

EFFECT OF VARIOUS DOSES OF PROGESTERONE
AND ESTROGENS ON EMBRYONIC DEVELOPMENT
IN OVARECTOMIZED RATS

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Experiments were carried out on pregnant noninbred rats. The animals were ovariectomized on the 8th day of pregnancy, continuation of which was ensured by injection of the principal ovarian hormones: estradiol dipropionate, folliculin, estriol, and progesterone in various combinations. The animals were killed on the 21st day of pregnancy, and the fetuses were removed and inspected externally. The results showed that ovariectomy, carried out on the 8th day of pregnancy, interrupted its course. Normal embryogenesis took place only after injection of all the principal ovarian hormones.

KEY WORDS: pregnancy; embryogenesis; ovariectomy; progesterone; estrogens.

The various ovarian hormones — estrogens and progesterone — perform different roles in the regulation of the reproductive function [3, 5] and a strictly definite combination of these hormones maintains the normal course of pregnancy and fetal development. If this combination is disturbed, pregnancy is interrupted or various malformations ensue [1, 6, 7, 9].

The writers showed previously that estradiol dipropionate and progesterone, if administered to ovariectomized pregnant rats separately or in combination, did not prevent the interruption of pregnancy and led to death of the embryos or to the formation of deformities [2].

In the present investigation an attempt was made to find a combination of the principal ovarian hormones which, when administered to pregnant ovariectomized rats, would ensure normal embryogenesis.

EXPERIMENTAL METHOD

Pregnant noninbred rats weighing 160–180 g were used. The rats were ovariectomized on the 8th day of pregnancy to exclude the action of ovarian hormones. The 8th day of pregnancy was chosen for the operation so that changes in embryogenesis could be studied from the early postimplantation period. The animals were divided into ten series.

In series I (control), 9 rats were subjected to ovariectomy only; in series II, 15 ovariectomized pregnant rats received 1 μ g estradiol dipropionate, 2 μ g folliculin, and 2 mg progesterone/100 g body weight from the day of the operation; in series III, 14 rats received the following hormones: 1 μ g estradiol dipropionate, 2 μ g folliculin, 10 μ g estriol, and 2 mg progesterone/100 g body weight; in series IV, 13 animals received 1 μ g estradiol dipropionate, 2 μ g folliculin, 10 μ g estriol, and 2 mg progesterone/100 g body weight; in series V, 14 rats received 1 μ g estradiol dipropionate, 2 μ g folliculin, 5 μ g estriol, and 1 mg progesterone; in series VI, 10 rats received 1 μ g estradiol dipropionate, 1 μ g folliculin, 20 μ g estriol, and 1 mg progesterone; in series VII, 9 rats received 1 μ g estradiol dipropionate, 2 μ g folliculin, and 20 μ g estriol; in series VIII, 9 rats received 2 μ g folliculin, 20 μ g estriol, and 2 mg progesterone; in series IX,

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TABLE 1. Number of Normal Fetuses on 21st Day of Development after Administration of Various Combinations of Ovarian Hormones to Ovariectomized Pregnant Rats

Series of experiments	Number of animals	Number of implanted embryos at time of operation	Normal embryos	
			absolute number	percent of number of implanted embryos
I. Pregnant rats ovariectomized on 8th day of pregnancy (control)	9	97	—	
II. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, progesterone 1 mg	15	99	65	65.7 \pm 4.7
III. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, estriol 20 μ g, progesterone 2 mg	14	102	96	94.1 \pm 2.3
IV. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, estriol 10 μ g, progesterone 2 mg	13	103	80	70.0 \pm 4.5
V. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, estriol 5 μ g, progesterone 2 mg	14	111	69	62.1 \pm 4.6
VI. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, estriol 20 μ g, progesterone 1 mg	10	83	45	53.0 \pm 5.5
VII. Ovariectomy + estradiol dipropionate 1 μ g, folliculin 2 μ g, estriol 20 μ g	9	72	12	16.6 \pm 4.2
VIII. Ovariectomy + folliculin 2 μ g, estriol 20 μ g, progesterone 2 mg	9	69	23	33.3 \pm 5.7
IX. Ovariectomy + estradiol dipropionate 1 μ g, estriol 2 μ g, progesterone 2 mg	8	69	42	63.7 \pm 5.8
X. Ovariectomy + estriol 20 μ g, progesterone 2 mg	7	62	—	—

8 rats received 1 μ g estradiol dipropionate, 20 μ g estriol, and 2 mg progesterone; in series X, 7 rats received 20 μ g estriol and 2 mg progesterone.

