# CHANGE IN CHARACTER OF THE ESTROUS CYCLE AND GAMETOGENESIS DURING SUPEROVULATION INDUCED IN RATS AT DIFFERENT STAGES OF THE CYCLE

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Hormonal action on reproductive function is widely used nowadays for the treatment of various forms of infertility in women [1] and also in livestock in order to obtain a large number of embryos from progenitors of valuable breeds [3]. Despite the widespread use of these methods the results of hormonal stimulation are often unpredictable because of the high degree of variability of the ovarian response to exogenous hormones, and also because of the great qualitative heterogeneity of the gametes. One cause of this phenomenon may be that exogenous hormones are not always given at a fixed and appropriate time, so that the ability of the reproductive organs to respond in a certain manner to gonadotropins may vary significantly in the course of the ovarian cycle [10]. There are contradictory data in the literature on the optimal time of injection of gonadotropins in order to obtain the maximal number of ovulating cells [7, 12]. The problem of the state of the gametes depending on the stage of the cycle at which stimulation is carried out, still remains unsolved. Accordingly the aim of this investigation was to study changes in the character of the estrous cycle and the frequency of ovulation, and also to make a cytogenetic study of the gametes of rats in which superovulation was induced at different stages of the cycle.

## EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred albino rats from the "Rappolovo" nursery, aged 3-4 months and weighing 180-200 g. The animals were kept under conditions of controlled illumination (light from 7 a.m. to 6 p.m.). Rats with an undisturbed 4-day cycle were used in the experiments. The animals were divided into five series. Series I (control) consisted of 10 intact animals killed in the morning in the stage of estrus (E). Series II consisted of 11-12 rats which received an intraperitoneal injection of 25 IU of lyophilized pregnant mare's serum (PMS) (Gestyla, from Organon, Oss, Holland) at 10 a.m. in the E stage; the same dose of hormone was injected at 10 a.m. into rats of series III-V, which were in the stages of metestrus (M, 15 animals), diestrus (D, 12 rats), and proestrus (P, 11 rats), respectively. The animals of series II-V were given an intraperitoneal injection of 20 IU of chorionic gonadotropin (CG, from Serva, West Germany). Throughout the experiments the cytologic fixture of vaginal smears from the animals was studied. Ovulating oocytes were removed from the oviducts 16 h after the last injection and counted. Total preparations were made by Tarkowski's method for cytogenetic analysis. The results were subjected to statistical analysis. The significance of differences was determined by Student's test with 0.05 confidence interval.

## EXPERIMENTAL RESULTS

The results of the cytologic study of vaginal smears, the number of ovulating cells, and their cytogenetic analysis in rats with induced superovulation on different days of the cycle are given in Table 1. They show that rats at different stages of the cycle react differently to stimulation of superovulation. The ovaries of rats stimulated in the E stage were most sensitive to the action of PMS with respect to frequency of ovulation, and those at the P stage were least sensitive.

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TABLE 1. Frequency of Ovulation, Cytologic Picture of Smears, and State of Gametes during Stimulation of Superovulation in Rats on Different Days of the Cycle

| Series of experiments | Stages of cycle | Frequency of ovulation | Number of animals with the given frequency of ovulation, % | Mean frequency of ovulation | Number of gametes with normal metaphase in stage P, % |
|---|---|---|---|---|---|
| I (Control, $n = 10$) | MDPE | 11±1 | 100 | 11±1 | 100 |
| II ($n = 12$) | EMP | 63±2* | 42 | 43±7 | 70±3 |
| | | 29±2* | 58 | | 58±3 |
| III ($n = 15$) | MDPE | 32±2* | 80 | 28±3 | 72±2 |
| | | 14±1 | 20 | | 13±4 |
| IV ($n = 12$) | DPPE | 42±2* | 58 | 35±4 | 53±3 |
| | | 22±1* | 42 | | 15±4 |
| V ($n = 11$) | PEM (P) E | 34±4* | 27 | 19±3 | 46±5 |
| | | 13±1 | 73 | | 42±5 |

Legend. *P < 0.05.

