Endometrial expression of immunomodulatory cytokines and their regulators during early pregnancy in bonnet monkeys (Macaca radiata)

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BACKGROUND: It is well established that endometrium undergoes extensive histological changes during implantation and subsequent stages of pregnancy in rodents as well as primates. Our previous investigation using a non-human primate model has demonstrated that morphological alterations are initiated even before the embryo invades the endometrium. The present study was undertaken to determine whether the embryo-induced morphological changes are accompanied by any alteration in the protein levels of the immunomodulatory cytokines and their regulators in the preimplantation stage endometrium. METHODS: The endometrial expression of immunosuppressive factors such as transforming growth factor β2 (TGFβ2), glycodelin (PP14), leukaemia inhibitory factor (LIF) and interleukin-6 (IL-6) were analysed on day 6 post-ovulation in pregnant and non-pregnant bonnet monkeys (Macaca radiata) using immunohistochemical methods. RESULTS: The endometrial expression of TGFβ2, TGFβ2 receptor, PP14 and IL-6 were significantly up-regulated (p < 0.05) in pregnant animals as compared to non-pregnant animals, whereas the expression of LIF and its receptor remained unaltered in pregnant animals. CONCLUSIONS: Expression levels of some immunomodulatory cytokines in endometrium are significantly increased even before the embryo invades the endometrium. The altered cytokine expression profile in endometrium probably contributes towards generating a conducive environment for the embryo survival, growth and development in the uterus.

Key words: bonnet monkeys/early pregnancy/endometrium/glycodelin/immunomodulation

Introduction
A significant proportion of pregnancy losses in humans have been linked to immune dysfunctions (Laird et al., 2003). Therefore, the immune functions, especially those executed by circulatory immune cells during pregnancy, have often interested reproductive immunologists. Studies pursued in this direction have demonstrated remarkable changes in the maternal cytokine profile in the circulation of pregnant women (Abrahams et al., 2004; Aluvihare et al., 2004; Dosiou and Giudice, 2005). Circulatory levels of several cytokines such as interleukin (IL)-1, IL-4, IL-6, IL-10 and transforming growth factor β1 (TGFβ1) have been found to be significantly higher in pregnant women (Austgulen et al., 1994; Makhseed et al., 2000; Power et al., 2002; Matthiesen et al., 2003). There is evidence to indicate an increase in the levels of immunosuppressive Th2 lymphocytes/cytokines and the decrease in pro-inflammatory Th1 lymphocytes/cytokine levels during pregnancy (Makhseed et al., 1999; Saito et al., 1999a; Ho et al., 2001; Wu et al., 2001; Holmes et al., 2003). Any deviation from this trend has been found to be associated with recurrent pregnancy loss (Reinhard et al., 1998; Jenkins et al., 2000; Raghupathy et al., 2000; Paradisi et al., 2003; Kwak-Kim et al., 2003). A higher Th1/Th2 lymphocyte (or cytokine) ratio was detected in patients presenting with implantation failures after IVF-embryo transfer, recurrent spontaneous miscarriages and pre-eclampsia (Saito et al., 1999b; Lim et al., 2000; Ng et al., 2002; Laird et al., 2003; Shimada et al., 2003). It is speculated that the increased levels of steroids, peptides such as relaxin, neuroendocrine mediators as well as embryonic stimuli contribute towards immunomodulation during pregnancy (Piccinni and Romagnani, 1996; Elenkov et al., 2000; Kidd, 2003; Ragusa et al., 2004).

