

Effect of Various Steroids on Gestation and Litter Size in Rats

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ALTHOUGH IT IS RECOGNIZED that estrogen and progesterone must be available in correct proportions for implantation and maintenance of the conceptus, very little is known concerning the optimal ratios, mode, or site of action of these hormones. Furthermore, almost nothing has been learned concerning the part played by the metabolites of these substances on reproductive function.

It appears likely that improper endometrial preparation may well account for implantation failures in many sterility problems. It is equally probable that even if implantation is achieved, an early and often unrecognized miscarriage may result from inadequacy of the implantation site. An abnormal metabolism of the ovarian hormones has received little emphasis in the past as a possible etiologic factor in unexplained sterility cases, miscarriages, and repeated abortions.

Recent reports^{4, 5} in the literature, however, indicate that certain metabolites of estrogenic and progestative substances that were previously con-

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sidered to be "weak" or inert may well play a role in the reproductive process. More specifically, these workers reported that various ratios of pregnanediol and pregnane-3- α -20- α -diol exerted a quantitative restriction on progesterone-induced decidual development in the albino rat. Also, working on the theory that certain metabolites of ovarian steroids act in concert, they further produced evidence showing that estriol vigorously inhibited estradiol-17- β -induced uterine growth. It thus appeared of interest to ascertain the action of various proportions of pregnanediol and pregnane-3- α -20- α -diol as well as estriol on the reproductive ability of laboratory animals.

MATERIALS AND METHODS

Sexually mature albino rats of the Charles River strain were used for all experiments. The females were selected at random, marked, and distributed 6 to a cage. Vaginal smears were studied for 2 to 3 weeks to ascertain the regularity of their estrus cycles. Two males were then placed in each cage of 6 females, a 3 ♀ : 1 ♂ ratio being maintained throughout all studies reported. In the first group of treated animals, the compounds were administered for 1 week to females of known fertility prior to cohabitation with the males. In the remaining experiments, the steroids were given in relation to the finding of spermatozoa and cornified cells in the vaginas. Both the pregnane-3- α -20- α -diol and the pregnanediol were suspended together in an aqueous vehicle containing 0.9% benzyl alcohol, a few drops of Tween 60, and 0.89% NaCl solution. The estriol was dissolved in purified U.S.P. propylene glycol. All injections were administered subcutaneously. Vaginal smears were taken daily and evidences of abortion when observed were carefully noted. The length of gestation as well as the number of viable young and stillbirths were recorded for each pregnant mother.

RESULTS

Control Experiments

Length of the Estrus Cycle. A total of 60 albino female rats were used for the purpose of ascertaining the regularity of their estrus cycles. Vaginal smears were taken daily, fixed in methyl alcohol, dried, stained with Giemsa, diagnosed, and recorded. These data showed that the length of the estrus cycles of this strain of rats is 4.5 days. No apparent difference

exists among the various highly inbred strains of albino rats used for most research in this area of investigation.

Length of Gestation. From the time the animals mate, as is evidenced by the presence of spermatozoa and cornified cells in the vaginal smear, a total of 22.0 days is required for parturition in this inbred strain of rats.

Litter Size. Thirty-three nontreated females were mated with fertile males. The average number of living young born in each litter was 9.6 ± 0.53 . Further, it is interesting to point out that the 10.5 ± 0.78 figure obtained from the second gestation is not significantly different from the number in the first gestation. In both instances, the mean gestation length was 22.0 days, and no stillbirths were found.

TABLE 1. Influence of Progesterone Metabolites on Gestation and Litter Size

<i>Treatment</i>	<i>No. of rats</i>	<i>Litter size</i>	<i>No. of stillbirths</i>	<i>Abortions</i>
Nontreated controls	33	$9.6 \pm 0.53^*$	0	0
Pregnanediol: pregnanedione 4:1 ratio (given 7 days before cohabitation)				
3.0 mg.	13	7.2 ± 1.4	2	3
6.0 mg.	13	7.2 ± 1.5	1	3
1:1 ratio				
4.5 mg. on day of mating	18	7.4 ± 0.62	13	3

* Average \pm standard error.

Influence of Aqueous Vehicle and Propylene Glycol on Mating and Pregnancy in the Rat. Both the aqueous vehicle and propylene glycol used as vehicles for our steroidal substances were without effect in modifying the mating response or pregnancy in our rats. These data are in agreement with the recent findings of Slechta, Chang, and Pincus.