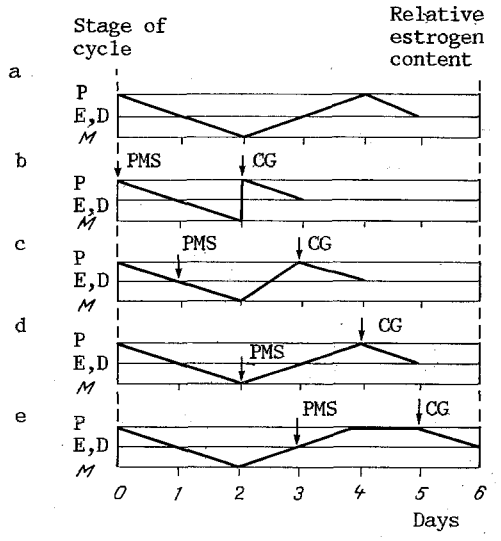


Fig. 1. Scheme showing sequence of stages of cycle in control and stimulated animals, corresponding to the character of change in the blood estrogen level. a) Control; b-e) experiment. P) Proestrus, E, D) estrus, diestrus, M) metestrus. PMS) Pregnant mare's serum; CG) chorionic gonadotropin. Arrows indicate time (stage of cycle) of injection of hormones.

An increase in the number of gametes during stimulation of superovulation is known to be due, on the one hand, to reduction of atresia in the developing follicles and, on the other hand, to involvement of an additional number of large preantral follicles in growth [5]. According to data in [8], about 30 small antral follicles appear in one ovary of a rat in the E stage in the morning, of which about five develop to the preovulatory stage. The rest undergo atresia, the first signs of which can be found as early as in the E stage.

A high frequency of ovulation during hormonal stimulation of rats in the E stage (about 60 cells per animal) can thus be explained on the grounds that injection of PMS completely prevents the initiated atresia. A decrease in the number of ovulating cells in rats stimulated in the final stages of the cycle is evidently connected with irreversibility of atresia in some follicles. In addition, unequal sensitivity of rats in the same stage of the cycle to hormonal stimulation was found. In each series two groups of rats could be distinguished: with a higher and lower frequency of ovulation. Both the frequency of ovulation and the relative proportions of these groups varied from one series to another (Table 1). The results are evidence of heterogeneity of the population of noninbred animals with respect to sensitivity to the action of gonadotropins, the causes of which require further study.

Recent investigations into the state of gametes in stimulated animals have shown that during superovulation the relative number of normal gametes is significantly reduced. In superovulating animals oocytes with signs of disturbance of formation of the meiotic spindle [2], growth of polyploidy with an increase in the dose of PMS [12], and also immature and fragmented oocytes [9, 6] have been described.

The cytogenetic study of ovulating oocytes also revealed an increase in their relative number with degenerative changes in the chromosomes: despiralization or condensation, progressing in some cases to the formation of amorphous masses of chromatin. The number of these oocytes was shown to depend on the stage of the cycle at which stimulation began. Thus if PMS was injected at the E and M stage about 60-70% of ovulating oocytes, or if at stage D and P, 56 and 46%, respectively, had no disturbances. In this case, direct correlation was observed in each series between the relative number of normal gametes and the number of ovulating oocytes. The patterns regularly observed are evidently linked with the spread of atresia in the growing follicles.

The pattern of change of the vaginal smears is known to reflect the degree of saturation of the body with estrogens. The sequence of stages of the estrous cycle in control rats with corresponding changes in the blood estrogen level is illustrated schematically in Fig. 1a. The sequence of stages of the cycle during stimulation of superovulation is shown in Fig. 1b-e with respect to the same criterion. It will be clear from Fig. 1 that, irrespective of the stage at which PMS was injected, P began 2 days later in the experimental rats, probably due to acceleration of follicle growth [5]. The results are indirect evidence in support of a significant change in the character of estrogen secretion in the experimental rats depending on the time of injection of PMS, which may have an influence both on the state of the gametes formed and on the functional readiness of the genital tract for an impending pregnancy [4].

The study of the frequency of ovulation in rats after injection of PMS on different days of the estrous cycle thus confirmed data in the literature [7] on the high sensitivity of the ovaries of rats in estrous to the action of PMS. The heterogeneity of animals in the same stage of the cycle, with respect to the absolute number of ovulating gametes, also was discovered. A relative increase in the number of gametes with chromosomal changes compared with the control and dependence of the frequency of the changes mentioned above on the state of the cycle at which PMS was injected were demonstrated. Finally, a study of the character of the estrous cycle in animals stimulated on different days of that cycle showed that hormonal stimulation has a significant effect on the subsequent sequence of its phases.

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