Review Article

Ultrastructural Modifications of Human Endometrium during the Window of Implantation

Maryam Kabir-Salmani, Ph.D.[1, 2*], Christopher R. Murphy, Ph.D.[3] , Ahmad Hosseini, Ph.D.[2], Mojtaba Rezazadeh Valojerdi, Ph.D.[4, 5]

1. Medical Genetics Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Molecular and Cellular Biology Research Center, Shahid Beheshti Medical University M.C, Tehran, Iran
3. Discipline of Anatomy & Histology, School of Medical Science and Bosch Institute, University of Sydney, Sydney, Australia
4. Embryology Department, Cell Sciences Research Center, Royan Institute, ACECR, Tehran, Iran
5. Anatomy Department, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Abstract

The endometrium is a highly dynamic tissue empowered with the capacity to undergo cyclic dramatic changes in response to ovarian steroid hormones, ultimately aiming to create a window of receptivity for blastocyst implantation. Intensive research has been performed to understand and establish morphological and molecular correlates of embryo implantation. However, it still remains a biological mystery particularly in the human, where ethical and moral constraints prohibit in vivo testing and the establishment of an ideal in vitro modeling. Rodent models of embryo implantation are largely irrelevant because the process varies significantly from that in humans. Even among primates, subtle differences exist among species. For maternal preparation of implantation, the endometrial epithelium which is surprisingly hostile towards the embryo implantation, acquires functional status receptive to blastocyst acceptance during a limited period of cycle days, termed as the 'window of implantation (WOI). This review provides currently available information concerned primarily with the various ultrastructural modifications of endometrium coordinated within the WOI that may signify endometrial receptivity. In the following sections, the dominant features of endometrial differentiation during WOI, including transformations of luminal epithelium, endometrial glands, and stromal decidualization will be discussed from the morphological points of view.

Keywords: Endometrium, Implantation, Receptivity

Introduction

The implantation process of the human embryo requires a subtle dialogue between the mother and the embryo. On the maternal side, a so-called receptive endometrium is a prerequisite and is considered as a main fertility-determining factor (1). Receptivity, as originally conceived, was functionally defined: that state of endometrial differentiation that is permissive for embryo attachment (2). A definition, which has frustrated efforts to establish morphological and molecular correlates, particularly in the human, because of ethical and practical constraints for both *in vivo* and *in vitro* studies. Considering the fact that up to 50% of embryos may possess chromosomal errors such as aneuploidy, triploidy, translocations and other genetic disorders; receptive endometrial layers may provide a natural gating mechanism that help screen out impaired embryos (3). Although there is still not full agreement as to the exact timing of a receptive endometrum in the human, clinical studies suggest that the window is temporally confined to days 20-24 of a normal menstrual cycle (day LH+7 to LH+11), termed as the window of implantation (WOI) (4). Consistently during a natural cycle, the endometrium becomes receptive to blastocyst implantation 6-8 days after ovulation and remains receptive for about 4 days (cycle days 20–24) (5, 6). During the WOI, the endometrial luminal epithelium, which is surprisingly hostile towards the embryo implantation, acquires functional status supportive to blastocyst acceptance (7). Moreover, luminal and glandular epithelium

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* Corresponding Address: P.O.Box: 14155-6343, Medical Genetics Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: Kabirs_m@yahoo.com

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as well as stromal components undergo functional and ultrastructural modifications to play a role in embryo nourishment, production of pregnancy recognition signals, immunoprotection, attachment, regulation of the trophoblast invasion and placentation (8, 9). Biochemical and molecular aspects of endometrium during WOI are explained in several reviews (3, 10-14). However to our knowledge, less study in far fewer reviews cover the morphological aspects of a receptive endometrium. In this review, the ultrastructural changes of endometrium during the period of WOI will be discussed according to currently available information mainly focus on human model. Before that, an overview of the structure of human endometrium will be briefly described.

Morphology and cellular composition of the human endometrium

The endometrium is a multilayered, dynamic organ overlaying the myometrium and is composed of several different cell types, including luminal and glandular epithelial cells, stroma with stromal fibroblastic cells, immunocompetent cells and blood vessels. Morphologically, the human endometrium contains two tissue layers with distinct response to hormonal stimuli and physiologic functions. These are the upper two-third "functionalis" layer and the lower one-third "basalis" layer (15). In the adult, endometrial morphology and cellular functions are strongly influenced both directly and indirectly by sex-steroids and exhibits unique properties of cyclical regeneration and remodeling throughout a woman's reproductive life. The histologic changes that occur during the pre-ovulatory phase of the menstrual cycle are neither specific of a given day nor of ovulation and are thus not useful for dating the endemetrium. Indeed, all of the tissue components including the glands, stromal cells and endothelial cells demonstrate proliferation which peaks on cycle days 8 to 10 (16). Moreover, the proliferative changes are significantly more pronounced in the functionalis than the basalis layer. The biologic rationale for the geographic variation in proliferative indices may lie in the different physiologic functions of the functionalis vs. the basalis layer (17). The former is the seat of blastocyst implantation, whereas the latter provides origin for the regenerative endometrium following menstrual degeneration of the functionalis. A typical feature of proliferative endometrium in epithelial cells is increased cilio and microvillogenesis. Ultrastructural evidence of tissue proliferation is evidenced by an increase in free and bound ribosomes, mitochondria, Golgi and primary lysosomes in gland cells and stromal fibroblasts. Finally, intranuclear estrogen and progesterone receptor concentrations are the highest during the preovulatory period of the menstrual cycle. The postovulatory rise in ovarian progesterone during luteal phase induces profound remodeling and differentiation of the estradiol-primed endometrium that make it receptive to embryo implantation during a narrow period of a menstrual cycle, termed as the WOI (18-22). Here, the dominant features of endometrial differentiation during WOI from the morphological points of view categorized as the ultrastructural transformations of luminal epithelium, morphological changes in the endometrial glands, and the stromal decidualization.

I. Ultrastructural transformations of luminal epithelium during WOI

Contact between the plasma membrane of luminal endometrial epithelial cells and that of the trophoblast is a common beginning to implantation in most species studied so far. On the basis of the fact that hatched embryos attach non-selectively to uterine stromal cells *in vitro* (23), it was concluded that the endometrial luminal epithelium confers upon the unique property of endometrial resistance to embryo implantation. Thus, although the endometrial stroma may also play a role, endometrial receptivity is mostly attributed to the endometrial epithelium (24). The luminal epithelium covering the human endometrial surface is composed of two cell types, the nonciliated cells, which bear microvilli, and the ciliated cells. The nonciliated cells strikingly undergo progestational secretory differentiation. Ultrastructural, biochemical and more general morphological data reveal that strikingly common phenomena occur in this plasma membrane during WOI and early pregnancy despite the diversity of placental types, from epitheliochorial to hemochorial. It has been suggested that these alterations be referred to collectively as "the plasma membrane transformation" (25-27). This term encapsulates the concept of a common and necessary process of changes in all compartments of the plasma membrane of uterine epithelial cells, including apical, lateral and basal domains.

I.A. Transformations of the apical plasma membrane

Experimentally tested traits of a receptive endometrium are lacking in the human. However, there are a number of candidate markers whose relevance to implantation is supported by animal studies and by their pattern of expression in humans. One such marker is the change in morphology of the apical plasma membrane of the endometrial epi-

thelial cells as seen in scanning electron microscopy (28). Ultrastructural, biochemical and more general morphological data reveal that strikingly common phenomena occur in the apical plasma membrane in response to ovarian hormones. In general, apical cell surfaces of epithelial cells contain numerous microvilli, which are covered by thick layer of carbohydrates called glycocalyx. Cell surface carbohydrates protect epithelial cells from proteolytic degradation and bacterial infection. In response to ovarian hormones, it gradually loses regular microvilli and becomes very flat, forming large, rounded, smooth-surfaced projections, which have been identified in the uterine epithelium of many species ranging from rabbits and rodents through camels to human beings (29-32). These structures are several micrometers wide and project into the uterine lumen above the microvilli level. Apical surface protrusions of the uterine luminal epithelium constitute part of the plasma membrane transformation (33) that occurs prior to implantation of the blastocyst in many mammalian species (34, 35). Because such smooth-surfaced projections occur during the receptive phase for blastocyst implantation in humans, these enigmatic structures have been the subject of many studies as important indicators of normal endocrine progression and biological markers of receptivity (36-39). Since the uptake of ferritin was demonstrated by apical protrusion of rats, the term 'pinopode' (Greek: drinking foot) was coined to signify their pinocytotic function (40). However, it was reported that pinopodes in humans have no significant pinocytotic function and it was suggested that a more descriptive term 'uterodomes' be referred to these structures in the human (41, 42). Uterodomes have been scored as: few (<20% of the endometrial surface covered), moderate (20%–50% covered), and abundant (>50% covered) (43), with further classification as developing (bulging cells still covered in microvilli), developed (bulging cells no longer covered in microvilli), or regressing (bulging cells covered in microvilli) (44). Consistently, in photomicrographs of scanning electron microscopy of our samples in Figure 1, panel A (a-c), uterodomes can be categorized according to their morphology to progressing, developed and regressing uterodomes in the biopsies from the early, mid, and late secretory phases of a regular menstrual cycle. According to studies by Nikas et al. (45), all cycles show a similar pattern in the evolution of surface morphology, as follows: During the proliferative phase, the cells vary greatly in size, and their shapes are either elongated or polygonal. Bulging is minimal, the intercellular clefts are barely marked, and the microvilli are short and slender. By contrast, during the early and mid-secretory phase, the morphologic changes are distinct and may allow dating of the tissue in a 24-48-hour interval. Taking an ideal 28-day cycle as reference, an increase in microvilli density and length is noticed on days 15 and 16, and the cells begin to bulge, mainly at the central part of their surface. Smooth apical projections, usually smaller than pinopodes, are occasionally seen in small groups in the endometrial folds. On day 17, bulging increases involving the entire cell apex and the microvilli reach their maximum development, being long, thick, and upright. On day 18, the microvilli start to diminish in size and their tips may appear swollen. On day 19, there is pronounced and generalized cell bulging. The microvilli decrease further in number and length by fusing together or disappear. Smooth and slender membrane projections begin to form, arising from the entire cell apex (developing pinopodes). By day 20, the microvilli are virtually absent and the membranes protrude and fold maximally (fully developed pinopodes). Fully developed pinopodes assume many shapes, resembling mushrooms or flowers. On day 21, bulging decreases and small tips of microvilli reappear on the membranes, which are now wrinkled, and the cell size starts to increase (regressing pinopodes). By day 22, the pinopodes have virtually disappeared and the microvilli have become more numerous. Day 23 is characterized by a further increase in the size of cells which by day 24 begin to appear dome-shaped and covered with short, stubby microvilli.

