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Human Endometrium in Cell Culture: A New Method for Culturing Human Endometrium as Separate Epithelial and Stromal Components

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Summary. The present study describes a simple method for culturing human endometrium as separate epithelial and stromal components. Fifty-two samples of normal human endometrium have been initiated in tissue culture: endometrium from both the proliferative and luteal phases of the menstrual cycle showed satisfactory growth in vitro wich a success rate of about 94%. Epithelial cultures remained viable for 60 days, while from stromal cells it was possible to establish cell lines. Both cell types possessed estrogen receptors. Epithelial cells showed no clear estrogen or progesterone response. Our observations suggest that this simple method for culturing human endometrium may serve as a tool in further investigations.

Key words: Endometrium – Culture – Estrogen receptors

Introduction

Research on human endometrium in tissue culture started at the beginning of the century. In 1926 an outgrowth of cells from endometrial explants was described [1]. Later studies dealt with the growth characteristics of these cells [2, 3]. These studies were limited in that separation between stromal and epithelial cells was not carried out. All results concerned cultures containing two cell populations. It was found that explants obtained during the follicular phase of the menstrual cycle grew better than explants obtained in the luteal phase, and greater success was achieved with samples from women of child bearing age [2. 3]. More recent studies have concentrated on efforts to separate and characterize the epithelial and stromal components in vitro, as well as to establish cell lines from the epithelial cells of the endometrium [1, 4, 5]. Studies on hormone sensitivity of the epithelial and stromal cells have shown various results. Different responses

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of endometrial cultures to different concentrations of estradiol and progesterone have been described [1, 4–6]. None of the techniques for culturing human endometrium described till now have become standard methods for growth and serial passaging of endometrial epithelium.

In the present study we describe a simple method for the establishment of an epithelial tissue culture of the endometrium. Using this in vitro system we have examined the influence of 17B-estradiol and progesterone on the growth rate of these cells and also the estradiol receptor level of cells from endometrial tissue obtained at various phases of the menstrual cycle.

Materials and Methods

Our experiments considered endometrium from the proliferative and secretory phase of the menstrual cycle and from menopausal women.

Endometrial Cultures

Endometrial samples were obtained at the time of hysterectomy or biopsy. Hysterectomy or biopsy were for reasons unrelated to this research including uterine prolapse, fibroids, endometriosis, and infertility problems, but not malignancy. The fresh tissue was put into sterile collection medium, Ham's F-12 enriched with 10% fetal calf serum (FCS), 2% glutamine and 200 µg/ml penicillin-streptomycin. All procedures were conducted in a sterile hood. Human endometrial tissue fragments were washed twice in sterile phosphate buffered saline (PBS) (pH 7.2–7.3) to remove blood and secretions, and then divided into two parts. The first was cut into small pieces, placed into T-25 flasks and allowed to adhere for 45 min. The second was incubated for 45 min in a trypsin EDTA solution. The cell suspension resulting was poured through two layers of sterile gauze to remove tissue fragments; FCS (15%) was added to inactivate the trypsin. Free cells were pelleted at 500 *g* (5 min) and suspended in Ham's F-12 containing 20% FCS, 2% glutamine, 100 µg/ml penicillin-streptomycin and 40 µg/ml of insulin. The cell suspension, at a concentration of 10^5 cells/ml, was poured into the T-25 flask containing explants, and the cultures were maintained as follows:

The cultures were fed twice with fresh culture medium during the first week. Then a low concentration of collagenase (0.20 mg/ml) was added to the growth medium, and the medium was changed every 2 days. After reaching clean epithelial cultures, the cells were subpassaged with trypsin EDTA solution.

Transmission Electron Microscopy

Endometrial cells growing in monolayer were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate HCl buffer (pH 7.4). After 1 h of fixation the monolayer was gently scraped from the bottom of the culture dish with a rubber policeman, postfixed with ice chilled 1% O_5O_4, dehydrated in a graded series of ethanol, and embedded in araldite. Ultrathin sections were cut with glass knives on a LKB ultramicrotime, stained with uranyl acetate and lead citrate and observed with a Philips 301 electron microscope.

