

# Histomorphometric aspects of adult castrated rat endometrium after the use of estrogen, progesterone and tamoxifen

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## Summary

The aim of this study was to analyse the morphologic and morphometric aspects of endometrium in rats receiving hormone replacement therapy with conjugated equine estrogen (CEE), medroxyprogesterone acetate (MPA) and tamoxifen (TMX).

Thirty-five adult rats, 2-3 months of age were ovariectomized four days prior to using the drugs. Rats were divided according to the following treatments for 60 days: CEE (50µg); CEE/MPA (50µg/2 mg); MPA (2mg); TMX (250µg); vehicle (propylene glycol). Fragments of endometrium were removed and analysed by light microscopy.

The endometrium suffered evident morphologic modifications under the action of hormones and TMX. The endometrium was significantly thicker in the CEE, CEE/MPA and TMX group when compared to the control, however the MPA group showed no differences when compared to the control group.

**Key words:** Endometrium; Tamoxifen; Menopause; Hormone replacement therapy.

## Introduction

In Brazil there are more than an estimated 10 million postmenopausal women and they will represent a significant part of the world's population until the end of this century [1, 2].

Hormone replacement therapy (HRT) is indicated with the aim of eliminating the adverse effects of hypoestrogenemia. HRT has been used more and more. Even though it brings about benefits, alleviating symptoms and clinical signs as well as preventing metabolic changes, it can produce undesirable side-effects, particularly in the endometrium [3, 4].

The carcinogenic potential of estrogen known since the second half of the 20<sup>th</sup> century through the research with animals corroborated by innumerable clinical studies has accumulated in the last two decades [5]. This fact highlights the importance of the endocrine aspects in gynecological neoplasias. Estrogen promotes cellular proliferation, induces the development of receptors, and increases DNA synthesis of stroma and epithelial cells. This effect is antagonized by progesterone [6, 7].

The most common estrogens used in hormone replacement are the natural ones. Conjugated estrogen hormones extracted from the urine of pregnant mares are highlighted among them [8, 9].

The benefits of estrogen replacement after menopause, fighting early symptoms and preventing late metabolic changes such as osteoporosis and cardiovascular diseases are unquestionable. However, a higher incidence of endometrial proliferation and even adenocarcinoma have been attributed to its isolated use [10, 11].

The use of progesterone or synthetic progestogens aims to prevent the development of hyperplastic lesions and endometrial carcinoma. The most utilized progestogens

are medroxyprogesterone acetate, administered orally or parenterally, norethindrone, cyproterone acetate and menegestrel [12, 13].

Tamoxifen was the first nonsteroidal antiestrogenic agent to be cleared by the Food and Drug Administration in the treatment of breast cancer patients in 1978 [14, 15].

Nowadays, tamoxifen is considered as a selective estrogen receptor modulator because in some target tissues it has an agonist action and in others an antagonist one. Tamoxifen does not constitute a pure antiestrogenic agent as studies showed that in rat uteri it has a partial agonist action, a well established fact in the eighties. However, its action in postmenopausal women has only started to be unveiled in the past decade [16, 17].

In the postmenopausal period, tamoxifen produces an estrogen-agonist effect. It maintains bone density and reduces the incidence of cardiovascular diseases, either by the reduction of low density lipoprotein circulating levels or by a vasodilator action on the coronary arteries. It also can have a partial agonist action in the endometrium [18, 19].

Therefore, due to the still existing questions and controversies about the effects of tamoxifen on the endometrium, we became interested in studying the histomorphometric effects of conjugated estrogens, isolated or associated, medroxyprogesterone acetate, and tamoxifen on the endometrium of oophorectomized adult rats.

Hence, we opted to utilize an experimental model in rats because these animals have short, regular and successive estrual cycles and are easy to handle with a low cost.

