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# Human Blastocyst Formation and Development

*Hilma Putri Lubis and Binarwan Halim*

## Abstract

The preimplantation period of human embryo development is remarkable and characterized by successive changes in terms of genetic control, physiology, and morphology of the embryo. Human preimplantation embryo development is characterized by the initial phase of embryo development, the phase before the embryo implantation process. In normal conditions, after fertilization, the embryo grows until the blastocyst stage. The blastocyst grows as the cells divide and the cavity expands, where it “hatches” from the zona pellucida to implant into the endometrium. Reprogramming and programming are continuous processes in the embryo that encompasses fusion of the egg and sperm pronuclei; epigenetic reprogramming and modification, an extensive wave of degradation of maternal transcripts, and activation of the nascent human embryonic genome and aneuploidy can occur in this stage. The embryo produces cytokines, growth factors, and receptors for endometrial signals in the apposition stage.

**Keywords:** preimplantation, embryo, epigenetic, reprogramming, blastocyst

## 1. Introduction

Preimplantation embryo development is characterized by a series of events after fertilization including the formation of the maternal and paternal pronuclei, followed by the formation of the zygote, which at  $\pm 20$  hours after insemination starts undergoing mitotic divisions every 12–18 hours (cleavage stage), reaching the morula (compaction), and the blastocyst grows as the cells divide and the cavity expands, until it arrives at the uterus, where it “hatches” from the zona pellucida to implant into the endometrium. The main objective of blastocyst culture was to increase the success rate of in vitro fertilization (IVF) because of better embryo selection. Blastocyst culture has also been used as a tool to select the most viable embryos in a cohort with a consequent reduction in the number of embryos transferred and the corresponding reduction in the incidence of multiple gestations. This chapter will review about human blastocyst formation and development and discuss about the physiology, morphology, and gene expression of the blastocyst.

