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Morphogenesis of the foetal membranes and placentation in the bat, *Miniopterus schreibersii fuliginosus* (HODGSON)

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Abstract. The development of foetal membranes and the changes in the structure of the placenta in the bat *Miniopterus schreibersii fuliginosus* is described. The study reveals that *M. schreibersii fuliginosus* exhibit developmental characters not matched by any other mammal let alone any other bat.

Keywords. *Miniopterus schreibersii fuliginosus*; morphogenesis; foetal membrane; placentation

1. Introduction

The information concerning the embryology of *Miniopterus schreibersii* is restricted to an erroneous account of the development of the amnion (Da Costa 1920) and brief descriptions of a part of the placenta during mid-pregnancy (Branca 1927; Grosser 1927; Kempermann 1929). Even the more recent information on the structure of the placenta of *M. schreibersii* (Malassine 1970) refers to a part of the placenta at about mid-gestation. These descriptions have failed to present the uniqueness of the development and the structure of the placenta of this bat. The present study has revealed that *M. schreibersii fuliginosus* exhibits developmental characters not matched by any other mammal, let alone any other bat. The peculiarities of the reproductive behaviour and the early development and the process of implantation of the blastocyst in this species have been described elsewhere (Chari and Gopalakrishna 1981).

2. Material and methods

The specimens of *M. schreibersii fuliginosus* were collected from the Robbers' cave, about 8 km from Mahabaleshwar, Maharashtra, India at frequent intervals from January to June 1978. Altogether 352 specimens at different stages of pregnancy were examined.

The female genitalia were dissected after killing the specimens with chloroform and fixed in various ways such as in neutral formalin, Bouin's, Carnoy's, Rossman's and Zenker's fixatives. In cases of advanced pregnancy the uterine wall was slit open to enable penetration of the fixative. After fixation for 24 hr the genitalia were preserved in 70 % ethanol where necessary. The tissues were dehydrated by passing through graded ethanol, cleared in xylol, embedded in paraffin and sectioned serially at a thickness of 3 to 8 μ. For routine histological study the sections were stained with Ehrlich's haematoxylin and counterstained with eosin. A few selected sections from each series were stained by the periodic acid-Schiff (PAS) procedure (Pearse 1968), some by Heidenhein's Azan technique and some by Mallory triple procedure.

3. Observations

The early formation of the primitive amniotic cavity has already been described (Chari and Gopalakrishna 1981). Further development of the foetal membranes and the changes in the structure of the placenta are described here.

3.1 *Neural groove stage*

The following descriptions refer to the structure of the gravid uterus containing an embryo in the early neural groove stage of development. Figures 1 and 6 illustrate the TS of the gravid uterus. The embryo was in contact with the uterine endometrium on all its sides, thus obliterating the uterine lumen at the nidation level. The embryonic plate with a shallow neural groove in its centre was oriented towards the antimesometrial side of the uterus. Definitive amniotic folds had grown dorsally for a short distance

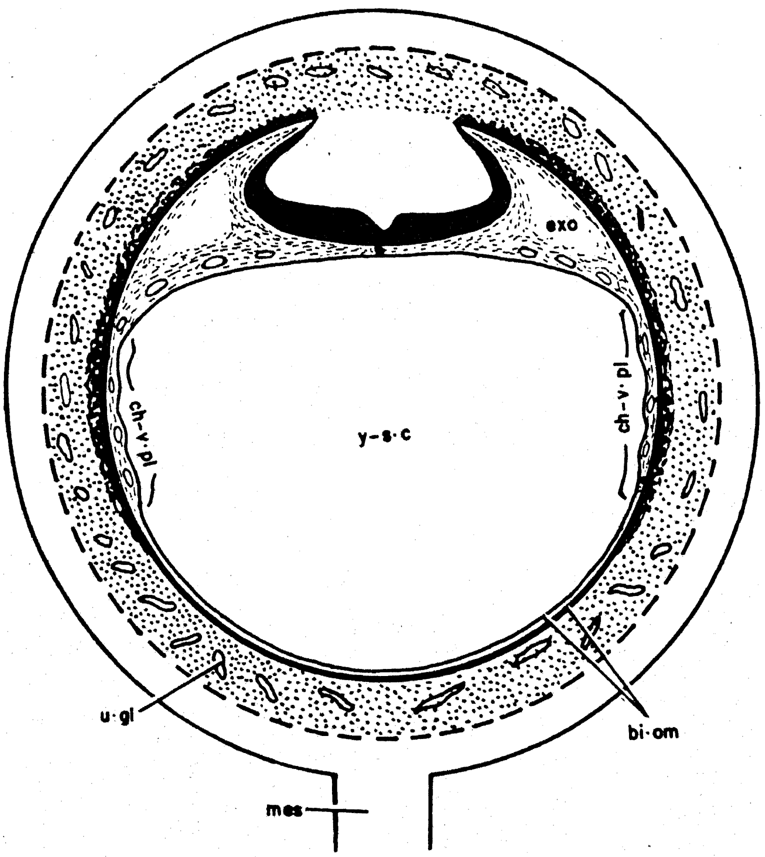

Figure 1. Semischematic diagram to illustrate the TS of the gravid uterus containing an embryo in the early neural groove stage of development. (bi. om: bilaminar omphalopleure; ch-v. pl: chorio-vitelline placenta; exo: excocoelom; mes; mesometrium; u.gl: uterine gland; y-s. c: yolk-sac cavity).

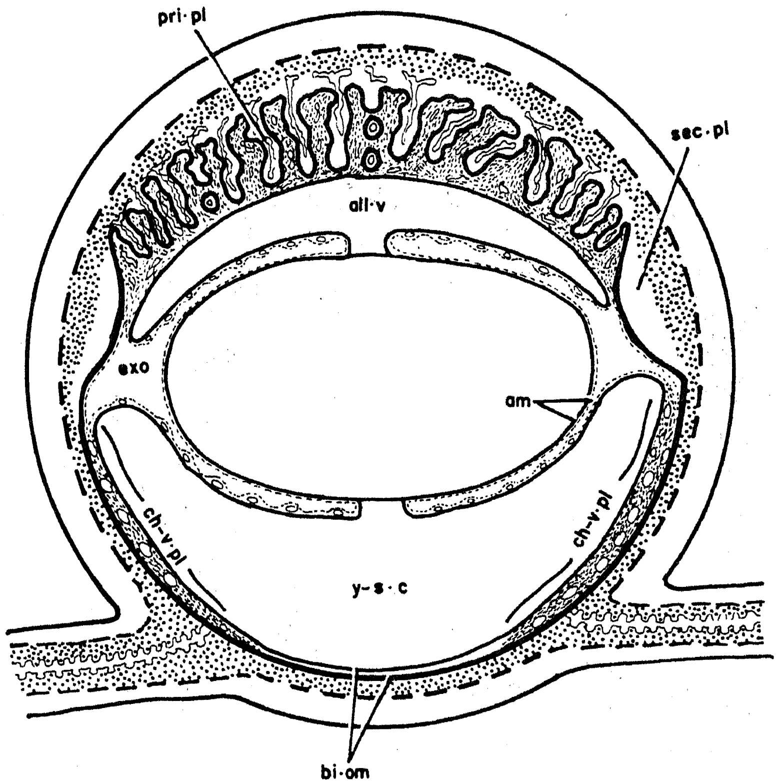

Figure 2. Semischematic drawing of a sagittal section of the gravid uterus containing an embryo at the early limb-bud stage of development. (all. v: allantoic vesicle; am: amnion; pri. pl: primary chorioallantoic placenta; sec. pl: secondary chorioallantoic placenta. The other legends are as in figure 1).

from the margins of the embryonic disc and exocoelom had extended into these folds. The amniotic cavity was widely open and was roofed over by the superficial layer of uterine endometrium. Mesoderm had extended into the yolk-sac wall on the lateral sides converting this segment of the yolk-sac wall into a trilaminar condition. Vitelline vessels had extended to about half the distance on the lateral sides of the trilaminar omphalopleure. Hence, there was a well formed chorio-vitelline placenta in relation to the proximal half and a non-vascular yolk-sac placenta in the distal half of the lateral wall of the yolk sac. A part of the chorio-vitelline placenta adjacent to the embryonic disc was, however, abolished due to the formation of the exocoelom which had separated the yolk-sac splanchnopleure from the chorion.

