ROUTE OF PROGESTAGEN ADMINISTRATION TO THE EWE AND SPERMICIDAL ACTION IN THE VAGINA AT THE ENSUING ESTRUS

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Summary

Parous ewes were treated orally with medroxyprogesterone acetate (MAP) or melengestrol acetate (MGA), subcutaneously with progesterone, or intra-vaginally with MAP, MGA or progesterone. Treatment was begun on day 8 of an estrous cycle and continued for 17 days. When the ewes were in estrus after progestagen withdrawal, they were inseminated artificially and their vulvo-vaginal junctions were ligated to prevent sperm cell drainage to the exterior. The ewes were killed 22 hrs later, their vaginas flushed, and intact sperm cells and tailless sperm heads counted.

The oral, subcutaneous or intravaginal administration of progestagen increased the numbers of sperm cells recovered as tailless heads, indicating stimulation of sperm-breaking mechanisms in the vagina at the ensuing estrus. Progestagen given intravaginally decreased the total number of sperm cells recovered, indicating increased sperm cell disappearance within the vagina.

Introduction

When ewes were treated with progestagen-impregnated intravaginal sponges and inseminated at the ensuing estrous period, sperm cell destruction in the vagina was much greater than it was in control ewes (Hawk and Conley, 1971). Much of the sperm cell destruction occurred as a break at the junction of the head and midpiece, resulting in the recovery from the vagina of large numbers of separated heads and tails. The sperm-breaking effect of the progestagen sponge was particularly evident when the vulvo-vaginal junction was ligated to prevent the drainage of semen to the exterior.

The intravaginal progestagen treatment has also tended to increase the disappearance of sperm cells within the ligated vagina (Hawk, 1972).

The progestagen which caused increased sperm breakage by intravaginal administration was medroxyprogesterone acetate. This experiment was done to determine the effect of two other progestagens and two other routes of administration of progestagen on the spermicidal activity of the vagina at the ensuing estrus.

Materials and Methods

The ewes were parous Rambouillets 6 to 8 years of age. They were checked twice daily for estrus by the use of vasectomized rams, and assigned to an experimental group as they came into estrus (day of estrus = day 0). Experimental treatments were begun on day 8 of the cycle, and the ewes were inseminated on the first day of estrus following treatment.

Control Ewes. Seven of these ewes were untreated (Group la, table 1). Five ewes were treated from day 8 to day 15 with a "blank" sponge, containing no progestagen (Group lb), and five were treated with a blank sponge plus a daily intravaginal infusion from day 8 to 15 of 0.8 ml of propylene glycol (Group lc); these ewes served as controls for ewes in Group 4 with intravaginal sponges. The sponges were removed from control ewes on day 15 because estrus was anticipated 2 or 3 days later; the ewes treated intravaginally with progestagen (Group 4) were generally expected to be in estrus 2 or 3 days after their treatments were ended.

The "blank" placebo sponges (The Upjohn Company) were cylindrical, 5 cm long and 2 cm in diameter. They were inserted into the anterior vagina. The propylene glycol was infused through polyethylene tubing (PE 160, Clay Adams), which was sutured to the blank sponge before the sponge was inserted into the ewe. One end of the tubing was positioned at the anterior end of the sponge and the other extended to the outside of the vulva. The propylene glycol was expelled from a syringe into the tubing, followed by a sufficient volume of air to push the propylene glycol into the vagina.