Notwithstanding, there are some important morphological differences between the ultrastructure in pinopodes of rodents and uterodomes of humans (41, 46). Of major importance is the observation that in animals other than rats and mice, the structures generally arise from the entire apical cell surface and essentially involve all of the apical plasma membrane. This seems particularly so in humans where it has been referred to smooth membrane projections that arise from the entire cell surface, (45, 47), while in rat and mouse pinopodes are connected to the apex by an stalk and more than one pinopode may arise from one cell (as schematically is shown in Figure 1Bc). There are other morphological distinctions between the known pinocytotic structures and uterodomes. For instance, while pinopodes are organell-free, uterodomes sometimes appear to contain numerous membranous cellular organelles and glycogen granules (as some are shown in our samples in Figure 1Ca, b).

Dockery et al. (48) showed transmission electron micrographs of membranous whorls and mitochondria in apical bulges of the human epithelial surface and the organelle content of these human structures has also been noted by other investigators (46, 49).

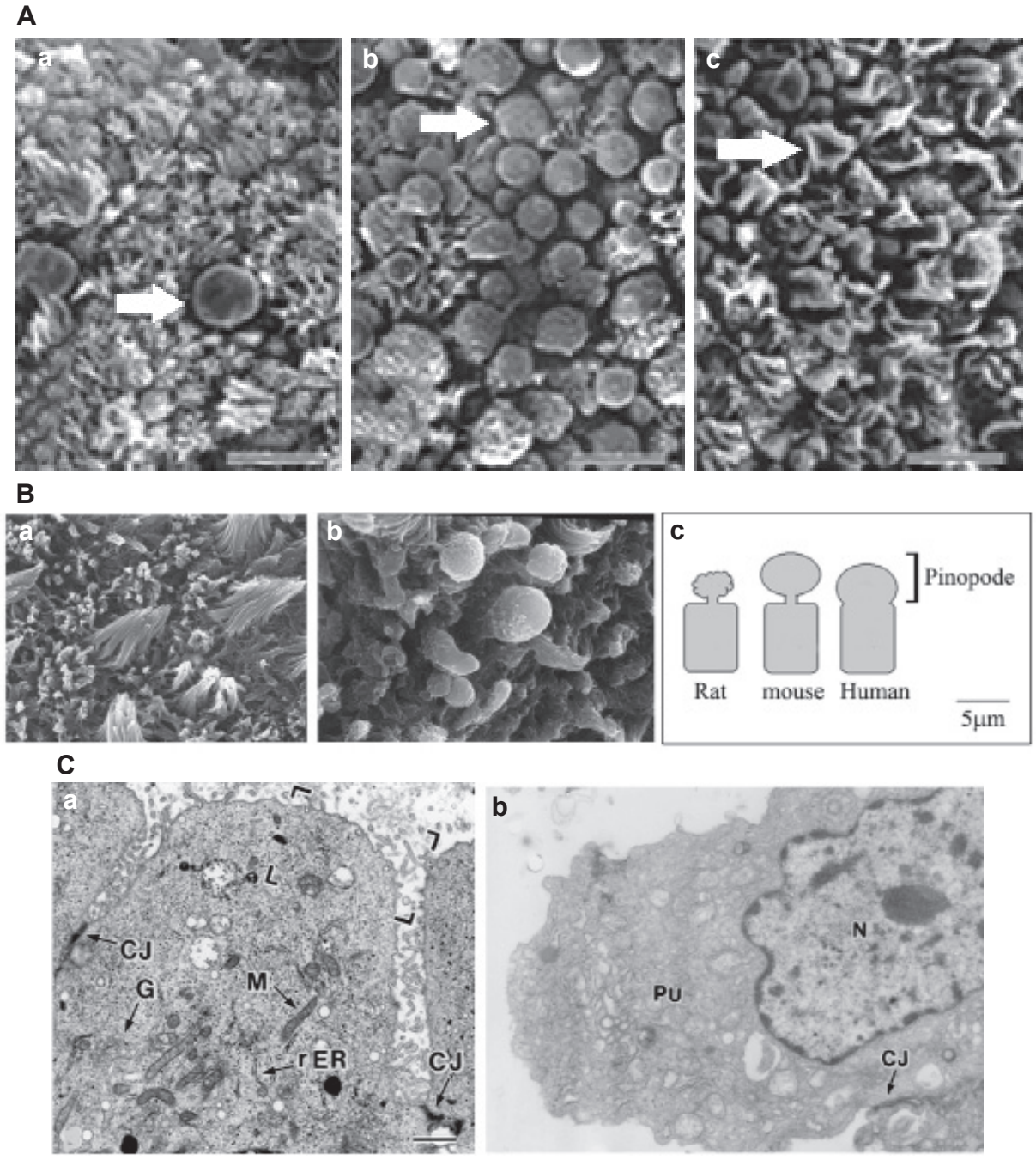

Figure 1. A) Scanning electron microscopy (SEM) photomicrographs of luminal surface of human endometrial biopsies were taken from (a) early, (b) mid, and (c) late luteal phases of normal menstrual cycle to identify developmental stages of uterodomes, pointed by white arrows. Notice that few isolated uterodomes are detectable in specimens from early luteal phase of menstrual cycle, whereas numerous developed uterodomes are detectable in the mid-luteal phase samples, and regressive uterodomes are dominant in late luteal phase specimens. Scale bars: 10μm. B) Shortening of microvilli and adhesion of cilia (a), formation of uterodomes (b), and schematic morphological differences between pinopodes extending from the apical surface of luminal epithelium cells in the rat, mouse, and human, as seen during their respective windows of receptivity. C) Some membranous organelles are detectable inside the uterodomes (a, b) N, nucleus; M, mitochondria; rER, rough endoplasmic reticulum; SV, secretory vesicle; G, Golgi complex; E, exocytosis; and CJ, cell junction Scale bar: 1μm.

Furthermore, in contrast to pinopodes of rat and mice, which contain many vaccules, Enders and Nelson commented on the lack of the typical pinopode vacuole in projections of the human uterine surface (50). There may also be a difference in the frequency of the known pinopodes and uterodomes. In rats and mice, pinopods were only observed on ~20% of cells (50, 51), depending on the exact time of pregnancy, while uterodomes seem to be visible on a majority of the non-ciliated cells in human studies (43, 45, 49). Although many groups have studied pinopodes, their actual function in the endometrium is unknown. Scanning electron microscopy of sequential endometrial biopsies in women shows that uterodomes form briefly (for 1-2 days) and their increased number correlates with the onset of WOI (52, 53). However, Usadi et al. postulated that pinopodes are observed first on luteal day 5, corresponding with the onset of mid-luteal phase increase in serum progesterone levels and persist for the entire duration of the secretory phase (54). It seems that uterodomes are the preferred sites of embryo-endometrial interactions and hypothetically, the receptors required for blastocyst adhesion are located on their surface (49, 55). It has been illustrated that the essential cell adhesion molecules and their ligands such as HB-EGF, trophinin, galectin, and osteopontin which are required for blastocyst initial adhesion are located on uterodomes (56-59). Furthermore, there is evidence that the glycocalyx is altered at the surface of the uterodomes in the mid-secretory, with loss of mucin epitopes, such as mucin-1 and mucin-16 (60, 61). Furthermore, we reported a secretory function for uterodomes in the human for the first time (46).

I.B. Transformations in the lateral plasma membranes

Prominent features of structural changes during WOI in these domains are mainly related to the junctional complexes. Regulation of epithelial organization, structure, and subsequent function is modulated by these structures, including tight junctions, adherens junctions, desmosomes and gap junctions (33, 62).