The trophoblast had penetrated 1/3 the thickness of the endometrium on the lateral wall of the uterus. In these regions the trophoblast could be distinguished into a basal layer of cytotrophoblast and a penetrating mantle of syncytiotrophoblast. Many maternal blood capillaries were markedly enlarged near the foetal border of the placenta on the lateral sides of the uterus and occurred in the form of large capillary loops surrounded by syncytiotrophoblast (figure 7). In many places along the foetal border of the chorio-vitelline placenta the cytotrophoblastic layer had pushed into the syncytiotrophoblastic zone in the form of numerous hollow inpushings, and extra-embryonic mesoderm had entered into these hollows. Hence, the foetal surface of the placenta had numerous indentations—the chorionic villi—between which lay the

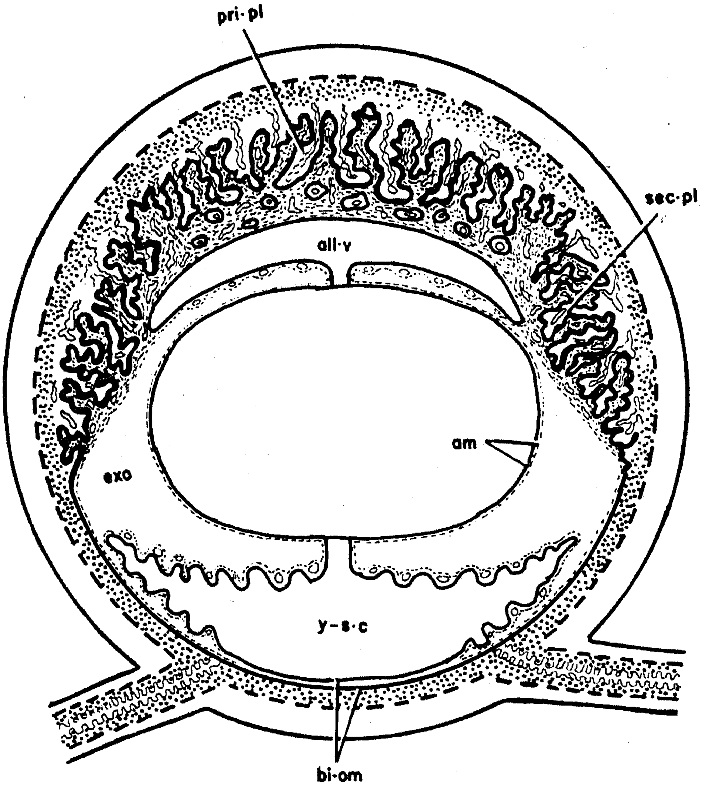

Figure 3. Semischematic diagram of the sagittal section of the gravid uterus to illustrate the arrangement of the foetal membranes at the late limb-bud stage of development of the embryo. (Legends as in previous figures).

enlarged maternal blood capillary loops surrounded by syncytiotrophoblast mentioned above. The abembryonic region of the yolk-sac wall remained bilaminar throughout gestation since mesoderm did not extend to this region. The trophoblastic layer in this region remained in contact with the uterine endometrial tissue but did not invade the endometrium.

At a slightly more advanced neural groove stage of development of the embryo (figure 8) the tail fold of the amnion had grown to about half the distance on the dorsal side of the embryonic plate, and the head fold had grown for a short distance. Mesoderm and exocoelom had entered the amniotic folds thus separating the amnion from the chorion. The trophoblastic layer of the chorion had invaded the endometrium and had formed a narrow zone of syncytiotrophoblast surrounding enlarged loops of maternal blood capillaries. Thus, a temporary chorionic placenta was established in these regions. The only change in the structure of the yolk sac was the extension of vitelline vessels further towards the distal region of the lateral walls of the yolk sac resulting in the establishment of the chorio-vitelline placenta on the entire lateral wall of the uterus.

3.2 *Early limb-bud stage*

The general arrangement of the foetal membranes at this stage of development is given in figure 2. The foetus had developed one pair of limb-buds, and was surrounded by a

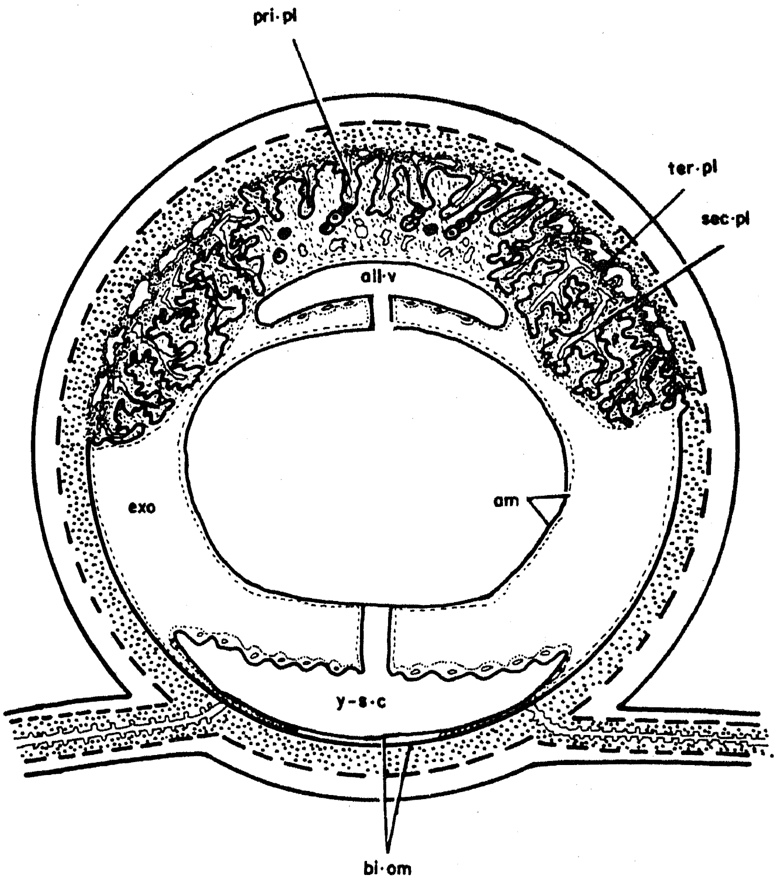

Figure 4. Semischematic diagram of the sagittal section of a gravid uterus to illustrate the arrangement of the foetal membranes at about mid-pregnancy. (ter.pl: tertiary chorio-allantoic placenta. Other legends are as in previous figures).

thin bilaminar amnion. The structure of the amnion did not change during the rest of gestation except that it expanded progressively as pregnancy advanced to accommodate the growing foetus.

The enlargement of the amnion and the extension of the exocoelom had separated a part of the vascular splanchnopleure from its association with the placenta on the proximal part of the yolk sac. Hence, the chorio-vitelline placenta was abolished from this region. However, it was present in relation to the distal half of the lateral sides of the yolk sac which was in contact with the uterine wall. The endodermal cells of the yolk sac were cubical and contained spherical nuclei in the regions where vitelline vessels had extended, while they were squamous in the abembryonic bilaminar omphalopleure.

The allantois had grown across the exocoelom carrying foetal blood vessels to the already existing chorionic placenta on the antimesometrial side of the uterus thus converting this into the chorio-allantoic placenta. There was a large allantoic vesicle which had expanded on the foetal surface of the placenta. This stage was characterised by the development of two caruncle-like protuberances (figure 9), which bulged into the exocoelom on the foetal side of the placental cup, one on the cranial and the other on the caudal side (with respect to the maternal uterus) at the margins of the chorio-allantoic placenta and between the chorio-allantoic and chorio-vitelline placentae.

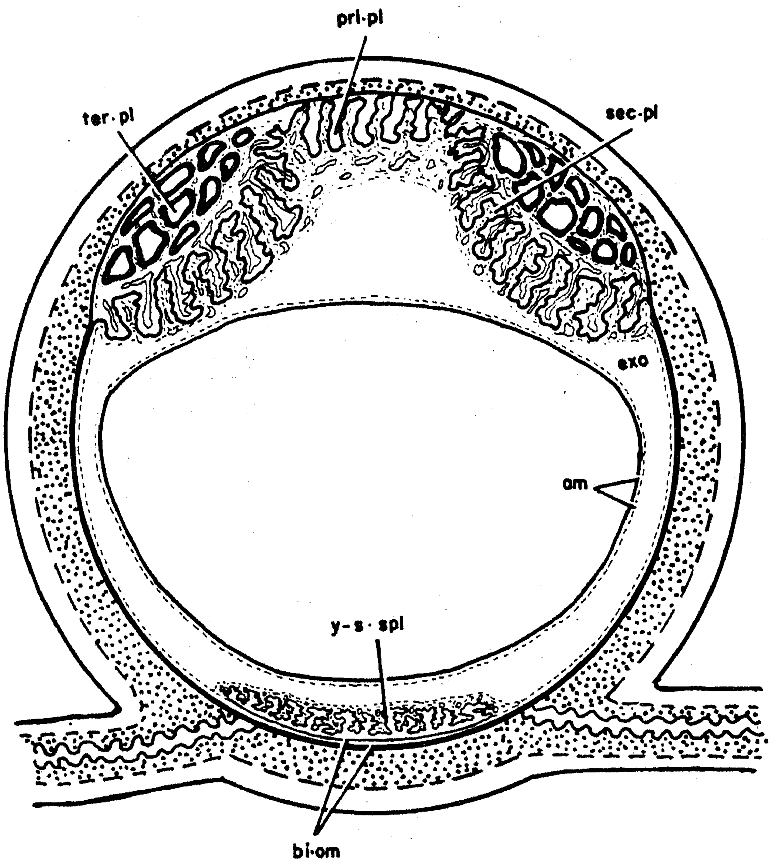

Figure 5. Semischematic diagram of the sagittal section of the gravid uterus to illustrate the arrangement of the foetal membranes at full term. (y-s. spl: yolk-sac splanchnopleure. Other legends as in previous figures).

