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Survival of Skin Homografts in Uteri of Pregnant and Progesterone-Estrogen Treated Rats* (32928)

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Since the fetus of viviparous forms has genetic characteristics of the father and mother, its implantation in the uterus of the mother can be considered a homograft with transplantation immunity. Billingham has reviewed several theories which attempt to explain transplantation immunity in the fetal–maternal relationship (1).

Lanman *et al.* (2) have shown that ova from *two* mated pairs survived and came to term when transplanted into a rabbit sensitized to the ova *one* of the donors. They suggested the protective mechanism to be the physical separation of the mother and fetus and the special properties of the trophoblast. These views were shared by Simmons and Russell (3) who showed that trophoblastic tissue was not antigenic while embryonic tissue was.

Recently, Hulka *et al.* (4) indicated that hormonal activity related to pregnancy could prevent immunological rejection of the fetus. They reported that two 19-nor steroids, norethynodrel and norethindrone, produced progestational changes in rabbit endometrium and prolonged survival of skin homografted to the rabbit ear.

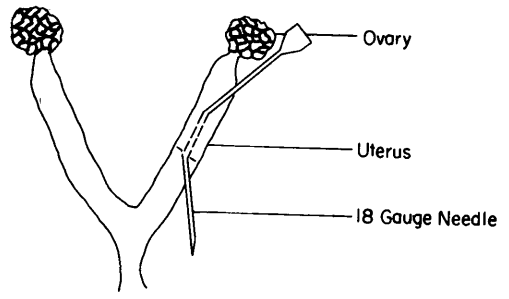
This present study determined the effect of progesterone and estrogen on uterine survival of homografts not protected by trophoblastic tissue.

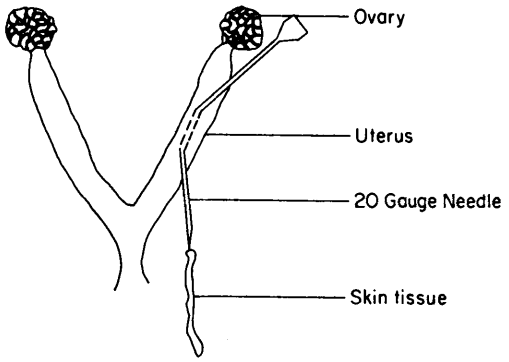
Materials and Methods. Adult Charles River female rats of the CD strain were used. The rats were housed in a room having 12 hours of light and 12 hours of darkness at alternating intervals. Food and water were given *ad libitum*.

Skin transplants. The dorsal surface of donor rats was shaved, and the fine fur removed with a depilatory. A skin flap was excised and excess subcutaneous fat removed. The skin was cut into strips approximately 16 mm long and 0.4 mm wide.

Two punctures were made in uteri of recipient rats 0.5 inch apart with an 18-gauge

* Since submission of this manuscript, a paper has been published supporting our findings and conclusions: Short, R. V. and Yoshinaga, K., J. Reprod. Fertility 14, 287 (1967).





FIGS. 1 and 2.

needle (Fig. 1). By means of a barbed 22-gauge needle, the transplant was pulled into the uterine lumen leaving both ends protruding through the punctures as anchors (Fig. 2).

The time of skin transplantation was designated as day 1. On days 3, 6, 9, and 12, groups of rats were sacrificed and the homografts evaluated according to the following categories: (a) intact, or partially rejected, and (b) completely rejected.

In pregnant rats, homografts were prepared 4 days after mating. In other experiments, progesterone (2 mg) and estrone (0.5 μg) were injected on Days 1 through Day 12.

Results. The skin protruding through the uterine wall did not degenerate at any time and the evaluation, therefore, of homograft rejection is concerned only with the skin within the uterine lumen (Table I).

After 3 days, skin homografts in uteri of nonpregnant intact or castrate rats appeared unchanged (Fig. 3). On day 6, the homograft was decreased in width and appeared to be degenerating. On day 9, the homograft was no longer visible.

During the period of degeneration, an inflammatory reaction, i.e., leukocytic infiltration, involving the myometrium, endometrium and the homograft was seen microscopically (Fig. 4). By day 12, the area between the points of attachment showed little or no inflammation.

In rats treated with *either* progesterone *or* estrone the same degenerative sequence was seen as outlined above. In pregnant rats or in nonpregnant rats treated with *both* progesterone *and* estrone, however, homografts survived for the entire experimental period of 12 days (Fig. 3). Histological examination of the homografts in these groups showed little or no inflammation (Fig. 4). The samples of the last two groups in Fig. 3 and Fig. 4 are from pregnant rats but are similar to nonpregnant rats treated with both hormones.

