OVINE UTERINE MORPHOGENESIS: EFFECTS OF AGE AND PROGESTIN ADMINISTRATION AND WITHDRAWAL ON NEONATAL ENDOMETRIAL DEVELOPMENT AND DNA SYNTHESIS1,2


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ABSTRACT

To determine effects of age and administration and withdrawal of a synthetic progestin (P) on endometrial development and DNA synthesis, ewe lambs were ovariectomized on d 0 (birth) and assigned to one of four groups (n = three/group) that provided (by means of hemihysterectomy) the following uterine tissue types: 1) d 0 control, 2) d 13 control, 3) d 26 control, 4) d 13 after 13 d exposure to P (13P) and 5) d 26 after P exposure from d 0 to 13 (26P). Uterine tissues were processed for histology or explanted with [methyl-3H] thymidine for autoradiography. Labeling index (LI) was determined for stroma and epithelium in caruncular and intercaruncular endometrial areas and for lumenal and glandular epithelium in uteri with glands. Endometrial glands were absent on d 0, evident at d 13 and well developed by d 26. Day 0 LI was greater (P < .05) in caruncular than in intercaruncular areas, and greater in stromal than in epithelial tissues. Relationships were reversed in d 13 endometrium (day x endometrial area, 19 < .07). Caruncular stromal LI was greater on d 0 than later (P < .02), whereas intercaruncular epithelial LI was greater after d 0 (P < .05), but decreased from d 13 to 26 (P < .05). Glandular epithelial LI was higher on d 13 than on d 26 (P < .03). Administration of P inhibited endometrial gland development and suppressed d 13P intercaruncular LI (P < .05). Withdrawal of P was followed by endometrial gland development and increased (P < .01) intercaruncular epithelial LI in d 26P uteri. Ovary-independent initiation of endometrial gland development involves age- and region-specific alterations in DNA synthesis and could involve negative control.

(Key Words: Sheep, Uterus, Neonates, Morphogenesis, DNA.)

Introduction

The endometrium of the adult ewe consists of raised, aglandular caruncular and intensely glandular intercaruncular areas. Caruncular morphogenesis is a predominantly fetal event (Wiley et al., 1987), whereas endometrial glands develop during early neonatal life in both intact and hemihysterectomized, bilaterally ovariectomized lambs (Bartol et al., 1988). Proliferation of neonatal ovine endometrial glandular epithelium was suggested to be a constitutive event that occurred consequent to removal of uterine tissues from inhibitory prenatal conditions (Bartol et al., 1988). Neonatal endometrial development may be steroid-independent (Ogasawara et al., 1983; Bigsby and Cunha, 1985, 1986). However, exposure of urogenital tissues to steroids during critical periods of development can alter adult endometrial histochi- n function (Sananes et al., 1980; Brannham et al., 1985a,b; Maier et al., 1985). Such developmental alterations may contribute to reduced reproductive success in adults. Consequently, conditions necessary for normal uterine development must be defined. The present studies were designed to examine pat-
ovine neonatal endometrial development and DNA synthesis autoradiographically in bilaterally ovariectomized neonatal lambs, and to determine the effect of administration and consequences of withdrawal of a potent synthetic progestin on neonatal endometrial responses.

Materials and Methods

Animals and Tissue Collection

Newborn ewe lambs (n = 12) of mixed breeding were obtained from a local supplier within 18 h of birth (d 0) and assigned at random to one of four groups (Table 1; n = three lambs/group). All lambs were bilaterally ovariectomized on d 0. One uterine horn was removed at random from each lamb in Groups I, II and III by hemihysterectomy at the time of ovariectomy on d 0. Earlier studies showed that bilateral ovariectomy and hemihysterectomy on d 0 did not affect ovine neonatal endometrial development as assessed histologically and histochemically on d 14 (Bartol et al., 1988). Lambs in Group IV remained uterine-intact on d 0. Thus, nine uterine horns were obtained from nine lambs on d 0. Bilaterally ovariectomized, hemihysterectomized animals assigned to Groups I and II served as developmental controls. Uterine tissue remaining in situ was recovered at hysterectomy from Group I lambs on d 13 and from Group II lambs on d 26.

