J. Anat. (1978), **126**, 2, pp. 385–392
With 6 figures
Printed in Great Britain

Structure of amniotic papillae in sheep

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(*Accepted* 5 *July* 1977)

INTRODUCTION

Villous and papilliform projections of various sizes and shapes on the fetal surface of the amnion have been reported in a number of animals including the dromedary camel (Morton, 1961), the Jamaican bat (Wislocki & Fawcett, 1941), cattle (Germain *et al.* 1974), and the spiny anteater, buffalo, okapi, llama, eland, duiker, steenbok, wildebeest, impala and blesbok (Young, 1975, personal communication).

The present authors saw 104 ovine placentae with amniotic papillae during the lambing seasons of 1974–5 and 1975–6. This represents approximately 65 % of all lambings attended by one of us (C.B.T.) in the course of practice. The breeds of sheep involved included the Border Leicester, Devon Closewool, Clun, Suffolk, Dorset Horn, and various crosses of these breeds. No special breed incidence was noted.

Such papillae appear not to have been fully reported or investigated previously in sheep, where they are to be found on the umbilical cord and the fetal side of the amnion – extending up to 35 cm from the base of the cord over the amniotic surface.

The papillae are white or yellow in colour and harder than the surrounding tissue. They may be up to 9 mm in height and 2 mm in diameter. They may have a simple 'drumstick' shape or exhibit a bifurcated head. Their numbers vary on each placenta from single figures to over 200. Small papillae may be found on the amnion of sheep from early mid-term onwards; where a gravid uterus contains two or more lambs, one or both placentae may be affected.

MATERIALS AND METHODS

Abattoir material was obtained within 2–3 minutes of the ewe being killed and fixation begun at once.

Selected papillae were removed from the amnion, fixed in formol saline, mounted on cavity slides, and examined with a stereo light microscope.

For transmission electron microscopy single papillae attached to pieces of amnion were fixed for 30 minutes in a 3 % solution of glutaraldehyde in 0·1 M phosphate buffer at pH 7·2 and at 18 °C. The pieces of amnion with the papillae still attached were washed in several changes of phosphate buffer and transferred for 1 hour to 1 % osmium tetroxide buffered to pH 7·2 with phosphate buffer. The preparations were dehydrated in alcohol, passed through propylene oxide and embedded in Araldite.

Semithin and ultrathin sections were cut with a diamond knife on a Sorvall MT2-B ultramicrotome. For light microscopy semithin sections were stained with 1 % toluidine blue in 1 % borax; ultrathin sections were mounted on Smethurst New 200

