Observations on some early stages of development and implantation of the blastocyst of *Rhinolophus rouxi* (Temminck)*

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ABSTRACT

The embryo enters the uterus at the morula stage of development. The progesterational response of the uterus is characterized by the enlargement of the uterine lumen near the centre of the right uterine cornu into a large spherical chamber in which the blastocyst undergoes nidation, and by a pronounced hypertrophy of the uterine glands on all the sides of the implantation chamber. The implantation of the blastocyst is circumferential, and the embryonic disc is directed towards the side of the uterus which is between the mesometrial and the lateral sides. The primitive amniotic cavity is formed by cavitation and the definitive amnion is later formed by the formation of folds from the margin of the embryonic disc.

1. INTRODUCTION

ALTHOUGH Chiroptera is one of the largest orders of mammals including over a hundred extant genera of bats incorporated in seventeen families, some details of embryology are available with reference to a few species belonging to a few families only and even amongst these, the details regarding the early development and placentation are lacking in most cases.

Among the Megachiroptera, which includes a single family, Pteropidae, details of the embryology of a few species are known through the work of Gohre, Selenka, Keibel, Van der Sprenkel, Kohlbrugge, Moghe, Wimsett and Karim. Among the Microchiroptera the embryology of members belonging to the family Vespertilionidae has been studied extensively. The other families of Microchiroptera, about which some details of embryology are known, are Rhinopomatidae, Emballonuridae, Noctilionidae, Megadermatidae, Hipposideridae, Phyllostomatidae, Desmodontidae and Molossidae.

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There is very little information concerning the embryology of Rhinolophidae. Van der Sprenkel made a brief mention about the structure of the yolk sac of *Rhinolophus hipposideros*. Hemmlett mentioned that the allantoic-placenta is discoidal and endotheliochorial in rhinolophids. Gopalakrishna and Bhiwgade gave a description of the arrangement of the foetal membranes in *Rhinolophus rouxi* at a late stage of gestation. Since there is practically no information concerning the early embryology and development of foetal membranes of any member of the family Rhinolophidae it was felt that a detailed study of the embryology of *Rhinolophus rouxi* would be of considerable interest and value. In the present paper, the early development and implantation of the blastocyst in this species are recorded. The development of the foetal membranes and placentation will be reported separately.

2. **Materials and Methods**

Specimens of *Rhinolophus rouxi* were collected at frequent intervals for two years (1971 and 1972) from a railway tunnel near Khandala in Maharashtra State. The specimens were killed either by decapitation or by chloroform, and the genitalia were fixed in various fixatives such as alcoholic Bouin’s fluid, neutral formalin, Carnoy’s fixative and preserved in 70% ethanol where necessary. After following the usual procedure of dehydration through graded series of ethanol and embedding in paraffin, the tissues were cut at a thickness of 6–8 μ. For routine histological examination the sections were stained with Ehrlich’s haematoxylin and counterstained with eosin. Some slides in each series were also stained by the PAS procedure. Altogether 95 pregnant females are examined. Table 1 includes the various stages studied with details of collection record.

3. **Observations**

**The Female Genitalia**

The breeding habits and the structure of the genitalia in the non-pregnant female of *Rhinolophus rouxi* have been described by Gopalakrishna and Rao. Only the right ovary releases a single ovum during each cycle and pregnancy is invariably borne in the right uterine cornu.

At oestrus the uterus (Plate I—figure 1) has an oblong outline in transverse sections, the mesometrial-antimesometrial axis being longer than the lateral axis. The muscle layers occupy nearly a quarter of the thickness of the uterine wall. The endometrium is nearly of uniform thickness on all the sides of the uterus, and the uterine glands are long and extend deep into the endometrium and are evenly distributed on all the sides. The uterine epithelium is made up of columnar cells about 30 μ in height. The uterine lumen is slit-like in consonance with the general shape of the uterus.
PREIMPLANTATION STAGES OF DEVELOPMENT

The earliest stage of development available for the present study is a free uterine morula of 48 cells and having a distinct envelope of zona pellucida (text-figure 1; Plate I—figure 2). The cells of the morula have fine granular cytoplasm, and vesicular nuclei each containing one or two nucleoli and darkly staining chromatin granules. A few small intercellular spaces, which are apparently the fore-runners of the blastocyst cavity, are present in the morula.

