Effect of Placental Tissue Implantation on the Morphology and Function of Rat Placenta

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Subcutaneous implantation of a fragment of cryopreserved placental tissue to pregnant rats stimulates the morphological and functional activity of the recipient's placenta and inhibits aging.

Key Words: *implantation; placenta; morphological study*

Placental dysfunction is one of the main causes of fetal and neonatal diseases, often eventuating in death [5]. Subcutaneous transplantation of cryopreserved placental tissues to pregnant women is used for treating intrauterine hypoxia of maternal placental origin.

We studied the mechanism underlying the effect of placental cryograft in pregnant rats in which cryopreserved fragments of placental tissue were implanted subcutaneously.

MATERIALS AND METHODS

Fragments of cryopreserved placental tissue (1/4 of disk) was implanted under the skin of 10 pregnant Wistar rats during the first week of gestation. Placentas for preparing the implantation material were removed under aseptic conditions from the uteri of normal Wistar rat on the eve of delivery, perfused, crushed, and frozen according to a special program [2]. The animals were sacrificed on days 7 and 14 after implantation. The morphology of implanted placental tissue and of the placenta removed from the uterus was studied. Full-term pregnancy placentas of intact Wistar rats (n=3) were the control. Fragments of the placentas (implanted under the skin and removed from the uterus) were examined by light and fluorescent microscopy under PZO and LUMAM-12 microscopes. The examined preparations were embedded in paraffin and stained with hematoxylin and eosin for nucleic acids, according to Einarson examined in the Schiff-iodine acid test, silver stained according to Bielschowsky's method, and primary fluorescence of unfixed and formalin-fixed placental tissue was studied.

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RESULTS

A fragment of placenta transplanted subcutaneously to a female was lyzed by adjacent tissues. Microscopic examination on day 7 after implantation showed abundant segmented nuclear infiltration and fragments of nuclei (caryorhexis). Later (day 14), villous chorion of the implanted placental fragment was completely lyzed and represented by homogenous eosinophilic conglomeration. The basal plate contained cell layers looking persistent despite infiltration by segmented nuclear leukocytes (Fig. 1, *a*). The nuclei of persistent cells of the donor placenta were large (8-20 μm in diameter), oval, with diffusely disposed finely dispersed chromatin. The cytoplasm was eosinophilic and had small pores. Staining for nucleic acids confirmed their presence in the nucleus and in small amounts in the cytoplasm. The cytoplasm of these cells fluoresced white-yellow in ultraviolet rays (primary fluorescence). The recipient placenta in full-term gestation was characterized by labyrinth hemochorial structure typical of rodents,

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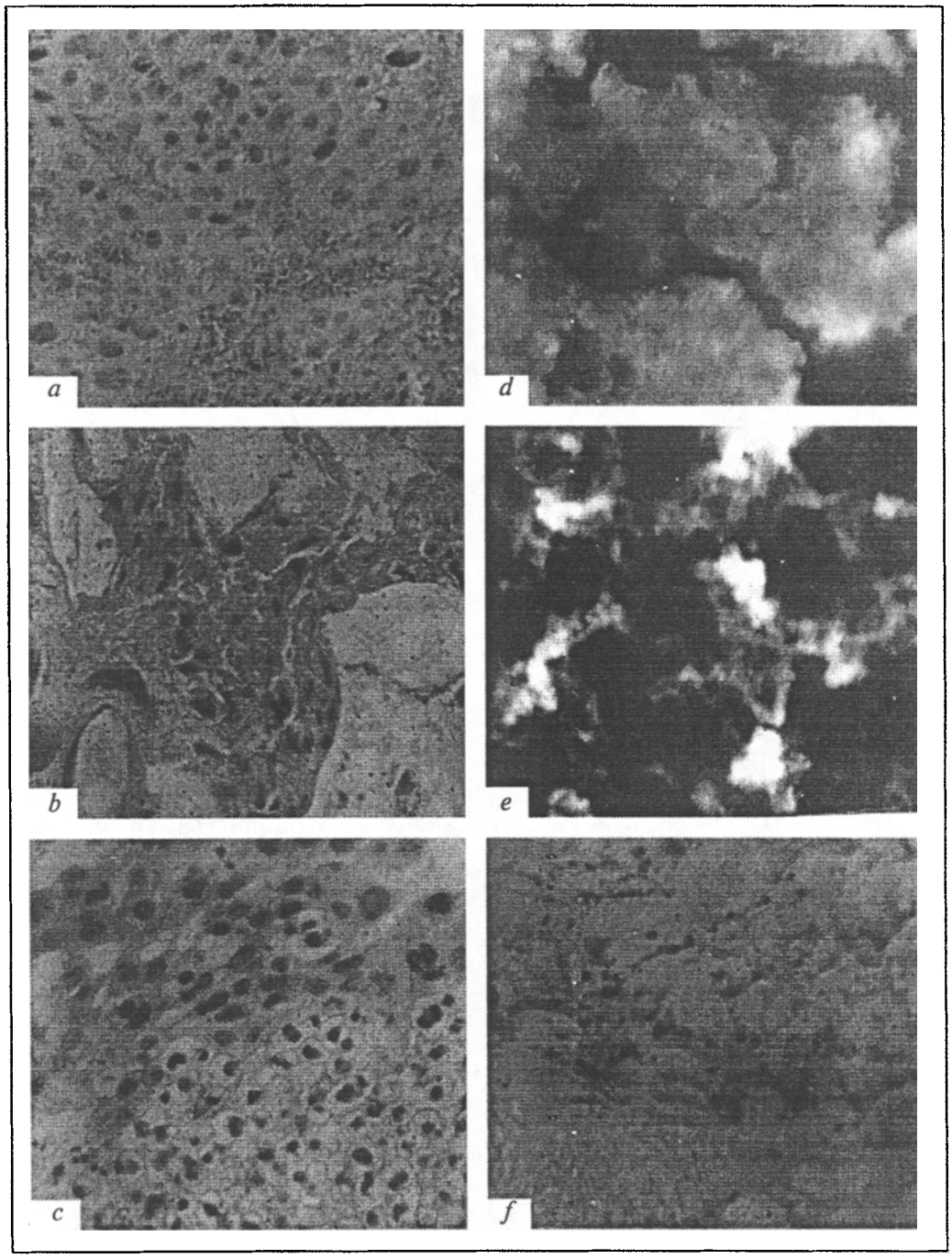

