INTRODUCTION

Muc-1 mucin is a large, extended glycoprotein that is expressed apically in a wide variety of epithelial tissues. The mouse (Muc-1) and human (MUC1) homologues are very similarly arranged and show the following features (Gendler et al., 1987, 1990; Lan et al., 1990; Ligtenberg et al., 1990; Spicer et al., 1991): (1) high molecular mass (200 to 10^3 kDa); (2) the presence of tandem repeats; (3) the high content of carbohydrate (50-90% by weight) O-linked to the core protein; and (4) an extended structure due to the presence of many prolines and O-linked glycans, projecting the extracellular domain far above the glycocalyx. Muc-1 differs from the other members of the mucin family by being an integral membrane protein predominantly found associated with the apical domain of epithelia and not mainly secreted.

The interest in the MUC1 molecule was initially due to its overexpression in many adenocarcinomas. In particular, over 90% of breast cancers express high levels of this molecule and an increase of at least 10-fold is often observed in tumors. Antibody studies on malignant mammary cell lines and adenocarcinomas showed that, besides the overexpression of MUC1 mucin during tumorigenesis, this molecule is also differentially glycosylated, presenting shorter carbohydrate chains compared to normal tissue (Burchell et al., 1987).

The glycosylation and expression levels of Muc-1 can also be modulated by variation in the developmental and physiological state of the normally expressing organ. The resting breast has very low levels of Muc-1 mRNA and protein correlate with higher levels of plasma estrogen in the estrus and proestrous phases. However, in ovariectomized mice without hormone replacement, the endometrium expressed high levels of this protein. These levels could not be substantially changed by estrogen, although progesterone reduced the levels of Muc-1 protein associated with the epithelium. These data together with the normal expression in the cycling mice suggest that progesterone might repress Muc-1 expression during the metestrus and diestrus phases. In cycling mice, when plasma progesterone is at its nadir and the estrogen level is elevated in estrus and proestrous phases, Muc-1 concentration would increase to its basal level, not because of estrogen stimulation, but due to lack of progesterone repression. The low level of expression is also observed in the endometrium during early pregnancy, where reduced levels of Muc-1 protein are seen at the time of implantation.

Key words: Muc-1, estrus cycle, peri-implantation period, granular metrial gland cells, early pregnancy, decidua, metrial gland, ovariectomy, endometrium, estrogen, progesterone

SUMMARY

Modulation of Muc-1 mucin expression in the mouse uterus during the estrus cycle, early pregnancy and placentation

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Key words: Muc-1, estrus cycle, peri-implantation period, granular metrial gland cells, early pregnancy, decidua, metrial gland, ovariectomy, endometrium, estrogen, progesterone
Muc-1 protein and mRNA appearance is under tight local and temporal control, usually correlating with epithelial sheet formation in the many organs known to express it (Braga et al., 1992). In all cases, during development in the mammary and salivary glands and in the lungs, pancreas, stomach and kidney, the induction of Muc-1 gene transcription is simultaneous with the morphological differentiation of the organs, and precedes their functional activity (Braga et al., 1992; Parry et al., 1992).

During mouse organogenesis, Muc-1 mRNA levels generally increase with development, but this increase might be a reflection of epithelial growth and not an upregulation of the Muc-1 synthesis in individual cells. These data show that once the organs complete their differentiation and maturation processes, the levels of Muc-1 protein reach a steady-state plateau, maintained by a balance between synthesis, secretion and degradation. However, no data are available on the turnover or induction of expression of Muc-1 during normal physiological conditions.

Several lines of evidence suggest that Muc-1 mucin expression can be modulated by hormones. In vitro studies using mouse mammary epithelial cells have shown that the levels of Muc-1 mRNA can be increased by addition of prolactin (Parry et al., 1992). An induction of expression of the Muc-1 gene occurs during mammary gland differentiation in pregnancy, an event that is hormonally coordinated. Many breast cancer cell lines that express high levels of Muc-1 are steroid responsive, and hormonal ablation or hormone antagonists are used as chemotherapy, particularly for metastatic breast cancer treatment. Furthermore, sequences similar to consensus sequences for estrogen and progesterone can be identified in the promoter region of the human MUC1 gene (Lancaster et al., 1990). However, to the best of our knowledge, no direct evidence for hormonal control of Muc-1 expression in vivo has been provided.

