Hormonal regulation and localization of estrogen, progestin and androgen receptors in the endometrium of nonhuman primates: effects of progesterone receptor antagonists*

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Summary. This article reviews the effects of estradiol (E_2), progesterone (P) and P receptor antagonists (PA) on the rhesus macaque endometrium. Ovariectomized macaques can be treated with implants of estradiol (E_2) and P to induce precisely controlled, artificial menstrual cycles. During these cycles, treatment with E_2 alone induces an artificial proliferative phase marked by extensive endometrial epithelial cell proliferation and increased expression of stromal and epithelial estrogen receptor (ER) and P receptor (PR). Androgen receptor (AR) is also upregulated by E_2 but is expressed only by the endometrial stroma. Progesterone acts on the E_2 primed endometrium to induce secretory differentiation and causes suppression of epithelial and stromal ER, epithelial PR, and stromal AR in the functionalis zone. However, epithelial ER and PR are retained in the basalis zone during the secretory phase. When potent P antagonists (PA) are administered acutely at the end of an E_2+P induced cycle, menses typically ensues similar to P withdrawal at the end of the menstrual cycle. When PAs are administered chronically there is significant blockage of all P-dependent effects including upregulation of ER, PR and AR and suppression of glandular secretory function. However, chronic PA administration also inhibits estrogen-dependent endometrial cell proliferation and growth. This endometrial antiproliferative effect is the basis of the clinical use of PA to control various diseases such as endometriosis.

Background

Progesterone is an essential reproductive hormone that acts on the estrogen primed endometrium to induce conditions favorable for embryo implantation (Navot et al., 1986; Ghosh and Sengupta, 1989), and the maintenance of pregnancy (Hodgen and Tullner, 1975). The genomic effects of P are mediated in target cells through interactions with specific intracellular progesterone receptors (PR). Compounds that bind to PR and antagonize the effects of P (progesterone antagonists; PA) have many clinically valuable roles such as inhibition of endometriosis, fibroids (Chwalisz et al., 2002; Olive, 2002) and excessive uterine bleeding (Slayden et al., 2001a). Some PAs have side effects due to lack of selectivity for PR and high cross reactivity for glucocorticoid receptor (GR) and androgen receptor (Giannoukos et al., 2001). Compounds with improved PR selectivity known as selective progesterone receptor modulators (SPRM) have recently been produced that may provide improved therapy for reproductive tract diseases including endometriosis (Chwalisz et al., 2002) and breakthrough bleeding (Williams et al., 1997). However, it is difficult to predict the action of these novel compounds from their chemical structure (Giannoukos et al., 2001). In vivo studies of new SPRMs are necessary to fully characterize the action of these compounds, and such studies require experimental models that accurately reflect hormone action in the human reproductive tract.

Ovariectomized rhesus macaques can be treated with implants of E_2 and P to induce menstrual cycles in which the hormonal environment is precisely controlled, and in
which PAs can be evaluated. This manuscript reviews the effects of E\(_2\) and P on the tissue architecture in the rhesus macaque endometrium and the expression of sex steroid receptors, and indicates the value of this model for preclinical research on PA compounds.

**Animal treatments**

All the studies described in this review were approved by the Oregon National Primate Research Center (ONPRC) Animal Care and Use Committee, and supervised by the ONPRC veterinary staff. Adult rhesus macaques (Macaca mulatta) were ovariectomized and artificial cycles (Rudolph-Owen et al., 1998; Slayden et al., 2001a) were initiated in the animals by inserting a subcutaneous (s.c.) capsule that released E\(_2\) to induce a 14 day proliferative phase, followed by insertion of a P capsule s.c. to induce a 14 day secretory phase. The levels of E\(_2\) and P produced by these capsules were within the normal range for macaques during the natural cycle. Figure 1 shows a diagram depicting an induced menstrual cycle. Removal of the P implant on day 28 completes the cycle and causes menstruation. We collected endometrium for analysis at the following phases: 1) the end of the proliferative phase, 2) the end of the secretory phase, and 3) during days 1–6 of the next cycle induced by P withdrawal. We refer to the transition between cycles as the luteal follicular transition (LFT) (Rudolph-Owen et al., 1998). We also evaluated the action of several PA compounds including mifepristone (Roussel UCLAF), ZK 137 316, and ZK 230 211 (Scher ing AG) administered chronically to artificially-cycled macaques (Hirst et al., 1992; Slayden et al., 1993, 2001a; Slayden and Brenner, 2003).

