BIOLOGY OF REPRODUCTION 30, 763–774 (1984)

Development of the Caruncular and Intercaruncular Regions in the Bovine Endometrium

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ABSTRACT

Uterine specimens from fetal and postnatal heifers were examined by various types of microscopy to determine when the endometrium differentiated into caruncular and intercaruncular regions and changes in the cell types as the uterus developed. The luminal surface of the fetal uterus was formed into prominent pedunculated or mushroom-shaped nodules. Glandular development was first observed at about 250 days of gestation as short invaginations of the epithelium of the internodular surface adjacent to nodular stalks. The basal profile of each epithelial cell in the deeper portions of developing glands formed cytoplasmic processes which extended into the stroma. Between birth and 3 months of age there was a tremendous increase in the glandular epithelium. Concurrent thickening of the connective tissue component caused expansion of the nodular peduncle and a gradual flattening of the nodule until, by 1 year of age, only slight elevations remained, with a discrete caruncle visible on each. By following the development of the glandular component it was concluded that only the apical portion of each fetal nodule will develop into a caruncle and the sides of the nodule, together with the internodular portion, are destined to be the intercaruncular region.

INTRODUCTION

On the luminal surface of the mature bovine uterus, four irregular rows of oval caruncles can be observed running the length of each horn. During early gestation, the epithelium of both the caruncular and intercaruncular regions becomes attached to the chorion by microvillous interdigitation to form the placenta (King et al., 1980, 1981; Wathes and Wooding, 1980). Each caruncle subsequently grows rapidly and develops crypts to increase the surface area of contact. The interlocking of chorionic villi into the crypts of the caruncles serves to maintain the physical position of the entire placenta. More importantly, the caruncles develop an extensive vascular bed and are the major sites of gaseous and small molecule exchange. The intercaruncular region is primarily involved in the supply of large molecules to the embryo. Morphogenetic studies on the development of the ungulate endometrium have been limited to a single report on the cow (Yamauchi, 1964) and several on the pig (Hadek and Getty, 1959; Bal and Getty, 1970; Crombie, 1972). Since there are structural and functional differences between the caruncular and intercaruncular regions of the bovine uterus, the early development has been studied in greater detail.

MATERIALS AND METHODS

Uteri were obtained from 21 fetuses of known gestational age ranging from 151 to 282 days and from 2 or 3 heifers at each of the following approximate ages: newborn (<24 h after birth), 3, 6, 9 and 12 months. The reproductive tract was removed immediately after exsanguination, opened along the mesometrial edge of the uterus and pinned endometrial side up on wax. The luminal surface was washed four times (10 min each) with Dulbecco's phosphate-buffered saline (PBS; 1X, pH 7.2) to remove superficial mucus and then fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 30 min to firm the tissue. Samples were then removed and processed as follows for light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Light Microscopy

Samples were fixed for 24–36 h in either 3% glutaraldehyde or Bouin's fluid, dehydrated through a

Accepted December 8, 1983.
Received August 22, 1983.
[1] Reprint requests.

graded series of alcohols and embedded in either paraffin or glycol methacrylate (GMA) (Sorvall JB-4 embedding medium). Sections were cut at 5 μm (paraffin) or 2 μm (GMA) and stained with hematoxylin and eosin (H&E).

Transmission Electron Microscopy

Samples of endometrium were minced into 1-mm cubes and fixed for 2 h in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), washed and postfixed for 2 h in 1% O_SO_4 in 0.1 M phosphate buffer containing 5.4% glucose (pH 7.2). Tissues were block-stained with uranyl acetate (U.A.), dehydrated through a graded series of alcohols and embedded in either Spurr's or Araldite CY212 resin. Thin sections were poststained with lead acetate and examined with a Philips 300 transmission electron microscope. Thick (1 μm) sections were also cut and stained with Paragon (King, et al., 1979) for light microscopy.

Scanning Electron Microscopy

Samples of endometrium were cut into 1-cm squares and fixed for 24 h in 3% glutaraldehyde in 0.1 M phosphate buffer, washed, postfixed 1 h in 1% O_SO_4, dehydrated through a graded series of alcohols, critical-point dried and sputter-coated with gold palladium, before examination in a Jeol JSM 35C scanning electron microscope.

RESULTS

Macroscopic Alterations of the Luminal Surface

The luminal surface of the fetal uterus was formed into prominent pedunculated or mushroom-shaped nodules. These nodules were organized into 4 irregular rows running the length of each horn (Fig. 1). The mean of total uterine nodules in 5 pre- and 3 postnatal specimens was 123.25 ± 20.9 (n=8) (range from 89 to 155), which is comparable to published figures for the number of caruncles in the mature bovine uterus (Amoroso, 1952).

Around the midpoint of gestation, the fetal nodules were generally so close together that they totally obscured the internodular surface (Fig. 6). Some expansion had occurred between the nodules in newborn heifers' uteri and this process continued until, by 6 months of age, the nodules had flattened so that all of the endometrial lining was visible. The outline of the original nodule was still preserved but a discrete round or elliptical caruncle was obvious at the apex of each swelling (Fig. 2). Both round and elliptical nodules and caruncles were observed on the luminal surface. To define the sizes of these, the shortest and longest axes of the largest and smallest nodules (newborn, n=2) or caruncles (6 month, n=2) were measured from photographs. In neonates the measurements of nodules ranged from 2.5 × 2.5 to 7 × 4 mm. At 6 months of age the caruncles ranged from 2 × 3 to 5 × 6 mm. Thus, although the entire uterus had grown considerably, the dimensions of the true caruncles at 6 months of age were similar to those of the nodular apices at birth. This is illustrated in Figs. 1 and 2 which are identical magnifications.