Groups III and IV were included in order to assess effects of chronic progestin administration and consequences of withdrawal on endometrial developmental responses described below. A commercially available progestin (P)-impregnated implant^6^, capable of continuous release of P throughout the experimental period (approximate release = 350 μg/d; E. A. Henderson, personal communication), was inserted subcutaneously in the periscapular area of all lambs assigned to Groups III and IV on d 0 and was removed on d 13 (d 13P; Table 1). Group III uterine tissue left in situ was recovered at hysterectomy on d 13P. Uterine-intact Group IV lambs were hemihysterectomized on d 13P. Consequently, two sets of three uterine horns were obtained that had been exposed to P for the first 13 d of neonatal life. Uterine tissue left in situ on d 13P in Group IV lambs was recovered at hysterectomy on d 26P, 13 d after P removal. Procedures applied to Group IV lambs permitted confirmation of d 13P responses and assessment of endometrial responses after P withdrawal (Table 1).

Collectively, these procedures provided the following uterine tissue types: 1) d 0 control, 2) d 13 control, 3) d 26 control, 4) d 13 after

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**Table 1. Experimental Procedures**

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutnatal age, d^b^</th>
<th>OvX^c^/Hemi^d^</th>
<th>Hystx^e^</th>
<th>Hystx^h^</th>
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<tr>
<td>I</td>
<td>13</td>
<td>Hystx</td>
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<td>II</td>
<td>26</td>
<td>OvX/Hemi</td>
<td>Hystx</td>
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<tr>
<td>III</td>
<td></td>
<td>OvX/Hemi +P^f^</td>
<td>Hystx-P^g^</td>
<td>Hystx</td>
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<tr>
<td>IV</td>
<td></td>
<td>OvX +P</td>
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^a^N = three lambs/group.
^b^d 0 = birth.
^c^OvX = bilateral ovariectomy.
^d^Hemi = Hemihysterectomy.
^e^Hystx = Hysterectomy.
^f^+P = insertion of progestin releasing implant (s.c.).
^g^--P = removal of progestin implant. Designated d 13P.
^h^Designated d 26P. Tissues recovered 13 d after P removal.

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^6^Compound SC-21009; 17α-acetoxy-11β-methyl-19-norpreg-3,20-dione (Norgestomet); Ceva Laboratories, Inc., Overland Park, KS.
13 d exposure to P (13P), and 5) d 26 after P exposure from d 0 to 13 (26P). Hemihysterectomy procedures allowed each animal to serve as its own developmental control.

For surgical procedures, neonatal lambs were induced to and maintained on a surgical plane of anesthesia with halothane (.5 to 1.5% in oxygen). Surgical procedures were described previously (Bartol et al., 1988). Following surgery, ambulatory lambs were returned to ewes and observed until they had nursed successfully. All lambs were treated with penicillin prophylactically at the time of surgery and for 2 d postoperatively (22,000 IU/kg). Lambs were observed daily during the period of study. All lambs were maintained with ewes under normal farm management conditions during intervals between surgeries. No surgically related complications were observed with any lambs assigned to the study.

