# THE MATURATION DIVISION OF OOCYTES IN MAMMALIAN OVARIES AT VARIOUS PHASES OF THE ESTRUS CYCLE, AND IN ACUTE RADIATION SICKNESS

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The ovaries are among the organs most sensitive to radiation, and structural and functional disturbances follow exposure to it. However, in recent years a number of investigations have been reported which indicate that large doses of x-rays exert a stimulating influence on the ovary. According to Russell and others [14-16], after a single total irradiation of mice with a dose of 400 r, there is no decrease but rather an increase in the number of corpora lutea in the ovaries. When these authors investigated ova in the oviducts, they found that the number of corpora lutea corresponded to the number of ova shed, and that in fertilized females, during the 1½-16½ days after irradiation there was an increase in the actual number of ova fertilized. E. A. Pozhidaev [8] also observed that compared to the ovaries of a control group the number of corpora lutea in rats exposed to 600 r increased considerably. He maintains that the reduction of the number of offspring in a litter from irradiated animals is not a consequence of a reduction in the number of ova shed (there is an excessive ovulation) but is due to a high mortality of the embryos in the early developmental stages.

In a study of the reactive and regenerative processes of the ovary we found [4, 5] that in animals with acute radiation sickness, the reduction division of the oocytes occurred especially often. There are a number of other references to the same effect [1, 6].

The first maturation division and the two phases of the second maturation division of the oocytes in mice and in other animals including man usually take place in the ovary in the pre-ovulatory period [9-13]. Odor [12, 13] made a particularly careful optical and electron-microscopical study of this process, and determined the duration of each phase for rats. There has been some discussion about the number and characteristics of the maturing oocytes at different phases of the cycle, because maturation is observed also in degenerating ova. However, in the principal Russian references [2, 3] it has been shown that the maturation division does not occur until after ovulation, and in many reports, each instance when a dividing cell has been found in the ovary, it has been thought that the effect was caused by some particular experiment.

The object of the present investigation has been to make a special study of the maturation of oocytes in control and in irradiated mammals.

## METHOD

Experiments were carried out on 100 sexually mature white rats which received a total irradiation of 400 r from a RUM-3 apparatus operating at a potential of 180 kv, current strength 10 ma, filter 0.5 mm Cu and 1 mm Al, focusing distance 30 cm, irradiation power 37 r per minute. The controls were the ovaries of non-irradiated mice. During the investigation, vaginal smears were made. The animals were killed in the daytime at various times after irradiation. The ovaries were fixed in Zenker formol, embedded in paraffin, cut in sections 6μ thick, and stained in hematoxylin-eosin, or by azan. The Feulgen reaction was used to indicate DNA. In all the ovaries, counts were made of the dividing oocytes. In addition, by the method of reconstruction, we determined the number of corpora lutea. The results were treated statistically by the Fisher-Student method.

## RESULTS

In the control animals, in 46 ovaries, 145 dividing oocytes were found. Only in three ovaries were no meioses found. The mean number of divisions per ovary was 3.1. The main mass of meioses (100) fell at estrus (on average by 5.3 per ovary). It was chiefly the oocytes of pre-ovular Graafian follicles without any signs of degeneration which were dividing. Usually it was the metaphase of the first and second maturation divisions which was found, while the polar body was still present. We also found that even in the pre-ovulatory period the first two phases of the second reduction division were occurring (Fig. 1).

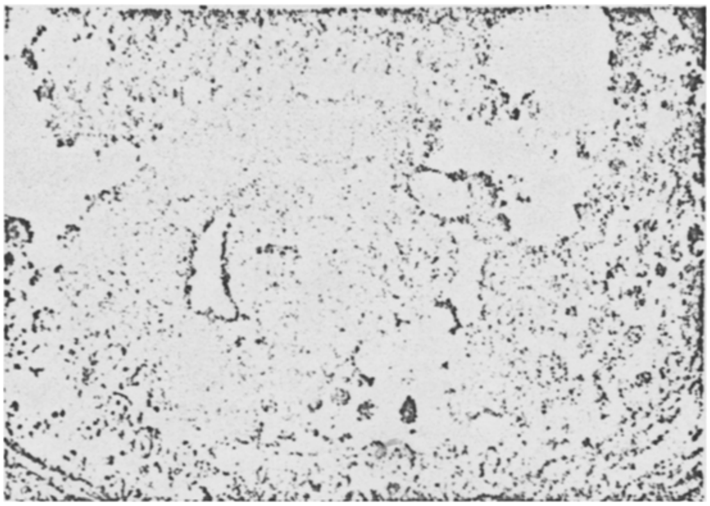

Fig. 1. First maturation division of the oocyte of a Graafian follicle from a control white mouse at estrus. Azan method. Objective 40 X, ocular 10 X.

In the remaining periods of the estrus cycle, occasional meioses were observed: in metestrus, there were on average 1.5 meioses per ovary, at diestrus, 1.8, and at proestrus there was 1 meiosis. The oocytes were dividing in the Graafian follicles and in the growing follicles which bore signs of deterioration (deformation of the cytoplasm and of the transparent membrane, breakdown of the follicular epithelium).