Emergence and Distribution of Uterine Glands

In the mature nonpregnant heifer, the epithelial cells were similar in the caruncular and intercaruncular regions (Wathes and Wooding, 1980), so the main feature differentiating the two areas was the presence or absence of gland openings. In the fetal uterus, the internodular surface had a relatively uniform cobblestoned appearance until about 250 days of gestation. At this stage, short invaginations into the stroma could be observed both from the surface and in cross section (Fig. 3). These invaginations initially formed in the internodular surface near its junction with a nodule. Ciliated cells frequently occurred near these invaginations. By 265 days of gestation occasional very short glands were distinguishable in this same region (Fig. 4) and these increased in length and frequency during the rest of gestation.

A surface view of the uteri from newborn calves showed more numerous invaginations as well as the larger, recognizable gland openings. In cross sections the glands were observed to have penetrated slightly further into the stroma.

Between birth and 3 months of age, there was a substantial increase in the glandular epithelium. Gland orifices and their associated ciliated cells were much more common throughout the internodular region and also on the sides of the nodules (Fig. 7). The development of the glands produced a distortion of the surface. While the nodular surface remained relatively smooth, the internodular regions had developed many folds and undulations around the glands. Not only were there more glands, but they had also developed well into the stroma, extending more than two-thirds of the distance to the myometrium.

Endometrial Remodelling

Since the glands commenced development before the nodules flattened, it was possible to use them as a marker to differentiate the caruncular from the intercaruncular regions on the nodules. During mid to late gestation (Fig.

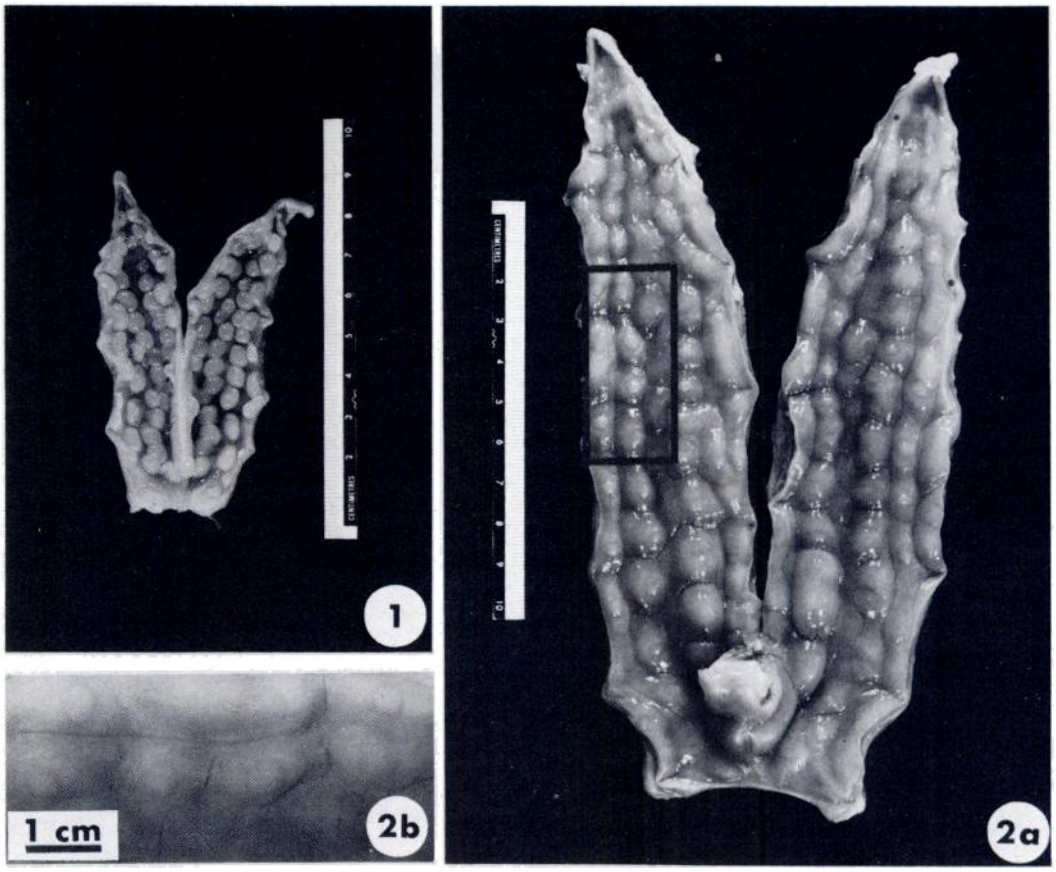


FIG. 1. Endometrial surface of a newborn calf uterus showing four irregular rows of nodules running the length of each horn.

FIG. 2a. Endometrial surface of a 6-month-old calf uterus.

FIG 2b. Enlargement of the outlined portion of Fig. 2a illustrating a discrete caruncle positioned at the apex of each swelling.

6) the epithelium was uniformly columnar in all regions with no undulations or glands. The base of the nodular stalk was narrow and there was also very little connective tissue separating the epithelium from the myometrium in the internodular regions.

At 3 months of age (Fig. 7), the nodules were still present, although the stalk had thickened dramatically through an increase in the stroma. The internodular region was now recognizably intercaruncular because the undulating surface was pitted by the many gland openings. These were also evident on the sides of the nodules, so that only the apices of the nodules were still smooth. The stromal component in the intercaruncular region had thickened considerably since birth, yet the glands had penetrated almost to the myometrium.