The tight junction (zonula occludens) is the most apical in the junctional complex and surmised their role in preventing or reducing paracellular movement of molecules. In addition, tight junctions maintain the proper distribution of 70 proteins and lipids within domains of the plasma membrane (63). In uterine epithelial cells of rats, Murphy et al showed that the tight junction on the lateral plasma membrane becomes much deeper during early pregnancy such that by the time of uterine receptivity it extends three-fold further down the lateral plasma membrane (64). These changes in structure are predominantly under the control of progesterone. This progesterone induced effect also results in the junction becoming more geometrically complex pattern of many interconnections between the strands which probably reflects a reduced paracellular flow. In the human endometrium too, freeze-fracture studies show alterations to tight junctions, which are geometrically more complex (65-67). It would seem likely that the increasing complexity and morphological “tightness” seen during early pregnancy is a reflection of the need to preserve the luminal contents specially developed by epithelial secretion for blastocyst development, from dilution or loss by unwanted flow into or out of lumen (29, 33).

The adherens junction (zonula adherens), which are usually seen as comparatively indistinct densities occurs immediately beneath the tight junction. A key component of the adherens junction on its cytoplasmic side is the terminal web, a layer of actin filaments which inserts into the lateral plasma membrane principally at the level of the adherens junction. The adherens junction as well as its associated terminal web is lost completely from the lateral plasma membrane by the time of the completed plasma membrane transformation (33). The loss of the terminal web is under maternal ovarian hormonal control and its dissolution at the time of endometrial receptivity does not rely on the presence of a blastocyst. However, such an event is likely to have considerable significance for the many changes seen in the various regions of the plasma membrane during early pregnancy. Disassociation of actin terminal web microfilaments could contribute to the apical loss of microvilli which is a key component of the plasma membrane transformation that seems to be essential for receptivity (26, 31, 68). It has been found that as the terminal web was lost, cellular vesicles and other organelles which previously were excluded from the submembranous cytoplasm, presumably by the dense terminal web of filaments, were able to approach the apical plasma membrane (69). An important agent in these processes is the characteristic large apical vesicles of uterine epithelial cells which are under progesterone control (70). These vesicles, which have cholesterol-rich membranes, approach and fuse with the apical membrane around the receptive phase for attachment as the terminal web is lost (71, 72). Such alterations in vesicular traffic could contribute to the well-known increase in apical plasma membrane

cholesterol by the time of attachment and may also contribute to the rearrangement and altered expression of the many molecules involved in the transformation of this membrane during early pregnancy (72, 73).

Desmosomes are the most basal component of the junctional complex and have a characteristic and distinctive appearance in cross section, making them easily identified using transmission electron microscopy (74). Desmosomes have also been examined in a few species and both the morphological desmosomes as well as key desmosomal proteins are reported to be similarly down-regulated in humans during WOI (75). While the terminal web and adherens junction are lost, plakoglubin, a key component of desmosomal-type junctions, become restricted to the apical quarter of the lateral plasma membrane whereas previously it had occurred all down the lateral plasma membrane. This suggests that the loss of these junctional components allows this molecule to focus along the apical-most portion of the lateral plasma membrane, where it might contribute to junction formation with the penetration shafts of trophoblasts which are known to transiently share junctions with uterine epithelial cells (76).

Gap junctions, made up of connexins (Cxs), play fundamental roles in coordinating a number of cellular processes through their ability to directly regulate cell-cell communication. Gap junction formation is one of the first maternal responses to a locally acting signal of the blastocyst and gap junctional connections are exquisitely modulated by embryonic signals in the implantation chamber (77). Connexins, a growing family of homologous proteins present in gap junctions, have been identified in the human endometrium (78). Gap junctional Cx are induced in the endometrium during implantation in the human and in response to trophoblast invasion. It has been reported that gap junctions, or at least certain connexins, are increased in the time leading to endometrial receptivity in humans (79).

Thus collectively, all the components of the lateral plasma membrane junctional complex are 'transformed' in one way or another during the period leading to endometrial receptivity. An interesting conclusion, based on this evidence of changes to uterine junctional structures in response to hormones, is that while uterine epithelial cells continue to a barrier to molecular movement between the cells, they are much less of barrier to physical trauma, whether that be a blastocyst from above or, in the case of uterine bleeding, blood from below.

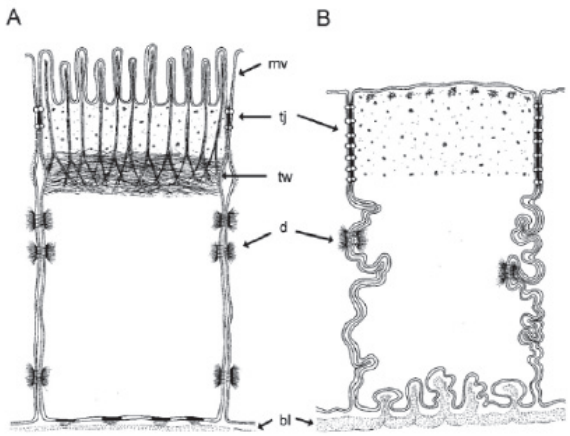

Figure 2. Diagrams of uterine the epithelial cells highlighting some of the membrane phenomena referred to in the text. (A) Epithelial cells are shown in response to predominantly estrogen stimulation and before receptivity (d 1). They display long microvilli (mv) and a relatively short tight junction (tj) down the lateral plasma membrane. A prominent terminal web is present (tw) and desmosomes (d) are comparatively numerous. (B) Epithelial cells are shown following the 'plasma membrane transformation' (d 6). The apical plasma membrane is smooth and flattened and the apical glycocalyx is lost which includes loss of Muc-1. Laterally, the tight junction extends three times as far down the lateral plasma membrane which is also more tortuous as is the basal plasma membrane which in addition has a thicker basal lamina (bl). Desmosomes (d) are considerably reduced along the lateral plasma membrane at this time and the terminal web is completely lost. (adopted with permission from.(30)

I.C. Transformations in the basal plasma membrane

The basal plasma membrane undergoes considerable change during WOI and early pregnancy. Several workers have found that the basal plasma membrane and basal lamina become more tortuous during early pregnancy (80-83). In humans, the thickness of the basal lamina increases during the reproductive cycle (81, 84). The increased tortuosity of the basal plasma membrane and increased thickness of the basal lamina reported in several studies are perhaps indicative for the tissue remodeling which occurs during early pregnancy and the thicker basal lamina may be a device to slow the blastocyst journey into the stroma. On the other hand, the loss of 'focal contacts' suggests that perhaps uterine epithelial cells are less adherent to the basal lamina by the time of blastocyst attachment and this is consistent with observations that the epithelium is more easily removed from the stroma at this time (26). The basement membranes are multilayered mats of extracellular matrix that underlie all epithelial cell sheets and tubes. The basal lalmina provides the interface between epithelial and mesenchymal environments and should not be considered simply as an inert

boundry. It plays a key role in the expression of the epithelial phenotype. It plays a role to determine cell polarity, influence cell metabolism, organize the proteins adjacent to plasma membranes, induce cell migration and serve as specific pathways for migration (85-88).
Collectively, some features of the plasma membrane transformations are schematically demonstrated in Figure 2.

"The plasma membrane transformation" concept

The alterations reviewed above which take place in the endometrial epithelium and in particular the plasma membrane of these cells during early pregnancy have been suggested to represent a loss of polarity by these cells (86) and this thought has been extended to suggest further that an epithelial-mesenchymal transition (EMT) may occur in the cells during this time (87). While EMT is effectively irreversible (89) and the changes in endometrial epithelial cells are reversed after only three days (90) this insight nonetheless highlights the critical importance of changes in endometrial epithelial cells for uterine receptivity. The EMT idea in one sense recalls earlier suggestions on events in endometrial epithelial cells which highlighted the apical plasma membrane flattening and led to the term 'attachment reaction' being used to describe some of the membrane changes which occur during early pregnancy (91). This term was used to describe those changes in the apical membrane of uterine epithelial cells upon contact with the blastocyst itself, or when opposing uterine epithelial cells came into physical contact at around the same time of early pregnancy in rats and mice and was thought to involve a differentiation of the epithelial cells (91, 92). In particular, the term indicated that in those species with an 'attachment reaction', closure of the endometrial lumen was involved such that little or no luminal space remained (91). However, as is now known, considerable change occurs in all compartments of the plasma membrane of uterine epithelial cells and these changes occupy most of early pregnancy in the rat and mouse with long, regular microvilli being converted into short, irregular structures, as well as the terminal web being lost, as early as day 3 of pregnancy, that is, two to three days before the blastocyst even enters the endometrium (69, 93, 94).
As we have also seen, in a wide diversity of species, there are changes in the apical plasma membrane which have features in common with those seen in rats and mice and in many of these other species, closure of the endometrial lumen does not occur. A common process is especially suggested by observations in animals with an epitheliochorial placenta like pigs and camels: here, as we have seen, the epithelium is not breached and the mature placenta consists of extensive interdigitation of very long trophoblastic and uterine epithelial microvilli throughout pregnancy. Nonetheless, before and during initial contact between uterine epithelial cells and the blastocyst, the regular microvilli of the endometrial epithelium flatten out, much as they do in rats and mice, after which they return (within the next 48 hours) to form the interface of the mature placenta.
Moreover, as we have also seen, there are molecular alterations in the plasma membrane during early pregnancy, which have common aspects across species and here the large apical carbohydrate MUC-1 is particularly instructive. Changes in the basal and lateral plasma membrane regions have also been documented in many species during early pregnancy in preparation for attachment, and these too show common aspects across species - especially some membrane junctional structures. Therefore, to bring a focus to the importance of the plasma membrane of uterine epithelial cells, to highlight that membrane alterations are a process during early pregnancy - not just an event at the time of attachment itself, to recognise that both apical and basolateral alterations occur, that molecular changes are also evident, and that moreover, there appears to be a degree of commonality across species, we have suggested that alterations in the plasma membrane of uterine epithelial cells during early pregnancy be referred to collectively as "the plasma membrane transformation" (25, 29, 31, 33, 41, 68). This term thus encapsulates the concept of a common and necessary process of change in all compartments of the plasma membrane of uterine epithelial cells as characteristic, across species, of the development of endometrial receptivity for implantation.
A fundamental property of simple epithelia like endometrial and trophoblast epithelium is to possess a polarized organization and, as one aspect of this, three distinct membrane domains, i.e, the apical, the lateral, and the basal plasma membrane domain (95-97). While basal and lateral membranes are studded with adhesion molecules so that they can mediate cell-to-cell and cell-to-matrix adhesion, apical plasma membranes normally lack most of these molecules and lack adhesive properties because apical surfaces must normally be non-self adhesive to prevent epithelial cavities from sealing up. At implantation initiation, we are confronted with the fact that uterine and trophoblast epithelium makes their first contact exactly via their apical cell membranes and this is what may be