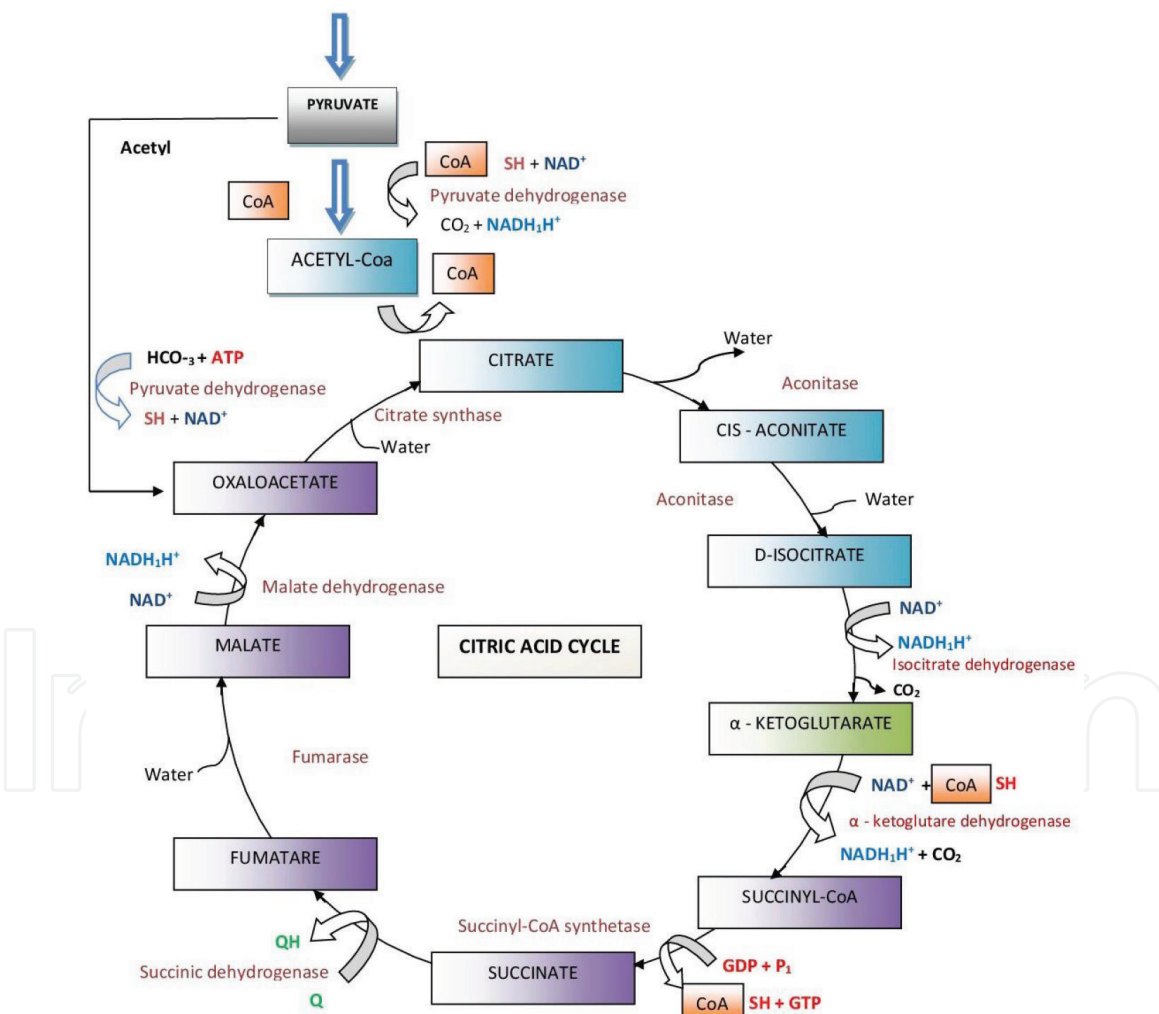
## 2. Physiology of the blastocyst

The transformation of the fertilized oocyte into the blastocyst is not only characterized by major morphological events but also by dramatic changes in its

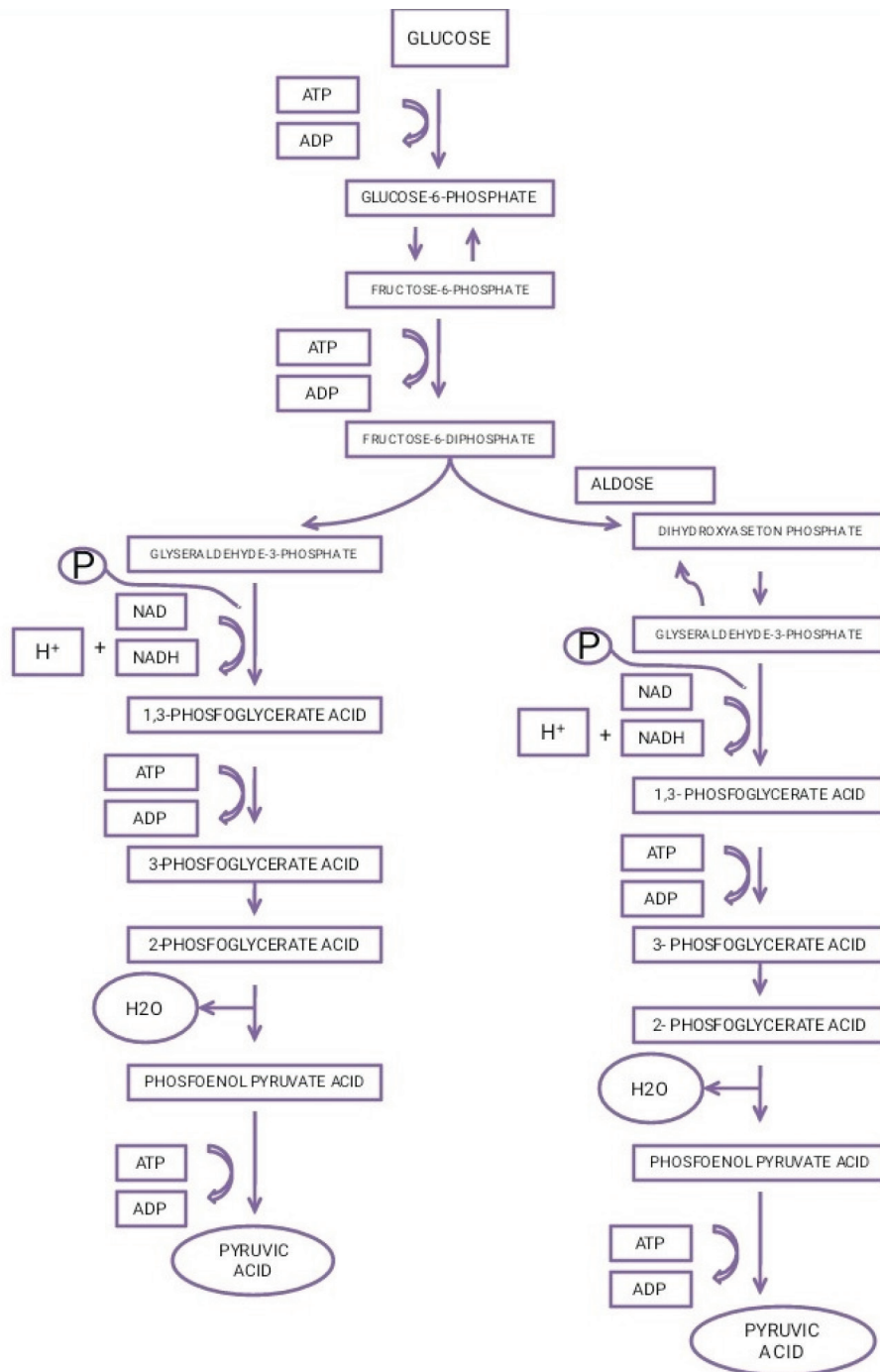
physiology, reflected in changes in the relative activity of the metabolic pathways which provide not only energy but also the biosynthetic intermediates required to support proliferation [1]. The ability of the cleavage stage embryo to react in the environment during early cleavage is limited because the human genome embryo is still inactive and the system that regulates the balance of the osmotic pressure is not fully functional [1, 2].

