

Minireview:

The role of progesterone in implantation and trophoblast invasion

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Abstract

By its genomic and non-genomic actions progesterone plays a role in preparing the endometrium for implantation and also in regulating trophoblast invasion and migration.

The genomic actions of progesterone are mediated by the classical nuclear progesterone receptors, PR-A and PR-B. In addition to their genomic actions, nuclear progesterone receptors may also trigger rapid cytoplasmic signalling events. Membrane-bound progesterone receptors have been implicated in rapid non-genomic actions of progesterone. Both genomic and extra-nuclear actions of progesterone are crucial for adequate decidualization and implantation.

Progesterone plays a role in establishing uterine receptivity by blocking the proliferative effect of estrogen, and by inducing genes that allow the endometrium to permit embryo attachment, and also acts as a negative regulator of trophoblast invasion by controlling matrix metalloproteinase (MMP) activity.

Introduction

During the first trimester of human gestation, the competent blastocyst adheres to the decidualized endometrium, to invade the uterine tissue and vessels and to establish pregnancy. To enable implantation the uterus undergoes tissue remodelling characterized by the appearance of specific adhesion molecules, vascular changes (i.e., transformation of uterine arteries into spiral arteries) and transient secretion of pro-inflammatory cytokines. This period is the implantation window that occurs on the 6th - 9th day after fertilization in humans.

Implantation is a strictly orchestrated equilibrium characterized by continuous fetomaternal dialogue. Implantation is only possible during the implantation window, and it is restricted in space to the endometrium and to the proximal third of the myometrium. Trophoblast invasion is a multi-step process. It involves the attachment of trophoblast cells to the extracellular matrix (ECM) components, degradation of the decidual and endothelial ECM and migration through the eroded connective tissue.

Among others, progesterone and progesterone-induced proteins play a crucial role in implantation. This review aims to discuss the multiple actions of progesterone via which it modulates implantation and trophoblast invasion.

1. Progesterone and progesterone-receptor isoforms

Progesterone is secreted by the ovaries and later on, by the fetoplacental unit in increasing concentrations throughout pregnancy, and acts in a paracrine or autocrine manner. Progesterone regulates not only the peri-implantation period and immune responses during pregnancy, but also the induction of labour and cervical ripening.

In the reproductive tract, progesterone exerts its effects via genomic and non-genomic actions that converge to produce tissue- and cell-specific responses (Gellersen et al., 2009).

The genomic actions of progesterone are mediated via the classical nuclear progesterone-receptors (PRs), PR-A (94 kDa) and PR-B (116 kDa) (Li and O'Malley, 2003). These are DNA-binding proteins that upon ligand binding recognize specific *cis*-acting progesterone response elements typically located in the promoter region of target genes and modulate their transcription (Savouret et al., 1990). The two types of nuclear PRs have distinct roles in reproduction. PR-A knockout mice show implantation failure and impaired decidualization

(Mulac-Jericevic et al., 2000), whereas both of these are normal in PR-B knockout mice, but mammary gland development suffers in the latter (Mulac-Jericevic et al., 2003).

In addition to the classic nuclear progesterone receptors, different intracellular progesterone-receptor variants have also been detected, including the PR-C (60 kDa), PR-M (38 kDa), PR-S and PR-T isoforms (Hirata et al., 2002). A putative mechanism of action of the truncated isoforms would be to antagonize PR-A or -B activation by sequestering progesterone. In line with this, recent evidence suggests a role for PR-C in parturition (Condon et al., 2006).

In addition to its genomic actions, PR-B has the capacity to activate rapid cytoplasmic signalling events; it can interact with the Src-homology 3 (SH3) domain of Src tyrosine kinases at the plasma membrane and trigger the Ras/Raf-1/MAPK pathway (Boonyaratanakornkit et al., 2001). These signalling pathways are critical for proliferation, differentiation and angiogenesis, and consequently, also for decidualization (Shimizu et al., 2005). The truncated intracellular progesterone receptor forms might also antagonize the cytoplasmic signalling activities of PR-B.

Membrane-bound progesterone receptors (Zhu et al., 2003) have been implicated in rapid non-genomic actions of progesterone (Thomas, 2008). The seven transmembrane domain mPR family (mPR α , mPR β , mPR γ , mPR δ and mPR ϵ) as well as the progesterone receptor membrane component 1 (PGRMC1) are distinct from the nuclear PRs and from the G-protein-coupled receptors (Thomas et al., 2007). Upon progesterone binding, mPRs may influence the activity of several signalling pathways, including mobilization of intracellular Ca²⁺ (Ashley et al., 2006), activation of MAPK cascades and inhibition of cAMP production (Hanna et al., 2006; Karteris et al., 2006; Thomas, 2008).

