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EFFECTS OF ESTROGEN AND/OR PROGESTERONE ON THE CHANGES OCCURING IN THE UTERINE LUMINAL EPITHELIUM OF OVARIECTOMIZED RATS

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This study examined the histological changes that occurred in the uterine luminal epithelium after ovariectomy and subsequent administration of ovarian hormones. Female Wistar-albino rats that were 6 weeks of age were used. The rats were subject to bilateral ovariectomy and then estrogen and progesterone (2,5 mg/kg) were given alone or together. Effects of ovarian hormones on the uterine luminal epithelium were examined under the light microscope. In the ovariectomized and progesterone injected animals, the uterine epithelium atrophied and the luminal epithelial cell height decreased. In the estrogen injected animals the uterine tissue was more hyperemic and filled with more luminal fluid than in the other groups. Estrogen treatment induced a significant increase in the epithelial cell height and a marked thickening in the basement membrane of epithelial cells. No histologically identifiable changes were observed in the uterine luminal epithelial cells after estrogen + progesterone treatment, except for well-developed endometrial glands. This study suggests that the uterine luminal epithelial cell height increases with estrogen treatment, while the uterine tissue atrophies after ovariectomy and progesterone treatment.

Key words : estrogen, microscopy, ovariectomy, progesterone

INTRODUCTION

The ovarian steroids (estrogen and progesterone) have a variety of activities in the female reproductive tract. The action of ovarian hormones under the stimulus of the anterior lobe of the pituitary causes the endometrium to undergo cyclic structural modifications during the menstrual cycle (Jungueira *et al.* 1986).

Uterine epithelial cells are the first site of contact between maternal and fetal tissue at implantation, and their surface undergoes specific changes mediated by ovarian hormones in preparation for implantation (Psychoyos 1991). In the oviduct, estrogen elevates estrogen receptor and progesterone receptor levels and stimulates differentiation of a fully ciliated-secretory epithelium primed for gamete transport. These effects are suppressed by progesterone, so that, at the

end of menstrual cycle, estrogens receptors are suppressed and the oviducts (especially the fimbriae and ampullae) become dramatically atrophied, deciliated and nonsecretory. In the endometrium, estrogen elevates estrogen receptor levels as well as epithelial and stromal proliferation during the proliferative phase. During the luteal phase, progesterone inhibits estrogen receptor, suppresses proliferation in most glandular and stromal cells (except in the basalis and spiral arteries), and converts the tissue into a hypertrophied and secretory state able to support implantation (Brenner *et al.* 1990, Jungueira *et al.* 1986).

The synthetic derivatives of ovarian hormones are used commonly in clinical conditions, including hormone replacement therapy and for contraception (Martel *et al.* 2000, Schmidt *et al.* 2000, Van Uem *et al.* 1989). However, chronic exposure to these hormones causes uterine leiomyomas (Burroughs et al. 2000), endometrial gland adenogenesis (Gray *et al.* 2000), endometrial cancer (Povlet *et al.* 1997) and decidual neoplasms (Zook *et al.* 2001).

The purpose of this study was to examine the histological changes that occurred in the uterine luminal epithelial cells after ovarian steroid administration (estrogen and progesterone) following ovariectomy.

MATERIAL AND METHODS

The experimental studies were performed on female Wistar-albino rats which were 6 weeks of age. The rats were kept in a room lit for 12 h daily and the temperature was maintained at 21 ^{o}C. They were fed on a pelleted diet with water *ad libitum* in the plastic cages. The animals were divided into groups as shown below, each with 5 animals.

Group I: sham-ovariectomized control rats injected with 0.1 ml sesame oil subcutaneously. Group II: ovariectomized rats injected 0.1 ml sesame oil subcutaneously. Group III: ovariectomized rats injected with estrogen (2.5 mg/kg) in 0.1 ml sesame oil subcutaneously. Group IV: ovariectomized rats injected with progesterone (2.5 mg/kg) in 0.1 ml sesame oil subcutaneously. Group V: ovariectomized rats injected with estrogen (1.25 mg/kg) + progesterone (1.25 mg/kg) in 0.1 ml sesame oil subcutaneously.

All hormones were purchased from Sigma Chemical Company (17 β-estradiol, progesterone). Rats were ovariectomized bilaterally under intraperitoneal rompun (5 mg/kg) - ketamine (60 mg/kg) combination anaesthesia. After 7 days from the surgery, ovarian hormones were given daily to the animals for three weeks. Vaginal smears of rats in Group I were taken before they were killed and the rats showing regular 5-day estrous cycles with vaginal estrus were used as the control. At the end of the study period, all rats were killed under general anaesthesia (rompun-ketamine) and the uterus was removed.

