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Early Development, Implantation of the Blastocyst and Amniogenesis in the Indian Molossid Bat, *Tadarida Aegyptiaca* (Geoffroy)

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The bilaminar blastocyst of *Tadarida aegyptiaca* implants in an enlarged pocket of the luminal slit at the cranial end of the right uterine cornu. Implantation is superficial and diffuse, and the embryonic mass is oriented towards the lateral side of the uterus. But, at later stages of development the embryonic plate faces the antimesometrial side of the uterus. The primitive amniotic cavity is formed inside the embryonic mass, but its roof is lost as the embryonic mass expands into a plate. Hence, for some period the primitive amniotic cavity is roofed over by the superficial region of the uterine endometrium. Definitive amniotic folds develop from the margins of the embryonic plate and grow dorsally to fuse in the mid-dorsal line. The trophoblast exhibits different intensities of proliferative activity in different regions. It is most proliferative in the abembryonic region where it forms a thick pad by the time the embryo develops into the neural groove stage of development.

Key Words: Early development, Implantation, Amniogenesis, Bat

Introduction

Reports on the early embryology of molossid bats are in the nature of descriptions of a few isolated stages of development of the embryo of a few species. Sansom (1932) described one free and a few implanted blastocyts of *Molossus rufus* and *M. obscurus* and noted that the blastocyst became attached to the uterine wall on all the sides and the embryonic mass was oriented towards the lateral side of the uterus. A primitive amniotic cavity was present in the center of the embryonic mass, and, as the blastocyst expanded and the embryonic mass became flat, the layer of cells of the embryonic mass, which formed the roof of the primitive amniotic cavity, disappeared with the result that the primitive amniotic cavity became roofed over only by the thinned out trophoblastic layer. Mossman (1937) and Stephens (1962) mentioned that implantation in *Tadarida* (*Nyctinomus*) *brasiliensis cynocephala is superficial* and diffuse, and the embryonic plate was oriented antimesometrially. They also mentioned that amniogenesis in this species occurred by development of folds. Pendharkar and Gopalakrishna (1982-1983) reported that in *Tadarida plicata plicata* the orientation of the embryonic mass during early stages of implantation was lateral and that a primitive amniotic cavity was formed within the embryonic mass. The trophoblastic layer, which formed the roof of the primitive amniotic cavity disappeared as the embryonic mass expanded into a plate. The definitive amnion was formed later by the development of folds from the margins of the embryonic plate.

It is evident from the foregoing that there are either significant differences in the early embryology of different molossid species or some

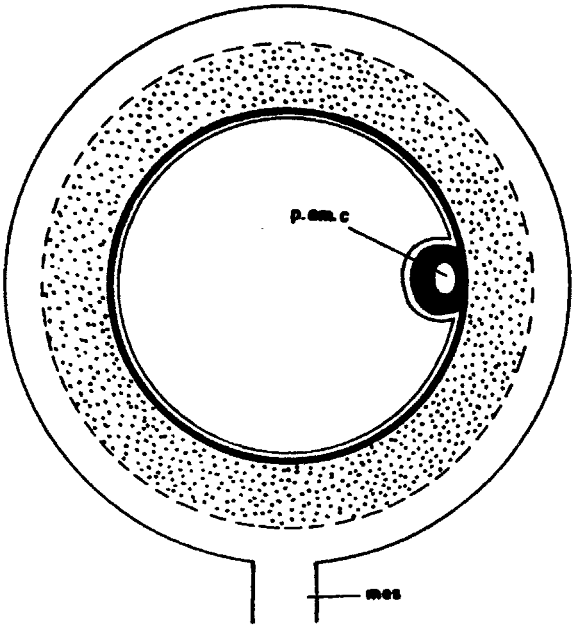


Figure 1 Semischematic representation of the topographical relationship between the implanted bilaminar blastocyst and the uterus (*Please see text for description*)
Mes, mesometrium; *p.am.c* primitive amniotic cavity

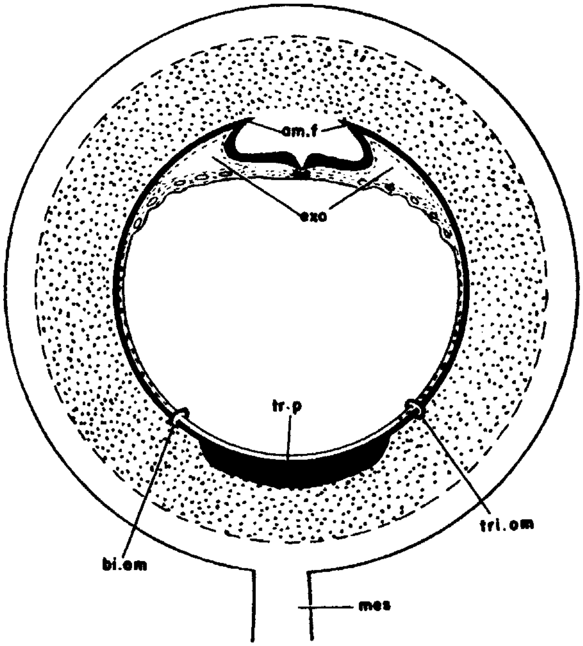


Figure 2 Semischematic representation of the transverse section of the uterus containing an embryo in advanced neural groove stage of development(*Please see text for description*)
am.f; amniotic folds; bi. om. bilaminar omphalopheure; *tr.p,* trophoblastic pad (*Other legends as in previous figure*)