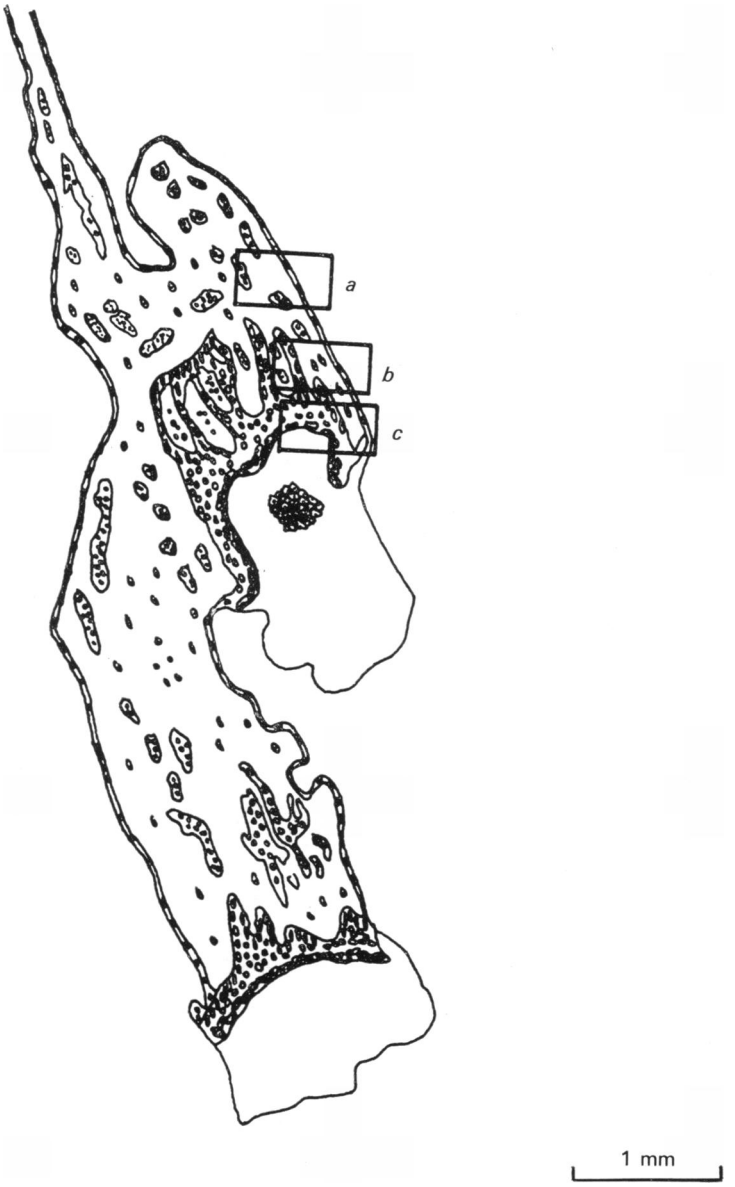
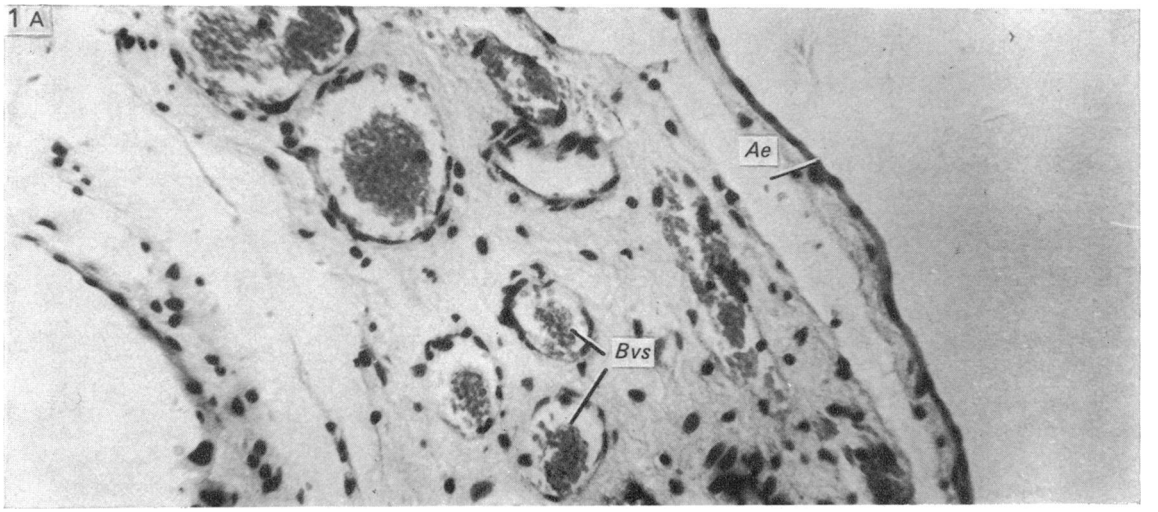


