

NAME: ONOSEME STEPHANIA OGHENEAKOPBO

COURSE: EMBRYOLOGY

DEPARTMENT: MBBS

MATRIC NUMBER: 18/MHS02/158

Following all the excitement associated with the first gestational week, the newly formed blastocyst is ready to settle into a supportive environment and continue the growth process. Week 2 is often referred to as the **week of twos**. It's the week when the embryoblast, extraembryonic mesoderm and trophoblast each separate into **two distinct layers**. Additionally, there are **two cavities** that develop within the embryonic unit at this time as well.

While every step is integral for adequate foetal development, one of the most important features of the second week is the completion of **implantation** and establishment of **fetomaternal interactions**. This article will follow the developing embryo through the completion of implantation and development of the non-embryonic components of the conceptus. It will also discuss some complications associated with implantation.

Implantation of the blastocyst

Implantation is a complex biochemical and mechanical process that begins in the first week of gestation and extends into the second week. There are many influencing factors that affect the process. These can be grouped into **maternal** and **embryonal factors**. However, both entities work synchronously in order to effectively achieve implantation. The process of implantation can be subdivided into three phases:

- There is a period of **apposition** where the blastocyst establishes weak interactions with the uterine wall.
- The **attachment** phase occurs when definitive binding of the blastocyst to the uterine [epithelium](#) is more established, such that the blastocyst cannot be flushed from the uterine cavity.
- Finally **invasion** occurs when the blastocyst begins to burrow into the endometrium.

This period usually occurs between the 19th and 24th day of the menstrual cycle. This coincides roughly with the 6th to 10th day following ovulation.

Maternal factors affecting implantation

In anticipation for successful fertilization each month, the inner uterine wall (**endometrium**) undergoes a series of changes in order to facilitate the blastocyst. Recall that there are three layers of the endometrium – the strata basalis, spongiosum and compactum.

Endometrium

The deepest layer is the **stratum basalis**, which functions as the regenerative layer and proliferates to form the stratum spongiosum and stratum compactum. **Stratum compactum** is the most superficial and the **stratum spongiosum** resides between the two. Together, the strata compactum and spongiosum form the **stratum functionalis**; which is the functional layer of the endometrium that facilitates implantation.

Functional layer of endometrium

The development of the stratum functionalis is mitigated by surges in **estrogen** (which are released from the maturing ovarian follicle, under the influence of follicle stimulating hormone). If fertilization did not occur during the previous menstrual cycle, the fall in reproductive hormones result in the degeneration of the stratum functionalis. The shedding of this layer during the menstrual phase of the cycle accounts for the vaginal bleeding known as **menstruation**.

In the subsequent cycle, as follicle stimulating hormone levels rise and stimulate maturation of another ovarian follicle, the resultant increase in estrogen levels leads to proliferation of the stratum basalis. This is known as the **proliferative phase**, during which time there is thickening of the endometrium. Following an increase in luteinizing hormone and the release of a secondary oocyte, the remaining corpus luteum continues to release **estrogen** and **progesterone** to maintain the stratum functionalis. The spiral arteries of the [uterus](#) become longer and more tortuous under the influence of the sex hormones.

Endometrium

Following successful fertilization the uterine epithelia, which is characterized by **ciliated columnar cells with microvilli**, undergoes morphological changes to accommodate the growing embryo. The expression of these cilia (extended processes located at the apical aspect of the cells) is regulated by both progesterone and estrogen. The underlying motile microtubules allow the cilia to move the developing embryo toward a favourable site on the uterine wall. This process usually starts around the end of the first gestational week, when the conceptus is classified as a blastocyst.

Simple columnar epithelium of the endometrium

The process of bringing the conceptus close to the uterine wall is referred to as **adplantation (apposition)**. As the blastocyst rolls along the surface of the uterus, the pole of the blastocyst with the inner cell mass is adjacent to the uterine wall. Early attachments to the **microvilli** (short, non-motile, apical epithelial processes) also facilitate the initiation of implantation.