The placenta at this stage was cup-shaped and can be recognised into three types (a) an extensive primary chorio-allantoic placenta on the antimesometrial side of the uterus, (b) the secondary allantoic placenta in the form of two caruncle- like structures mentioned above, and (c) the chorio-vitelline placenta on the lateral sides of the gestation sac. Whereas the foetal mesenchyme and foetal blood vessels entered the

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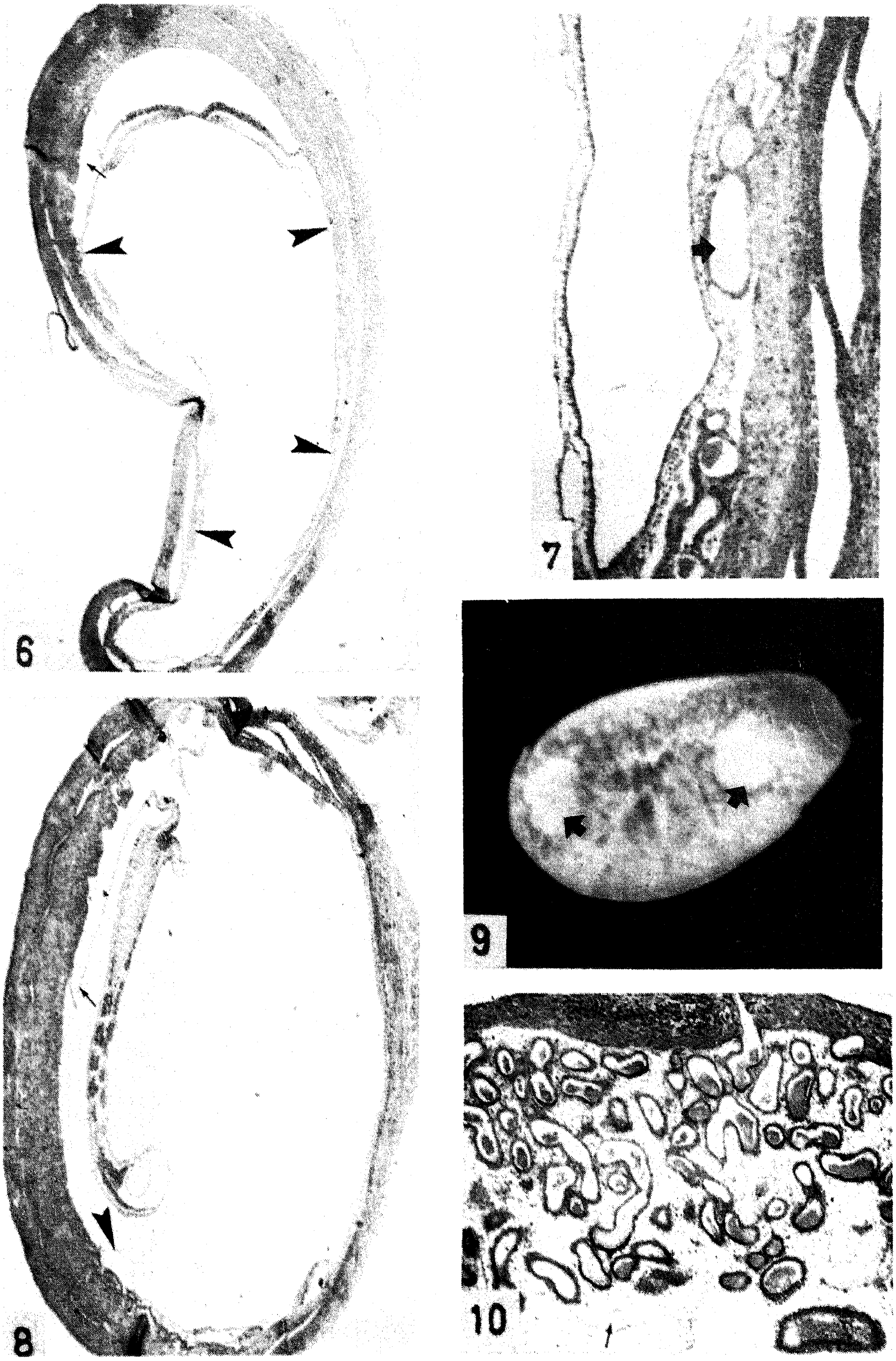
Figures 6–10. 6. TS of the uterus containing an embryo in the early neural groove stage of development. Arrow points to the exocoelom and the region between the arrowheads constitute the chorio-vitelline placenta (× 50). **7.** Part of the choriovitelline placenta at neural groove stage of development of the embryo. Note the large cut ends (arrow) of maternal blood capillary loops surrounded by trophoblast adjacent to the region of the chorio-vitelline placenta (× 145). **8.** Sagittal section of the uterus containing an embryo at very advanced neural groove stage of development. Note the amniotic folds growing from the margin of the embryonic disc. The tail fold (arrow) has grown to about half the distance and the head fold (arrowhead) to a short distance. The exocoelom has separated the amniotic folds from the chorion (× 52). **9.** Dissected uterus to expose the foetal surface of the antimesometrial side of the uterus containing an embryo in the early limb-bud stage of development. Note the presence of two secondary placental discs (arrow) (× 6). **10.** Part of the primary chorioallantoic placenta on the antimesometrial side of the uterus at early limb-bud stage. Note the presence of cut ends of numerous primary placental tubules which are all nearly of the same diameter. The placental tubules lie within a mass of allantoic mesenchyme containing foetal capillaries (arrow) (× 120).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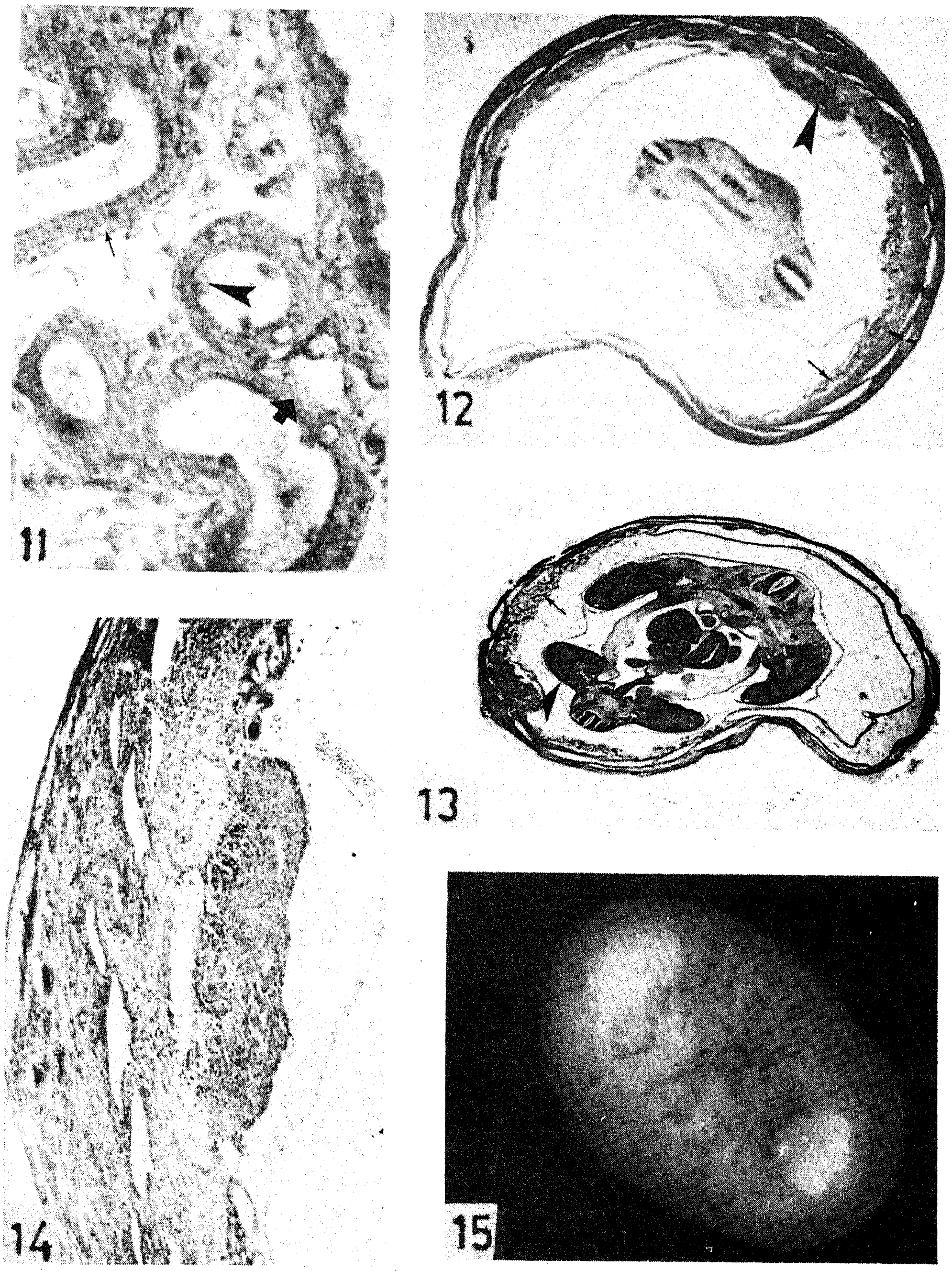
placental complex in the primary chorio-allantoic placenta, they remained on the foetal surface in the secondary chorio-allantoic placenta and the chorio-vitelline placenta.