The tendency of metabolism to produce energy from pronucleate oocytes until blastocyst stage can be assessed from mitochondrial forms. In the stage of pronucleate oocytes and cleavage stage, their mitochondrial form is still immature, and the production of energy in oocytes is usually low and will be increased tremendously from cleavage stage embryo until blastocyst stage. In the stage of pronucleate oocytes, the type of metabolism is oxidative phosphorylation (OXPHOST); then in the cleavage stage embryo, the metabolism uses lactate, pyruvate, specific amino acids, and fatty acids [1–3].

In the blastocyst stage, the metabolism produces energy that mainly depends on the process of glycolysis, with anabolic dominantly seen in the mitochondria [3, 4].



**Figure 1.** Tricarboxylic acid (TCA) cycle or the Krebs cycle. The citric acid cycle begins with one acetyl-CoA molecule reacting with one molecule of  $\text{H}_2\text{O}$ , releasing a coenzyme-A group, and donating the remaining two carbon atoms in the form of an acetyl group to oxaloacetic acid which has molecules with four carbon atoms, to produce citric acid with six carbon atoms. The outcome products of the first turn of the cycle are one GTP (or ATP), three NADH, one  $\text{QH}_2$ , and two  $\text{CO}_2$ . Because two acetyl-CoA molecules are produced from each glucose molecule, two cycles are required per glucose molecule. Therefore, at the end of two cycles, the final products are two GTP, six NADH, two  $\text{QH}_2$ , and four  $\text{CO}_2$ .