2. Regulation of endometrial receptivity and trophoblast attachment to the endometrial surface by progesterone

Implantation starts with apposition and attachment of the blastocyst. Under the influence of steroid hormones, the endometrium becomes receptive for a limited period of time, called the implantation window. Progesterone plays a key role in establishing uterine receptivity, by blocking the proliferative effect of estrogen on uterine epithelial cells, and inducing genes that allow the normally refractory endometrium to respond to and permit attachment of the embryo (Fig.1).

During the implantation window, progesterone induces the expression of the basic helix-loop-helix transcription factor *Hand2* in the decidual stroma, which in turn suppresses the production of fibroblast growth factors, e.g., FGF-1,-2,-9,-18 - that act as paracrine mediators of mitogenic effects of estrogen on the epithelium (Li et al., 2011). In the uterine stroma of mutant mice lacking *Hand2* gene in PR-positive cells, there is a continued induction of FGFs, together with epithelial proliferation and active estrogen-induced signalling pathways, resulting in impaired implantation and infertility (Li et al., 2011).

Progesterone induces the expression of Indian hedgehog (Ihh) in the endometrial epithelium (Takamoto et al., 2002) that activates stromal COUP-TFII (NR2F2). Lower induction of Ihh is seen in response to progesterone in the uteri of mutant mice lacking nuclear PRs (Matsumoto et al., 2002). COUP-TFII mediates progesterone-induced suppression of epithelial estrogen action through reducing epithelial steroid receptor coactivator-1 (SRC-1) as well as estrogen receptor α (ER α), and inhibiting phosphorylation of ER α during the peri-implantation period. Uterine-specific *COUP-TFII* knockout female mice are infertile due to impaired embryo attachment and uterine decidualization. Furthermore, the expression of ER α and SRC-1 are upregulated in conditional *COUP-TFII* knockout mutants (Kurihara et al., 2007).

COUP-TFII activates the bone morphogenetic protein 2 (Bmp2) to allow decidualization. Bmp2 mediates FKBP chaperones, and activates Wnt4 and Cox2. Though embryo attachment is normal in uterine-specific *Bmp2* knockout female mice, the uterine stroma does not undergo decidual reaction to support further embryonic development (Lee et al., 2007).

The transcription factor forkhead box O1 (FOXO1) engages in transcriptional crosstalk with progesterone receptor to coordinate cell cycle regulation and differentiation of human endometrial stromal cells into decidual cells. FOXO1-dependent transcriptional targets are Wnt signalling-related genes (*Wnt4*, *Wnt16*), the insulin receptor, differentiation markers (e.g., *PRL*, *IGFBP1*), and the cyclin-dependent kinase inhibitor p57 (Takano et al., 2007). Analysis of FOXO1-dependent down-regulated genes uncovered several factors involved in cell cycle regulation (e.g., cyclin B1, cyclin B2, cyclin-dependent kinase-1). siRNA inhibition of FOXO1 significantly attenuated the effects of progestin to inhibit endometrial epithelial cell growth (Kyo et al., 2011).

Wnts are implicated in implantation and early trophoblast development as well as in the pathogenesis of trophoblastic diseases. Recent studies reported that various Wnts (e.g., Wnt5a, Wnt11) are present in the pre-implanting embryo and a shift was demonstrated from non-canonical signalling in the pre-implantation period towards canonical signalling in

activated blastocysts during implantation (Sonderegger et al., 2010). Dickkopf-related protein-1 (Dkk1), a major secreted Wnt signalling antagonist is upregulated by progesterone in the endometrium during the implantation window, furthermore, progesterone-dependent induction of Dkk1 inhibited Wnt signalling (Li et al., 2010; Liu et al., 2010), suggesting that repression of the pathway plays a role in decidualisation. In Wnt-activated Ishikawa cells, progesterone inhibits Wnt signalling by inducing Dkk1 and FOXO1. Furthermore, progesterone inhibition of Wnt signalling was partially circumvented in both Dkk1 and FOXO1 knockdown cells (Wang et al., 2009).

The progesterone-mediated basic leucine zipper CCAAT/enhancer binding protein β (C/EBP β) regulates stromal cell proliferation during the peri-implantation period. C/EBP β binds to the promoter of cyclin B2 and induces its expression while C/EBP β inhibits p53 and cell cycle inhibitors p21 and p27. Uteri of mice lacking C/EBP β did not respond with stromal cell proliferation to decidual stimulation, because of altered expression of cell cycle regulatory factors that control the G₂ to M transition of the proliferating uterine stromal cells (Wang et al., 2010).

