BLASTOCYST

Blastodermic Vessicle

The mammalian blastula formed during development where the inner cell mass can be found which forms the embryo.

🎀 Reproductive cells 🌸 Male & Female

About Blastocyst

Structure

The blastocyst is a structure formed in the early development of mammals. The structure formed when cells in the mammalian blastula separate into an inner and outer layer. In humans, blastocyst formation begins about 5 days after fertilization, when a fluid-filled cavity opens up in the morula. The human blastocyst is composed of 70-100 cells (Pic. 1).

There are two types of blastomere cells (Pic. 2):

- The **inner cell mass (ICM)**, also known as the **embryoblast**. The ICM cells are non-differentiated and pluripotent and should form a compact and oval formation. Outer membranes of ICM don’t have junctions with other cells and communicate directly with blastocoeal fluid. They are under the regulation of specific growth factors from the blastocoeal that regulate their differentiation into the primitive endoderm.

- The **trophoblast (TE)** is a layer of cells forming the outer ring of the blastocyst that combines with the maternal endometrium to form the placenta. During implantation, the trophoblast differentiates into two distinct layers: the inner cytotrophoblast (is the inner layer of the trophoblast, composed of stem cells which give rise to cells comprising the chorionic villi, placenta, and syncytiotrophoblast), and the outer syncytiotrophoblast (the outermost layer of the trophoblast). Trophodermal cells are associated with frequent small area of tight junctions and desmosomes, which forms a seal between the cells and maintaining cell polarity.

Blastocoeal prevent liquid spills and keep the sodium ions in blastocoeal. These ions cause osmotic gradient, which consequently results in the passive diffusion of water molecules into the blastocoeal causing the blastocoeal to start to grow. The expansion of the blastocoeal plays an important role in differentiation between TE and ICM. One of the functions of TE is also in reducing the oxygen concentration in the blastocoeal fluid and enabling hypoxic conditions that are required for normal gene transcription in ICM cells.

Development

Compaction:

In humans, blastocyst formation begins about 5 days after fertilization (Pic. 3). The blastocyst is a structure formed in the early development of vertebrates. An early embryo begins to divide without increasing its volume. After the third mitotic division, a substantial protein biosynthesis is restored and the embryo starts growing. Consequently, the junctions between blastomeres change, leading to the formation of the compact stage. Compaction should normally be completed on the fourth day of its development when the embryo reaches the morula stage. The junctions are dynamic and change during mitosis. Compaction represents the beginning of differentiation, followed by polarization of peripheral blastomeres and lost of totipotency. The polarization is induced by junctions with neighbouring blastomeres. Due to embryo growth, the cells lose their oval shape and become more tightly connected to each other. Compaction is therefore a process of forming gap junctions, adherens junctions, tight junctions and desmosomes between blastomeres. Gap junctions are especially important for transport of metabolites and molecules that regulate mitotic divisions. The membranes between
the cells are difficult to observe with a light microscope. The abnormally tight junctions lead to exclusion of blastomeres from the formation of a compact embryo.

Cavitation:

After about 1 day (5–6 days post-fertilization), which is the time usually required to reach the uterus, the blastocyst begins to embed itself into the endometrium of the uterine wall where it will undergo later developmental processes, including gastrulation (Pic. 4). After compaction, the cells start forming a fluid-filled cavity - blastocoel. During cavitation, the cells differentiate into the trophoderm (TE) and inner cell mass (ICM). The blastocyst is completely embedded in the endometrium only 11–12 days after fertilization.

Implantation

Implantation is critical to the survival and development of the early embryo. It establishes a connection between the mother and the early embryo which will continue through the remainder of the pregnancy. Implantation is made possible through structural changes in both the blastocyst and endometrial wall. It is by this adhesion that the fetus receives oxygen and nutrients from the mother to be able to grow. In humans, implantation of a fertilized ovum occurs between 6 to 12 days after ovulation. In preparation for implantation, the blastocyst sheds its outside layer, the zona pellucida, which binds sperm during fertilization. The zona pellucida degenerates and decomposes, and is replaced by a layer of underlying cells called the trophoblast. The trophoblast will give rise to the placenta after implantation.

