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Fibroblast Growth Factor-10: A Stromal Mediator of Epithelial Function in the Ovine Uterus

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ABSTRACT

Fibroblast growth factor-10 (FGF-10) is a stromal-derived paracrine growth factor considered to be important during embryogenesis; however, its expression by cells in the female reproductive tract has not been investigated. Therefore, an ovine FGF-10 cDNA was cloned from an ovine endometrial cDNA library to investigate expression and potential paracrine characteristics of FGF-10 in the ovine uterus. The ovine FGF-10 cDNA encodes a protein of 213 amino acids and possesses an unusually long 5′ untranslated region (UTR). In situ hybridization demonstrated that ovine FGF-10 mRNA was expressed by endometrial stromal cells and by mesenchymal cells of the chorioallantoic placenta. The mRNA for FGF-7, a homologue of FGF-10, was localized in the tunica muscularis of blood vessels in endometrium and myometrium. In contrast, FGF receptor 2IIIb, the high-affinity receptor for both FGF-10 and FGF-7, was expressed exclusively in luminal epithelium, glandular epithelium, and placental trophectoderm. The in vivo spatial expression pattern suggests that FGF-10 is a novel endometrial stromal cell-derived mediator of uterine epithelial and conceptus trophectodermal functions. The nonoverlapping spatial patterns of expression for FGF-10 and FGF-7 in ovine uterus and conceptus suggest independent roles in uterine function and conceptus development.

conceptus, growth factors, uterus

INTRODUCTION

Epithelial-mesenchymal interactions have been implicated in development, growth, differentiation, and adult function of the uterus, a steroid hormone-responsive organ [1, 2]. In the uterus, several cell types, including luminal epithelium (LE), glandular epithelium (GE), stromal fibroblasts, myometrium, endothelium, and immune cells are involved in paracrine interactions with one another. Stromal cells regulate proliferation, differentiation, morphogenesis, and secretion of epithelia. Reciprocal interactions are required to ensure the correct organization and function of the endometrium as well as myometrium [2].

Ovarian hormones, progesterone (P_4) and estrogen, are important regulators of uterine function during the estrus cycle and pregnancy. The action of P_4, the hormone of pregnancy, on the uterus is an absolute and universal requirement for establishment and maintenance of pregnancy in all mammals. Receptors for progesterone (PR) and estrogen (ERα) are expressed in all uterine cell types during early stages of the estrus cycle; however, P_4 negatively autoregulates expression of its own receptor, particularly in uterine LE and GE [3, 4]. Indeed, it is now clear that loss of PR in LE and superficial GE occurs before implantation in a number of species, including sheep [4–6]. In sheep, PR in LE and GE declines to undetectable levels during the preimplantation period and for the remainder of pregnancy [4]. ERα is also absent from endometrial epithelia throughout pregnancy, and ERβ is undetectable in ovine uterus (unpublished observation). Therefore, uterine epithelial responsiveness to ovarian steroids is likely to be mediated by PR-positive and ERα-positive stromal and myometrial cells. Stromal-derived growth factors, which bind receptors that are unique to epithelial cells, fibroblast growth factor-7 (FGF-7) and hepatocyte growth factor (HGF) in particular, have been proposed to mediate effects of P_4 or estrogen on epithelial cells [7].

FGF-7 and FGF-10 are the two members of the FGF family with closest structural and functional similarities [8, 9]. FGF-7 is an established paracrine growth factor of mesenchymal origin unique to epithelial cell proliferation and differentiation [10], and has been proposed to mediate effects of P_4 in primate endometrium [11]. FGF-10 was originally isolated from rat lung mesenchyme and identified as a mesenchymal-derived growth factor that is essential for patterning the early branching and morphogenic events, including embryonic lung and limb bud formation [12–18]. Like FGF-7, FGF-10 is a specific mitogen for epithelial cells [9, 15]. FGFR2IIIb, the established receptor for FGF-7, is also the high-affinity receptor for FGF-10 [9]. Both FGF-10 and FGF-7 have the same binding affinity to FGFR2IIIb [15].

Expression of the FGF-10 gene in the female reproductive tract has not been described. In order to test the hypothesis that FGF-10 is expressed in uterus and is a mediator of uterine and conceptus epithelial-mesenchymal interactions, we cloned ovine cDNAs for FGF-10, FGFR2IIIb, and FGF-7 and examined temporal and spatial aspects of their expression in the ovine uterus.

MATERIALS AND METHODS

Animals and Tissue Collection

All experimental and surgical procedures involving animals were approved by the Agricultural Animal Care and Use Committee, Texas A&M University (Animal Use protocols 7-286 and AG-239AG). Mature Rambouillet ewes were observed daily for estrus using vasectomized rams. After exhibiting at least two estrus cycles of normal duration (16–18 days), ewes were assigned randomly on Day 0 to cyclic or pregnant status. Ewes assigned to pregnant status were mated to intact rams beginning at estrus (Day 0). Ewes were then hysterectomized (n = 4 ewes/day) on Days 1, 3, 5, 7, 9, 11, 13, or 15 of the estrus cycle and Days 11, 13, 15, 17, and 19 of pregnancy. At hysterectomy, sections

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of the uterine wall from the mid-portion of each uterine horn and, if present, placentomes were fixed in fresh 4% paraformaldehyde in PBS (pH 7.2). The remaining endometrium was physically separated by dissection from myometrium, frozen in liquid nitrogen, and stored at −80°C for RNA isolation and analyses. Additional uteri were collected from Days 25 and 40 pregnant ewes (term = 147 days) for in situ hybridization analyses.

