

THE MITOTIC BEHAVIOR OF THE EPITHELIUM OF THE UTERUS AT DIFFERENT STAGES OF THE SEXUAL CYCLE IN MICE

O. I. Epifanova

From the Laboratory of the Histophysiology (Director – Candidate of Biological Sciences
V. N. Dobrokhotoy) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii)
of the AMN SSSR, Moscow

(Received December 28, 1957. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

At the present time great attention is being given to the study of the laws governing the mitotic behavior of the body. However, so far too little attention has been paid to the study of the extent to which the mitotic behavior may reflect the functional state of a particular organ.

In this connection it appears essential to study the character of the changes in mitotic activity which are associated with certain rhythmic processes. One of these processes, as is known, is the sexual cycle, brought about by hormonal changes in the body. In this respect it would be of particular interest to study the mitotic behavior of the epithelium of the uterine mucous membrane, where physiological regeneration is completed in a short time and attains a high degree of morphological expression.

In the literature there are only isolated and scanty details on this subject. In some textbooks, in the description of the changes in the epithelium of the uterus in rodents during the sexual cycle it is observed that a large number of cellular divisions is present in proestrus, and that these are absent in diestrus and metestrus. I. D. Rikhter [1] draws attention to the sudden appearance of a large number of mitoses in the proestrus stage and stresses that the process of mitotic division of the nuclei pursues an energetic course in estrus also, but this gradually weakens and continues to diminish in metestrus.

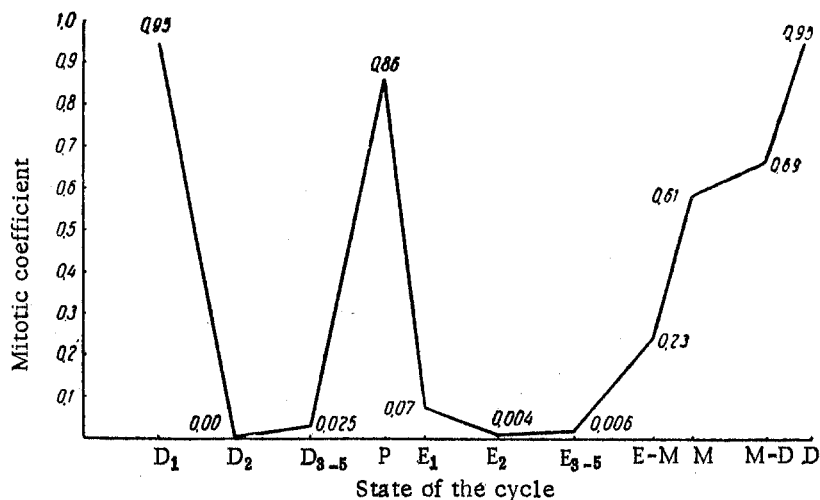
The quantitative characteristics of the mitotic activity of different organs in mice during the sexual cycle was the subject of an investigation by Bullough [5], who showed that the number of mitoses in the epithelium of the uterine mucosa is increased at the end of diestrus and in the stage of metestrus, and also after the injection of estrogens into the animal.

An increased number of dividing cells in the epithelium of the uterus in mice after injection of estrone was observed by I. A. Alov. In control experiments the peak period of cell divisions was found in the stage of diestrus and a smaller number was observed in estrus. Unfortunately, in this paper there is no distribution of the numbers of mitoses according to days during the longer stages (diestrus, estrus). Furthermore, none of the authors mentioned, with the exception of I. D. Rikhter, made a comparison of the mitotic activity of the epithelium of the uterus with its functional state. The lack of any quantitative counting of the mitoses in I. D. Rikhter's research prevents the formation of any definite conclusions on this question.

In the present work our aim was to demonstrate the character of the mitotic activity of the epithelium of the mucous membrane of the uterus throughout the sexual cycle and to compare the results obtained with the morphological changes in the endometrium.

EXPERIMENTAL METHOD

The investigation was carried out on 80 female white mice weighing 25 g. The stages of the sexual cycle were determined by taking vaginal smears twice daily for 10-11 days, after which the animals were



The mitotic coefficient in the epithelium of the mucous membrane of the uterus in mice at different stages of the sexual cycle.

