

ROLE OF PROGESTERONE IN STRUCTURAL AND BIOCHEMICAL REMODELING OF ENDOMETRIUM

Gracy Rosario ¹, Geetanjali Sachdeva ¹, William C. Okulicz ², Christopher I. Ace ², Rajendra R. Katkam ¹ and Chander P. Puri ¹

¹ National Institute for Research in Reproductive Health, Indian Council of Medical Research, Jehangir Merwanji Street, Parel, Mumbai-400012, Maharashtra, India, ² Department of Obstetrics and Gynaecology, UMass Medical College, 55 Lake Avenue N, Worcester, MA 01655, U.S.A.

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Role of Progesterone in Endometrial Growth and Development
 - 3.1. Structural Remodelling of Endometrium
 - 3.2. Biochemical Remodelling of Endometrium
 - 3.2.1. Steroid Hormone Receptors
 - 3.2.2. Transforming Growth Factor Beta and its Receptor
 - 3.2.3. Leukaemia Inhibitory Factor
 - 3.2.4. Cyclooxygenase-2
 - 3.2.5. Prostaglandins
 - 3.2.6. Cell Adhesion Molecules/Integrins
4. Perspective
5. Acknowledgement
6. References

1. ABSTRACT

The endometrial response to the varying levels of ovarian steroids is exhibited as alterations in its form and function. These changes in endometrial morphology and physiology, especially those observed during the implantation window are prerequisites to support embryo attachment and invasion. However the state of endometrial receptivity to embryo results from an operative network of several molecular events triggered by estrogen, progesterone and probably some other factors, yet to be discovered. It is well established that estrogen and progesterone are the critical endocrine determinants of endometrial functions. However the precise delineation of hormone driven events and their interaction is yet to be ascertained. Several attempts have been made to understand these cascades, however most of these studies have been conducted *in vitro* using one or the other component of endometrial tissues. We have attempted to investigate *in vivo* morphological and biochemical/molecular changes in endometrium in response to neutralization of progesterone synthesis/ function in two primate animal models. In one of the models, ovariectomized rhesus monkeys, artificial menstrual cycles were simulated and subsequent effects on the expression of various genes were investigated in presence and absence of sufficient progesterone levels. The results coincided with those observed in the endometrium of the other model, bonnet monkeys presenting normal hypothalamus-ovarian-pituitary function but displaying retarded endometrial growth due to blocked progesterone receptor. A significant decline was observed in the expression of transforming growth factor beta, transforming growth factor beta receptor, leukaemia inhibitory factor, whereas no remarkable changes were

observed in the expression of estrogen receptor and progesterone receptors in response to neutralization of progesterone synthesis/function in these two animal models. Taking support from the inferences drawn from previously published *in vitro* studies and our data from *in vivo* studies conducted in these two models, we propose a hypothesis supporting a potential link between the expressions of transforming growth factor beta, leukaemia inhibitory factor, cyclooxygenases and integrins.

2. INTRODUCTION

Endometrium has been a focus of extensive research investigations because of its crucial functional relevance in various female reproductive events i.e. menstruation, implantation and maintenance of pregnancy. It has attracted the attention of not only those investigators who are interested in deciphering the causes of various endometrial pathologies and infertility but also of those engaged in developing the new strategies for female fertility regulation.

The endometrium undergoes several structural and biochemical changes in every menstrual cycle to facilitate embryo implantation. Implantation is a precisely coordinated but highly intricate, multifactorial and multistep process mediated via bi-directional molecular signaling between a receptive endometrium and a healthy embryo (1). This state of receptivity by the endometrium is achieved during the mid-secretory phase of menstrual cycle and is called the "implantation window" or "window of receptivity". In humans, endometrial receptivity lasts for

Role of progesterone in endometrium

only 5-6 days i.e. from day 19 to 24 of a normal menstrual cycle (2,3). During this period, endometrium displays a distinct morphological and functional profile.

It has been now unequivocally established that ovarian steroids – progesterone and estrogen are the major determinants of morphological and functional maturation of endometrium. Both estrogen and progesterone are known to play a critical role in regulating the endometrial growth and development in a cyclic manner. While estrogen induces growth and proliferation of endometrium, progesterone induces endometrium to undergo differentiation and maturation. Any aberration in hormonal availability resulting from pituitary-hypothalamus/ovarian disorders or their functional activity due to target refractoriness adversely affects endometrial functions. There exists enough clinical data to support an association between insufficient hormonal levels and implantation failure (4,5). Conception rate is often lower in the women presenting luteal phase defect with normal follicular maturation than in the women with normal progesterone levels (6). Poor progesterone surge during the luteal phase may cause impaired endometrial receptivity and subsequent infertility. Progesterone also plays a crucial role in the co-ordination of endometrial breakdown, regulation of menstrual blood loss and endometrial transudation of fluid (7). Progesterone supplementation is routinely used in the treatment of various endometrial disorders like secondary amenorrhea and dysfunctional premenopausal bleeding (8).

Although the estrogen-progesterone relationship needs to be investigated in detail, the relative concentrations of these two hormones appear to have important effects on endometrial physiology. It has been shown that the long term exposure to estrogens unopposed by progesterone leads to increased mitotic activity of endometrial cells, increased number of DNA replication errors, and somatic mutations resulting in malignant phenotype or carcinomas (9). It has also been shown that high serum estradiol levels associated with controlled ovarian hyper-stimulation in IVF patients are detrimental to embryo implantation as evident by a threefold increase in implantation rates in donor IVF programs as compared to standard IVF programs (10,11). Further, endometria of women using antiprogestins as a fertility regulating measure are often devoid of the characteristics secretory features observed in normal mid secretory endometrium (12). These observations are suggestive of the well orchestrated functional co-ordination between estradiol and progesterone and also of the regulatory role of progesterone in estradiol induced endometrial growth.

Progesterone induces differentiation and maturation by acting on the estrogen-primed endometrium through progesterone receptors. This results in switching on of several progesterone dependent genes. Genes, negatively regulated in the estrogen dominant phase of menstrual cycle, may also be expressed. This results in synthesis of a number of endometrial proteins and other factors and consequently alters the structural and molecular profiles of endometrium (13-17). There may occur number of such events in response to a common trigger i.e. progesterone and these events may regulate one another.

However the number, nature, sequence, inter or intra relationship of the progesterone induced molecular events are yet to be elucidated.

3. ROLE OF PROGESTERONE IN ENDOMETRIAL GROWTH AND DEVELOPMENT

3.1. Structural Remodelling of Endometrium

The primate endometrium has been characterized by Bartelmez (18), using histological criteria, as composed of four horizontal zones: the transient functionalis is composed of zone I, the luminal epithelia (ciliated and secretory) and densely packed stroma, and zone II, the upper third segment of the glands; the germinal basalis is composed of zone III, the middle third of the glands, and zone IV, the deepest portion of the glands adjacent to the myometrium. In addition, the endometria's complexity is further defined by the number of different cell types that it harbors. These cell types include: luminal and glandular epithelia, stromal fibroblasts, vascular smooth muscle cells and cells of the lymphocytic system (19,20). Different cell types in the uterus have been shown to respond differently to the same hormonal stimulation (21,22). Therefore, a differential cell type response to hormonal stimuli is likely to play an important role in the coordination of hormonal signals that permits the endometrium to achieve appropriate endpoints in reproduction.

