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Osteopontin Is Synthesized by Uterine Glands and a 45-kDa Cleavage Fragment Is Localized at the Uterine-Placental Interface Throughout Ovine Pregnancy[1]

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ABSTRACT

Osteopontin (OPN) is a phosphorylated and glycosylated, secreted protein that is present in various epithelial cells and biological fluids. On freezing and thawing or treatment with proteases, the native 70-kDa protein gives rise to 45- and 24-kDa fragments. Secreted OPN functions as an extracellular matrix (ECM) protein that binds cell surface receptors to mediate cell-cell adhesion, cell-ECM communication, and cell migration. In sheep and humans, OPN is proposed to be a secretory product of uterine glandular epithelium (GE) that binds to uterine luminal epithelium (LE) and conceptus trophectoderm to mediate conceptus attachment, which is essential to maintain pregnancy through the peri-implantation period. Cell-cell adhesion, communication, and migration likely are important at the interface between uterus and placenta throughout pregnancy, but to our knowledge, endometrial and/or placental expression of OPN beyond the peri-implantation period has not been documented in sheep. Therefore, the present study determined temporal and spatial alterations in OPN mRNA and protein expression in the ovine uterus between Days 25 and 120 of pregnancy. The OPN mRNA in total ovine endometrium increased 30-fold between Days 40 and 80 of gestation. In situ hybridization and immunofluorescence analyses revealed that the predominant source of OPN mRNA and protein throughout pregnancy was the uterine GE. Interestingly, the 45-kDa form of OPN was detected exclusively, continuously, and abundantly along the apical surface of LE, on conceptus trophectoderm, and along the uterine-placental interface of both interplacentomal and placentomal regions through Day 120 of pregnancy. The 45-kDa OPN is a proteolytic cleavage fragment of the native 70-kDa OPN, and it is the most abundant form in uterine flushes during early pregnancy. The 45-kDa OPN is more stimulatory to cell attachment and cell migration than the native 70-kDa protein. Collectively, the present results support the hypothesis that ovine OPN is a component of histotroph secreted by the uterine GE that accumulates at the uterine-placental interface to influence maternal-fetal interactions throughout gestation in sheep.

placenta, pregnancy, uterus

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INTRODUCTION

All mammalian uteri contain endometrial glands that secrete ‘‘histotroph.’’ Histotroph is a complex mixture of a variety of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins, and other substances [1] that plays a role in conceptus nourishment, attachment, implantation/placentation, and immunoprotection [2, 3]. Studies of ewes in which uterine glands have been epigenetically ablated by neonatal progestin exposure confirm that histotroph is required to maintain pregnancy through the peri-implantation period, when conceptus trophectoderm must adhere to the luminal epithelium (LE) [4].

Osteopontin (OPN) is a component of histotroph [5, 6] that is hypothesized to be involved in ovine implantation [7, 8]. This highly phosphorylated and glycosylated, secreted protein is present in various biological fluids [9]. It also has been found on epithelial cells of the gallbladder, pancreas, lung, sweat ducts, placenta, gastrointestinal, urinary, mammary, and reproductive tracts [10–14]. On freezing and thawing or treatment with proteases, the native 70-kDa protein gives rise to 45- and 24-kDa fragments [15]. Secreted OPN functions as an extracellular matrix (ECM) protein that regulates the activity of a variety of cells by signaling through integrin or CD44 receptors [16, 17]. Reported effects of OPN include mediation of cell-cell adhesion, cell-ECM communication, and cell migration [16, 18].

Uterine OPN and its integrin receptors have become the focus of studies concerning conceptus adhesion for implantation in a number of species, including humans, in which OPN expression is increased by progesterone during the menstrual cycle, starting on Days 16–17, and secretory glandular epithelium (GE) appears to be a source of OPN localized on the apical LE surface [19]. In pregnant sheep, OPN protein is a component of histotroph that binds to the apical surface of LE and GE where several integrins are apically expressed and could form known OPN receptors, including $\alpha_v\beta_3$, $\alpha_v\beta_1$, $\alpha_4\beta_5$, $\alpha_4\beta_1$, and $\alpha_5\beta_1$ [6, 7]. The form of OPN protein that increases in uterine flushings from pregnant ewes between Days 11 and 17 is predominantly a 45-kDa cleavage fragment that binds with higher affinity to integrins and has greater biological activity than the native 70-kDa OPN [20]. The OPN protein is also present on trophectoderm of Day 14–19 ovine conceptuses despite the absence of conceptus OPN mRNA [6]. The OPN mRNA is expressed in the GE of pregnant ewes and is induced when ovariectomized ewes are given exogenous progesterone [5, 21]. Indeed, uterine gland knockout ewes lack uterine glands and exhibit a peri-implantation defect that is associated with the absence of OPN in uterine secretions [4,