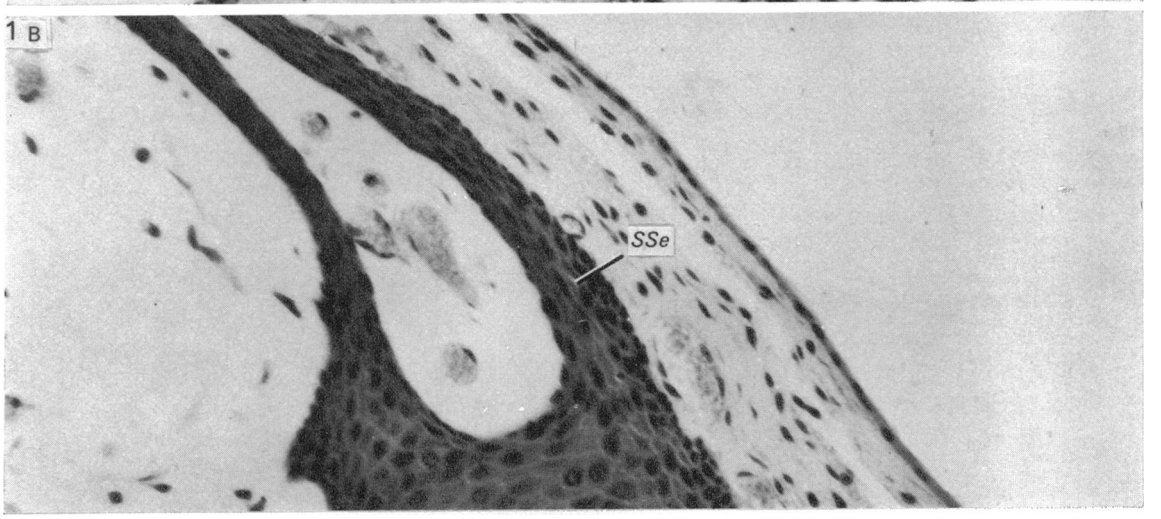
Fig. 1. Diagram of semithin section of a small papilla showing position of Figs. 1 *a*, *b* and *c* in relation to the total structure.

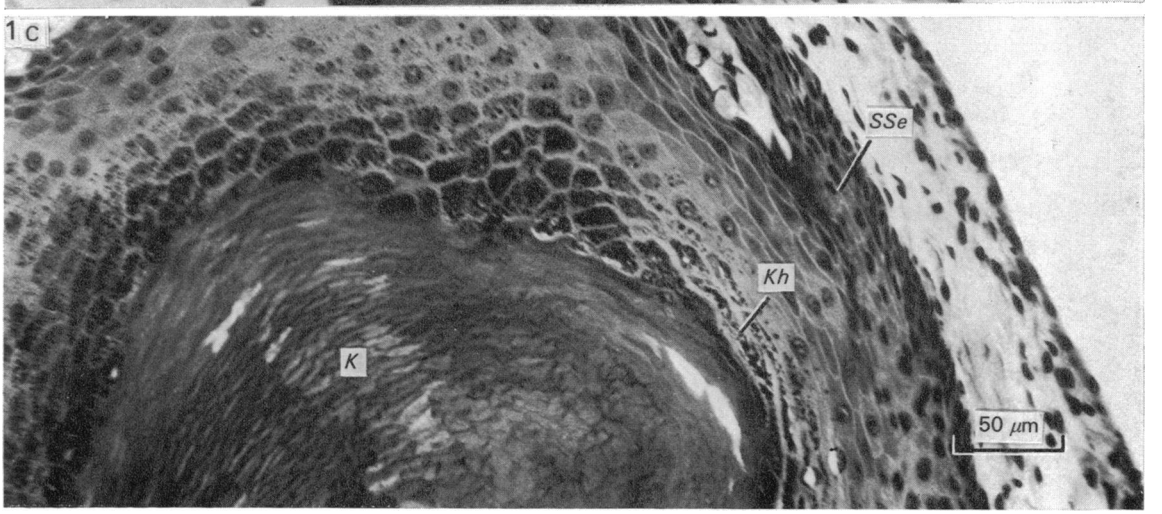
Fig. 1 (*a*). Light micrograph showing amniotic epithelium (*Ae*) and loose connective tissue containing blood vessels (*Bvs*).

Fig. 1 (*b*). Light micrograph showing stratified squamous epithelial cells (*SSe*).

Fig. 1 (*c*). Light micrograph showing progression of stratified squamous epithelial cells (*SSe*) from granular layer containing keratohyalin (*Kh*) to the keratinized layer (*K*).


1 A
Ae
Bvs


1 B
SSe


1 C
SSe
Kh
K
50 μm

grids and doubly stained, first with a saturated solution of uranyl acetate in 50 % ethanol for 20 minutes, and then with lead citrate for 2 minutes. Sections were examined with an AEI EM 801 electron microscope.

For scanning electron microscopy material was fixed and dehydrated as for transmission electron microscopy, passed through several changes of amyl acetate, critical point dried, and coated with 50 nm of gold. Specimens were examined in a Cambridge S600 Stereoscan microscope.

