HISTOGENESIS OF THE OVINE UTERUS

A. A. Wiley, F. F. Bartol and D. H. Barron

Auburn University
Auburn, AL 36849

University of Florida
Gainesville 32610

ABSTRACT

Reproductive tracts from fetal (d 55 to 150) and neonatal (d 9 to 3 mo) lambs were examined by light microscopy in order to describe ovine uterine histogenesis. Morphologically, d-55 and -60 fetal uteri were bipartite, though characteristic external cornual morphology was not apparent until d 100 to 110. Histologically, lumina of d-55 and -60 fetal uteri were tubular and smooth with no indication of caruncular or glandular areas. In d-90 to -100 fetal uteri, luminal clefts were visible along the mucosal surface and a band of eosinophilic, elongated, smooth muscle-like cells was distinguishable. By d 100 to 110, nodular and internodular areas were clearly defined as luminal clefts deepened. Expansion of deep internodular areas, parallel to the luminal nodular surface, progressed in fetal uteri from d 118 to 150. Slight invaginations along deep internodular areas were visible in d-135 fetal uteri and were regular features of d-150 fetal uteri. In contrast, shallow, slightly coiled simple tubular glands were visible in the endometrium of internodular (intercaruncular) areas in a d-9 neonatal uterus. Older neonatal uteri (d 26 to 3 mo) contained coiled tubular glands which extended to the myometrium. Data indicate a systematic pattern of ovine uterine histogenesis involving dynamic morphogenetic alterations, especially between d 80 to 130 of fetal life. The observation that uterine glands were absent in fetal uteri but present in neonatal uteri suggests that glandular induction must occur during the periparturient/neonatal period.

(Key Words: Sheep, Uterus, Histology, Fetus, Embryonic Development, Neonates.)

Introduction

The uterus of the adult ewe has been described histologically during various reproductive phases, including the estrous cycle (Casida and McKenzie, 1932; Cole and Miller, 1935; McKenzie and Terrill, 1937; Hadek, 1958; Cloud and Casida, 1969; Brinsfield et al., 1974) and pregnancy (Wimsatt, 1950; Ryle, 1962; Brinsfield et al., 1974). Ovine fetal and neonatal uterine development have not been investigated as thoroughly. Bryden (1969) described para- mesonephric duct development through d 34 in the ovine embryo. Kennedy et al. (1974) described the neonatal lamb uterus between birth and 33 wk. Information pertaining to histogenesis of ovine uterine tissues during the fetal period between d 55 and birth is entirely lacking.

Events responsible for organization of the fetal ovine uterus could affect adult uterine function. Additionally, the dichotomous nature of the adult ovine endometrium, consisting of both aglandular (caruncular) and glandular (intercaruncular) areas (Mossman, 1980), provides a model for study of mechanisms underlying establishment of divergent structural and functional areas within a single, mesodermally derived organ. Since baseline data are needed, the objective of this study was to describe ovine uterine histogenesis during fetal and neonatal development, with particular emphasis on development and differentiation of caruncular and intercaruncular endometrial areas.

Materials and Methods

Uteri were collected from 29 ovine fetuses between d 55 and 150 of gestation. Twenty- two uteri were obtained following hysterectomy of time-mated pregnant crossbred ewes (estrus/mating = d 0), while seven were obtained from fetuses aged by crown-rump measurement (Barcroft, 1946). Additionally, uteri were...
obtained from six lambs between d 9 and 3 mo: two were taken at hysterectomy and four were collected from normal lambs immediately postmortem. All uteri were fixed immediately in neutral, buffered 10% formalin for at least 48 h before further processing for light microscopy. Tissue was taken from one uterine horn of each fixed uterus, dehydrated in graded changes of ethanol (80 to 100%) and embedded in paraplast. Sequential cross-sections of 3 to 7 μm were obtained, mounted and stained with either Mayer's or Harris' Hematoxylin and counterstained with eosin (H&E). Selected sections were also stained with Masson's Trichrome (Thompson, 1966). Micrometry was accomplished using a calibrated ocular micrometer. Photomicrographs were obtained using a Zeiss Photomicroscope, an Olympus BH Photomicroscope and Panatomic-X black and white film.

