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The ovarian stimulation effects on Muc1 expression of the mouse endometrium before implantation

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Abstract

Background: Acceptance of uterus and reaction between endometrium and embryo has an important role for implantation. Muc1, an integral membrane mucin, is expressed on the apical surface of uterine epithelial cells and could have effects on its receptivity.

Objective: The aim of this study was to evaluate the changes in Muc1 expression of gravid mouse endometrium with and without hyperstimulation before implantation.

Materials and Methods: Adult female NMRI mice were divided into control and experimental groups. Experimental group superovulated using an intraperitoneal injection of Pregnant Mare's Serum Gonadotrophin (PMSG) followed 48 hours later by another injection of Human Chorionic Gonadotropic hormone (HCG). The female mice have mated with normal male mice. All control and hyperstimulated groups subdivided into six groups. After mating, female mice were examined by vaginal plaque as day of zero and in 0-5 days after copulation, they were sacrificed by cervical dislocation. Then the middle 1/3 parts of their uterine horns were obtained and stained by immunohistochemicaly technique for Muc-1 detection.

Results: Our results showed that in the control and hyperstimulated groups, the Muc1 expression is markedly reduced in the luminal uterus epithelium at the time of implantation. Furthermore, luminal and glandular uterus epithelium did not exhibit the same decrease in Muc1 expression during the receptive phase.

Conclusion: Ovarian hyperstimulation didn't alter the Muc1 expression markedly in surface and glandular epithelium of endometrium, which could affect on its receptivity.

Key words: *Endometrium, Muc1 expression, Ovarian stimulation.*

Introduction

The implantation process involves complex and synchronized molecular and cellular events between the uterus and the implanting embryo. Implantation occurs only during a certain time in pregnancy referred to as the window of implantation (1). The opening of this window and process of implantation are known to be controlled by ovarian steroid hormones (2).

The receptive status of the Endometrium in embryonic implantation is a balance between the activation of adhesion molecules and the presence of a barrier that the embryo may encounter on the endometrial epithelium (3). The nonreceptive uterus maintains a thick glycocalyx on the apical surface of luminal epithelial cells (4). Within this carbohydrate mixture is a transmembrane mucin, mucin-1 (Muc1).

Muc1 is an extremely large (>200 kDa), heavily glycosylated molecule that is proposed to extend much farther from the luminal surface than other components of the apical glycocalyx (5). Muc1 may inhibit the interaction between trophoblast and apical epithelium adhesion molecules at the time of implantation, giving the possibility of forming a uterine barrier for implantation.

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It is suggested that Muc1 acts as an antiadhesive molecule on the uterine surface, thus preventing embryo implantation (6). Several lines of evidence suggest that Muc1 expression can be modulated by hormones (7).

Sex steroids can be involved in the regulation of Muc1 transcription either by directly interacting with the Muc1 promoter or indirectly by stimulating or repressing of the transcription factors (3).

Muc1 could allow a local mechanism to contribute to the receptivity of the endometrium. In the endometrium, Muc1 extends beyond the glycocalyx and is probably the first molecule that the embryo encounters on its route to attachment. Muc1 acts as an anti adhesive molecule in the uterus (14).

There is hypothesis that ovarian stimulation (OS) would induce different biological and molecular profiles of endometrium that might lead to altered endometrial receptivity and implantation outcome (19).

The purpose of this study was to investigate the alterations on Muc1 expression of the mouse endometrium after hyperstimulation using HMG and HCG injections. Therefore, a careful evaluation of the regulation of Muc1 at the endometrial surface is necessary.

On the basis of the presence of Muc1 related epitopes in the uterine epithelium we speculated that Muc1 gene expression could be modulated by the physilogical changes in the mouse uterus. Reports on the presence of the Muc1 molecule in the endometrium led us to examine the pattern of Muc1 expression in gravid mouse endometrium with and without hyperstimulation before implantation.

Materials and methods

In this experimental research, female virgin NMRI mice, aged 10-12 weeks, were cared for and used according to the guide for the care and use of laboratory animals. They were housed under 12 h light: 12 h dark condition. They were randomly divided into experimental and control groups. Experimental group (group A) superovulated using an intraperitoneal injection of 7.5 i.u Pregnant Mare's Serum Gonadotrophin (PMSG) followed by another injection of 7.5 i.u Human Chorionic Gonadotropic hormone (HCG) 48 hours later. Control group (group B) weren't superovulated and were subdivided into six subgroups. After mating, the female mice were examined by vaginal plaque as day zero and in 0-5 days after copulation, they were sacrificed by cervical dislocation. The middle 1/3 parts of their uterine horns were removed in control and experimental groups (8). Both experimental and control group include 6 subgroup and every subgroup include 3 mice.