Estradiol Receptor Assay

Estradiol receptor levels were measured as previously described [7]. The assay was performed with cells growing for 4 weeks in culture. Because of the limited number of cells in individual culture, five cultures from the same type and stage were combined for each assay. Since most of the receptors

were found in the cytosol and not in the nuclear fraction, all measurements were done on the cytosolic fraction. The tissue was washed in PBS, scraped from the bottle, suspended in Ted buffer (containing 0.01 M Tris-HCl, 15 mM EDTA, 0.5 mM dithiothreitol, pH 7.4) and homogenized. The homogenate was centrifuged at 35,000 *g* for 35 min at 4° C to obtain the supernatant cytosolic fraction. To analyze saturation kinetics we incubated 200 µl cytosol (about 2 mg/ml soluble protein) in duplicate with increasing quantities of H^3-17B-estradiol added in 50 µl homogenization buffer (0.1–3 nM final concentration). Parallel incubations contained 100 fold excess nonradioactive hormone. After overnight incubation at 4° C, 500 µl of a charcoal suspension was added (250 mg Nurit A, 25 mg dextran in 100 ml buffer) for 30 min added to 6 ml of counting mixture and counted. Protein was assayed by the method of Lowry et al. [8].

Hormones

The effect of hormones on cell proliferation was monitored with cells grown for 21 days at 37° C in F-12. Two days before the start of growth experiments cells were transferred to tubes in a concentration of about $3-6 \times 10^4$ cells/ml. At the start of the experiment 17B-estradiol in three concentrations was added to the media (E_2–17B was concentrated in ethanolic solution). The final concentration of ethanol in the media never exceeded 0.5%. Eight days after the addition of the estradiol, cells were scraped from the tube and counted.

Results

General Growth Form in vitro

In our experiments endometrium from both stages of the menstrual cycle and from menopausal women showed satisfactory cellular growth in vitro (Table 1). Attachment of the free cells was complete in about 24 h, at that time primary outgrowth of epithelial cells from the tissue fragments was also observed in endometrium of women of childbearing age. Explants which were taken from menopausal women showed cell initiation only 5 days post culturing and free cells did not adhere to the flask. One week following culture initiation colonies of epithelial and stromal cells were observed with phase microscopy.

Following the addition of collagenase to the medium at this stage the fibroblasts stopped growing and became granulated while the epithelial cells appeared healthy and continued to multiply. Three weeks after the initiation of the incubation with a low collagenase medium the fibroblasts rolled off the flask and we had clean epithelial cultures (Fig. 1). The epithelial cells showed rapid spread during the first 3 weeks of culture. Thereafter, a phase of non spreading was observed. In this stage epithelial cells remained alive for about 60 days

Table 1. Epithelial cell culture success rate, including colony diameter and viable period

Average viable period (days)	% Success	Average colony diameter (cm)	Phase	No. of cultures
60 ± 2.9	100	0.76 ± 0.08	Unidentified	14
70 ± 1.5	100	1.04 ± 0.90	Proliferative	13
65 ± 1.4	100	0.83 ± 0.05	Secretory	14
45 ± 2.3	72	0.59 ± 0.08	Menopausal	11
	94		Total	52

(Table 1). The epithelial colonies diameter ranged from approximately 0.59–1.04 cm depending on the phase of the menstrual cycle in which the biopsy was taken (Table 1). We were able to transfer the epithelial culture once, after the second passage cells died and did not adhere to the flask. Fibroblasts which had been scraped with a rubber policeman prior to treatment with collagenase grew well and formed a monolayer in a non or low collagenase medium (Fig. 2).