called a cell biological paradox (86, 87, 98). Solutions for the paradox are found when taking a side view to processes in embryology that involve interaction of two epithelia, typically combined with epithelium-to-mesenchyme (EM) transformation, a process that is also being discussed to be involved in tumor cell invasion. Thus, it was proposed that a destabilization of the apico-basal polarity of the endometrial epithelial cells is important in creating a receptive endometrium for implantation and the hypothesis was arose that implantation might occur in the context of altered maternal epithelial polarity. However, it must be pointed out that application of this concept to uterine receptivity is still very hypothetical.

A recent hypothesis postulates that epithelial cells turn off genes for apical-basal polarity and turn on genes for a more mesenchyme-like phenotype allowing cell-cell interaction with trophoblast. Consistently, it has been proposed that some of the molecular events involved in EM transformation can also be found in both, the acquisition of receptivity by the endometrial epithelium and the expression of the invasive phenotype by the trophoblast. The many changes which take place in the endometrial epithelium during early pregnancy have been suggested to represent a loss of polarity in these cells (87). In wild-type animals, luminal epithelial cell polarity becomes less marked in the peri-implantation period when latero-basal markers become detectable in the apical membrane (99, 100). Mouse uterine endometrium shows signs of transformation into non-polarized and adhesive structures and an upregulation of cadherin in peri-implantation uterine epithelial cells, with the apical membranes showing enrichment of cadherin moieties in comparison to the basal and lateral membrane (101). Thie et al. (102), demonstrated random distribution of cell adhesion molecules like E-cadherin and α6, β1 and β4 integrin subunits during the modulation of the epithelial phenotype of uterine cells, i.e. loss of apical-basal polarity, which prepare the apical cell pole for cell-to-cell contact with trophoblast *in vivo*. Taken together, the incidence of such phenomenon during human embryo implantation deserves further studies especially in human model.

II. Ultrastructural aspects of the glandular epithelium during WOI

The ultrastructural transformation of the glandular epithelium throughout the menstrual cycle depends on different distribution patterns of cytoplasmic and nuclear receptors for progesterone and estrogen in the endometrium (84, 103-105). Under the influence of progesterone in luteal phase, the cells are transformed from relatively inactive cells full of free ribosomes to very active polarized cells, containing giant mitochondrial profiles, intracellular deposits of glycogen/glycoprotein-rich material and a complex intranuclear channel system (84). A number of studies which focused on structural differences in the glandular epithelium have described this feature as the 'post-ovulatory triad', marked by the presence of giant mitochondria, subnuclear glycogen, and the nuclear channel system during the secretory phase (106). It has been reported that formation of the nuclear channel system (NCS) is initiated during the mid- to late-proliferative phases of the cycle and it remains until the late secretory phase and the development of this organelle seems to be stimulated by progesterone (84, 103, 107). The nucleolar channel system is a well-established ultrastructural hallmark of the postovulation endometrium and for close to half a century, the NCS has been known as an ultrastructural hallmark of the postovulation human endometrium (108). It consists of several layers of tubular membrane cisternae in the nuclei of endometrial epithelial cells and is often associated with the nuclear envelope and nucleoli, hence the name (106). Its transient presence has been associated with human fertility (109). The work of More et al., (110) provided a clear insight into the organization of this structure, which exists as an elaboration of the inner nuclear envelope and forms a spherical, or coneshaped, stack of interdigitating membranous tubules each surrounded by a granular intranuclear matrix. The function of the NCS is not yet known. It has been suggested that it is involved in the rapid transport of new mRNA (111), but more recently Buchwalow et al. (112) localized ribonucleoproteins and nucleoside phosphatases within the NCS. What messages these mRNA may convey remains to be elucidated; furthermore, the mechanisms of transport via such a route remain obscure. Also, given that the lumen of the tubules is continuous with that of the nuclear envelope, which is itself linked to the cavity of the endoplasmic reticulum, the role of mRNA within the space remains puzzling. There remains the possibility that the NCS somehow feeds back information concerning the progress and products of transcription within the rough endoplasmic reticulum to the nucleus and so refines its control at a time of rapid and extensive secretory activity. It is also of interest to note that Ca^{2+} release channels have been reported to be associated with the inner nuclear envelope (113), which may suggest an alternative role for the NCS in the dynamic secretory activity of these cells.

The morphology of the endometrial glands has been studied extensively during the various phases of the menstrual cycle and the accumulation of glycogen appears to be a prominent marker of secretory activity (84, 114). The electron density of the glandular cells varies according to amount of glycogen accumulation. Accordingly, two main cell types with structural diversity are reported in the glandular wall: glycogen rich cells (dark cells) and clear glycogen poor cells (114). Glycogen-rich cells show an advanced progression of glycogen formation, and their apical regions are filled almost entirely with glycogen accumulations. Further, some clear cells with some signs of early glycogen synthesis are interpreted as 'intermediate' between clear and dark cells. Clear cells are in close proximity to the basal lamina and show more metabolic activity with a well-developed endoplasmic reticulum, Golgi apparatus, mitochondria and abundant cytoplasm. Mitochondria are numerous in clear glandular cells but their number decrease in dark cells. The nuclear structure of glandular cells was often elliptical, regular, euchromatic, and finely granular containing electron dense nucleoli. Due to their active components and organelle structures, clear cells are presumed to be main (stem) cells for further proliferation, differentiation and transformation of glandular epithelium. Details of the organelles of the dark epithelium cells are difficult to identify in gland units. Myelin figures, with different configurations and contents, are seen both in dark cells and in intermediate cells. Dark cells contain a small number of microvilli on their luminal surface and irregular, hill-shaped short projections of cytoplasm are also seen, representing glycogen discharge. Intermediate cells have moderate amounts of glycogen particles and active rough endoplasmic reticulum (rER) cisternae, but have fewer polysome accumulations and do not have a dense dark cytoplasm. Three days after the LH surge, glycogen begins to accumulate within the cytoplasm, initially in a subnuclear location but by 6 days large aggregations are seen in the apical regions of the cell. The mechanism of release of glycogen and the formation of the classical secretions is not yet clear but by day 23 of the normal cycle, the deposits begin to disperse and by day 24 only small isolated cytoplasmic aggregates are visible. These findings indicate that for at least the first 6 weeks of pregnancy the uterine glands resemble those during the luteal phase of the cycle, when progesterone concentrations are high. Glycogen particles are clearly present in the uterine secretions at this stage, and undoubtedly contribute to the PAS reactivity of the secretions in the archival sections (115).