Cloning and Sequence Analysis

A partial cDNA for ovine FGFR2IIIb was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) from Day 1 cyclic ovine endometrial tRNA using primers based on the human FGFR2IIIb sequence (forward, 5′-TCTGTTCAATGTGACCGAGG, reverse, 5′-TCGGTCACATTGAACAGAGC) using methods described previously [19]. The PCR conditions were as follows: 1) 94°C for 6 min; 2) 1 min at 94°C, 2 min at 55°C, and 1 min at 72°C for 34 cycles; and 3) 72°C for 10 min. The 298-base pair (bp) PCR product containing FGFR2IIIb-specific exon K was cloned into the PCR-II vector using the T/A cloning Kit (Invitrogen, Carlsbad, CA). Automated DNA sequencing was performed to confirm identity. A 450-bp partial cDNA for porcine FGF-10 (unpublished results) and a 700-bp partial cDNA for porcine FGF-7 (GenBank accession number AF217463) were used for screening a Day 15-pregnant ovine endometrial Lambda ZAP II cDNA library (Stratagene, La Jolla, CA) for ovine FGF-10 and FGF-7. The library was screened with low stringency using [^{32}P] random-labeled cDNA probes. One clone containing the entire ovine FGF-10 coding sequence and one clone containing a partial ovine FGF-7 cDNA were obtained and fully sequenced in both directions. A 766-bp ovine FGF-10 *Sac*I and *Bam*HI restriction fragment from the earlier FGF-10 clone was subcloned into pCRII cloning vector as a template for making riboprobes for Northern blot and in situ hybridization analyses.

Northern Blot Analysis

Polyadenylated mRNA was purified from endometrial tissues using a Poly(A)$^+$ Pure kit (Ambion, Austin, TX). Poly(A)$^+$ RNA (2 μg) from cyclic or pregnant ewes or both was separated by electrophoresis through a denaturing agarose gel, and transferred to a Nytran Plus nylon membrane (Schleicher & Schuell, Keene, NH) by capillary blotting. The Northern blots were then hybridized with radiolabeled antisense riboprobes specific for ovine FGF-10 and FGFR2IIIb. Washing procedures, including RNase A digestion, were performed under high stringency as previously described [19]. Autoradiographs were prepared using X-OMAT x-ray film (Kodak, Rochester, NY).

Semiquantitative RT-PCR

Semiquantitative RT-PCR analysis was used to determine steady-state levels of FGF-10, FGFR2IIIb, and FGF-7 mRNA in endometrial total RNA from cyclic and pregnant ewes. First-strand cDNA was synthesized from total endometrial RNA isolated from each cyclic and pregnant ewe as described previously [20]. The cDNA was diluted with water (1:10 and 1:100) for use as a template in PCR reactions. PCR reactions contained 100 ng of each primer, 1 U AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA), 1 mM dNTPs, 5 μl RT cDNA (1:10 for FGF-10, FGFR2IIIb, and FGF-7 and 1:100 for β-actin) in optimized buffer (Invitrogen, Carlsbad, CA; Buffer F for FGF-10, FGFR2IIIb, and FGF-7; Buffer D for β-actin). The dilution and volume of cDNAs used in PCR reactions were determined to be in the linear range to ensure that the amount of PCR product derived from a given amount of total RNA in a sample was directly proportional to the concentration of target mRNA in the sample. Amplification of β-actin was used as an internal control to correct for differences in amount of RT cDNA used in each reaction. Primer sequences were as follows: for FGF-10 forward, 5′-CTTCTTGGTGTCTTCCGTCC, reverse, 5′-CTCCTTTTCCATTCAATGCC; for FGFR2IIIb forward, 5′-TCTGTTCAATGTGACCGAGG, reverse, 5′-TCGGTCACATTGAACAGAGC; for FGF-7 forward, 5′-GCTTGCAATGACATGACTCC, reverse, 5′-TGCCATAGGAAGAAAGTGGG; for β-actin forward, 5′-CATCCTGACCCTCAAGTACCC, reverse, 5′-GTGGTGGTGAAGCTGTAGCC. PCR conditions were 30 cycles at 95°C for 30 sec, 55°C for 1 min, and 72°C for 1 min. The PCR products were separated by 1.5% agarose gel electrophoresis and stained with ethidium bromide. DNA fluorescence in correctly sized bands was densitometrically quantitated using a video documentation system and image analysis software (Alpha Innotech Corporation, San Leandro, CA). The β-actin values were used as a covariate in statistical analyses to correct for differences in amounts of cDNA used for each PCR reaction and are expressed as relative light units.

In Situ Hybridization Analysis

In situ hybridization was performed as previously described [21] with antisense or sense [^{35}S]-riboprobes for ovine FGF-10, FGFR2IIIb, and FGF-7. After hybridization and washing procedures, slides were dipped in Kodak NBT-2 liquid photographic emulsion and stored at 4°C for 2 wk. Slides were then developed in Kodak D-19 developer and counterstained with hematoxylin.

Photomicroscopy

Digital photomicrographs of in situ hybridization slides were captured using a Zeiss Axioplan2 photomicroscope (Carl Zeiss, New York, NY) fitted with a Hamamatsu chilled 3CCD color digital camera (Hamamatsu Corporation, Bridgewater, NJ). Digital images were assembled using Adobe Photoshop 4.0 (Adobe Systems, Seattle, WA). Black and white photomicrographs were electronically printed using a Kodak DS8650 color printer.

