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Distribution of Integrins and the Extracellular Matrix Proteins in the Baboon Endometrium during the Menstrual Cycle and Early Pregnancy[1]

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ABSTRACT

Integrins are heterodimeric glycoproteins that have been found to undergo dynamic temporal and spatial changes in distribution in the endometrium during the menstrual cycle in women. Likewise the extracellular matrix (ECM) ligands for these receptors are likely to play a role in the establishment of a receptive endometrium. To develop primate models to study the role of these molecules in the cascade of molecular events leading to implantation, integrin expression and associated changes in ECM were investigated during the menstrual cycle and in early pregnancy in the baboon. Antibodies specific for the integrins (α_{1-6} and α_v; β_1, β_3, and β_4) and ECM (laminin, collagen IV, fibronectin) were utilized. In addition, cytokeratin and α-smooth muscle actin were used as epithelial, stromal, and smooth muscle cell markers, respectively. Endometrium was obtained in duplicate or triplicate during the menstrual cycle and early pregnancy. Changes observed during the natural menstrual cycle were confirmed using ovariectomized, steroid-treated animals. Constitutively expressed integrins on the endometrial epithelium included the collagen/laminin receptors: α_2, a_3, α_6, and β_4. The pattern of expression correlated well with the distribution of ECM in this tissue. Collagen IV was confined to the basement membrane of glandular epithelium and blood vessels. Laminin immunostaining was found in the basement membrane, mostly in the stroma of the basal region, in the glandular endometrium and vasculature. Fibronectin was present throughout the stroma but not in the basement membrane. The collagen receptor $\alpha_1\beta_1$ and fibronectin receptor $\alpha_4\beta_1$ appeared in the glandular epithelium in the luteal phase. As in the human, α_1 and α_4 disappeared from the glandular epithelium with the establishment of pregnancy. In contrast, the $\alpha_v\beta_3$ vitronectin receptor appeared in the glandular epithelium only in pregnancy or following long-term steroid treatment with estrogen and progesterone but not during the time of uterine receptivity associated with the initial period of embryo attachment. Osteopontin, an ECM ligand for $\alpha_v\beta_3$, was coexpressed with this integrin in invading cytotrophoblasts, glandular epithelium, and decidualizing stromal cells. Decidualization in the baboon was associated with changes in integrin expression similar to those found in humans: there was an increase in α_1, α_3, α_6, β_1, and $\alpha_v\beta_3$ in the decidualized stromal cells. Laminin and collagen IV expression also increased at the implantation site and throughout the endometrium. In contrast, fibronectin expression was most evident at the implantation site and corresponded to α_5 expression on the invading cytotrophoblasts. In summary, marked similarities were found in the expression of ECM and the integrin receptors between the baboon and the human endometrium throughout the menstrual cycle and in pregnancy. Cycle-specific integrins, α_1, and α_4, were present on epithelial cells during the secretory phase. Delayed expression of $\alpha_v\beta_3$ in baboon endometrial glands correlated closely with the time of enhanced glandular secretory activity in this primate. The baboon appears to be an excellent model for the investigation of the role of integrins and ECM leading to successful implantation.

INTRODUCTION

Despite years of intensifying research, the molecular basis for normal implantation remains poorly understood. The timing of embryo attachment in the human was initially suggested in the 1950s by the work of Hertig and colleagues [1], who evaluated luteal phase hysterectomy specimens for the presence of early gestations. Only free-floating embryos were found in the uteri of women undergoing hysterectomy before cycle Day 19 to 20, whereas implanted embryos were found from cycle Day 21 onward. These and other subsequent studies in animal models led Finn [2] to suggest that the endometrium undergoes a defined period of receptivity. Studies in hormonally prepared recipients from assisted reproductive technology cycles have also documented a period of receptivity for the transfer of human embryos [3, 4]. Recent studies in the human have demonstrated many potential markers of this time in the woman's cycle [5, 6], but little progress has been made in defining the role of these endometrial proteins on trophoblast/endometrial interactions. One of the impediments has been the lack of suitable models with which to approach this question.

The baboon is an excellent primate model for studying the events surrounding endometrial receptivity and implantation (see [7–9] for reviews). Although there are differences between the baboon and human in the timing and cell-specific expression of the major endometrial proteins [7, 9, 10], they are all induced by progesterone and up-regulated during pregnancy [9, 11–13].

Recently we demonstrated that epithelial integrins may be good marker proteins of the window of implantation in the human [14, 15], and it has been shown that decidualization of the endometrial stroma is associated with specific shifts in integrin expression as well [15, 16]. The presence of cycle-dependent changes in both epithelial and stromal integrins has prompted us and others to suggest a possible role for integrins in the cascade of molecular events leading to successful implantation. Two of these integrins have been noted to be missing or their expression delayed in the endometrium of women with infertility and potential defects in uterine receptivity [17–19]. The purpose of the present investigation was to document the expression of integrins and their extracellular matrix (ECM) ligands in

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the endometrium of the baboon during the menstrual cycle and in early pregnancy. By comparing these results with the pattern seen in human females, we hope to better understand the role of integrins in implantation and to develop the baboon as a potential model for the study of ways to improve or block implantation in the human.

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the Animal Care Committee of the University of Illinois. Uterine tissue was obtained at laparotomy from cycling baboons during the proliferative (n = 3) and secretory (n = 8) phases of the menstrual cycle [20]. The day of ovulation in the baboon was determined by monitoring the preovulatory estradiol surge. Two days after the peak of estradiol was designated as Day 1 postovulation. Ovariectomized, steroid-treated baboons (n = 2–3 per group) were used to confirm whether changes observed during the menstrual cycle were hormonally modulated [21]. Mature cycling baboons were mated with fertile males during the periovulatory period as determined by sex skin tumescence, and uterine samples (n = 4) were obtained between Days 21 and 30 of pregnancy [22]. For integrin immunolocalization, tissues were frozen in liquid propane as previously described [23, 24]. Adjacent blocks of tissue were fixed in 4% paraformaldehyde/0.5% glutaraldehyde and embedded in paraffin for immunolocalization of ECM proteins.

For purposes of comparing baboon and human endometrium during the period of uterine receptivity, previously acquired human tissue is used (see Fig. 10). These luteal phase endometrial biopsies were obtained from women presenting to the reproductive endocrine service at the University of Illinois. Informed consent was obtained from all patients. Retention for this study of a portion of the biopsy done for routine diagnostic purposes was approved by the Institutional Review Board of the University of Illinois.

