CooperSurgical Fertility Companies

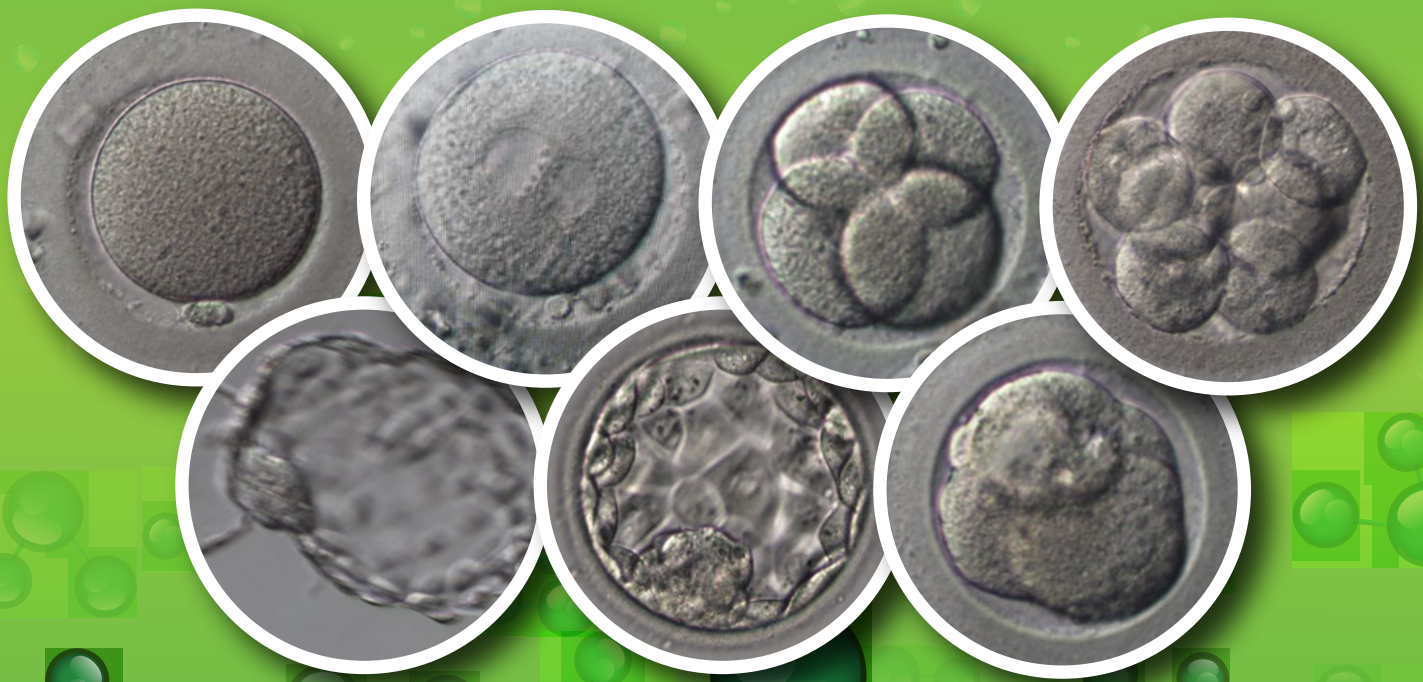
CooperGenomics a CooperSurgical company

Volume 11 (June, 2019)

NEXUS

Indian Fertility Society & ORIGIO India Initiative

EMBRYO GRADING



Series Editor
Dr. (Col) Pankaj Talwar

Dr M Gouri Devi
President - IFS

Embryo grading is a technique that allows physicians and embryologists to observe the rate of development of embryos created through In-vitro fertilization in a lab setting. This information along with the patient's health, age, fertility and medical history, helps to determine the optimal day of transfer, the appropriate number of embryos to transfer, and exactly which embryos to transfer.

The grading is based on subjective assessment of the embryo's potential to develop. These grading systems help us to determine which embryos to transfer and/or freeze. Generally in many fertility centers, embryo transfers occur either 3 days or 5 days after egg retrieval. Because embryos are developmentally different on these days, we have different grading systems for Day 3 (Cleavage stage) embryos and Day 5 (Blastocyst stage) embryos. Each will be discussed in detail, in this issue of Nexus E-bulletin. Hope all of you will be enlightened and use this academic bonanza to enhance your laboratory results.

Prof (Dr) Pankaj Talwar
Secretary General - IFS
Series Editor - NEXUS

At the very onset, the editorial team would like to thank all of you for positively appreciating our previous Nexus E-bulletins. Your encouragement motivates us to present more advancements in the field of Assisted Reproduction Techniques.

Our present edition is focused on simplifying the process of Oocyte and Embryo Grading and covers all essential details. The aim of this E-bulletin is to educate the ART professionals about the universally accepted light microscopic parameters used for grading of oocytes, zygotes and embryos.

Embryo grading is done to ensure that patient have a better success rate of conceiving through In-vitro fertilization and for this it is important that only the best quality embryo is selected for transfer. The remaining good quality embryos are frozen and stored. They are used if the first embryo transfer is not successful. The process of grading begins after oocyte (egg) recovery.

It is a topic that has had its fair share of debate over the years. Each IVF lab may have a slightly different way of grading an embryo. For the most part, however, they are all observing the same features, which are: appearance of the embryo, cell number, fragmentation, inner cell mass, trophectoderm and the degree of expansion.

I am thankful to Ms Sapna for her inputs and proofreading the document.

M: +91 9810790063
E: pankaj_1310@yahoo.co.in

Dr Sarabpreet Singh
Guest Editor

- **MD (AIIMS)**
- **Senior Consultant & Chief Embryologist at the Artemis Health Institute**
- **IFS and ESHRE-certified Senior Clinical Embryologist**
- **Convenor, IFS SIG (Clinical Embryology)**
- **Executive member of Academy of Clinical Embryologists, India**
- **Area of Interest: Cryobiology and Male Infertility**

The most important factor determining the success of an ART program is embryo selection. The process of embryo selection begins right from oocyte collection and goes upto embryo transfer. In this review, we provide the guidelines for selecting as well as deselecting an oocyte prior to fertilization and an embryo post fertilization.

It is imperative for every embryologist to know the basic science of the development of embryonic milestones. Appropriate embryo selection predicts the implantation potential of an embryo, thereby, enhancing the success rate and decreasing multiple pregnancy rate.

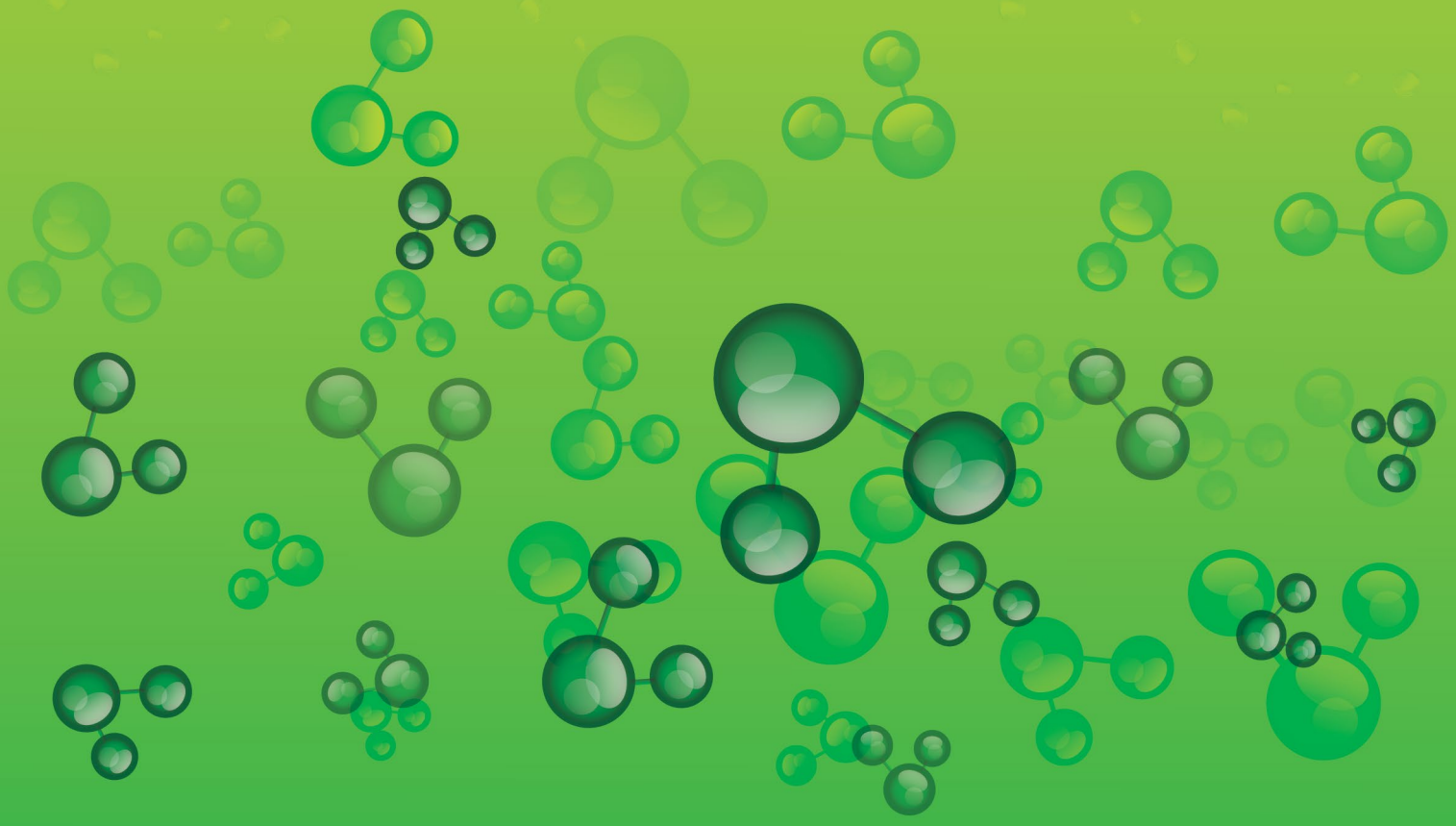
It has taken months of hardwork to be able to present this article on 'Oocyte and Embryo Grading'. Besides the relevant text, we have made efforts to provide clear and well labelled photographs. It is comprehensive and insightful. An article of this standard could never have been possible without the untiring efforts of Dr Aneesha Grover and Dr Mona Sharma.

I would like to thank the Artemis management who allowed me to use the pictures from IVF lab. My sincere thanks to Mr Varun for his efforts to ensure a final product of this quality.

INDEX

Part : 1

Oocyte & Embryo Grading



Sub-Index - Part :1

1 Introduction

Embryo quality is currently the most important predictor of pregnancy. [1,2] Many invasive and non-invasive techniques to select human embryos have been developed but embryo selection based on embryo morphology still remains the most widely used method worldwide. Correct embryo selection helps achieve high implantation rates and avoids multiple gestation and associated complications.

In many countries, there are laws defining the number of embryos that can be transferred in a single IVF cycle, making the technique of precise embryo selection extremely important. Embryo selection should be based on a cumulative scoring over the course of embryonic development and not on a single observation or a *'snap shot analysis'*. It is of utmost importance for any ART program to follow a standardized timeline for observing the developmental milestones of embryos and grading them.

The embryologist should take a note of the morphological features, which might positively or negatively impact embryonic development and implantation. Based on these observations, the embryos are either transferred, frozen or discarded.

We shall review the universally accepted light microscopic parameters used for grading oocytes, zygotes and embryos.

2 Oocytes

An ideal mature human oocyte, based on morphological characteristics, should have a homogenous cytoplasm, a single polar body, an appropriate zona pellucida (ZP) thickness and proper perivitelline space **(Fig. 1)**.[3]

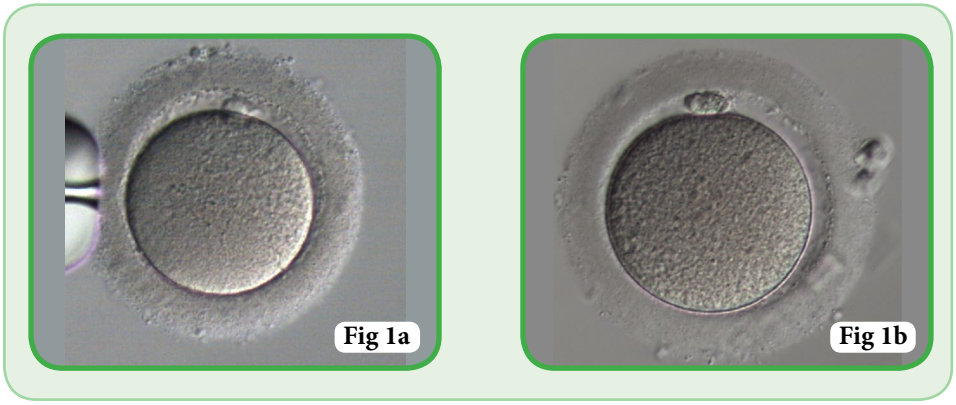


Fig 1a & b. Ideal oocytes

During ART cycles, the ovarian hormonal stimulation allows for the maturation of many oocytes which were destined to be atretic and hence, compromises oocyte quality. The developmental competence of the oocyte depends upon the nuclear and cytoplasmic maturation of the oocyte and the synchronization between the two. Post-insemination, subsequent embryonic development depends a lot on the oocyte's competence and the inherent defects present in the oocyte. The dysmorphic oocytes, which fail to fertilize by IVF might fertilize by ICSI and develop normally **(Fig. 2)**.

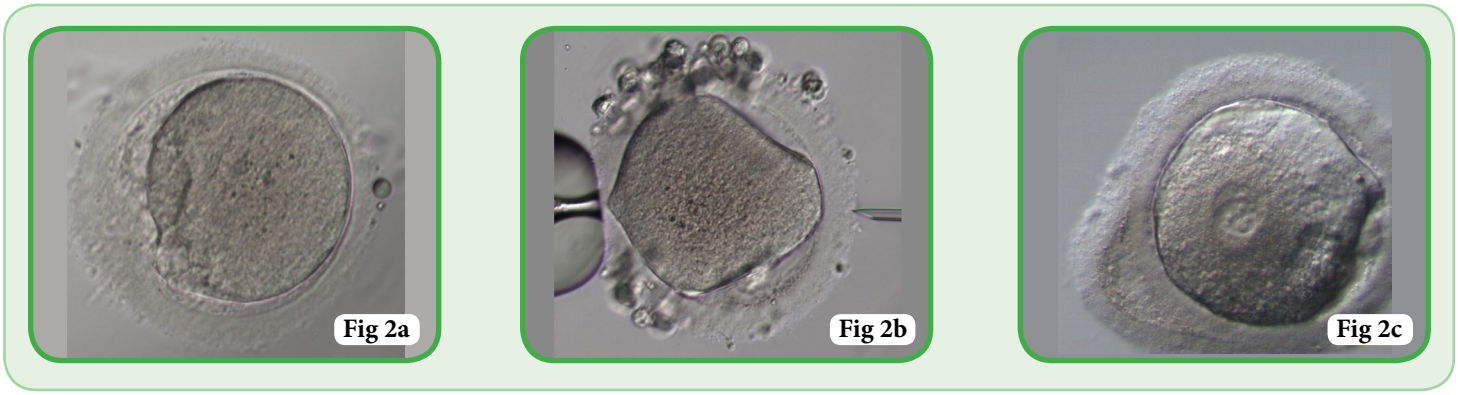


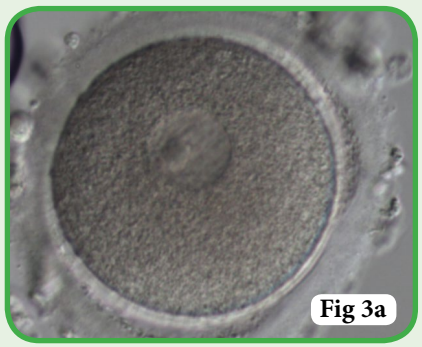
Fig 2a & b. Dysmorphic oocytes

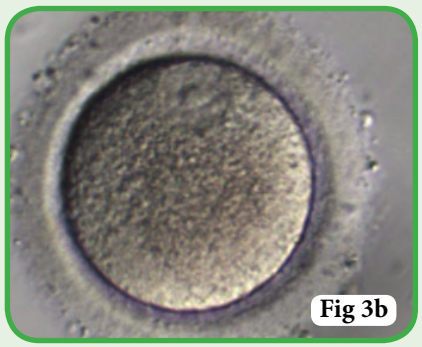
Fig 2c. Fertilized dysmorphic oocyte

Usually, these embryos arrest either before the blastocyst stage or during the first few weeks after embryo transfer. Therefore, knowledge of the morphological abnormalities of oocytes, which are known to compromise developmental competence, is essential. This also helps in deselecting the oocytes for ICSI.

Based on the nuclear maturation, the oocytes can be classified as:

1. **Germinal Vesicle (GV)**: In the meiotically arrested oocytes, the chromatin is encapsulated by a nuclear structure. **(Fig. 3)**
2. **Metaphase-I oocyte (M-I)**: Neither a visible GV nor first polar body is visible **(Fig. 4)**
3. **Metaphase-II oocyte (M-II)**: First polar body is visible in the PVS **(Fig. 5)**





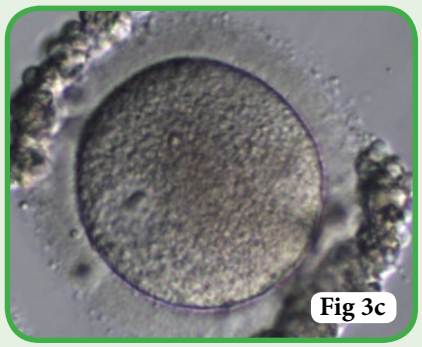
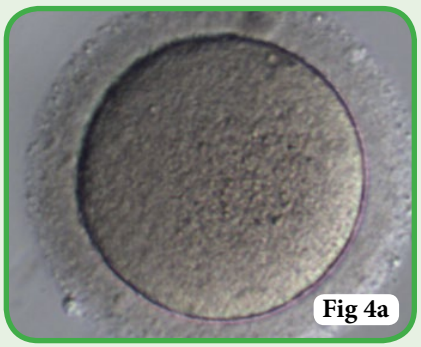
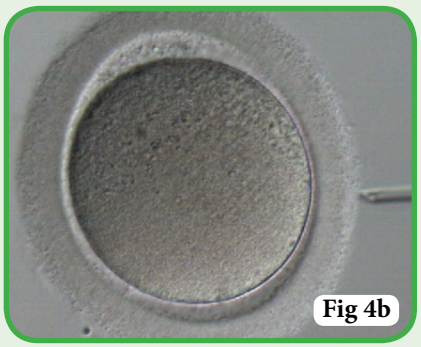


Fig 3a & b. Germinal Vesicle with a prominent single nucleolus.
Fig 3c. Germinal Vesicle with a fading nuclear membrane (GVBD)





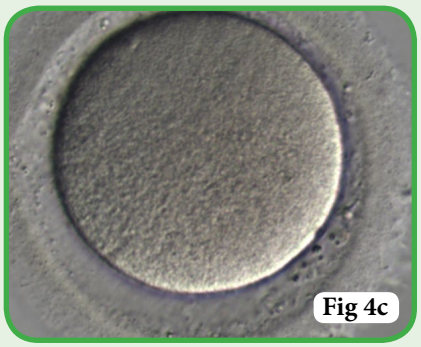
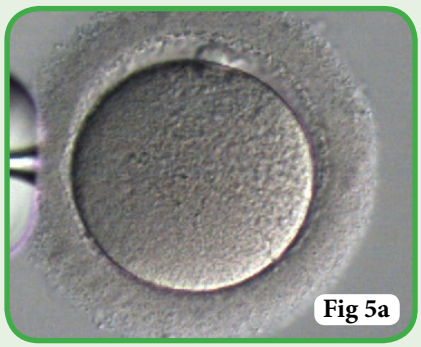


Fig 4. Denuded MI oocytes – neither nucleus nor polar body is appreciated



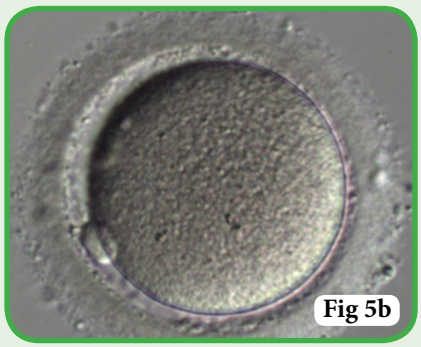


Fig 5. Denuded MII oocytes. First polar body is seen in the perivitelline space (PVS)

A. Morphological Assessment

I. Size and Shape

a. Giant oocyte: These oocytes are almost twice the size of a normal oocyte (about 200 microns) **(Fig. 6a)**. It results from cytokinetic failure where nuclear division takes place in the absence of cytoplasmic division.[4] That is the reason why they have a binucleate appearance **(Fig. 6b)**. It leads to digynic triploidy, if inseminated. Therefore, these oocytes should never be injected if ICSI is to be done or transferred if IVF is carried out.

b. Dysmorphic shape: Alterations in shape and size which are visible only after denudation (ICSI) and go unnoticed in conventional IVF **(Fig. 2)**.

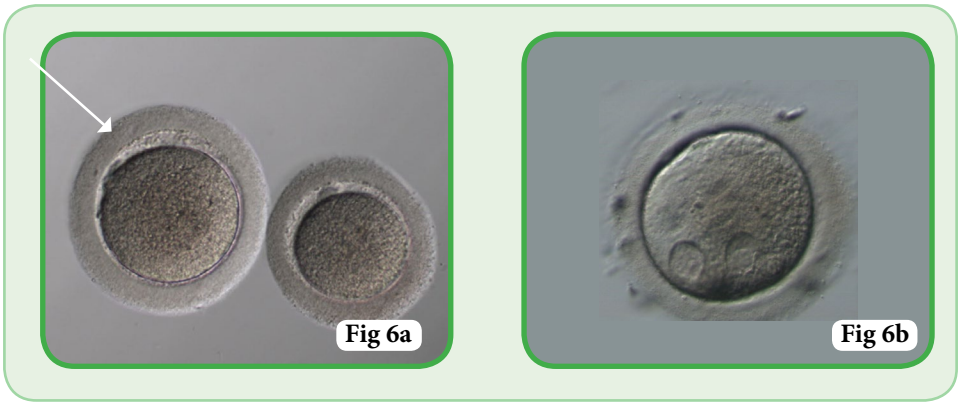


Fig 6b. Giant oocyte (arrow) in relation to normal sized oocyte

Fig 6a. Giant oocyte (note the binucleate appearance)

c. Oval oocytes: Oval oocytes deserve special mention as the shape influences future embryonic development (explained later) **(Fig. 7)**.

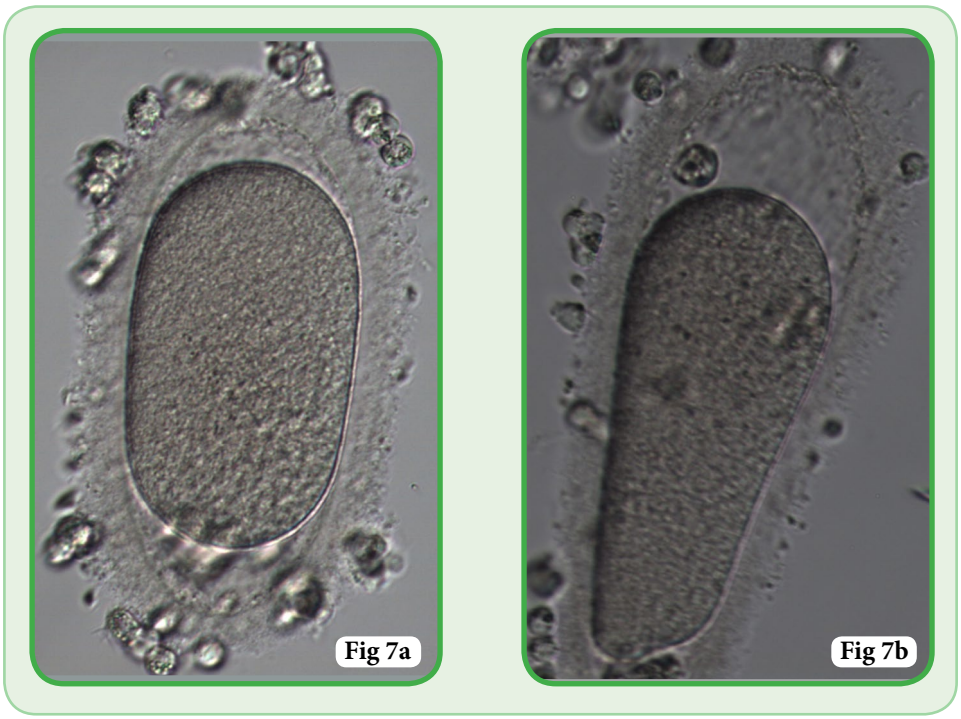


Fig 7a & b. Oval oocytes or oocytes with ovoid zonae

d. Conjoined oocytes: Two oocytes are surrounded by a common zona **(Fig. 8).** The oocytes might show different nuclear maturity. Both the oocytes have to be injected separately. No pregnancies have been reported from such oocytes. [5]

Fig 8a & b. Conjoined oocytes

e. Atretic oocytes: Are also encountered occasionally **(Fig. 9).**

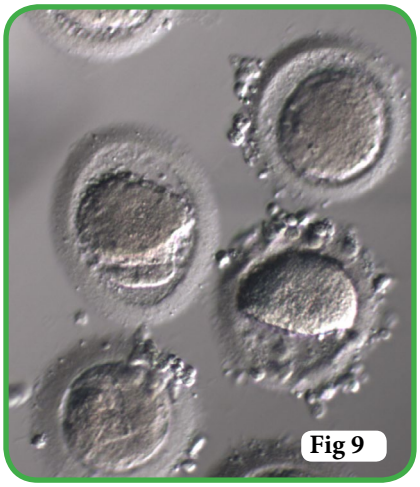


Fig 9. Atretic oocytes

II. Intracytoplasmic Anomalies

a. Granulations: It is the most common cytoplasmic variation. Its severity is judged by the depth and diameter of the granular area. It can range from mild to severe granulation **(Fig. 10)**.

