Placental protein 14 in endometrium during menstrual cycle and effect of early luteal phase mifepristone administration on its expression in implantation stage endometrium in the rhesus monkey

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Placental protein 14 (PP14) is a glycoprotein which is secreted by secretory phase endometrium and decidua in women. Despite the suggestion that PP14 is involved in the process of endometrial maturation for blastocyst implantation, our understanding in this regard is poor. In the present study, the concentrations and distribution patterns of immunodetectable PP14 in the endometrium during proliferative and secretory phases of normal ovulatory menstrual cycles, as well as in implantation stage endometrium in naturally mated ovulatory cycles with or without early luteal phase mifepristone treatment, were investigated using the rhesus monkey as a primate model. Immunopositive PP14 was observed mainly in epithelial cells of glands and it was detected in one major immunopositive band at M, 28 kDa in tissue homogenate and spent medium. The area of immunopositive precipitation of PP14 in glands was minimal in follicular phase endometrium, and was higher (P < 0.01) in early, mid- and late luteal phase endometrium compared with that in pre- and peri-ovulatory phases of the cycle, but there was no change in its area profile in the glandular compartment throughout the luteal phase. Immunopositivity for PP14 in luminal contents of gland displayed an increasing profile from early to late secretory phases. Thus, the concentrations and the distribution of immunodetectable PP14 in luteal phase endometrium of the rhesus monkey showed marked similarity with those of human endometrium during the natural menstrual cycle. Although there was no marked change in the band characteristics for the protein in implantation stage endometrium following early luteal phase mifepristone treatment, it was markedly decreased (P < 0.01) in tissue homogenate and in vitro spent medium along with a lesser (P < 0.02) degree of immunoprecipitation in the glands in implantation stage samples of mifepristone treatment group compared with that in control group samples. Thus, the contragestational effect of early luteal phase mifepristone treatment appears to be associated with a decrease in the concentration of immunodetectable PP14 in implantation stage endometrial glands and its secretion in the rhesus monkey. It remains to be seen whether this decline is caused from direct antiprogestosterone action on endometrial glands during progesterone dominance, or secondarily from associated retarded development of endometrium.

Key words: immunoblot/immunohistochemistry/mifepristone/PP14/rhesus monkey

Introduction

Placental protein 14 (PP14) is a glycoprotein and is secreted by secretory endometrium and decidua in women (Julkunen, 1986; Julkunen et al., 1986, 1990). PP14 has been implicated in the process of endometrial preparation for blastocyst implantation under progesterone dominance in women (see reviews: Seppala et al., 1991, 1997; Edwards, 1995; Seppala and Tiitinen, 1995). There is evidence to suggest that PP14 may mediate immunomodulatory and immunosuppressive effects in the endometrium around the time of implantation in the human (Bolton et al., 1987; Pockley et al., 1988; Okamoto et al., 1991). Furthermore, it may transport small hydrophobic molecules across tissue boundaries and thus may influence the conceptus before the development of the placental circulation (Huhtala et al., 1987). However, our understanding about the involvement of endometrial PP14 during blastocyst implantation in primates is poor. In the present study, our aim was to examine the concentrations and distribution patterns of immunodetectable PP14 in the endometrium during the proliferative and secretory phases of normal ovulatory menstrual cycles in the rhesus monkey. Furthermore, we have investigated the association between PP14 and endometrial receptivity for blastocyst implantation during the mid-luteal phase by examining the profile of its immunopositive expression in the implantation stage endometrium on day 6 after ovulation in naturally mated ovulatory cycles with or without mifepristone (RU486) treatment on day 2 after ovulation. Mifepristone is a potent anti-progesterone and a promising endometrial contraceptive agent. A single dose application of this antiprogestin agent on day 2 after ovulation inhibits blastocyst implantation, but with no notable changes in menstrual cyclicity in women and in monkeys (Gemzell-Danielsson et al., 1993; Ghosh and Sengupta, 1993). The antinidatory action of mifepristone is associated with desynchronization of the endometrium along with repression of glandular secretory differentiation and vascular maturation (Li et al., 1988a; Johannisson et al., 1989; Gemzell-Danielsson et al., 1994, 1997; Ghosh et al., 1996, 1998a; Dockery et al., 1997) and differential profiles of proteins in endometrial samples around the time of implantation (Beier et al., 1994; Ghosh et al., 1997).
Materials and methods