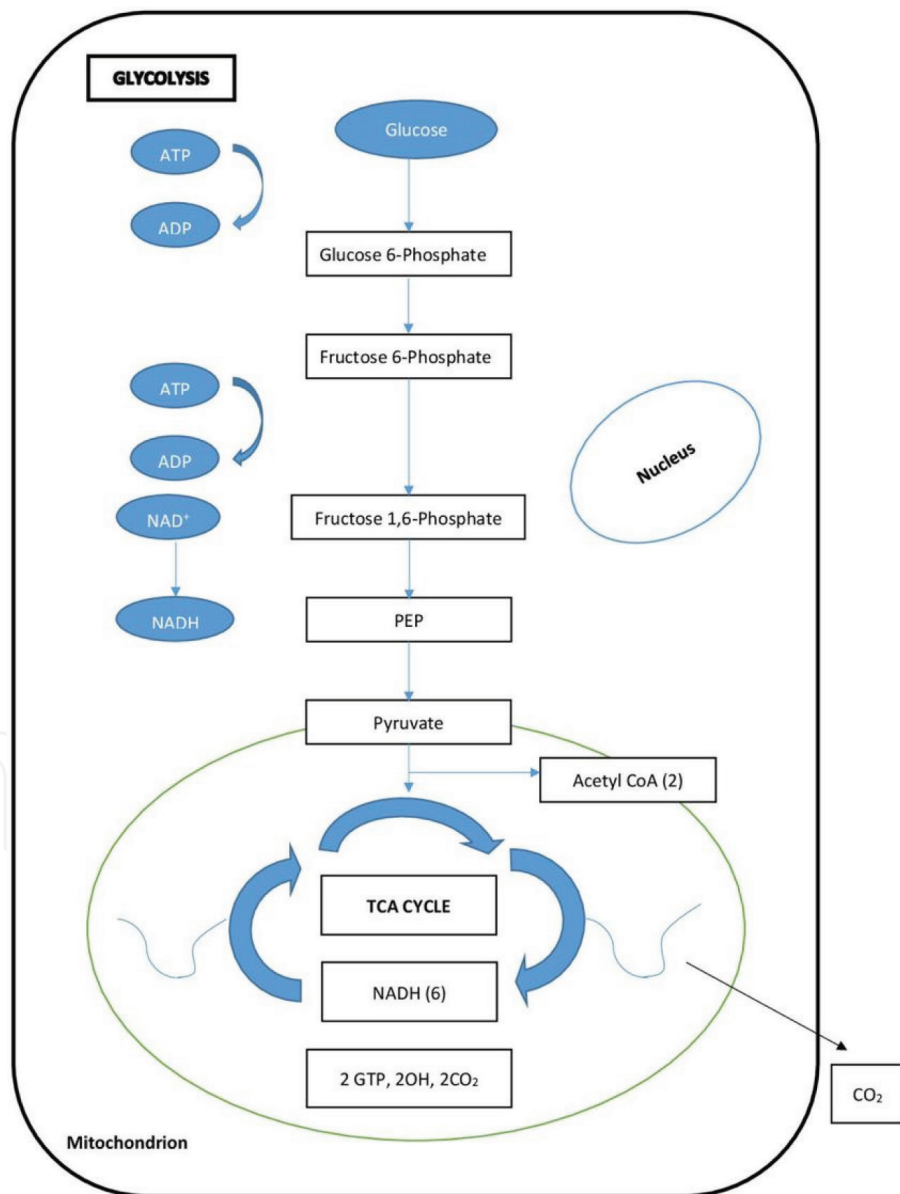
**Figure 2.**

Glycolysis pathway or Embden-Meyerhof-Parnas (EMP) pathway. (1) In the first stage, glucose will be converted to glucose 6-phosphate by the hexokinase enzyme. This stage requires energy from adenosine triphosphate (ATP). The ATP that has released the stored energy will change to ADP. (2) Glucose 6-phosphate will be converted to fructose 6-phosphate which is catalyzed by the enzyme phosphohexose isomerase. (3) Fructose 6-phosphate will be converted to fructose 1,6-bisphosphate; this reaction is catalyzed by the enzyme phosphofructokinase. In this reaction, energy from ATP is needed. (4) Fructose 1,6-bisphosphate (6 C atoms) will be broken down into glyceraldehyde 3-phosphate (3 atoms C) and dihydroxy acetone phosphate (3 atoms C). The reaction is catalyzed by the enzyme aldolase. (5) One molecule of dihydroxy acetone phosphate that is formed will be converted to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase. The enzyme works back and forth, meaning it can also convert glyceraldehyde 3-phosphate to dihydroxy acetone phosphate. (6) Glyceraldehyde 3-phosphate will then be converted to 1,3-bisphosphoglycerate by the enzyme glyceraldehyde 3-phosphate dehydrogenase. In this reaction NADH will be formed. (7) 1,3 bisphosphoglycerate will be converted to 3-phosphoglycerate by the phosphoglycerate kinase enzyme. These reactions will be released as energy in the form of ATP. (8) 3-phosphoglycerate will be converted into 2-phosphoglycerate by the phosphoglycerate mutase enzyme. (9) 2-phosphoglycerate will be converted to phosphoenolpyruvate by the enzyme enolase. (10) Phosphoenolpyruvate will be converted to pyruvate which is catalyzed by the pyruvate kinase enzyme. In this stage also produced energy in the form of ATP.

In the cleavage stage embryo, pyruvate uptake increases continuously until the blastocyst stage. In the blastocyst stage, glucose uptake is higher than pyruvate uptake, and  $O_2$  consumption will increase in the initial development stage before compaction (pre-compaction). In the pre-compaction stage, we can observe low biosynthetic activity, low  $O_2$  consumption, and ovoid form of mitochondria; the main nutrition is pyruvate, with dominant maternal genome where cells divide in the similar shape [1–3].

In the post-compaction stage, we can observe high biosynthetic activity, higher oxygen consumption, and elongated form of mitochondria; the main nutrition is glucose, and with dominant human embryo genome. In this stage, cells will be differentiated into trophoblast (TE) and inner cell mass (ICM) [1–3, 5].

Amino acids in the metabolism of blastocyst can be used as a source of energy, and some amino acids such as aspartate through malate aspartate shuttle enter in the tricarboxylic acid (TCA) cycle to produce energy. However, glutamine can also



**Figure 3.** Metabolism of the blastocyst. After compaction, there is an increase in oxygen consumption and utilization of glucose as a source of energy (glycolysis). The increase in oxygen consumption reflects the considerable energy required for the formation and maintenance of the blastocoel, but the increase in glucose utilization reflects an increased demand for biosynthetic process. In TCA cycles, it produces NADH, GTP, QH, and  $CO_2$  and 34 ATP. PEP, phosphoenolpyruvate.

enter as glutamate in the TCA cycle to produce energy. Amino acid in the blastocyst stage also plays a role in the regulation of intracellular pH buffer, as a material development process and as antioxidants and chelators [1–3, 5].

After compaction, the embryo exhibits increased  $O_2$  consumption and glucose usage capacity as an energy source. This oxygen consumption increase shows that the energy is needed for the formation and maintenance of blastocoel [1–3, 5].

Increased metabolism of the blastocyst occurs due to the increased release of blastomere to 150–200 cells with the formation and maintenance of blastocoel through the activity of  $Na^+/K^+$  ATPase pump which produces energy. Energy is needed for the degradation of the zona pellucida with protease enzyme. Pyruvate as a source of energy reserves other than carbohydrates also functions as an antioxidant [1–3, 5].

The human blastocyst uses amino acids as a source of energy in the catabolism process and produces ammonium 30 pmol/hour. The most used amino acid is aspartate, besides consuming arginine, serine, methionine, valine, and leucine [1–3, 5].

Metabolism of the blastocyst occurs in two different places: in trophoctoderm (TE) cells where glucose consumption occurs and half is converted to lactate, whereas glycolysis process occurs in inner cell mass (ICM) (**Figures 1–3**) [1–3, 5].