Previous studies in the primate endometrium have shown different zonal patterns of proliferation, during estrogen versus progesterone dominance in both natural menstrual cycles (23,24) and artificial menstrual cycles (25,26). These studies showed that different zonal patterns of proliferation under the same hormonal milieu are a property of the primate endometrium. Of particular importance was the observation that the deepest glandular portion of the basalis (adjacent to the myometrium) increased 10-fold in proliferation during the secretory phase (24). Concomitant with this increase in proliferation in zone IV of the basalis, proliferation was dramatically reduced in epithelial cells in the upper zones of the endometrium as serum progesterone rises during the secretory phase of the menstrual cycle. Thus, zone IV of the endometrial basalis becomes the dominant proliferating tissue during progesterone dominance.

In concert with these cell-type and zonal changes in proliferation there are also concomitant changes in endometrial estrogen receptor expression. Immunohistochemical analysis of the estrogen receptor showed a dramatic down-regulation in zones I, II and III during peak serum progesterone levels whereas strong positive immunoreactive staining for estrogen receptor is retained in the glandular epithelium of zone IV (25,26). These results show that there is a zonal-dependent regulation of the estrogen receptor by progesterone in the primate endometrium and provide further support for a cell-type specific and zonal-dependent regulation of the estrogen receptor during the secretory phase.

The maintenance of strong positive immunoreactive staining for the estrogen receptor in zone IV

Role of progesterone in endometrium

of the basalis in combination with the maintenance of cellular proliferation might suggest that estrogen continues to exert a proliferative stimulus on glandular epithelial cells in this endometrial zone. Although glandular estrogen receptor in zone IV escape progesterone-induced down-regulation, no direct evidence to date supports a role for estrogen as the mitogenic stimulus in this zone. Indeed, proliferation of glandular epithelia in zone IV increases during P-dominance and this proliferation was shown to be independent of secretory estrogen (25). These data suggest a potential role for progesterone either directly or indirectly as a mitogen for glandular epithelia in zone IV. Overall, these latter studies together with recent studies using laser capture microdissection/differential display further underscore the complex cell-type and spatial-temporal regulation of gene expression that appears to be a hallmark of the primate endometrium (27,28).

The precise role of progesterone in structural and functional maturation of endometrium can only be understood by blocking progesterone action. This can be achieved by inhibiting either its synthesis or its function. Since it is not feasible and ethical to curtail the progesterone action in humans without compromising other systemic processes, nonhuman primates serve as an ideal model to carry out such studies under controlled experimental conditions. Two such models-rhesus and bonnet monkeys have been developed to gain an insight into the molecular events associated with the structural and functional maturation of endometrium.

In one model (rhesus monkeys), synthesis of endogenous progesterone is shut off by ovariectomy and exogenous progesterone at different doses is administered to evaluate the effect on endometrial growth, development and differentiation. In second model (bonnet monkeys), endometrial progesterone receptors are blocked using an antiprogesterin at specific doses. This selectively renders the endometrium refractory to progesterone action, without causing any adverse effect on pituitary, ovarian and adrenal functions.

The ability to artificially program a menstrual cycle in ovariectomized rhesus monkeys offers an added advantage to evaluate the endometrial response/sensitivity to subtle changes in peripheral hormonal levels. Menstrual cycle can be artificially stimulated in ovariectomized rhesus monkeys by controlled administration of estradiol followed by progesterone using silastic implants. The artificially stimulated cycles mimic the natural cycles and are usually of 28-day duration with peak estrogen and progesterone levels in serum reaching 250-350 pg/ml and 5-12 ng/ml respectively (29,31). Hormonal replacement approach in these ovariectomized monkeys is effective in supporting implantation, gestation and full term delivery (30).

Endometrial biopsies are collected on days 9, 11 and 13 of adequate proliferative phase (Estrogen dominant-proliferative phase), days 21 and 23 of adequate secretory phase (PcDNA) and days 21 and 23 of progesterone inadequate secretory phase (IcDNA). These biopsies are

then evaluated for their morphological and biochemical profile in response to varying amounts of estradiol and progesterone during different phases of the cycle (29,32).

Neutralization of progesterone actions in normally cycling ovulatory bonnet monkeys is achieved by treating the animals with antiprogesterins. These antiprogesterins, 19-norsteroids, block progesterone action by binding to progesterone receptor (PR). Several antiprogesterins have been developed to regulate female fertility by virtue of their ability to disrupt pituitary-hypothalamic-ovarian functions. These antiprogesterins are of two types – type I and type II showing different modes of action. Type II antiprogesterins such as mifepristone (RU486) promote dimerization of PR and its binding to DNA (34). This class of antiprogesterins acts as agonist as well as antagonist. Type I antiprogesterins i.e. onapristone (ZK 98.299) impairs binding of progesterone-PR complexes to progesterone responsive elements of DNA and act as pure antagonists (35). In our study model, onapristone was used as a tool to block the progesterone action on endometrium.

Our previous studies demonstrated an early decline in the progesterone concentration and premature menstruation following treatment with ZK 98.299 or ZK 98.734 in bonnet monkeys (36,37). Treatment with these antiprogesterins induced early menstruation during HCG induced pseudopregnancy in bonnet monkeys (37). Treatment of bonnet monkeys with high doses of onapristone for 7 consecutive cycles led to decreased LH secretion, impaired folliculogenesis, anovulation and endometrial retardation (38).

In our study model, regularly cycling bonnet monkeys were administered with low doses- 2.5 mg and 5.0 mg of onapristone dissolved in vehicle [benzoyl benzoate: castor oil (1:15)], on every third day of the menstrual cycle. Endometrial biopsies from control and treated bonnet monkeys were collected on day 8 when the serum progesterone levels reached a value of 3-5 ng/ml after the mid cycle peak in estradiol levels. However, pituitary-hypothalamo-ovarian functions remained unperturbed in the animals treated with low doses of onapristone as evident by normal histomorphology and functions of the adrenals, ovary and corpus luteum (39). Nonetheless, endometria from these animals exhibited delayed morphological maturation and subsequent non-receptivity, which could contribute to infertility (40).

In contrast to the characteristic histological features of a control mid-secretory phase endometria i.e. large and tortuous corkscrew shaped secretory glands, an increased edema, vascularity, coiled spiral vessels and stromal predecidual reaction, edema, mitosis, endometria from the animals treated with 5.0 mg onapristone displayed retarded growth with drastic reduction in diameter and height of glandular epithelial cells. Lumen of the endometrial glands had scanty secretions. Increase in vacuolization and glandular volume fraction was frequently observed. Pseudo stratification was also observed. Presence of mitotic figures in glandular epithelial cells reflected a

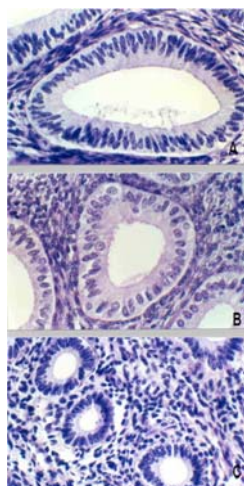
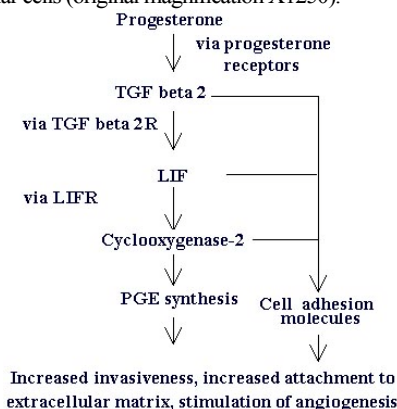


Figure 1. Histomorphological analysis of representative endometrial tissues from bonnet monkeys treated with vehicle (a) or onapristone - 2.5 mg (b) and 5.0 mg (c) onapristone. Mitoses (arrow) leading to pseudostratification were observed in endometrial glands in 2.5 mg treated animals (b). No mitoses were observed within shrinking glands in endometria from 5.0 mg treated animals (c). However, secretory material (arrows) was seen in glandular cells (original magnification X1250).