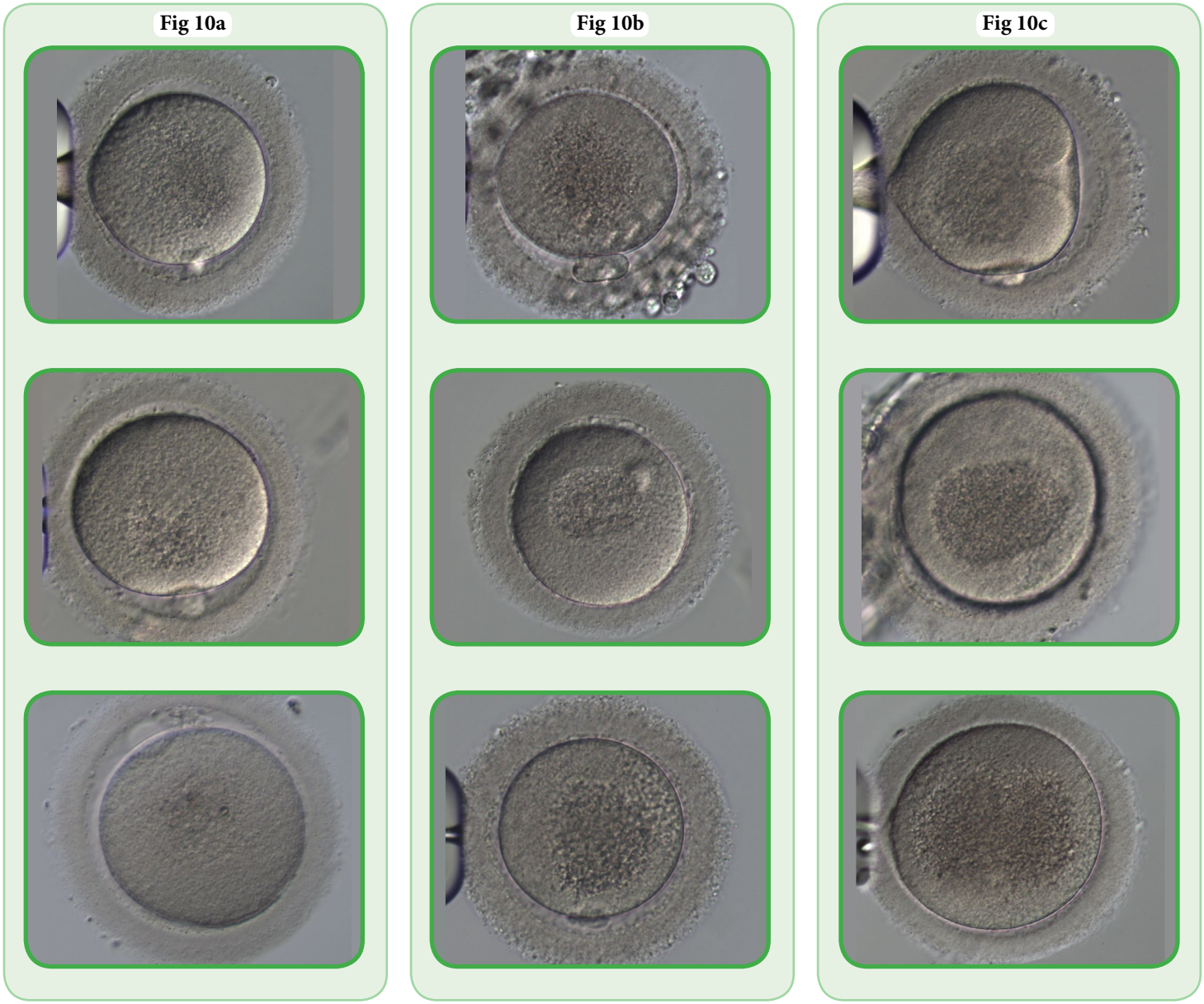


Fig 10. Oocytes showing granulation of varying degree.
Fig 10a. Mild Granulation.
Fig 10b. Moderate granulation.
Fig 10c. Severe granulation

The consensus is that granulations are the result of organelle clustering and associated with lower implantation potential. It can be either:

1. **Diffuse cytoplasmic granularity:** 50% of the oocytes are aneuploid and associated with decreased cryosurvival **(Fig. 11)** [6]
2. **Centrally located cytoplasmic granular area**: Presence of blood clots in COC are associated with dense central granulation of oocytes, negative effect on fertilization and blastocyst rates. [7] **(Fig. 12)**
3. **Diffuse peripheral granulation:** Associated with compromised pronuclear morphology. [8] **(Fig. 13)**

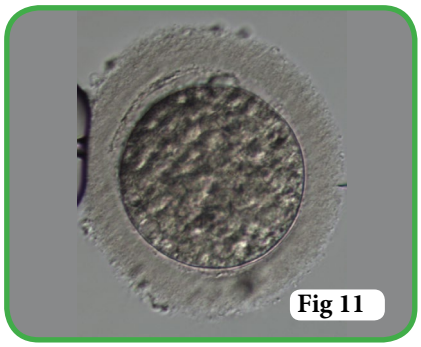
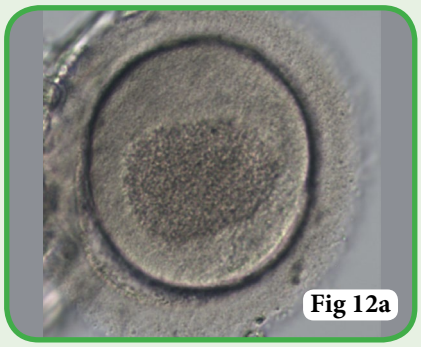
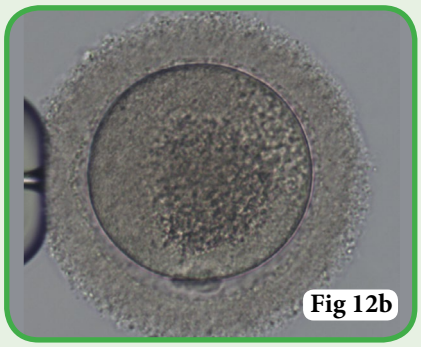


Fig 11. Oocyte with diffuse cytoplasmic granularity which is typical of organelle clustering





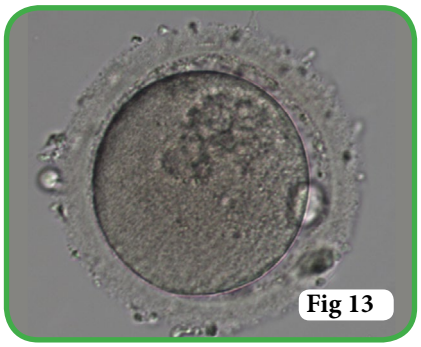
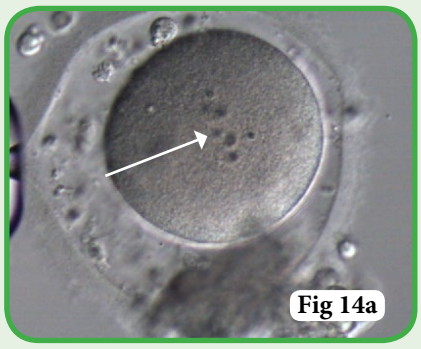


Fig 13. Oocytes with peripheral granulation

Fig 12a & b. Oocytes with centrally located cytoplasmic granular area

b. Inclusions & Refractile bodies: Inclusions might get stuck in the injection pipette while aspirating ooplasm during ICSI **(Fig. 14 a & b)**. So, aspiration during ICSI should be done away from inclusions. Refractile bodies can be occassionally seen **(Fig. 15)**. These are morphological variations with no prognostic significance.



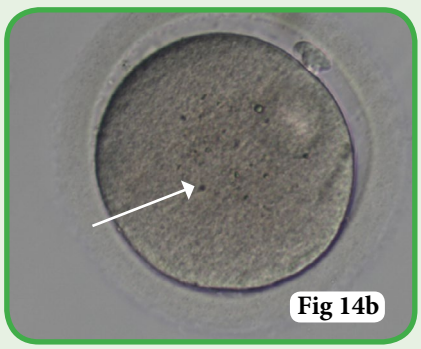
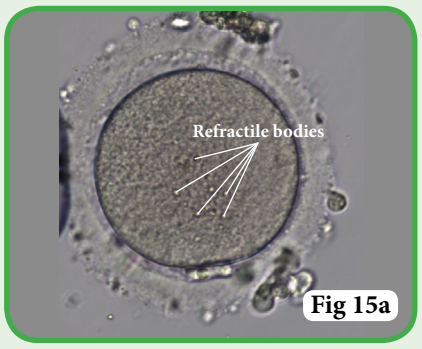


Fig 14. Cytoplasmic inclusions



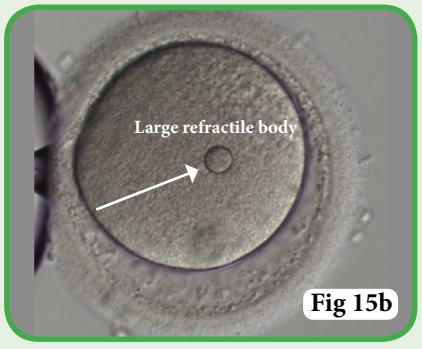


Fig 15a & b. Refractile bodies

c. Vacuoles: are fluid filled structures, which are easily appreciated (Fig. 16-18). A vacuole of >14 µ diameter **(Fig. 17)** results in significantly decreased:

a. Fertilzation rates by displacing the MII spindle from its polar position.
b. Cryosurvival.[9]

If such oocytes are fertilized and the vacuoles persist beyond syngamy, they interfere with cleavage planes resulting in low blastulation rates.[10]

Three types of vacuoles can be identified:

1. Present at oocyte collection, which develop during maturation (day 0);
2. Artificially created by ICSI (day 1); and
3. Vacuoles accompanied with developmental arrest (day 4).

The time of vacuole development is important: if they arise at a later stage they affect the rate of blastocyst formation adversely.

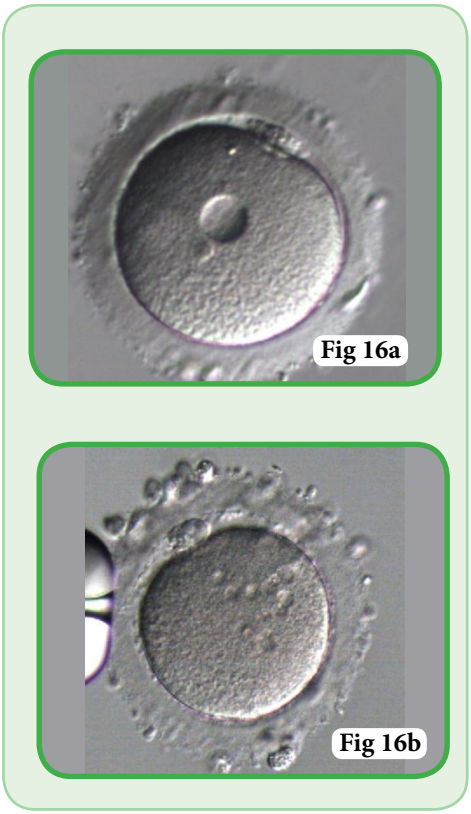


Fig. 16a. Denuded oocyte with a vacuole**. Fig. 16b**.Denuded oocyte with multiple small vacuoles

Fig. 17 a & b. Oocytes with a single large vacuole > 14µ

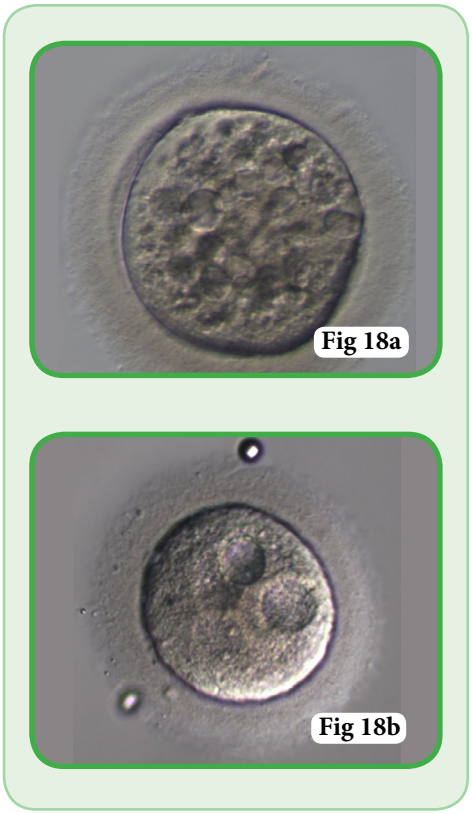


Fig: 18a & b. Oocyte with multiple small and big vacuoles associated with poor prognosis

d. Smooth Endoplasmic Reticulum (SER) Clusters/Discs: it appears like a translucent disc like structure, which has to be differentiated from a vacuole **(Fig. 19)**. It is strongly recommended not to inseminate oocytes with SER discs and to re-examine all sibling oocytes before inseminating. [10] SER clusters are associated with poor obstetric outcome.[11] Studies have shown association with imprinting disorders such as Beckwith-Wiedemann Syndrome.

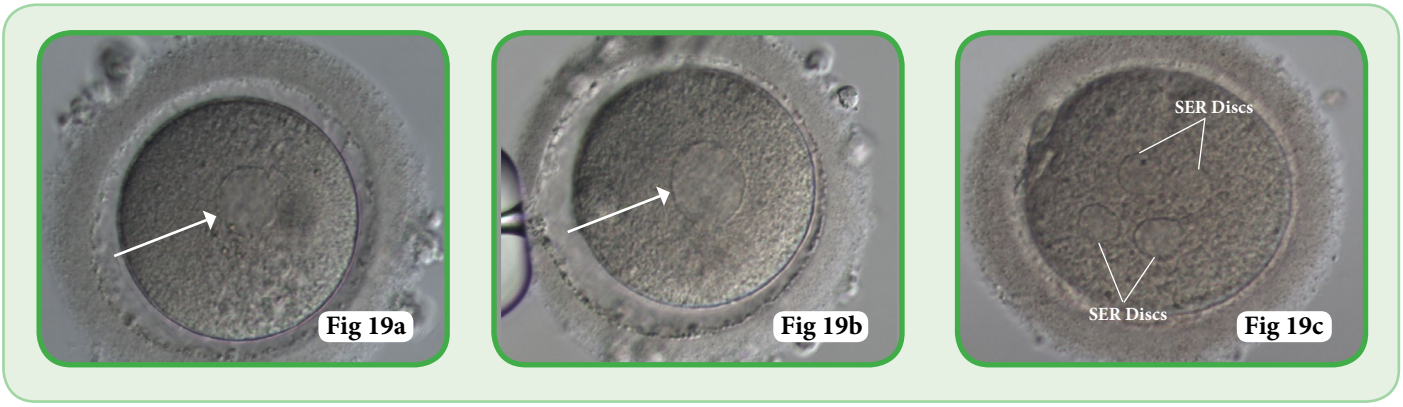


Fig: 19a & b. SER disc or clusters. **Fig: 19c.** Oocyte with multiple SER discs

e. **Dark Cytoplasm:** Oocytes with dark cytoplasm **(Fig. 20)** have a 83% lower chances of forming good quality embryo and the resulting embryos have decreased implantation rate (IR).[13] However, Esfandiari et al. in 2006 showed no difference in the lab & clinical outcome.[14]

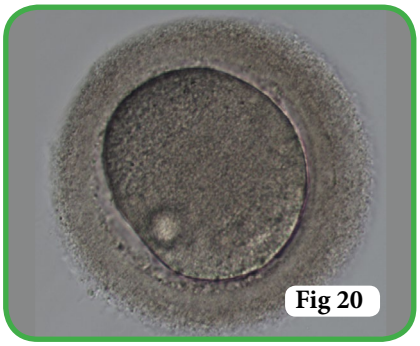


Fig 20. Oocyte with thick zona and dark cytoplasm

III. Extracytoplasmic Anomalies:

These are considered phenotypic deviations.

a. First polar body morphology: (Fig. 21)

i. Smooth PB
ii. Disc like PB
iii. Duplicate PB
iv. Fragmented PB
v. Giant PB

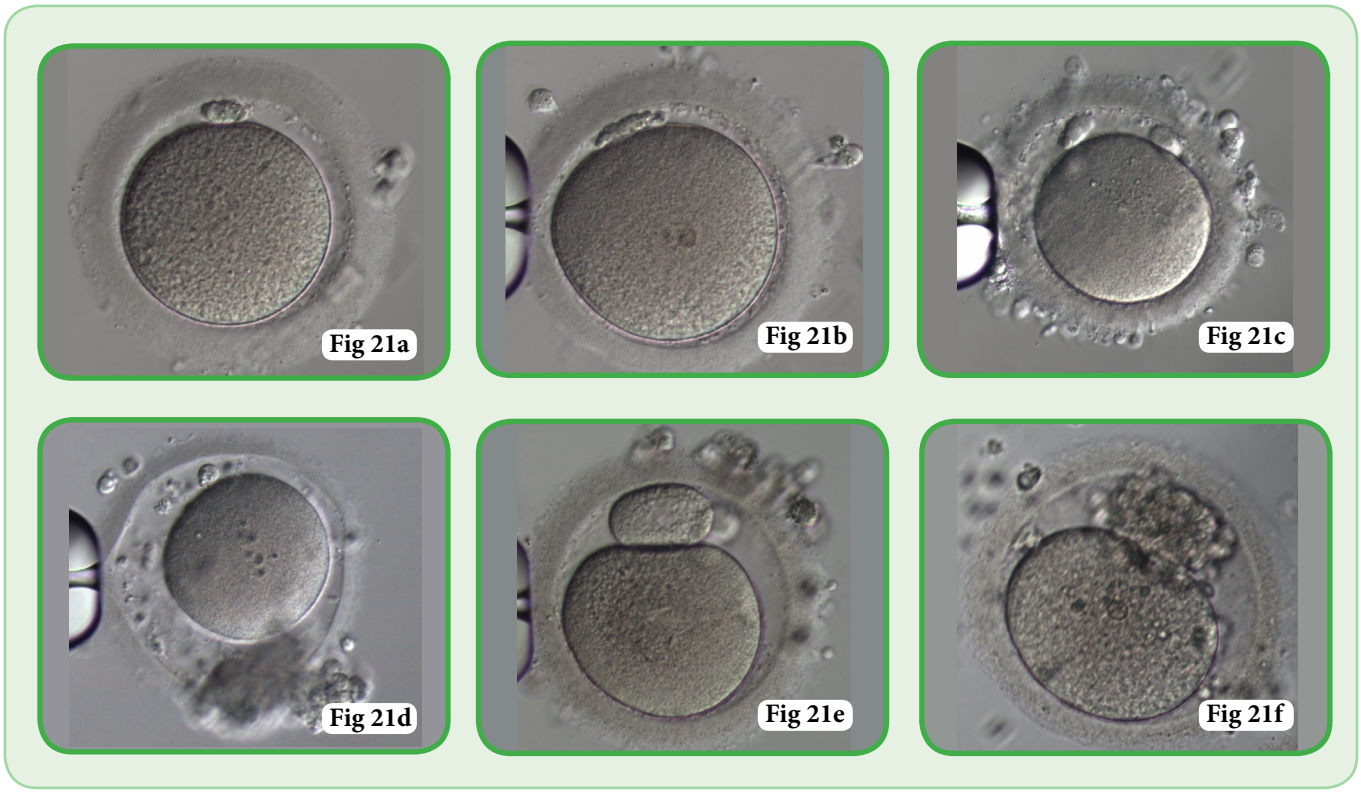


Fig. 21a to f. Variations in the morphology of first polar body or PB1 **Fig 21a.** Smooth PB **Fig 21b.** Disc like PB **Fig 21c.** Duplicate PB **Fig 21d.** Fragmented PB **Fig 21e.** Giant PB **Fig 21f.** Fragmented Giant PB

Oocytes with a large polar body **(Fig. 21e & f)** are associated with a poor prognosis. [15] Insemination of oocytes with giant polar body should be avoided and if inseminated, it should be cultured separately and documented.

b. Perivitelline space (PVS)

i. **Large PVS:** Seen in one third of all ova.[16] It might result due to overmature eggs or a large PB. **(Fig. 22a)**
ii. **Granulated PVS:** Can be physiological or enhanced by exposure to high doses of hMG. [17] Granularity in the PVS may also be due to the over-maturity of oocytes[18] as well as extrusion of a large polar body. **(Fig. 22b)**

The morphological variations in the size of the PVS as well as the presence of fragments or granules in the PVS don't affect either the fertilization & cleavage rates or the embryo quality. However, a note should be made of an exceptionally large PVS.

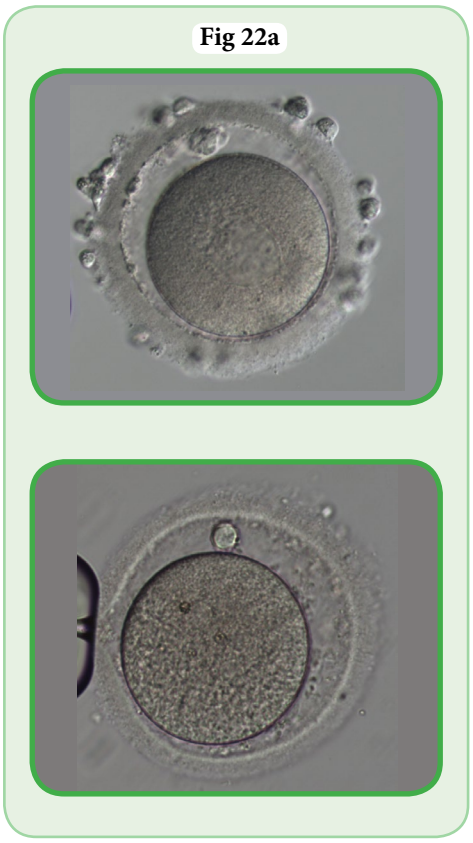


Fig 22 a. Enlarged PVS seen in oocytes

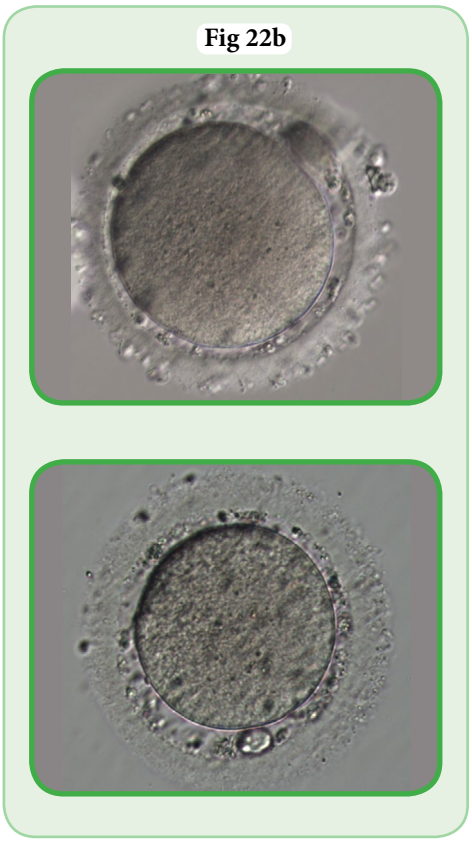


Fig 22 b. Denuded oocytes with perivitelline dust or fragments

c. Zona Pellucida (ZP):

i. Variations in thickness

- Thin zona pellucida **(Fig. 23)**
- Thick zona pellucida **(Fig. 24)**

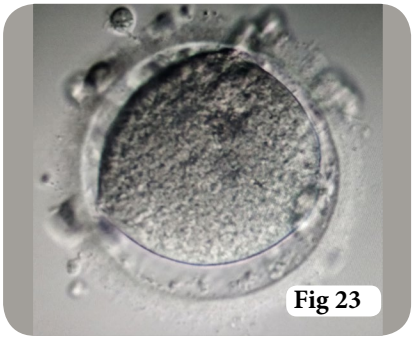


Fig 23. Denuded oocyte with thin zona

Fig 24. Denuded oocyte with thick zona

ii. Irregular zona pellucida (Fig. 25)

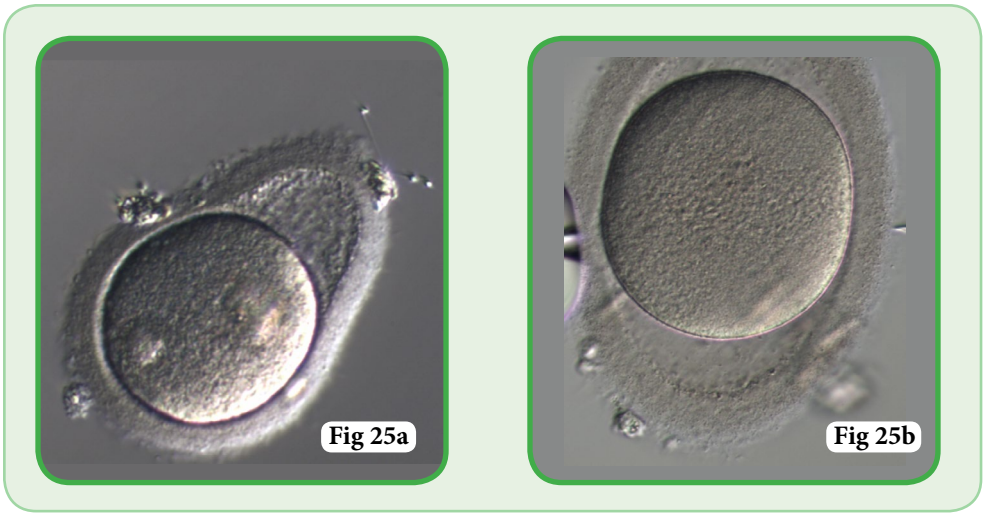


Fig 25a & b. Irregular Zona

iii. Duplication/tears of inner layer: Intrazonal space is created due to duplication of inner layer of zona pellucida **(Fig. 26)**

