0013-7227/07/$15.00/0
*Printed in U.S.A.*

Endocrinology 148(8):3922–3931
Copyright © 2007 by The Endocrine Society
doi: 10.1210/en.2007-0321

# Tight and Adherens Junctions in the Ovine Uterus: Differential Regulation by Pregnancy and Progesterone

M. Carey Satterfield, Kathrin A. Dunlap, Kanako Hayashi, Robert C. Burghardt, Thomas E. Spencer, and Fuller W. Bazer

*Center for Animal Biotechnology and Genomics, Department of Animal Science (M.C.S., K.A.D., K.H., T.E.S., F.W.B.), and Image Analysis Laboratory, Department of Veterinary Integrative Biosciences (R.C.B.), Texas A&M University, College Station, Texas 77843*

**In species with noninvasive implantation by conceptus trophectoderm, fetal/maternal communications occur across the endometrial epithelia. The present studies identified changes in junctional complexes in the ovine endometrium that regulate paracellular trafficking of water, ions, and other molecules, and the secretory capacity of the uterine epithelia. Distinct temporal and spatial alterations in occludin, tight junction protein 2, and claudin 1–4 proteins were observed in the endometrium of cyclic and early pregnant ewes. Dynamic changes in tight junction formation were characterized by an abundance of tight junction proteins on d 10 of the estrous cycle and pregnancy that substantially decreased by d 12. Early progesterone administration advanced conceptus development on d 9 and 12 that was associated with loss of tight-junction-associated proteins. Pregnancy increased tight-junction-associated proteins between d 14–16. Cadherin 1 and β-catenin, which form adherens junctions, were abundant in the endometrial glands, but decreased after d 10 of pregnancy in the luminal epithelium and then increased by d 16 with the onset of implantation. Results support the ideas that progesterone elicits transient decreases in tight and adherens junctions in the endometrial luminal epithelium between d 10–12 that increases selective serum and tissue fluid transudation to enhance blastocyst elongation, which is subsequently followed by an increase in tight and adherens junctions between d 14–16 that may be required for attachment and adherence of the trophectoderm for implantation. The continuous presence of tight and adherens junctions in the uterine glands would allow for vectorial secretion of trophic substances required for conceptus elongation and survival. (*Endocrinology* 148: 3922–3931, 2007)**

ESTABLISHMENT AND MAINTENANCE of pregnancy in mammals involves dynamic changes in the uterine epithelia that are regulated by steroid hormones, cytokines, and growth factors. These changes establish receptivity of the uterine luminal epithelium (LE) to the developing embryo, differentiated function of glandular epithelium (GE) required for subsequent secretion of uterine histotroph, and protection of the developing semiallogenic conceptus (embryo/fetus and associated extra-embryonic membranes) from the maternal immune system (1–6). In domestic animals, the implantation cascade is characterized by preattachment elongation of the blastocyst followed by apposition, adhesion, and attachment of the trophectoderm to the LE (7). Uterine-dependent conceptus elongation ensues in response to cues from the endometrial LE and GE in the form of histotroph (8) and possibly through stromal and serum-derived factors that bypass the epithelia via trafficking through the paracellular space into the uterine lumen to act directly on the conceptus.

Regulation of epithelial organization, structure, and subsequent function is modulated by two forms of junctional complexes, tight junctions, and adherens junctions. Tight junctions are located on the plasma membrane and facilitate cellular polarity, cell-cell contact, and adhesion. Tight junctions function as barriers that regulate the passage of ions, water, and molecules through the paracellular space. In addition, tight junctions maintain the proper distribution of proteins and lipids within domains of the plasma membrane (9). A growing class of proteins is known to be associated with the formation of tight junctions. These proteins are classified into one of three families of molecules: junctional adhesion molecules, occludins (OCLN), and claudins (CLDNs). Transepithelial paracellular permeability can be regulated by both OCLN and CLDNs (10). Zona occludens contain members of a submembranous class of proteins [tight junction proteins (TJP)] that bind both CLDNs and OCLN (11) and function as a scaffold to bring structurally diverse proteins into close proximity at tight junctions (12). The amount of OCLN and TJP1 protein present in a tissue is related inversely to the permeability of that tissue (13). Regulation of tight-junction-dependent transepithelial paracellular permeability is mediated by multiple factors including calcium (14, 15), growth factors, kinases, and second messengers (16), as well as hormones including progesterone (P4), prolactin, and placental lactogen (17).

Adherens junctions formed by classical cadherin/catenin complexes mediate epithelial organization and function. E-cadherins (CDH1) facilitate cell to cell adhesion within epithelia through homodimeric attachment to other CDH1 molecules on adjacent cells. These cadherins are first bound by

**First Published Online May 3, 2007**

Abbreviations: CDH1, Cadherin 1; CLDN, claudin; CO, corn oil; CTNNA1, α-catenin; CTNNB1, β-catenin; GE, glandular epithelium; IFNT, interferon τ; LE, luminal epithelium; OCLN, occludin; P4, progesterone; PGR, P4 receptor; TJP, tight junction protein.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**



Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

intracytoplasmic β-catenin (CTNNB1) (18, 19). α-Catenin (CTNNA1) is subsequently recruited to the complex (20) and binds to the actin cytoskeleton to facilitate cellular organization and shape (21). Stable cell to cell adhesion is mediated by the phosphorylation state of CTNNB1 (for review see Ref. 22). Homodimeric CDH1 interactions as well as other cell adhesion molecules have also been implicated in the relative invasive capabilities of tumors (for review see Refs. 23 and 24). During establishment of pregnancy, CDH1 and CTNNB1 may be important for maintaining the LE in a receptive state for implantation and blastocyst trophectoderm integrity during its rapid elongation to form a filamentous conceptus.

Our working hypotheses are that dynamic changes occur in assembly of tight junctions and adherens junctions in the ovine endometrium during the periimplantation period of pregnancy to change paracellular permeability, and that assembly of these junctional complexes is regulated by hormones of pregnancy. As a first step in testing this hypothesis, we determined effects of pregnancy and P4 on tight junction and adherens junction components in the ovine uterine endometrium.

## Materials and Methods

### *Animals*

Mature ewes (*Ovis aries*) were observed for estrus (designated as d 0) in the presence of a vasectomized ram and used in experiments only after exhibiting at least two estrous cycles of normal duration (16–18 d). All experimental and surgical procedures were in compliance with the Guide for the Care and Use of Agriculture Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M University.

### *Experimental design*

*Study 1.* At estrus (d 0), ewes were mated to either an intact or vasectomized ram as described previously (25) and then hysterectomized (n = 5 ewes/d) on either d 10, 12, 14, or 16 of the estrous cycle or d 10, 12, 14, 16, 18, or 20 of pregnancy. Pregnancy was confirmed on d 10–16 after mating by the presence of a morphologically normal conceptus(es) in the uterus. At hysterectomy, several sections (~0.5 cm) from the midportion of each uterine horn ipsilateral to the corpus luteum were fixed in fresh 4% paraformaldehyde in PBS (pH 7.2). After 24 h, fixed tissues were changed to 70% ethanol for 24 h and then dehydrated and embedded in Paraplast-Plus (Oxford Labware, St. Louis, MO). Several sections (1–1.5 cm) from the middle of each uterine horn were embedded in Tissue-Tek OCT compound (Miles, Oneonta, NY), frozen in liquid nitrogen vapor, and stored at −80 C. The remaining endometrium was physically dissected from myometrium, frozen in liquid nitrogen, and stored at −80 C for subsequent RNA or protein extraction. In monovulatory pregnant ewes, uterine tissue samples were marked as either contralateral or ipsilateral to the ovary bearing the corpus luteum. No tissues from the contralateral uterine horn were used in this study.