of the conclusions of earlier workers were erroneous due to non-availability of appropriate stages of development. The present report, based on the examination of closely graded stages of development of *Tadarida aegyptiaca,* presents evidence for the proper interpretation of the early embryology of members of this large and cosmopolitan family of bats.

Material and Methods

Pregnant specimens of *Tadarida aegyptiaca* carrying early stages of development were collected at frequent intervals during July in two successive years (1983 and 1984) from Khandwa, Madhya Pradesh. The specimens were killed by *chloroform, their genitalia fixed in various fixatives such as Bouin's, Rossman's, Carnoy's and neutral* formalin for 24 hr and then processed for histological studies following the usual procedure. The paraffin embedded tissues were serially sectioned at 5 to 8 μm *thickness,* stained with Ehrlich's or Harris' haematoxylin, counterstained with eosin, dehydrated by passing through graded ethanol, cleared in xylol and mounted in Canada balsam or DPX.

Results

Breeding Habits

The species has a strictly defined annual breeding cycle. Copulation occurs during the first week of July and fertilisation and pregnancy follow immediately. Ovulation, releasing a single oocyte, occurs invariably from the right ovary and the conceptus is carried in the right uterine cornu. The uterus *is* oblong in transverse sections, and the uterine luminal slit is expanded in the dorso-ventral (mesometrial-antimesometrial) axis in consonance with the shape of the uterus. Deliveries in the colony occur during the last week of September or early in October.

Pre-implantation Stages of Development

Tubal stages: An unfertilised oocyte (figure 3) was located at the ovarian end of the Fallopian tube. The oocyte was surrounded by a mass of small cells containing darkly staining nuclei. These were the cells of cumulus oophorus which had escaped with the oocyte during ovulation. The zona pellucida was thin and the cytoplasm of the oocyte was highly *eosinophilic* and finely granular. The spherical nucleus was located in the centre of the oocyte. Fertilisation occurs when the oocyte is still near the ovarian end of the Fallopian tube in this species. In a two-celled stage (figure 4), which was present in the middle segment of the Fallopian tube, the two cells were so placed that parts of both the cells could be only in one or two sections. The zona pellucida was considerably thicker than in the previous stage. The cytoplasm was vacuolated.

Uterine stages: A free blastocyst with a thin zona pellucida (figure 5) was located in the uterine lumen near the cranial end of the uterus. The embryonic mass, made up of compactly arranged cells with vesicular nuclei, occupied nearly half of the cavity of the blastocyst. The trophoblastic layer was composed of widely separated flat cells with lightly staining oval or spherical nuclei. Thin strands of cytoplasm connected adjacent cells in the trophoblast layer. A more advanced blastocyst (figure 6) than the one described above was present in the middle of the luminal slit near the cranial end of the utcrus. The blastocyst had undergone a little artifactual distortion in shape caused during the preparation of the stained sections. The embryonic mass was composed of a bunch of compactly arranged small cells with darkly stained nuclei. The trophoblast layer was made up of fusiform (in sectional views) cells inter-connected by cytoplasmic processes. A thin zona pellucida could be identified in some places.

A free unilaminar blastocyst (figures 7 & 8) was present in an enlarged pocket of the lumen near the cranial end of the uterus adjacent to the tubo-uterine junction. The zona pellucida had disappeared altogether. The blastocyst was artifactually distorted in shape. The cells of the trophoblast were flat and were widely separated, adjacent cells being connected by thin cytoplasmic strands. Their nuclei were fusiform and darkly staining. The cells of the embryonic mass were loosely arranged and most of them were spherical. Several small inter-cellular spaces were present in the embryonic mass. Some of the cells of the embryonic mass were in mitosis.

The most advanced unilaminar blastocyst available for the present study was a large blastocyst which had established contact with the uterine wall on all the sides of an enlarged pocket of the luminal slit near the cranial end of the uterine cornu (figures 9 & 10). The blastocyst was so oriented that the embryonic mass was facing the lateral side of the uterus. The cells of the trophoblast overlying the embryonic mass were low cuboidal and contained vesicular nuclei. In the rest of the regions the trophoblast layer was composed of widely separated flat cells with mostly oblong darkly staining nuclei. The embryonic mass had expanded laterally and had become slightly discoid and was made up of round cells with large spherical nuclei. Some of the cells were in mitosis. The uterine epithelium was present on all the sides of the implantation chamber although much attenuated in some places, especially adjacent to the abembryonic surface of the blastocyst. In this region, only a thin strip of cytoplasm containing small fusiform pycnotic nuclei was all that remained of the uterine epithelium.

