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## EFFECT OF ANTI-PROGESTROGEN ON THE EPITHELIAL CELL HEIGHT OF RAT ENDOMETRIUM

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### ABSTRACT

**Objective:** To investigate the effect of mifepristone on height of the epithelial cells of the endometrium.

**Study Design:** Laboratory based randomized controlled trials

**Place & Duration of the study:** Department of Anatomy, Army Medical College Rawalpindi from Jan. 2007-march 2007.

**Method:** Sixty adult female rats were divided randomly into two groups, comprising of 30 animals in each group. In group A one ml of normal saline was given orally daily for three months while in group B mifepristone was given orally in a dose of 1 mg/kg body weight daily for three months.

**Results:** In the experimental group reduction was observed in epithelial cell height. Significantly lower level of progesterone while higher level of estrogen level in experimental group was noted as compared to the control group.

**Conclusion:** Long term mifepristone administration suppresses the endometrial proliferation and epithelium height was decreased. It also lowered the plasma concentration of progesterone. While the plasma concentration of the estrogen was raised

**Keywords:** Mifepristone, estrogen, progesterone, epithelium

### INTRODUCTION

The progesterone and estrogen hormones induce proliferation and differentiation in the endometrium<sup>1</sup>. The inner mucosa, or endometrium, consists of a surface columnar epithelium overlying a thick lamina propria containing numerous blood vessels and endometrial glands<sup>2</sup>.

Mifepristone acts on the receptors just like the progesterone but may produce different conformational changes.<sup>3</sup> With the rising trend of the use of antiprogesterin and insufficient knowledge regarding its effects on histomorphology of uterus, this study has been designed to see the long term effects of mifepristone treatment on rat endometrium.

### MATERIAL AND METHODS

These laboratory based randomized controlled trials were conducted at the department of Anatomy, Army Medical College Rawalpindi from Jan. 2007-march 2007. Sixty healthy adult female Sprague Dawley rats weighing 200-300 g were procured from the National Institute of Health Sciences Islamabad. The animals were randomly divided into two groups of 30 each.

#### Group A (Control)

Thirty female rats were given one ml of normal saline orally daily for three months.

### Group B (Experimental)

Thirty female rats were given the drug (Mifepristone) orally in a dose of 1 mg/kg body weight daily for three months.

All the animals were sacrificed next day after the last oral dose. Two ml blood was taken directly from the heart for measurement of estrogen and progesterone levels. Uterine horns along with a portion of vagina was removed, trimmed and placed into 10% Formalin for 24 hours. About ½ cm piece of tissue was taken from the middle of the right uterine horn. Approx five microns thick sections were cut stained with hematoxylin and eosin for light microscopic study.

## MICROSCOPIC OBSERVATIONS

### Height of Epithelium

The height of the epithelium was taken from the basement membrane up to the upper limit of the cell facing the lumen under high power field (x40 objective) from three regions and their mean was taken as the reading for that animal.

### Statistical analysis

Data had been analyzed using SPSS version 15. Descriptive statistics were used to describe the data. P-value < 0.05 was considered as significant.

## RESULT

Total sixty animals were included in the study, 30 in each group. The tubular sections showed three distinct layers (inner, middle and outer). In control group the luminal side of inner layer (*Endometrium*) was lined by single regular row of cylindrical cells. These closely packed cells having rectangular outlines had round ovoid or elongated nuclei. Their nuclei tended to be aligned at the center of the cells and the cytoplasm was basophilic reflecting the microscopic picture of simple columnar epithelium (Fig. 1b). In experimental group the inner layer was folded giving it an overall ruffled appearance as a result lumen was much reduced

as compared to normal group. The epithelium appeared pseudostratified with decrease in height as compared with control group (Fig. 1d).

The mean epithelium height in experimental group was  $12.8 \pm 0.40 \mu\text{m}$  which was lower than that of the control group. The difference was regarded highly significant statistically ( $p = 0.001$ ) when compared with the control group. Average progesterone level was significantly lower in experimental group as compared to control group ( $p = 0.001$ ) while average estrogen level was significantly higher in experimental group as compared to control group ( $p = 0.001$ ). (Table-1)

## DISCUSSION

In this study of 3 months we have studied the long term effects of mifepristone treatment on rat endometrium. Uterine epithelium height was decreased in the experimental group after administration of mifepristone compared with control animals. This may be due to effect of drug on the uterus. It has also been reported by others that mifepristone blocks estrogen-induced increase in uterine growth inhibits true uterine growth. 25 hrs, following an injection of progesterone, a significant increase in the height of cells of the luminal epithelium is observed<sup>4</sup>. Our data indicated decrease in the height of the cells of the luminal epithelium after mifepristone treatment.