**Results**

Uterine horns of d-55 and -60 fetal sheep were slightly curved and fused caudally, forming a common uterine body as confirmed histologically. Further light microscopic examination showed the lumina to be tubular and, therefore, without irregularities suggestive of caruncular or glandular areas (figure 1A, B). The mucosal surface consisted of a simple columnar epithelium, apparent in all uteri examined (figure 1B). Inter- and intra-cellular vacuoles were observed occasionally in the epithelium at this stage of development, as well as in d-90 fetal uteri (figure 1A). Two subepithelial cell layers were visible within the mesenchyme in uteri from d 55 and -60 fetal sheep. Cells just beneath the mucosal surface were packed densely and contained differentially rounded to elongated nuclei (inner layer). Below this layer, closer to the serosal surface, was a layer of loosely packed cells with elongated nuclei. All subepithelial mesenchymal cells were spindle shaped with little cytoplasm and prominent nuclei. Mesenchymal differentiation into endometrial stroma and myometrial layers was not apparent in uteri from d 55 and 60.

Day-90 to -100 fetal uteri had curved uterine horns characteristic of the adult uterus. Histological examination revealed slight, symmetric luminal clefts, involving several adjacent cells, located at regular intervals along the uterine mucosal surface (figure 1C). Clefts deepened in d-100 uteri such that areas of epithelium and underlying mesenchyme protruded into the uterine lumen (figure 1D). These early morphogenetic events established clearly defined nodular (mesenchymal-epithelial protrusions) and internodular areas (deepening luminal clefts). The inner mesenchymal cell layer of uteri from this stage (d 90 to 100) was stratified into three sublayers (figure 1C). Cells in the immediate subepithelial area were most densely packed and contained rounded nuclei. Beneath this dense layer was a more loosely arranged intermediate layer of cells with elongated nuclei. A band of distinctly eosinophilic cells was visible beneath the intermediate loose layer of mesenchymal cells. These cells contained elongated nuclei and were arranged in a circular pattern parallel to the uterine lumen. This cell layer likely represents differentiating smooth muscle cells destined to develop into the inner circular layer of the myometrium. Definitive evidence for presence of smooth muscle would require electron microscopic and immunocytochemical investigations. Between this eosinophilic band and the serosa, the outer mesenchymal layer contained loosely packed cells with elongated nuclei. Vascular elements were observed in all subepithelial areas.

Luminal clefts were more prominent by d 100 to 115, making nodular and internodular areas clearly distinguishable (figure 1D). Associated with the basal aspect of the columnar epithelium of both nodular and internodular areas were cells with rounded, intensely basophilic nuclei and clear cytoplasm, measuring 8 to 10 μm in diameter. Such histological characteristics suggest that these spherical cells are lymphocytes, although this was not confirmed histochemically. In addition to those cells described for d-90 to -100 fetal uteri, red blood cells were seen regularly at the mesenchymal-epithelial interface.

From d 118 through 150 the deepest portion of the internodular areas expanded parallel with the nodular surface (figures 1E and 2A). Slight epithelial undulations, confined to the deep internodular mucosal surface, were seen by d 135. These undulations were never more than one or two cells deep, nor did they involve more than two or three mucosal epithelial cells (figure 2A, B). Cellular relationships within the...
Figure 1A. Photomicrograph of a d-55 fetal ovine uterus in cross-section showing both uterine horns. Note smooth uterine mucosal surface, columnar epithelial cells and inter/intracellular vacuole (arrow). Mesenchymal cells are arranged into inner (I) and outer (O) layers. (×140; Masson’s trichrome).

Figure 1B. Photomicrograph of a d-55 fetal ovine uterus. Detail of uterine epithelium and inner mesenchymal cell layer. (×450; H&E).

Figure 1C. Photomicrograph of a d-90 fetal ovine uterus in cross-section. Note luminal cleft (arrow), the first sign of ovine endometrial remodeling. Mesenchyme is arranged into three sublayers including a dense subepithelial layer (DL), an intermediate loose layer (IL) and a band of eosinophilic differentiating smooth muscle cells (SM). Perimetrial outer mesenchymal layer is indicated (O). (×350, H&E).

Figure 1D. Photomicrograph of a d-100 (crown-rump length) fetal ovine uterus in cross-section. Note prominent luminal cleft (arrow), well developed nodules (N) and differentiating smooth muscle cells (SM). (×140, H&E).

Figure 1E. Photomicrograph of a d-123 fetal ovine uterus in cross-section. Note expanded internodular areas (arrows) and well vascularized (BV) nodules (N) with loosely arranged mesenchyme. Differentiating smooth muscle layer denoted (SM). (×140, H&E).

Discussion

This paper describes, for the first time, histogenesis of the fetal ovine uterus, especially during the period associated with early nodular (caruncular) and internodular endometrial differentiation and development, and adds to the observations of Kennedy et al. (1974) relative to neonatal ovine uterine development.

