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Localization and regulation of estrogen and progestin receptors in the Macaque oviduct

Localización y regulación de receptores de estrógenos y progestinas en el oviducto de macaca

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In non-human primates, the oviduct undergoes dramatic, hormonally regulated changes in morphological structure. In spayed animals, the epithelium is undifferentiated, and estrogen treatment can stimulate differentiation to a fully ciliated and secretory state. This state can be maintained indefinitely by estrogen, but if progestin treatment is initiated the epithelium will atrophy, dedifferentiate, and remain so as long as combined progestin-estrogen treatment is continued. Oviductal estrogen receptors increase in number during estrogen treatment and decrease during combined progestin-estrogen treatment. Consequently we have concluded that progestins act as estrogen antagonists by suppressing estrogen receptors below physiologically effective levels. However, our immunocytochemical studies show that in estrogen-treated animals, estrogen and progestin receptors are present only in secretory cells, stromal cells and smooth muscle cells, not in ciliated cells. This suggests that the effects of estrogens and progestins on ciliogenesis must be mediated indirectly, through other cell types that contain both receptors. We suggest that the cell type directly involved in mediating these effects is the stromal cell, because after combined progestin-estrogen treatment, only stromal cells retain progestin receptors. Consequently only stromal cells can directly mediate the sustained antagonistic effects of progestin treatment. We propose a model of steroid hormone action in the oviduct in which estrogens and progestins act directly on oviductal stromal cells to regulate the secretion of various growth factors or other mediators which are the proximal agents that regulate epithelial cell differentiation in the primate oviduct.

INTRODUCTION

An extensive literature indicates that the primate oviduct is a target organ for the ovarian steroids (1-3). Estrogens stimulate growth of all the cellular components, and progestins inhibit this effect. These events are particularly dramatic in the oviductal epithelium of macaques and other non-human primates. In these species, there are natural cycles of differentiation and dedifferentiation (Fig. 1) which can be mimicked in spayed animals through appropriate administration of estradiol (E_2) and progesterone (P). For example, in rhesus monkeys during the two weeks after ovariectomy, the oviductal epithelium

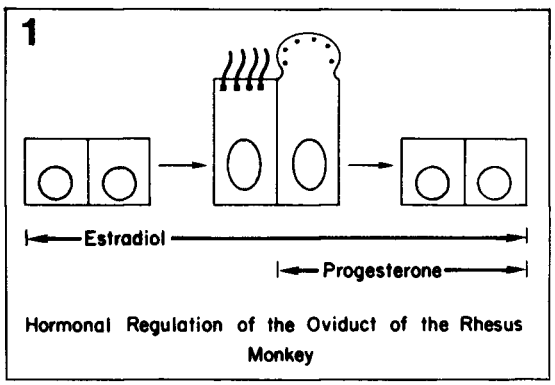

Fig. 1: A diagram depicting the effects of E_2 and P on the oviductal epithelium. During either natural on hormonally manipulated cycles, E_2 stimulated differentiation to a ciliated-secretory state: P treatment suppresses the effects of E_2.

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dedifferentiates into a low cuboidal, undifferentiated epithelium that lacks ciliated and secretory cells (Fig. 2). In such animals, a two week treatment with E_2 can induce a fully differentiated, ciliated and secretory epithelium which will be maintained as long as the E_2 is continued (Fig. 3). If P treatment is begun after two weeks of E_2 treatment, the oviductal epithelium will undergo a dramatic loss of ciliation, and secretory activity will cease even though E_2 treatment is continued. If the P treatment is stopped after two weeks and the E_2 continued, the epithelium will redifferentiate to a ciliated and secretory state.

Similar cycles of ciliation and secretion occur during the natural menstrual cycle of several primates; ciliogenesis and increased secretion occur during the follicular phase when E_2 is elevated, and deciliation and decreased secretion occur during the luteal phase when P rises, even though E_2 levels remain substantial. In all primate species, these phenomena are more dramatic in the fimbriae and ampullae and least in the isthmic region of the oviduct. In women, similar hormonally regulated oviductal cycles occur, but a smaller percentage of the cellular population undergoes complete ciliation-deciliation cycles (4). The human tube is at its most dedifferentiated state during late pregnancy because of the long term suppressive effects of P (5).

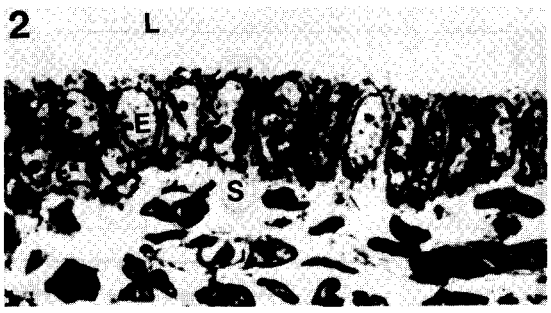


Fig. 2: Oviductal epithelium (fimbriae) in a state of full regression at the end of the menstrual cycle. A similar state can be induced by ovariectomy or by sequential E_2-P treatment. The epithelium is atrophied, deciliated and nonsecretory. No basal bodies, cilia or secretory granules are evident. Original magnification, 630X.

