The most profound dilemma in assisted reproduction to date is the inability to recognize potentially viable embryos before their replacement into the reproductive tract. Application of increasingly advanced new technology has allowed the field of embryo evaluation to evolve rapidly and dramatically over the past five years.

Human Preimplantation Embryo Selection assembles the leaders in the field of assisted reproduction to discuss their visions of dealing with the topic of embryo selection in the light of the limitations involving the fragility of the early human embryo. The purpose of this book is to shed light on the ever-expanding techniques that are being employed and evaluated with regard to embryo selection. A comprehensive overview may guide the ART practitioner in choice of techniques and evaluation of benefits and drawbacks.

This book will provide an up-to-date overview and reference source: an invaluable tool for assisted reproductive practice worldwide.
Human Preimplantation Embryo Selection
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Human Preimplantation
Embryo Selection

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Preface

Assisted reproductive technology (ART) is a numbers game, with permutations that involve the transfer of multiple embryos... but the most important number in IVF is of course the number one. One embryo, one sac, one fetus and one healthy baby – the ability to choose just one embryo that will lead to the successful birth of a baby is what we all crave in our profession. Seeking just this is the name of the game, the “Holy Grail of IVF”, as suggested by some of the authors in this book.

The early pioneers of human IVF very quickly observed that not all gametes and embryos had the same potential to establish an ongoing pregnancy, and only a small proportion of oocytes that fertilized in vitro was truly viable. This was quickly followed by noting that, contrary to established experience in animal models such as the mouse, there is an obvious diversity in human embryo morphology and implantation potential. Although a correlation could be seen between outcome and morphological phenomena such as fragmentation, it was generally accepted that aesthetic appreciation – ‘embryonic looks’ – could be deceiving, and even the ‘ugliest’ embryo of a cohort can sometimes develop into a beautiful healthy baby. After 30 years of clinical IVF treatment, we have learned a great deal about human embryos – but there is still so much left to explore.

The absence of absolute criteria that can predict the implantation potential of an embryo brings to mind the proverbial principle illustrated by the threesome of the Japanese Wise Monkeys – ‘to see no evil, hear no evil, and to speak no evil’. The practice of blindly compensating for lack of appropriate embryonic viability testing by transferring large groups of embryos is now all but gone; the debate surrounding embryo viability has changed instead to one of aptitude – the partial failure of new tests to predict implantation has become the norm. This notion has recently been transformed into a new and exciting science, and the search for the ultimate test has begun: the race is on to achieve the happy retirement of two words: ‘success rate’.

This book was planned as a means of exploring this new and exciting science, and experienced authors who specialize in embryo testing were invited to contribute their expertise. Some of the authors have their background in basic science, other are dedicated to clinical IVF; they all share the common goal of finding this ‘holy grail’ with differing approaches and strategies. Our aim was to produce a book that is comparable to a peer-reviewed work, and the authors graciously allowed us to mingle with their text as editors, patiently providing explanations and further data if it was required. Although it is difficult to cover all aspects of gamete and embryo testing in one text, we tried to make it as comprehensive and up to date as possible.

It is divided into four main sections, with chapters dealing with morphology determinations, immunology and metabolism, genetic aberrations, and pre-fertilization parameters. With respect to morphology assessment, there appears to be no real consensus on how to grade human embryos based on their morphology, and it is therefore relatively easy to criticize this most basic tool. It is generally accepted that there is a correlation between cell number and implantation, yet the absolute nature of this correlation is unknown; prospectively randomized trials have never been contemplated in order to determine the real value of morphological parameters or embryo development rate. We feel that use of microscopy is not over, and the morphology debate is becoming of increasing interest, with obvious but ethically challenging work yet to be undertaken.

The second section on embryo metabolism offers an exciting glimpse into the feasibility of scoring embryos by examining spent culture media, using non-invasive tests. Although large randomized trials have not been carried out in this area of research, retrospective data shows promise, and more research is needed to expand the use of this tool for embryo assessment. The third section of the book explores ways of assessing the genetic status of embryos. Some conditions such as aneuploidy and mosaicism may be associated
PREFACE

with adverse conditions during follicular growth and gamete preparation, and also correlated with clinical outcome. Cell analysis using gene expression or imprinting are exciting approaches that may one day be available as clinical tools. Mutations in mitochondria, or changes in their patterns of activity provide another potential tool for single cell or whole embryo analyses. The fourth and final section covers examples of pre-fertilization parameters: aspects of sperm function, including DNA and centriolar integrity, and investigations of follicle-specific factors that influence oocyte competence.

