INDUCTION OF EMBRYONIC DEATH IN SHEEP BY INTRAUTERINE INJECTION OF A SMALL VOLUME OF NORMAL SALINE

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Summary. Normal saline (1-0 ml) was injected into the lumen of the uterus of ewes on Days 8, 12, 13, 14, 15, 16 and 20 of pregnancy. Treatment caused embryonic death in 100% of ewes on Day 14, and in 50% or more of ewes on Days 13 and 15. Treatment on other days did not affect the viability of the embryo, nor did sham operations performed on the 14th day of pregnancy. The possible means by which embryonic death results from treatment on Days 13 to 15 are discussed.

In recent unpublished experiments, attempts have been made to produce developmental anomalies in the sheep embryo by injection of small volumes (1-0 ml) of teratogens suspended in normal saline into the lumen of the uterus on the 14th day of pregnancy. It was found that this procedure caused termination of pregnancy in 100% of pregnant ewes.

The aim of the present experiment was to determine whether intrauterine injections of normal saline alone at different times early in gestation would result in termination of pregnancy.

Twenty-two mature Merino ewes kept under standard indoor conditions were mated to a mature Merino ram fitted with a Sire-sine harness (Radford, Watson & Wood, 1960). Ewes were inspected for time of mating at 12-hr intervals (day of mating = Day 0).

Ewes were anaesthetized with intravenous pentobarbital sodium, and a mid-line laparotomy was performed to expose the reproductive tract. Normal saline (0-5 ml) was injected into the lumen of each horn of the uterus after a 2-in. 18-gauge hypodermic needle, from which the point had been removed, had been passed through the wall of the uterus about 1 cm distal to the uterotubal junction. Injections were made at 8, 12, 13, 14, 15, 16 and 20 days of gestation, and sham operations were performed at 14 days. In the latter case, the needle was passed into the lumen of each horn of the uterus but no fluid was injected.

Embryos were recovered and examined between the 17th and 40th days of gestation.

The results of the experiment are summarized in Table 1. It can be seen from the table that pregnancy is terminated in ewes by injection of 1-0 ml normal saline into the uterus at 14 days' gestation. Injection of the same volume of
saline into the uterus at 13 and 15 days' gestation caused embryonic death in more than 50% of the ewes. Pregnancy was unaffected by intrauterine injections of saline before the 13th day or after the 15th day of pregnancy. Similarly, sham operations on the 14th day did not affect pregnancy.

In earlier experiments, 1·0 ml of teratogen suspended in normal saline was injected into the lumen of the uterus in thirty-six ewes on Day 14 of pregnancy. The procedure resulted in return to service within 44 days in seventeen of the ewes, and no sign of pregnancy as judged by laparotomy and examination of the reproductive tract at 48 to 59 days after treatment in the remaining nineteen ewes. Of six control ewes injected with 1·0 ml normal saline on the 14th day of gestation, one returned to service 6 days later and five proved to be non-pregnant 15 to 26 days after treatment. It was concluded that intrauterine injection of 1·0 ml of fluid on the 14th day of pregnancy caused either embryonic death, or abortion, or both, in 100% of pregnant ewes.

Table 1. The effect of intrauterine injection of 1·0 ml normal saline at different stages of early pregnancy on subsequent pregnancy in ewes

<table>
<thead>
<tr>
<th>Day of treatment (day of mating = Day 0)</th>
<th>No. of ewes</th>
<th>Day of recovery (day of mating = Day 0)</th>
<th>Pregnancy status at recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>2</td>
<td>18</td>
<td>2 pregnant with viable embryos</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>19</td>
<td>3 pregnant with viable embryos, 1 not pregnant, new CL†</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>19</td>
<td>2 pregnant with viable embryos, 1 with resorbing membranes only, 1 with resorbing membranes and 14- to 15-day embryo</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>20</td>
<td>4 with resorbing membranes only</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>21</td>
<td>1 pregnant with viable embryos, 2 with resorbing membranes only, 1 with resorbing membranes and 15- to 16-day embryo</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>25</td>
<td>4 pregnant with viable embryos</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>25</td>
<td>3 pregnant with viable embryos</td>
</tr>
<tr>
<td>14 (sham)</td>
<td>4</td>
<td>20</td>
<td>4 pregnant with viable embryos</td>
</tr>
</tbody>
</table>

* In all ewes, except the 12-day animal which was not pregnant on the day of recovery, one or two large corpora lutea were observed at recovery.
† Because ovulation had occurred recently, it was assumed that this ewe had not conceived, or the embryonic death had occurred before Day 12.

The results of the present experiment demonstrate that pregnancy is terminated by the intrauterine injection of 1·0 ml of saline, but this occurs only during the critical period between Days 13 and 15 of gestation.

A number of important intrauterine events take place during this period. The first stages of embryonic attachment occur on Day 15 or soon after (Boshier, 1969, 1970), and it is on Days 13 to 15 that the presence of the embryo in the uterus becomes essential for the normal maintenance of the corpus luteum of pregnancy (Moor & Rowson, 1966a, b). Significant increases in endometrial prostaglandin F$_2$ concentration and content occur in cyclic ewes 14 days after mating to vasectomized rams (Wilson, Cenedella, Butcher & Inskeep,
inhibition, venous release produced stance of the embryonic exerting due saline

The embryonic saline was supported by the endometrium, and hence prevented luteolysis. Possibly intrauterine injection of saline on Days 13 to 15 of gestation results in dilution of the inhibitor substance produced by the embryo. The effect of slight distention of the uterine wall at this time should not be discounted, although it is unlikely that it is important. The effect of the sham operations demonstrated that embryonic death was not due to manipulation of the reproductive tract.

It is postulated that the intrauterine injection of saline on Day 14, and in some cases on Days 13 and 15, of pregnancy in the ewe prevents the embryo from exerting its protective action on the corpus luteum: luteal failure leads to embryonic death.

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REFERENCES

tome formation in sheep. J. Reprod. Fert. 19, 51.