Six free unilaminar blastocysts at progressively advanced stages of development, and which had hatched out of the zona pellucida, were noticed in different specimens collected on February 1, 1971. In each case the blastocyst was present in an enlarged pocket of the uterus—the prospective implantation chamber—at about the middle of the length of the uterine cornu. The youngest blastocyst (Plate I—figures 3 and 4) had a nearly spherical inner cell mass composed of 68 cells. Two blastocysts, which were slightly more advanced than the one described above, had each a slightly flattened inner cell mass made up of 80 cells. The most advanced of these blastocysts (Plate II—figures 5 and 6) had a nearly biconvex inner cell mass composed of numerous cells. In all the blastocysts the trophoblast layer is composed of

Table 1. Collection record and pertinent notes

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of specimens</th>
<th>Stage of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-1-1971</td>
<td>1</td>
<td>Morula</td>
</tr>
<tr>
<td>1-2-1971</td>
<td>9</td>
<td>Preimplantation stages</td>
</tr>
<tr>
<td>4-2-1971</td>
<td>12</td>
<td>Preimplantation stages</td>
</tr>
<tr>
<td>12-2-1971</td>
<td>2</td>
<td>Preimplantation stages</td>
</tr>
<tr>
<td>23-2-1971</td>
<td>2</td>
<td>Implanted bilaminar blastocyst</td>
</tr>
<tr>
<td>26-2-1971</td>
<td>1</td>
<td>Implanted bilaminar blastocyst</td>
</tr>
<tr>
<td>16-3-1971</td>
<td>18</td>
<td>Neural groove stages and early limb-buds stages</td>
</tr>
<tr>
<td>23-3-1971</td>
<td>12</td>
<td>Late limb-buds stages and mid-pregnancy stages</td>
</tr>
<tr>
<td>1-4-1971</td>
<td>9</td>
<td>Mid-pregnancy stages</td>
</tr>
<tr>
<td>21-4-1971</td>
<td>1</td>
<td>Mid-pregnancy stage</td>
</tr>
<tr>
<td>26-4-1972</td>
<td>20</td>
<td>Advanced pregnancy stages</td>
</tr>
<tr>
<td>25-5-1971</td>
<td>8</td>
<td>Full term pregnancy stage</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
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</tr>
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</table>
nearly cubical cells each with a vesicular nucleus, and the inner cell mass is composed of small polygonal or irregularly shaped compactly arranged cells with darkly staining nuclei. In the most advanced of these blastocysts a few flat endodermal cells underlie the embryonic disc.

Six free bilaminar blastocysts are available for the present study (Plate II—figures 7 and 8; Plate III—figures 9 and 10). In all the cases the blastocyst wall is composed of an outer layer of trophoblast made up of nearly cubical cells and an inner layer of endoderm composed of squamous cells. The embryonic mass, which is composed of round or polygonal cells, becomes progressively flattened into a disc.

Preimplantation Changes in the Uterus

The progestational changes are more pronounced in the middle third of the length of the uterus than in the rest of regions. This segment of the uterus increases in its diameter across the dorso-ventral axis from 895 μ at the morula stage to 1580 μ at the bilaminar blastocyst stage. The uterine lumen in this region expands into a large spherical chamber at this level to accommodate the rapidly enlarging blastocyst (Plate II—figures 7 and 8; Plate III—figures 9 and 10). The progressive expansion of the implantation chamber is accompanied by a reduction in the height of the epithelium, especially on the lateral side of the uterus. Table 2 gives the measurements of the various parts of uterus during the growth of the embryo during the preimplantation period.
Figures 1–4. (1) Transverse section of the uterus at cestrus. The uterine lumen is slit-like, × 54. (2) Section of the uterus containing a morula, × 54. (3) Section of the uterus containing a free unileminar blastocyst. The long, coiled glands with wide lumina, × 54. (4) Magnified view of the blastocyst shown in figure 3, × 290.

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Figures 5-8. (5). Section of the uterus to show a free unilaminar blastocyst in a large implantation chamber of the uterine lumen, × 54. (6). Magnified picture of the blastocyst shown in figure 5. Note the endodermal cells (arrow), × 300. (7). Section of the uterus containing a free bilaminar blastocyst in a large implantation chamber. A distinct, but collapsed endodermal layer is seen in the blastocyst cavity (arrow), × 75. (8). Section of the uterus containing a free bilaminar blastocyst lying in the implantation chamber, × 75.
Table 2. Measurements of the uterus and its part during the preimplantation stages of the development of the embryo.