Antibodies

Monoclonal antibodies (mAbs) P1H5, P1B5, P1D6, and 3E1 specific to α_2, α_3, α_5, and β_4 subunits were purchased from Gibco/BRL Life Sciences (Gaithersburg, MD). Dr. Martin Helmer kindly supplied mAbs TS2/7 and B-5H10 directed against the α_1 and α_4 subunits, respectively. GoH3, a specific mAb directed against α_6, was donated by Dr. Arnoud Sonnenberg. AP3, a mAb directed against the β_3 subunit of $\alpha_v\beta_3$ vitronectin receptor, was a gift from Dr. Peter Newman (Milwaukee, WI); mAb LM142 against α_v was provided by Dr. David Cheresh (Scripps Research Institute, LaJolla, CA). The mAbs were titrated to maximize the staining efficiency on baboon tissues.

Monoclonal antibodies against ECM proteins were obtained from the following sources: laminin and collagen IV from Sigma Chemical Company (St. Louis, MO), cytokeratin from Becton Dickenson (San Raphael, CA), α-smooth muscle actin from Dako (Carpenteria, CA), and osteopontin from Hybridoma Bank (Iowa City, IA). The fibronectin antibody was a polyclonal antibody obtained from Dako.

Immunohistochemistry

Immunoperoxidase staining on cryostat sections was performed using primary mAbs as previously described [15]. Serial cryosections 5 μm thick were placed onto poly-L-lysine-coated slides and air dried overnight prior to being fixed in −20°C acetone for 10 min; they were stained using Vectastain Elite ABC kits (Vector Laboratories, Burlingame, CA). Diaminobenzidine (DAB; Sigma) was used as the chromogen. Primary antibody was placed on cryosections after blocking with 1% BSA in PBS (pH 7.2 to 7.4) and allowed to bind at room temperature for 1 h. Following a PBS rinse, a secondary antibody consisting of biotinylated goat anti-mouse antibody was allowed to bind for 30 min. Following another PBS rinse, the endogenous peroxidases were quenched with a 30-min incubation with 0.3% H_2O_2 in absolute ethanol, followed by a 30-min rehydration in PBS. Avidin/biotinylated horseradish-peroxidase macromolecular complex (ABC) was then incubated on the sections for 30 min before the addition of DAB for 3 min to complete the reaction. Samples were subsequently washed in PBS and mounted. The resulting staining was evaluated with use of a Nikon (Tokyo, Japan) microscope by an observer who was uninformed regarding the physiological state of the endometrium.

The pattern and distribution of integrin staining were estimated by means of the HSCORE as calculated using the following equation: HSCORE = Σ Pi (i+1), where i = intensity of staining with a value of 1, 2, or 3 (weak, moderate, or strong, respectively) and Pi is the percentage of stained epithelial or stromal cells for each intensity, varying from 0% to 100%. Previous studies using this technique have yielded low inter- and intraobserver variation [25]. Photomicrographs were made with Kodak 100 ASA (Eastman Kodak, Rochester, NY) film.

TABLE 1. Distribution of integrin subunits by ligand specificity.

Ligand specificity	
Collagen/laminin	Fibronectin/vitronectin
α_1/β_1	α_4/β_1
α_2/β_1	α_5/β_1
α_3/β_1	α_v/β_1
α_6/β_1	α_v/β_3
α_6/β_4	

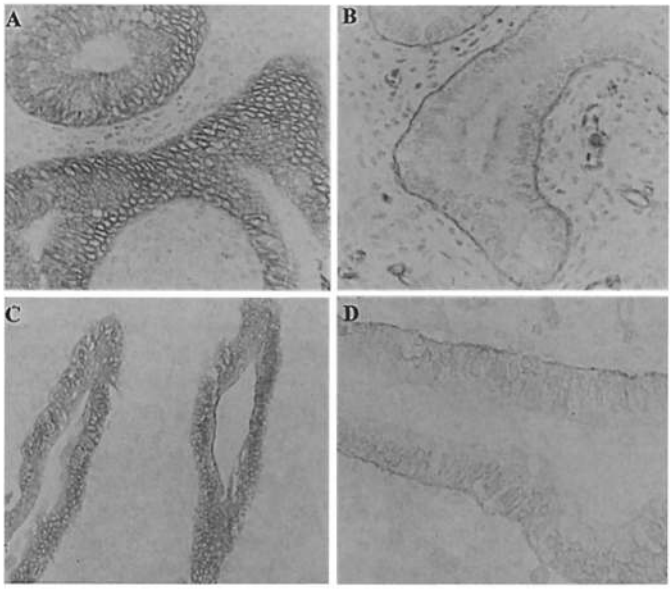

FIG. 1. Immunocytochemical localization of constitutively expressed epithelial integrins α_2 (**A** and **C**) and α_6 (**B** and **D**). Note the similarity of expression in the glandular epithelial cells during both the follicular (**A** and **B**) and midluteal (**C** and **D**) phases of the menstrual cycle. ×200.