Flowchart: A Cascade of One of the Endometrial Events Facilitating Implantation: A Hypothesis

delay in endometrial maturation. Stromal cells also demonstrated retarded histological features. Predecidual reaction, edema, mitosis in the stromal cells were reduced significantly in the treated animals (Figure 1).

However the endometria from animals treated with 2.5 mg onapristone presented no signs of drastic alterations in histological features. All the morphological parameters like gland cell height, amount of secretions in the glandular lumen remained unchanged, although the gland diameter was reduced. However this did not rule out the possibility of antiprogesterin induced changes in endometrium at ultrastructural level.

Ultrastructural analysis of receptive endometria from control animals revealed the presence of a well-defined nucleus with prominent nucleoli, well-developed nuclear envelope-endoplasmic reticulum system and golgi apparatus. Rough endoplasmic reticulum (RER), represented by short and

dilated cisternae enclosed the electron lucent material. RER was surrounded by numerous small and large mitochondria with well-organized internal structure with transverse cristae. The endometrial stroma also showed well developed RER. Lipid droplets were also seen within the large mitochondria in the stromal cell cytoplasm (Figure 2). However these ultrastructural features of secretory endometrium were significantly altered in 2.5 mg treated animals thereby indicating an adverse effect of antiprogesterins on subcellular organelles of both epithelial and stromal compartments. A general loss of cell organelles such as cell nuclei, endoplasmic reticulum, mitochondria, golgi system, vacuoles and glycogen bodies was observed. Increased intercellular spaces were observed. The desmosomes were absent. The stromal cells showed degeneration with vacuolated appearance and edematous intercellular space (Figure 3). Endometria from the animals treated with 5 mg onapristone showed a total degeneration of cellular architecture. Indented nuclei showed a segmented appearance without nucleoli. The stromal cells also showed degeneration and accumulation of secretory material in the intercellular spaces. These observations support a significant role of progesterone in maintaining the cytoarchitecture of endometrial tissue (Figure 4) (41).

Histomorphological and ultrastructural analysis of non-receptive endometria from antiprogesterin treated animals clearly indicated that blocking the progesterone action induces retardation in endometrial development. However it still remained to be ascertained whether this retardation in endometrial development was because of the change in expression profiles of specific genes. This prompted us to analyze the expressions of molecular markers that could play a significant role in endometrial growth, development and thereby receptivity.

3.2. Biochemical Remodelling of the Endometrium

The attachment of the blastocyst to the extracellular matrix, followed by its invasion to endometrial stroma is mediated through extracellular matrix proteins, adhesion molecules and prostaglandins. It is speculated that endometrial receptivity is the outcome of expression and function of several factors, which play significant role in the synthesis of extracellular matrix proteins, cell adhesion molecules, prostaglandins and probably several other proteins (42-45). Here we propose a hypothesis to explain one of such progesterone driven events, which may endow the endometrium with receptivity. This hypothesis is based on our findings obtained using two nonhuman primate models and also on those reported by others (Flowchart).

3.2.1. Steroid Hormone Receptors

Ovarian hormones, estradiol and progesterone, mediate their activity via specific receptors on endometrium. Estradiol is known to induce the synthesis of both estrogen (ER) and progesterone receptors (PR) while progesterone down-regulates the expression of ER and PR (46). The expressions of these receptors are downregulated in the endometrium during the mid-secretory phase as compared to that in the proliferative phase. This is supported by a report demonstrating PR upregulation in the endometria of females with luteal phase defect (47). However, there seems more consensus on these receptors

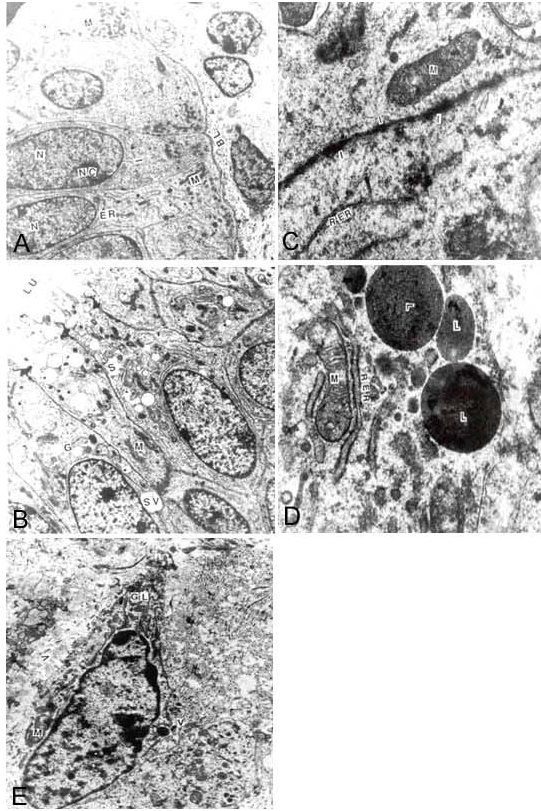


Figure 2. Electronmicrographs showing ultrastructural features of representative peri-implantation endometria from control bonnet monkeys showing well developed organelles. A and B: Apical positions of the gland cell show glycogen (g) extend into the lumen (LU). e Golgi apparatus (G) and mitochondria (M) are abundant. Secretory vesicles (SV) and granular endoplasmic reticulum (ER) are seen in the cytoplasm. Basal cells show prominent nuclei (N) with nucleolus (NC). I = desmosomes, BL = basal lamilla, s = secretory materials (X 1,400). C: Giant mitochondria (M) are seen in glandular cell cytoplasm with organised cristae and desmosomes (d) between two gland cells (X 3,000). D: Glandular epithelial cell cytoplasm with mitochondria surrounded by rough endoplasmic (RER) and lipid droplets (L) were also seen. (X 3,000). E: Shows endometrial stromal cells with well developed RER, several phagocytic vacuoles (v) with cross-sectioned collagen fibres together with some small glycogen particles (GL). Extracellular matrix is edematous. M = Mitochondria (X 2,400).

being better indicators of the change in hormonal levels than that of endometrial receptivity. Studies conducted in two primate models also support this view. The expressions of both ER and PR remain unchanged during the peri-implantation period in antiprogesterone treated bonnet monkeys showing normal hormonal levels but impaired fertility (48). In ovariectomized rhesus monkeys also, no change in the expressions of endometrial ER and PR was observed in the E-dominant proliferative and P-adequate secretory phases (29). Interestingly women with endometriosis had normal downregulation of PR but still failed to conceive (47). This again suggests that the

expression of progesterone receptors during mid secretory phase does not serve as a marker of endometrial receptivity. Progesterone once bound to PR, initiates a series of events, which leads to synthesis of various cytokines, growth factors etc. Transforming growth factor beta is one of such factors.