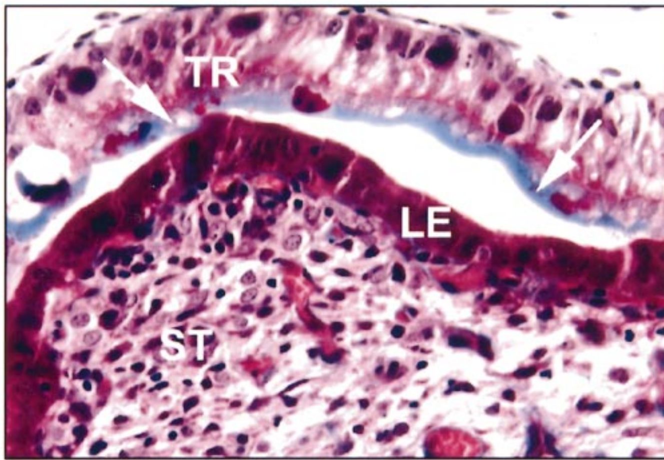

FIG. 1. Representative photomicrograph of endometrium and placenta from a Day 35 pregnant ewe subjected to Masson trichrome staining. This procedure stains nuclei black, cytoplasm and muscle fibers red, and ECM blue. Note the presence of ECM along the interface between endometrial LE and placental tissue (white arrows). ST, Uterine stroma; TR, conceptus trophectoderm. Width of each field is 250 μm.

8]. Therefore, OPN is proposed to be a progesterone-induced secretory product of the GE that binds to uterine LE and conceptus trophectoderm to mediate conceptus elongation and attachment, which is essential to maintain pregnancy through the peri-implantation period in ewes.

Although cell-cell adhesion, cell-ECM communication, and cell migration undoubtedly continue to be critical at the interface between uterus and placenta throughout pregnancy, to our knowledge endometrial and/or placental expression of OPN beyond the peri-implantation period has not been documented in ewes. Our working hypothesis is that OPN is a component of histotroph that is secreted by the endometrial GE throughout pregnancy in the ewe and contributes to an ECM that supports maternal-conceptus adhesion and communication. Therefore, the present study determined temporal and spatial alterations in OPN mRNA and protein expression in the ovine uterus and placenta between Days 25 and 120 of pregnancy.

MATERIALS AND METHODS

Animals and Tissue Collection

Experimental and surgical procedures complied with the Guide for the Care and Use of Agricultural Animals and were approved by the Texas A&M University Institutional Agricultural Animal Care and Use Committee.

Mature, western-range ewes were observed daily for estrous behavior. After experiencing at least two estrous cycles of normal duration (16–18 days), ewes were assigned randomly on Day 0 (estrus/mating) to cyclic or pregnant status. Fifty-two ewes were ovariohysterectomized (n = 4 ewes/day) on Days 14, 20, 25, 30, 35, 40, 45, 50, 55, 60, 80, 100, or 120 of pregnancy. At hysterectomy, uteri from Day 14 pregnant ewes were flushed with 0.9% NaCl to verify pregnancy by recovery of an apparently normal conceptus. Pregnancy was verified on all later days by visual observation of conceptus tissues undergoing attachment and placentation. Several sections (thickness, ~1–1.5 cm) from the middle of each uterine horn were fixed in fresh 4% paraformaldehyde in PBS (pH 7.2) for 24 h and then embedded in Paraplast Plus (Oxford Labware, St. Louis, MO). Several sections were also embedded in Tissue-Tek Optimal Cutting Temperature Compound (Miles, Oneonta, NY), snap-frozen in liquid nitrogen, and stored at −80°C. The remaining endometrium was dissected from the myometrium, frozen in liquid nitrogen, and stored at −80°C for RNA extraction.