*Study 2.* At estrus (d 0), ewes were mated to intact rams and then assigned randomly to receive daily im injections from d 1.5–9 with either: 1) corn oil vehicle (CO; n = 6) or 2) 25 mg P4 (Sigma Chemical Co., St. Louis, MO; n = 6) in CO vehicle as described previously (26). All ewes were hysterectomized on d 9. Uteri were processed as described in study 1.

*Study 3.* At estrus (d 0), ewes were mated to intact rams as described previously (26) and then assigned randomly to receive daily intramuscular (im) injections of either: 1) CO vehicle from d 1.5–12 (CO, n = 8); 2) 25 mg P4 (Sigma Chemical Co.) from d 1.5–12 (P4, n = 7); or 3) 25 mg P4 (d 1.5–8, n = 5) followed by 75 mg of RU486 (Sigma Chemical Co.), a P4 receptor antagonist, from d 8–12 (P4 + RU). All ewes were hysterectomized on d 12, and the uteri were processed as in study 1.

### *Immunohistochemistry*

Immunohistochemical localization of immunoreactive proteins in the ovine uterus were performed as described previously (27). Rabbit antibodies against tight-junction-associated proteins were purchased from Zymed Laboratories (San Francisco, CA) including: CLDN1 (no. 51–9000) used at a final concentration of 2.5 μg/ml, CLDN2 (no. 51–6100) used at a final concentration of 1 μg/ml, CLDN3 (no. 34–1700) used at 0.1 μg/ml, CLDN4 (no. 36–4800) used at 1 μg/ml, OCLN (no. 71–1500) used at 1 μg/ml, and TJP2 (no. 71–1400) used at 1 μg/ml. A Vectastain ABC antirabbit kit was used for detection of the aforementioned proteins after antigen retrieval with boiling citrate buffer as described previously (28). Negative controls included substitution of the primary antibody with rabbit IgG at the same concentration. Immunoreactive CTNNB1 protein was detected using an anti-β-catenin (610153) antibody (BD Biosciences) at a final dilution of 1:250. Mouse IgG was substituted for the primary antibody as a negative control. A Vectastain ABC antimouse kit was used for detection of CTNNB1 protein after antigen retrieval with boiling citrate buffer. Immunoreactive proteins were visualized using diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) as the chromagen. Sections were subsequently dehydrated and coverslips were affixed with Permount.

### *Immunofluorescence analyses*

Frozen sections (8 μm) of uteri embedded in OCT compound were cut with a cryostat and mounted on Superfrost/Plus microscope slides (Fisher Scientific, Pittsburgh, PA). Using methods described previously (29, 30), sections were fixed in −20 C methanol, permeabilized with 0.3% Tween 20 in 0.02 M PBS, blocked in antibody dilution buffer (two parts 0.02 M PBS, 1.0% BSA, and 0.3% Tween 20, and one part glycerol) containing 10% normal goat serum, and incubated overnight at 4 C with anti-E-cadherin (CDH1) rabbit antiserum (no. 07-697; Upstate Cell Signaling Solutions, Lake Placid, NY) at a dilution of 1:350 or nonimmune rabbit serum at the same concentration. Immunoreactive protein was detected using a fluorescein-conjugated goat antirabbit IgG (Molecular Probes, Eugene, OR). Sections were then rinsed and overlaid with a coverslip and Prolong Antifade mounting reagent (Molecular Probes).

### *RNA isolation*

Total cellular RNA was isolated from frozen endometrium from the uterine horn ipsilateral to the corpus luteum (studies 2 and 3) using TRIzol reagent (Life Technologies, Inc., Bethesda, MD) according to the manufacturer's recommendations. The quantity and quality of total RNA was determined by spectrometry and denaturing agarose gel electrophoresis, respectively.

### *Slot blot hybridization analysis*

Steady-state levels of *CDH1* and *CTNNB1* mRNA in endometria were assessed by slot blot hybridization as described previously (31). Briefly, radiolabeled antisense cRNA probes were generated by *in vitro* transcription using linearized plasmid templates containing partial cDNAs, RNA polymerases, and [α-$^{32}$P]UTP. Denatured total endometrial RNA (20 μg) from each ewe in studies 2 and 3 was hybridized with radiolabeled cRNA probes. To correct for variation in total RNA loading, a duplicate RNA slot blot membrane was hybridized with radiolabeled antisense 18S cRNA (pT718S; Ambion, Austin, TX). After washing, the blots were digested with ribonuclease A, and radioactivity associated with slots was quantified using a Typhoon 8600 MultiImager (Molecular Dynamics, Piscataway, NJ). Data are expressed as relative units.

### *In situ hybridization analysis*

Location of *CDH1 and CTNNB1* mRNAs in the ovine uterus was determined by radioactive *in situ* hybridization analysis as described previously (31). Radiolabeled antisense and sense cRNA probes were generated by *in vitro* transcription using linearized partial plasmid cDNA templates, RNA polymerases, and [α-$^{35}$S]UTP. Deparaffinized, rehydrated, and deproteinated uterine tissue sections were hybridized with radiolabeled antisense or sense cRNA probes. After hybridization, washing, and ribonuclease A digestion, slides were dipped in NTB-2 liquid photographic emulsion (Kodak, Rochester, NY), and exposed at

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

4 C for 10 d. Slides were developed in Kodak D-19 developer, counterstained with Gill's hematoxylin (Fisher Scientific, Pittsburgh, PA), and then dehydrated through a graded series of alcohol to xylene. Coverslips were then affixed with Permount (Fisher Scientific).

### *Photomicroscopy*

Photomicrographs of *in situ* hybridization and immunocytochemistry slides were taken using a Nikon Eclipse E1000 photomicroscope (Nikon Instruments, Melville, NY). Digital images were captured using a Nikon DXM 1200 digital camera with ACT-1 software and assembled using Adobe Photoshop 7.0 (Adobe Systems, Seattle, WA). Fluorescence images of representative fields were recorded using a Zeiss Axioplan2 microscope (Carl Zeiss, Thornwood, NY) with a Axiocam HR digital camera and Axiovision 4.3 software and assembled as previously described (32).

### *Statistical analyses*

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). Slot blot hybridization data were corrected for differences in sample loading by using the 18S rRNA data as a covariate. Data are presented as the least-squares means with overall SE.

## Results

### *Tight-junction-associated proteins in ovine endometrium (study 1)*

Immunoreactive TJP2 and OCLN proteins were abundant in the endometrial LE and GE on d 10 of the cycle and pregnancy (Fig. 1). In both cyclic and pregnant ewes, TJP2 and OCLN protein was markedly decreased in the LE on d 12. In cyclic ewes, TJP2 and OCLN protein remained low in the LE and GE on d 14 and then increased on d 16; however, in pregnant ewes, TJP2 and OCLN protein increased in both LE and GE on d 14 and remained abundant in those epithelia on d 16. Immunoreactive TJP2 and OCLN proteins were also observed in the conceptus trophectoderm from d 18 and 20 pregnant ewes.