**Laboratory methods**

*Endometrial sampling:* The macaque uterus is anatomically similar to the human uterus, and consists of three parts, the fundus (dome-shaped top) the corpus (body) and the isthmus (neck), which leads into the cervix. In these studies we removed the oviducts, separated the uterus from the cervix at the isthmus and cut the uterus in half longitudinally from fundus to isthmus. After the first longitudinal cut, the uterine halves were again cut longitudinally to create equal quarters. From two of these quarters, cross-sections (2 mm thick) of the endometrium from lumen to myometrial border were cut for ICC and morphological study. The endometrium from the remaining two quarters of the uterus was blunt dissected from the myometrium with iris scissors and prepared for RNA isolation or receptor binding studies. Separation of the uterus into quarters, as described, facilitates the precise separation of endometrium from myometrium.

*Immunocytochemistry:* Samples of fresh tissue for immunocytochemistry (ICC) were microwave stabilized (Slayden et al., 1995) for 7 seconds in an Amana Radarrange Touchmatic microwave oven (Amana, Iowa) mounted in Tissue Tek II OCT (Miles Inc., Elkhart, IN) and frozen in liquid propane cooled by liquid nitrogen. Cryostat sections (5 μm) were thaw-mounted on Superfrost Plus (Fisher Scientific Pittsburgh, PA) slides, placed on wet ice at 5°C, and microwave irradiated again for 2 sec. ICC for steroid receptors and markers of cell proliferation was done as recently described (Slayden et al., 1998, 2001b). Briefly, microwave-treated sections were lightly fixed (0.2% picric acid, 2% paraformaldehyde in PBS) and the ICC was conducted with specific monoclonal or

![Artificial Menstrual Cycle](image)

**Fig. 1.** A timeline depicting the induction of artificial menstrual cycles with implants of E\(_2\) and P in ovariectomized rhesus macaques. These implants produce 60–100 pg E\(_2/\text{ml}\) and 5–6 ng P/ml.
polyclonal antibodies. In each case primary antibody was reacted with biotinylated second antibody and detected with an avidin-biotin peroxidase kit (Vector Laboratories, Burlingame, CA). The monoclonal antibodies used in the studies presented in this review included: anti-ER (1D-5; Biogenex, San Ramon CA), anti-AR (F-39; Biogenex), anti-PR (PR Ab-8; Neomarker Inc., Fremont CA) anti-Ki-67 antigen (Dako Corp. Carpinteria, CA); and anti Br(d)U (Cat# 691991). A polyclonal antibody to phosphorylated histone H3 (phospho H3, a marker of mitotic cells) was purchased from Upstate Biotechnology, Waltham MA (Cat. 06-570) (Brenner et al., 2003).

Representative tissue samples for morphological study were fixed in 2% glutaraldehyde and 3% paraformaldehyde, embedded in glycol methacrylate (GMA), sectioned (2 µm) and stained with Gill’s Hematoxylin. Photomicrographs were prepared from digital images captured with an Optronics DEI-750 CCD camera through Zeiss planapochromatic lenses. In several studies we assessed the abundance of proliferating cells in the endometrium of hormone-treated macaques by either counting mitotic cells, Ki-67 positive cells or Br(d)U labeled cells (Slayden et al., 1998; Slayden and Brenner, 2003). These counts were done either by manual counting by a trained observer who used an ocular micrometer grid to define microscope fields, or by computer assisted cell counting (Brenner et al., 2003). We further utilized computer image analysis with Image Proplus (Media Cybernetics Inc., USA) to quantify endometrial gland and arterial areas (Slayden et al., 2000). In each case cell counts and morphometric values were analyzed by ANOVA followed by Fisher LSD (Petersen, 1985).