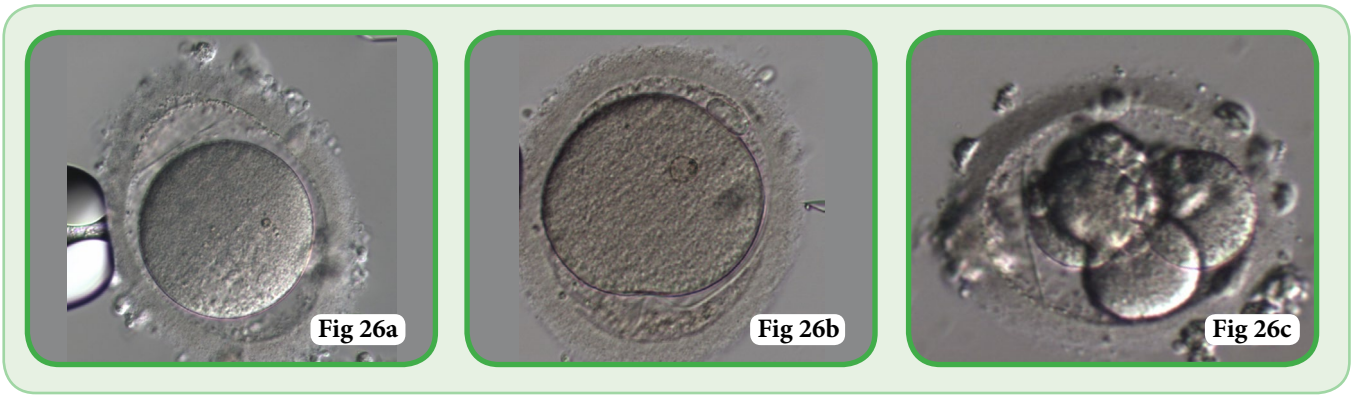


Fig 26a, b & c. Duplication of zona / septate zona

iv. Dark zona pellucida: Unusual thickness or color **(Fig. 27)** should be documented.[10]

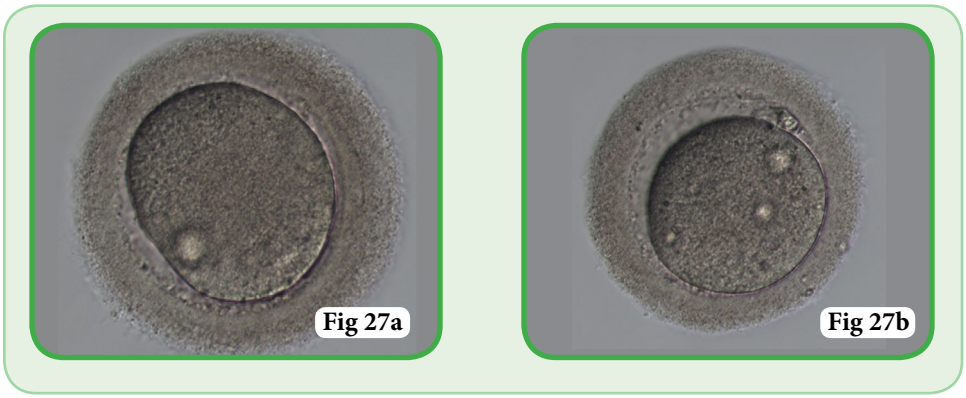


Fig: 27a & b. Dark zona pellucida. The thickness of zona is not uniform

B. Oocyte ageing:

This may be due to in vivo or in vitro acquired cellular, biochemical and morphological changes of oocytes leading to reduced fertilization rates, polyspermy, digyny, parthenogenesis, chromosomal disorders & poor developmental potential.

The noticeable features of oocyte ageing are dark ooplasm and zona pellucida & large PVS with granularity **(Fig. 28).**

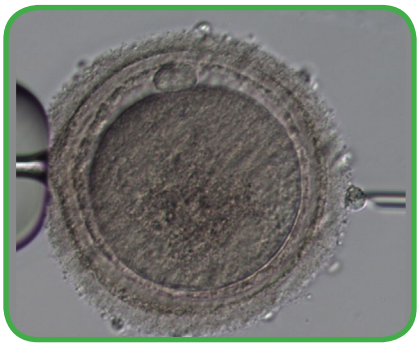


Fig: 28. Aged oocytes. Note the dark zona and ooplasm and the fragments present in the wide PVS

It has been observed that most of the oocytes exhibit multiple cytoplasmic and extracytoplasmic anomalies. **(Fig. 29 to 33)**

Fig 29. Oval oocyte with elongated zona & wide PVS

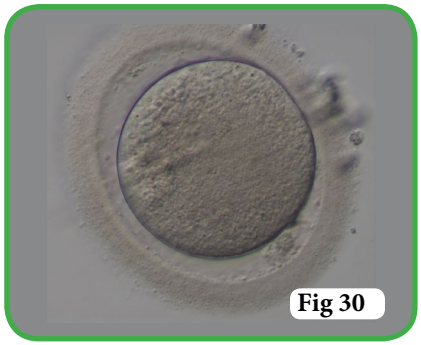


Fig 30. Elongated zona with wide PVS & fragmented PB1

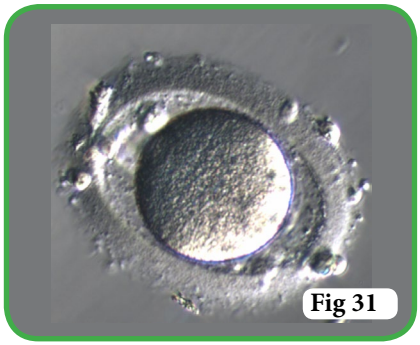


Fig 31. Elongated zona with splitting of inner layer & fragments in the wide PVS

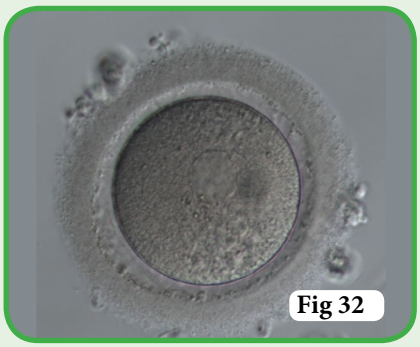


Fig 32. SER disc and fragments in the wide PVS

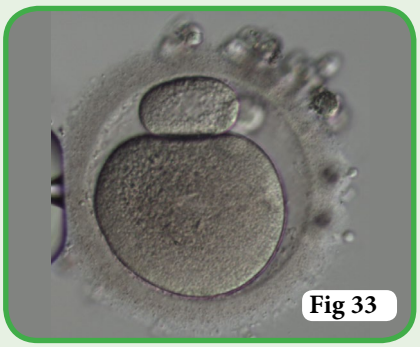
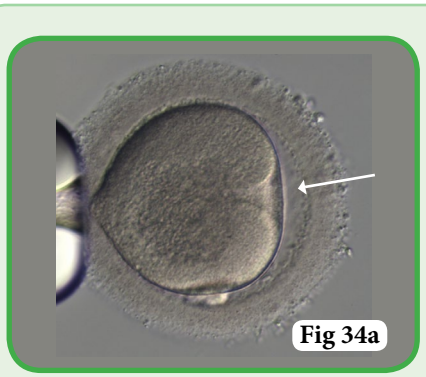


Fig 33. Oocyte with moderate granulation and giant fragmented PB1

C. Viscosity of the ooplasm & resistance of cell membrane during ICSI:

These are not morphological parameters but are properties appreciated on injection **(Fig. 34)**. The resistance of the oolemma and viscosity of the ooplasm have a direct impact on FR, embryo quality & blastulation rates.[19]



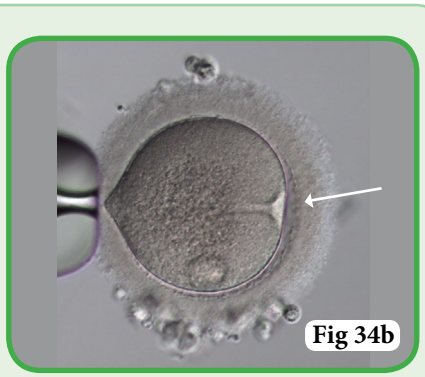


Fig 34a & b. Resistance of the oolemma at time of injection (note the funneling effect of oolemma (arrow) after injection).

3 Embryonic Development and Grading:

A. Milestones and Timing of Obeservation:

The developmental stages relative to timings of observation are as follows (Table 1):

Table : 1. Stages of embryonic development relative to timings of observation.

Day & types of observation	Time of observation (hours post insemination)	Likely stage of development
Day 1 Fertilization check	**17 ± 1**	**Pronuclear stage**
Day 1 Syngamy check	**23 ± 1**	**Syngamy**
Day 1 Early cleavage check	**26 ± 1 h post-ICSI 28 ± 1 h post-IVF**	**2-cell stage**
Day 2 Embryo assessment	**44 ± 1**	**4-cell stage**
Day 3 Embryo assessment	**68 ± 1**	**8-cell stage**
Day 4 Embryo assessment	**92 ± 2**	**Morula**
Day 5 Embryo assessment	**116 ± 2**	**Blastocyst**

The kinetics of cell division is not only influenced by the time of observation but also by the culture conditions such as temperature, pH and culture media.

B. Asessment of fertilization:

At 16-18 hrs post insemination, one expects to see a spherical oocyte with 2 even-sized centrally located, juxtaposed pronuclei with distinct membranes and 2 polar bodies. Ideally, the 2 pronuclei have nucleolar precursor bodies (NPBs) equivalent in number and size and aligned at the region of membrane juxtaposition. **(Fig. 35)**

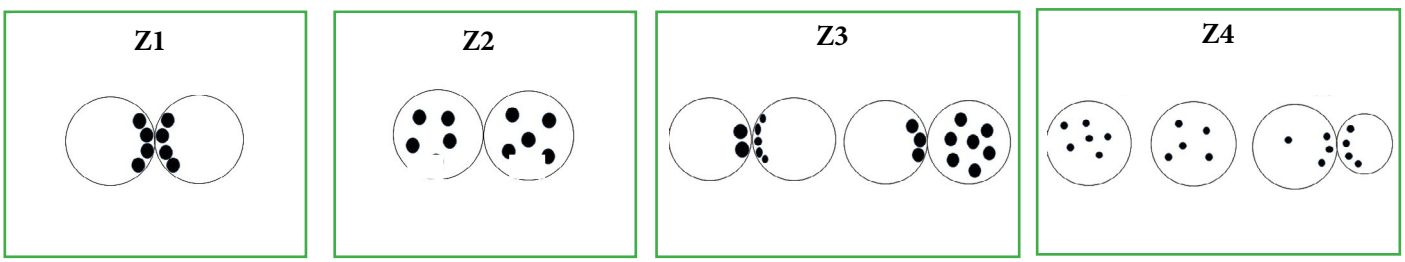


Fig: 35. Zygote scoring system of Scott et al. [21]

Pronuclear scoring (Table 2) (Fig. 35, 36):

It is performed at the same time as fertilization check. Three categories are defined:

1. **Category 1:** Symmetrical: Equivalent to Z1 or Z2 **(Fig. 35, 36a)**
2. **Category 2:** Non-symmeterical: Any other arrangement e.g Z3 or Z4 **(Fig. 35, 36b)**
3. **Category 3:** Abnormal: Pronuclei with 0 (Ghost Pronuclei), (Fig 36c i) or 1 PB (Bull's Eye Pronuclei), **(Fig 36c ii)** have been shown to be associated with poor outcome in animal studies. **(Fig. 36c)**

Table : 2. Consensus scoring system for pronuclei

Catagory	Rating	Discription
1	Symmetrical	Similar to Z1 and Z2
2	Non - symmetrical	Peripherally located pronuclei and other arrangements
3	Abnormal	Pronuclei with 0 or 1 NPB

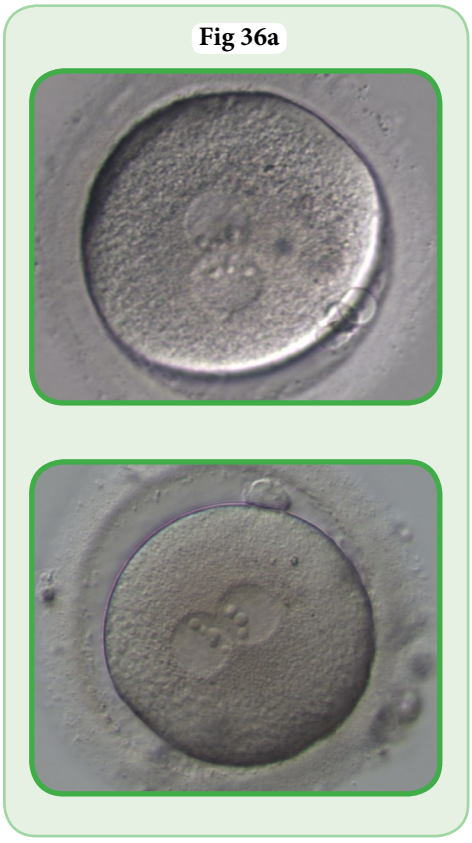


Fig 36a. PN scoring - Category 1

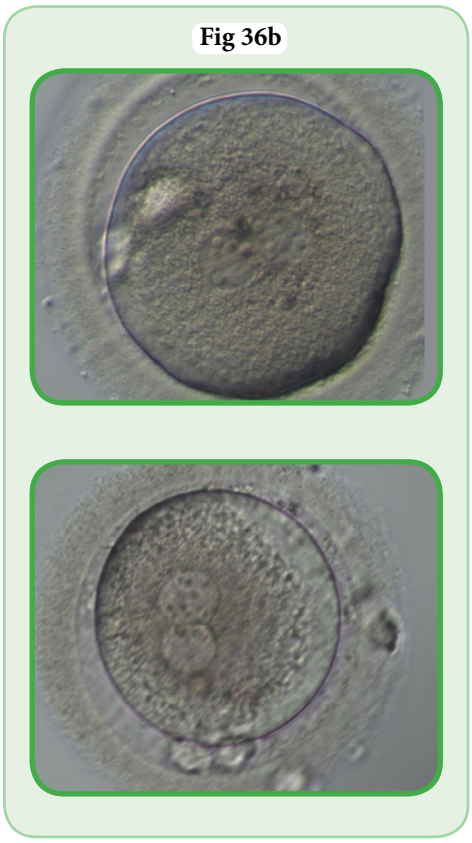


Fig 36b. PN scoring - Category 2

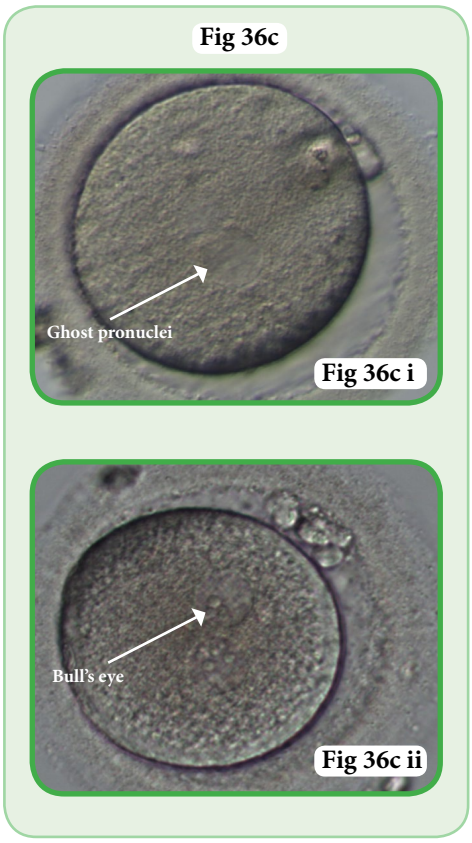


Fig 36c. PN scoring - Category 3

The atypical features of pronuclei are:

i. Widely separated pronuclei **(Fig. 37)**
ii. Pronuclei of grossly different sizes **(Fig. 38)** and
iii. Micronuclei **(Fig. 39a & b)**
iv. More than 2 PN: due to polyspermy **(Fig. 40a & b)**

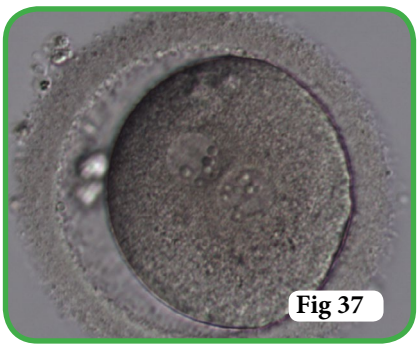


Fig 37. Widely separated PN

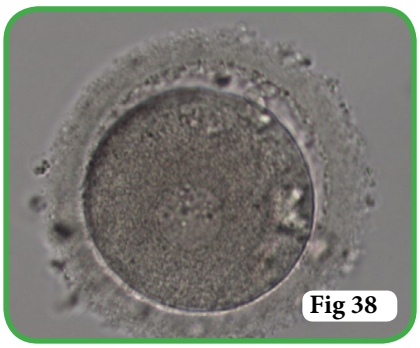


Fig 38. Discrepancy in size of PN

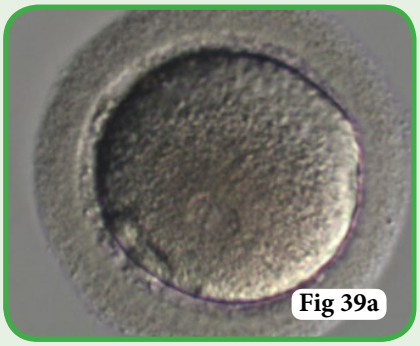


Fig 39a & b. Micronuclei

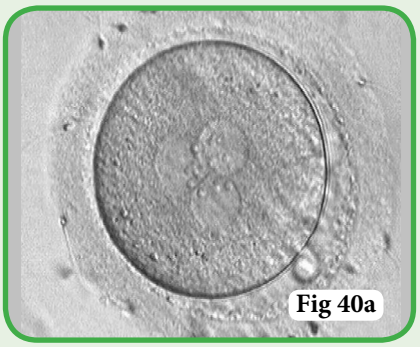


Fig 40a. 3PN zygote

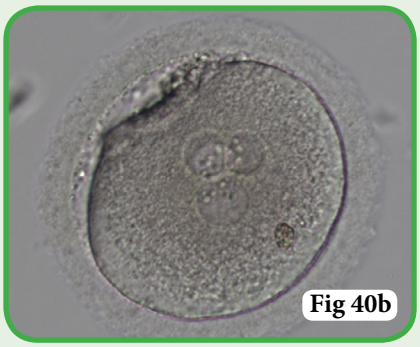


Fig 40b. 4PN zygote

The presence of a 'cytoplasmic halo' has a positive influence on further embryonic development including blastocyst formation and implantation. **(Fig. 41)** [20, 21]

Zygote scoring is important in countries where embryo selection is mandatory at zygote stage. It excludes embryos with poor outcome. Embryos with poor zygote scoring result in slow development & poor blastocyst formation. [21]

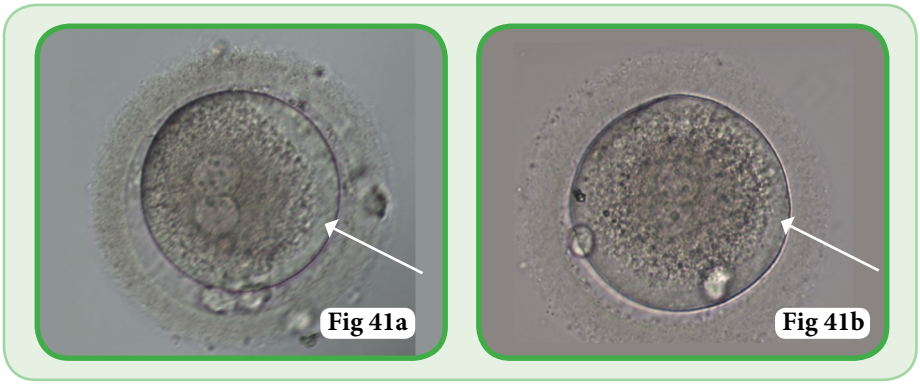


Fig: 41a & b. Zygote at 2PN stage and cytoplasmic halo

C. Early Cleavage – Day 1 Embryos (Fig 42)

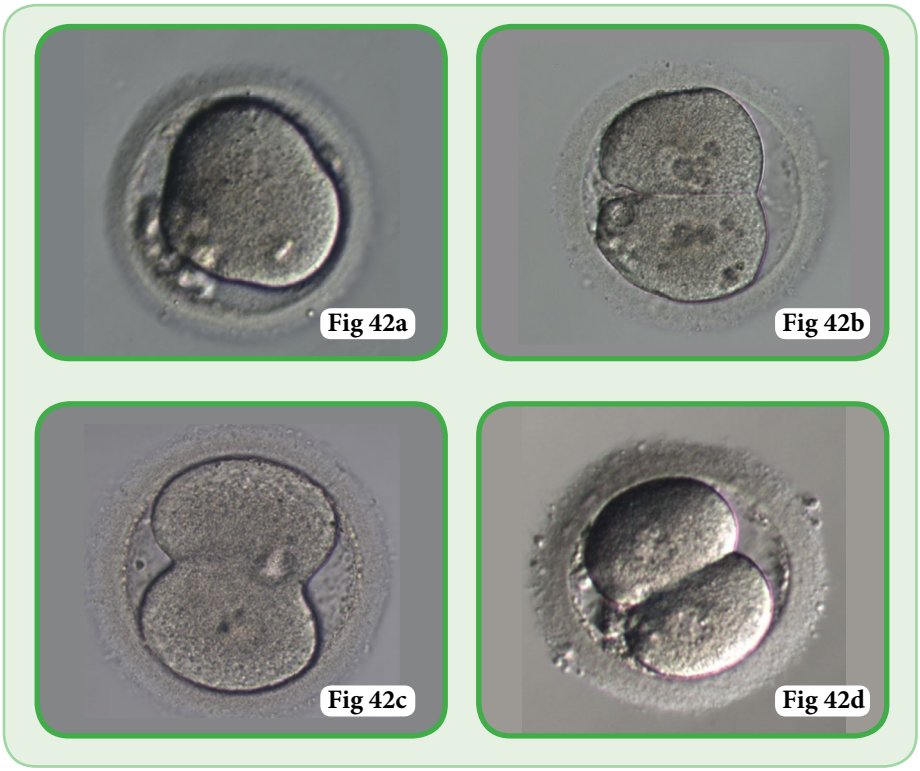


Fig: 42a, b, c & d. Early cleaving embryos at various stages of development (25 hours post insemination)

The identification of early cleavers is important due to the following reasons:

1. They form into more even sized blastomeres with lower chromosomal anomalies. [22]
2. They have been shown to have higher blastocyst formation and pregnancy rates. [23]
3. It helps exclude embryos which directly cleave into 3 or more cells which have higher incidence of chromosomal anomalies [24]
4. Early cleavage before 20 hours have been shown to have a poor prognosis.

