Placental Development in the Mongolian Gerbil (*Meriones unguiculatus*)

I. EARLY DEVELOPMENT TO THE TIME OF CHORIO-ALLANTOIC CONTACT [1]

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ABSTRACT   Early gerbil development was studied from days 4 through 12 of gestation. Implantation occurs on day 8 in a shallow antimesometrial implantation crypt. The proamniotic cavity forms from an invagination of basal trophoblast and a folding together of the rim of the cup thus produced. The approximation of this rim gives rise to the ectoplacental cone. The further development and expansion of the ectoplacental cone is much like the rat. Amniogenesis is by folding into the proamniotic cavity. Between large glycogen-filled decidual cells occur many PAS-positive eosinophilic granular cells. A massive subplacental gland forms from proliferating uterine epithelium within the decidua basalis. Epithelial proliferation begins shortly after implantation, and soon short thick cellular processes extend from the antimesometrial side of the lumen whereas long branching cords penetrate the decidua basalis mesometrially. The function of this structure is unknown.

The rodent family, Cricetidae, has long been an important group for the study of reproduction. However, until recently one of its subfamilies, Gerbillinae (the jirds and gerbils), has been neglected. Even though the use of these animals in reproductive studies is increasing, surprisingly little has been published on placental development in this group.

Basic studies in gerbil reproductive biology have been done by Marston and Chang ('65, '66) in the Mongolian gerbil (*Meriones unguiculatus*) and by Salzmann ('63) in *Meriones shawi*, a North African gerbil. These authors have reported data on estrous cycle lengths, ovulation rates, time of ovulation and mating, preimplantation development, duration of gestation and sex ratios at birth. In addition, histological studies on reproductive organs and implantation have been done in *M. shawi* (Salzmann, '63).

This research is intended to provide a descriptive basis for subsequent investigation of early development in the gerbil, and to compare developmental events in these species with phenomena in the more widely studied rat, mouse, and hamster.

MATERIALS AND METHODS

Twelve pregnant gerbils, from days 4 through 12 of gestation, were autopsied. Uterine horns of the earlier stages were straightened and pinned flat before fixation in alcohol-formalin-acetic acid, Carnoy's fluid or neutral buffered formalin (10%). Individual gestation sacs from the later stages were removed from the rest of the uterine horn and fixed in like manner.

Serial sections of the tissues were prepared by the paraffin technique and mounted. Staining methods employed were hematoxylin and eosin, periodic acid — Schiff (PAS), a stain for specific demonstration of DNA and RNA (A. D. Floyd, unpublished), and naphthol yellow S for demonstration of protein.

OBSERVATIONS

Preimplantation. Because of work already done on cleavage stages in the gerbil (Marston and Chang, '66), only one representative specimen was examined in this period. The embryos at 10:00 AM of day 4 are all in the eight-cell cleavage stage, clustered together in the ampulla of the oviduct. This agrees with previous observations (Marston and Chang, '66).

Several specimens were examined throughout day 7, and until 3:00 AM of day 8. In all cases, unattached uterine blastocysts in their zonae pellucidae are distributed along the uterine horns. There is as yet no consistent orientation of the inner cell mass to the uterus, but the blastocysts themselves are always located on the antimesometrial side of the lumen. The blastocysts of the later periods are partially enclosed in shallow crypts formed by the intact uterine epithelium (fig. 3).

At this time the mesometrial endometrium at the implantation sites is being thrown up into narrow longitudinal folds, each composed of two layers of tall columnar epithelium covering a thin central stromal lamina. These folds cross the lumen and block the openings of the shallow implantation crypts (fig. 3).

Implantation. The penetration of the trophoblast processes between uterine epithelial cells was not observed. The first stage available (early on day 8) demonstrates the blastocyst implanted beneath the uterine epithelium of an antimesometrial crypt (fig. 1), but a PAS-positive membrane separates it from the decidual tissue. The abembryonic trophoblast cells have begun their transformation into primary giant cells. The inner cell mass, now oriented mesometrially, consists of two layers of round ectodermal cells. A few scattered endoderm cells occur on the central surface of the inner cell mass.

At this stage the maternal decidual reaction is already quite advanced, swelling the endometrium surrounding the implantation crypt. The decidual cells are uniformly large, with large, apparently polyploid nuclei. Except for several layers of decidual cells immediately surrounding the implanted blastocyst, the decidua is packed with glycogen.

