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ASSOCIATION OF EXTRACELLULAR MATRIX FIBRILS WITH INVASIVE TROPHOBLASTIC CELLS OF THE MOUSE

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During pregnancy, the cells of the connective tissue of the endometrial stroma, morphologically classified as fibroblasts, acquire new structural and functional characteristics and result in an epithelioid tissue named decidua. The decidual cells become large and round. The cells stablish contacts and the intercellular space becomes greatly reduced (C. A. Finn, 1977, The implantation reaction, p. 245-308. In R. M. Wynn, ***Biology of the uterus***. New York, Plenum Press, 2nd edition; H. M. Weitlauf, 1988, Biology of implantation. In E. Knobil et al. (eds) ***Physiology of Reproduction,*** Raven Press, 2,540 p.; P. A. Abrahamsohn, 1989, Morphology of Decidua, p. 127-133. In K. Yoshinaga ***Blastocyst Implantation***. Boston, Adams Publ. Group).

At the maternal-embryonic interface there is a close proximity between the decidual cells and the embryonic trophoblastic cells. Due in part to the arrangement of the decidual cells and in part to the close proximity between these cells and the trophoblastic cells, the study of the invasiveness of the trophoblast during pregnancy is very difficult.

The transplant of trophoblast tissue to host tissues that have characteristics that are similar to those of the endometrial stroma previous to decidualization is an alternative way to try to understand the invasive mechanisms of the trophoblast cells in connective tissues in general.

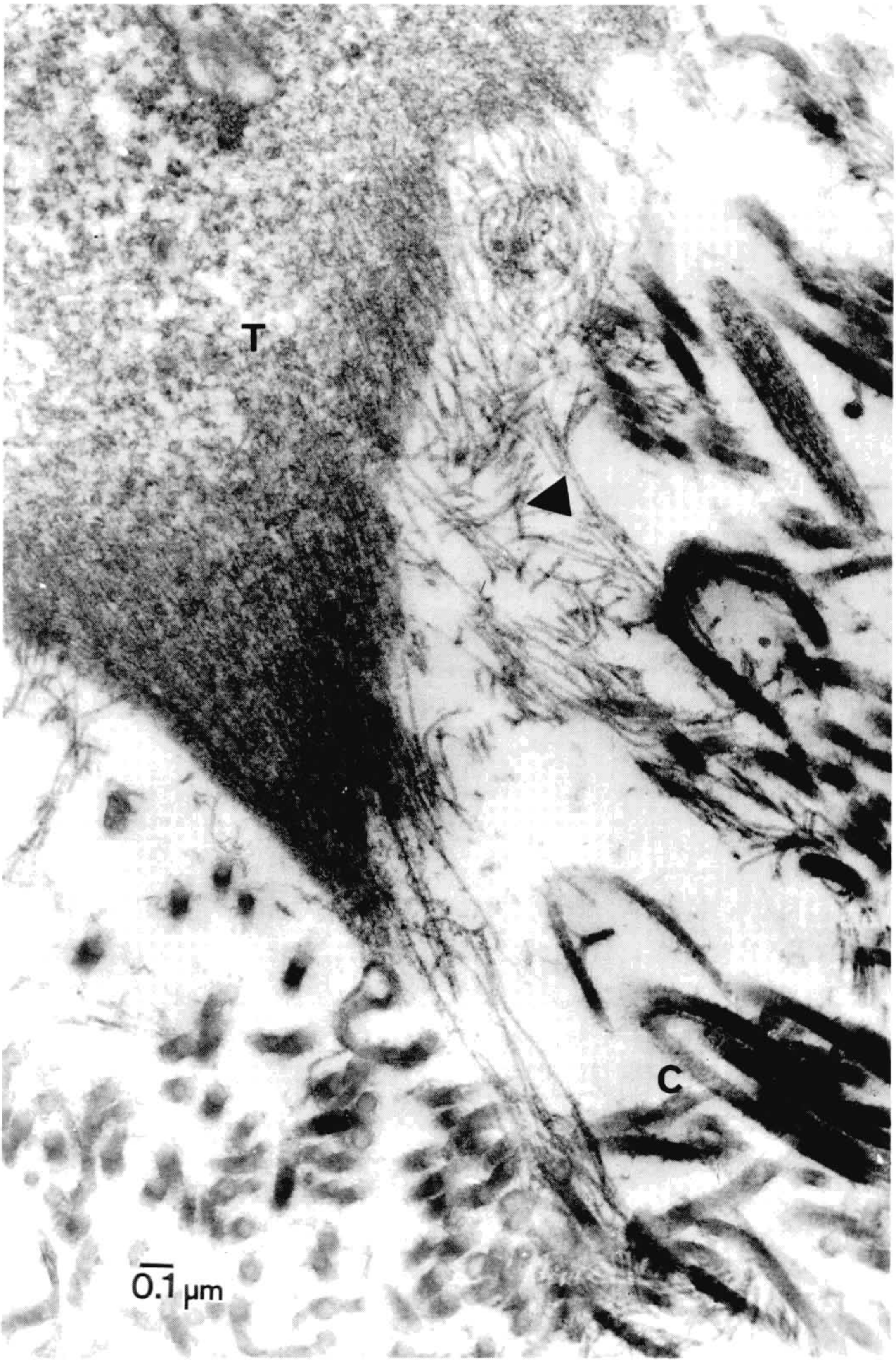
Swiss mice aged between three and four months were used. Virgin females were mated on the evening and they were examined on the following morning for the presence of vaginal plugs. The day on which a vaginal plug was found was called day one of pregnancy.

