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THE PHYSIOLOGY OF PLACENTATION

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ABSTRACT. Placentation is a complex spatial and temporal process, involving manu local and general factors, both placental and maternal. The vasculogenesis of the placenta is evident after 21 days of amenorrhea and ends at 10 weeks. Trophoblastic invasion, ranging from 8 to 18 weeks, has two spatial pathways, interstitial and endovascular. During the second trimester, the second stage of endovascular trophoblastic invasion extends into the lumen of spiraling arteries deeper in the myometrium, and the vessels turn into dilated vessels with thin walls, which are passive pathways for increased uteroplacental blood flow from pregnancy. This process is guided and controlled by enzymes, proliferation markers, adhesion molecules, extracellular matrix degradation, oxygen tension, immunological factors, insulin-like growth factor, placental fibroblasts and placental macrophages, vasoactive factors, and angiogenic factors.
KEYWORDS: placenta, trophoblast, pregnancy, endometrium, decidua

1. TROPHOBLASTIC INVASION

1.1 Temporal continuity

In the first trimester of a normal pregnancy, the proliferative trophoblast invades the deciduous segment of the maternal spiral arteries, replacing the endothelium and destroying the muscular and elastic layers, placental vasculogenesis being evident after 21 days of amenorrhea (Karumanchi et al, 2014) and ending at 10 weeks (Lain et al, 2002; ACOG, 2002; Chambers et al, 2001; Pijnenborg et al, 1983) the arterial wall is replaced by a fibrinoid. Trophoblastic invasion from 8 to 18 weeks has two spatial pathways: interstitial and endovascular; endovascular cells adopt an endothelial-like phenotype (Pijnenborg et al, 1983; Pijnenborg et al, 1981; Pijnenborg et al, 1983). The trophoblastic invasion of the decidua basalis and myometrium begins at the center of the implantation site and spreads to the edges of the placental bed; in the second trimester, the trophoblast often has an annular shape with lower cellularity in the center (Pijnenborg et al, 1983; Pijnenborg et al, 1981; Pijnenborg et al, 1983). Interstitial trophoblastic mononuclear cells reveal agglomeration and fusion in giant multinuclear cells that are no longer invasive; some interstitial cells can surround spiral arteries and adopt a perivascular position (Pijnenborg et al, 1983; Pijnenborg et al, 1981; Pijnenborg et al, 1983).

1.2 Stages

The first step in altered spiral arteries involves the vacuolization of endothelium and media without the participation of the trophoblast. The second step consists in the additional disorganization of the media, resulting in intercellular spaces augmented by a disrupted muscular and discontinuous organization (Pijnenborg et al, 2006); the interstitial trophoblast, which induces extracellular matrix alterations through the secretion of enzymes that degrade it (the early vascular remodeling associated with the interstitial trophoblast) is present (Pijnenborg et al, 2006). In the third step, the endovascular trophoblast appears in the lumen of the vessels, which is incorporated intramurally in the fourth step by penetrating vascular endothelium via intercellular loopholes (Pijnenborg et al, 2006) so that the endothelium does not completely disappear even if the trophoblast can induce apoptosis of the endothelium (Ashton et al, 2005); in this fourth step a fibrinoid material is seized, which includes the other trophoblasts while they are still in the luminal position (Pijnenborg et al, 1996). This trophoblast included in fibrinoid is mononuclear and has the appearance of a "spider" due to cellular prolongations, unlike interstitial trophoblasts. The fifth step is the repair of maternal endothelium, with thickening of the intima by the proliferation of myo-intimal cells (Pijnenborg et al, 2006).

1.3 Importance

During the second trimester, the second stage of endovascular trophoblastic invasion extends into the lumen of spiraling arteries deeper in the myometrium, and the vessels turn into dilated vessels with thin walls, which are passive pathways for increased uteroplacental blood flow from pregnancy; there is a difference of at least one month (4-6 weeks) between the deciduous and myometrial endovascular invasion phase, but the process is considered to be continuous, not two separate processes (Pijnenborg et al, 1983).

2. ENZYMES INVOLVED

The trophoblast expresses NO endothelial synthetase (eNOS), but not inducible (iNOS, which occurs only in infections and inflammation) or neuronal (nNOS), nitric oxide being a powerful vasodilator (Lyall, 2003).

The eNOS isoform is expressed after estrogen stimulation, and NO release is inhibited by a specific estrogen receptor antagonist (ICI 182,170) (Russell et al, 2000).

NOS has two natural inhibitors: NG-monomethyl-L-arginine (L-NMMA) and NG, NG-dimethyl-L-arginine or asymmetric dimethyl-arginine (ADMA); the isoform of the latter, symmetrical dimethyl arginine (SDMA) does not inhibit NOS (Vallance et al 1992). ADMA is metabolised by DDAH I and II enzymes, the latter also found in the placenta (Leiper et al, 1999). Another important enzyme in trophoblast proliferation is HO (hemooxygenase), which produces carbon monoxide, with three isoforms (1, 2, 3); the HO-2 isoform interferes with NOS for vasodilatation (Zakhary et al, 1996; Lyall et al, 2000).