### 3. Morphology of the blastocyst

In in vitro fertilization (IVF), the blastocyst culture was important to increase the success rate of IVF because of better embryo selection after better genomic activation and endometrial receptivity [6]. The blastocyst comprises two cell types: the inner cell mass (ICM, from which the fetal tissues develop) and the trophoctoderm (which will form mostly extraembryonic tissues such as the placenta [1]). This morphological differentiation was thought to represent the developmental capability of the blastocyst [6, 7].

#### 3.1 Degree of expansion

The fluid accumulation presence between cells at the morulae stage is the phase that determines embryonic development. The accumulated fluid will form blastocoel gradually which usually occurs on day 4 and/or on the beginning of day 5 human embryo stage which marks the development of new embryos known as the blastocyst stage. An increase in fluid volume and the number of cells in the blastocyst causes an enhancement in the size cavity of the blastocyst and its cavity with depletion of the zona pellucida (ZP) [6]. The number of cells that comprise a blastocyst can vary considerably as shown in one study to range between 24 and 322 cells, which is often reflected in the blastocyst's morphology [8].

The stages of blastocyst embryo development are divided into four grades. A grade of 1 is given to the embryo with blastocoel cavity less than 50% of the embryo volume. A grade of 2 is given to the embryo with blastocoel cavity as much as 50% of the embryo volume or even more than that. A grade of 3 was given to the embryo that had a blastocoel cavity which had fulfilled that all embryos and zona pellucida (ZP) appeared to be thinner than embryo on day 3. A grade of 4 is given to the embryo that has successfully hatched from ZP [6, 9].

#### 3.2 ICM morphology

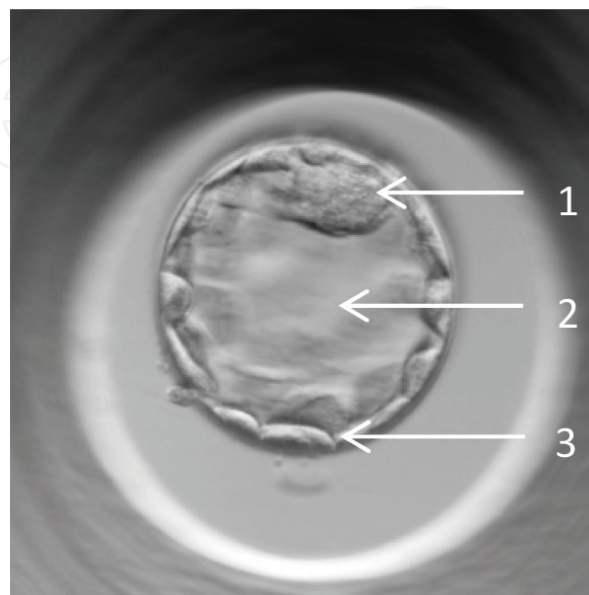
A collection of cells located within the blastocoel in one pole of the blastocyst cavity is called ICM. ICM will develop into fetal tissue. ICM consists of tightly

packed cells and loosely bound cells that cause the size of the ICM very large and/or small morphologically [6]. The morphological form of ICM is assessed based on how much the cell compaction is until there is no cell clot at all. A grade of 1 is given to ICM with a very large and dense form of cell clots. A grade of 2 is given to ICM with a slightly diffuse cell form. A grade of 3 is given to ICM with very few cells and which does not even form clots. However, the best ICM grade (A) contains tightly packed and many cells; the middle ICM grade (B) is composed of loosely grouped and several cells, and the worst grade (C) describes an ICM that contains very few cells that are loosely bound [6, 9].

