Morphological changes and protein secretion induced by progesterone in the endometrium during the luteal phase in preparation for nidation

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Progesterone induces morphological modifications and protein secretion during the luteal phase to permit implantation/nidation. It acts through specific receptors which are regulated by oestrogens. The proliferative phase is thus a prerequisite for progesterone activity. Changes in morphology are first located in the glandular component, with peak secretion occurring during the implantation window. The various components of the stroma are involved during the second part of the luteal phase, resulting in decidualization and the formation of spiral arteries. The subsequent fall-off in progesterone leads to menstruation. The next cycle begins with a regenerative process in which oestrogens induce the modifications typical of the proliferative phase. The luteal phase is usually assessed on morphological criteria but should also be accompanied by protein secretion profiles of glands and stroma. Key words: endometrium/morphology/progesterone/proteins

Introduction

Morphological and physiological changes of the endometrium are triggered by oestrogens and progesterone secreted cyclically by the ovary, and the morphological appearance of the endometrium is indicative of the functional status of the hypothalamus–pituitary–ovary axis. The endometrium undergoes physiological changes characterized by growth, secretory differentiation and, in the absence of fecundation, menstruation and subsequent regeneration. Proliferation, differentiation and menstruation involve the upper two thirds or functional component of the endometrium. The lower third constitutes the basal component of the mucosa and undergoes only minimal morphological change. This basal component is, however, responsible for mucosa regeneration (Ferenczy and Bergeron, 1991). These cyclical modifications allow the creation of a hospitable environment for nidation. At menopause, the absence of oestrogens leads to progressive endometrial involution and subsequent atrophy.
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The mechanism of action of progesterone: progesterone receptors

Progesterone acts on the epithelium and stroma of the endometrium by means of specific receptors, which are proteins located in the nucleus of endometrial cells and have specific affinity for progesterone. The two subtypes of receptors A and B have been underscored recently in the endometrium (Wang et al., 1998).

Immunohistochemical assays show these proteins in the nuclei of glands and stroma (Bergeron et al., 1988; Garcia et al., 1988; Lessey et al., 1988). Progesterone receptors are not found in the endothelium of the vessels. Progesterone receptor synthesis is regulated by oestrogens through oestrogen receptors. It follows that the presence of progesterone receptors is a good marker for endometrial oestrogen dependence. Progesterone receptor levels are highest during the preovulatory and immediate post-ovulatory periods during which serum oestrogen titres are highest (Figure 1). The two subtypes are present in the glandular and stromal nuclei during the proliferative phase (Wang et al., 1998). The proliferative phase during which progesterone receptor synthesis takes place is therefore essential for good secretory differentiation. Maturation disorders during the secretory phase may be due to an inadequate proliferative phase.

Progesterone inhibits the synthesis of oestrogen receptors. As a result, progesterone receptors are no longer regulated. Consequently, both oestrogen and progesterone receptors (subtypes A and B) in the epithelium fall sharply after ovulation, with oestrogen receptors disappearing completely from the stroma. Predecidual cells, while not containing oestrogen receptors, do contain progesterone receptors (Figure 2). However the new subtype B antibody fails to stain stromal nuclei during the secretory phase (Wang et al., 1998). Progesterone-induced changes in the stroma during the luteal phase appear to be mediated primarily by the subtype A and the absence of oestrogen receptors suggests that the subpart A is part of the normal make-up of the stroma in the intermediate and late secretory phase.

Progestosterone-induced changes during the luteal phase

The luteal or secretory phase starts after ovulation and lasts from the 14–28th day of the cycle. Progesterone induces a secretory differentiation and has a twofold anti-oestrogen effect: inhibiting the synthesis of oestrogen receptors and synthesizing 17β hydroxydehydrogenase in the glands which convert oestradiol into oestrone (Satyaswaroop et al., 1982). Oestrone has a weak affinity for oestrogen receptors.

