The ultrastructure of implantation in the golden hamster (Cricetus auratus)

By M. P. YOUNG\(^1\), J. T. WHICHER\(^1\) & D. M. POTT S\(^1\)

From the Anatomy Department, Cambridge

Previous work on the early development of the golden hamster includes the investigation of Ochs (1908), Graves (1945) and Ward (1948), all at the level of the light microscope. Austin (1963) has examined the ultrastructure of the oocytes of the golden hamster while Enders & Schlafke (1965) have observed the pre-implantation stages of pregnancy.

The ultrastructure of implantation has been studied in two other species of myomorph rodents: the mouse (Potts, 1966\(a\); Reinius, 1967) and the rat (Enders & Schlafke, 1967).

Implantation is taken as beginning when the zona pellucida is lost and the trophoblast is in contact with the uterine epithelium throughout its circumference. This takes place at between 80–100 h post coitum. Previous studies have been made on specimens embedded in paraffin, and the shrinkage which occurs with this method of preservation has caused implantation to appear to begin considerably later than this: Graves (1945) gives it as beginning at 5 days, Ward (1948) as 4 days 8 h.

Implantation is antimesometrial and the embryonic pole is orientated towards the mesometrium.

MATERIALS AND METHODS

The hamsters were anaesthetized with ether and perfused through the abdominal aorta with 2 % glutaraldehyde at 4 °C and fixed for 2 h. The material was washed twice and stored overnight in cacodylate buffer at 4 °C, post-fixed in Dalton's solution for 1.5 h at 4 °C, and dehydrated, embedded and cut according to a double sectioning technique (Potts, 1966\(b\)). The sections for electron microscopy were double stained with uranyl acetate and lead citrate.

Six specimens were studied in detail, taken from 20 mated animals at selected intervals between 82 and 116 post coitus (p.c.). Copulation was observed and taken as time zero.

Of the specimens, one is at 82 h p.c., one at 88 h p.c., one at 94 h p.c., 2 at 95 h p.c. (from same litter) and one at 116 h p.c.

\(^1\) Authors' address: Anatomy School, Downing Street, Cambridge, England.
Embryo

The zona pellucida is retained in one specimen at 88 h p.c. (probable post fertilization age 82 h). The blastocyst is about 80 μ in diameter and consists of about 16 cells. From the outer surface of the trophoblast a number of balloon-like masses of cytoplasm protrude into the space between the blastocyst and zona pellucida. The balloons contain an amorphous granular material, devoid of organelles, and the limiting membrane is continuous with that of the cell from which it protrudes. Where the balloon impinges on the zona pellucida it is associated with a band of osmophilic material in the zona.

The remaining specimens, including that at 82 h p.c., are about to implant or are already implanted. The zonae are lost and the blastocysts, tightly enclosed by maternal epithelium, are spaced at intervals along the antimesometrial side of the uterus.

The trophoblast cells are flattened against the uterine epithelium (Plate 1, fig. 1). Cytoplasmic fibrous material (consisting of double lines 35 mμ apart with interconnecting units also at 35 mμ) is present in both the trophoblast and inner cell mass during the early stages of implantation. Mitochondria are round or oval with numerous cristae. The trophoblast nuclei possess a dense reticular centre surrounded by a less osmophilic granular material. The inner layer of the nuclear membrane is more electron-dense than the outer. The cells are united by (1) small desmosomes (maculae adherens), in which the apposed cell membranes are thickened and a dark granular material is found between the membranes and on the cytoplasmic sides of the cellular contact, and (2) junctional complexes, in which dark granular material obscures the apposed membranes.

Maternal tissues

Before implantation, when the blastocyst is still free in the uterine lumen, the maternal endometrium consists of cuboidal epithelium cells with centrally placed nuclei, separated from loose stromal tissue by a prominent basement membrane. The epithelial cells have a villous border, the villi having a filamentous core.

Plate 1

Fig. 1. Transverse section of implanting blastocyst 95 h p.c. The uterine lumen is occluded. A decidual reaction has begun in the subepithelial tissue near the embryo. Light micrograph of glutaraldehyde-fixed, double-embedded specimen. Stain: toluidene blue and methylene blue. × 300.

Fig. 2. Trophoblast overlying uterine epithelium at 95 h p.c. Note the cytoplasmic fibrous material (arrow). e, Uterine epithelium; t, trophoblast. × 12500.

Fig. 3. Maternal–embryonic junction 95 h p.c. Note the electron-dense deposit in the extracellular space. × 30000.

Fig. 4. Microvillous surface of apposed uterine epithelial cells in line of uterine occlusion 95 h p.c. × 12500.
Fig. 5. Detail maternal-embryonic junction 95 h p.c. × 30000.

Fig. 6. Implantation site 95 h p.c. There is a large inclusion in the trophoblast cell. s, Stromal tissue. × 3000.

M. P. YOUNG, J. T. WHICHER & D. M. POTTS
Implantation in golden hamster

passing up to 2.0 μ into the apical cytoplasm (Plate 1, fig. 2). Desmosomes are present between the epithelial cells. In certain areas the epithelium may be multi-layered. Striking differences can be seen between adjacent cells, some staining lightly, some darkly and others containing numerous vacuoles in the cytoplasm.