Histology and Autoradiography

At hysterectomy, a segment (.5 to 1.0 cm) of the caudal portion of each uterine horn was fixed in 10% neutral buffered formalin (NBF). Remaining excised tissue was placed into approximately 20 ml of warm (37°C) defined medium (HDMEM; pH 7.4), consisting of Ham's F-12 and Dulbecco's Modified Eagles media (1:1, v/v), with insulin (10 µg/ml), fatty acid-free bovine serum albumin (.05%, w/v) and penicillin-streptomycin and transported to the laboratory. Tissue from each uterine horn then was minced into small pieces (2 x 3 mm), blotted quickly on sterile gauze and transferred aseptically into a tared, sterile, 25-ml Erlenmeyer flask containing 4 ml HDMEM. Explant rate did not exceed 65 mg wet tissue per milliliter of HDMEM (mean ± SE = 186 ± 8.2 mg wet uterine tissue/explant). Each explant was supplemented with 20 µCi of [methyl-3H] thymidine (5 H) TdR; 80 Ci/mmol) and incubated 4 h at 37°C in a 5% CO2 atmosphere with continuous, gentle agitation in a Dubnoff shaking incubator.

After the 4-h labeling period, HDMEM was poured off, tissue slices were washed thoroughly with 60 to 100 ml of cold, phosphate-buffered NaCl (.9% w/v; pH 7.4; PBS), transferred immediately to BNF and allowed to fix for at least 48 h prior to embedding. Both [3H] TdR-labeled and unlabeled, BNF-fixed uterine tissues were embedded in Paraplast-Plus as described previously (Wiley et al., 1987). For [3H] TdR-labeled tissues, two or three small pieces of uterine tissue were embedded in each block. Consequently, sections on each slide represented random areas of a uterine horn. Sections (5 to 6 µm) were mounted on glass slides, cleared in xylene, brought to distilled water through graded changes of ethanol and allowed to dry at 37°C. Slides of tissues from each animal and each day then were dipped in NTB-2 autoradiographic emulsion diluted 1:1 (v/v) with distilled water (dH2O), allowed to air dry in total darkness for 2 h, and placed into a light-tight microscope slide box. Complete sets of [3H] TdR-labeled uterine tissue sections, representing tissue from all day, group and animal categories, were exposed together for 14 d at 4°C. Exposure time was established following preliminary studies conducted to identify conditions necessary to obtain repeatable results. Unlabeled, NTB-2-coated tissue sections served as negative controls. Exposed slides were developed for 4 min in D-1912 diluted 1:1 (v/v) with dH2O, stopped in dH2O, fixed and stained lightly with hematoxylin prior to analysis. Procedures for in vitro [3H] TdR labeling of uterine tissues and preparation of autoradiograms were modified from those described by Bigsby and Cunha (1985).

General histological relationships and responses of neonatal endometrial tissues were assessed in BNF-fixed tissues stained with Mayer's hematoxylin and counterstained with eosin for light microscopy (Thompson, 1966). Representative cross-sections from throughout each uterine horn were examined individually.

Labeling index (LI) was determined for stromal and epithelial tissues in caruncular and intercaruncular areas of neonatal uterine tissues from which autoradiograms were prepared. Labeled cells (>5 grains/nucleus) were identified at a total magnification of 1000x. Labeling index was determined by counting at least 1,000 cells (labeled and unlabeled) for each tis-
sue type (epithelium and stroma) in each endometrial area (caruncular and intercaruncular) of each uterine horn. In all cases, LI was expressed as the proportion of the total number of cells counted that contained labeled nuclei, multiplied by 100.

Because orientation of explanted uterine tissue was difficult, cells were counted only in those sections in which caruncular and intercaruncular areas were clearly identifiable. Counts were performed on multiple tissue sections until minimum totals were reached for each tissue and area of each uterine horn. Whenever possible, epithelial counts were made on sections that were approximately midsagittal. Entire segments of caruncular epithelium (apical and lateral borders) and intercaruncular epithelium were counted. When uterine glands were present in intercaruncular areas, both lumenal and glandular epithelial cell counts were conducted. When glandular proliferation was partial (d 13, 26P), all epithelial cells were counted. When endometrial glandular proliferation was extensive (d 26), epithelial counts were confined to cells in the bottom 100 μm of the base of counted glands. Caruncular stroma was defined as that tissue lying beneath the epithelium, but within approximately 115 μm of the stromal-epithelial interface, as determined using a calibrated grid micrometer. Individual caruncular stromal cell counts were made systematically by counting 1,000 cells in three defined 115-μm² areas: one immediately adjacent to the caruncular apex and two within the caruncular matrix perpendicular to the first and on opposite sides of the caruncle. Intercaruncular stroma was defined as that tissue lying beneath the intercaruncular epithelium and above the inner circular layer of myometrial smooth muscle. When intercaruncular areas contained endometrial glands, intercaruncular stroma was defined to include mesenchymal tissue lying beneath the deepest glandular epithelium. Inter glandular mesenchymal nuclei were not counted because objective delineation of stroma and epithelium was not possible in these areas. Consequently, when endometrial glandular proliferation was extensive (d 26), intercaruncular stromal LI was not determined.