**Effects of estradiol and progesterone on endometrial morphology**

In several reviews we described the morphological changes within the macaque endometrium during the menstrual cycle (Brenner et al., 1990, 1991; Brenner and Slayden, 1994a). In macaques, like women, E2 secreted by the ovary during the follicular (proliferative) phase stimulates cell proliferation in both the endometrial glands and stroma. The length of the proliferative phase in the natural cycle is normally 10–14 days but can be highly variable (Gilardi et al., 1997). After ovulation, rising P levels suppress mitotic activity in the glands of the functionalis, induce secretory changes in the endometrium (the secretory phase) and prepare the uterus for embryo implantation. If fertilization and implantation do not occur, the luteal phase ends after 12 to 14 days.

Four distinct zones have been characterized in the rhesus (Bartelmez, 1951) and human (Bartelmez, 1957) endometrium. Zone 1 is characterized as the luminal surface epithelium and an underlying band of stromal cells. Slightly deeper, Zone II contains glands that run perpendicular to the surface. Deeper still, Zone III contains glands that are branched, and the deepest zone, Zone IV is the basal layer that is adjacent to the myometrium, where the glands terminate. Secretory differentiation and menses occur in Zone I–III, and these zones combined are frequently termed the functionalis zone. In women, Zone IV, the basalis zone, is relatively unresponsive to cyclic hormonal changes, but in the macaque, the basalis proliferates during the luteal phase under the influence of P (Padykula et al., 1989; Okulicz et al., 1993; Brenner et al., 2003). Hodgen (Hodgen et al., 1983) first reported that a secretory endometrium and pregnancy could be established in ovariectomized monkeys treated with Silastic implants of estradiol (E2) and P in sequential fashion to recreate the pattern of serum E2 and P that occurs during the fertile menstrual cycle. In both the artificial and natural cycle the proliferative phase begins when menstruation ends and the glands and stroma begin a period of E2-dependent growth. After 14 days of E2 priming the upper layers of the functionalis zone display tubular glands (Fig. 2) with abundant mitotic cells (Fig. 2c). To detect and quantify endometrial cell proliferation we and others have used immunocytochemistry (ICC) for several proliferation-associated markers including Ki-67 (Gerdes et al., 1984; Gerdes et al., 1991; Slayden et al., 1993; Okulicz et al., 1993), phospho H3 (Brenner et al., 2003) (a marker of mitotic cells) and in vivo labeling with bromodeoxyuridine (Br(d)U) (Apte, 1990). Figure 3 shows representative sections stained with these markers. Counts of cells stained with these methods reveal that during the late proliferative phase, almost all of the glandular growth occurs in the mid and upper functionalis zones, with minimal proliferation in the basalis (Brenner et al., 2003). In contrast, during the early luteal phase, the basalis zone displays an intense period of cell proliferation (Fig. 3d) and mitotic figures are clearly evident in histological specimens (Fig. 2f). Stromal cell proliferation occurs throughout the endometrium. After E2 priming the glands in the upper zones also contain abundant apoptotic cells (Fig. 2c), suggesting that during the proliferative phase there is a balance of cell birth and cell death. New growth of small vessels in the upper functionalis zone appears to peak on Day 8 of the proliferative phase (Nayak and Brenner, 2002). In the proliferative phase, spiral arteries are found primarily in the deep zones.

