EFFECT OF LH RELEASING HORMONE ON THE STATE OF THE GAMETES AND MATURATION OF RAT FOLLICULAR OOCYTES *IN VITRO*

É. M. Kitaev, O. N. Savchenko,
M. N. Pimenova, and F. I. Proimina

UDC 615.357:577.175.829].015.4:[612.621.9

KEY WORDS: ovulation; oocyte; culture; chromosomal aberrations; LH releasing hormone.

Both natural hormones and preparations with follicle-stimulating and luteinizing gonadotrophic activity are in current use for the treatment of anovulatory states. The list of these preparations includes, in particular, synthetic analogs of LH releasing hormone (LHRF) [8]. By means of these preparations and natural pituitary gonadotrophins it is possible to obtain ovulation in 80-90% of patients, but the frequency of subsequent pregnancy still remains low. Meanwhile hormone therapy as a rule is accompanied by high frequency of early abortion, insufficiency of the luteal phase, and other complications [3, 7]. Accordingly, it is important to study the character of ovulation and the state of the developing gametes during induction of ovulation.

The aim of this investigation was to study these processes in intact sexually mature rats after single and repeated administration of LHRF.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rats aged 5-6 months with a normal estrous cycle. Altogether 89 rats were used, divided into three groups: 1) control (n = 27); 2) animals (n = 35) receiving a single intramuscular injection during proestrus of 50 mg LHRF (from Ayerst Laboratories, Canada), 3) rats (n = 27) receiving LHRF in a dose of 500 mg 6 times at intervals of 4 days. The animals were killed 24 h after injection of LHRF. For cytogenetic analysis, ovulating oocytes were removed by an incision in the ampulla of the oviducts, fixed in a mixture of methanol and acetic acid, and stained by Giemsa's method. The surface of the ovaries was carefully examined under the MBS-1 microscope to determine the number of unovulated follicles. Oocytes were then removed from follicles visible to the eye and cultured by the ordinary laboratory method in HAM P-10 medium with the addition of 10-50% calf embryonic serum at 36.8°C in an atmosphere of 5% CO_2 for 42-46 h. Total preparations were then obtained by Tarkowski's method [6]. The frequency of chromosomal disturbances was calculated relative to the number of cells with no morphological signs of chromosome degeneration after culture.

EXPERIMENTAL RESULTS

After injection of LHRF into the experimental animals an increase in the number of unovulated follicles was observed. When such follicles, which were over 600 μ in diameter, were opened, typical "preovulatory" oocytes surrounded by a loose, mucificated follicular epithelium, could be removed. Cytogenetic analysis of these oocytes showed that most of them were in metaphase stage II and frequently showed signs of degeneration. It was found that the number of animals in the control group with unovulated follicles depended on the season when the experiments were done, for whereas in the spring and summer it did not exceed 10%, in the fall and winter unovulated follicles were found in almost 50% of the animals. Meanwhile the number of these rats in the experimental group was 50-70% irrespective of the season of the year.

The results of cytophysiological and cytogenetic analysis of the population of ovulating and follicular oocytes in animals of the control and experimental groups show that whereas

Laboratory of Early Human Embryogenesis, Institute of Obstetrics and Gynecology, Academy of Medical Sciences of the USSR. Laboratory of Physiology and Pathology of the Human Endocrine System, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad.
(Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Baranov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 88-89, August, 1983. Original article submitted July 28, 1982.

0007-4888/83/9608- 1137$07.50

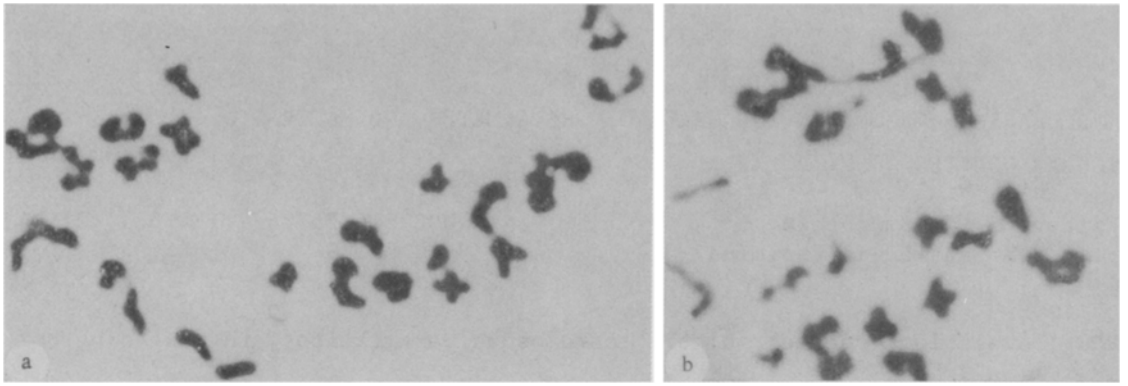

Fig. 1. Ovulating oocytes of intact rats (control): a) normal (n = 21); b) initial signs of degeneration of chromosomes. Here and in Fig. 2, staining by Giemsa's method. 900 ×.

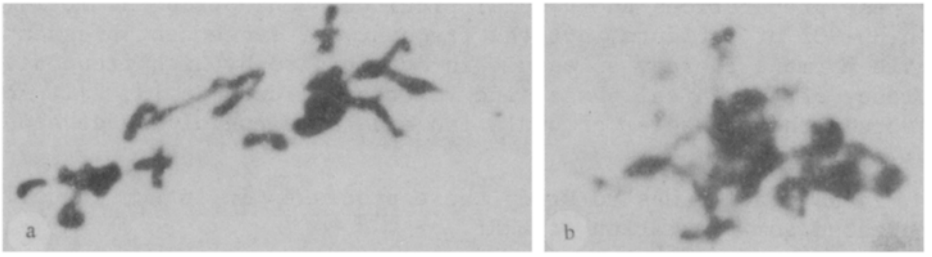

Fig. 2. Ovulating oocytes of rats receiving LHRF: a) fusion of several chromosomes; b) more advanced fusion of chromosomes and loss of distinct outlines.

the number of ovulated cells with signs of chromosomal degeneration at the metaphase II stage in the control group was about 30%, and it increased only very slightly after a single dose of LHRF, after repeated injections of LHRF the number of these cells increased significantly.