The uterine epithelium at the implantation site is degenerate, but elsewhere along the length of the crypt and the original lumen (now displaced mesometrially) the uterine epithelium is composed of tall columnar cells, several in mitosis (fig. 1).

Egg-cylinder stage. In the two days following implantation (to day 10), the entire conceptus elongates greatly in the mesometrial-antimesometrial axis, as is typical of muroid rodents. The "egg cylinder" is formed from the elongating inner cell mass (figs. 2, 4) just as has been described in the rat (Huber, '15). Endoderm rapidly proliferates to cover the egg cylinder and line the entire blastocoele, converting it into the yolk sac cavity. The visceral endoderm, covering the egg cylinder, is low columnar, while the parietal endoderm cells sparsely lining the yolk sac cavity, are squamous or rounded.

This endoderm, the newly formed Reichert's membrane, and the loose layer of primary giant cells surrounding the conceptus constitute the bilaminar omphalopleure (fig. 2).

The trophoblast cells basal to the egg-cylinder are initially rather inactive. However, those at the junction of the bilaminar omphalopleure and egg cylinder multiply and extend mesometrially as an open cylinder (figs. 2, 4). Very soon the edges of this cylinder meet and fuse to form the ectoplacental cone. The space thus enclosed (which contains debris from extravasated maternal blood) is the proamniotic cavity. A very small intercellular space within the ectodermal node will also coalesce with this proamniotic cavity (fig. 2).

Hereafter the ectoplacental cone continues its development in typical myomorph fashion. The actively dividing central cells of the cone are small, round and basophilic. They form a compact plate, in contrast to the loosely organized cells of the rest of the ectoplacental cone (fig. 5). These peripheral cells disrupt maternal capillaries and venules, allowing blood to extravasate between them (figs. 4, 5).

The maternal decidual reaction is at its height of development at this time, filling the endometrium around the conceptus. The antimesometrial decidua is composed of large, vacuolated cells, most of which

are packed with glycogen (fig. 6). The basal decidua is still but little developed. Its small cells contain only moderate amounts of glycogen.

The uterine epithelium in the implantation area is now rapidly proliferating. The epithelial cells of the implantation crypt have multiplied to form a thick columnar mass. Mitotic figures are also numerous in the original uterine luminal epithelium. These cells, unlike those of the neighboring decidua, are devoid of glycogen, but are rich in RNA.

Expansion stage. In the eleventh and twelfth days of gestation, the general shape of the conceptus changes from cylindrical to almost spherical (fig. 7). During this time the embryo passes through the primitive streak stage and begins formation of the first somites. The bilaminar omphalopleure remains as before. The visceral and parietal endodermal layers are approximated, allowing only a small yolk sac cavity.

The amnion forms exactly as it does in the rat (Mossman, '37). Somatopleuric folds bulge into the proamniotic cavity meet and fuse to give rise to the amniotic and chorionic membranes. Once formed, amnion and chorion separate, leaving a large, central cavity, the exocoelom (fig. 7). The amnion stretches across the dorsal surface of the cup-shaped embryonic disc, enclosing the amniotic cavity (fig. 7).

The chorion constitutes the roof of the newly formed epamniotic cavity, whose floor is the base of the ectoplacental cone and whose cylindrical wall is the visceral yolk sac segment not invaded by mesoderm. The epamniotic cavity is only transitory, for the chorion begins almost immediately to sag onto the surface of the ectoplacental cone and fuse with it (fig. 7).

The allantois is an angiogenic mesenchymal bud projecting into the exocoelom from the caudal limit of the embryonic disc. It never contains an endodermal diverticulum (fig. 7).

The ectoplacental cone soon flattens around the mesometrial pole of the conceptus (fig. 7). Its base, the floor of the epamniotic cavity, is a compact layer of 5 to 15 cells in thickness. These cells are small, basophilic, and mitotically active

(fig. 8). The rest of the cone is a loose reticulum of small stellate trophoblast cells (fig. 8). These cells apparently phagocytose the maternal blood cells which fill the spaces between them. The peripheral cells of this area enlarge to differentiate into secondary giant cells.

The central zone of the decidua capsularis, next to the conceptus, now begins to become necrotic, but all the peripheral decidual areas still consist mostly of hypertrophied, glycogen-rich cells, which are smallest in the decidua basalis. At this time, granular cells become numerous in the peripheral decidual areas, particularly in the decidua basalis. They are large, round cells with eccentric nuclei, and their fragile cytoplasm is packed with large, PAS-positive, intensely eosinophilic granules (fig. 9).