All histological observations and cell counts were made using an Olympus BH-2 microscope.

Photomicrographs were prepared using an Olympus AH-2, Vanox-S photomicroscope and Panatomic-X black and white film.

Statistical Analyses

All LI data were subjected to ANOVA using GLM procedures available from SAS (1985). Within-day analyses (d 0, 13, 26, 13P and 26P) considered variation due to endometrial area (caruncular and intercaruncular), tissue (epithelium and stroma) and their interactions. Effects of stage of development were examined using a statistical model that considered variation due to day (d 0, 13 and 26), animal within day (A(d)), endometrial area, tissue and interactions. Error terms for tests of significance of all main effects and interactions included the random effect A(d) according to the expectation of the mean squares. For each area and tissue, orthogonal day contrasts (d 0 vs d 13 and d 26; d 13 vs d 26) were performed. Effects of P on endometrial development were examined directly by comparing histological and LI responses of d 13 (Group I) to d 13P (Group III) tissues. Analysis of variance for these data considered variation due to treatment (d 13 vs d 13P), animal within treatment (A(T)), endometrial area, tissue and interactions. Error terms for tests of significance of main effects and their interactions included the random effect A(T) according to the expectation of the mean squares. Effects of P withdrawal on endometrial LI responses were examined by analyzing Group IV data alone. Analysis of variance considered variation due to day (d 13P vs d 26P), endometrial area, tissue and interactions. Individual endometrial area tissue responses were compared between Groups III and IV for d 13P in order to assess effects of day of first hemihysterectomy.

Results

Representative photomicrographs of endometrial tissues from d 0, 13 and 26 are shown in Figure 1, together with histograms depicting LI responses for caruncular and intercaruncular epithelium and stroma. Histograms depicting LI responses of d 13 and 26 intercaruncular lumenal and glandular epithelium are shown in Figure 2. Data in figures and text are presented as least squares means (± SE).

Endometrium from d 0 contained prominent, aglandular caruncles supported on narrow stalks of stromal tissue, separated by aglandular...
intercaruncular areas. Simple cuboidal or columnar epithelium covered both caruncular and intercaruncular areas (Figure 1A). Overall, LI was greater \((P < .05)\) in caruncular than in intercaruncular areas of d 0 uteri \((LI: 11.6 \pm 1.4\% \text{ vs } 6.9 \pm 1.6\% ; \text{Figure 1B})\). Moreover, d 0 stromal LI exceeded epithelial LI \((P < .05)\) regardless of endometrial area \((LI: 11.3 \pm 1.4\% \text{ vs } 6.8 \pm 1.6\% ; \text{Figure 1B})\).

In contrast to d 0 endometrium, intercaruncular endometrial areas of all d 13 lambs contained distinct, simple, coiled tubular glands that had developed approximately one third of the distance to the inner circular layer of the myometrium (Figure 1C). Also, unlike d 0 responses, d 13 LI was lower \((P < .07)\) in caruncular than in intercaruncular areas \((LI: 5.1 \pm 3.0\% \text{ vs } 13.2 \pm 2.6\% )\), and a day \((0 \text{ and } 13) \times \text{ endometrial area (caruncular and intercaruncular)}\) interaction was detected \((P < .05)\). Differences in LI between d 13 endometrial areas were largely attributable to the high epithelial LI of newly

Figure 1. Representative photomicrographs of neonatal uterine tissues in cross-section (left; hematoxylin and eosin) and corresponding labeling index responses (right; least squares means + SE) for endometrial caruncular (CR) and intercaruncular (IC) epithelium (EP) and stroma (ST) on d 0 (A and B), 13 (C and D) and 26 (E and F).