During the luteal phase, P gradually suppresses cell proliferation in the glandular epithelium of the functionalis and induces secretory changes including hypertrophy and accumulation of glycogen. By day 14 of E2+P treatment the functionalis glands are dilated and the epithelium has a
Fig. 2. GMA-embedded, hematoxylin-stained sections showing the histological effects of E2 and E2+P on the endometrium of artificially cycled monkeys. a, b: Full thickness section of endometrium. A dark line has been drawn to shown myometrial border. Treatment with E2+P induces expansion of the endometrial stroma and saccula-
tion of the glands typical of the secretory phase.
c, d: Endometrial functionalis zone. In c, abundant mitotic figures (M arrows) were observed in the glands during E treatment whereas treatment with P blocked mitosis and induced secretory differ-
entiation (d). e, f: Endometrial basalis zone. No mitotic cells were observed after 14 days of E2 alone, whereas treatment with P stimulated mitotic activity in the basalis glands. g, h: Spiral arteries. 
E2+P treatment resulted in hypertrophy of the spi-
Steroid receptors in the primate endometrium

sacculated, appearance (Fig. 2b). By day 7 of E₂+P treatment the endometrial stroma becomes highly edematous and the spiral arterioles begin to enlarge. By day 14 of E₂+P the spiral arteries are strikingly hypertrophied compared to 14 days of E₂ alone (Fig. 2g, h). Spiral artery growth is associated with a striking P-dependent increase in cell proliferation in endothelial, smooth muscle and perivascular stromal cells. The macaque endometrium does not fully decidualize until pregnancy occurs or if P treatment is maintained for much longer than 14 days. Treatment for 5 months with E₂+P induces a highly decidualized endometrium with enlarged stromal cells, excessive amounts of extracellular matrix in the functionalis, enlarged spiral arteries and a greatly reduced number of glands (Fig. 4). Decidualization of the endometrial stroma and glandular atrophy has also been reported in macaques treated for 14 weeks with intrauterine devices that released the synthetic progestin levonorgestrel (Wadsworth et al., 1979).

**Fig. 3.** Immunostaining for Ki-67 antigen (a) Phospho H3 (b) and Br(d)U (c) in macaque endometrium during the artificial proliferative phase. Gl: gland. Dark stained cells are positive. Counts of phospho H3 stained cells (d) revealed that cell proliferation in the functionalis zone occurs primarily during E₂ (14dE=E₂ implant for 14 days) and ceases during P treatment (3dP, 7dP and 14dP=3, 7, and 14 days of E₂+P treatment in the secretory phase). In contrast, mitotic activity in the basalis zone is low after 14dE₂, increases during the early secretory phase (3dP and 7dP) and returns to baseline by 14 days of E₂+P.
In macaques, like women, the decline of luteal phase P at the end of the cycle induces menses and the upper third of the endometrium sloughs off as the next cycle begins (Corner, 1963; Rogers, 1999). In artificial cycles, during the first 48 h after P implant removal the endometrium shrinks, and by 72 h, the upper regions of the functionalis slough away as menstruation ensues. In both the human and macaque endometrium, several MMP enzymes that facilitate breakdown of the extracellular matrix increase dramatically in the functionalis zone before and during menses. Once the sloughing is complete, expression of these MMPs declines. The precise effects of the different MMPs on menstrual bleeding is not clearly established, but it is clear that many of them can breakdown collagen and other fibrillar components of the extracellular matrix (Marbaix et al., 1995; Marbaix et al., 1992) which leads to the disruption of tissue integrity associated with sloughing (Galant et al., 2000).
Effects of E₂ and P on steroid receptors

Two functional estrogen receptors ERα and ERβ, transcribed from different genes, occur in the endometrium and other tissues. The two ERs are regulated differently and show cell type-specific expression in the endometrium. During the cycle, ERα mRNA and protein increase under E₂ influence and decrease under P influence in the endometrium of women and nonhuman primates (Brenner et al., 1990). In macaques, at the end of the proliferative phase staining for ERα is strongly positive in the nuclei of glands and stroma in both the functionalis and basalis zones. P treatment during the artificial secretory phase suppresses ERα staining in the glands and stroma of the functionalis zone (compare Figure 5 a through d, but not in the glands and stroma of the basalis zone. In macaques, ERβ staining is evident in the glands and stroma but changes minimally during the cycle (not shown). However in women, glandular, but not stromal ERβ decreases somewhat during the late luteal phase of the menstrual cycle (Critchley et al., 2000). In both macaques and women, throughout the follicular and luteal phases, the vascular endothelium expresses only ERβ, not ERα, (Critchley et al., 2001). This means that E₂ could act on the vascular endothelium through ERβ during the entire cycle.