Histologically, the primary chorio-allantoic placenta was composed of a large number of simple placental tubules, which lay in a mass of foetal mesenchyme in which foetal blood capillaries occurred (figure 10). Each placental tubule consisted of an enlarged maternal blood capillary surrounded by two sheaths of trophoblast—an inner syncytiotrophoblast and an outer cytotrophoblast (figure 11). The maternal blood capillary had a distinct endothelial lining with squamous cells with darkly staining nuclei. The endothelial cells of the maternal capillaries within the placental tubules became progressively sparse as the tubules reached the foetal borders of the placenta. The syncytiotrophoblast had basophilic, darkly staining cytoplasm and irregularly scattered nuclei, whereas the cytotrophoblastic layer consisted of a regular row of cells with lightly staining cytoplasm and dark nuclei. A distinct PAS-positive membrane occurred between the endothelial lining and the syncytiotrophoblast. Examination of serial sections revealed that a large number of maternal blood capillaries emerged out of the endometrial tissue and became surrounded by the trophoblastic sheaths as they passed into the placental zone. While most of the capillaries remained unbranched, a few of them broke into one or two branches. Hence, most of the placental tubules were very nearly of the same calibre. The capillaries within the placental tubules returned to the endometrium after passing through the mass of foetal mesenchyme and joined large maternal channels in the endometrium. Hence, the placenta appeared to be composed of numerous capillary loops surrounded by trophoblastic layers and embedded in foetal mesenchyme. Some of the nuclei of the syncytiotrophoblast in a few placental tubules were in the process of disintegration, and the disintegrated nuclei appeared in stained sections of the placental tubules as a band of darkly staining row of granules embedded in the syncytiotrophoblastic lamina in line with the circle of the intact nuclei of the syncytiotrophoblastic layer.

The two small caruncle-like structures mentioned earlier were the forerunners of the secondary chorio-allantoic placental discs which were formed by the hyperplasia of the periplacental syncytiotrophoblast and the underlying endometrial stromal tissue. The caruncles bulged towards the foetal side of the placental cup (figure 12). The cells of the endometrium had also undergone hypertrophy and vacuolation and there were numerous small fluid-filled intercellular spaces in this region. The layer of cytotrophoblast, which was in continuation with the cytotrophoblastic layer of the primary placenta and the chorio-vitelline placenta, formed the superficial coat on the foetal surface of the caruncle. There was a mass of allantoic mesenchyme with a few foetal blood capillaries on the foetal surface of the caruncle (figure 13).

3.3 *Late limb-bud stage*

The arrangement of the foetal membranes at this stage is illustrated in figures 3 and 14. The foetus had developed two pairs of limb-buds. Exocoelom had expanded and extended to the lateral sides of the yolk-sac wall resulting in the complete separation of the vascular splanchnopleure from the chorion resulting in the abolition of the chorio-vitelline placenta. The separated yolk-sac splanchnopleure had undergone partial collapse and was pushed towards the abembryonic bilaminer omphalopleure. The histological structure of the yolk-sac wall had not undergone any noticeable change



Figures 11–15. **11.** A few of the primary placental tubules at early limb-bud stage to show the presence of maternal endothelial cells (arrowhead), syncytiotrophoblast (thick arrow) and cytotrophoblast (thin arrow) (× 550). **12.** TS of the gravid uterus containing an embryo in the early limb-bud stage of development. The choriovitelline placenta (arrow) occurs on the lateral sides of the gestation sac. Arrowhead points to the secondary chorio-allantoic placental caruncle. (× 43). **13.** TS of the uterus containing an embryo in late limb-bud stage of development. Arrow points to the primary placenta and the arrowhead to the secondary chorio-allantoic placenta. (× 20). **14.** The secondary placental caruncle at early limb-bud stage. Note the presence of foetal mesenchyme and allantoic capillaries lying on the foetal side of the secondary placental bulge. (× 84). **15.** Foetal surface of the dissected uterus at late limb-bud stage to show the presence of two secondary placental caruncles at late limb-bud stage of development (× 4·5).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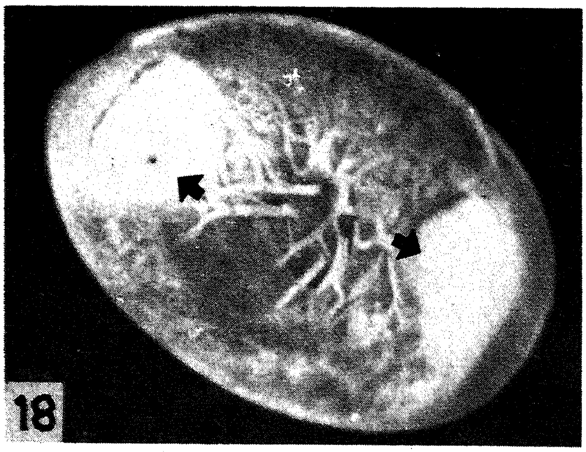
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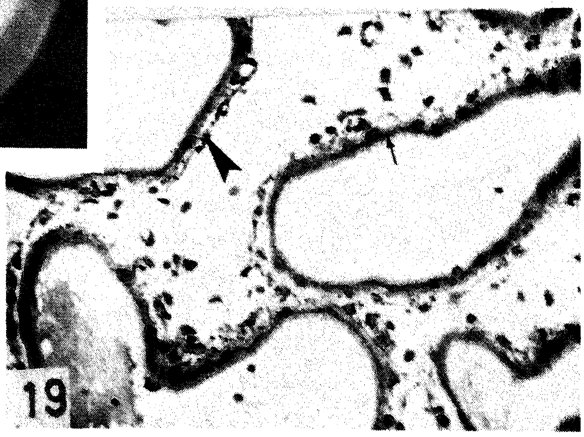
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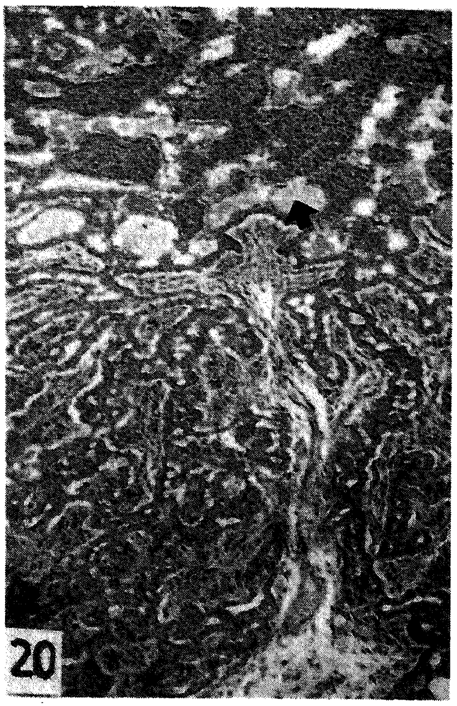
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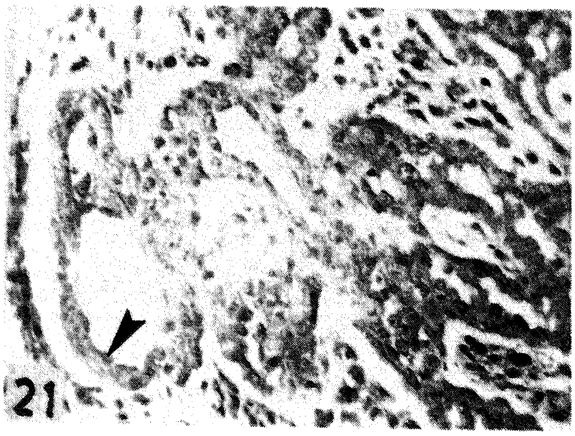
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over the previous stage. In the bilaminer segment of the yolk-sac wall the cells of the trophoblast, which were in close apposition with the uterine endometrial tissue, had hypertrophied and had become cubical to columner.