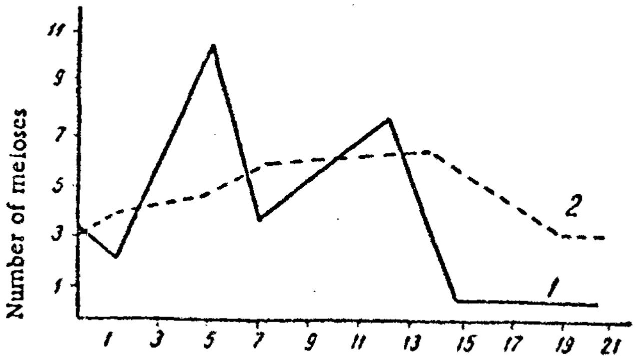


Fig. 2. Number of meioses and corpora lutea in irradiated white mice. 1) Number of meioses; 2) number of corpora lutea.

Statistical treatment of the results showed a significant difference between the number of meisoses at estrus and other phases of the cycle (P = 0.000). The difference in the number of meioses in the other phases was not statistically significant.

It has been shown [7] that even relatively small doses of ionizing radiation cause considerable disturbances of the estrus cycle, and affect ovarian hormonal function. In our experiments, after irradiation we frequently observed a polymorphism of the vaginal smears, which made it difficult to determine the phases of the estrus cycle. Therefore, in ovaries of such animals the dividing oocytes were counted in terms of the number of days after irradiation, and the results obtained were compared with the mean number of meioses in the control (Fig. 2).

Usually, on the first day after irradiation, the cytoplasm of the dividing oocytes was vacuolized, and chromosomes were fragmented. From the second day onwards, after irradiation there was an increase in the number of maturationdivisions up to 11.5 per ovary (P = 0.0000). During the development of signs of acute radiation sickness which occurred on the 6-8th days, the number of meioses was reduced on average to 3.7, but the reduction was not statistically significant (P = 0.1). On the following days and until the 14th day, the number of dividing oocytes was much higher than in the contol, reaching 7.6 per ovary (P = 0.0003). During the third and fourth weeks after irradiation the number of meioses fell to 0.9 per ovary.

Thus, from the counts made it was shown that between the 2nd and the 14th days after irradiation the number of maturing ova increased considerably above the values in the control animals, and the difference was significant at a level of P = 0.001. However, most divisions were observed to occur in degenerating ova and in the oocytes of follicles showing signs of atresia. The corona radiata of the dividing cells was often disrupted, the membrana pellucida and the cytoplasm of the oocytes was crenated, and the follicular epithelium had degenerated. Chromosomal aberrations consisting of fragmentation, breakdown or loss of chromosomes, and bridge formation were observed. If ovulation occurred, such ova would be unlikely to give rise to viable embryos (Fig. 3).

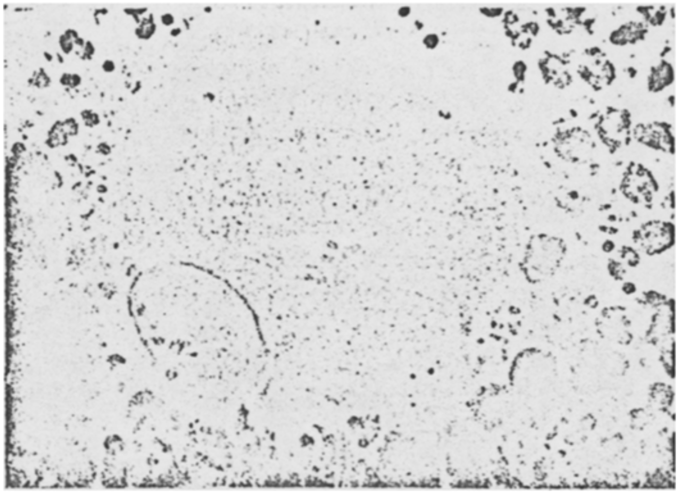

Fig. 3. Second maturation division of the oocyte of a Graafian follicle from a white mouse three days after total irradiation with 400 r. Chromosomal aberrations. Breakdown of the follicular epithelium. Treated with Feulgen. Objective 90 X, ocular 10 X.

To describe ovulation after irradiation we counted the number of corpora lutea. In 24 control ovaries, 72 corpora lutea were found, on average three per organ. In the 32 ovaries of the irradiated animals 149 corpora lutea were found, representing 4.7 per ovary.

On the first day after irradiation, the number of corpora lutea was close to the control value of 3.4. From the second to the fourteenth days, in the irradiated animals the number of corpora lutea increased considerably to 4.7 on the fifth day, 5.7 on the eighth, and 6.1 on the fourteenth. The increase was statistically significant (P = 0.001). For 3-4 weeks after irradiation, the number of corpora lutea fell until it approached the control value of 3.3. The number of ovulations after irradiation therefore increased.

Thus, our investigation has shown that the increase in the number of maturing oocytes between the second and fourteenth days of acute radiation sickness results from ovarian damage by x-rays, and from an increase of the process of atresia. The greater number of ova shed is probably the result of neurohumoral changes induced by the ionizing radiation.


## SUMMARY

A study was made of the oocyte maturation division in albino mice at various phases of the estrus cycle after exposure to 400 r of x-irradiation. From the 2nd to the 24th day after irradiation, the number of maturing oocytes and corpora lutea increased. However, the maturation division was observed mainly in the degenerating oocytes. This process was therefore the result of radiation injury of the ovaries and of increased atresia. The increase in the number of ova shed was the result of neurohumoral changes induced by the ionizing radiation.


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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.