The epithelium of the original uterine lumen is now extremely hyperplastic. Cords of epithelial tissue penetrate in all directions from the original luminal lining into the decidua basalis (fig. 10). On the antimesometrial side of the lumen these cords may attain 15 cells in thickness, but narrow branching cords only two cells thick extend mesometrially. The entire epithelial structure becomes very massive, displacing the surrounding decidua, and compressing the uterine lumen. The cells are small, round and basophilic, very rich in RNA and mitotically active. The cell cords soon develop an intimate relation with surrounding capillaries and venous sinusoids (fig. 11). The original epithelial mass is spherical, but upon growth of the conceptus, it becomes flattened into a concave discoid shape. Because of its general endocrine appearance, it has been named the "subplacental gland" (Fischer and Floyd, '72).

DISCUSSION

Implantation. The timing of implantation, as well as other developmental events, is delayed from the pattern known in other muroid rodents (see Fischer and Floyd, '72, for discussion). Delay of implantation due to suckling does occur in gerbils (Salzmann, '63; Marston and Chang, '65), but is not of relevant influence in this study. Because of the difficulty in following estrous cycles with smears,

all pregnancies were timed from post-partum estrous matings, but all young were removed from the mother either the morning of birth or that of sperm-positive smear (day 1 of pregnancy). The occurrence of a previous pregnancy does not seem to influence events, for Marston and Chang ('65), using virgin animals, were able to flush intact uterine blastocysts until midway through day 7. This agrees with our histological finding of implantation early on day 8.

Implantation occurs within a relatively small uterine crypt, a blastocyst sized indentation of the antimesometrial uterine lining. In other myomorph forms, where the implantation crypt is deep, the blastocyst is secured in position by apposition of uterine cryptal epithelium mesometrial to it (Enders and Schlafke, '67). Stabilization of the gerbil blastocyst differs in that it appears to be due to a complex folding of the mesometrial uterine epithelium, which approximates the shallow implantation crypt and acts as its "plug" (fig. 3).

The ectoplacental cone. The ectoplacental cone and the proamniotic cavity form in a manner different than that which has been described for the rat (Huber, '15). In that species the ectoplacental cone develops from a proliferation of trophoblast cells basal to the egg cylinder ectoderm, and the proamniotic cavity is formed by the coalescence of two intercellular spaces which develop within the ectodermal node and the extraembryonic ectoderm of the egg cylinder.

In the more primitive Geomyoid rodents, where no ectoplacental cone exists, the epamniotic cavity is open to the uterine lumen (Mossman and Strauss, '63). This is somewhat similar to the early condition in *Meriones* (figs. 1, 2, 4; see also Salzmann, '63), where the extraembryonic ectoderm invaginates from the surface to form a cup, whose concavity, the proamniotic cavity, is also open to the uterine lumen. But, whereas the epamniotic cavity

in *Geomys* remains open (Mossman and Strauss, '63), in the gerbil the mesometrial edges of the cup meet and fuse very soon to enclose a definitive proamniotic cavity. The fusion of these edges brings together a mass of cells indistinguishable from the ectoplacental cone of the rat.

It is of interest that the most widely studied cricetid rodent, the hamster, also has a slit-like proamniotic cavity which in the earliest stages is continuous with the uterine lumen (Ward, '48). This character, though different in degree between gerbil and hamster, is one that appears to distinguish the early development of Cricetidae from that of Muridae.