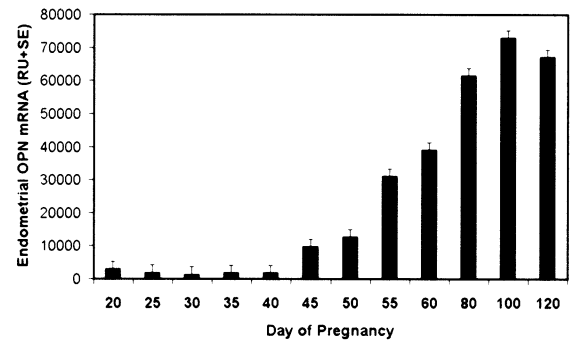

FIG. 2. Slot-blot hybridization analysis of steady-state levels of OPN mRNA in ovine total endometrium from Day 20 through Day 120 of pregnancy. The OPN mRNA levels are expressed as relative units (RU), normalized for differences in sample loading using the 18S rRNA, in 20 μg of total endometrial mRNA. Data are presented as least-square-means RU with overall SEM.

Histological Analysis

Ovine uteri were sectioned (thickness, 5 μm), deparaffinized in CitraSolv (Fisher Scientific; Pittsburgh, PA), and rehydrated through a graded alcohol series to distilled water. Tissues were then exposed to a Masson trichrome staining procedure as previously described [8]. This staining procedure results in black nuclei, red cytoplasm and muscle fibers, and blue ECM components.

RNA Isolation and Analyses

RNA isolation. Total cellular RNA was isolated from endometrial tissue samples using TRIzol reagent (Invitrogen, Carlesbad, CA) according to the manufacturer's recommendations.

Slot-blot analysis. Steady-state levels of OPN mRNA were assessed in endometrial total RNA samples by slot-blot hybridization analysis as described previously [22] using 20 μg of endometrial total RNA and ^{32}P-labeled antisense ovine OPN cRNA probe [5]. To correct for variation in total RNA loading, a duplicate RNA slot membrane was hybridized with ^{32}P-labeled antisense 18S rRNA cDNA (pT718S; Ambion, Austin, TX). The radioactivity in each slot was quantified by a Typhoon 8600 Imager (Molecular Dynamics, Piscataway, NJ) and expressed as total counts.

In situ hybridization analysis. Osteopontin mRNA expression in ovine uterine tissues was localized by in situ hybridization as previously described [5]. Deparaffinized, rehydrated, and deproteinated uterine cross-sections (thickness, 5 μm) were hybridized with ^{35}S-radiolabeled antisense or sense cRNA probes for ovine OPN [5]. Following washes and RNase A digestion, slides were dipped in Kodak NTB-2 liquid photographic emulsion (Kodak, Rochester, NY), stored at 4°C for 5 days, developed in Kodak D-19 developer, counterstained with Harris modified hematoxylin (Fisher Scientific), dehydrated, and protected with coverslips.

Immunofluorescence Analyses

Antibodies used for immunofluorescence staining included rabbit anti-OPN amino terminal (LF-124) and carboxyl terminal (LF-123) [23] and a fluorescein-conjugated goat antibody against rabbit immunoglobulin G (Chemicon International, Temecula, CA). Proteins were localized in frozen ovine uterine cross-sections as previously described [6]. Briefly, tissues were fixed in −20°C methanol, permeabilized with 0.3% Tween 20 in PBS, blocked in 10% normal goat serum, incubated overnight at 4°C with 2 μg/ml of primary antibody, and detected with fluorescein-conjugated secondary antibody. Slides were overlaid with a coverglass and Prolong antifade mounting reagent (Molecular Probes, Eugene, OR).

Photomicrography

Photomicrographs of representative fields of in situ hybridization, Masson trichrome staining, and immunofluorescence staining were evaluated with a Zeiss Axioplan2 microscope (Carl Zeiss, Thornwood, NY) fitted with a Hamamatsu chilled 3CCD color camera (Hamamatsu Corporation, Bridgewater, NJ). Digital images were captured using Adobe Photoshop 4.0 (Adobe Systems, Seattle, WA) and an Apple PowerMac G3 computer (Apple Computer, Cupertino, CA).