The first modifications occur in the glands where DNA synthesis and mitosis slow and subsequently stop. Sub-nuclear vacuoles appear in each gland cell at the 17th day of the cycle and the cell nuclei take on a palisade-like appearance (Figure 3). On day 18, glycogen vacuoles are sub- and supra-nuclear. A nucleolar channel system (NCS) in the glands is a specifically female feature of the post-
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ovulatory period (More et al., 1974; Ferenczy and Guralnick, 1983). It is assumed that this system derives from the progesterone-induced invagination of the nuclear membranes into the nucleus thereby facilitating nuclear RNA transport into the cytoplasm. From day 19, the vacuoles are only supra-nuclear and glycoprotein-rich apocrine secretion from cytoplasm takes place, evidenced by protrusion and detachment of the apical part of the cells. Secretion peaks on day 21 of the cycle and coincides with the date of implantation of the blastocyst in the event of fertilization (Figure 4). This period has been called the implantation window and is the short period during which implantation is possible. After this, the endometrium becomes refractory. Ultrastructurally, pinopodes are evidenced during this period, indicating that the endometrial surface epithelium is receptive to the eventual implantation of a blastocyst (Nikas and Psychoyos, 1997). This period is also associated with the secretion of a certain number of progesterone-regulated proteins. Immunohistochemical studies have located these proteins exclusively in the epithelial component. They are: progesterone-associated endometrial protein (PEP) (Joshi, 1983), insulin growth factor binding protein 2 (IGFBP2) or protein 14 or glycodegin (Waites et al., 1988a; Seppala and Tiitinen, 1995) (Figure 5), crystalloglobulin (Guild et al., 1997), the integrins (Lessey et al., 1995) and glycoproteins or type 1 mucins (Ravn et al., 1992; Serle et al., 1994; Aplin et al., 1996; Carson et al., 1998) which are secreted into the cytoplasm of the glands. The physiological role played by these various proteins is still not fully understood. PP14 would seem to have an immunodepressive function, facilitating embryo implantation. The uterine flushings of patients who have had a miscarriage show reduced PP 14 secretion (Dalton et al., 1995). Retarded maturation is also observed very frequently in these patients. Mucin, on the other hand, particularly muc-1, has important functions at the luminal surface and plays a dominant role in maintaining a functionally non-receptive uterine surface with regard to blastocyst attachment. It may inhibit implantation at the maternal cell surface and it has been suggested that mucin deficiency may enhance the chances of lower quality embryos implanting successfully (Hey et al., 1995).

The stroma of the endometrium is made up of specialized fibroblasts. They too react to hormone stimulation through receptors. Stroma presents few modifications before day 20. From day 20, however, the stroma of the endometrium appears oedematous. This is in response to prostaglandins (PG) E2 and F2 secreted by the stromal cells and regulated by progesterone (Smith et al., 1984; Neulen et al., 1988). PGE2 stimulates capillary permeability either directly or by means of increased histamine release which enhances the appearance of oedema in the stroma. PGE2 also enhances endothelial mitosis and clumping of perivascular filaments. Endothelial proliferation leads to an enrolment of the arterial system of the endometrium, present in the form of spiral or coiled arteries located in the functional component of the mucosa (Figure 6). Progesterone receptors have been reported in the muscular walls of vessels (Perrot-Applanat et al., 1988) and progesterone probably directly influences endometrial vascular proliferation. The role of fibroblast cells is to become transformed into decidual
cells by means of the predecidual cells. These latter contain vimentin and desmin but not cytokeratin, an immunohistochemistry finding which confirms their stromal origin. Predecidualization is induced by progesterone and may take place in the ovaries, pelvis or abdomen during progesterone stimulation, especially during pregnancy. Predecidualization begins on the 23rd day of the cycle around the spiral arteries and extends after the 24th day to all the stroma of the whole functional component (Figure 7). Predecidualization presents the characteristic appearance of extended cytoplasms with an increase in nuclear DNA synthesis, mitotic activity and the formation of a basal membrane with abundant pericellular laminin (Kearns, 1983; Wewer et al., 1985). This pericellular laminin is specific to epithelial cells but it is also found around adipose and muscle cells. It is not known by what mechanism the growth factors present in the decidua actually induce mitosis. Oestrogens probably do not have a role in stromal replication since oestrogen receptors are no longer present in the stroma during this period of the cycle. That oestrogens do not influence predecidualization is also borne out by the fact that ovariectomized women whose menstrual cycle is induced by oestrogen and progesterone administration present similar predecidualization whether or not they receive oestrogens during the luteal phase (de Ziegler et al., 1992).

Predecidual cells secrete numerous substances that may be nutritious, metabolic or immunosuppressive. Among these is an immunohistochemically documented insulin growth factor binding protein 1 (IGFBP1), otherwise known as protein 12 (Waites et al., 1988b) (Figure 8), along with prolactin and relaxin (Huang et al., 1987) (Figure 9) The extracellular matrix plays a role in ensuring cell cohesion, growth and differentiation. The extracellular matrix of the endometrium and decidua has been studied in early-stage pregnancies (Aplin et al., 1988). Type IV collagen facilitates the attachment of trophoblastic cells and the clumping of decidual cells. Collagen is also involved in the mechanisms regulating the permeability and nutrition of trophoblastic and endometrial cells. A fall in the synthesis and deterioration of type IV collagen due to increased activity of collagenase present in the decidual cells (Iwahashi et al., 1996) may be the cause of metabolic anomalies for the fetus and trigger miscarriage.