The allantoic mesenchyme carrying foetal blood vessels had spread on the entire foetal surface of the placenta thereby converting the entire placenta into the chorio-allantoic placenta. In sectional views the allantoic vesicle appeared like a crescentic space in the allantoic mesenchyme between the amnion and the placenta.

The chorio-allantoic placenta could be recognised into two types (a) the bowl-shaped primary chorio-allantoic placenta occupying a wide area of the uterine wall on the antimesometrial side of the uterus, and (b) two secondary chorio-allantoic placental discs, one on each side of the primary allantoic placenta (figure 15). The structure of the primary placenta was similar to that in the previous stage.

The secondary placental caruncles had enlarged and had become discoid. One or two afferent maternal blood vessels coming from the uterine endometrium passed through the entire thickness of the disc and, after reaching the foetal border of the disc, broke up into several branches which ran in all directions along the foetal border of the disc. All along their length they opened into small capillaries which returned the blood to large veins deep in the endometrium. The cytotrophoblastic layer had been pushed from the foetal surface of the placental disc deep into the syncytiotrophoblastic zone of the disc in the form of numerous hollow villi into which foetal mesenchyme had entered (figure 16).

3.4 *Mid-pregnancy*

The general topography of the foetal membranes at this stage is illustrated in figure 4. The yolk sac had undergone further collapse and the endodermal cells of the vascular splanchnopleure had hypertrophied and had become cubical, each with a large centrally placed nucleus. The mesodermal cells also had hypertrophied, but to a lesser extent, and its wall on the foetal side was adhering to the amnion in some places.

The chorio-allantoic placenta could be recognised into three kinds at this stage (a) the primary placenta on the antimesometrial side of the uterus, (b) two secondary placental discs, and (c) a tertiary placenta embedded within each of the secondary placental discs. The primary and the secondary placentae bore the same topographical relationships as

Figures 16–21. **16.** Part of the foetal surface of the secondary placental disc at late limb-bud stage of development. Note the invasion of foetal mesenchyme into the disc (× 280). **17.** Sagittal section of the antimesometrial part of the uterus at mid-pregnancy to show the primary placenta (arrow) and two secondary placental discs on either side of it (× 10). **18.** Foetal surface of the placenta at about mid-pregnancy. Note the two secondary placental discs (arrow) (× 4·5). **19.** Primary placental tubules at mid-pregnancy. Note the absence of the endothelial lining and the disintegration of most of the nuclei of syncytiotrophoblast which occur in the form of numerous darkly staining granules forming a dark band (arrow). Arrowhead points to the layer of cytotrophoblast (× 550). **20.** The junction between the secondary placental disc and the remnants of the uterine endometrium at mid-pregnancy to show the formation of the tertiary placenta in the form of large lacunae (arrow). The secondary placenta is composed of a network of tubules (× 120). **21.** Part of the secondary placenta at mid-pregnancy. Note the presence of maternal endothelial cells (arrowhead) inside the placental tubules (× 160).


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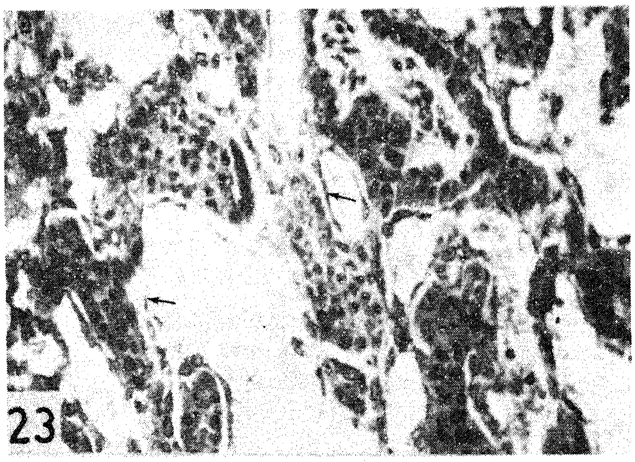
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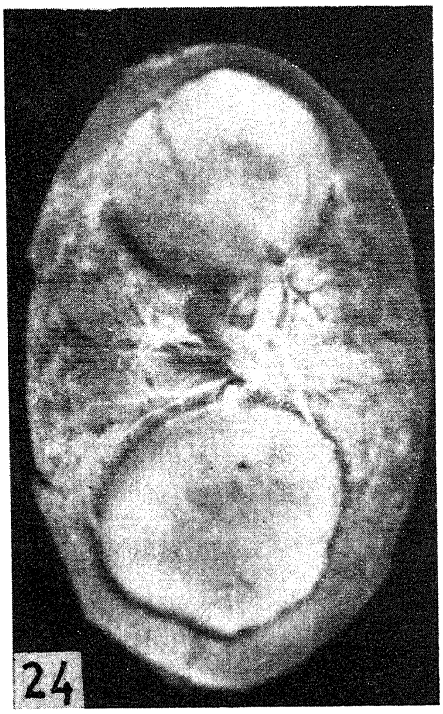
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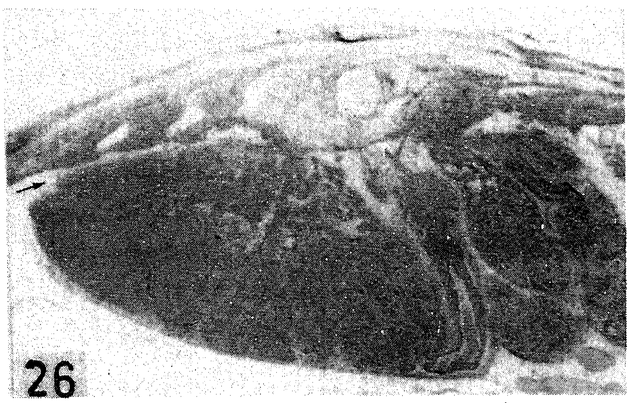
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in the previous stage except that the secondary placental discs had enlarged and had partly encroached on the primary placenta (figures 17 and 18). The beginnings of the tertiary placenta were noticeable deep inside the secondary placental discs in the junctional zone between the placental discs and the uterine endometrium.

The only apparent change in the histology of the primary placenta was the disintegration of most of the nuclei of the syncytiotrophoblast to form darkly staining granules, which, in stained sections of most of the tubules, occur as a dark band formed by the juxtaposition of these granules in the center of the cytoplasm of the syncytiotrophoblast with almost no nucleus or only a few nuclei spaced far from one another (figure 19). The cytotrophoblastic sheath had also become considerably attenuated in most of the tubules and occurred in some places as a layer of lightly stained widely separated cells surrounding the tubules.

The histology of the secondary placental discs had undergone considerable change over the previous stage. Numerous chorionic villi, which had penetrated deep into the mass of the disc from the foetal surface of the disc, had widened and had become branched, and these branches had become inter-connected (figure 20). Consequently, the intervening maternal channels invested by syncytiotrophoblast had become compressed into tubules—the placental tubules of the secondary placenta—which had become invested by an outer sheath of cytotrophoblast belonging to the invading chorionic villi. Thus, was formed a three-dimensional network of placental tubules each having a central maternal blood capillary surrounded by an inner syncytiotrophoblastic and an outer cytotrophoblastic sheath. Endothelial cells were present in the maternal capillaries within the placental tubules but they were reduced in number and were placed far apart (figure 21). The meshes of the network of placental tubules were occupied by foetal mesenchyme and foetal blood capillaries.

The syncytiotrophoblastic mantle on the maternal border of the secondary placental discs had undergone hyperplasia and had formed a large mass of cytoplasm in which darkly stained clusters of nuclei occurred randomly (figure 22). Numerous maternal blood capillaries with distinct endothelial lining of flat cells with dark fusiform nuclei occurred in the syncytiotrophoblastic mass (figure 23). This entire complex was the forerunner of the tertiary placenta. The blood capillaries in the tertiary placenta were continuous with the maternal capillaries in the placental tubules in the secondary placental discs.

At a slightly more advanced stage of pregnancy the enlargement of the secondary placental discs had reduced the extent of the primary placenta considerably (figure 24).