3.2.2. Transforming Growth Factor beta 2 and its receptor

Transforming growth factor betas (TGF betas) are a family of multifunctional growth factors that regulate many aspects of cellular growth and differentiation. Any aberrations in the expression of this growth factor may cause cells to function erratically. Women with endometriosis show increased secretion of TGF beta in the peritoneal fluid, which may be one of the major causes of infertility in these patients (49). The expression of ebf (endometrial bleeding associated factor), a member of the TGF beta super family, is found downregulated in secretory phase endometria of a subset of women with infertility (50). These reports support the role of TGF betas in endometrial physiology. The human endometrium has been shown to express TGF beta 1, TGF beta 2, TGF beta 3 and TGF beta type II receptors maximally in the secretory phase, thereby indicating the expression of these proteins is progesterone dependent (51). This was corroborated by our studies in two animal models. TGF beta 2 was found up regulated in P adequate secretory endometria than in the progesterone inadequate secretory endometria in ovariectomized rhesus monkeys (32). Further TGF beta 2 expression was found downregulated in onapristone treated animals (52). Progesterone is also required for functional maturity of TGF betas. Progesterone is known to regulate the expression of plasminogen activator, which acts on plasminogen to produce plasmin, that in turn converts inactive latent TGF beta 2 to active TGF beta 2 (53-55).

TGF beta 2 actions are mediated via its receptor (TGF beta 2R). The expression of TGF beta 2R is downregulated in bonnet monkeys following onapristone treatment (52). Studies conducted in ovariectomized rhesus monkeys also demonstrate higher expression of TGF beta 2R in P adequate secretory endometria as compared to that in P inadequate secretory endometria (32). This suggests the possibility of impaired TGF beta functions in absence of an optimal primary inducer (progesterone). Endometrial maturation may be affected because of suboptimal synthesis or inefficient maturation of TGF betas. Modulation in the synthesis of TGF betas may also affect the pathways, which promote embryo-endometrial interactions.

The above data suggest that appropriate progesterone action is required for the expression of TGF beta 2 and its receptor. Because proliferation generally ceases as differentiation begins, the decreased expression of TGF beta 2 and its receptor as a result of inadequate progesterone action could potentially lead to retarded endometrial differentiation.

There exists enough data to demonstrate the differential pattern of gene expression for various

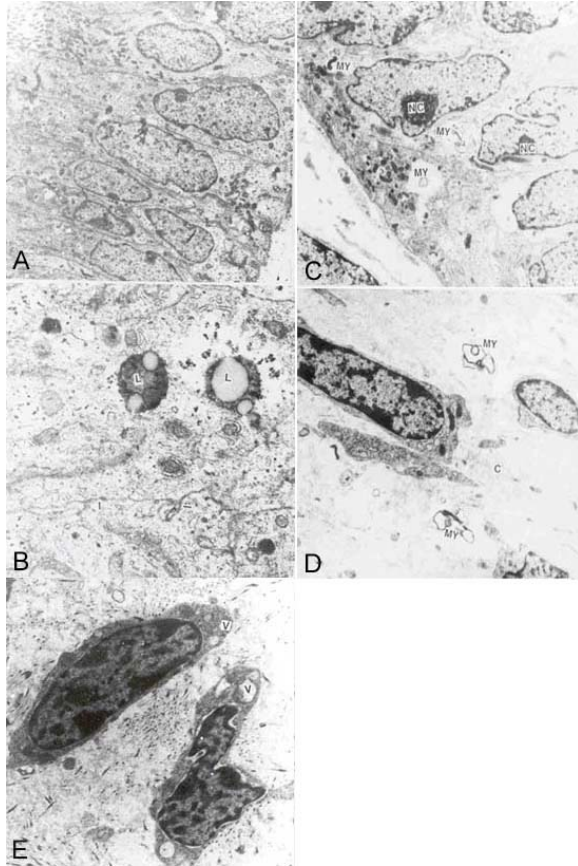


Figure 3. Electronmicrographs showing ultrastructural features of representative peri-implantation phase endometria from the bonnet monkeys treated with 2.5 mg dose of ZK 98.299 for multiple cycles. A and B: There is general loss of cell organelles with scattered lipid droplets (L). Apical ends of gland cells show fewer microvilli. I = desmosomes (X 1,400). C: show the nuclei of glandular cells showing prominent nucleoli NC) and myelin bodies (MY) in the cytoplasm (X 3,000). D and E: show the degenerative stromal cell with myelin bodies (MY) and collagen fibres (c). (X 2,400).

cytokines, growth factors and secretory proteins in the peri-implantation endometria under the influence of rising progesterone levels. It is obvious that none of these factors function in isolation. These factors act in concerted manner. However, the key players in these progesterone induced events need to be identified.

In vitro studies have demonstrated upregulation of leukaemia inhibitory factor (LIF) in human stromal cells on supplementation with TGF beta 2. Interestingly progesterone alone failed to cause an upregulation of LIF in stromal cells (56). Our preliminary studies have also revealed up regulation of LIF expression in endometrial explants on supplementation with recombinant TGF beta 2. This suggests that progesterone regulates LIF expression via TGF beta 2. Further there have been reports to demonstrate TGF beta 2 induced expression of alpha v beta 3 expression in glioma cells (57). However the possibility

of similar interactive relationship between alpha v beta 3 and TGF beta 2 in endometrium needs to be validated.

3.2.3. Leukaemia Inhibitory Factor

Leukemia inhibitory factor (LIF) is a cytokine involved in hematopoiesis, neurogenesis, embryogenesis and embryonic stem cell differentiation. Normal embryo development but failed implantation in LIF knockout mice demonstrated a critical role of LIF in implantation (58). LIF expression was found deregulated in endometria of majority of the infertile women whose eggs did not implant despite their oocytes having undergone a successful IVF (59). LIF concentration in uterine flushings was found low in women with unexplained infertility (60).

LIF mRNA and protein expression were found significantly more in the human endometrium during the mid-secretory phase when progesterone levels are high (61). In the majority of infertile women, LIF production was downregulated in the endometrium during both the proliferative and the secretory phases of the cycle (62). The dysfunction of cytokine production was found more profound in patients with multiple failures of implantation (63). There are reports to suggest that heterozygous point mutations in the LIF gene at receptor binding regions could give rise to decreased availability or biological activity of LIF in the uterus and cause implantation failure (64).

Further LIF expression was found significantly low in peri-implantation endometria from our animal model - antiprogesterin treated bonnet monkeys (51). Rhesus monkeys treated with low doses of RU486 also demonstrated reduced intensity of LIF in peri-implantation endometria (65). In addition, it has also been shown that LIF expression is also greatly under-represented in rhesus monkey endometria during an inadequate secretory phase (32,33).

Endometrial glandular LIF expression declined significantly in women using RU486 (66). In artificially stimulated cycles of ovariectomized rhesus monkeys LIF mRNA could only be detected in the P dominant phase indicating again that the LIF expression is regulated by progesterone (29).

The action of LIF is mediated through two receptors-LIFR beta and gp130. However, LIF receptor beta and gp130 are constitutively expressed both in the proliferative and secretory phases of the normal menstrual cycle of humans (67). This suggested that LIFR beta and gp130 expressions are not progesterone regulated. However our studies in bonnet monkeys suggest that the antiprogesterin treatment leads to down regulation of LIFR in the endometria during the peri-implantation phase (Figure 5).