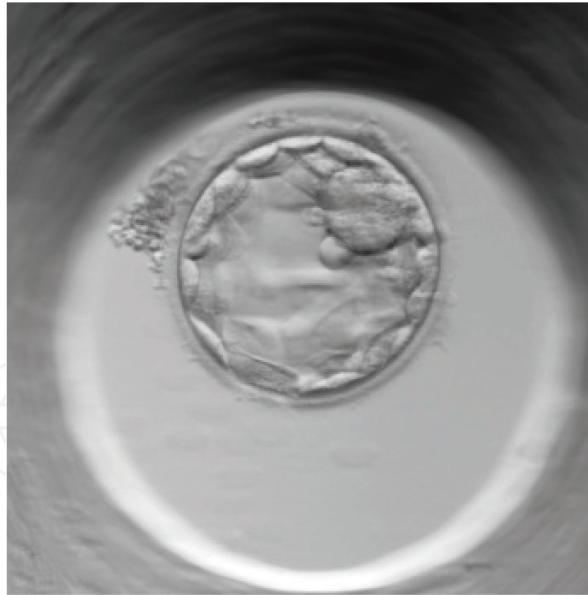
### 3.3 TE morphology

The outer cells of the blastocyst, forming the blastocyst structure itself, are called the trophoblast (TE) cells. TE cells play a role in the formation of fluid accumulation in the blastocoel which can be a key in ICM determination, but in the early stages of the blastocyst stage, the role of TE cells is unclear. TE cells will develop into extraembryonic tissue such as the placenta. In addition, TE cells are thought to play a role in the implantation process which the TE cells have contributed in the phase of apposition, adhesion, and invasion of the endometrium which can support the implantation of the blastocyst in the uterus. Molecular factors are also produced by TE cells that play a role in the embryo implantation process [6–9]. The TE cells have traditionally been graded in a similar manner to the ICM, i.e., by their number and cohesiveness according to three different grades (A, B, C). According to blastocyst grading by Gardner and Schoolcraft, the best TE cell grade (A) contains many cells that form a cohesive epithelium; the middle TE category (B) is composed of few cells forming a loose epithelium, and the worst category (C) describes a TE that contains very few large cells that struggle to form a cohesive epithelium. However, other publications have found no relationship between TE grade and viability (**Figure 4**) [6, 8, 9].

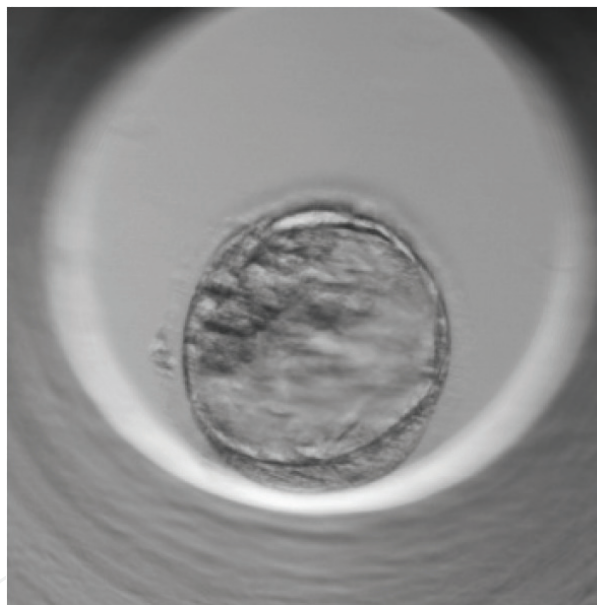
Gardner and Schoolcraft in 1999 introduced the blastocyst grading system which is adopted by the majority of IVF laboratories in the world. In the blastocyst grading system, they classified the degree of blastocyst into three category,



**Figure 4.** Blastocyst morphology. (1) inner cell mass (ICM), (2) blastocoel, and (3) trophoblast.



**Figure 5.**  
*Blastocyst (grade 4: A: A) means that the blastocyst has been expanded (grade 4), has a large ICM and compact (grade A), and has TE which consists of many cells which are mutually binding (grade A).*



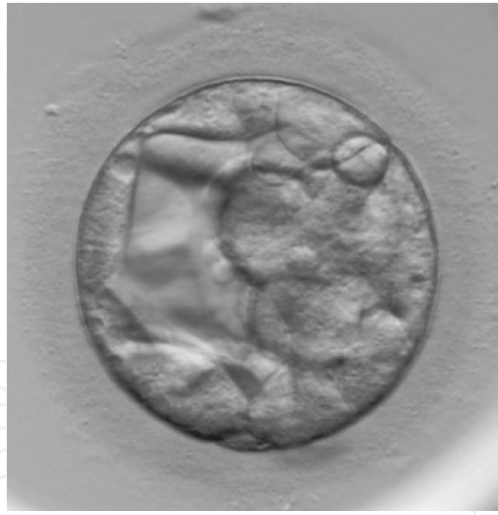
**Figure 6.**  
*Blastocyst (grade 4: C: C) means that blastocyst embryo has been expanded (grade 4), has ICM that is difficult to see and has very few cells (grade C), and has TE which has very little cell (grade C).*

i.e., degree of expansion, inner cell mass (ICM) quality, and the trophoblast (TE) cell quality [6, 10].

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology states that a good embryo on day 5 which is examined at  $116 \pm 2$  hours must be in the form of blastocyst [9].

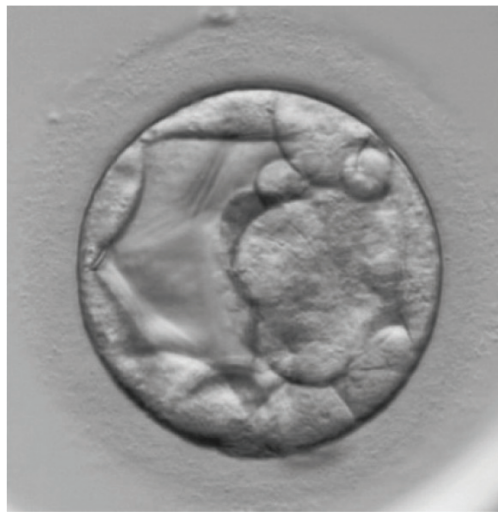
Embryo, according to the Istanbul consensus, is given a grade based on three things; the first thing is the embryo development stage, the second is ICM, and the third is TE. So that the order of the fifth day embryo assessment is based on the stages of development, then based on ICM morphology, and the last is based on TE. The following is a presentation of the fifth day embryo according to the Istanbul consensus (**Figures 5–9; Table 1**) [6, 9].