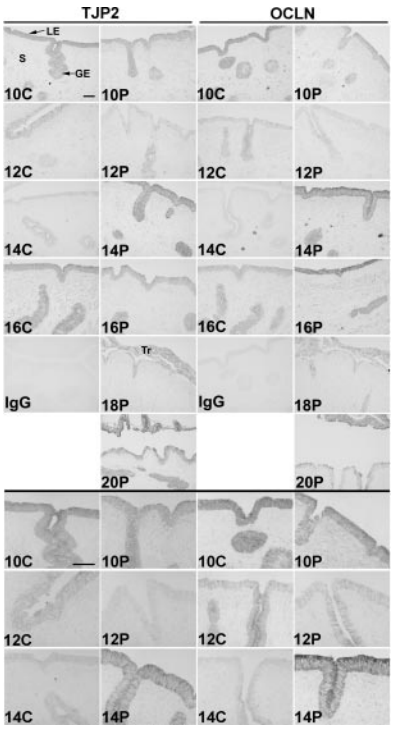


FIG. 1. Immunolocalization of TJP2 and OCLN proteins in the ovine endometrium during the estrous cycle and pregnancy (study 1). Immunoreactive proteins were detected using specific rabbit polyclonal antibodies against the respective proteins. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. Sections in *lower panels* are from areas of *upper panels* shown at higher magnification. S, Stroma; Tr, trophectoderm. *Bar*, 10 μm.

Temporal and spatial alterations in the CLDN proteins were detected in uteri of cyclic and pregnant ewes (Fig. 2). In cyclic ewes, immunoreactive CLDN1 protein decreased in the LE from d 10–14 of the cycle and then increased on d 16. There was also a decline in CLDN1 protein abundance in the LE between d 10–12 of pregnancy, but CLDN1 protein was more abundant in the LE beginning on d 14–18 and there was a marked increase in CLDN1 protein in both LE and GE on d 20 of pregnancy. CLDN1 protein was also detected in the trophectoderm of conceptuses on both d 18 and 20 of pregnancy.

The patterns of change in abundance of CLDN2, CLDN3, and CLDN4 proteins were similar in uteri of cyclic and pregnant ewes (Fig. 2). The abundance of these proteins in LE and GE decreased between d 10–12 in both cyclic and pregnant ewes. The remainder of the estrous cycle was characterized by low levels of CLDN2, CLDN3, and CLDN4 proteins on d 14 followed by an increase on d 16. However, in pregnant ewes, there was an increase in abundance of CLDN2, CLDN3, and CLDN4 proteins on both d 14 and 16. A more punctate expression pattern for these proteins was observed on d 18 and 20 of pregnancy for CLDN2 and CLDN4 while CLDN3 protein abundance increased throughout the LE and GE. CLDN2, CLDN3, and CLDN4 proteins were detected in trophectoderm of conceptuses on both d 18 and 20 of pregnancy. For all of the proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium that lacks endometrial glands.

### *CDH1 and CTNNB1 mRNA (study 1)*

*CDH1* mRNA was localized to LE and GE of the ovine endometrium and was abundant on d 10 of the estrous cycle and pregnancy (Fig. 3). In both cyclic and pregnant ewes, *CDH1* mRNA declined between d 10 and 14 and then increased between d 14 and 16 of pregnancy. Abundant *CDH1* mRNA remained in endometrial LE and GE as well as trophectoderm of the conceptuses on d 18 and 20 of pregnancy.

Temporal and spatial changes in *CTNNB1* mRNA were also observed in the ovine uterus throughout the estrous cycle and early pregnancy (Fig. 3). *CTNNB1* mRNA was abundant in LE, GE, and stroma on d 10 of the estrous cycle. A similar pattern was observed on d 10 of pregnancy, but mRNA levels were lower in all cell types. By d 12 of the cycle and pregnancy, less *CTNNB1* mRNA was detected in GE and stratum compactum stroma and, to a lesser extent, in LE. *CTNNB1* mRNAs then increased in LE and GE on d 14 of both the cycle and pregnancy. In cyclic ewes, there was a marked decline in *CTNNB1* mRNA on d 16 in both the LE and GE. In contrast, *CTNNB1* mRNA was abundant in uterine LE and GE of ewes between d 16 and 20 of pregnancy, as well as in trophectoderm of the conceptus on d 18 and 20 of pregnancy. For

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

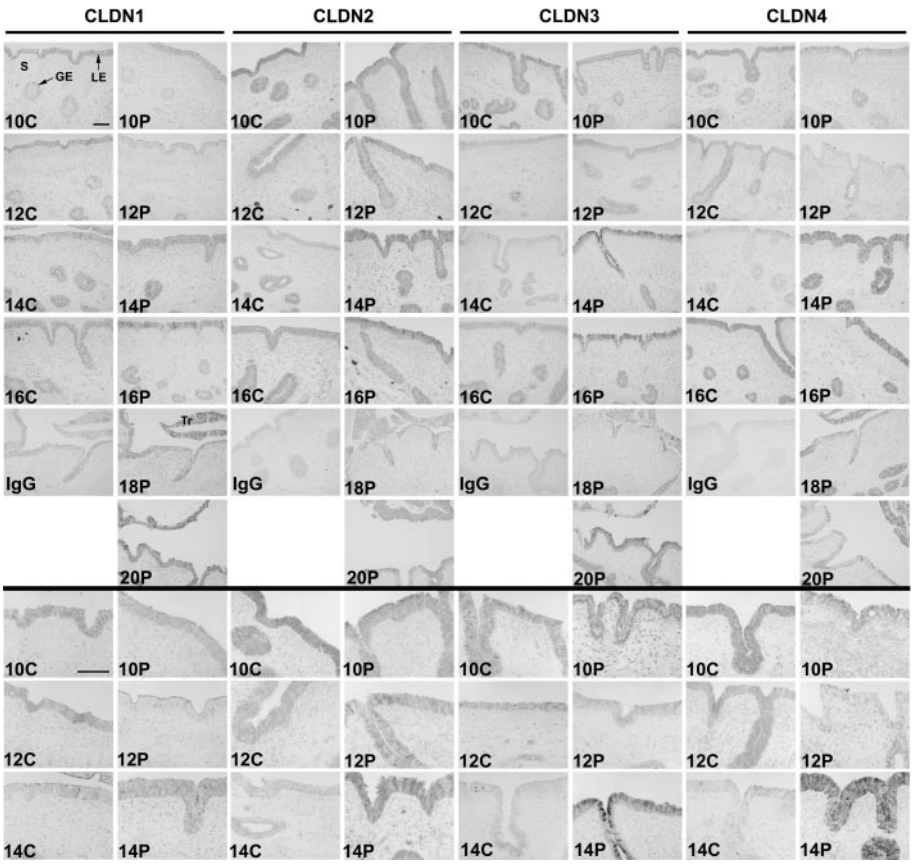


FIG. 2. Immunolocalization of CLDN1, CLDN2, CLDN3, and CLDN4 proteins in the ovine endometrium during the estrous cycle and pregnancy (study 1). Immunoreactive proteins were detected, each using specific rabbit polyclonal antibodies against the respective proteins. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. Sections in *lower panels* are from areas of *upper panels* shown at higher magnification. S, Stroma; Tr, trophectoderm. *Bar*, 10 μm.

all of the mRNAs and proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium.

### *CDH1 and CTNNB1 protein (study 1)*

Overall, levels of CDH1 protein in the endometrium paralleled changes in *CDH1* mRNA in both cyclic and pregnant ewes (Fig. 4). Immunoreactive CDH1 protein was particularly abundant in the endometrial GE of both cyclic and pregnant ewes and was located primarily at the apical region of epithelial cells. In cyclic ewes, CDH1 protein decreased in abundance from d 10–14 and then increased to d 16. Indeed, immunoreactive CDH1 protein was not detectable in the LE of d-14 cyclic ewes. In pregnant ewes, CDH1 protein in the LE also decreased after d 10, was almost undetectable on d 14 and 16, and then increased on d 18. Some of the LE in the uteri of d 18 and 20 pregnant ewes was not present due to assimilation of the LE by the trophoblast giant binucleate cells of the conceptus, which begin to differentiate on d 14–15 of pregnancy.