Implanted Bilaminar Blastocysts

In an early implanted bilaminar blastocyst (figures 11 & 12) the embryonic mass had expanded to form a small disc which faced the lateral side of the uterus. The trophoblast was composed of a layer of cuboial cells which lay in close apposition with the endometrial tissue as the uterine epithelium had disappeared from the implantation chamber. Endoderm had become differentiated and underlay the embryonic disc and the trophoblastic layer, and was composed of flat widely scattered cells with fusiform nuclei. Thin cytoplasmic strands connected the adjacent endodermal cells.

An advanced implanted bilaminar blastocyst (figures 13 and 14) was present near the cranial end of the uterus. The embryonic mass faced the lateral side of the uterus. Many inter-connected spaces-the forerunners of the primitive amniotic cavity had formed in the embryonic mass. The trophoblastic layer consisting of cuboidal cells was in contact with the endometrium. The endodermal layer formed a lining of flat cells closely underlying the trophoblastic layer and the embryonic mass. The topographical relationship between the blastocyst and the uterus at this stage of development is schematically shown in figure 1.

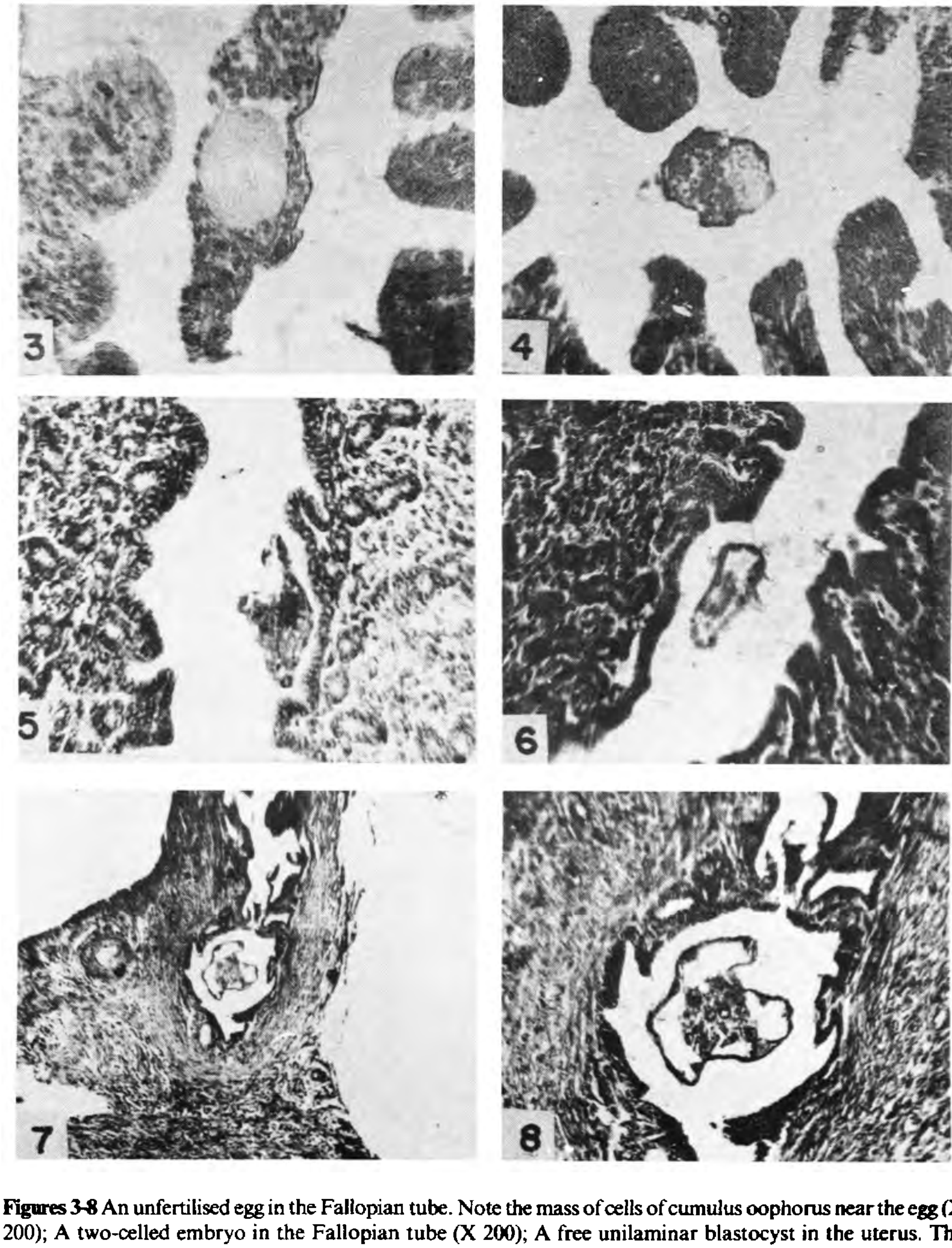
A blastocyst in which the embryonic mass had expanded into a plate composed of three to four layers of cells, was located in an enlarged chamber of the uterine lumen near the cranial end of the uterus (figures 15 & 16). The embryonic plate had expanded towards the antimesometrial side. There was a wide primitive amniotic cavity, for which the embryonic plate formed the floor and a layer of trophoblast formed the roof. In the region of the roof and the lateral sides of the implantation chamber the trophoblastic layer, composed of low cuboidal cells with small spherical nuclei, was in apposition with the superficial layer of uterine endometrium. However, on the abembryonic side, that is, on the mesometrial side of the uterus adjacent to the tubo-uterine junction, the trophoblast had undergone proliferation and had entered the uterine endometrium engulfing and cytolysing the cells of the endometrium on the border of the still persisting uterine luminal slit. Thus, a trophoblastic mass, which was several cells thick, was formed on the mesometrial side of the uterus, and within the mass was incorporated the cavity of the uterine luminal slit containing cell debris of the uterine epithelium and superficial region of endometrium. The endodermal layer was in the form of a thin unilaminar membrane of flat cells closely underlying the embryonic disc and the trophoblast layer.

