

EXPERIMENTAL BIOLOGY

CHANGES IN THE NUMBER OF OOCYTES IN THE TRANSPLANTED OVARY

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It is being increasingly accepted in the literature that no new oocytes appear during the postnatal development of mammals [7-10]. This conclusion is based mainly on the results of counting the oocytes in animals of different ages, and also after certain experimental procedures. However, some authors [1-6] have observed experimentally an increase in the number of oocytes in adult animals.

The object of the present investigation was to study the changes taking place in the number of oocytes in the ovaries after transplantation.

EXPERIMENTAL METHOD

The experiments were carried out on 120 sexually mature rabbits weighing 2-3 kg. The rabbits were castrated and one of the ovaries was transplanted into the broad ligament of the uterus, the second into the anterior chamber of the eye. The times after transplantation varied from 1 to 180 days. The material was fixed in Carnoy's fluid, Zenker-formol, and formol-Ca, and treated by the usual methods.

The primordial and growing follicles were counted in every 5th section in the transplanted ovary. The mean number of sex cells per unit area and per unit weight of the ovary was calculated. Because of the slight increase in size of the transplanted ovaries and their frequent attachment to the surrounding tissues, their weight and area were measured by means of an Edinger's drawing apparatus, by projecting every 5th section onto paper and then weighing the cut-out pieces on analytical scales.

The significance of the quantitative results obtained was assessed by the methods of variance analysis.

EXPERIMENTAL RESULTS

The process of survival of the grafts took place as two consecutive phases—a phase of destruction and a phase of regeneration. In the degenerative phase (mainly from the 5th to the 14th day) all the elements of the ovary underwent some degree of degeneration. The organ swelled, the connective tissue became loose, the blood vessels around the degenerating follicle became dilated. In the cytoplasm of the oocytes degenerative changes were observed; it either developed large granules or became homogeneous in structure. The nuclei of the oocytes lost their normal structure and became oxyphilic, homogeneous masses, gradually becoming indistinguishable from the cytoplasm. The growing follicles and Graafian vesicles were the first to die.

During the first 5 days after the operation most of the oocytes died. The medulla became essentially a continuous zone of necrosis. The connective tissue of the cortex became loose, and the number of cells was reduced because of pycnosis of the nuclei and subsequent destruction of the cells. The germinal epithelium in some grafts died while in others it showed little change.

Alongside the processes of degeneration, on the 3rd day after transplantation signs of proliferative processes were observed in the connective tissue of the ovaries, arising in two ways. On the one hand, fibroblastic tissue with bands of capillaries grew in from the tissues bordering the ovary (the iris or peritoneum), and on the other hand, a reorganization of the connective tissue of the cortex began to take place: the number of cells increased, they assumed the typical arrangement for the ovary, and they invaded the medulla in bands.

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On the 5th day after the operation single primordial follicles could be clearly distinguished for the first time in the regenerating ovary among the actively proliferating connective tissue, usually where blood vessels were growing into it. Most frequently the follicles lay close to the surface of the ovary. Later the number of viable follicles increased.

During the study of the transplanted ovaries attention was drawn to the early onset of processes of atresia in the generative cells. On the 14th day after transplantation into the abdomen, vacuolation of the cytoplasm was observed in the primordial and growing follicles and of the follicular epithelium, and the regular arrangement of the chromatin in the nucleus was disturbed, ending in the total destruction of the cell and the formation of a corpus albicans. This atresia mainly affected the growing follicles in the early stages of maturation. The result of this atresia was disturbance of the processes of maturation in the transplanted ovary, as shown by the absence of growing and vesicular follicles.

A manifestation of disturbance of maturation of the follicles was atypical development of the cells. Growth of the follicular epithelium lagged behind growth of the oocyte; it lost its regular pattern so that the cells changed in shape and size. In the ovaries occasional oocytes could be seen which were 2-3 times larger than the normal primordial follicles, but they were partly or completely without follicular epithelium. In the ovaries transplanted into the anterior chamber of the eye the disturbance of regular growth and maturation of the follicles was revealed by cystic degeneration of most of the vesicular follicles, leading to death not only of the Graafian follicles, but often of the whole transplanted ovary. Long survival of the grafts was not observed. In most of them frank degenerative processes were present on the 90th-180th day, terminating in death.

