Ultrastructural aspects of the postmenopausal endometrium after oral or transdermal estrogen administration

E. C. Baracat, *M.D., Ph.D.*; **M. J. Simões,** *Ph.D.*; **J. M. Soares Jr.,** *M.D.*; **M. A. Haidar,** *M.D., Ph.D.*; **G. Rodrigues de Lima,** *M.D., Ph.D.*

Department of Gynecology, Federal University of São Paulo, Paulista Medical School, São Paulo (Brazil)

Summary

In this report we examined the ultrastructural features of the postmenopausal endometrial cells of women treated with different doses of conjugated equine estrogen (CEE), or transdermal 17ß-estradiol. Eight women with uterine prolapse and at least 5 years of menopause were randomly divided into four groups and treated as follows: (I) no hormonal treatment; (II) 0.625mg/day of CEE orally; (III) 1.25mg/day of CEE orally; (IV) 50µg/day of 17ß-estradiol transdermally. Hormones were administered for 28 days followed by vaginal hysterectomy. Fragments of the endometrium were prepared for transmission electron microscopic analysis. We observed that the postmenopausal endometrium of the untreated group was atrophic with lined superficial epithelial cuboidal cells. The presence of gland and stroma cells with clear cytoplasm containing few organelles and heterochromatin nuclei were also observed. On the contrary, the endometrium of the group that received 0.625mg/day of CEE showed signs of proliferative cells such as the presence of numerous organelles in the cytoplasm and euchromatic nuclei. All of the proliferative effects on the endometrium were more pronounced in the groups that received 1.25mg/day of CEE and 50µg/day of transdermal 17ß-estradiol. We concluded that the ultrastructural proliferative changes of the postmenopausal endometrium induced by 1.25mg/day of CEE were similar to 50µg/day of transdermal 17ß-estradiol.

Key words: Postmenopause; Estrogen replacement therapy; Endometrium; Endometrial ultrastructure.

Introduction

Estrogen replacement therapy is known to improve the quality of life and to prevent severe diseases such as osteoporosis and cardiovascular morbidity in postmenopausal women [1]. However, undesirable consequences of this therapy can occur. For instance, endometrial cancer in estrogenically conditioned patients has been reported since 1960, and this effect seems to be related to high doses and long periods of treatment [2]. It is crucial to understand how different doses of estrogen and routes of administration can affect the endometrial morphology.

The endometrium may be regarded as one of the most spectacular and dynamic target tissues in the body, in which morphologic changes occur in rhythmic fashion throughout the reproductive lifetime of women and after menopause [3]. The morphology of postmenopausal endometrium, in comparison with premenopausal tissue, is characterized by an atrophic mucosa lined by a thin cubical epithelium with few endometrial glands and a dense stroma [4-9]. However, Baracat *et al.* described that after replacement therapy the postmenopausal endometrium showed signs of tissue growth, such as mucosa with a large number of glands and a considerable development of the surface and glandular epithelia, and also abundant loose stroma with numerous mitosis [9]. The authors reported that postmenopausal endometrium was not only responsive to estrogenic action but also underwent a proliferative stimulus dependent on the dose, route and type of estrogen administered. This study also concluded that the effects of 1.25 mg/day conjugated equine estrogens (CEE) and 50 µg/day transdermal 17 ß-estradiol are equivalent for endometrial stimulation [9].

The effects of estrogen on the morphology of postmenopausal endometrium have been also studied by transmission electron microscopy. Untreated postmenopausal endometrium has short microvilli and electrodense cytoplasm epithelium and poorly developed stromal cells. After estrogen treatment two types of epithelial cells clearly can be observed: ciliated and glandular cells together with an increased number of endometrial stromal cells [10, 11]. However, little is known about the effect of transdermal 17ß-estradiol on postmenopausal endometrial cells.

In this study we examined ultrastructural features of the postmenopausal endometrial cells of women treated with different doses of CEE or transdermal 17ß-estradiol using electron microscopy.

Material and Methods

Patients and treatment schedules

Eight 45-55 year-old women with a prolapsed uterus and at least five years after menopause were selected for this study at the Division of Endocrynologic Gynecology and Climacterium in the Department of Gynecology, at the Federal University of São Paulo - Escola Paulista de Medicina. The experimental protocol was pre-approved by the Brazilian Institutional Review Board, and all patients gave informed consent for their participation in the study. Postmenopausal women receiving any other hormonal treatment, suffering from any endocrinological disease or taking other medication were excluded. The eight women were randomly divided into four groups of treatment: (I) - no hormonal treatment (control), (II) - 0.625 mg/day of oral

Revised manuscript accepted for publication November 13, 2000

CEE, (III) 1.25 mg/day of oral CEE and, (IV) - 50 µg/day of transdermal 17 ß-estradiol. Hormones were administered for 28 days before vaginal hysterectomy to correct the prolapsed uterus.