Several specimens containing embryos in progressively advanced stages of development of the bilaminar blastocyst were available for study. As the embryo advanced in development the embryonic plate expanded considerably towards the antimesometrial side. and the trophoblastic roof of the primitive amniotic cavity disappeared thereby exposing the embryonic plate to the potential uterine lumen. The trophoblastic mass, mentioned in the description of the previous stage, became progressively thicker to form a pad of trophoblast and the cavity, containing cell debris within the pad, became progressively smaller. The endodermal layer, which formed the inner lining to the trophoblast layer, was made up of flat cells with darkly staining fusiform nuclei.

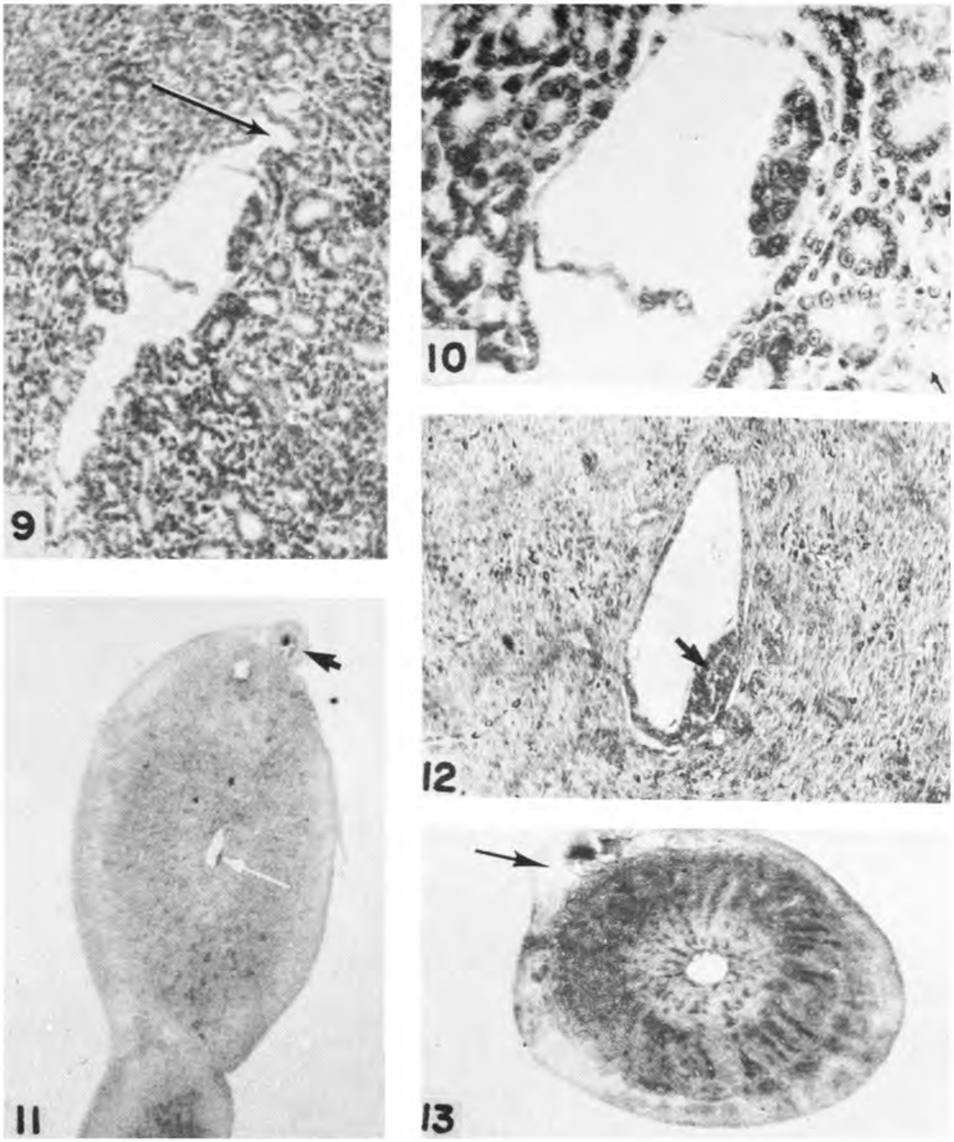
Trilaminar Blastocysts

Figure 17 illustrates the transverse section of the uterus containing an early trilaminar blastocyst. The blastocyst had expanded considerably and the embryonic plate was wide and faced squarely the mesometrial side of the uterus. Since the roof of the primitive amniotic cavity had disappeared, the embryonic plate was exposed to a large cavity the potential uterine