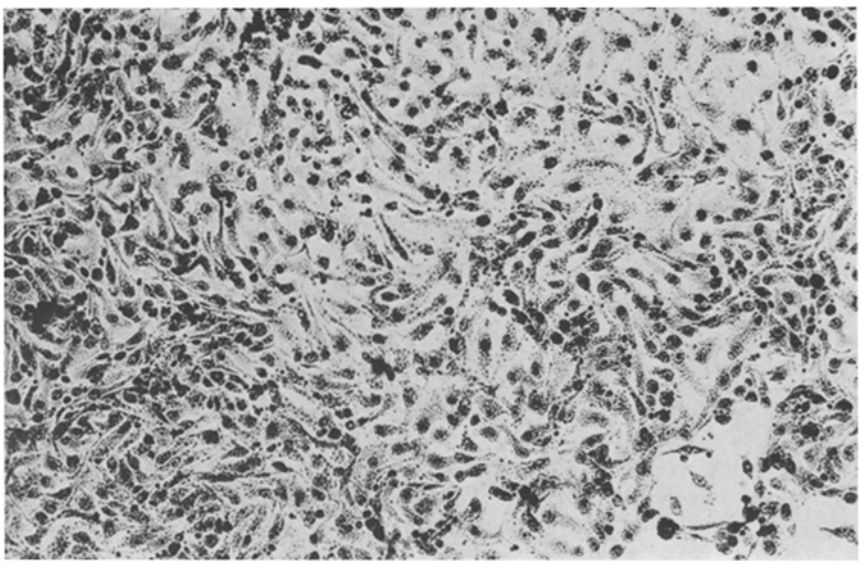

Fig. 1. Clean epithelial culture 3 weeks after the initiation of incubation with a low collagenase medium. Phase contrast × 100 (Giemsa stain)

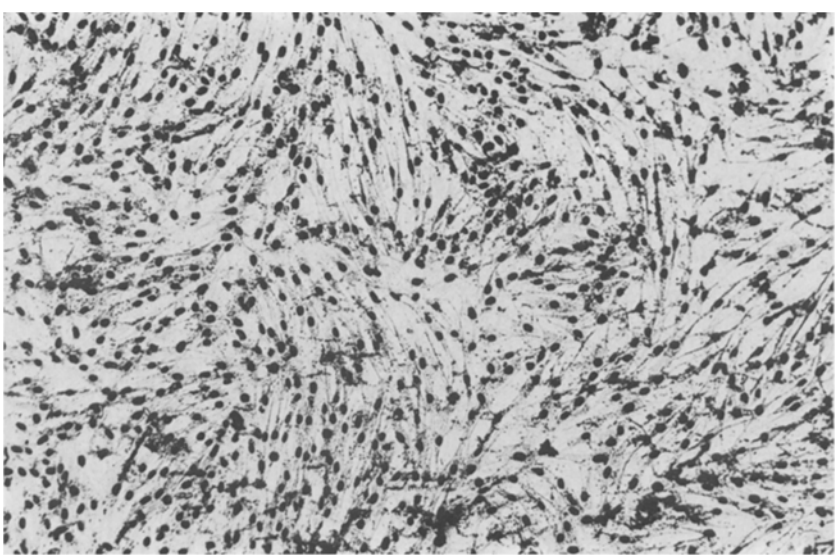

Fig. 2. Stromal cells growing in monolayer. Phase contrast × 100 (Giemsa stain)

Cell Characterization

Epithelial Cells. Epithelial colonies contained large curled cells with large nuclei and prominent nucleoli (Fig. 1). The surface of the tissue culture cells was covered with microvilli, and epithelial type junctional complexes were observed