Ultrastructural analysis

Immediately after surgical removal, fragments of the endometrium were placed in vials containing 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, at 4°C, and then transferred to the laboratory. The endometrial tissue was rinsed and fixed in fresh 2.5% glutaraldehyde for 3 hours, then washed three times in phosphate buffer, and postfixed for 90 minutes in phosphate-buffered 1% osmium tetroxide, pH 7.3, at 4°C. After fixation, this tissue was washed rapidly in cold phosphate buffer, dehydrated in a graded series of alcohol and finally embedded in araldite. Sections of approximately 60 nm thick were stained with uranyl acetate and lead citrate and examined and photographed using a Zeiss 9S2 transmission electron microscope.

Results

In order to compare the effects of different doses of oral CEE with 50 µg/day of transdermal 17ß-estradiol on the endometrial ultrastructure, we examined three different cells: superficial epithelium, glandular epithelium and stroma.

The effect of estrogen treatment on the superficial epithelium of postmenopausal women

The endometrium of the control group (no estrogen treatment) was lined by a single cubical epithelium as a result of the atrophic phenomenon (Figure 1a). These cells showed electrodense nuclei and their cytoplasms were small with few organelles and some isolated electrodense bodies. Tissue specimens from estrogen-treated patients in this study exhibited changes when compared with the control group. The epithelial cells of the second group treated with 0.625 mg/day of oral CEE (Figure 1b) were higher than the control group. In addition the nuclei were voluminous, euchromatic with a conspicuous nucleoli and occupied almost the whole cytoplasm. The cytoplasm contained numerous organelles, such as mitochondria, rough endoplasmic reticule (RER), Golgi complex and electrodense bodies. When the dose was raised to 1.25 mg/day of CEE (third group, Figure 1c), the epithelium appeared as one layer of large columnar cells, containing a great amount of cytoplasmic volume. The nuclei were voluminous, euchromatic, and arranged in several levels resembling a pseudo-stratified epithelium as a result of a rapid proliferation of cells. One or more nucleoli were conspicuous in all cells. The cytoplasm was richer in organelles than in the group treated with 0.625 mg/day of CEE. Furthermore slender microvilli were distributed densely on the luminal surfaces of the cells. Similar results were found when we analyzed the fourth group treated with 50 µg/day of transdermal 17 ß-estradiol (Figure 1d). The superficial epithelium was lined by single columnar ciliated epithelium with large cells showing an eliptical nucleus and evident nucleoli. The nuclei were voluminous, euchromatic and were located in different levels resembling pseudo-stratified epithelium. The cytoplasm was rich in organelles such as mitochondria, RER, Golgi complex and electrodense bodies.