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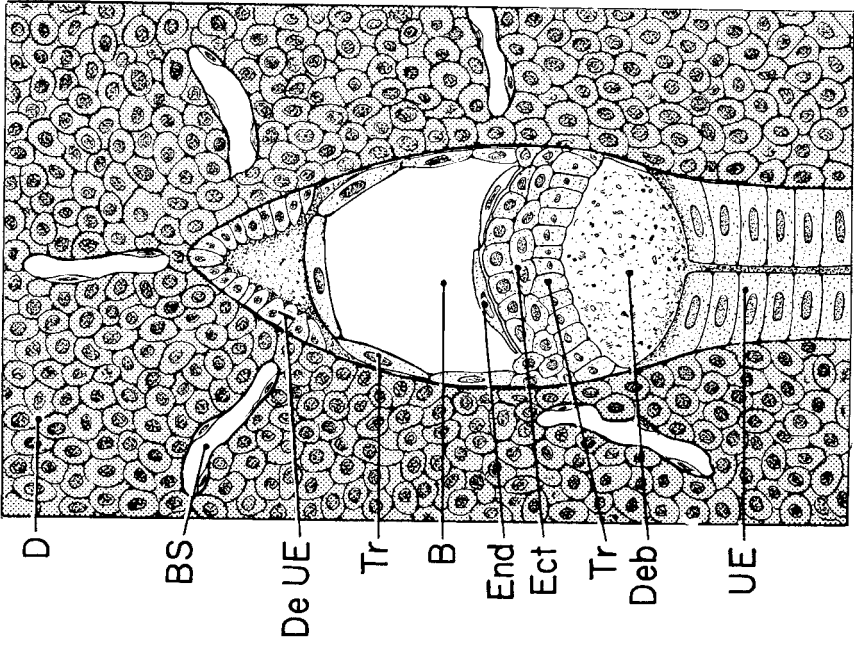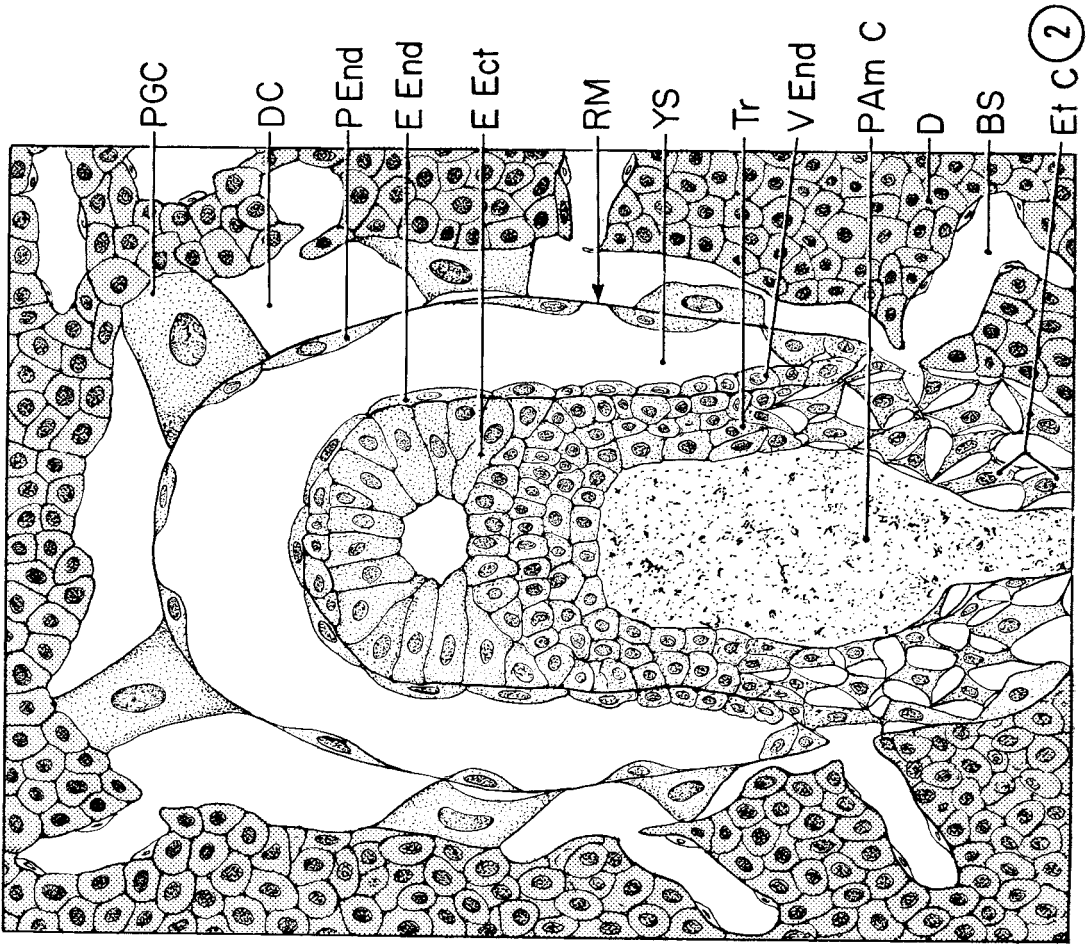
PLATES

PLATE 1

EXPLANATION OF FIGURES

All figures oriented with the mesometrial side down.