Kay Elder
Jacques Cohen
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1. Human oocyte and embryo assessment for ART

A Henry Sathananthan and Sulochana Gunasheela

INTRODUCTION AND METHODS

The human oocyte, the female germ cell, is a unique cell equipped to fuse with and incorporate the sperm cell at fertilization and to sustain early embryonic development. It needs to be assessed for maturational status and normality for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in assisted reproductive technologies (ART). It is desirable to obtain a fresh, mature oocyte for insemination, usually after ovarian stimulation with gonadotropins or after down-regulation using gonadotropin-releasing hormone (GnRH) agonists/follicle stimulating hormone (FSH). With improved methods of ovarian stimulation and better timing of human chorionic gonadotropin (hCG), the majority of oocytes approach metaphase II (MII) and could be easily harvested for ART by ultrasonography. The trend now is to harvest a single oocyte in the natural cycle with minimal stimulation. The ripe MII oocyte is ovulated in a natural ovarian cycle around day 14. As much as we assess oocytes and sperm for ART, the embryo has to be assessed for embryo transfer in ART and currently for embryonic stem (ES) cell technology, a logical progression of ART. The fertilized ovum is the embryo, which undergoes cleavage by repeated mitoses to form a blastocyst during the first week of preimplantation embryogenesis (Figure 1.1). The embryonic genome is activated between the 4- and 8-cell stages in humans and the blastocyst implants in the uterus during the second week of development. The reader is referred to atlases of ART and other selected websites and references for images of gametes and embryos.1-9 All embryologists are advised to follow any embryology textbook to appreciate the highlights of development during the embryonic period (the first 8 weeks of development), when most of the tissue and organ rudiments are laid down in the embryo.

This chapter presents images supported by point-form assessments of the relevant stages of development. These include gross morphology, assessed in the laboratory using the inverted light microscope (LM), digital images of epoxy sections (LM), as well as fine structural assessments that may not be seen routinely, visualized by transmission electron microscopy (EMTEM). For surface observations in scanning electron microscopy (SEM), the reader is referred to atlases by Sathananthan3 and Makabe et al.10 Fluorescent microscopy (FM) is dealt with elsewhere in this book (see Chapter 26). The author’s website6 has images relevant to this chapter.

OOCYTE ASSESSMENT

MATURATIONAL STATUS

Preovulatory oocytes, collected from multiple follicles after ovarian stimulation have commenced the final stages of meiotic maturation, ranging from germinal vesicle breakdown (GVBD) through metaphase I (MI), to MII.11-13 Nuclear maturation goes hand-in-hand with cytoplasmic and cortical maturation. Furthermore, changes also occur in the egg vestments, particularly the zona pellucida (ZP), increasing receptivity to sperm binding and penetration. Significantly, GVBD heralds the resumption of meiosis and initiates the expansion of the cumulus during maturation. This usually occurs in the culture medium prior to insemination (IVF) or sperm injection (ICSI) and may take 2-6 hours to complete, depending on the timing of oocyte pickup after administration of hCG. The process might be completed after insemination with washed sperm during IVF. Since the oocyte is
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Denuded of cumulus cells before ICSI, it is possible to precisely identify the mature oocyte, which has the first polar body (PB1) at the animal pole (AP). Whatever technique is used, the oocyte should not age in culture, becoming postmature, which could lead to abnormal fertilization and development, particularly aneuploidy and polyploidy.

The mature oocyte is one of the largest cells (100–120 μm in diameter), surrounded by a gelatinous, glycoprotein shell, the ZP, and several layers of follicular cells, composing the cumulus oophorus. The female germ cell carries the 23 maternal chromosomes (n = 23) for procreation. The sperm cell contributes the 23 paternal chromosomes (n = 23) and the dominant centrosome (cell center) that initiates embryonic development after fertilization. Both sperm and egg contribute to the embryonic genome establishing diploidy (2n = 46), the essence of fertilization.