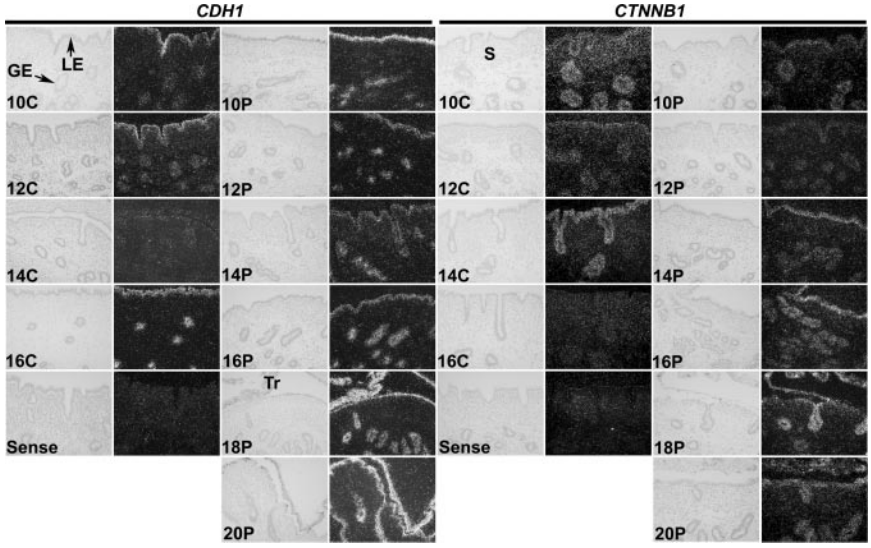


FIG. 3. *In situ* localization of *CDH1* and *CTNNB1* mRNAs in the ovine endometrium during the estrous cycle and pregnancy (study 1). Cross-sections of uteri were hybridized with radiolabeled antisense or sense ovine cRNA probes for each respective gene. The *left panel* is bright-field photomicrographs, and the *right panel* is dark-field photomicrographs. S, Stroma; Tr, trophectoderm. *Bar*, 10 μm.

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

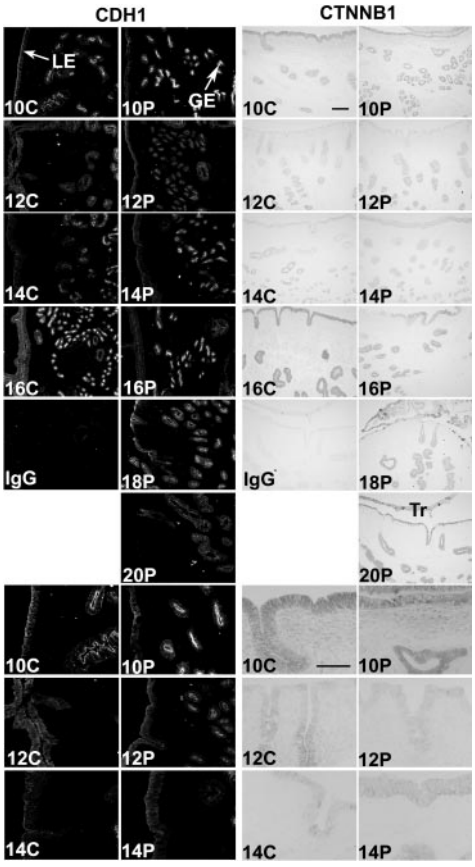


FIG. 4. Immunolocalization of CDH1 and CTNNB1 proteins in the ovine endometrium during the cycle and pregnancy (study 1). Immunoreactive proteins were detected each using specific rabbit polyclonal antibodies against the respective proteins. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. S, Stroma; Tr, trophectoderm. *Bar*, 10 $\mu$m.

Immunoreactive CTNNB1 protein was evaluated during the estrous cycle and early pregnancy by immunohistochemistry (Fig. 4). On d 10 of the cycle or pregnancy, CTNNB1 protein was observed predominantly in the LE and GE. By d 12 of both the cycle and pregnancy, there was a marked reduction in CTNNB1 protein, particularly in the LE and to a lesser extent in the GE. The pattern was maintained through d 14; however, abundant levels of CTNNB1 protein were detected in the LE and GE on d 16 of the estrous cycle. In uteri from d 16 pregnant ewes, CTNNB1 protein increased in the GE and to a lesser and more varied extent in the LE. By d 18 of pregnancy, more abundant levels of CTNNB1 protein were detected in the endometrial GE and LE. Both mononuclear and binucleate cells of the conceptus trophectoderm contained high levels of CTNNB1 protein on d 18 and 20 of pregnancy. In particular, nuclear CTNNB1 protein was observed in the trophoblast giant binucleate cells (data not shown). For all of the proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium.

*Progesterone regulation of tight-junction-associated proteins (studies 2 and 3)*

To determine whether P4 regulates tight-junction-associated proteins, ewes were treated with either vehicle or P4 beginning on d 1.5 postmating and then hysterectomized on either d 9 or 12. This treatment regimen advances development of the conceptus and induces changes in gene expression in the uterine LE (26). Immunoreactive TJP2 protein was most abundant in the LE and GE of uteri from d 9 and 12 CO-treated ewes (Fig. 5). Treatment with P4 decreased the abundance of TJP2 protein in the LE compared with CO treatment, whereas treatment with P4 and RU486 (P4 + RU) increased TJP2 protein, particularly in the GE. Similar results were found for immunoreactive OCLN protein (Fig. 5), although the P4-induced loss of OCLN protein was more pronounced in the LE and GE on d 12. Furthermore, treatment of ewes with P4 and RU486 increased OCLN protein in both the LE and GE. Immunoreactive TJP2 and OCLN proteins were most abundant at the apical portions of the epithelial cells.

CLDN1 protein was detected at low levels in the LE and GE on d 9 in both CO- and P4-treated ewes (Fig. 6). On d 12, P4-treated ewes had less CLDN1 protein compared with CO controls, whereas administration of RU486 slightly increased CLDN1 protein in both the LE and GE. CLDN2, CLDN3, and CLDN4 proteins were most abundant in uterine LE of d-9 CO-treated ewes, decreased in LE of d 12 ewes treated with P4 and were most abundant in endometrial glands of RU486-treated ewes on d 12. For all of the proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium.

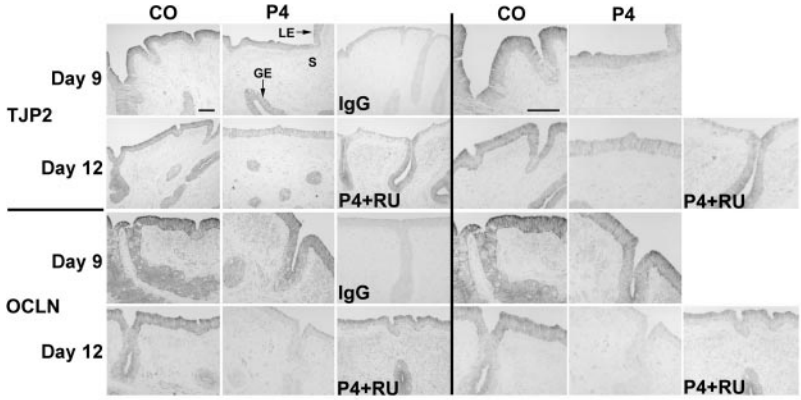


FIG. 5. Effects of CO, P4, or P4 and RU486 (P4 + RU) on TJP2 and OCLN protein in endometria from d 9 (study 2) and d 12 (study 3) ewes. Immunoreactive OCLN and TJP2 proteins were detected using specific rabbit polyclonal antibodies. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. S, Stroma. *Bar*, 10 $\mu$m.