lumen the floor of which was formed by the embryoic plate and the roof by the superficial surface of the uterine endometrium (figure 18). Mesoderm had become differentiated and occurred as a band of loosely arranged cells, three to four cells-thick, beneath the embryonic plate and had extended to a little distance on the lateral sides of the blatocyst. In the rest of the regions the blastocyst was bilaminar, and the trophoblast, composed of cuboidal cells, was in apposition with the uterine endometrium.

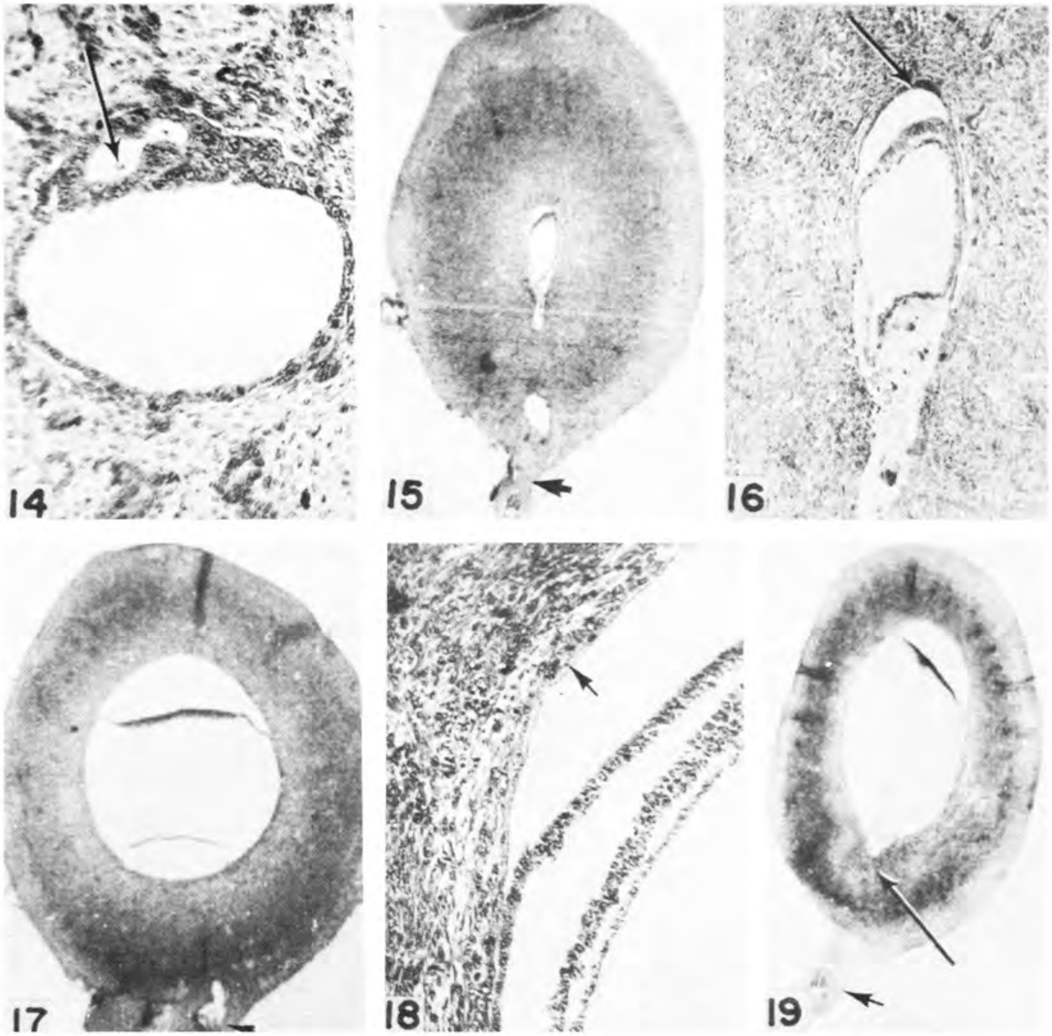
In the most advanced trilaminar blastocyst available for this study (figure 19) the embryonic plate was broad and faced the antimesometrial side of the uterus. Mesoderm had extended to the lateral side of the blastocyst. Only a small area on the abembryonic side was still bilaminar. The large primitive amniotic cavity was roofed by the superficial surface of the uterine endometrium (figure 20). On the lateral sides of the blastocyst the trophoblast was made up of a single layer of cuboidal cells with vesicular nuclei. However, in the abembryonic region the trophoblast had proliferated (figure 21) and had entered the uterine