Once the uterine lining is receptive for implantation, the endometrium is said to be in the **implantation window**. However, this is a complex process that depends on the presence of numerous cytokines, immunomodulators, and increased binding capacity of the epithelium in order for implantation to work. Recall from the first week of gestation that the resulting conceptus is genetically unique when compared with its parents. Therefore, the mother's immune system should identify the blastocyst as a parasitic entity that should be destroyed.

A key element in the implantation phase that is thought to modify this anticipated immune response (and other parts of implantation) is known as **leukaemia inhibitory factor (LIF)**. This is a member of the interleukin – 6 (IL-6) family that has the ability to influence several unrelated gene expressions (i.e. it's a pleiotropic entity). Within the reproductive system, it is produced by both the endometrial epithelium as well as the developing blastocyst. Leukaemia inhibitory factor has the following functions with regards to endometrial receptivity:

- The **decidualization** of the stromal cells (discussed below) is by LIF. This response is generated by way of a cyclic **adenosine monophosphate (cAMP)** pathway that activates a biochemical cascade involving estrogen and progesterone, IL-5, IL-6, prostaglandins, and cyclooxygenase 2 (COX-2). The resultant cellular morphological change creates a more **favourable implantation environment**. This particular reaction is important because it plays a significant role in immunomodulation. It is particularly difficult for thymocytes (T-cells) to invade decidual tissue; similarly, decidual cells have a hard time recruiting T-cells from the percolating blood. Therefore, the probability of the maternal immune system mounting a cytotoxic T-lymphocyte (CTL) response against the allogeneic conceptus is markedly reduced.
- In addition, LIF upregulates numerous epidermal growth factors such as **heparin – binding epidermal growth factor like protein (HB-EGF)**, **epiregulin (EPR)**, and **amphiregulin (AREG)**. These proteins act as ligands for the epidermal growth factor receptors and subsequently stimulate the proliferation of the endometrial epidermis.
- LIF also stimulates the activity of implantation genes, including members of the **Wnt family** (Wnt 4, 5a, and others) and **MutS homolog homebox 1 gene (MSX 1)**. These and other implantation genes result in differentiation of the luminal and glandular epithelia (decidualization), proliferation of the stromal cells, and decrease in the polarity of the epithelium to foster adhesion.
- **Uterodomes** (also known as pinopods) are micro-protrusions of the luminal surface of the uterine epithelium that interdigitate with the blastocyst during adplantation and attachment. While they are present throughout the luteal phase of the menstrual cycle, they increase in number under the influence of LIF. In lower other mammals, pinopods are thought to play a significant role in pinocytosis (cell drinking). However, this is not its primary role in humans. As a result, they are referred to as uterodomes based on their appearance.
- Under normal circumstances, the luminal surface of the uterus is coated with a thick layer of **glycocalyx**. This is a carbohydrate, glycoprotein rich layer that promotes repulsion of foreign entities from the cell membranes. It also has a large quantity of **mucins** (MUC-1 and MUC-4). LIF mediates significant local downregulation of MUC-1 in the area of the epithelium adjacent to the hatched blastocyst, which reduces repulsion of the blastocyst.

However, relatively high levels of mucins are normally present in unfavourable implantation sites to prevent attachment.

- Finally, the presence and activity of a member of the **junctional adhesion molecule family** (JAM-2) is also noted to be increased within the uterus during the 1st to 2nd gestational weeks. Junctional adhesion molecules have the ability to interact within the family (i.e. JAM-JAM binding) as well as with other integrins on non-uterine cells. They therefore promote **cellular attachment** to the epithelial membrane. Both LIF and progesterone have been shown to upregulate the expression of these adhesion molecules.

As mentioned earlier, the luminal uterine stroma undergoes morphological transformation under the influence of LIF, estrogen and progesterone. This process – known as decidualization – involves accumulation of **lipids** and **glycogen** at a cellular level. The cells (i.e. **decidual cells**) subsequently acquire a polyhedral shape (as opposed to their previous columnar appearance). The process begins locally at the site of fetomaternal attachment, but gradually radiates until the entire endometrium has been transformed. On a molecular level, bone morphogenetic protein 2 (BMP-2) is needed for this transformation to occur; its deficiency is a known cause of **infertility**. The decidua provides an immunologically safe space for the embryo, in addition to providing nutrition for its development.