<table>
<thead>
<tr>
<th>Stage of development of the embryo</th>
<th>Diameter of the uterus in µ</th>
<th>Thickness of the myometrium in µ</th>
<th>Height of the uterine epithelium in µ</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mesometrial side</td>
<td>Lateral side</td>
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<tr>
<td>1. Morula</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Unilaminar blastocyst</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>(I)</td>
<td>900</td>
<td>230</td>
<td>30</td>
</tr>
<tr>
<td>(II)</td>
<td>975</td>
<td>230</td>
<td>30</td>
</tr>
<tr>
<td>(III)</td>
<td>1000</td>
<td>230</td>
<td>25</td>
</tr>
<tr>
<td>(IV)</td>
<td>1050</td>
<td>190</td>
<td>25</td>
</tr>
<tr>
<td>(V)</td>
<td>1050</td>
<td>180</td>
<td>25</td>
</tr>
<tr>
<td>(VI)</td>
<td>1050</td>
<td>160</td>
<td>25</td>
</tr>
<tr>
<td>3. Bilaminar blastocyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(I)</td>
<td>1100</td>
<td>110</td>
<td>25</td>
</tr>
<tr>
<td>(II)</td>
<td>1200</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>(III)</td>
<td>1200</td>
<td>96</td>
<td>20</td>
</tr>
<tr>
<td>(IV)</td>
<td>1350</td>
<td>80</td>
<td>18</td>
</tr>
<tr>
<td>(V)</td>
<td>1350</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>(VI)</td>
<td>1380</td>
<td>60</td>
<td>15</td>
</tr>
</tbody>
</table>

During the morula stage the uterine epithelium is composed of tall columnar cells with nuclei situated in the middle of the cells. As the embryo reaches the unilaminar blastocyst stage the cells of the uterine epithelium becomes closely crowded, and their nuclei occur at different levels, and at the bilaminar blastocyst stage they are cuboidal on the lateral sides and columnar on the rest of the sides of the uterus, and the nuclei occur near the basal regions of the cells. A noteworthy feature of the stage is the accumulation of considerable quantity of fluid between the epithelial lining and the subjacent endometrial tissue making this region loose and edematous.

The most prominent preimplantation change in the uterus, as seen in stained sections, seems to occur with respect to the uterine glands. During the morula stage (Plate I—figure 2) the uterine glands occur evenly on all the sides of the uterus although their number appears to be more on the antimesometrial side than on the other sides. Most of the uterine glands are nearly straight, and extend to about three-fourths of the thickness of
the endometrium. The epithelium of the glands consists mostly of cubical cells with homogeneous cytoplasm and vesicular nuclei situated near the basal regions of the cells. Progressively the glands increase in length and number, become coiled and undergo progressive widening (Plate I—figure 3; Plate II—figure 5) and their lumina contain copious amount of eosinophilic secretion. The cells of the gland epithelium become tall columnar and vacuolated with basally situated vesicular nuclei except towards their fundic regions, where the cells remain cubical and contain darkly staining nuclei.

Concomitant with the widening of the uterine glands the endometrial stroma between the glands gets progressively compressed into thin strands composed of fusiform cells having darkly staining nuclei, fine fibres and blood capillaries. The myometrium is progressively reduced in its thickness from 230 μ in the morula stage to 60 μ in the bilaminar blastocyst stage.

**FURTHER DEVELOPMENT OF THE BLASTOCYST AND IMPLANTATION**

The following descriptions are based on the examination of the three bilaminar blastocysts at different stages of implantation. The bilaminar blastocyst establishes its contact with the uterine wall commencing from the embryonic region and progressively extending to the lateral side of the blastocyst. In the earliest stage (Plate III—figures 11 and 12) the embryonic mass faces the lateral side of the uterus, and processes emanating from the cells of the trophoblast overlying the embryonic mass extend towards the uterine epithelium (Plate III—figure 12). The uterine epithelium is intact on all the sides except on the side adjacent to the embryonic mass, where the cells of the uterine epithelium appear to be undergoing destruction as evidenced by the fact that the cell boundaries have disappeared, and the nuclei have become fragmented into small darkly staining bodies. The uterine glands become further coiled, and undergo further widening resulting in the further reduction of the endometrial connective tissue.

At a slightly more advanced stage of development the embryonic disc is oriented between the lateral and the mesometrial sides of the uterus, and occurs as a multilayered nearly flat disc, which forms the floor of a spacious cavity—the primitive amniotic cavity (Plate IV—figures 13 and 14). The edges of the embryonic disc are curved dorsally in the form of folds underlying the trophoblast. These are apparently the folds of the definitive amnion. The endoderm consisting of squamous cells underlies the embryonic disc and the trophoblast.