Apposition and adhesion of the blastocyst are integrin-dependent processes and take place in a chemokine- and cytokine-rich microenvironment. Embryo attachment is mediated by cytokines (LIF, IL-1, CSF), epidermal growth factor (EGF) family members (EGF, HB-EGF, amphiregulin), cell adhesion molecules and different glycoproteins. Progesterone alters the expression of these regulators.

PR-A upregulates the uterine cell-surface glycoprotein Muc1 that acts as a barrier to trophoblast invasiveness. The anti-adhesive Muc-1 prevents blastocyst attachment to the uterine epithelium until 3 days after entrance to the uterus. In infertile patients with polycystic ovary syndrome PR expression together with Muc-1 expression was found to be significantly higher than in fertile patients (Margarit et al., 2007).

Progesterone is responsible for the induction of fibronectin-receptor expression via calcitonin. Calcitonin acts on the preimplantation embryo by binding a G-protein-coupled receptor, triggering an increase in intracellular calcium, activation of adenyl cyclase and expression of the fibronectin receptor (Staun-Ram and Shalev, 2005).

Progesterone regulates the expression of HB-EGF and amphiregulin. HB-EGF regulates decidualization by upregulating the cyclin D3 mediated polyploidization of uterine stromal cells (Jessmon et al., 2009). Progesterone maintains EGFR expression during pregnancy and promotes growth of the decidua.

In response to the increasing progesterone level, the expression of homeodomain transcription factor HoxA-10 rises during the implantation window. Lower induction of Hoxa10 is seen in response to progesterone in the uteri of PR-defective mice (Matsumoto et al., 2002)

During the implantation window, expression of $\alpha_v\beta_3$ and $\alpha_4\beta_1$ integrins increase in endometrial epithelium in response to progesterone. β_3 subunit expression is upregulated by HoxA-10. Pinopodes are progesterone-dependent apical epithelial projections and their appearance is induced by HoxA-10. In vitro studies showed that blastocysts tend to attach to pinopode rich areas of cultured endometrial epithelium. The abundance of pinopodes relates to implantation success and patients with multiple implantation failures fail to produce pinopodes (Nikas and Makrigiannakis, 2003). Recently, pinopodes have been demonstrated throughout the luteal phase of the menstrual cycle in human, which question the clinical usefulness of these structures as markers of endometrial receptivity (Quinn and Casper, 2009).

HoxA-10 mediates progesterone regulation of Cox2 and the prostaglandin receptor EP₃ and EP₄. EP₃ and EP₄ are activated by PGE₂ that has been implicated in both endometrial decidualization and embryo implantation by regulating mitogenic and angiogenic processes. EP₃ and EP₄ are aberrantly expressed in the uterine stroma in *HoxA-10* knockout mice (Lim et al., 1999).

Human decidualized endometrial stromal cells express insulin-like growth factor-binding protein 1 (IGFBP-1) that is transcriptionally regulated by FOXO1 and HoxA-10, which together upregulate the IGFBP-1 promoter activity (Kim and Fazleabas, 2004). IGFBP-1 is a soluble protein modulating the bioavailability of IGF-I and IGF-II.

Mice lacking HoxA-10 demonstrate a defective stromal cell proliferation, together with altered expression of two cyclin-dependent kinase inhibitor genes, p57 and p15 (Lu et al., 2008). HoxA-10 deficiency also leads to severe local immunological disturbances, characterized by polyclonal T cell proliferation that occurs in contrast to the progesterone-mediated immunosuppression normally present in the peri-implantation uterus (Yao et al., 2003).

Uterine expression of chemokines is hormonally regulated; progesterone upregulates endometrial IL-8 and MCP-1 as well as CXCL1 and CXCR4 during the receptive phase of the cycle (Hess et al., 2007).

During the adhesion and invasion processes, the semi-allogeneic embryo confronts the maternal immune system. Progesterone has the capacity to influence the maternal immune system via the progesterone-induced blocking factor (PIBF). PIBF alters the arachidonic acid

metabolism (Par et al., 2003), inhibits NK activity (Szekeres-Bartho et al., 1997, Faust et al., 1999) and results in Th2 dominant cytokine production by maternal lymphocytes (Szereday et al., 1997). PIBF has an anti-abortive effect in mice. Higher rates of fetal loss were found in mice treated with anti-PIBF compared to untreated controls (Szekeres-Bartho et al., 1997).