Day 0: (A; \(\times 25\)) Note absence of uterine glands in IC area and prominent CR. (B) LI: CR > IC \((P < .05)\); ST > EP \((P < .05)\). Day 13: (C; \(\times 25\)) Note uterine glands in IC area. (D) LI: CR < IC \((P < .05)\); IC-EP* mean includes both lumenal and glandular data (see Figure 2). Day 26: (E; \(\times 25\)) Note extensively coiled uterine glands in IC area. (F) LI: IC-EP* mean includes both lumenal and glandular data (see Figure 2). ND = not determined (see text). Effects of day on individual LI responses for each endometrial area (CR and IC), by tissue (EP and ST), are illustrated by comparison of individual bars in vertical columns by day (B, D and F). Bars in vertical columns with different letters \((a, b, c)\) differ \((P < .05)\) according to results of orthogonal day contrasts \((d 0 \text{ vs } 13 \text{ and } 26; \text{d } 13 \text{ vs } 26)\).
formed endometrial glands. Intercaruncular glandular epithelial LI was over three times greater ($P < .001$) than lumenal epithelial LI in d 13 tissues (Figure 2).

In uterine tissues obtained from hemihysterectomized, bilaterally ovariectomized lambs on d 26, intercaruncular endometrial glandular development was extensive and advanced compared with that seen in d 13 tissues (Figure 1C, E). Simple, coiled, tubular endometrial glands extended to the myometrium throughout intercaruncular areas. Because of the extensive nature of intercaruncular endometrial glandular development in d 26 uteri, delineation between glandular and stromal cell nuclei in intercaruncular areas was not possible. Consequently, intercaruncular stromal LI data were not obtained from these tissues. Caruncular stromal and epithelial LI did not differ in d 26 lambs (Figure 1F). However, as observed for d 13 tissues, LI in d 26 intercaruncular glandular epithelium (17.4 ± 1.0%) was greater ($P < .001$) than that found for intercaruncular (4.9 ± 1.0%) and caruncular (6.7 ± 1.0%) lumenal epithelium (Figure 2).

Orthogonal contrasts of day responses for each tissue (epithelium and stroma) in each area (caruncular and intercaruncular) revealed developmentally related changes in endometrial LI (Figure 1B,D and F). Caruncular epithelial LI was not affected by stage of development (7.8 ± 1.4%). However, caruncular stromal LI was greater on d 0 than later (d 13 and 26; $P < .02$). In contrast, intercaruncular epithelial LI was lowest in tissues from d 0 ($P < .02$) and was higher in d 13 than in d 26 tissues ($P < .05$; Figure 1B,D,F). Because intercaruncular areas of uteri from d 13 and 26 contained both lumenal and glandular epithelium, epithelial data from this area were analyzed separately. A day × intercaruncular area (lumenal and glandular) interaction ($P < .03$) indicated that patterns of lumenal and glandular epithelial DNA synthesis differed between d 13 and d 26 tissues (Figure 2A,B). The developmentally related reduction in intercaruncular epithelial LI was pronounced for glandular epithelium in d 26 tissues (Figure 2). Intercaruncular stromal LI did not differ between d 0 and d 13 (LI: 9.2 ± 1.4%; Figure 1B,D).