The samples were collected at different gestational ages (day 0 to 5, vaginal plug designated day 0) from pregnant mice and were immediately fixed in methacarn (60% methanol, 30% chloroform, 10% acetic acid) (9).

Immunohistochemical analysis

The paraffin-embedded tissues were sectioned to a thickness of 5 µm. Sections were then deparaffinized in xylene, dehydrated in a series of ethanol solutions and stained using standard immunohistochemistry procedures.

Tissue sections were pretreated by boiling in 10 mmol/L citrate buffer (pH 6.0) for 15 min as recommended by the supplier. For immunohistochemical detection of Muc1, CT1 polyclonal antibody (sigma, USA) at dilution of 1:200 was used, then incubated with alkalin phosphatas conjugated secondary antibody (abcam, ab5746) (1:100 dilution in TBS) for 1 hour. The antibodies were visualized by incubating with NBT/BCIP cromogen (Roche) for 10 min. Staining intensity of tissue sections was evaluated and graded. The sections were then counterstained with hematoxylin rinsed in tap water and mounted (10). The positive controls were used by breast cancer samples (9).

In this study, Immunoreactivity was scored according to the tensity of staining and statistic analysis didn't perform.

Results

Immunoreactivity was graded as – (negative), ± (trace positive), + (positive), ++ (moderately positive) or +++ (strongly positive) (10).The samples were scored by two independent observers, and slides with discordant interpretations were examined by both observers together until a consensus was reached. As expected, staining was restricted to the apical aspects of luminal and glandular epithelial cells. Our data showed that the levels of Muc1 associated with the uterine epithelia are reduced by the time of implantation of the blastocyst (Table I, Figure 1).

Ovarian hyperstimulation didn't alter the Muc1 expression markedly in surface and glandular epithelium, which could affect on its receptivity (Table I ، Figure 1).