Discussion. Skin homografted in the uterine lumen evokes an inflammatory response which usually precedes immunological rejection (3). The hormonal effects on uterine inflammation have been well documented. Broome *et al.* (5) and Hawk (6) have shown that bacteria grow better in uteri of pseudopregnant rabbits than

TABLE I. Survival Time of Uterine Skin Transplants.

Group	No. of transplants, intact or still visible/No. of total transplants			
Days:	3	6	9	12
Intact controls	5/5	5/5	0/5	0/3
Castrate controls	5/5	5/5	0/5	0/3
Pregnant	5/5	5/5	5/5	5/5
Progesterone (2 mg/day)	5/5	4/5	0/5	0/3
Estrone (0.5 μg/day)	5/5	5/5	0/5	0/3
Progesterone (2 mg/day) + estrone (0.5 μg/day)	5/5	5/5	5/5	5/5

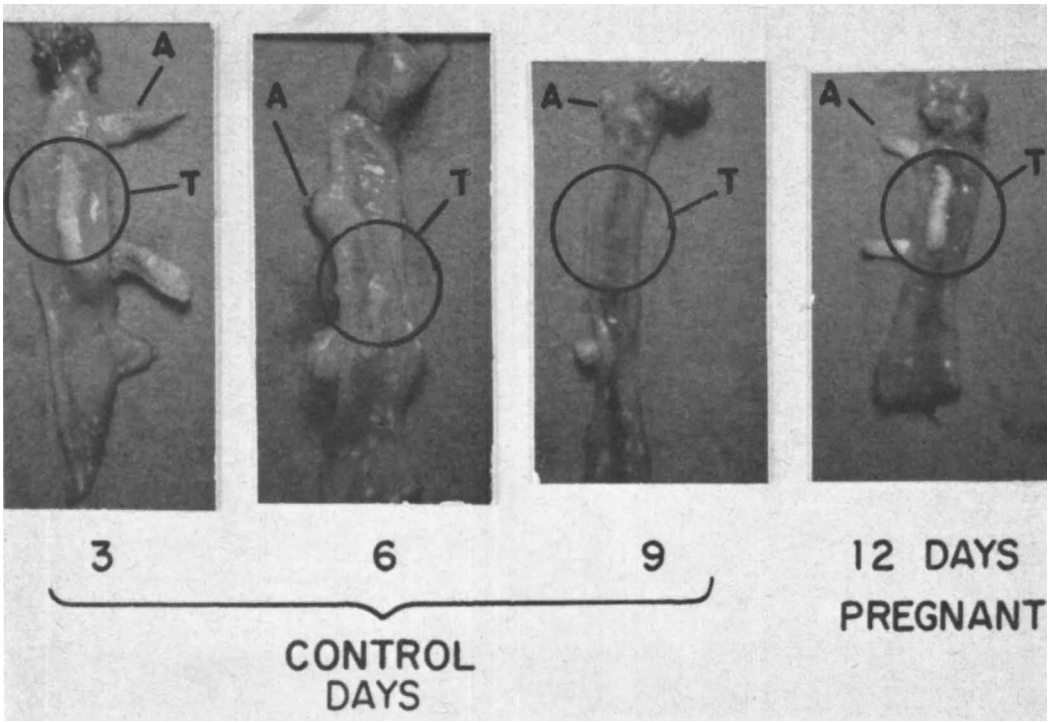


FIG. 3. Photographs of uteri grafted skin; T = area of transplantation; A = skin protuding into peritoneum, anchoring transplant in uterine lumen. In control uteri: day 3 the graft is intact; day 6 partially degenerated graft; day 9 graft is no longer present. In pregnant uterus, 12 days after transplantation, graft is intact.

in the uteri of estrous rabbits. Both investigators consider that the difference in bacterial viability is due to secretion of progesterone during pseudopregnancy which inhibits both cellular and noncellular inflammatory actions. Similar effects have been seen by Marcus (7) in ovariectomized hamsters. He reported that greater leukocytic response to spermatozoa occured with estrogen treatment than with combined progesterone–estrogen treatment. In our studies, progesterone and estrogen, given in combination, prevented graft-induced inflammation and rejection. This inhibition may be one of the factors which increases homograft survival time.