Abbreviations: B=basal bodies, C=Ciliated cells, E=epithelium, L=lumen, S=Stroma, SC=secretory cell.

Fig. 3: Oviductal epithelium (fimbriae) in the fully ciliated-secretory state in the late follicular phase. A similar state can be induced by 10-14 days of E_2 treatment of a spayed animal. Note the basal bodies at the base of each cilium in the ciliated cells. The tips of the secretory cells are filled with glycoprotein-rich granules and they bulge into the lumen beyond the tips of the cilia. Original magnification, 630X.

We hypothesized that such dramatic steroid effects on cell differentiation involved action at the genomic level, mediated by steroid receptors, and our research has been aimed at evaluating the role of receptor regulation in these effects. We have measured estrogen receptor (ER) and progestin receptor (PR) levels in the oviduct through binding and immunoassays during the natural cycle and under various hormonal conditions, and we have localized these receptors to specific cell types by immunocytochemical techniques with monoclonal antireceptor antibodies. The following is a review of these studies.

METHODS

Animals and Hormonal Treatments: We have used rhesus (*Macaca mulata*) and cynomolgus (*Macaca fascicularis*) macaques and found no substantial species differences. Some animals were laparotomized and the reproductive tracts were removed at different times during the menstrual cycle (6). Other animals were spayed and then treated by either injecting E_2 alone or with P intramuscularly (7) or by implanting Silastic capsules filled with crystalline steroids in subcutaneous sites (8, 9). Fim-

briae, ampullae and isthmus were sperated and some pieces were either frozen for immunocytochemistry (ICC) or fixed for histology. The remaining tissue was homogenized for ER and PR assays.

Antibodies: The monoclonal antibody H222 was originally prepared against purified ER obtained from a human breast cancer cell line (MCF-7) by Greene *et al.* (10). The antiPR antibody (B39) had been prepared against human PR (11). These antibodies cross-react strongly with the rhesus monkey ER and PR (12). A monoclonal antibody of the same immunoglobulin subclass (IgG_{2a}), anti-Timothy grass pollen (AT) was used as a nonspecific control antibody, courtesy of Dr. Arthur Malley, Oregon Regional Primate Research Center (Beaverton, OR).

Receptor Assays: Nuclear and cytosolic ER were analyzed with binding assays as previously described (8). We have also used sucrose gradients to quantify the level of cytosolic ER (7), and developed a quantitative gradient shift assay (GSA) for activated nuclear ER which uses ^3H-E_2 and the anti-ER monoclonal antibody H222 to effect a physical separation on a high salt sucrose gradient of the ^3H-E_2-ER: anti-ER complex from nonspecific binders. The specificity and sensitivity of this GSA have been reported (13 14). Progesterone receptor was measured with a binding assay as previously described (15).

Immunocytochemistry (ICC): Receptors were localized with the immunocytochemical method previously described (9) and subsequently modified (16). Frozen blocks were sectioned (5 μm) on a cryostat, sections were mounted on gelatin coated glass slides, lightly fixed, washed, and incubated overnight at 4°C with either H222 (10 μg/ml), B39 (1 μg/ml), or AT (10 μg/ml) as a control for nonspecific staining. The primary antibodies were detected with an avidin-biotin peroxidase kit from Vector Laboratories. In our most recent work we have placed the freshly cut frozen sections in absolute acetone for 48 h at −80°C to freeze-substitute them before fixation, and included 1.5% polyvinylpyrrolidone in the fixative, wash and incubation solutions. We have found these steps improve cell morphology and retain more protein in the frozen sections (16).

Morphology: Samples of tissues were fixed in a glutaraldehyde-formaldehyde mixture, and embedded in Araldite for light and/or electron microscopy (2).

RESULTS

Artificial cycles

In our first study (7), two consecutive artificial menstrual cycles were produced in nine spayed monkeys by daily injection of a sequential E_2-P regimen as follows: Cycle I: E_2 benzoate (20 μg/day for 10 days) then P (1.5 μg/day) and E_2 benzoate (20 μg/day) for 19 days. Cycle II: E_2 benzoate (20 μg/day for 14 days) then P (1.5 mg/day) and E_2 benzoate (20 μg/day) for 21 days. One oviduct was removed during Cycle I and the remaining oviduct was removed during Cycle II. In addition, other animals were treated similarly to produce two cycles and were sampled at three critical times: 1st, after 14 days of E_2 alone; 2nd, after a subsequent 21 days of E_2 plus P; and 3rd, after 14 more days of E_2 alone.

The results showed that ciliogenesis and the development of secretory activity occurred when E_2 acted alone, and atrophy, deciliation and cessation of secretion took place when P was administered, even though E_2 levels remained constant at approximately 200 pg/ml. Reciliation and secretion occurred after the P treatment was stopped. This was the first demonstration that P could antagonize the effects of E_2 on oviductal epithelial differentiation in primates.