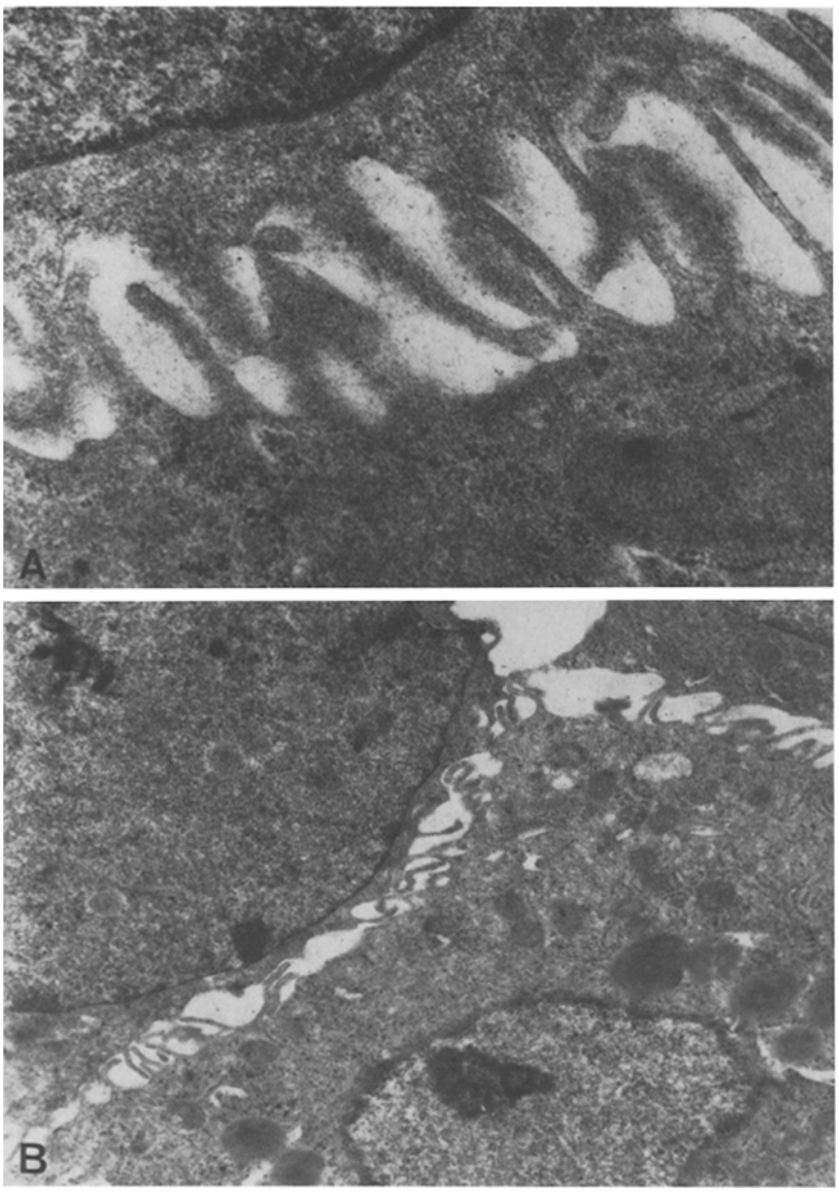

Fig. 3A, B. Electron micrographs of cultured epithelial cells from luteal endometrium growing 21 days in culture. **A** Epithelial microvilli; × 20,000. **B** Epithelial cells with glycogen particles; × 7,000

between the cells (Fig. 3). Glycogen particles were observed in the cytoplasma of the epithelial cells (Fig. 3).

Stromal Cells. Stromal colonies contained elongated cells with small nuclei and indistinct nucleoli (Figs. 2 and 4). No microvilli were observed on the surface of the cells and the junctions seen between the cells were similar to those observed in vitro in fibroblasts. Between the cells there was dense material (Fig. 4).