1 Newly implanted blastocyst, day 8. A single cellular layer of trophoblast (Tr) surrounds the blastocyst, but abembryonic cells are enlarged. The inner cell mass is differentiated into ectoderm (Ect) and a few endodermal cells (End) facing the blastocoel (B). The mesometrial epithelium (UE) of the implantation crypt is columnar, but degenerating epithelium (DeUE) and cellular debris (Deb) surrounds the blastocyst. Decidual cells (D) already pack the stroma between radial blood sinusoids (BS). × 280.

2 Egg cylinder, day 10. The inner cell mass has differentiated into an embryonic disc composed of an ectodermal node (E Ect) covered by endoderm (E End). The basal trophoblast (Tr) has proliferated and invaginated, partially enclosing the proamniotic cavity (PAm C), and producing the stellate cells of the ectoplacental cone (ETC). Visceral endoderm (VEnd) covers the walls of the egg cylinder, and parietal endoderm (PEnd) lines Reichert's membrane (RM), converting the blastocoele into the yolk sac cavity (YS). Primary trophoblast giant cells (PGC) sparsely cover Reichert's membrane and extend across the blood-filled decidual cavity (DC) to contact the decidua (D). Blood sinusoids (BS) freely open into the decidual cavity. × 280.

PLATE 1

EARLY GERBIL DEVELOPMENT
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PGC

DC

P End

E End

E Ect

RM

YS

Tr

V End

PAm C

D

BS

Et C ②

D

BS

De UE

Tr

B

End

Ect

Tr

Deb

UE

①

PLATE 2

3   Unimplanted blastocyst, day 7. Antimesometrial fold of decidua (D) covered by obliquely cut uterine epithelium (UE) holding a blastocyst (*arrowhead*) *in a shallow implantation crypt.* Note the lateral orientation of the inner cell mass at this time. × 132.

4   Beginning of egg cylinder elongation, day 9. Trophoblast at edges of the bilaminar omphalopleure (arrows) has begun to proliferate, causing a central invagination, the proamniotic cavity (PC). Reichert's membrane is visible as a thin line (arrowhead) separating yolk sac cavity below from blood filled decidual cavity above. Decidua (D). × 330.

5   Ectoplacental cone, day 12. A relatively solid germinal plate of trophoblast cells borders the epamniotic cavity (EC). Stellate cells of the cone arise from this plate and enclose maternal blood spaces (BS). × 132.

