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Crescent Journal of Medical and Biological Sciences
Vol. 4, No. 1, January 2017, 28–31
eISSN 2148-9696

The Assessment of the Ultrastructure of Luminal Epithelium in the Endometrium After the Application of Antiprogesterone

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Abstract

Objective: There is growing interest in the use of progesterone receptor modulators (e.g., mifepristone) for the treatment of gynecologic and other conditions. The aim of this study was to investigate the effects of antiprogesterone (RU486) on the ultrastructure of endometrial in the super ovulated mice at implantation window.

Materials and Methods: Adult male and female mice were used for the induction of pseudopregnancy. The mice were divided into two experimental and control groups. The Experimental group was further subdivided into two groups based on hormone injection: 1) antiprogesterone and 2) hyperstimulation + antiprogesterone. The control group did not get any hyperstimulation. All groups' uteruses were collected after 4.5 days of pregnancy and were prepared for the assessment of the histological changes with light microscopy (LM) and Transition Electron Microscopy (TEM).

Results: The results showed that antiprogesterone injection decreases the height of microvilli and pinopods in the apical cell in comparison to the control group. Antiprogesterone injection increases the inflammatory cells in the lamina propria of endometrium. The histomorphometrical results indicated that endometrial luminal epithelium height in the hyperstimulation + antiprogesterone group was significantly different compared to the control group ($P<0.01$).

Conclusion: These findings demonstrate that the use of mifepristone is associated with histological changes in the endometrium.

Keywords: Endometrium, Antiprogesterone, Superovulation, Ultrastructure.

Introduction

Implantation of the blastocyst in the endometrium starts with blastocyst attachment and ends with the formation of placenta (1). Successful implantation depends on the embryo quality, endometrial receptivity, synchronization of the embryo development, and endometrial maturation. Failure in the endometrial receptivity is responsible for the two-thirds of infertilities (2). Pinopods are the ultrastructural of luminal epithelium that appear in the implantation window and have been first discovered in rats and mice (3). They appear on the 19th day and become fully developed on the 22nd day (4). Research has shown that the expression of HOX-10 is a homo-box gene required for maturation and growth of pinopods (5). Endometrial receptivity for blastocyst implantation is controlled by ovarian steroid hormones. In the endometrial receptivity phase, morphological and biochemical changes occur in luminal epithelium (6). In the adult mouse, estrogen causes the proliferation of luminal epithelial cells and inflammation of the stroma, and progesterone has an antiproliferative role in the epithelium and proliferative and anti-inflammatory roles in the stroma. Antiprogesterones (e.g., mifepristone, onapristone) inhibit progesterone functions. The use of antiprogesterone indicates the progesterone receptor role in the regulation molecules such as growth factors, peptide hormones, metabolic enzymes, protease inhibitors, molecules involved in the immune, skeletal proteins and cell adhesion molecules (7).

Antiprogesterone mifepristone is used for abortion in the first trimester of pregnancy. A low dose of it serves as a contraceptive and is used in bleeding therapy in woman. It also inhibits the fertilization and transfer of embryos from the uterine tubes to endometrial (8). Studies on monkeys and rats show that the injection of anti-progesterone in the Rhesus monkey could reduce the fetal growth and development especially in early pregnancy, as monoclonal antibodies against progesterone injections in rats can cause fetal growth (9). The drug also causes the cervix to dilate, releasing prostaglandins and increasing androgen sensitivity of the myometrium to the contractile effects of prostaglandins (8). Mifepristone delays the development of uterine glandular epithelium, reduced Insulin-like

Received 24 February 2016, **Accepted** 30 August 2016, **Available online** 28 September 2016

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growth factor (IGF), glycodelin, integrin 3β, ultimately preventing implantation by inhibiting endometrial maturation (10).
The present study aims at assessing the effects of mifepristone on mice endometrium after hyperstimulation.