Some efforts have also been invested in exploring the potential changes in local immune network at the feto-maternal interface (Kruse et al., 1999). Secretion of IL-10 and TGFβ by γδT cells increases during pregnancy in human decidua (Nagaeva et al., 2002). Further, the proportion of IL-4 secreting Th2 cells has been found to be significantly higher than interferon-γ secreting Th1 cells in the human decidua during pregnancy (Ho et al., 2001). The secretion of Th2 cytokines such as IL-6 and IL-10 by phytohaemagglutinin-stimulated lymphocyte subsets was decreased in the decidua of pre-eclamptic births (Saito et al., 1999).
women as compared to women with normal pregnancy (Wilczynski et al., 2003). Further, the leukaemia inhibitory factor (LIF) production by CD4+ decidual clones, obtained during miscarriage from women with recurrent miscarriage, was lower compared with CD4+ clones obtained from women undergoing pregnancy terminations (Piccinni et al., 2001). These studies provide strong evidence of functional alterations in the local immune network in the gestational endometrium in humans. However, as only a small percentage of endometrial cells are the immune cells, they may not account for the dramatic changes in the circulating levels of various cytokines during pregnancy (Laird et al., 2003). It is now reported that the endometrial epithelial and stromal cells as well as the decidual cells synthesize several cytokines (Laird et al., 2003). Th2 cytokines such as IL-6, IL-1, LIF and IL-10 are produced in large amounts by the decidual cells during pregnancy (Vigano et al., 2002; Sengupta et al., 2003). These studies have, however, focused on analysing the expression levels of these factors after embryo implantation. There exists no report on the endometrial expression of the immunomodulatory factors before embryo invasion in primates. The knowledge gathered by pursuing studies in this direction may help to identify the causes of early pregnancy losses in women and implantation failures in assisted reproductive technologies.

The present study was initiated to analyse the endometrial expression of two cytokines, LIF and IL-6, and their regulators, TGFβ2 and glycodelin (PP14) during very early stages of pregnancy (before embryo invasion) in primates. As it is difficult to obtain human samples at these stages of pregnancy because of ethical and moral limitations, we chose to study these events in bonnet monkeys. Bonnets show close similarity to humans in terms of their reproductive parameters such as menstrual cycle length, endometrial receptivity and implantation (Katkan et al., 1995; Beier and Beier-Hellwig, 1998; Sachdeva et al., 2001; Lessey, 2003; Rosario et al., 2003; Creinin et al., 2004). Also like humans, implantation in bonnet monkeys is initiated approximately on day 6–7 post ovulation (Lindenberg et al., 1986; Jayaprakash et al., 1997). In addition, the high fecundity of bonnets and the ready availability of the assay (preimplantation factor bioassay) to detect very early pregnancy in bonnets were the other advantages that prompted us to select these species as the subjects for the present study.

Materials and methods

The present study was approved by the Institutional Animal Ethics and Human Ethics Committees and the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Bonnet monkeys (Macaca radiata)

Adult bonnet monkeys were housed singly under controlled conditions in the animal house facility of the institute and were fed with a diet composed of semi-formulated Indian bread, fresh seasonal fruits, eggs and sterile water.

The cyclicity of female bonnet monkeys was monitored as described previously (Puri et al., 2000; Rosario et al., 2005). Regularly cycling female bonnet monkeys (n = 9) showing normal hormonal profiles were mated with males of proven fertility for 6 consecutive days starting from 2 days prior to the expected estradiol (E2) peak. Two regularly cycling female bonnet monkeys were treated with 1.25 mg of an antiprogestin onapristone, (ZK 98,299) (a kind gift from Schering, Berlin, Germany) in 0.5 ml benzyl benzoate: castor oil (1:15) on every third day of the menstrual cycle until the day of biopsy collection. These animals were also mated with males of proven fertility. The control group (n = 5) included regularly cycling non-mated female bonnet monkeys (Rosario et al., 2005).

Radioimmunoassays for estradiol and progesterone

Serum estradiol and progesterone concentrations were measured by specific radioimmunoassays as described previously (Puri et al., 1987).

Preimplantation factor bioassay

Preimplantation factor (PIF) bioassay was performed on a single serum sample collected on day 6 post E2 peak to detect early pregnancy in the mated bonnet monkeys as detailed previously (Rosario et al., 2005). PIF-positive mated animals were designated as pregnant, non-mated animals as non-pregnant.