Statistical Analysis

All quantitative data were subjected to least-squares ANOVA using the general linear models procedures of the Statistical Analysis System [22]. Analyses of RT-PCR data included the β-actin data as a covariate. All tests of statistical significance were performed using the appropriate error terms according to the expectation of mean squares of error. Data are presented as least square means with standard errors (SEM).

RESULTS

A 2.9-kilobase (kb) cDNA encoding ovine FGF-10 (GenBank accession number AF213396) was isolated by screening a Day 15 pregnant ovine endometrial cDNA library with a partial porcine FGF-10 cDNA. This ovine FGF-10 cDNA possesses a predicted open reading frame encoding a 213-amino acid (~24 kDa) protein that shares

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ovine      1 MWKWILTHCASAFPHLSG-CCCCFLLLFLVSSVPVTCQALDQDMVSPGATNSSSSSSS-
rat        1 ................P.-.....................G......E..........S
human      1 ................P.C.....................G......E......------
mouse      1 ................P.-.............F.......G.....QE...C..------
chicken    1 .C.....NG....S..PC---..L.............HD.G...L..E..........-

ovine     59 SSSSVSLPSSAGRHVRSYNHLQGDVRWRKLFSFTKYFLKIE-NGKVSGTKKENCPYSILE
rat       60 ....F.S.................................K.................
human     55 -..-F.S.................................K.................
mouse     54 ....F.S.....................R.........T..K........N.D....V..
chicken   57 FP..F.S...................K...Y.YN.......K.............F....

ovine    118 ITSVEIGVVAVKAINSNYYLAMNKKGKLYGSKEFNNDCKLKERIEENGYNTYASFNWQHN
rat      120 ............................................................
human    113 ............................................................
mouse    114 ............................................................
chicken  117 ............S.K............V.......S..................L..K..

ovine    178 GRQMYVALNGKGAPRRGQKTRRKNTSAHFLPMVVHS
rat      180 ..................................
human    173 ..................................
mouse    174 ................................TIQT
chicken  177 ....F.....R..TK...................M.
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FIG. 1. Amino acid sequence alignment of ovine FGF-10 (GenBank accession number AF213396) with that of rat (GenBank accession number D79215), human (GenBank accession number AB002097), mouse (GenBank accession number D89080), and chicken (GenBank accession number D86333). The protein sequence of ovine FGF-10 is most homologous to rat FGF-10 (97%) and human FGF-10 (94%).

high sequence identity with FGF-10 of rat (97%), human (94%), mouse (90%), and chicken (84%; Fig. 1). A unique feature of the 2.9-kb ovine FGF-10 cDNA is an exceptionally long (1.4 kb) 5′ UTR. The cloned FGF-10 cDNA also contained an 844-bp full-length 3′ UTR. To determine the presence of FGF-10 receptor in ovine endometrium, using RT-PCR we cloned a 298-bp partial cDNA for FGFR2IIIb (GenBank accession number AF213380), an alternative spliced variant of the *bek* receptor gene [23]. An 813-bp partial cDNA for ovine FGF-7 (GenBank accession number AF241265) was also cloned by screening a Day 15 pregnant ovine endometrial cDNA library.

Northern blot analyses detected a 4.5-kb mRNA for FGF-10 (Fig. 2A) and a 4.7-kb mRNA for FGFR2IIIb (Fig. 2B) in ovine endometrium. The ovine FGF-10 mRNA was similar in size to that of rat and mouse [12, 24] and the size of ovine FGFR2IIIb mRNA was similar to that of human [25].

As illustrated in Figure 3, semiquantitative RT-PCR analyses using β-actin as a covariate were performed to examine the steady-state levels of mRNA for FGF-10, FGFR2IIIb, and FGF-7 in ovine endometrium during the estrus cycle and early pregnancy. Steady-state levels of

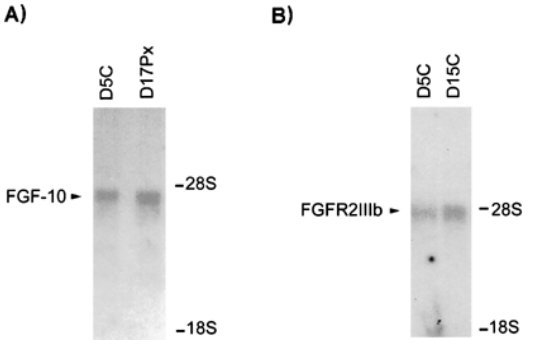

FIG. 2. Northern blot analysis of FGF-10 (**A**) and FGFR2IIIb (**B**) mRNA in cyclic (C) and pregnant (Px) ovine endometrium. Each lane represents 2 μg poly(A)$^+$ endometrial RNA. Autoradiographs developed after 48 h are shown. Positions of the 28S (4.7 kb) and 18S (1.8 kb) rRNAs are indicated.

FGF-10 mRNA (Fig. 4A) were low on Day 1, increased to Day 5, and remained similar through Day 15 of the estrus cycle ($P < 0.1$, quadratic). In early pregnant ewes, FGF-10 mRNA expression was consistent ($P > 0.1$). On Days 11, 13, and 15, endometrial FGF-10 mRNA expression was not different between cyclic and pregnant ewes ($P > 0.1$). As illustrated in Figure 4B, FGFR2IIIb mRNA was constitutively expressed in ovine endometrium and was not affected ($P > 0.1$) by day or pregnancy status. Similarly, steady-state levels of FGF-7 mRNA were not affected ($P > 0.1$) by day or pregnancy status (Fig. 4C).