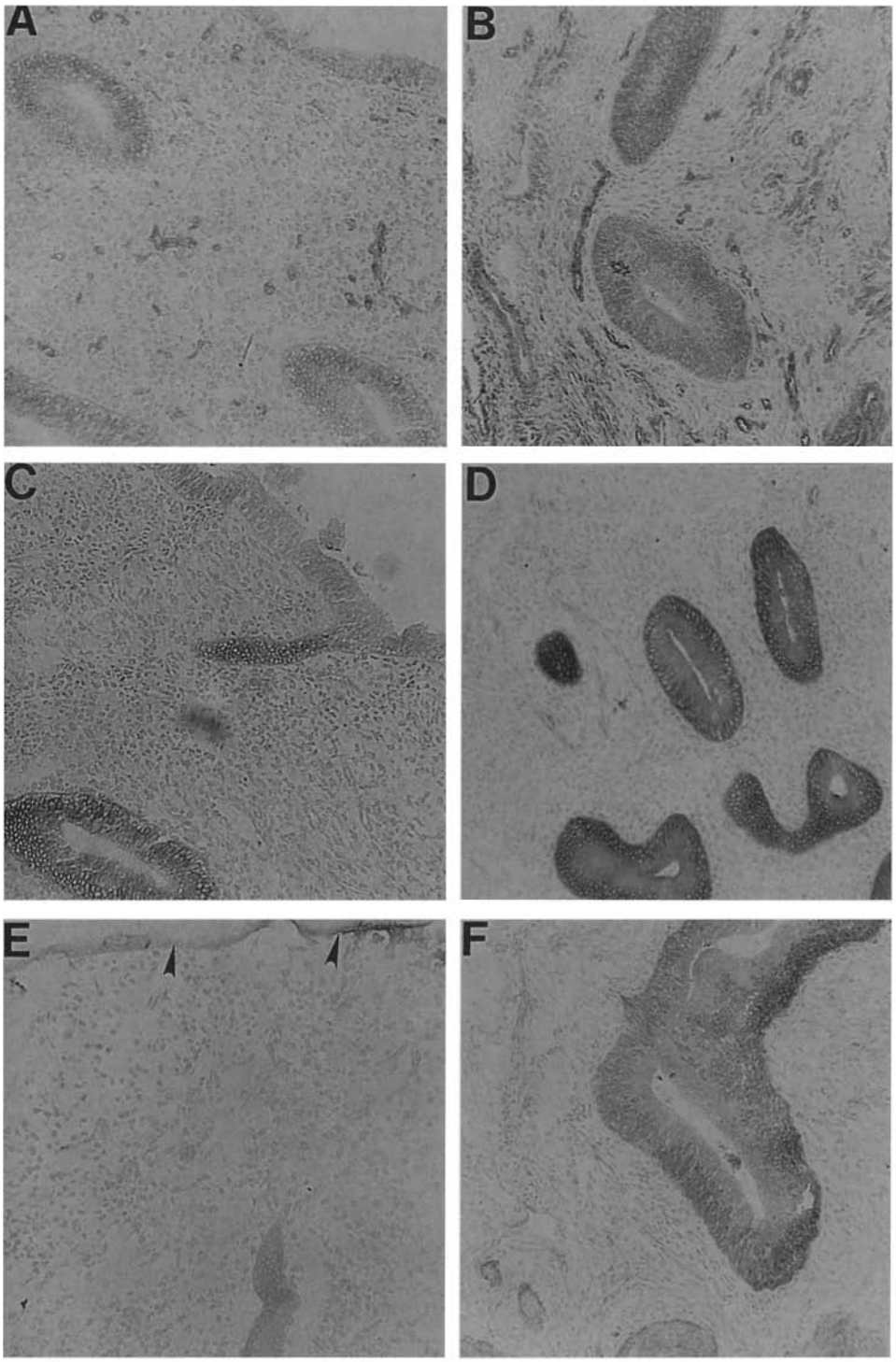

FIG. 2. Low-power micrographs of mid-luteal tissues showing the immunocytochemical localization of the cycle-specific epithelial integrin α_1 (**A** and **B**) and constitutive epithelial integrins α_2 (**C** and **D**) and α_3 (**E** and **F**). **A**, **C**, and **E** are the upper functionalis regions, and **B**, **D**, and **F** are the basal glands. Note the very minimal staining for both α_1 and α_2 in the surface epithelium compared to the glands. In contrast, α_3 shows a very distinct subcellular localization (arrowheads). ×158.

ECM proteins were immunolocalized using 5-μm paraffin sections [26, 27]. The sections were predigested for 30 min at room temperature with 0.4% pepsin in 0.01 N HCl. The sections were rinsed in three changes of Tris-buffered saline. Endogenous peroxidase was quenched with 0.3% H_2O_2, and sections were incubated overnight at 4°C with either fibronectin (1:1500), laminin (1:500), collagen IV (1:500), α-smooth muscle actin (1:1000), or commercially prediluted cytokeratin antibodies. The immunoreactive products were visualized using the ABC Vectastain kit and DAB.

For co-localization of β_3 and osteopontin in pregnant tissues, adjacent paraffin-embedded sections were predigested with 1% trypsin in Tris-buffered saline for 30 min at room temperature. Sections were incubated with the respective antibodies, and immunoreactive product was visualized using the ABC Vectastain kit and DAB. In order to identify cytotrophoblasts, glandular epithelia, and blood vessels, adjacent sections were also stained with cytokeratin and α-smooth muscle actin. The sections were photographed using Kodak Tech Pan 25 ASA film.

RESULTS

Cycling Animals

The ligand preference for each integrin examined in this study is presented in Table 1. Several integrins primarily