It must be noted that degenerative changes in chromosomes (despiralization, indistinctness of outlines, the formation of interchromosomal bands) in animals receiving LHRF were more pronounced in character (Figs. 1 and 2). Moreover, in animals with induced ovulation, immature oocytes at the diplonema stage were found among the ovulating oocytes (up to 1.2%). The number of chromosomal aberrations in the experimental animals did not differ significantly and was between 0.5 and 1.2% compared with 0.9% in the control.

During culture of follicular oocytes removed from rat ovaries after injection of LHRF significant differences were found in the character of maturation compared with the control. For instance, the frequency of resumption of meiosis in the population of follicular oocytes of the experimental animals increased significantly (82.5 and 86.9% respectively in rats of groups 2 and 3 compared with 66.2% in the control). Under these circumstances the number of spontaneously cleaving oocytes was increased (up to 50% in rats of group 3). After culture for 42-46 h a regular decrease in the number of oocytes at the metaphase II stage with no signs of chromosomal degeneration was observed in the experimental animals, together with a significant increase in the total number of oocytes degenerating in the course of culture (70.0 and 83.5% compared with 36.5% in the control). Finally, a tendency for the number of oocytes with chromosomal anomalies to increase (17% compared with 12.4% in the control) was observed in the group of animals receiving a single injection of LHFR.

LHRF thus has an unfavorable effect both on the population of follicular oocytes and on ovulating oocytes. LHRF probably does not stimulate growth of the follicles sufficiently, and this leads to desynchronization of ovulation and maturation of the oocytes. The latter, in turn, makes intrafollicular maturation or liberation of immature gametes possible, as is confirmed by the discovery of degenerating oocytes at the metaphase II stage and of ovulating oocytes at the diplonema stage in unovulated follicles, and also by the increase in the degree of heterogeneity of the population of newly formed gametes. These data agree with the observations of Long [2], who found similar changes in mice during superovulation and of Mori

et al. [4], who stimulated ovulation in mice by serum gonadotrophin and also by chorionic gonadotrophin and found, besides an increase in the number of ovulating eggs a decrease in their percentage fertility and an increase in the number of stillbirths.

The results of culture of follicular oocytes from ovaries of animals stimulated by LHRF indicate that in their cytogenetic characteristics these oocytes correspond in general to the oocyte population from ovaries of control animals in the estrus–metestrus stage [1]. However, the increase in the number of cells resuming meiosis, the number of spontaneously cleaving oocytes, the increase in the number of degenerating cells, and also some increase in the number of chromosomal anomalies must be taken into consideration. These particular features are probably associated primarily with the predominantly luteotrophic action of LHRF, which raises the blood LH level and can intensify the processes of follicular atresia in the ovaries [5].

The study of the effect of single and repeated injections of LHRF on the state of the follicular oocytes and on the population of ovulating oocytes in intact rats thus shows that LHRF, which simulates follicle formation, leads to some increase in the intensity of follicular atresia, with a consequent increase in the degree of heterogeneity of the resulting gamete population, without, however, having any significant effect on the frequency of chromosomal aberrations among the ovulating oocytes.

LITERATURE CITED

1. É. M. Kitaev and M. N. Pimenova, Byull. Éksp. Biol. Med., No. 12, 721 (1980).
2. F. Longo, Gamete Res., 3, 379 (1980).
3. A. McGregor, J. Johnston, and C. Bunde, Fertil. Steril., 19, 616 (1968).
4. H. Mori, N. Tsukamoto, R. Tanaka, et al., Sci. Rep. Fac. Agr. Ibaraki Univ., 28, 31 (1980).
5. J. Richards and A. Midgley, Biol. Reprod., 14, 82 (1976).
6. A. Tarkowski, Cytogenetics, 1, 394 (1966).
7. C. Wu., Fertil. Steril., 30, 617 (1978).
8. S.-Y. Ying, N. Leing, and R. Guillemin, in: Recent Advances in Fertility Regulation (Symposium), Geneva (1981), pp. 28-42.

EFFECT OF THE THYMUS ON ENDOCRINE FUNCTIONS OF THE GONADS AND ADRENALS IN MICE

V. M. Chesnokova, L. N. Ivanova, and E. V. Gruntenko

UDC 612.616.31+612.621.31+612.45.018]-06:612.438

KEY WORDS: nude mice; transplantation of the thymus; 11-hydroxycorticosteroids; sex hormones.

The thymus is closely interconnected with the glands of internal secretion [4, 7, 10]. This fact is demonstrated particularly vividly by experiments on mutant nude mice with genetically determined absence of the thymus. In such animals the onset of sexual maturity is delayed, fertility is reduced [10], and the morphology [5] and function [2, 7] of many endocrine organs are disturbed.

The aim of this investigation was to study whether these changes in the endocrine sphere of genetically athymic mice are directly connected with absence of the thymus, or whether it is an independent pleiotropic effect of the nude mutation. It was interesting to study mutant animals into which the thymus was transplanted from normal mice during the first days of life.

Laboratory of Physiological Genetics and Laboratory of Genetics of Cancer, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk.

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 89-91, August, 1983. Original article submitted November 16, 1982.