The embryonic uterus or paramesonephric duct is first seen on d 24 in the sheep (Bryden, 1969). Fusion of paramesonephric ducts, the event responsible for species specific uterine morphology (Davies, 1967), is partial in sheep and occurs in the fetus between d 34, a stage at which Bryden (1969) observed separate ducts, and d 55 to 60, a stage in the present study in which caudally fused separate uterine horns were observed. Histologic examination of the fused portion of the uterus revealed a single uterine lumen lined by a simple columnar epithelium. Thus, characteristic ovine bipartite uterine morphology is attained between d 34 and 55 of fetal life.

The fetal mesenchyme, which becomes the endometrium and myometrium of the adult, has already begun to differentiate in the d-55 fetal ovine uterus. At this stage two distinct cell layers were apparent, the outer layer destined to become the myometrium. Uterine mesenchymal differentiation is also initiated during fetal life in other species, including the cow (Atkinson et al., 1984), horse (Ginther, 1979), guinea pig (Gulino et al., 1984) and human (Davies, 1967; O’Rahilly, 1977; Konishi et al., 1984). Like the sheep, these mammals also have a relatively long gestation period and produce young that are comparatively mature at birth. In animals such as the rat, hamster and mouse, that have a short gestation period and produce young that are comparatively immature at birth, endometrial and myometrial differentiation proceeds during the neonatal period (Davies et al., 1985) but still follows a pattern similar to that described for fetal sheep.

In some species, restructuring of the endometrium during either fetal or early neonatal development results in formation of fixed mucosal surface structures that persist as characteristic features of adult uteri. These features include raised areas of aglandular endometrium (caruncles) found in both sheep and cows and longitudinal ridges (folds) of glandular endometrium found in both horses.
and pigs (Mossman, 1980). Yamauchi (1964) observed that caruncles first appeared during the fourth month of fetal life in cattle. A subsequent study by Atkinson et al. (1984) substantiated the findings of Yamauchi (1964) and showed that only the most apical portion of the raised endometrium or nodule became the aglandular caruncle. In the present study, alterations in mucosal surface topography were first observed in d-90 fetal ovine uteri as slight epithelial invaginations (luminal clefts). These clefts became more prominent as development proceeded, due in part to nodular development on either side of the clefts. Histologically, d-118 fetal ovine uteri, with expanded internodular areas and well-developed nodules, resembled the d-169 fetal bovine uterus (Atkinson et al., 1984). Proliferation of uterine glands along the sides of nodules in ovine neonatal (d 26) uterine tissues suggests that, like the cow (Atkinson et al., 1984), only the more apical portion of the fetal ovine nodule becomes the adult aglandular caruncle.

Uterine glands, a characteristic feature of all adult mammalian uteri, are notably absent for much of fetal development in most mammals. In the present study, slight mucosal epithelial invaginations were observed in internodular areas of d 135 to 150 fetal ovine uteri. Hence, no uterine glandular development, as defined by significant invasion of subepithelial mesenchyme, was noted in uteri at any stage in ovine fetal development described in this study. However, shallow uterine glands were clearly visible in the deep intercaruncular areas of the d-9 neonatal ovine uterus. Uterine glandular development proceeds rapidly, such that by d 26 in the neonatal lamb, numerous coiled glands extend to the myometrium, a condition similar to that found in adult ovine uteri. Neonatal uteri obtained between d 26 and 3 mo contained similarly well-developed glands with no evidence of glandular involution. Present observations agree with those of Kennedy et al. (1974), who found extensive glandular development in uteri from 4-wk-old lambs that was maintained in 33-wk-old lambs but was absent in newborn lambs.

In contrast to the circumstances in sheep, formation of uterine glands was initiated during the last month of fetal development in the cow, as indicated by the appearance of short epithelial invaginations in the d-250 fetal bovine uterus (Atkinson et al., 1984). However, extensive glandular development occurred in the cow between birth and 3 mo when glands increased in number and nearly reached the myometrium (Atkinson et al., 1984). Bal and Getty (1970) described the presence of slight epithelial invaginations in the newborn pig and extensive glandular development by 1 mo when glands had reached the myometrium. In all three species (sheep, cattle and pigs) uterine glandular development was progressive and appeared to be completed, in terms of growth toward the myometrium, prior to onset of puberty.