The doses of progesterone and estrogen which maintain the homograft are similar to those used by Cochrane and Meyer (8) to demonstrate survival and implantation of ova in castrated rats. They showed that progesterone alone could maintain the ova in a viable condition *in utero,* but in our studies, only the estrogen–progesterone combination permitted skin survival. This difference could be due to greater antigenic stimulus by the skin compared to a trophoblast-surrounded ovum.

Homograft survival at the points of exit from the uterus remains unexplained. The survival may be due to the fact that this tissue protrudes into the peritoneum and therefore is not in contact with as many antibody-containing cells. Another possibility is that the disappearance of the homograft from the uterine lumen is not an example of immunological rejection. Parr and co-workers (9) have shown that a foreign body in the uterus will evoke an inflammatory reaction causing an increase in lysozyme activity. This enzyme or other leukocyte-connected enzymes may lead to homograft digestion.

It is concluded that in addition to the nonantigenic makeup of the trophoblast, hormone influences acting on the uterus can play a role in preventing the immunologic rejection

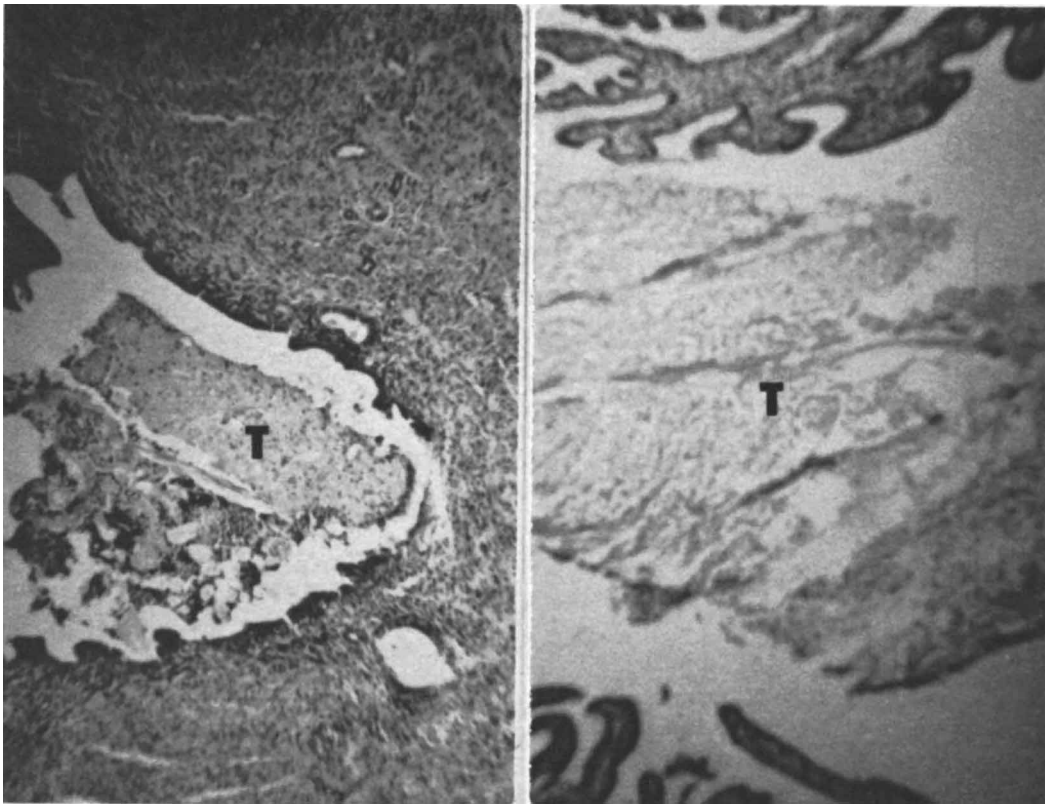


FIG. 4. Photomicrografts of uteri grafted skin. T = transplant. Left (40 ×), 9 days after transplantation into control uterus. Leukocytes have invaded myometrium, endometrium, and transplant. Right (100 ×), 12 days after transplantation in pregnant uterus. Transplant is intact with no inflammation.

of the fetus. Further, at least in these studies, the interaction of progesterone and estrogen, as well as pregnancy, prevented uterine inflammation evoked by a homograft.

Summary. Skin homografts placed in uteri of nonpregnant intact or castrate rats evoke a local inflammatory reaction followed by rejection with 9 days. Similar results are obtained with rats treated with either progesterone or estrone alone. Homografts in pregnant or in nonpregnant rats treated with both progesterone and estrone, however, survive for the 12-day duration of the experiment.

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