D. Cleavage stage embryos:

Cleavage stage embryos range from 2-cell stage to mórula which is a compacted mass of 12-16 cells. The important parameters are:

I. Growth rate / cleavage rate (cell number)
II. Degree of fragmentation
III. Additional parameters

a. Symmetric and asymmetric cleavage
b. Synchronous and asynchronous division
c. Nucleation
d. Spatial Distribution of Cells and Compaction
e. Cytoplasmic Anomalies: Pitting, Granularity
f. Vacuolization

I. Growth rate / Cleavage rate:
This refers to the number of blastomeres relative to the time of observation and is the parameter with the highest predictive value. [25, 26]

Embryos with 'normal cell number' i.e. 4 cells at 44 ± 1 h post insemination[27, 28] and 8 cells at 68 ± 1 h post insemination (was 4 cell at day 2) have significantly higher implantation and pregnancy rates compared with the transfer of embryos with higher or lower cell numbers. [29]

The 'right for the day embryos' i.e. 4 cells at day 2 and 7 or 8 cells at day 3 have higher chances of being euploid. [30, 31]
It has been reported that too slow or too fast embryo cleavage rate has a negative impact on implantation rate [32, 33]
It has been shown that embryos with five or less blastomeres on Day 3 lead to early pregnancy loss. [34]

II. Degree of fragmentation

A fragment is defined as anuclear structures of blastomeric origin surrounded by a plasma membrane. It is generally <45 µm in diameter on Day 2 and <40 µm in diameter on Day 3.[35]
The degree of fragmentation is an important parameter of embryo evaluation and is part of all the scoring systems. It is evaluated in relation to the cytoplasmic volume and is classified as:

a. **Mild Fragmentation** (< 10% of cytoplasmic volume) **(Fig. 43a)**
b. **Moderate Fragmentation** (10-25% of cytoplasmic volume) **(Fig. 43b)**
c. **Severe Fragmentation** (>25% of cytoplasmic volume). **(Fig. 43c)**[10]

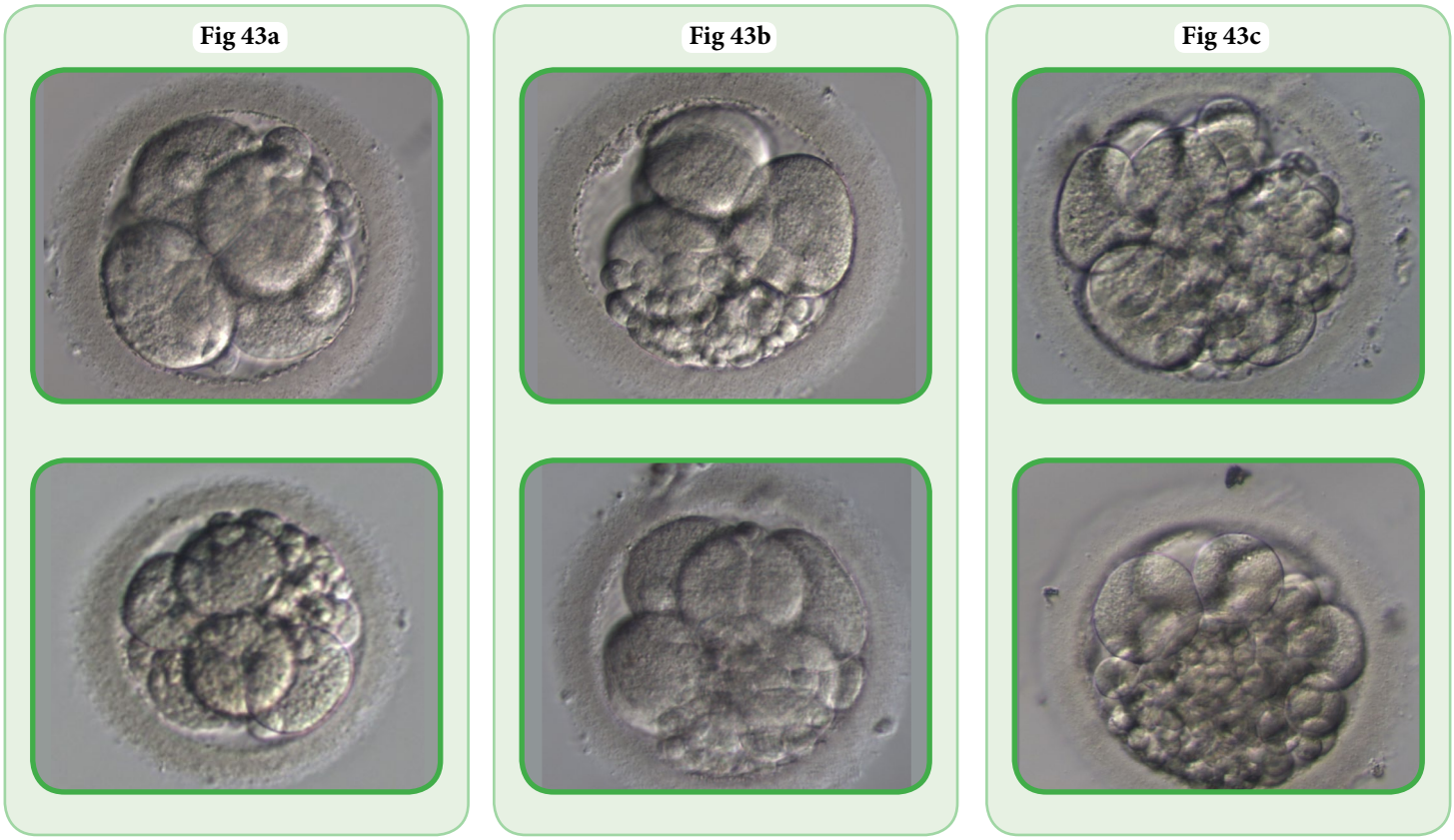


Fig 43. Varying degrees of fragmentation
Fig 43a. Mild Fragmentation (<10%) **Fig 43b.** Moderate Fragmentation (10-25%) **Fig 43c.** severe (>25%) fragmentation

As it is a dynamic phenomenon, it can be either definitive where stable fragments are seen clearly detached from the blastomeres or transient fragments, which are seen initially and then may disappear during later development. **(Fig. 44a & b)** [36]

Fig: 44a. 2 cell embryo with Definitive fragmentation (indicated by arrow)
Fig: 44b. 2 cell embryo with Transient fragmentation (indicated by arrow)

The distribution of fragments in perivitelline space can be either **scattered** or **localized (Fig 45a & b).** The scattered fragmentation is associated with an increased incidence of chromosomal abnormality. [33]

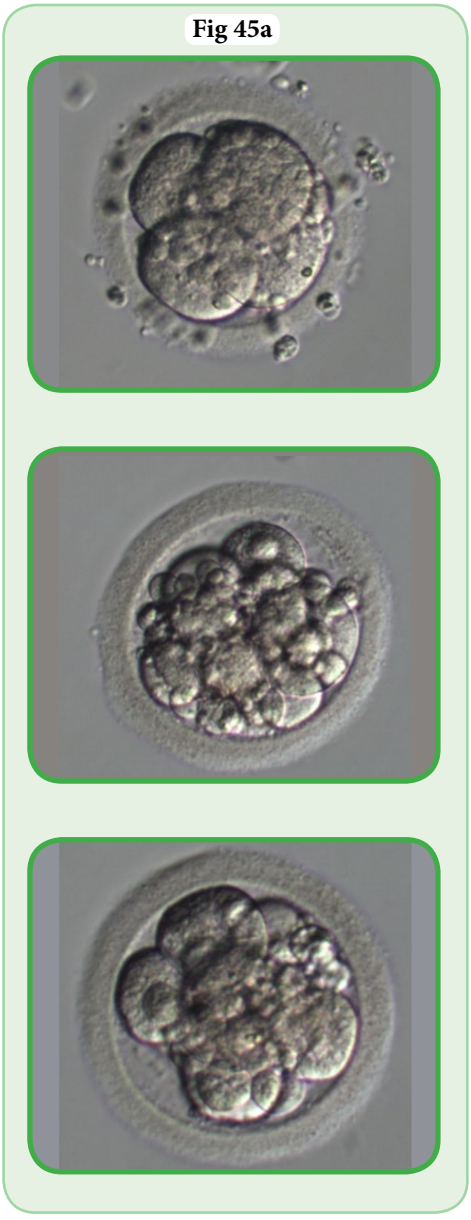


Fig 45a. Scattered Fragmentation

Fig 45b. Localized Fragmentation

Some studies have proven that mild degree of fragmentation has a negligible impact on implantation and pregnancy rates [37] whereas high degrees of fragmentation correlate negatively. [38] Fragmentation should be viewed at different focal planes to accurately assess the number of cells and degree of fragmentation. **(Fig. 46)**

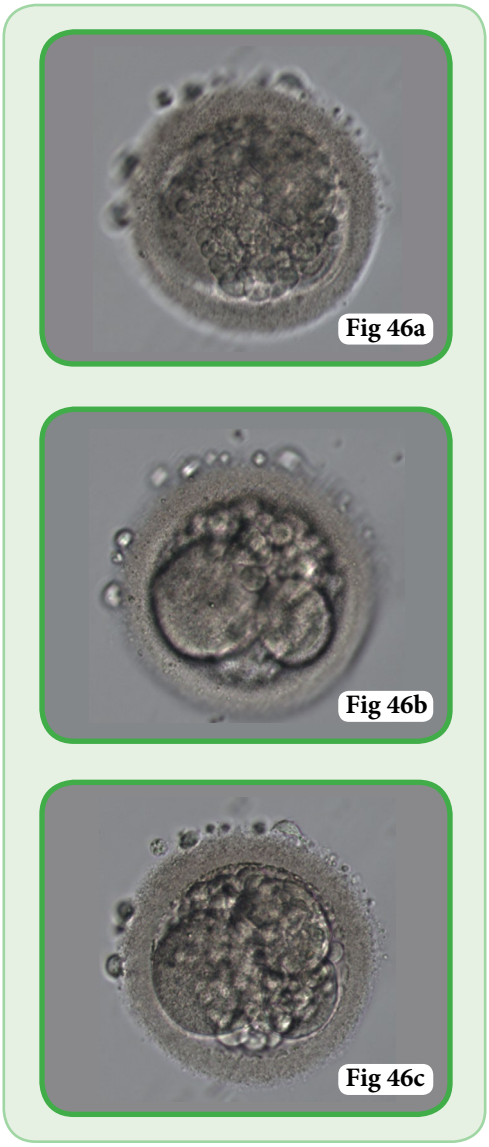


Fig 46. Fragmentation should be assessed after viewing at different planes. In **Fig. 46a,** the embryo looks like a severely fragmented embryo where number of cells can't be deciphered. When viewed at different planes, it appears to be a moderately fragmented 2-cell embryo in **Fig. 46b** and a 3 cell embryo in **Fig. 46c**

III. Additional parameters

a. Symmetric and Asymmetric division:

i. **Symmeteric division** – Zygote divides into two equally sized daughter cells **(Fig. 47)**

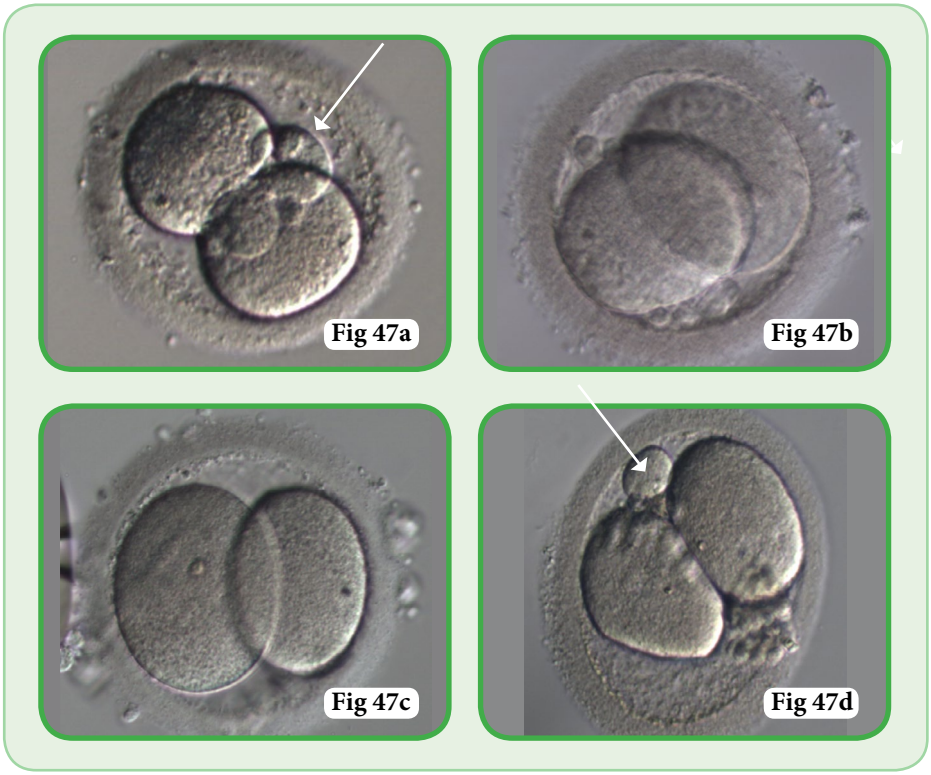


Fig 47a. Symmetrically divided two-cell embryo (definitive fragment indicated by arrow)
Fig 47b. The two blastomeres arranged at 90° to each other
Fig 47c. The blastomeres next to each other
Fig 47d. An oblong embryo with almost equal blastomeres and a large fragment (arrow)

ii. **Asymmeteric division/Uneven cleavage** – Cell cleaves into two unequal sized cells and one of the blastomeres of the next generation will inherit less than half the amount of cytoplasmic molecules from the parent blastomere leading to a defective lineage in the embryo **(Fig. 48).** There is a high degree of concurrence between uneven cleavage, multinucleation, fragmentation and aneuploidy [24, 39] **(Fig. 49).**

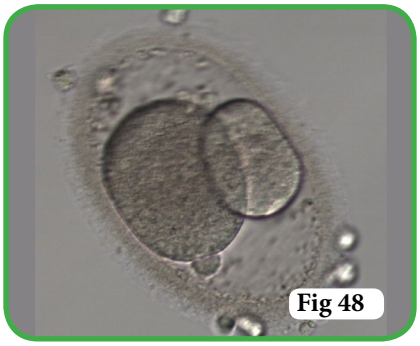


Fig 48. Asymmetrically divided two-cell embryo

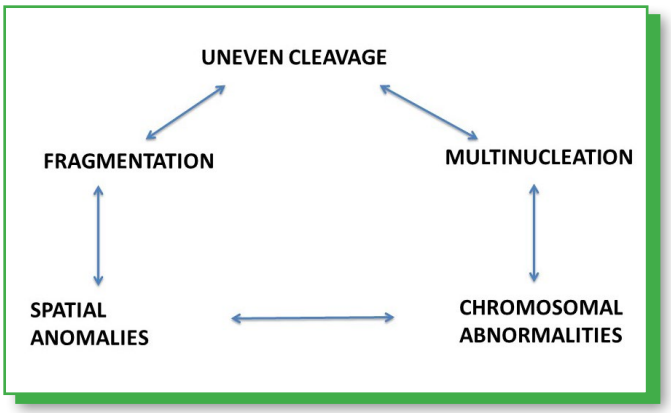


Fig. 49

Fig: 49. Pentad of uneven cleavage, multinucleation, chromosomal anomalies, spatial anomalies and fragmentation

b. Synchronous and Asynchronous Division:

If the blastomeres divide synchronously, 2, 4 and 8 cell embryos with equal sized blastomeres are observed. These are known as **stage specific embryos.** If the blastomeres in such embryos are unequal they are known as **non-stage specific embryos.** However, if the blastomeres divide asynchronously, 3, 5, 6 or 7 cell embryos are observed. Depending on the size of the blastomeres in these embryos, they are either labelled stage or non-stage specific **(Table 3, Fig. 50)**

Table. 3: Arrangement of blastomeres in stage specific and non stage specific embryos

CELL NUMBER	STAGE SPECIFIC	NON-STAGE SPECIFIC
3 Cell Embryo	1 Large and 2 small blastomeres **(Fig 51a)**	All blastomeres equal **(51b i & b ii)** or unequal in size **(Fig 51b iii)**
4 Cell Embryo	All blastomeres equal **(Fig 52a)**	One or two blastomers much larger than the others **(Fig 52b)**
5 Cell Embryo	3 Large and 2 small blastomeres **(Fig 53a)**	5-cell embryo: two large and three smaller blastomeres **(Figs 53b I)** or one small and four larger blastomeres **(Fig 53b ii)** or 5 equal blastomeres **(Fig 53b iii)**
6 Cell Embryo	2 Large and 4 small blastomeres **(Fig 54a)**	All blastomeres equal **(Fig 54bi & ii)** or extremely different in size **(Fig 54b iii)**
7 Cell Embryo	1 Large and 6 small blastomeres **(Fig 55a)**	Five large and two small blastomeres **(Fig 55b)**
8 Cell Embryo	All blastomeres equal **(Fig 56a)**	Five large and three small blastomeres **(Fig 56b)**

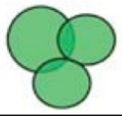
	Stage Specific	Non - Stage Specific
Three Cell Embryos		
Four Cell Embryos		
Five Cell Embryos		
Six Cell Embryos		
Seven Cell Embryos		
Eight Cell Embryos		

Fig: 50. Schematic representation of Stage Specific (green) and Non-Stage Specific (Yellow) embryos at different stages of development.

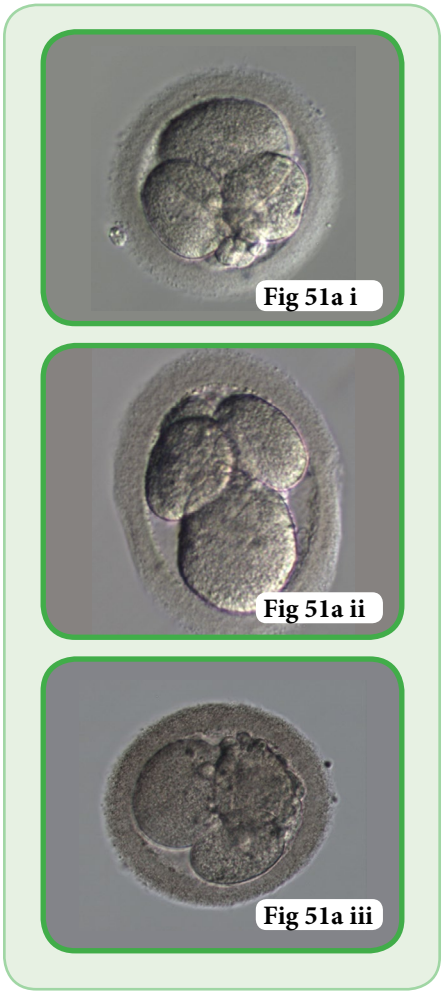


Fig 51a. Stage specific 3 cell embryos

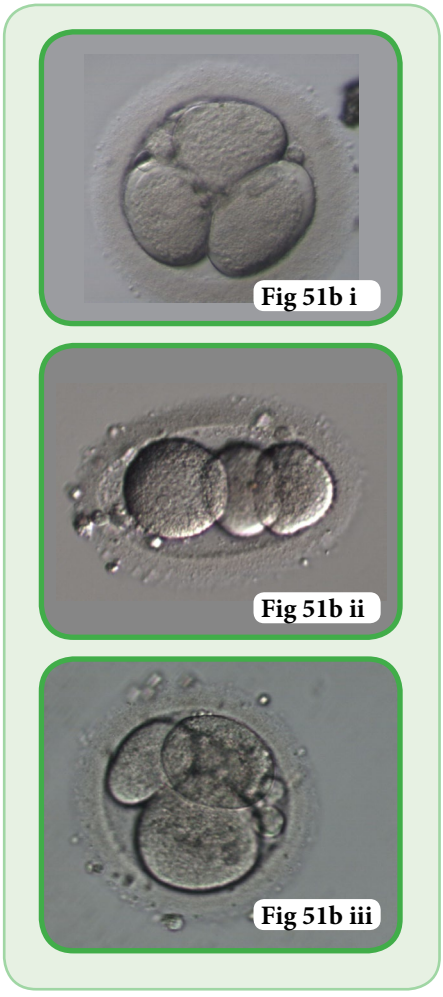
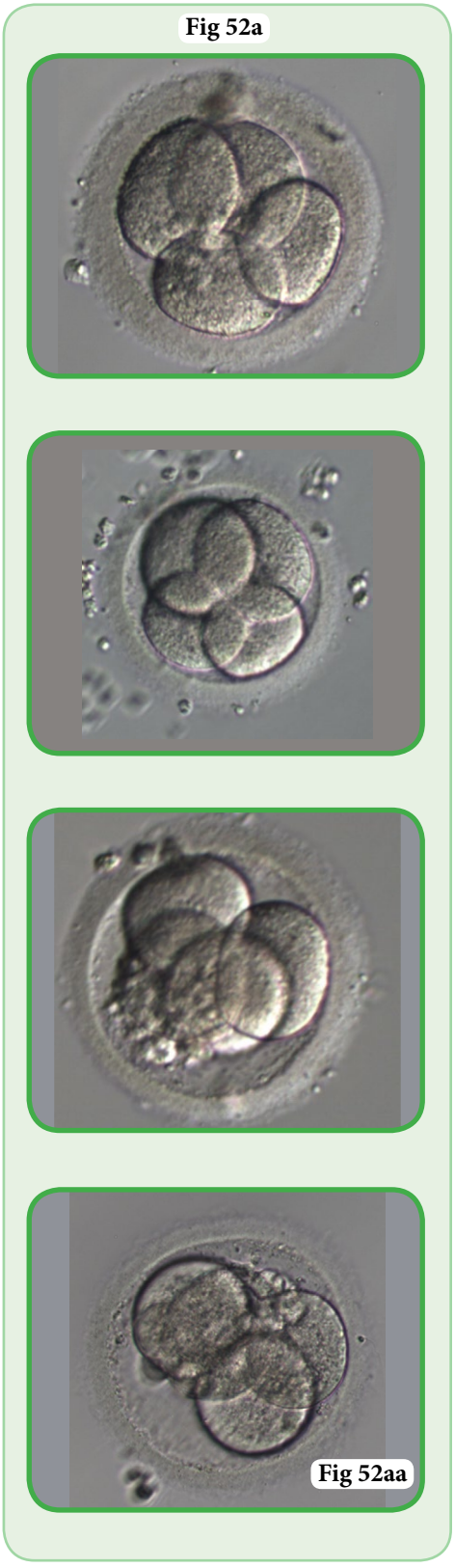


Fig 51b. Non-stage specific 3 cell embryos



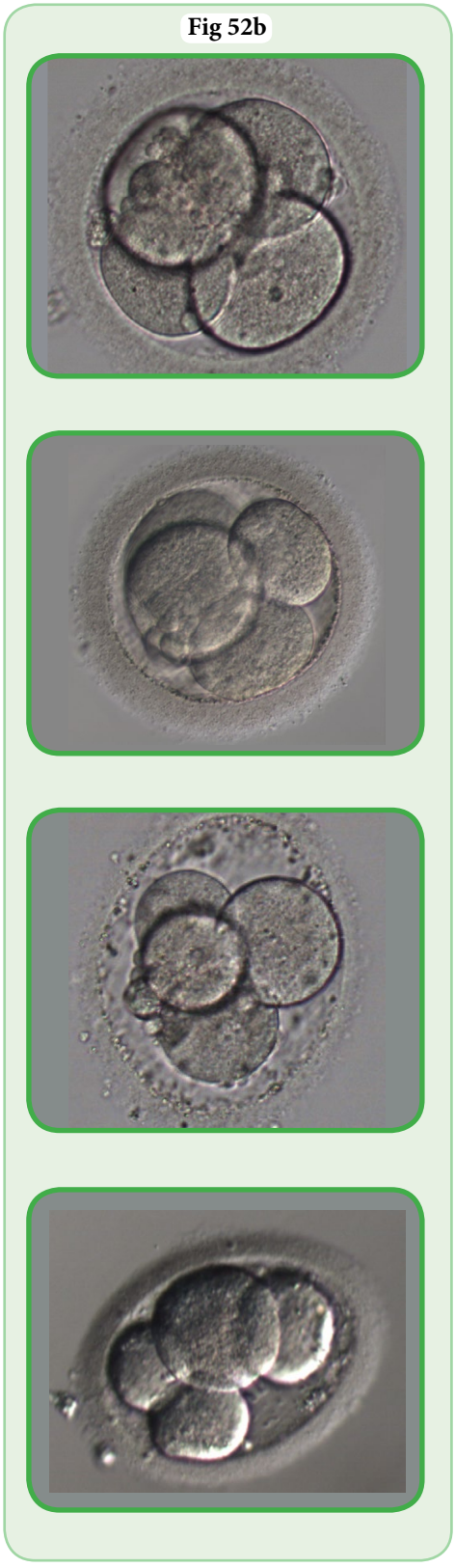


Fig 52a. Stage specific 4 cell embryos
Fig 52aa. Even-sized blastomeres with wide PVS

Fig: 52b. Non-stage specific 4 cell embryos

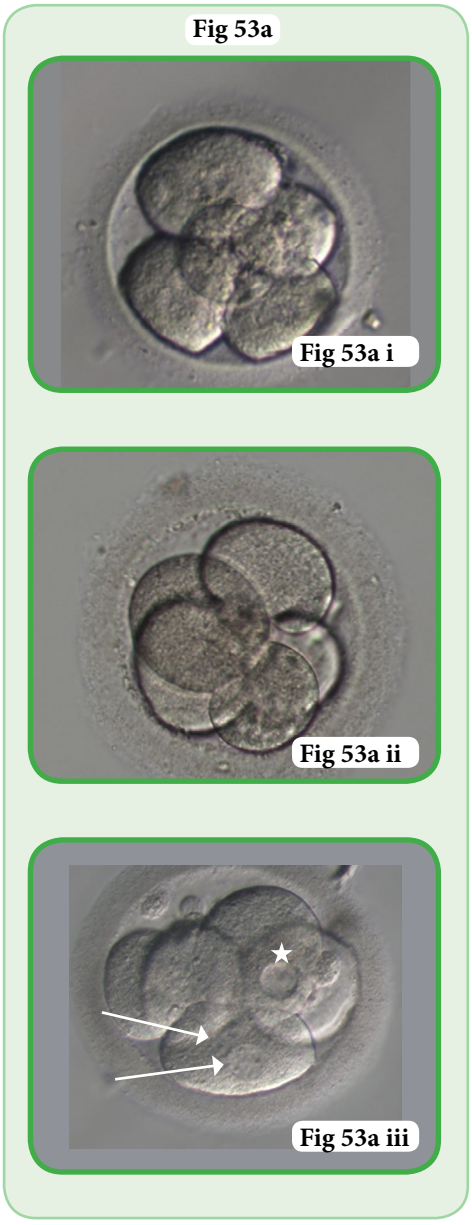
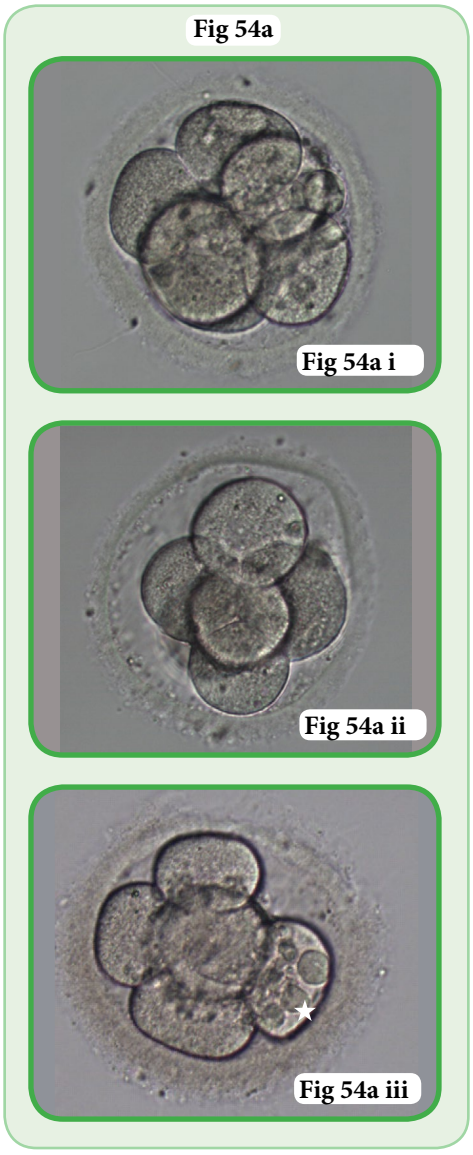