As the trophoblastic layer progressively establishes contact with the uterine stroma on all the sides, the uterine epithelium disappears from all the sides of the implantation chamber, and the trophoblastic layer undergoes proliferation and enters the endometrium in the form of conical inpushing
Figures 9-12. (9). Section of the uterus containing a late free bilaminar blastocyst, × 54. (10). Part of the blastocyst shown in figure 9, × 380. (11). Section of the uterus with an early implanting bilaminar blastocyst (arrow points mesometrically), × 80. (12). Enlarged part of the blastocyst shown in figure 11. The uterine epithelium which shows signs of destruction (arrow), × 380.

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Figures 13-17. (13). Section of the uterus containing an implanted bilaminar blastocyst (arrow points mesometrically). The endodermal layer (arrow head) has been artfactually peeped away from its original position, × 54. (14). Magnified view of the embryonic region of the blastocyst shown in figure 13. Note the early amniotic folds (arrows), × 350. (15). Part of the uterine wall at implanted bilaminar blastocyst stage shown in figure 13. Solid cords of trophoblast (arrow) push into the endometrium, × 180. (16). Section of the uterus containing an implanted bilaminar blastocyst (arrow points mesometrically), × 54. (17). Enlarged part of the uterine wall to show the finger-shaped intrusions (arrow) of the trophoblast into the endometrium, × 360. (Facing page 207)
(Plate IV—figure 15). Thus, the blastocyst gets firmly anchored to the uterine wall on its entire surface. The uterine glands seem to undergo rapid disintegration in the proximal region (that is near the uterine lumen). Only the fundic parts of the glands persist near the myometrial border of the endometrium.

A late implanted bilaminar blastocyst is shown in Plate IV—figure 16. The embryonic disc lies facing between the lateral and the mesometrial sides of the uterus. The endodermal lining, both in the region of the embryonic disc and the extraembryonic region, is a distinct layer of lightly staining cells, which are nearly cubical in the region of the embryonic disc, but squamous in the extra embryonic regions. The primitive trophoblast on the mesometrial and the lateral sides of the uterus has entered the endometrium in the form of finger-shaped cords (Plate IV—figure 17) which, in many places, are branched and the branches are interconnected. The endometrial tissue is loose and spongy near the myometrial border, and contains numerous patches of necrotic tissue. A few remnants of the uterine glands are present in the deeper regions of the endometrium.

4. DISCUSSION

An unusual feature of the early development of Rhinolophus rouxi is that the embryonic mass, which is oriented towards the lateral side of the uterus during early stages, appears to change towards the mesometrial side as the embryo advances in development. The position of the embryonic disc in relation to the uterus is constant for a species, and in many mammalian orders, it is constant for members of the family. While evaluating the phylogenetic importance of the foetal membranes, Mossman31 stated: “the orientation of the embryonic disc with reference to the uterus is usually constant in all groups known to be closely related”. In a later review Mossman42 reiterated the same hypothesis and mentioned, “In most major taxonomic groups of eutherian mammals, available data show that at the time of first attachment of the blastocyst to the endometrium the inner cell mass or the embryonic disc has an almost constantly specific directional orientation to the uterus.” Regarding the bats he mentioned, “The literature on the Microchiroptera (Insectivorous bats) indicated wide variation similar to those in Insectivores. It is particularly confusing since it often indicates lack of consistency even within smaller groups, that is superfamilies and families.” Rhinolophus rouxi, therefore, appears to differ from all the other wild mammalian species so far studied except Megaderma lyra lyra22 where the embryonic mass has a variable orientation.

The most prominent progestational reaction of the uterus in all the bats so far studied concerns the formation of an edema in a specific region
of the uterus, or circumferential on all the sides of the uterus. In *Rhinolophus rouxi*, on the other hand, the most prominent progestational uterine reaction appears to concern the glands which undergo enormous hypertrophy on all the sides of the uterus around the implantation chamber. By the time the blastocyst begins to implant the endometrial stromal tissue is reduced to thin strands pressed between the large uterine glands. Perhaps, the circumferential implantation of the blastocyst is in some way related to the diffuse progestational changes.

**Acknowledgement**

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**References**

DEVELOPMENT AND IMPLANTATION OF *Rhinolophus Rouxi*