# Breast histomorphometry of rats treated with estrogen and/or progestogen

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## Summary

**Objective:** To evaluate the breast histomorphometric changes in rats treated with estrogen and/or progestogen for a short period of time. **Methods:** Forty oophorectomized rats were divided into four groups: GC, vehicle; GE, treated with estradiol benzoate (37.6 mg/animal); GP, treated with medroxyprogesterone acetate (11.2 mg/animal); and GEP, treated with estradiol benzoate (37.6 mg/animal) plus medroxyprogesterone acetate (11.28 mg/animal). In GE group, estradiol was administered subcutaneously for seven days; in GEP group, estradiol was administered once in a day for the first seven days and the progestogen over the next 23 days both subcutaneously. Twenty-four hours after the last hormone administration, the animals were killed upon deep anesthesia and the first inguinal breasts were removed, fixed in 10% formaldehyde and processed to be included in paraffin, with the sections being stained by hematoxylin-eosin. Morphology and the area occupied by mammary parenchyma were assessed, with the data undergoing analysis of variance followed by the Kruskal-Wallis test ($p < 0.05$). **Results:** The control group breasts were found atrophic and, in GE and GEP group animals, typical alveoli with secretion inside are present; in progestogen-treated animals (GP), alveoli formed by large cells occupying almost the entire alveolar lumen are noted. Morphometric analysis showed a larger mammary parenchyma area in hormone-treated animals ($GE = GP > GEP > GC$; $p < 0.05$). **Conclusion:** Estradiol and progestogen had a proliferative effect on mammary parenchyma. However, prior estradiol administration changes the progestogen action on rat mammary tissue.

**Keywords:** Breast; rats; estradiol; medroxyprogesterone 17-acetate; hormone replacement therapy.

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## Introduction

The transition from the reproductive period into the non-reproductive period in women, which is known as climacteric, is marked by important endocrine, somatic, and psychic changes due to the reduced ovarian hormone production. This period landmark is menopause, where the last menstruation date was 12 months before[1,2].

For most women, this phase can bring symptoms such as hot flashes, neuropsychiatric findings, vaginal dryness and others[3,4].

Replacement hormone therapy (HT) can provide several benefits for women, especially in post-menopause, and has been shown effective in alleviating the main symptoms resulting from hypoestrogenism[5-7]. However, the occurrence of long-term adverse effects by using estroprogestative therapy is reported, especially an increased breast cancer risk[8]. Actually, many epidemiological studies have reported an increased breast cancer risk, with this risk being higher when estrogen-progestogen combinations are used than with estrogens alone[9]. The breast is a structure responding variably to steroid hormones. Its hormonal interaction is complex; sexual steroids can act by autocrine, paracrine or endocrine routes. Under the action of these steroid hormones, mammary tissue reaches its maximum development[10,11]. The hormone action on target-organs results from their binding intracellular receptors, modulating gene expression and hence specific protein synthesis[12].

There is evidence that estrogens stimulate ductal growth[13,14]. However, for some authors, estrogens produce breast epithelium atrophy[15]. Progestogens added to estrogenic therapy do not reduce breast cancer risk. There is further evidence that the combination might increase the breast epithelial cell proliferation[16].

However, whether the acute treatment with either estrogens and progestogens alone or the combined therapy changes the breast proliferative pattern is debated. Therefore, this paper aims at evaluating the histomorphologic and histomorphometric effects of estrogen and/or progestogen therapy on the mammary tissue of oophorectomized adult rats.

## Methods

Forty Wistar (*Rattus norvegicus albinus*) EPM-1 lineage, adult, virgin and 90-day-old rats provided by the *Centro de Desenvolvimento de Modelos de Experimentação* (CEDEME) [Experimental Model Development Center] at the *Universidade Federal de São Paulo – Escola Paulista de Medicina* (Unifesp/EPM) were used. This study was approved by Unifesp/EPM Research Ethics Committee (Report no. 0820/07).

The animals were maintained restricted to plastic cages in the Histology and Structural Biology Discipline vivarium (Unifesp/EPM) with a controlled room temperature of 22°C and artificial lighting from fluorescent lamps, with a 12-hour light photoperiod (7 AM to 7 PM) and a 12-hour dark photoperiod (7 PM to 7 AM); food and water *ad libitum*.

