VARIOUS TYPES OF EMBRYO-ENDOMETRIAL CONTACTS DURING DELAY OF IMPLANTATION IN THE MOUSE

S. BERGSTROM AND O. NILSSON

Department of Anatomy, University of Uppsala, S752 20 Uppsala, Sweden

(Received 29th September 1972)

Summary. A gradual closure of the uterine lumen occurs during progesterone treatment of mice spayed in early pregnancy. This causes an appearance of various imprints by the endometrium on the blastocyst. Imprints bounded by a polygonal circumference are caused by the slightly protruding intercellular borders of the endometrium. Shallow imprints appearing later are produced by bulging epithelial cells. Deep, rounded imprints are caused by fungus-like protrusions of the luminal epithelium. Generally, imprints are more frequent on the blastocyst surface than on the endometrial surface, suggesting that the trophoblast cells are more malleable than uterine epithelial cells in delayed implantation. The good correlation between structures in scanning and transmission electron micrographs indicates that the preparation techniques used are satisfactory.

Blastocyst implantation can be experimentally delayed in some rodents by ovariectomy in early pregnancy (Canivenc, Laffargue & Mayer, 1956; Yoshinaga & Adams, 1966). By giving exogenous progesterone to pregnant mice from the day of ovariectomy (usually Day 3), a steady state in blastocyst metabolism and morphology is gradually achieved (Weitlauf & Greenwald, 1968; Bergström, 1972b). This steady state runs parallel to an epithelial luminal closure (Martin, Finn & Carter, 1970), and such blastocysts reside in intimate relation with the surrounding epithelium (Hedlund & Nilsson, 1971). Although various embryo-endometrial contacts are present, the blastocysts are not functionally attached but can be flushed out of the uterus.

The structural characteristics of the surface of blastocysts in delayed implantation can conveniently be examined with the scanning electron microscope (SEM). Since this necessitates a flushing of blastocysts out of the uterus, preferably by a fixative (Bergström, 1972a), the mutual relations between blastocyst and uterine epithelium are distorted and it remains to be established whether the blastocyst surface, as seen under the SEM, really represents the blastocyst surface in situ. To answer this question, blastocysts in various stages of delayed implantation were studied both in situ (by transmission electron microscopy—TE microscopy) and after flushing out of the uterus (by SE microscopy).
Virgin female albino mice of the NMRI strain and weighing 20 to 25 g were mated with males of the same strain. The day on which a vaginal plug was found was designated Day 1 of pregnancy. All animals were bilaterally ovariectomized under ether anaesthesia between 12.00 and 15.00 hours on Day 3. From the day of ovariectomy until laparotomy, all animals received daily injections of 1 mg progesterone in 0.04 ml peanut oil (A.B. Leo, Helsingborg, Sweden).

Laparotomies were performed under sodium pentobarbital anaesthesia on Days 5, 6, 7, 9 and 18. The uteri were either excised and flushed with fixative to recover blastocysts for SE microscopy or fixed in situ for TE microscopy by perfusion through the abdominal aorta. Glutaraldehyde (2.5%) in Soerensen’s phosphate buffer (pH 7.4, 390 mosmol/kg water) was used as the primary fixation solution throughout.

Blastocysts that had been flushed from the uterus were collected in watchglasses, in which they were post-fixed in a solution of 1% osmium tetroxide in Soerensen’s buffer and washed as previously described (Bergström, 1972a). After freeze-drying at −79°C and subsequent gold-palladium coating, the specimens were observed in a JEOL JSM-U3 SEM, usually run at 10 kV.

Perfused uteri were carefully dissected out, post-fixed in osmium tetroxide as above, embedded in Epon and sectioned for light microscopy. When a blastocyst was found in situ, the blastocyst site was trimmed, sectioned for electron microscopy, and observed in an RCA-3B or JEOL 100B electron microscope.

The blastocyst was usually free-floating in the lumen before Day 6 and its surface was characterized by a regular arrangement of microvilli. This was observed both on SE and TE microscopy. A gradual closure of the uterine lumen accompanied by structural changes of the epithelial cells was observed from Day 6 and the structure of the ensuing contacts between blastocyst and endometrium was observed to change during the first days of delayed implantation.

Imprints bounded by a polygonal circumference appeared on Day 6 (Pl. 1, Fig. 1). They were best observed in the centre of the moderately convex trophoblast cells and were not deep. As seen on TE microscopy, the polygons were caused by the slightly protruding intercellular borders on the surface of the uterine epithelium (Pl. 1, Fig. 2).

On Day 7, the whole apical area of the now bulging uterine epithelial cells was involved in the contacts and the epithelial cells caused shallow imprints all over the blastocyst surface (Pl. 2, Fig. 5). This closer relationship between the bulging epithelial cells and the blastocyst was confirmed by TE microscopy (Pl. 2, Fig. 6).

A deeper, rounded and crater-like imprint appeared about Day 7. It had a well-demarcated border (Pl. 1, Fig. 3). These imprints usually overlapped the shallow imprints caused by the uterine cell surface and were seen by TE microscopy to correspond to the fungus-like protrusions from the apical surface of the epithelial cells (Pl. 1, Fig. 4). A steady state of blastocyst surface ultrastructure then occurred from Day 7 onwards, at least up to Day 18.

The embryo-endometrial contacts during artificially induced delay of im-
Fig. 1. Imprint bounded by a polygonal circumference and situated in the centre of a mouse trophoblast cell. × 8000.

Fig. 2. Protruding apical areas of intercellular regions in the mouse endometrium. These areas protrude into an adjacent trophoblast cell and cause an imprint. × 8000.

Fig. 3. Crater-like imprint on a mouse trophoblast cell. Note the sharply bounded edges. × 9000.

Fig. 4. Fungus-like protrusions from a uterine epithelial cell producing an imprint in the adjacent trophoblast cell. × 9000.

(Facing p. 532)
Fig. 5. Shallow imprints on a mouse trophoblast cell. ×6500.

Fig. 6. Protruding uterine epithelial cells in contact with the surface of an overlying blastocyst and producing shallow imprints. ×6500.

(Facing p. 533)
plantation cause imprints on the trophoblast surface but hardly at all on the epithelial surface. The endometrial cells and their protrusions would thus appear to constitute a less malleable tissue than the trophoblast cells. This property of the blastocyst wall might have a functional significance in establishing the attachment to the uterine epithelium which occurs at implantation.

The fungus-like protrusions formed the closest contact with the trophoblast cells; the protrusions even being surrounded by the cytoplasm of the trophoblasts. The interior of the protrusions had an ultrastructure suggesting a protein content (Nilsson, 1972). Since the blastocyst seems to need protein as a nutrient (Brinster, 1971), the close apposition between the protrusions and the trophoblast tissues might be a structural indication of a route for transfer of nutrients to the blastocyst. This assumption is further strengthened by the finding that, in experiments with progesterone deprivation, a low viability of the blastocysts is associated with a decreased number of protrusions (Bergström & Nilsson, 1972).

Since SE microscopy of blastocysts shows a close correspondence to results obtained by TE microscopy, the flushing out of blastocysts in the delayed state, by the fixative used, does not distort the appearance of the blastocyst surface. The present preparation technique is, therefore, suitable for SEM observations of blastocysts.

REFERENCES