Materials and Methods

Adult male and female mice were used for the induction of pseudopregnancy. The mice were divided into two experimental and control groups. The male mice in the experimental groups were superovulated by injection of a single dose of 10 IU PMSG (pregnant mare serum gonadotropin), and after 48 hours, 10 IU hCG (human chorionic gonadotropin). The mice were mated with the vasectomies mice to produce pseudopregnancy. The experimental group was subdivided into two groups based on hormone injection: 1) Antiprogesterone group: the pseudopregnant mice that were not superovulated and received daily injection of antiprogesterone 1 mg for 4 days. 2) Hyperstimulation + antiprogesterone group: the pseudopregnant mice that were superovulated and received daily injection of antiprogesterone 1 mg for 4 days.
The control group did not get any hyperstimulation. Female mice in the control and experimental groups were housed over night with vasectomised males, and the presence of vaginal plaque was checked the on following morning.

Tissue Preparation

The animals in all groups were sacrificed by cervical dislocation after 4.5 day of pseudopregnancy. The samples were obtained from 1/3 middle part of uterine horns and were fixed in the formaldehyde and embedded in paraffin wax. After preparation of 5 µm sections, the sections were stained with hematoxylin and eosin (H & E) and studied by light microscopy (LM).
To assess the ultrastructural changes using transition electron microscopy (TEM), the samples were fixed in the glutaraldehyde and osmium tetroxide 1% and were embedded in the resin.

Statistical Analysis

The data obtained from each group were fed into SPSS software and analyzed by using the one-way analysis of variance (ANOVA) test.

Results

The results obtained from the study were presented in two parts: LM and TEM (Figures 1 and 2).

Histomorphometrical Results

The analysis of the endometrial luminal epithelium height in the control and experimental groups through ANOVA test indicate that there is a significant interaction between the control and experimental groups ($P=0.001$). The endometrial luminal epithelium height in the hyperstimulated + antiprogesterone group was significantly different in comparison to control group ($P=0.001$), but in the antiprogesterone group there was no significant correlation in comparison to control group ($P=0.90$; Table 1).

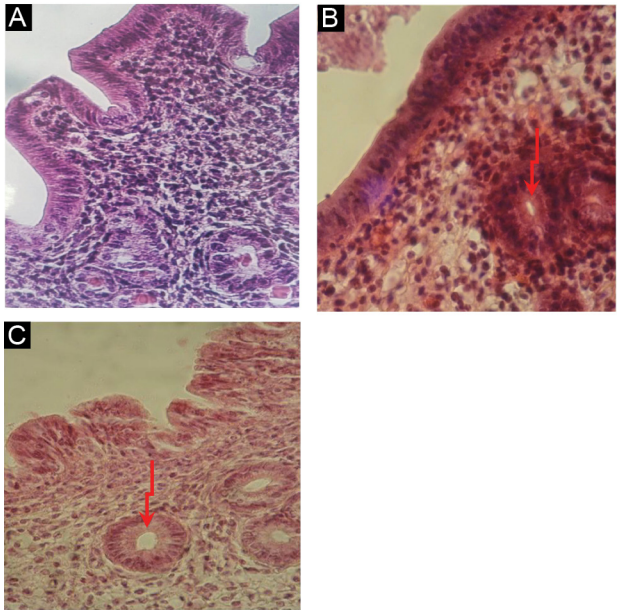

Figure 1. The Light Microscopic Photographs of Endometrium in the Groups of Study. A) Control group. The luminal epithelium is columnar. Pay attentaion to glandular secratio (H&E staining ×400). (B) Antiprogestron group: Pay attentaion to glandular epithelium and decreasing of glandular secration (H&E staining ×400). C) Hyperstimulated + antiprogesterone group: The luminal epithelium is pseudostratified. Pay attentaion to luman if uterine glands.