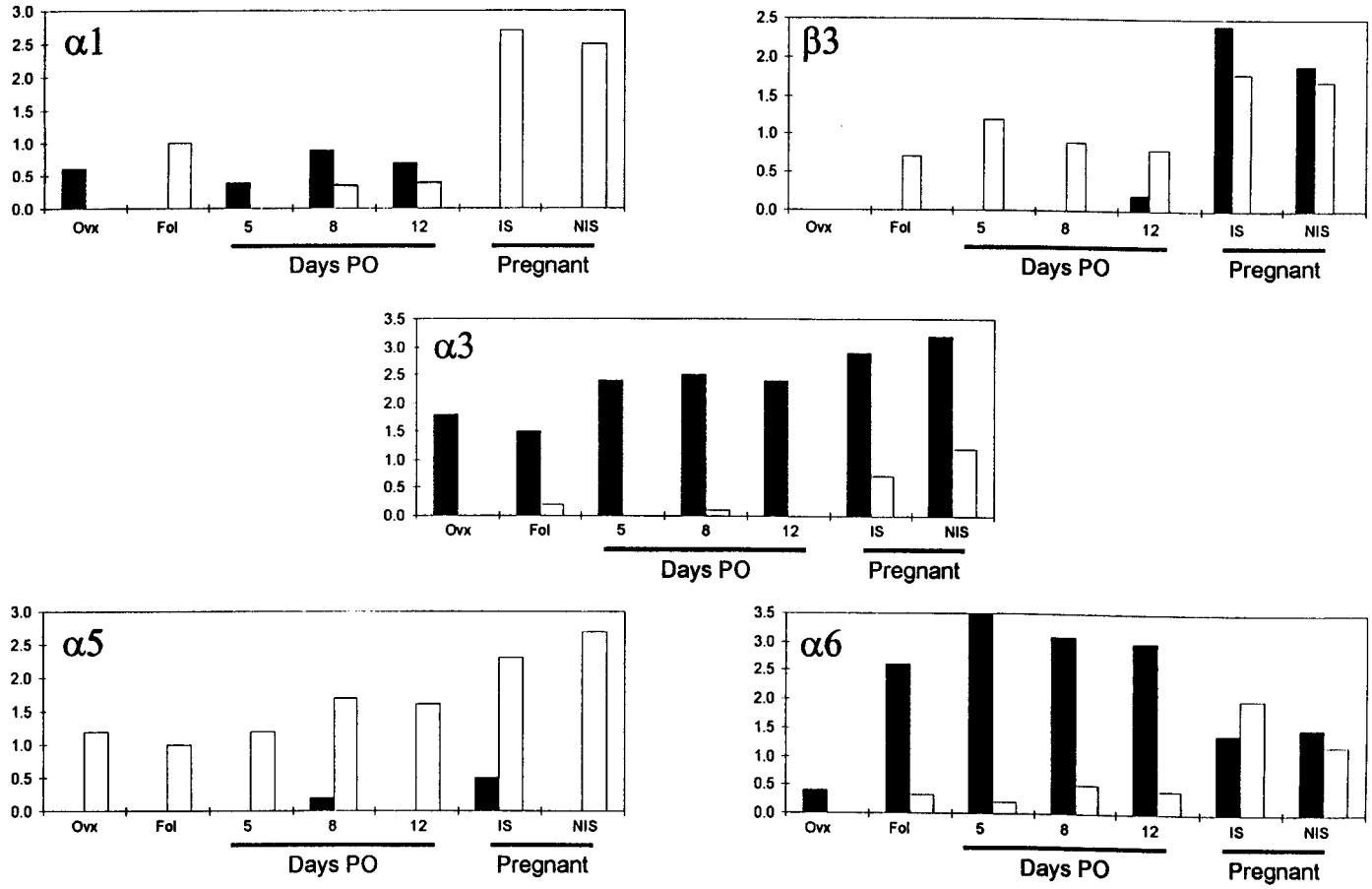

FIG. 3. HSCORE analysis of constitutively expressed (α_3, α_5, and α_6) and cycle- and pregnancy-specific (α_1 and β_3) integrins during the cycle and early pregnancy. Values represent means of HSCOREs obtained in duplicate or triplicate. Glandular expression is depicted in the dark bars and stromal expression in the open bars. Abbreviations: Ovx, ovariectomized; Fol, follicular; PO, postovulation; IS, implantation site; NIS, nonimplantation site.

recognized collagen and laminin, found in the basement membrane surrounding the glandular epithelium and vessels. Other integrins recognized fibronectin or vitronectin, more commonly associated with the mesenchymal regions of the endometrium. Immunostaining patterns of integrins and ECM in the baboon were similar to those seen in humans. Of note, all the antibodies that are specific for human integrin subunits and that were used in previous studies recognized epitopes in the baboon endometrium with patterns of staining that resembled those of the human endometrium. Constitutively expressed integrins seen on glandular epithelial cells of the baboon included α_2, α_3, α_6, and β_4 subunits. Luminal epithelial staining was evident only with antibodies against α_3 and α_6. The staining appearance of these subunits is shown in Figure 1, A–D, and Figure 2, C–F; the distribution is summarized in Figure 3. The α_5 integrin was not seen on glandular epithelial cells of any animal. However, α_5 expression was evident on stromal cells during the cycle and pregnancy (Figs. 3 and 4B) and also on invading cytotrophoblasts (Fig. 5B).

For two specific integrins, the pattern of epithelial integrin staining changed throughout the normal menstrual cycle. The α_1 subunit increased during the luteal phase (Fig. 2, A and B, Fig. 6C) and was also present in animals treated with estrogen and progesterone (data not shown). No glandular staining was seen in proliferative-phase samples (Fig. 6A) or in ovariectomized animals treated with estrogen alone (data not shown). Another integrin with a cycle-specific pattern of expression was α_4, seen in the early but not

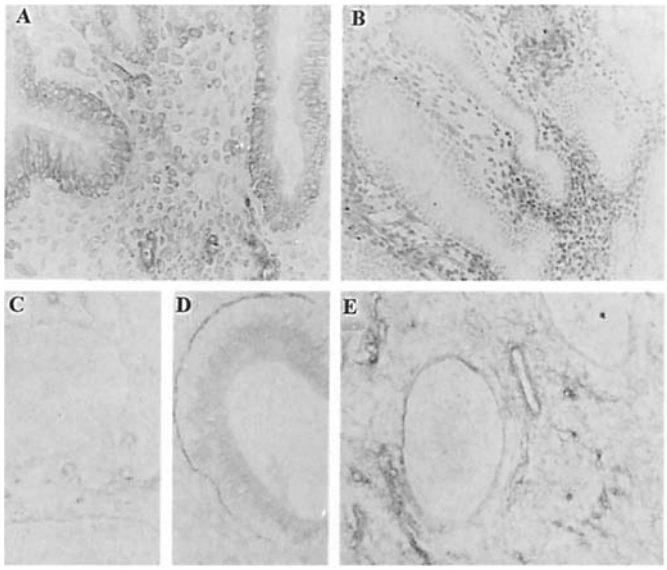

FIG. 4. Immunocytochemical localization of specific integrins and their corresponding ECM ligands during the midluteal phase. Collagen IV (**C**) and laminin (**D**) are present in the basement membrane of the glandular epithelial cells that express α_1 and α_4 (**A**). Fibronectin (**E**) is present primarily in the stroma and blood vessels and corresponds to the expression of its ligand α_5 in stromal cells (**B**). ×200.

