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CONFRONTATION OF RABBIT BLASTOCYSTS AND ENDOMETRIUM IN ORGAN CULTURE

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Embryo implantation in the uterus involves attachment of the apical side of the trophoblast to the apex of uterine epithelial cells, and it starts when the invasive phase of the trophoblast coincides with a "receptive state" of the endometrium, the latter being controlled by estrogens and progesterone. The molecular basis for the receptive state is completely unknown so far. In the present communication we are reporting results from a series of investigations in which we try to develop an in vitro culture model for the study of molecular mechanisms of this process. In the first series, it was tried to find conditions under which rabbit endometrium can be maintained in vitro in organ culture in a quasi-physiological condition as judged by morphological transformation of the endometrium as typical for the preimplantation phase. In a subsequent series of experiments, we are confronting this endometrial tissue with preimplantation rabbit blastocysts under varying conditions.

Fragments of rabbit endometrium are explanted during early pseudopregnancy and are cultured on a gyratory shaker for 2 to 6 days. During the first 2 days the epithelium is found to grow over the former wound so that a complete epithelial covering with a morphologically normal apico-basal polarity and a well-developed basal lamina is restored. There is no central necrosis in the stroma under the conditions used. Under progesterone substitution (10^{-5}mg/ml, similar to plasma levels found at corresponding pregnancy stages), our cultured endometrial fragments are found to show extensive fusion of epithelial cells. This mimics impressively the in vivo situation where large symplasms form in the uterine epithelium at implantation sites, from 7 d p.c. on in the antimesometrial and one day later also in the mesometrial part of the endometrium. It appears that only the superficial parts of the original (not the regenerated) uterine epithelium are capable of progesterone-induced fusion.

On the other hand, detailed freeze-fracture studies of cell membranes reveal that only a proportion of uterine epithelial cells shows the proliferation of tight junctions which is typically seen in the progesterone-dominated uterus in vivo. The epithelium of cultured specimens seems to be a more heterogeneous population of cells than in vivo, which is also documented in the formation of gap junctions.

Blastocysts grown in vitro in co-culture with precultured endometrial fragments show a remarkable degree of differentiation both in the embryo proper and in the trophoblast (typical trophoblastic knobs as well as the horseshoe-shaped syncytiotrophoblastic thickening surrounding the embryo proper). Our experiments show that in order to achieve an attachment of blastocysts to endometrial fragments it is critical that both are kept in close contact as they are in the uterus. Random collision in simple culture systems (e.g. during gyration in Erlenmeyer flasks, Fig. 1) is not sufficient, even if the material is assembled in a cavity formed in an agar disk(Fig.2)and is left stationary for 1-2 hours. Better contact is provided by co-culture in plastic tubes closed with dialysis membranes (Fig. 3), or in dialysis tubings (Fig. 4). In these latter types of experiments, we were able to observe attachment and also invasion although so far only in a minority of specimens. In addition to these difficulties with maintaining apposition of specimens throughout in vitro cultivation, fluctuations in the concentration of progesterone seem to pose a problem which cannot be solved by changing media daily. We are experimenting, therefore, with a continuous medium exchange system (Fig. 5) which we hope will allow us to condition the uterine epithelium for trophoblast attachment in a way that is more comparable to the in vivo situation.