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

*CDH1 and CTNNB1 mRNA in studies 2 and 3*

Steady-state levels of *CDH1* mRNA were not affected ($P > 0.10$) by treatment on d 9 or 12 (Fig. 7A). On d 9, endometrial CTNNB1 mRNA was lower ($P < 0.05$) in P4-treated compared with CO-treated ewes (Fig. 7B). On d 12, P4 treatment did not affect ($P > 0.10$) *CTNNB1* mRNA in the endometrium; however, there was a 2-fold increase ($P < 0.01$) in CTNNB1 mRNA in endometria of RU486-treated ewes. *In situ* hybridization analysis identified CTNNB1 mRNA in the endometrial LE, GE, and stroma of ewes (Fig. 7C). The reduction in *CTNNB1* mRNA in endometria from d 9 P4-treated ewes appeared to result from an overall decline in the LE, whereas RU486 treatment resulted in an increase in *CTNNB1* mRNA in d 12 ewes in all endometrial cell types. For all of the mRNAs and proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium.

*CDH1 and CTNNB1 protein in studies 2 and 3*

Immunoreactive CDH1 protein was less abundant in the endometrial LE of P4-treated ewes on d 9 (Fig. 8). However, CDH1 protein abundance in CO-treated compared with P4-treated ewes was not different on d 12. Interestingly, the abundance of CDH1 protein was decreased in LE of ewes treated with P4 and RU486 to d 12 after onset of estrus.

Immunoreactive CTNNB1 protein was observed predominantly in the endometrial LE and GE of both CO- and P4-treated ewes on d 9 (Fig. 8). For CO-treated ewes on d 12, immunoreactive CTNNB1 protein was observed in GE, but was not detectable in LE. However, CTNNB1 protein was very low to undetectable in GE of P4-treated ewes on d 12. In contrast, CTNNB1 protein was abundant in GE and detectable in LE of RU486-treated ewes on d 12. For all of the proteins analyzed in this study, no differences were observed in their abundance in the LE of the intercaruncular endometrium and the caruncular endometrium.

## Discussion

Domestic animals are characterized as being nonmenstruating and having conceptuses that have a noninvasive type of implantation. These two distinguishing characteristics indicate the unique importance of and requirement for elasticity of function of the uterine epithelia. The epithelia must adapt to establish the appropriate microenvironment based on the physiological status of the animal. These adaptations are acutely monitored in response to appropriately timed cues from the ovary and paracrine effectors from the adjacent stroma and conceptus. In sheep, the spherical hatched blastocyst on d 8 expands to d 10 and then begins a morphological change to a tubular conceptus by d 12 and subsequently undergoes rapid elongation into a filamentous conceptus by d 14 (33, 34). The developing ovine conceptus undergoes elongation while maintaining superficial contact with the receptive LE before firm adhesion by d 18 at which point the conceptus can no longer be easily flushed from the uterine lumen. Elongation of the conceptus trophectoderm is required for the production of sufficient quantities of interferon $\tau$ (IFNT) to elicit maternal recognition of pregnancy and inhibit development of the luteolytic mechanism (34–38). Beginning on d 14–15, trophoblast giant binucleate cells begin differentiating from the mononuclear trophectoderm

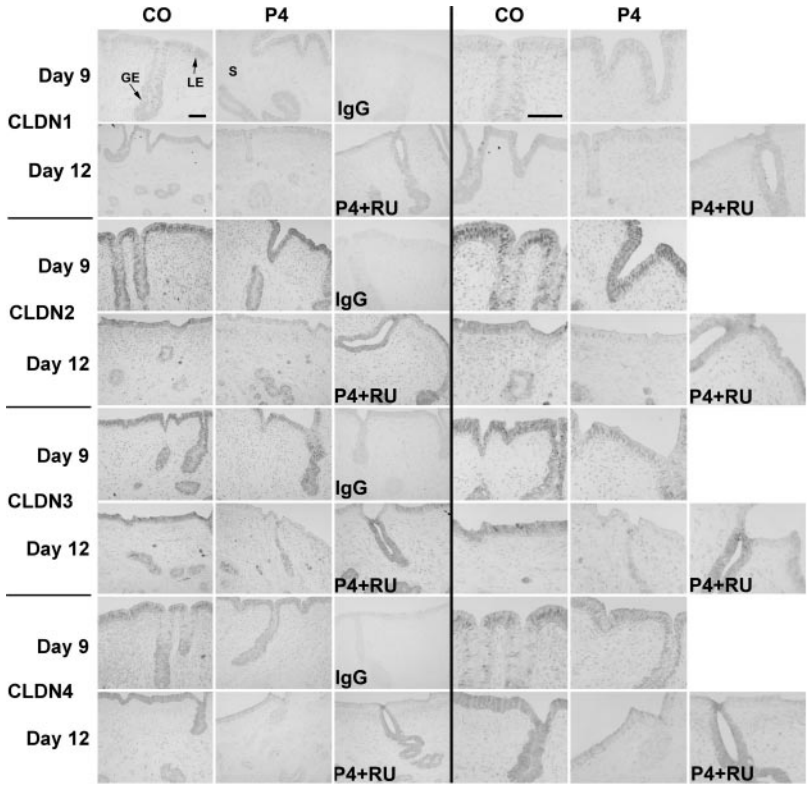


FIG. 6. Effects of corn CO, P4, or P4 and RU486 (P4 + RU) on CLDN proteins in endometria from d 9 (study 2) and d 12 (study 3) ewes. Immunoreactive CLDN 1, 2, 3, and 4 proteins were detected using specific rabbit polyclonal antibodies. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. S, Stroma. *Bar*, 10 $\mu$m.

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

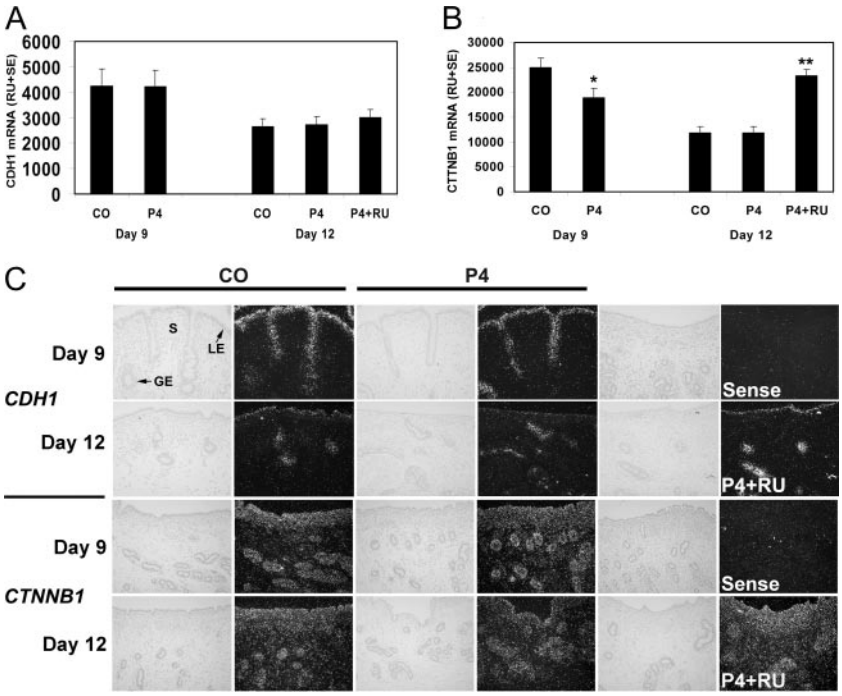


FIG. 7. Effects of CO, P4, or P4 and RU486 (P4 + RU) on *CDH1* and *CTNNB1* mRNA in endometria from d 9 (study 2) and d 12 (study 3) ewes. A and B, Steady-state levels of mRNAs were determined by slot blot hybridization analysis and are presented as relative units (RU) with SE. C, Cross-sections of uteri were hybridized with radiolabeled antisense or sense ovine cRNA probes for each respective gene. S, Stroma. *Bar*, 10 $\mu$m.

cells in the conceptus and then migrate and fuse with the endometrial LE in both the caruncular and intercaruncular areas of the uterus and with each other to form multinucleated syncytia (39). These periimplantation events begin synepitheliochorial placentation in ruminants.