Collection of endometrial biopsies

Endometrial biopsies were collected on day 6 post ovulation from pregnant (n = 5), non-pregnant (n = 5) and onapristone treated pregnant (n = 2) animals as described previously (Katkan et al., 1995) and processed for routine paraffin embedding and sectioning. The endometrial sections were stained with haematoxylin and eosin to analyse the histological alterations induced in response to embryonic stimuli (Rosario et al., 2005). Endometrial aspirates were also collected from proven fertile women (n = 3) during the mid-secretory phase. Informed consent was obtained from all the women prior to collection of endometrial biopsies.

Immunohistochemical localizations

LIF, LIF receptor and PP14

Immunohistochemical localizations of LIF, LIF receptor (LIFR) and PP14 in endometria from non-pregnant, pregnant (in both groups; n = 5 for LIF and LIFR and n = 3 for PP14) and onapristone-treated pregnant animals (n = 2 for LIF) were carried out. In brief, sections were deparaffinized and rehydrated. For the sections to be probed with antibodies against PP14, endogenous peroxidase activity was quenched by incubating the sections with 0.3% H2O2 for 30 min. For LIF and LIFR, endogenous alkaline phosphatase activity was inhibited by levamisole. For LIF and LIFR localizations, the sections were blocked in 1% normal rabbit serum in 0.01% bovine serum albumin phosphate-buffered saline (BSAPBS) for 1 h and for PP14 localization, in 1% mouse serum prepared in PBS for 30 min. Sections were incubated at 4°C for 16 h with goat recombinant anti-human LIF or LIFR antibodies (diluted 1:20 in 0.01% BSAPBS) (R&D Systems, Minneapolis USA). PP-14 antigen was immunolocalized using monoclonal antibody against PP-14, diluted 1:20 in PBS (a kind gift from Dr Anjali Karande, IISc, Bangalore, India). In the negative control sections for LIF and LIFR, the primary antibodies were replaced by goat serum and for PP14, by SP-20 myeloma cell culture supernatant. Following washings in PBS, sections were incubated with biotinylated rabbit anti-goat secondary antibody (diluted 1:100 in 0.01% BSAPBS) for LIF and LIFR and biotinylated goat anti-mouse secondary antibody (diluted 1:50 in PBS) for PP14 (Vector Laboratories, Burlingham, CA, USA) for 2 h at room temperature and washed in PBS twice for 10 min. Detection of LIF/LIFR immunoprecipitates was carried out using the alkaline phosphatase kit according to the manufacturer’s protocol (Vectastain alkaline phosphatase-AP kit, Vector Laboratories) while for PP-14, avidin: biotin peroxidase system (Vector Laboratories) was used. The sections for LIF/LIFR immunostaining were incubated with freshly prepared substrate p-nitrophenol.
phosphatase (Vector alkaline phosphatase substrate kit 1, Vector Laboratories) for 5 min, whereas for PP14, the sections were incubated with diaminobenzidine (Sigma St Louis, Missouri, USA) and H\textsubscript{2}O\textsubscript{2} for 10 min. The endometrial sections were counterstained lightly with haematoxylin. The sections were dehydrated, cleared in xylene and viewed under the Olympus BX60 microscope. The experiments were repeated three times for each animal tissue. In all runs, endometrial samples from pregnant, non-pregnant and appropriate negative controls were included.