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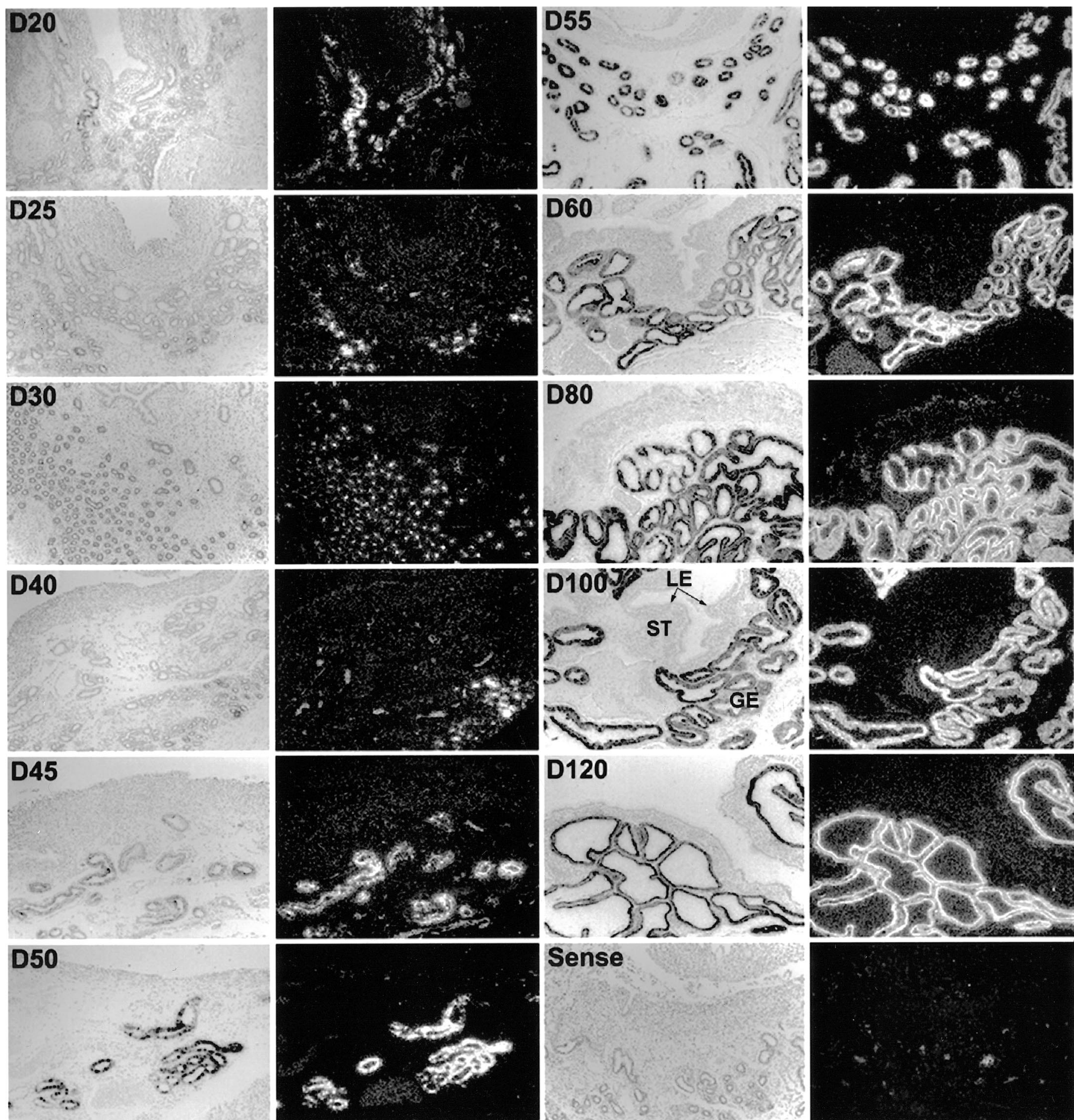

FIG. 3. In situ hybridization analysis of OPN mRNA in endometrial glandular epithelium (GE) of pregnant ewes. Corresponding bright- and dark-field images of endometrium from different days of pregnancy are shown. A representative section from Day (D) 40 hybridized with radiolabeled sense cRNA probe (Sense) serves as a negative control. LE, luminal epithelium; ST, Uterine stroma. Width of each field is 940 μm.

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Statistical Analysis

Data from slot-blot hybridization analyses were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System (SAS Institute, Cary, NC). Effects of day on steady-state levels of endometrial OPN mRNA were examined by regression analysis. Hybridization data (relative units) were normalized for differences in sample loading using the 18S rRNA data as a covariate in ANOVA. Data are presented as least-square-means relative units with overall SEM.