After a seven-day period, all animals underwent bilateral oophorectomy and 30 days later they were randomly separated into four groups with 10 rats each, namely: GC, the animals were treated with corn oil (vehicle); GE, the animals were treated with estradiol benzoate (37.6 mg/animal) subcutaneously for seven consecutive days; GP, animals were treated with medroxyprogesterone acetate (11.28 mg/animal) subcutaneously for 23 consecutive days; GEP, animals were treated with estradiol benzoate (37.5 mg/animal) for seven days followed by medroxyprogesterone acetate (11.28 mg/animal) for further 23 consecutive days. The hormones were administered in doses similar to those used in post-menopaused women[17].

Twenty-four hours after the last administration, all the animals were given a xylazine (20 mg/kg) and ketamine (100 mg/kg) mixture intraperitoneally injected and the first inguinal pair of breasts was removed. Then the breasts were fixed in 10% formaldehyde and processed 24 hours later to be included in paraffin according to routine histological methods. Four-micrometer (microtome LEICA – RM 2145) serial sections were obtained from the blocks and stained by hematoxylin and eosin for further histomorphologic and morphometric analysis. The area occupied by mammary and adipose tissues was assigned for morphometric analysis. The imaging retrieval system used was made up by a light microscope (Carl Zeiss) attached to a high resolution camera (AxioCam- MRC, Carl Zeiss) and color video monitor (Samsung). The images acquired were evaluated by the imaging analysis software AxionVision REL 4.6 by Carl Zeiss. For this purpose, the portion occupied by mammary parenchyma and fat tissue was delimited in an area of 380 x $10^3$ mm each section. Four near serial section were evaluated in each experimental animal, with a total area of 152 x $10^3$ $mm^2$ being evaluated in each study group. The analysis of variance (ANOVA) and the Kurskal-Wallis test were used to analyze the results achieved. The analyses were carried out by using the software Prisma (California, USA) and Excel (Microsoft). In all statistical testing, significance levels of 5% were used ($\alpha \leq 0.05$).

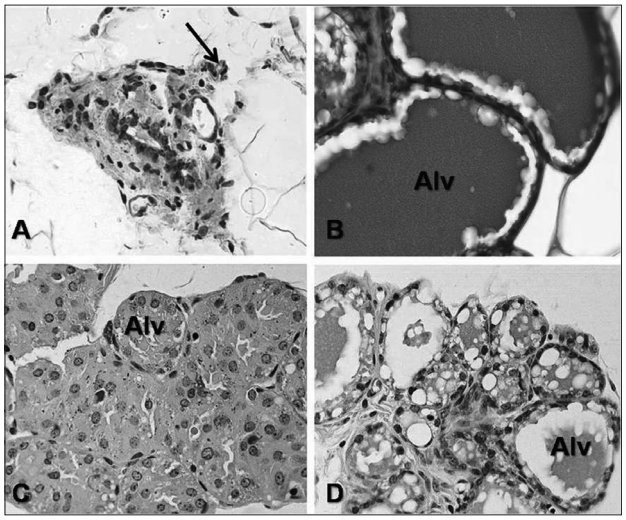
## Results

A) Histomorphologic results: control group (GC) animal breast showed a high fat tissue concentration and scanty parenchyma where rudimentary ducts and alveoli could be identified (Figure 1A). Within the latter, yellow pigment-laden macrophages could be identified. Yet in the estradiol-treated group (GE), a further developed parenchyma was observed, with dilated ducts and alveoli presenting, the vast majority of them having an eosinophilic material (secretion) inside (Figure 1B).

In the group treated with progesterone alone (GP), we noted numerous mitosis figures in the mammary parenchyma, with the cells being irregularly arranged. The ducts were lined by either a single-layer or a stratified cylindrical epithelium with dense connective tissue surrounding them. On the other hand, alveoli were lined by a single-layer cubic epithelium, whose rounded nuclei were displaced down to the basal region and the cytoplasm was eosinophilic. No secretion was identified inside the ducts and/or alveoli (Figure 1C).

In the group treated with estradiol plus medroxyprogesterone (GEP), the histological feature was quite similar to that in the estrogen-treated group, i.e., it was made up by dilated ducts and alveoli containing a secretion inside. In this group (GEP), we noticed the alveoli had a lower size and the secretion present in the alveolus lumen was scarcer (Figure 1D).

**Figure 1 –** Photomicrographs showing a portion of mammary tissue from oophorectomized rats (A), estradiol-treated rats (B), medroxyprogesterone-treated rats (C) or rats treated with a estradiol plus medroxyprogesterone combination (D). In A, a mammary duct and a rudimentary alveolus are observed (arrow). Otherwise, in B and D, estradiol is noticed to induce the formation of alveoli containing a secretion inside, while medroxyprogesterone alone (C) induces proliferation with no typical alveoli (Alv). H.E. 400X.