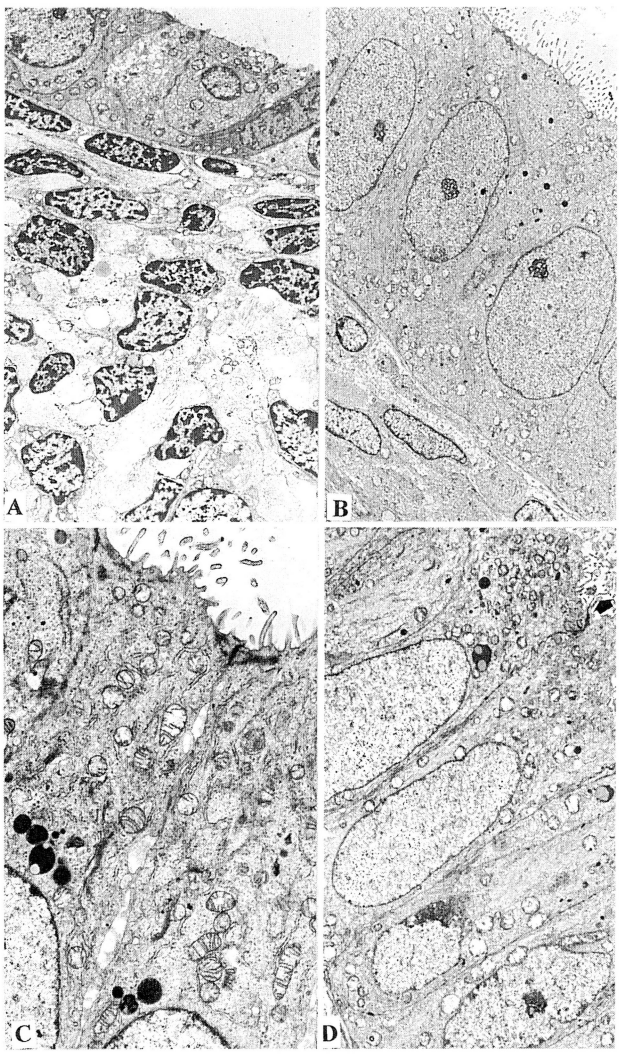

Figure 1. — Representative electromicrographical superficial epithelial cells of endometrial sections from postmenopausal women (n=8) treated with: (a) no hormone (n=2, control) showing a simple cylindrical superficial epithelium (2800x), (b) 0.625 mg/day of oral CEE (n=2) showing a single ciliated cylindrical superficial epithelium. The nuclei are rich in euchromatin with evident nucleoli (2800x), (c) 1.25 mg/day of oral CEE (n=2) showing the apical region of superficial epithelial cells with numerous microvilli and cytoplasm rich in organelles (3400x) and, (d) 50µg/day of transdermal 17 ß-estradiol (n=2) showing a well developed single cylindrical superficial epithelium with numerous long cilia and microvilli (arrow) (2800x).

The effect of estrogen treatment on endometrial glands of postmenopausal women

The endometrial glands of the control group were small in number and lined by a single cubic or cylindrical epithelium with some presenting cystic dilatation

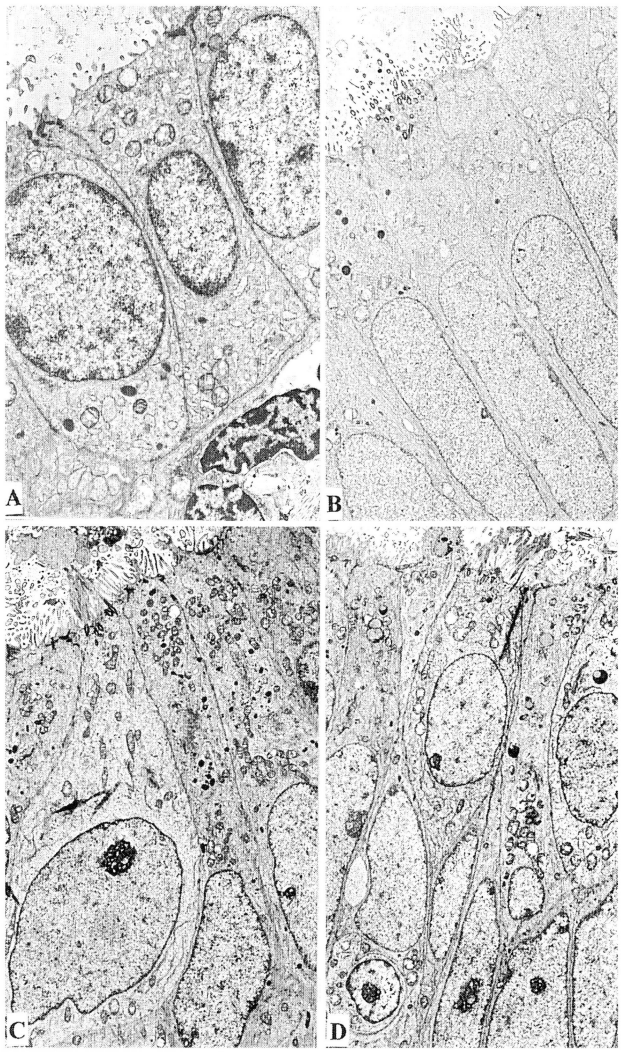

Figure 2. — Representative electromicrographical glandular epithelial cells of endometrial sections from postmenopausal women (n=8) treated with: (a) no hormone (n=2, control) showing a simple cylindrical glandular epithelium which has some microvilli in the apical region (3800x), (b) 0.625 mg/day of oral CEE (n=2) showing well developed cilia and microvilli in the apical region of a single cylindrical glandular epithelium (2800x), (c) 1.25 mg/day of oral CEE (n=2) showing numerous long cilia and microvilli in the apical region of the glandular epithelium (2800x) and, (d) 50 µg/day of transdermal 17 ß-estradiol (n=2) showing a well developed glandular epithelium with numerous cilia and microvilli in the apical region (2800x).