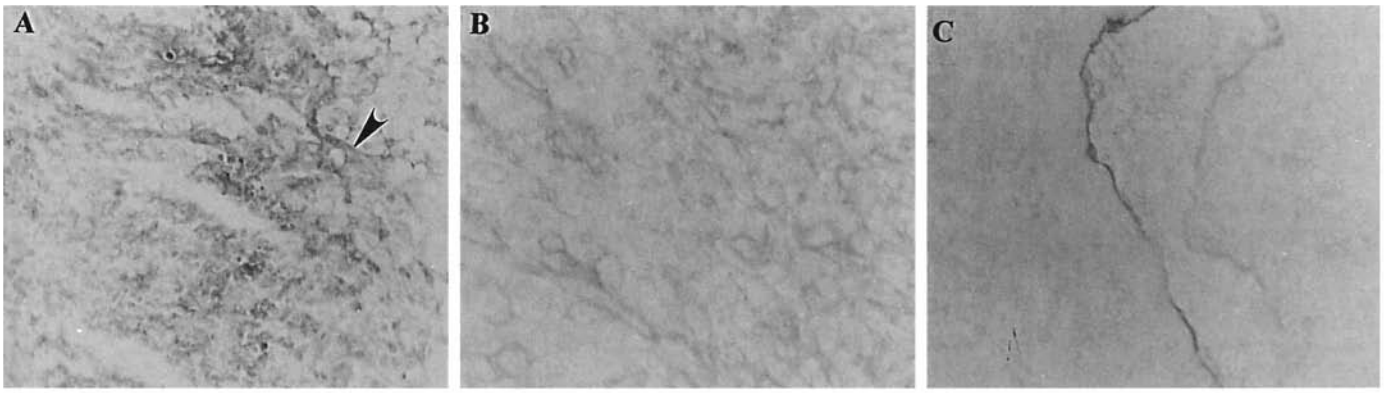

FIG. 5. Immunocytochemical localization of α_1 (**A**), α_5 (**B**), and α_6 (**C**) at the site of implantation. Note that the invading cytotrophoblasts (arrowhead) express α_1 and α_5 while α_6 is confined to the basement membrane of the placental villi. ×275.

the late secretory endometrium. This pattern of staining was also consistent with hormonally treated animals, being absent in ovariectomized or estrogen-treated baboons but present after 7 days, although not after 14 days, of estrogen and progesterone treatment (data not shown). Finally, the $\alpha_v\beta_3$ integrin, which has been found to be present in humans approximately 5–6 days after ovulation [5], was not seen in the glandular epithelium of cycling baboons (Fig. 6D). It did appear, however, to be strongly expressed in the glandular epithelium from pregnant animals (Fig. 7B). Figure 3 summarizes the staining patterns in the proliferative and secretory phases of the cycle and during pregnancy.

As shown in Figure 4, the ECM components collagen IV, laminin, and fibronectin were present in discrete locations within the endometrium and were coexpressed with the appropriate integrin ligand. Collagen IV and laminin were expressed in the basement membranes of epithelial cells together with epithelial expression of α_1, α_2, α_3, α_6, and β_1. Fibronectin was localized on stromal cells together with α_5 (Fig. 4, B and E).

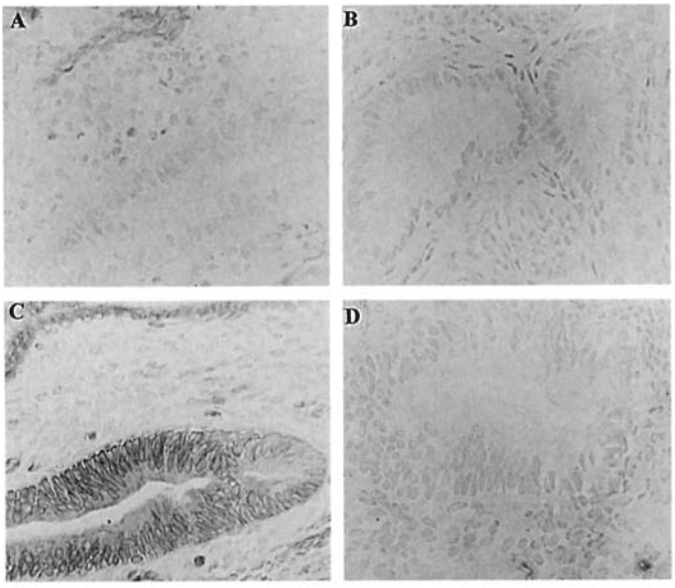

FIG. 6. Immunocytochemical localization of cycle-specific integrins α_1 (**A** and **C**) and β_3 (**B** and **D**). Note that α_1 (**C**) and α_4 (data not shown) are specifically expressed in the glandular epithelium during the midluteal phase. In contrast to what occurs in the human (see Fig. 10), β_3 is not expressed in the baboon uterus at the time of uterine receptivity (**D**). **A** and **B** are tissues obtained during the follicular phase of the cycle. ×200.

Pregnant Animals

Invading cytotrophoblasts at the site of placental attachment stained positively for α_1 (Fig. 5A) and α_5 (Fig. 5B). In contrast, the integrin α_6 was localized specifically to the basement membrane of the placental villi (Fig. 5C). Laminin and collagen IV (which interact with α_1) and fibronectin (which interacts with α_5) were also expressed at the site of implantation (Fig. 8, A–C).

Stromal cells stained primarily for $\alpha_5\beta_1$, the classic fibronectin receptor during the cycle (Fig. 4B), and increased dramatically during pregnancy (Fig. 3). Increased staining for α_1, α_3, α_4, α_6, $\alpha_v\beta_3$, and β_1 were all noted in the decidua of pregnancy (Figs. 3, and 7, A and B). This increase in several collagen/laminin receptors ($\alpha_1\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$) paralleled the increase in these ECM molecules as shown in Figure 8, D and E. The expression of luminal and glandular $\alpha_v\beta_3$, which became evident to a limited extent during the late luteal phase, increased markedly during pregnancy (Figs. 3 and 7B).

Since osteopontin is a known ligand for $\alpha_v\beta_3$ in many tissues [28] including the endometrium [29, 30], adjacent sections were stained with both antibodies. Figure 9 shows the extensive co-distribution of both these molecules in invading cytotrophoblasts (Fig. 9, A and B). The presence of cytotrophoblasts was confirmed by cytokeratin staining (Fig. 9C). Both the glandular epithelium and decidualizing stromal cells in the pregnant endometrium also expressed both the receptor ($\alpha_v\beta_3$) and the ligand (osteopontin; Fig. 9, D and E). In addition, cytotrophoblasts that had invaded the spiral arteries also stained positively (Fig. 9, G–I).