For light microscopy, uteri were fixed in 10% formaldehyde, dehydrated in alcohol, embedded in paraffin and sliced at 5-6 μm thickness. Serial sections were stained with hematoxilen-eosin, Masson's trichrome and periodic acid Schiff (PAS)-Alcian blue staining. All the sections were examined under a BH-2 Olympus photomicroscope.

RESULTS

In the control groups it was observed that the uterine luminal epithelium was composed of nonciliated secretory columnar cells and ciliated cells. There were rare apoptotic cells between the luminal epithelial cells (Figures 1, 2).

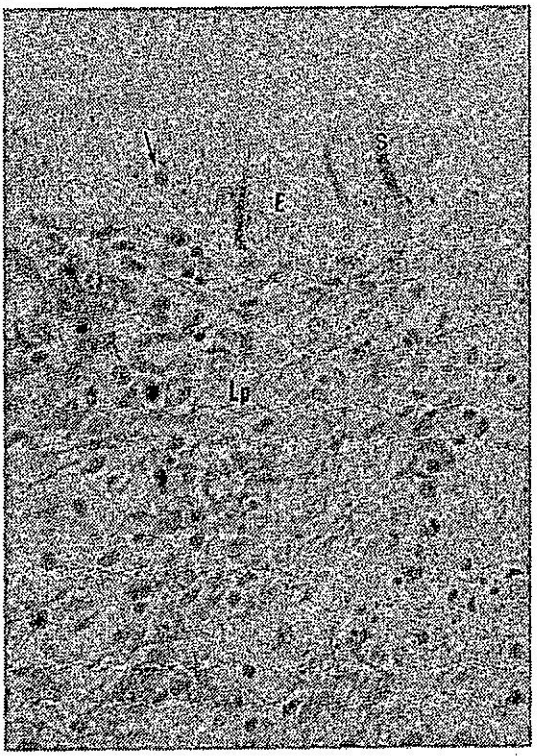

Figure 1. Luminal epithelial cells (E) of the rat uterus in control groups. →: apoptotic cells, s: secretory cells, Lp: Lamina propria. Masson's trichrome stain X 20.

In the ovariectomized and progesterone-injected animals, the uterus atrophied, luminal epithelial cell height decreased, and the uterine epithelium included low cuboidal cells with oval or ellipsoidal nuclei (Figures 3, 4).

In the uterine luminal epithelium of estrogen-injected animals, a significant increase in cell height was observed. The boundary between the epithelial cells could not be distinguished. Since the epithelial cell nuclei were at various levels, the uterine epithelium showed similarity to pseudostratified epithelium. The uterine tissue was more hyperemic and was filled with more luminal fluid than in the other groups. The uterine epithelium possessed well-developed secretory cells. There were numerous empty small vesicles between the epithelial cells (Figure 5), and marked thickening was observed in the basement membrane of epithelial cells (Figure 6).

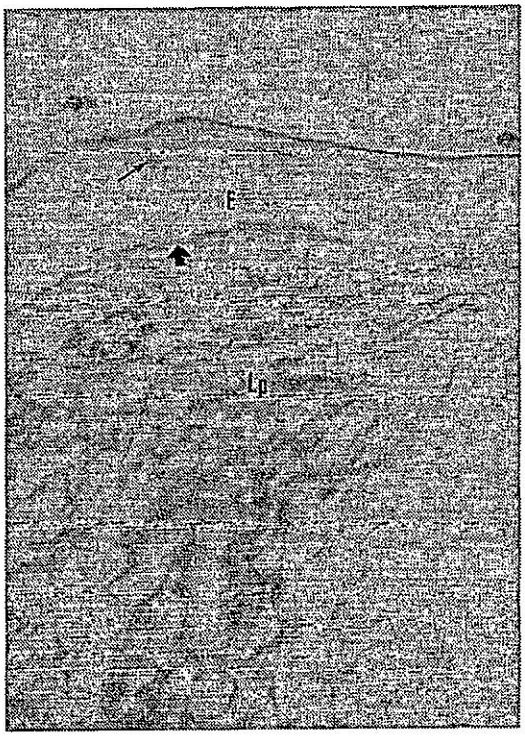

Figure 2. Luminal epithelial cells (E) of the rat uterus in control groups. : apoptotic cells, : Basement membrane, Lp: Lamina propria. PAS-Alcian blue stains X 20.