By 6 months of age (Fig. 8) the stalk expanded to a width greater than the apex, and gland orifices had appeared over all but the actual top of the original nodule. The glands which opened on the sides of the nodule had grown deep into the stroma so only the connective tissue directly under the apical epithelium was aglandular. By this stage the nodular apex, in cross section, resembled a true caruncle.

By 1 year of age (Fig. 9), discretely outlined round or elliptical caruncles were recognizable on the slight elevations that remained of the original nodules. Glands were present throughout the connective tissue except for the region immediately under the caruncular epithelium. As is typical of the mature caruncle, this region of the connective tissue was more intensely stained than the glandular region.

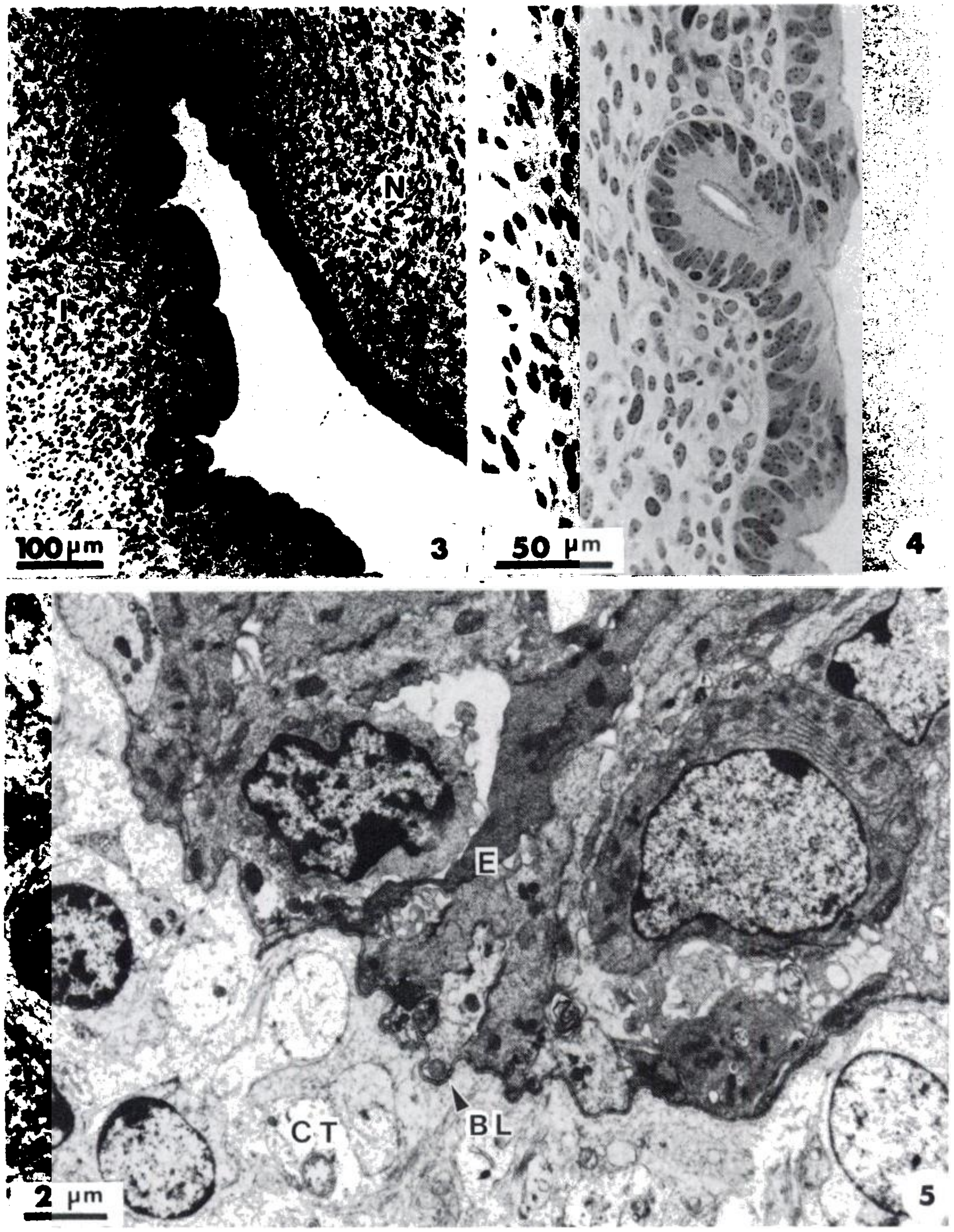


FIG. 3. Invaginations representing the earliest stage of gland development. These are developing on the internodular (I) surface near its junction with a nodule *(N)*; 250 days gestation (LM).

FIG. 4. Cross section through a gland primordium. Note the dense apical band in glandular cells, at the invagination and in occasional cells of the internodular surface; 265 days gestation (LM).

FIG. 5. Relationship between the connective tissue *(CT)* and epithelial cells *(E)* at the deepest point of a gland primordium. Cytoplasmic processes from the epithelial cells have induced undulations in the basal lamina *(BL)*; 280 days gestation (TEM).

Initiation of Glandular Development

Ultrastructural examination of perinatal samples revealed differences between the epithelium of the developing glands and the rest of the surface. The typical surface epithelial cell was columnar with basal nucleus, small mitochondria and rough endoplasmic reticulum. The apical membrane was formed into a border of uneven microvilli and, just below the apex, adjacent cells were connected laterally by the typical junctional complex (Fig. 10). Throughout all regions of the epithelium occasional lymphocytes were observed between the epithelial cells. The basal lamina was regular and relatively straight beneath the surface epithelium.