3. PROLIFERATION MARKERS

Proliferation markers studied for the trophoblast include Ki-67, MIB-1, 3H-thymidine and PCNA (antibodies against the nuclear proliferative nuclear antigen) (Kosanke, 1994, Műlhauser et al, 1993).

Cytokeratin is also involved in the differentiation and proliferation of epithelial cells, including trophoblasts (Műlhauser et al, 1993).

Transforming growth factor (TGF-b1, b2 and b3) also plays a role in trophoblastic invasion. The expression of TGF-β3 in the viral trophoblast begins at weeks 5-6, maximal at 7-8 weeks and undetectable at 9 weeks; the serum level of the three isoforms is unchanged between weeks 7 and 19. TGF-b 2 is the isoform that would play a role in trophoblastic invasion (Lala et al, 1996; Caniggia et al, 1999).

4. ADHESION MOLECULES

Integrins and adhesion molecules (CAM) play an important role in trophoblastic invasion, being grouped into four families:

- cadherin family (E, N, P, R, B, VE)

-immunoglobulin family: PECAM-1 (endothelial cell adhesion molecule), VCAM-1 (vascular cell adhesion molecule), ICAM-1, 2 and 3 (intercellular adhesion molecule), NCAM (neural cell adhesion molecule)

-selectine family (E, P, L)

-family of integrins (α and β subunits) (Zhou et al, 1997; Alberts et al, 1994; Birk et al, 1991; Kreis et al, 1993).

CAMs have a wide range of functions, including maintaining tissue integrity, transducing cellular signals and regulating the immunological and inflammatory response. The villous cytotrophoblast expresses cadherin E, and the invasive one cadherin VE (the first inhibits the invasion, the second supports it). VCAM, PECAM, selectin E and integrins exhibit a change in expression so that the invasive trophoblast acquires the endothelium like phenotype. All invasive cells have altered phenotypes of CAMs and enzymes for lysis MEC (Zhou et al, 1997; Alexander et al, 1991).

The cytotrophoblast expresses the integrins α1 / b1, α5 / b1, α6 / b4 and α3 / b1, and the decidum expresses in this region α1 / b1 and α6 / b1 and intercalates with the maternal cells by fibronectin A + B +, collagen IV and laminins A), B1 (b1), B2 (g1) and M (a2); the cytotrophoblast is no longer connected to the basal membrane, type IV collagen and fibronectin no longer expressed. Collagen IV, laminin, fibro and vitronectins, but not collagen I, III, VI or fibrin are detected. (Damsky et al, 1992; Aplin, 1993; Korhonen et al, 1991; Castellucci et al, 1991).

5. EXTRACELLULAR MATRIX DEGRADATION (ECM)

ECM is composed of a variety of proteins and polysaccharides assembled in an organized network, being mainly produced by cells inside the matrix (Alberts et al, 1994, Birk et al, 1991; Kreis et al, 1993).

For a successful successful invasion, the trophoblast must induce a series of genes involved in digesting MEC: proteinases together with their activators and inhibitors (Hutin et al, 1983).

The most studied are TIMP and plasmin-activated matrix metalloproteinases (MMPs) which, in turn, arise from activation of plasminogen by plasminogen activators: uPA (urokinase PA) and tPA (tissue type PA) . MMP 1, 2, 3, 7, 9 and 11 are associated with the invasive trophoblast phenotype; Types 2 and 9 appear early, but not on time. TIMP-1 to 3 are deciduous in the first quarter, 1 and 3 suffering up-regulation in the third quarter (Graham et al, 1996).

6. OXYGEN TENSION

Differentiation of the trophoblast from the incipient pregnancy occurs in a low oxygen environment, increasing it in weeks 10-12, with the opening of the interstitial space for the maternal circulation; this increase corresponds to the maximal phase of trophoblastic invasion in the spiral arteries (Pijnenborg et al, 1983).

Trophoblastic invasion is 5-10 times more effective at an oxygen concentration of 20% than 2% (Ashton et al, 2005).

Oxygen pressure increases from 17.9 mmHg to 8-10 weeks at 60.7 mm Hg at 12-13 weeks following spiral artery remodeling (Rodesch et al, 1992), then at 45-50 mm HG at term (Soothill et al, 1986).

In normal oxygen medium, the invasive trophoblast phenotype includes inhibition of HIF-1a and TGF-β3, reduced proliferation and increased expression of MMP 9 and integrin α1 (Caniggia et al 2000; Caniggia et al 2000b).

Pregnancy is a state of moderate oxidative stress (placental oxidative changes and increased generation of superoxide oxygen species and other reactive oxygen species), countered by a number of factors such as SOD or superoxide-dismutase, catalase, GPX or glutathione peroxidase and GSTPi or glutathione S-transferase, together with cysteine and glutathione cofactors (Jauniaux et al, 2000; Raijmakers et al, 2001).