Decidual cells

Fetal factors affecting implantation

As the blastocyst is hatched from the zona pellucida, it comes into contact with the endometrium. Surface proteins on the **trophoblast** known as **trophinin** interact with similar trophinin molecules on the endometrial surface. This plays a role in the differentiation of the trophoblast into two distinct layers:

- In the periphery, there are multinucleated cells that have no discernible cell walls. This area is referred to as the **syncytiotrophoblast**. There is no mitotic activity in this area.
- Deep to the syncytiotrophoblast is a cluster of mitotically active cells known as the **cytotrophoblast**. This cluster of cells rapidly replicates and migrates to the periphery in order to support the growing syncytiotrophoblast.

Cytotrophoblast

Of note, by the end of week 2, the bilaminar disc develops a localized region of thickened cells in the hypoblast. This area is referred to as the **prechordal plate**. Not only does the prechordal plate help with the organization of the head region during development, but it also indicates the future head region and the location of the mouth.

The **syncytiotrophoblast** is a highly invasive structure that penetrates the endometrium in order for the blastocyst to become embedded. The trophinin-trophinin binding also induces endometrial epithelial apoptosis in the mother in order to accommodate the invading cell mass. The blastocyst then utilizes the nutrients from the recently destroyed maternal cells as it continues to grow. Furthermore, the syncytiotrophoblast (under the influence of LIF) begins to produce **human chorionic gonadotropin hormone** (β -hCG). The presence of β -hCG prevents corpus luteum degeneration, resulting in the continued release of estrogen and progesterone. The detection of the glycoprotein in maternal circulation and urine also forms the basis of the pregnancy test.

Syncytiotrophoblast

Another recently discovered element that contributes to implantation is the protein **preimplantation factor**. It is derived from the embryo and has been shown to aid in maternal immunomodulation, embryo-endometrial adhesion, and endometrial apoptosis.

- **Endometrial development** occurs each month in anticipation of successful fertilization.
- Successful fertilization occurs and the zygote develops into a **blastocyst**.
- The blastocyst rolls along the endometrium towards a favourable **implantation site**.
- Concurrently, there is **maternal immunomodulation**, downregulation of repelling entities and upregulation of adhesion moieties.
- The blastocyst hatches from the zona pellucida.
- **Adplantation** occurs when weak carbohydrate binding occurs between the embryo and the glycocalyx.
- As the embryo gets closer to the endometrium, stronger bonds are formed. These involve interactions with integrins, L-selectin, osteopontin, cadherins, trophinin, as well as CD44 and CD98.
- Attachment stimulates **maternal decidualization** and **embryonal trophoblast differentiation**.
- **Trophoblastic invasion** coincides with desmosomal detachment of maternal cells, as well as apoptosis of adjacent decidua.

Blastocyst implantation

Amniotic cavity

While implantation ensues, the embryoblast also undergoes differentiation to form a **bilaminar disc**. The flat, circular disc is comprised of a thicker **epiblast** with high columnar cells and a thinner **hypoblast** with small cuboidal cells. A small space develops relative to the epiblast; it is the precursor of the amniotic cavity.

Epiblastic cells forming the floor of the cavity subsequently separate to form **amnioblasts** that will form the amnion (surrounding the amniotic cavity). The sac and cavity will eventually become filled with **amniotic fluid** later on in the pregnancy. They provide shock absorption and facilitate movement of the foetus during development.

Amniotic sac

Umbilical vesicle

Peripherally, the hypoblast is continuous with another structure known as the **exocoelomic (Heuser's) membrane**. It also forms the roof of the enclosed exocoelomic cavity. Combined, the membrane and the hypoblast form the visceral lining of the yolk sac. However, since the human embryo does not possess a yolk, it is more appropriate to refer to it as the **primary umbilical vesicle**.