On the basis of the above suggestions and the presence of Muc-1-related epitopes in the uterine epithelium and cervix (Koldovsky et al., 1985; Zotter et al., 1988), we speculated that Muc-1 gene expression could be modulated by the physiological changes in the mouse uterus as has been previously suggested for the breast. Reports on the presence of the Muc-1 molecule in the endometrium (Zotter et al., 1988) and in the placenta (Braga et al., 1992) led us to examine the pattern of expression of Muc-1 during the implantation and placentation periods. In this context, we investigated whether the Muc-1 gene could be hormonally regulated in the uterus of normal cycling mice.

**MATERIALS AND METHODS**

**Animals**

Ovaries, oviducts, uteri and cervix were collected at different gestational ages (day 1 to 7; vaginal plug designated day 1) from pregnant mice (C57Bl/ICRF) killed by cervical dislocation and were immediately fixed in methacarn (60% methanol, 30% chloroform and 10% acetic acid). The reproductive tracts were cut in serial sections in order to find the embryos and implantation sites. Samples where the embryos could not be found were discarded. Older embryos (days 8 to 12) were not dissected from the uterus and embryonic membranes; they were processed individually as described above.

Vaginal smears were performed to determine the stage of the estrus cycle previous to killing the animals. Samples of uteri at different stages of the estrus cycle were collected and immediately frozen in liquid nitrogen or fixed in methacarn. The determination of the stage of the cycle was later confirmed by histological changes in the vaginal epithelium that normally occur in cycling mice.

**Immunostaining**

Paraffin blocks containing the samples were sectioned (5 µm thick) and routinely stained with hematoxylin and eosin. Immunostaining was performed as described previously (Bartek et al., 1985). The polyclonal antiseraum CT1, raised to a synthetic peptide corresponding to the 17 C-terminal amino acids in the cytoplasmic tail of human MUC1, was used as described (Parmberton et al., 1992). The timing of the substrate reaction was carefully measured to avoid product saturation and to allow for comparison of the samples. As negative control, the CT1 antiserum was blocked with the peptide to which it was raised prior to incubation with the sections.

**RNA extraction and processing**

RNA samples from frozen uteri were extracted by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczyski and Sacchi, 1987). RNA was resuspended in sterile water, aliquoted and kept at −70°C until used. Concentration and purity were estimated by absorbance at 260 nm. Northern blots were performed as described previously (Braga et al., 1992).

**Reverse transcription-polymerase chain reaction (RT-PCR)**

The RT-PCR technique was performed as described by Rappolee (Rappolee et al., 1988) and as optimized for Muc-1 amplification (Braga et al., 1992). Cycling conditions using a thermal reactor (Hybaid) were: 1 minute at 94°C, 30 seconds at 62°C, and 1 minute at 72°C. To assure that we were in the linear range of the exponential amplification, the number of cycles was titrated using 5 µg of uterus RNA samples and determined to be 33 cycles for both Muc-1 and β-actin primers. Lactating mammary gland RNA (diluted 1:10) and L-cell RNA were used as positive and negative controls for Muc-1 expression, respectively. PCR fragments (15% of the reaction) were fractionated in 2% agarose gels, stained with ethidium bromide and photographed using a positive/negative Polaroid film (Polaroid, type 55). Negatives were scanned in a laser scanner (LK Bromma - Ultralaser Scanner X L), and the area of each peak calculated and expressed in arbitrary units. The data were not expressed as relative mRNA abundance, i.e. corrected for the levels of a reference molecule (β-actin), because we have observed that β-actin levels vary during the estrus cycle. Therefore, a calculation using β-actin levels as a reference would produce misleading results.

**Hormonal treatment**

Mice were ovariectomized and treated with hormones was started 4 days later. Subcutaneous injections of different hormones were performed daily for 7 days. Groups of five animals received 0.1 µg/day estrogen (1,3,5(10)-estradiene-3,17β-diol; Steraloids Ltd.), 1 mg/day progesterone (4-pregnene-3,20-dione; Sigma), 0.1 µg/day estrogen plus 1 mg/day of progesterone and the control group received only the diluent (0.9% NaCl, 1% gelatin in 10% ethanol). Three hours after the last injection, animals were killed by cervical dislocation. Uteri were removed, and the horns separated; one was immediately frozen in liquid nitrogen and the other was fixed in methacarn. RNA and protein analyses were performed.
Muc-1 protein expression in the mouse uterus