In this work, we used sucrose gradient assays to quantify the amount of cytosolic ER. We found that E_2 increased the level of ER, that sequential P administration greatly suppressed the cytosolic ER, and that when P treatment ceased, ER levels recovered. Overall, the waxing and waning of the oviductal epithelium, as measured by the height of the ciliated cells and the percent of ciliation, was mirrored by the increase and decrease in the level of

TABLE 1

The effects of estradiol and progesterone on ciliation, cell height and cytosolic ER in the oviducts of spayed rhesus monkeys[a]

Time Sampled	Estradiol (pg/ml)	Progesterone (ng/ml)	Cytosolic ER[d]	Percent ciliation	Cell height (μm)
E-14-I[b]	160 ± 12	1.5 ± 0.2	30 ± 3	63 ± 1.4	25 ± 2.4
P-21[b]	93 ± 19	10.3 ± 0.8	No peak	14 ± 3.0[d]	16 ± 0.8[d]
E-14-II[b]	259 ± 54	0.6 ± 0.5	25.7 ± 7	57 ± 2.0	31 ± 1.1

[a] All data are expressed as means ± standard errors. The table is based on data in reference N^{o} 7.

[b] E-14-I: estradiol benzoate, 20 μg/day for 14 days (n=3).
P-21: Same as above plus 21 additional days of progesterone (1.5 mg/day) and estradiol benzoate (20 μg/day) (n=4).
E-14-II: Same as above plus 14 additional days of estradiol benzoate (20 μg/day) (n=3).

[c] DPM of ^{3}H estradiol in 8S peak/μg DNA.

[d] Significantly different from E-14-I or II, $p < 0.001$.

cytosolic ER (Table 1). Additionally, measurements of the actual tissue concentrations of E_2 showed that there was significantly less E_2 bound to the oviductal tissues when P was administered even though serum E_2 levels remained constant (7, 17). Consequently, we concluded that the suppressive effects of P on E_2-dependent oviductal differentiation were due to the suppression of cytosolic ER to levels so low that the amount of E_2 bound in the oviduct was below the threshold for E_2 action.

In our more recent studies, we controlled hormone levels by implanting steroid-filled, silastic capsules into subcutaneous sites. Such capsules release relatively constant levels of steroids over long periods, and serum hormone levels are easily manipulated by inserting or removing the capsules. In one study (18) oviducts were removed from spayed cynomolgus macaques that had been either untreated, treated with a 2.0 cm E_2 implant for two weeks, or treated first with a 2.0 cm E_2 implant for 2 weeks and then with an additional 6.0 cm P implant for 2 more weeks.

As before, ciliation/secretion was induced by the E_2 treatment and suppressed by the sequential E_2-P treatment. In this work we used binding and exchange assays to measure both cytosolic and nuclear ER, and expressed them per mg DNA. Total ER was the calculated sum of the nuclear and cytosolic values. The binding assays (Fig. 4, tabular data) showed that ER levels (total, cytosolic and nuclear) were elevated by the E_2 treatment and suppressed by the P treatment. These data expanded our previous results to include nuclear and total ER levels. However, our conclusion remained the same, namely that the mechanism by which P antagonized the effects of E_2 on the oviduct was by suppressing the level of oviductal ER below the threshold required to mediate E_2 action.

The binding assays indicated that in untreated spayed animals most of the receptor was in the cytosolic fraction, but the ICC studies indicated that all of the specific staining was in the nuclei of stromal and epithelial cells (Fig. 4A) as well as the nuclei of smooth muscle cells (not shown). After E_2 treatment more ER was found in both the cytosolic and nuclear fractions and there was an increase in both the intensity of staining of cell nuclei and the number of positively stained cells (Fig. 4B). Sequential P treatment lowered cytosolic and nuclear ER in the oviduct significantly below the amount present in E_2 treated animals, and lowered the cytosolic, but not the nuclear levels significantly below the levels found in spayed animals. After such sequential P treatment,