7. IMMUNOLOGICAL ASPECTS

According to some studies, invasive trophoblastic cells undergo an antigenic modification to be similar to endothelial vascular antigens, which protects them from recruitment and rejection by deciduous NK cells, this stage seems to be missing in preeclampsia (ACOG, 2002; Duley, 2002; Esplin eta al, 2001; Sibai et al, 1997).

HLA-G, a class 1B antigen with low specific tissue and polymorphism, but not classical HLA classes I and II (A, B, DR, DQ, DP), is expressed in the maternal trophoblast (Kilpatrick et al, 1990). The strong expression of HLA-G could at least partially explain the maintenance of the semiallograft represented by the conception product by inhibiting T lymphocytes and NK cells from decidua, which is particularly important due to the association of trophoblast and NK cells (Le Gal et al, 1999; McMaster et al, 1995).

It seems that HLA-G is also a preinvasive condition necessary for a physiological trophoblast invasion (Brunori et al, 2000). The trophoblastic cells express HLA-G, ALK-1, 2 and 3 surface receptors and interact with TGF-β, the immunoreactivity being maximal at weeks 5-9 and much reduced at weeks 12-13. HLA-G would help trophoblastic cells escape IL-2, a decoy cytotoxin (Graham et al, 1996; Caniggia et al 2000).

8. INSULIN-LIKE GROWTH FACTOR

Insulin-like growth factors (IGFs) and specific binding proteins (IGFBPs) could play a role in regulating trophoblastic invasion (Crossey et al, 2002).

9. PLACENTAL FIBROBLASTS AND PLACENTAL MACROPHAGES

In the first trimester, these cells synthesize a wider range of ECM components than in the term, extracellular ECM being produced by them (Garcia-Lloret et al, 1994). Macrophages also play a role in the transport of stromal substances, angiogenesis regulation and immune fetal defense, and can be found in fetal villities from week 4 to term (Khan et al, 2000). Together with placental macrophages, they produce CSF-1 and GM-CSF80, which regulates the survival, activation and growth of macrophages, which in turn produce PGE2 and TXA2 following stimulation with LPS (lipopolysaccharides) (Wetzka et al, 1997).

10. VASOACTIVE FACTORS

Table II. Vasoactive factors involved in placentation (Schneider et al, 2001; Wetzka et al, 1997)

Type	Effect
NO	Vasodilation Inhibits platelet aggregation Adjusts myocardial contractility Regulates endothelium-leukocyte interaction Adjusts endothelial integrity Regulates vascular cell proliferation
PGI2	Vasodilatation
Hyperpolarizing factors	Vasodilatation
Endothelin-1	Vasoconstriction
TXA2, PGH2	Vasoconstriction
Angiotensin II	Vasoconstriction; AT1 and 2 receptors, the first being responsible for most pressures
Urotensin II	Vasoconstriction
VEGF, sFlt-1	Angiogenesis Control
tPA, PAI-I	Fibrinolysis regulation; increase in pregnancy
Cytokines (IL-1, IL-6, CSF)	Lymphocyte activation
Bradykinin	Vasodilatation (including COX and NOS inhibition)
Acetylcholine	Vasodilation (including partial effect in the presence of L-NMMA)

Endothelin receptors include ETA, ETB 1 and ETB 2, ETB 1 being the one that mediates vasodilatation, and the other vasoconstriction (Molnar et al, 1995).

Peripheral vasodilation in pregnancy is evident at 5 weeks and becomes complete at 16 weeks. Cardiac output increases by 45% at 24 weeks (Robson et al, 1989).

At the end of the first trimester, peripheral vascular resistance decreases by 40%, so the renal vascular flow increases by 80% to 24 weeks, the flow of the extremities increases by 200% and the uterine arteries by 1000%. By comparison, cerebral and hepatic blood flow remains constant (Davison et al, 1984).

cGMP also contributes to Vasodilatation, whose level increases in early pregnancy and remains elevated to term and acts by NOS induction, the effect being complementary (Verma et al, 1993).

Between weeks 10 and 30, maternal TA drops, returning to pre-pregnancy values after week 30 (Savvidou et al, 2000).

11. ANGIOGENIC FACTORS

VEGF (Vascular Growth Factor) presents the A-D, A and C * isoforms present in syncytiotrophoblast. They act through VEGFR-1 (flt-1), 2 (KDR / flk-1) and 3 (flt-4) receptors; 1 and 3 are present in invasive cytotrophoblast (Zhou et al, 2002; Ahmed et al, 1995; Dunk et al, 2001).

Placental growth factor (PIGF) has elevated values up to the end of Q2, and tyrosine kinase-1 fms-like (sFlt-1) is low, this proangiogenic trend then changing to moderate placental growth (Ahmed et al, 1995; Dunk et al, 2001).

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