(Figure 2a). The glandular cells presented voluminous nuclei, few organelles and some electrodense bodies. There were few microvilli in the apical pole of the ciliated cells. After 0.625 mg/day of CEE, the endometrial glands turned into ciliated columnar epithelium, showing euchromatic elliptic nuclei and conspicuous nucleoli (Figure 2b). Mitochondria, RER, Golgi complex and electrodense bodies were also observed in the apical pole. In addition, after increasing the dose to 1.25 mg/day of CEE, the changes were much more intense than others above (Figure 2c). The blood vessels were dilated and congested, and well-developed endometrial glands were also observed next to the superficial epithelium in this group. The secretory cells were large and voluminous with euchromatic nuclei and conspicuous nucleoli. The cytoplasm showed numerous mitochondria and well developed RER. Some microvilli were observed in the apical pole. Areas of pseudo-stratification as well as epithelial buds were also observed. However, after 50 µg/day of transdermal 17 ß-estradiol treatment we did not see areas of pseudo-stratification. In this group, the endometrial glands were lined by ciliated columnar epithelium (Figure 2d) as in 0.625 mg/day of CEE group (Figure 2b), and the cells showed euchromatic elliptic nuclei and conspicuous nucleoli. Mitochondria, RER, Golgi complex and electrodense bodies were also observed in the apical pole.

The effect of estrogen treatment on the stromal cells of postmenopausal women

The atrophic stroma of the control group consisted of cellular connective tissue with a predominance of fibroblasts and collagen fibers. These cells showed few organelles and their nuclei were rich in heterochromatin (Figure 3a). However, this pattern changed after estrogen treatment (Figure 3b, c, d). The stroma of 0.625 mg/day of the CEE treated group had many fibroblasts with voluminous euchromatic nuclei and evident nucleoli. Numerous collagen fibers and intercellular substance occupied the intercellular space (Figure 3b). This effect was more accentuated in the 1.25 mg/day of CEE treated group; the stroma contained numerous fibroblasts with fusiform vesicular nuclei and conspicuous nucleoli. The cytoplasm showed projections which touched one another establishing cell gap junctions. Mitochondria, RER and electrodense bodies were observed (Figure 3c). Some fibroblasts displayed one or more cilium. The intercellular substance was much richer in amorphous substance than 0.625 mg/day of the CEE treated group (Figure 3b, c). In the 50 µg/day of transdermal 17 ß-estradiol treated group the stroma was highly developed with a great number of fibroblasts which showed fusiform vesicular nuclei with one or two nucleoli. The cytoplasm revealed numerous projections which touched one another. Mitochondria, RER and electrodense bodies were also observed (Figure 3c). The intercellular space was rich in intercellular substance like in the 1.25 mg/day CEE treated group (Figure 3c, d).

Discussion

Estrogen is widely used in peri- and postmenopausal women with symptoms related to its deprivation. However, this replacement can cause a risk of endometrial cancer [2]. Therefore, the knowledge of dose, type and route of administration is fundamental for preventing risks such as endometrial cancer and some side-effects such as postmenopausal bleeding.

Most studies show that postmenopausal endometrial morphology is characterized by atrophic mucosa lined with cubical epithelium and few endometrial glands and the presence of dense stroma. This histological picture is a feature of an endometrial hypoestrogenic state [5-7, 9].

Some studies of the postmenopausal endometrium using electron microscopic analysis confirm these features [10-12]. In this study the control group presented similar features (Figure 1a, 2a, 3a).

Estrogen has a proliferative effect on postmenopausal women. Independent of the dosage, type or route of administration, the ultrastructural changes on superficial and glandular epithelium and stroma confirm this action. In addition some studies compared these changes with the proliferative phase of the menstrual cycle [10-12]. In contrast, there are some characteristics identified in postmenopausal endometrium under the effects of estrogen which are similar to those observed in adenocarcinoma of the endometrium: an accumulation of lipid goticules, irregular nuclear form and the presence of perinuclear microfibrils [11]. We also detected these characteristics in patients who received 1.25 mg/day CEE and 17 ß-estradiol 50 µg/day (data not shown), but not in the 0.625mg/day group.

The 1.25mg/day CEE treated group presented more developed superficial and glandular cells than the 0.625 mg/day treated group. We observed well developed cytoplasm and numerous microvilli and long cilia in the 1.25mg/day treated group. This effect is dependent on the dosage of CEE and can be compared with 50 µg/day of the transdermal ß-estradiol treated group. Although the changes on the glandular epithelium were most accentuated in the 1.25mg/day CEE treated-group (Figure 2c, Table 1), both groups presented nuclear pseudo-stratification, and tortuous, large and juxtaposed glands (Figure 2c, d).