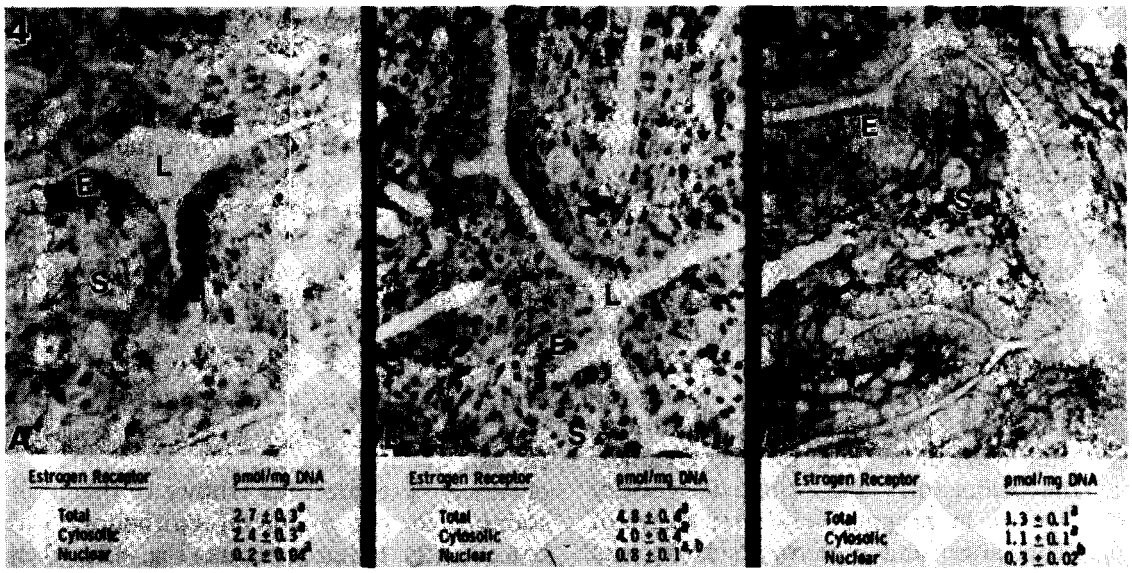


Fig. 4: A composite diagram that shows the effects of three treatments on oviductal estrogen receptor (ER). Panel A = spayed, untreated; Panel B = spayed, treated with E_2 for 14 days; Panel C = spayed, treated with E_2 for 14 days and E_2 plus P for additional 14 days. The top part of each panel is a photomicrograph of oviductal epithelium (ampulla) stained for ER. The bottom part of each panel is a tabulation of the mean amount of ER measured by binding assays in oviducts from the same treatment groups. Values with the same superscript letter are significantly different between treatment groups ($p < 0.01$). Note that in spayed animals, a small number of stromal and epithelial cells are positive for ER. After E_2 treatment this number increases, and after P treatment, the number of ER-positive cells in the stroma and the epithelium decreases to less than in spayed animals. The staining evident in the stroma is nonspecific background staining that was also evident in anti-Timothy grass pollen stained controls. Total receptor levels follow the same pattern. Note that all positive staining is confined to cell nuclei. Original magnification: A = 630X, B = 400X, C = 630X.

staining for ER was no longer detectable in stromal or epithelial cell nuclei (Fig. 4C) and was greatly suppressed in smooth muscle nuclei. Thus the changes in the staining for ER detected by ICC in cell nuclei paralleled the changes in the total amount of ER measured by binding assays in the combined cytosolic and nuclear fractions. These findings support the current view (19) that the ER is a nuclear protein which is synthesized in the cytoplasm and rapidly enters the nucleus in spayed as well as in hormone-treated animals.

Therefore, in target cells of spayed animals, all of the ER is present and can be stained in the cell nuclei in frozen sections, but a large amount may enter the cytosolic fraction when the tissue is homogenized. In E_2 treated animals a part of this population of ER binds E_2 and develops a higher affinity for chromatin. Consequently, when cells from E_2-treated animals are ruptured in dilute buffers, some ER remains in the nuclear fraction, and some enters the cytosolic fraction; the ratio between these depends on the number of ER molecules with high affinity for chromatin. ER is always detected in cell nuclei in frozen sections by ICC regardless of the hormonal state of the animal because the protein is trapped there by the freezing technique. Also, the intensity of staining as well as the number of positively stained cells is directly proportional to the total amount of ER detected in nuclear and cytosolic fractions by binding assays. Similar conclusions as to the nuclear localization of the ER and PR have been reached by many laboratories who have compared immunocytochemical and biochemical studies (20-22).

The natural cycle

We have also measured cyclic changes in oviductal ER during the course of the natural menstrual cycle (23). In that study we removed the ovaries, uteri and oviducts from 27 cynomolgus monkeys at intervals

during the cycle, and correlated oviductal histology with ovarian and endometrial histology and serum E_2 and P levels. Consequently we were able to define the changes that occur during the natural oviductal cycle very precisely. We found that oviducts could be classified into the following stages, depending on the degree to which the cycle had progressed: preciliogenic-ciliogenic (menses, early follicular), ciliogenic-ciliated (midfollicular), ciliated-ciliogenic (late follicular), ciliated-secretory (periovulatory), early regression (early luteal), late regression (midluteal), and full regression (late luteal). Oviducts in the ciliogenic and ciliated-secretory stages were in progressively later stages of cilia formation and secretory activity and were from animals in which E_2 was acting unopposed by P; oviducts in the various stages of regression were in progressively later stages of atrophy, deciliation and cessation of secretion and were from animals with elevated serum P levels. Secretory cells became maximally tall and filled with granules during the follicular phase; they atrophied and became degranulated during the luteal phase. Cytosolic and nuclear ER levels were significantly higher in oviducts that were in various stages of ciliogenesis than in those that were in stages of late and full regression (Table 2). Consequently we concluded that in the natural cycle, as in hormonally treated animals, P could antagonize the effects of E_2 on oviductal differentiation by suppressing ER levels below the threshold required to facilitate E_2 action.