Experimental Data

Steroidal Treatment 1 Week before Cohabitation

EFFECTS OF 10 μ G. ESTRIOL DAILY FOR 7 DAYS PRIOR TO MATING. This metabolite of the naturally occurring estrogen produced a goodly number of cornified cells in the vaginal lavage while the treatment was in effect, but was found to be without effect in modifying the mating response of the treated female rats in this series. Likewise, this substance did not affect the length of gestation (22.0 days) in this group of animals. The influence

on the average number of living young born in this group, however, was reduced (Fig. 1). These data indicate that 7 daily injections of 10 μ g. of estriol given to female rats 1 week before cohabitation with males of proved fertility reduced the number of living young to approximately 66 per cent of the control figure (9.6 ± 0.53 for the controls as opposed to 6.1 ± 1.1 for the estriol-treated group—see Table 2). Of particular significance is the fact that none of the 33 nontreated or vehicle-treated females aborted after mating, whereas 7 of the 22 rats given estriol aborted. Laparotomies

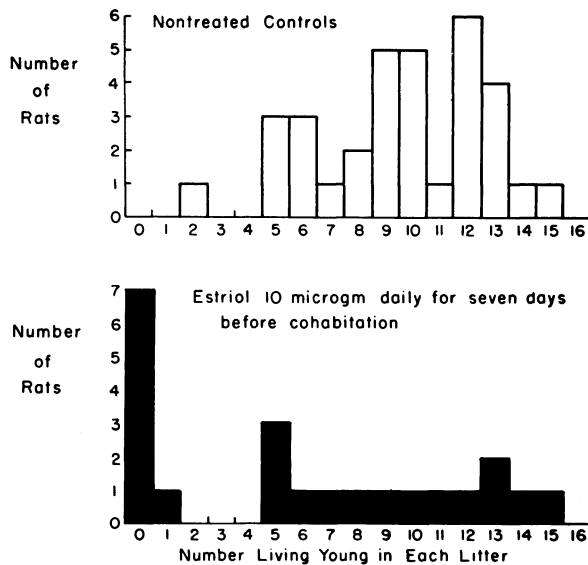


Fig. 1. The influence of 10 μ g. estriol daily for 7 days before cohabitation on litter size, compared with untreated controls.

performed on these 7 females showed placental scars in only 3 animals, thus suggesting that the abortions in the other 4 probably took place prior to implantation.

In addition to decreasing the number of living young born in this group, a total of 15 stillbirths were produced by another 7 females. The 15 dead young were distributed among 32 live young. Only 1 mother had as many as 5 stillbirths, the remaining 6 were as follows: 1 with 3 dead, 2 with 2 dead, and 3 with 1 dead. One mother died in the process of giving birth, and upon postmortem examination, she was found to have 15 dead young, all of them exhibiting undeveloped features.

EFFECTS OF 4:1 PREGNANE-3- α -20- α -DIOL: PREGNANEDIONE. Seven daily subcutaneous injections of 3 mg. of these compounds given to 13 females effected a somewhat smaller decrease in the number of living young than in the animals which received the estriol for 1 week (Fig. 2). Only 3 rats completely aborted, 2 having placental scars and 1 without any evidence

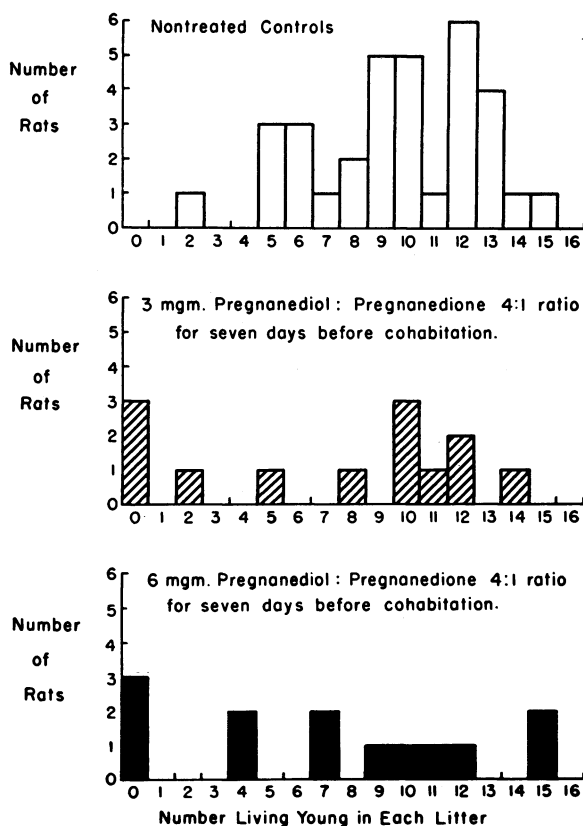


Fig. 2. The effect of a 4:1 ratio of pregnanediol and pregnanedione for 7 days before cohabitation on litter size.

of placentation on laparotomy. The young born alive averaged 7.23 ± 1.4 per litter, thus showing a decrease of 25 per cent in contrast to the normal control value, and only 2 stillbirths were observed (Table 1).

