OVUM Fertilization and Embryo Survival in Ewes Treated with Estradiol Immediately Prior to Mating

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SUMMARY

Eight ewes in each of four groups were injected subcutaneously immediately prior to natural mating with 0, 30, 150, or 750 μg of estradiol-17β. The ewes were necropsied 3 days after mating and the ova were examined. None of the three doses of estradiol reduced ovum fertilization significantly, but the 750-μg dose decreased the number of accessory sperm cells in the zona pellucidae. Eight other ewes in each of four groups were treated with the same doses of estradiol-17β and necropsied 25 days later. None of the estradiol treatments significantly reduced the rate of pregnancy.

The results indicated that doses of 30 or 150 μg of estradiol, injected immediately prior to mating, were not detrimental to sperm transport, ovum fertilization, or embryo survival for 25 days. The dose of 750 μg of estradiol may have reduced the efficiency of sperm transport. (Key Words: Estradiol, Ovum Fertilization, Embryo Survival.)

INTRODUCTION

Recent experiments in this laboratory indicated that 30 μg of estradiol-17β administered to ewes a few hours before or at the time of mating increased the number of sperm cells in the cervix by twofold to threefold and in the oviducts by about 10-fold at 24 hr post-mating (Hawk and Cooper, 1975). Dosages of 10 or 100 μg of estradiol increased the numbers of sperm cells in the cervix but not in the oviducts.

The administration of estrogen to laboratory animals at or soon after the time of mating hastened or delayed ovum transport through the oviducts and caused a high rate of embryonic death (Greenwald, 1957, 1967). Because estradiol or a related compound might have potential usefulness for improving sperm transport in domestic animals, a study was conducted to determine whether the administration of estradiol at the time of mating would detrimentally affect ovum fertilization or embryo survival in the ewe.

MATERIALS AND METHODS

Parous Rambouillet ewes about 6 years of age were checked for estrus twice daily by the use of vasectomized rams. Ewes that were detected in estrus in the morning were used in these experiments.

In the experiments on ovum fertilization (table 1) and embryo survival (table 2), eight estrous ewes were assigned to each of four groups (estradiol-17β doses of 0, 30, 150, or 750 micrograms). The control ewes (tables 1 and 2) were given 1 ml of corn oil by subcutaneous injection. The 30-μg and 150-μg doses were given subcutaneously in 1 ml of corn oil. The 750-μg dose was given subcutaneously in 3 ml of corn oil. Immediately after the injection each ewe was mated to two fertile rams.

The 30-μg dose of estradiol appears to be near optimum for improving sperm transport (Hawk and Cooper, 1975). In order to test for detrimental effects of estradiol, particularly at the 30-μg dose, without using large numbers of ewes, the two consecutive fivefold increases to 150 and 750 μg were made in the 30-μg dose. If the 30-μg dose had detrimental effects on ovum fertilization or embryo survival that were not detectable with eight ewes per group, it was assumed that the 150-μg dose would likely have a considerably greater detrimental effect and that the 750-μg dose would probably inhibit fertility almost completely.

Ovum Fertilization. The ewes were sacrificed 72 hr after mating. Oviducts adjacent to developing corpora lutea were dissected free from the broad ligament and flushed with physiological saline solution. The ova recovered by this
procedure were examined with a phase contrast microscope for cleavage and for number of blastomeres. Gentle pressure was then exerted on the cover slip to crack the zona pellucida and expel its contents, and the sperm cells attached to the zona pellucida were counted.

Proportions of ewes in the four groups with cleaved ova were compared by chi-square. Numbers of accessory sperm cells were compared by analysis of variance and calculation of confidence intervals.

Embryo Survival. The ewes were necropsied 25 days after mating. The corpora lutea were counted, the uterus was opened and embryonic membranes carefully recovered. The membranes and embryos were examined and the crown-rump length of each embryo that appeared to be morphologically normal was measured. In two instances, membranes appeared to be normal but the embryos had degenerated to the point that they were no longer recognizable as embryos and were not measured.

The proportions of ewes pregnant in the four groups were compared by chi-square.

RESULTS AND DISCUSSION

Ovum Fertilization. Forty-one of 50 ova (82%) recovered from ewes of the four treatment groups had cleaved (table 1). Of the nine uncleaved ova, eight did not contain accessory sperm cells, but one, recovered from a control ewe, contained 205. Thirty of the 32 ewes had at least one cleaved ovum. No significant differences were found among the four groups of ewes in the proportion of ova that had cleaved or the proportion of ewes that had cleaved ova. These results indicated that an estradiol dose as high as 750 μg did not inhibit ovum fertilization.