Immediately below the basement membrane there is a layer of flattened, close-packed stromal cells and blood vessels. Farther away from the epithelium the stromal tissue is much looser and consists of an approximately equal mass of extracellular ground substance and of rounded stromal cells. Cells of the leucocyte series are present. There is a capillary network beneath the uterine epithelium. At approximately 80 h p.c. the uterine lumen is obliterated and the villi of the apposed epithelial cells interdigitate (Plate 1, fig. 4).

Maternal-embryonic junction

At the implantation site in the 82 h specimen and in one 95 h specimen (Plate 1, fig. 2) the epithelial microvilli lie against the regular surface of the trophoblast cells, often bent over and flattened against them.

In the second 95 h specimen the plasma membranes of the epithelial cells in contact with the embryonic pole of the blastocyst are irregular, while in areas not directly related to the trophoblast the microvilli persist. At the abembryonic pole the process has gone a step farther and the microvilli are totally replaced by irregular cytoplasmic processes without any internal structure. In some areas an electron-dense deposit is found in the extracellular space between trophoblast and maternal cells (Plate 1, fig. 3). Large complex inclusions are found in association with these changes in the plasma membranes (Plate 2, fig. 6). The inclusions contain tangled plasma membranes, lipid droplets, vacuoles and areas of electron-dense material.

The basement membrane is intact in all the specimens studied but a decidual reaction is recognizable in the sub-epithelial tissues from 95 h p.c. The extracellular space is reduced and the endoplasmic reticulum is more prominent than in undecidualized cells (Plate 2, fig. 6).

DISCUSSION

The exact age post coitum is of limited value when studying the details of the sequence of ultrastructural changes occurring in implantation. Embryos in the same uterus can be in different stages of development.

Development in the hamster is rapid. The zona pellucida is lost 70–90 h p.c. and implantation takes place between 80 and 100 h p.c. The times are in advance of those for the mouse and rat, and the hamster is the only one of the three in which the fibrous material present in the cytoplasm of the oocyte and cleavage stages persists until after implantation has begun (unpublished observations). It is suggested that the fibrous material consists of sheets of interconnecting
meshwork forming a cellular structure. According to Enders & Schlafke (1965) it is not apparent in permanganate-fixed material and is thus suggested to be of a protein nature. Enders & Schlafke (1965) point out that the fibrous material has a distribution inversely related to the distribution of ribosomes.

In the cleavage and morula stages there are no specialized cell contacts between embryonic cells, but desmosomes and junctional complexes appear at the blastocyst stage, as in other species that have been studied (Enders & Schlafke, 1965). The cytoplasmic balloons seen in the preimplantation stage are associated with fine structural changes in the zona pellucida and may be associated with its dissolution.

During implantation in the mouse and rat the microvillous border of the endometrial cells is replaced by a flatter, more regular surface (Potts, 1966a; Enders & Schlafke, 1967; Reinius, 1967), and in the rat Potts & Psychoyos (1967) have shown that the ultrastructural changes taking place depend on the sequential release of progesterone and oestrogen. The alterations in fine structure of the maternal–embryonic junction in the hamster parallel those in the myomorph rodents already studied, suggesting that oestrogen may be involved. Orsini & Psychoyos (1965) found that implantation occurred after egg transfer to ovariectomized hamsters maintained on progesterone alone, but they were unable to exclude the possibility of an adrenal source of oestrogen.

An electron-dense extracellular deposit between the maternal and embryonic cells during the later phases of attachment is also found in the mouse (Potts, 1966a).

Complex, electron-dense, cytoplasmic inclusions can be distinguished in the trophoblast and epithelial cells at the time when the trophoblast is attaching to the uterine epithelium. These inclusions have a similar ultrastructure to the W-bodies which have been described in mouse trophoblast at the time of implantation (Wilson, 1963; Finn & McLaren, 1967; Potts, unpublished observations). It is difficult to determine whether an inclusion, such as that illustrated in Plate 2, fig. 6, is evidence of phagocytosis of the maternal epithelium by the trophoblast or a specialized cytoplasmic organelle produced in the trophoblast and associated with trophoblast invasion.

**SUMMARY**

1. The pre-implantation blastocyst of *Cricetus auratus* is rich in cytoplasmic fibrous material which persists until implantation has begun.

2. Implantation takes place between 80 and 100 h post coitum.

3. The uterine lumen is obliterated at the time of implantation. The maternal–embryonic junction is characterized by a close association between the trophoblast and the microvilli of the epithelial cells. As implantation proceeds the microvilli are replaced by irregular cytoplasmic processes. Complex cytoplasmic inclusions are found in the trophoblast at the time of implantation.
Implantation in golden hamster

Résumé

L'ultrastructure de l'implantation chez Cricetus auratus

1. Dans la période précédant l'implantation, le blastocyste de Cricetus auratus est riche en matériel cytoplasmique fibreux qui persiste jusqu'au début de l'implantation.

2. L'implantation se produit entre 80 et 100 h post coitum.


This research was partly supported by a grant from the Medical Research Council. We are grateful to Mrs. Sheila Barton for her assistance at all stages of this work.

References


(Manuscript received 31 July 1967, revised 2 November 1967)