Spatial and temporal alterations in FGF-10 mRNA expression in uteri from cyclic and pregnant uteri were examined by in situ hybridization (Fig. 5). The FGF-10 mRNA was expressed only by stromal cells of the intercaruncular and caruncular endometrium in uteri from both cyclic and pregnant ewes. Hybridization signals above the sense control were not detected in LE, GE, or myometrium. In pregnant ewes, FGF-10 mRNA was expressed in the maternal caruncular stroma adjacent to placental cotyledons in placentomes (see first Day 40 pregnancy photomicrograph). FGF-10 mRNA was also localized to the chorioallantoic mesenchyme but not to the trophectoderm (see second Day 40 pregnancy photomicrograph).

As illustrated in Figure 6, in situ hybridization revealed that FGFR2IIIb mRNA was expressed exclusively in endometrial LE and GE of both cyclic and pregnant ewes. Hybridization signals above sense control were not found in stroma, myometrium, or blood vessels. In pregnant ewes, FGFR2IIIb mRNA was localized to epithelium of chorionic villi (see first Day 40 pregnancy photomicrograph) in placentomes. FGFR2IIIb mRNA was also expressed by trophectoderm of the chorioallantoic placenta as indicated on the second Day 40 pregnancy photomicrograph.

Because FGF-7 also signals through FGFR2IIIb and exhibits close structural and functional similarity with FGF-10, we also examined the expression of FGF-7 mRNA in ovine uterus (Fig. 7). In situ hybridization revealed that FGF-7 mRNA was expressed predominantly in the mesenchymal component (tunica muscularis) of blood vessels in endometrium and myometrium of both cyclic and preg-

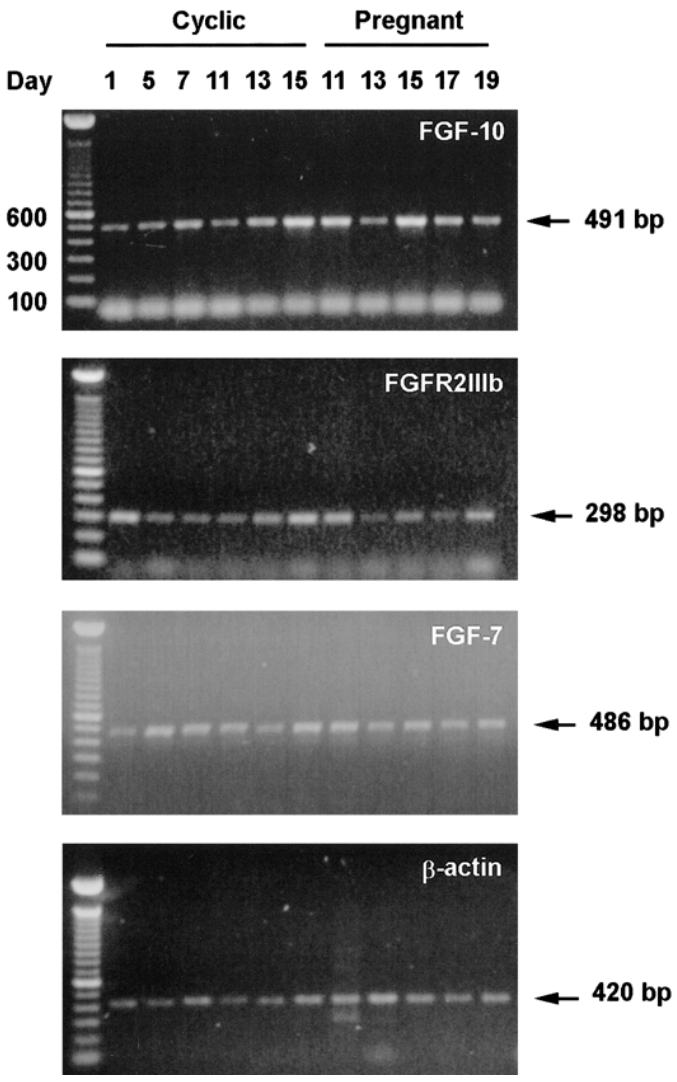

FIG. 3. Representative RT-PCR analyses of FGF-10, FGFR2IIIb, and FGF-7 mRNA in endometrial total RNA isolated from uteri of cyclic and pregnant ewes. The PCR products for FGF-10 (491 bp), FGFR2IIIb (298 bp), FGF-7 (486 bp), and internal control β-actin (420 bp) are indicated. Positions of the 100-bp DNA marker ladder are shown on the left.

nant ewes. The expression of FGF-7 mRNA in LE, GE, or stroma was not detected compared with sense control.