Fig. 1. Muc-1 protein expression pattern as determined by immunohistochemistry using the antiserum CT1 (reaction with substrate for 4 to 5 minutes).
(A) Decidua basalis (12 day of pregnancy);
(B) control slide for the decidua basalis is shown as stained with the antiserum blocked with its immunizing peptide.
Muc-1 expression in endometrium during early pregnancy: (C) day 2;
(D) day 5, showing a blastocyst (inner cell mass is not present in this section) in close contact with the uterine epithelia.
Expression of Muc-1 protein in the endometrium of cycling mice: (E) estrus;
(F) metestrus;
(G) diestrus;
(H) proestrus. Stronger specific Muc-1 staining is observed up to day 3 of pregnancy and in the estrus and proestrus phases of the cycle.
with pools of five uteri per group, and analyses were done at least twice.
The time-course experiment of hormone treatment was performed as above. Four days after ovariectomy, subcutaneous injections of the hormone solutions using the same dose were given to the groups of five mice. Mice were killed 24 hours later. Collection of the samples and analyses were performed as described.

RESULTS

We have previously analysed the expression of the Muc-1 gene and protein in mouse post-implantation embryos. In the developing mouse, staining for Muc-1 can first be detected by day 12 in the lung, pancreas and stomach epithelia (Braga et al., 1992). In the present study, the expression pattern of Muc-1 was extended and examined: (1) in the early embryo; (2) during the formation of placenta; (3) in the endometrium during early pregnancy and during the estrus cycle; and (4) in the endometrium of ovariectomized mice, after hormone replacement.

Expression of Muc-1 protein in the early mouse embryo and during placentation
We have approached the early embryo Muc-1 expression in situ using immunohistochemistry, to avoid dissections and any possible contamination with either endometrium or placental tissues in our samples. Our data show that no specific staining for Muc-1 was observed in embryos or in extraembryonic membranes from day 1 to day 7, as determined by the antiserum CT1 (data not shown).

In the present study, an unusual pattern of expression of the Muc-1 protein was observed in the decidua basalis: intracellular granules present in large and scattered cells reacted strongly with CT1 antiserum (Fig. 1A). This staining pattern was shown to be specific, as it disappeared completely when blocked antiserum was used (Fig. 1B). The expressing cell population is the granular metrial gland cell (Dr S. Peel, personal communication; reviewed by Peel, 1989; Stewart, 1991). The cytoplasm is palely stained by hematoxylin and contains a large number of granules that can be readily identified by staining with glycoprotein reactive dyes (periodic acid/Schiff staining technique).

Although the granular metrial gland cells appear in the uterus as early as day 6 of pregnancy, the first time Muc-1 expression could be detected was at day 8, when a few cells were found expressing low levels of this protein. With time, both the number of cells expressing it, the size of the cells and the intensity of staining of the granules increased considerably, as would be expected by the mituplication and differentiation of the granular metrial cells (Stewart, 1983; Stewart and Peel, 1977). On the last day of examination by immunohistochemistry, day 12, we found expression by the largest number of cells. Since we have found that Muc-1 mRNA is expressed both at day 14 and at term (unpublished data), it appears that Muc-1 starts to be expressed in the decidua by day 8 and is continually expressed until term. This pattern of Muc-1 expression is quite unusual, considering that: (1) this is the first time that Muc-1 has been demonstrated intracellularly in normal tissues and not associated with the plasma membrane; and (2) granular metrial gland cells are not epithelial cells but differentiate in situ from a lymphocyte-like precursor (see below).

Expression of Muc-1 protein by the endometrium during early pregnancy and estrus cycle
When the expression in the endometrium during early pregnancy was analysed, it appeared that strong staining specific for Muc-1 was found at the beginning of pregnancy, on days 1 to 3 (vaginal plug designated day 1) (Fig. 1C, day 2) as opposed to the intensity of staining observed by days 4, 5 and 6 (Fig. 1D, day 5). Although the reaction of the conjugated peroxidase with the substrate has been carefully timed for all samples, it is very difficult to quantify using immunostaining. Nevertheless, these data suggest that the levels of Muc-1 protein associated with the uterine epithelia are reduced by the time of implantation of the blastocyst. The decrease in apical staining might result from increased secretion of the molecule and/or reduction of the Muc-1 protein/RNA synthesis.