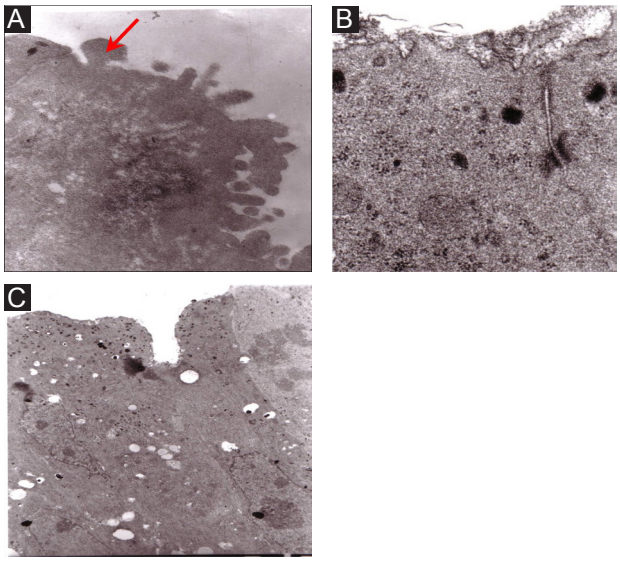

Figure 2. The EMT Microscopic Photographs of Endometrium in the Groups Involved in the Study. (A) Control group: The luminal epithelium contains of pinopods. (B) Antiprogesterone + hyperstimulated group. (C) Antiprogesterone group: The microvillus disapeared in the apical cells.

Discussion

In the menstrual cycles, the endometrium is changed for implantation. These changes that prepare the uterus for implantation are referred to as the implantation window. During the implantation, the apical membrane of the lu-

Table 1. Morphometrical Assessment of the Luminal Epithelium in the Control and Experimental Groups

Morphometrical Parameter	Control Group	Antiprogesterone	Antiprogesterone After Hyperstimulation	*P* Value
Height of luminal epithelium	22.23 ± 1.46[a]	22.54 ± 0.80[a]	28.74±4.04[b]	<0.05

Note: Different superscripts in the table show significantly difference ($P<0.05$) between groups.

minal epithelium is changed (11).

On the first and second days of gestation in rats and mice, the microvillus in the apical membrane of epithelium is thin and long, and then gradually become shorter and irregular. On the fourth day of pregnancy, pinopods appear in the apical membrane of luminal epithelium (12). In the rat and mouse, the implantation window lasts for only about 24 hours on days 4–5 of pregnancy, and endometrial receptivity is dependent on the estrogen and progesterone hormones (13). The duration of the implantation window in the natural cycle is 20-22 days, and in the IVF technique, 17-18 cycles (14). The roles of pinopodes in the humans are unknown, although it appears that they are involved in binding the blastocyst to endometrial (15). Administration of exogenous gonadotropic hormones such as human menopausal gonadotropins (hMG) and hCG leads to increased secretion of estrogen and progesterone. Steroid hormones and their receptors have been suggested to be involved in the regulation of pinopod formation (16).

The results of the present study showed that ovarian hyperstimulation induced changes in the endometrium, increasing the luminal epithelium in comparison to the control group. The morphology of endometrial in the control and experimental groups are demonstrated in Figure 1. Doing morphological and morphometrical analyses in the RU 486 group, the inflammatory and pyknotic cells were spotted in the stroma. This result confirms that decidual reaction is dependent on progesterone and estrogen. Qamar et al showed that there was an increase in the number of infiltration of granulocytes and eosinophils in the endometrial stroma after the application of antiprogesterone in the rat (17).

The use of RU 486 after hyperstimulation showed increased height of luminal epithelium in comparison to RU 486 group. The shape of luminal epithelium was irregular and the endometrial stroma contained inflammatory cells. This suggests that ovarian hyperstimulation could induce morphological changes in the luminal epithelium. On the other hand, Nikas showed that ovulation could not have an influence on the formation of pinopod in the endometrium (15). The findings of this study showed that progesterone is essential for growth and development of endometrial stromal cells. The results obtained from ultrastructural assessment of luminal epithelium in the different groups showed redundancies of pinopods in the luminal epithelium in the control group (A). Ultrastructure study of the hyperstimulated + antiprogesterone group indicated that the hight of microvilli decreased in comparison to the control group (B). In the antiprogesterone group, the microvillus disapeared in the apical cells (C).

Conclusion

The results of this study showed that the use of antiprogesterone and hyperstimulation could decrease the growth of pinopod and change the shape of luminal epithelium. Also, antiprogesterne could decrease the glandular diameters and glandular secretions.

Ethical Issues

The Ethical Committee of Islamic Azad University approved this study.

Conflict of Interests

The authors declare no conflict of interests.

Financial Support

None.

Acknowledgments

This research was done in Histology Department of Faculty of Medicine, Islamic Azad University, Tabriz and Shahid Beheshti University. We are grateful to the staff for their help and support.

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