Animals and general procedures

Healthy, mature and proven fertile male and female rhesus monkeys (Macaca mulata) kept in a semi-natural condition at the Primate Research Facility of the All India Institute of Medical Sciences (New Delhi, India) were used in this study. The details of animal selection, housing, management, monitoring of cycles, mating, laparotomy, endometrial sampling, serum collection and radioimmunoassays for serum oestradiol-17β and progesterone have been described elsewhere (Ghosh and Sengupta, 1989, 1992, 1993; Ghosh et al., 1996, 1997, 1998a). The study design was approved by the Ethics Committee for the Use of Primates in Biomedical Research of the All India Institute of Medical Sciences.

Treatment groups

Females showing at least two consecutive ovulatory menstrual cycles of normal length (26–32 days) were allocated in two groups. Animals (n = 20) in group 1 were used for obtaining endometrial samples during the proliferative and secretory phases of normal menstrual cycles. In group 1, endometrial samples were collected on cycle day (cd) 5 (group 1a; n = 4), cd12 (group 1b; n = 4), cd16 (group 1c; n = 4), cd20 (group 1d; n = 4) and cd24 (group 1e; n = 3). In group 2 (n = 12), females were allowed to cohabit with males during days 8–16 of their ovulatory cycles. Vaginal smears were checked daily for the presence of spermatozoa. Female monkeys of group 2 were injected s.c. with 2 ml vehicle (1:4, benzyl benzoate:olive oil, v/v; group 2a, n = 6) only, or with a single dose of RU486 (2 mg/kg body weight) in the same volume of vehicle (group 2b; n = 6) on day 2 after ovulation in mated cycles. In group 2, endometrial samples were collected on day 6 after ovulation and samples only from mated, ovulatory cycles that yielded reimplantation stage embryos were used for further examination in this study. The days of ovulation in both groups were assessed from the daily profiles of oestradiol and progesterone in peripheral serum samples. Steroid radioimmunoassays were performed according to the method of Sufi et al. (1988) using antisera and chemicals obtained from the WHO Matched Reagents Programme. The methodological details are described elsewhere (Ghosh and Sengupta, 1992, 1993; Ghosh et al., 1993, 1996).

Tissue collection and processing

The procedural details of collection and processing of endometrial samples for Western immunoblotting and immunohistochemistry have been described elsewhere (Ghosh et al., 1993, 1996, 1998a). Briefly, endometrial samples were collected on specific days of menstrual cycles (group 1) and on day 6 after ovulation (group 2) by performing laparotomy and fundal hysterotomy following ketamine (12 mg/kg, Vetal, Parke-Davis, Mumbai, India) anaesthesia. The tissue samples were quickly washed in ice-cold phosphate-buffered saline and then fixed in phosphate-buffered neutral formaldegre (4%) and finally embedded in paraffin wax by a routine procedure. Paraffin-embedded tissue samples were used for immunohistochemistry. Furthermore, tissue samples were homogenized in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) with 1 mM phenylmethylsulphonyl fluoride and also processed for incubation in phenol red-free Eagle’s minimum essential medium for 16 h at 37°C in a humidified environment of 5% carbon dioxide and 95% air. Tissue homogenate and spent media were used for Western blot analysis. All chemicals were purchased from Sigma Chemical Co., St Louis, MO, USA.

Antibodies

In initial experiments, two antibodies were employed; one was a polyclonal antibody raised against rhPP14 in rabbits and the other was a mouse monoclonal antibody (B7B10) raised against bovine β-lactoglobulin, cross-reactive with hPP14 (Kumar Reddy et al., 1992; Dutta et al., 1998); both were IgG. Since both of them yielded similar results, further experiments were performed with monoclonal antibody only. Dilutions of stock were precalibrated based on 3–5 titration points; the working dilutions for Western blotting and immunohistochemistry are given in following sections. The details of the antibodies are given elsewhere (Kumar Reddy et al., 1992; Dutta et al., 1998).