The 750-μg dose of estradiol apparently had adverse effects on ovum morphology and numbers of accessory sperm cells in the zona pellucida. In three of the seven ewes with cleaved ova, the zonae pellucidae were wrinkled and ova were partly flattened. No ova from ewes of the other groups were flattened or had wrinkled zonae pellucidae. These observations suggest that the 750-μg dose of estradiol had harmful effects either directly on the ovum or on the oviducal environment. The number of accessory sperm cells in the zona pellucida (table 1) was significantly lower for the 750-μg group than for any of the other groups (750-μg group vs controls or vs 150-μg group, P<.05; 750-μg group vs 30-μg group P<.025). These

<table>
<thead>
<tr>
<th>Amount of estradiol (μg)</th>
<th>Ova recovered</th>
<th>Ova cleaved</th>
<th>Cleaved ova with</th>
<th>Cleaved ova with</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>15</td>
<td>8</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>10</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>150</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>750</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

a Ewes were necropsied and ova examined 3 days after mating.

b Means (±SE for accessory sperm) calculated from one value per ovum. The average of two values was used for each ewe with two ovum.

c For accessory sperm numbers, 750-μg group vs controls or 150-μg group, P<.05; 750-μg group vs 30-μg group, P<.025.

d The zonae pellucidae were wrinkled and the ovum partly flattened in three of the seven ewes with cleaving ovum.
TABLE 2. EFFECT OF ADMINISTERING ESTRADIOL IMMEDIATELY PRIOR TO MATING ON EMBRYO SURVIVAL TO 25 DAYS POST-MATING

<table>
<thead>
<tr>
<th>Amount of estradiol (μg)</th>
<th>Number of Ewes</th>
<th>Number of Corpora lutea</th>
<th>Number of Normal embryos</th>
<th>Crown-rump length of embryos (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>7</td>
<td>10</td>
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<tr>
<td>150</td>
<td>8</td>
<td>7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>750</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

aThe five ewes that were not pregnant returned to estrus 16, 17, or 18 days after mating.
bNumbers of corpora lutea and embryos in pregnant ewes only.
cMeans ± SE calculated from one value per pregnant ewe. The average of two values was used for each ewe with two embryos.

Differences suggest that sperm transport may have been inhibited in ewes treated with 750 μg of estradiol so that relatively few sperm cells reached the oviducts. Other explanations could be made, including the possibility that the 750-μg dose of estradiol might have caused ova to be held longer than usual in the anterior oviducts where sperm cell numbers would be expected to be low.

The ewes given 30 μg of estradiol had the most accessory sperm cells (table 1). This observation is consistent with previous results in which increased numbers of sperm cells were found in oviducts of ewes treated with 30 μg of estradiol (Hawk and Cooper, 1975). The number of accessory sperm cells for control ewes was similar to that for ewes treated with 150 μg of estradiol. This finding is consistent with the lack of effect of 100 μg of estradiol on sperm transport to the oviducts (Hawk and Cooper, 1975).

Embryo Survival. Twenty-seven of the 32 ewes in the experiment were pregnant with one or two embryos at 25 days after mating (table 2). Five ewes returned to estrus at 16 to 18 days after mating. A degenerating embryo along with a normal embryo was recovered from one ewe treated with 150 μg of estradiol and from one ewe treated with 750 micrograms.

The estradiol treatments failed to cause a significant reduction in the proportion of ewes pregnant at 25 days, although three of eight ewes treated with 750 μg of estradiol returned to estrus compared to none of eight control ewes.

The growth of embryos, as measured by crown-rump length at 25 days, was not significantly affected by estradiol treatment (table 2).

Estrogen given to laboratory animals at or soon after mating may hasten or delay the transport of ova through the oviducts, cause the degeneration of ova in the oviducts or uterus, or cause the expulsion of ova from the uterus (Greenwald, 1957, 1959, 1963, 1967; Chang and Yanagimachi, 1965; Chang and Harper, 1966; Chang, 1974). The present experiments with ewes indicated that 30 or 150 μg of estradiol-17β administered at the time of mating had no apparent effects on sperm transport, ovum fertilization or embryo survival. A dose of 750 μg of estradiol decreased the numbers of accessory sperm cells and caused morphological changes in some ova. However, the 750-μg dose was apparently only marginally detrimental to the entire reproductive process; embryos survived in five of eight ewes treated with 750-μg and were developing normally at 25 days after mating. The 750-μg dose of estradiol that seemed to be slightly detrimental to reproduction is 25 times the dose of 30 μg that appears to be near the optimum for improving sperm transport (Hawk and Cooper, 1975).

LITERATURE CITED
Greenwald, G. S. 1959. The comparative effectiveness...
of estrogens in interrupting pregnancy in the rabbit. Fertil. and Steril. 10:155.