RESULTS

Examination of semithin sections of a small 4 mm papilla attached to the amnion shows the main structural characteristics of these papillary growths. The vascular complex within the connective tissue at the base of the papilla can be seen underlying the amniotic epithelium (Fig. 1 *a*).

The stratified squamous epithelial cells progress in shape from cuboidal, through irregular polyhedral forms, to thin flattened squamous cells, terminating in the keratinized stratum corneum. The granular layers contain irregular shaped densely staining granules, thought to be keratohyalin (Figs. 1 *b*, *c*).

Using scanning electron microscopy the tip and stem of small 4 mm papillae were seen to be covered by stratified squamous epithelial cells (Fig. 2). In a larger (7 mm) papilla, the tip showed no evidence of such an epithelial covering except for a distinct 'lip' towards its outer edge (Fig. 3). The centre of the tip was composed of bundles of connective tissue.

The stem wall of the larger growth was almost devoid of any organized squamous epithelial cells and had a coral-like appearance (Fig. 4).

Microscopic examination of affected amnii showed that some papillae arose from a slight depression on a raised 'plaque' on the amnion. This can be seen at the bottom of Figure 3.

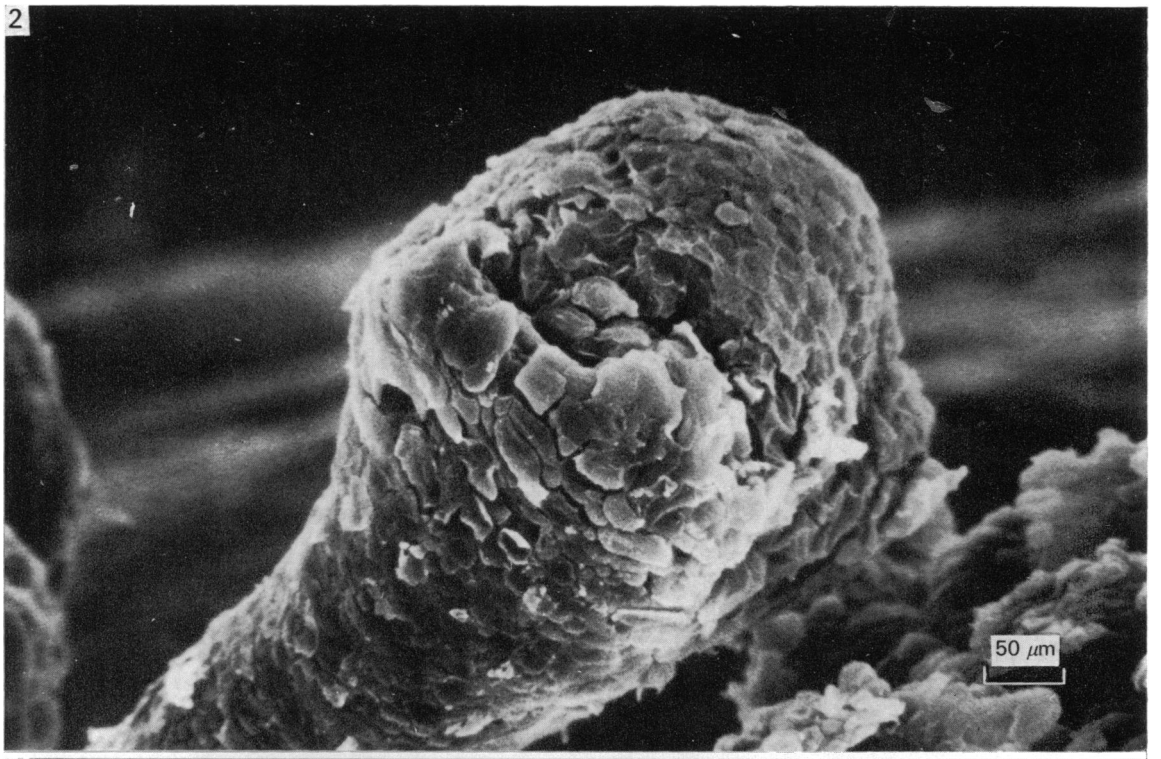
A mid-stem section of a 4 mm papilla examined by transmission electron microscopy showed a stratified squamous epithelium over a basic connective tissue ultrastructure, the connective tissue varying in distribution in different specimens. There were random arrangements of fibrils and banded collagen fibres in the stem and tip (Fig. 5). The presence of mitochondria and myelin was noted. Electron-dense material resembling glycogen deposits was present in some fields.