Changes caused in the luminal epithelium in response to antiprogesterone are usually attributed to the direct affect of the hormone.<sup>5</sup> Most of the glandular epithelial cells had sub nuclear vacuoles in various studies. Ultra structurally there is noted the presence of large vacuoles, membrane inclusions and myelin like bodies in the endometrial taken from treated animals, features usually suggestive of the disintegration of cytoplasm proteins.<sup>6</sup> In treated animals, vacuolization is one of the structural indicators of energy deficit and permeability disorder of membranes in the endometria<sup>7</sup>. This

hypothesis, that protein synthesis is proceeding at a faster rate in these cells in response to progesterone, is thus consistent with the morphometric data<sup>8</sup>.

Endometrial cells require a balance between estrogen and progesterone production. The absence of progesterone removes the progesterone brake leading to persistent estrogenicity and constant endometrial proliferation. The endometrium can become disordered, although the ratio of stroma to glands remains normal, and vascular abnormalities such as dilated capillaries become apparent. It is unlikely to be solely due to the effect of high levels of unopposed estrogen because it occurred in some women, e.g. in Shanghai where there were low estrogen levels with complete suppression of ovarian follicular development<sup>9</sup>

In a study on ovariectomized rhesus macaques were treated for 5 months with either estradiol (E<sub>2</sub>) alone, E<sub>2</sub> + progesterone (two doses) or E<sub>2</sub> + ZK (0.01, 0.05 or 0.25 mg/kg) all doses of ZK blocked endometrial proliferation and induced endometrial atrophy.<sup>10</sup> The absence of progesterone removes the 'progesterone brake', leading to persistent estrogenicity and constant endometrial proliferation., Though usually there is normal ratio of stroma to glands, vascular abnormalities such as dilated capillaries become apparent and the endometrium can become disordered<sup>11</sup>.

Rats receiving long-term PR antagonist treatment show endometrial stimulation under the effect of estrogen<sup>12</sup>. High doses of mifepristone (25 and 50 mg/d) lead to variable effects in women, such as atypical cystic changes, as have been described in eutopic endometrium<sup>13</sup>. Serum progesterone levels declined in experimental groups after mifepristone administration. Level of estrogen hormone was elevated in the experimental group as compared with the control group. Less congruency has been found in the earlier reports about the effect of mifepristone on progesterone secretion.<sup>14</sup> No statistically significant change in

the progesterone levels was observed in the 2-day follow-up using 200 mg of mifepristone<sup>15</sup>. With administration of 600 mg mifepristone, there was observed an increase in progesterone levels on day 1 followed by a significant decrease in another study<sup>16</sup>

## CONCLUSION

We concluded that in long term mifepristone affected the endometrial proliferation and induced histomorphological changes in the uteri of the experimental rats. Significant reduction in epithelial height was observed in epithelium height. It also lowered the plasma concentration of progesterone .While the plasma concentration of the estrogen was raised.

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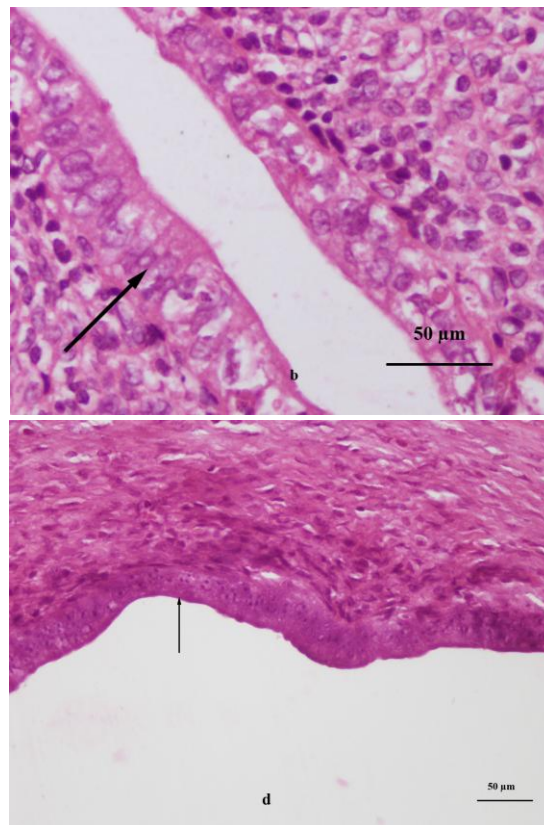
**Table-1: Comparison of Study variables between control and experimental groups**

Parameters	Control (n=30)	Experimental (n=30)	p value
Epithelium height ( $\mu\text{m}$ )	$15.9 \pm 0.99$	$12.8 \pm 0.40$	0.001*
Progesterone ng/ml	$5.5 \pm 0.8$	$2.8 \pm 0.09$	p =0.001
Estrogen pg/ml	$41.7 \pm 0.66$	$83.6 \pm 1.2$	p =0.001

Values are expressed as mean  $\pm$  SD

NS = Insignificant

- = Significant



**Figure 1: Photomicrograph of a cross section from uterine horn of animal no 13 of control group a, showing simple columnar epithelium b. (arrow). Animal no 11 of experimental group b, showing pseudo-stratified epithelium (arrow) d. H&E stain. Bar =50 $\mu\text{m}$ .**