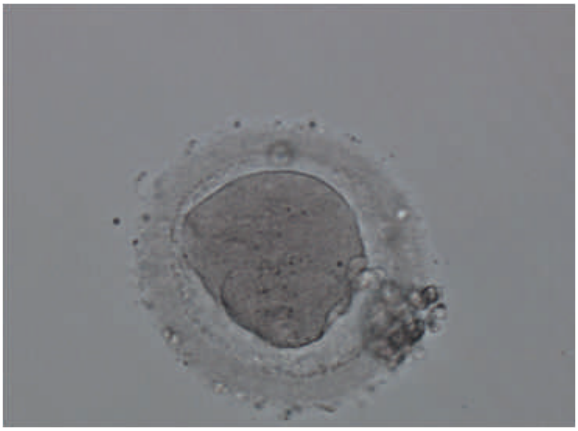


Fig 14. Abnormal shape and textured cytoplasm

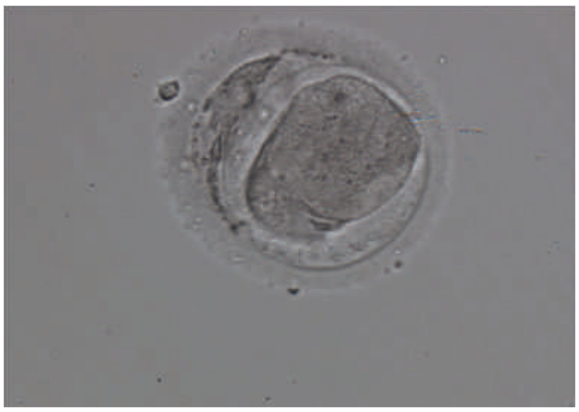


Fig 15. Abnormal shaped cytoplasm

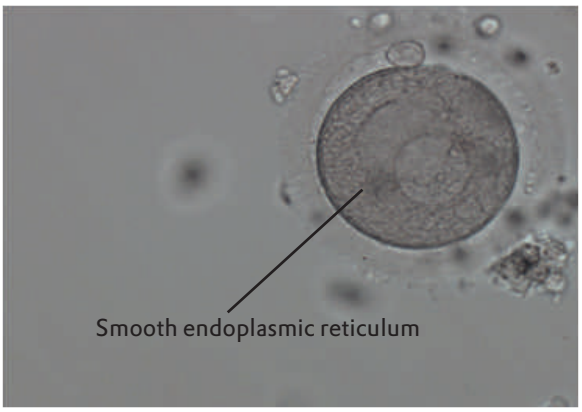


Fig 16. Smooth endoplasmic reticulum aggregation

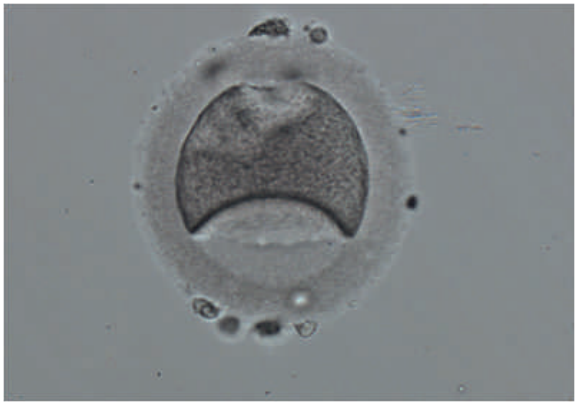


Fig 17. Crescent shaped cytoplasm

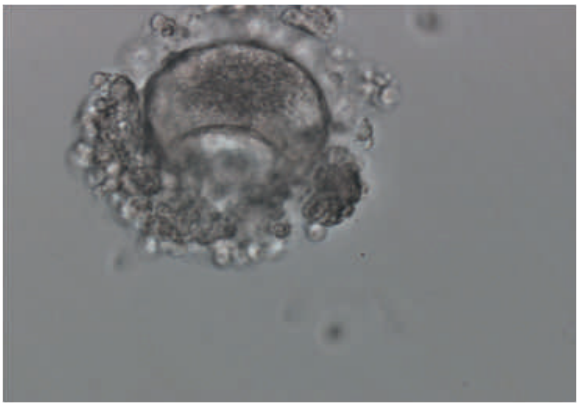


Fig 18. Abnormal cytoplasm with tight corona cells

Abnormal shape

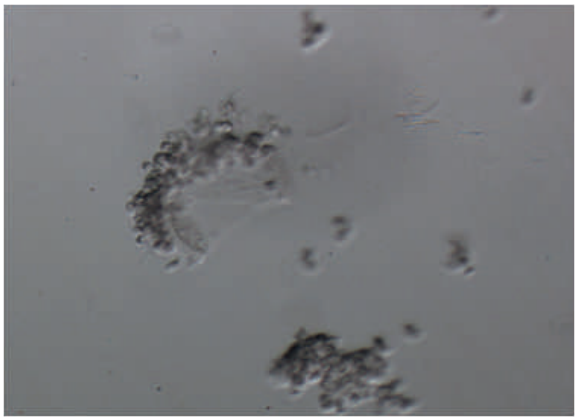


Fig 19. Broken zona

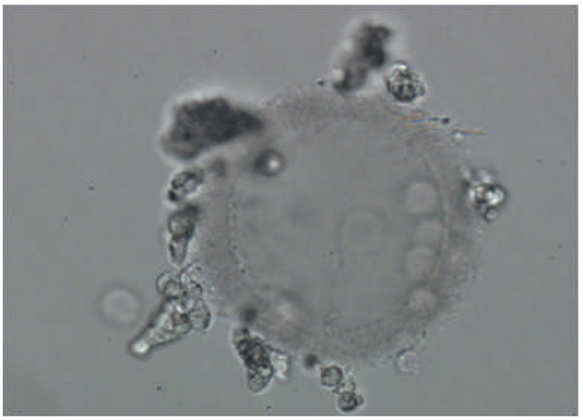


Fig 20. Empty zona

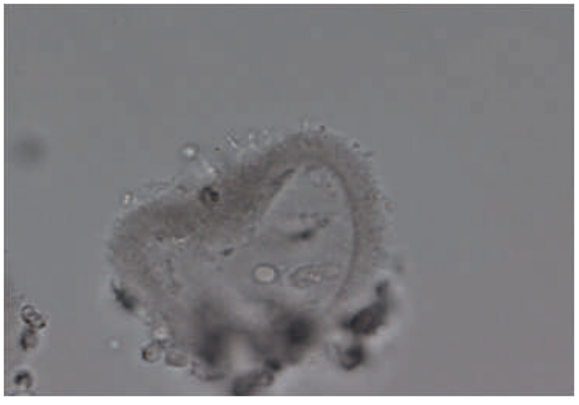


Fig 21. Heart shaped empty zona

Fig 22. Giant empty zona

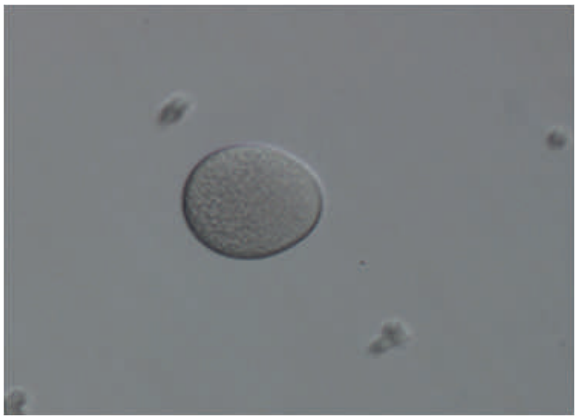


Fig 23. Zona free oocyte cytoplasm

Fig 24. Giant oocyte

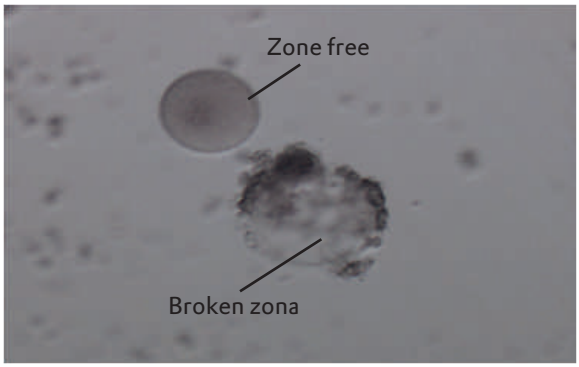


Fig 25. Broken zona with zona free cytoplasm

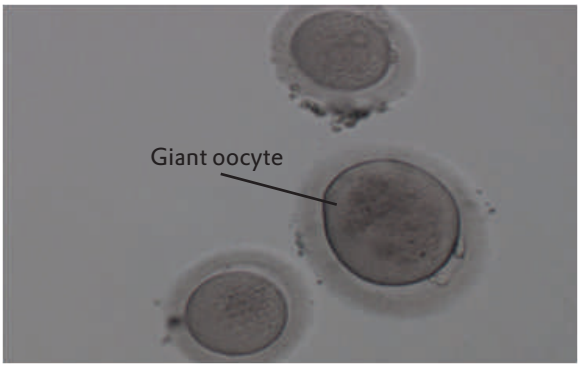


Fig 26. Giant oocyte

Discussion:

An oocyte is considered normal if it is spherical in shape and is enclosed by a uniform zona pellucida, with a uniform translucent cytoplasm free of inclusions and a size-appropriate polar body.

Nuclear Maturation