**FINE STRUCTURE OF THE MATURE EGG**

To appreciate the processes of oocyte maturation, fertilization, and development we need to briefly review the structure of organelles in the oocyte. Basic cellular organelles found in most somatic cells are found in oocytes (Figure 1.2). These include the mitochondria, smooth endoplasmic reticulum (SER), lysosomes, annulate lamellae, few Golgi complexes, microtubules (MT), and microfilaments (MF). The SER consists of isolated vesicles or aggregates of tubular elements. Ribosomes are rare and rough endoplasmic reticulum (RER) is absent. Cortical granules (CG), unique to oocytes, are located beneath the oolemma (plasma membrane) and play an important role in fertilization. The human oocyte has no lipid or yolk inclusions, but survives in the oviduct and uterus during the first week of development.

The metaphase II spindle, located at the AP, is barrel-shaped, anastral, and aligned perpendicular to the surface (Figure 1.3). It is composed of MT but lacks a functional maternal centrosome at each pole. The spermatozoon provides the dominant, centrosome (centriole) for embryo development in humans. The layer of follicle cells just outside the ZP is termed the corona radiata (CR). The CR is composed of typical somatic cells with the usual complement of cellular organelles. The oocyte has a

**Figure 1.1** Normal whole embryos – 1-cell stage to blastocyst (LM). (A) Activated oocyte; (B) fertilized ovum (2PN); (C) 2-cell; (D) 4-cell; (E) 6-cell; (F) 8-cell; (G) compaction; (H) morula; (I) blastocyst; (J) hatching blastocyst. (Courtesy Dr. S. Gunasheela.)
HUMAN OOCYTE AND EMBRYO ASSESSMENT FOR ART

The maturing, metaphase I oocyte has:  
- No polar body (LM)  
- No germinal vesicle (LM)  
- An expanding cumulus and corona cells (LM)  
- A metaphase I spindle with homologous chromosomes (FM, EM)  
- One or two layers of CG beneath oolemma (LM, EM).

(This stage is transient, there being no interphase.)

The immature oocyte (Figure 1.4) at prophase I shows:  
- No polar body (LM)  
- A GV or nucleus with a dense nucleolus (LM)  

---

Figure 1.2 Human oocyte fine structure. The illustration incorporates cellular organelles of immature and mature oocytes, as well, and two follicle cells that play an important role in oocyte maturation. A = aggregate of SER; C = caveolus; CCP = CR process; CG = cortical granules; Ch = chromosomes; CR = corona radiata; En = endocytosis; Ex = exocytosis; G = Golgi complex; L = primary lysosome; M = mitochondria; MB = multivesicular body; MF = microfilaments; MT = microtubules; MV = microvilli; N = nucleus; PR = polyribosome; PVS = perivitelline space; RB = residual body; RER = rough endoplasmic reticulum; S = vesicular SER; Sp = meiotic spindle; Z = zona pellucida. Modified from Sathananthan et al. (1993).
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- A compact, unexpanded cumulus and corona (LM)
- A discontinuous layer of CG beneath oolemma (LM, EM)
- An agranular cortex with Golgi membranes that secrete CG (LM, EM).

(Oocytes about to mature will show an eccentrically located GV at one pole.)

Oocytes during GVBD (Figure 1.4) show: \(^2,\!^{11}\)

- A disappearing GV or nucleus (LM)
- Breakdown of the nuclear envelope (LM, EM)
- Condensation of chromosomes (FM, EM)
- Formation of a spindle with MT (FM, EM)
- Uncoupling of cell junctions between CR cells and oocyte (EM).

(This stage heralds the resumption of meiosis after its arrest at the GV stage.)