Animals were killed on day 8 of pregnancy and the uteri were dissected. The implantation sites were isolated and kept in sterile phosphate buffered saline containing 0.3% of bovine serum albumine (PBS-BSA) at 37°C. Each implantation site was then placed in a dish containing PBS-BSA. Under a stereo microscope the endometrial tissue and the embryoblastic parts of the embryo were carefully pulled off with the aid of microscissors and embryological needles. The remaining ectoplacental cones were transferred to small wells containing PBS-BSA at 37°C where they were kept until being transferred to the host animals. Recipient animals were males, virgin and pregnant female mice. These animals were kept under anaesthesia with chloral hydrate. The skin of their back was shaved and a small hole was made in the middle of the lombar region. The needle was introduced in this hole and was pushed towards the left and right side so as to open two channels within the subcutaneous tissue.

Two or three ectoplacental cones were collected at a time into a glass cappilary connected to a microsyringe. The cappilary was inserted through the channel at the left side and the ectoplacental cones flushed out at the end of the channel together with about 20 μl of medium. After withdrawing the cappilary it was inserted in the right side channel and a similar amount of medium was deposited. A total of 43 ectoplacental cones were used in these experiments. The recipients were killed from the third up to the fifth day after embryo-transfer. The subcutaneous dorsal regions were cut and were fixed in buffered 2% glutaraldehyde containing either Rutheniun Hexamine Trichloride, Safranine O or tannic acid, and processed for electron microscopy.

When the skin of the flanks was dissected, the subcutaneous tissue was examined and either did not show any alterations as compared to a normal subcutaneous tissue or presented nodules, indicating that the implant had been successfull. Macroscopical examination of sham-operated skins failed to show any alterations.

The ultrastructural analysis of the nodules showed that two distinct trophoblast cell populations developed in the connective tissue of the host animals: A) Invasive trophoblast cells associated with blood vessel damage and local hemorrhage, and B) Non-invasive trophoblast cells.



Invasive trophoblastic cell (T) developed in host connective tissue. C : collagen fibrils; (↟) : microfibrils. Fixed with glutaraldehyde containing tannic acid 56000X.

Many surface areas of invasive cells were surrounded by microfibrils, without any cross-banding, measuring 10 nm in diameter.

One end of the microfibrils seemed to be attached to the trophoblast cell surface whereas the opposite end seemed to attach to collagen fibrils.

The cell surface of macrophages and mast cells, that surrounded the implant also showed a similar association with microfibrils. However, these microfibrils were not close to collagen fibrils.

Our results showed that invasive trophoblastic cells that developed in the host tissue maintained close proximity with microfibrils which seemed to be attached to collagen fibrils. The non-invasive trophoblast cells which also developed in some transfers did not present association with microfibrils.

The nature of these microfibrils is unknown, but their morphology is similar to the fibrotubules that belong to the elastic system (see S. Inoué & C. P. Leblond, 1986, The microfibrils of connective tissue. *I. Ultrastructure. Amer. J. Anat., 176*: 121-138).

Curiously, the macrophages and mast cells which surrounded the implant region also showed close proximity with these microfibrils.

As these three types of cells are migratory, we are studying the posibility whether their association with microfibrils might be related to their movement within the connective tissue.

To our knowledge, the attachment of this kind of microfibrils to cellular surfaces was not reported in the litterature before.