Two functional PRs (PR-A and PR-B) have also been identified. However, unlike the two ERs, both PR-A and PR-B are encoded by a single gene, and are differentially regulated by transcription of two distinct promoters and by translation at two distinct promoter sites. PR-B is a larger molecule than PR-A due to the presence of an additional 165 amino acids in the N-terminal domain, and these two isoforms are differentially expressed in the human

Fig. 5. Endometrium stained for ERα. Strong staining was observed in the glands and stroma of the functionalis (a) and basalis (c) after E₂ treatment. After treatment with E₂+P (b), P blocked E₂-upregulation of ERα in the glands and stroma of functionalis zone, whereas considerable ERα staining was maintained in the basalis zone (d). Gl: glands, S: stroma
Slayden and R. M. Brenner: endometrium (Mangal et al., 1997). As shown by SDS-PAGE and Western blotting between days 2 and 8 of the menstrual cycle, PR-B is almost undetectable and the A:B ratio is >10:1. From days 9 to 13, the ratio is about 5:1, and it is about 2:1 between days 14 and 16. Thereafter, PR-B dwindles rapidly and is virtually undetectable at the end of the cycle. Radioligand binding studies show that E₂ increases total PR binding in the macaque endometrium. Immunocytochemistry with antibodies that recognize both PR forms show that E₂ treatment increases PR nuclear staining in all cell types except vascular smooth muscle and endothelium. Figure 6 (a–d) shows staining for PR in the macaque endometrium. Strong staining is observed in both the glands and stroma during the proliferative phase. In the luteal phase or after cotreatment with P, glandular PR staining is strikingly decreased, but PR is retained in the functionalis stroma, basalis glands and basalis stroma. PR staining is strong in perivascular stromal cells, but there is no specific PR staining in either the endothelium or the smooth muscle cells of the endometrial vasculature (not shown). Therefore, any effects of P on the vascular endothelium are likely to be indirectly mediated by the perivascular stroma.

Androgen receptor (AR) is also present in the macaque and human endometrium (West et al., 1990; Adesanya et al., 1999; Slayden et al., 2001b). Under normal cyclic conditions, AR mRNA (Fig. 7) and protein (Fig. 8) are only expressed by endometrial stroma, not the glands or vascular endothelium. Like ERα and PR, E₂ increases and P decreases expression of endometrial AR (West et al., 1990; Slayden et al., 2001b).

Extensive changes in the expression and localization of

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Fig. 6. Endometrium stained for PR by ICC. After E₂ alone, strong PR staining was observed in the glands and stroma of both the functionalis (a) and basalis zones (c). Cotreatment with E₂+P suppressed glandular PR in the functionalis zone but not the basalis (compare b and d). PR staining was retained in the stroma after E₂+P treatment in both zones. Gl: glands, S: stroma.
ER and PR occur during the transition between the luteal and follicular phases (luteal-follicular transition, LFT). For instance, classic binding studies (West and Brenner, 1983) and ICC (McClellan et al., 1986) indicate that total ER levels are minimal at the end of the secretory phase of the cycle, and increase >3 fold by the end of the LFT, 4 days after P withdrawal in the presence of E2 and very strong ER staining can be detected in the glands and stroma after 14 days of E2 alone (Fig. 9). However, we recently found that the recovery of ER after P withdrawal does not wholly depend on E2. For example, throughout days 1–4 of the LFT, whether or not E2 is present, ER expression increases first in the endometrial stroma and then in the glandular epithelium. ER staining is readily detectable in the glandular epithelium by day 6 of the LFT and continues to be expressed on day 14 whether or not E2 is present (Fig. 9). However, only when E2 is present does the intensity of ER staining increase and strengthen throughout the proliferative phase (Fig. 9).