D₁) 1st day of diestrus; D₂) 2nd day of diestrus; D₃₋₅) 3rd-5th days of diestrus; P) proestrus; E₁) 1st day of estrus; E₂) 2nd day of estrus; E₃₋₅) 3rd-5th days of estrus; E-M) estrus-metestrus; M) metestrus; M-D) metestrus-diestrus.

decapitated. The last smear was taken from each mouse immediately before decapitation. The uterus and ovaries were fixed in Zenker's fluid. In every case the upper third of the right cornu of the uterus was cut off with a razor for further treatment and embedded in paraffin wax. Transverse serial sections through the cornu of the uterus, 7-8 μ in thickness, were stained with hematoxylin and by Mallory's method.

In every fourth section (counting from the upper part of the cornu), the contours of the epithelium were traced under an Edinger drawing apparatus with a magnification of 178. For one case 10 such tracings were made. The mitoses were examined under the microscope (ocular 7 \times , objective 90 \times) and their distribution was indicated at the same time on the tracing of their particular section, mentioning the phase of division. The outlines of the tracing were next transferred on to standard paper, cut out with scissors and weighed on a torsion balance. From a knowledge of the weight of 1 cm² of paper the area of the epithelium could be determined. The intensity of cell division was judged by the changes in the value of the mitotic coefficient (the number of mitoses per unit area of epithelium).

EXPERIMENTAL RESULTS

The frequent taking of vaginal smears enabled it to be demonstrated that the overwhelming majority of experimental mice had a regular cycle, i. e. the appearance of all the stages of the sexual cycle in the animals was observed in sequence. However, the variations in the times of the various stages in different animals were very considerable. In addition in almost half the animals with a regular cycle, estrus lasted for as many or even for more days than diestrus.

The results obtained are shown on the Figure, where along the ordinate axis are represented the values of the mitotic coefficient (MK) and the abscissa represents the stages of the sexual cycle, with observation so far as possible of the time relationships between them. In consequence of variations in the times of the cycle in the mice, we grouped together the animals killed on the 3rd-5th days of diestrus and on the corresponding days of estrus. Besides this, on the abscissa are indicated signs for certain transitional stages of the cycle.

As the Figure shows, the mitotic activity in the epithelium of the mucous membrane of the uterus is not the same at different stages of the sexual cycle, and is expressed by a two-peaked curve with maximum cell division in proestrus and at the beginning of diestrus. The differences pointed out above in the duration of the separate stages of the cycle in the mice are not reflected in the character of the curve.

At the end of diestrus, in the later stages of which the mitotic coefficient was close to zero ($MK = 0.025$), a sharp increase takes place in the number of cell divisions in the epithelium of the mucosa (in proestrus $MK = 0.86$), to be replaced by an equally rapid fall at the beginning of estrus. In the 1st day of estrus the mitotic coefficient is 0.07, and later on it falls still further, after which it remains practically unchanged throughout the whole period of estrus ($MK = 0.004$ and 0.006). During the transition to metestrus a gradual increase appears in the mitotic coefficient ($MK = 0.23$), which continues right up to the 1st day of diestrus, when the number of cell divisions is at its highest ($MK = 0.95$). However, on the 2nd day of diestrus the mitotic coefficient is already down to zero.

The changes observed in the mitotic activity correspond to those morphological changes which are undergone by the uterine mucosa during the sexual cycle.

The first rise in mitotic activity corresponds in time to the intensive proliferation of the uterine epithelium in the stage of proestrus. This stage is characterized by a rapid increase in the size of the uterus and widening of its cavity, the contours of which become broken up. At this stage the epithelial layer is clearly distinguished from the underlying tissue, the epithelial cells being high prismatic, with elongated and evenly stained nuclei, displaced to the basal part of the cell. The stroma is friable and contains numerous small glands with a well-marked lumen. The rapid course of the processes of proliferation of the uterine epithelium is replaced by an equally sharp fall in the tempo of cell division, which then remains low throughout the whole period of estrus. In this stage the uterus is still large, and its cavity is widened as before. The uterine glands attain a considerable degree of development. At the end of estrus the process of destruction of the epithelium begins: the nuclei become irregular in shape, they no longer stain evenly everywhere, and the phenomena of pyknosis and rhexis are often observed.

The second rise in mitotic activity in the transition to metestrus coincides in time with the process of regeneration of the epithelium and is accompanied by the gradual desquamation of its older areas. In metestrus the basal membrane, which sharply delineates the stroma from the epithelium, is visible only in isolated areas in the section. The nuclei of the epithelium become round, and move from their basal position to the center of the cell, sometimes even closer to its apical part.

At first the phenomena of pyknosis and rhexis of the nuclei are seen. The borders between the epithelial cells often cannot be made out, and in places the epithelium becomes stratified. At this stage the stroma is compact and consists mainly of round cells. The glands diminish in number and their lumen is narrow. The desquamation of areas of epithelium into the cavity of the uterus continues. The restorative processes in the uterine mucosa proceed more slowly than the proliferative processes in the epithelium in the stage of proestrus, and the rise in mitotic activity also takes place gradually.

