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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: IN ECTOPIC ENDOMETRIUM

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ABSTRACT

Under progesterone regulation, blastokinin can be synthesized by rabbit endometrium grown in ectopic sites as well as in its normal location as lining for the uterine lumen. Transplanted to the ear, endometrial tissue exhibits both growth and secretory activity in response to the progestational states of pregnancy and pseudopregnancy or to the administration of exogenous progesterone to ovariectomized does. Conversely, growth and blastokinin secretion are reduced to absent in grafts to animals with low ovarian steroids as during anestrous or after ovariectomy without hormonal supplement. Estrogen supplementation gives conflicting growth patterns but never results in blastokinin secretion.

INTRODUCTION

One of the principal distinguishing characteristics of the progestational rabbit uterus is the synthesis of the unique protein blastokinin by the endometrium (Krishnan and Daniel, 1967). In a series of papers published in the Journal of the Tennessee Academy of Science we have reported our attempts to identify a variety of conditions which relate to blastokinin synthesis by endometrial tissue in its normal relationship to the uterus. (Booher and Daniel, 1977; Daniel, 1980a, b, c; Daniel and Booher, 1977) Our interest has now been directed to the question of whether endometrium can be grown in abnormal locations (ectopic sites) in the animal's body and synthesize blastokinin under the same conditions. The answer to this question is especially important because the techniques developed could also provide an insight into, and methodology for, artifically inducing endometrioses in the rabbit as a means of studying this medical mystery which mainly afflicts human females.

Although our earlier attempt to grow endometrial tissue ectopically was successful (Boyce and Daniel, 1978), it was not convenient as a research system because the graft was made to the parietal peritoneum and necessitated repeated surgical procedures each time sampling or measurement was required. A different site was necessary where the growth of the graft could be supported and measured, and secretions removed non-surgically and without discomfort to the animal. Because of its easy acessibility, good vascularity, and two layers of skin covering an inner core of cartilage and connective tissue, the ear was selected as an especially appropriate site. We report here the growth of endometrium tissue grafts in the ears of female rabbits, secretion of blastokinin by these grafts and how their growth and secretory activity are affected by the endocrinological state of the animal.

METHODS AND MATERIALS

Twenty young adult female rabbits of mixed breeds were used in this study. Four does were made pregnant by natural mating, four were pseudo-pregnant, three were anestrous animals and nine were ovariectomized. Of the ovariectomized does three were given 100 micrograms of estradiol-17β/kilogram of body weight subcutaneously daily, three were given three miligrams progesterone/kilogram per day, and three were left untreated as controls.

The animals were anesthetized with Ketamine HCl (Vetalar, Parke-Davis) (25 mg/kg) followed ten minutes later with Xylazine (Rompun, Haver-Lockhart) (15 mg/kg). The abdomen was opened by a two inch incision, and one horn of the uterus removed after the cervical end, the utero-tubual junction and all major blood vessels had been ligated. The horn was transferred to a sterile dish where a longitudinal incision exposed the endometrium and permitted it to be scraped free with a flat scalpel blade. A small cut (¼ inch) was made through the shaved, dorsal skin of the ear and a pocket formed by stretching the skin below the cut free of underlying tissue with blunt tipped forceps. Pieces of endometrium were inserted into the pocket with forceps or small-bore hypodermic syringe and secured by a single suture to close the cut opening. The procedure was repeated on the second ear. The abdomen was closed using routine suturing techniques with special care not to traumatize the intact horn. After awakening from the anesthesia the does were returned to their cages and maintained as before with food and water *ad libitum* and a twelve-hour photo-period.

The grafting was performed on the fifth day following breeding for both the pregnant and pseudo-pregnant animals, and coincidental with ovariectomy for the animals in the hormone treatment categories. Growth of the grafts was determined by using calipers to measure the greatest length, width and height of the swelling and calculating the approximate volume. Measurements were taken at convenient intervals but never exceeding one week.

Secretions were collected by inserting a 22gauge needle into the accumulated fluid that pooled in the lumen which invariably formed in the successful grafts. The fluid was withdrawn into a hypodermic syringe and put into Spectrapor 3 membrane tubing to be dialyzed against two changes of distilled water for 24 hours at four degrees centigrade. The samples were concentrated by the use of aquacide powder, protein concentration determined by the folin-phenol reaction of Lowry *et. al.* (1951) and fractionated by poly acrylamide gel electrophesis, details of which procedures have been given earlier (Booher and Daniel, 1977). Blastokinin was identified as a prominent post albumen band which has been clearly distinguished from all other uterine fluid and serum proteins.

RESULTS AND DISCUSSION

Figure 1, shows typical grafts of the size obtained in the ears of pregnant does after three weeks compared to the lack of graft development characteristically seen in anestrous rabbits. In ovariectomized animals the grafts did not grow well usually having completely disappeared by four to six weeks, but in pseudo-pregnant animals, and in the ones given progesterone supplementation after ovariectomy the grafts resembled those in pregnant does. The grafts did not follow a consistent pattern in ovariectomized does given estradiol; about one-third of them increased slightly in size over a one month period, one-third decreased to disappearance in the same time and the remainder showed no measurable change, staying the same size as initially throughout the test period.