Figures 3-8 An unfertilised egg in the Fallopian tube. Note the mass of cells of cumulus oophorus near the egg (X 200); A two-celled embryo in the Fallopian tube (X 200); A free unilaminar blastocyst in the uterus. The embryonic mass occupies nearly half the blastocyst cavity, (X 120); A free unilaminar blastocyst located in a slightly enlarged pocket of the uterine luminal slit, (X 90); A free unilaminar blastocyst lodged in an enlarged pocket of the uterine luminal slit (*Please see text for description*) (*X* 100 & 240 respectively)



Figures 9-13 An advanced unilaminar blastocyst which is in contact with the uterine wall. Uterine epithelium is present on all the sides of the implantation chamber. The embryonic mass is laterally oriented. Arrow points to the remnant of the uterine lumen on the mesometrial side(X 128 & 280 respectively); An implanted bilaminar blastocyst. Please see text for description. White arrow in figure 11 and black arrow in figure 12 point to the embryonic mass. Black arrrow in figure 11 points to mesometrium, (X 24 & 140 respectively); An implanted bilaminar blastocyst with a small primitive amniotic cavity in the embryonic mass. Arrow points to the mesometrium (*Please see text for description*) (X 24)



Figures 14-19 Enlarged part of figure 13 to show the implanted blastocyst and primitive amniotic cavity (shaded arrow) within the embryonic mass. Uterine epithelium has disappeared from all the sides of the implantation chamber, (X 180); Section of uterus containing an advanced implanted bilaminar blastocyst. The embryonic plate is oriented antimesometrially. The large primitive amniotic cavity is roofed over by a layer of trophoblast (shaded arrow). The trophoblast on the abembryonic side has proliferated. Black short arrow in figure 15 points to mesometrium. (X 24 & X 100 respectively); Section of the uterus containing an early trilaminar blastocyst with a large primitive amniotic cavity whose roof is formed by the superficial layer of uterine endometrium (arrow in figure 18). Arrow in figure 17 points to mesometrium (X 24 & X 180 respectively); Section of the uterus containing an advanced trilaminar blastocyst. (*Please see text for description*). Shaded arrow points to the trophoblastic pad. Black arrow points to the mesometrium, (X 24)