Establishment and maintenance of the microenvironment within the uterine lumen to support pregnancy requires the epithelia to serve as a selective transporter and/or barrier. Junctional complexes within the epithelia maintain this unique microenvironment by regulating the passage of water, ions, and other small molecules, regulating localization of various transporters within the epithelia to maintain appropriate molecular gradients, and changing cell shape and polarity to alter cell to cell communication and function. Results from the present studies on changes in endometrial tight-junction and adherens-associated proteins provide insight into the functional requirements of the epithelia for the maintenance of pregnancy in sheep. All tight-junction- and adherens-associated proteins were moderately to abundantly present in the endometrial epithelia on d 10 of the estrous cycle and pregnancy; however, by d 12, the junctional proteins in the LE decreased to very low or undetectable levels, resulting in a potentially "leaky" paracellular space that would allow transport of molecules from the uterine stroma and/or selective movement of serum transudate directly to the conceptus. The observed decline in tight junction and adherens junction-associated proteins would theoretically decrease tight junctions and increase the availability of stromal-derived factors, such as hepatocyte growth factor, fibroblast growth factor 7, and insulin-like growth factors (IGF1 and IGF2), as well as factors from serum, such as glucose, insulin, and essential amino acids, in the uterine lumen. Indeed, many of the proteins present in the uterine lumen are not directly synthesized by the endometrial epithelia or conceptus (40). During early pregnancy, the tubular blastocyst begins to elongate on d 12 to form a filamentous conceptus, which involves the proliferation and migration of the trophectoderm. Thus, accelerated blastocyst growth may result, in fact, from the influx of factors into the uterine lumen through the paracellular space as integrity of tight and adherens junctions decreases. Indeed, blastocyst elongation and formation of a filamentous conceptus has not been achieved *in vitro*.

The changes in tight junction and adherens junction-associated proteins observed in the present study are hypothesized to be promulgated by P4. In both cyclic and pregnant ewes, P4 receptors (PGR) decline to undetectable levels in the endometrial LE and GE between d 10–12 after onset of estrus (33, 41–43). This loss of PGR is induced by exposure of the endometrium to P4 for 8–10 d. Indeed, early P4 administration accelerates the loss of PGR from the LE and GE, advances onset of expression of P4-stimulated genes such as galectin-15, and enhances blastocyst growth and development (26). In studies 2 and 3, we analyzed tight-junction-associated proteins in that model and found that early P4 stimulated a decline in tight junction and adherens junction-associated proteins in the uterine epithelia. This decline occurs in both cyclic and pregnant ewes because maternal recognition of pregnancy does not occur until d 12–13. In fact, Guillomot *et al.* (44, 45) observed that horseradish peroxidase injected into the uterine lumen of pregnant ewes and cows accumulated in the intracellular spaces beneath the basement membrane in the stroma. This transport was mediated via transepithelial endocytotic activity (vesicles) as well as paracellular permeability and passage through intercellular spaces between tight junctions. These phenomena were especially marked when circulating P4 concentrations were high during late diestrus when PGR would be absent from the endometrial LE and GE.

In addition to P4, the presence of the conceptus-affected

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

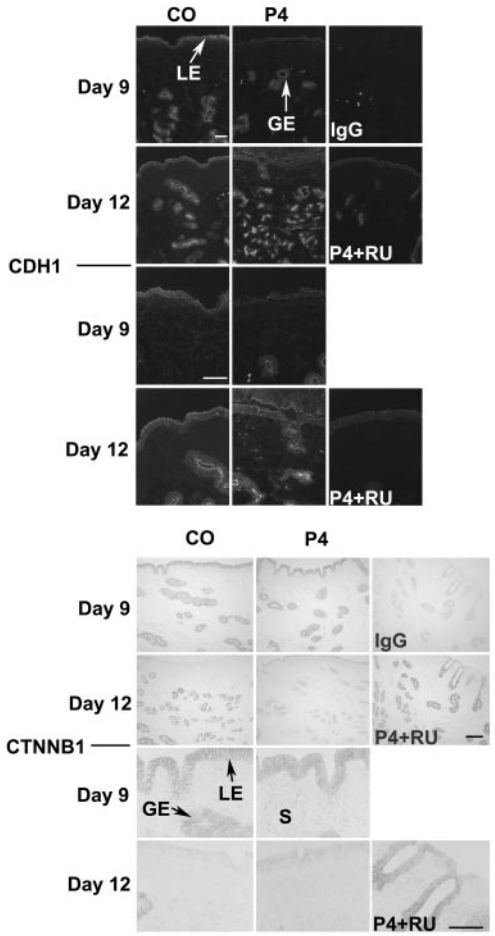


FIG. 8. Effects of CO, P4, or P4 and RU486 (P4 + RU) on CDH1 and CTNNB1 protein in endometria from d 9 (study 2) and d 12 (study 3) ewes. Immunoreactive protein was detected using specific rabbit polyclonal antibodies. For the IgG control, normal rabbit IgG was substituted for the primary antibody. Sections were not counterstained. S, Stroma. *Bar*, 10 $\mu$m.

tight-junction-associated proteins in the uterine LE. Consistently, tight-junction-associated proteins were much more abundant in the LE of d-14 pregnant ewes compared with cyclic ewes, suggesting that a conceptus-derived factor stimulates tight-junction-associated proteins within the LE. During decidualization in mice, a stromal barrier surrounding the invading blastocyst uncharacteristically expresses tight-junction-associated proteins, which are believed to maintain an immunologically privileged environment assisting in the maintenance of pregnancy (46, 47). Indeed, a similar mechanism may exist in sheep, whereby the developing conceptus signals its presence and produces a factor that stimulates tight junction formation that acts as a functional barrier to the maternal immune system. Simultaneously, reducing paracellular permeability of the LE may facilitate pregnancy recognition, allowing for sufficient quantities of IFNT to act on the LE and GE to inhibit luteolysis and induce genes encoding secreted proteins, such as galectin-15, cathepsin L, WNT7A, and cystatin C that may stimulate conceptus development and implantation. Furthermore, tight junction formation may maintain IFNT-induced or stimulated proteins at high levels in the uterine lumen. Expression of these tight-junction-associated proteins remains high to d 20. The conceptus trophectoderm also expresses high levels of tight-junction-associated proteins, which is not unexpected because a sophisticated cellular organization is required to facilitate the rapid morphological and developmental changes during this period.