Western immunoblotting

Profiles of experimental proteins in tissue homogenates and spent media were characterized by the sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)/Western immunoblotting method as described by Ausubel et al. (1994) using electrophoresis and trans-blot equipments from Bio-Rad Laboratories (USA). The materials and chemicals were purchased from Bio-Rad and Sigma. The methodological details have been described elsewhere (Ghosh et al., 1998a). Samples of same protein content (25 µg) were separated on 15% SDS–PAGE gels; prestained molecular markers (Amersham, UK) were run with each gel as standard. Electrophoretically separated proteins were transferred to nitrocellulose membranes which were then incubated with primary monoclonal antibody at a working dilution of 4×10⁻³ (stock: 4 mg/ml). Final visualization was achieved by using Vectastain ABC immunoperoxidase kits (Vector Laboratories, Burlingham, CA, USA) and diaminobenzidine hydrochloride (Sigma). Respective primary antibody control and secondary antibody control were run to examine the specificity of the procedure. The molecular weights of individual bands were determined based on profile analysis with calibrated molecular weight standard curve, and semi-quantitative densitometric analysis was performed based on profile analysis and peak integration of immuno-positive bands using the Molecular Analyst image analysis software and a Gel Scanner GS 670 (Bio-Rad). The results are shown as Mᵢ of individual bands and semi-quantitative measures (optical density, nm) of profiles of bands.

Immunohistochemistry

Paraffin sections 5 µm thick were employed for immunohistochemical localization of PP14 using specific antibody at a working dilution of 4×10⁻². The details of the antibody have been described elsewhere (Kumar Reddy et al., 1992; Dutta et al., 1998). Visualization was achieved using Vectastain ABC Peroxidase Elite Kit (Vector) and 3,3’-diaminobenzidine hydrochloride (Sigma) as substrate following a method described previously (Ghosh et al., 1996, 1998a,b). Respective primary antibody control and secondary antibody control were run simultaneously.

Morphometry

The areas of immunoprecipitate in endometrial glandular epithelial cells, surface epithelial cells and stromal cells were morphometrically determined as described earlier (Ghosh et al., 1993, 1996, 1998a,b). Briefly, the areas of immunoprecipitation in different endometrial compartments were examined in the functionalis zone using a Leica microscope at ×25 and a precalibrated computer-aided video image analysis system (Quantimet 500 C+ image analysis system, Leitz GmbH, Cambridge, UK) and percentage immunopositive area was calculated from the immunoprecipitate total areas in the experimental compartment. Only glands which were in true cross-section were measured using a model system described by Schipper et al. (1989). Semi-quantitative scoring was done based on a 5-scale subjective assessment system: 0, nil (0%); 1, very weak (<5%); 2, weak (5–25%); 3, moderate (25–75%); 4, strong (>75%), as described by
Press et al. (1988). It was assumed that these measurements reflect the concentrations of the experimental protein in different endometrial compartments.

**Statistical analysis**

Statistical analyses of quantitative measurements were performed using Kruskal–Wallis test followed by multiple comparisons test using calculated critical differences for comparing values between groups (Siegel and Castellan, 1988) for group 1 and matched t-test for group 2 (Samuels, 1991). The probability level of $P = 0.05$ was taken as the limit of significance. The data are shown as mean ± SEM.

**Results**

**Menstrual cycle**

Figures 1 and 2 show typical distribution of immunopositive PP14 in epithelial and stromal compartments of the functionalis zone of endometrial samples collected on cycle days 8 (group 1a), 12 (group 1b), 16 (group 1c), 20 (group 1d) and 24 (group 1e) of ovulatory menstrual cycles. As shown in Figure 3, PP14 was detected in one major immunopositive band at $M_r$ 28 kDa in tissue homogenate and spent medium of the endometrium, and in immunohistochemical analysis it was observed mainly in the epithelial compartment (Figures 1 and 2). Positive stainings for PP14 were seen both inside cells and within the lumen of glands with variable patterns and intensities. Generally, a higher degree of immunoprecipitation was observed in the apical border of epithelium compared with that in the basal region, and staining patterns were variable, from discrete, granular to diffuse in appearance. Furthermore, nuclear staining was occasionally observed without any obvious pattern. Vascular contents were generally positive for PP14. However, no further analysis was performed in this regard. Figure 4 attempts to summarize the observed changes in different endometrial compartments during pre-ovulatory, peri-ovulatory, early luteal, mid-luteal and pre-menstrual phases of cycle. The respective phases of menstrual cycle in relation to ovulation were determined based on serum concentrations of oestradiol-17$\beta$ and progesterone.

