

## Full Length Research Paper

# Effect of anti-progestogen mifepristone on the endometrium of rat uterus

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To investigate the effect of mifepristone on of rat uterus, 60 adult female rats were divided randomly into two groups, comprising of 30 animals in each group. In group A, 1ml 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. All animals were sacrificed next day after the last oral dose. 2ml blood was taken directly from the heart for measurement of estrogen and progesterone levels. About ½cm piece of tissue was taken from the middle of the right uterine horn. Sections were stained with hematoxylin and eosin for light microscopic study. In the experimental group reduction was observed in epithelial cell height and endometrial area. Significantly lower level of progesterone, while higher level of estrogen was noted in the experimental group as compared to control group. Long-term mifepristone administration suppresses the endometrial proliferation, endometrial area and decreases epithelium height.

**Keywords:** Mifepristone, receptors, estrogen, progesterone, endometrium.

## INTRODUCTION

The progesterone and estrogen hormones induce proliferation and differentiation in the endometrium (Connelly. 2001). The effects of estrogen and progesterone are mediated through interaction with specific intracellular receptors that are members of the nuclear receptor super family of transcription factors (Benakanakere et al., 2009). Estradiol acts as a cell mitosis inducing key genes involved in replication, whereas progesterone has reverse effects when compared to estradiol and acts as a differentiation factor. The cross talk between the endocrine system, growth factors, and neurotransmitters take place both at the receptor level.

Mifepristone, 17, hydroxyl 11--(4-dimethylaminophenyl)-17-(prop-1-ynyl)-estra-4, 9-dien-3-one, is derived from the estrane progestin. It is also named as RU486 (Sukjum. 2005). Mifepristone acts on the receptors just like the progesterone but may produce different

conformational changes (Antoniou et al., 1997). With the rising trend of the use of antiprogestin 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.

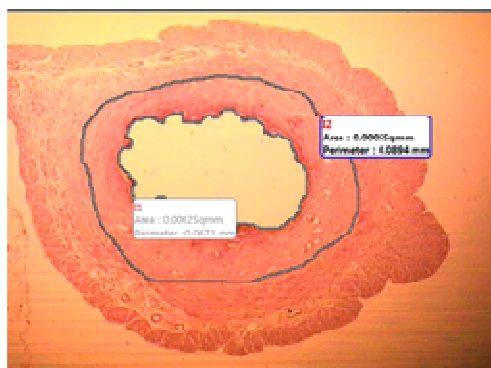
## MATERIAL AND METHODS

These laboratory based randomized controlled trials were conducted at the department of Anatomy, Army Medical College Rawalpindi. 60 healthy adult female Sprague Dawley rats weighing 200-300g 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 1ml of normal saline orally daily for three months.

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**Figure 1.** Photomicrograph of a cross section from uterine horn of animal of control group showing area of the endometrium. haematoxylin and eosin 40X

## Group B (Experimental)

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

All animals were sacrificed next day after the last oral dose. 2ml 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 ½cm piece of tissue was taken from the middle of the right uterine horn. Approximately 5 thick sections were cut and 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.

### Area of Endometrium

It was calculated by using the Motic Images Plus 2.0 ML software on the computer. The software was properly calibrated for x4 objective with the calibration slide provided with the microscope, Model: DMB3:223. The slides of endometrium were then focused on the screen. The images of the slides were captured by capture command using motic live imaging module. The images were automatically transferred into the computer, where they were saved in tagged image file format. Each image was opened in morphometric computer software, Motic