The count confirmed an increase in the number of follicles in the transplanted ovary. Whereas in the control animals the mean number of primordial follicles was 139 ± 10 /mg weight of the ovary,* in the first five days after transplantation their number fell to 24.4 ± 4.5 /mg weight in the ovaries transplanted into the abdomen and to 26.7 ± 7.4 in the ovaries transplanted into the anterior chamber of the eye. The number of growing follicles fell sharply in the first five days, and the Graafian follicles were completely destroyed. On the 7th-24th day the number of primordial follicles increased to 105.9 ± 25.7 /mg weight of ovary in the anterior chamber of the eye and 54.5 ± 12.5 in the abdomen, as shown in the table.

The mean number of primordial follicles continued to rise in the later periods (on the 21st-30th days). It increased to 212.8 ± 68 /mg weight in the abdomen and 115.6 ± 24 in the anterior chamber of the eye, a number considerably (4-5 times) higher than the number of follicles in the first five days after transplantation, and it eventually equalled the number of primordial follicles in the control ovaries.

If all the counts were expressed in relation to the whole ovary, and not per milliliter of tissue, the results were in principle the same.

The increase in the number of primordial follicles in the abdomen in the period from 14 to 30 days preceded the increase in their number in the anterior chamber of the eye. The sharp fall in the number of primordial follicles in the anterior chamber on the 21st day after transplantation could be attributed to an increase in the number of Graafian follicles showing cystic degeneration at this time. On the 60th day

* A conventional unit equivalent to the weight of paper on which sections of the ovary were traced with a magnification of 13 times.

Number of Primordial Follicles in Intact Ovaries and Ovaries Transplanted into the Abdomen and Anterior Chamber of the Eye

Time after operation (in days)	Transplantation into abdomen				Transplantation into anterior chamber of eye		
	No. of animals	Mean weight of ovary (in mg)	Mean number of primordial follicles in the whole ovary	Mean number of primordial follicles/mg weight of ovary	No. of animals	Mean weight of ovary (in mg)	Mean number of primordial follicles/mg weight of ovary
Control	8	763 ± 46	105 431 ± 13 405	1 390 ± 10.0	8	763 ± 46	139 ± 10.0
5	6	1 165 ± 230	33 205 ± 10 443	14.4 ± 4.5	7	592 ± 40	26.7 ± 7.4
7	5	607 ± 34	24 108 ± 5 608	41.5 ± 12.5	7	305 ± 23	41.8 ± 12.8
14	6	675 ± 75	34 567 ± 11 767	54.9 ± 18.1	8	111.5 ± 13	105.9 ± 25.7
21	5	499 ± 37	88 957 ± 11 826	170.4 ± 51.8	5	234 ± 21	45.7 ± 15.0
30	5	465 ± 45	91 409 ± 26 286	212.8 ± 68.0	7	355 ± 28	115.6 ± 24.0
60	2	457 ± 41	126 610 ± 23 342	2 550 ± 19.0	6	187 ± 30	28 ± 11.4
180	3	354 ± 11	10 250 ± 4 756	28.9 ± 5.3	5	234 ± 19	40.9 ± 11.8

after transplantation the number of primordial follicles in the ovary transplanted into the abdomen remained large ($255 \pm 19/\text{mg}$ weight), whereas very few growing and vesicular follicles were present, namely 5-6 in the whole ovary. In the ovaries transplanted into the anterior chamber of the eye the number of primordial follicles fell sharply on the 60th day. On the 180th day after transplantation they also became few in number in the ovaries transplanted into the abdomen.

Hence the number of primordial follicles increased considerably between the 14th and 60th days. Although no signs of the formation of oocytes from cells remaining viable after transplantation could be seen, the increase in their number from the 14th to the 60th day demonstrated that new oocytes were in fact formed.

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