Though LIF seems to play a role in implantation, it may not be the sole determinant of endometrial receptivity as evident by implantation failure in cyclooxygenase-2 (COX-2) knockout mice showing normal production of LIF. Interestingly, the expressions of both LIF and COX-2 were found impaired in LIF knockout

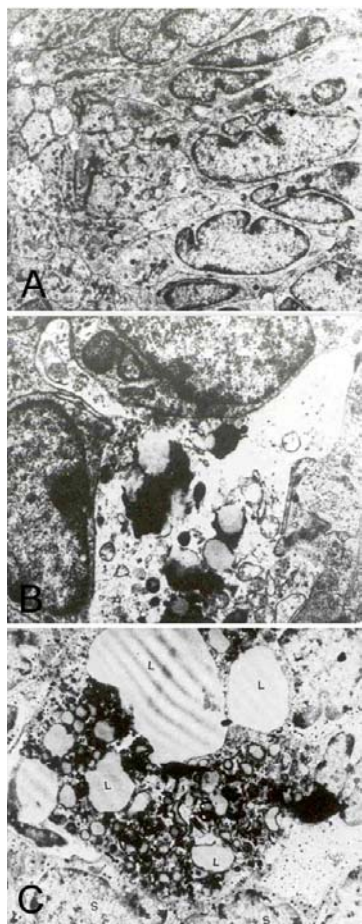


Figure 4. Electromicrographs showing ultrastructural features of representative peri-implantation phase endometria from the bonnet monkeys treated with 5.0 mg of ZK 98,299 for multiple cycles. A: Glandular epithelial cell shows disintegrated nuclei without nucleoli with increased inter and intracellular space. B: Shows the accumulation of secretory materials with marked vacuolation between glandular cells. C: Shows degenerative stromal cells filled with large lipofuscin bodies (L). s = secretory material (X 1,400).

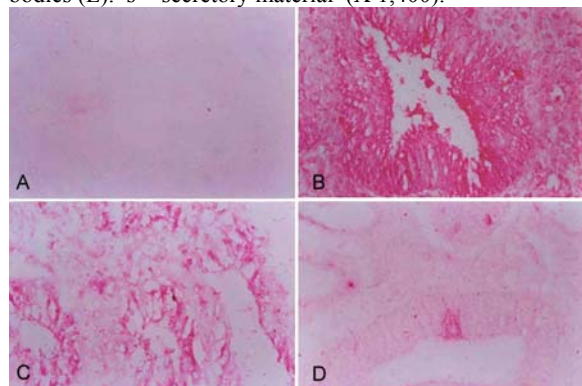


Figure 5. Immunohistochemical localization of leukaemia inhibitory factor (LIFR) in the peri-implantation phase endometria from control (B) and onapristone treated 2.5 mg (C) and 5.0 mg (D) bonnet monkeys. Negative control is shown in A.

mice, thereby implying that the LIF gene product may regulate the synthesis of Cyclooxygenase-2, an enzyme catalyzing the rate limiting steps in prostaglandins synthesis (68,71).

3.2.4. Cyclooxygenase-2

Cyclooxygenases are of two types: constitutively expressed cyclooxygenase-1 (COX-1) and inducible cyclooxygenase-2 (COX-2). COX-2 is induced by variety of molecules like chorionic gonadotropins, cytokines or tumor promoters (69). Expressions of COX-1 in glandular epithelium and COX-2 in luminal epithelium decreased significantly in peri-implantation phase endometria of women using RU486 (70). Similar observations were noted in onapristone treated bonnet monkeys. A downregulation of COX-2 expression was observed in the peri-implantation endometria of treated animals as compared to control animals (Figure 6). This indicates that the expressions of endometrial COX-1 and COX-2 may be progesterone-regulated. Data supporting the critical role of COX-2 in endometrial receptivity come from knockout studies. Targeted disruption of COX-2 but not of COX-1 in female mice resulted in multiple failures such as ovulation, fertilization, implantation and decidualization (71). Further LIF knockout mice showing implantation failure had also impaired production of COX-2 (68).

Interestingly, human COX-2 gene contains a TGF beta response element (72). It is likely that TGF beta may have a regulatory role in the expression of COX-2. This was indirectly supported by our studies demonstrating downregulation of both TGF beta 2 and Cyclooxygenase-2 in the peri-implantation phase endometria of bonnet monkeys treated with antiprogesterin.

3.2.5. Prostaglandins

Prostaglandins are end products of arachidonic acid metabolism catalyzed by the action of the rate limiting cyclooxygenase (73). Prostaglandins act as inflammatory mediators and therefore are important regulators involved in the success of pregnancy (74). Prostaglandin F 2 alpha (PGF2 alpha) is responsible for luteolysis whereas prostaglandin E2 (PGE2) has luteoprotective action (69). PGE2 regulates the production of prolactin that has been shown to have a role in stromal cell differentiation (75). The production of endometrial PGE2 and PGF2 alpha are significantly higher during the mid-secretory phase as compared to the proliferative phase (76). Defects in PGE2 production in the pre- and post implantation stages of pregnancy are known to cause clinical miscarriage. Further PGF 2 alpha concentration was found high in the peri-implantation endometria from women using RU486 (77). These reports suggest more significant role of PGE2 than PGF2 alpha in endometrial receptivity and implantation. It has been noted that women using Nonsteroidal anti-inflammatory drugs (NSAIDs), known inhibitors of cyclooxygenases for the treatment of arthritis, are more likely to develop infertility (78,79).

3.2.6. Cell Adhesion Molecules/Integrins

Integrins are a family of ubiquitous heterodimeric glycoproteins involved in cell-cell and cell-substrate

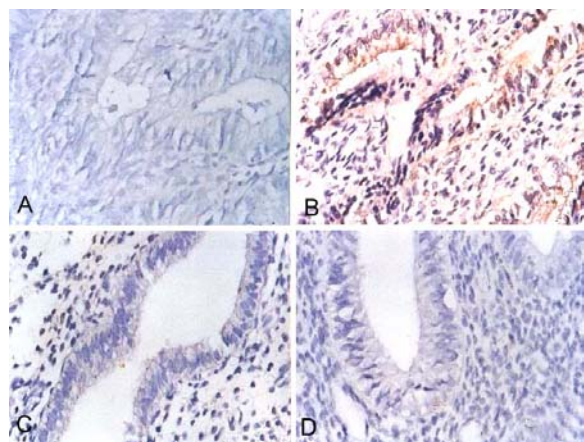


Figure 6. Immunolocalization of cyclooxygenase-2 (COX-2) in the peri-implantation phase endometria from control (B) and onapristone treated (2.5 mg, C; 5.0 mg, D) animals. Negative control is shown in A.

adhesion by binding to a specific RGD (Arg-Gly-Asp) or related sequences in their ligands (80). These integrins display spatial and temporal expression in the endometrium during the menstrual cycle and are required for attachment of the embryo to the extracellular matrix (81). Various integrins are cycle dependent i.e. these are expressed only during the peri-implantation period. The α v β 3 integrin/ vitronectin receptor appears in the human endometrium on days 19-24 of the menstrual cycle i.e. the peri-implantation period (82-84). This integrin is also aberrantly expressed in women with luteal phase deficiency thereby suggesting that α v β 3 is progesterone dependent (85). The integrin α 1 β 1 is expressed in Ishikawa cells (a cell line constitutively expressing PR) in response to progesterone and this expression is blocked by the anti-progestin RU4 (86), which indicates that this integrin is progesterone dependent (86). Further decreased expression of the integrin subunit α 1 in human endometrial stromal cells during the late secretory phase may be associated with poor fertility outcome in unexplained fertility cases (43). Our study also showed down-regulation of α v β 3 and α 1 β 1 integrins in the peri-implantation phase endometria from onapristone treated animals (87).

However the exact mechanism by which the expression of these integrins is regulated is not understood. *In vitro* treatment of human tumor cells with LIF led to upregulation in the expression of α v β 1, suggesting that LIF may be an important regulator of integrin expression (87). The inhibition of COX-2 by NSAIDs also suppressed the activation of α v β 3 (88). Similar *in vitro* studies in endometrial cells may help establish the regulatory network between LIF, TGF β 2, COX2 and integrins.