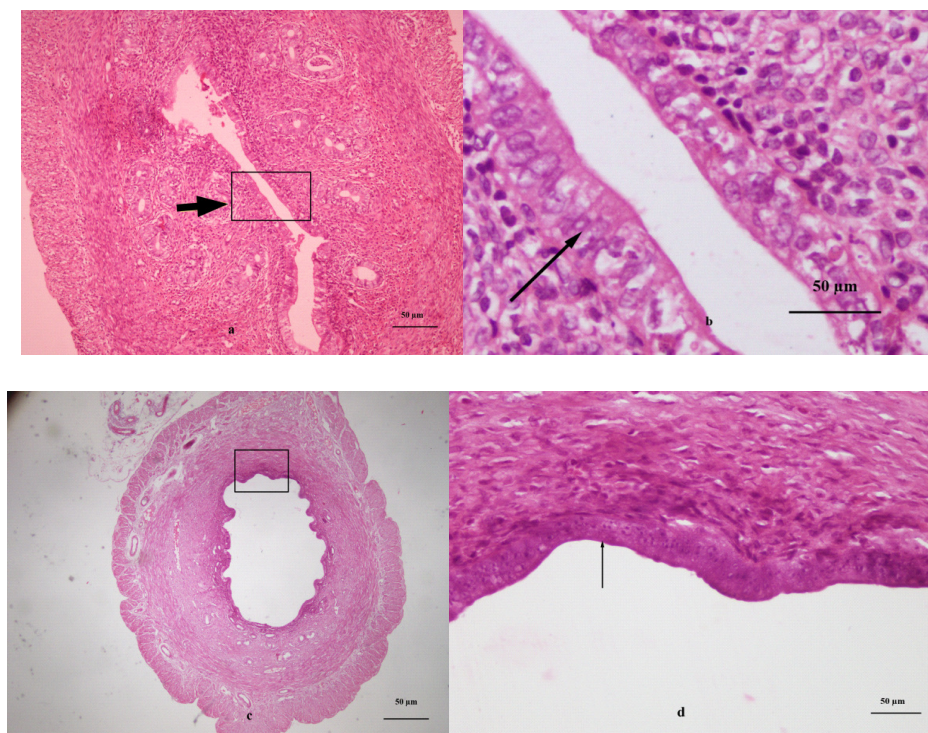
Images Plus 2.0 ML software by Motic Incorporation Ltd Hong Kong. The images were properly zoomed in to show the area of interest. After that the “irregular” tool for measurement of area was selected. The luminal area was outlined on the luminal surface of the endometrium and marked as area 1. The “irregular” tool was again selected to outline the border of the endometrium at the junction with myometrium and marked as area 2. The area of the endometrium then calculated by subtracting the area 1 from area 2. The final reading was recorded as the area of the endometrium for that slide (Figure 1).

## Statistical analysis

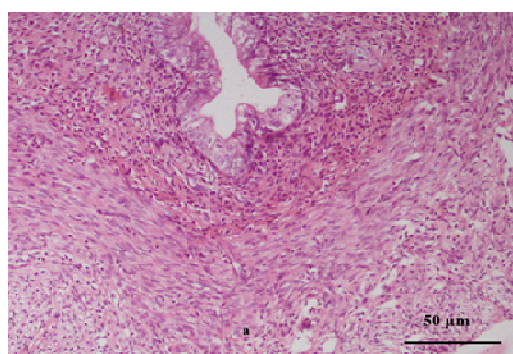
Data had been analyzed using SPSS version 15. Descriptive statistics were used to describe the data. An independent sample t-test was applied for the comparison of quantitative variables and chi-square test was applied for qualitative variables for the comparison of control and experimental groups. P-value < 0.05 was considered as significant.

## RESULT

Total 60 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 (Figure 2b). Vacuolization of the simple columnar epithelium was noted in the basal aspect of the cells. Some cells were larger in size and form balloon like bulges on the luminal surface. While 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



**Figure 2.** 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 =50µm.



**Figure 3.** Photomicrograph of a cross section from uterine horn of animal no 27 of experimental group a, showing increased amount of connective tissue, increased number of infiltrating cells in the stroma. H&E stain. Bar =50µm

pseudostratified with decrease in height as compared with control group (Figure 2d). The superficial part of endometrium consisted of few glands and abundant stroma and a deeper layer had many glands and relatively less stroma. The stromal cells were tightly packed, having basophilic cytoplasm. The infiltration of the stroma with the eosinophil was observed (Figure 3). Glands varied in size, some of them were dilated and

cystic. The glandular epithelium was pseudostratified. The lowest fraction of secretory vacuoles was observed in the glandular epithelial cells.

Average epithelium height in experimental group was significantly lower than that of the control group ( $p = 0.001$ ). Average area of endometrium in experimental group was lower in as compared to control group but this difference was not statistically significant ( $p = 0.063$ ).