Table I shows the mean morphometric values of areas of immunopositive precipitation for PP14. In the glandular compartment it varied significantly ($P < 0.001$) during different phases of the menstrual cycle. On further analysis, it was found that area of immunopositive precipitation for PP14 in glands was higher ($P < 0.01$) in early, mid- and late luteal phase endometrium compared with that in the pre- and peri-ovulatory phases of the cycle; however, there was no change in the area profile of immunopositive PP14 in the glandular compartment throughout the luteal phase. On the other hand, glandular lumen contents showed an increasing immunopositivity for PP14 from early to late secretory phases. When total areas of immunoprecipitation in glandular cell body and lumen were measured, it was found to increase steadily throughout the luteal phase (Figure 4). Surface epithelium was highly positive on cd16 (days 4–6 after ovulation), following which it decreased markedly; however, this could not be analysed using two-dimensional morphometry for the lack of adequate sample fields. During pre- and peri-ovulatory periods, PP14 in the epithelial compartment was only marginal (Table I, Figure 4). Immunopositive area for PP14 in the stromal compartment was found to be generally low in any phase of the menstrual cycle without any discernible pattern (Table I).
Figure 2. Immunohistochemical staining for placental protein 14 in endometrial glands and stroma of pre-ovulatory (a), peri-ovulatory (b), early luteal (c), mid-luteal (d) and pre-menstrual (e) stages of normal menstrual cycle. Primary antibody control (f) shows no staining. Bar = 30 µm.

Figure 3. Western immunoblot profiles for placental protein 14 (PP14) in media incubated with endometrial samples (A) and in homogenate of endometrial samples (B) collected on day 6 after ovulation from control (group 2a, C) and mifepristone-treated (group 2b, R) animals.

Implantation stage endometrium following early luteal phase mifepristone

As shown in Figure 3, PP14 was detected in one major immunopositive band at Mr 28 kDa in tissue homogenate and spent medium, and there was no marked change in the band characteristics for the protein between the two treatment groups. As shown in Table II, total densitometric scores of band profiles from Western blot analysis revealed a marked decrease ($P < 0.01$) in tissue homogenate concentrations and in-vitro secretory concentrations of PP14 in the mifepristone group (group 2b) compared with control (group 2a). Table III shows the morphometric analysis of the area of immunopositive precipitate for PP14 in the glandular and stromal compartments in the functionalis zone on day 6 after ovulation with or without early luteal phase mifepristone treatment. Representative microphotographs of immunodetectable PP14 for both treatment groups are shown in Figure 5. Early luteal phase mifepristone treatment resulted in significant ($P < 0.02$) decrease in glandular immunopositive area profiles for PP14. However, there was no change in the PP14 positive area in stromal compartments on day 6 with or without early mifepristone treatment (Table III). A very high degree of immunostaining was noted in the surface epithelium (63.1 ± 6.9%) of control (group 2a) endometrium; however, it could not be compared with mifepristone-exposed samples, since the number of fields for surface epithelium was not sufficient in mifepristone-treated (group 2b) endometrial samples.
Pregnancy-associated endometrium is termed glycodelin A. It is also known as not endometrium specific and are expressed in other tissues.