To check whether the commercially available antibodies against human LIF/LIFR react with monkey LIF/LIFR, antibodies preadsorbed against bonnet endometrial tissues were used to immunostain human endometrial tissues. However, for this study the Vectastain ABC system was used instead of the vectastain alkaline phoshphatase system (Vector Laboratories). The deparaffinized and dehydrated endometrial tissue sections from bonnet monkeys were treated with 0.3\% H\textsubscript{2}O\textsubscript{2} for 30 min, blocked in 1\% rabbit sera in PBS for 30 min and then incubated at 4\textdegree C for 16 h with goat recombinant anti-human LIF (diluted 1:25 in PBS) or LIFR (diluted 1:20 in PBS) antibodies or normal goat sera. The spent antibodies were collected and stored at –20\textdegree C for 4 h. After washing in PBS, the sections were incubated with biotinylated rabbit anti-goat secondary antibody (diluted 1:100 in PBS) for 2 h at room temperature, followed by incubation in avidin–biotin complex for 30 min. Detection of the immunoprecipitates was carried out using diaminobenzidine (Sigma, USA) and H\textsubscript{2}O\textsubscript{2} for 10 min. The endometrial sections were counterstained lightly with haematoxylin. Further, immunolocalization of LIF/LIFR was carried out on human endometrial tissues in a similar fashion. Human endometrial tissues were also immunostained with LIF/LIFR antibodies preadsorbed previously with bonnet endometrial tissues.

**TGF\textsubscript{\beta}, TGF\textsubscript{\beta} receptor and IL-6**

Endometrial sections from non-pregnant, pregnant [in both groups; \( n = 5 \) each for TGF\textsubscript{\beta2}, TGF\textsubscript{\beta}2 receptor (TGF\textsubscript{\beta}2R) and \( n = 3 \) for IL-6] and from onapristone-treated pregnant animals (\( n = 2 \) for TGF\textsubscript{\beta2}, TGF\textsubscript{\beta}2R) were deparaffinized in xylene and rehydrated through descending grades of alcohol. Endogenous peroxidase activity was quenched by incubating the sections with 0.3\% H\textsubscript{2}O\textsubscript{2} in methanol for 30 min. The sections were then blocked with either 2\% normal goat serum in PBS (for TGF\textsubscript{\beta2} and TGF\textsubscript{\beta}2R) or 1\% normal goat serum (for IL-6) for 30 min. The sections were further incubated at 4\textdegree C for 16 h with the respective primary antibodies (1:20 dilution of polyclonal rabbit antibodies against TGF\textsubscript{\beta2} and TGF\textsubscript{\beta}2R; 1:10 dilution of polyclonal rabbit antibody against IL-6) procured from Santa Cruz Biotecnology Inc. (CA, USA). In the negative control sections, the primary antibodies were replaced by normal rabbit. The next day, sections were washed twice in PBS for 10 min each and incubated with 1:200 diluted goat anti-rabbit biotinylated antibody (Santa Cruz Biotecnology) prepared in blocking solution for 2 h at room temperature. After washing in PBS, the sections were incubated with avidin–biotinylated horseradish peroxidase complex for 30 min followed by a PBS rinse and addition of diaminobenzidine and H\textsubscript{2}O\textsubscript{2} (Santa Cruz Biotecnology) in PBS for 10 min. The endometrial sections were counterstained lightly with haematoxylin only for TGF\textsubscript{\beta2} and TGF\textsubscript{\beta}2R. The sections were dehydrated, cleared in xylene, mounted in DPX and viewed under the Olympus BX60 microscope. The experiments were repeated three times for each animal tissue. In all runs, endometrial samples from pregnant, non-pregnant and appropriate negative controls were included.

**Image analysis**

The immunoprecipitates of the various antigens were quantified in four randomly selected areas from endometrial sections for each animal by image analysis software BioVis 1.42. The integrated optical density (IOD) in each area was calculated using the software. Negative control slides were analysed in a similar fashion. The IOD values of the negative control (without antibody) were subtracted from the IOD values for each animal in the two groups. The mean, SD and SEM were calculated for each group. The variation between the individual runs of the various immunolocalization procedures was also calculated using BioVis 1.42. Statistical analysis was carried out using the Student’s \( t \)-test.