DISCUSSION

Previous studies of FGF-10 suggested that its expression is very restricted compared with other FGF family members in terms of tissue expression. FGF-10 is expressed primarily by a limited number of embryonic tissues and ductal organs such as fetal lung, limb bud, and developing prostate, and by even fewer adult tissues [12–18]. In the present

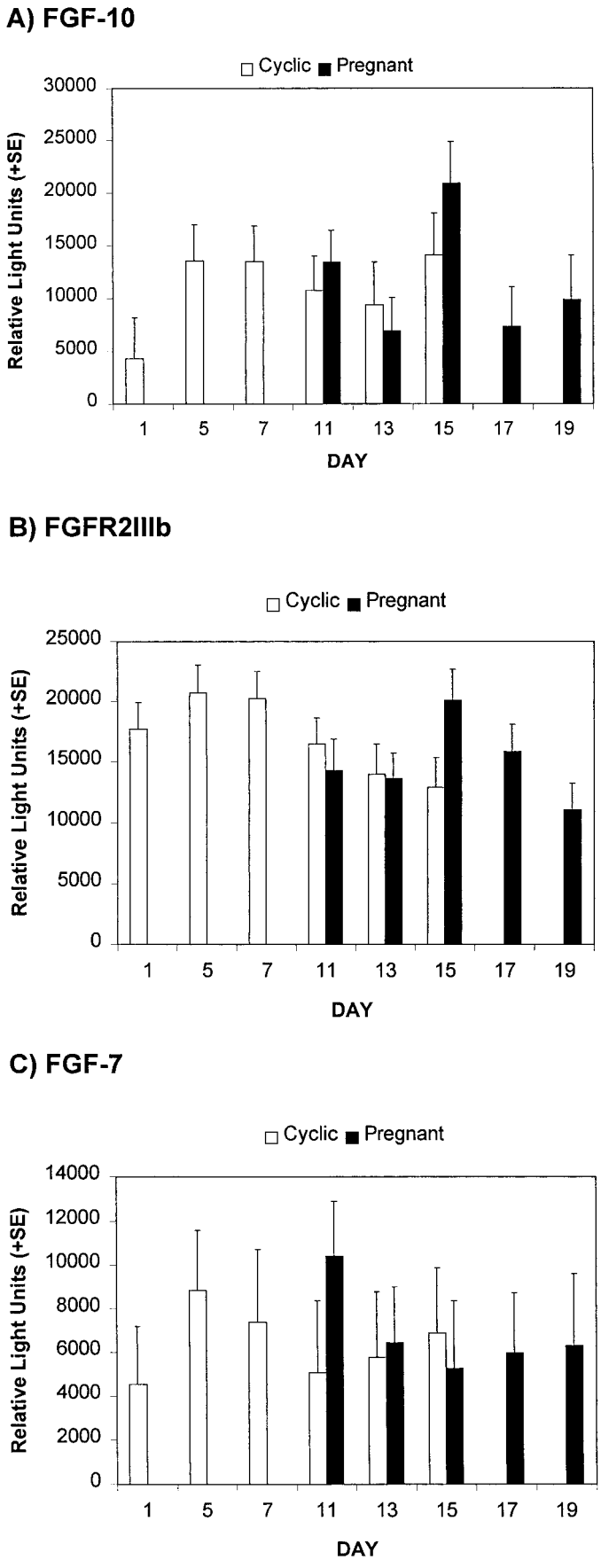

FIG. 4. Semiquantitative RT-PCR analysis of FGF-10, FGFR2IIIb, and FGF-7 mRNA abundance in ovine endometrium during the estrous cycle and early pregnancy. **A**) There was an effect of day ($P < 0.1$) on steady-state levels of FGF-10 mRNA. FGF-10 mRNA expression was not affected by pregnant status or day × status ($P > 0.1$). **B**) FGFR2IIIb mRNA was constitutively expressed in ovine endometrium during the estrus cycle and early pregnancy ($P > 0.1$). **C**) There was no effect of day, status, or day × status on steady-state levels of endometrial FGF-7 mRNA ($P > 0.1$).