We have also investigated the protein and mRNA expression pattern of the Muc-1 gene during the estrus cycle in the mouse. The protein analysis was performed using immunohistochemistry, and the results are shown in Fig. 1 (E to H). The Muc-1 protein levels appeared to be higher in the estrus (Fig. 1E) and proestrus (Fig. 1H) phases as compared to metestrus (Fig. 1F) and diestrus (Fig. 1G). Estrus and proestrus are considered the estrogenic phases of the cycle; in contrast, progesterone plasma levels are highest at the diestrus and metestrus phases. Strongest Muc-1 positive immunostaining of the uterine glands was observed in the estrus stage (Fig. 1E).

The RNA levels in the endometrium from cycling mice were investigated using the RT-PCR technique. In Fig. 2A, the titration of the number of PCR cycles used in the RT-PCR analysis is shown, demonstrating that the conditions used in our experiments (33 cycles) are in the linear range of the exponential amplification of the PCR reaction. The Muc-1 RNA expression pattern throughout the estrus cycle is in accordance with the protein data, as the highest levels of mRNA are found in the estrus and proestrus phases (Fig. 2B). The differences observed in the Muc-1 mRNA levels during the different phases of the cycle are significant ($P=0.025$). These data were confirmed by northern blots (Fig. 2C).

Expression of Muc-1 protein in the uterus after hormonal treatment of ovariectomized mice
In order to define whether variations in hormone concentrations in the plasma during the estrus cycle could be responsible for this oscillation of Muc-1 mRNA and protein levels, mice were ovariectomized and supplemented with estrogen, progesterone or combinations of both hormones for 7 days. The levels of Muc-1 mRNA appeared to be upregulated by the addition of estrogen, and this effect could not be antagonized by the simultaneous addition of progesterone (Fig. 3). Progesterone injections appeared to reduce Muc-1 levels, although this result is not statistically significant.

When the Muc-1 protein expression in the same samples was analysed by immunohistochemistry, a fairly strong
expression of Muc-1 was detected in the apical portion of the epithelium following treatment with estrogen (Fig. 4A) or estrogen plus progesterone (Fig. 4C). Progesterone injections produced a more cuboidal epithelium as compared to the other groups, with negligible Muc-1 protein associated with it (Fig. 4B). However, control animals yielded a striking result, as their epithelium and glands showed the highest expression of this mucin (Fig. 4D). A large amount of secretion can be observed in the luminal uterine cavity in the estrogen-treated samples (Fig. 4A,C), although hormonal treatment may alter retention of material by affecting luminal volume. This secretion does not react with the Muc-1-specific antiserum, CT1. However, it should be pointed out that this antiserum was raised to the Muc-1 cytoplasmic tail (Pemberton et al., 1992), and it has been shown that at least one form of secreted Muc-1 lacks the epitope for the CT1 antiserum (Boshell et al., 1992). Thus, we cannot exclude the possibility that the secretion of this molecule might be induced by estrogen and progesterone.

The protein analysis results for Muc-1 expression (Fig. 4A to D) after hormone replacement do not correlate with the RNA data (Fig. 3): highest message levels are observed in the estrogen-treated animals, while highest protein levels associated with the epithelium are observed in the control animals. Two possible explanations can be postulated. The first one is that uteri from estrogen-treated mice have higher levels of Muc-1 message, and as the mRNA is translated part of the synthesized protein is secreted. However, this suggestion is difficult to confirm in vivo, due to a lack of antibodies reactive with the extracellular domain of the mouse Muc-1 protein. There is a panel of monoclonal antibodies directed to carbohydrate epitopes on the extracellular domain of the human MUC1 mucin that cross-react with the mouse lactating mammary gland mucin (Parry et al., 1992). Staining of the uterus samples with these antibodies, however, showed almost no specific reaction at the apical domain of the endometrium and no staining of the

**Fig. 2.** Muc-1 RNA expression in the uterus of cycling mice. (A) Titration of the PCR cycle number with uterus RNA samples; (B) RT-PCR using Muc-1-specific oligonucleotides and RNA from uterus at different stages of the estrus cycle (5 µg of RNA used in each reaction); (C) northern blot of uterus total RNA samples (10 µg; Lact. mammm. gl., 1 µg) probed with the tandem repeat fragment of the Muc-1 cDNA. The same blot was stripped and reprobed with the β-actin probe.