Immunocytochemical heterogeneity

In all of our studies, the immunocytochemical preparations showed that ER staining in the oviductal epithelium was consistently heterogeneous (Fig. 4B). That is, not all epithelial cells were positive for ER. Close examination of large numbers of E_2-treated oviducts showed that the stromal cell population and the secretory cell population were consistently positive for ER but that the ciliated cell population was consistently ER-negative (Figs. 5 and 6). After P treatment, it was the stromal and secretory cells that lost all detectable staining for ER in parallel with

TABLE 2

Comparison of serum steroids with oviduct estrogen receptor levels in the early follicular and late luteal phases of the natural menstrual cycle[a]

	Stage of the menstrual cycle		
	Late luteal (n=9)	Early follicular (n=9)	P
Serum steroids:			
E_2 (pg/ml ± SEM)	70 ± 12	98 ± 16	NS
P (ng/ml ± SEM)	3.6 ± 1.4	0.38 ± 0.11	< 0.05
Estrogen receptor levels:			
Cytosol (dpm/μg protein ± SEM)	15 ± 1	29 ± 2	< 0.001
Nuclear (dpm/μg DNA ± SEM)	32 ± 3	68 ± 7	< 0.001
Morphological stage:	Late and full regression	Preciliogenic-ciliogenic Ciliogenic-ciliated	

[a] In this tabulation, data on nine animals in the late luteal phase (last two oviductal stages) are specifically compared with data on nine animals in the early follicular phase (first three oviductal stages). The table is based on data in reference N° 23. The two major columns in the table under the heading "Stage of the menstrual cycle" compare various data from the late luteal and early follicular phases. These data are serum estradiol (E_2), serum progesterone (P), nuclear and cytosolic estrogen receptor, and morphological stage. For each parameter, a Student's t test was used to determine whether the differences between the follicular and luteal phases were significant. The P values for each comparison are presented in the last column on the right side of the table.

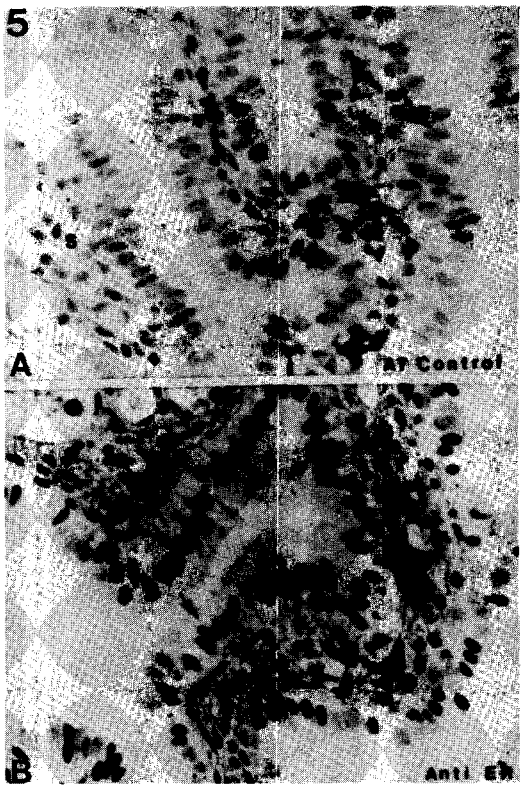


Fig. 5: Estrogen receptor (ER) staining in oviductal epithelium (ampulla) in fully ciliated-secretory state. Panel A: stained with AT, a control antibody and hematoxylin to show nuclei. Panel B: stained with H222 for ER, and hematoxylin for nuclei. Comparison of the two panels shows that not all nuclei in the epithelium are positive for ER. This heterogeneity is highly consistent, and careful examination shows that the ER-positive nuclei are in stromal and secretory cells, not ciliated cells. This heterogeneity can also be seen in Fig. 4B. The ciliated cells are all ER-negative; this is best seen in Fig. 6. Original magnification: 400X. The arrows point to ER-positive secretory cells.

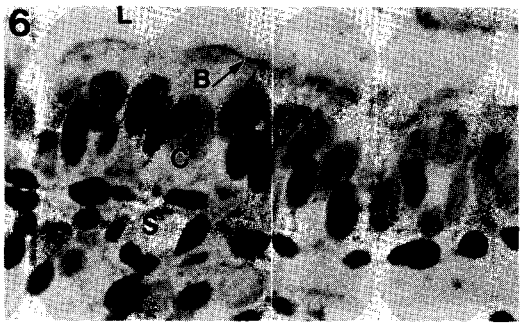


Fig. 6: Estrogen receptor (ER) staining in oviductal epithelium (fimbriae) in the ciliated-secretory state. This micrograph also shows that the ciliated cells are ER-negative and the stromal and secretory cells are ER-positive. The cilia are difficult to discern in photomicrographs of frozen sections but the characteristic basal bodies are readily apparent. Original magnification: 900X.