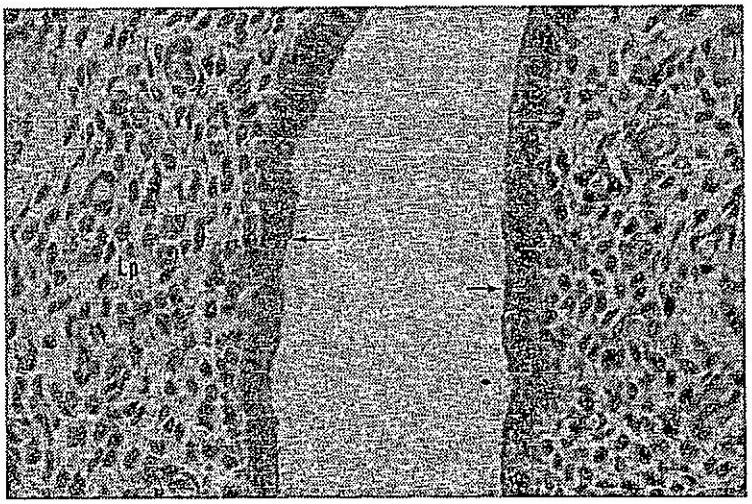

Figure 3. Luminal epithelial cells (→) of the ovariectomized rats. Lp: Lamina propria. Hematoxylin and eosin stains X 20.

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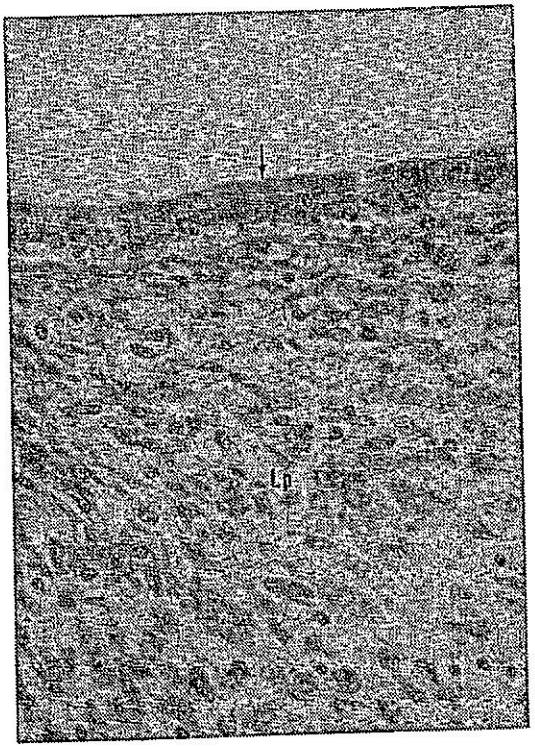

Figure 4. Luminal epithelial cells (→) of the progesterone-injected rats. Hematoxylin and eosin stains X 20.

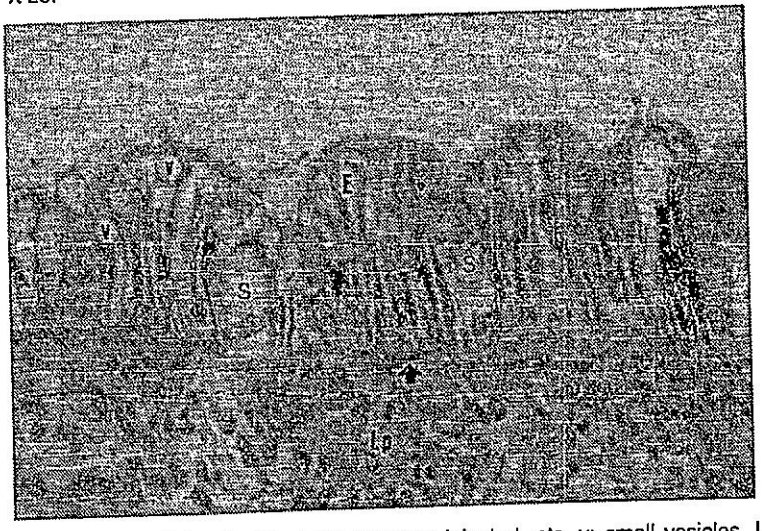

Figure 5. Luminal epithelial cells (E) of the estrogen-injected rats. v: small vesicles, Lp: Lamina propria, s: secretory cells, →: Basement membrane. Masson's trichrome stain X 20.