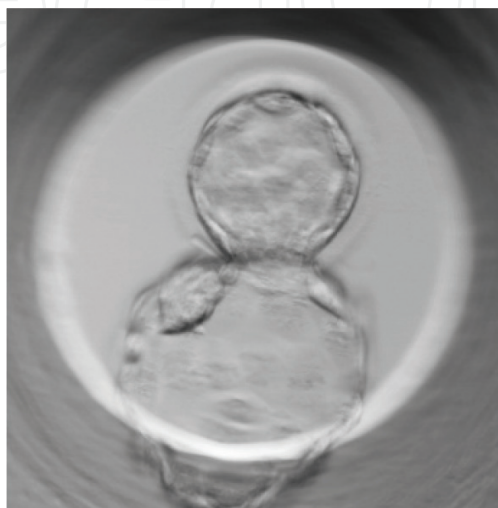
**Figure 7.**

*Early blastocyst is the fifth day embryo development stage which has a blastocoel less than 50% of the embryo volume, and the formation of ICM and TE is not clear.*



**Figure 8.**

*Blastocyst (grade 2: A: A) embryos that have embryo development stage with a grade of 2 are blastocyst embryos which still have thick ZP.*



**Figure 9.**

*Blastocyst (grade 5: A: A) means that blastocyst embryo which has come out of its shell (grade 5) has ICM whose cells are large and easy to observe (grade A) and has TE consisting of many cells (grade A).*

<b>Expansion grade</b>	<b>Blastocyst development and stage status</b>
1	Early blastocyst—Blastocoel cavity less than half the volume of the embryo
2	Blastocyst—Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst—Blastocoel completely filling the embryo
4	Expanded blastocyst—Blastocoel volume is now larger than that of early embryo and zona is thinner
5	Hatching out of the shell
6	Hatched out of the shell
<b>ICM grade</b>	<b>Inner cell mass quality</b>
A	Tightly packed and many cells
B	Loosely grouped and several cells
C	Very few cells
<b>TE grade</b>	<b>Trophectoderm quality</b>
A	Many cells forming a cohesive epithelium
B	Few cells forming a loose epithelium
C	Very few large cells

**Table 1.**  
 The blastocyst grading system. Modified from Gardner and Schoolcraft.

#### 4. Gene expression of human preimplantation embryo

The human embryo preimplantation development is characterized by reprogramming and programming that encompasses fusion of the egg and sperm pronuclei, epigenetic reprogramming, an extensive wave of degradation of maternal transcripts, and activation of the nascent human embryonic genome [11, 12].

There are 1909 genes expressed in only oocytes (maternal genome) and 3122 genes expressed in only blastocyst (embryonic genome). The main difference in observed profile expression between oocytes and embryos is reflected in blastocyst and oocyte specific gene expression analysis. Specific oocyte and blastocyst genes are separated in groups by expression levels. There are 270 specific oocyte genes and 308 blastocyst-specific genes that show high levels of expression. In the panther pathway analysis of high expression genes, the MII oocyte pathway is Wnt signaling pathway, where blastocyst-specific genes are expressed according to integrin signaling pathway, cytoskeletal regulation. The scarcity of the materials, however, both in size (10.1 mm diameter) and in quantity (only a few to tens of oocytes from each ovulation), have been almost the molecular analysis of preimplantation embryos [11–13].

Some interesting patterns in embryo preimplantation based on transcriptional genome-wide analysis include: (1) several genes that experience up- or downregulation during human oocyte maturation from immature germinal vesicle oocyte to the oocyte metaphase II stage; (2) partially expressed transcripts of the MII oocytes will be partially downregulated or degraded during the development to the 4-cell stage; (3) many genes are upregulated after the 4-cell stage, which reflects the main wave Embryonic Genome Activation (EGA); (4) genes involved in lineage commitment are regulated in the development of preimplantation; and (5) many expressed genes dynamically encode transcription factors, epigenetic modifying factors, and chromatin remodeling [12–16].

Human genome activation begins in the 4- and 8-cell stages or even in the early 2-cell stage. Data suggest that TE or ICM cell lineage-associated genes are expressed in human embryos later than in mice at around early blastocyst stage, but it is still unclear. Human embryos can be cultured in vitro for 7–8 days post-fertilization. Genes unique in the blastocysts included annexins A2 and A3 (ANXA2, ANXA3), gap junction protein alpha 1 (GJPA1), guanosine triphosphate-binding protein 4 (GTPBP4), and adenosine triphosphatase H.–transporting, lysosomal accessory protein 1 (ATP6AP1). The blastocyst-specific genes were associated with oxidative phosphorylation, glycolysis, and sterol metabolism and were rich in RNA-binding proteins, methyltransferases, gap junction proteins, and intermediate filaments. Oxidative phosphorylation and glycolysis processes control ATP generation during pre-compaction and cavitation stages, respectively. Highly expressed blastocyst genes were involved in the Rho GTPase, control pathway that regulates cytoskeletal changes occurring during cell growth and development; the PDGF pathway, which plays a critical role in cellular proliferation and metabolism, and the integrin signaling pathway are also important in actin reorganization [12–19].