Aging, postmature oocytes in culture (Figures 1.5–1.7) will show: \(^2,\!^{11},\!^{13}\)

- A dense ooplasm with vacuoles (swollen vesicular SER) (LM, EM)

---

**Figure 1.3** Normal and aging oocytes – metaphase II spindles (TEM). The normal MII spindle is barrel-shaped, has no centrosomes at either pole and is attached to the egg cortex. The ageing spindle is displaced centripetally and has disorganized chromosomes at its MII plate. CG = cortical granules, p = polar body; S = smooth endoplasmic reticulum; Z, zona. ×27,300, ×3500. From Sathananthan (2002),\(^6\) (2007).\(^22\)

**Figure 1.4** Preovulatory oocyte maturation (phase-contrast and LM). (A) and (D) germinal vesicle (GV) stage, (B) and (C) metaphase II, (E) GV breakdown, (F) telophase I, are depicted. Note retraction of cumulus cells in (C) and (E). ×400, ×1000. (A), (B) courtesy Dr. D. Payne, Adelaide, (C)–(F) From Sathananthan et al. 2003.\(^2\)
Figure 1.5 Changes in aging oocyte ultrastructure. A, aggregate of smooth endoplasmic reticulum (SER) (hypertrophic); CG, cortical granules (crowded, displaced); Ch, chromosomes (scattered); G, Golgi; L, lysosome; Lb, lipofuschin body; M, mitochondria (dense); MV, microvilli (short); S, vesicular SER (swollen); Sp, MII spindle (displaced); Z, zona pellucida (hardened). From Sathananthan 1997.

Figure 1.6 Meiotic and mitotic spindles – chromosome scatter (TEM). The MII spindle (A) and that at syngamy (B) are disorganized. Some chromosomes have scattered outside the spindle zone, which can cause aneuploidy in embryos. A ×17 000, B ×10 000. From Sathananthan 2002.

Figure 1.7 Aging oocyte – cortical granules (CG) and smooth endoplasmic reticulum (SER) (TEM). CG crowd beneath the surface with large aggregates of SER. Hypertrophy of SER is primarily induced by gonadotropin stimulation, during maturation. ×35 500. From Sathananthan 2002.

- Normal or abnormal MII spindles, displaced from the surface (LM, FM, EM)
- Loss of spindle MT causing chromosome scatter (LM, FM, EM)
- Crowding of CG beneath oolemma or their centripetal migration (LM, EM)
- Few lipofuschin bodies with aging pigment (EM)
- Large hypertrophic aggregates of tubular SER (EM).

**ASSESSMENT OF FERTILIZATION**

Fertilization begins with sperm–egg membrane fusion and culminates at syngamy, when the genetic constitution of the embryo is established. The oocyte is activated to become an embryo, the beginnings of life.

The early events of fertilization cannot be visualized in the laboratory, except for the appearance of the second polar body (PB2), usually alongside PB1. These events, however, can be seen by TEM and FM, which are both invasive procedures. About 12 hours after insemination or ICSI it is easy to confirm fertilization in the laboratory, when two distinct pronuclei (PN), male and female, appear in the ooplasm. This stage is currently used to predict
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normal development and prospective implantation. Each PN has about eight dense nucleoli that align adjacent to apposing pronuclear membranes in normal pronuclei. Apart from pronuclei, the alignment of nucleolar-associated chromatin is equally important in PN assessment, since this condenses to form the maternal and paternal, homologous chromosomes later at syngamy. This chromatin, however, is more difficult to see in the laboratory but is clearly visible in sections of ova, particularly by TEM (Figure 1.8).

The dominant sperm centrosome has now been released from the sperm neck and is activated to form a sperm monoaster, that will eventually duplicate to establish a bipolar spindle (bipolarization) at the onset of mitosis. The zygote centrosome in the sperm aster has now duplicated centrioles and has become functional by attracting maternal $\gamma$-tubulin, which nucleates MT, best visualized by FM. We believe that the restoration of the functionality of the dominant sperm centrosome is the most significant event of oocyte activation that initiates embryo development.15,17

A normally fertilized ovum 3 hours after insemination shows:2,3

- Abstriction of PB2 into the PVS (LM, EM)
- An incorporated, decondensing sperm head in the ooplasm (FM, EM)
- A developing female pronucleus in ooplasm beneath PB2 (FM, EM)
- A sperm tail, midpiece, or sperm centriole in the ooplasm (FM, EM)
- Evidence of CG exocytosis all around the oocyte (EM).