Leucocytes make up a sizeable proportion of the endometrial stromal cells, comprising 7% of all stromal cells during the proliferative phase and 30% at the outset of pregnancy (Rebello et al., 1975). Macrophages are present in the premenstrual endometrium and in the decidua and may well play a role in implantation and sustaining of pregnancy through some immunosuppressive mechanism and by the production of cytokines. Lymphocytes in the endometrium are type T lymphocytes expressing CD8 antigen considered as a variant of NK cells, although their role in normal pregnancy is still not fully understood (Bulmer and Johnson, 1985). There may be an immunological mechanism triggering early miscarriage although there is little morphological evidence to support this hypothesis. As a rule no inflammatory infiltrate containing plasma cells is found in biopsies taken from patients with recurrent miscarriage. In contrast, the decidua
from early miscarriages often contain inflammatory infiltrate; however, it is not certain whether this is the cause of miscarriage or a secondary phenomenon.

The menstrual phase

Menstruation is the result of enzymatic autodigestion and ischaemic necrosis. In the first part of the secretory phase, acid phosphatases and lytic enzymes are confined to the lysosomes. Progesterone stabilizes the lysosomal membranes. During the second part of the secretory phase, these lysosomal membranes are disrupted and the enzymes are released into the cytoplasm and intercellular spaces. The lytic enzymes digest cell elements, including intercellular bridges and desmosomes. Matrix metalloproteases have a particularly important role, causing the breakdown of the extracellular matrix and basal membranes. As their action is localized, sloughing is limited to the function of layer only. The basal membranes of the vessels are also altered by these matrix metalloproteases and by plasmin, resulting in endothelial cell breakdown and extravasation of red blood cells. The predecidual cells also have a role in the disruption of the endometrium during menstruation: being phagocytic they digest the extracellular collagen (Lawn et al., 1971).

Haemostasis is the result of a balance between coagulation and fibrinolysis. Since progesterone maintains coagulation, any fall in progesterone levels will engender fibrinolysis. Plasminogen activators are present in the menstrual endometrium and originate from the endometrial vascular endothelium. They convert plasminogen into plasmin which in turn prevents the menstrual blood from clotting. Fibrinolysis increases progressively, leading to menstrual bleeding.

Vasomotor phenomena bring about vasoconstriction and vascular relaxation. They are regulated by PGF and oestrone both of which increase during the secretory phase and reach peak levels during the menstrual phase. PGF cause vasoconstriction of the basal arteries which gives rise to circulatory arrest in the spiral arteries and contraction of the myometrium at the boundary between endometrium and myometrium. These phenomena induce ischaemia of the upper part of the endometrium. Endothelins also play an important role in vasoconstriction. They are regulated negatively by progesterone and oestrogens and positively by tumour growth factor (TGF)-β. Finally, there is apoptosis, a phenomenon regulated by the gene bcl-2 and by tumour necrosing factor (TNF)-α, causing gland cell death (Rango et al., 1998).

These tissue and vascular phenomena together lead to tissue desquamation or sloughing and the shedding of menstrual blood.

The regenerative phase

The regenerative phase starts at the moment of menstruation and constitutes the first few days of the proliferative phase. Menstruation is interrupted by clotting
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mechanisms once again prevailing at the end of the menstrual period and by vasoconstriction of both the arteries in the basal component of the denuded mucosa and the radial and arcuate arteries of the myometrium.

Tissue necrosis provokes a marked inflammatory response with the production of pro-inflammatory, chemotactic and anti-inflammatory cytokines. Regeneration also depends on macrophages and polymorphonucleocytes which help to clean up the necrotic area. DNA synthesis only recommences 2 or 3 days after sloughing of the functional component (Ferenczy, 1980; Padykula, 1989). The stromal cells of the basal component of the mucosa proliferate to replace the shed endometrium and subsequently are active in re-establishing the endometrium. This synthesis is associated with a regeneration of the epithelium with gland proliferation, starting from the basal component and in the surface epithelium around the tubes and isthmus. This post-menstrual regenerated epithelium binds to the fibroblasts of the underlying stroma, with the stromal cells forming clumps onto which the surface epithelium can migrate. Epithelial growth may in fact be stimulated by the underlying fibroblasts. Tenascin, a fibronectin-inhibitor synthesized by the fibroblasts of the endometrial stroma, may also play a role in facilitating epithelial migration (Chiquet-Ehrismann et al., 1989; Vollmer et al., 1990).