Fig. 53a Stage-specific 5-cell embryos
Fig. 53a iii 5 cell embryo with vacuole (*) and binucleate blastomere (arrow)

Fig. 53b Non-stage specific 5-cell embryos
Fig. 53b ii 5 cell embryo with localized moderate degree of fragmentation



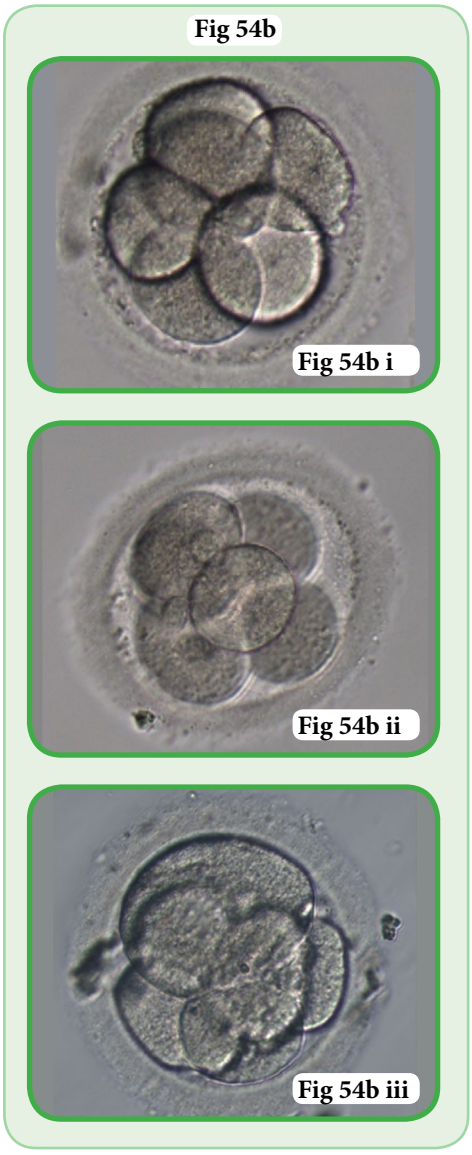


Fig. 54a i. Stage specific 6 cell embryos
Fig. 54a ii. Wide PVS
Fig. 54a iii. Vacuoles in blastomere (*)

Fig: 54b. Non-stage specific 6 cell embryos

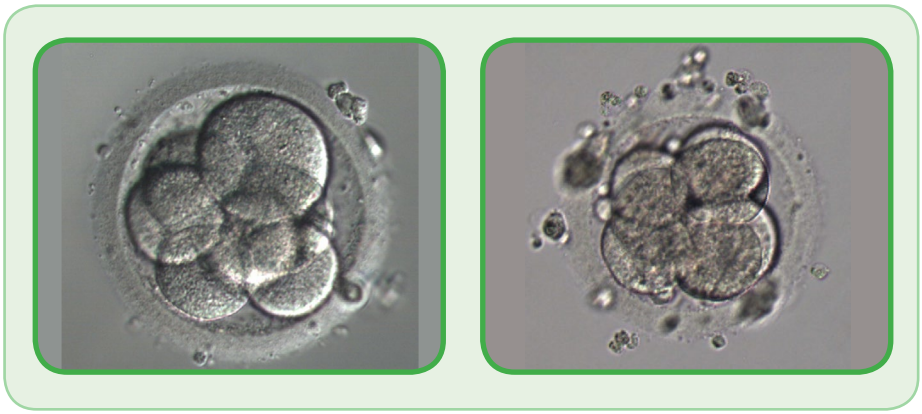


Fig: 55a. Stage specific 7 cell embryos

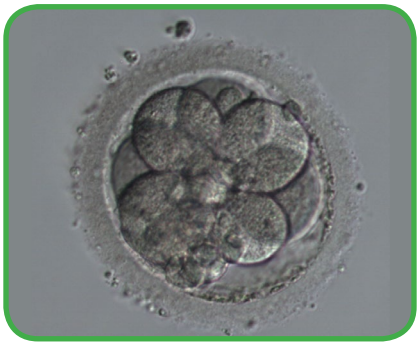


Fig: 55b. Non-stage specific 7 cell embryos

Fig 56a i. Stage specific spherical 8 cell embryo.
Fig. 56a ii. Stage specific oval 8 cell embryo.
Fig. 56a iii. Stage specific 8 cell embryo with mild scattered fragmentation.

Fig 56b. Non-stage specific 8 cell embryo

Embryos at different stages of development can be encountered. **Fig. 57, 58 and 59** show 9, 10 and 16 cell embryos respectively.

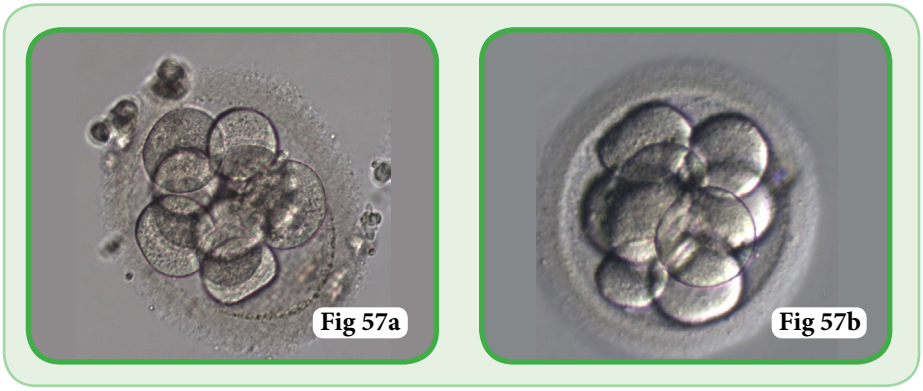


Fig 57a & b. 9-cell embryos

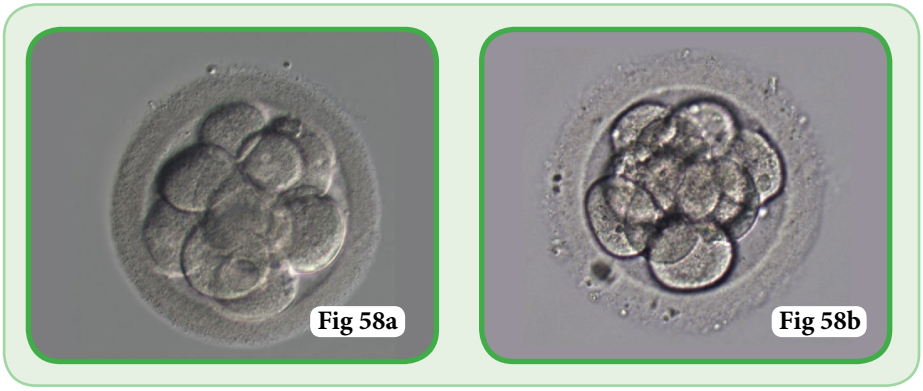


Fig. 58a & b. 10-cell embryos

Fig 59. 16-cell embryo

c. Nucleation:

In a cleavage embryo, presence of more than one nucleus in any of the blastomere is called multinuceation.[42] It is better appreciated in 2 cell or 4 cell embryos compared to 8 cell embryos. **(Fig. 60 to 63)** It has been shown to be associated with culture media, [43] and inaccurate temperature control especially during oocyte retrieval. [44]

Multinucleation is associated with:

i. High degree of chromosomal aberration. [24]
ii. Higher degree of fragmentation. [42]
iii. Uneven cell size. [24]
iv. Lower implantation, pregnancy and birth rates [24, 42]
v. Higher incidence of spontaneous abortions [30]

Multinucleated embryos should be excluded from transfer if a better embryo is available for transfer.

Fig 60a, b & c. 2-cell multinucleated embryos (indicated by *)

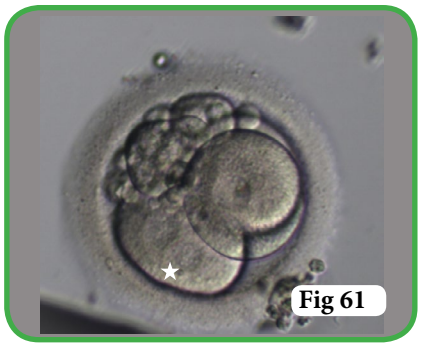


Fig 61. 3-Cell multinucleated embryos (indicated by *)

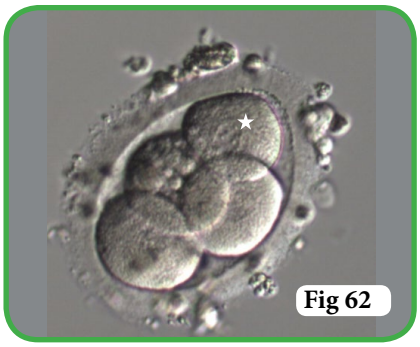


Fig 62. 4-cell multinucleated embryo

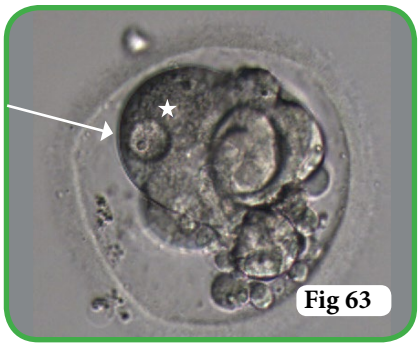


Fig 63. Day 4 compacting embryo showing binucleation (*) and vacuole indicated by (arrow).

d. Spatial distribution of cells

Human oocytes are polarized and consist of an animal and vegetal pole.[45]
The unique pattern of first and second cleavage divisions results in a typical pyramidal or tetrahedral structure with 4 cells with different polarity. **(Fig 64)** Any deviation from this pattern of cleavage will lead to non-tetrahedral or 'clover' shaped 4 cell embryo.

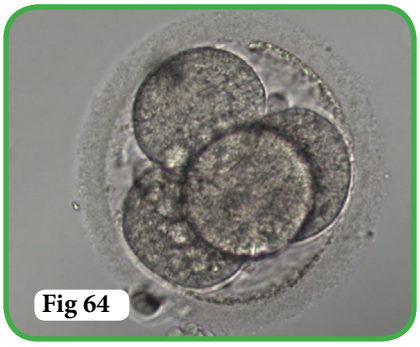


Fig 64. 4 cell embryo with tetrahedral structure

The cleavage planes influence embryo development even at later stages of development, thereby, influencing implantation potential of an embryo. [46]
In ovoid embryos, resulting from oval oocytes, the spatial distribution of embryos is entirely different **(Fig. 65 & 66)**. This leads to reduced number of cell to cell contacts resulting in delayed preimplantation development.

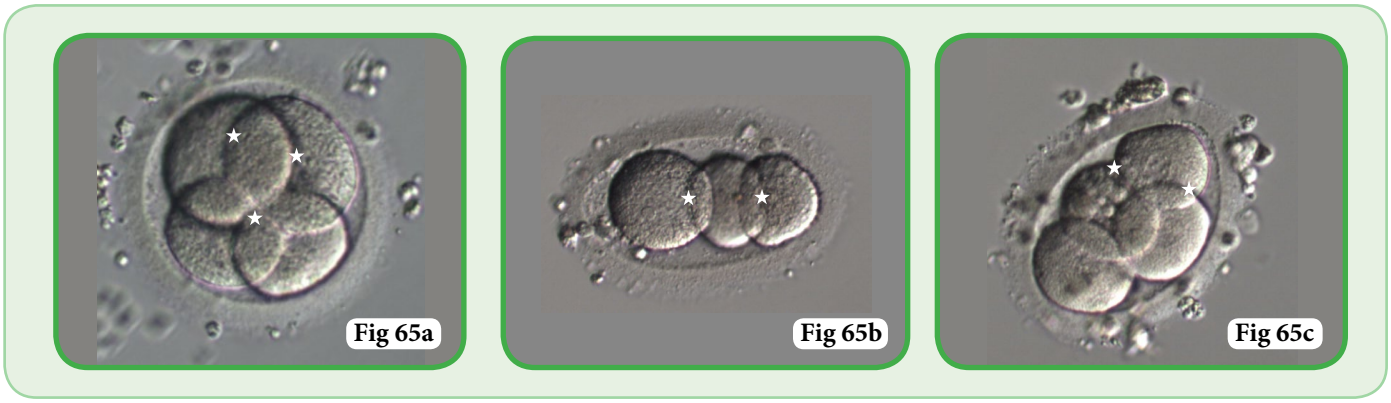


Fig 65. Cell to cell contacts in 4 cell embryo. In a tetrahedral structure, one blastomere communicates with 3 other blastomeres **(fig 65a)** whereas in an oval embryo, each blastomere communicates with either 1 **(Fig 65b)** or 2 blastomeres **(Fig 65c)**

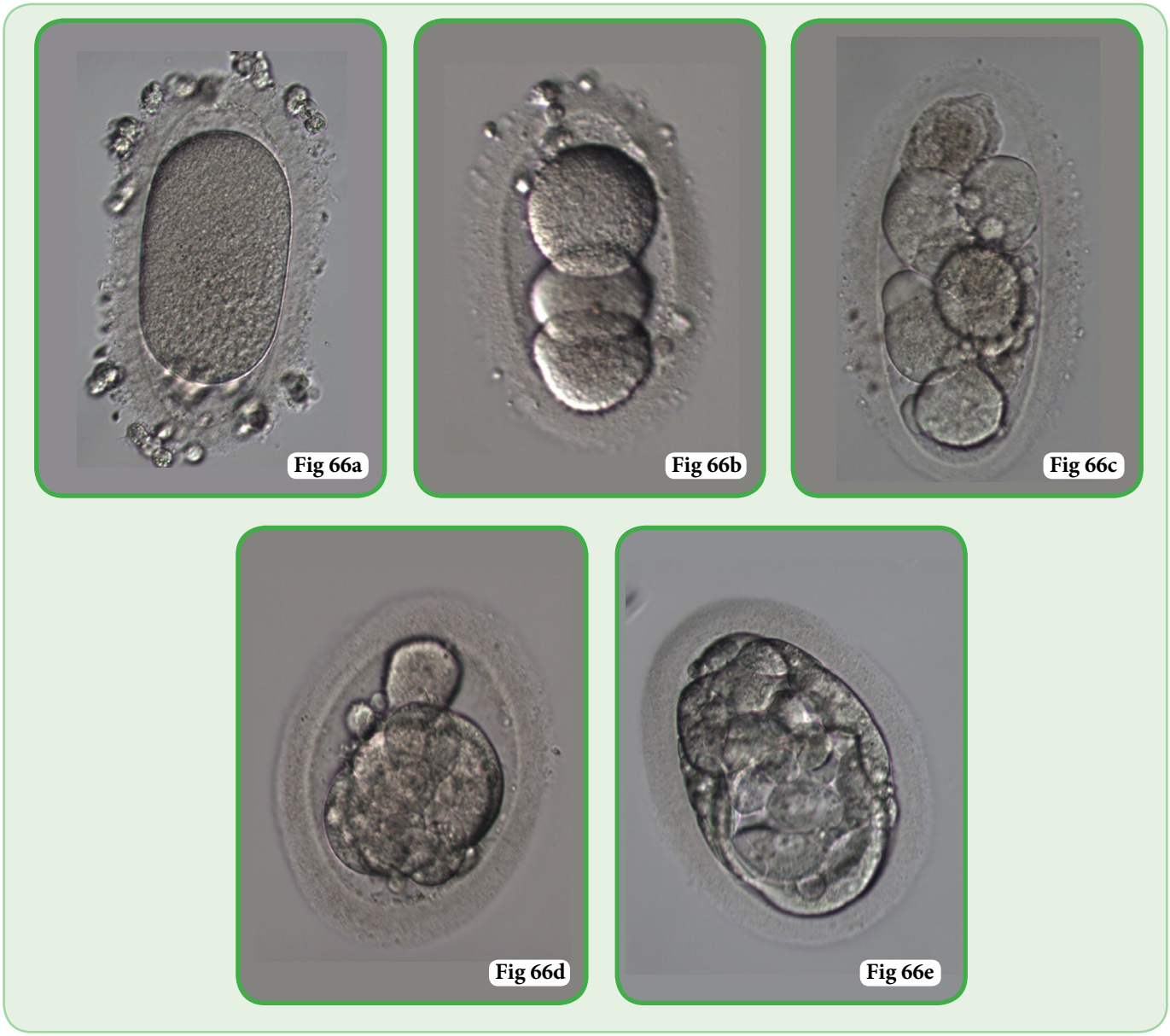


Fig 66 a to e. Development of an oval oocyte. Note that due to limited intercellular interactions, compaction may not be complete

e. Cytoplasmic anomalies

i. **Cytoplasmic Pitting:** Numerous pits with an approximate diameter of less than 1.5 microns present on the surface of cytoplasm. Some studies have shown pitting to be associated with improved blastocyst formation [47] whereas Ebner et al., 2005 demonstrated that extreme degree of cytoplasmic pitting is associated with early loss of gestational sacs. **(Fig 67)** [48]

ii. **Cytoplasmic Granularity:** The organelles retract towards the centre of the cell leading to a centralized granularity. These embryos are associated with reduced implantation potential and are likely to degenerate. **(Fig 68)**[49]

iii. **Cytoplasmic Vacuoles:** Vacuoles are membrane bound cytoplasmic inclusions filled with fluid **(Fig. 69).** They can be seen in retrieved oocytes after oocyte collection as well as can be induced by ICSI. Few, small vacuoles can be ignored but extensive vacuolization should be recorded as it is detrimental to the spatial development.Vacuoles arising at the compaction stage may lead to developmental arrest with a grievous effect on blastocyst formation. **(Fig. 63)** [50]

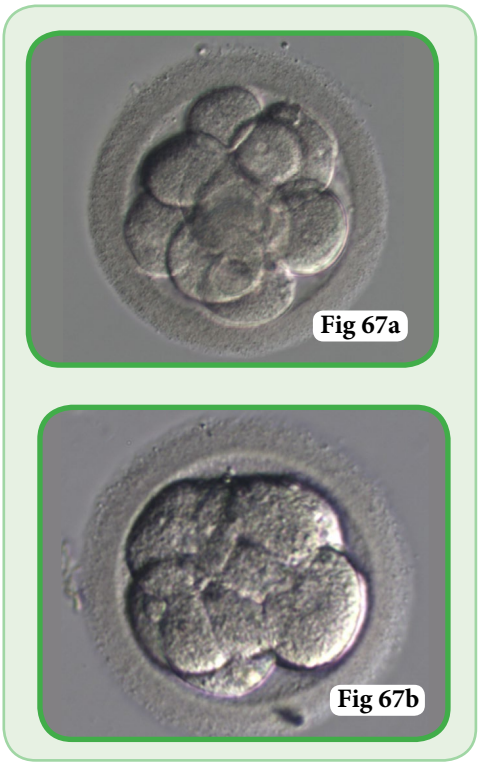


Fig 67. Cleavage stage embryos with cytoplasmic pitting

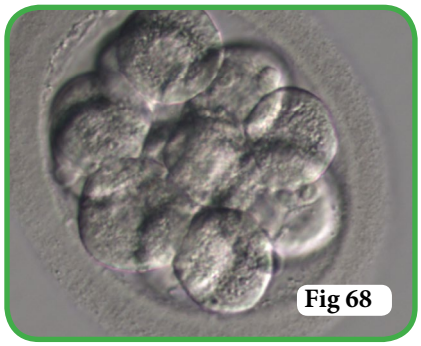


Fig 68. Cleavage stage embryo with cytoplasmic granularity

Fig 69a & b. Cleavage stage embryos with vacuoles (marked by *)

The consensus system for cleavage stage embryos is shown in the table (Table 4)

Table 4. Consensus scoring system for cleavage-stage embryos

GRADE	FRAGMENTATION	CELL SIZE	MULTINUCLEATION
1	<10%	Stage-specific	Not seen
2	<10-25%	stage-specific cell size for majority of cells	Not seen
3	>25%	Non stage-specific	Seen

E. Assessment of day 4 embryos (morula) (92±2h)

Usually, a day 4 human embryo or morula, assessed at 92 ± 2 hours, should comprise of 16-32 cells and looks like a mass of cells with indistinguishable cell boundaries.
The intercellular adherence begins at the 8-cell stage, steadily increasing with time **(Fig. 70).** It is considered a good sign of implantation. [51]

Fig: 70. Day 3 embryos with signs of compaction
Fig 70a & b. 8 cell day 3 embryos
Fig 70 c. 16 cell day 3 embryo

A good quality morula has entered into fourth round of cleavage & all the cells are included in the morula (Fig. 71). A fair quality morula will have compaction involving a majority of embryo volume **(Fig. 72)** whereas a poor quality morula is one where 2 to 3 blastomeres remain outside the compacted mass **(Fig. 73; Table 5)**

Table 5. Consensus scoring system for day-4 embryos

GRADE	4TH ROUND OF CLEAVAGE STARTED	STATUS OF COMPACTION
1	Yes	Involving the whole embryo
2	Yes	Involving majority of embryo
3	±	Disproportionate compaction involving less than half of the embryo, with two three cells remaining as discrete blastomeres

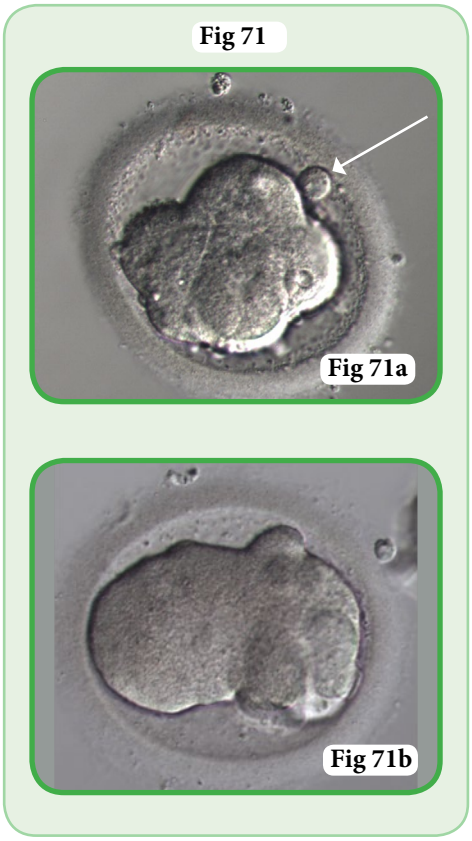


Fig 71. Grade I (Good) morulas
Fig 71a. Well compacted morula (Fragment indicated by arrow)

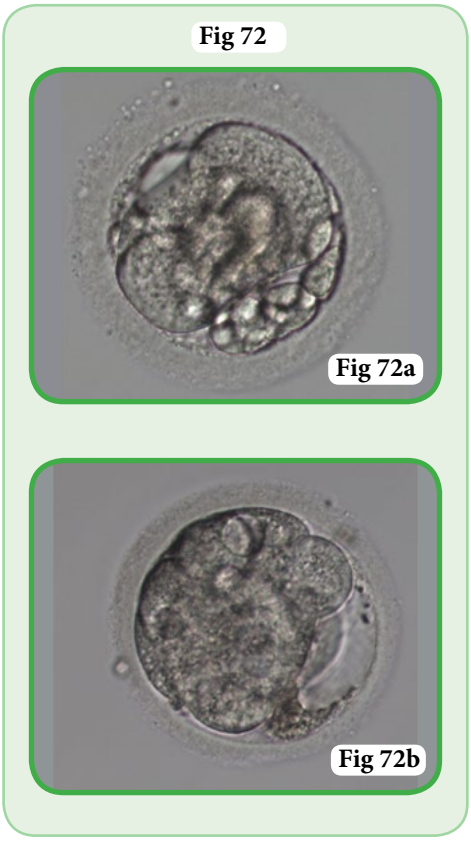


Fig: 72. Grade II (Fair) morulas with externalization of fragments

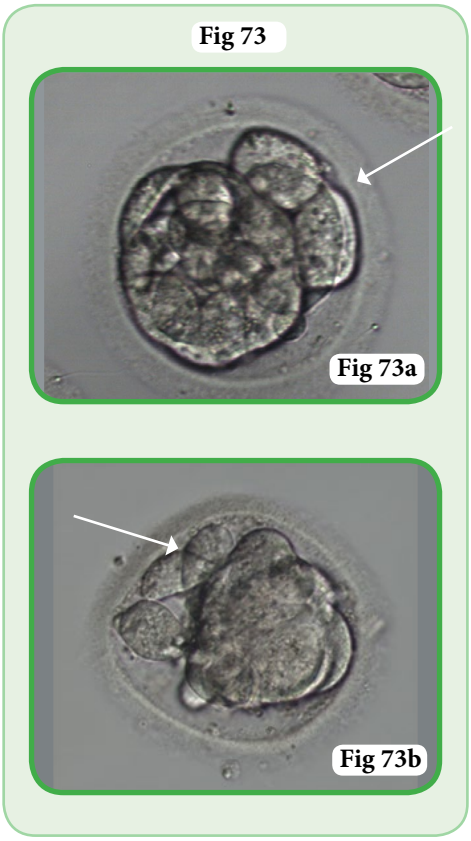


Fig 73. Grade 3 or poorly compacted morulas: 2-3 blastomeres are left out (marked by arrow)

It is seen that if more than half of the embryo is excluded, the prognosis is likely to be poor. [52]

Though similar rates of chromosomal abnormalities are seen in ovoid as well as spherical embryos,[39] ovoid embryos usually don't compact well **(Fig. 66d)** and blastocysts developing from spherical oocytes are preferred for embryo transfer.