Interactions between integrins and the ECM are important for regulating mammary epithelial differentiation and secretory activity [31]. Expression of $\alpha_v\beta_3$ in the glandular epithelial cells in the human [15] and baboon (this study) coincides with increased glandular secretory activity. Figure 10 shows that in the human endometrium, $\alpha_v\beta_3$ expression was associated with placental protein 14 (PP_{14}) synthesis by glandular epithelial cells (Fig. 10, B and D). In contrast, both $\alpha_v\beta_3$ and PP_{14} expression in the baboon increased markedly only after the establishment of pregnancy (this study and [11]).

DISCUSSION

The patterns of integrin expression in the baboon were remarkably similar to those seen in humans during the cycle and in pregnancy [14–16, 32, 33]. In addition, the patterns

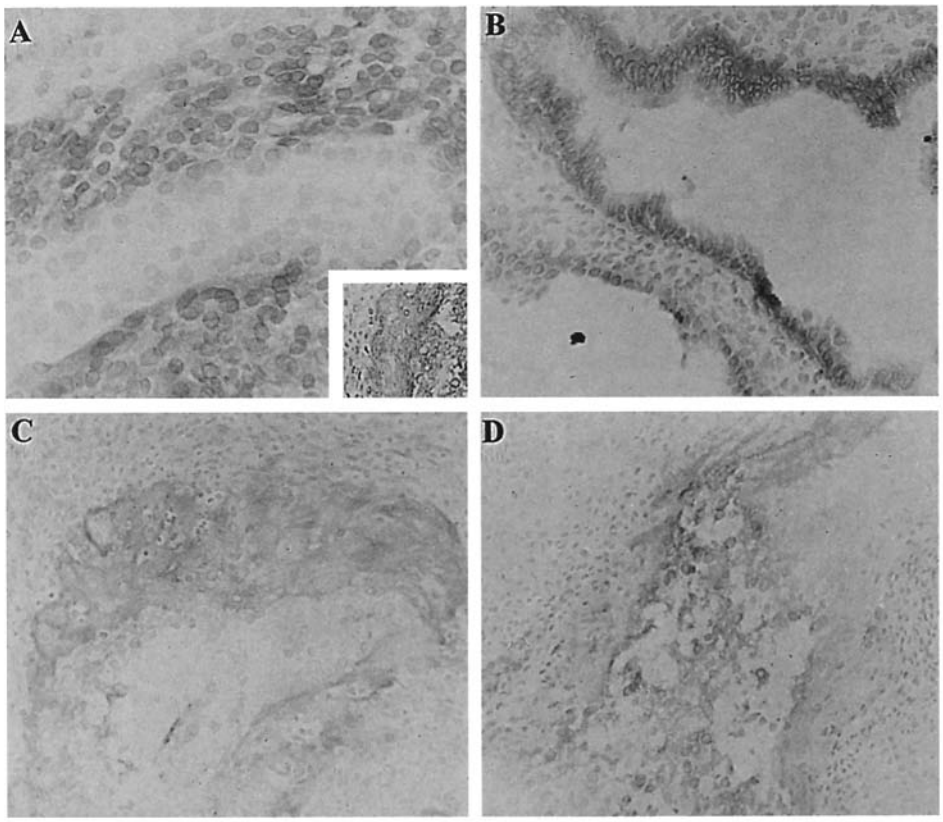

FIG. 7. Immunocytochemical localization of α_1 (**A** and **C**), α_4 (inset, **A**), and β_3 (**B** and **D**) in the endometrium from the implantation site on Day 22 of pregnancy. Note the dramatic switch from glandular to stromal expression of α_1 and α_4 (**A**) during pregnancy. The α_1 also continues to be expressed in cytotrophoblasts that have invaded the spiral arteries (**C**). The β_3 expression becomes readily evident with the establishment of pregnancy in both glandular epithelial and stromal cells (**B**). **D** shows β_3 expression on invading cytotrophoblasts and surrounding decidualizing stromal cells. ×285.

of constitutive and stromal integrin expression were identical, reflecting the role of integrins in cell-substratum attachment and maintenance of cellular phenotype. Parallel ECM staining was noted as well; this finding was similar to previously reported results in the human endometrium [34]. Thus, ligands for collagen/laminin receptors are localized to the basement membrane cells, while their respective integrin receptors are located on the epithelial and vascular cells. The patterns of α_6 and β_4, which comprise the primary laminin receptor, were found confined to the basal pole of the epithelial cells; this is similar to the pattern seen in human endometrium [14, 32] and other human epithelial cells [35–38]. While $\alpha_6\beta_1$ may also be present on epithelial cells, the expression of this integrin pair was difficult to assess because of the ability of β_1 to interact with other α subunits.

Many of the integrins found in the baboon are constitutively expressed throughout the menstrual cycle yet maintain regional differences within the endometrium proper. Like the changes noted in the epidermis, these patterns of expression are likely to be involved in maintenance of structural/functional differences or in the regulation of the developmental process of endometrium growth [39]. Based on studies from the human [40], certain integrins are known to be very uncharacteristic for the epithelial cell. These include $\alpha_4\beta_1$ and $\alpha_v\beta_3$, neither of which has been found to be expressed on most epithelial cells. Further, $\alpha_v\beta_3$ is localized on the apical surface and found primarily on the