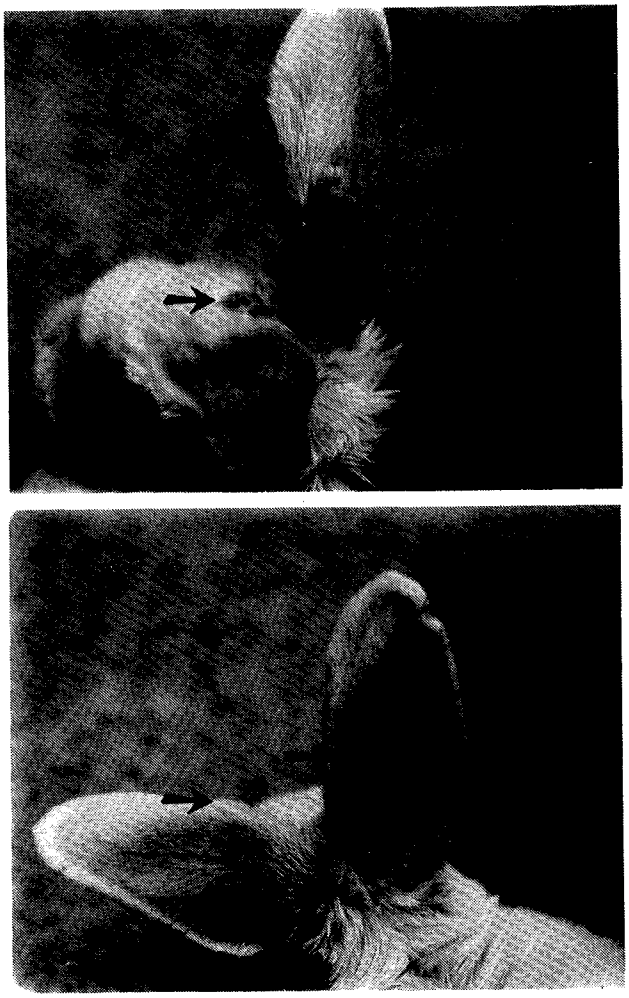

Fig. 1. *Endometrial graft sites three weeks after transfer to the ears of A. a pregnant doe and B. an anestrous doe.*

The growth of grafts in three of the experimental groups described above are illustrated in Fig. 2, namely the pregnant, pseudo-pregnant and ovariectomized series. Although there is at least one devient in each series it is clear that the majority of the grafts follow consistent patterns. In the pregnant animals, the grafts initiated growth immediately, obtained their maximum size in 3 to 4 weeks and then diminished thereafter back to a small and usually constant size by about 2 weeks *post-partum*. In the pseudo-pregnant series the grafts followed much the same pattern even though the animals usually returned to estrus within 3 to 4 weeks. Two of the pseudo-pregnant does and one from the pregnant series were bred again after their grafts had receeded (5 to 6 weeks after the start of the first part of the experiment). In each of these cases the grafts reinitiated their growth and continued to enlarge again for 2 to 4 weeks, at least half of them attaining sizes significantly greater than achieved during their first growth period. Lack of any graft growth with immediate regression, characterized the untreated ovariectomized animals.

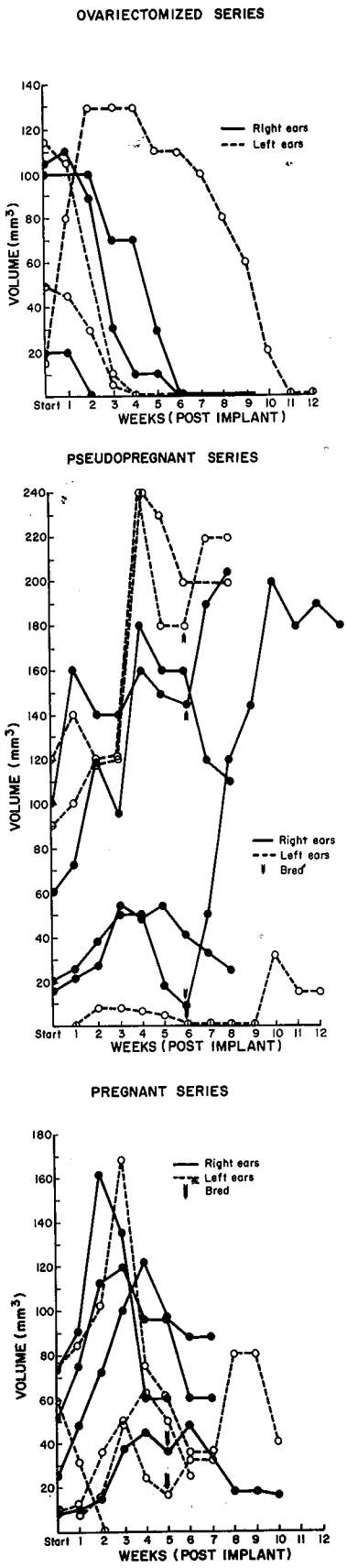

Fig. 2. *Growth of endometrial grafts to the ears of pregnant, pseudo-pregnant and ovariectomized does.*