Mouse Endometrial Glycocalyx Alteration After Ovarian Hyperstimulation and Progesterone Injection

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Abstract

Introduction: The aim of this study was to determine mouse endometrium glycocalyx cell surface alteration after ovarian hyperstimulation using hMG, hCG and daily progesterone injection at implantation time.

Materials and Methods: For this purpose adult NMRI mice were superovulated using hMG, hCG and then daily injections of progesterone (1 mg/mouse) performed in one group. The animals were sacrificed by cervical dislocation 3.5 and 4.5 days after hCG injection. Tissues were obtained from 1/3 middle part of uterine horns and processed for light and transmission electron microscopic studies.

Results: Our results showed that the intensity of PAS reaction on surface epithelium of the control and the hyperstimulated groups increased 4.5 days after mating or hCG injection, but in the hyperstimulated and progesterone injected group there were intense PAS reaction on the 3.5 day after hCG injection. A well defined glycocalyx was observed on the epithelial cell surface of the control group and the hyperstimulated group, there were a lot of long cylindrical microvilli on their epithelium but after progesterone injection some of the microvilli and glycocalyx disappeared and there were some cytoplasmic projections.

Conclusion: Thus ovarian hyperstimulation caused alteration in the glycocalyx and these changes may affect the electrical charge of the endometrium at the implantation period. Thus these changes could have influenced endometrial receptivity.

Key words: Ovarian hyperstimulation, Glycocalyx, mouse endometrium, Progesterone

Introduction

During embryo implantation, uterine epithelial cells are the first sites of contact between maternal and fetal tissues. Both of these surfaces undergo specific morphological and ultrastructural changes, which are mediated by ovarian hormones (1, 2). There are a lot of interdigitations between the microvilli of trophoblast and the endometrium during adhesion and attachment stages of implantation (3). In preparation of the endometrium for blastocyst adhesion and attachment, not only the microvilli alter in shape, size and number, but also the glycocalyx on the surface of this structure undergoes some changes (4, 5, 6).

The endometrial microvilli are long, thin and regular in shape at the preimplantation period (oestrus of rat or mice), but they transform to short and irregular projections at implantation time (7). This alteration is due to actin microfilament changes which occupy their axis (8, 9). The endometrium becomes more adhesive during the reception of blastocyst. Cell adhesion molecules such as integrins and some carbohydrates and mucins are involved in this attachment (10).

Previous studies demonstrated that under the influence of ovarian hormones, the endometrium undergoes changes in the distribution and synthesis of carbohydrates (5, 11). It was shown that some negatively charged glycocalyx of trophoblast and uterine epithelial cell surfaces decreased at implantation (12), whereas some data showed that synthesis and expression of specific carbohydrate such as lactosaminoglycan, heparan sulfate proteoglycan and glycosaminoglycans increased during the period of attachment (13,14).

The synthesis of these carbohydrates was stimulated by estrogen (13). After ovarian hyperstimulation, estrogen secretion was in the supraphysiological levels, and the high level of these hormones may affect the expression of carbohydrates on the endometrial surface. Alterations in the levels of ovarian hormones may alter uterine receptivity (15).

The aim of this study was to determine glycocalyx alteration of mouse endometrium during preimplantation and implantation after ovarian hyperstimulation and progesterone injection using Periodic Acid Schiff´s reaction and transmission electron microscopic studies.

Materials and Methods

* *Animals*

Females NMRI mice aged 6-10 weeks were housed under 12h light: 12h dark condition.These were randomly divided into three groups:

Group 1: Hyperstimulated mice: The female mice in this group were superovulated using an intraperitoneal injection of 10 i.u. human menopasual gonadotropic hormone (hMG), which was followed 48h latter by another injection of 10 i.u. human chorionic gonadotropic hormones (hCG). In the evening of the second injection, the mice were rendered pseudopregnant by mating artificially using a plastic soup, which was turned on the vaginal orifice several times.

Group 2: Hyperstimulated mice with progesterone administration: After mice superovulation in the same manner as the previous group, daily subcutaneous injections of progestrone (1 mg/mouse) were performed (16).

Group 3: Control group: The female mice were rendered pseudopregnant by mating artificially similar to the hyperstimulated group.