(A fertilization cone may be evident at site of sperm incorporation.)

A normally fertilized ovum 12–14 hours after insemination (Figure 1.8) has:2,3

- Two pronuclei, male and female, associated in the central ooplasm (LM)
- Two polar bodies – PB1 with chromosomes and PB2 with a nucleus (LM)
- Nucleoli aligned close to apposing PN nuclear envelopes (LM, EM)
- No CG or few beneath oolemma after IVF (EM)
- Crowding of organelles, mostly mitochondria, around PN (LM, EM).

(Delayed CG exocytosis has been observed after ICSI by TEM.)

An abnormally fertilized ovum (dispermy) during IVF (Figure 1.9) has:2,3,9

- Three pronuclei (two male and one female) (LM)
- Two polar bodies (PB1 and PB2) in PVS (LM)

Figure 1.8 Normal bipronuclear ova (LM and TEM). These bipronuclear ova, after monospermic fertilization, seem normal. Note alignment of nucleoli adjacent to apposing pronuclear membranes. What is more significant is the alignment of chromatin, associated with nucleoli, which would condense to form the male and female chromosomes at syngamy. ×35 700. From Sathananthan, 2003.6
HUMAN OOCYTE AND EMBRYO ASSESSMENT FOR ART

- A triploid chromosome complement 69XXY or 69XYY (LM)
- Two male centrosomes and two sperm asters (LM, FM, EM)
- A bipolar or tripolar spindle at syngamy (LM, FM, EM).

(May cleave into two or three cells, show normal early cleavage; and even develop to term.)

An abnormal fertilized ovum (digyny) after ICSI shows:

- Three pronuclei (two female and one male) caused by suppression of PB2 (LM)
- A single polar body – PB1 (LM)
- A triploid chromosome complement 69XXX or 69XXY (LM)
- One male centrosome and one sperm aster (FM, EM).

(Will not cleave normally beyond the 6–8-cell stage.)

Structurally abnormal PN ova usually have:

- PN with fuzzy irregular outlines (LM)
- PN of unequal size located peripherally (LM)
- PN not closely associated in the central ooplasm (LM, FM)
- Nucleoli not aligned against apposing PN envelopes (LM, EM)
- PN showing incomplete incorporation of chromatin with micronuclei (EM).

‘Silent fertilization’ (Figure 1.10) may occur when:

- Sperm nuclear decondensation is arrested after IVF or ICSI (FM, EM)
- Sperm head remains unexpanded and does not form a male PN (EM)
- Sperm decondense chromatin but do not release its centrosome (FM, EM)
- The ovum has proceeded to syngamy after rapid PN formation (FM, EM)
- The ovum has arrested at syngamy – centrosomal dysfunction (FM, EM).

(Will not cleave normally beyond the 6–8-cell stage.)

(The acrosome has to be discarded before spermhead decondensation during ICSI.)

Figure 1.9 Dispermic tripronuclear (3PN) ova (TEM). Classical images of 3PN ova at the pronuclear stage and syngamy. Note chromatin (dark specks) and nucleoli located toward adjacent membranes of the pronuclear envelopes. The spindle is tripolar enabling the ovum to divide into three cells, instead of two. ×5000, ×8000. From Sathananthan et al. 1995.
HUMAN PREIMPLANTATION EMBRYO SELECTION

EMBRYO ASSESSMENT

The first week of preimplantation development begins at fertilization and proceeds to blastocyst hatching (Figure 1.1). The first 2 or 3 days are critical in assessing normal development for embryo transfer (ET) in routine ART. The rate and timing of cleavage and development are important in assessing normality (Table 1.1). On day 1 the pronuclear ovum is assessed for normal or abnormal fertilization. The most important morphological parameters to assess in the laboratory are blastomere appearance, fragmentation, and multinucleation. The latter can be assessed non-invasively by using superior optical lenses, combined with digital microphotography recorded on video. Those with equal blastomeres, minimal cytoplasmic fragmentation, and few multinucleated cells have a better prospect of implantation. The fate of each embryo could be monitored right up to blastocyst hatching. Embryos are graded accordingly for ET (Table 1.2). Occasionally, embryonic blocks may occur at the 1-cell, 8-cell, or at any stage depending on culture conditions and embryo quality. It is advisable to let early embryos continue to develop at their own pace to overcome blocks. Totally arrested embryos should be discarded as they will eventually degenerate. Arrests could be caused by mitotic disturbances involving both chromosomal and centrosomal dysfunction. Aneuploidy, polyploidy, and mosaicism are the chief causes of early embryonic loss, apart from extensive fragmentation, which is now regarded as an apoptotic phenomenon. Several