Polar body (PB): The first polar body is the marker of nuclear maturation. An oocyte with 1st polar body (Metaphase II) is at a stage in meiosis, when it is receptive to fertilisation for a period of 12-24 hours.The first polar body in human remains intact for more than 20 hr afte ovulation, while in mammals it has a shorter life. We can postulate that the first polar body morphology gives information not only on the nuclear maturation of the oocytes but on the age of the oocyte also.

Oocyte abnormalities

Ebner suggests classifying oocyte anomalies as intracytoplasmic and extracytoplasmic (Ebner T. 2001)[1]. He suggested that more than a half of the collected oocytes will show at least one anomaly. In our study, however we found that 29.06% of the oocytes collected were abnormal. The below table-2 shows the different forms of oocyte abnormalities.

Table 2. Different forms of oocyte abnormalities

Intracytoplasmic abnormalities	Extracytoplasmic abnormalities	Abnormal Oocyte shape
1.Variations in colour or granularity of the cytoplasm	1.Wide perivitelline space size	1.Giant oocyte
2.Presence of inclusions, vacuoles or retractable bodies (Ebner et al. 2003,)[2]	2.Perivitelline space granularity	2.Small oocyte
3.Aggregations of the smooth endoplasmic reticulum (Otsuki J. 2004)[3]	3.Fragmented, multiple and big first polar body	3.Zona free
4.Parthenogenetic	4.Anomalies in Zona pellucida layer- thick, thin and abnormal shape	4.Empty zona
		5.Oval or irregular shape

In our study the most commonly found oocyte abnormalities were empty and broken zona. These were found in 57 oocytes which contributes 18.3% of the total 311 abnormal oocytes. These oocytes were found to have no cytoplasm and cannot be used for ART procedure. Loutradis et al., (1999)[4] reported from his study that drastic morphological alterations (broken or empty zona pellucidae) were regarded as unsuitable for ICSI.

The second most common oocyte abnormality was found to be fragmented polar body in 49 oocytes (15.7%) from total of 311 abnormal oocytes. However De Santis et al. (2005)[5] did not find any correlation between surface characteristics, fragmentation and fertilization rate, embryo quality and blastocyst formation and Ten et al. (2007)[6] found fertilization rates and embryo quality were not related to the shape (normal, fragmented or irregular) of first polar body. In contrast to these observations, Ebner et al. (2000)[7] found a strong correlation between all observed morphological features of first polar body (intact versus rough surface, fragmented or enlarged) and fertilization rates/embryo quality.

