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DEVELOPMENT OF AN IN-VITRO MODEL FOR EMBRYO IMPLANTATION AND TROPHOBLAST INVASION

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Invasion of the trophoblast into the endometrium is a characteristic element of the process of embryo implantation in most mammalian species. Implantation initiation involves the astonishing phenomenon of contact formation between the apical parts of two epithelia, i.e. trophoblast and uterine epithelium, starting with superficial attachment and formation of intercellular junctions and followed by penetration of the trophoblast through the epithelium.

Our previous studies on the mechanisms of implantation initiation had concentrated on analytical work on special proteinase systems (e.g. blastolemmase) as well as on experiments on blockage of implantation initiation by intrauterine administration of proteinase inhibitors in vivo. Here we are reporting on preliminary results of our attempts to develop an in-vitro model for the study of trophoblast attachment and invasion. The model uses confrontation cultures of three dimensional endometrial fragments plus blastocysts.

Our first series of experiments has concentrated on the formation and on the maintenance, in vitro, of three dimensional fragments of endometrium which are completely covered by uterine epithelium, in order to allow the trophoblast to interact with its physiological counterpart in the final co-cultures. Endometrium is obtained from pseudopregnant rabbits and cultured on a gyratory shaker for 2 days according to suggestions made by M.M. Mareel. Light and electron microscopical studies of the material reveals that the epithelium usually grows over the cut surface of the fragments completely during the 2 day incubation period. At the former wound, the epithelium is at first flat but nevertheless well polarized, and it later grows in height. Even after a total incubation period of 4 days there is usually no central necroses in the stroma, diffusion apparently being aided by the existence of wide uterine crypts. However, there is evidence for shedding of uterine epithelial cells, particularly those located superficially, if no hormones are added to the medium. It appears, therefore, that a rapidly proliferating population of uterine epithelial cells takes over by growing out from the depths of the crypts (a population that may be more similar to nonpregnant uterine epithelium) if no hormones are provided in vitro. In order for our model to be representative of normal implantation, however, it appears essential to maintain the epithelium of the superficial part of the endometrium with which the trophoblast would normally interact in vivo. Certain hormonal regimens (progesterone, estradiol-17β) were found to maintain, in vitro, a superficial epithelium which shows most morphological criteria typical for the late preimplantation phase including e.g. formation of symplasms by cell fusion. First results on co-culture of blastocysts with the endometrial fragments, during which a remarkable development of the embryos was observed, will also be reported.

This model may allow us to study cell biological changes in the uterine epithelium which form an essential element of the "receptive state" during which the trophoblast can succeed in attaching to the otherwise non-adhesive surface of the epithelium, and in penetrating it.