B) Morphometry: the achieved data regarding the mammary parenchyma area in rats included in the several study groups are expressed in Table 1.

## Discussion

The information concerning the risks and benefits of post-menopausal hormone replacement creates questions and uncertainties. In spite of quantitative assessments for signs, symptoms, psychosocial aspects and hormone treatment use, there is little information about the individual significance and Brazilian woman experience with these issues. The current paper tried to reproduce this hormone therapy effects by using estrogen and progestogen doses equivalent to those usually used in post-menopausal hormone replacement. For this purpose, an allometric calculation was made by equating the dose according to the rats' weight and metabolism[17].

From the hormone measurements used, our results showed there is a smaller mammary parenchyma and proliferation area in oophorectomized animals that were not given hormones. However, a larger mammary parenchyma was found in animals treated with either estradiol or medroxyprogesterone over the group receiving estradiol followed by medroxyprogesterone.

In animals receiving estradiol (all over the trial period or only on the first days on hormone therapy), we noticed alveoli containing a secretion inside. This fact would result from prolactin release by the pituitary gland under an estrogenic action; there would still be a gonadotropin secretion inhibition[18-20]. Actually, our results bear out biopsy studies in post-menopausal woman breasts demonstrating higher epithelium proliferation in HT users compared with a non-hormone user group[21,22]. In these studies, most changes are found in the duct-terminal lobule unit where most breast tumors develop.

Experimental studies have also proved proliferative changes in 17-beta estradiol-treated rat breasts[23]. Some patients are known to report breast symptoms, such as breast pain, breast enlargement or increased consistency on palpation, resulting from estrogen action, when they are in the premenstrual phase. These phenomena are likely due to the trophic stimulus occurring in the post-menopausal atrophic fibroglandular tissue[24]. Premenopausal women are prone to have more ducto-glandular and fibrous tissue in the breasts rather than fat tissue. This ratio is inverted with time, reaching a postmenopausal near complete replacement by fat tissue[25].

**Table 1 –** Means, standard-deviations, and percentage of the area occupied by mammary parenchyma in oophorectomized rats treated with estradiol, medroxyprogesterone or an estradiol and medroxyprogesterone combination

| | Study groups | | | |
|---|---|---|---|---|
| | GC | GE | GP | GEP |
| Total occupied area $10^3$ $\mu m^2$ | 1,915.20 ± 133.30 | 11,948.72 ± 287.28 | 10,488.00 ± 342.21 | 7,546.80 ± 260.52 |
| % occupied area | 12.16 | 78.61 | 69.00 | 49.65 |

GC, control group; GE, estradiol group; GP, medroxyprogesterone group; GEP, estradiol and medroxyprogesterone-treated group (GE = GP > GEP > GC; $p < 0.05$).

In our study, the finding that rat breasts had a different morphologic behavior in animals receiving estradiol followed by medroxyprogesterone and in animals treated with medroxyprogesterone alone was outstanding. Progestogen alone induced increased mammary parenchyma, but with no typical alveolus formation. In contrast, the behavior was quite different in animals previously treated with estradiol, as the alveoli were found more regular. These data are in line with those achieved by molecular and proteomic biology techniques, showing hormone signaling serial activation on mammary epithelium is required for morphogenesis progression, i.e., for mammary alveolus formation[26].

Concerning the association between sexual hormones and breast cancer before menopause, its assessment is exceedingly difficult and hindered by the hormonal level fluctuation occurred over the menstrual cycle. The relationship between breast cancer and estrogen levels in premenopausal women could not be clearly demonstrated, possibly because the latter are constantly above the limit required to stimulate a breast cancer growth[27]. In contrast, the short-term relationship between hormone replacement in postmenopausal women and breast cancer development is uncertain based on inconsistent results in several studies. In studies indicating increased risk, it is limited to the long term (> 5-10 years), being relatively small (RR: 1.2-1.5)[28].

Thus, new studies following this line are warranted and their purpose should be to clarify the relationship between breast cancer and postmenopausal hormone therapy dose and duration.

## Conclusion

Estradiol and medroxyprogesterone have a proliferative effect on mammary parenchyma, although prior estradiol administration changes the progestogen action on mammary tissue in adult rats.

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