the suppression in total ER measured by binding and exchange assays (Fig. 4C). This suppressive effect of P on stromal and secretory cell ER occurred within 24 hours of P treatment (18). Fig. 7 shows a series of immunocytochemical preparations of oviducts from animals treated for 14 days with E_2 (Fig. 7A) and then sampled 1 h (Fig. 7B), 3 h (Fig. 7C) and 12-24 h (Fig. 7D) after onset of P treatment, with E_2 treatment maintained throughout. The first clearly detectable evidence of suppression of ER staining occurred between 12-24 h. Thus one day after P treatment began ER was suppressed to very low levels in stromal and secretory cells. The first evidence of deciliation and suppression of secretion was usually evident within 48-72 hours of the onset of P treatment, thus the decline in ER preceded the biological effects of P which supports the role of this decline as a causal factor in P antagonism of E_2 action.

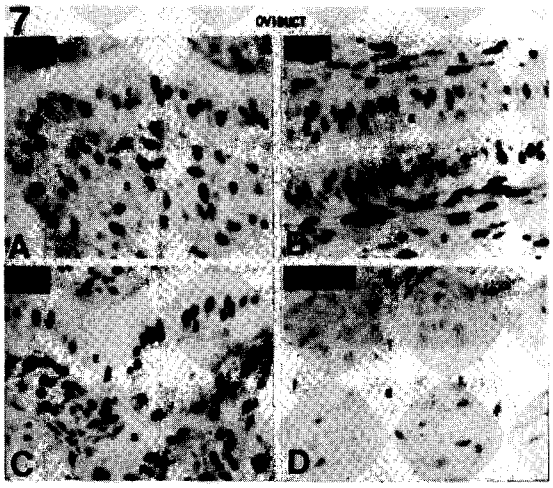
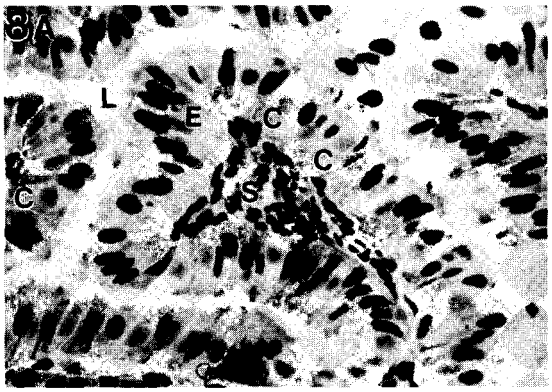


Fig. 7: A composite figure which shows the effect of progesterone (P) on oviductal (fimbriae) estrogen receptor (ER) at 0 h (A), 1 h (B), 3 h (C) and 12-24 h (D) after implantation of a P capsule into animals that had been treated with an E_2 implant for the previous 14 days. Staining for ER was not clearly reduced until 12-24 hours after P treatment, and it was suppressed in stromal and secretory cells at approximately the same rate. Binding assays for cytosolic and nuclear ER were also done at the same time intervals (see reference 18). Nuclear ER was significantly lowered within 1-3 h, but cytosolic and total ER were not significantly lowered until 12-24 h. This supports the view that the amount of nuclear staining revealed by this immunocytochemical methods is proportional to the total amount measured in binding assays, not just the amount measured in the nuclear fraction.

Immunocytochemistry also showed that in spayed animals, PR staining was minimal. In the E_2 treated animals, there was a dramatic increase in nuclear staining for PR in the smooth muscle, the stromal and the secretory cells, but again, the ciliated epithelial cells were negative for PR just as they were for ER (Fig. 8A, B). In the animals treated with the sequential E_2-P regimen, staining for PR was completely suppressed in the epithelium to nondetectable levels but remained readily detectable in the stroma (Fig. 9A, B). As a check on the sensitivity of the ICC technique, we prepared very thick frozen sections (30 μm, in contrast to the usual thickness of 5 μm) to increase the concentration of the PR antigen in the sections. When we performed ICC on these thicker sections, the epithelium remained negative for PR while the stromal cells stained very darkly for PR (Fig. 10). This confirmed that the



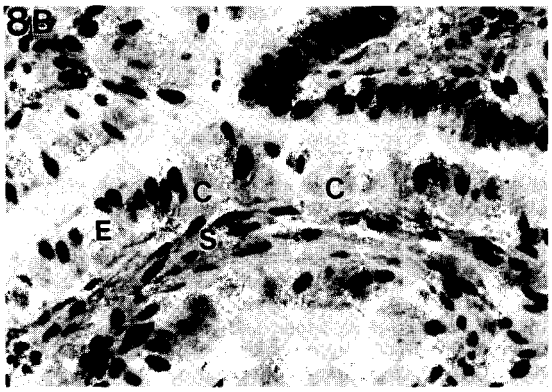
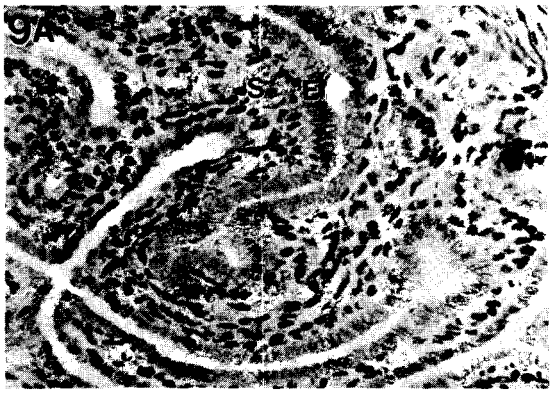