* *Tissue preparation*

Ten mice from each group were sacrificed by cervical dislocation on 3.5 and 4.5 day after hCG injection or mating. The samples were obtained from the 1/3 middle part of uterine horns immediately. For light microscopic study, the tissues were fixed in formaldehyde and embedded in paraffin wax. Six micrometers sections were prepared and stained with Periodic Acid-Schiff´s (PAS) reaction.

Other tissues segments were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer (pH. 7.2) at 4°C for 1.5 hour and post fixed in 1% osmium tetroxide for 1 hour. After the tissues were dehydrated in ascending ethanol concentration follwed by acetone they were embedded in epon812 resin. Semi-thin and ultra-thin sections were stained with 1% toluidine blue, and alcoholic uranyl acetate as well as aqueous lead citrate, respectively. Then they were examined using

Zeiss transmission electron microscope.

Results

At the light microscopic levels, the PAS reaction of endometrium was evaluated on the surface and glandular epithelium, and on the stroma of the endometrium. The summary of these reactions are shown in table 1.

Table 1: Evaluation of PAS Reaction in the mouse endometrium.

Treatment	Days after hCG or Mating	Surface Epithelium	Glandural Epithelium	Strom	Secretion
Control	3.5	1+	1+	1+	not visible
	4.5	4+	3+	4+	not visible
Hyperstimulatied	3.5	1+	1+	1+	not visible
	4.5	4+	3+	2+	not visible
Hyperstimulatied and pro. injected	3.5	2+	3+	1+	not visible
	4.5	2+	2+	2+	visible

The intensity of PAS reaction scored between 1+ to 4+ according to the colorless to pink, Pro. (Progestrone).

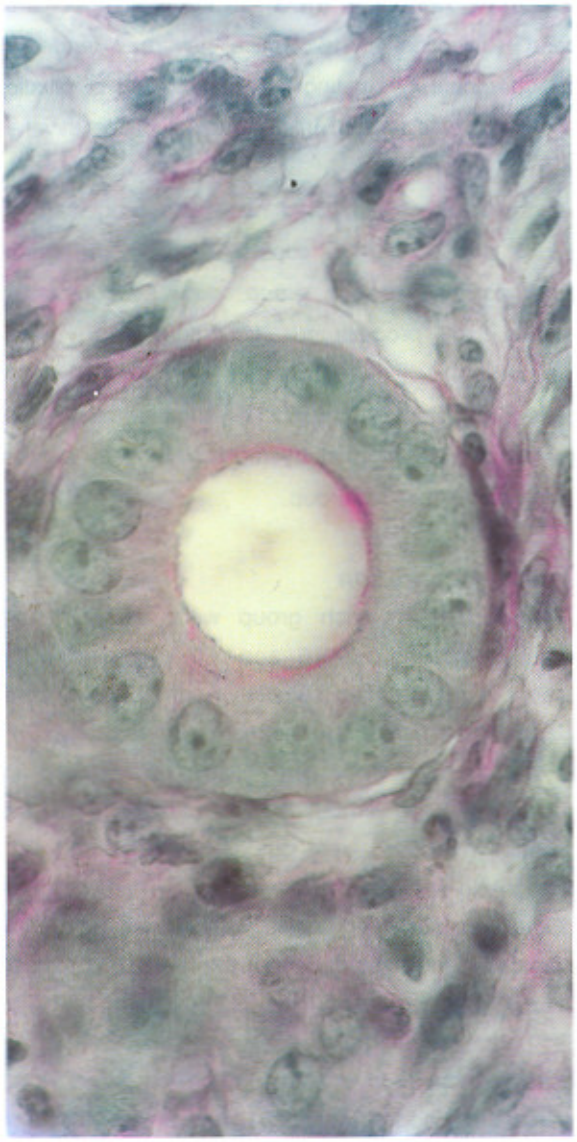

Fig 1: PAS Reaction in the control mouse endometrium on 4.5 days after pseudopregnancy (X 1000).