Figures 22–27. **22.** Part of the utero-placental junction in the region of the secondary chorio-allantoic placenta at mid-pregnancy. Note the presence of large masses of syncytiotrophoblast (arrow) at the maternal border of the secondary placenta. Note also large lacunae (arrowhead) of the tertiary chorio-allantoic placenta (× 112). **23.** Part of the syncytial area at the margin of the secondary placental disc at mid-pregnancy. Note the large pools of maternal blood and the presence of flat endotholial cells (arrow) bordering the lacunae (× 550). **24.** Foetal surface of the chorio-allantoic placenta at an advanced stage of pregnancy. Note the enlarged secondary placental discs (× 5). **25.** A few degenerating tubules of the primary chorio-allantoic placenta at advanced pregnancy. See text for description. Arrow points to the cytotrophoblastic layer (× 500). **26.** Section of the uterus showing the relationship between the secondary placental disc and the uterine wall at advanced pregnancy. Arrow points to the chorionic layer which has deeply undercut the placental disc. (× 22). **27.** Part of the tertiary chorio-allantoic placenta at advanced pregnancy. (× 100).

The primary placental tubules had undergone rapid degeneration, and most of the cells of the maternal capillary endothelial lining, and nearly all the nuclei of the syncytiotrophoblast, had disappeared leaving only the cytotrophoblastic layer with vesicular nuclei (figure 25). An endothelial cell, however, could be located in a few of the tubules. However, in all the primary placental tubules there was a prominent PAS-positive lamina lining the lumina of the tubules.

Each secondary placental disc was deeply undercut by a layer of chorion carrying foetal capillaries from all the sides. Hence, in stained sections the disc appeared to be attached to the uterine wall by a wide peduncle (figure 26). There was no histological change in the secondary placenta except for the attenuation of the cytotrophoblastic layer covering the placental tubules. This layer was absent from many of the tubules.

There was a marked change in the histology of the tertiary placenta. The enlargement and confluence of adjacent maternal capillaries had resulted in the formation of an interconnected labyrinth of large blood lacunae (figure 27) surrounded by an inner sheath of syncytiotrophoblast and an outer sheath of cytotrophoblast (figure 28), the latter being continuous with the trophoblastic layer of the chorion which undercut the placental disc. Due to the expansion of the maternal blood spaces the syncytiotrophoblastic layer was compressed to form a sheath to the blood spaces. The endothelial cells had disappeared from most of these blood channels, and a thick layer of homogeneous eosinophilic cytoplasm formed the inner lining of these channels. In a few lacunae the endothelial cells were present on the maternal border of the eosinophilic cytoplasmic layer. A PAS-positive dark scarlet discontinuous membrane lay embedded within the thick cytoplasmic layer in the wall of all the lacunae. Allantoic mesenchyme carrying foetal capillaries invaded the tertiary placenta from the chorion, which undercut the secondary placental discs, and occurred between the walls of adjacent lacunae, thus effecting the foetal vascularisation of the tertiary placenta.

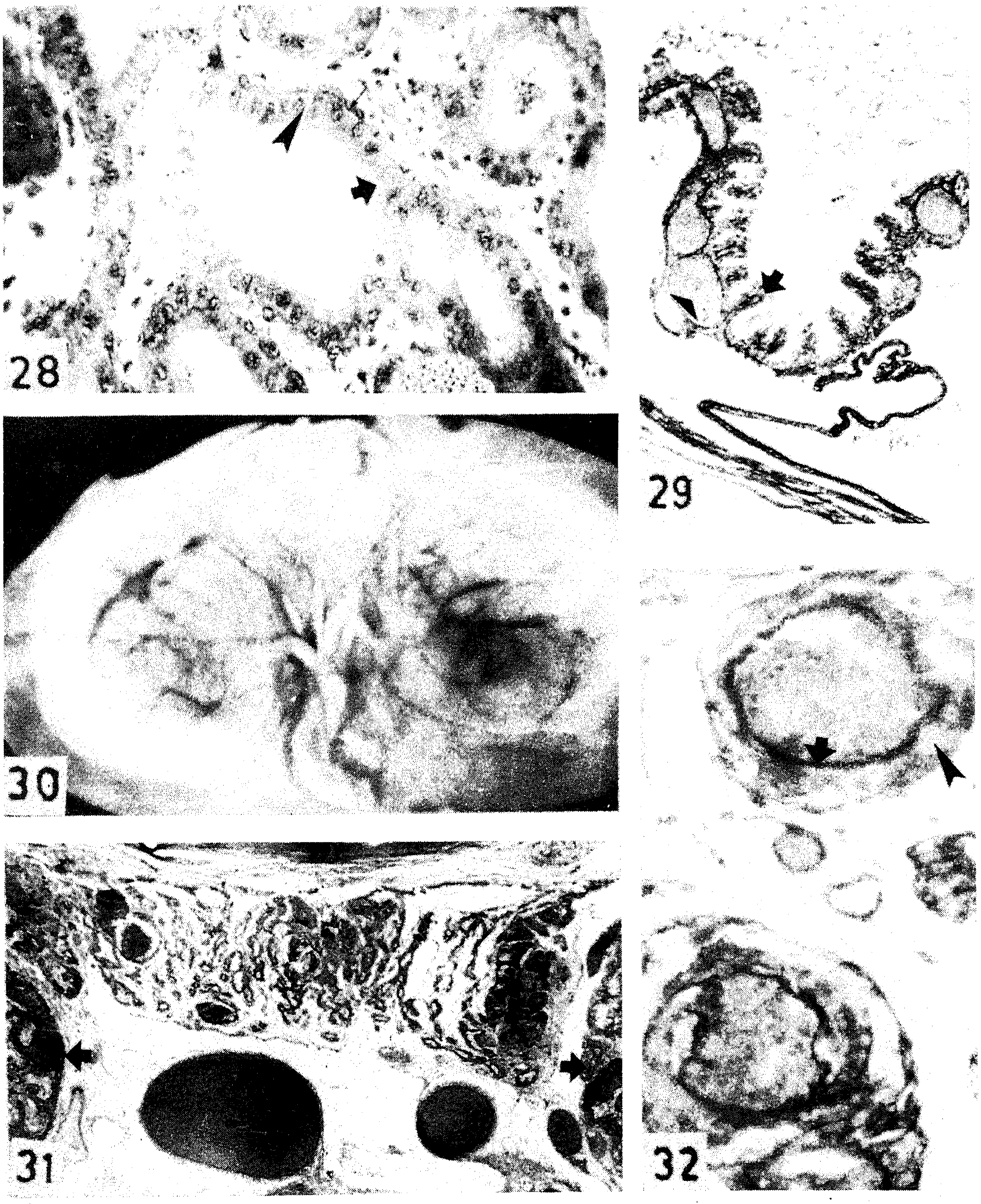
3.5 *Full term*

The general topography of the foetal membranes is illustrated in figure 5. The amnion closely adhered to the body of the foetus except in the region of the cervical flexure where a small fluid-filled amniotic cavity persisted.

The yolk sac occurred on the mesometrial side in the form of a collapsed bag with highly folded splanchnopleure resulting in the yolk-sac lumen becoming reduced to narrow spaces. Apart from the general folding of the splanchnopleure, the mesodermal layer was thrown into numerous secondary folds which projected into the exocoelom (figure 29). The endodermal layer was composed of flat cells with darkly staining fusiform nuclei in these regions whereas the mesodermal cells were large, polygonal, vacuolated and contained spherical nuclei. In the abembryonic region, where the bilaminer omphalopleure was in contact with the endometrium, the trophoblast layer was composed of tall columner cells.

The chorio-allantoic placenta consisted of two large secondary placental discs lying one behind the other in the cranio-caudal axis of the uterus, and these were separated by a narrow gap which was all that remained of the primary placenta. The umbilical cord was inserted in the region of the primary placenta (figure 30). There was no allantoic vasicle.

The primary placenta (figure 31) was composed of a few placental tubules at different



Figures 28–32. **28.** Part of the tertiary placenta at advanced pregnancy. Note the absence of maternal capillary wall within the lacunae. Arrowhead points to syncytiotrophoblast and thin arrow points to cytotrophoblast, thick short arrow points to the homogeneous cytoplasmic layer (× 550). **29.** Part of the free yolk-sac splanchnopleure at full term. Note the secondary folds of the mesodermal layer (arrow) which projects into the exocoelom. Arrowhead points to the cut end of a vitelline blood capillary (× 140). **30.** Foetal surface of the placenta at full term to low the large secondary placental discs. The large dark nearly circular patch in the middle of each disc indicate the presence of the tertiary placenta in the deeper regions of the secondary placenta (× 4). **31.** Part of the primary placenta occurring between the secondary placental discs (arrow) at full term. Note the degenerating tubules of the primary placenta. (× 80). **32.** Cut ends of two of the partially degenerated tubules of the primary placenta at full term. Note the thick dark membrane (arrow) forming the inner membrane of the tubule. Arrowhead points to a nucleus of ιhe cytotrophoblast (PAS staining) (× 550).

stages of degeneration. The less degenerated tubules had on their maternal border a thick eosinophilic, PAS-positive, homogeneous, discontinuous membrane surrounded by a sheath of cytotrophoblast consisting of large vacuolated cells containing darkly stained irregularly shaped nuclei (figure 32). The eosinophilic PAS-positive membrane represented the remnants of the maternal endothelium and the syncytiotrophoblastic layer of the placental tubules. In the tubules, which were in an advanced stage of degeneration, the wall was made of a layer of cytotrophoblast composed of large vacuolated cells. The inter-tubular areas were occupied by foetal mesenchyme and foetal blood capillaries.