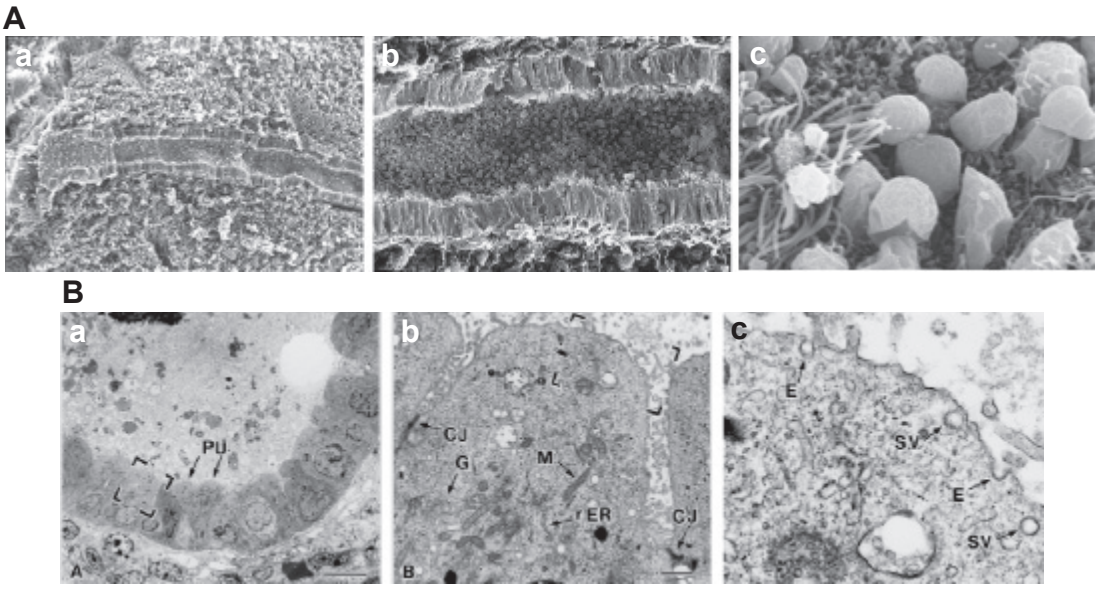

Figure 3. Panel A exhibits photomicrographs of scanning electron microscopy (SEM) from human endometrium in mid-luteal phase of a normal menstrual cycle reveal existence of uterodomes on the surface of the endometrial glandular epithelium, a and b are longitudinal section of an endometrial gland with different magnifications and c demonstrates covering of apical surface of some glandular epithelial cells with uterodomes. Panel B: Photomicrographs of transmission electron microscopy (TEM) from horizontal section of the mid-luteal uterine gland show uterodomes on the apical surface of some glandular epithelial cells (a). Some membranous organelles are detectable inside the uterodomes (b). Secretory vesicles are detectable in different phases of exocytosis and fusion with the cell membrane (c). N, nucleus; M, mitochondria; rER, rough endoplasmic reticulum; SV, secretory vesicle; G, Golgi complex; E, exocytosis; and CJ, cell junction.

Proteins are also a major component of the glandular secretions. Quantitatively the major protein is a dimeric glycoprotein referred to by various synonyms in the past, most commonly PP14 or α_2-2PEG, but is now termed glycodelin A (115). Moreover, MUC-1 is a large glycoprotein whose expression in the glandular epithelium is also progesterone dependent. In the normal cycle, its secretion begins 3-4 days after the LH surge and continues into the late secretory phase (116).

The basal lamina associated with the glandular epithelium increases in thickness during the luteal phase of the menstrual cycle reaching maximum thickness at LH+8 (84, 117). An immunocytochemical study showed that collagen type IV, fibronectin, and laminin were associated with the basement membrane of the glandular epithelial cells during the luteal phase (118). Changes of these components were reported around LH+10 (118).

Morphological studies by our group using transmission and scanning electron microscopes demonstrated that similar projections to luminal uterodomes existed on the glandular epithelium (Figure 3Aa-c). The release of secretory vesicle from these projections is clearly shown in Figure 3, panel Ba-c.

III. Ultrastructural aspects of the decidual cells during WOI

Decidualization is a complex series of biochemical and morphological changes in the uterine stroma, which prepare the uterine lining for maintenance and growth of the implanted blastocyst. Although there are many studies that have defined the morphological and biochemical end points of a decidual cell (119), the sequence of cellular and molecular events associated with the transformation of a stromal fibroblast to a secretory decidual cell have yet to be elucidated. The term decidua (L. deciduus, a falling off) refers to the gravid endometium, that is, the functional layer of the endometrium in a pregnant woman. This term is appropriate because this part of the endometrium separates (falls away) from the remainder of the uterus after parturition (childbirth). Three regions of deciduas called deciduas basalis, capsularis, and parietalis respectively are part of the deciduas deep to the conceptus that forms the maternal part of the placenta. By the time of implantation, the predominant morphologic alteration is edema of the endometrial stroma. The stroma of the endometrium is composed of highly specialized stromal cells, which are not simply fibroblasts, but respond to hormonal stimuli and have receptors for both estrogen and progesterone (120). In response to increasing progesterone levels in the maternal blood, they enlarge and differentiate to decidual cells, in which glycogen and lipid accumulate. Decidual cells undergo hypertrophy, becoming rounder and more polygonal in shape. Their nuclei become fully euchromatic with distinctive nucleoli. Decidualization starts around day 23 in stromal cells adjacent to the spiral arteries and occurs independently of the presence of a blastocyst. However, it is more robust and complete in conception cycles (121). As the conceptus implants, decidual cells and vasculature undergo a transformation called “the decidual reaction”. Progesterone-induced decidualized human endometrial stromal cells form a hemostatic envelope that protects against hemorrhage during invasion of endometrial capillaries by implanting blastocyst-derived cytotrophoblasts (122). Furthermore, the decidual cells of the endometrium fulfill paracrine, nutritional, immunoregulatory, and embryoregulatory functions throughout pregnancy (123). Although there are many studies that have defined the morphological and biochemical end points of a decidual cell, the sequence of cellular and molecular events associated with the transformation of a stromal fibroblast to a secretory decidual cell have yet to be elucidated. Differentiation of endometrial stromal cells into predecidual cells occurs in the late secretory phase of the cycle (124, 125). In humans, decidualization includes stromal hyperplasia and marked changes in the uterine stromal cell phenotype. Stromal edema is maximal around LH+8. This is correlated with increased intedigitations between basolateral membranes of adjacent glandular epithelial cells. It may indicate some resorption of water from the from the gland lumen (84, 126). This may also be related to ion transfer across the epithelium so that water follows. Associated with this edema, focal loss of collagen type VI and deposition of glycosaminoglycans in the stromal matrix is reported (85). They suggested that the loss of type VI collagen may help in providing a suitable environment for the infiltration of the trophoblast into the maternal substratum. The edema and changing matrix compositon may help in the migration of the trophoblast (127).

Briefly, ultrastructural studies on the human decidual cell indicate that they possess all the characteristics of a secretory cell, such as a euchromatic nucleus, numerous profiles of Golgi cisternae, dilated rough endoplasmic reticulum and dense membrane bound secretory granules (128). There is an increase in number and complexity of cytoplasmic organelles, such as rough endoplasmic reticulum and Golgi complexes, indicating an increase in synthetic and secretory activity. Another

characteristic of the true decidual cell is the acquisition of an external lamina, which is composed of dense fibrillar material closely resembling the basement membrane of epithelia (129). Smaller, less differentiated decidual cells are only partially covered by an external lamina, which suggests that this feature is typical of the mature decidual phenotype (130). Among the most distinguishing ultrastructural features of the differentiated decidual cell is the presence of the membrane-bound, osmiophilic bodies contained in the tips of club-shaped processes at the decidual cell periphery (129, 131). The greater abundance of secretory bodies observed at the cell periphery than in the cytoplasm suggests that the membrane-bound granular material is stored in club-shaped processes prior to release into the extracellular space (131). Prior to implantation, the ECM surrounding the stromal cells is composed of fibrillar collagens (types I and III), collagens V and VI, and fibronectin (132, 133). Besides cellular changes, decidualization creates an ECM composition of utmost importance. This matrix is composed mainly of laminin, heparan sulphate proteoglycans and type IV collagens (134). During decidualization, the collagen matrix becomes less fibrous and collagen VI is removed (133), and the decidual cells produce basement membrane components such as laminin, entactin, and collagen type IV (132, 135). These same changes in matrix composition occur during artificially induced decidualization, indicating that this tissue remodeling is independent of an embryonic signals (133).

Discussion

The endometrium is a highly dynamic tissue empowered with the capacity to undergo dramatic changes in response to steroid hormones, ultimately aiming to create a window of receptivity for blastocyst implantation. Endometrial maturation is regulated by the ovarian steroid hormones, cytokines, growth factors, and is modulated by embryonic signals during implantation to modify the endometrium transiently receptive to implanting embryos. Receptive endometrium is defined during a limited period spanning cycle days 20-24 (day LH+7 to LH+11), termed as the window of implantation (136, 137). Generally, the dominant features of a receptive phase endometrium in this period can be categorized as: i. The plasma membrane transformation of luminal epithelium, ii. Glandular secretion, iii. Stromal decidualization and changes of the immune cell populations. The aim of this review was to outline the current understanding of endometrial receptivity according to these features in humans from the morphological points of view.