1 Animal Physiology and Genetics Institute, A.R.S., Agricultural Research Center, Beltsville, Maryland 20705. The authors express appreciation to Dr. R. G. Zimbelman, The Upjohn Company, Kalamazoo, Michigan, for supplying the synthetic progestagens. Mention of products or companies in the report does not constitute endorsement by the U.S. Department of Agriculture to the exclusion of others not mentioned.
<table>
<thead>
<tr>
<th>Group, route of progestagen administration and subgroups</th>
<th>No. of ewes</th>
<th>No. of sperm cells deposited (millions)</th>
<th>No. of sperm cells recovered (millions)</th>
<th>% tailless of total sperm recovered&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Tailless</td>
<td>Total</td>
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<tr>
<td>1. Controls (total)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>685</td>
<td>456&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>a. Untreated</td>
<td>7</td>
<td>688</td>
<td>437</td>
<td>38</td>
</tr>
<tr>
<td>b. Blank sponge</td>
<td>5</td>
<td>697</td>
<td>546</td>
<td>39</td>
</tr>
<tr>
<td>c. Blank sponge + vehicle</td>
<td>5</td>
<td>669</td>
<td>394</td>
<td>24</td>
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<td>2. Oral (total)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16</td>
<td>636</td>
<td>461&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>a. MAP</td>
<td>9</td>
<td>627</td>
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<td>77</td>
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<td>b. MGA</td>
<td>7</td>
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<td>3. Subcutaneous</td>
<td>12</td>
<td>682</td>
<td>338&lt;sup&gt;c&lt;/sup&gt;</td>
<td>86&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>4. Intravaginal (total)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26</td>
<td>641</td>
<td>196&lt;sup&gt;d&lt;/sup&gt;</td>
<td>131&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>a. MAP sponge</td>
<td>12</td>
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<td>137</td>
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<tr>
<td>b. MGA</td>
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<td>662</td>
<td>217</td>
<td>110</td>
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<tr>
<td>c. Blank sponge + MGA</td>
<td>4</td>
<td>640</td>
<td>227</td>
<td>107</td>
</tr>
<tr>
<td>d. Blank sponge + progesterone</td>
<td>6</td>
<td>652</td>
<td>179</td>
<td>150</td>
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</table>

<sup>a</sup>Each figure is the mean of individual ewes for the group or subgroup. The figures calculated from the means for tailless and total sperm recovered are 7%, 18%, 20% and 40% for Groups 1 through 4.

<sup>b</sup>The total for the group includes all ewes in the subgroups.

<sup>c,d,e</sup>Within a column, any two means which have no superscript letter in common differ significantly (P < .01 or P < .05).
Oral Progestagen Treatment. Nine ewes (Group 2a) were fed 60 mg daily of medroxy-progesterone acetate (MAP; The Upjohn Company), and seven ewes (Group 2b) were fed 0.3 mg daily of melengestrol acetate (MGA; The Upjohn Company). From day 8 to 25 of a cycle, the ewes were fed the progestagens in dry soybean meal in a gelatine capsule.

Subcutaneous Progesterone Treatment. Twelve ewes were injected daily from day 8 to 25 with 20 mg of progesterone per day in 0.8 ml of corn oil (Group 3).

Intravaginal Progestagen Treatment. Twelve ewes were treated with an intravaginal sponge containing 60 mg of MAP (Group 4a). The sponge was inserted on day 8 and removed on day 25. Four other ewes were treated daily by infusing 0.3 mg of MGA in 0.8 ml of propylene glycol through a catheter inserted daily into the anterior vagina (Group 4b). No evidence was seen of expulsion of the propylene glycol from the ewe. Four ewes were treated with an intravaginal blank sponge, to which was attached polyethylene tubing; they were also infused daily with 0.3 mg of MGA (Group 4c). Six other ewes treated with a tube-bearing intravaginal blank sponge were infused daily with 20 mg of progesterone in 0.8 ml of propylene glycol (Group 4d). The treatments were begun on day 8, and the sponge and tubing were withdrawn from all ewes following the last daily infusion on day 25.