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Oehninger et al., 1995; Seppala and Tiitinen, 1995). It belongs to a family of glycopolypeptides known as glycodelins. Glycodelins refer to various splicing variants and glycoforms of PP14 having diverse functions (Garde et al., 1991; Morrow et al., 1994; Dell et al., 1995). It is now obvious that glycodelins are not endometrium specific and are expressed in other tissues (Seppala et al., 1997). The glycodelin variety present in endometrium is termed glycodelin A. It is also known as pregnancy-associated α2-globulin (α2-PEG). The Human Gene Mapping Nomenclature Committee (Winnipeg, Manitoba, Canada) has suggested that PP14 should be named as progesterone-associated endometrial protein (PAEP) (Kamarainen et al., 1991). It bears significant NH2-amino acid sequence homology with that of β-lactoglobulins of various animal species (Seppala et al., 1991). There is N-terminal amino acid sequence homology between PP14 and human retinol binding protein, but PP14 binds neither retinol nor retinoic acid (Seppala et al., 1988). On the basis of sequence analysis, PP14 appears to contain 180 amino acids, including an 18 amino acid-containing putative signal peptide; the predicted molecular weight of the mature protein is 18787 (Julkunen et al., 1988). In fluorographic studies with 35S-labelled and 125I-labelled PP14, an apparent molecular weight of ~28 kDa was observed (Julkunen et al., 1986). Earlier the same mAb (B7B10) was observed to detect PP14 in human amniotic fluid at Mr 24 kDa in Western blot analysis (Dutta et al., 1998). In the present study using the rhesus monkey, a single major band of PP14 at Mr 28 kDa was detected in secretory phase endometrial tissue homogenate and in the secretory product of endometrium in vitro.

Julkunen et al. (1986, 1990) and Waites et al. (1988) studied the profile of endometrial PP14 throughout the menstrual cycle in women, and observed that this protein was minimal during the peri-ovulatory period and increasingly expressed in glands throughout the luteal period; maximum concentrations were seen during the late secretory stage, while the luminal surface was found positive only during the mid-secretory and late secretory stages; the stroma was weakly stained without any obvious pattern. Klementzis et al. (1994) also observed that there was an increasing profile in the total area of precipitate for immunopositive PP14 in the endometrium throughout the secretory phase in women. A similar profile was observed in the concentration of PP14 in uterine luminal fluid (Li et al., 1993). We now report that the pattern of PP14 expression in the rhesus monkey endometrium throughout the ovulatory menstrual cycle is similar to that in the human.

It is to be noted that the concentration of endometrial PP14 during the peri-ovulatory period was observed to be very low in the present study, similar to women (Julkunen et al., 1990). It has been suggested that the absence of endometrial PP14 during the peri-ovulatory period characterizes the ‘fertilization window’, because endometrial PP14 inhibits oocyte-spermatozoa adhesion in a potent and dose-dependent manner (Oehninger et al., 1995; Seppala et al., 1997). On the other hand, the percentage area of immunopositive precipitate in glandular cells reached a maximum around the time blastocyst implantation is initiated, i.e. days 7–8 after fertilization; thereafter it remained unchanged until the late luteal period. A comparable observation was made in an in-vitro study using isolated human endometrial cells; it was observed that highest (2.0–3.0 times) stimulation of PP14 synthesis by endometrial epithelial cells under progesterone stimulation occurred in early secretory phase samples compared with other stages of cycle (Laird et al., 1993). Julkunen et al. (1986) also observed that repression of PP14 synthesis and secretion by cycloheximide in tissue culture was maximum with early luteal phase (LH+4 day) human endometrium, less in mid-luteal phase (LH+7–10 days) endometrium, and least in late luteal phase (LH+12 day) endometrium. Also, significant increase in the Golgi complex, along with increased synthesis and secretion of procollagen molecules in glandular epithelium of human endometrium is noted during the early- to mid-secretory phases of the cycle (Themann and Schunke, 1963; Dallenbach-Hellweg, 1981; Dockery et al., 1988; Brenner and Maslar, 1988; Ferenczy, 1994). Wahlstrom et al. (1985) observed that the peri-implantation stage human endometrium was PP14 positive, and it may appear in endometrium as early as 24 h after follicle aspiration.
Table I. Morphometric analysis of immunopositive placental protein 14 in endometrium during different phases of menstrual cycle