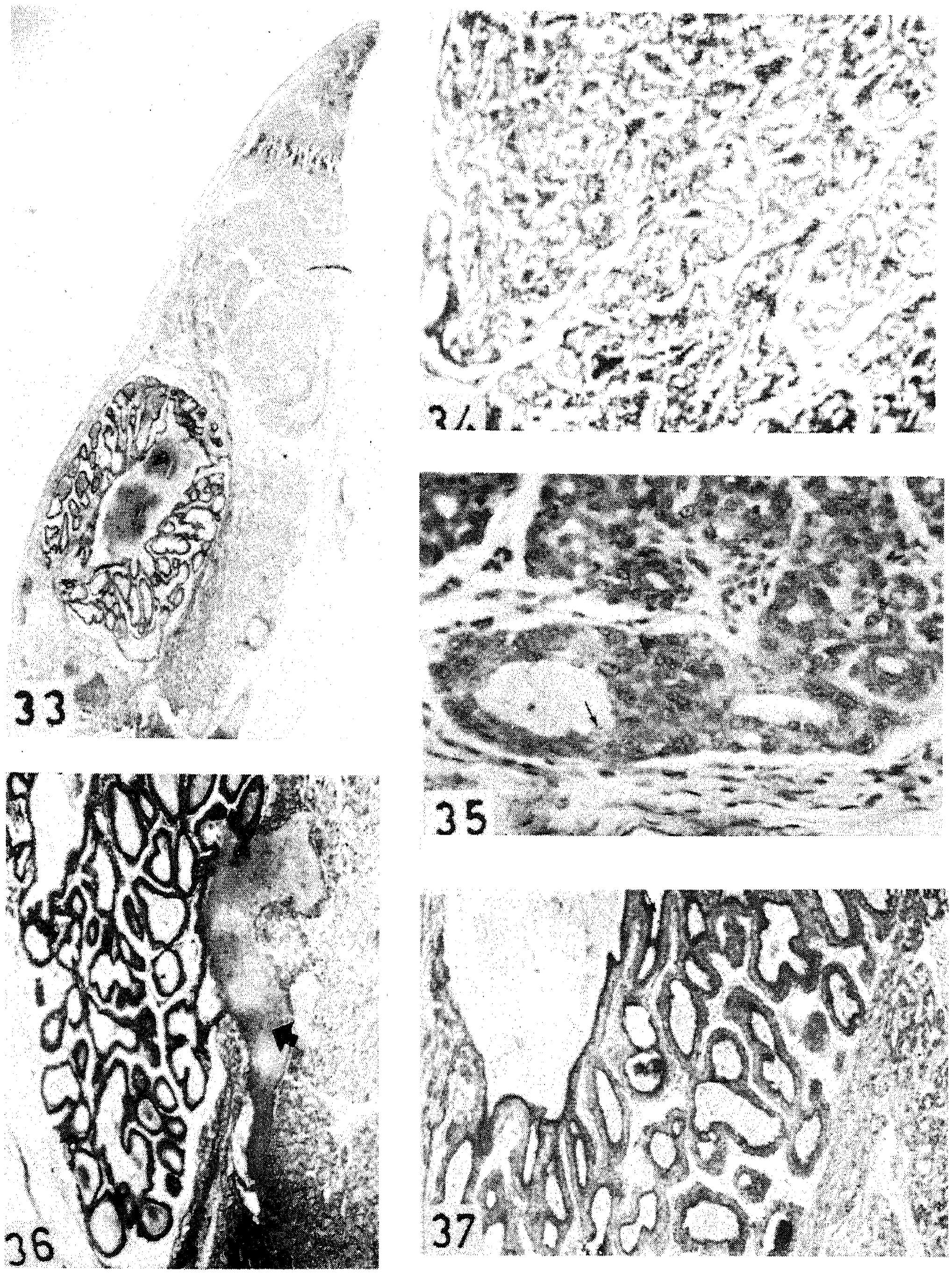
Each secondary placental disc (figure 33) was made up of a three-dimensional network of closely arranged placental tubules (figure 34) each composed of a central maternal blood capillary in which the endothelial cells occurred as widely separated fusiform cells with small dark nuclei (figure 35) except in the large vessels at the maternal border of the disc where the endothelial cells were large and close together. Each tubule was surrounded by a layer of syncytiotrophoblast, which occurred as a homogeneous zone of cytoplasm with irregularly scattered lightly staining nuclei. The inter-tubular areas were occupied by foetal mesenchyme and foetal capillaries.

There were two or three large pools of maternal blood surrounded by a thin endothelial lining and syncytiotrophoblast at the junction between each secondary placental disc and the tertiary placenta embedded in it (figure 36). Maternal blood capillaries in the placental tubules of the secondary placenta opened into these large pools which, in their turn, opened into the lacunar system of the tertiary placenta.

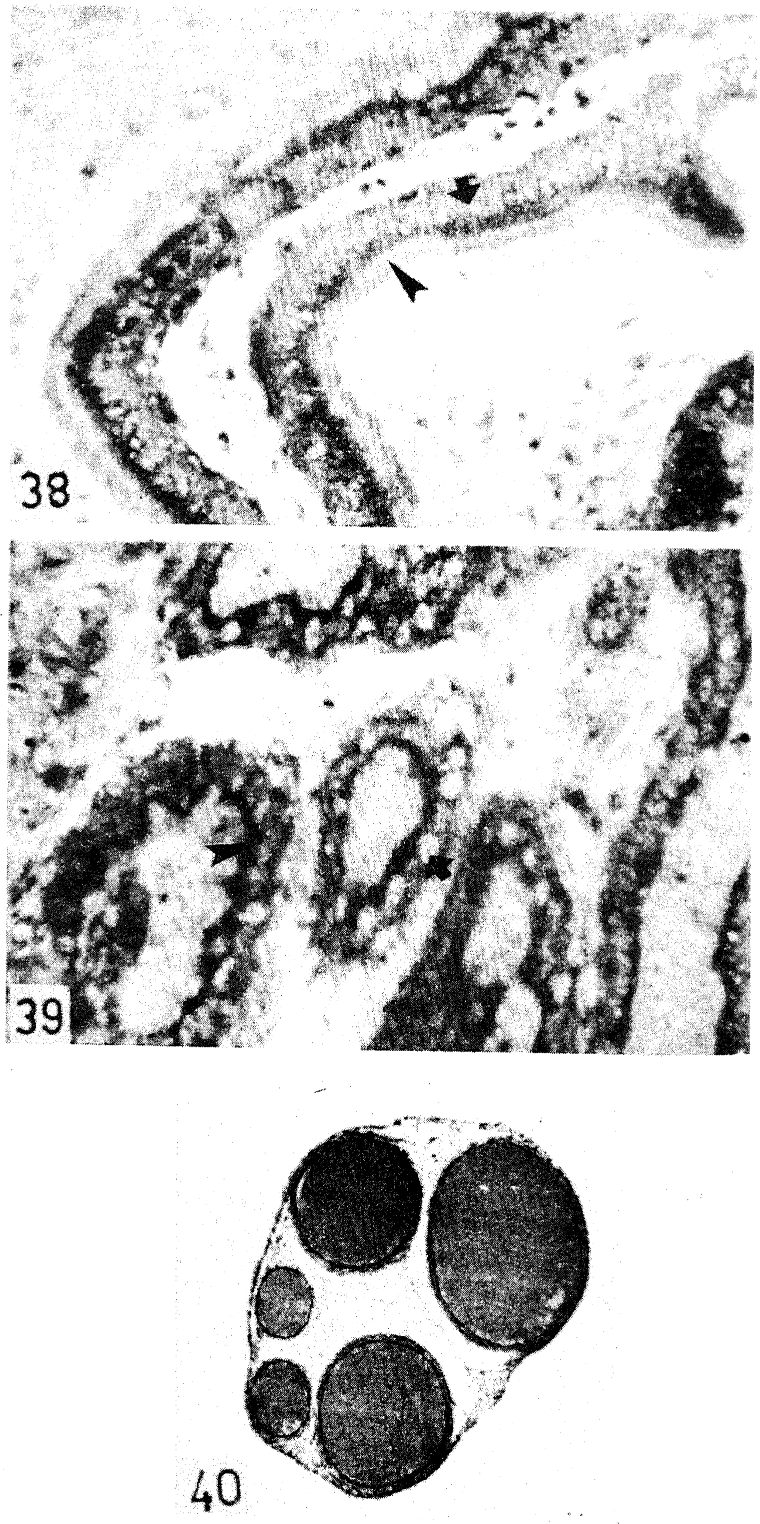
The tertiary placenta lay embedded deep within each secondary placental disc near its myometrial border, and consisted of a labyrinth of inter-connected large lacunae containing maternal blood (figures 33 and 37). The lacunae were surrounded by thick walls composed of a layer of darkly staining syncytiotrophoblast with large spherical nuclei arranged close together in a row (figure 38). The cytotrophoblastic layer was absent from the walls of the lacunae. A few lightly staining cells abutting against the outer surface of the syncytiotrophoblastic sheath between the placenta and the thin remnants of endometrium at the utero-placental junction, and which were continuous with the cells of the periplacental layer of trophoblast, were the only remnants of cytotrophoblast. However, no cytotrophoblast was present in the lacunar system of the tertiary placenta. Foetal mesenchyme and foetal blood capillaries occurred in the regions between the walls of the adjacent lacunae. In sections stained by PAS-procedure, a dark, thick, scarlet, homogeneous layer became evident in the inner surface of the lacunae (figure 39). This layer is also intensely eosinophilic.