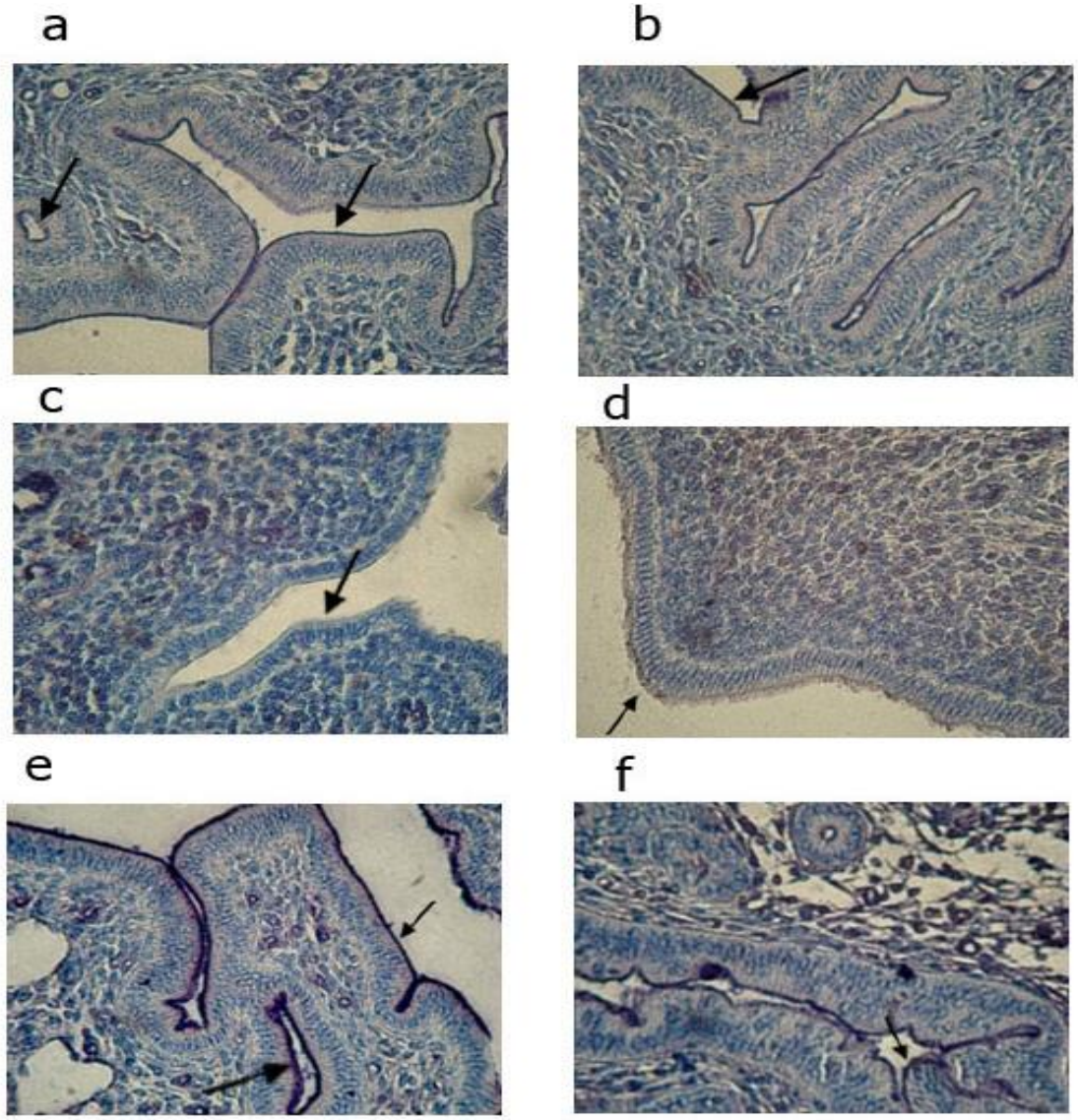

Figure 1. Immunohistochemical staining for the expression of endometrial Muc1 using the antibody CT1. a, Pregnancy day 0, hyperstimulated group; b, Pregnancy day 0, control group; c, Pregnancy day 2, hyperstimulated group; d, Pregnancy day 2, control group; e, Pregnancy day 5, hyperstimulated group; f, Pregnancy day 5, control group. Note that the Muc1 immunostaining is present within the glandular and luminal epithelial cell. Variation was apparent in the staining intensity between endometrium of pregnancy day 0 and endometrium of pregnancy day 5. Magnification: c and d, X400; a, b, e and f, X100.

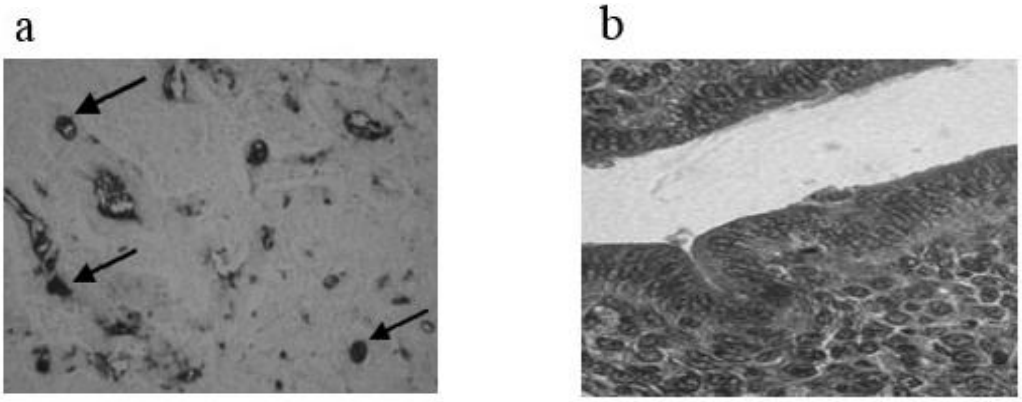

Figure 2. Negative (a) and positive (b) control of IHC of mouse endometrium Muc1. The mammary gland is used for positive control. Magnification is X400.

Table I. Summary of Muc1 immunohistochemistry using CT1 polyclonal antibody in control group (without hyperstimulation) and experimental (hyperstimulated) group. (Number with positive staining/ total number of specimens = 3/3).

Day of pregnancy	Muc1 expression in control group		Muc1 expression in hyperstimulated group	
	Endometrial epithelium	Endometrial glands	Endometrial epithelium	Endometrial glands
0	++	++	++	++
1	++	++	++	++
2	+++	+++	+++	+++
3	++	++	++	+++
4	±	+	±	++
5	±	+	±	+

Discussion

Implantation failure remains an unsolved problem in reproductive medicine and is considered as a major cause of infertility in otherwise healthy women (13).