<table>
<thead>
<tr>
<th>Group</th>
<th>Cycle day (n)</th>
<th>Phase of menstrual cycle in relation to day of ovulation</th>
<th>Surface epithelium (score range)</th>
<th>Area of immunopositive precipitation (%) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>8 (4)</td>
<td>Pre-ovulatory (~2 to ~4)</td>
<td>1</td>
<td>24.7 ± 3.2</td>
</tr>
<tr>
<td>1b</td>
<td>12 (4)</td>
<td>Peri-ovulatory (0 to +2)</td>
<td>1</td>
<td>16.2 ± 2.7</td>
</tr>
<tr>
<td>1c</td>
<td>16 (5)</td>
<td>Early luteal (+4 to +6)</td>
<td>4</td>
<td>48.3 ± 4.7</td>
</tr>
<tr>
<td>1d</td>
<td>20 (4)</td>
<td>Mid luteal (+8 to +10)</td>
<td>3</td>
<td>40.4 ± 5.2</td>
</tr>
<tr>
<td>1e</td>
<td>24 (3)</td>
<td>Pre-menstrual (+12 to +14)</td>
<td>2</td>
<td>38.8 ± 5.8</td>
</tr>
</tbody>
</table>

*Endometrial samples were collected on cycle days 8, 12, 16, 20 and 24 for groups 1a, 1b, 1c, 1d and 1e respectively.

*Based on day of ovulation as day 0.

*P*, 0.001 in Kruskal–Wallis test. For statistical analysis between groups, see text.

Table II. Densitometric analysis of immunopositive band of PP14 in Western blots

<table>
<thead>
<tr>
<th>Source</th>
<th>Total immunopositive score (OD, mm) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Group 2a, n = 6)</td>
</tr>
<tr>
<td></td>
<td>Significance in difference between groups (P)</td>
</tr>
<tr>
<td>Tissue homogenate</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Spent medium</td>
<td>0.26 ± 0.01</td>
</tr>
</tbody>
</table>

OD = optical density.

Table III. Morphometric analysis of immunohistochemical staining for PP14 in group 2 endometrial samples

<table>
<thead>
<tr>
<th>Area of immunopositive precipitation (%) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gland (n = 6)</td>
</tr>
<tr>
<td>Control (Group 2a)</td>
</tr>
<tr>
<td>50.5 ± 5.3</td>
</tr>
</tbody>
</table>

*P*, 0.02, bnon-significant between groups.

PP14 in monkey endometrium

in induced superovulatory cycles. Furthermore, a strong expression of PP14 was observed in the surface epithelium and glands during the peri-implantation stage in the present study. Collectively, results from the present study and earlier studies suggest that the sensitivity of PP14 induction in endometrial epithelium is highest around the time of implantation in primates. However, similar to the human (Julkunen et al., 1986, 1990; Waites et al., 1988; Klentzeris et al., 1994), the total score in glands (precipitates in cell body plus luminal secretion) showed an increasing profile throughout the luteal phase, because of increasing gland area and increased luminal secretion (Noyes et al., 1950; Li et al., 1988b; Ferenczy, 1994). Furthermore, the concentrations of PP14 in uterine flushings closely correlate with the volume fraction measurement of glands of secretory stage endometrium (Li et al., 1993).

To our knowledge this is the first report showing the expression of immunodetectable PP14 in luteal phase endometrium in a non-human primate species with marked similarity with that of human in its characteristics and profiles in the glandular compartment during the natural menstrual cycle. In previous studies, although PP14 could be immunodetected in placental extract and blood samples of pregnant baboons (Siniosich et al., 1990), it could not be detected in baboon endometrium during the menstrual cycle (Fazleabas and Verhage, 1987). Recently, Okulicz et al. (1996) could detect the cDNA for PP14 in adequately progesterone-stimulated endometrium from ovariectomized rhesus monkeys. Thus, it appears that the rhesus monkey can be used as a primate model in order to further examine the biology of endometrial PP14.