**Results**

Six of the nine mated animals were found to be pregnant on the basis of serum PIF positivity (Rosario et al., 2005). Of these six animals, endometrial biopsies from five animals were used for the present study. These animals showed the presence of pre-epithelial plaque reaction in the luminal and glandular epithelium, decreased secretory activity and adluminal vacuolation in the glands and increased compaction in the stroma (Rosario et al., 2005). However, no significant differences were noted in the circulating levels of estradiol and progesterone during the cycle as well as on the day of biopsy in pregnant and non-pregnant animals (Rosario et al., 2005).

**Immunolocalizations of LIF and LIFR**

The representative photographs of immunolocalization patterns of LIF and LIFR are shown in Figures 1 and 2 respectively. The antigens were localized in the cytoplasm of glandular epithelial cells and stroma. The antibodies localized the antigens in the endometrial tissues from both human and bonnet monkeys (Figures 1c, d and 2c, d). However, there was significant reduction in the intensities of LIF and LIFR immunoprecipitates in human tissue, immunostained with rhLIF/rhLIFR antibodies preadsorbed with monkey endometrial tissue (Figures 1e and 2e). This indicated that the antibodies against human LIF/LIFR cross-reacted with the respective antigens in monkey tissues. However, no significant differences were observed in the expression of immunoreactive LIF and LIFR in the endometria of pregnant animals as compared to non-pregnant animals (\( p > 0.05 \)) (Figures 3A and 4A; Table I). The semiquantitative analysis is shown in Figures 5A and B.
Interestingly, the expression of endometrial LIF was found to be down-regulated in two pregnant animals treated with 1.25 mg dose of onapristone (Figure 3A,d).

Immunolocalization of IL-6

The expression of IL-6 in the endometrium was localized in the stroma underlying the glands. In the non-pregnant animals, the expression of IL-6 was faint while its expression increased significantly during early pregnancy \( (p < 0.05) \) (Figures 4B and 5C; Table I).

Immunolocalization of PP14

The expression of PP14 was found only in the cytoplasm of glandular epithelium of endometrium. The expression of immunoreactive PP14 was higher in endometria of the pregnant animals as compared to non-pregnant animals \( (p < 0.05) \) (Figures 4C and 5D; Table I).
Immunolocalizations of TGFβ2 and TGFβ2R

Immunoreactive endometrial TGFβ2 and TGFβ2R were mainly localized in the glandular epithelial compartment, the stroma showing minimal expression. In pregnant animals, the endometrial glandular epithelium showed increased expression ($p < 0.05$), while stroma showed no alteration in the expression of TGFβ2 and TGFβ2R when compared to non-pregnant animals (Figures 3B, C and 5E, F; Table I). The expression of immunoreactive TGFβ2 and TGFβ2R in 1.25 mg onapristone-treated pregnant animals was similar to that observed in the pregnant animals (Figures 3B,d and C,d).

Discussion

Immunomodulation during pregnancy has been implicated to play a role in preimplantation embryo development, implantation and fetal allograft tolerance. Differential distributions of lymphocyte subsets and cytokine levels have been reported in pregnant and non-pregnant women (Makhseed et al., 1999; Saito et al., 1999a; Ho et al., 2001; Wu et al., 2001; Holmes et al., 2003). However, the majority of these studies have invariably focused on analysing the immune alterations in circulation at the post-implantation stages of pregnancy.

Our previous studies have demonstrated significant morphological changes in the endometrium, prior to embryo attachment in bonnet monkeys (Rosario et al., 2005). These studies were indicative of the embryonic influences on endometrial preparation for decidualization and angiogenesis. We extended these investigations to determine whether endometrium modulates its cytokine profile during very early stages of pregnancy; in order to create an immunosuppressive environment within the uterine cavity. The expression of immunosuppressive factors...
such as LIF, IL-6, TGFβ2 and PP14 were analysed in gestational endometrium collected from bonnet monkeys on day 6 post ovulation.