## Material and Methods

Albino rats (*Rattus norvegicus albinus*, Rodentia, Mammalia) of the EPM 1-Wistar family, virgins, adults, weighing from 180 to 210 grams were provided by the Experimental Models Deve-

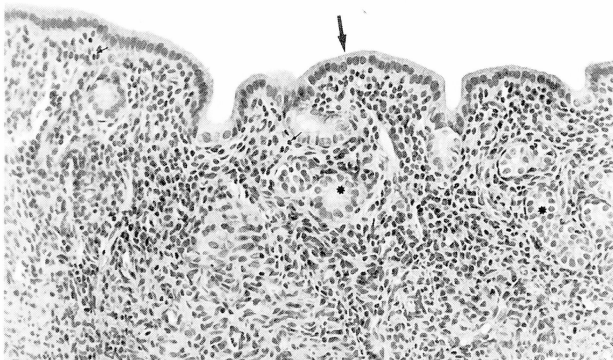


Figure 1.

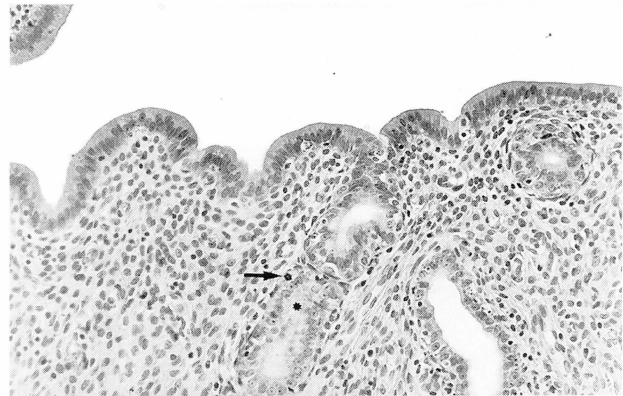


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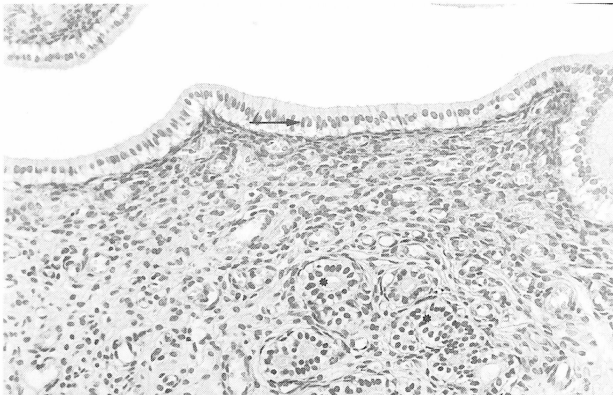


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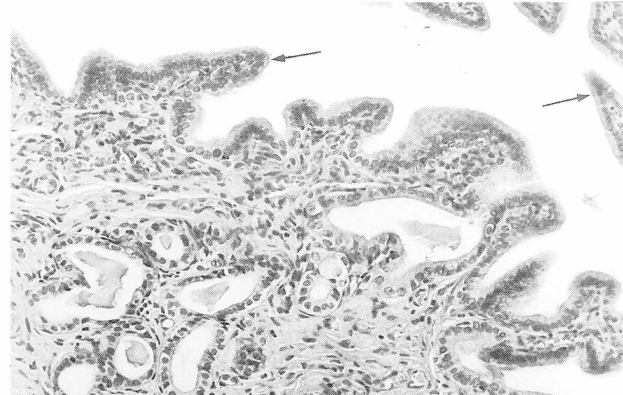


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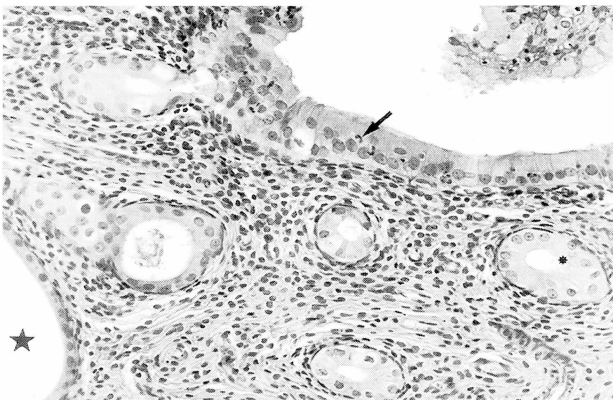


Figure 5.

lopment Center for Medicine and Biology of the Federal University of São Paulo.