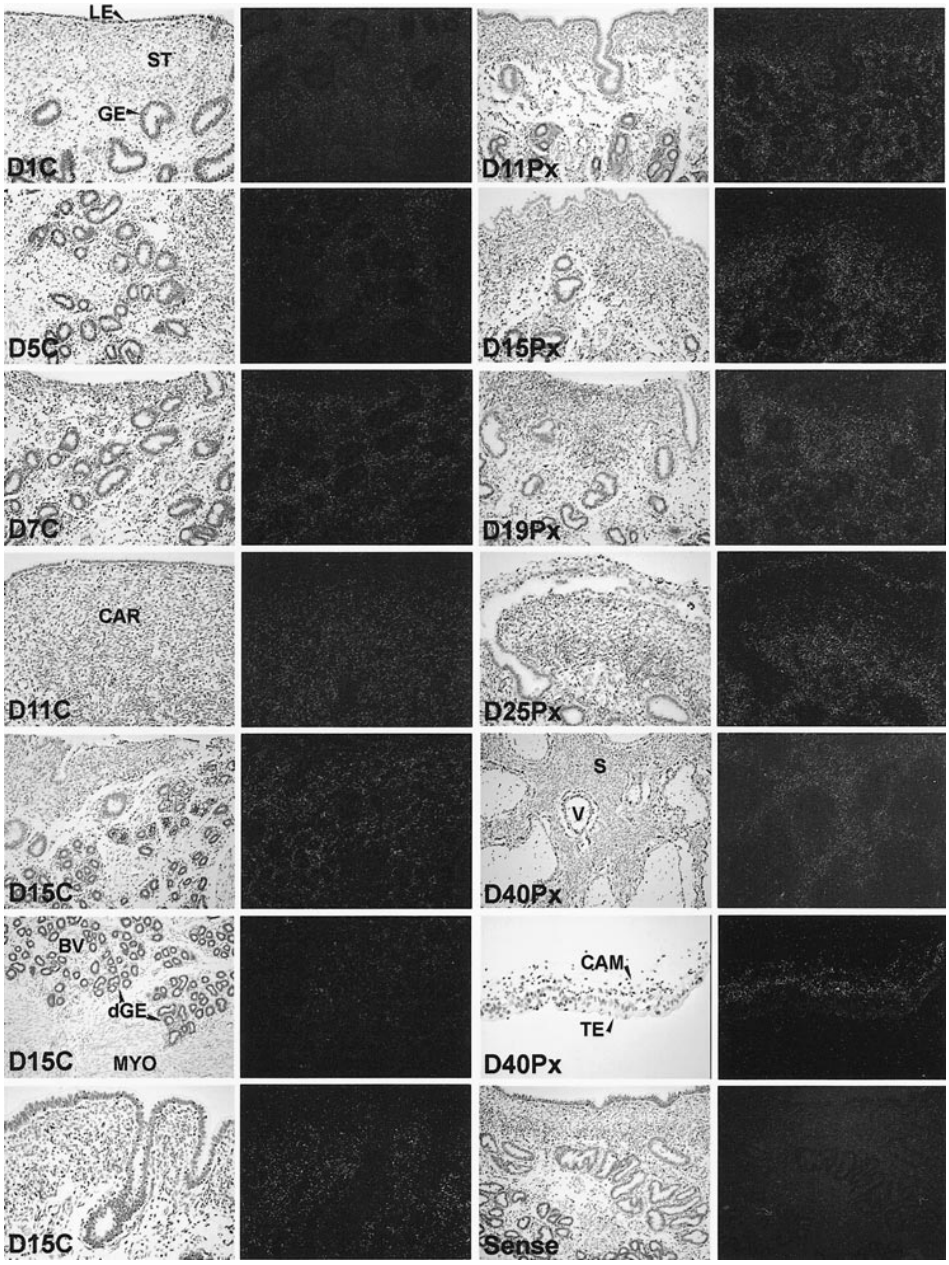

FIG. 5. Localization of FGF-10 mRNA in endometrium of cyclic (C) and early pregnant (Px) ewes by in situ hybridization. Cross-sections of the uterine wall from cyclic and pregnant ewes were hybridized with ^{35}S-Labeled antisense and sense ovine FGF-10 cRNA probes. Hybridized sections were digested with RNAse A, and protected transcripts visualized by liquid emulsion autoradiography. Developed slides were lightly counterstained with hematoxylin. Representative brightfield and darkfield photomicrographs for each day are shown. LE, Luminal epithelium; GE, glandular epithelium; ST, stroma; Car, caruncular area; MYO, myometrium; dGE, deep glandular epithelium; S, maternal stroma; V, chorionic villi; TE, trophectoderm; CAM, chorioallantoic mesenchyme. All photomicrographs are shown at a magnification of ×260 except for the last Day 15 cyclic and the second Day 40 pregnant panels, which are shown at a magnification of ×531.

study, we provide the first evidence for expression of FGF-10 in adult uterus.

A striking feature of ovine FGF-10 cDNA is an exceptionally long 5′ UTR. Northern blot analysis indicated that the 4.5-kb FGF-10 transcript in endometrium is 1.6 kb larger than the cDNA clone isolated by library screening, suggesting that the 5′ UTR of the full-length ovine FGF-10 mRNA could be at least 3 kb in length, compared with the normal length of 5′ UTR for approximately 90% of vertebrate mRNAs between 10 and 200 nucleotides [26]. However, similar to FGF-10, some growth factors and proto-oncogenes related to cell proliferation and differentiation, such as insulin-like growth factor-2, transforming growth factor beta-1, platelet-derived growth factor, vascular endothelial growth factor, and c-*myc* contain an unusually long 5′ UTR [27]. Indeed, another member of the FGF family, FGF-2, also has a long 5′ UTR. Both FGF-2 and FGF-3 exhibit alternative initiation of translation [28, 29], resulting in different protein isoforms. A long 5′ UTR usually suggests complex control of expression at the translational level [26, 27]; therefore, complicated mechanisms for translational regulation may exist for the ovine FGF-10 gene as well as other FGF family members.

In situ hybridization revealed that FGF-10 mRNA could be detected only in endometrial stromal cells, whereas its receptor is localized only to epithelial cells in the ovine