The nonciliated cells lining the developing gland contained similar organelles, except at the apex. A high proportion of these cells exhibited an extremely dense filamentous band extending out from the zonula adherens and often stretching completely across the cell (Fig. 11). This band was much more concentrated than the terminal web usually associated with an epithelial cell and was easily recognizable at the LM level (Fig. 4). This feature was evident in most cells in the early glands and in occasional cells of the internodular surface, but was never observed in cells covering the nodular apex.

Cells in various stages of ciliogenesis were observed frequently in the developing glands and also occurred on the internodular surface. These cells generally had a paler cytoplasm and fewer organelles than the adjacent nonciliated cells. The cilia were usually sparser than in a fully developed cell and often the basal bodies were not yet lined up evenly under the apical membrane. Ciliated cells were never observed, by SEM or TEM, on the apical surface of a nodule.

The basal profile of each epithelial cell in the deeper portions of developing glands formed cytoplasmic processes which extended into the stroma (Fig. 5). This created an undulating but still intact basal lamina. In contrast, the junction between the epithelium and stroma remained smooth in all other regions.

The connective tissue component was also organized into two distinctive regions. The LM examination showed a denser staining pattern in the internodular region and extending up the sides of each nodule (Fig. 6b). In the center of the nodule and directly under the apical epithelium the connective tissue stained less intensely.

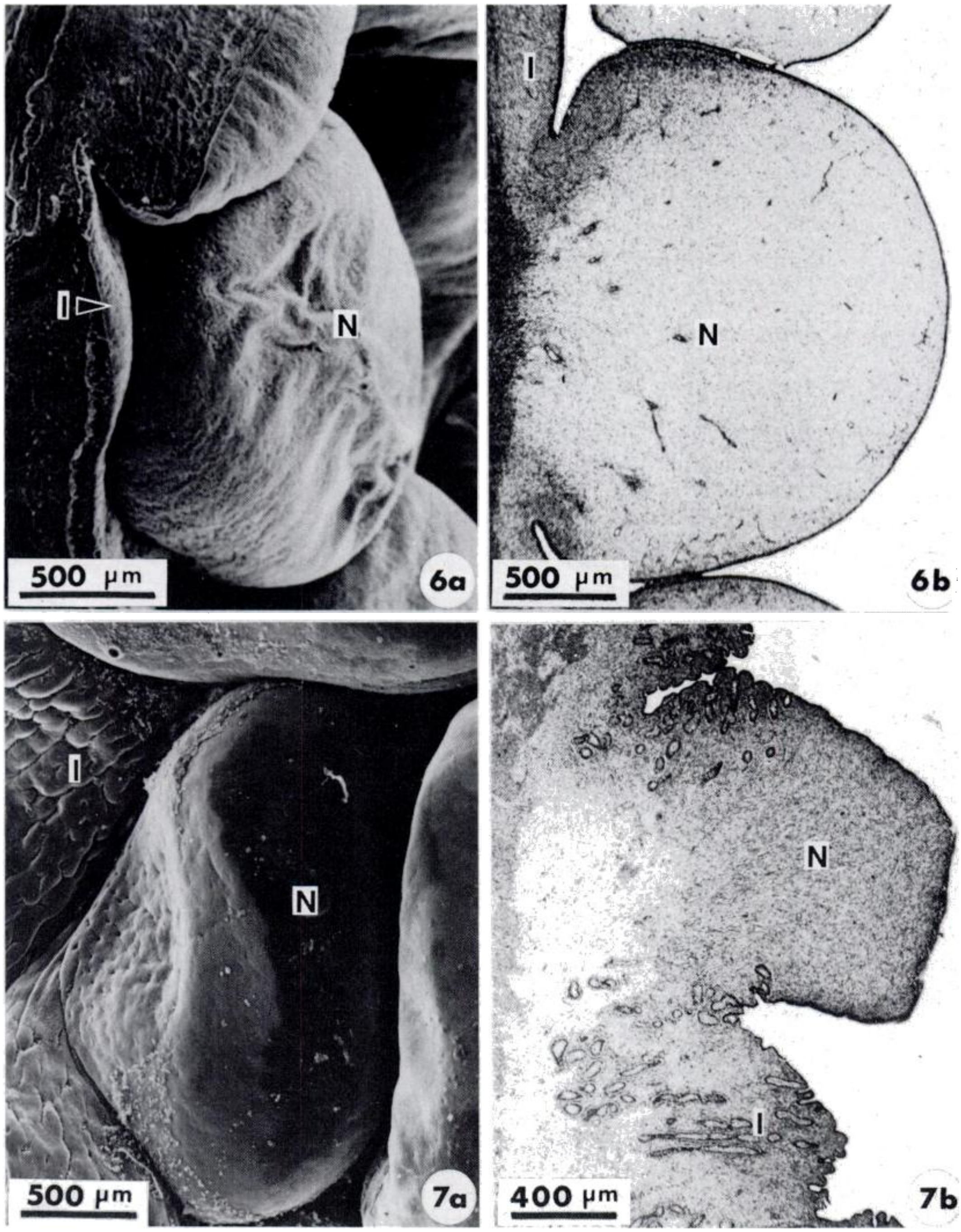
The pale nodular connective tissue was more diffuse than that observed in the mature caruncle. The fibroblasts appeared typical, although not as frequent, and the matrix was also more diffuse with sparse distribution of collagen (Fig. 12). The internodular connective tissue was completely different (Fig. 13). The fibroblasts were larger and more densely packed together. Their nuclei were paler and more oval than those of mature fibroblasts. The cytoplasm was also more abundant, paler and contained microtubules. The collagen fibrils were more prominent and densely organized than in the apex of the nodules. Small nonmyelinated nerve fibers were also very common in this region, in contrast to the mature uterus, where nerves were only infrequently observed.