Fig. 8: Two examples, A and B, of oviductal epithelium (ampulla) in the ciliated-secretory state stained for progesterone receptor (PR). As with estrogen receptor (ER), only the stromal and secretory cells are PR-positive. Original magnification: 630X.



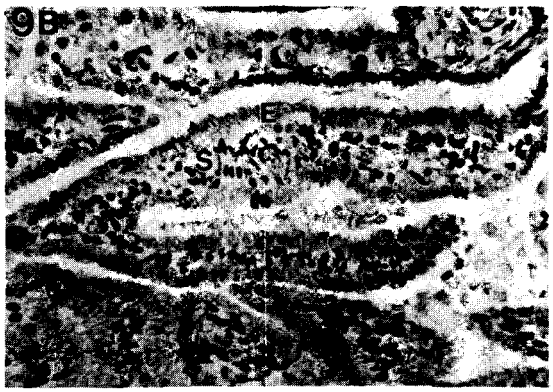


Fig. 9: Two examples, A and B, of progesterone receptor (PR) staining of oviductal epithelium in the fully regressed state induced by sequential E_2-P treatment. PR remains easily detectable in the stroma but is undetectable in the majority of epithelial cells. Original magnification: 630X.

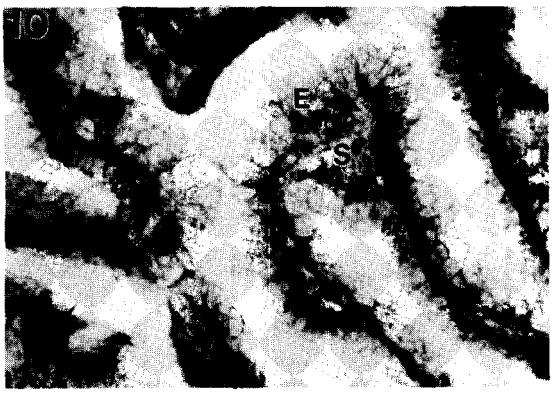


Fig. 10: Progesterone receptor (PR) staining in a thick section (30 μm) of oviductal epithelium (fimbriae) in the fully regressed state induced by sequential E_2-P treatment. In such a thick section, the antigen concentration would be increased 5-6 fold over the amount normally in a 5 μm section. Yet PR remains undetectable in the epithelium while it overstains in the stroma. Original magnification: 630X.

absence of staining for PR in the epithelium in routine sections was not due to insensitivity of the technique.

Measurements of PR by binding assays confirmed that sequential P treatment downregulated total PR. For example (15), in E_2 treated animals the levels of PR in pmol/mg DNA were as follows: cytosolic, 12.8 ± 2.2; nuclear, 1.95 ± 0.34; total, 14.8 ± 2.6 (N=6). In sequential E_2-P treated animals, PR levels were significantly reduced ($P<0.001$) for each category as follows: cytosolic, 0.57 ± 0.20; nuclear, 0.46 ± 0.14; total, 1.03 ± 0.31 (N=8). This suppression by P of its own receptor has been found in various P target organs in various species (24). Our immunocytochemical results suggest, however, that the suppression of PR is much more complete in the epithelium than in the stroma (and the smooth muscle), and that it is the amount of PR remaining in the stromal cells that mediates the continuing effect of P on the oviductal epithelium.

DISCUSSION

A new model of hormone action in the oviductal epithelium

These results have raised numerous questions concerning the mode of action of E_2 and P at the cellular level. First, ER and PR are both lacking from ciliated cells yet E_2 stimulates ciliogenesis and P inhibits it. How can E_2 and P affect cells that lack ER and PR? Second, P suppresses its own receptor, yet as long as its injection is continued, it will continue to act as an estrogen antagonist in tissues with greatly reduced levels of PR. How does P maintain its effects while suppressing its own receptor?

We suggest that many of the effects of E_2 and P on the state of differentiation of the oviductal epithelium are mediated indirectly, through soluble growth factors (or unknown mediators) secreted by stromal cells. Stromal cells are separated from the epithelium by a definite basement membrane, so it is unlikely that such effects are mediated by direct junctional contact between stromal and epithelial cells. In earlier electron microscopic studies, we never observed such contacts (2), and to our knowledge, none have been reported.