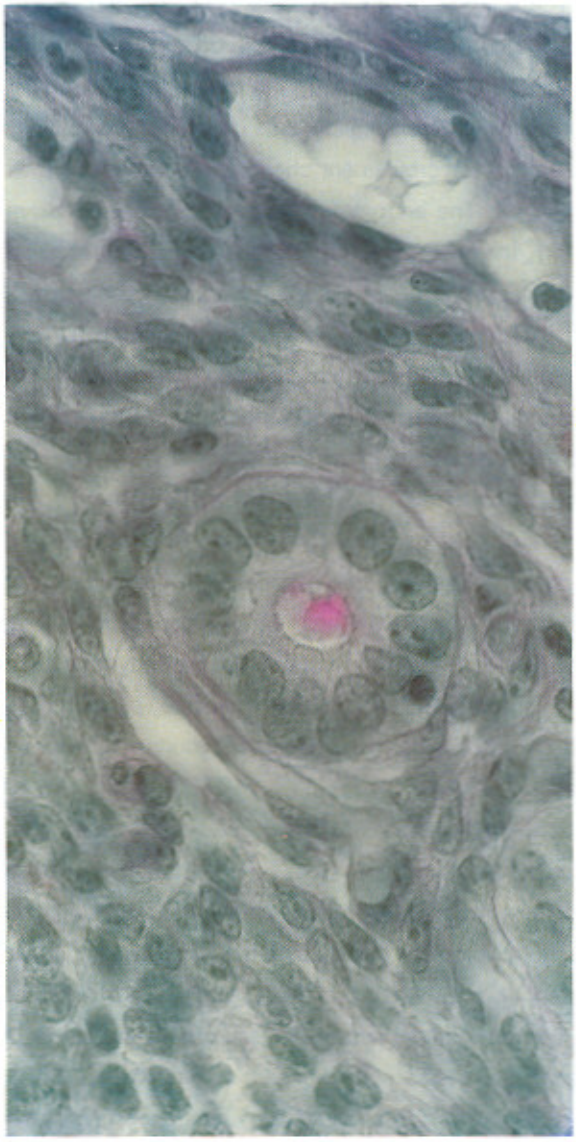

Fig 2: PAS Reaction in the hyperstimulated progesterone injected mouse endometrium on 4.5 days after pseudopregnancy (X 1000).

Our results showed that on the 3.5 day after mating or hCG injection in the three groups, the reaction was weak in the stroma and surface epithelium but on the glandular epithelium of the progesterone injected group the reaction was stronger than in the other groups. In contrast on 4.5 day after mating or hCG injection, which was due to implantation time in mice, the intensity of PAS reaction in the controls (Fig 1) and the hyperstimulated groups was stronger than that in the hyperstimulated and progesterone injected groups (Fig 2).

Electron micrographs showed that there were dark and clear types of cells in surface and glandular epithelium. These cells had a lot of projections, some were typical microvilli and the others were irregular swollen projections, which assumed pinopodes.

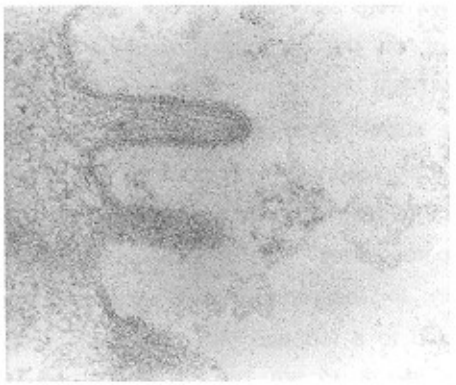

Fig 3: Electron micrograph of surface of control mouse endometrium on 3.5 days after pseudopregnancy (X 144,500).

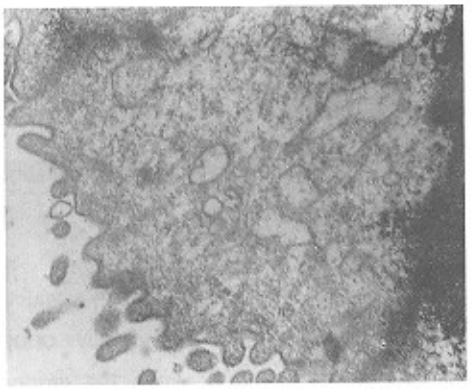

Fig 4: Electron micrograph of surface of hyperstimulated progesterone injected mouse endometrium on 3.5 days after hCG injection (X 51000).

In all groups the normal microvilli were seen (Fig 3), but 3.5 day after hyperstimulation and progesterone injection these structures disappeared although some smooth and swollen projections were abundant (Fig 4).