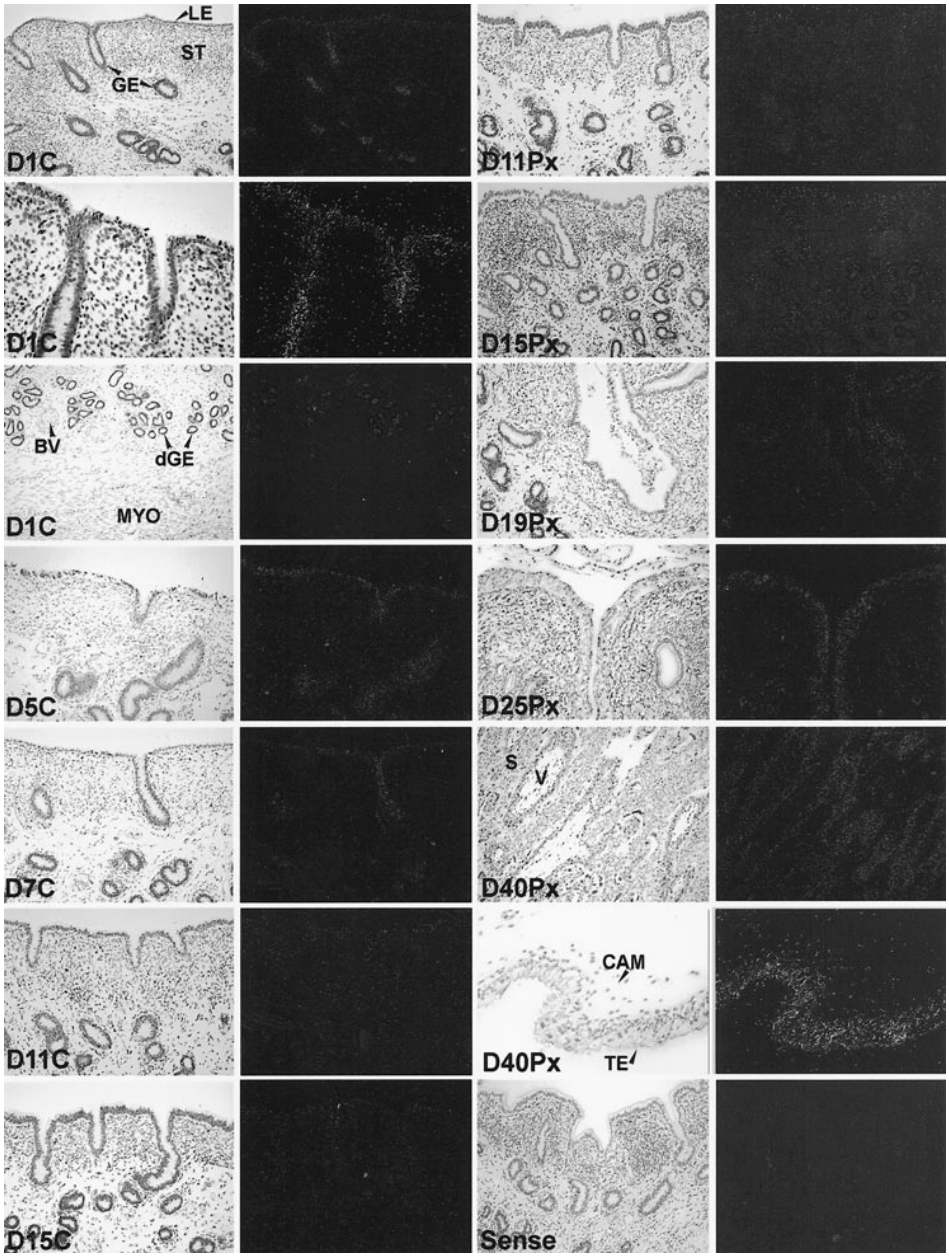

FIG. 6. Localization of FGFR2IIIb mRNA in endometrium of cyclic (C) and early pregnant (Px) ewes by in situ hybridization. Cross-sections of the uterine wall from cyclic and pregnant ewes were hybridized with ^{35}S-labeled antisense and sense ovine FGFR2IIIb cRNA probes. Hybridized sections were digested with RNAse A, and protected transcripts visualized by liquid emulsion autoradiography. Developed slides were counterstained lightly with hematoxylin. Representative brightfield and darkfield photomicrographs for each section are shown. LE, Luminal epithelium; GE, glandular epithelium; ST, stroma; BV, blood vessel; MYO, myometrium; dGE, deep glandular epithelium; S, maternal stroma; V, chorionic villi; TE, trophectoderm; CAM, chorioallantoic mesenchyme. All photomicrographs are shown at a magnification of ×260 except for the second Day 1 cyclic and the second Day 40 pregnant panels, which are shown at a magnification of ×531.

uterus. These in vivo expression patterns suggest that FGF-10 is a strong candidate for a paracrine mediator of uterine mesenchymal-epithelial interactions. Because FGF-10 is a secreted, physiologically significant epithelial mitogen that is important for epithelial branching and morphogenesis [8, 13, 30], FGF-10 may play a role in regulating ovine uterine epithelial proliferation, differentiation, and function, which occur primarily during early pregnancy and in neonates [31, 32]. Indeed, FGF-10 and its receptor are expressed in neonatal ovine uterus during endometrial gland development [33], implicating a role for FGF-10 in postnatal uterine gland morphogenesis. Another interesting finding was FGF-10 expression by chorioallantoic mesenchyme and expression of its receptor by the adjacent trophectoderm. These results indicate that FGF-10 may also mediate placental mesenchymal-trophectodermal interactions to stimulate proliferation and differentiation of the placenta. Thus, expression of FGF-10 in the conceptus, neonatal uterus, and adult uterus suggest that FGF-10 may be a critical factor required for uterine epithelial morphogenesis and cytodifferentiation.