Fig. 1. Implanted placental tissue and recipient placenta. *a*) implanted placental tissue on day 14 after implantation; *b*) basal plate of a control rat; loss of cells, "suppressed" morphofunctional state; *c*) basal plate of the placenta in an experimental rat (day 7 after implantation of placental tissue): morphologically and functionally active trophoblast cells of two types of specialization; *d*) intense autofluorescence of trophoblast cells of the basal plate in experimental rat (day 7 after implantation of placental tissue); *e*) the same for the chorion; *f*) glycogen granules in decidual cells of experimental rat (day 14 after implantation of placental tissue). *a-c*: hematoxylin and eosin staining, ×140; *d*, *e*: fluorescence, ×140; *f*: Schiff-iodine acid test, ×280.

which makes its histological section look small-cell. Fetal capillaries were large, endotheliocyte nuclei were oval, and chorionic stroma was multicellular (histiocytes). The fetal part of the placenta contained numerous, in comparison with the control, trophoblastic cells with large dark nuclei (chromatin was dispersed), of irregular shape, the nucleolus was usually discernible, while in the control it was not seen. Unlike in the control, fibrinoid was almost completely absent from fetal tissues and the labyrinth lumen. The basal plate trophoblast was preserved and looked much more active: in the control

(Fig. 1, *b*) the nuclei were more often elongated and intensively hyperchromatic, while in experiment (Fig. 1, *c*) they were more bulky and larger; chromatin was finely dispersed, the nucleolus was discernible; trophoblast cell cytoplasm contained high amounts of RNA (Fig. 1, *d, e*). These cells of the basal plate trophoblast may be specialized in hormonal protein production. In contrast to the control, there were binuclear trophoblastic cells. There were oval cells with dark round nuclei and large optically transparent vacuoles in the cytoplasm (Fig. 1, *c*). They looked like fetal adrenocortical corticocytes. Yellowish fluorescence of these trophoblastic cells in ultraviolet light indicates their probable steroid-producing specialization. In the control the content of then cells is much lower, and they look dying in general. Decidual cells detached from the placenta were enlarged, being morphologically and functionally more active than in the control. The Schiff-iodine acid test detects more glycogen granules in their cytoplasm than in the control (Fig. 1, *c, f*). Silver staining according to Bielschowsky revealed reticuline fibers round decidual cells. A younger (7-day-old) recipient placenta was characterized by a very potent basal plate with bulky trophoblasts (types 1 and 2) with signs of high morphofunctional activity.

The basal plate containing the trophoblast and decidual cells persisted in cryopreserved rat placental tissue implanted subcutaneously to pregnant rats. Apparently, decidual cells prevent rapid autolysis of the basal plate trophoblast, which is observed in the fetal part of transplanted placental tissue. Moreover, before transplantation of the placenta, villous chorion was adapted to exceptionally favorable conditions: the vicinity of maternal and fetal circulations which are absent from the basal plate. The cytoplasm of persistent cells of the placental tissue fragment on day 7 after implantation contained a small amount of RNA; this cytoplasm was capable of primary fluorescence (yellowish-white) in ultraviolet light, which is typical of steroids [1]. "Territorial" closeness to implanted fragment of lipid metabolites is important, because the graft was implanted under the skin. The placental graft may be involved in general hormonal regulation in the mother-placenta-fetus system. After an increase in the level of steroids, their production in the implant and concentration in the blood decreased, due to the substitute role of the implant, which may stimulate the hypothalamus-pituitary-ovarian system of the female by the feedback principle. Intensified hormone production in this system has an pronounceded "rejuvenating" effect, which was observed in the placentas implanted in the uterus. Conditions for the exchange between the mother and fetus improve, and hormone-producing activity of such a placenta increases, as evidenced by the trophoblast status in the villous chorion and in the basal plate.

Implantation of placental fragment under the skin of a pregnant rat exerts a long-lasting effect. The basal plate of placental tissue is more stable to lysis than villous chorion. The effect of implantation is apparently hormonal, stimulating the production of steroid and protein hormones in the recipient placenta, presumably through maternal hypothalamus-pituitary-ovarian axis after the feedback mechanism. Necrobiosis in the placenta is delayed, judging from the low volume of fibrinoid, which replaces dead placental structures. The placental barrier retains its optimal state till the end of gestation: 21-25 days in rats.

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