Another group of 13 females received a daily subcutaneous injection of 6.0 mg. of the above steroids for 7 days. The results obtained (see Fig. 2) demonstrate no difference whatsoever between the two groups (7.2 ± 1.4 vs. 7.2 ± 1.5 (Table 1).

Steroidal Treatment on the Day of Mating and Prior to Implantation

EFFECTS OF 10 μ G. ESTRIOL ON DAY OF MATING. The results produced in this group of 18 female rats showed at best only a slight trend towards a decrease in the number of living young, 8.7 ± 1.2 (Fig. 3). A total of 4 stillbirths was encountered, and these were distributed among 3 litters.

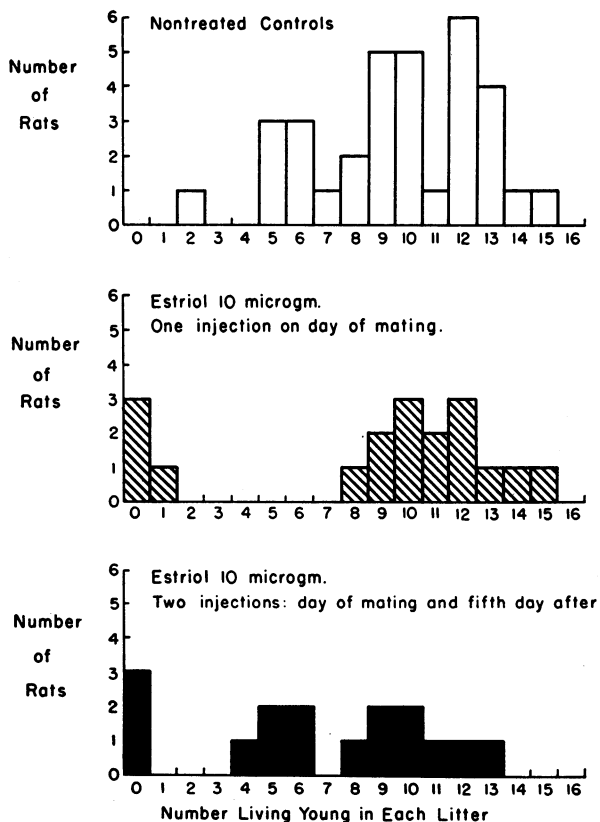


Fig. 3. The influence of estriol when given on the day of mating as well as on the fifth day thereafter on litter size.

Three of the 18 treated females aborted, all showing evidence of placental scars on laparotomy.

EFFECTS OF 10 μ G. ESTRIOL ON DAY OF MATING AND ONCE AGAIN 4 DAYS LATER. In addition to an injection on the day of mating, a second injection was given 4 days later, or approximately 12 hours prior to anticipated implantation. A decided reduction was observed in the average number of living

young born in this group— 6.1 ± 1.1 in contrast with the 9.6 ± 0.53 arithmetic mean for the control series (see Fig. 3). Moreover, 3 of the 16 treated females, receiving the two injections of 10 μ g. estriol 96 hours apart, completely aborted prior to implantation. These data are parallel with the results obtained in the group of animals receiving estriol for 7 days prior to cohabitation (Table 2).

EFFECTS OF 4.5 M.G. 1:1 PREGNANE-3- α -20- α -DIOL: PREGNANEDIONE ON DAY OF MATING. In this group of 18 females the ratio of the 2 pregnane com-