The umbilical cord was about 2 cm long and was gently twisted two or three times along its length. It was inserted to the region of the primary placenta and broke up into two main branches, one to each secondary placental disc, and to a few small branches irrigating the remnants of the primary placenta. Sections of the umbilical cord (figure 40) revealed that there was no remnant of the allantoic duct. There were five blood vessels comprising of a vitelline artery, a vitelline vein, two allantoic arteries and an allantoic vein, the last being the widest vessel in the umbilical cord.

Four to five large maternal blood vessels coming from the uterine wall pass through the entire thickness of each secondary placental disc and after reaching the foetal border of the disc, broke into several branches which raidated in all directions along the foetal surface of the placental disc. Blood from the radial vessels trickled into the placental tubules through fine ostia all along their length. Blood from the placental



Figures 33–37. 33. Section showing the relationship between the secondary placental disc and the tertiary placenta at full term. Please see text for description (× 34). **34.** Part of the secondary placenta at full term to show its labyrinthine nature (× 60). **35.** Cut ends of a few tubules of secondary placenta at full term. Arrow points to the endothelial cells of the maternal capillary inside the tubule (× 210). **36.** Part of the secondary and tertiary placentae at full term. Arrow points to a large pool of maternal blood at the junction between the secondary and tertiary placentae (× 100). **37.** Part of tertiary placenta at full term. Please see text for description (× 130).



Figures 38–40. **38.** Part of the tertiary placenta at full term to show the composition of the walls of the lacunae surrounding maternal blood spaces. Arrowhead points towards the homogeneous cytoplasmic layer; arrow points to the syncytiotrophoblastic layer. (× 680). **39.** A few of the lacunae of the tertiary placenta. Note the dark homogeneous layer (arrowhead) on the inner surface of the lacunae. Arrow points towards the nucleus of the syncytiotrophoblast. (PAS staining) (× 550). **40.** Section of the umbilical cord at full term to show the presence of five blood vessels (× 410).

tubules collected into large pools (mentioned earlier) in the junction between the secondary placenta and the tertiary placenta and then passed into the lacunar system of the tertiary placenta. Blood from the tertiary placenta returned to efferent maternal veins in the junctional zone between the placenta and the myometrium. It was evident that there were two places in the placental disc where maternal blood accumulated before it returned to the maternal circulation. Perhaps, the formation of these two pools of maternal blood in the placenta is an adaptation in this bat to retain maternal blood in the placenta as long as possible so that maximum physiological exchange can take place.

The parietal layer of chorion, which lay closely abutting against the lateral wall of the uterus, consisted of hypertrophied tall columnar cells with lightly staining spherical nuclei and a thin layer of extra-embryonic mesoderm on its foetal surface. The trophoblastic layer of the parietal chorion was in continuity with the trophoblastic layer of the abembryonic bilaminar omphalopleure and also with the periplacental trophoblastic layer between the placenta and the myometrium.

4. Discussion

The foregoing study has revealed that *Miniopterus schreibersii fuliginosus* presents some unique embryological features. It differs from all other members of the family Vespertilionidae (in which *Miniopterus* has been included so far) in the circumferential attachment of the embryo to the uterine wall resulting in the obliteration of the uterine lumen at the level of gestation, whereas in all other vespertilionids so far studied the embryo is attached to the antimesometrial side of the uterus leaving a part of the uterine lumen persisting on the mesometrial side (Duval 1894–1896; Ramaswami 1933; Wimsatt 1944, 1945; Gopalakrishna 1949, 1950; Phansalkar 1972; Gopalkrishna and Sapkal 1974; Luckett 1979).

The literature on the development of the amnion in bats has been reviewed by several workers (Gopalakrishna 1949; Wimsatt 1954; Gopalakrishna and Khaparde 1978; Gopalakrishna and Karim 1979, 1980; Jeevaji 1979). Amniogenesis in *M. schreibersii fuliginosus* appears to be unusual. While the primitive amniotic cavity arises as a small space within the embryonic mass (Chari and Gopalakrishna 1981) as in all other bats (Wimsatt 1944; Gopalakrishna 1949; Bhiwgade 1976; Gopalakrishna and Karim 1979, 1980; Jeevaji 1979; Luckett 1979) the rapid expansion of the embryonic disc tears the roof of the primitive amniotic cavity, and, since the primitive amniotic cavity is roofed by the trophoblastic layer overlying the embryonic disc, the precocious loss of this layer before the trophoblast proliferates and invades the endometrium results in exposing the embryonic plate to the superficial layer of uterine endometrium, the uterine epithelium having disappeared earlier. The amniotic cavity, therefore, becomes confluent with the potential uterine lumen until the definitive amniotic folds develop and fuse on the dorsal aspect of the embryonic plate. A parallel situation has not been noticed in any other bat except perhaps in *Eumops* sp (Hamlett 1947) in which the amniotic cavity was reported to be roofed over by the superficial layer of uterine endometrium for a short period.

The most unique feature of the embryology of this bat is the mode of development and the ultimate structure of the chorio-allantoic placenta. The early stages of development of the placenta are similar to those of other bats so far described (Wimsatt

1945, 1954, 1958; Gopalakrishna 1950, 1958; Gopalakrishna and Moghe 1960; Phansalkar 1972; Sapkal 1973). But the later development of the chorio-allantoic placenta with the development of the secondary and the tertiary placentae in an almost chronological sequence and their ultimate structure are unmatched by any mammal, let alone any bat. The primary chorio-allantoic placenta is progressively replaced by two secondary placental discs, and the tertiary placenta is developed within each of the secondary placental discs. The secondary chorio-allantoic placenta is typically labyrinthine, and endotheliochorial (Vasochorial) and the tertiary chorio-allantoic placenta, which has no parallel in any other mammal, should be considered as haemomomochorial since only the syncytiotrophoblast is present in the walls of the lacunar system of the tertiary placenta.

The placenta of *Miniopterus schreibersii* of Europe was described by Branca (1927), Grosser (1927), Kempermann (1929) and more recently by Mallassine (1970). From these descriptions it is evident that each author described only the histology of the chorio-allantoic placenta at one or two stages of development of this animal. None of the authors, however, gave a complete description of the gestation sac they studied nor did any earlier worker describe the full term placenta of this animal. From the descriptions of Branca (1927) and Kempermann (1929) it is evident that they were describing the pregnant uterus a little earlier than mid-gestation. Apparently, Kempermann's (1929) "Haupt Placenta" refers to the secondary placenta described here. Malassine's (1970) description also appears to refer to a stage corresponding to about mid-pregnancy before the development of the tertiary placenta. His descriptions pertain only to the histological structures of the secondary placenta (which he called "Disque placentarie") corresponding to a stage a little before mid-gestation. None of the authors knew about the development of the tertiary placenta. It is most unlikely that the structure of the placenta of *Miniopterus schreibersii* of Europe can be so different from that of the Indian sub-species of *Miniopterus schreibersii*. The differences between the descriptions of the earlier authors and those of the present are due to the fact that the earlier descriptions were based on the examination of the placenta either before or at about mid-pregnancy. Hence, the earlier authors missed to notice the uniqueness of the development and the histological structure of the definitive placenta of *Miniopterus schreibersii*.

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