Galectin-1 is also a progesterone induced molecule that has a pivotal role in conferring fetomaternal tolerance. The ratio of resorbed fetuses was significantly higher in mice lacking galectin-1 than that of wild-type mice. Galectin-1 treatment prevented fetal loss by restoring tolerance through the induction of tolerogenic dendritic cells, which in turn promoted the expansion of interleukin-10 (IL-10)-secreting regulatory T cells in vivo (Blois et al., 2007).

3. Regulation of trophoblast invasion and migration

Trophoblast plays an important role in implantation and interaction of the embryo with the decidualized endometrium. Once the embryo is attached to the endometrium, the cytotrophoblast undergoes an epithelial-mesenchymal transition, initially forming multilayered cell columns and then infiltrating deeply in the maternal decidual stroma and blood vessels. Interstitial trophoblast cells destroy the tunica media then endovascular trophoblast cells invade the arteries and replace the maternal endothelial lining by fibrinoid material. The endoglandular trophoblast cells break through the basement membrane of uterine glands to open their lumen towards the intervillous space. At the inner myometrium, trophoblast cells fuse to become placental-bed giant cells (Red-Horse et al., 2004, Moser et al., 2010).

Invasive growth of extravascular trophoblast is strictly controlled by proteolytic enzymes, among others, by matrix metalloproteinases (MMPs). Progesterone acts as a negative regulator of trophoblast invasion by controlling MMP activity, thus preventing excessive extravillous trophoblast invasion (Fig. 2.) (Bischof et al., 1998; Staun-Ram and Shalev, 2005).

MMP-2 and MMP-9 - that digest type IV collagen, the main component of basal membranes - are highly expressed by trophoblast in early gestation, and activation of these MMPs indicates the invasive property of trophoblast cells (Staun-Ram et al., 2004). Progesterone treatment was found to decrease MMP-9 secretion in primary cytotrophoblast cells (Shimonovitz et al., 1998) as well as cell migration, MMP-2 and -9 activities and proliferation in HTR-8/SVneo cells (Chen et al., 2011). Studies on short-term human endometrial organ cultures, as well as those using isolated stromal cells, have demonstrated

that physiological concentrations of progesterone can suppress MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9 mRNA or protein expression. Furthermore, progesterone stimulates the expression of TIMP-3, -a tissue inhibitor of MMPs (Higuchi et al., 1995).

Progesterone affects MMP activity by transcriptional modulation either directly or indirectly via local mediators, e.g., cytokines, leptin.

Progesterone receptors normally act by binding to specific progesterone response elements in the promoter region of the target genes (Li and O'Malley, 2003), but they can also regulate promoters, that have no progesterone response elements (Cheng et al., 2006; Zhang et al., 2003; Christian et al., 2002).

Transcription of *MMP-2* is mediated by binding of the transcription factor SP4 to the *MMP-2* promoter. Progesterone suppresses *MMP-2* transcription, by inhibiting the binding of the progesterone receptor/SP4 transcription factor complex to the promoter region of *MMP-2* and also, by promoting the proteasomal degradation of SP4 (Goldman et al., 2009).

The promoter region of *MMP-9*, *MMP-1* and *MMP-3* contains NF- κ B binding sites. Progesterone prevents NF- κ B entering the nucleus by stimulating the expression of the inhibitory I κ B α (Wissink et al., 1998), thus inhibits binding of NF- κ B to the promoter regions of *MMP-9*, *MMP-1* and *MMP-3* which in turn results in decreased MMP-9, MMP-1 and MMP-3 activity (Jabbour et al. 2006).

Progesterone stimulates TGF- β in stromal cells (Bruner et al., 1995) which in turn inhibits MMP-7 expression by endometrial epithelium and activates the expression of TIMPs (Schroen and Brinckerhoff, 1996). Progesterone-mediated suppression of MMP-7 requires local TGF-beta (Bruner et al., 1995) production, shown by the fact, that neutralization of TGF-beta activity abrogated the effect of progesterone on MMP-7 activity (Bruner et al., 1995). On the other hand, in human endometrial stromal cells progesterone inhibits TGF β 1-induced stimulation of *MMP-2* and *MMP-11* via its nuclear receptor. TGF β 1 has been shown to decrease the expression of nuclear progesterone receptors in this cell type (Itoh et al., 2012).

Progesterone inhibits IL-1 α -induced MMP-3 activation in human endometrial stromal cells. Pro-inflammatory cytokines produced by epithelial cells have been shown to facilitate focal expression of MMPs by down-regulating PR expression in the neighbouring stromal cells, even in the presence of progesterone (Bruner et al., 2005). These data suggest a regulatory crosstalk between progesterone and cytokines in order to maintain the fine tuning of MMP activity.