**Fig. 3.** Determination of Muc-1 message levels in mouse uteri taken after ovariectomy and following hormone treatment for 7 days. E, estrogen; P, progesterone; E+P, estrogen plus progesterone; S, saline. LMG, lactating mammary gland. Estrogen treatment appears to induce higher levels of Muc-1 mRNA expression; progesterone and saline administration yielded slightly higher levels than the negative control, L-cell RNA.
Fig. 4. Muc-1 immunostaining pattern of endometrium from ovariectomized mice treated with hormones for 7 days (A to D) and 24 hours (E to H) as follows: estrogen (A and E), progesterone (B and F), estrogen and progesterone (C and G). Controls (D, H) were injected with saline (0.1% gelatine, 0.9% NaCl, 10% ethanol). Substrate reaction was for 4 minutes in A to D and for 2 minutes in E to H.
Muc-1 protein expression in the mouse uterus

Human MUC1 mucin has been previously shown to be present in the endometrium (Zotter et al., 1988), and changes in the levels of the carbohydrate antigens associated with the mucus in the cervix and endometrium have been reported during the normal menstrual cycle (Gilks et al., 1989; Koldovsky et al., 1985). However, no quantitative studies have yet been done. The modulation of expression of Muc-1 mRNA and protein in the mouse endometrium and during placentation is reported here for the first time.

The multiple changes that occur in the mouse uterus during the estrus cycle are coordinated by the levels of ovarian steroids in the plasma. Our results show that Muc-1 mRNA and protein levels are altered in the uterine epithelium during the estrus cycle in the mice. The observed increases in Muc-1 message and protein levels during the estrus and proestrus phases are coincident with the highest levels of estrogen in the plasma (Bergman et al., 1992; Walmer et al., 1992).

There are other molecules reported in the literature, whose expression in the mouse uterus/vagina during the estrogentic phases of the cycle provides evidence for modulation by estrogen (Bergman et al., 1992; Huet-Hudson et al., 1989, 1990; McMaster et al., 1992; Walmer et al., 1992). For Muc-1 expression, however, hormone replacement experiments did not demonstrate the significance of the temporal correlation between higher levels of message in the endometrium with higher levels of plasma estrogen. During a 24 hour induction test, levels of Muc-1 mRNA and protein remained unchanged in all groups. These data suggest that the endometrium expresses Muc-1 at basal levels, and this level is not altered by estrogen. The higher Muc-1 mRNA levels obtained with estrogen in the long-term experiments might reflect the proliferation of the epithelium (Mukku et al., 1982; Walmer et al., 1992) and/or a greater stabilization of the message by estrogen during the treatment.

The failure of the expected estrogen action cannot be attributed to the conditions of the experiments, as other parameters were indicative of the effectiveness of estrogen treatment: the morphology of the uterus (swollen in the estrogen-treated groups, data not shown) and the height of the epithelium in the histological sections. In a recent report, it was noted that different levels of administered estrogen could result in different intensities of its effects in an ovariectomized animal (Bergman et al., 1992). However, the amount of hormones injected in our experiments is known to produce physiological effects in the uterus and was similar to others in which specific estrogen stimulation was obtained (Huet-Hudson et al., 1989; McMaster et al., 1992).

If estrogen is not regulating the Muc-1 levels during the estrus cycle, which molecule is? The high levels of Muc-1 protein seen in the saline-injected groups suggest that the basal level of expression of this molecule in the endometrium is quite high, comparable to that observed in the stomach and pancreas, for instance. In 3-week-old virgin mice (before sexual maturation), before the onset of puberty, both glandular and luminal uterine epithelia already express Muc-1 protein (data not shown). The most likely explanation for the modulation of expression in the uterus of intact mice seems to be a down-regulation by progesterone during the metestrus and diestrus phases and during early pregnancy. After the progesterone plasma concentration decreases in cycling mice, Muc-1 would return to its basal level, which is not significantly affected by estrogen. A possible stabilization of the Muc-1 message by estrogen in estrus and proestrus, however, cannot be excluded.

It is worthwhile mentioning that MUC1 levels seem to vary differently in adult human endometrium (our unpublished observation; J. Aplin, personal communication). However, it should be noted that human samples in these studies were usually obtained from fertility clinics and although the patients are considered normal, they do not easily become pregnant. If this modulation in humans is correct, then the uterus will be the first place where MUC1 is regulated differently than in the mouse model.