The outer cells of compacted embryos have probably lost their totipotency as they are bound to form the trophectoderm. [53] The next phase of development is the beginning of cavitation that leads to the formation of the blastocyst **(Fig. 74).**

Cell junctions especially tight junctions, begin to spread leading to the activation of the embryonic genome. [54, 55] This is considered a good sign of the developmental capacity of the embryo. It may be influenced by the composition of culture media and culture conditions.
Some authors have reported equivalent results with Day 4 vs Day 5 embryo selection and transfers. [56]

F. Assessment of day 5 embryos (blastocysts, 116 ± 2 h):

Extended culture of cleavage stage embryos to day 5 or blastocyst stage increases the success rate of IVF by virtue of:

1. Eliminating embryos with chromosomal translocations, thereby, selecting the most viable embryos in a cohort
2. Better embryo selection after genomic activation
3. Better endometrial synchronicity
4. Reduction in the incidence of multiple gestations.

For a very long time, Gardner and Schoolcraft's system of blastocyst grading was followed. [57] The Istanbul consensus blastocyst grading system is modeled on the Gardner and Schoolcraft system with some exceptions. [10]

For grading a blastocyst, the following parameters need to be understood:

I. Degree of expansion:

A watershed line during the embryonic development is the accumulation of fluid in the morula forming a cavity known as blastocoel. (Fig. 74) As the cavity expands, the zona thins out, and the blastocyst comes out of the zona and is now known as a hatched blastocyst. The size of the blastocysts, in terms of expansion of cavity is graded quickly on a stereo microscope.

The following 6 grades were given by Gardner and Schoolcraft, 1999: [57]

i. **Grade 1:** Blastocoel cavity < 1/2 the volume of the embryo **(Fig: 74a)**
ii. **Grade 2:** Blastocoel cavity ½ or > ½ **(Fig: 74b)**
iii. **Grade 3:** Blastocoel cavity completely fills the embryo **(Fig: 75)**
iv. **Grade 4:** Blastocyst cavity is > original volume of the embryo and the ZP is thinned **(Fig: 76)**
v. **Grade 5:** Blastocysts or hatching blastocysts i.e. is herniating through a natural breach in the ZP **(Fig: 77a)**
vi. **Grade 6:** Blastocyts: blastocyst has completely escaped from a natural breach in the ZP **(Fig: 77d)**

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011 [10] have simplified the grading by combining the first two and last two grades of Gardner and Schoolcraft (1999).

i. **Grade 1:** Early **(Fig: 74)**
ii. **Grade 2:** Blastocyst **(Fig: 75)**
iii. **Grade 3:** Expanded **(Fig: 76)**
iv. **Grade 4:** Hatched/Hatching **(Fig: 77)**

The outer cells of the blastocyst are called the **trophoectoderm (TE)** cells whereas the cells usually forming a clump of cells at one pole of the blastocyst, are called the **inner cell mass (ICM)** cells.

The ICM cells are destined to form the embryo proper and associated extraembryonic structures whereas the TE cells form the fetal extra-embryonic membranes as well as the placenta.

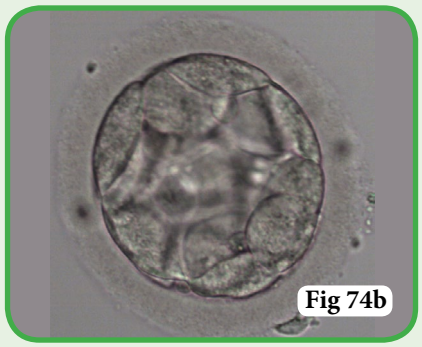
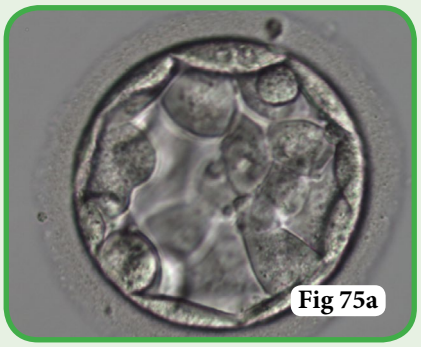


Fig 74a. Early blastocyst with cavity occupying less than half of embryonic volume.

Fig 74b. Early blastocyst with cavity occupying half of embryonic volume.



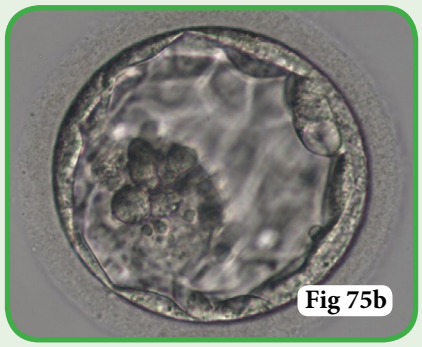
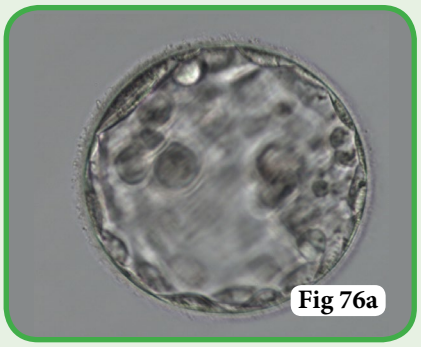


Fig 75. Grade 2 Cavity filling the complete embryonic volume.



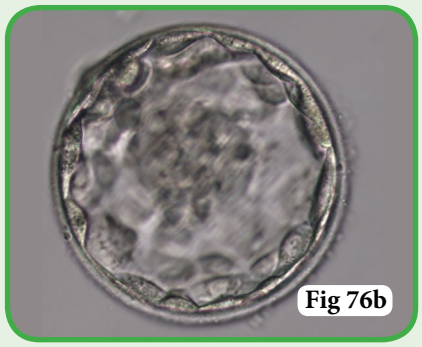
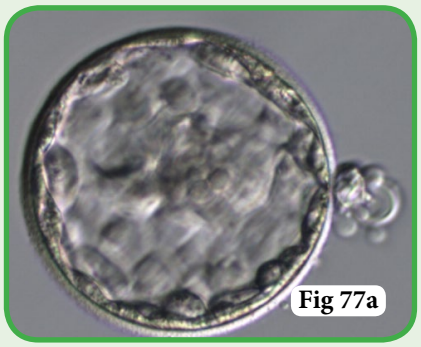
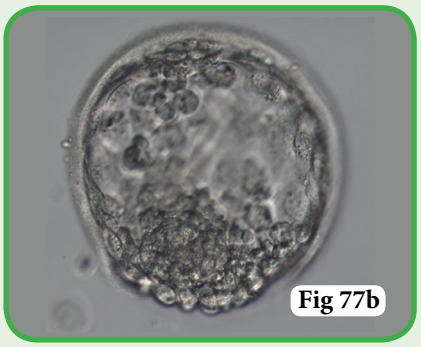
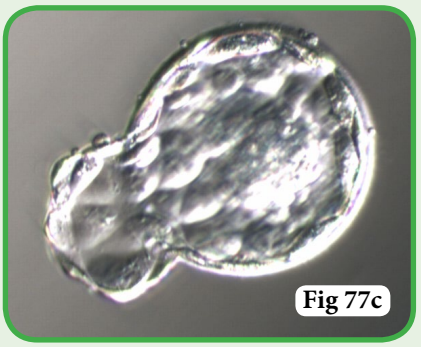


Fig 76. Expanded Blastocysts: Cavity > Original embryonic volume with zona thinning.







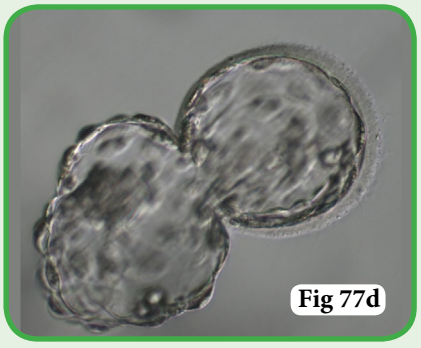


Fig 77a to d. Hatching / Hatched Blastocysts

The blastocysts undergo regular cycles of collapse and re-expansion **(Fig 78a)**. A collapsed blastocyst cannot be graded reliably **(Fig. 78c).** The collapsed blastocysts should be allowed to re-expand and then re-evaluated 1–2 h later **(Fig. 78d).**

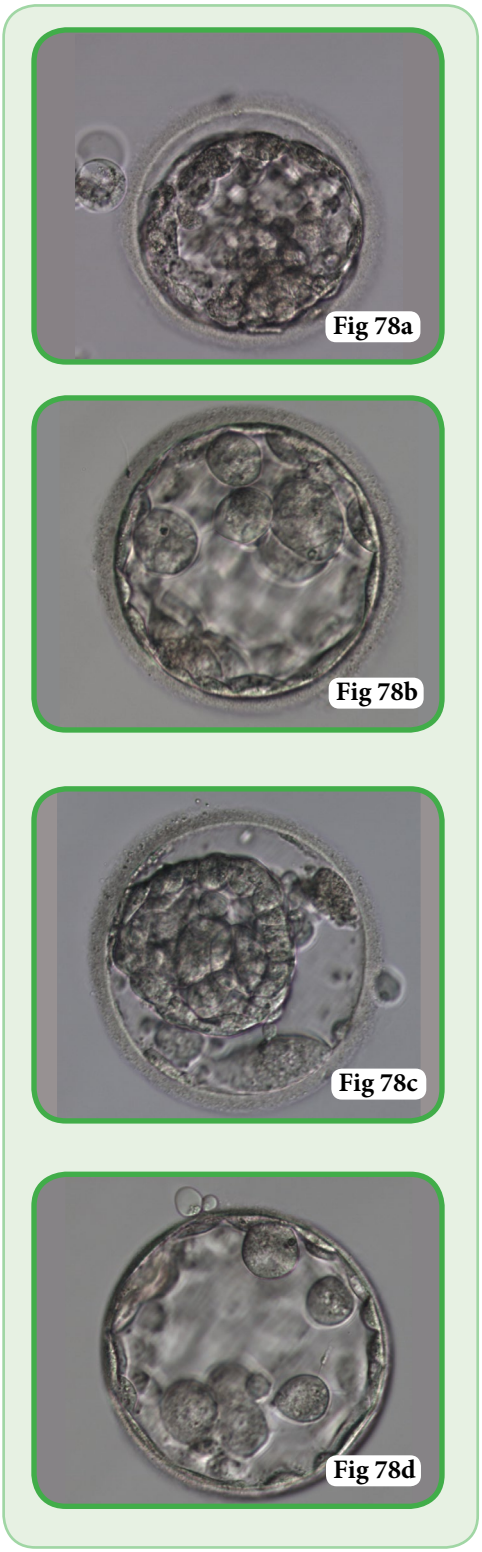


Fig 78a. Collapsed Blastocyst
Fig 78 b, 78c and 78d: Pictures taken at 2 hour intervals showing alternate cycles of collapse and expansion.
Fig 78c: It is not possible to grade the blastocyst as ICM and TE are not visualised clearly in a collapsed blastocyst.

ii. Inner cell mass:

The ICM can vary from a large clump of tightly packed cells to loosely bound cells that are difficult to discern. [10]

a. **Grade 1:** Multiple cells tightly packed together & prominent **(Fig. 79)**
b. **Grade 2:** Several cells loosely grouped but easily discernible **(Fig. 80)**
c. **Grade 3:** Loosely bound few cells **(Fig. 81)**

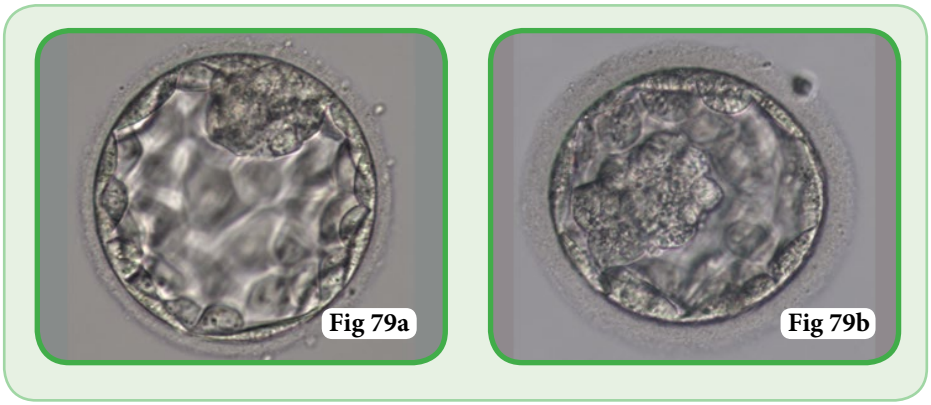


Fig 79. Blastocysts with prominent ICM

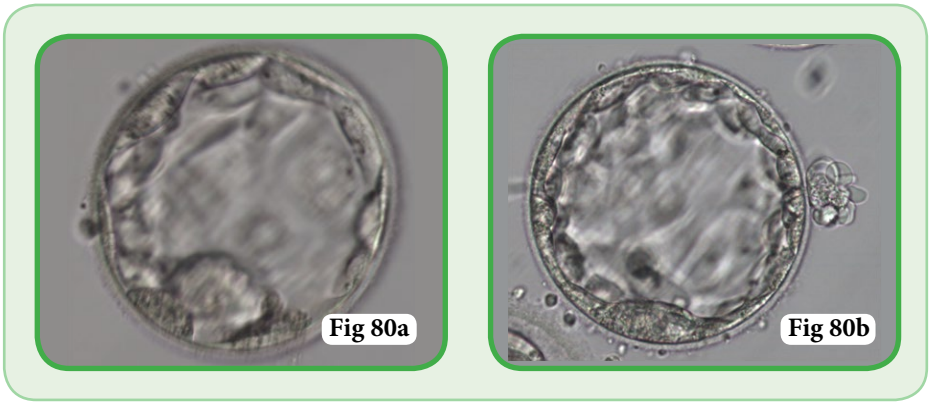


Fig 80. Blastocysts with loosely grouped ICM

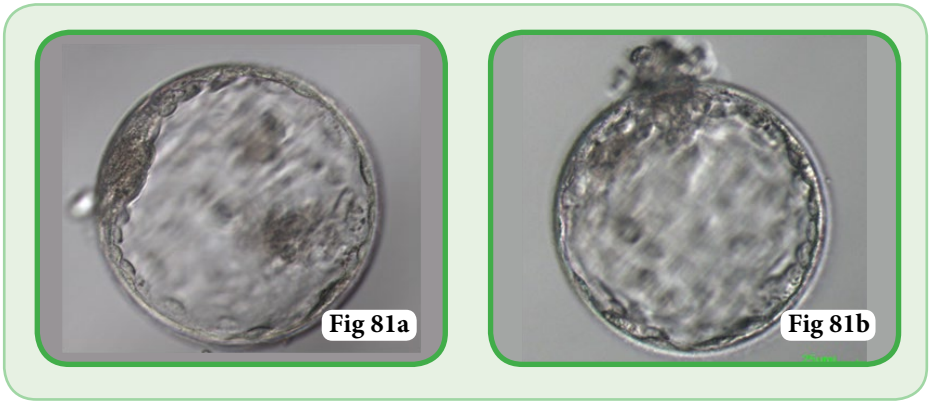


Fig 81. Blastocysts with loosely bound few cells in ICM

Shape of the ICM can vary from:

a. Mushroom-shaped **(Fig. 82)**
b. Stellate-shaped **(Fig. 83)**
c. Crescent-shaped **(Fig. 84)**

Double ICM: Double ICM was significantly higher in in vitro fertilized blastocysts compared to in vivo fertilized blastocysts (0.6%) leading to a higher incidence of monozygotic twinning.

Fig. 82 Expanded blastocyst with Grade 1 Mushroom-shaped ICM

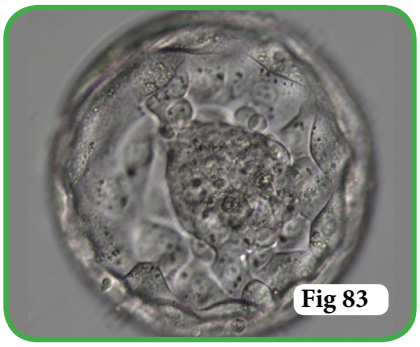


Fig. 83 Expanded Blastocyst with Grade 1 Stellate-shaped ICM

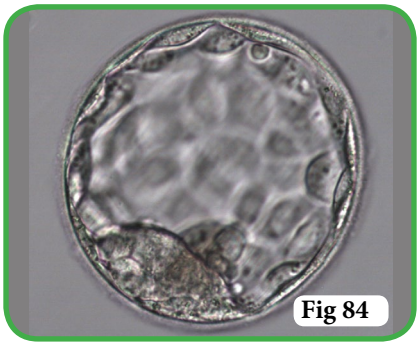


Fig. 84 Expanded blastocyst with Grade 2 Crescent-shaped ICM

III. Outer cell mass/trophoectoderm:

a. **Grade 1:** Cohesive epithelium composed of many cells **(Fig. 85)**
b. **Grade 2:** Few cells forming a loose epithelium **(Fig. 86)**
c. **Grade 3:** Few, large cells that struggle to form a cohesive epithelium **(Fig. 87)**

Therefore, the right way to grade a blastocyst is to document its degree of expansion along with grade of inner cell mass and trophoectoderm. For example a 3.1.1 blastocyst would signify an expanded blastocyst with grade 1 inner cell mass and grade 1 trophoectoderm.

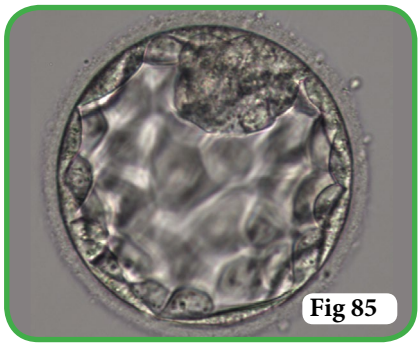


Fig: 85. Expanded Blastocysts with Grade 1 TE & Grade 1 ICM (3.1.1 blastocyst)

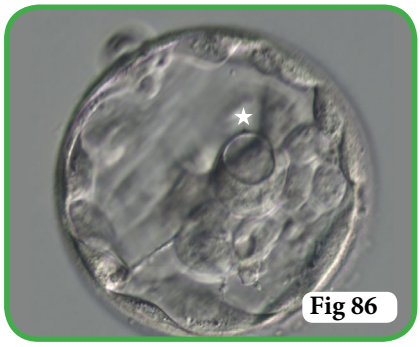


Fig: 86. Expanded Blastocysts with Grade 2 TE & Grade 1 ICM (3.1.2 blastocyst). Vacuole in the ICM is marked by *

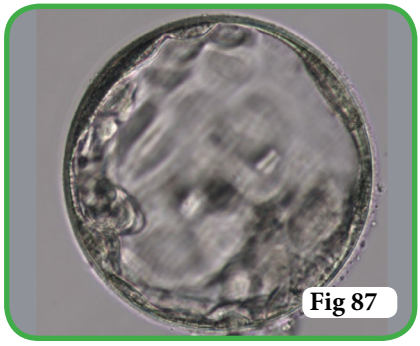


Fig: 87. Expanded Blastocysts with Grade 3 TE & Grade 3 ICM (3.3.3 blastocyst)

As per the Istanbul Consensus, it was agreed that ICM morphology carries a higher prognostic value for implantation and fetal development though a functional TE is also essential. [10]
Different authors have made different observations regarding prognostic value of TE, ICM or degree of expansion in terms of implantation potential & pregnancy outcome. [58, 59, 60, 61, 62]

The correct way of grading ICM & TE is by focusing it at different levels so that one can visualize and grade ICM & TE (Fig. 88).

Fig 88. Day 6 Blastocyst (4.1.1) : same blastocyst focused at different planes

Multiple sites of hatching can be seen in blastocysts especially ICSI generated where the breach created by microinjection pipette fails to close completely (Fig. 89).

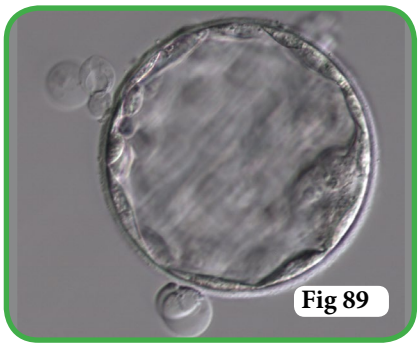


Fig 89. Blastocyst (4.2.2) with multiple sites of breach

At times, a cytoplasmic string connecting ICM with TE can be appreciated **(Fig. 90)**. This might contribute to the higher incidence of monozygotic twinning in in-vitro generated blastocysts.
Examples of different grades of blastocysts are shown in **(Fig. 91 to 93).** Please appreciate large ICMs of different shapes in **(Fig. 94)**