4. PERSPECTIVE

Progesterone action is essential for maturation of the endometrium into a receptive state for implantation in humans and nonhuman primates. We envisage that

coordinated, steroid-induced activation and repression of many genes during the changeover from E to P-dominance during the secretory phase is essential for this process. Many of these genes and gene networks that are involved are likely to be induced or inhibited in a temporal, spatial, and cell-type specific context within the endometrium. The orchestration of these latter events by progesterone is essential for reproductive competence. Only continued future research efforts will allow us to understand details of the complex hormonal regulation of primate endometrial function. Such studies are expected to serve as an important basis for treatments of fertility/infertility in women.

5. ACKNOWLEDGEMENTS

The authors thank the Indo-US Program for Contraceptive and Reproductive Health Research for partly funding the research work (NIH HD-31620). The authors also thank Mr Hemant Karekar, National Institute for Research in Reproductive Health, India for the artwork.

6. REFERENCES

1. Satyaswaroop P. G. & S. Tabibzadeh: Progestin regulation of human endometrial function. *Hum Reprod* 15 (Suppl 1), 74-80 (2000)
2. Navot D., R. T. Scott, K. Droesch, L. L. Veeck, H. C. Liu & Z. Rosenwaks: The window of embryo transfer and the efficiency of human conception *in vitro*. *Fertil Steril* 55, 114-118 (1991)
3. Lessey B.A.: The role of the endometrium during embryo implantation. *Hum Reprod* 15 (suppl 6), 39-50 (2000)
4. Soules M. R., R. L. McLachlan, E. K. Marit, K. D. Dahl, N. L. Cohen & W. J. Bremner: Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. *J Clin Endocrinol Metab* 69, 804-812 (1989)
5. Wentz A. C., L. R. Kossoy & R. A. Parker: The impact of luteal phase inadequacy in an infertile population. *Am. J. Obstet Gynecol* 162, 937-43 (1990)
6. Check J. H., M. M. Vaze, R. S. Raikar, K. Nourozi, S. G. Joshi, R. Epstein & B. Vetter: Late luteal phase progesterone-dependent endometrial protein level in women with in-phase biopsies -- can levels predict a sub fertile group? *Int J Fertil* 37, 350-353 (1992)
7. Fraser I. S.: Regulating menstrual bleeding. A prime function of progesterone. *J Reprod Med* 44, 158-164 (1999)
8. Fitzpatrick L. A. & A. Good: Micronized progesterone: clinical indications and comparisons with current treatments. *Fertil. Steril* 72, 389-397 (2000)
9. Akhmedkhanov A., A. Zeleniuch-Jacquotte & P. Toniolo: Role of exogenous and endogenous hormones in

Role of progesterone in endometrium

endometrial cancer: review of the evidence and research perspectives. *Ann N Y Acad Sci* 943, 296-315 (2001)

10. Pellicer A., D. Valbuena, P. Cano, J. Remohi & C. Simon: Lower implantation rates in high responder: evidence for an altered endocrine milieu during the pre-implantation period. *Fertil Steril* 65, 1190-1195 (1996)

11. Simon C., F. Cano, D. Valbuena, J. Remohi & A. Pellicer: Clinical evidence for detrimental effect on uterine receptivity of high serum oestradiol levels in high and normal responder patients. *Hum Reprod* 10, 242-247 (1995)

12. Cameron S. T., H. O. D. Critchley, C. H. Buckley, R. W. Kelly & D. T. Baird: Effect of two antiprogestins (mifepristone and onapristone) on endometrial factors of potential importance for implantation. *Fertil Steril* 67, 1046-1053 (1997)

13. Beier H. M.: Uteroglobulin and other endometrial proteins: Biochemistry and biological significance in beginning pregnancy. In: Beier H.M. and P. Karlson Eds: *Proteins and Steroid in Early Pregnancy*, Springer Verlag, Berlin, 38-71 (1982)

14. Hegele-Hartung C. & H. M. Beier: Distribution of uteroglobulin in the rabbit endometrium after treatment with anti-progesterone (ZK 98.734): an immunocytochemical study. *Hum Reprod* 1, 497-505 (1986)

15. Hegele-Hartung C., D. Mootz & H. M. Beier: Luteal control of endometrial receptivity and its modification by progesterone antagonist. *Endocrinol* 131, 2446-2460 (1992)

16. Beier-Hellwig K., B. Bonn, K. Sterzik et al: Uterine receptivity and endometrial secretory protein pattern. In: Dey. S.K. Eds: *Molecular and Cellular Aspects of Peri-implantation Process*, Sereno Symposia, U.S.A. Springer-Verlag, New York, 87-102 (1994)

17. Gemzell-Danielsson K., M. L. Swahn, P. Svalander, E. Johannisson & M. Bygdeman: Effect of single post-ovulatory dose of RU486 on endometrial maturation in the implantation phase. *Hum Reprod* 9, 2398-2404 (1994)

18. Bartelmez G.W., G. W. Corner & C. G. Hartman: Cyclic changes in the endometrium of the rhesus monkey (*Macaca mulatta*). *Contrib Embryol* 34, 99-144 (1951).

19. Tabibzadeh S: Proliferative activity of lymphoid cells in human endometrium throughout the menstrual cycle. *J Clin Endocrinol Metab* 70, 437-443 (1990).

20. Tabibzadeh S: Immunoreactivity of human endometrium: Correlation with endometrial dating. *Fertil Steril* 54, 624-631 (1990)

21. McCormack S. A. & S. R. Glasser: Differential response of individual uterine cell types from immature rats

treated with estradiol. *Endocrinology* 106, 1634-1649 (1980).

22. King R. J. B., G. Dyer, W. P. Collins & M. I. Whitehead: Intracellular estradiol, estrone and estrogen receptor levels in endometria from postmenopausal women receiving estrogens and progestins. *J Steroid Biochem* 13, 377-382 (1980).

23. Padykula H. A., L. G. Coles, J. A. McCracken, N. W. King, C. Longcope & I. R. Kaiserman-Abramof: A zonal pattern of cell proliferation and differentiation in the rhesus endometrium during the estrogen surge. *Biol Reprod* 31, 1103-1118 (1984).

24. Padykula H. A., L. G. Coles, W. C. Okulicz, S. I. Rapaport J. A. McCracken, N. W. King Jr., C. Longcope & I. R. Kaiserman-Abramof: The basalis of the primate endometrium: A bifunctional germinal compartment. *Biol Reprod* 40, 681-690 (1989).

25. Okulicz W. C., M. Balsamo & J. Tast: Progesterone regulation of endometrial estrogen receptor and proliferation during the late proliferative and secretory phase in artificial menstrual cycles in the rhesus monkey. *Biol Reprod* 49, 24-32 (1993).

26. Okulicz W. C. & M. Balsamo: A double immunofluorescent method for the simultaneous analysis of progesterone-dependent changes in proliferation (Ki-67) and the estrogen receptor in the rhesus endometrium. *J Reprod Fertil* 99, 545-549 (1993)

27. Torres M.T., Ace C.I. & W. C. Okulicz: Assessment and application of laser microdissection for analysis of gene expression in the rhesus endometrium. *Biol. Reprod* 67, 1067-1072 (2002)

28. Okulicz, W. C., Ace C. I. & M. T. Torres: Gene expression in the Rhesus monkey endometrium: differential display and laser capture microdissection. *Frontiers in Bioscience* 8, 551-558 (2003)

29. Ace C. I. & W. C. Okulicz: Differential gene regulation by estrogen and progesterone in the primate endometrium. *Mol Cell Endocrinol* 115, 95-103 (1995)

30. Hogden G. D.: Surrogate embryo transfer combined with estrogen-progesterone therapy in monkeys: Implantation, gestation and delivery without ovaries. *J Am Med Assoc* 250: 2167-2171 (1983)

31. Bhartiya D., S. R. Choudhary & V. K. Bajpai: Stromal cell interaction and relevance to predecidual events and menstruation. *Hum Reprod* 11, 850-856 (1996).