Endometrial PP14 and receptivity for implantation

A single dose application of a potent antiprogestin, mifepristone on day 2 after ovulation-induced desynchronization of endometrium, repressed glandular secretory differentiation and vascular maturation (Li et al., 1988a; Johannisson et al., 1989; Gemzell-Danielsson et al., 1994, 1997a; Ghosh et al. 1996,
observed that the contragestional effect of early luteal phase mifepristone treatment was associated with a marked decrease in the concentration of immunodetectable PP14 in endometrial glands and its secretion around the time when implantation is initiated. In human studies, serum concentrations of PP14 at the time of menstruation was found to be decreased following low weekly doses of mifepristone treatment (Gemzell-Danielsson et al., 1996) while endometrial expression of PP14 was decreased during the mid luteal phase of ovarian cycles subjected to low daily doses of mifepristone treatment (Gemzell-Danielsson et al., 1997b). It remains to be seen whether this decline is caused from direct antiprogestosterone action on endometrial glands during progesterone dominance, or secondarily from retarded development of the endometrium. Glucocorticoid/progesterone regulatory elements (PRE) have been found in the PP14 gene, and these elements bind progesterone receptors in vitro (Vaisse et al., 1990). Thus, it is possible that mifepristone may inhibit PP14 transcription by blocking PRE. While there is evidence to suggest that endometrial PP14 could be regulated by progesterone (Seppala et al., 1987a,b), several studies indicate that the regulation of PP14 by progesterone could be a complex phenomenon (Ren et al., 1990; Critchley et al., 1990, 1992; Batista et al., 1996). It has been suggested that progesterone regulation of endometrial PP14 may be indirect, and linked with secretory differentiation of the endometrium under progesterone dominance (Bell and Drife, 1986). Previously, it was observed that the amount of immunodetectable PP14 in uterine flushing, as well as the area of precipitate for immunopositive PP14, were strongly associated with normal endometrial development, and were low when endometrial maturation was retarded (Li et al., 1993; Kletzeris et al., 1994; Dalton et al., 1995). Also, there was no up-regulation of the cDNA population for PP14 in inadequately progesterone-stimulated secretory phase endometria of ovariectomized monkeys (Okulicz et al., 1996). Since the secretory maturation of endometrium is significantly retarded with early luteal phase mifepristone treatment in women and monkeys (Li et al., 1988a; Johannisson et al., 1989; Gemzell-Danielsson et al., 1994, 1997a; Ghosh et al., 1996, 1998a,b; Dockery et al., 1997), it is likely that the decline in endometrial PP14 following early luteal phase mifepristone treatment resulted from the inhibition of endometrial maturation. However, it is interesting to note that endometrial retardation following tamoxifen treatment for 3 days after ovulation was not associated with any decrease in plasma PP14 in women, while mifepristone treatment had led to a significant decrease in plasma PP14 concentrations, as well as to endometrial retardation (Swahn et al., 1993). The functional significance of PP14 in relation to the secretory differentiation of endometrium during the implantation window under progesterone dominance remains to be examined. Meanwhile, there is evidence that immunohistochemical detection of PP14 in mid-secretory phase endometrium may be a promising approach in the assessment of endometrial maturation (Manners, 1990; Rizk et al., 1992).

Acknowledgements
This study was supported by the Rockefeller Foundation (RF 95021 #34) and Department of Biotechnology, India. We thank the Special

1998a,b; Dockery et al., 1997), resulting in inhibition of blastocyst implantation, with no notable changes in menstrual cyclicity in women and in monkeys (Gemzell-Danielsson et al., 1993; Ghosh and Sengupta, 1993). It was also observed that a single dose administration of antiprogestin (onapristone) on day 2 after ovulation resulted in significant decrease in plasma concentrations of PP14 on day 12 after ovulation in women (Cameron et al., 1996). In the present study, we

Figure 5. Immunohistochemical localization of placental protein 14 in surface epithelium (a, c), glands and stroma (b, d) of endometrium collected on day 6 after ovulation from control (group 2a; a, b) and mifepristone-treated (group 2b; c, d) fecund cycles. Bar = 30 µm.

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Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Switzerland for providing us with the reagents for oestradiol and progesterone radioimmunoassays, and Roussel-Uclaf, France for their kind gift of RU486.

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Received on April 29, 1998; accepted on September 15, 1998