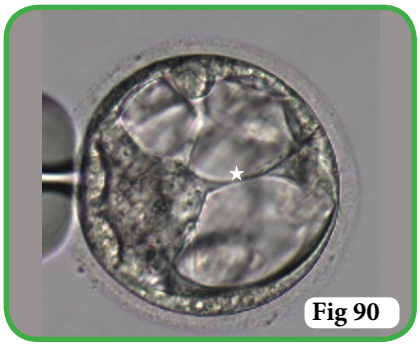


Fig 90. 3.1.2 Blastocyst with a prominent cytoplasmic string joining ICM & TE

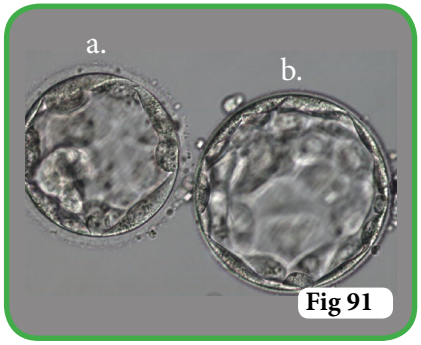


Fig 91a. 3.2.2 Blastocyst
Fig 91b. 3.1.2 Blastocyst

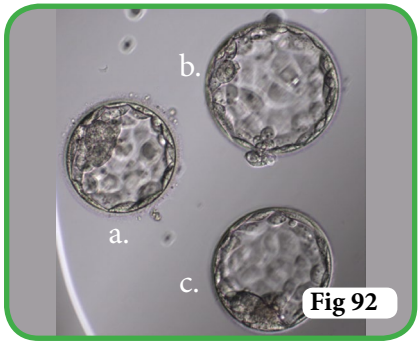


Fig 92a. 3.1.1 Blastocyst
Fig 92b. 4.2.1 Blastocyst
Fig 92c. 3.2.1 Blastocyst

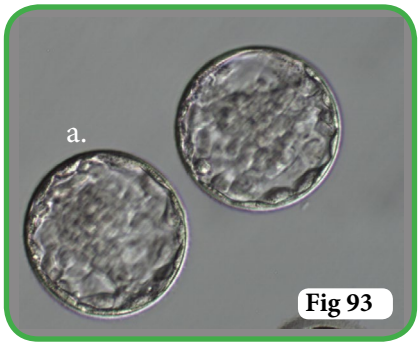


Fig: 93 a 3.1.3 Blastocyst
Fig: 93 b 3.1.2 Blastocyst

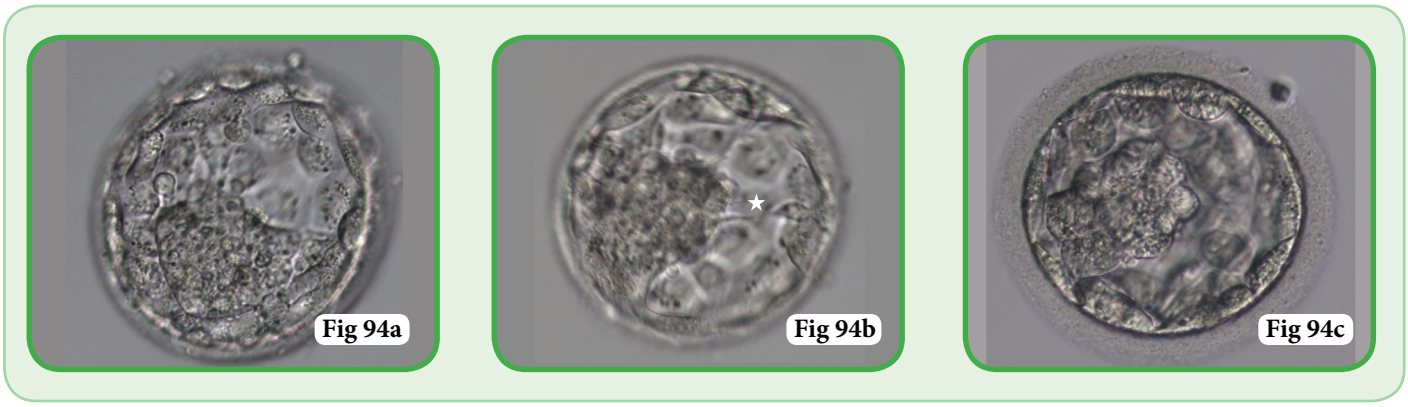


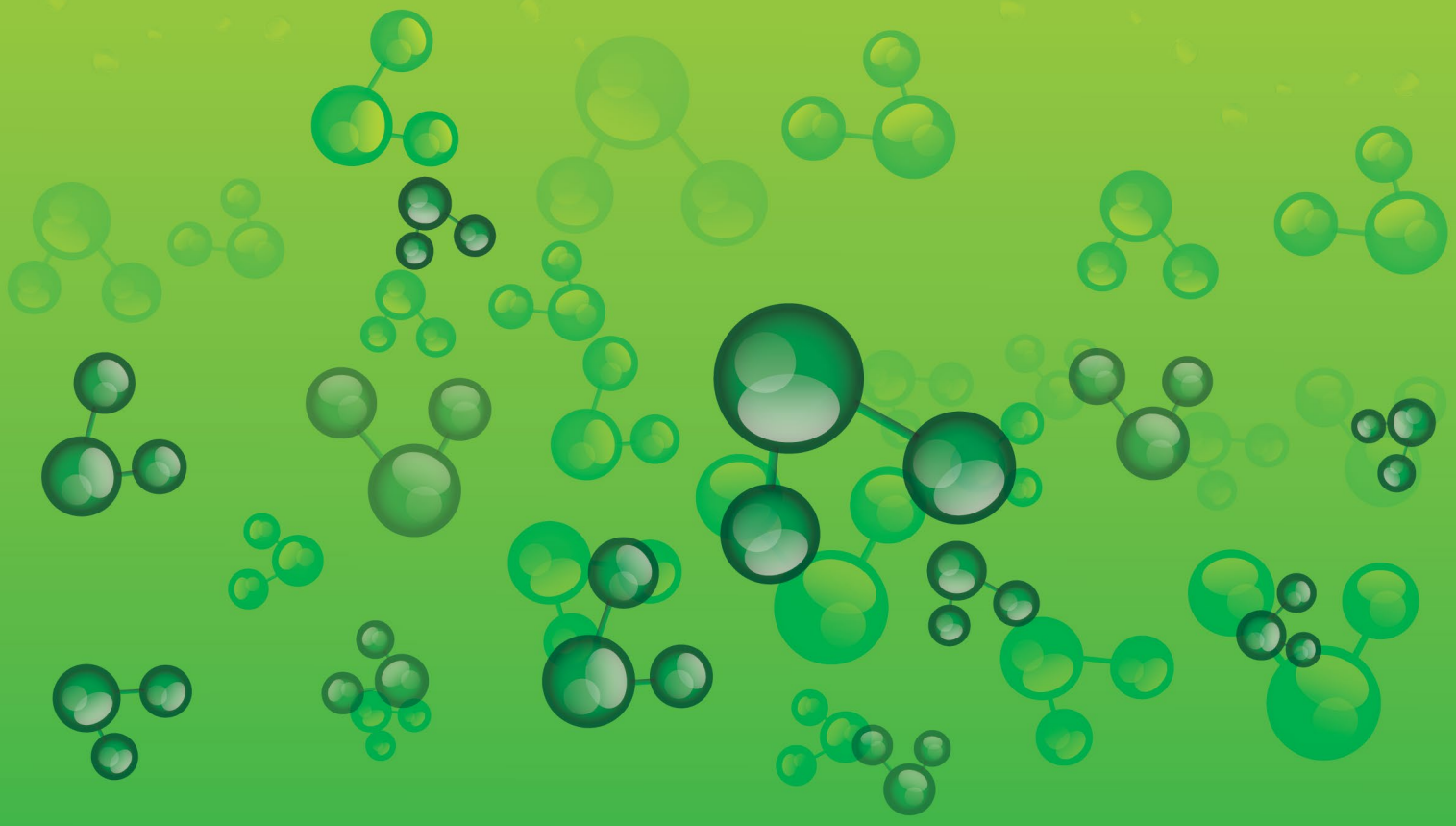
Fig 94. Expanded blastocysts with large ICM
a. Stellate shaped ICM (3.1.1 blastocysts)
b. Cytoplasmic string (*) connecting large ICM to TE (3.1.2 blastocyst)
c. Large mulberry shaped ICM (2.1.2 blastocyst)

Cellular degeneration in blastocysts can be appreciated. (Fig. 95) These blastocysts are irredeemable, continue to degenerate further and have no potential to implant and develop to term.

Fig 95. Cellular degeneration in blastocysts can be isolated or total.

Part : 2

Frequently Asked Questions (FAQs)



Index - Part : 2

1 How important it is to understand embryonic development and embryo quality?

Many studies have proven that embryo quality is currently the most important predictor of pregnancy. It helps achieve high implantation rates and avoids multiple gestation, Therefore, it is of utmost importance for any ART program to follow a standardized timeline for observing the developmental milestones of embryos as well as grading them.

2 Is it important to understand oocyte morphology?

During ovarian hormonal stimulation, the natural selection procedure is bypassed resulting in the maturation of many oocytes & compromising the oocyte quality. The embryonic development post insemination is dependant on oocyte's competence. The oocytes with morphological anomalies or alterations should be cultured separately and preference should be given to embryos resulting from oocytes with normal morphology during embryo transfer. Therefore, it is important to understand oocyte morphology.

3 Can all oocytes be injected during ICSI? Are there any contraindications?

After denudation, the oocyte morphology should be assessed and in the following morphological deviations, insemination is contraindicated:

1. **Giant oocyte:** Results from cytokinetic failure and leads to digynic triploidy if injected
2. **Oocytes with vacuoles more than 14 microns or multiple small vacuoles** occupying a significant part of ooplasm: can interfere with cleavage planes
3. **Oocytes with SER Discs:** Associated with poor obstetric outcome and imprinting disorders
4. **Oocytes with Giant PB:** Interferes with cleavage planes and associated with poor prognosis

4 What can be reasons if no 2PN are seen on Day 1 after ICSI? Should the oocytes be discarded or cultured further?

It is imperative to review the following aspects before deciding the fate of oocytes not showing 2PN on day 1.

a. Is it a partial fertilization failure, which is commonly seen, or is it an absolute failure of fertilization.
b. Status of embryos belonging to other patients in the same incubator.
c. Can we appreciate 1PN or >2PN.

Depending on these 3 points, the following reasons can be postulated:

A. Absolute failure:

a. Incorrect timing of observation post insemination
b. Incorrect handling of oocytes that is prolonged exposure to altered pH and temperature while preparing the oocytes for IVF/ICSI
c. Incubator malfunction:
    1. Altered temperature
    2. Interrupted gas supply and
    3. Interrupted power supply issues
d. Check the injector
e. Check the proficiency of operator

The data logs have to be reviewed for the last 24 hours. Other patient's embryos in the same incubator should be analysed for growth arrest.

B. Partial Failure: The oocytes with no signs of fertilization should be segregated, cultured and observed. The following possibilities are to be considered:

a. Total number and percentage of mature oocytes amongst the total retrieved oocytes should be documented.
b. The embryonic development can be either slow or fast in the embryos not showing 2PN. The fast growing embryos might show early cleavage at or before 25 hrs post insemination. The embryos should be examined on day 2 for cleavage.

In both the situations, embryos should never be discarded and examined on day 2 for any cleavage and depending on

5 How relevant is zygote scoring?

Zygote scoring is important as it helps us in isolating oocytes with polyspermy or zygotes showing more than 2PN. Zygote scoring is important in countries where embryos to be eventually transferred are selected at zygote stage and cultured further. Rest of the embryos are frozen. It excludes embryos with poor outcome. Embryos with poor zygote scoring result in slow development & poor blastocyst formation.

6 Once fragmentation appears, can it decrease over time?

Fragmentation can either be:
a. Definitive: Stable fragments are seen clearly detached from the blastomeres or
b. Transient: Fragments: appear to be part of blastomere and may disappear & decrease during later development.

7 After cell number, which is the most important parameter of embryo grading: fragmentation/ cell size/evenness of cells?

As per the ACE/NEQAS embryo grading system, the embryos should be graded in a stage specific way that is after cell number the cell size is analysed. Whereas the ASEBIR embryo assessment criteria, SART and Alpha consensus on embryo grading emphasize on fragmentation ahead of cell size.

8 Which grade embryos should be transferred? What are the chances of pregnancy with poor quality embryos?

The dictum is to transfer top, good and fair grade embryos and in that order of preference. Top embryos are defined as follows:

Day 2: 4 equal mononucleated blastomeres in a 3-dimensional tetrahedral arrangement, with <10% fragmentation
Day 3: 8 equal mononucleated blastomeres, with <10% fragmentation
Day 4: An optimal embryo at this stage would be compacted or compacting, which has entered into the fourth round of cleavage. Compaction should include virtually all the embryo volume.
Day 5: A fully expanded through to hatched blastocyst:

- ICM: Prominent, easily discernible, many cells;
- TE: Many cells forming a cohesive epithelium

The timing of observation should be followed as defined in the Consensus Workshop on Embryo Assessment.[10]

There are sufficient reports to demonstrate that low quality blastocysts can lead to live births with normal obstetric and perinatal outcomes.[63, 64] It has also been shown that grade C blastocysts are often euploid and result in live births, although they may also result in more miscarriages.[65]

9 What grade embryos should be vitrified on Day3/5?

As a rule of thumb, top and good embryos should be vitrified. A top embryo on day 3 is defined as an embryo with atleast 7 cells and less than 10 percent fragmentation. A good embryo is an embryo with 6 cells and upto 20% fragmentation or 8 cells with 20-50 percent fragmentation. An expanded blastocyst with Grade 1 ICM and Grade 1 TE is considered a top grade blastocyst.

10 Is there a hierarchy amongst different embryo morphology criteria ?

The process of embryo selection begins on Day 0 as soon as we retrieve the oocytes. It is advised that embryos developing from abnormal oocyte morphology be cultured separately. During further embryonic development any deviation from normal development invites a lower score. These developmental features help us in selecting or deselecting embryos if many are available at the time of embryo transfer with similar grades.
Various scientists and embryo grading systems have tried to relate the morphology parameters with the implantation potential.

Holte et al., 2007 studied **2266 embryos** and developed an integrated morphology cleavage score with parameters such as cell number, equal blastomere size and the number of mononucleated blastomeres on Day 2. These parameteres had a significant predictive value for implantation.[66]

Guerif et al. (2007) studied 4042 embryos and found that cell number at Day 2 and the incidence of early cleavage were the most predictive parameters for good blastocyst quality, while combining all parameters (pro-nuclear morphology, early cleavage, cell number, and incidence of fragmentation) gave a relatively poor prediction of embryo viability. It was also observed that the Day 2 morphology did not correlate with implantation potential once an embryo had reached the blastocyst stage and had good morphology.[67]

Rehman et al. (2007) also substantiated Guerif 's findings. He found that later stages of embryo development had higher sensitivity and specificity for predicting the implantation potential of an embryo. These observations suggest that there is an additional value in assessing blastocyst development for the prediction of embryo potential.[68]

In almost all the grading systems, cell number remains the most important criteria for cleavage stage embryos followed by fragmentation & symmetery. Multinucletaion and vacuolation as the other criteria for grading embryos in terms of their ability to implant. **Alpha consensus outlines some disqualifications for oocytes and embryos such as:**

1. Giant oocyte
2. Oocyte with exceptionally large PB
3. Oocytes with SER disks
4. Oocytes with vacuoles more than 14 micron
5. Zygote showing more than 2PN
6. Multinucleated embryos
7. Embryo with more than 50% fragmentation
8. Non-viable embryo: An embryo in which development has been arrested for at least 24 h, or in which all the cells have degenerated or lysed.

11 What should we do with excess blastocysts on Day 5?

The available blastocysts should be graded. An expanded blastocyst with Grade 1 ICM and Grade 1 TE is considered a top grade blastocyst. Many studies have shown that the minimal grade for blastocyst usability is 3.2.2. Any blastocyst stage 1 or 2, or ≥3 with a grade 'C' ICM or TE, would be deemed low quality. A blastocyst with degenerative foci (Fig. 95) is considered grade 3. While grade 'C' blastocysts are often transferred in fresh cycles, they are often not frozen or biopsied. Since low quality blastocysts can lead to live births with normal obstetric and perinatal outcomes this bias against freezing grade 'C' blastocysts has limited the establishment of a lower threshold for viability [67]

12 Can hatched blastocysts be transferred / vitrified?

Studies have shown that hatched blastocysts can be vitrified and warmed successfully followed by expansion after warming. Such embryos have resulted in pregnancies followed by delivery of healthy children. Some studies have shown a better survival in expanded blastocysts with open zona vs intact zona. [69, 70]

13 Is timed evaluation of embryo for grading sufficient or should other methods be employed for embryo selection?

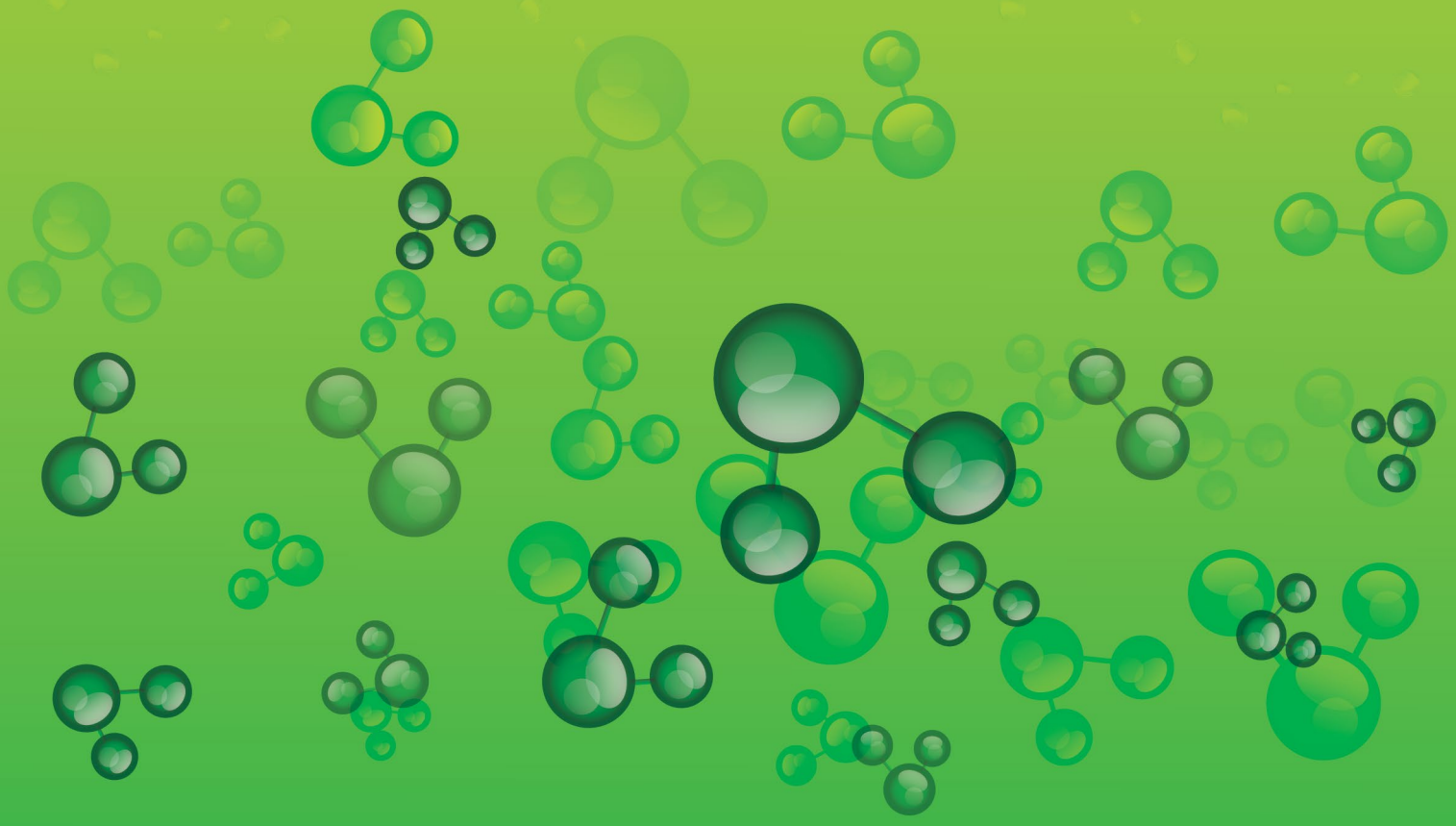
The most commonly used method is the microscopic or morphological evaluation of embryos. So, embryologists should be able to correlate the optical features of an embryo with its implantation potential.
There are many other methods available for grading embryos such as:

- **High-resolution videocinematography**
- **Computer-assisted morphometric analysis or Time Lapse analysis**
- Preimplantation genetic screening / diagnosis (PGS/PGD)
- **Culture of cumulus cells**
- Oxygen levels in follicular fluid / **perifollicular vascularization**
- Distribution of mitochondria and ATP levels in blastomeres
- Metabolic assessment of culture media **(amino acid profiling, metabolomics)**
- **Gene expression/expression of mRNA in cumulus cells and/or embryos**

Most of these methods are unaffordable, expensive and inconclusive.

Part : 3

Daily Practice Points



Index - Part : 3

Recommended guidelines on Oocyte and Embryo Grading

(Adapted from "The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction. 2011; 26 (6):1270–1283")

The Alpha Executive and ESHRE Special Interest Group of Embryology convened a two day workshop on 26 and 27 February 2010 in Istanbul, Turkey. The ultimate aim of this workshop was to establish the common criteria and terminology for grading oocytes, zygotes and embryos that would be accepted to the routine application in any IVF laboratory.

Consensus Points:

Following discussions related to each of the presentation, following consensus points were developed. These points are the first set of consensus recommendations for oocyte and embryo scoring and also represent the **'minimum standards'** for oocyte and embryo morphology scoring.

1. Timing and reporting of observation of fertilized oocytes and embryos

It was noted that standardized timing of observations is critical to compare the results between different laboratories, and that this should be relative to the time of insemination and uniformly presented in assessment reports as hours post insemination.

Consensus was that these must be reported separately, in association with the time of post-insemination. **(Table 1)**

Type of Observation	Timing (hours post- insemination)	Expected stage of development
Fertilization check	17 ± 1	Pronuclear stage
Syngamy check	23 ± 1	Expect 50 % to be in syngamy (up to 20% may be at the 2- cell stage)
Early cleavage check	26 ± 1 h post- ICSI 28 ± 1 h post-IVF	2-cell stage
Day 2 embryo assessment	44 ± 1	4-cell stage
Day 3 embryo assessment	68 ± 1	8-cell stage
Day 4 embryo assessment	92 ± 2	Morula
Day 5 embryo assessment	116 ± 2	Blastocyst

Table 1: Timing of observation of fertilized oocytes and embryos (adapted from Istanbul consensus 2011)

2. Oocyte scoring

The optimal oocyte morphology is that of spherical structure enclosed by a uniform zona pellucida, with a uniform translucent cytoplasm free of inclusions and a size-appropriate polar body. Detailed scoring is summarised in **Table 2**

Oocyte components	Consensus points	Scoring
Cumulus-oocyte complex (COC)	COC scoring provides an important tool for troubleshooting	**Binary score (0 or 1) with a 'good' COC (score 1)** defined as having expanded cumulus and a radiating corona.
Zona pellucida scoring	There was no specific benefit of measuring zona thickness was observed, and it was agreed that there is insufficient evidence for any effect on outcome.	Colour and thickness of the zona pellucida should be made in case of patient- specific effects.
Perivitelline space (PVS)	Presence of inclusions in the PVS is anomalous but there was insufficient evidence to support this observation.	Inclusions in PVS should be noted but there is no requirement to count or measure them. Also, a note of PVS should only be made if it is exceptionally large
Polar body scoring (PBS)	**Presence or absence of the first polar body** should be noted in the uninseminated oocyte, where possible.	Oocytes with an abnormally **large polar body** should not be inseminated, due to risk of oocyte aneuploidy
Cytoplasm scoring	Homogenous cytoplasm is expected, and that non-homogeneous cytoplasm is of unknown biological significance and based on current evidence, may represent variability between oocytes rather than a 'dysmorphism' of developmental significance	**Granularity** of cytoplasm is ill-defined and distinctly different from clustering of organelles. **Clustering** is associated with the risk of a serious, significantly abnormal outcome. **sER** disks are associated with the risk of a serious, significantly abnormal outcome.
Vacuolization	In fertilized oocytes, **vacuoles** that persist **past syngamy** can interfere with cleavage planes resulting in lower blastocyst rate. Hence, large vacuoles in the oocyte should be noted.	**Few small vacuoles (5-10 µm in diameter)** that are fluid filled but transparent are of no biological consequence. **Large vacuoles (more than 14 µm in diameter)** are associated with fertilization failure.

Table 2: Oocyte scoring

3. Fertilization check

The optimal fertilized oocyte should be

- Spherical,
- Have two polar bodies, with two centrally located, juxtaposed and even sized pronuclei with distinct membranes.
- The pronuclei should have equivalent numbers and size of Nucleolus Precurssor Bodies (NPBs) that are ideally equatorially aligned at the region of membrane juxtaposition.

S. No.	Consensus Points
1.	Both pronuclear size and location should be assessed at fertilization check
2.	Following features of pronuclei are severely atypical: widely separated pronuclei; pronuclei of grossly different sizes; micronuclei.
3.	Presence of sER disks should be assessed as part of the fertilization check (if IVF, rather than ICSI was performed) and such oocytes should not be transferred.
4.	There is insufficient evidence to support a prognostic value for the observation of a peripheral cytoplasmic translucency in the fertilized oocyte (a 'halo')

Table 3: Consensus points on Fertilization check

i. Pronuclear scoring

Pronuclear scoring provides additional information to the fertilization check, and that both should be performed at the same time. There should be three categories: symmetrical; non-symmetrical; and abnormal. The abnormal category includes pronuclei with no NPBs (so-called 'ghost pronuclei'), and those with a single NPB ('bulls-eye pronuclei"), which have been associated with abnormal outcomes in animal models.

Category	Rating	Description
1.	Symmetrical	Equivalent to Z1 and Z2
2.	Non-symmetrical	Other arrangements, including peripherally sited pronuclei
3.	Abnormal	Pronuclei with 0 or 1 NPB

Table 4: Consensus scoring system for pronuclei (adapted from Istanbul consensus)

4. Cleavage-stage embryos

i. Assessment of cell number : Table 5

Rate of cleavage	Significance
Embryos cleaving more slowly than the expected rate	Have reduced implantation potential
Embryos cleaving faster than the expected rate	These embryos are likely to be abnormal and have reduced implantation potential

Hence, the current expected observation for embryo development is 4 cells on Day 2 and 8 cells on Day 3, depending on the time elapsed post-insemination.

ii. Fragmentation

A fragment was defined as an extracellular membrane-bound cytoplasmic structure that is less than 45 µm diameter in a Day-2 embryo and less than 40 µm diameter in a Day-3 embryo.

Grade	Rating	Description
1.	**Mild**	• Less than **10% fragmentation** • Stage specific cell size • No multinucleation
2.	**Moderate**	• **10– 25% fragmentation** • Stage-specific cell size for majority of cells • No evidence of multinucleation
3.	**Severe**	• **Severe fragmentation (more than 25%)** • Cell size not stage specific • Evidence of multinucleation

Table 6: The relative degrees of fragmentation for cleavage stage embryo (adapted from Istanbul consensus)

The definition of the impact of fragment localization could not be included, as this can be a dynamic phenomenon, i.e. the fragments can move within the embryo.

iii. Multinucleation

- Multinucleation is the presence of more than one nucleus in a blastomere, and includes micronuclei.
- Multinucleation is associated with a decreased implantation potential, and that multinucleated embryos are associated with an increased level of chromosome abnormality and, as a consequence, increased risk of spontaneous abortion.
- Multinucleation assessment should be performed on Day 2 (i.e. 44+1 h post-insemination), and that the observation of multinucleation in one cell is sufficient for the embryo to be considered to be multinucleated.
- Laboratories should record the incidence of multinucleation in each embryo, and ideally, the nucleation status of each blastomere in each Day-2 embryo.
- The grading scheme for multinucleation should be binary, noting its presence or absence.

iv. Cell size

For embryos at the 2, 4 and 8-cell stages, blastomeres should be even sized. For all other cell stages, one would expect a size difference in the cells, as the cleavage phase has not been completed.