32. Okulicz W. C., C. I. Ace, C. Longscope & J. Tast: Analysis of differential gene regulation in adequate versus inadequate secretory-phase endometrial complementary deoxyribonucleic acid from the rhesus monkey. *Endocrinol* 137, 4844-4830 (1996)

Role of progesterone in endometrium

33. Okulicz, W.C. & C. I. Ace: Progesterone-regulated gene expression in the primate endometrium. *Seminars in Reproductive Endocrinology* 17, 241-255 (1999).
34. Meyer M. E, A. Pornon, J. W. Ji, M. T. Bocquel, P. Chambon & H. Gronemeyer: Agonistic, antagonistic activities of RU 486 on the functions of the human progesterone receptor. *E. M. B. O.* 12, 3923-3932 (1990)
35. Klein-Hitpass L., A. C. Cato, D. Henderson & G. U. Ryffel: Two types of antiprogesterins identified by their differential action in transcriptionally active extracts from T47D cells. *Nucleic Acid Res* 19, 1227-1234 (1991)
36. Puri C. P. W. A. G. Elger & J. M. R. Pongubala: Induction of menstruation by antiprogesterone ZK 98.299 in cycling bonnet monkeys. *Contraception* 35, 409-421 (1987)
37. Puri C. P., R.R. Katkam, A. D'Souza, W. A. G. Elger & R. K. Patil: Effects of a progesterone antagonist lilepristone (ZK 98.734) on the induction of menstruation, inhibition of nidation and termination of pregnancy in bonnet monkeys. *Biol Reprod* 43, 437-443 (1990)
38. Puri C. P., R. K. Patil, W. A. G. Elger, A. D. Vadigoppula & J. M. R. Pongubala: Gonadal and pituitary responses to progesterone antagonist ZK 98.299 during the follicular phase of the menstrual cycle in bonnet monkeys. *Contraception* 39, 227-243 (1989)
39. Ishwad P. C., R. R. Katkam, I. N. Hinduja, S. Chawlisz, W. A. G. Elger & C.P. Puri: Treatment with a progesterone antagonist ZK 98.299 delays endometrial development without blocking ovulation in bonnet monkeys. *Contraception* 48, 57-70 (1993)
40. Katkam R. R., K. Gopalkrishnan, S. Chawlisz, E. Schillinger & C. P. Puri: Onapristone (ZK 98.299): A potential antiprogesterin for endometrial contraception. *Am J Obstet Gynecol* 173, 779-787 (1995)
41. Gopalakrishnan K., R. R. Katkam, G. Sachdeva, S. D. Kholkute, V. Padwal & C. P. Puri: Effects of an antiprogesterin onapristone on the endometrium of bonnet monkeys: morphometric and ultrastructural studies. *Biol Reprod* 2003 (in Press)
42. Bilalis D. A., L. D. Klentzeris & S. Fleming: Immunohistochemical localization of extracellular matrix proteins in luteal phase endometrium of fertile and infertile patients. *Hum Reprod* 11, 2713-2718 (1996)
43. Gonzalez R. R., A. Palomino, A. Boric, M. Vega & L. Devoto: A quantitative evaluation of alpha 1, alpha 4, alpha V and beta 3 endometrial integrins of fertile and unexplained infertile women during the menstrual cycle. A flow cytometric appraisal. *Hum Reprod* 14, 2485-2492 (1999)
44. Lessey B. A., A. J. Castelbaum, S. W. Sawin & J. Sun: Integrins as markers of uterine receptivity in women with primary unexplained infertility. *Fertil Steril* 63, 535-542 (1995)
45. Psychoyos A., G. Nikas & A. Gravanis: The role of prostaglandins in blastocyst implantation. *Hum Reprod* 10 (Suppl 2), 140-150 (1995)
46. Fujishita A., P. K. Nakane, J. Koji, H. Masuzaki, R. O. Chavez, T. Yamake & T. Ishimara: Expression of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: an immunohistochemical and in situ hybridization study. *Fertil Steril* 67, 856-864 (1997)
47. Lessey B. A., A. P. Killam, D. A. Metzger, A. F. Haney, G. L. Greene & K. S. McCarty Jr: Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab* 67, 334-340 (1988).
48. Puri C. P., R. R. Katkam, G. Sachdeva, V. Patil, D. D. Manjramkar & S. D. Kholkute: Endometrial contraception: Modulation of molecular determinants of uterine receptivity. *Steroids* 65, 783-794 (2000)
49. Oosterlynck D. J., C. Meuleman, M. Waer & P. R. Kononcks: TGF-beta activity is increased in the peritoneal fluid of women with endometriosis. *Obstet Gynecol* 83, 287-292 (1994)
50. Tabibzadeh S., J. M. Mason, W. Shea, Y. Cai, M. J. Murray & B. Lessey: Dysregulated expression of ebaf, a novel molecular defect in the endometria of patients with infertility. *J Clin Endocrinol Metab* 85, 2526-2536 (2000)
51. Chegini N., Y. Z hao, R. S. Williams & K. C. Flanders: Human uterine tissue throughout the menstrual cycle expresses transforming growth factor-beta 1 (TGF beta 1), TGF beta 2, TGF beta 3, and TGF beta type II receptor messenger ribonucleic acid and protein [125] TGF beta1-binding sites. *Endocrinol* 135 (1), 439-449 (1994).
52. Sachdeva G., V. Patil, R. R. Katkam, D. D. Manjramkar, S. D. Kholkute & C. P. Puri: Expression profiles of endometrial leukaemia inhibitory factor, transforming growth factor beta 2 (TGF beta 2) and TGF beta 2 receptor in infertile bonnet monkeys. *Biol Reprod* 65, 1-8 (2000)
53. Massague J.: The transforming growth factor \square family. *Annu Rev Cell Biol* 6, 597-641 (1990)
54. Lyons R. M., L. E. Gentry, A. F. Purchio & H. L. Moses: Mechanism of activation of transforming growth factor beta 1 by plasmin. *J Cell Biol* 106, 1659-1665 (1990)
55. Casslen B., A. Andersson, I. M. Nilsson & B. Astedt: Hormonal regulation of the release of plasminogen activators and of a specific activator inhibitor from endometrial tissue in culture. *Proc Soc Biol Med* 182, 419-424 (1986)