RESULTS

Localization of ECM at the Uterine-Placental Interface

In support of our working hypothesis that OPN is a component of histotroph contributing to an ECM that supports maternal-conceptus adhesion and communication throughout pregnancy, Masson trichrome staining of paraffin-embedded, Day 35 cross-sections revealed the deposition of

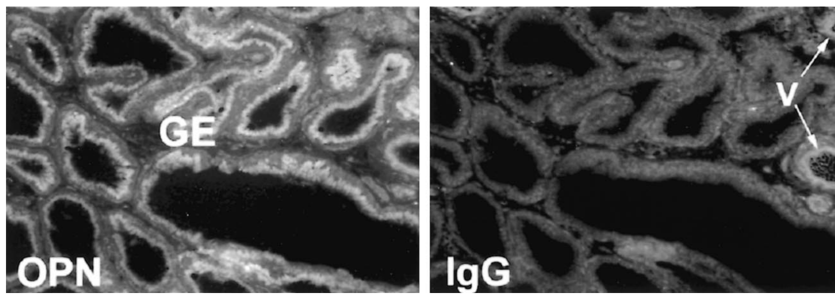

FIG. 4. OPN protein expression in ovine endometrial GE from a Day 100 pregnant ewe. The uterine cross-section shown represents Days 20 through 120 of pregnancy, because the staining pattern did not differ as a result of the day of pregnancy. The OPN was detected using immunofluorescence staining of frozen sections. Compare the absence of antibody staining in Day 100 endometrium when rabbit immunoglobulin G (IgG) was used to detect immunoreactive proteins. V, Vasculature. Width of each field is 940 μm.

ECM (blue staining) along the interface between uterine LE and placental tissue (Fig. 1). The following results indicate that OPN is an important constituent of this ECM.

Expression of OPN mRNA in Total Endometrium

Temporal changes in steady-state levels of OPN mRNA in total ovine endometrium from Days 25 through 120 of pregnancy were quantified by slot-blot hybridization (Fig. 2). The OPN mRNA remained constant through Day 40. Steady-state levels of endometrial OPN mRNA were affected ($P < 0.001$) by day of pregnancy. Overall, OPN mRNA levels were lowest on Days 20–40, increased 39-fold from Days 40 to 100, and remained maximal thereafter (cubic effect of day, $P < 0.0001$, $r^2 = 0.94$) (Fig. 2).

Localization of OPN mRNA Within Endometrial Glands

In situ hybridization analysis of endometrial cross-sections revealed expression of OPN mRNA in the middle to deep GE from Days 20 through 120 of pregnancy (Fig. 3). An increase in total hybridization was evident concomitant with the hypertrophy in endometrial glands that occurs between Days 50 and 60 of pregnancy (Fig. 3). As previously reported, OPN mRNA was also present in endometrial stratum compactum stroma between Days 25 and 120 of pregnancy [24]. However, hybridization was much less in these cells than in GE and is not readily apparent in Figure 3 because of the relative intensity of the GE signal.

Localization of OPN Protein in the Uterus and Placenta

The location of OPN protein in pregnant ovine uterus and placenta was assessed by immunofluorescence staining of frozen cross-sections using the LF-123 rabbit anti-OPN antibody that detects both native and the carboxyl half of OPN starting at the Arg-Ser (RS) thrombin-cleavage site or the LF-124 antibody that detects both native and the amino half of OPN ending at the RS site (see Fig. 5A).

OPN protein in endometrial glands. In agreement with in situ hybridization results, immunoreactive OPN protein (LF-123 antibody) was present in the endometrial GE of all ewes between Days 20 and 120 of pregnancy (Fig. 4).

OPN protein at the uterine-placental interface. Immunolocalization of OPN protein by immunofluorescence staining of serial cross-sections from Days 14 through 120 of pregnancy with LF-123 and LF-124 antibodies is illustrated in Figure 5B. Previous studies have established that LF-124 antibody detects a 45-kDa OPN cleavage product that contains the Arg-Gly-Asp (RGD) integrin-binding sequence and is secreted into the uterine lumen of ewes during early pregnancy, whereas LF-123 antibody does not detect 45-kDa OPN [6]. Both antibodies immunoreact with native 70-kDa OPN in immunofluorescence staining. Importantly, however, clear differences exist in the endometrial distribution of native 70-kDa OPN and the secreted 45-kDa fragment, which is exclusively, continuously, and abundantly present along the apical surface of LE, on trophectoderm, and along the entire uterine-placental interface of both interplacentomal and placentomal regions through Day 120 of pregnancy (Fig. 5B). As previously reported [24], OPN protein is also expressed in uterine stroma between Days 30 and 120 of pregnancy (Fig. 5B). Stromal OPN is detectable with either the LF-123 or LF-124 antibody, indicating expression of native 70-kDa OPN.