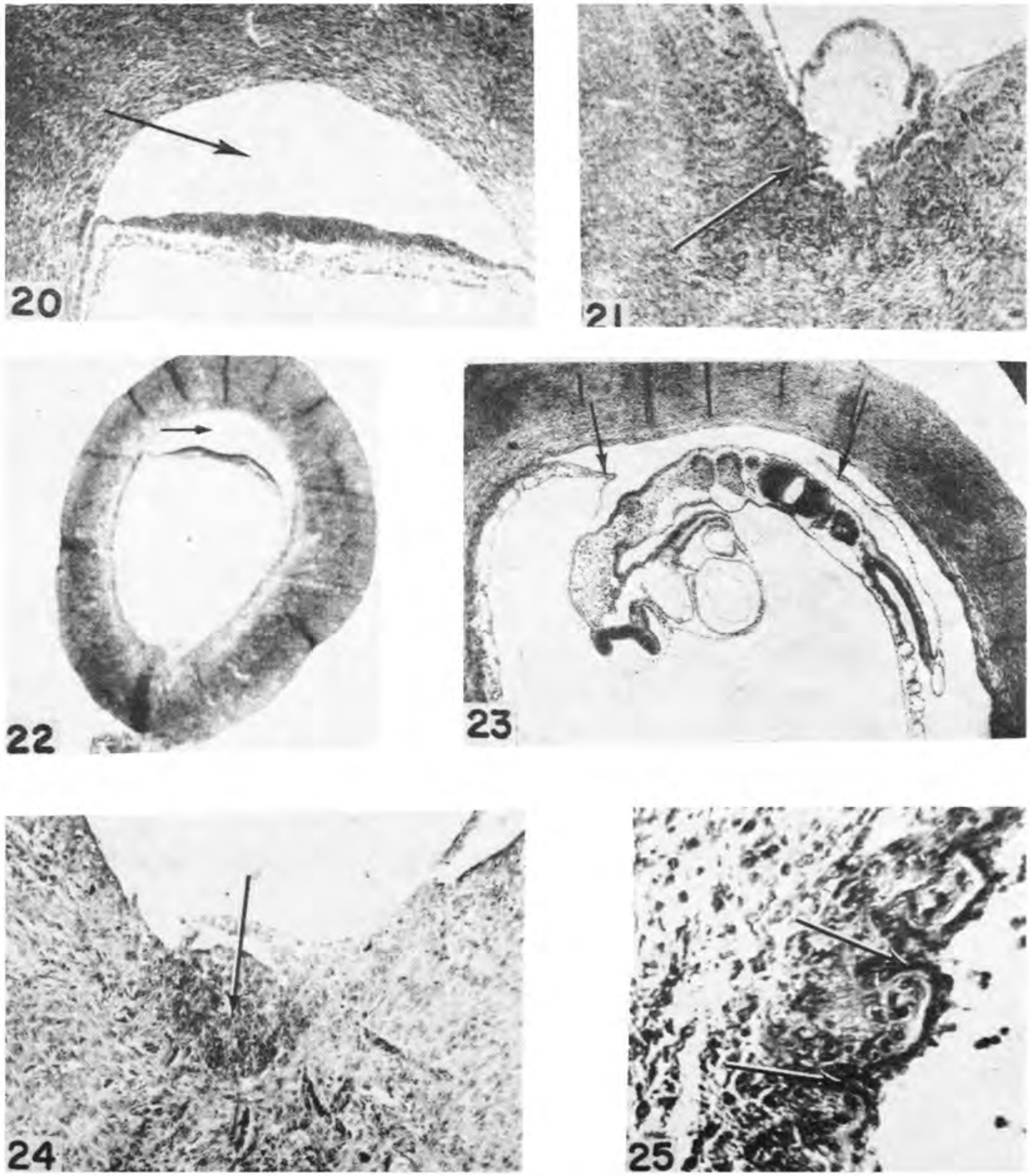


Figure 20-25 Part of figure 19 to show the details on the embryonic region. Note that the primitive amniotic cavity (arrow) is roofed over by the uterine endometrium (X 130); Mesometrial part of figure 19 enlarged to show the trophoblastic pad (shaded arrow) (X 130); Section of a gravid uterus containing an embryo in early neural groove stage of development. Note the large open primitive amniotic cavity on the antimesometrial side (arrow) and the trophoblastic pad on the mesometrial side, (X 24); Part of a section of a gravid uterus containing an embryo in the advanced neural groove stage of development. Arrows point to the amniotic folds (*Please see text for description*) (X 60); Mesometrial part of the gestation sac at advanced neural groove stage to show the trophoblastic pad (shaded arrow) (X 120); The lateral wall of the yolk sac at advanced neural groove stage. Note the formation of finger-shaped and hollow projections of trophoblastic inpushings into the endometrium (shaded arrow) (X 200)

endometrium destroying progressively deeper regions of the endometrium. Hence, a thick pad of cytotrophoblast was formed adjacent to the utero-tubal junction on the mesometrial side. Cellular debris of cytolysed endometrial cells was present in a cavity, the still persisting mesometrial remnant of the uterine luminal slit, in the centre of the trophoblastic pad.

Neural Groove Stage

In an embryo in the early neural groove stage of development (figure 22) the ectodermal plate composed of six to seven layers of compactly arranged cells had a shallow depression on its dorsal side. The embryonic plate tapered towards its margin. Mesoderm, composed of several layers of polygonal, loosely arranged cells, underlay the embryonic plate, and had extended to the blastocyst wall, hereafter referred to as the yolk-sac wall, to about half the distance on the lateral sides. Vitelline vessels extended to a short distance into the lateral wall of the yolk sac. The embryonic plate formed the floor of the large primitive amniotic cavity, the roof of which was formed by the superficial layer of uterine endometrium. On the lateral sides of the uterus the trophoblastic layer had undergone proliferation and had pushed into the endometrium in the form of short conical projections. In the abembryonic region the trophoblastic pad had become thicker than in the previous stage.

Four specimens with progressively advanced stages of development of the neural groove were available for this study. Figure 2 illustrates the general structure of the pregnant uterus containing an embryo at a more advanced neural groove stage of development than the one described above. During these stages the following changes were noticed. The embryonic plate became progressively thicker and expanded both laterally and along the cranio-caudal axis. Amniotic folds of the definitive amnion had grown dorsally but had not fused in the mid-dorsal line. Even in the most advanced of these stages (figure 23), in which the neural groove had closed in some places, the dorsal surface of the embryo faced the naked superficial surface of the uterine endometrium on the antimesometrial side. The yolk-sac wall was in contact with the uterine wall on its sides. Extra-embryonic mesoderm had entered the yolk-sac wall to about three fourths of its area from the embryonic side leaving only the distal quarter of the yolk-sac wall still bilaminar. Vitelline vessels had extended to a little beyond the equator of the yolk sac. On the sides of the gestation chamber the trophoblast had invaded the uterine endometrium in the form of short finger-shaped or conical root-like projections (figure 25). Some of these had become hollow and carried extra-embryonic mesoderm. On the abembryonic (mesometrial) side the trophoblast had proliferated to form a thick pad which had completely plugged the uterine luminal slit (figure 24). The endodermal cells were cuboidal in the trilaminar regions of the yolk sac and flat (fusiform in sectional views) in the bilaminar regions.