Table 1.1 Normal embryonic growth from day 2–6. An embryo that develops to this timetable is likely to be more viable than the one which shows delayed growth

<table>
<thead>
<tr>
<th>Day</th>
<th>Embryo</th>
<th>Appearance/hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fertilized ovum</td>
<td>2 PN (12 hours) and syngamy (18–24 hours)</td>
</tr>
<tr>
<td>2</td>
<td>Cleaving embryo</td>
<td>2-6 cell rounded blastomeres</td>
</tr>
<tr>
<td>3</td>
<td>Cleaving embryo</td>
<td>8–10 cells or rounded blastomeres</td>
</tr>
<tr>
<td></td>
<td>Compacting embryo</td>
<td>Blastomeres show evidence of adhesion</td>
</tr>
<tr>
<td>4</td>
<td>Compacted morula</td>
<td>Blastomeres show increased adhesion</td>
</tr>
<tr>
<td></td>
<td>Early cavitating</td>
<td>Beginning of blastocoel formation</td>
</tr>
<tr>
<td>5</td>
<td>Early blastocyst</td>
<td>Blastocoel formed</td>
</tr>
<tr>
<td></td>
<td>Mid-blastocyst</td>
<td>ICM, trophoblast and blastocyst clearly seen</td>
</tr>
<tr>
<td></td>
<td>Expanding blastocyst</td>
<td>Trophoblast expanding, zona thinning out</td>
</tr>
<tr>
<td></td>
<td>Early hatching</td>
<td>Embryo growing, blastocoel much increased</td>
</tr>
<tr>
<td>6/7</td>
<td>Late blastocyst</td>
<td>Expanded ~150–200 cells; diameter ~215 μm</td>
</tr>
<tr>
<td></td>
<td>Hatching blastocyst</td>
<td>Trophoblast hatching out of zona</td>
</tr>
<tr>
<td></td>
<td>Hatched blastocyst</td>
<td>Trophoblast and ICM hatched out of empty zona</td>
</tr>
</tbody>
</table>

PN, pronuclei; ICM, inner cell mass. Modified from Gunasheela.24

Table 1.2 Embryo grading for embryo transfer in the laboratory. Grades 1 and 2 have a greater potential of establishing a clinical pregnancy

<table>
<thead>
<tr>
<th>Grade</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blastomeres of equal size and no cytoplasmic fragmentation</td>
</tr>
<tr>
<td>2</td>
<td>Blastomeres of equal size and minor cytoplasmic fragmentation (&lt;10%)</td>
</tr>
<tr>
<td>3</td>
<td>Blastomeres of unequal size and variable fragmentation</td>
</tr>
<tr>
<td>4</td>
<td>Blastomeres of equal or unequal size and significant fragmentation (&gt;10%)</td>
</tr>
<tr>
<td>5</td>
<td>Few blastomeres of any size and severe fragmentation (50%)</td>
</tr>
</tbody>
</table>

Modified from Veeck.4
HUMAN OOCYTE AND EMBRYO ASSESSMENT FOR ART

laboratories are now culturing embryos to blastocysts to select the most viable and vigorous for ET on day 6 or 7. Blastocyst culture is expensive, time-consuming, and suitable for larger IVF centers.19–22
Normal cleavage stage embryos (Figures 1.1, 1.11–1.13) usually have:2,4,12
• Rounded equal-sized blastomeres, except when cells are dividing (LM)
• Blastomeres with well defined outlines or cell membranes (LM)
• Cells with centralized, single nuclei, or metaphases (LM, EM)
• No fragments or show minimal fragmentation (<10%) (LM, EM).
(Embryos should develop according to the time frame in Table 1.1.)
Common abnormalities (Figures 1.14–1.17) in early embryos include:1,2,4
• Extensive cytoplasmic fragmentation of blastomeres (30–50%) (LM)
• Spontaneous fragmentation of whole blastomeres – apoptosis? (LM)
• Some unequal or fused blastomeres with eccentric nuclei (LM)
• Multinucleation of blastomeres – polyploidy (LM, FM, EM)
• Micronuclei in blastomeres beside normal nucleus – aneuploidy (LM, FM, EM).