Endodermal cells arising from the exocoelomic membrane extends circumferentially to enclose the embryonic disc and both cavities. It is subsequently referred to as the **extraembryonic mesoderm**. Both cavities facilitate embryonic folding as growth and morphological changes occur. The umbilical vesicle may play a role in nutrient transfer to the embryo. Furthermore, it is an important source of primordial germ cells.

Early fetomaternal circulation

While the aforementioned cavities and bilaminar disc develop, the syncytiotrophoblast began to lacunate (I.e. form **lacunae**). The lacunae are filled with an amalgam of cellular debris and maternal blood, known as the **embryotroph**. This fluid gains access to the embryonic disc via diffusion and delivers nutrients as well as oxygen to the embryo. The lacunae subsequently become confluent, forming lacunar networks, which serves as the **primordial uteroplacental circulation**. As the networks continue to fuse, the syncytiotrophoblast has a sieve-like appearance, particularly around the embryonic pole of the conceptus. This will subsequently give rise to the **intervillous spaces** of the placenta.

The capillaries around the implanted embryo become engorged, dilated and their walls become thin. From here onwards, they are known as **sinusoids**. The syncytiotrophoblast continues to erode the walls of the sinusoids, resulting in more maternal blood flowing freely into the lacunar networks. Much of the derived nutrients is conveyed to the embryo by the trophoblast. However, the trophoblast grows a lot faster than the embryo in the early phases. As such, it is likely to have a higher nutritional requirement than the embryo.

Chorionic sac

As time progresses, the extraembryonic mesoderm increases in size. Numerous cavities known as the **extraembryonic coelomic spaces** begin to appear deep to the cytotrophoblast and superficial to the exocoelomic membrane. These spaces coalesce to form the **extraembryonic coelom**. This coincides with a decrease in the volume of the primary umbilical vesicle; which is then referred to as the **secondary umbilical vesicle**. The cells of the secondary umbilical vesicle arise from migratory extraembryonic endodermal cells of the hypoblast. There is a remnant of the primary umbilical vesicle within the extraembryonic coelom that is referred to as an **exocoelomic cyst**.

Chorion

Cellular columns, lined with syncytial coverings, extending into the syncytiotrophoblast indicate the ending of the second gestational week. This phenomenon marks the formation of the **primary chorionic villi**. Splanchnic and somatic derivatives of the extraembryonic mesoderm line the umbilical vesicles, and the trophoblast and amnion, respectively. The somatic extraembryonic mesoderm, along with both trophoblastic layers, gives rise to the **chorion**. The space enclosed by the chorion is the chorionic sac; it contains the embryo, as well as the amniotic sac and umbilical vesicle. The latter three structures are attached to the chorion by the connecting stalk. The former extraembryonic coelom is now referred to as the chorionic cavity.

Chorionic villi

Implantation completed

By the **10th** day following fertilization, the embryo is completely embedded in the endometrium. There is a small defect in the epithelium that is sealed by a fibrin-based blood clot known as the **closing plug**. By day 12, the area is completely healed. The 14 day embryo maintains the form of a flat **bilaminar embryonic disc** at the end of second week. The thickened **prechordal plate** develops as a localized thickening of hypoblast and indicates the future site of mouth and organizer of the head region.

Summary of 2nd Week

Here is a simplified summary of the events of the second week:

- The hatched **blastocyst** moves along the endometrium towards a favourable point of implantation.
- The endometrium is converted into an **immunologically privileged site** with optimum blood supply

- Weak attachments are strengthened.
- Differentiation of the **trophoblast** and **endometrial stroma** facilitate **invasion** of the endometrium.
- Several **cavities** and **membranes** are formed.
- **β-hCG** production is stimulated.
- Complete **implantation** with endometrial scarring is achieved by day 10.
- Complete **regeneration** around the scar results around day 12.
- At the end of week 2, the **chorionic cavity** is formed.