Another mechanism, through which progesterone restricts trophoblast invasion and migration is; by altering the plasminogen activator system. Progesterone activates the specificity protein transcription factor 1 (SP-1) which in turn enhances the transcription of tissue factor (TF), a hemostatic agent and plasminogen activator inhibitor-1 (PAI-1) that inhibits the fibrinolytic pathway (Norwitz et al., 2007). TF inhibits urokinase-type plasminogen activator (uPA) which might participate in activation of TGF β .

Progesterone-regulated genes, e.g., leptin, may use the STAT3 pathway which is known to activate the transcription of MMP-2 and MMP-9. Progestins act via nuclear progesterone receptors to suppress functional leptin-receptor mRNA expression, and may thereby alter the sensitivity of the endometrium to leptin (Koshiba et al., 2001). In line with this, in the trophoblast there is an inverse relationship between the expression of the progesterone induced PIBF and the leptin/leptin-receptor system. In the normal trophoblast - molar pregnancy - choriocarcinoma transition there is an increasing leptin/leptin-receptor expression together with decreasing PIBF expression in the trophoblast (Miko et al., 2011).

After digesting the extracellular matrix, extravillous trophoblast cells migrate through the eroded connective tissue as far as the proximal myometrium. Progesterone has the capacity to regulate the migratory properties of extravillous trophoblast cells by genomic (e.g., Dkk1, IGFBP-1) and non-genomic (e.g., RhoA/ROCK) actions (Fig.2.).

Trophoblast migration involves the FAK, RhoA/ROCK and Wnt signaling pathways. Focal adhesion kinase (FAK) is a non-receptor protein tyrosine kinase that has a growth and migration-promoting role. FAK has an impact on GTPase Rho proteins (RhoA, Rac1, Cdc 42) that regulate diverse biological processes such as cell cycle, cell-cell/focal adhesions, polarization and cell migration (Knöfler, 2010; Spencer et al., 2004). The downstream effectors of Rho include p21-activated kinase (PAK), which cross-talks to the MAPK pathway by modulating Raf and Rho-associated, coiledcoil containing protein kinase (ROCK).

The progesterone-regulated IGFBP-1 stimulates the cell migratory properties of extravillous trophoblast cells by binding to $\alpha_5\beta_1$ integrins on the surface of EVT, leading to activation of FAK and MAPK pathways (Gleeson et al., 2001).

Progesterone stimulates vascular endothelial cell migration by Tyr-phosphorylation of FAK in a dose- and time-dependent manner. Progesterone enhances the activation of actin-binding protein moesin, resulting in actin cytoskeleton remodelling. In the presence of progesterone, PR-A interacts with G α protein which activates the RhoA/ROCK-2 cascade (Zheng et al., 2012).

Progesterone upregulates Dkk1 during the implantation window thus might inhibit the actions of Wnt3a that induces trophoblast migration and MMP-2 secretion through canonical Wnt signalling and Akt activation in trophoblast cell lines and in primary first trimester extravillous trophoblast cells (Pollheimer et al., 2006).

Conclusions

Taken together, these data show the complexity of progesterone action in early pregnancy. In addition to inhibiting the proliferative effect of estrogen on uterine epithelial cells, progesterone facilitates uterine receptivity by inducing stromal cell proliferation, decidual growth and the expression of adhesion molecules via its genomic actions, allowing the embryo to implant. Progesterone acts as a negative regulator of trophoblast invasiveness by controlling matrix metalloproteinase activity, and modulates trophoblast migration via diverse pathways. Moreover, progesterone suppresses the deleterious maternal immune responses.

Via these effects progesterone is a key for the establishment and maintenance of pregnancy. Any disturbances in its actions may contribute to pathological pregnancies, e.g. early fetal loss, habitual abortions, preterm delivery.

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Figure Legends

Figure 1. Progesterone regulates endometrial receptivity and embryo attachment.

Progesterone blocks the proliferative effect of estrogen on uterine epithelial cells and facilitates uterine receptivity by its genomic actions. Progesterone modulates stromal cell proliferation, decidual growth, expression of adhesion molecules and suppresses the maternal immune system to allow embryo implantation. (Progesterone induced molecules are shown **in bold**.)

Figure 2. Progesterone modulates extracellular matrix remodelling.

Progesterone acts as a negative regulator of trophoblast invasion by controlling matrix metalloproteinase (MMP) activity via its genomic actions. (Progesterone regulated molecules are shown **in bold**.)

Figure 3. Progesterone promotes trophoblast migration.

Progesterone modulates migratory properties of trophoblast cells by genomic (IGFBP-1, Dkk1) and non-genomic (RhoA/ROCK) actions. (Progesterone induced molecules are shown **in bold**.)