The tip of some small papillae showed a smooth stratified squamous surface with underlying fibrillar material and degenerate cells. Conversely, the tip of a different specimen demonstrated an irregular surface, with fibrillar material much in evidence within the core.

The high degree of vascularity of the stem as seen in the semithin sections of the larger papillae was an extension of the greatly increased number and size of blood vessels on the amnion bearing such papillae.

From the material examined it would appear that the small papillae have no obvious supply from the amniotic blood vessels. However, a stage is reached in the development of a papilla when amniotic capillaries adjacent to its base extend bud-like projections towards the base. In the larger papillae the base area is surrounded by a complex convoluted capillary system. Capillaries can be seen extending from this complex into the papilla base and continuing up its stem (Fig. 6).

Dissection of a placenta exhibiting this gross hyperaemia revealed that these distended vessels originated from the chorion in regions where the amnion and chorion were in close apposition.



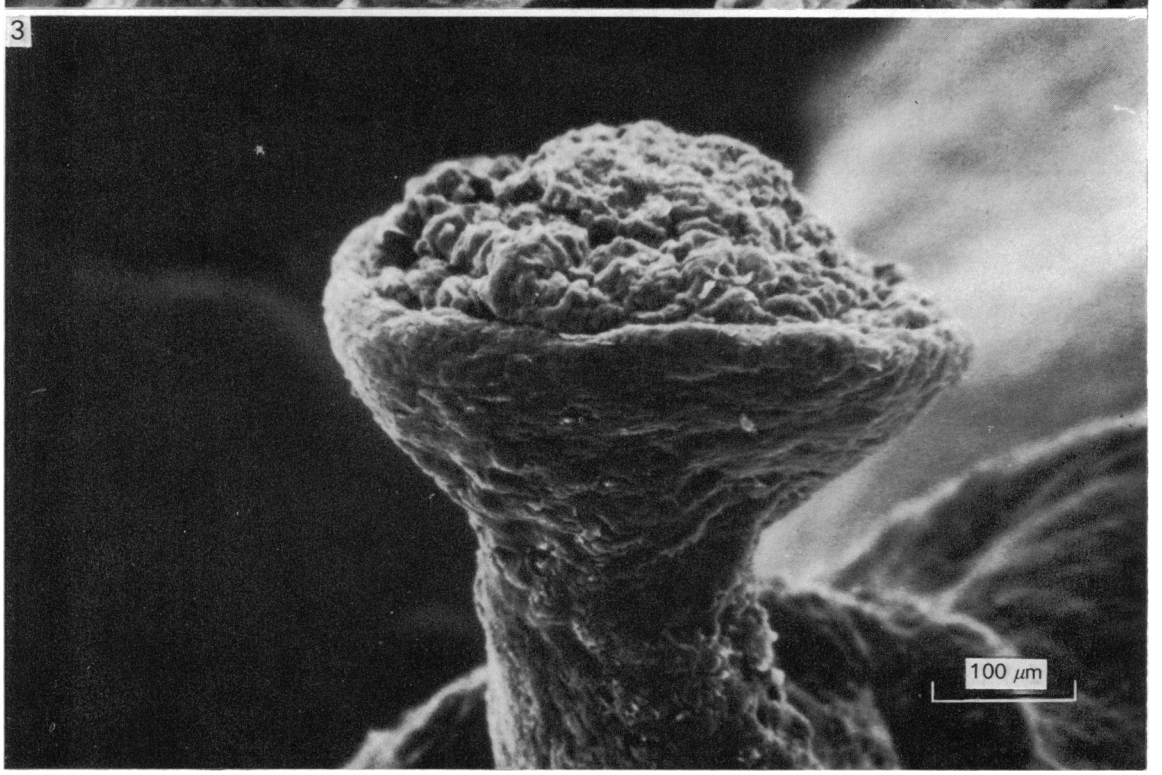
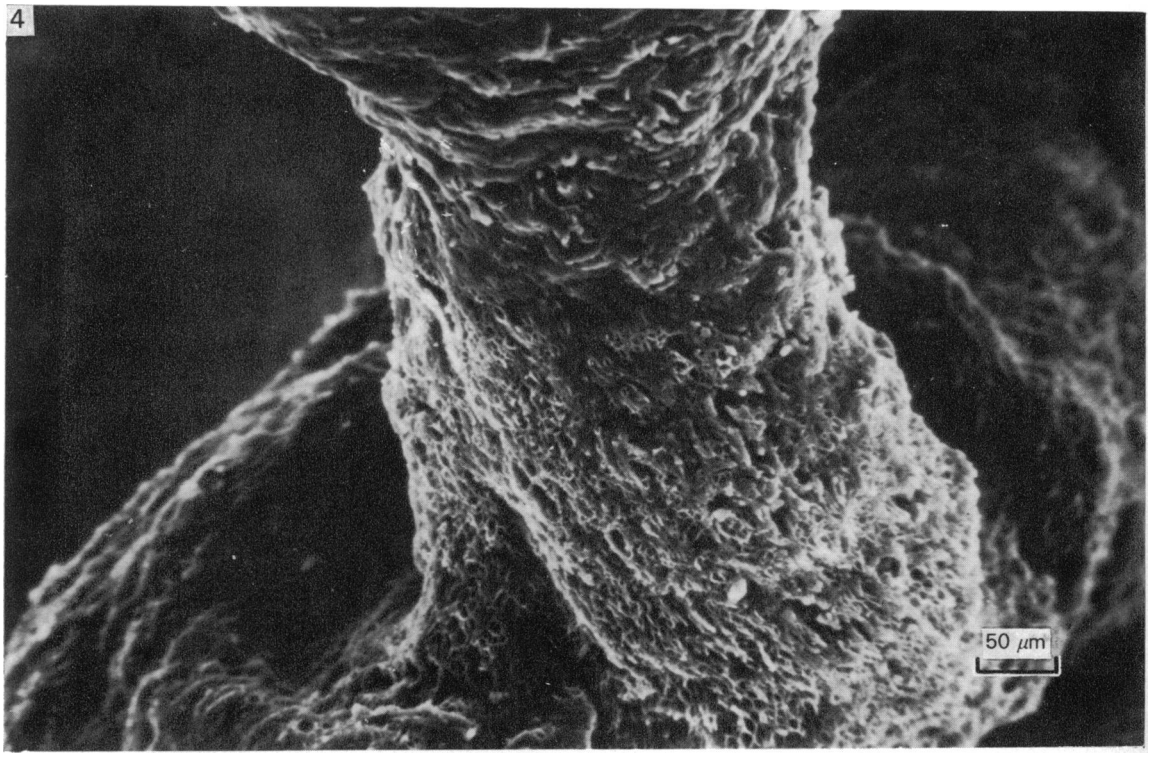