Arrested, degenerating embryos (Figures 1.16 and 1.17) show:1,2
• Dark granular blastomeres and aggregation of organelles (LM)
• Extensive vacuolation of blastomeres – increases density (LM, EM)
• Cells with eccentrically located nuclei (LM, FM, EM)
• Multinucleated cells, many fragments and uneven cells (LM, FM, EM)
• Lack of compaction in later embryos and morulae (LM, EM).

Figure 1.11  Diagrams of normal (A) and abnormal (B) embryos. Both normal and abnormal blastomeres are seen in abnormal embryos. Cytoplasmic fragmentation, multinucleation, and micronucleation are the main abnormalities. Fragments may be internal or external in the PVS, few to many. F, fertilization; C, cell; PN, pronuclei. From Sathananthan et al 1993.9
Normal blastocysts on day 5/6 (Figure 1.18) have

- Distinct trophoblast, ICM and blastocoel (LM)
- Well-defined, compact ICM with many cells and cell junctions (LM, EM)
- Trophoblast forming a continuous, flat epithelium with cell junctions (LM)
- A large fluid-filled blastocoel, when expanded (LM)
- Few cleavage stage fragments in the blastocoel and PVS (LM, EM).

(Healthy blastocysts usually have 150–250 cells after DAPI, 4′,6-diamidino-2-phenylindole, staining.)

Hatching blastocysts on day 6/7 (Figures 1.1 and 1.19) show:

- A fully expanded trophoblast and blastocoel (LM)
- A thinned-out zona (LM)
- Evidence of early hatching – trophoblast emerging at one pole (LM, EM)

Figure 1.12 Two-cell embryos – normal and fragmented. Both normal and abnormal embryos are evident. Fragments appear over the cleavage furrow or a whole cell can fragment totally. ×400. From Sathanathan et al.25

Figure 1.13 Normal human embryos – one cell to morula (phase-contrast). The cleavage embryos have equal blastomeres and minimal cytoplasmic fragmentation, except the 3-cell embryo. ×400. From Menezes (2005).25
Figure 1.14 Fragmented dispermic embryos (LM and TEM). Fragmentation is a common occurrence in early human embryos. Cytoplasmic fragments are devoid of nuclear material (D) compared with a normal blastomere (E). Four–8-cell and 5-cell (left) and a 10-cell embryo (right). ×400, ×3500, ×6000. From Sathananthan et al. 1999b.6,9

Figure 1.15 Abnormal multinucleated dispermic embryos (TEM). (A) 1-cell (fragmented), (B) 2-cell (micronucleated), and (C) and (D) 3-cell embryos (multinucleated). ×6000, ×4000. From Sathanathan 2004.8
HUMAN PREIMPLANTATION EMBRYO SELECTION

- Plump ‘zona-breaker’ (trophoblast) cells at hatching point (LM, EM)
- A layer of endoderm cells beneath ICM (LM, EM).

(Apoptotic cells are usually found in the ICM associated with phagocytic cells.)

Abnormal blastocysts (Figures 1.18 and 1.20) may: 4,20,21

- Have no ICM or have a small or dispersed ICM (LM)
- Fail to expand and hatch on day 6 – are moribund or unable to break zona (LM)
- Arrest in development and often degenerate (LM)
- Have cleavage stage fragments in PVS – interferes with hatching (EM)
- Show many multinucleated cells in ICM, trophoblast, and endoderm (EM).