Blastocyst scoring systems

The introduction of blastocyst culture into an IVF program offers the possibility for reducing the number of transferred embryos. But the replacements of more than one blastocyst still results in a very high proportion of multiple pregnancies. For this reason, the tendency for single blastocyst transfer arises and various methods of selecting the optimal blastocyst with good developmental potential have been published. Numerous biochemical studies showed that selection between the blastocysts can be made by evaluating their metabolic activity.

Tripartite scoring of blastocysts:

Gardner and Schoolcraft gave six numerical scores (1-6) to blastocysts regarding the degree of blastocoel expansion and status of hatching. The early blastocysts with the beginning of blastocoel formation are scored as 1 and hatched blastocysts as 6 (Pic. 5).

Grading of blastocysts:

Too little attention has been given to individual grading parameters, and the main question in the selection process is which of the blastocyst structures is more important for achieving normal pregnancy. A tripartite scoring system is therefore difficult to use in evaluating the implantation ability of various morphological types of blastocysts. It is not helpful in cycles in which the blastocysts for transfer have to be selected between suboptimal blastocysts. From this reason, the simple blastocyst grading system was developed. This system took four morphological parameters into consideration: expansion of blastocoels, morphology of ICM, cohesiveness of TE and presence of excluded blastomeres or fragments from the formation of blastocysts (Pic. 6). This system does not distinguish between different degrees of blastocoel expansion. It described eight morphological types of day-5 embryos that are most frequently found in the cohort of vital embryos after prolonged cultivation in vitro (B1 - Optimal blastocysts: full or expanded blastocysts with blastocoel filling the entire blastocyst, oval shaped and compact inner-cell mass (ICM) and multicellular cohesive trophoderm (TE). B2 - Expanded blastocysts with normal ICM, but non-optimal (fragmented or necrotic) TE. B3 - Unexpanded blastocysts and compact morulae with beginning of cavitation. B4 - Expanded blastocysts with normal TE, but non-optimal (non- compact or fragmented) ICM. B5 - Expanded blastocysts with non-optimal ICM and TE. B6 - Slightly smaller blastocysts with up to 20% excluded blastomeres or fragments from the formation of blastocyst. B7 - Necrotic blastocysts without ICM and with large vacuole instead of blastocoel. B8 - Small blastocysts with less than 80% of embryonic mass transformed into compact morulae or blastocysts). All eight types were ranked for their implantation abilities and live birth rates from B1 to B8 (live birth rates: 45.2%, 32.8%, 26.9%, 23%, 17.7%, 16.7%, 7.7%, 1.2%). ICM was found to be the most important factor for successful implantation.

Other morphological characteristics with impact on implantation:

Individual grading parameters were further studied by various groups.

Using morphometry of ICM, Richter et al. defined optimal blastocysts even more precisely. The authors discredited the previously described tripartite scoring systems, stating that the observed differences using this system reflect differences in developmental timing rather than differences in actual quality. More attention in
their own study was given to measuring of ICM size and shape. They found that the ICMs of implanting blastocysts were significantly larger than ICMs of non-implanting ones. A linear positive relationship between ICM size and implantation ability was revealed. Optimal ICM size was defined as measuring >4500 μm² and poor blastocysts with ICM size of <3800 μm² (implantation rates 45% vs. 32%). The ICM shape seems to play an important role in further embryo development as well, since blastocysts with optimal ICM sizes and oval shapes implanted in a higher proportion (60%) than the blastocysts with ICMs that were only optimally sized (29%) or shaped (32%).

Ebner et al. found a significant difference in blastocyst implantation rates when the location of herniation during hatching process is positioned in the ICM region or TE region (67% vs. 41%). Between various morphological characteristics with possible influence on further embryodevelopment, cytoplasmic strings that connect ICM with TE (Scott, 2000) and vacuoles in the ICM region were found to decrease implantation ability. The proportion of cytoplasm excluded from the formation of blastocyst either as blastomeres or fragments is also in correlation with embryo ability to reach a morphologically optimal blastocyst.