After its reconstruction is complete, the epithelium of the uterus enters the stage of diestrus, i. e. one of relative rest. Its lumen becomes narrow and the epithelial layer is clearly divided by a basal membrane from the underlying tissue. The epithelial cells are cubical, and the nuclei are round or slightly elongated and stain evenly. Glands are present in extremely small numbers. Mitotic division of the epithelial cells is hardly observed at this stage.

In the course of the investigation it was found that the vaginal smears do not always accurately reflect the changes in the cycle taking place in the uterus. Thus the phase of proliferation begins in the uterus evidently sooner than in the vagina, and when the smear gives a picture of diestrus the endometrium is already showing the typical picture of proestrus. Other authors too [2] have drawn attention to this feature. At the end of estrus the changes in the vagina, on the other hand, precede the process of reconstruction of the epithelium of the mucosa; the smear is characteristic of diestrus whereas the uterus has not in fact entered the resting phase. It appears to us that this can explain the high mitotic activity of the epithelium in the first day of diestrus as determined by the smear.

In the study of the factors regulating cell division, great attention has been paid by investigators to the action of the sex hormones, in particular estrogens [1, 5]. The opinion is held that estrogens play an important role in the regulation of cell division in the reproductive organs and in organs directly connected with the reproductive system (mammary gland, pituitary).

It may be postulated that the rise in mitotic activity, which we demonstrated in the epithelium of the uterus at the stage of proestrus, is also due to an increase in the concentration of estrogens in the blood. However,

it is difficult to explain from this point of view the second wave of growth of cell division, beginning during the transition to metestrus.

An analysis which was made of the number of early and late phases of mitosis

$$\frac{EP + P + M}{A + T + LT}$$

where EP is the early prophase, P is the prophase, M the metaphase, A the anaphase, T the telophase and LT is the late telophase, showed predominance of early phases over late at all stages of the cycle. If, however, in the first day of diestrus and in proestrus, when the absolute number of cell divisions was at a maximum, the ratios between early and late phases of mitosis were 3 and 2.4 respectively, then during the transition to metestrus, and in metestrus they were 5 and 4.3, although the total number of dividing cells was several times smaller. Under these circumstances the relative number of metaphases hardly altered, varying in all stages between 30 and 40%. This increase in the number of early phases with an overall reduction in the number of mitoses suggests that besides a factor stimulating cell division there is apparent, at these stages, the action of an inhibitor, restraining the course of early phases of mitosis. Consequently, in this case the value of the mitotic coefficient does not evidently fully reflect the true mitotic activity of the particular meristem. This problem of the nature of the factors responsible for the peculiarities of cell division in the period of reconstruction of the epithelium of the uterus calls for special investigations. A stimulating effect of products of disintegration of the degenerating portions of the epithelial layer on this process is not excluded.

We may generalize from the results obtained to state that the changes in the mitotic activity of the epithelium of the mucous membrane of the uterus in the course of the sexual cycle bear a regular and rhythmic character and coincide in time with the morphological reorganizations of the mucous membrane which take place at the various stages of the sexual cycle.

It may be concluded from this that the mitotic behavior may reflect changes in the functional state of the organ.

SUMMARY

The mitotic activity of the epithelium of the uterine mucous membrane in mice differs at various stages of the sexual cycle and may be expressed by a double-peaked curve with the maximum cellular divisions in the proestrus and the initial stage of diestrus. The changes in the mitotic activity coincide in time with the morphological reconstructions of the mucous membrane at various stages of the sexual cycle. Consequently, the mitotic activity may reflect the functional condition of the organ.

LITERATURE CITED

- [1] I. A. Alov, Transactions of the Khabarovsk Medical Institute, Collection 15, 31-43 (1957).*
- [2] I. D. Rikhter, Special Histophysiological Features of the Mucous Membrane of the Genital Tract in Female Rats and Mice. Author's Abstract of Dissertation for Doctorate (1949).*
- [3] B. Zondek, The Hormones of the Ovary and the Anterior Lobe of the Pituitary (Russian Translation) Moscow (1938).
- [4] N. A. Shereshevskii, O. A. Steppun and A. V. Rumiantsev, The Basis of Endocrinology, Moscow-Leningrad (1936).*
- [5] W. S. Bullough, Biological Sc. v. 231, 453-516 (1946).

* In Russian.