In the present studies, CDH1 and CTNNB1 mRNAs and proteins were abundant in the endometrial LE on d 10 of the estrous cycle and pregnancy and then decreased in subsequent days corresponding with conceptus elongation. The abundance of *CDH1* and *CTNNB1* mRNAs increased on d 16 and 14 of pregnancy, respectively. However, the levels of CDH1 protein within the LE did not parallel that of the mRNA suggesting posttranscriptional regulation of CDH1 levels. This phenomenon, based on discordant levels of mRNA and protein, began on d 10 and continued on d 12 when CDH1 protein levels were significantly lower than mRNA levels within the LE, but not the GE. The mechanism responsible for this decline in CDH1 protein is not known, but may be physiologically important for the initiation of the implantation process. Indeed, in rats, CDH1 is lost from the epithelium before invasion of the blastocyst into the endometrial stroma. These studies indicated a role for calcitonin in both *in vitro* and *in vivo* regulation of CDH1 expression during the period of implantation (48). However in mice, the LE expresses abundant CDH1, which is highly localized to the apical domain at the implantation sites, whereas CDH1 is more diffusely localized at the inter-implantation sites (49). As the blastocyst begins to invade into the underlying stroma, these cells also uncharacteristically express CDH1 (46, 49). Similarly, CTNNB1 protein declines from d 10–14 within the LE and to a lesser extent within the GE, further supporting the decline in adherens junctions during the elongation process. In mice, inhibition of CTNNB1 signaling in both the developing blastocyst and uterine endometrium may have a role in synchronizing preimplantation embryonic development (50). A similar mechanism may exist in sheep to synchronize events associated with the elongating conceptus and endometrial functions during this critical morphological transition. Results from this study indicate that two components of adherens junctions decline during the elongation process only to increase as the conceptus initiates implantation. The decline in adherens junction proteins also corresponds with loss of the PGR from the LE and the subsequent loss of the antiadhesive glycocalyx molecule, mucin 1 (2). These events may act in concert to allow the LE to become receptive to contact by the conceptus trophectoderm enabling the elongation process to be initiated. The presence of abundant quantities of CDH1 protein and moderate levels of CTNNB1 protein within the GE throughout the estrous cycle and pregnancy results in a polarized epithelium that can more efficiently secrete uterine histotroph. Previous research using the ovine uterine gland knockout ewe model established the requirement for secretions from the ovine uterine GE for conceptus survival beyond d 12–14 (8). Increases of CDH1 and CTNNB1 proteins in the LE from d 16–20 of pregnancy may occur in response to more aggressive adhesion and attachment of the elongating ovine conceptus to restrict invasion of the trophectoderm, thus maintaining appropriate contact for placental development and

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

inhibiting an adverse immunological reaction by the maternal endometrium.

Adherens junction proteins, in a manner similar to tight junctions, return on d 16 of the cycle as the ewe returns to estrus. A highly polarized epithelium exhibiting a high degree of cell-to-cell contact may be necessary to provide a microenvironment suitable for capacitating sperm, as well as structural stability to withstand the high contractile rate of this tissue and the high degree of water imbibition occurring at estrus. Results of studies 2 and 3 illustrate a subtle, but potentially physiologically relevant discord between *CDH1* mRNA and protein. Although *CDH1* mRNAs are not regulated by administration of P4 or blockade of its action, clearly P4 treatment resulted in a decline in CDH1 protein within the LE on d 9 and a decline after RU486 administration on d 12. *CTNNB1* mRNA and protein decreased in response to P4 administration to d 9 and this may result in adherens junction breakdown and a decline in CDH1 protein as well. A decline in CDH1 and CTNNB1 within the LE on d 9 could be hypothesized to result as part of the cascade of epithelial alterations after loss of the PGR from the LE.

Collectively, results of the present study identify regulated changes within the epithelial architecture that modulate various functions of an epithelial cell layer. These organizational changes are under the control of both steroid hormones and conceptus-derived factors. Future studies will aim to identify additional constituents of the uterine microenvironment derived from nonepithelial sources via epithelial paracellular transport to enhance conceptus growth and development. Identification of critical constituents of the uterine milieu of nonepithelial origin will give further insight into the role of the underlying stromal compartments in supporting growth and survival of the conceptus and ultimately decreasing periimplantation pregnancy losses.

## Acknowledgments

We thank Mr. Kendrick LeBlanc and other members of our laboratory for assistance with animal husbandry and surgeries.

Received March 8, 2007. Accepted April 20, 2007.

Address all correspondence and requests for reprints to: Fuller W. Bazer, Center for Animal Biotechnology and Genomics, 442 Kleberg Center, 2471 TAMU, Texas A&M University, College Station, Texas 77843-2471. E-mail: fbazer@cvm.tamu.edu.

This work was supported in part by National Institutes of Health Grant 5 P30 ES09106 and National Research Initiative Competitive Grant 2005-35203-16252 from the U.S. Department of Agriculture Cooperative State Research, Education and Extension Service.

Disclosure Statement: The authors have nothing to disclose.

## References

1. **Burghardt RC, Johnson GA, Jaeger LA, Ka H, Garlow JE, Spencer TE, Bazer FW** 2002 Integrins and extracellular matrix proteins at the maternal-fetal interface in domestic animals. Cells Tissues Organs 171:202–217
2. **Johnson GA, Bazer FW, Jaeger LA, Ka H, Garlow JE, Pfarrer C, Spencer TE, Burghardt RC** 2001 Muc-1, integrin, and osteopontin expression during the implantation cascade in sheep. Biol Reprod 65:820–828
3. **Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K** 2000 Embryo implantation. Dev Biol 223:217–237
4. **Spencer TE, Johnson GA, Burghardt RC, Bazer FW** 2004 Progesterone and placental hormone actions on the uterus: insights from domestic animals. Biol Reprod 71:2–10
5. **Kirby DR, Billington WD, Bradbury S, Goldstein DJ** 1964 Antigen barrier of the mouse placenta. Nature 204:548–549
6. **Muggleton-Harris AL, Johnson MH** 1976 The nature and distribution of serologically detectable alloantigens on the preimplantation mouse embryo. J Embryol Exp Morphol 35:59–72
7. **Guillomot M, Flechon JE, Leroy F** 1993 Blastocyst development and implantation. In: Thibault C, Levasseur MC, Hunter RHF, eds. Reproduction in mammals and man. Paris: Ellipses; 387–411
8. **Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE** 2001 Endometrial glands are required for preimplantation conceptus elongation and survival. Biol Reprod 64:1608–1613
9. **Gonzalez-Mariscal L, Betanzos A, Nava P, Jaramillo BE** 2003 Tight junction proteins. Prog Biophys Mol Biol 81:1–44
10. **Tsukita S, Furuse M, Itoh M** 2001 Multifunctional strands in tight junctions. Nat Rev Mol Cell Biol 2:285–293
11. **Wittchen ES, Haskins J, Stevenson BR** 1999 Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. J Biol Chem 274:35179–35185
12. **Gonzalez-Mariscal L, Betanzos A, Avila-Flores A** 2000 MAGUK proteins: structure and role in the tight junction. Semin Cell Dev Biol 11:315–324
13. **Gonzalez-Mariscal L, Namorado MC, Martin D, Luna J, Alarcon L, Islas S, Valencia L, Muriel P, Ponce L, Reyes JL** 2000 Tight junction proteins ZO-1, ZO-2, and occludin along isolated renal tubules. Kidney Int 57:2386–2402
14. **Gonzalez-Mariscal L, Chavez de Ramirez B, Cereijido M** 1985 Tight junction formation in cultured epithelial cells (MDCK). J Membr Biol 86:113–125
15. **Gonzalez-Mariscal L, Contreras RG, Bolivar JJ, Ponce A, Chavez De Ramirez B, Cereijido M** 1990 Role of calcium in tight junction formation between epithelial cells. Am J Physiol 259:C978–986
16. **Balda MS, Gonzalez-Mariscal L, Contreras RG, Macias-Silva M, Torres-Marquez ME, Garcia-Sainz JA, Cereijido M** 1991 Assembly and sealing of tight junctions: possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. J Membr Biol 122:193–202
17. **Nguyen DA, Parlow AF, Neville MC** 2001 Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation. J Endocrinol 170:347–356
18. **McCrea PD, Turck CW, Gumbiner B** 1991 A homolog of the armadillo protein in *Drosophila* (plakoglobin) associated with E-cadherin. Science 254:1359–1361
19. **McCrea PD, Gumbiner BM** 1991 Purification of a 92-kDa cytoplasmic protein tightly associated with the cell-cell adhesion molecule E-cadherin (uvomorulin). Characterization and extractability of the protein complex from the cell cytostructure. J Biol Chem 266:4514–4520
20. **Aberle H, Butz S, Stappert J, Weissig H, Kemler R, Hoschuetzky H** 1994 Assembly of the cadherin-catenin complex in vitro with recombinant proteins. J Cell Sci 107(Pt 12):3655–3663
21. **Rimm DL, Koslov ER, Kebriaei P, Cianci CD, Morrow JS** 1995 $\alpha$1(E)-Catenin is an actin-binding and -bundling protein mediating the attachment of F-actin to the membrane adhesion complex. Proc Natl Acad Sci USA 92:8813–8817
22. **Lilien J, Balsamo J** 2005 The regulation of cadherin-mediated adhesion by tyrosine phosphorylation/dephosphorylation of $\beta$-catenin. Curr Opin Cell Biol 17:459–465
23. **Okegawa T, Li Y, Pong RC, Hsieh JT** 2002 Cell adhesion proteins as tumor suppressors. J Urol 167:1836–1843
24. **Cowin P, Rowlands TM, Hatsell SJ** 2005 Cadherins and catenins in breast cancer. Curr Opin Cell Biol 17:499–508
25. **Spencer TE, Bartol FF, Bazer FW, Johnson GA, Joyce MM** 1999 Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. Biol Reprod 60:241–250
26. **Satterfield MC, Bazer FW, Spencer TE** 2006 Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. Biol Reprod 75:289–296
27. **Spencer TE, Stagg AG, Ott TL, Johnson GA, Ramsey WS, Bazer FW** 1999 Differential effects of intrauterine and subcutaneous administration of recombinant ovine interferon $\tau$ on the endometrium of cyclic ewes. Biol Reprod 61:464–470
28. **Taylor KM, Gray CA, Joyce MM, Stewart MD, Bazer FW, Spencer TE** 2000 Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone, and prolactin. Biol Reprod 63:1192–1204
29. **Choi Y, Johnson GA, Burghardt RC, Berghman LR, Joyce MM, Taylor KM, Stewart MD, Bazer FW, Spencer TE** 2001 Interferon regulatory factor-two restricts expression of interferon-stimulated genes to the endometrial stroma and glandular epithelium of the ovine uterus. Biol Reprod 65:1038–1049
30. **Johnson GA, Stewart MD, Gray CA, Choi Y, Burghardt RC, Yu-Lee LY, Bazer FW, Spencer TE** 2001 Effects of the estrous cycle, pregnancy, and interferon $\tau$ on 2′,5′- oligoadenylate synthetase expression in the ovine uterus. Biol Reprod 64:1392–1399
31. **Spencer TE, Stagg AG, Joyce MM, Jenster G, Wood CG, Bazer FW, Wiley AA, Bartol FF** 1999 Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. Endocrinology 140:4070–4080
32. **Johnson GA, Burghardt RC, Spencer TE, Newton GR, Ott TL, Bazer FW** 1999 Ovine osteopontin: II. Osteopontin and $\alpha_v\beta_3$ integrin expression in the uterus and conceptus during the periimplantation period. Biol Reprod 61:892–899
33. **Rowson LE, Moor RM** 1966 Development of the sheep conceptus during the first fourteen days. J Anat 100:777–785