These ultrastructural differences between the nodular apex and the rest of the endometrium were most dramatic in the animals sampled just before and after birth, when glandular development was initiated. By 3 months of age, the apical terminal web in both glandular and surface epithelium was only rarely denser than in the mature uterus. Ciliogenesis was still occurring and ciliated cells were much more frequent in the glandular epithelium. The basal projections had disappeared from the cells in the deeper portions of the glands leaving a relatively smooth outline.

DISCUSSION

Yamauchi (1964) has described the morphogenesis of the fetal bovine uterus from 1 to 8 months of gestation and claimed that the caruncles first appeared around 4 months. The earliest samples for the current study were obtained at 5 months and the general appearance of the distinctly nodular surface agreed with the previous report. However, subsequent examination of the tissues did not support the contention that entire nodules were caruncles.

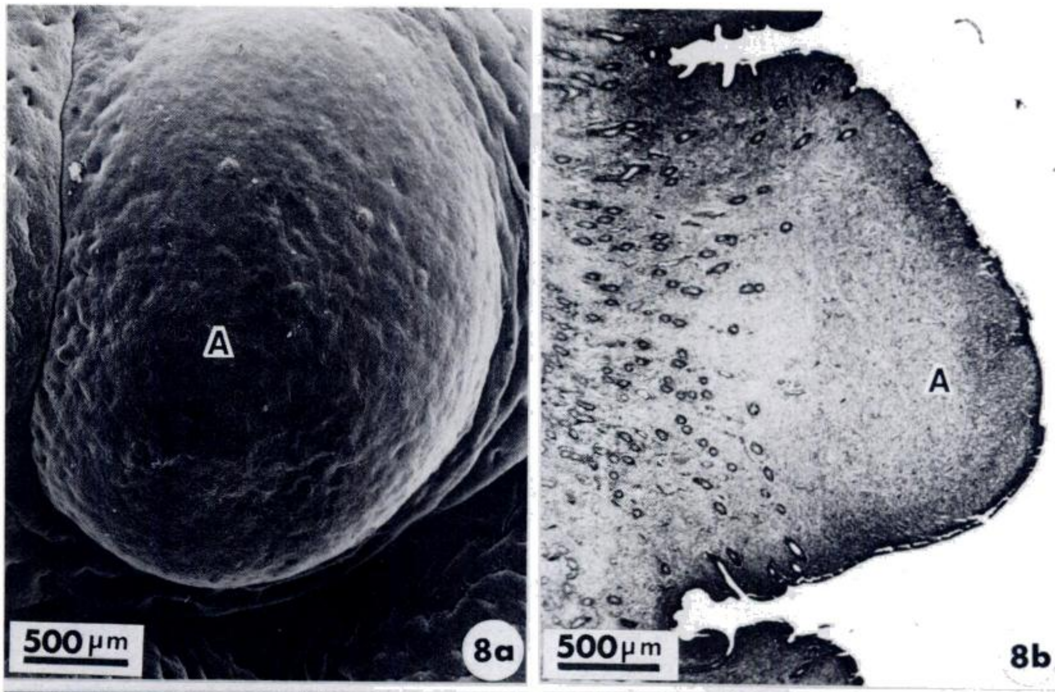
Fig. 14 is a schematic comparison between two fetal nodules in late gestation and two caruncles at 6 months of age illustrating that the dimensions of the nodular apices in newborn animals and the recognizable caruncles at 6 months of age were similar. If, as Yamauchi claimed, the entire nodule was a caruncle, the epithelium of the apex *(solid)* and sides *(hatched)* of the nodule would form the mature caruncle. This would require an actual reduction in the amount of caruncular epithelium from the fetal to the mature stage. Concurrently, there would have to be a tremendous



FIGS. 6–9. Nodular and internodular surface relationships and glandular development in pre- and postnatal uteri.

FIG. 6. Prenatal endometrium without glands on the prominent nodules *(N)* or internodular *(I)* regions. Note intensely stained stroma beneath internodular epithelium and extending up the nodular sides in 6b. *6a)* One hundred and sixty-nine days gestation (SEM); *6b)* 201 days gestation (LM).

FIG. 7. Postnatal endometrium showing thickened nodular stalk plus glandular development in internodular *(I)* regions and sides of nodules *(N)*; 3 months. *7a)* SEM; *7b)* LM.