56. Arici A., Engin-O, E. Attar & D. L. Olive: Modulation of leukaemia inhibitory factor gene expression and protein biosynthesis in human endometrium. *J Clin Endocrinol Metab* 80, 1908-1915 (1995)
57. Platten M., W. Wick, C. Wild-Bode, S. Aulwurm, J. Dichgans & M. Weller: Transforming growth factors beta (1) (TGF-beta(1)) and TGF beta (2) promote glioma cell migration via up-regulation of alpha (V) beta (3) integrin expression. *Biochem Biophys Res Commun* 268, 607-611 (2000)
58. Stewart C. L., P. Kaspar, J. Brunett, H. Bhatt, I. Gadi, F. Kongten & S. J. Abbondanzo: Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* 359, 76-79 (1992)
59. Delage G, J-F. Moreau, J-L. Taubin, S. Freitas, E. Hambartsoumian, F. Olivennes, R. Fanchin, H. Letur-Konirsch, R. Frydman & G. Chaouat: In vitro endometrial secretion of human interleukin for DA cells/leukaemia inhibitory factor by explant cultures from fertile and infertile women. *Hum Reprod* 10 (9), 2483-2488 (1995)
60. Laird S. M., E. M. Tuckerman, C. F. Dalton, B. C. Dunphy, T. C. Li & X. Zhang: The production of leukaemia inhibitory factor by human endometrium: Presence in uterine flushings and production by cells in culture. *Hum Reprod* 12, 569-574 (1997)
61. Charnock-Jones D. S. A. M. Sharkey, P. Fenwick & S. K. Smith: Leukaemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. *J Reprod. Fertil* 101, 421-6 (1994).
62. Tsai H. D., C. C. Chang, Y. Y. Hsieh & H. Y. Lo: Leukaemia inhibitory factor expression in different endometrial locations between fertile and infertile women throughout different menstrual phases. *J Assist Reprod Genet* 17, 415-418 (2000)
63. Hambartsoumain E.: Endometrial leukaemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. *Am J Reprod Immunol* 39, 137-143 (1998)
64. Giess R., I. Tanasescu, T. Steck & M. Sendtrur: Leukaemia inhibitory factor gene mutations in infertile women. *Mol Hum Reprod* 5, 581-586 (1999)
65. Jimenez-Moreno V., M. Billeter, C. Q. Liu, K. Gordon & M. Mahony: Local effects of mifepristone on the nonhuman primate endometrium. *Fertil Steril* 74, 134-140 (2000)
66. Danielsson K. G., M. L. Swahn & M. Byderman: The effect of various doses of mifepristone on endometrial leukaemia inhibitory factor expression in the midluteal phase – an immunohistochemical study. *Hum Reprod* 12, 1293-1297 (1997)
67. Cullinan C. B., S. J. Abbondanzo, P. S. Anderson, J. W. Pollard, B. A. Lessey & C. L. Stewart: Leukaemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *Proc Natl Acad Sci U.S.A.* 93, 3115-3120 (1996)
68. Song H., H. Lim, S. K. Das, B. C. Paria & S. K. Dey: Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reaction of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Endocrinol* 14, 1147-1161 (2000)
69. Asselin E., D. Lacroix & M. A. Fortier: IFN- tau increases PGE2 production and COX-2 gene expression in the bovine endometrium in vitro. *Mol Cell Endocrinol* 132, 117-126 (1997)
70. Marions L.& K. G. Danielsson: Expression of cyclooxygenase in human endometrium during the implantation period. *Mol Hum Reprod* 5, 961-965 (1999).
71. Lim H., B. C. Paria, S. K. Das, J.E. Dinchuk, R. Langenbach, J. M. Trzaskos & S. K. Dey: Multiple female reproductive failures in cyclooxygenase 2- deficient mice. *Cell* 91, 197-208 (1997)
72. Yang X., F. Hou, L. Taylor & P. Polgar: Characterization of human cyclooxygenase Z gene promoter localization of a TGF beta response element. *Biochem Biophys Acta* 1350, 287-292 (1997)
73. Sitruk-Ware R.: Anti-progesterones. *Presse Med* 28, 2123-2131 (1999).
74. Boone DL, Currie WD & Leung PC: Arachidonic cid and cell signalling in the ovary and placenta. *Prostaglandins Leukot Essent Fatty acid* 48, 79-87 (1993)
75. Frank G. R., K. Brar, M. I. Cedars & S. Handwerger: Prostaglandin E2 enhances human endometrial stromal cell differentiation. *Endocrinol* 134, 258-263 (1994)
76. Ishihara O., O. Tsutsumi, M. Mizuno, K. Kinoshita & K. Satoh: Metabolism of arachidonic acid and synthesis of prostanoids in human endometrium and decidua. *Prostaglandins Leukot Med* 24, 93-102 (1986)
77. Nayak N. R, J. Sengupta J. & D. Ghosh: Antinidatory effect of luteal phase administration of mifepristone (RU486) is associated with changes in endometrial prostaglandins during the implantation window. *Contraception* 58, 111-7 (1998)
78. Mendonca L. L., M. A. Khamashta, C. Nelson-Piercy, B. J. Hunt & G. R. Hughes: Non-steroidal anti-inflammatory drugs as a possible cause for reversible infertility. *Rheumatology (oxford)* 39, 880-882 (2000)

Role of progesterone in endometrium

79. Calmels C., J. J. Dubost, C. Jasmin-Lebrun & B. Sauvezie: A new case of NSAID-induced infertility. *Rev Rheu Engl Ed* 66, 167-168 (1999)

Mumbai-400012, Maharashtra, India, Tel: 9122-2413211, Fax: 9122-24132412, E-mail: dirirr@vsnl.com

80. Hii L. P. P. & P. A. W. Rogers: Endometrial vascular and glandular expression of integrin alpha v beta 3 in women with and without endometriosis. *Hum Reprod* 13, 1030-1035 (1998)

81. Grosslinsky C. M., C. W. Yowell, J. Sun, L. V. Parise & B. A. Lessey: Modulation of integrin expression in endometrial stromal cells in vitro: *J Clin Endocrinol Metab* 81, 2047-2054 (1996)

82. Lessey B. A., L. Damjanovich, C. Coutifaris, A. Castebaum, S. M. Albeda & C. A. Buck: Integrin adhesion molecules in human endometrium: Correlation with the normal and abnormal menstrual cycle. *J Clin Invest* 90, 188-195 (1992)

83. Lessey B. A., A. Castelbaum, C. A. Buck, Y. Lei, C. W. Yowell & J. Sun: Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. *Fertil Steril* 62, 497-506 (1994)

84. Dou Q., R. S. Williams & N. Chegini: Expression of integrin messenger ribonucleic acid in human endometrium: a quantitative reverse transcription polymerase chain study. *Fertil Steril* 71, 347-353 (1999)

85. Lessey B. A., I. Yeh, A. J. Castelbaum, M. A. Fritz, A. O. Ilesanmi, P. Korzeniowski, J. Sun & Z. K. Chwalisz: Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. *Fertil Steril* 67, 184-186 (1996)

86. Lessey B. A., A. O. Ilesanmi, A. J. Castelbaum, L. Yuan, S. G. Somkuti, Z. K. Chwalisz & P. G. Satyaswaroop: Characterization of the functional progesterone receptor in an endometrial adenocarcinoma cell line (Ishikawa): progesterone induced expression of alpha 1 integrin. *J Steroid Biochem Mol Biol* 59, 31-39 (1996)

87. Heymann D, J. Harb, S. Ringeard, F Blanchard, D. Lassort, S. Raher & A. Godard: Modulation of integrin expression on human tumor cells by leukaemia inhibitory factor (LIF) and oncostatin M (OSM). *Bull Cancer* 83, 13-21 (1996)

88. Dormond O., A. Foletti, C. Paroz & C. Ruegg: NSAIDS inhibit alpha v beta 3 integrin mediated and cdc42/ Rac dependent endothelial-cell spreading, migration and angiogenesis. *Nat Med* 7, 1041-1047 (2001)

Key Words: Progesterone, Endometrium, Antiprogestins, Growth Factors, Cytokine, Ultrastructure, Review

Send correspondence to: Dr Chander P. Puri, Director, National Institute for Research in Reproductive Health, Indian Council of Medical Research, Jehangir Merwanji Street, Parel,