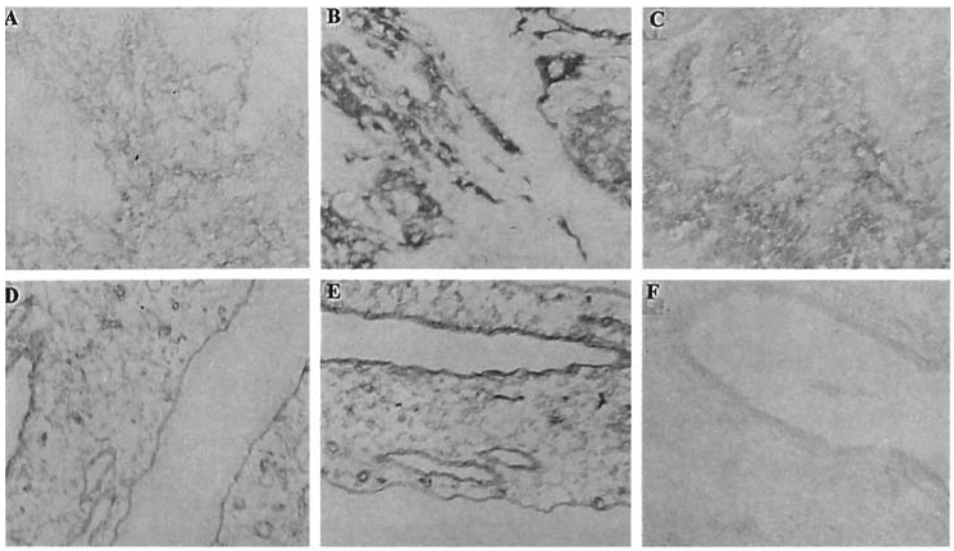

FIG. 8. Immunolocalization of laminin (**A** and **D**), collagen IV (**B** and **E**), and fibronectin (**C** and **F**) at the implantation site (**A–C**) and in deeper endometrium (**D–F**) during early pregnancy. Note that the appropriate ligands for integrins expressed on invading cytotrophoblasts are all expressed at the site of implantation. Associated with the shift in epithelial to stromal expression of α_1 and α_4, both laminin and collagen IV are now readily evident in the pregnant endometrium (**D** and **E**). ×200.

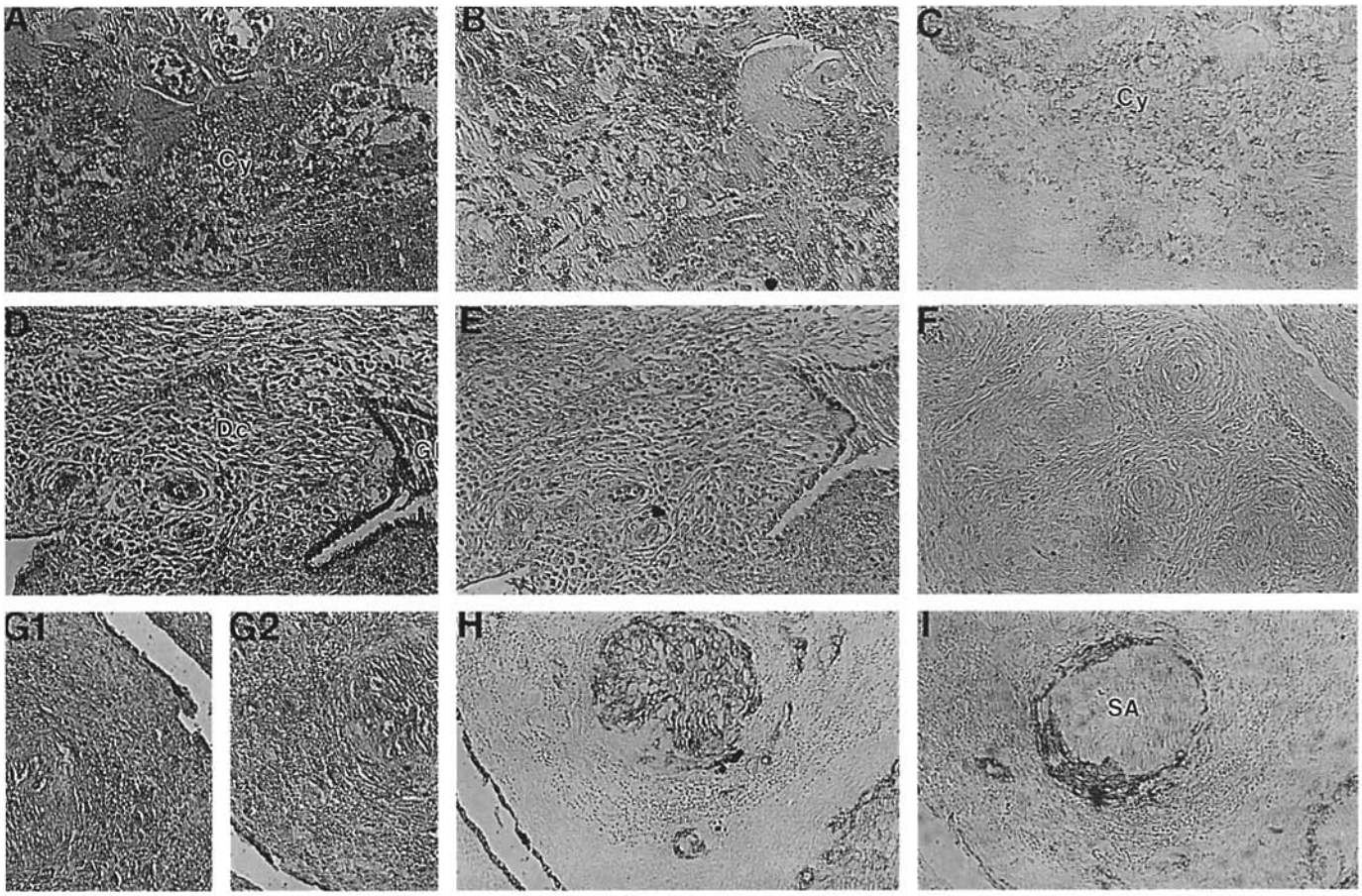

FIG. 9. Co-localization of β_3 and osteopontin at the site of implantation on Day 25 of pregnancy. **A** and **B** are invading cytotrophoblasts, and **C** is stained with cytokeratin for orientation. **D** and **E** show the intense co-localization of both receptor ($\alpha_v\beta_3$) and ligand (osteopontin) in the glandular epithelium and stroma. G_1 and G_2 show the presence of both β_3 and osteopontin on cytotrophoblasts that have invaded the spiral arteries. **H** and **I** are stained with cytokeratin and α-smooth muscle actin, respectively, for orientation. **F** is a preimmune control. Cy, cytotrophoblasts; Dc, decidua; Gl, glands; SA, special artery. ×125.

