### Initial differentiation of the trophoblast – overview

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Objectives

The purpose of this Chapter is to review:

1) The initial differentiation of primitive trophoblast into cytotrophoblast and syncytiotrophoblast.
2) Formation of anchoring and floating villi.
3) Further differentiation of the villous and non-villous trophoblast into various types.
4) Processes like apoptosis and syncytia formation that result in trophoblast turnover.

Initial differentiation of trophoblast – an overview

On the seventh day postconception, the blastocyst consists of a single layer of mononuclear trophoblast called the trophectoderm and an inner cell mass called the embryoblast. During further growth, embryoblast form the embryo proper and trophectoderm form the trophoblast and later the placenta.

Trophectoderm initially differentiates into mononuclear trophoblast called the cytotrophoblast (CTB). These cells are the stem cells of the trophoblastic cell line. During the apposition phase of implantation when CTB comes in close contact with the endometrial luminal epithelium, a series of events lead to implantation. As a first step, CTB fuses to form multinuclear syncytiotrophoblast (STB) (BOYD AND HAMILTON, 1970). At this stage extravillous cytotrophoblast (evCTB) and STB are the two types of trophoblast in the implanted embryo (Fig. 5-1) (JANATPOUR, ET AL., 1999; KINGDOM, ET AL., 2000; KUNATH, ET AL., 2004; HARUN, ET AL., 2006; KIMURA, ET AL., 2007).

Actual invasion of the endometrium by the trophoblast starts with the formation of invadopodia (Figs. 5-1, 2). These are thin folds of STB cytoplasm and cellular membrane. These extensions advance between adjacent endometrial luminal epithelial cells and reach the underlying basement membrane. This process is facilitated by the separation of the luminal epithelial cells during early stages of implantation. Thus the initial contact between the trophoblast and endometrium occurs mostly on the lateral borders of the luminal epithelial cells. This is where cell adhesion systems essential to implantation are upregulated (See Chapter 4). Cell junctions and especially desmosomes are decreased to facilitate implantation. STB then spreads along the epithelial surface of the basement membrane and forms a partially flat trophoblastic plate.
FIGURE 5-1. The implantation and invasion sequence of the human blastocyst.

A) Blastocyst enters the uterine cavity approximately 72 hours after fertilization and starts rolling in the uterine cavity. After hatching, the trophectoderm becomes exposed to the uterine milieu and among other factors starts to express L-selectin. Although the endometrial luminal lining is covered with MUC-1, which does not allow implantation, as the time for actual implantation approaches, this substance disappears from a focal area and ligands of L-selectin, which were not accessible before this step, become accessible (See Chapter 4, Fig. 4-5).

B) As the blastocyst approaches to the implantation site, embryonic plate is positioned parallel to the surface.

C) When STB forms the invadopodia the invasion starts. 1) Trophoblast. 2) L-selectin expressed by the trophoblast. 3) Blastocyst is in a rolling motion after it enters the uterine cavity. 4) MUC-1 covering the endometrial lining. MUC-1 hides the ligands for L-selectin except a designated area, so implantation can occur only at that location. 5) Epithelial lining of the endometrium. 6) Ligands of L-selectin are exposed to facilitate implantation. 7) Endometrial glands and stroma. 8) Lacunae filled with maternal blood. 9) CTB. 10) STB. 11) Invadopodia of STB.

D) The invasion progresses and blastocyst burrows deeper into the decidua.
The differentiated mononuclear CTB form the STB by I. So STB have multiple nuclei and no discernible cytoplasmic borders. They initiate the invasion towards the implantation site, erode the basement membrane and enter the maternal decidua. They have an affinity towards maternal capillaries. Endometrial glands at this stage contain abundant secretions. CTB continue to proliferate. Later they push through the STB layer and take over the uterine invasion, establishing the placenta. 1) Blastocoele. 2) Trophoblast layer covering the blastocyst. 3) Differentiated mononuclear CTB. 5) STB. 6) Invadopodia. 7) Basement membrane. 8) Embryoblast.
Initially trophoblastic growth is only by STB, which slowly surrounds the blastocyst *(Fig. 5-3)*. This growth and later primary invasive functions of STB are possible because of rapid proliferation of mononuclear CTB and their fusion to form this type of trophoblast.

When STB growth reaches a critical point, the basement membrane is digested allowing the STB to reach the endometrial stroma. STB continues to expand and covers the entire embryo and its developing structures. The expanding STB invades all the structures in its path, which include maternal capillaries and endometrial glands.

STB also grows around congested endometrial blood vessels and endometrial glands. STB erodes these structures and causes them to break down creating large spaces filled with maternal blood and endometrial glandular secretions. These are called *lacunae*. Opening of uterine vessels into the lacunae and the STB represents the beginning of the *uteroplacental circulation*. Adjacent lacunae soon fuse to form *lacunar networks*, which give the STB a sponge-like structure. The lacunar networks are the primordia of the *intervillous spaces* of the placenta. When maternal blood flows into the lacunae, its nutritive substances become available to the embryonic tissues. As both arterial and venous maternal blood vessels come into the lacunae, blood circulation becomes more efficient. Oxygenated blood passes into the lacunae from the spiral arteries, and deoxygenated blood is removed from them via the veins of the uterus.

By 10 to 12 days after fertilization, the embryo is completely embedded in the endometrium. The site of penetration is initially marked by a plug of blood clot and cellular debris. By day 12 an almost completely regenerated epithelium covers the defect *(Fig. 5-3)*. The end of the second week is characterized by the first appearance of chorionic villi.