The effect of delay in development to blastocyst by one day was also analyzed by several groups. It can frequently occur that embryos reach blastocyst stage only on day 6. The reasons for this phenomenon are not exactly known. However, the implantation rates can be improved when day 6 blastocysts are frozen and replaced during one of the next fresh cycles.

**Blastocyst culture**

Most of the blastocyst culture media enable the embryos to reach the blastocyst stage in more than 50% of cases. The blastulation rate and quality of developed blastocysts can be improved by reducing the oxygen concentration in the incubator atmosphere.

**Blastocyst selection for transfer**

Multiple gestation represents the most significant complication of assisted reproductive treatment. By improving the culture conditions, developing culture media for prolonged cultivation of embryos in vitro and by introducing the blastocyst culture, reduction of the number of embryos for transfer was enabled. Moreover, it has been proved in many studies that the transfer of only one blastocyst in a group of patients with the highest probability for conception can result in a similar pregnancy rate as the transfer of two blastocysts, but the proportion of multiple pregnancies is significantly reduced.

Traditional procedures for embryo evaluation and selection are based on the morphological characteristics observed with a microscope at several discrete time points of embryonic development. At the early cleavage stage, morphological parameters including cell number, proportion of fragmentation, presence and number of nuclei, size and symmetry of blastomeres are used to evaluate and select embryos for transfer. At the blastocyst stage, the degree of blastocyst expansion and morphology of inner cell mass (ICM) and trophoderm (TE) are commonly used to evaluate and grade the blastocysts. Nevertheless, transfer of the top grade embryos often fails to establish a viable pregnancy, while replacement of embryos with poor morphological scores sometimes results in a live birth (Pic. 7). Thus, there are obvious shortcomings with traditional methods of evaluation and selection of embryos for transfer based on morphological characteristics alone.

One of the main reasons for doubts about the reasonability of single blastocyst transfer in the past was in the relatively low success of the blastocyst-freezing program. The pregnancy rate in European countries was around 15% per one thawing cycle. This was only half of the pregnancy rate achieved by fresh blastocysts, but the modification of vitrification techniques in the last couple of years have much improved the survival and live birth rates.

**Single blastocyst transfer:**

Single embryo transfer (SET) has been advocated as a strategy to reduce the frequency of multiple births after in vitro fertilization. The success rate of SET mainly depends on the ability of selection of the best embryo from all those available. The selection made among blastocysts is easier than selection of early cleavage stage embryos. Nevertheless, the morphology of blastocysts is very heterogeneous and a decision for single or double blastocyst transfer is sometimes very difficult, especially if only morphologically suboptimal blastocysts are available.

**Double and multiple blastocyst transfer:**

After double blastocyst transfer (DET) the twin pregnancy rate was 62%, compared with only 3.2% after a single blastocyst transfer. Placement of multiple embryos carries the risk of multiple pregnancy. In the past, physicians have often placed too many embryos in the hope to establish a pregnancy. The appropriate number of embryos to be transferred depends on the age of the woman, whether it is the first, second or third full IVF cycle attempt and whether there are top-quality embryos available. The number of embryos transferred in a
Blastocyst morphology after cryopreservation

Blastocyst is not the optimal stage for cryopreservation. It contains a large amount of liquid in the blastocoele, which must be eliminated before the embryo undergoes cooling. To achieve this, a high concentration of cryoprotectants must be used, despite that they can become toxic to an embryo after a longer exposure time. Vitrification is a cryopreservation method for surplus blastocysts. It seems that non-expanded blastocysts are a more optimal stage for vitrification than expanded blastocysts, since the former survive vitrification at higher rates. Nevertheless, the implantation abilities of devitrified early blastocysts or expanded blastocysts were comparable, but significantly lower when compared to fresh blastocysts.