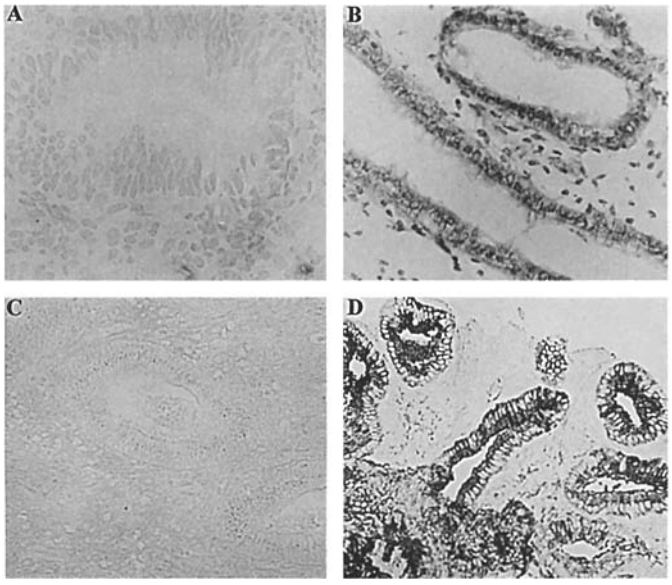

FIG. 10. Co-localization of β_3 and PP_{14} in the human (**B** and **D**) during the period of uterine receptivity. Note that the absence of β_3 and PP_{14} in the baboon uterine endometrium at a comparable time of the menstrual cycle (**A** and **C**). ×200.

microvilli of these epithelial cells. Such specialized temporal and spatial distribution argues for a role of this integrin in the implantation process in primates, though evidence to support such a role is not yet available.

The stroma, a site of fibronectin deposition, was also the site of the fibronectin receptor, $\alpha_5\beta_1$. Fibronectin receptors were found on the epithelial cells, but only during the early and midluteal phase ($\alpha_4\beta_1$) and in pregnancy ($\alpha_v\beta_3$). In humans, there are no reported studies to date examining the effect of steroid hormones on these changes in integrin expression seen in vivo during the natural cycle. Thus, it was of interest to note that many of the same changes that occurred in cycling baboons were seen in steroid-treated animals. Both α_1 and α_4 were found to be increased in the glands of estrogen- and progesterone-treated baboons [21], suggesting that these changes are directly inducible by progesterone treatment of an estrogen-primed endometrium. In addition, these integrins, together with α_5 and α_6, increased markedly in decidualizing stromal cells during pregnancy. Long-term exposure to estrogen and progesterone did result in $\alpha_v\beta_3$ expression, suggesting that these late effects seen in the baboon are also regulated by the sex steroids. As discussed below, these integrins may bind ligands associated with the ECM of the endometrium as well as sites associated with the conceptus.

The changing patterns of integrins in the decidua of pregnancy is intriguing and entirely consistent with previously

noted changes in integrin expression seen in human pregnancy [15, 16, 33]. These changes in cellular integrin expression are also coincident with previously reported cell-specific changes in insulin-like growth factor-binding protein-1 (IGFBP-1) and type I insulin-like growth factor (IGF) receptor expression in the pregnant baboon [13, 24, 26]. The switch in cell-specific expression of IGFBP-1 and the type I IGF-receptor could not be induced in simulated-pregnant baboons, suggesting that the presence of the conceptus may be required for the induction of these proteins in decidual cells [13]. In contrast, the cytoskeletal changes together with the changes in integrin and ECM expression associated with initiation of the decidualization process could be induced by hormone treatments that simulate pregnancy ([13, 27]; A.T. Fazleabas and B.A. Lessey, unpublished results). Thus, the hormonal induction of α-smooth muscle actin; the integrins α_1, α_3, α_5, and α_6; and ECM proteins in decidualizing stromal cells may be essential for cell proliferation and differentiation associated with this process [41, 42].

As previously reported, there are increases in α_5 expression and de novo increases in several collagen/laminin receptors—$\alpha_1\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_1$—associated with the establishment of pregnancy. The "pericellular" basement membrane seen in prior studies [34] demonstrates that new ECM molecule deposition is associated with a new cellular phenotype. This phenomenon, coined "dynamic reciprocity" by Bissell et al. [43], suggests that the cells that are induced to lay down new ECM are shaped by exposure to this substrate. Hormonal or paracrine signals contribute to induce decidual changes [44]. The ECM may induce specifically noted increases in integrin expression as well or may participate in signal transduction. The interaction between integrins and the ECM induces changes in the cell cytoskeleton that are thought to be critical for signal transduction [41]. In the baboon uterus, stromal cells undergoing decidualization express α-smooth muscle actin and smooth muscle myosin II [27]. Thus, the increased stromal expression of specific integrins during pregnancy and the accompanying changes in the ECM may be essential for stromal cell proliferation and differentiation associated with the process of decidualization, since the actin cytoskeleton appears to play a central role in integrin-mediated signal transduction [42]. In addition, trophoblast invasion into the decidua may also be responsible for some of these changes [13, 44]. The decidua not only supports trophoblast invasion by supplying structural integrity but also secretes proteins that correspond to collagen/laminin receptors found on invading cytotrophoblast [45, 46]. Thus, the dynamic changes in integrin expression and the ECM may also regulate trophoblast invasion by directing signal transduction mechanisms within the trophoblast cells [47, 48].

The expectation for a role of $\alpha_v\beta_3$ in endometrial function surrounding implantation was heightened by the observation that this vitronectin receptor appeared in normal fertile women around the time of initial embryo attachment to the endometrium [14, 15]. It was therefore somewhat unexpected to find that this integrin appeared well after the time of initial embryo attachment in the baboon. The expression of this integrin does correlate, however, with the time of enhanced glandular secretion in both species [29, 49]. Since endometrial glandular secretions may be important for maintaining embryo viability prior to initial attachment to the uterus, we suggest that the difference in the time of β_3 expression between the human and baboon may reflect differences between implantation (interstitial and superficial) in these two species.

In conclusion, subhuman primates such as the baboon are known to have endometrial architecture and histologic changes analogous to those of the human. These similarities are supported by almost identical patterns of integrin expression on both glandular epithelium and stroma during the menstrual cycle. Patterns of integrin expression seen in pregnancy reflect a shift from epithelial to stromal dominance and are associated with parallel changes in ECM distribution. Differences in cycle-dependent integrin expression were noted for the $\alpha_v\beta_3$ vitronectin receptor and may reflect a delay in the pattern of secretion or in the mechanics of trophoblast invasion in this species as compared to those associated with implantation in the human. Since integrin-mediated signaling pathways via the cytoskeleton overlap with those induced by growth factors, the interaction between these two signaling pathways may play a critical role in the establishment of a receptive endometrium and the initiation and maintenance of pregnancy. Cellular studies on these interactions are currently underway in our laboratories.

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