DISCUSSION

Results of the present study are the first, to our knowledge, to demonstrate that OPN is present at all sites of intimate contact between conceptus and maternal tissues between Days 30 and 120 of pregnancy in sheep. Furthermore, the results strongly suggest that OPN protein is synthesized and secreted from the GE as a constituent of histotroph that specifically binds to the uterine-placental interface throughout gestation in sheep. These findings are significant, because they definitively localize a secretory product of the GE to regions of contact between conceptus and uterus where OPN can potentially influence fetal/placental development and growth and mediate communication between placental and uterine tissues to support pregnancy.

Steady-state levels of total endometrial OPN mRNA increased approximately 30-fold between Days 40 and 80 of gestation. Although increases in total OPN mRNA in the sheep are confounded by a complex temporal and spatial pattern of expression that includes both an increase in OPN gene expression in stratum compactum stroma that begins between Days 20 and 25 of pregnancy [24] as well as constitutive expression within the GE, even a perfunctory examination of mRNA localization (Fig. 3) clearly indicates that the major source of OPN is GE. This is readily seen by comparing the relative magnitude of hybridization between stroma and GE on Days 80 and 100 of pregnancy.

Changes in OPN mRNA correlate with growth of endometrial glands that occurs as the uterus remodels to accommodate rapid conceptus development and growth during the last two-thirds of pregnancy [25]. During ovine gestation, GE undergoes hyperplasia through Day 50, followed by hypertrophy and maximal production of histotroph after Day 60 [26]. A similar correlation between GE development and gene expression during pregnancy has been shown for the well-defined secretory product of GE, uterine milk protein (UTMP) [26]. Endometrial gland morphogenesis and, by inference, increases in GE gene products, such as OPN and UTMP, likely are influenced by sequential uterine exposure to estrogen, progesterone, interferon-tau, pla-

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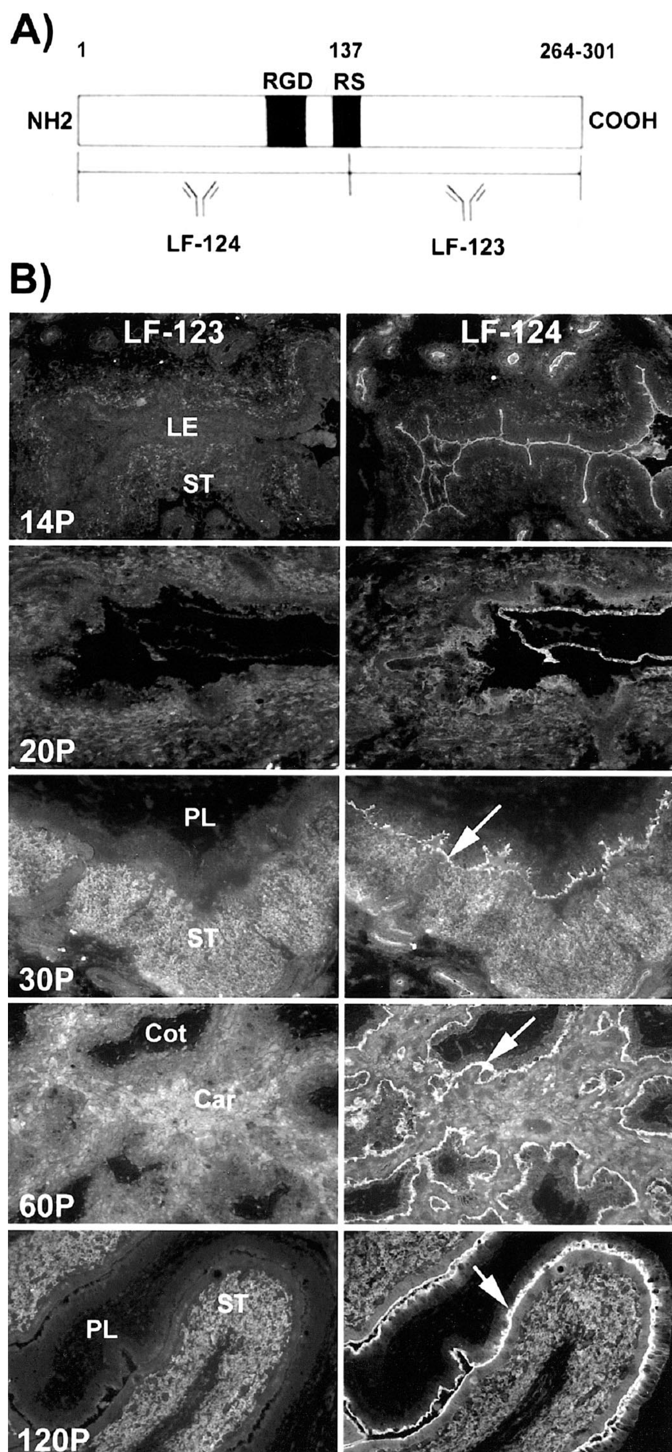