It is important to report that both groups with 1.25mg/day of ECC and 50 µg/day of transdermal 17ß es showed a larger concentration of organelles, mainly mitochondria, RER, a conspicuous Golgi complex and numerous large electrodense bodies in the epithelial cells. This may be due to the estrogen effect of increased cellular metabolism like in the proliferative phase of endometrium in the menstrual cycle [10-12]. However the presence of irregular nuclear forms, a large amount of euchromatin and evident nucleoli is similar to features described for hyperplasia and adenocarcinoma [11].

The presence of cilia in some fibroblasts in the stroma could be dependent on estrogenic action [13]. These ultrastructural changes in stroma were observed in our study and were more intense in the patients who received 1.25 mg/day of CEE or 50 µg/day of transdermal 17 ß-estradiol (Figure 3d, Table 1). The results are similar between the 1.25 mg/day CEE treated group and 50 µg/day transdermal 17 ß-estradiol groups.

Table 1. — *Summary of the proliferative effects of estrogen on the endometrium of postmenopausal patients, comparing dosage, type and route of administration.*

Group	Cellular type		
	Superficial epithelium	Glandular epithelium	Stroma
Untreated	–	–	–
0.625mg/day Oral CEE	+	+	+
1.25mg/day Oral CEE	+++	+++	+++
50µg/day Transdermal 17ß-estradiol	+++	++	+++

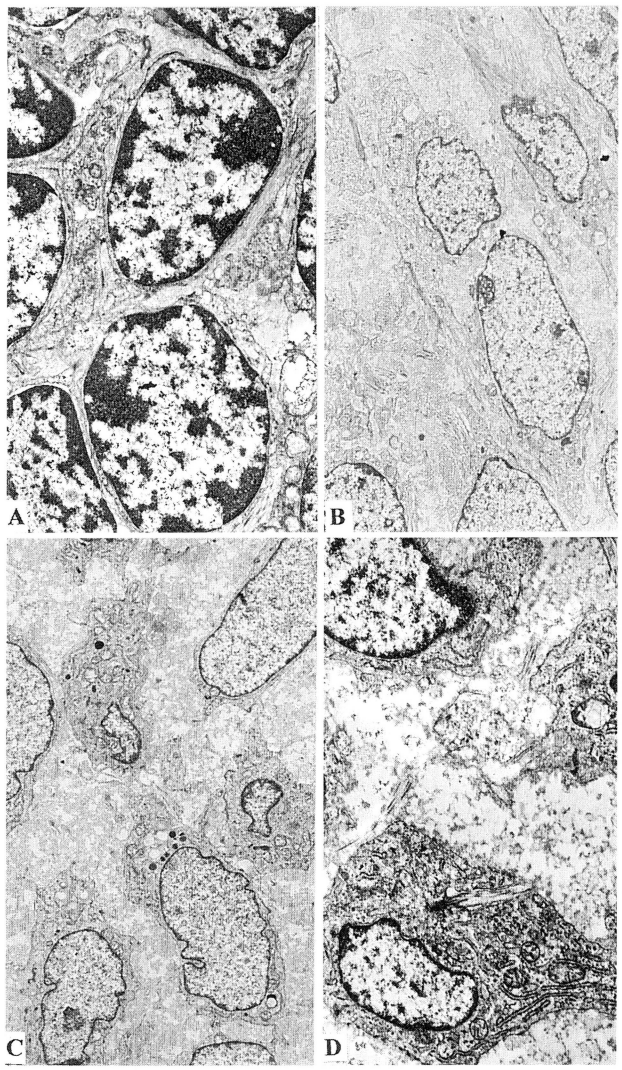

Figure 3. — Representative electromicrographical stromal cells of endometrial sections from postmenopausal women (n=8) treated with: (a) no hormone (n=2, control) showing stroma rich in heterochromatic nuclei of fibroblasts (5300x), (b) 0.625 mg/day of oral CEE (n=2) showing stroma with rich nuclei in euchromatin of fibroblasts (5000x), (c) 1.25 mg/day of oral CEE (n=2) showing stroma with well developed fibroblasts (5200x) and, (d) 50 µg/day of transdermal 17ß-estradiol (n=2) showing a stroma with well developed fibroblasts some of which have cilia (5200x).