PR expression does, however, depend on E2. PR expression, which is initially low at the beginning of the LFT, strengthens in the stroma and becomes detectable in the glands by day 5 if E2 is present, but in the absence of E2, PR staining fades to nondetectable or barely detectable by days 6–14 (Fig. 10). In sum, PR, but not ER, requires E2 action during the LFT to recover from the suppression induced by P in the luteal phase. The basalis zone behaves differently, as neither ER nor PR are greatly suppressed by P action during the luteal phase.
PR blockade with progesterone antagonists

The effects of various regimens of mifepristone and some Schering PA compounds including ZK 137 316 and ZK 230 211 on the endometrium of rhesus macaques was recently reviewed (Brenner and Slayden, 1994b; Brenner et al., 2002). Acute administration of these compounds during the secretory phase blocks the action of P and induces menses similar to P withdrawal. When an effective, P-blocking dose of a potent PA like ZK 230 211 is administered daily throughout the artificial cycle, progestational differentiation of the endometrium is completely suppressed, but in addition, the effects of E\textsubscript{2} are also suppressed: the glandular epithelium becomes atrophied, the stroma becomes dense, mitotic activity is suppressed and endometrial thickness is greatly reduced (Fig. 11). These endometrial antiproliferative effects also occur when ovariectomized animals are treated only with E\textsubscript{2} plus PA (in the absence of P) indicating that the effect is “antiestrogenic”, even though PAs do not bind to the ER (Wolf et al., 1989). However, the antiestrogenic effect is limited to the endometrium, as it does not appear to occur in the oviduct or vagina (Slayden et al., 1998; Slayden et al., 2001a). In addition, PA treatment leads to stromal cell atrophy, stromal compaction and hyalinizing degeneration of the spiral arteries (Fig. 11i), all in the presence of physiologically adequate serum E\textsubscript{2} levels. Similar effects were found after long-term treatment of intact cycling macaques with low doses of RU 486 for 8 years (Grow et al., 1996). Also, in the endometrium of women treated chronically with low doses of RU 486, glandular mitosis was inhibited and stromal

**Fig. 8.** Endometrium stained for AR by ICC. Unlike ER and PR, AR was localized by ICC and ISH only in the stroma. Compared to E\textsubscript{2} treatment alone (a, c) co-treatment with E\textsubscript{2}+P (b, d) decreased stromal AR protein. The suppressive effect of P on AR expression was greatest in the functionalis (b). Gl: glands, S: stroma
compaction was induced (Baird et al., 2003). Rodents do not show the endometrial antiproliferative effects of PA. Rather, PA treatment of cycling rats induces unopposed E$_2$ effects, marked by hyperplasia of the uterine luminal epithelium (Chwalisz et al., 2000).

Paradoxically, treatment with PAs induced striking increases in endometrial ER$\alpha$, PR and AR (Fig. 12). AR in particular was elevated in both the endometrial stroma and in the glandular epithelium of the functionalis zone. The mechanism through which PA suppresses E$_2$ effects on cell proliferation has not been fully explained (Slayden et al., 1998, 1993, 2001a; Slayden and Brenner, 1994). Hodgen’s laboratory reported that RU 486 treatment resulted in excessively elevated ER, and they suggested that elevated