The role of oestradiol in endometrial regeneration is only evident after the necrotic area has been cleaned up. Oestradiol serum titres are very low during the menstrual phase and regeneration seems due initially just to a repair mechanism. In fact, it has been observed that endometrial regeneration in ovariectomized rabbits is identical to that of rabbits with normal ovaries. A very short DNA regulating phase would explain the very swift regeneration of the endometrium. On day 5 of the cycle a new surface epithelium is already observed.

Figure 1. Immunohistochemistry using antibody against progesterone receptors (Beckman-Coulter, Loissy, France) shows evidence of progesterone receptors in the nuclei of glands and stroma during the early secretory phase (peroxidase-antiperoxidase stain; original magnification ×250).

Figure 2. Several progesterone receptors are still evident in the stromal cells but have disappeared from the nuclei of the glands during the late secretory phase [specific antibody (Beckman-Coulter) and peroxidase-antiperoxidase stain; original magnification ×250].

Figure 3. During the early secretory phase, the glands contain vacuoles of glycogen in the basal component of each cell (haematoxylin and eosin stain; original magnification ×250).

Figure 4. During the intermediate secretory phase, the glands are dilated and the stroma is oedematous (haematoxylin and eosin stain; original magnification ×250).

Figure 5. The glands secrete protein 14 into the cytoplasm during the intermediate secretory phase, as shown here by immunohistochemical study (specific antibody to PP12 kindly donated by Dr S.C. Bell, University of Leicester, and peroxidase-antiperoxidase stain; original magnification ×250).

Figure 6. As of day 23 of the cycle the stroma contains well developed spiral arteries (arrows) surrounded by predecidual stromal cells (haematoxylin and eosin stain; original magnification ×400).

Figure 7. Predecidualization of the stroma (small arrows) extends throughout the stroma of the functional component from day 24 of the cycle. Large arrow shows spiral artery (haematoxylin and eosin stain; original magnification ×250).

Figure 8. Predecidual cells secrete protein 12 into the cytoplasm during the late secretory phase as shown in this immunohistochemical assay (specific antibody to PP12 kindly donated by Dr S.C. Bell, and peroxidase-antiperoxidase; original magnification ×400).

Figure 9. Immunohistochemical study evidences predecidual cells and decidual cells synthesizing prolactin (specific antibody to prolactin was a gift from Dr Keller, (University of McGill, Montreal, Canada) and peroxidase-antiperoxidase; original magnification ×400).
From day 5, serum oestradiol levels increase and the endometrial mucosa proliferates under the influence of oestrogen by means of the oestrogen receptors which appear once again in gland and stroma nuclei.

**Inadequate luteal phase**

Endometrial biopsy is a useful tool to investigate the endometrial mucosa and form an opinion of endometrial receptivity according to the criteria set down by Noyes which forms the basis of the description of endometrial differentiation set out above (Noyes et al., 1950). Retardation of 2 days is considered incompatible with viable implantation. Reproducibility of the histological finding is much more likely if the biopsy is performed at the outset of the luteal phase when the diagnostic criteria used apply to both the glandular and stromal components. Biopsy carried out around day 22 of the menstrual cycle or luteinizing hormone (LH) + 7 provides the most reliable morphological dating when intra- and interobserver variations are taken into account. It is vital to provide the pathologist with accurate information, especially as to the day of the biopsy. Since the duration of the luteal phase is constant, the onset of the menstrual phase should in theory allow prediction of the ovulation date. However, ovulation is best assessed by LH peak levels rather than with the date of menses (Shoupe et al., 1989). Another source of error is the variable cycle length in the same patient (Li et al., 1989; Gibson et al., 1991). These cycle variations are frequently due to a variable proliferative phase and are another reason why ovulation is best assessed with the LH peak. Moreover a diagnosis of maturation retardation should be made only after two biopsies have been taken at different cycles.

Inadequate luteal phase is a clear histopathological condition. Progesterone treatment corrects the histological anomalies, although a certain number of cases of luteal insufficiency has been observed in patients with normal progesterone levels. Retarded endometrial development may therefore be due to suboptimal response by the endometrium to progesterone. Moreover, oestrogen impregnation during the follicular phase is essential to ensure response to progesterone during the luteal phase. The management of inadequate luteal phase by inducing artificial cycles has often proved more effective than administering progesterone by itself (Li et al., 1994), a finding which confirms the role of oestrogens or other factors in regulating the endometrium’s response to progesterone.

**Conclusions**

Morphological modifications of the endometrium are regulated by oestrogens and progesterone secreted by the ovary, either directly or by means of proteins synthesized by these hormones. Understanding these morphological changes to the endometrium during the menstrual cycle is the key to predicting normal implantation of the embryo. This study should be conducted, together with
biochemical and molecular biological assessments of secretory activity, to provide better understanding of the mechanisms of action of hormones on the cells.

References


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