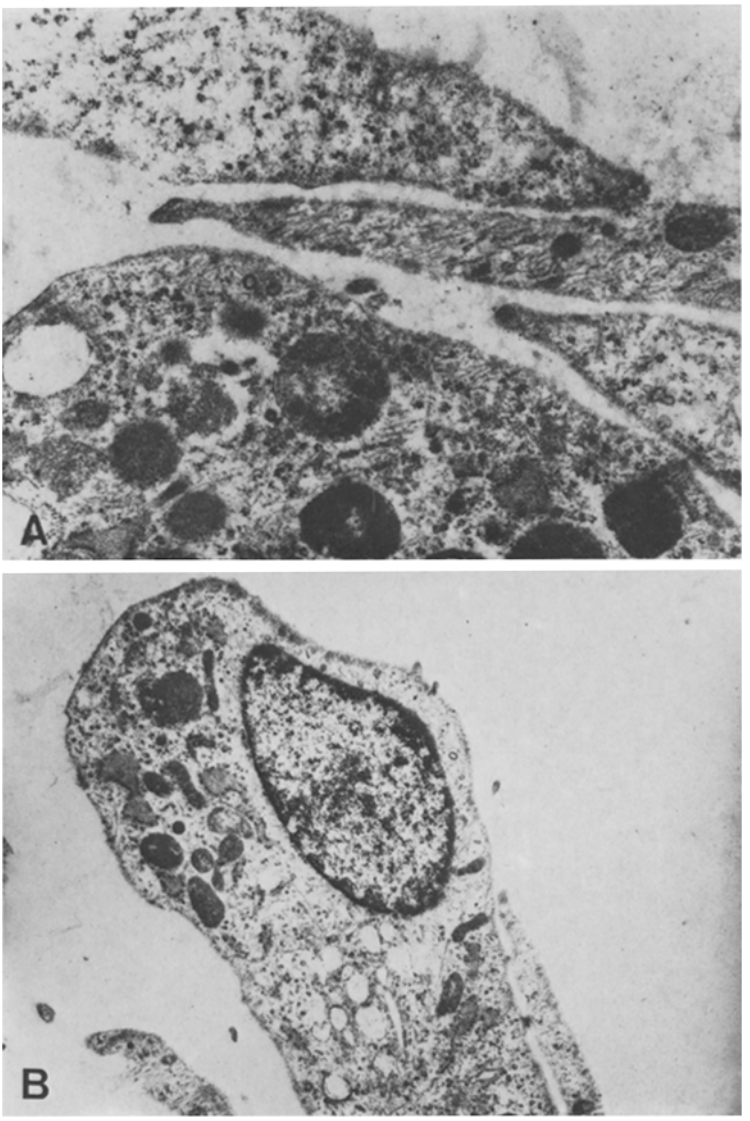

Fig. 4A, B. Electron micrographs of cultured stromal cells from luteal endometrium growing 21 days in culture. **A** The surface of these cells is devoid of microvilli; × 20,000. **B** Elongated stromal cell; × 7,000

Estradiol Receptors Levels in Epithelial and Stromal Cells

Cytosolic estradiol receptors were assayed in epithelial and stromal cultures taken from the follicular and luteal phase of the menstrual cycle.

Cytosols of seven epithelial cultures and three of stromal cultures were evaluated for the presence of estradiol receptors (Table 2). There was a marked difference between estradiol receptor levels in the epithelial and stromal cultures. In epithelial cultures receptor level was 54.8 ± 3.0 (mean ± SEM) while in stromal cultures the mean level was 6.6 ± 0.2.

There was no difference between receptor levels of the epithelial cells taken at various stages of the menstrual cycle.

Table 2. Cytosolic estradiol receptors in epithelial and stromal cultures taken at the follicular or luteal phase

No.	Type of culture	Stage	f/moles/mg[a]	Mean ± SEM
1	Epithel	Follicular	52.1	
2	Epithel	Follicular	46.6	55.1 ± 6.0
3	Epithel	Follicular	66.7	
4	Epithel	Luteal	50.7	
5	Epithel	Luteal	50.2	55.5 ± 5.1
6	Epithel	Luteal	65.6	
7	Epithel	Unidentified[b]	51.7	
8	Stroma	Follicular	6.9	
9	Stroma	Follicular	6.8	6.6 ± 0.2
10	Stroma	Luteal	6.2	

[a] Five separate cultures were combined for each result
[b] Stage of menstrual cycle unidentified because of irregular bleeding

Table 3a. Effect of 17 B estradiol on epithelial cellular replication

Culture no.	Inoculum (cells/ml)	Control (cells/ml)	Increase (%)	$E_2 = 10^{-8}$ M (cells/ml)	Increase (%)	$E_2 = 10^{-7}$ M (cells/ml)	Increase (%)
1 – DC	4.2×10^4	6.3×10^4	1.50	5.8×10^4	1.38	6.1×10^4	1.45
2 – TAH	7.0×10^4	9.3×10^4	1.33	9.4×10^4	1.34	1.0×10^5	1.43
4 – F	2.0×10^4	3.0×10^4	1.50	3.3×10^4	1.65	3.2×10^4	1.60
6 – L	6.1×10^4	8.2×10^4	1.34	7.4×10^4	1.21	9.0×10^4	1.47
7 – L	4.0×10^5	5.0×10^5	1.25	4.8×10^5	1.20	5.0×10^5	1.25
5 – F	3.2×10^4	3.7×10^4	1.16	3.7×10^4	1.16	3.9×10^4	1.22
3 – TAH	4.1×10^4	5.3×10^4	1.29	7.2×10^4	1.76	6.4×10^4	1.56
8 – L	5.2×10^4	6.2×10^4	1.19	8.4×10^4	1.61	8.2×10^4	1.58
9 – L	4.8×10^4	5.8×10^4	1.21	6.1×10^4	1.27	6.3×10^4	1.31

Table 3b. Summary

Mean cell growth	Mean	SD	SEM	p
Control	1.30	0.12	0.04	
$E_2 = 10^{-8}$ M	1.40	0.24	0.07	n.s.
$E_2 = 10^{-7}$ M	1.43	0.13	0.05	$p > 0.05 < 0.1$