The role of LIF in endometrial receptivity and implantation has been extensively investigated (Stewart et al., 1992; Yang et al., 1995; Cullinan et al., 1996; Ghosh et al., 1998; Vogiagis and Salamonsen, 1999; Rosario et al., 2003). LIF, a pleiotropic cytokine of the IL-6 superfamily, displays multiple biological activities with its expression being dependent on cellular localization, steroid hormones and a local cytokine network (Kondera-Anasz et al., 2004). LIF regulates the growth and differentiation of embryonic stem cells, peripheral neurons and several other cells. LIF is also known to play an important role in implantation by affecting the trophoblast differentiation pathway towards the adhesive phenotype (Senturk and Arici, 1998). Further, LIF production by decidual T cells is believed to contribute to the maintenance of pregnancy, since in women with unexplained recurrent abortions, decidual T cells produce lesser amounts of LIF (Piccinni et al., 1998; Piccinni, 2002).

To detect endometrial LIF/LIFR in bonnet monkeys, we used commercially available antibodies against recombinant human LIF/LIFR. These antibodies could immunolocalize endometrial LIF/LIFR proteins in bonnet monkeys also. Further, the intensities of the LIF/LIFR immunoprecipitates in human endometrial sections probed with antibodies previously preadsorbed with bonnet endometrial tissues were lesser as compared to those reacted with the antibodies without any pretreatment. We did not observe any significant change in the endometrial expression of LIF and its receptor on day 6 post ovulation in pregnant animals as compared to non-pregnant animals. This is in contrast to a previous report demonstrating up-regulation in the endometrial expression of LIF and its receptor on day 3–9 of pregnancy in rhesus monkeys (Yue et al., 2000). However, the study by Yue et al. (2000) did not provide strong evidence of successful conception, as just the sperm presence in the vaginal smear was considered sufficient to designate the mated rhesus monkeys as pregnant animals whereas in our study, pregnancy on day 6 post ovulation was confirmed by the PIF bioassay. Also, remarkable histological changes were observed in endometrium from the pregnant bonnet monkeys (Rosario et al., 2005). Thus our study demonstrates that the expression of endometrial LIF/LIFR is not changed before embryo invasion in pregnant animals.

Two animals that were treated with a dose of onapristone (1.25 mg), too low to prevent the initiation of pregnancy, showed lesser endometrial LIF expression. This was indicative of the endometrial LIF expression being progesterone dependent. As these two animals were PIF positive (and pregnant), it may be concluded that embryonic stimuli do not regulate the LIF expression on day 6 post ovulation in pregnant animals. Since endometriectomy was done on day 6 post ovulation and pregnancy terminated, it remained a matter of speculation whether the reduced LIF expression due to antiprogestin treatment affects the subsequent stages of implantation in pregnant animals. Nonetheless, these observations provide a clue in favour of the possibility that endometrial LIF production is controlled predominantly by progesterone, rather than by embryonic stimuli during early pregnancy. It is likely that the levels of endometrial LIF and its receptor expressed during the implantation window are sufficient for receptivity to the embryo and that there occurs no further modulation by embryonic stimuli in pregnant animals. The support for this speculation comes from studies indicating the similar levels of LIF in the conditioned media from endometria cultured in the presence and absence of embryos. Also, similar levels of LIF have been detected in non-pregnant females and females with anembryonic pregnancy (Spandorfer et al., 2001; Chen et al., 2004). On the other hand, there exists a possibility that the endometrial LIF expression in primates is biphasic as seen in mice; implying that though it remains unaltered before embryo invasion (Bhatt et al., 1991; Song et al., 2000), it may change during subsequent stages of pregnancy in primates.