Studies on gene expression during the preimplantation period have identified transcription factors from housekeeping genes, transcription and growth factor genes, sex-determining genes, tissues specific, novel genes, and genes of unknown functions [12, 13].

#### **4.1 Housekeeping gene**

Housekeeping gene is a gene that plays a role in regulating basal cell function which is important in cell maintenance and also has a specific role in tissues or organisms. Therefore, housekeeping gene is estimated to be expressed in all cells of organism normally, including tissue, external signal, cell cycle, or cell development stage [12, 13, 20].

Beta-actin, keratin-18, ubiquitous cytoskeletal elements, cell adhesion molecules, and alpha tubulin have been detected, as well as such housekeeping genes as hypoxanthine phosphoribosyl transferase (HPRT), adenosine phosphoribosyl transferase (APRT), glucose-6-phosphate dehydrogenase, hexokinase I, and adenosine deaminase. Overall, out of the 536 housekeeping genes investigated, 427 were detected in the oocyte and 452 in the blastocyst [12, 13, 20].

#### **4.2 Transcription and growth factor genes**

Transcription regulators, growth factors, proto-oncogenes cycle gene cells, and receptors are several genes that expressed during the preimplantation period, including CD44, a cell surface glycoprotein that can play a role in implantation; OCT 4 and OCT 6, transcriptional regulators; cyclin B1, a cell cycle gene; colony stimulating factor 1 receptor, c-fms; tumor necrosis factor and its receptors; interleukin-1 type I receptor (IL-1R tI); growth factors such as insulin and their receptors; epidermal growth factor receptor (EGF-R), epidermal growth factor (EGF), and transformation of growth factor-alpha (TGF- $\alpha$ ) [12, 13, 17–19].

#### **4.3 Tissue-specific genes, novel genes, and genes of unknown function**

A major histocompatibility complex molecule was detected in preimplantation human embryos and implicated tissue-specific genes as globin and interleukin-10 and also human transposable element, LINE-1, and known expressed sequence tags

(ESTs) in the GenBank and dbEST databases. Of the 46 peptide hormone genes investigated, 13 were detected in the human blastocysts (SPAG9, TMSB10, OXT, POMC, PPY, SCT, LHB, TRH, PRLH, GNRH2, GIP, CCK, and GPHB5) of which four, namely, SPAG9, TMSB10, OXT, and POMC, showed high expression levels. Thirty-two out of the 162 investigated genes coding for human HRs were detected in the human blastocysts, of which NPM1, ATP6AP2, LEPROTL1, and HTR1D showed high expression levels [12, 13, 15–17, 21].

Aneuploidy is common in humans and is the leading cause of all human birth defects as well as miscarriage; errors can arise in meiosis during generation of the oocyte and sperm and in the mitotic divisions of the nascent embryo. A recent study used array-based technology to examine the genome-wide copy number of distinct loci in the cleavage stage human embryos. This study identified several types of chromosomal abnormalities that occurred in human embryos and observed that mosaicism for whole chromosomes (aneuploidies) in one or more blastomeres occurred in more than 80% of embryos [11, 12, 17, 22].

## **5. Conclusions**

Human preimplantation embryos will become an integral and essential part of such endeavors by setting the genetic foundation that determines the course of human development. It is characterized by reprogramming and programming that encompasses fusion of the egg and sperm pronuclei, epigenetic reprogramming, an extensive wave of degradation of maternal transcripts, and activation of the nascent human embryonic genome. The main objective of blastocyst culture was to increase the success rate of in vitro fertilization (IVF) because of better embryo selection after endometrial synchronicity and/or better genomic activation. Metabolism of the blastocyst occurs in two different places: in trophoblast cells where glucose consumption occurs and half is converted to lactate, whereas glycolysis occurs in ICM. Morphology of the blastocyst depends on the degree of expansion and ICM and TE morphology. This morphological differentiation was thought to represent the developmental capability of the blastocyst. Gene expression during the preimplantation period has identified transcription factors from housekeeping genes, transcription and growth factor genes, sex-determining genes, tissues specific, novel genes, and genes of unknown functions. Genes unique in human blastocysts included annexins A2 and A3 (ANXA2, ANXA3), gap junction protein alpha 1 (GJPA1), guanosine triphosphate-binding protein 4 (GTPBP4), and adenosine triphosphatase H-transporting, lysosomal accessory protein 1 (ATP6AP1). The blastocyst-specific genes were associated with oxidative phosphorylation, glycolysis, and sterol metabolism.

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## **Conflict of interest**

There is no conflict of interests in this manuscript.

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