Figure 1.16 Three to 6-cell dispermic embryos (LM). Both normal and abnormal embryos are shown. Vacuolated blastomeres are degenerating. Note variation in cell size, few fragments, and multinucleated blastomeres. ×100. From Sathananthan et al. 1999b.9

Figure 1.17 Eight to 10-cell dispermic embryos (LM). Both normal and abnormal embryos are evident. Blastomeres with clear vacuoles are degenerating. Note unequal-sized blastomeres and few fragments. ×200. From Sathananthan et al. 1999b.9
Figure 1.18 Normal and abnormal blastocysts after ICSI (LM). (A) Normal blastocyst with trophoblast (T), inner cell mass (ICM), and blastocoel (B); (B) disorganized ICM; (C) disorganized endoderm (E); (D) Failed hatching – degenerating. ×400. From Sathananthan et al. 2003a,b.20,21

Figure 1.19 Hatching blastocyst – zona breakers (LM). The blastocyst has hatched halfway. The inner cell mass is elsewhere. Zona breakers (ZB) at hatching point. A ×400, B ×1000. From Sathananthan et al. 2003b.21

Figure 1.20 Fragments in a blastocyst (TEM). Fragments (F) are found between the trophoblast and zona and are discarded at hatching or remain in the blastocoel (not shown). Note dense mitochondria in fragment of early cleavage embryo. ×3400. From Sathananthan et al. 2003b.21
HUMAN PREIMPLANTATION EMBRYO SELECTION

Chromosome abnormalities in embryos (LM, FISH, PGD):

- Are higher than 50% irrespective of maternal age
- Aneuploidy increases with maternal age (>35 years)
- Aneuploidy is unrelated to embryo dysmorphism
- Polyploidy, mosaicism, chaoticism, and haploidy
- Most arrested embryos are abnormal
- Slow developing embryos also show more abnormalities
- Mosaicism is common in blastocysts – will not implant
- Most dispermic ova are mosaics compared to digynous ova.

Morphological abnormalities correlate well with chromosomal aberrations and decreased implantation potential.9,10,18,22,23

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Figure 7.5 Non-invasive analysis of human embryo viability. Individual blastocysts are incubated in 0.5-5.0 µl volumes of defined medium. Serial or endpoint samples of medium can then be removed for analysis. An indirect measurement of metabolic pathways, i.e. glycolysis and transamination can be obtained by measuring specific nutrients in combination, such as glucose uptake and lactate production, or amino acid turnover with ammonium production. As well as testing for known molecules, surface enhanced laser desorption/ionization time-of-flight mass spectrometry can also now be used to identify novel peptides and proteins. 68 LDH, lactate dehydrogenase; sHLA-G, soluble histocompatibility antigen class I G; HOXA10, homeobox A10; PAF, platelet activating factor.


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mind that embryo development is a dynamic process and that the kinetics involved can yield additional information about embryo competence. A number of studies have demonstrated that the timing of cell cleavage is a significant indicator of embryonic competence. The embryo needs not only to develop to the 4-cell stage, but also it needs to do so at the correct time. Cleavage that occurs too rapidly or too slowly is an indication of impaired competence. Likewise, the onset of mitoses and the appearance/disappearance of the pronuclei after fertilization need to take place during a narrow time interval (22–25 hours) in high quality embryos, as suggested by Fancsovits et al. 36 It has also been suggested that the interval between pronuclear breakdown and the first cleavage division should be relatively constant, about 3 hours. 37,38 Other studies have demonstrated that the occurrence of early cleavage may be a good prognostic factor. However, the specific timing of early cleavage seems to be related to the method of fertilization, suggesting that different kinetics are involved in the processes of ICSI vs regular IVF. 39

CONCLUSION

In the past, embryo evaluation has been based mainly
on subjective evaluation of morphological parameters considered to be important markers of quality. However, a number of drawbacks are associated with this type of analysis. One example is the differentiation between large fragments and blastomeres, and another is imprecise estimation of the degree of fragmentation. The introduction of computer-based morphometric analysis has allowed us to enter a new level of embryo evaluation. These techniques open an array of possibilities for standardization and more precise measurements, including total cytoplasmic reduction as a new means of describing fragmentation, and detection of multinucleation based on blastomere size. In the final analysis, the combination of kinetics and morphometrics that include detailed information retrieved over several days is a new and fascinating aspect. The ‘3-dimensionality’ of multilevel


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