6   Decidua capsularis, day 12. Decidua near the decidual cavity (DC) is necrotic, but large, clear, glycogen-filled cells pack most of the field. Note the variation in size of the decidual cell nuclei. × 132.
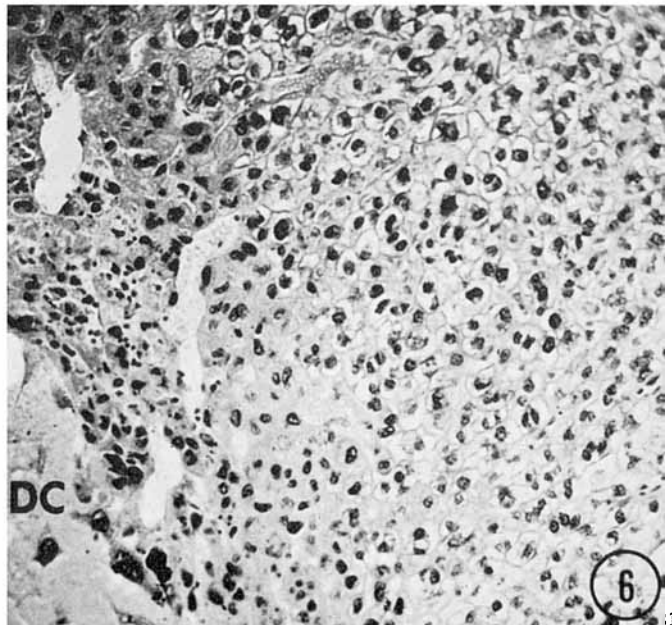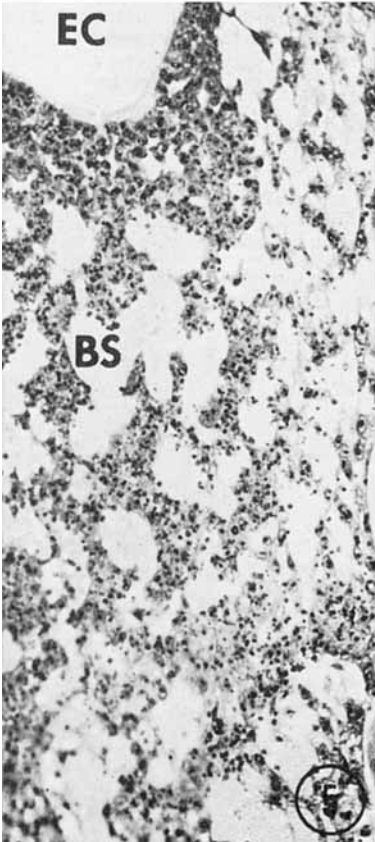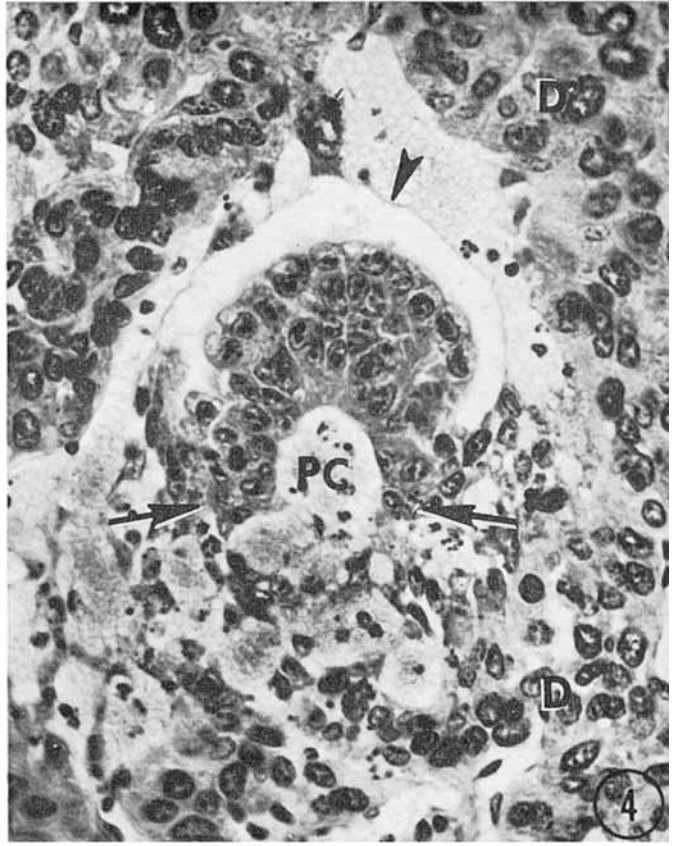
PLATE 3

EXPLANATION OF FIGURES

7 Conceptus in the expansion stage, day 12 (late). The bilaminar omphalopleure (arrowhead) almost completely surrounds the conceptus, separating outer decidual cavity and yolk sac cavity. The thick, trilaminar embryonic disc (top) attaches to the amnion (A), which is now separated by the exocoelom from the chorion (C). A mesenchymal allantoic bud (arrow) protrudes into the exocoelom. The flattened ectoplacental cone (bar) consists of a thick central plate and the stellate peripheral cells. × 33.

8 Detail of ectoplacental cone of the expansion stage, day 12 (late). The chorion (arrowhead) which separates the exocoelom (Ex) from the epamniotic cavity (EC) is sagging toward the surface of the ectoplacental cone (E), with which it will soon fuse. An extensive blood space (BS) separates this central, compact plate from the peripheral stellate cells of the cone. These peripheral cells have a marked tendency to phagocytose leucocytes from the blood which percolates between them. Yolk sac cavity (YS). × 132.

9 Decidua, day 12. Scattered among normal vacuolated decidual cells are many eosinophilic granular cells (arrow). × 1015.

10 Subplacental gland, day 12. Extensive cellular masses are present on the anti-mesometrial aspect of the gland, but typical glandular cords occur mesometrially. Contrast the appearance of the gland with normal decidua basalis (D). × 132.

11 Subplacental gland, day 12. Note the continuity of the glandular elements with the uterine epithelium. Uterine lumen (L). Note also the extensive blood sinuses (BS) which drain into the subplacental gland. × 330.

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