Localization of ovine FGF-7 mRNA to tunica muscularis of uterine blood vessels is consistent with expression of FGF-7 mRNA in spiral arteries of primate endometrium

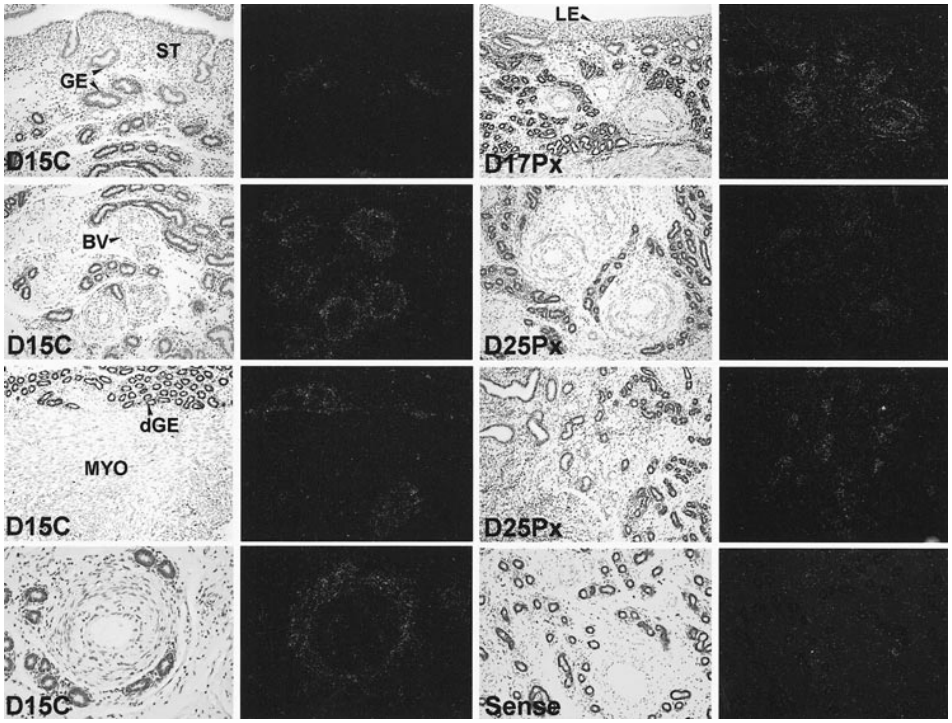

FIG. 7. Localization of FGF-7 mRNA in endometrium of cyclic (C) and early pregnant (Px) ewes by in situ hybridization. Cross-sections of the uterine wall from cyclic and pregnant ewes were hybridized with ^{35}S-labeled antisense and sense ovine FGF-7 cRNA probes. Hybridized sections were digested with RNAse A, and protected transcripts visualized by liquid emulsion autoradiography. Developed slides were lightly counterstained with hematoxylin. Representative brightfield and darkfield photomicrographs for each section are shown. LE, Luminal epithelium; GE, glandular epithelium; ST, stroma; MI, media intima; MYO, myometrium; dGE, deep glandular epithelium; S, maternal stroma; V, chorionic villi; TE, trophectoderm; CAM, chorioallantoic mesenchyme. All photomicrographs are shown at a magnification of ×260 except for the last Day 15 cyclic panel, which is shown at a magnification of ×531.

[7]. However, FGF-7 expression by stromal cells proximal to LE and shallow GE was not detected, as has been described for primates [7]. In the ovine uterus, cells that expressed FGF-7 are a stromal cell subtype that are different from those that express FGF-10. Furthermore, expression of FGF-7 in ovine uterus is very restricted compared with FGF-10, which is expressed throughout the stromal compartment proximal to endometrial LE and GE. On the basis of spatial patterns of expression of FGF-10 and FGFR2IIIb mRNAs, a paracrine mode of action of stromal cell-derived FGF-10 on LE and GE is suggested to regulate function. The restricted expression FGF-7 mRNA to tunica muscularis does not rule out a potential role as a paracrine growth factor for glandular epithelia, given that FGF-7 is expressed near deep GE. Nonetheless, the unique temporal and spatial patterns of expression for FGF-10 and FGF-7 suggest that these two stromal-derived growth factors may have distinct and nonoverlapping roles in ovine uterine functions.

In adult ewes, steady-state levels of FGF-10 mRNA were relatively high in uterine stromal cells during the luteal phase of the estrus cycle and peri-implantation period of early pregnancy, when there are high levels of circulating P_4, but an absence of PR expression in uterine LE and GE [4]. Available evidence from our laboratory indicates that P_4 induces expression of osteopontin and uterine milk protein mRNAs in uterine GE, which do not express PR [34]. This strongly supports the hypothesis that P_4 can indirectly regulate epithelial cell function by paracrine growth factors produced in response to effects of P_4 on PR-positive stromal cells. Results from this study suggest that FGF-10 is a strong candidate for such a mediator; however, exclusively on the basis of temporal aspects of FGF-10 mRNA expression, it cannot be concluded that FGF-10 is a progestamedin in the ovine uterus. Both in vivo and in vitro experiments on steroid hormone effects on endometrial stromal FGF-10 mRNA must be conducted to answer this question. Semiquantitative RT-PCR analyses indicate that expression of FGFR2IIIb and FGF-7 mRNA is constitutive during the estrous cycle and pregnancy. Based on these results, it is not obvious that P_4 or estrogen directly regulates the expression of these two genes.

This is the first report identifying FGF-10 as a growth factor in the mammalian uterus and placenta. Collectively, results indicate that FGF-10 is a candidate stromal mediator of epithelial cell function with the potential to affect three types of mesenchymal-epithelial interactions in the ovine uterus: 1) endometrial stromal-epithelial, 2) endometrial stromal-trophectodermal, and 3) chorioallantoic mesenchymal-trophectodermal communications. We propose that FGF-10 may act during pregnancy on maternal and fetoplacental tissues to stimulate epithelial proliferation and differentiation in support of the establishment and maintenance of pregnancy.

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Veterinary Medicine Image Analysis Laboratory, which is supported, in part, by NIH grant P30 ES09106.

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