ACTION OF OESTROGENS AND ANTI-OESTROGENS ON EARLY PREGNANCY IN THE RABBIT

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Summary. Mated female rabbits were injected subcutaneously on Days 5 to 7 or 7 to 9 of pregnancy with various substances in nut oil, and examined on Days 9 to 11. Approximately 50% failure of normal implantation was caused by 30 μg of oestradiol, 25 mg of dimethylstilboestrol or 10 mg of 17-ethinyl-19-nortestosterone daily. MER 25 in doses of up to 25 mg, and 17-ethyl-19-nortestosterone in doses of up to 10 mg daily were ineffective.

INTRODUCTION

A number of studies has recently been made on the effects of oestrogens and anti-oestrogens on early pregnancy in the mouse (Emmens, Cox & Martin, 1959, 1960) and the mouse and rat (Emmens & Finn, 1962). The anti-oestrogen, MER 25, has also been shown by Lerner, Holthaus & Thompson (1958) to inhibit early pregnancy in both rats and rabbits. Other recent studies with rabbits include those of Greenwald (1957, 1959), who determined the effects of various natural and synthetic oestrogens on very early pregnancy, and of Allen & Wu (1959), who, in investigating the possible substitution of 17-α-ethinyl-19-nortestosterone for progesterone in pregnancy found effects that may have been due to its anti-oestrogenic properties.

The present studies were undertaken because of the relative paucity of data from the rabbit, and to widen our knowledge of the actions of these compounds in early pregnancy. A fuller review of present knowledge of the actions of oestrogens and anti-oestrogens in preventing implantation, the mechanism of particular interest here, is to be found in Emmens & Finn (1962).

MATERIALS AND METHODS

Rabbits of various breeds weighing 2 to 4 kg were mated naturally and the females injected with substances under test in 1 ml of nut oil or, for intravenous injection, in 0.2 ml of propylene glycol. Controls received nut oil or propylene glycol alone. Subcutaneous injections were given on Days 4, 5 and 6; 5, 6 and 7; or 7, 8 and 9, counting the day of copulation as Day 1. Intravenous injections were given on Day 6. Laparotomy or killing was done on Days 9 to 11 after mating, and the numbers of corpora lutea and implanting embryos recorded. Animals not showing recent ovulation were discarded. A few groups were left to produce litters.

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As few animals were available at any one time, most of the comparisons in Table 1 are confounded with time-to-time variation in response. This, however, can hardly be of serious consequence in present circumstances, where the results are of a preliminary nature.

The compounds used were those described by Emmens et al. (1960).

### Results

The experimental findings are shown in Tables 1 and 2. Table 1 lists the numbers of rabbits that ovulated and that became pregnant in response to various treatments, together with the total numbers of corpora lutea and implantation sites per group. In addition, a separate account is given in the rows in italics of those animals exhibiting apparently normal implantation.
This was sometimes much less than the total of all implantations. No attempt will be made, however, to compare groups on the basis of percentage implantations, since the occurrence of implantation is clearly from the data a quantal phenomenon, and statistically valid comparisons can only be made of the numbers of rabbits exhibiting implantation or not, a much reduced series numerically as compared with numbers of implanting ova. Thus, very few instances occurred in which the number of ova implanting was much less than the number of corpora lutea found, unless it was zero. In a few cases, one or two more implantation sites were counted in a group than the total of corpora lutea found, due either to miscounting or to multiple ovulation. Table 2 lists the groups of rabbits allowed to litter normally if they would.

### Table 2

<table>
<thead>
<tr>
<th>Substance</th>
<th>Daily dose</th>
<th>No. rabbits</th>
<th>No. litters</th>
<th>Young per litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nut oil</td>
<td>1 ml</td>
<td>9</td>
<td>8</td>
<td>5.4</td>
</tr>
<tr>
<td>Dimethylstilboestrol</td>
<td>25 mg</td>
<td>7</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>17-ethinyl-19-nortesterone</td>
<td>10 mg</td>
<td>5</td>
<td>4</td>
<td>6.3</td>
</tr>
</tbody>
</table>

It will be apparent from the Tables that 17-ethyl-19-nortestosterone and mer 25 were ineffective in the doses given, and that 100% inhibition of normal implantation was achieved only with oestradiol. However, the corrected $\chi^2$ value for the results in Table 1 with 25 mg of dimethylstilboestrol (DMS) given on Days 7 to 9 was 5.4, significant at the 2% level, and that for 10 mg of 17-ethinyl-19-nortestosterone was 8.4, significant at the 1% level, in comparison with controls. In Table 2, a significant reduction in the number of females littering was again seen with DMS, but not with 17-ethinyl-19-nortestosterone.

With effective doses of oestradiol or of the above anti-oestrogens, it was particularly noticeable that implantation might occur but appear abortive, thus reducing the count of apparently normal implants. With 30 µg of oestradiol given on Days 5 to 7, for example, all five rabbits showed implantation sites, but only one of them showed normally-appearing sites, the remainder were smaller, with blood clots and apparently undergoing resorption.

### DISCUSSION

Since few positive results were obtained in this series, it is impossible to decide whether the period 5 to 7 days or 7 to 9 days after mating is the best for injection, but there would seem to be little in it. Implantation in the rabbit commences during the 6th and 7th day after copulation (Boyd & Hamilton, 1952) and so might well be inhibited by either course of injections, although the earlier might be expected to take better effect. Emmens et al. (1960) and Emmens & Finn (1962) have discussed the difficulties in deciding, with the mouse or rat, whether the interruption of early pregnancy is in all their
experimental cases attributable to prevention of implantation and if so, to oestrogenic, anti-oestrogenic or some other property of the substances injected. There is evidence that anti-oestrogenicity may well be the mechanism in some instances. In this connexion, it is of interest that about 30 µg of oestradiol daily is an effective dose in the rabbit, and that about 25 mg daily of DMS has similar potency. In the mouse, (Martin, Emmens & Cox, 1960) DMS has 0.56 × 10⁻³ times the oestrogenic potency of oestradiol, hence 25 mg is equivalent to about 14 µg of oestradiol — too close a figure to suppose that the action of DMS in the rabbit is other than oestrogenic, although Martin et al. (1960) found that the relative potencies of oestradiol and DMS as anti-fertility agents and as oestrogens in the mouse are significantly different. We are thus left undecided, from these present data, about the action of DMS in the rabbit.

As with the mouse (Emmens & Finn, 1962) 17-ethinyl-19-nortestosterone was effective in inhibiting implantation, while 17-ethyl-19-nortestosterone was not. This, again, points to a correlation of oestrogenic (or pro-oestrogenic) activity and anti-fertility effects, but the doubtfulness of this conclusion has already been discussed elsewhere (Martin et al., 1960; Emmens & Finn, 1962).

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REFERENCES