Effects of administration and consequences of withdrawal of P on ovine neonatal endometrial histology are illustrated in Figure 3. Chronic administration of P to neonatal lambs inhibited formation of endometrial glands, which

**Figure 2.** Intercaruncular endometrial lumenal and glandular epithelial (EP) labeling index (LI) responses (least squares means ± SE) for neonatal uterine tissues obtained on d 13 and 26 (d 0 = birth). Overall: d 13 > d 26 ($P < .03$); d × EP ($P < .03$). For each day: lumenal < glandular ($P < .001$).
were uniformly absent from intercaruncular areas of d 13P uteri (Figure 3A). Histologically, d 13P endometrium most nearly resembled d 0 uterine tissue (Figure 3A vs Figure 1A). In some instances, however, intercaruncular epithelial cells appeared crowded and the intercaruncular epithelial surface uneven, suggestive of some degree of morphogenetic change in this area of d 13P uteri. Endometrial response to P in Group III d 13P tissues was histologically indistinguishable from that of Group IV d 13P tissues.

Comparisons of individual tissue LI responses for each endometrial area between Group III and IV d 13P uteri indicated that tissue LI was not affected by day of initial hemihysterectomy (Table 1). Effects of P on d 13 endometrial LI are illustrated in Figure 4. Overall endometrial LI was lower (P < .10) in d 13P compared with d 13 uteri (LI: 7.6 ± .50% vs 9.2 ± .62%). However, responses of d 13P tissues were not uniform throughout the endometrium. A treatment x endometrial area interaction (P < .01) indicated that LI relationships differed between caruncular and intercaruncular areas in d 13 and 13P uteri. Although caruncular LI were similar between d 13 and 13P groups, P-induced suppression of endometrial LI was pronounced in intercaruncular areas of d 13P uteri (Figure 4).

Comparison of Figure 3B (d 26P) with Figure 3A (d 13P) illustrates changes in endometrial histology seen following withdrawal of P in Group IV lambs. Endometrial glands, or gland-like structures, and lumenal epithelial corruga-

![Figure 4](image)

Figure 4. Least squares means (+ SE) of labeling index (LI) responses for caruncular (CR) and intercaruncular (IC) epithelial (EP) and stromal (ST) endometrial tissues from untreated (d 13) and progesterone (P)-treated (d 13P) lambs. Overall: d 13P < d 13 (P < .10); Treatment x endometrial area (CR and IC; P < .01). Day 13P IC < d 13 IC (P < .04).

Overall, caruncular and intercaruncular LI did not differ between d 13P and d 26P tissues from Group IV (Figure 5A). Consistent with other post-d 0 observations, however, d 26P caruncular LI was less than intercaruncular LI (P < .10; 8.4 ± 2.7% vs 14.8 ± 2.4%), and epithelial LI was greater than stromal LI (P < .03; 15.9 ± 2.4% vs 7.2 ± 2.7%). Because, in

![Figure 5](image)

Figure 5. Least squares means (+ SE) of labeling index (LI) responses for uterine caruncular (CR) and intercaruncular (IC) epithelial (EP) and stromal (ST) tissues illustrating effects of progesterone (P) withdrawal after 13 d of exposure (d 13P) on d 26P endometrial LI. (A) Individual endometrial area responses (CR and IC) by tissue (EP and ST). No overall effect of P withdrawal on d 26P LI. Mean for d 26P IC-EP includes both lumenal and glandular EP data. (B) Intercaruncular lumenal (L) and glandular (G) epithelial LI responses for d 13P (NG = no glands developed) and d 26P (G > L, P < .01).
contrast to d 13P uteri, intercaruncular areas of d 26P tissues contained developing endometrial glands, epithelial LI data from this area were analyzed separately. Relationships between d 26P intercaruncular luminal and glandular epithelial LI data are illustrated, relative to d 13P intercaruncular epithelial LI data, in Figure 5B. Intercaruncular luminal epithelial LI did not differ between d 13P and d 26P. However, d 26P intercaruncular glandular epithelial LI was approximately twice that of luminal epithelium (P < .01). Thus, initiation of intercaruncular endometrial epithelial morphogenesis, following withdrawal of P on d 13P, was accompanied by a substantial increase in DNA synthesis by glandular, but not luminal, epithelial cells in d 26P uteri (Figure 5B).