After an adaptation period, all animals were submitted to a dorsal bilateral oophorectomy following ethyl ether anesthesia. Four days after castration, the 50 rats were randomly distributed in five groups of ten each, as follows:

**G I** - Control group that received propyleneglycol separately;

**G II** - Group that separately received conjugated estrogens from mares in a dosage of 50 µg/animal/day;

**G III** - Group that separately received medroxyprogesterone acetate in a dosage of 2.0 mg/animal/day;

**G IV** - Group that received conjugated estrogen from mares, in the same dosage as group II, but associated continuously to medroxyprogesterone acetate, in a dosage of 2.0 mg/animal/day;

**G V** - Group receiving tamoxifen separately in a dosage of 250 µg/animal/day.

The drugs utilized were diluted in 0.5 ml of propyleneglycol in order to standardize the offered volume. The gavage procedure was performed for 60 consecutive days. After this period, sacrifices were performed for the excision of the medium portion of the right uterine horns. The uterine fragments were analyzed by light microscopy.

A histologic evaluation through image digitalization was performed for the morphometric study, with a graphic analysis program of an IMAGELAB (SOFTIUM-Brazil) computer. The digital images were analyzed by image subtraction, separated colorimetrically.

## Results

### LIGHT MICROSCOPY

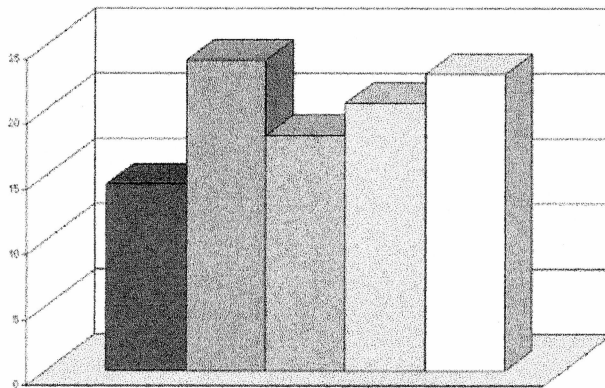
#### 1 - Morphologic

**(Figure 1) G I:** Photomicrography showing part of the endometrium of the group of control rats. Observe the luminal epithelium (bigger arrow), endometrial glands (asterisk) and, in the stroma, innumerable heterochromatic nuclei (smaller arrows). H.E. ± 280 X.

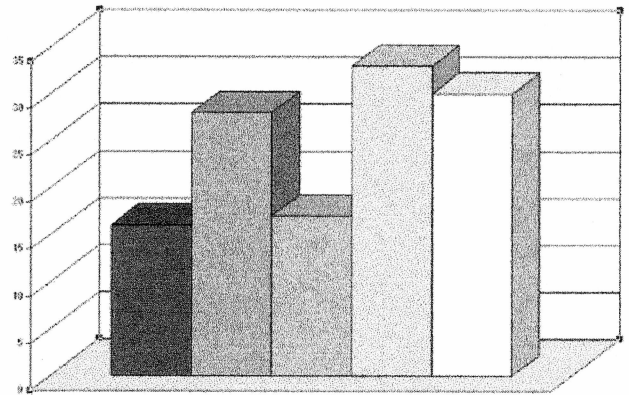
**(Figure 2) G II:** Photomicrography showing part of the endometrium of a rat treated with conjugated estrogens. Observe in the endometrial gland (asterisk), typical pictures of mitosis (arrow). H.E. ± 280 X.