For some time it has been known that the uterine epithelium undergoes careful preparation for the implantation of the blastocysts, which occurs under the tight control of ovarian steroids (reviewed by Psychoyos, 1986). This receptivity can be caused by inducing the expression of genes or modulating glycosylation processing of certain proteins in the epithelial cells, creating a window where implantation is allowed (Kimber and Lindenberg, 1990; Lindenberg et al., 1988). The down-regulation of the levels of genes like Muc-1 and the lactoferrin gene (McMaster et al., 1992) in the uterus during the peri-implantation period may also contribute to this preparation of the epithelium. A role for Muc-1 protein is still unclear, although some suggestions can be found in the literature (Braga et al., 1992; Coidtong et al., 1973; Ligenberg et al., 1992; Steck and Nicolson, 1983). Among these are the anti-adhesive properties that have been attributed to this molecule, based on
its biochemical and structural features. The large number of prolines and the many O-glycosylation sites in the large extracellular domain lead to a molecule with a rod-like shape, extending far beyond the glycocalyx (Jentoft, 1990). Epithelia that express high levels of the Muc-1 protein are predicted to have their apical domains shielded, preventing other molecular interactions most probably by steric hindrance. This prediction is supported by experimental data in which: (1) the metastatic potential of tumor cells was correlated with high expression of mucins (Steck and Nicolson, 1983); and (2) reduced in vitro aggregation ability was shown for transfecteds with the human MUC1 gene (Ligtenberg et al., 1992). It is interesting to speculate that these anti-adhesive properties provide a possible reason for the reduced levels found in the normal endometrium by implantation. In this sense, the lower levels of the Muc-1 protein at the apical domain might facilitate the exposure of the relevant molecule(s) mediating the blastocyst attachment.

The observation of Muc-1 protein expression by granular metrial gland cells during placentation was surprising in view of previous reports. The striking feature of Muc-1 expression by granular metrial gland cells is the intracellular pattern of expression observed and the cell type identified. The precursors of these cells migrate to the uterus where they differentiate in situ. They can be found mainly in the decidua basalis and inside the blood vessels of the decidua and around trophoblastic giant cells. Muc-1-specific staining was observed in round cytoplasmic granules, although membrane localization cannot be excluded at the moment. In other normal tissues where Muc-1 is expressed, it is found apically in the simple epithelia (Braga et al., 1992; Zotter et al., 1988). The presence of this molecule has been associated with the differentiation of various epithelia (Braga et al., 1992; Parry et al., 1992). This is the first report where Muc-1 is demonstrated to be intracellular and not associated with the plasma membrane of polarized cells.

In addition, granular metrial gland cells are not derived from epithelial cells, but have a lymphocyte-like precursor that migrates from the bone marrow to the uterus and differentiates in situ (Stewart and Peel, 1977). These cells express lymphocyte proteins like perforin (Parr et al., 1987) and NK lineage antigens like Thy-1 and asialo-GM1 (Mukhtar et al., 1989). The function of these cells is unknown, but their characteristic granules are thought to be relevant to their function (reviewed by Peel, 1989; Stewart, 1991). Recent evidence indicates the ability of granular metrial gland cells to kill layer one trophoblast, by behaving like NK killer cells (Stewart and Mukhtar, 1988). The finding of an epithelial-specific protein in lymphocyte-derived cells and the fact that the role of granular metrial gland cells has been an enigma for years only add more confusion to the establishment of a function for Muc-1. It will be interesting to investigate further whether this unusual expression of Muc-1 during placentation is of any relevance to the maintenance of pregnancy. A mouse model where the Muc-1 gene has been efficiently disrupted would be extremely useful for this purpose.

In summary, we have shown an unusual pattern of expression of the Muc-1 protein during placentation and a modulation of expression of Muc-1 in the uterus of normal cycling mice and during early pregnancy. Although the highest Muc-1 protein and RNA levels correlate temporally with the highest concentration of plasma estrogen during the cycle, we were unable to show the significance of this correlation. On the contrary, higher levels of protein expression were observed in the ovariectomized control animals. It seems that the normal endometrium shows a high basal level of the Muc-1 protein, and that during early pregnancy, metestrus and diestrus phases, these levels may be repressed by progesterone. Therefore, during the estrogenic phases of the cycle, the levels of this molecule would increase not because of a stimulation by estrogen, but due to a lack of progesterone repression. The implications of these results for the receptivity of the uterus for implantation and for a successful pregnancy remain to be established.

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