Clinical significance

Human chorionic gonadotropin: Pregnancy test

Implantation spotting can be misinterpreted as the beginning of the menstrual period. Consequently, most pregnant females may not realize that they are pregnant. However, home pregnancy kits rely on the presence of β-hCG to determine whether or not the individual is pregnant.

hCG (human chorionic gonadotropin) is a glycoprotein that is made up of alpha and beta subunits. As hCG accumulates in the **maternal serum**, it is **metabolized** into the constituent alpha and beta subunits, filtered by the [kidneys](#) and excreted in urine. Unlike the alpha subunit, which is homologous to the alpha units in other reproductive hormones (FSH, LH, etc), the **beta (β) moiety** is unique to hCG and therefore can be used as the basis of the **pregnancy test**. Throughout the pregnancy, the β-hCG levels will continue to increase and then decrease as the pregnancy comes closer to term. This can be used to monitor the viability of the conceptus throughout the gestational period.

Implantation ideally occurs within the **body of the uterus**; usually either on the anterior or posterior wall. However, there are instances when the blastocyst may implant in an area other than the corpus uteri. This process is referred to as an **ectopic pregnancy**. These are obstetric emergencies that regrettably require termination of the pregnancy as they threaten the life of the mother and the implantation site cannot facilitate the projected fetal growth.

Of the numerous inappropriate sites that implantation can occur, **tubal ectopic pregnancies** are the most common. Within the [fallopian tubes](#), an ectopic pregnancy is most likely to happen in the ampulla, then at the isthmus, followed by the infundibulum. Implantation in the **ovary**, and abdominopelvic regions have also been reported. **Uterine scarring** (as a result of pelvic inflammatory disease, aggressive dilation and curettage, etc.), early **loss of the zona pellucida**, reduced motility of the cilia (caused for example, by smoking) can result in abnormal implantation.

Sites of abnormal implantation

Placenta previa

There are other cases where the blastocyst implants within the uterine cavity, but the placenta may cover the internal cervical os. Unlike ectopic pregnancies, these pregnancies may actually progress to term. But they are considered **high risk** pregnancies as they are likely to progress to preterm labor. This process is referred to as placenta previa.

Placenta previa can be subdivided into marginal and complete types. In **marginal placenta previa**, the leading edge of the placenta is within 2 cm of the internal vaginal os; while in **complete placenta**, the entire internal os is covered by the placenta. The leading theory behind the onset of placenta previa is that the cranial part of the corpus uteri is unfavorable for implantation. As a result, the blastocyst implants in the caudal part of the uterus, and the placenta develops near or over the internal os. As the **cervix** undergoes changes to facilitate delivery during the third trimester, the placenta loses its attachment to the endometrium.

Digital vaginal examination or coitus can result in painless bleeding from the vagina. This inevitably has unfavorable effects on both the mother and fetus. These patients should be admitted for maternal and fetal monitoring. The goal is to keep the mother hemodynamically stable and ascertain if an emergency cesarean delivery is indicated.

Uterine fibroids

Leiomyomata uteri or uterine fibroids are benign stromal tumours of the uterus. They are quite common among women of **reproductive age**. Clinical manifestation spans a spectrum of **asymptomatic** existence to **dysmenorrhea** and **infertility**. The impact of the leiomyoma is dependent on its location and size. Essentially, large leiomyomas can distort the uterus, making implantation more challenging. Furthermore, they can obstruct the proximal fallopian ostium, preventing the zygote from entering the uterus.

Endometriosis

Endometriosis is another relatively common gynaecological disorder that has been demonstrated to negatively affect **fertility**. Numerous mechanisms have been proposed in the effect of endometriosis on fertility. These include problems with follicular maturation, defective fertilization, and issues with implantation. Patients with endometriosis have demonstrated resistance to **progesterone** as well as decreased levels of **LIF** and **integrins**. These entities are known to positively influence implantation and as such, their absence would be detrimental to the process.

Hydrosalpinx

A hydrosalpinx is a mechanical obstruction of the **distal fallopian tube** associated with **fluid accumulation** in the tubular lumen. It is also relatively common among women with tubal disease and fertility issues. One of the proposed mechanisms by which hydrosalpinx interferes with implantation is **integrin** and **LIF deficiency**. Another is based on the fact that there is intermittent release of hydrosalpinx fluid into the uterine cavity. This coats the blastocyst and results in impaired attachment.