Representative autoradiographs depicting typical labeling patterns in intercaruncular areas of d 13, 26 and 26P uteri, in which uterine glands formed, are presented in Figure 6. No attempt was made to quantify labeling intensity. Greater density of silver grains can be due to increased cell density in a section, but it can also indicate areas of increased DNA synthetic activity. However, relationships depicted here support the idea that proliferation of endometrial glands requires an increase in both the proportion of epithelial cells synthesizing DNA (LI) and an increased rate of DNA synthesis by glandular epithelial cells.

Discussion

Histological relationships reported here for d 0 and 13 uteri were essentially identical to those reported earlier (Bartol et al., 1988) and support the ovary-independent nature of this period of ovine endometrial development. Glandular development was more extensive in d 26 than in d 13 uteri, and resembled that reported for intact lambs of the same age (Wiley et al., 1987). Thus, endometrial gland proliferation can proceed to d 26 without ovarian support. However, the possibility that ovarian factors may begin to affect uterine development after wk 2 of postnatal life cannot be eliminated based on present data. Although ovariecotomy of neonatal lambs on or before d 4 did not affect uterine wet weights 14 d later (Foster et al., 1972), d 44 uterine wet weights were reduced in neonatal lambs castrated on d 5 (Liefer et al., 1972). Thus, ovarian factors do affect ovine uterine growth within the first 50 d of postnatal life.

Endometrium of the newborn (d 0) ewe lamb is histoarchitecturally and developmentally unique relative to later neonatal stages studied. Uterine glands were absent on d 0 and, in contrast to later stages, overall caruncular and stromal LI were greater than intercaruncular and epithelial LI (Figure 1). Although caruncles first appear and develop during the fetal period (Wiley et al., 1987), present data support the idea that caruncular morphogenesis continues and may be completed during the early postnatal period. Data can be interpreted to suggest that normal perinatal conditions support or stimulate neonatal caruncular endometrial hyperplasia preferentially, and that this hyper-
plastic growth is largely attributable to proliferation of endometrial stromal cells.

A general, development-related, regional transition in endometrial DNA synthesis, from caruncular and stromal predominance on d 0 to intercaruncular and epithelial predominance on d 13, was associated with appearance of uterine glands (Figure 1). This developmental transition involved a significant, site-specific increase in LI and an apparent increase in DNA synthetic activity in epithelium in the distal regions of newly formed uterine glands (Figures 1, 2 and 6A). Thus, intercaruncular areas, morphogenetically quiescent at birth, support intense epithelial mitotic activity with onset of gland formation, noted to begin prior to d 10 (Wiley et al., 1987). Data are consistent with earlier observations that showed epithelial-mesenchymal interface (EMI) alcianophilia (at .3 M MgC12) in ovine uterine tissues to be uniform at birth but attenuated or lost in distal regions of developing uterine glands on d 14 (Bartol et al., 1988). These relationships support the idea that mesenchymal degradation of EMI proteoglycans and glycosaminoglycan turnover are necessary to support intense, localized epithelial mitotic activity in morphogenetically active regions of the developing ovine endometrium (Bernfield et al., 1984; Spooner and Thompson-Pletscher, 1986).

Although glandular development was more extensive in d 26 than d 13 uteri (Figure 1C,E), the decreased LI and apparent reduction in labeling intensity in d 26 intercaruncular glandular epithelium (Figure 1D,F; Figure 6A,B) suggests a general reduction in overall uterine glandular morphogenetic activity at this stage. Studies of neonatal mice (Ogasawara et al., 1983) and rats (Branham et al., 1985a,b) indicate that rapid proliferation of uterine glands during a defined period of infancy was followed by a quiescent period that lasted only until puberty in intact animals, but persisted in ovariec tomized animals. A decline in both whole uterine [125I]iodo-2'-deoxyuridine uptake and epithelial LI was associated with the end of the ovary- and adrenal-independent, autonomous phase of epithelial proliferation in neonatal mice (Ogasawara et al., 1983). Decreased DNA synthesis in d 26 glandular epithelium might, therefore, signal the end of ovary-independent epithelial proliferation in the neonatal ovine uterus.