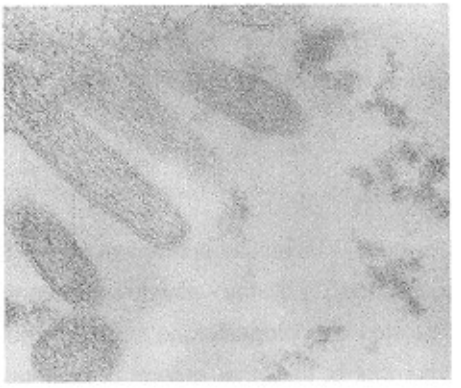

Fig 5: Electron micrograph of surface of hyperstimulated progesterone injected mouse endometrium on 4.5 days after hCG injection (magnification 144,500).

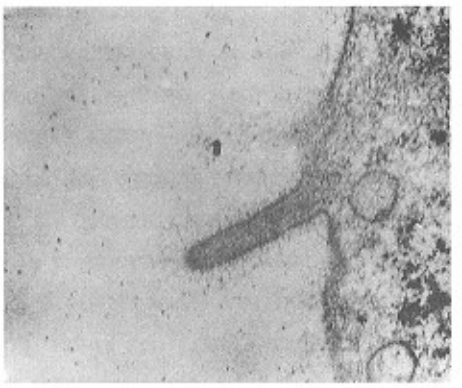

Fig 6: Electron micrograph of surface of control mouse endometrium on 4.5 days after pseudopregnancy (X 85,000).

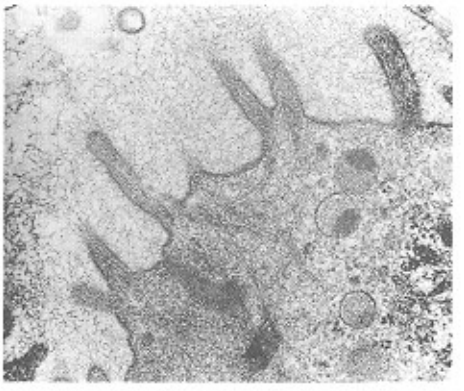

Fig 7: Electron micrograph of surface of hyperstimulated mouse endometrium on 4.5 days after hCG injection (X 85000).

It seems that in this group after 4.5 days of hCG injection microvilli reformed (Fig 5), on the other hand,

the life span of smooth projections was short time (about 24 h).

The glycocalyx on the endometrial microvilli were seen in the controls (Fig 6) and the hyperstimuated groups (Fig 7), but in the hyperstimulated and progesterone injected groups they were not visible (Fig 5).

Discussion

In this study, our results showed that PAS reaction on the surface and the glandular epithelium of hyperstimulated and progesterone injected groups was higher than that of the other groups at preimplantation time (3.5 days after hCG injection or mating). The intensity of this reaction was almost similar to that at the implantation time in all groups. Thus, it seems that the presence of carbohydrate or mucus substances on the endometrial surface is more dependent on the progesterone than the estrogen. Thus, the progesterone may affect the premature expression of mucuidal substances on the endometrium surface. No noticeable differences were seen between the presence of PAS positive substance in the stroma of all groups, although this reaction was stronger on the control group at the implantation time. Thus in this group, the decidual reaction may have occurred better than the other groups.

The comparison of apical membrane of all groups showed that the microvilli were present in the all groups although the number of these structures had some changes but on the hyperstimulated and progesterone injected groups, the microvilli disappeared and other projections were seen without any glycocalyx on their surfaces. Our scanning electron microscopic studies showed that these projections were typical pinopodes or fungiform projections, which are implantation markers (unpublished data). It seems that in this group the microvilli decreased in number and length by fusion of these microvilli. Expression of smooth projections (pinopodes) is depended on the progesterone. Because, in the hyperstimulated groups without any progesterone injection, these structures were not seen 3.5 days after hCG injection. The number of these projections was lower than that of the other groups 4.5 days after hCG injection thus the ratio of estrogen to progesterone is critical for their expression. In agreement with our results, some investigators showed that pinopodes were progesterone dependent structures (17, 18), high dose of estrogen inhibits both pinopodes expression and implantation (19). This phenomenon occurred during ovarian hyperstimulation.