Accordingly, in this model of hormone action (see Figs. 11, 12), E_2 would act directly at the genomic level through

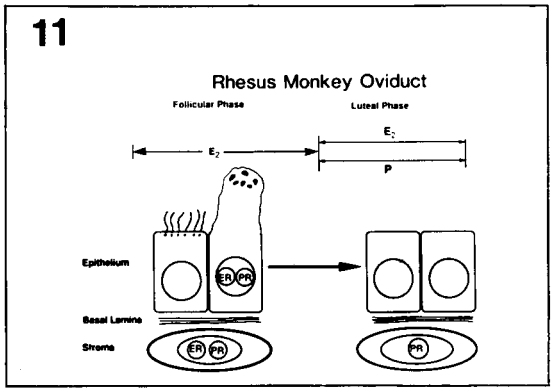


Fig. 11: A summary diagram which indicates that although E_2 induces the ciliated-secretory state, estrogen receptor (ER) is only present in stromal and secretory cells. E_2 also induces progesterone receptor (PR), but only in stromal and secretory cells. When P acts, it suppresses the effects of E_2 on ciliated and secretory cells, even though PR is only present in stromal and secretory cells when P action begins. After P action is completed, only stromal cells maintain PR, so these cells must be responsible for the maintenance of the P effect.

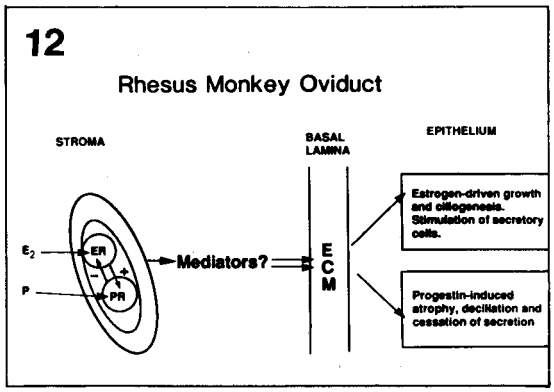


Fig. 12: A summary diagram which indicates a new model of steroid hormone action in the oviductal epithelium. E_2 and P act through their respective receptors (ER and PR) in stromal cells to regulate the secretion of mediators, probably growth factors. It is these mediators that directly influence the state of differentiation of the oviductal epithelium. P acts through PR in stromal cells to lower stromal ER and this leads to lowered secretion of the E_2-dependent mediators. Withdrawal of these mediators leads to oviductal dedifferentiation, as if E_2 itself had been withdrawn.

the ER in stromal cells to induce the secretion of growth factors that would diffuse across the basal lamina and stimulate growth and differentiation in the ER-negative ciliogenic epithelial cells. ER is present in secretory cells, so E_2 could act directly at the genomic level in secretory cells to stimulate secretion of specific oviductal glycoproteins. During E_2 actions, PR is induced in stromal and secretory cells only, not ciliated cella. Consequently when P levels rise, P can act directly in PR-positive stromal and secretory cells to suppress ER in both cell types and thus inhibit the secretion of E_2 dependent oviductal glycoproteins from the secretory cells and growth factors from the stromal cells. In addition, P might stimulate the release of specific growth of differentiation inhibitors from stromal cells. The effects are the same as observed after ovariectomy or E_2 withdrawal, namely atrophy, deciliation and cessation of secretion.

As P continues to act it eventually down-regulates PR completely in the secretory cells but only partially in the stromal cells. Because the suppressive effects of P on E_2 action in the epithelium continue indefinitely until P treatment is stopped, and because only stromal cells retain PR during sustained P action, we conclude that the sustained antagonism of the epithelium by P is mediated indirectly by the stroma. Specifically, we suggest it is the suppression of ER by P in stromal cells that is responsible for the inability of E_2 to act in the presence of E_2 and P. Once P treatment is stopped, ER can recover spontaneously (as it does in spayed animals) to levels adequate to mediate E_2 actions in stromal and secretory cells. These cells can then increase their ER and PR levels in response to E_2, the stromal cells can secrete their specific mediators, and the cycle of differentiation of the oviductal epithelium can begin anew.

What are these presumed stromal mediators? Are they well known growth factors such as epidermal growth factor or insulin-like growth factor or are they unknown, oviduct-specific peptides? At the moment we cannot answer such questions. However, our laboratory is now pursuing these and other possibilities with the techniques of immunocytochemistry, *in situ* hybridization and RNA blot analysis. We hope to localize various known peptide growth factors and/or their mRNAs to the different cell types of the primate oviduct under controlled, hormonal conditions. Our goal is to test the predictions of our current model and refine it into a new, definitive analysis of steroid hormone action in the oviductal epithelium of primates. Ovum transport, fertilization, early embryo development and the normal delivery of the embryo into the uterus all depend on a functional oviductal epithelium. A more refined model of steroid hormone action in the oviduct would greatly increase our understanding of the key events that occur within the tubal environment, and could lead to new methods to regulate human fertility.

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