Steroid-induced alterations of neonatal endometrial development can have adverse effects on adult reproductive performance. Although studies of this type have focused primarily on effects of estrogens (Sheehan et al., 1981; Branham et al., 1985a,b), administration of progestrone to neonatal rats between d 3 and 9, prior to onset of epithelial proliferation, reduced or abolished the capacity of adult uteri to form deciduoma (Sananes et al., 1980). Present data indicate that the ovine endometrium is susceptible to developmental modification by exposure to P between birth and d 13. Endometrial gland formation was inhibited, and intercaruncular LI was suppressed, by chronic administration of P from d 0 to d 13P in neonatal lambs. Data reflect responses of tissues to a potent 19-norprogesterone with no known estrogenic activity (E. A. Henderson, personal communication). Although glands were absent from d 13P uteri, intercaruncular lumenal epithelial surfaces were more irregular than those seen for d 0 uteri and contained occasional undulations, histologically similar to epithelial corrugations described for ovariec tomized mice treated chronically with progestin (Martin et al., 1970). Uterine epithelial corrugation was suggested to be a specific morphogenic response to chronic administration of natural and synthetic progestins (Martin et al., 1970).

The ability of progestins to inhibit uterine epithelial proliferation is well established (Martin, 1980; Bigsby and Cunha, 1985). However, effects of P on suppression of LI in d 13P uteri, although pronounced for intercaruncular epithelium, were seen in both stromal and epithelial components of this endometrial area. Consequently, effects of P were not tissue-specific (epithelial vs stromal), but were area-specific. The mechanism of this suppression cannot be determined at present. Data relative to the distribution of steroid receptors in neonatal ovine endometrium are lacking. Although these tissues were shown to be estrogen-sensitive (Lieber et al., 1972), steroid requirements for neonatal ovine endometrial development and epithelial proliferation (Bigsby and Cunha, 1985) have not been defined.

Presence of glands, or gland-like epithelial specializations, in intercaruncular areas of d 26P uteri, 13 d after P withdrawal, indicated that epithelial proliferation could occur upon removal of tissues from the influence of a steroid inhibitor. Remodeling of the intercaruncular epithelial surface following P withdrawal involved both an increase in the proportion of epithelial cells synthesizing DNA and an
apparent increase in rate of DNA synthesis by epithelium in newly formed glands and clefts. Patterns of change in intercaruncular endometrial LI from d 13P to d 26P were similar to those identified to occur between d 0 and 13 in untreated tissues. Thus, localized increases in endometrial DNA synthetic activity appear to be necessary for intercaruncular epithelial morphogenesis. Sugimura et al. (1986) observed that regional heterogeneity of DNA synthesis, similar to that observed here, was a characteristic feature of morphogenesis in a number of developing epitheliomesenchymal organs and supported the idea that regulation of these events involved local rather than systemic control.

Collectively, data support the idea that initiation of ovine endometrial glandular morphogenesis could involve local negative control. This hypothesis suggests that proliferation is a constitutive property of cells, and that cell proliferation is controlled by locally produced, cell-type specific inhibitory molecules. Therefore, cells would proliferate only when local concentrations of inhibitor(s) were lowered (Soto and Sonnenschein, 1987). The possibility that prenatal endocrine conditions or postnatal administration of P support production of such inhibitors, and that removal of tissues from these conditions ultimately reduces inhibitor levels and permits site-specific epithelial proliferation, warrants investigation.

**Literature Cited**