Fig. 2. Scanning electron micrograph of the papilla tip showing the covering of epithelial cells.

Fig. 3. Scanning electron micrograph of a larger papilla showing the underlying connective tissue and the epithelium covered lip.



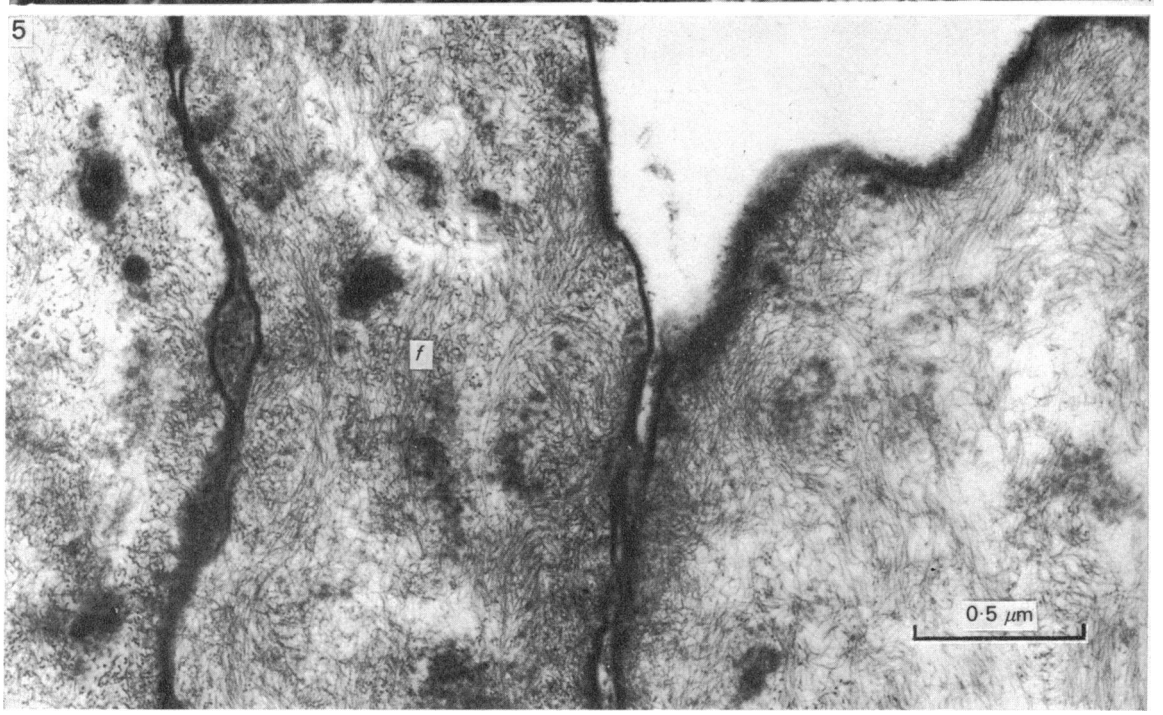


Fig. 4. Scanning electron micrograph of the stem wall of a large papilla showing the absence of epithelial cells.

Fig. 5. Transmission electron micrograph of the papilla tip showing fibrillar material (*f*).

Fig. 6. Stereo light micrograph of a papilla attached to the vascular amnion showing numerous blood vessels (*Bvs*).

DISCUSSION

In attempting to compare the structure of papillae it is difficult to carry out both scanning electron microscopy and transmission electron microscopy on the same structure. Any comparison has to be made between structures of the same shape and/or size, but one cannot be sure that they are at an identical stage of development.

We suggest that the papillae exist in different developmental stages – even on the same placenta. The smaller papillae are characterized by a stratified squamous epithelial covering of the stem and, especially, the tip. The correlation between the results of the scanning and transmission electron microscopy on this point is very good.

In slaughterhouse material examined macroscopically and microscopically, papillae are present from early mid-term onwards. In the earlier stages of gestation all the papillae are small and have a simple 'drumstick' appearance (as in Figure 2).

As pregnancy advances an increasing number of larger papillae are observed. We suggest that these larger papillae with their reduced epithelial covering and greater vascularity, have developed from the smaller, simpler forms.

The amnion in sheep is not notably vascular, and yet the papillae-bearing amnii were usually grossly hyperaemic. The vascular complex shown in Figure 6 is unusual for an ovine amnion. Dissection showed that the capillaries originate from the blood vessels of the chorion: this is probably one reason why the majority of papillae are

situated in an area around the umbilical cord and its base where there exists a close relationship between chorion to amnion.

SUMMARY

The structure of amniotic papillae in sheep was investigated by light transmission and scanning electron microscopy. Papillae were found on the amnion near the umbilical cord in a majority of the sheep examined, from early mid-term onwards. The papillae possessed a basic connective tissue core with a varying degree of vascularity, the whole being sheathed in squamous epithelial cells in the earlier stages of development; but in larger, and presumably older, papillae, squamous epithelium was absent over the tips. The blood supply to these papillae was shown to originate from the chorion and to pass into the amnion at sites where the two members were closely applied to each other.

Tentatively we conclude that amniotic papillae are complex organized structures which develop near amniotic plaques, or, in some instances, from the plaques themselves. The stimuli responsible for their growth and development are unknown.

The authors wish to record their thanks to Professor D. Nichols and Dr H. Stebbings for making facilities available and for their help and encouragement throughout this study, Mr D. H. Steven for his help in the interpretation of micrographs, Dr A. Young for making available his records of placental dissection and Mr P. G. Hignett for his never-failing help and interest.

This work was supported by a grant from the Wellcome Trust.

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