**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
Area of endometrium ( $\mu\text{m}$ ) <sup>2</sup>	14125337 $\pm$ .51491	1680475 $\pm$ 0.1168956	0.063
Progesterone ng/ml	5.5 $\pm$ 0.8	2.8 $\pm$ 0.09	0.001
Estrogen pg/ml	41.7 $\pm$ 0.66	83.6 $\pm$ 1.2	0.001

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 long, we have studied the long-term effects of mifepristone treatment on rat endometrium. Average epithelium height was significantly lower in experimental group as compared to control group. 25 hours, following an injection of progesterone, a significant increase in the height of cells of the luminal epithelium is observed (Sonmez et al., 2000). 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 effect of the hormone (Ozcan et al., 1998). Most of the glandular epithelial cells had subnuclear vacuoles in various studies. Ultrastructurally the presence of large vacuoles, membrane inclusions and myelin like bodies was noted in the endometrial cells taken from treated animals, suggesting the disintegration of the cytoplasmic proteins (De Vivo et al., 2003). In treated animals, vacuolization is one of the structural indicators of energy deficit and permeability disorder of membranes in the endometrial cells (Gopalkrishnan et al., 1959). 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 (Brenner and Slayden, 1994).

Administration of antiprogesterone affected the morphology of the uterine glands. These glands were lined with pseudostratified or stratified epithelium in a large number of animals. The cells containing vacuoles were observed in the glandular cells in the experimental group. Mitotic figure was also found to be reduced in these glandular cells. In our study, morphometric analysis revealed a drastic reduction in the height of the endometrial glandular cells in experimental animals. The

nucleus in these animals, thereby indicating impaired cells had scanty cytoplasm with round oval or elongated glandular function following antiprogesterone treatment. 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 was low estrogen levels with complete suppression of ovarian follicular development (Jeffrey et al., 1998). This should have led to increased cell proliferation in the endometrial epithelial cells. However endometria in the experimental group showed decreased mitotic activity in the glandular and luminal epithelium (Van Look and Venherizen, 1994). Ovarian endometrial receptivity and reserves change with age (Yilmaz, 2010).

The area of the endometrium was larger in the control group than it is in the experimental group. As the epithelial cell height was reduced, the area of the endometrium was also reduced in the experimental group. In the present study, the long-term effect of exposure to mifepristone was decrease in height of luminal epithelium. Disruption in the nature of the extracellular matrix that mediates epithelial – stromal cell interactions and growth factor action required for uterine development are likely to cause these changes in the uterine wall (Baird and Gasied, 1999). Reduction in endometrial thickness and suppression of endometrial cell proliferation are seen with the use of mifepristone in women (Hargrov et al., 1989). Cyclical endometrial proliferation and differentiation and secretion during the human menstrual cycle are strictly controlled by estrogen and progesterone (Maxson, 1987). A balance between estrogen and progesterone production is required in endometrial cells (Schaison et al., 1985). In a study on ovariectomized rhesus macaques were treated for 5 months with either estradiol ( $E_2$ ) alone,  $E_2$  + progesterone (two doses) or  $E_2$  + onapristone.

0.01, 0.05 or 0.25 mg/kg) all doses of ZK blocked endometrial proliferation and induced endometrial atrophy (Murphy, 1994). 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 (Emre et al., 2010). Unopposed estrogenic stimulation, due to the long-term use of progesterone antagonists, may lead to endometrial hyperplasia and possible malignancies (Kafkasu et al., 1999). Female cancers under the age of 40 are the most common cancer of women during reproductive period (Ishwad et al., 1993).

Rats receiving long-term PR antagonist treatment show endometrial stimulation under the effect of estrogen (Edwards et al., 1995). Tamoxifen acts as an estrogen antagonist in the presence of high endogenous estrogen levels (Camerone et al., 1996). The nonhuman primate endometrium, however, demonstrates endometrial atrophy and evidence of antiestrogenic effects. High doses of mifepristone (25 and 50mg/d) lead to variable effects in women, such as atypical cystic changes, as have been described in eutopic endometrium (Grow, 1996). 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 (Slayden et al., 1998). No statistically significant change in the progesterone levels was observed in the 2-day follow-up using 200mg of mifepristone (Schreiber et al., 1983). 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 (Ishwad et al., 1993). The paradoxical effects (Mifepristone both raises and lowers progesterone levels) have also been explained by the hypothesis that mifepristone can act either by preventing the progesterone effect or in a way that is similar to that of progesterone, which always stimulates its own secretion by auto regulation. Depending on the duration of pregnancy, it is possible that these effects differ (Sitruk, 2000). The study on a model for human blastocyst-endometrial interactions showed anti-attachment properties after an antiprogesterone drug (Edward et al., 1995).

## CONCLUSION

Significant reduction in epithelial height was observed, but reduction in area was not significant in rats treated with mifepristone. Plasma concentration of estrogen was increased, while the plasma concentration of the progesterone was decreased. Our results suggest that long-term treatment of mifepristone affects the endometrial proliferation and induces histomorphological changes in the uteri of the experimental rats.

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