The gap-type junctions are important communications for the maintenance of homeostasis in multicellular organisms. Of various communication systems, gap junctional intercellular communication is the only means for cells to directly exchange signals. Substantial progress has recently been made in studies on the role of gap junctions both in experimental and human tumorigenesis [14]. It was reported that ultrastructural evidence of interaction between the glandular epithelium and superficial stromal cells during the menstrual cycle in the late proliferative and initial secretory phases occurs through a cellular gap [15]. However, we only observed these cellular gap junctions among the fibroblasts.

Conclusion

We conclude that the ultrastructural proliferative changes in postmenopausal endometrium under estrogen replacement are dependent on dosage. The action of 50 µg/day of transdermal 17 beta-estradiol was similar to 1.25mg/day of CEE in our study (Table 1).

References

[1] Geisler J., Omsjo I. H., Helle S. I., Ekse D., Silsand T., Lonning P. E.: "Plasma oestrogen fractions in postmenopausal women receiving hormone replacement therapy: influence of route of administration and cigarette smoking". *J. Endocrinol.*, 1999, *162*, 265.

[2] Grady D., Gebretsadik T., Kerlikowske K., Ernster V., Petitti D.: "Hormone replacement therapy and endometrial cancer risk: a meta-analysis". *Obstet. Gynecol.*, 1995, *85*, 304.

[3] Ferenczy A.: "Superface ultrastructural response of the human uterine lining epithelium to hormonal environment. A scanning electron microscopic study". *Acta Cytol.*, 1977, *21*, 556.

[4] Cramer D. W., Knapp R. C.: "Review of epidemiologic cancer and exogenous estrogen". *Obstet. Gynecol.*, 1979, *54*, 521.

[5] Cajander S., Holst J., Carlstrom K., Damber M. G., Schoults B.: "Endometrial response following percutaneous estrogen replacement therapy: A morphometric investigation". *Acta Obstet. Gynecol. Scand.*, 1983, *62*, 97.

[6] Trévoux R., De Brux J., Castanier M., Nahoul K., Soule J. P., Scholler R.: "Endometrium and plasma hormone profile in the peri-menopause and post-menopause". *Maturitas*, 1986, *8*, 309.

[7] Parks R. D., Scheerer R. P., Greene R. R.: "The endometrium of normal postmenopausal women". *Surg. Gynecol. Obstet.*, 1988, *106*, 13.

[8] Paganini-Hill A., Ross R. K., Henderson B. E.: "Endometrial cancer and patterns of use of estrogen replacement therapy: A study". *Br. J. Cancer*, 1989, *59*, 445.

[9] Baracat E. C., Simões, M. J., Haidar M. A., Evencio Neto J., Novo N. F., Rodrigues de Lima G.: "Histomorphometry of the postmenopausal endometrium after oral and transdermal estrogen replacement therapy". *Menopause*, 1996, *3*, 44.

[10] Flowers C. E., Wilborn W. E., Hyde B. M.: "Mechanisms of bleeding in postmenopausal patients receiving estrogen alone or with a progestin". *Obstet. Gynecol.*, 1983, *61*, 135.

[11] Aycock N. R., Jollie W. P.: "Ultrastructural effects of estrogen replacement on postmenopausal endometrium". *Am. J. Obstet. Gynecol.*, 1979, *135*, 461.

[12] Amso N. N., Crow J., Lewin J., Shaw R. W.: "A comparative morphological and ultrastructural study of endometrial gland and Fallopian tube epithelia at different stages of the menstrual cycle and the menopause". *Human. Reproduction.*, 1994, *9*, 2234.

[13] Simões M. J., Mora O. A., Oliveira-filho, R. M.: "Resorption of endometrial collagen in oophorectomized rats". *Acta. Anat.*, 1984, *20*, 142.

[14] Sulkowski S., Sulkowska M., Skrzydlewska E.: "Gap junctional intercellular communication and carcionogenis". *Pol. J. Pathol.*, 1999, *50* (4), 227.

[15] Roberts D. K., Walker N. J., Lavia L. A.: "Ultrastructural evidence of stromal/epithelial interactions in the human endometrial cycle". *Am. J. Obstet. Gynecol.*, 1988, *158*, 854.

Address reprint requests to:
EDMUND C. BARACAT, M.D., Ph.D.
Dept. of Gyn.-Fed. Univ. of São Paulo
Escola Paulista de Medicina
Rua Dr. Diogo de Faria, 1087-cj 610
04037-003 São Paulo (Brazil)