The plasma membrane transformation highlights the alterations in all domains of the plasma membrane of luminal epithelium during WOI and early pregnancy. During this transformation, luminal epithelium gradually loses regular microvilli and becomes very flat, forming large, rounded, smooth-surfaced projections termed as pinopode or uterodome (29, 32, 41). In the human, uterodomes are shown to have a role in early attachment and secretions of uterine fluids (46, 49). Modificationes of the junctional complexes of lateral domain constitute part of the plasma membrane transformation in this period (29, 33). Different cell types, which interact to allow a co-ordinated invasion of embryo, form the endometrial net. Four groups of resident endometrial cells can be differentiated: stromal cells, epithelial and endothelial cells, and non-resident immune cells. Stromal cells primed by estrogen will differentiate to decidual cells under the influence of progesterone. Stromal decidualization is defined as the differentiation of the elongated fibroblast-like mesenchymal cells in the endometrium to the large, round decidual cells and the coordinated expression of numerous new cellular products of the ECM, cytokines, hormones, and various other peptides such as insulin-like growth factor binding-protein-1 (138, 139) and the influx of specific leukocyctes (140). Studies on structural modifications of the glandular epithelium have described this feature as the ‘post-ovulatory triad’, marked by the presence of giant mitochondria, subnuclear glycogen, and the nuclear channel system. Under the influence of progesterone in luteal phase, the cells transform from relatively inactive cells full of free ribosomes to very active polarized cells, containing giant mitochondrial profiles, intracellular deposits of glycogen/glycoprotein-rich material and a complex intranuclear channel system. Understanding the morphological aspects of receptive endometrium may help to introduce series of promising morphological biomarkers of the window of implantation.

References

1. Strowitzki T, Germeyer A, Popovici R, von Wolff M. The human endometrium as a fertility-determining factor. Hum Reprod Update. 2006; 12: 617-630.
2. Psychoyos A. Uterine receptivity for nidation. Ann N Y Acad Sci. 1986; 476: 36-42.
3. Edwards RG. Human implantation: the last barrier in assisted reproduction technologies? Reprod Biomed Online. 2006; 13: 887-904.

4. Navot D, Bergh P. Preparation of the human endometrium for implantation. Ann N Y Acad Sci. 1991; 622: 212-209.
5. Croxatto HB, Ortiz ME, Diaz S, Hess R, Balmaceda J, Croxatto HD. Studies on the duration of egg transport by the human oviduct. II. Ovum location at various intervals following luteinizing hormone peak. Am J Obstet Gynecol. 1978; 132: 629-634.
6. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. Fertil Steril. 1992; 58: 537-542.
7. Diedrich K, Fauser BC, Devroey P, Griesinger G. The role of the endometrium and embryo in human implantation. Hum Reprod update. 2007; 13: 365-377.
8. Bazer FW. Uterine protein secretions: Relationship to development of the conceptus. J Anim Sci. 1975; 41: 1376-1382.
9. Roberts RM, Bazer FW. The functions of uterine secretions. J Reprod Fertil. 1988; 82: 875-892.
10. Aplin JD. Embryo implantation: the molecular mechanism remains elusive. Reprod Biomed Online. 2006; 13: 833-839.
11. van Mourik MS, Heijnen CJ, Macklon NS. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol. 2008.
12. Kabir-Salmani M, Hosseini A, Rezazadeh valojerdi M. Review Article: Endometrial Receptivity to Implantation in Humans: Biochemical and Molecular Aspects. Yahkhteh Med J. 2008; 10: 1-24.
13. Carson DD. Molecular and cell biology of embryo-uterine interactions: mammalian embryo implantation. Semin Cell Dev Biol. 2008; 19: 160.
14. Lim HJ, Dey SK. HB-EGF: A unique mediator of embryo-uterine interactions during implantation. Exp Cell Res. 2008.
15. Ferenczy A, Bergeron C. Histology of the human endometrium: from birth to senescence. Ann N Y Acad Sci. 1991; 622: 6-27.
16. Ferenczy A, Bertrand G, Gelfand MM. Proliferation kinetics of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol. 1979; 133: 859-867.
17. Hawkins SM, Matzuk MM. The menstrual cycle: basic biology. Ann N Y Acad Sci. 2008; 1135: 10-8.
18. Fawcett DW. The development of mouse ova under the capsule of the kidney. Anat Rec. 1950; 108: 71-91.
19. Kirby DR. The development of mouse blastocysts transplanted to the scrotal and cryptorchid testis. J Anat. 1963; 97: 119-30.
20. Kirby DR. Development of the mouse blastocyst transplanted to the spleen. J Reprod Fertil. 1963; 5: 1-12.
21. Finn CA, Martin L. The control of implantation. J Reprod Fertil. 1974; 39: 195-206.
22. Paria BC, Reese J, Das SK, Dey SK. Deciphering the cross-talk of implantation: advances and challenges. Science. 2002; 296: 2185-2188.
23. Carver J, Martin K, Spyropoulou I, Barlow D, Sargent I, Mardon H. An in-vitro model for stromal invasion during implantation of the human blastocyst. Hum Reprod. 2003; 18: 283-290.
24. Giudice LC. Potential biochemical markers of uterine receptivity. Hum Reprod. 1999; 14 Suppl 2: 3-16.
25. Murphy CR, Shaw TJ. Plasma membrane transformation: a common response of uterine epithelial cells during the peri-implantation period. Cell Biol Int. 1994; 18: 1115-1128.
26. Murphy CR. The plasma membrane of uterine epithelial cells: structure and histochemistry. Prog Histochem Cytochem. 1993; 27: 1-66.
27. Singh MM, Chauhan SC, Trivedi RN, Maitra SC, Kamboj VP. Correlation of pinopod development on uterine luminal epithelial surface with hormonal events and endometrial sensitivity in rat. Eur J Endocrinol. 1996; 135: 107-117.
28. Nikas G, Makrigiannakis A, Hovatta O, Jones HW, Jr. Surface morphology of the human endometrium. Basic and clinical aspects. Ann N Y Acad Sci. 2000; 900: 316-324.
29. Murphy CR. Uterine receptivity and the plasma membrane transformation. Cell Res. 2004; 14: 259-267.
30. Tachi S, Tachi C, Lindner HR. Ultrastructural features of blastocyst attachment and trophoblastic invasion in the rat. J Reprod Fertil. 1970; 21: 37-56.
31. Murphy CR. The cytoskeleton of uterine epithelial cells: a new player in uterine receptivity and the plasma membrane transformation. Human Reprod Update. 1995; 1: 567-580.
32. Murphy CR. The plasma membrane transformation of uterine epithelial cells during pregnancy. J Reprod Fertil. 2000; 55: 23-28.
33. Murphy CR. Junctional barrier complexes undergo major alterations during the plasma membrane transformation of uterine epithelial cells. Hum Reprod. 2000; 15 Suppl 3: 182-188.
34. Guillomot M, Guay P. Ultrastructural features of the cell surfaces of uterine and trophoblastic epithelia during embryo attachment in the cow. Anat Rec. 1982; 204: 315-322.
35. Abd-Elnaeim MM, Pfarrer C, Saber AS, Abou-Elmagd A, Jones CJ, Leiser R. Fetomaternal attachment and anchorage in the early diffuse epitheliochorial placenta of the camel (Camelus dromedarius). Light, transmission, and scanning electron microscopic study. Cells Tissues Organs. 1999; 164: 141-154.
36. Ferenczy A, Richart RM. Scanning and transmission electron microscopy of the human endometrial surface epithelium. J Clin Endocrinol Metab. 1973; 36: 999-1008.
37. Psychoyos A, Martel D. Problems related to the implantation of the human egg after *in vitro* fertilization. Acta Eur Fertil. 1985; 16: 107-109.
38. Nikas G, Drakakis P, Loutradis D, Mara-Skoufari C, Koumantakis E, Michalas S et al. Uterine pinopodes as markers of the 'nidation window' in cycling women receiving exogenous oestradiol and progesterone. Hum Reprod. 1995; 10: 1208-1213.
39. Adams SM, Murphy CR. A successful pregnancy following SEM fine tuning of hormonal priming. BMC Pregnancy Childbirth. 2001; 1: 3.
40. Nilsson O. Ultrastructure of the process of secretion in the rat uterine epithelium at preimplantation. J