FIG. 5. Detection of OPN protein in ovine uterus and placenta using immunofluorescence staining of frozen sections. **A**) Immunoreactive OPN proteins were detected using immunoglobulin (Ig) G (LF-123; [24]) against the carboxyl half of human recombinant OPN (rhOPN) starting at the thrombin (RS) cleavage site and using IgG (LF-124; [23]) against the amino half of rhOPN ending at the RS site. **B**) White arrows denote the uterine-placental interface. Refer to Figure 4 for an example of representative background staining for the rabbit IgG control. Car, Caruncle; Cot, cotyledon; PL, placenta; ST, uterine stroma. Width of each field is 940 μm.

cental lactogen, and placental growth hormone [27, 28]. Using an ovariectomized ewe model, administration of progesterone increased GE expression of OPN and UTMP [21–27], and injection of a progesterone-receptor antagonist prevented progesterone effects on OPN mRNA [21]. Furthermore, intrauterine infusion of placental lactogen to progesterone-treated ovariectomized ewes increased OPN and UTMP levels over those with progesterone alone [27, 28]. Interestingly, regulation of OPN and UTMP genes diverge in regard to exposure to growth hormone. Whereas infusion of growth hormone into progesterone-treated ovariectomized ewes increased GE expression of UTMP mRNA, OPN mRNA was not affected [27, 28]. It is important to note that sequential treatment of ovariectomized ewes with progesterone, interferon-tau, placental lactogen, and growth hormone resulted in GE development similar to that observed under the influence of placental lactogen from the placenta during pregnancy[28].

The differential staining observed when serial cross-sections were immunolabeled with LF-123 and LF-124 antibodies provides significant insights regarding the mechanistic role of OPN during pregnancy. These antibodies were generated against the amino (LF-124) and carboxyl (LF-123) terminal fragments of recombinant human OPN that form when the native 70-kDa protein is cleaved by thrombin at its RS sequence [23]. Both immunoglobulins detect the native 70-kDa OPN, but only the LF-124 antibody recognizes the 45-kDa cleavage fragment that contains the integrin-binding RGD sequence and has increased cell attachment and spreading properties through increased accessibility of the RGD to integrin receptors [20]. Only the 45-kDa OPN supports $\alpha_9\beta_1$- and $\alpha_4\beta_1$-mediated cell migration and adhesion [29, 30] as well as attachment through several other non-RGD sites [31]. This fragment is the predominant form of OPN in uterine flushes from pregnant ewes [5, 6]. The 45-kDa OPN fragment is clearly present at the apical surface of uterine LE on Day 14 of pregnancy, on Day 20 conceptus trophectoderm, and along the entire uterine-placental interface of both placentomal and interplacentomal regions through Day 120 of gestation. Because OPN mRNA is synthesized by GE, but not by LE or conceptus, immunodetection of 45-kDa OPN protein at these locations confirms that OPN is secreted by GE and binds to all sites of direct attachment between conceptus and uterus. The source of OPN protein that localizes at the interface between caruncular epithelium and chorion likely is OPN secreted from neighboring GE in the intercaruncular endometrium. However, stromal and immune cells of placentomes express OPN, which may be transported to the interface between caruncular epithelium and chorion [24, 32].