Fundamental histological data presented here are necessary to identify critical periods of ovine uterine development, and set the stage for more detailed studies of events and mechanisms responsible for ovine uterine morphogenesis. It is suggested from the present data that ovine uterine morphogenetic events during the fetal period (d 55 to 150) are associated with endometrial remodeling and establishment of clearly defined fetal nodular and deep internodular areas. Genesis of endometrial glands in the early neonatal period enables glandular (intercaruncular) and aglandular (caruncular) endometrial
areas to be defined histologically, and to remain
as characteristic features of the adult ovine uterus.

Ordered morphogenetic events characterized
by histological associations seen in developing
fetal and neonatal ovine uteri include: 1) strata-
fication and reorientation of subepithelial
mesenchymal layers, 2) differentiation of an
eosinophilic presumptive myometrial layer, 3)
endometrial restructuring and formation of
fixed mucosal surface structures (uterine carun-
cles/folds) and 4) development and prolifera-
tion of uterine glands. These events are
common temporal features of mammalian
uterine morphogenesis and involve movement,
proliferation, remodeling and adhesion of cells
and cell layers, are thought to require or to be
potentiated by specific extracellular matrix
conditions (Toole, 1981), and may be facili-
tated by endocrine factors. Further, Cunha et
al. (1985) observed that, as in other urogenital
organs, uterine epithelial morphogenesis and
cytodifferentiation are induced and specified
by stromal tissues. Additionally, recent studies
of the developing neonatal murine uterus
provided evidence for gonadal steroid-indepen-
dent epithelial proliferation, differential prolif-
erative responses between stromal and epithelial
tissues following administration of steroids, and
stromal mediation of hormonal effects in
uterine epithelium (Bigsby and Cunha, 1985,
1986; Cooke et al., 1986). Such events and
mechanisms are likely to be involved in ovine
uterine morphogenesis, epithelial proliferation,
cytodifferentiation and function, but remain to
be defined.

Literature Cited

Atkinson, B. A., G. J. King and E. C. Amoroso. 1984. Development of the caruncular and intercarunc-
ular regions in the bovine endometrium. Biol.
Reprod. 30:763.

34:15.

Barcroft, J. 1946. The growth of the body and of its constituent parts. In: Researches on Pre-natal
Life. pp 29–41. Blackwell Scientific Publications,
Oxford.

Bigsby, R. M. and G. R. Cunha. 1985. Effects of progestins and glucocorticoids on deoxyribonu-
cleic acid synthesis in the uterus of the neonatal

Bigsby, R. M. and G. R. Cunha. 1986. Estrogen stimulation of deoxyribonucleic acid synthesis in
uterine epithelial cells which lack estrogen

the ewe during the estrous cycle and early pregnancy.

Bryden, M. M. 1969. Postnatal developmental anatomy of the sheep, with particular reference to the
period of the embryo (11 to 34 days). D.V.M.

Casida, L. E. and F. F. McKenzie. 1932. The oestrous
cycle of the ewe: histology of the genital tract.

changes in the uterine horns during the estrous
cycle in the ewe in relation to the proximity of


Cooke, P. S., F.-D. A. Dean, D. K. Fuji, H. A. Bern
morphology and estrogen responsiveness in
cultured vaginal and uterine epithelia transplanted

Cunha, G. R., R. M. Bigsby, P. S. Cooke and Y.
Sugimura. 1985. Stromal-epithelial interactions
in the determination of hormonal responsiveness.
In: J. A. McLachlan (Ed.) Estrogens in the
Environment. pp 273–287. Elsevier/North-Hol-
land, New York.

Wynn (Ed.) Cellular Biology of the Uterus. pp

Effects of maternal administration of diethylstil-
bestrol and estradiol on the newborn guinea pig.

Ginther, O. J. 1979. Placentation and embryology. In:
Reproductive Biology of the Mare Basic and
Applied Aspects. pp 255–320. McNaughton and
Gunn, Inc., Ann Arbor, MI.

Differential estrogen and antiestrogen responsive-
ness of the uterus during development in the
fetal, neonatal and immature guinea pig. Biol.
Reprod. 31:371.


1974. The post-natal development of the ovari
and uterus of the merino lamb. J. Reprod. Fertil.
36:275.

Development of smooth muscle in the human fetal
139:239.

McKenzie, F. F. and C. E. Terrill. 1937. Estrous,
ovulation and related phenomena in the ewe.

Mossman, H. W. 1980. Comparative morphology of
the endometrium. In: F. A. Kimball (Ed.) The
New York.

O’Rahilly, R. 1977. Prenatal human development. In:
R. M. Wynn (Ed.) Biology of the Uterus. pp

Ryle, M. 1962. Early reproductive failure of ewes in a