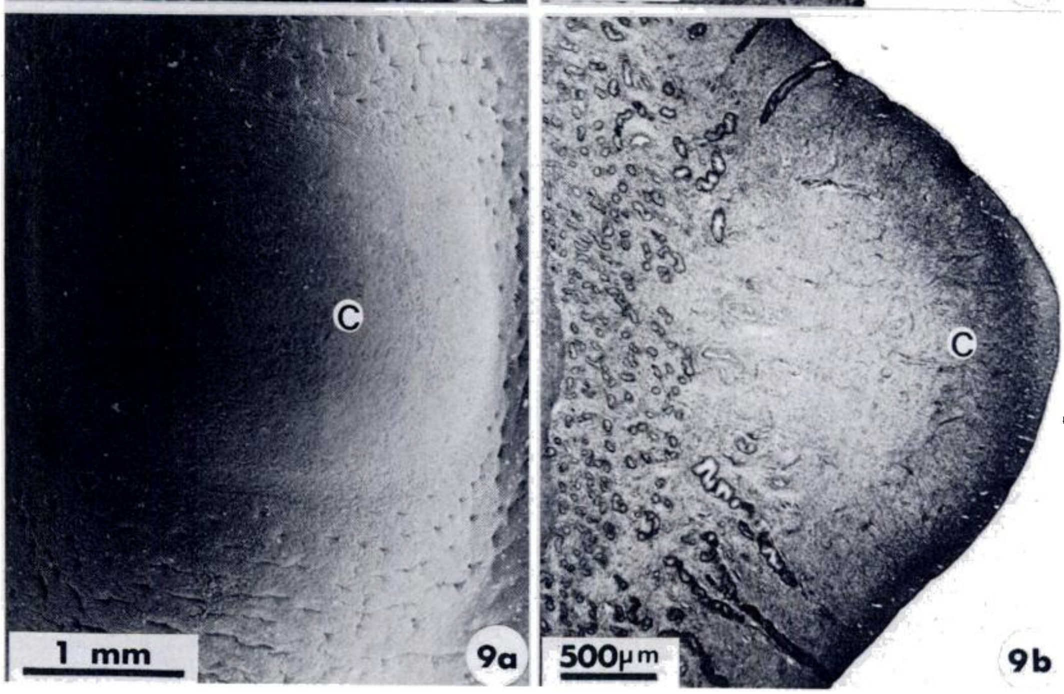


FIG. 8. By 6 months of age the nodular stalk has expanded to a width greater than the apex *(A)*. Glandular development has progressed beneath the presumptive caruncle. *8a)* SEM; *8b)* LM.

FIG. 9. At 1 year of age discretely outlined aglandular caruncles (C) are slightly elevated above the intercaruncular surface. 9a, SEM. 9b, LM.

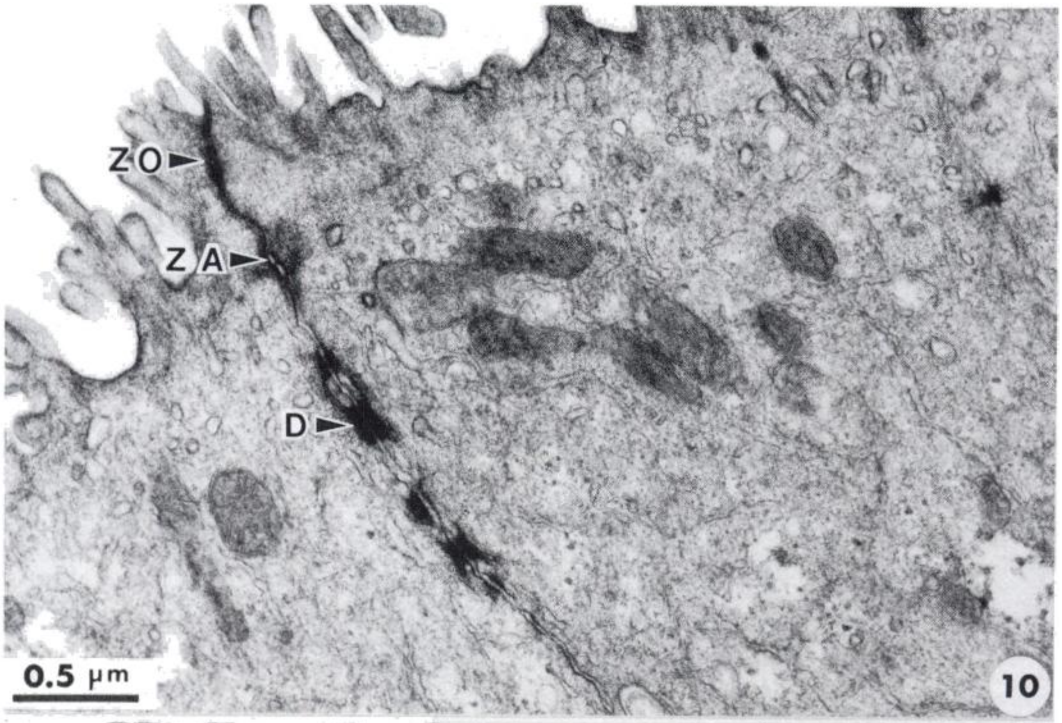


FIG. 10. Apical junctions between 2 epithelial cells near the apex of a nodule. *ZO*=Zonula occludens; *ZA*= zonula adherens; *D*=desmosome; 280 days gestation (TEM).

FIG. 11. Dense filamentous band extending between the zonulae adherens in the apices of epithelial cells lining a gland primordium; 280 days gestation (TEM).