Table 4a. Effect of progesterone on epithelial cellular replication

Culture no.	Inoculum (cells/ml)	Control (cells/ml)	Increase (%)	$P_2 = 10^{-8}$ M (cells/ml)	Increase (%)	$P_2 = 10^{-7}$ M (cells/ml)	Increase (%)
10 – F	2.0×10^4	2.8×10^4	1.40	2.7×10^4	1.35	2.8×10^4	1.40
11 – F	3.4×10^4	4.1×10^4	1.20	4.2×10^4	1.23	3.9×10^4	1.14
12 – F	1.7×10^4	2.7×10^4	1.58	2.9×10^4	1.70	2.6×10^4	1.52
13 – L	5.3×10^4	8.3×10^4	1.56	8.1×10^4	1.52	8.0×10^4	1.50
14 – L	4.8×10^4	7.2×10^4	1.50	7.3×10^4	1.52	7.1×10^4	1.48
15 – L	4.3×10^4	8.5×10^4	1.97	7.9×10^4	1.83	8.4×10^4	1.95

Table 4b. Summary

	Mean	SD	SEM	*p*
Prog 10^{-8} M	1.52	0.20	0.09	n.s.
Prog 10^{-7} M	1.50	0.24	0.11	n.s.

Hormone Sensitivity

The effect of 17B-estradiol and progesterone on epithelial cellular replication was not clear cut (Tables 3 and 4). In our experiments estradiol at 10^{-7} M and 10^{-8} M produced a slight enhancement in cell proliferation, although it was not significant (Table 3). The growth rate in vitro was not dependent on the phase of the menstrual cycle at which the biopsy was taken. Progesterone experiments with epithelial cells caused no change in cellular replication at concentrations of 10^{-7} M and 10^{-8} M (Table 4).

Discussion

The present study describes a simple method for culturing human endometrium as separate epithelial and stromal components. The study was performed on a relatively large number of different endometrial samples including 52 tissue cultures.

Endometrium from both the proliferative and luteal phase of the menstrual cycle showed satisfactory growth in vitro with a success rate of about 94% (Table 1). Morphologic patterns of epithelial and stromal cells cultured in vitro resembled those seen in fresh biopsies of the endometrium. These features are similar to those described by others [1, 4, 5]. In order to preserve the closest possible correlation to in vivo conditions only primary or secondary cultures were used in the experiments.

In the present study we have demonstrated the presence of specific estrogen receptors both in epithelial and stromal cells of the endometrium. The estrogen receptor level which we found in the cultured epithelial cells was comparable (in the same range as) to the levels found in whole endometrium in vivo as well as to

that observed by others in cultures of endometrial epithelium [9, 10]. However, receptor levels in our stromal cell culture were lower than in the epithelial and therefore lower than the receptor level of stromal cells in vivo. This suggests that our stromal cells after 4 weeks in culture do not completely represent the situation of stromal cells in vivo.

Previous work has shown that the concentration of estrogen receptors in human endometrium varies during the menstrual cycle; the high levels found in proliferative endometrium decline drastically during the luteal phase in both cytosolic and nuclear fractions [11]. In our study estrogen receptor level in the tissue culture was not dependent on the stage of the menstrual cycle at which the tissue was removed. This is not in accordance with the results of Flehmig et al. [9] which in a 1-week-old culture showed higher estrogen receptor levels in the follicular than in the luteal phase. The difference might be due to the time interval between plating of the cells and the measurement of the receptor level. Since the receptor measurements were carried out after 4 weeks in culture the receptor level reflects the conditions in culture and not the conditions of the cells in vivo at the time of their removal.

Despite their possesion of receptors to estrogen, epithelial cells showed no reproducible proliferative response to the hormone in concentrations of 10^{-7} M and 10^{-8} M. Epithelial cells also did not respond to progesterone at concentrations of 10^{-7} and 10^{-8} M. Our experience in hormonal manipulation of epithelial tissue culture of the endometrium is in agreement with previous studies [1, 4, 5]. Since epithelial cells are known to respond to estrogen in vivo, it would suggest that some other conditions which exist in vivo are necessary for the hormonal response of endometrium to estrogen in addition to the presence of receptors. Another possibility is that blood vessel cells and not the epithelia or stroma responding to hormonal stimulation. A similar situation is found in the uterine cervix in which the influence of hormones concerning organ function is on blood vessels and epithelial cells [12].

We are currently investigating better conditions for the hormonal response of endometrial cells in culture.

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