**(Figure 3) G III:** Photomicrography showing part of the endometrium of a rat belonging to the group treated

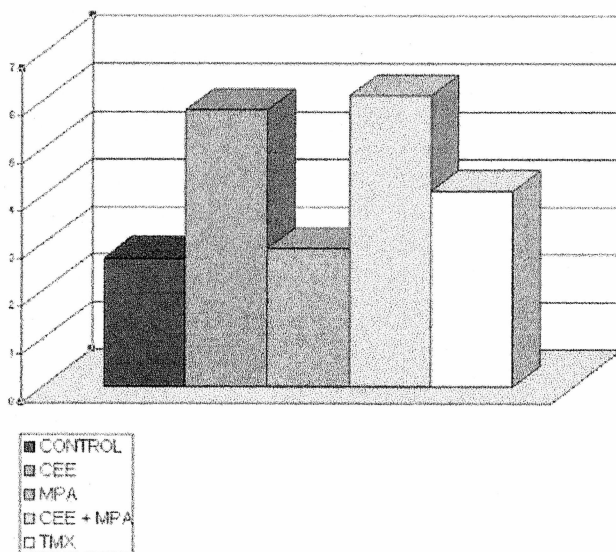
GRAPHIC 1



GRAPHIC 2



GRAPHIC 3



with medroxyprogesterone acetate. Observe the superficial epithelium with elliptical nuclei dislocated to the central part of the cells (arrows), and innumerable endometrial glands (asterisk). H.E.  $\pm$  280 X.

(Figure 4) **G IV:** Photomicrography showing part of the endometrium of a rat treated with conjugated estrogens and medroxyprogesterone acetate. Observe a great concentration of folds (arrows) in the endometrial lining. H.E.  $\pm$  280 X.

(Figure 5) **G V:** Photomicrography showing part of the endometrium of a rat treated with tamoxifen. Observe the luminal epithelium infiltrated by leucocytes (arrows), endometrial glands (asterisk) and part of the cyst (star). H.E.  $\pm$  280 X.

## 2 - Morphometric:

The morphometric results are expressed in graphics 1, 2 and 3

**Graphic 1:** Micrometry, in the mean of three measurements in micra, of slender regions of the endometrium according to the groups: control (C), conjugated estrogens (CEE), conjugated estrogens and medroxyprogesterone

acetate (CEE+MPA), tamoxifen (TMX), medroxyprogesterone acetate (MPA), statistical means and results.

**Graphic 2:** Comparison between the glandular lumen area and the endometrial stroma, according to the groups: C, CEE, CEE+MPA, TMX, MPA, statistical means and results.

**Graphic 3:** Endometrial areas in  $\mu\text{m}^2$ , according to the groups: C, CEE, CEE+MPA, TMX, MPA, statistical means and results.

## Discussion

In our study, we observed significant histologic changes when associating medroxyprogesterone acetate. Evident secretory changes were observed in the group that received the combined therapy, unlike the one that used estrogen only.

As to the group medicated with tamoxifen, it was observed that, despite the atrophic results of the colposcopic exam, the endometrium was found to be more developed than that of the control group.

It is important to highlight that, through light microscopy, significant morphologic changes in the endometrium were observed in all the groups studied, except the control group.

In order to improve these histologic findings, we performed a histomorphometric study through computer graphic analysis by image digitalization.

Through graphic analysis, we observed that at the end of hormonal replacement, or after 60 days, the group that received equine conjugated estrogens separately presented much thicker endometrial epithelia and a larger luminal area than the other groups. These data indicate that the equine conjugated estrogens would act especially in the endometrium superficial epithelium as a whole.

In the group receiving conjugated estrogens associated with medroxyprogesterone acetate, besides the evident endometrial thickening, we also observed irregularities in the epithelium, forming treelike structures resulting from the secretory action created by the medroxyprogesterone acetate. Furthermore, in this group the endometrial stroma area was also larger compared to the other groups (control, conjugated estrogens, medroxyprogesterone acetate and tamoxifen).

The group that received tamoxifen also presented endometrial thickness, but much smaller than the one induced by the equine conjugated estrogens separately. The fact that tamoxifen is an agonist estrogen substance in the endometrium would explain this trophic action. When tamoxifen is used in elevated doses or in smaller doses for a long period of time, it would act as an endometrial agonist. It is known that, when used chronically for more than five years tamoxifen, due to its agonist action in the endometrial tissues of postmenopausal women, can induce neoplasias.

Yet, in the control group and in the one receiving only medroxyprogesterone acetate, the endometrium was atrophic. The graphic analysis did not reveal any significant differences between these two groups.

### Conclusion

Based on our preliminary results and extrapolating them to the human being, we could suggest that either the isolated estrogenic replacement or the chronic use of tamoxifen in the postmenopausal period, might determine different levels of endometrial proliferation. Thus, the need for adequate monitoring of the endometrium when using this kind of therapy.

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