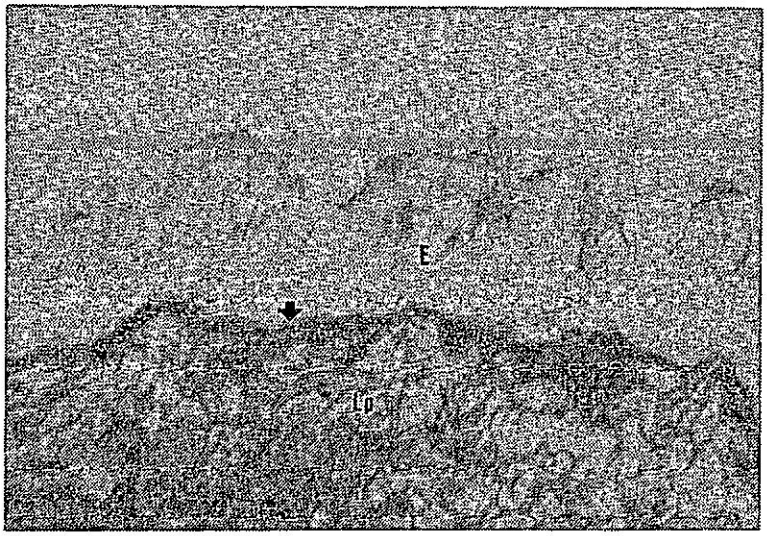

Figure 6. A thick basement membrane (➔) observed between epithelial cells (E) and lamina propria (Lp) of the estrogen-injected rats. PAS-Alcian blue stains X 20.

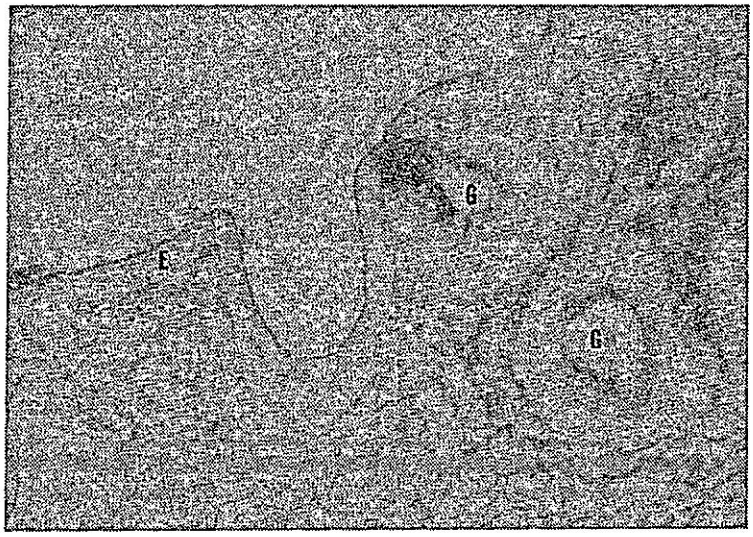

Figure 7. The endometrial glands (G) situated close to epithelial cells (E) and opened into uterine lumen observed after estrogen + progesterone treatment. PAS-Alcian blue stains X 20.

The uterine epithelial cell height of the groups given estrogen+ progesterone was similar to that seen in the control group. In this group it was observed that the endometrial glands were well developed, situated close to epithelial cells and opened into the uterine lumen (Figure 7).

DISCUSSION

It is well known that the ovarian hormones cause cyclic changes in cell morphology and histology of the female genital tract in mammals (Alkhalaf *et al.* 1992, Corbeil *et al.* 1985, Spornitz *et al.* 1994, Spornitz *et al.* 1999). To investigate the effects of these hormones, experimental studies have been made by numerous authors (Hosie and Murphy 1995, Murray 1992, Pastore *et al.* 1992, Williams and Rogers 1980).

Malini and Vanithakumari (1993) reported that the uterine weight, RNA, DNA and protein concentration increased significantly with estrogen administration following ovariectomy. They observed that the uteri of rats treated with estrogen were more distended with luminal fluid and more hyperemic than those of progesterone treated rats. In the present study, estrogen administration, but not progesterone administration, also induced hyperemia and luminal fluid accumulation in the uterine tissue.