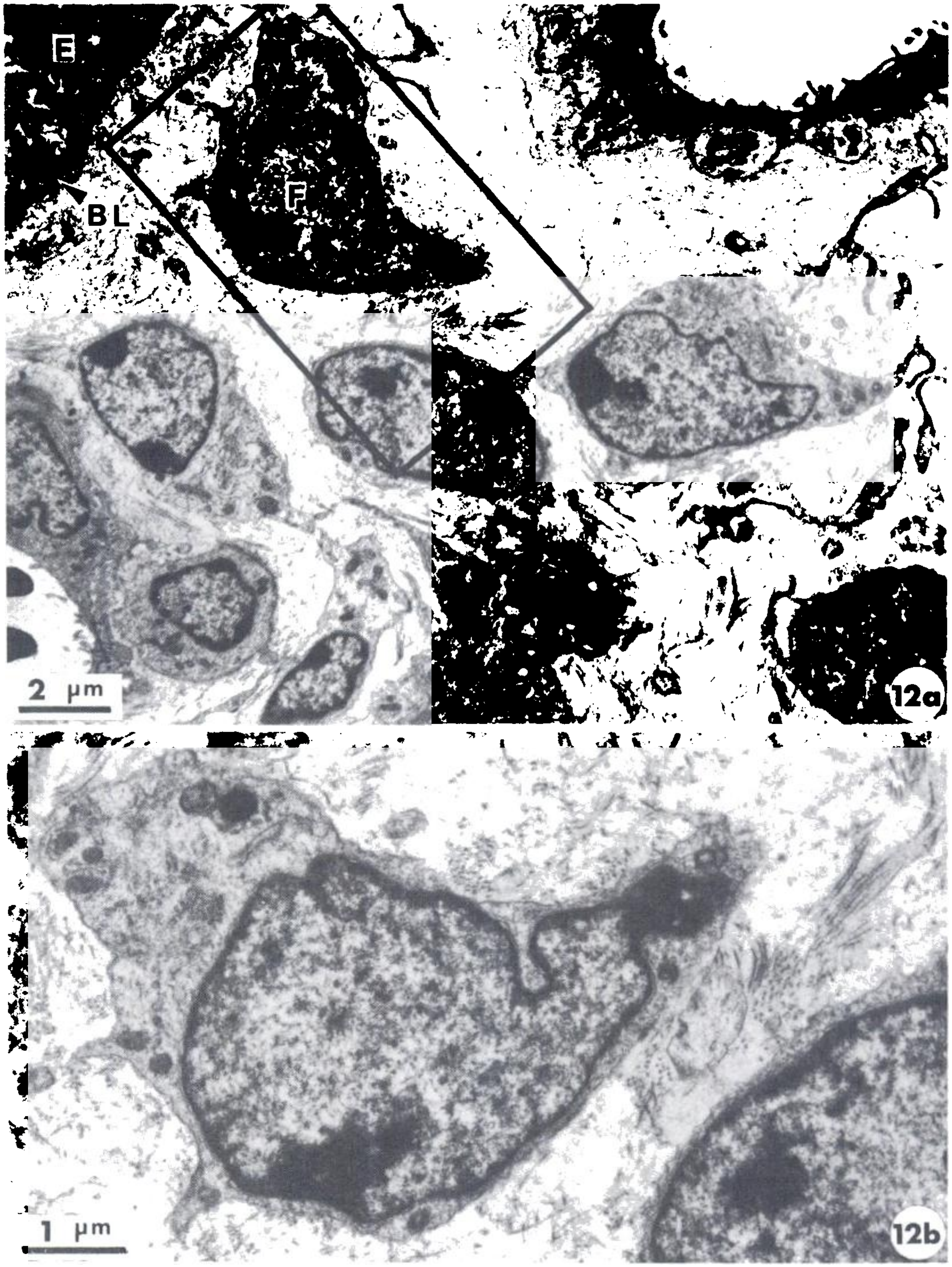


FIG. 12. Typical connective tissue below the epithelium *(E)* of a nodular apex. Note smooth basal lamina *(BL)*, normal fibroblasts *(F)* and sparse collagen. *b)* Higher manification of a typical fibroblast; 280 days gestation (TEM).