Implantation/placentation is a progressive process involving adhesion molecule-dependent remodeling of endometrium and conceptus trophectoderm. Recently, OPN and its integrin receptors have become the focus of studies concerning adhesion for implantation. Lessey et al. [33] have established that transient endometrial expression of $\alpha_v\beta_3$ and $\alpha_4\beta_1$ integrins is cycle-dependent and defines the implantation window in women. Altered expression of these integrins is correlated with several causes of infertility [34]. Null mutations of α_v, α_5, β_1, or β_5 integrin genes in mice lead to peri-implantation lethality and failure of chorion-allantois fusion [35], whereas functional blockade of α_v and β_3 integrins in mice reduces the number of implantation sites [36]. Endometrial integrin expression has also been reported in several species that exhibit noninvasive implantation, including the sheep, pig, goat, and cow [6, 7, 37–39]. Uterine, placental, and/or conceptus expression of OPN has been demonstrated in cows, pigs, mice, baboons, humans, and sheep. Preliminary studies in cows suggest that OPN protein is localized to the apical surface of GE,

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to stroma, and on trophectoderm cells of midpregnancy cows [40]. In pigs, OPN mRNA is synthesized in discrete regions of the uterine LE between Days 12 and 15 of pregnancy, is present throughout the entire LE thereafter, and appears in GE by Day 30 [41]. In the mouse, OPN is transcribed by trophectoderm, metrial gland cells of decidua, and placenta [42], whereas OPN protein is found in GE and decidualized stroma of baboons [43]. In women, high levels of OPN mRNA were first localized by Young et al. [10] to decidual stromal cells as well as endometrial glands of nonpregnant secretory uterus. The OPN transcript was later detected in hypersecretory endometrial GE of pregnant women [11, 44]. Omigbodun et al. [45] subsequently determined that progesterone increased OPN protein on cytotrophoblasts of the chorionic villus, but not syncytial trophoblasts, and concluded that OPN mediates adhesive and/or signaling events between these trophoblast cell types via α_v integrins. Importantly, endometrial expression of OPN is increased by progesterone during the menstrual cycle, starting on Days 16–17, and secretory GE appears to be the source of OPN protein that is localized on the apical LE [19, 44].

In sheep, OPN is a component of histotroph that increases in uterine flushings from pregnant ewes between Days 11 and 17 [6]. Exposure to progesterone induces OPN mRNA expression only in endometrial GE, but OPN protein is present on the apical aspect of the endometrial LE and trophectoderm [5, 6, 21]. Indeed, OPN has been proposed to bridge integrin receptors expressed by trophectoderm and uterus to induce adhesion between LE and trophectoderm essential for initial conceptus attachment in sheep and humans [6, 7, 44, 46]. Two recent reports using high-density microarray screening of human endometrium during the receptive phase for implantation have confirmed that OPN increases markedly during the window of implantation. A comparison of gene expression between endometrium from Days 8 to 10 of the cycle with Days 8–10 postmidluteal LH surge showed an 8.1-fold increase in OPN mRNA [47]. Similarly, microarray comparison of endometrium from Days 2 to 4 with Days 7 to 9 post-LH surge showed a 12.3-fold up-regulation [48]. Results from the present study support the results from studies of pigs [41] and cows [40] and firmly establish that glandular OPN continues to have a role in maternal-fetal interactions well beyond the receptive phase for implantation. The role of OPN at the uterine-placental interface is not clear, but this ECM protein has potential to mediate adhesion, remodeling, and cell-cell/cell-ECM communication at sites of contact between maternal- and fetal-derived tissues. The presence of the highly adhesive [20] and flexible [49] 45-kDa OPN cleavage fragment containing multiple binding sequences for interaction with different proteins [50] and capable of multimer formation [46] supports the hypothesis that OPN continues to serve as an adhesive ligand that bridges receptors on uterus and placenta throughout gestation. However, other roles for OPN are possible. For example, OPN fragments are known to initiate cell proliferation and migration [51] and may be involved in cytoskeletal reorganization for placentation. In addition, ECM proteins, such as OPN, can bind integrins to promote survival in cells that would otherwise undergo apoptosis [52], and OPN sequesters Factor-H, a plasma protein that binds and inactivates complement pathway components, to the cell surface and inhibits complement-mediated cell lysis [53]. Interestingly, both OPN and another endometrial secretory protein, decay-accelerating factor, which functions in a similar way to inhibit complement-mediated cell lysis, exhibit comparable temporal and spatial increases of expression in the peri-implantation human endometrium [54]. Therefore, OPN may serve to stabilize cells at the uterine-placental interface.

Collectively, OPN mRNA and protein localization in the present study confirm that OPN is a component of ovine histotroph that binds to the uterine-placental interface, where it could have profound influence throughout gestation. Experiments that address physiological, cellular, and molecular mechanisms involved in OPN-mediated attachment, proliferation, migration, and signal transduction using in vitro, ex vivo, and in vivo models are warranted to define the role of OPN during pregnancy.

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