IL-6, another cytokine of the interleukin-6 superfamily, shares a signal transducing receptor subunit gp130 with LIF (Sherwin et al., 2002). The expression of IL-6 is reported to be significantly higher in the primary implantation site of rhesus monkey (Sengupta et al., 2003). Data also demonstrate that endometrial IL-6 expression is significantly less in women presenting with habitual abortion (von Wolff et al., 2002). Decidual stromal cells from early human pregnancies in culture produce significant amounts of IL-6 (Montes et al., 1995). In the present study also, the expression of endometrial IL-6 on day 6 post ovulation was found to be significantly higher in pregnant animals as compared to non-pregnant animals. It is likely that embryonic stimuli modulate endometrial IL-6 production. Our findings are in agreement with a previous report which demonstrated significant stimulation of IL-6, but not of LIF, in endometrial cells treated with hCG (Uzumcu et al., 1998). However, in contrast to a previous report (Laird et al., 1993), IL-6 was not detected in the epithelial cells in the present study. This difference in the spatial localization of IL-6 in these two studies cannot be attributed to embryonic influences as a similar pattern was observed in non-pregnant animals.

We next investigated the endometrial expression of glycoprotein (PP14), known for its ability to regulate IL-6 production in vitro. PP14 induces a dose-dependent increase in IL-6 production by human endometrial epithelial cells (Laird et al., 1994). In addition, PP14 has been studied extensively for its immunosuppressive role in pregnancy (Seppala et al., 2002). Some of the immunosuppressive functions of PP14 are probably due to its ability to stimulate apoptosis in activated T cells (Mukhopadhyay et al., 2004) and to suppress the activity of CD56+ NK cells (Okomo et al., 1991). We observed a significant increase in the immunolocalization of PP14 in the glandular epithelium of endometria from pregnant animals. Our results corroborate the previous observations in baboons and humans (Fazleabas et al., 1997; Hausermann et al., 1998; Seppala et al., 2002). It is likely that PP14 expression is one of the regulatory steps in endometrial IL-6 production during early pregnancy. Enhanced synthesis of IL-6 and PP14 in endometrium before embryo invasion in primates has not been reported so far.

A significant increase in endometrial IL-6 synthesis observed during early pregnancy prompted us to analyse the
expression of TGFβ2, another regulator of IL-6 expression. TGFβ2 suppresses generation of cytotoxic T cells in vitro, reduces the activity of decidual CD16-CD56 bright NK cells and CD4 and CD8 membrane expression on peripheral blood leucocytes (Saito et al., 1993; Clark et al., 1994; Ouellette et al., 1999). A significant decrease in the levels of TGFβ2 mRNA was observed in the utero-placental units of mice with pregnancy loss as compared to control mice, thereby implicating the potential role of TGFβ2 in the maintenance of pregnancy. The patients with unexplained recurrent miscarriage were found to have a deficiency of decidual immunosuppressor cells that produce TGFβ2 (Lea et al., 1995). We observed a significant increase in endometrial TGFβ2 and its receptor in the glandular epithelium in pregnant animals. Increased endometrial TGFβ2 synthesis may be responsible for enhanced IL-6 synthesis in gestational endometrium before embryo invasion. Further, TGFβ2 and TGFβ2R expression in endometrium were also up-regulated in two onapristone-treated pregnant animals. This up-regulation, despite anti-progesterin treatment, could have been induced by embryonic stimuli.

The present study investigated the level of protein expression of immunosuppressive factors, i.e. LIF, TGFβ2, PP14, IL-6 in each sample from at least three animals in each group. The expression of endometrial TGFβ2, PP14 and IL-6 were significantly higher in pregnant animals as compared to non-pregnant animals. It may be hypothesized that the increase in endometrial TGFβ2 and PP14 due to embryonic stimuli enhances endometrial IL-6 production and subsequent action during early pregnancy. It is also possible that these events are independent of one another. Nonetheless, it can be concluded that the factors endowed with immunosuppressive functions show significantly higher expression in endometrium even before embryo invasion and probably contribute towards modulation of the immune environment within the uterine cavity. It remains to be investigated how these molecules synthesized in different endometrial compartments interact to modulate the uterine milieu and facilitate implantation.

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