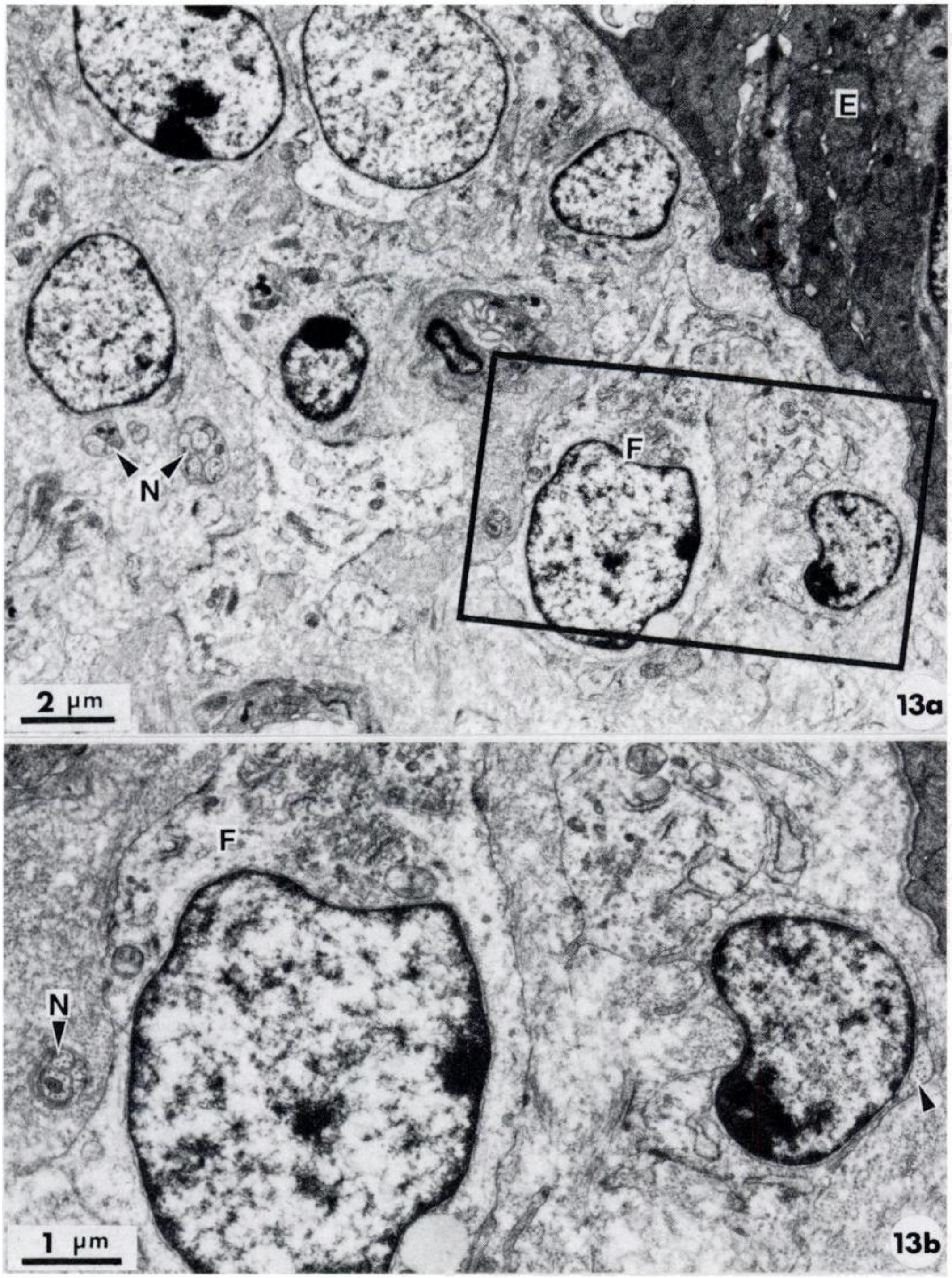


FIG. 13. Connective tissue near the epithelium *(E)* of a developing gland. Fibroblasts *(F)* have large pale nuclei and diffuse cytoplasm. Small nerves *(N)* occur frequently and the collagen matrix is more densely organized than in Fig. 12. *b)* The unusual nuclei, cytoplasm and microtubules (▲) of the fibroblasts; 280 days gestation (TEM).

FIG. 14. A schematic comparison between *(A)* 2 fetal nodules in late gestation and *(B)* 2 caruncles at 6 months of age. The *solid* epithelium at the apex of the nodule eventually becomes caruncular surface while the remainder *(hatched and unshaded in A)* is incorporated into the intercaruncular regions.

increase in the internodular *(unshaded)* epithelium to produce the large intercaruncular areas.

A more logical explanation was that only the most apical *(solid)* portion of each nodule was destined to develop into a caruncle. The epithelium of the sides *(hatched)* of the nodule, together with the internodular region *(unshaded)* would form the glandular intercaruncular areas. This hypothesis was confirmed by observations on glandular development. Although the first recognizable glands developed in the internodular region, these were generally near the junction with a nodular stalk. During the first 6 months after birth, gland openings appeared over the entire internodular surface as expected, but also over the sides of the nodules (Fig. 14, *hatched portion*) so that only the most apical portion remained to form the caruncle.

As glandular development progressed, the luminal surface changed from the nodular configuration to relatively flat with only slight undulations formed by the caruncles. Most of this alteration occurred through proliferation of the connective tissue component which caused the nodular stalk to thicken. Stromal expansion also occurred in the internodular region between the myometrium and the epithelium, so that the whole epithelial layer was pushed upwards and stretched out until it was almost level with the original nodular apices. This expansion would allow the uterus to almost double in length with very little increase in the cells of the epithelium.

Cunha et al. (1980) reviewed the evidence for stromal-epithelial interactions in sex differentiation and demonstrated, by tissue recombinations, that it is the mesenchyme which determines the type of differentiation of the overlying epithelium. In the present study, the cytoplasmic processes of the developing glands were associated with the regions of connective tissue containing a denser matrix, unusual fibroblasts and many small nerves. It may therefore be hypothesized that some type of interaction is necessary between the stroma and the epithelium to initiate glandular development.

Hadek and Getty (1959), and Bal and Getty (1970) have described some of the morphological changes in the developing porcine uterus. They noted shallow depressions in the uterine epithelium of the newborn pig which were thought to foreshadow the glands which were recognizable by 2 weeks of age. However, since they had no material from fetal uteri, there is no indication of when these may have first appeared. Crombie (1972) described the cells of the glandular epithelium of the late porcine fetus (Days 100–113) as being similar to those of the nonpregnant adult. However, the description was confined to subcellular organelles with no indication of the number or size of glands present at that stage, so it is difficult to correlate these studies.

Yamauchi's (1964) examination of the fetal bovine uterus included a description of the development of nodules but no glands were observed since his latest specimens were presumed to be about 8 months of gestation (Yamauchi, 1961). In the present study, glandular development was initiated during the final month of gestation and followed a pattern similar to that described for the neonatal pig (Hadek and Getty, 1959).

The concentration of unconjugated estrogens in the maternal plasma increased dramatically during the final 2 weeks of pregnancy (Robertson and King, 1979; Thatcher et al., 1980). Since estrogen stimulates the growth of endometrial glands in ovariectomized and immature rats (Anderson et al., 1975) and in ovariectomized cats (Bareither and Verhage, 1980), it is postulated that the high maternal estrogens in late gestation may be involved in the initiation of glandular development in the bovine fetus.

ACKNOWLEDGMENTS

Thanks are extended to D. Bellissimo and J. Keys for technical assistance. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Agriculture and Food.

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