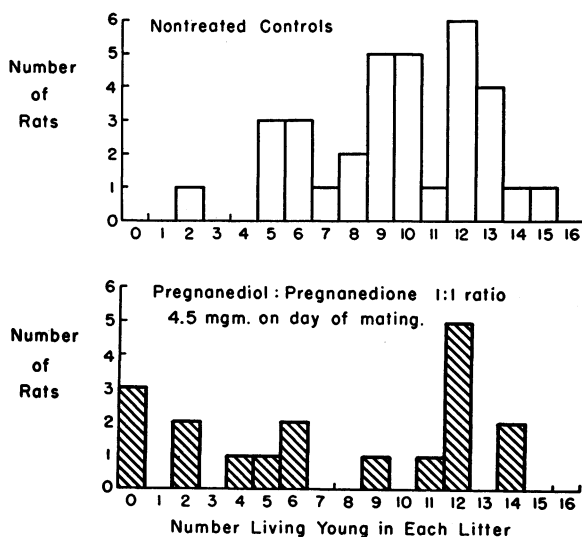


Fig. 4. The action of a 1:1 ratio (4.5 mg.) of pregnanediol and pregnanedione when given on the day of mating on the size of litters.

pounds was changed to 1:1 and a total of 4.5 mg. was given. The results obtained closely paralleled those of the previous pregnane series (Fig. 4; see Fig. 2). The average number of living young born was 7.4 ± 0.62 and, of the 18 pregnant females in this group, 3 aborted, 2 with placental scars and 1 without. No statistical difference was noted in the three groups on the pregnane regimen (see Table 1).

It is of interest that the pregnanediol-pregnanedione treatment did not seem to have any influence on the estrus cycles or mating response, nor did these steroids affect the length of gestation of 22.0 days. In this treatment group, 3 abortions occurred, and 13 stillbirths were delivered by 7 mothers.

Steroidal Treatment after Mating and through Calculated Implantation Day

EFFECTS OF 10 μ G. ESTRIOL ON FIRST DAY OF MATING AND THEN DAILY FOR A TOTAL OF 5 DAYS. In 17 animals the litter size was reduced to 4.8 ± 0.7 , one half the mean value of the controls. One stillbirth was produced, and

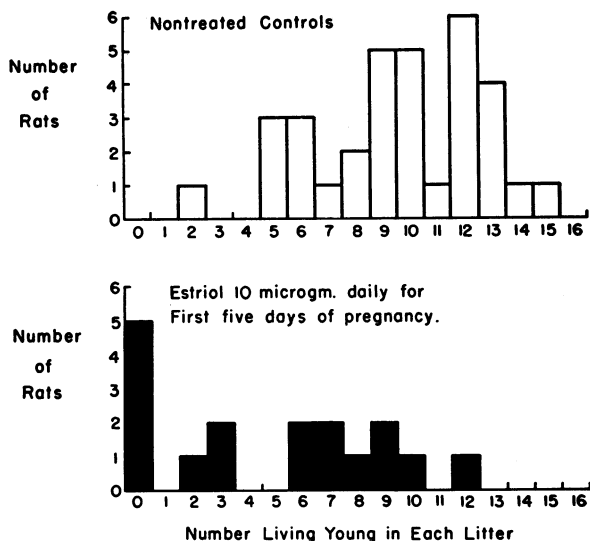


Fig. 5. The effect of 10 μ g. estriol given on day of mating and daily for 5 days thereafter.

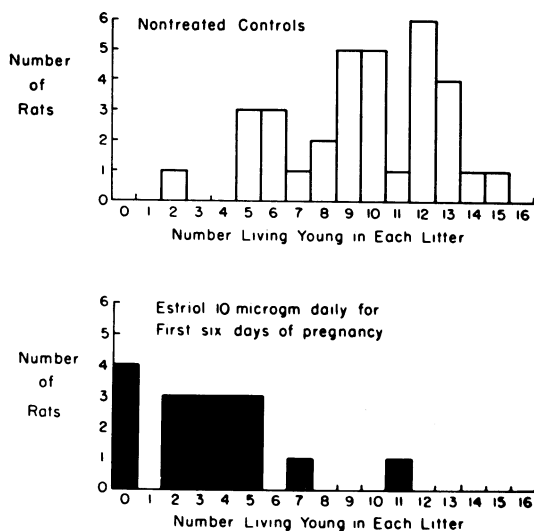


Fig. 6. The effect of 10 μ g. estriol given on day of mating and daily for 6 days thereafter.

5 rats aborted *in toto* (Fig. 5). The uteri of 2 of the latter showed placental scars at necropsy.

EFFECTS OF 10 μ G. ESTRIOL ON FIRST DAY AFTER MATING AND THEN DAILY FOR A TOTAL OF 6 DAYS. There were 18 animals in this group. The average litter size, 3.2 ± 0.68 , showed a dramatic reduction from control values (Fig. 6). There were 5 stillbirths and 5 animals again totally aborted.

