SUPPORT OF DECIDUOMA FORMATION AND GROWTH BY THE TRANSPLANTED OVARY OF THE RAT

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Summary. The luteal function of ovaries transplanted into the anterior abdominal musculature was judged by evaluation of the decidual cell response to uterine trauma, and by determining the number of days of vaginal di-oestrus.

The weight of the deciduomatous uterine horns in rats with transplanted ovaries was similar to those of the controls, indicating that neither the normal vasculature nor the normal innervation of the ovary is necessary for the induction of pseudopregnancy and that the steroid hormones necessary for deciduoma formation and growth reach the uterus by way of the systemic circulation.

After the induction of pseudopregnancy, the number of days until the subsequent pro-oestrous vaginal smear was prolonged in the rats bearing ovarian autotransplants. However, the vaginal cytology was suggestive of a prolonged pro-oestrus rather than a prolonged di-oestrus.

Transplantation of guinea-pig ovaries with subsequent resumption of their function as shown by successful pregnancy and parturition was first reported by Castle & Phillips (1909). Long & Evans (1922) reported that autotransplantation of rat ovaries resulted in the re-establishment of oestrous cycles and in the induction of pseudopregnancy after coitus with vasectomized males. More recently, Anderson, Melampy & Chen (1967) have demonstrated that after the induction of pseudopregnancy, the number of days of vaginal di-oestrus is prolonged in rats bearing ovarian autotransplants as compared with intact controls. Although it is reasonable to suppose that such vaginal di-oestrus reflects luteal function, this possibility was tested by evaluation of the decidual response to uterine trauma.

Adult rats were housed, four to five per cage, in rooms with a 14 hr light/10 hr dark lighting schedule (05.00 to 19.00 hours). They were allowed free access to rat chow and water. Charles River rats were used in the first experiment and Sprague Dawley rats were used in the second experiment.

Under ether anaesthesia, the ovaries and tubes were removed, and after trimming, the ovaries were placed in separate, surgically created pouches in the anterior abdominal musculature. The control animals were subjected to sham operation, in which a suture was placed around the utero-tubal junction.

Pseudopregnancy was induced by mating, either with fertile (first experiment) or vasectomized (second experiment) males. The morning on which either spermatozoa or a copulation plug could be observed in the vagina was designated Day 1.

In the first experiment, vaginal cycles were followed by daily lavage for 2 weeks before operation, and the animals were permitted to recover for a minimum of 2 weeks before being placed in cages with males. Half of the animals in each group were then subjected to uterine trauma, while the vaginal cycles of the remainder were followed until the subsequent pro-oestrous smear. In the second experiment, the animals were permitted to recover for a minimum of 3 weeks after operation before being caged with vasectomized males. All vaginal smears were fixed in 95% ethanol and stained with 1% toluidine blue before final evaluation.

On Day 5, between 11.00 and 13.00 hours, the mid-ventral abdominal incision was re-opened and the anti-mesometrial lining of the uterus was scratched with a barbed needle as described by De Feo (1963a, b). Autopsy was performed on Day 10 and the weights of the uterine horns subjected to trauma were recorded.

Operations were performed with no consideration given to the stage of the oestrous cycle. In the first experiment, all rats had shown at least 1 day of vaginal cornification by the 5th day after the operation. Following the induction of pseudopregnancy, the number of days until the subsequent pro-oestrous smear was 16.2 ± 0.6 S.E. ($n = 9$) in the group with transplanted ovaries, while in the control, it was 13.0 ± 0.5 S.E. ($n = 6$), the difference being statistically significant ($P < 0.05$). The vaginal cytology characteristic of pro-oestrus was taken as the required end point. However, in many of the animals with transplanted ovaries we observed that 1 to 3 days before the characteristic picture of vaginal pro-oestrus was seen, the vaginal cytology presented a picture which differed from either the predominant leucocytes seen during the earlier stages of di-oestrus or the predominant small nucleated cells seen during pro-oestrus, in that all of the cellular elements were present, but were extremely sparse.

The left uterine horn which had been subjected to trauma in the animals with transplanted ovaries weighed 983 ± 30 mg S.E. ($n = 9$) while that in the control group weighed 1142 ± 102 mg S.E. ($n = 9$), a nonsignificant difference.

In the second experiment, the uterine horns subjected to trauma weighed 1897 ± 215 mg S.E. ($n = 6$) in the animals with transplanted ovaries while they weighed 1972 ± 65 mg S.E. ($n = 7$) in the animals with tubal ligation. These differences were not statistically significant.

These experiments demonstrate the luteal function of transplanted ovaries by virtue of their ability to support the formation and growth of deciduomata which were quantitatively similar in both groups of animals. Further, these observations demonstrate that the ovarian steroid hormones necessary for this process reach the uterus by way of the systemic circulation.

With respect to the number of days until the subsequent pro-oestrous smear, we have confirmed the observations of Anderson *et al.* (1967). However, the technique of fixing and staining the daily vaginal smear has enabled us to

recognize an unusual pattern of the vaginal cytology seen only in the animals bearing transplanted ovaries (although not in all of them), and seen for 1 to 3 days before the characteristic cytology of pro-oestrus. This cytological picture seems to be akin to what Astwood (1939) called "pre-estrus". Studying the "short" oestrous cycle of the rat, he sought to determine whether or not an animal in di-oestrus was about to enter the pro-oestrous stage. He observed a recognizable cytological picture which was notable for its sparsity of formed elements and which was identified by the presence of loosely formed groups of small oval and rounded cells of varying size admixed with a few leucocytes. Within several hours, the appearance of the typical nucleated cells of pro-oestrus marked the conclusion of this "pre-estrus" stage. Our observations may therefore indicate that, in animals with transplanted ovaries, there is a prolonged 'pre-estrus' rather than a prolonged 'di-oestrus', stage of the cycle. We are investigating this possibility by measuring peripheral plasma progesterone concentrations in the two groups of animals.

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