Embryos developing from giant oocytes were reported to have increased chance for (Digynic Triploidy) (Digynic Triploidy is the result of fertilization of a diploid ovum by a single sperm, with the diploid ovum being the result of either an error in the first (MI) or second (MII) meiotic division). This is in spite of the normal in vitro development as reported by Rosenbusch et al and Balakieretal (2002)[8,9].

According to a review by Laura Rienzi et al[10], out of the 92 studies of different parameters (including both single features and cumulative scores) investigating direct association of oocyte morphology with the further embryo prognosis, 57 studies resulted in a significant correlation with good embryo outcome, whereas in 35 studies no predictive value of the microscopic feature was found and the diversity of observations and results did not allow statistical comparison. However, there was no clear tendency of improved accuracy regarding the predictive value in recent publications compared with the earlier ones. 24 of 42 study observations performed between 1997 and 2005 have found correlations with the embryo outcome, while 33 out of 50 studies between 2006 and 2009 found correlations.

However we suggest degenerated, parthenogenetic, giant oocytes, empty or broken zona and some forms of oocytes with multiple oocytes (abnormal shaped with granular cytoplasm) should be considered unsuitable for ICSI.

We found 90 (74.28%) of our patients out of 121 patients had one or more oocyte abnormalities.

This can be due the following reasons

1. Underlying infertility
2. Effect of ovarian hyper stimulation
3. Advanced maternal age

Conclusion:

From the above discussion it is clear that there have been numerous studies, which have attempted to correlate oocyte morphology with embryo developmental competency. There is no consensus, with some studies supporting some relationship with oocyte abnormalities and compromised embryo development and, in contrast, some studies which do support an association with implantation potential and oocyte abnormalities. To date there are too many confounding factors in the various papers, which have been reviewed in part by Rienz et al., (2011)[10].

The morphological evaluation of oocytes and its impact on embryo quality has been controversial. However, abnormal oocyte morphology can be directly influenced by the follicular environment, ovarian function and the effects of ovarian hyper stimulation used in assisted reproductive technology. Furthermore, 70.94 % of morphologically normal oocytes can give rise to a small percentage of pregnancies, while morphological abnormalities appear to be associated with compromised oocyte quality, their precise identification and impact on embryo development is currently lacking, suggesting that most of the problems leading to poor embryonic development and implantation failure cannot be detected using standard microscopic evaluation. Further work is therefore required to assess both the morphological characteristics as related to oocyte developmental competence and the cellular and molecular findings of the oocyte for understanding the pathophysiology, will help design strategies to improve fertilization and embryo development.

Acknowledgements

We gratefully acknowledge the help and guidance from Dr.N.Pandiyan; comments and helpful discussions from Dr. Thilaka Muthiah and Dr.G.Kavitha for her support.

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GIRLS SHOULD REMAIN SLIM & TRIM EVEN AT BIRTH

In a cohort study carried out on 1053 seventeen - year olds, Rae-Chi Huang and his associates of The University of Western Australia found that those women, who were heavier at birth, were at a greater risk of developing diabetes and related metabolic risks. At seventeen years, these women were found to have greater waist circumference, higher levels of triglycerides and insulin, and lower HDL-cholesterol (good cholesterol). However, similar association between birth weight and the metabolic risk was not observed in males. These findings are particularly significant taking into consideration increasing incidence of maternal obesity and gestational diabetes. The latter means that there will be rise in heavier female newborns. The results of this study have been accepted for publication in The Endocrine Society's Journal of Clinical Endocrinology and Metabolism (JCEM). In related medical news, a controversial study has been undertaken in Britain to enable pregnant women to deliver slim babies

(http://www.medindia.net/healthnews/Women-Child-Health-News.asp)

- Dr. K. Ramesh Rao

House boat - Kerala