TABLE 2. Influence of Estriol on Gestation and Litter Size

<i>Treatment</i>	<i>No. of rats</i>	<i>Litter size</i>	<i>Total no. of stillbirths</i>	<i>Total no. of abortions</i>
Nontreated controls	33	9.6 ± 0.53	0	0
Estriol 10 μ g. daily for 7 days prior to mating	22	6.1 ± 1.1	30 ^a	7
Estriol 10 μ g. on day of mating only	18	8.7 ± 1.2	4	3
Estriol 10 μ g. on day of mating and fifth day thereafter	16	6.7 ± 1.1	3	3
Estriol 10 μ g. daily first 5 days after mating	17	4.8 ± 0.99	1	5
Estriol 10 μ g. daily first 6 days after mating	18	3.2 ± 0.68	5	5

^a One female died at parturition; autopsy showed 15 dead or undeveloped fetuses.

Table 2 summarizes the effects of estriol on litter size, stillbirths, and abortions.

DISCUSSION

The effects of various substances on progestational reactions, mating behavioral patterns, gestation, and parturition have been reported in the recent literature.^{4, 5, 8} With the exception of one study, this previous research has utilized naturally occurring hormones or synthetic compounds rather than the metabolites of the hormones involved in reproductive processes. We have been impressed with the probability that any endocrine receptor-organ response is not accomplished by the independent action of one hormone alone. It appears more likely that such response is the physiologic expression of the sum total of the biologic hormones and their metabolites acting in concert on the receptor organs. The early observations from Hisaw's laboratory^{3, 9} and other reports are consistent with this principle. It seemed worth while, therefore, to study the effect of estriol and certain

of the C₂₁-metabolites related to progesterone in experiments on gestation, parturition, and live births per litter in the albino rat. The observations of Haines, suggesting that variations in levels of pregnanediol and pregnandione might be responsible for miscarriage and toxemia, gave impetus to the current investigation.

Early abortions can be ascribed primarily either to inadequacy of the implantation site or to abnormalities of the embryo, or perhaps a combination of the two. Our interest in these studies was to see whether certain of the naturally occurring metabolites of ovarian steroids might cause an increase in abortion rate and a reduction of litter size.

Previous work⁵ presented evidence that certain combinations of progesterone metabolites interfered with the development of deciduomas in the rat. Animals treated with the same compounds in this series either before or on the day of mating were found to produce more abortions and stillbirths and fewer live young than the controls.

The effect of estriol on the birth rate of these rats was more dramatic. This steroid is considered one of the less active metabolites of estradiol-17- β , yet it had previously been found capable of inhibiting the usual growth-stimulating effect of estradiol on the nonpregnant rat uterus.⁴ In the present investigation, when estriol was used before mating, it reduced the litter size to 66 per cent of the controls. This of course might represent either a deleterious effect on ovulation, or a "tube-locking" of the ova. However, when the same dose was employed from the day of mating and daily thereafter beyond the time of usual implantation, 6 days later, a reduction of live births to 33 per cent of the controls was produced. In this experiment the medication was withheld until after ovulation had presumably occurred. The presence of placental scars and an increased incidence of abortions and stillbirths argues against the possibility that the fertile ova might have been "locked" by the estrogen in the tubes. It appears probable that the uterine bed may have been inadequate for proper implantation or maintenance of the embryos.

This study suggests that faulty metabolism of progesterone or estradiol-17- β may interfere with the normal development of the implantation site, thus causing an increase in abortions and stillbirths in the albino rat.

SUMMARY

The purpose of this investigation was to ascertain the action of estriol

and various ratios of pregnane-3- α -20- α -diol: pregnanedione on gestation and the number of living young born in each treatment group of rats.

These substances, in the doses here used, did not affect the mating response or length of gestation. Combinations of the two pregnanes tended to reduce the size of the litters compared with untreated controls both when given for a week before mating and also on the day of mating only.

When estriol was given prior to mating, it caused a more marked reduction in live births, and when this steroid was employed after mating and daily through time of implantation, the litters decreased to 33 per cent of the control size. In the same animals, the incidence of placental scars, abortions, and stillbirths further bears witness to the possibility that the steroids employed interfered with the optimum differentiation of progestational endometrial changes, rather than affecting any suppression of ovulatory mechanisms.

It is postulated that faulty metabolism of either estrogenic or progestational hormones may play a role in certain clinical problems of miscarriage and abortion by a similar interference with the development of the maternal bed.

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