Vitrification is a two-step technique. In the first step, the blastocyst must be exposed to an equilibration medium, which causes partial dehydration and decreasing in the blastocyst volume and its re-expansion after a couple of minutes. The second step must be performed in one minute. After putting the blastocyst into the vitrification medium, its blastocoele quickly looses liquid. Blastocysts are therefore vitrified in a collapsed stage. When it is warmed, its embryonic mass fills only 50% of the volume within zona pelucida (Pic. 9). By decreasing the concentration of cryoprotectants stepwise, the blastocyst should recover the volume of trophoderm cells that they had before cryopreservation. The intracellular organelles must be redistributed forming polarized cells and a functional Na/K pump, responsible for filling the blastocoele with liquid. Two hours after warming, a blastocyst should partially or completely re-expand to the dimensions it had before vitrification. If the blastocyst survives, its ICM must be equally shaped and sized as before cryopreservation.

Live birth rates after vitrification of variously expanded blastocysts:

Meta analysis of studies comparing transfer outcomes of slowly frozen/thawed and vitrified/warmed embryos and blastocysts showed significantly better clinical results in the vitrification group. Vitrification is becoming an increasingly popular method of cryopreservation due to simplification of the procedure. It is evident that this method improved survival and implantation rates, especially in the blastocyst cryopreservation program. There are some technical details with great impact on blastocyst survival. First, expanded blastocysts are sometimes more difficult to equilibrate with cryoprotectant than other stage embryos, thus, exposure to equilibration solution should be modified from embryo to embryo depending on its expansion rate. However, all procedures are time limited due to possible toxic effects in cases of longer incubation of embryos in cryoprotectant. Secondly, faster rates of cooling and warming can be achieved by minimizing the volume of the cryoprotectant with which embryos are vitrified.

**Gallery**

**Pic**
A - many cells, tightly packed; B - several cells, loosely grouped; C - very few cells.

**Pic**
1 The blastocoeel cavity < half the volume of the embryo; 2 The blastocoeel cavity = half the volume of the embryo; 3 Full blastocyst, 4 Expanded blastocyst, 5 Hatching out of the shell; 6 Hatched out of the shell.

**Pic**
The eight morphological types of day-5 embryos.

**Pic**
Delivery and twins rate in a younger patient group after the transfer of one or two blastocysts of poor quality.

<table>
<thead>
<tr>
<th></th>
<th>Single blastocyst transfer</th>
<th>Double blastocyst transfer</th>
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<tbody>
<tr>
<td>Blastocyst quality</td>
<td>BS - BS</td>
<td>Both BS-BS</td>
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<tr>
<td>No. of transfers</td>
<td>172</td>
<td>368</td>
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<tr>
<td>Clinical pregnancies</td>
<td>57 (33.1)</td>
<td>90 (32.1)</td>
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<tr>
<td>Deliveries</td>
<td>50 (29.1)</td>
<td>83 (26.8)</td>
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<tr>
<td>Singleton</td>
<td>50 (100)</td>
<td>65 (78.5)</td>
</tr>
<tr>
<td>Twins</td>
<td>0 (0)</td>
<td>15 (21.7)</td>
</tr>
<tr>
<td>Triplets</td>
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The appropriate number of embryos to be transferred depends on the age of the woman.

<table>
<thead>
<tr>
<th>Age</th>
<th>Attempt No.</th>
<th>Embryos transferred</th>
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<tbody>
<tr>
<td>&lt;37 years</td>
<td>1st</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>if top-quality</td>
</tr>
<tr>
<td></td>
<td>3rd</td>
<td>No more than 2</td>
</tr>
<tr>
<td>37–39 years</td>
<td>1st &amp; 2nd</td>
<td>1 if top-quality</td>
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<tr>
<td></td>
<td>3rd</td>
<td>if no top-quality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>40–42 years</td>
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devitrification procedure
A Blastocysts in the collapsed stage immediately after devitrification. B Re-expanded blastocysts two hours later.

The blastocyst stage occurs between 5 and 9 days of conception.

Blastocyst just before implantation.

Sources

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