Discussion

From the foregoing descriptions of the early developmental stages of *Tadarida aegyptiaca* and from whatever information is available of early development of other molossid bats (Sansom 1932, Hamlett 1947, Pendharkar & Gopalakrishna 1982-1983) it is evident that molossid bats present some unique features. The blastocyst implants in the cranial segment of the uterus near its tip and becomes attached to the uterine wall on its entire surface. Hence, the uterine lumen becomes obliterated at the level of implantation. During early stages the embryonic mass of the blastocyst is oriented laterally, but as the embryonic mass expands to form a disc and then a plate, it grows towards the antimesometrial side. Hence, from late trilaminar blastocyst stage onwards the embryonic plate faces the antimesometrial side. Secondly, a cavity, the primitive amniotic cavity, is formed within the embryonic mass, and the roof of this cavity, which becomes progressively thin due to stretching, finally disappears by the time the embryonic mass expands into a broad plate. Hence, for some period the primitive amniotic cavity is roofed by the superficial layer of endometrium. Later, folds of the definitive amnion develop from the margins of the embryonic plate and arch over the embryonic plate and fuse dorsally to form the definitive amnion. Evidently, Sansom (1932), who mentioned that the amniotic cavity was arched over by the primary trophoblastic layer, did not examine more advanced stages to determine the disappearance of the trophoblast layer nor did Mossma (1937) and Stephens (1962) examine earlier stages to determine whether a primitive amniotic cavity was formed within the embryonic mass. The non-availablity of the critical stages of development, evidently, led these authors to draw divergent and erroneous conclusions regarding amniogenesis in molossid bats. Hemlett (1947), in *Eumpos* (sp. ?), another molossid bat, the roof of the primitive amniotic cavity disappeared with the result that the embryonic pate faced the superficial layer of endometrium. *Miniopterus schreibersii fuliginosus* (Gopalakrishna & Chari 1983; & Gopalakrishna 1984) belonging to Vespertilionidae (Simpson 1945) or Miniopteridae (Gopalakrishna & Karim 1980) is the only other bat in which such a method of amniogenesis has been reported so far.

Although there are no demonstrable physical differences among the trophoblastic cells in the different regions of the blastocyst, there is a distinct physiological differentiation of the trophoblast layer into three regions even at the implanted blastocyst stage. There appears to be an ascending gradient of proliferation and invasiveness of the trophoblast into the endometrium from the embryonic to the abembryonic pole. The trophoblast overlying the embryonic disc is non-proliferative and does not invade the endometrium. However, that it takes part in removing the uterine epithelium form this region seems to be unquestionable. This region of the trophoblast layer disappears as the embryonic disc expands. The trophoblast on the lateral sides is not only responsible for the removal of the uterine epithelium but invades the endometrium in the form of small blunt conical projections, and cytolyses its superficial regions. On the abembryonic pole the trophoblast is highly proliferative and forms a thick pad of cytotrophoblast after cytolysing the uterine epithelium and the superficial region of the endometrium. Such a regional physiological differentiation of the trophoblast in molossid bats leads to far reaching results in later development. On the embryonic side is formed a trophoblastic placenta, which becomes converted to form a part of the diffuse chorio-allantoic placenta at later stages. On the lateral sides is formed the chorio-vitelline placenta which later forms a part of the chorio-allantoic placenta, and the mesometrial side develops the definitive chroio-allantoic placenta. These have been described in detail in two other molossid bats (Stephens 1962, Gopalakrishna et al. 1989). Although a precisely similar kind of physiological differentiation of the trophoblast has not been noticed in any other family of Chiroptera, the concept of physiological polarity of the trophoblast in chiropteran development was reported by Gopalakrishna and Badwaik (1988).

In the light of the above discussion, Sansom's (1932) observation, that the trophoblast on the mesometrial side was more proliferative than in the other regions in the implanted blastocysts of *Molossus rufus* and *M. obscurus,* assumes considerable significance. It has also been shown by later workers (Stephens 1962, Pendharkar & Gopalakrishna 1982-83, Gopalakrishna et al. 1989) that the final chorio-allantoic placenta becomes located in relation to this area in all molossid bats. Evidently, physiological differentiation of the trophoblast into a precociously proliferative region on the mesometrial side is an unique feature, but common to all molossid bats.

Acknowledgements

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