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**Fig. 9.** ER$\alpha$ staining in the endometrium during the LFT, and after both E$_2$+P withdrawal. ER expression is almost undetectable at the end of the luteal phase (d0). After P withdrawal with E$_2$ maintained in the LFT, ER$\alpha$ increased first in stromal nuclei (day 1–4) and then in the glandular epithelium (d4–6 and 14). After withdrawal of both E$_2$ and P (No E$_2$), the pattern of recovery of ER$\alpha$ expression was similar to that seen in the LFT, but this intensity of ER$\alpha$ staining was notably reduced.
ER could produce a super-estrogenized state (Neulen et al., 1990, 1996). Since superphysiological doses of estrogen are reported to be inhibitory to endometrial growth (Neulen et al., 1987) then overexpression of ER could result in a similar antiproliferative action. However, as mentioned above, PA treatment also elevates endometrial AR. Because androgens are known to inhibit estrogen-dependent endometrial growth, elevations in AR induced by PA could allow endogenous androgens to suppress proliferation. In support of this hypothesis, we found that treatment
Fig. 11. GMA-embedded, hematoxylin-stained sections of endometrium comparing the histological effects of $E_2 + P$, $E_2$ alone or $E_2 + P + ZK$ 230 211 (Schering PA). In a–c, a black line indicates the endometrial (Endo)-myometrial (Myo) border (Bar shows scale). Treatment with ZK 230 211 blocked the effects of P and suppressed endometrial thickness to levels thinner than seen in animals treated with $E_2$ alone for 28 days. This reduction in endometrial thickness was associated with an increase in stromal compaction and a lack of mitotic cells (compare e and f). Gl: glands, S: stroma, M: mitotic cell. Treatment with $E_2 + P + ZK$ 230 211 also resulted in hyalinizing degeneration of the spiral arteries (compare g–i, see arrow in i). High power photos were made at $250 \times$ original magnification (Bar shows scale).
with flutamide, an androgen receptor antagonist, prevented the endometrial antiproliferative effect in animals treated with E2 and PA (Brenner et al., 2002; Slayden and Brenner, 2003). Additional research on the role of AR as a mediator of endometrial PA effects is needed.

As noted, PAs can induce degeneration of the spiral arteries (Slayden et al., 1998, 2001a). These vessels, which are unique to the primate endometrium, are primary targets for PA action even though the endothelium and smooth muscle of the arteries lack PR. Only the perivascular stromal cells express PR and AR and these may be the cells most affected by PAs. The damage to the spiral arteries, which includes narrowed lumens and hyalinized walls, probably leads to reduced vascular perfusion which could play an important role in the endometrial antiproliferative effect (Chwalisz et al., 2000).

Fig. 12. ERα, PR and AR staining in rhesus macaque endometrium from animals treated with E2+P compared to E2+P+ZK 230 211. In animals treated with E2+P, ER (a), PR (c), and AR (e) expression was minimal. Co-treatment with E2+P+ZK 230 211 resulted in a striking increase in ERα (b), PR (d) and AR (e) staining. In ZK 230 211 treated animals AR was increased in both the stroma (S) and the glands (Gl) (compare e and f).
**Conclusion**

In the primate endometrium, ER, PR and AR undergo dynamic changes during the menstrual cycle, and there are striking zonal and cellular differences in receptor regulation and hormonal responsiveness. During the proliferative phase, under E_2_ treatment, ER is expressed in glands and stroma throughout the functionalis and basalis, but E_2_ treatment only stimulates mitotic activity in the functionalis. It is therefore likely that specific factors required for estrogen-driven mitosis are lacking from the basalis. During the luteal phase, in macaques, P inhibits mitosis in the functionalis but stimulates proliferation in the basalis. Therefore, progestin-dependent mitosis requires factors unique to the basalis. In the functionalis, P suppresses ER in both the glands and stroma, but suppresses PR only in the glands. Therefore the progestational effects of P are probably mediated by stromal PR. P stimulates spiral artery growth by inducing mitosis in the smooth muscle and endothelial cells of the vessels, but PR is only expressed by the perivascular stromal cells. It is likely therefore that progestin-dependent spiral artery growth is mediated by the perivascular stroma. Unfortunately, the intracellular and/or paracrine mechanisms that underlie these various effects are not yet understood at a molecular level. Current in vitro models do not faithfully preserve the normal in vivo relationships among endometrial cell types in the different endometrial zones. Progress in understanding these interactions will be slow until more refined in vitro systems that preserve these relationships are developed.

The paradoxical ability of PA to both block P action and have an antiestrogenic effect on endometrial proliferation occurs uniquely in women and nonhuman primates. Rodents do not show these effects. These compounds are extremely valuable for control of endometriosis and bleeding disorders, and their endometrial antiproliferative effects protect the endometrium against the cancer potential of unopposed E_2_ action. In the future, development of new PAs and SPRMS to improve women’s health will require both the increased use of nonhuman primates and a deeper understanding of stromal-epithelial interactions and the factors that regulate zonal differences in the primate endometrium.

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