The grading scheme for cell size should be binary, noting whether all cell sizes are stage appropriate.

v. Other morphological features of Day-2 and -3 embryos

- Other morphological features, such as **cytoplasmic granularity, membrane appearance** and the **presence of vacuoles,** can also be scored as part of the morphological assessment of Day-2 and Day-3 embryos.
- It is important to understand that these features can vary between a patient's embryos and between patients.
- At this stage, there is no significant body of evidence to support a clear biological effect of these features on implantation potential. Therefore, more research is required to identify which, if any, of these features are correlated with (or indicative of) implantation potential.
- Also, embryos with **apparent spatial disorganization,** i.e. those that do not have the expected three dimensional arrangement of blastomeres, there is no conclusive evidence that they are abnormal.
- In addition, it was noted that while **early compaction** on Day 3 is atypical, this observation is of unknown biological significance.

vi. Cleavage-stage embryo scoring system

An optimal **Day-2 embryo (44+1 h post-insemination)** would **have 4 equally sized mononucleated blastomeres in a three-dimensional tetrahedral arrangement, with 10% fragmentation** whereas an optimal **Day-3 embryo (68+1 h post-insemination) would have 8 equally sized mononucleated blastomeres, with 10% fragmentation.** The consensus scoring system for cleavage-stage embryos is presented in **Table 7.**

Stage of development	Timing (hours post insemination)	Cell number	Degree of fragmentation
Day 2 embryo (4 cell)	44 ± 1 h	4 equally sized mononucleated blastomeres in a 3D tetrahedral arrangement	Less than 10% fragmentation
Day 3 (6-8 cell)	68 ± 1 h	8 equally sized mononucleated blastomeres	Less than 10% fragmentation

Table 7: Cleavage stage embryo scoring system

5. Day 4 assessment (Morula stage)

An optimal embryo at this stage (92+2 h) would be compacted or compacting, and have entered into a fourth round of cleavage. Compaction should include virtually all the embryo volume. Also morphology of Day- 4 embryo will include apparently excluded cells, the effect of which is unclear. The exception is that if more than half of the embryo is excluded, it was agreed that this is likely to be associated with a poor prognosis. Scoring system for Day-4 embryos is presented in **Table 8.**

Grade	Rating	Description
1.	**Good**	• Entered into a fourth round of cleavage • Evidence of compaction that involves virtually all the embryo volume
2.	**Fair**	• Entered into a fourth round of cleavage. • Compaction involves the majority of the volume of the embryo
3.	**Poor**	• Disproportionate compaction involving less than half of the embryo, with two or three cells remaining as discrete blastomeres

6. Day 5 assessment (Blastocyst stage)

An optimal embryo at this stage (92+2 h) would be compacted or compacting, and have entered into a fourth round of cleavage. Compaction should include virtually all the embryo volume. Also morphology of Day- 4 embryo will include apparently excluded cells, the effect of which is unclear. The exception is that if more than half of the embryo is excluded, it was agreed that this is likely to be associated with a poor prognosis. Scoring system for Day-4 embryos is presented in **Table 8.**

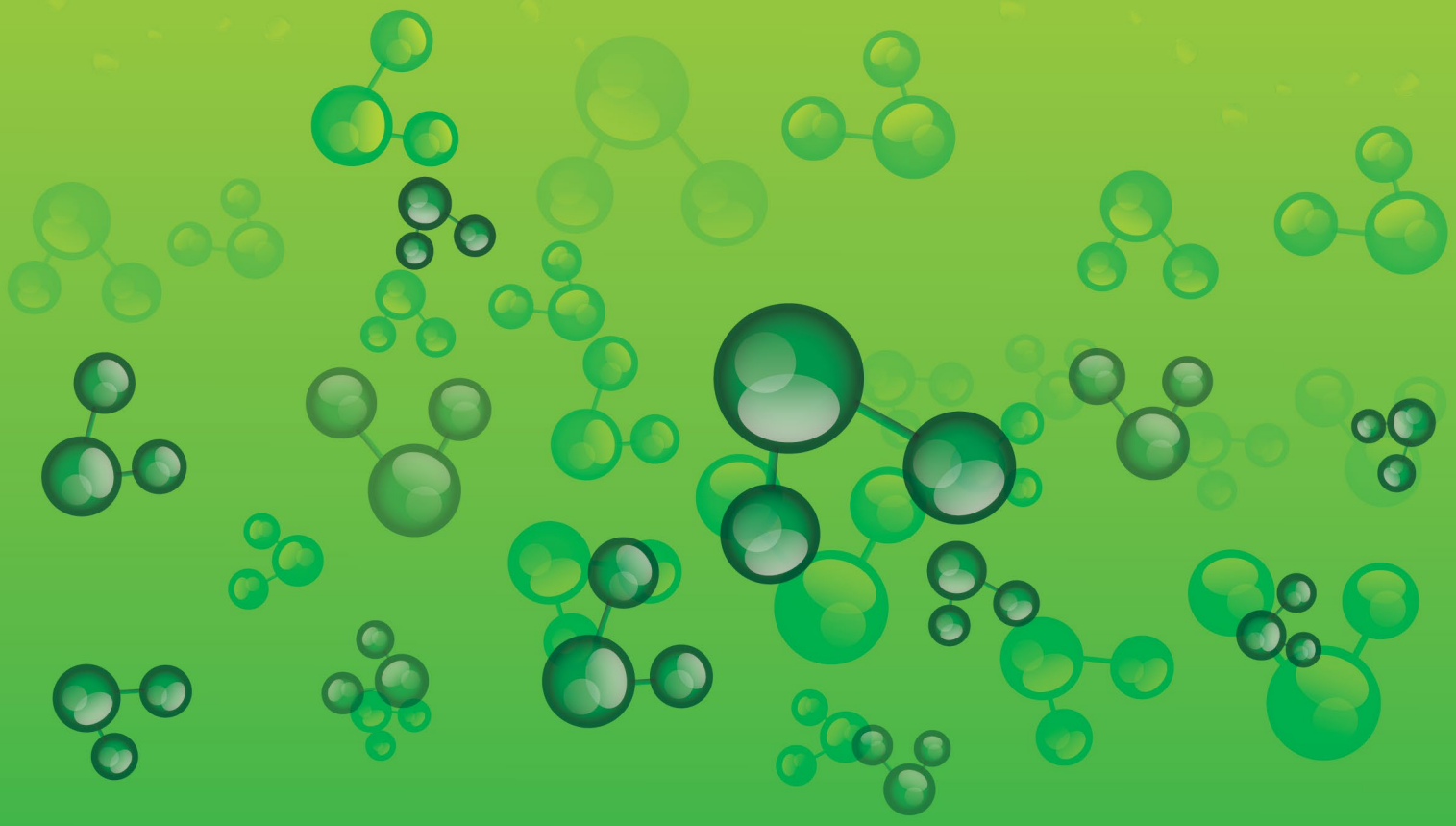
	Grade	Rating	Description
Stage of development	**1.**	-	Early
	2.	-	Blastocyst
	3.	-	Expanded
	4.	-	Hatched/hatching
Inner Cell Mass (ICM)	**1.**	**Good**	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2.	**Fair**	Easily discernible, with many cells that are loosely grouped together
	3.	**Poor**	Difficult to discern, with few cells
Trophoectoderm (TE)	**1.**	**Good**	Many cells forming a cohesive epithelium
	2.	**Fair**	Few cells forming a loose epithelium
	3.	**Poor**	Very few cells

Table 9. The consensus scoring for a Day 5 embryo

- It was also noted that while the ICM has a high prognostic value for implantation and fetal development, a functional TE is also essential.
- 'Hatching' is defined as the obvious emergence of the TE with enclosed blastocoel through a thinning zona pellucida. Also, hatching cannot be reliably assessed in embryos with an artificially breached zona pellucida (with the exception of the breach made during ICSI).
- If a blastocyst is collapsed at the time of assessment, it cannot be graded reliably. These blastocysts should be re-evaluated 1–2 h later, as regular cycles of collapse and re-expansion of blastocysts is normal.

Part : 4

References



References

1. Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age PhilippeTerriou, ChristopheSapin, ClaudeGiorgetti. EricHansaJean-LouisSpach, RogerRoulier. Fertility and Sterility. Volume 75, Issue 3, March 2001, Pages 525-531.
2. Fei Wan, Dina Appleby, Linli Hu, Hanwang Zhang. Quality of embryos transferred and progesterone levels are the most important predictors of live birth after fresh embryo transfer: a retrospective cohort study. Journal of Assisted Reproduction and Genetics. February 2014, Volume 31, Issue 2, pp 185–194.
3. Swain JE, Pool TB. ART failure: oocyte contributions to unsuccessful fertilization. Hum Reprod Update 2008; 14:431–446.
4. Balakier H, Bouman D, Sojecki A, Librach C, Squire JA. Morphological and cytogenetic analysis of human giant oocytes and giant embryos. Hum Reprod 2002;17:2394–2401.
5. Rosenbusch B, Hancke K. Conjoined human oocytes observed during assisted reproduction: description of three cases and review of the literature. Rom J Morphol Embryol 2012;53:189–192.
6. S. Kahraman K. Yakın E. Dönmez H. Şamlı M. Bahçe G. Cengiz S. Sertyel M. Şamlı N. İmirzalıoğlu. Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. Human Reproduction, Volume 15, Issue 11, 1 November 2000, Pages 2390–2393.
7. T Ebner, M Moser, O Shebl, M Sommergruber, C Yaman, G Tews. Blood clots in the cumulus–oocyte complex predict poor oocyte quality and post-fertilization development. RBM online Vol 16 No 6. 2008 801-807.
8. Rienzi L, Ubaldi FM, Iacobelli M, Minasi MG, Romano S, Ferrero S, Sapienza F, Baroni E, Litwicka K, Greco E. Significance of metaphase II human oocyte morphology on ICSI outcome. Fertil Steril 2008; 90:1692 – 1700.
9. Thomas Ebner, , Marianne Moser, Michael Sommergruber,, Ute Gaiswinkler, Omar Shebl, Klaus Jesacher, Gernot Tews. Occurrence and developmental consequences of vacuoles throughout preimplantation development. June 2005Volume 83, Issue 6, Pages 1635–1640.
10. Alpha Scientists in Reproductive medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011; 26:1270 – 1283.
11. Ebner T, Moser M, Shebl O, Sommerguber M, Tews G. Prognosis of oocytes showing aggregation of smooth endoplasmic reticulum. Reprod Biomed Online 2008;16:113–118.
12. Otsuki J, Okada A, Morimoto K, Nagai Y, Kubo H. The relationship between pregnancy outcome and smooth endoplasmic reticulum clusters in MII human oocytes. Hum Reprod 2004;19:1591–1597.
13. Ten J1, Mendiola J, Vioque J, de Juan J, Bernabeu R.. Donor oocyte dysmorphisms and their influence on fertilization and embryo quality. Reprod Biomed Online. 2007 Jan;14(1):40-8.
14. Esfandiari N, Burjaq H, Gotlieb L, Casper RF. Brown oocytes: implications for assisted reproductive technology. Fertil Steril 2006;86:1522–1525.
15. Fancsovits P, Tothne Z, Murber A, Takacs FZ, Papp Z, Urbancsek J. Correlation between first polar body morphology and further embryo development. Acta Biol Hung 2006;57:331–338.
16. Laura Rienzi, Basak Balaban, Thomas Ebner and Jacqueline Mandelbaum. The oocyte. Human Reproduction, Vol.27, No.S1 pp. i2–i21, 2012.
17. Hassan-Ali H, Hisham-Saleh A, El-Gezeiry D, Baghdady I, Ismaeil I, Mandelbaum J. Perivitelline space granularity: a sign of human menopausal gonadotrophin overdose in intracytoplasmic sperm injection. Hum Reprod 1998;13:3425–3430.
18. Miao YL, Kikuchi K, Sun QY, Schatten H. Oocyte aging: cellular and molecular changes, developmental potential and reversal possibility. Hum Reprod Update 2009;15:573–585.
19. Wilding M, Di ML, D'Andretti S, Montanaro N, Capobianco C, Dale B. An oocyte score for use in assisted reproduction. J Assist Reprod Genet. 2007;24:350–358.
20. Scott L. Pronuclear scoring as a predictor of embryo development. Reprod Biomed Online. 2003 Mar;6(2):201-14.
21. Scott L1, Alvero R, Leondires M, Miller B. The morphology of human pronuclear embryos is positively related to blastocyst development and implantation. Hum Reprod. 2000 Nov;15(11):2394-403.
22. Hardarson T, Hanson C, Sjo ¨ gren A, Lundin K. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. Hum Reprod 2001;16:313 – 318.
23. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. Hum Reprod 2001;16:2652–2657.

24. Hardarson T, Selleskog U, Reismer E, Wannerström S, Westin C, Ahlstro¨ m A, Hanson C. Zygotes cleaving directly into more than two cells after 25 – 27 h in culture are predominantly chromosomally abnormal. Hum Reprod 2006;21:i102.
25. Alikani M, Calderon G, Tomkin G, Garrisi J, Kokot M, Cohen J. Cleavage anomalies in early human embryos and survival after prolonged culture in-vitro. Hum Reprod 2000;15:2634–2643.
26. Fisch JD, Rodriguez H, Ross R, Overby G, Sher G. The Graduated Embryo Score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. Hum Reprod 2001;16:1970–1975.
27. Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, Bergh T. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod 2007;22:548–557.
28. Scott L, Finn A, O'Leary T, McLellan S, Hill J. Morphologic parameters of early cleavage-stage embryos that correlate with fetal development and delivery: prospective and applied data for increased pregnancy rates. Hum Reprod 2007;22:230–240.
29. Finn A, Scott L, O'leary T, Davies D, Hill J. Sequential embryo scoring as a predictor of aneuploidy in poor-prognosis patients. Reprod Biomed Online 2010;21:381–390.
30. Almeida PA1, Bolton VN. The relationship between chromosomal abnormality in the human preimplantation embryo and development in vitro. Reprod Fertil Dev. 1996;8(2):235-41.
31. Magli MC, Gianaroli L, Ferraretti AP, Lappi M, Ruberti A, Farfalli V. Embryo morphology and development are dependent on the chromosomal complement. Fertil Steril 2007;87:534–540.
32. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. Hum Reprod 1997;12:1545 – 1549.
33. Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W, Gerris J. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999;14:2345–2349.
34. Hourvitz A, Lerner-Geva L, Elizur SE, Baum M, Levron J, David B, Meirow D, Yaron R, Dor J. Role of embryo quality in predicting early pregnancy loss following assisted reproductive technology. Reprod Biomed Online 2006;13:504–509.
35. Johansson M, Hardarson T, Lundin K. There is a cut off limit in diameter between a blastomere and a small anucleate fragment. J Assist Reprod Genet 2003;20:309–313.
36. Van Blerkom J, Davis P, Alexander S. A microscopic and biochemical study of fragmentation phenotypes in stage-appropriate human embryos. Hum Reprod 2001;16:719–729.
37. Van Royen E, Mangelschots K, De Neubourg D, Laureys I, Ryckaert G, Gerris J. Calculating the implantation potential of day 3 embryos in women younger than 38 years of age: a new model. Hum Reprod 2001;16:326 – 332.
38. Racowsky C, Jackson KV, Cekleniak NA, Fox NH, Hornstein MD, Ginsburg ES. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. Fertil Steril 2000; 73:558 – 564.
39. Magli MC1, Gianaroli L, Ferraretti AP. Chromosomal abnormalities in embryos. Mol Cell Endocrinol. 2001 Oct 22;183 Suppl 1:S29-34.
40. Kirkegaard K, Agerholm I, Ingerslev HJ. Time-lapse monitoring as a tool for clinical embryo assessment. Hum Reprod 2012;27:1277–1285.
41. Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod 2011;26:2658–2671.
42. Van Royen E, Mangelschots K, Vercruyssen M, De Neubourg D, Valkenburg M, Ryckaert G, Gerris J. Multinucleation in cleavage stage embryos. Hum Reprod 2003;18:1062–1069.
43. Winston NJ, Braude PR, Pickering SJ, George MA, Cant A, Currie J, Johnson MH. The incidence of abnormal morphology and nucleocytoplasmic ratios in 2-, 3- and 5-day human pre-embryos. Hum Reprod 1991;6:17–24.
44. Pickering SJ, Taylor A, Johnson MH, Braude PR. Diagnosing and preventing inherited disease: an analysis of multinucleated blastomere formation in human embryos. Human reprod 1995; 10: 1912-22.
45. Edwards RG, Beard HK. Oocyte polarity and cell determination in early mammalian embryos. Mol Hum Reprod 1997;3:863–905.
46. Edwards RG, Hansis C. Initial differentiation of blastomeres in 4-cell human embryos and its significance for early embryogenesis and implantation. Reprod Biomed Online 2005;11:206–218.
47. Rienzi L, Ubaldi F, Minasi MG, Iacobelli M, Martinez F, Tesarik J, Greco E. Blastomere cytoplasmic granularity is unrelated to developmental potential of day 3 human embryos. J Assist Reprod Genet 2003; 20:314–317.
48. Ebner T, Tews G, Sommergruber M, Moser M. Cytoplasmic pitting has a negative influence on implantation outcome. J Assist Reprod Genet 2005b; 22:239 – 244.

49. Veeck LL. Preembryo grading and degree of cytoplasmic fragmentation. In: An Atlas of Human Gametes and Conceptuses: An Illustrated Reference for Assisted Reproductive Technology. New York, USA: Parthenon Publishing, 1999, 46–51.
50. Ebner T, Moser M, Sommergruber M, Gaiswinkler U, Shebl O, Jesacher K, Tews G. Occurrence and developmental consequences of vacuoles throughout preimplantation development. Fertil Steril 2005a;83:1635–1640.
51. Skiadas CC, Jackson KV, Racowsky C. Early compaction on day 3 may be associated with increased implantation potential. Fertil Steril 2006; 86:1386 – 1391.
52. Tao J, Tamis R, Fink K, Williams B, Nelson-White T, Craig R. The neglected morula/compact stage embryo transfer. Hum Reprod 2002; 17:1513 – 1518.
53. Cauffman G, De Rycke M, Sermon K, Liebaers I, Van de Velde H. Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos. Hum Reprod 2009; 24:63 – 70.
54. Alikani M. Epithelial cadherin distribution in abnormal human pre-implantation embryos. Hum Reprod 2005;20:3369–3375.
55. Desai NN, Goldstein J, Rowland DY, Goldfarb JM. Morphological evaluation of human embryos and derivation of an embryo quality scoring system specific for day 3 embryos: a preliminary study. Hum Reprod 2000;15:2190–2196.
56. Feil D, Henshaw RC, Lane M. Day 4 embryo selection is equal to Day 5 using a new embryo scoring system validated in single embryo transfers. Hum Reprod 2008;7:1505–1510.
57. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In Jansen R, Mortimer D (eds). Toward Reproductive Certainty: Fertility and Genetics Beyond 1999. London: Parthenon Publishing 1999a,378–388.
58. Chen X1, Zhang J, Wu X, Cao S, Zhou L, Wang Y, Chen X, Lu J, Zhao C, Chen M, Ling X. Trophectoderm morphology predicts outcomes of pregnancy in vitrified-warmed single-blastocyst transfer cycle in a Chinese population. J Assist Reprod Genet. 2014 Nov;31(11):1475-81. doi: 10.1007/s10815-014-0317-x. Epub 2014 Aug 16.
59. Stephanie Marshall Thompson, Ndidiamaka Onwubalili, Kelecia Brown, Sangita K. Jindal, and Peter G. McGovern. Blastocyst expansion score and trophectoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer (eSET): a national study. J Assist Reprod Genet. 2013 Dec; 30(12): 1577–1581.
60. Subira J1, Craig J1, Turner K1, Bevan A1, Ohuma E2, McVeigh E1, Child T1, Fatum M1. Grade of the inner cell mass, but not trophectoderm, predicts live birth in fresh blastocyst single transfers. Hum Fertil (Camb). 2016 Dec;19(4):254-261. Epub 2016 Sep 14.
61. Almagor M1, Harir Y2, Fieldust S2, Or Y2, Shoham Z2 Ratio between inner cell mass diameter and blastocyst diameter is correlated with successful pregnancy outcomes of single blastocyst transfers. Fertil Steril. 2016 Nov;106(6):1386-1391. doi: 10.1016/j.fertnstert.2016.08.009. Epub 2016 Aug 24.
62. Du QY1, Wang EY1, Huang Y1, Guo XY1, Xiong YJ1, Yu YP1, Yao GD1, Shi SL1, Sun YP2. Blastocoele expansion degree predicts live birth after single blastocyst transfer for fresh and vitrified/warmed single blastocyst transfer cycles. Fertil Steril. 2016 Apr;105(4):910-919.e1. doi: 10.1016/j.fertnstert.2015.12.014. Epub 2016 Jan 8.
63. Irani M, Reichman D, Robles A, Melnick A, Davis O, Zaninovic N, Xu K, Rosenwaks Z. Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates. Fertil Steril 2017;107:664–670.
64. Wirleitner B, Schuff M, Stecher A, Murtinger M, Vanderzwalmen P. Pregnancy and birth outcomes fol-lowing fresh or vitrified embryo transfer according to blastocyst morphology and expansion stage, and cul-turing strategy for delayed development. Hum Reprod 2016;31:1685.
65. Dean E. Morbeck. Blastocyst culture in the Era of PGS and FreezeAlls: Is a 'C' a failing grade? Human Reproduction Open, pp. 1–6, 2017
66. Holte L. Berglund K. Milton C. Garello G. Gennarelli A. Revelli T. Bergh. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval J. Human Reproduction, Volume 22, Issue 2, 1 February 2007, Pages 548–557,
67. F. Guerif A. Le Gouge B. Giraudeau J. Poindron R. Bidault O. Gasnier D. Royere. Limited value of morphological assessment at days 1 and 2 to predict blastocyst develop-ment potential: A prospective study based on 4042 embryos Human Reproduction, Volume 22, Issue 7, 1 July 2007, Pages 1973–1981, https://doi.org/10.1093/humrep/dem100
68. Khurram S. Rehman, Orhan Bukulmez, Martin Langley, Bruce R. Carr, Anna C. Nackley, Kathleen M. Doody, Kevin J. Doody. Late stages of embryo progression are a much better predictor of clinical pregnancy than early cleavage in intracytoplasmic sperm injection and in vitro fertilization cycles with blastocyst-stage transfer Fertility and Sterilty. May 2007Volume 87, Issue 5, Pages 1041–1052.
69. Zech NH[1], Lejeune B, Zech H, Vanderzwalmen P. Vitrification of hatching and hatched human blastocysts: effect of an opening in the zona pellucida before vitrification. Reprod Biomed Online. 2005 Sep;11(3):355-61.

70. Hiraoka K[1], Hiraoka K, Kinutani M, Kinutani K. Vitrification of human hatched blastocysts: a report of 4 cases. J Reprod Med. 2007 May;52(5):413-5.

Notes

Notes

Organised by

INDIAN FERTILITY SOCIETY

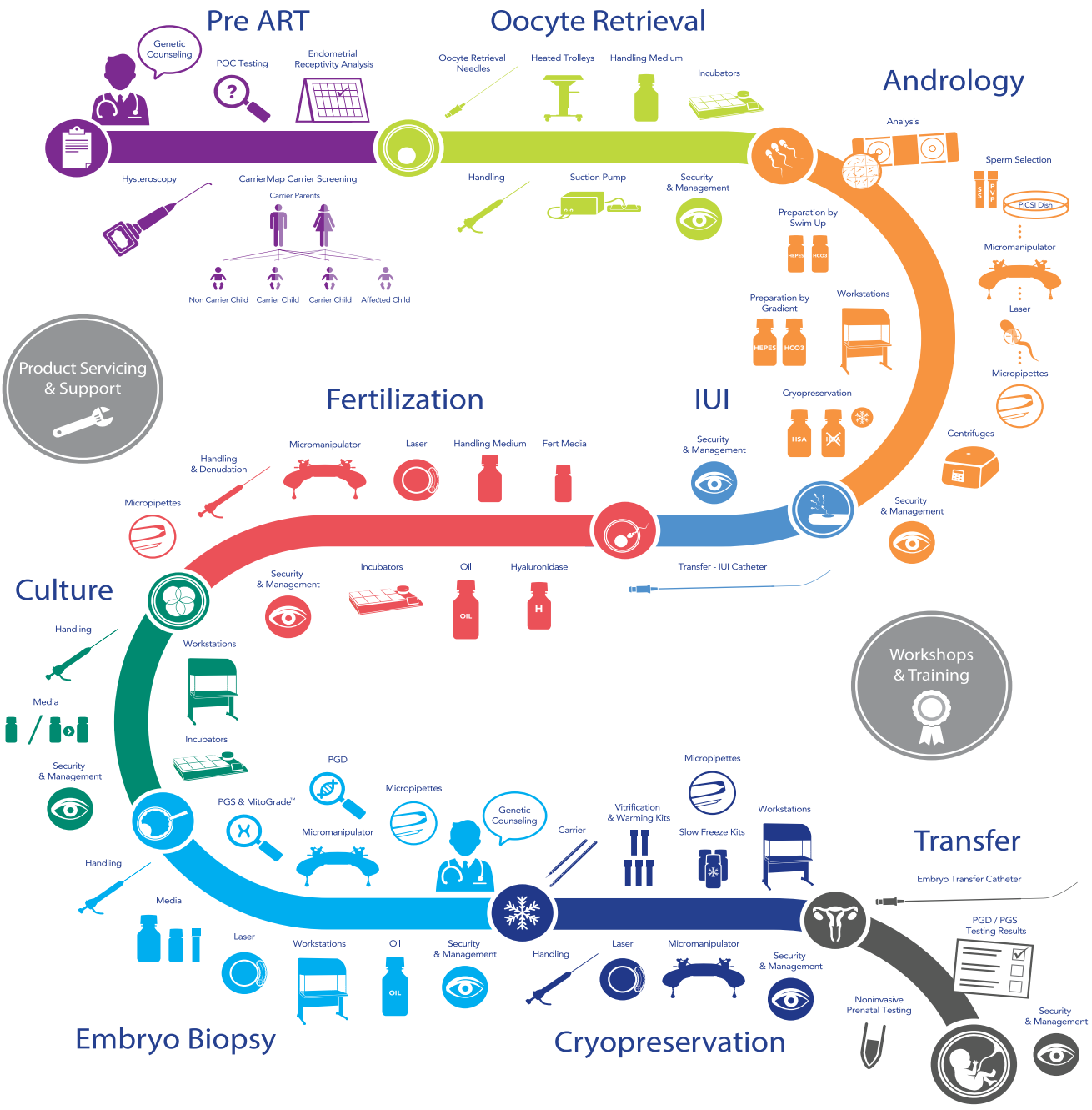
15th Annual Congress of Indian Fertility Society

FERTIVISION 2019

6-8 December
New Delhi | India

Theme: *Beyond Tomorrow*

www.fertivision2019.com



Next Generation ART Solutions

ORIGIO India Private Limited
C-401, Delphi, Hiranandani Business Park, Powai, Mumbai-400 076, Maharashtra, India
Tel.: +91 22-49280000 | Fax : +91 22-49280010
Website : www.coopersurgical.com / www.origio.com

For queries and feedback

Dr (Prof) Pankaj Talwar
Editor Nexus
Secretary General - Indian Fertility Society
E: pankaj_1310@yahoo.co.in, M: +91 9810790063