Ovarian stimulation (OS) with gonadotrophins is an important approach in IVF. However, the impact of different OS on endometrium receptivity remains controversial (17, 18). Many studies in human have shown that the periovulatory endometrial characteristics in ovarian stimulation cycles are considerably different compared with the natural cycle (20). This difference could affect luteal phase function and alter endometrial receptivity (20, 21).

It has long been hypothesized that gonadotrophins and GnRH agonist/antagonist used to induce multifollicular development in controlled ovarian hyperstimulation (COH) might also affect endometrial receptivity, either directly or indirectly. It is suggested that Muc1 acts as an antiadhesive molecule on the uterine surface, thus preventing embryo implantation (6). The present study used a human IVF-mimicked mouse model to investigate the effects of ovarian stimulation on the endometrial receptivity and embryonic implantation. Using immunohistochemical analysis, we demonstrated that Muc1 was expressed in the endometrium of mature female mice during the implantation windows. There was a not significant difference in the expression of endometrial Muc1 between ovarian stimulation groups and the control group during the implantation window of mice. Immunohistochemical analysis showed that the Muc1 was mainly located in endometrial glandular epithelial and luminal epithelial cells.

The immunohistochemical location of Muc1 in the mouse uterus at the time of implantation window was similar to that in previous studies in mice (8, 15, 16). This observation is in agreement with the results shown in the previous studies, which reported that Muc1 expression in the endometrium was under maternal control and was regulated by circulating steroids hormone levels (7).

Fossum *et al* reported a significant decrease in the implantation rates after embryo transfer to ovarian stimulated mice using Pregnant Mare Stimulating Gonadotropin (PMSG) and HCG and suggested that this failure was caused by changes in uterine receptivity (22). In Karmer *et al* study a high luteal phase oesteradiol/progesterone ratio has been associated with implantation failure in mice (23). Basir *et al* concluded that excessive high concentration of oestradiol leads to suboptimal endometrial environment for implantation and this may explain the finding regarding the decreased implantation and pregnancy rates in IVF (24). Disagreement between these studies and our observation could be attributed to the experimental design. They reported changes in the endometrial morphology; these changes are not reflected in the gene expression pattern of the endometrial biopsy. These studies showed that the implantation rate was significantly less in the superovulated mice. A decreased implantation rate was supposed to be due to the changes in the uterine milieu, especially due to the change in endometrial receptivity. Unfortunately, all of these studies did not provide detailed information on the change in endometrial receptivity after OS treatment. The present study, for the first time, reported the ovarian stimulation effects on Muc1 expression of the mouse endometrium before implantation. Further studies in this aspect are needed to provide more definitive answers.

Although there are considerable similarities amongst mammals in the early stage of development, it is difficult to extrapolate the information obtained from this mouse model directly to the human IVF clinics. Indeed, numerous differences exist in this aspect between

the human and the mouse (11, 12, 27). Hence the extrapolation mentioned above should be done with caution. Moreover, because of a relatively small size of each group and the uncertain roles of Muc1 in endometrial receptivity and embryonic implantation in either human or mice, further studies are needed to clarify the reality of all inferences and extrapolations on the basis of the present results. Other studies showed that in IVF cycles with either GnRH agonists or antagonists, no deleterious effect of the endometrial biopsy on clinical pregnancy was recorded (25).

In agreement with our results, Mirkin and co-worker concluded that although ovarian stimulation causes structural and functional changes compared with natural cycles, small changes were found when gene expression patterns were compared, and that ovarian stimulation may therefore do not have a major impact on endometrial receptivity (26).

Up to now, the effects of ovarian stimulation on Muc1 expression of the mouse endometrium in human and other mammals are still unclear. In the present study we demonstrated, in the control and hyperstimulation groups the Muc1 expression is markedly reduced in the luminal uterus epithelium at the time of implantation. Our results are consistent with the existing viewpoint that endometrial expression of Muc1 positively correlates with endometrial receptivity and embryonic implantation. This loss of Muc1 protein is potentially due to the action of steroid hormones. In addition, our results showed that ovarian hyperstimulation didn't alter the Muc1 expression markedly in surface and glandular epithelium, which could affect on its receptivity.

The obtained results have been demonstrated that endometrial receptivity is an equilibrated, complex and active process involving hundreds of up- and down- regulated genes and a key molecule with the capacity to regulate endometrial receptiveness by itself does not exist. However, some molecules are more relevant than others in the development of receptiveness. Further unraveling of molecules involved in the implantation mechanism is needed for a better comprehension of the link between altered endometrial development and receptivity in IVF cycles.

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