Also our results showed that the glycocalyx developed on the surface of the control and the hyperstimulated groups at the implantation time and there were no carbohydrates in the progesterone injected groups. Thus, our results showed that the glycocalyx were seen only on the microvilli and they were not on the smooth projections. Farach and Murphy (1988) showed that synthesis of proteoglycan, heparan sulfate and some carbohydrates increased during implantation (14). In contrast previous reports showed that exogenous administration of gonadotropin as superovulatory agents was associated with reduction in the endometrium glycocalyx and it contributed to a reduced the endometrium receptivity for blastocyst (5, 12, 20). The decrease in carbohydrate binding to the epithelial surface is believed to be due to disruption in the progesterone:estradiol ratio, which was reported in animals undergoing hyperstimulation. Some investigators believed that disappearance of negatively charged glycocalyx from the apical epithelial membrane could be effective in negative charge reduction of the endometrium during implantation, also carbohydrates have essential role in the cell-cell adhesion especially during implantation; they were important for embryo attachment. The reduction or changes in the carbohydrates or glycocalyx of cell coat could affect the interaction between the embryo and the uterus and their attachment, thus this reduction after ovarian hyperstimulation and progesterone injection is due to decrease in uterine receptivity.

References

1. Leesey BA: The role of the endometrium during embryo implantation. Hum Reprod 2000;15: 36-50

2. Janne OA: Progesterone action in mammalian uterus. Acta Obstet Gynecol Scan Suppl 1981;101: 11-16
3. Lessey BA: Endometrial receptivity and the window of implantation. Bailieres Best Pract Res Clin Obstet Gynaecol 2000;14: 775-880.
4. Enders AC, Schlafke S: A morphological analysis of the early implantation stages in the rat. Am J Anat 1967; 120: 185-226
5. Chavez DJ, Anderson TL: The glycocalyx of mouse uterine luminal epithelium during oestrus, early pregnancy, the peri implantation period and delayed implantation. I: Acquisition of Ricinus Communis I binding sites during pregnancy. Biol Reprod 1985; 32: 1135-1142
6. Duncan DA, Mazur MT, Younger JB: Glycocongugate distribution in normal human endometrium: lectin histochemical study. Int J Gyn Path 1988; 7: 236-241.
7. Nikas G, Makringlannakis A, Hovatta O, Jones HW: Surface morphology of the human endometrium: Basic and clinical aspects. Ann New York Acad of Sci 2000; 900: 316-324
8. Luxford KA, Murphy CR: Reorganization of the apical cytoskeleton of uterine epithelial cells during early pregnancy in the rat: a study with myosin subfragment 1. Biol Cell 1992; 74: 195-202
9. Murphy CR, Luxford KA: Plasma membrane transformation: A common response of uterine epithelial cells during the peri-implantation period. Cell Biol Int 1994; 18: 1115-1128
10. Lessey BA, Catelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, et al.: Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab 1994; 79: 643-649
11. Lee HC, Damjanor I: Pregnancy related changes in the human endometrium revealed by lectin histochemistry. Histochem 1985; 82: 275-280
12. Peverini S, Kramer : Superovulation affects glucosamine trisaccharides in the glycocalyx of the rat endometrium at the time of implantation. J Anat 1995; 187: 487- 490
13. Murphy CR, Rogers AW: Effects of ovarian hormones on the cell membrane in the rat uterus III. The surface carbohydrates at the apex of the luminal epithelium. Cell Biophys 1981; 3: 305-320
14. Murphy CR, Turner VF: Glycocalyx carbohydrates of uterine epithelial cells increase during early pregnancy in the rat. J Anat 1991; 177: 109-115.
15. Ertzeid G, Storeng R: The impact of ovarion stimulation on implantation and fetal development in mice. Hum Reprod 2001; 16: 221-5
16. Miller BG: Delayed interactions between progesterone a low doses of 17 ß-estradiol in the mouse uterus. Endocrinol 1979;104: 26-33
17- Develioglu OH, Hsiu JG, Nikas G, Toner JP, Oehninger S, Jones HW:Endometrial estrogen and progesterone receptor and pinopodes expression instimulated cycles in oocyte donation. Fertil Stril 1999;71: 1040-1047
18. Stavreus-Evers A, Nikas G, Sahlin L, Eriksson H, Landgren BM: Formation of pinopodes in human endometrium is associated with the concentrations of progesterone and progesterone receptors. Fertil Steril 2001; 26: 782-791
19. Martel D, Monier MN, Roche, D, Psychoyos A: Hormonal dependence of pinopodes formation at the uterine luminal surface. Hum Reprod 1991;6: 597-603
20. Kramer B, Wet G: Exogenous gonadotropin administration affects the glycocalyx of rat endometrial epithelial cells during the period of implantation. J Assis Reprod Gen 1994; 11: 504-509