Hosie and Murphy (1995) studied the uterine luminal epithelium of ovariectomized rats treated with a single minimal physiological dose of estrogen and progesterone under scanning electron microscope. They observed in the uterine tissue that progesterone administration resulted in an appearance similar to that seen in control groups However, estrogen administration caused hypertrophy and the cells appeared large, rounded and bulging with numerous long microvilli, while gland openings were more obvious than in the control groups. We noted marked gland openings in the estrogen + progesterone treated rats.

Slayden *et al.* (1993) showed that the oviduct atrophied after estrogen + progesterone treatment in ovariectomized monkeys while estrogen treatment stimulated the differentiation of both ciliated and secretory cells. They observed that the uterine endometrium attained a pseudopregnant state after estrogen + progesterone treatment while estrogen administration resulted in changes similar to the proliferative phase of the menstrual cycle. Our findings are in agreement with these results.

Grunert *et al.* (1982) reported a decrease in luminal epithelial cell height 6 hours after estrogen treatment but an increase in luminal epithelial cell height 24 hours after estrogen treatment in immature rats. In the present study also, the high doses of estrogen induced an increase in the uterine luminal cell height. An increase in luminal epithelial cell height following estrogen administration was also reported by Murray (1992). The morphologically low cuboidal epithelial cells of the uterine glands appeared to be synthetically inactive. Estrogen administration to ovariectomized sheep showed ultrastructurally that protein synthesizing organelles were well developed and the Golgi complex and rough endoplasmic reticulum were abundant when compared to progesterone treated animals. Epithelial cells of ovariectomized rats, both young and aged, which were polygonal in outline, flattened or even somewhat concave and had short microvilli were also found Craig and Jollie (1984). They observed that, following estrogen treatment, cells of both groups became bulged into the lumen and more round or oval than those of progesterone-treated rats. Moreover, they observed that cells in aged rats had longer microvilli than those in young rats after estrogen administration.

In addition to the above results, we observed a marked thickening in the basement membrane of epithelial cells after estrogen administration.

Hosie and Murphy (1992) noted hyperplasia and hypertrophy of the luminal epithelium, an increase in the length and density of microvilli and the formation of gland "hillocks" after estrogen treatment of ovariectomized rats. After progesterone treatment, they observed the development of pinopods and secretion droplets in the luminal epithelial cells. In the present study the formation of gland "hillocks" after estrogen administration and the development of pinopods and secretion droplets after progesterone administration were not observed. In contrast to our results, Williams and Rogers (1980) described an increase in cell volume and a dramatic increase in secretory granules after progesterone administration to ovariectomized rats.

Spornitz *et al.* (1994) studied the endometrial ultrastructure of the rat at exactly dated cycle stages. They described the most distinctive cycle stage as estrus. They observed ultrastructurally the secretory granules, the relatively large cell volume, the high number of autophagic vacuoles and the small number of relatively small lipid vacuoles during this stage of the cycle. They also supported their studies by monitoring the changes occuring during the cycle in the rat uterine surface epithelium under scanning electron microcope. They reported that the secretion rate of the uterine epithelium was the highest during estrus when characteristic cylindrical cells covered with long, dense microvilli were present. In the present study, we observed similar results in the estrogen injected animals. They determined biochemically that the estrogen concentration reached a peak during proestrus and not during estrus. This condition seems to be paradoxical when compared to our results. These sometimes conflicting results may come from investigators using ovariectomized rats supplemented with different amounts of exogenous hormones.

In conclusion, these findings indicate that the uterine epithelial cells of ovariectomized rats undergo the changes with estrogen treatment but regress after ovariectomy and progesterone treatment. Thus, there is a requirement for both estrogen and progesterone for the development of uterine glands.

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PROUČAVANJE UTICAJA ESTROGENA I/ILI PROGESTERONA NA PROMENE EPITELA UTERUSA OVARIJEKTOMISANIH ŽENKI PACOVA

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja histoloških promena u materici ženki pacova posle ovarijektomije i tretmana estrogenom i/ili progesteronom. Kod ovarijektomisanih jedinki, tretman progesteronom je dovodio do atrofije endometrijuma i smanjivanja visine epitelnih ćelija. Tretman estrogenima je imao za posledicu hiperemiju, povećanje visine epitelnih ćelija i zadebljanje bazalne membrane. Nakon istovremenog tretmana progesteronom i estrogenima uočeno je samo uvećanje endometrijalnih žlezdanih ćelija.