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022

34. **Spencer TE, Johnson GA, Bazer FW, Burghardt RC** 2004 Implantation mechanisms: insights from the sheep. Reproduction 128:657–668
35. **Guillomot M, Michel C, Gaye P, Charlier N, Trojan J, Martal J** 1990 Cellular localization of an embryonic interferon, ovine trophoblastin and its mRNA in sheep embryos during early pregnancy. Biol Cell 68:205–211
36. **Farin CE, Imakawa K, Roberts RM** 1989 In situ localization of mRNA for the interferon, ovine trophoblast protein-1, during early embryonic development of the sheep. Mol Endocrinol 3:1099–1107
37. **Gray CA, Burghardt RC, Johnson GA, Bazer FW, Spencer TE** 2002 Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. Reproduction 124:289–300
38. **Vallet JL, Bazer FW, Fliss MF, Thatcher WW** 1988 Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandins F-2$\alpha$ and E and of 13,14-dihydro-15-keto prostaglandin F-2$\alpha$ in cyclic ewes. J Reprod Fertil 84:493–504
39. **Wooding FB** 1984 Role of binucleate cells in fetomaternal cell fusion at implantation in the sheep. Am J Anat 170:233–250
40. **Lee RS, Wheeler TT, Peterson AJ** 1998 Large-format, two-dimensional polyacrylamide gel electrophoresis of ovine periimplantation uterine luminal fluid proteins: identification of aldose reductase, cytoplasmic actin, and transferrin as conceptus-synthesized proteins. Biol Reprod 59:743–752
41. **Spencer TE, Bazer FW** 1995 Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol Reprod 53:1527–1543
42. **Wathes DC, Lamming GE** 1995 The oxytocin receptor, luteolysis and the maintenance of pregnancy. J Reprod Fertil Suppl 49:53–67
43. **Wathes DC, Hamon M** 1993 Localization of oestradiol, progesterone and oxytocin receptors in the uterus during the oestrous cycle and early pregnancy of the ewe. J Endocrinol 138:479–492
44. **Guillomot M, Betteridge KJ, Harvey D, Goff AK** 1986 Endocytotic activity in the endometrium during conceptus attachment in the cow. J Reprod Fertil 78:27–36
45. **Guillomot M, Flechon JE, Wintenberger-Torres S** 1981 Conceptus attachment in the ewe: an ultrastructural study. Placenta 2:169–182
46. **Paria BC, Zhao X, Das SK, Dey SK, Yoshinaga K** 1999 Zonula occludens-1 and E-cadherin are coordinately expressed in the mouse uterus with the initiation of implantation and decidualization. Dev Biol 208:488–501
47. **Wang X, Matsumoto H, Zhao X, Das SK, Paria BC** 2004 Embryonic signals direct the formation of tight junctional permeability barrier in the decidualizing stroma during embryo implantation. J Cell Sci 117:53–62
48. **Li Q, Wang J, Armant DR, Bagchi MK, Bagchi IC** 2002 Calcitonin down-regulates E-cadherin expression in rodent uterine epithelium during implantation. J Biol Chem 277:46447–46455
49. **Jha RK, Titus S, Saxena D, Kumar PG, Laloraya M** 2006 Profiling of E-cadherin, $\beta$-catenin and $Ca^{2+}$ in embryo-uterine interactions at implantation. FEBS Lett 580:5653–5660
50. **Li J, Zhang JV, Cao YJ, Zhou JX, Liu WM, Fan XJ, Duan EK** 2005 Inhibition of the $\beta$-catenin signaling pathway in blastocyst and uterus during the window of implantation in mice. Biol Reprod 72:700–706

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

## Erratum

In the article "Tamoxifen-Induced Rapid Death of MCF-7 Breast Cancer Cells Is Mediated via Extracellularly Signal-Regulated Kinase Signaling and Can Be Abrogated by Estrogen" by Aiping Zheng, Anu Kallio, and Pirkko Härkönen (*Endocrinology* **148:**2764–2777), reference 85 was incomplete. The correct reference 85 is Toran-Allerand CD, Guan X, MacLusky NJ, Howarth TL, Diano S, Singh M, Connolly Jr ES, Nethrapalli IS, Tinnikov AA 2002 ER-X: a novel, plasma-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. J Neurosci 22:8391–8401. *The authors apologize for any problems this error may have caused.*

Downloaded from https://academic.oup.com/endo/article/148/8/3922/2502346 by guest on 20 August 2022