The choice of doses in these experiments was based on the fact that during pregnancy the excretion of estrogens rises particularly sharply, chiefly on account of estriol [4, 5, 8].

The injections of the estrogens and progesterone in all the experimental groups were alternated: estrogens were given on even days and progesterone on odd days.

The rats were killed on the 21st day of pregnancy. Some animals from each series were kept in order to study the fertility of the progeny; the fetuses were removed from the remaining animals and inspected externally.

As an index to assess the effect of treatment, the number of normal embryos was expressed as a percentage of the total number of implanted embryos. The numerical results were subjected to statistical analysis. To calculate the mean error the equation for alternative variation was used:

$$m = \pm \sqrt{\frac{P(100-P)}{n-1}},$$

where P denotes the percentage obtained from the experiment and n is the number of fetuses or implantations studied.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

In the experiments of series I, ovariectomy interrupted the pregnancy in all the animals. In series II, normal embryogenesis was observed in $65.7 \pm 4.7\%$ of cases, but developmental anomalies or death of the fetuses in 34.3% of cases. In series III, 5 of the 102 embryos had anomalies and one was dead, but the remaining 96 embryos ($94.1 \pm 2.3\%$) were normally developed. In series IV, the number of normal embryos was somewhat lower than in the preceding series ($70.0 \pm 4.3\%$) on account of an increase in the number of abnormal embryos (30). In series V, normal embryogenesis occurred in $62.1 \pm 4.6\%$ of cases and the remaining embryos were damaged (anomalies, death, deformities in 3 embryos). In the animals of series VI there were $53.0 \pm 5.5\%$ of normal embryos. The percentage of normal embryos was much lower in the animals of series VII, for which $83.4 \pm 4.2\%$ of the embryos were damaged. In series VIII, 23 of the 69 implanted embryos ($33.3 \pm 5.7\%$) were normal. In series IX, normal embryogenesis took place in $63.7 \pm 5.8\%$ of cases. In series X, pregnancy was interrupted in all 7 ovariectomized pregnant rats.

The investigations showed that the operation of ovariectomy, when carried out on the 8th day of pregnancy, caused interruption of pregnancy in all the rats. This confirmed the necessity for ovarian hormones for normal embryogenesis. In the experiments of series II 34.3% of the embryos were damaged. This fact can be explained by the absence of estriol, which is mainly responsible for maintaining the normal course of pregnancy. A fall in the estriol titer is known to lead to early abortion [5].

To examine the role of estriol in the maintenance of pregnancy, in the next series estriol was added to the combination of hormones in various doses (Table 1). The experiments of series III, in which a high proportion of normal embryos (94.1%) was obtained, are interesting. When the dose of estriol was reduced, the number of normal embryos decreased. The reduced dose of estriol was thus insufficient for the maintenance of normal embryogenesis. The combination of hormones given in series III was the most favorable for the normal course of pregnancy.

A decrease in the dose of progesterone in series VI led to disturbance of the normal course of pregnancy but in series VII, when progesterone was left out and the animals were given estrogens only, the number of normal embryos fell sharply. It was thus confirmed that progesterone is an important hormone of pregnancy and is essential for normal embryogenesis.

Analysis of the results of series VIII, IX, and X showed that the presence of all three fractions of estrogens is essential for normal embryogenesis. However, the observations show that a strictly definite quantitative combination of estrogens and progesterone is important for the maintenance of pregnancy and normal embryogenesis in ovariectomized rats, and that a leading role cannot be ascribed to any one of them.

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