Ultrastruct Res. 1972; 40: 572-580.
41. Murphy CR. Understanding the apical surface markers of uterine receptivity: pinopods-or uterodomes? Hum Reprod. 2000; 15: 2451-2454.
42. Adams SM, Gayer N, Hosie MJ, Murphy CR. Human uterodomes (pinopods) do not display pinocytotic function. Hum Reprod. 2002; 17: 1980-1986.
43. Nikas G. Pinopodes as markers of endometrial receptivity in clinical practice. Hum Reprod. 1999; 14 Suppl 2: 99-106.
44. Nikas G. Endometrial receptivity: changes in cell-surface morphology. Semin Reprod Med. 2000; 18: 229-235. 45. Nikas G. Cell-surface morphological events relevant to human implantation. Hum Reprod. 1999; 14 Suppl 2: 37-44.
46. Kabir-Salmani M, Nikzad H, Shiokawa S, Akimoto Y, Iwashita M. Secretory role for human uterodomes (pinopods): secretion of LIF. Mol Hum Reprod. 2005; 11: 553-559.
47. Develioglu OH, Hsiu JG, Nikas G, Toner JP, Oehninger S, Jones HW, Jr. Endometrial estrogen and progesterone receptor and pinopode expression in stimulated cycles of oocyte donors. Fertil Steril. 1999; 71: 1040-1047.
48. Dockery P, Ismail RM, Li TC, Warren MA, Cooke ID. The effect of a single dose of mifepristone (RU486) on the fine structure of the human endometrium during the early luteal phase. Hum Reprod. 1997; 12: 1778-1784.
49. Bentin-Ley U, Sjogren A, Nilsson L, Hamberger L, Larsen JF, Horn T. Presence of uterine pinopodes at the embryo-endometrial interface during human implantation *in vitro*. Hum Reprod. 1999; 14: 515-520.
50. Enders AC, Nelson DM. Pinocytotic activity of the uterus of the rat. Am J Anat. 1973; 138: 277-299.
51. Parr MB, Parr EL. Uterine luminal epithelium: protrusions mediate endocytosis, not apocrine secretion, in the rat. Biol Reprod. 1974; 11: 220-233.
52. Stavreus-Evers A, Nikas G, Sahlin L, Eriksson H, Landgren BM. Formation of pinopodes in human endometrium is associated with the concentrations of progesterone and progesterone receptors. Fertil Steril. 2001; 76: 782-791.
53. Stavreus-Evers A, Hovatta O, Eriksson H, Landgren BM. Development and characterization of an endometrial tissue culture system. Reprod Biomed Online. 2003; 7: 243-249.
54. Usadi RS, Murray MJ, Bagnell RC, Fritz MA, Kowalik AI, Meyer WR et al. Temporal and morphologic characteristics of pinopod expression across the secretory phase of the endometrial cycle in normally cycling women with proven fertility. Fertility Steril. 2003; 79: 970-974.
55. Bentin-Ley U, Pedersen B, Lindenberg S, Larsen JF, Hamberger L, Horn T. Isolation and culture of human endometrial cells in a three-dimensional culture system. J Reprod Fertil. 1994; 101: 327-332.
56. Stavreus-Evers A, Aghajanova L, Brismar H, Eriksson H, Landgren BM, Hovatta O. Co-existence of heparin-binding epidermal growth factor-like growth factor and pinopodes in human endometrium at the time of implantation. Mol Hum Reprod. 2002; 8: 765-769.
57. Sugihara K, Kabir-Salmani M, Byrne J, Wolf DP, Lessey B, Iwashita M et al. Induction of trophinin in human endometrial surface epithelia by CGbeta and IL-1beta. FEBS Lett. 2008; 582:1 97-202.
58. Lessey BA. Two pathways of progesterone action in the human endometrium: implications for implantation and contraception. Steroids. 2003; 68: 809-815.
59. Shimizu Y, Kabir-Salmani M, Sugihara K, Sakai K, Iwashita M. Expression and Localization of Galectin-9 in the Human Uterodome. Endocrin J. 2008; 55: 879-887.
60. Gipson IK, Blalock T, Tisdale A, Spurr-Michaud S, Allcorn S, Stavreus-Evers A et al. MUC16 Is Lost from the Uterodome (Pinopode) Surface of the Receptive Human Endometrium: *In Vitro* Evidence That MUC16 Is a Barrier to Trophoblast Adherence. Biol Reprod. 2008; 78: 134-142.
61. Horne AW, Lalani EN, Margara RA, Ryder TA, Mobberley MA, White JO. The expression pattern of MUC1 glycoforms and other biomarkers of endometrial receptivity in fertile and infertile women. Mol Reprod Dev. 2005; 72: 216-229.
62. Satterfield MC, Dunlap KA, Hayashi K, Burghardt RC, Spencer TE, Bazer FW. Tight and adherens junctions in the ovine uterus: differential regulation by pregnancy and progesterone. Endocrinology. 2007; 148: 3922-3931.
63. Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE. Tight junction proteins. Prog Bioph Mol Biol. 2003; 81: 1-44.
64. Murphy CR, Swift JG, Mukherjee TM, Rogers AW. The structure of tight junctions between uterine luminal epithelial cells at different stages of pregnancy in the rat. Cell Tissue Res 1982; 223: 281-286.
65. Iwanaga S, Inokuchi T, Notohara A, Higashi R, Murakami M, Kato T. Alterations in tight junctions of human endometrial epithelial cells during normal menstrual cycle--freeze-fracture electron microscopic study. Nippon Sanka Fujinka Gakkai Zasshi. 1985; 37: 2847-2852.
66. Rogers PA, Murphy CR. Morphometric and freeze fracture studies of human endometrium during the peri-implantation period. Reprod Fertil Dev. 1992; 4: 265-269.
67. Murphy CR, Rogers PA, Hosie MJ, Leeton J, Beaton L. Tight junctions of human uterine epithelial cells change during the menstrual cycle: a morphometric study. Acta Anat. 1992; 144: 36-38.
68. Murphy CR. Commonality within diversity: the plasma membrane transformation of uterine epithelial cells during early placentation. J Assist Reprod Gen. 1998; 15: 179-183.
69. Luxford KA, Murphy CR. Reorganization of the apical cytoskeleton of uterine epithelial cells during early pregnancy in the rat: a study with myosin subfragment 1. Biol Cell. 1992; 74: 195-202.
70. Ljungkvist I. Attachment reaction of rat uterine luminal epithelium. II. The effect of progesterone on the morphology of the uterine glands and the luminal epithelium of the spayed, virgin rat. Acta Soc Med Ups. 1971; 76: 110-126.
71. Parr M. Apical vesicles in the rat uterine epithelium during early pregnancy: a morphometric study. Biol Reprod. 1982; 26: 915-924.

72. Murphy CR, Martin B. Digitonin cytochemistry reveals cholesterol-rich vesicles in uterine epithelial cells. Acta Histochem. 1987; 81: 143-147.
73. Hyland RA, Shaw TJ, Png FY, Murphy CR. Pan-cadherin concentrates apically in uterine epithelial cells during uterine closure in the rat. Acta Histochem. 1998; 100: 75-81.
74. North AJ, Bardsley WG, Hyam J, Bornslaeger EA, Cordingley HC, Trinnaman B et al. Molecular map of the desmosomal plaque. J Cell Sci. 1999; 112: 4325-4336.
75. Sarani SA, Ghaffari-Novin M, Warren MA, Dockery P, Cooke ID. Morphological evidence for the 'implantation window' in human luminal endometrium. Hum Reprod. 1999; 14: 3101-3106.
76. Schlafke S, Enders AC. Cellular basis of interaction between trophoblast and uterus at implantation. Biol Reprod. 1975; 12: 41-65.
77. Grummer R, Hewitt SW, Traub O, Korach KS, Winterhager E. Different regulatory pathways of endometrial connexin expression: preimplantation hormonal-mediated pathway versus embryo implantation-initiated pathway. Biol Reprod 2004; 71: 273-281.
78. Granot I, Dekel N, Bechor E, Segal I, Fieldust S, Barash A. Temporal analysis of connexin43 protein and gene expression throughout the menstrual cycle in human endometrium. Fertil Steril. 2000; 73: 381-386.
79. Jahn E, Classen-Linke I, Kusche M, Beier HM, Traub O, Grummer R et al. Expression of gap junction connexins in the human endometrium throughout the menstrual cycle. Hum Reprod. 1995; 10: 2666-2670.
80. Blankenship TN, Given RL. Penetration of the uterine epithelial basement membrane during blastocyst implantation in the mouse. Anat Rec. 1992; 233: 196-204.
81. Shion YL, Murphy CR. The basal plasma membrane and lamina densa of uterine epithelial cells are both altered during early pregnancy and by ovarian hormones in the rat. Eur J Morphol. 1995; 33: 257-264.
82. Keys JL, King GJ. Effects of topical and systemic estrogen on morphology of porcine uterine luminal epithelia. Biol Reprod. 1992; 46: 1165-1175.
83. Larsen JF. Electron microscopy of the uterine epithelium in the rabbit. J Cell Biol. 1962; 14: 49-64.
84. Dockery P, Li TC, Rogers AW, Cooke ID, Lenton EA. The ultrastructure of the glandular epithelium in the timed endometrial biopsy. Hum Reprod. 1988; 3: 826-834.
85. Mylona P, Kielty CM, Hoyland JA, Aplin JD. Expression of type VI collagen mRNAs in human endometrium during the menstrual cycle and first trimester of pregnancy. J Reprod Fertil. 1995; 103: 159-167.
86. Denker HW. Implantation: a cell biological paradox. J Exp Zool. 1993; 266: 541-558.
87. Denker HW. Endometrial receptivity: cell biological aspects of an unusual epithelium. A review. Ann Anat. 1994; 176: 53-60.
88. Palmer JA, Lau TM, Hickey M, Simbah M, Rogers PA. Immunohistochemical study of endometrial microvascular basement membrane components in women using Norplant. Hum Reprod. 1996; 11: 2142-2150.
89. Hay ED. The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn. 2005; 233: 706-720.
90. Png FY, Murphy CR. The plasma membrane transformation does not last: microvilli return to the apical plasma membrane of uterine epithelial cells after the period of uterine receptivity. Eur J Morph. 1997; 35: 19-24.
91. Nilsson O, ed. Some ultrastructural aspects of ovo-implantation. Basel: Karger, 1970; 52-72.
92. Pollard RM, Finn CA. Influence of the trophoblast upon differentiation of the uterine epithelium during implantation in the mouse. J Endocrinol. 1974; 62: 669-74.
93. Jones BJ, Murphy CR. A high resolution study of the glycocalyx of rat uterine epithelial cells during early pregnancy with the field emission gun scanning electron microscope. J Anat 1994; 185 (Pt 2): 443-446.
94. Bucci M, Murphy CR. Differential alterations in the distribution of three phosphatase enzymes during the plasma membrane transformation of uterine epithelial cells in the rat. Cell Biol Int. 1999; 23: 21-30.
95. Hay JG, Geddes DM. Transepithelial potential difference in cystic fibrosis. Thorax. 1985; 40: 493-496.
96. Simons K, Fuller SD. Cell surface polarity in epithelia. Ann Rev Cell Biol. 1985; 1: 243-288.
97. Rodriguez-Boulan E, Salas PJ, Sargiacomo M, Lisanti M, Lebivic A, Sambuy Y et al. Methods to estimate the polarized distribution of surface antigens in cultured epithelial cells. Methods Cell Biol. 1989; 32: 37-56.
98. Thie M, Harrach-Ruprecht B, Sauer H, Fuchs P, Albers A, Denker HW. Cell adhesion to the apical pole of epithelium: a function of cell polarity. Eur J Cell Biol. 1995; 66: 180-191.
99. Illingworth IM, Kiszka I, Bagley S, Ireland GW, Garrod DR, Kimber SJ. Desmosomes are reduced in the mouse uterine luminal epithelium during the preimplantation period of pregnancy: a mechanism for facilitation of implantation. Biol Reprod. 2000; 63: 1764-1773.
100. Kimber SJ. Cell Biology of Implantation and Plancentation. Introduction. Semin Cell Dev Biol. 2000; 11: 61-65.
101. Jha RK, Titus S, Saxena D, Kumar PG, Laloraya M. Profiling of E-cadherin, beta-catenin and Ca(2+) in embryo-uterine interactions at implantation. FEBS Lett. 2006; 580: 5653-5660.
102. Thie M, Fuchs P, Butz S, Sieckmann F, Hoschutzky H, Kemler R et al. Adhesiveness of the apical surface of uterine epithelial cells: the role of junctional complex integrity. Eur J Cell Biol. 1996; 70: 221-232.
103. Dockery P, Pritchard K, Taylor A, Li TC, Warren MA, Cooke ID. The fine structure of the human endometrial glandular epithelium in cases of unexplained infertility: a morphometric study. Hum Reprod. 1993; 8: 667-673.

104. Li TC, Dockery P, Cooke ID. Effect of exogenous progesterone administration on the morphology of normally developing endometrium in the pre-implantation period. Hum Reprod. 1991; 6: 641-644.
105. Verma V. Ultrastructural changes in human endometrium at different phases of the menstrual cycle and their functional significance. Gynecol Obstet Invest 1983; 15: 193-212.
106. Spornitz UM. The functional morphology of the human endometrium and decidua. Adv Anat Embryol Cell Biol. 1992; 124: 1-99.
107. Kohorn EI, Rice SI, Hemperly S, Gordon M. The relation of the structure of progestational steroids to nucleolar differentiation in human endometrium. J Clin Endocrinol. 1972; 34: 257-264.
108. Terzakis JA. The nucleolar channel system of human endometrium. J Cell Biol. 1965; 27: 293-304.
109. Kittur N, Zapantis G, Aubuchon M, Santoro N, Bazett-Jones DP, Meier UT. The nucleolar channel system of human endometrium is related to endoplasmic reticulum and R-rings. Mol Biol Cell. 2007; 18: 2296-2304.
110. More IA, McSeveney D. The three dimensional structure of the nucleolar channel system in the endometrial glandular cell: serial sectioning and high voltage electron microscopic studies. J Anat. 1980; 130: 673-682.
111. More IA, Armstrong EM, McSeveney D, Chatfield WR. The morphogenesis and fate of the nucleolar channel system in the human endometrial glandular cell. J Ultrastruct Res. 1974; 47: 74-85.
112. Buchwalow IB, Belyaeva LA, Zavalishina LE. Localization of nucleoside phosphatases (ATPase and 5'-nucleotidase) and nuclear ribonucleoproteins in the human endometric glandular cells during the secretory phase. Acta Histochem. 1985; 77: 205-208.
113. Gerasimenko OV, Gerasimenko JV, Tepikin AV, Petersen OH. ATP-dependent accumulation and inositol trisphosphate- or cyclic ADP-ribose-mediated release of Ca2+ from the nuclear envelope. Cell. 1995; 80: 439-444.
114. Demir R, Kayisli UA, Celik-Ozenci C, Korgun ET, Demir-Weusten AY, Arici A. Structural differentiation of human uterine luminal and glandular epithelium during early pregnancy: an ultrastructural and immunohistochemical study. Placenta. 2002; 23: 672-684.
115. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J Clin Endocrinol Metab. 2002; 87: 2954-2959.
116. Hey NA, Li TC, Devine PL, Graham RA, Saravelos H, Aplin JD. MUC1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients. Hum Reprod. 1995; 10: 2655-2662.
117. Dockery P, Khalid J, Sarani SA, Bulut HE, Warren MA, Li TC et al. Changes in basement membrane thickness in the human endometrium during the luteal phase of the menstrual cycle. Hum Reprod update. 1998; 4: 486-495.
118. Bilalis DA, Klentzeris LD, Fleming S. Immunohistochemical localization of extracellular matrix proteins in luteal phase endometrium of fertile and infertile patients. Hum Reprod. 1996; 11: 2713-2718.
119. Enders AC. Current topic: structural responses of the primate endometrium to implantation. Placenta. 1991; 12: 309-325.
120. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS, Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab. 1988; 67: 334-340.
121. Christian M, Mak I, White JO, Brosens JJ. Mechanisms of decidualization. Reprod Biomed Online. 2002; 4 Suppl 3: 24-30.
122. Lockwood CJ, Krikun G, Rahman M, Caze R, Buchwalder L, Schatz F. The role of decidualization in regulating endometrial hemostasis during the menstrual cycle, gestation, and in pathological states. Semin Thromb Hemost 2007; 33: 111-117.
123. Tabibzadeh S, Babaknia A. The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. Hum Reprod. 1995; 10: 1579-1602.
124. Finn C, Porter D. The Uterus: Publishing Sciences Group, 1975; 18-132.
125. Hulboy DL, Rudolph LA, Matrisian LM. Matrix metalloproteinases as mediators of reproductive function. Mol Hum Reprod. 1997; 3: 27-45.
126. Li TC, Rogers AW, Dockery P, Lenton EA, Cooke ID. A new method of histologic dating of human endometrium in the luteal phase. Fertil Steril. 1988; 50: 52-60.
127. Edwards RG. Physiological and molecular aspects of human implantation. Hum Reprod. 1995; 10 Suppl 2: 1-13.
128. Wynn RM. Ultrastructural development of the human decidua. Am J Obstet Gynecol. 1974; 118:6 52-70.
129. Lawn AM, Wilson EW, Finn CA. The ultrastructure of human decidual and predecidual cells. J Reprod Fertil. 1971; 26: 85-90.
130. Wewer UM, Faber M, Liotta LA, Albrechtsen R. Immunochemical and ultrastructural assessment of the nature of the pericellular basement membrane of human decidual cells. Lab Invest. 1985; 53: 624-633.
131. Herr JC. Reflexive gap junctions. Gap junctions between processing arising from the same ovarian decidual cell. J Cell Biol. 1976; 69: 495-501.
132. Wewer UM, Damjanov A, Weiss J, Liotta LA, Damjanov I. Mouse endometrial stromal cells produce basement-membrane components. Differentiation. 1986; 32: 49-58.
133. Mulholland J, Aplin JD, Ayad S, Hong L, Glasser SR. Loss of collagen type VI from rat endometrial stroma during decidualization. Biol Reprod.1992; 46: 1136-1143.
134. Ferenczy. The endometrial cycle: morphologic and biochemical events. New York: Raven Press, 1995; 171-194.
135. Glasser SR, Lampelo S, Munir MI, Julian J. Expression of desmin, laminin and fibronectin during in situ differentiation (decidualization) of rat uterine stromal cells. Differentiation. 1987; 35: 132-142.
136. Psychoyos A. Hormonal control of uterine re-

ceptivity for nidation. J Reprod Fertil. 1976: 17-28.
137. Sharkey AM, Smith SK. The endometrium as a cause of implantation failure. Best Practice Res. 2003; 17: 289-307.
138. Tabanelli S, Tang B, Gurpide E. *In vitro* decidualization of human endometrial stromal cells. J Steroid Biochem Mol Biol. 1992; 42: 337-344.
139. Tang B, Guller S, Gurpide E. Mechanism of human endometrial stromal cells decidualization. Ann N Y Acad Sci. 1994; 734: 19-25.
140. Petraglia F, Tabanelli S, Galassi MC, Garuti GC, Mancini AC, Genazzani AR et al. Human decidua and *in vitro* decidualized endometrial stromal cells at term contain immunoreactive corticotropin-releasing factor (CRF) and CRF messenger ribonucleic acid. J Clin Endocrinol Metab. 1992; 74: 1427-1431.