Estrus, Insemination and Sperm Cell Recovery. Of the 10 control ewes that were treated with a blank sponge or with a blank sponge and propylene glycol, nine came into estrus 2 days and one 3 days after the sponge was removed on day 15. Of the 42 ewes that showed heat after oral or intravaginal treatment with progestagen (Groups 2 and 4), two ewes were in heat 1 day after the last day of treatment, seven were in heat on the second day, 24 on the third day, seven on the fourth day, and two on the fifth day. Of 12 ewes injected subcutaneously with progesterone, two were in heat on the third day, seven on the fourth day, and two on the fifth day after the end of treatment. No ewes showed heat during treatment. Six ewes that had not shown estrus within 5 days after the end of treatment were not used.

Each ewe was inseminated with 0.25 ml of raw semen from pooled ejaculates of two or more rams. The semen was collected in an artificial vagina and used for insemination within 30 minutes. The semen had good motility, and less than 2% of the sperm cells were broken into heads and tails.

Immediately before insemination, the tissue around the vulvo-vaginal junction was anesthetized by injections of 2% procaine solution. The vulva was held open by forceps and a ligature was placed in the mucosa at the vulvo-vaginal junction. Particular care was taken not to puncture or occlude the urethra. The ligature was tied loosely, the insemination tube placed in the anterior vagina, the ligature tightened around the tube, and the semen deposited. The ligature was tightened and tied as the tube was withdrawn. The reproductive tract was ligated to retain sufficient numbers of sperm cells in the vagina that spermicidal effects of the progestagens could be detected with reasonably small numbers of ewes (Hawk and Conley, 1971).

The ewes were killed 22 hr. after insemination. The reproductive tract was removed, the vagina was flushed with saline solution, and intact and tailless sperm cells were counted as described previously (Hawk, 1972).

Statistical Analysis. The data were subjected to analysis of variance and Duncan's multiple range test. The percentages of sperm cells recovered as tailless heads were transformed to angles before analysis.

Results

Control Ewes. There was no evidence that sperm cell disappearance or breakage was increased by the use of a blank sponge or by the infusion of propylene glycol (Group 1, table 1). The three control subgroups were therefore pooled. For the 17 control ewes, the proportion of sperm recovered from the vagina averaged about 70% of the number deposited at insemination; about 90% of the recovered sperm cells were intact.

Oral Progestagen. The rate of sperm breakage did not differ significantly between ewes treated with MAP or with MGA (Group 2), so the subgroups were pooled. The oral administration of progestagen increased both the number and percentage of sperm recovered as tailless heads (P ≤ .02) for each comparison with control ewes.

Subcutaneous Progesterone. This treatment increased both the number and percentage of sperm recovered tailless as compared to control ewes (Group 3 vs. Group 1). Considerable variation was seen in Group 3 in the number of sperm cells recovered as tailless heads; six ewes had more than 100 million and four ewes had less than 30 million.

Intravaginal Progestagen. Differences among the four subgroups within Group 4 were not
statistically significant, so data were pooled for statistical comparisons with other main groups. This was the only progestagen treatment which caused a significant reduction in the number of sperm cells recovered intact (table 1). When compared to the controls, the intravaginal progestagen treatment also increased both the number and proportion of sperm recovered as tailless heads. Compared to the other progestagen treatment groups, the intravaginal treatment group had a higher percentage of sperm recovered tailless.

The main effect of intravaginal as compared to oral progestagen treatment was an increase in sperm disappearance. Comparing Group 2a with Group 4a for total sperm cells recovered (508 vs. 311 million), it appears that MAP caused a greater disappearance of sperm cells when given intravaginally than when given orally. Similarly, by comparing Group 2b with Groups 4b and 4c (631 vs. 330 million sperm cells recovered), it is apparent that MGA also caused greater sperm disappearance when given intravaginally than orally.

By comparing the subgroups of Group 1 with the subgroups of Group 4, it is clear that the spermicidal effects of intravaginal progestagen treatment were caused by the progestagen and not by the sponge which was placed in the vaginas of most of the treated ewes. Previous results also failed to implicate the sponge itself as a factor in spermicidal action (Hawk, 1972).

Discussion

Two general types of sperm cell loss occur in the ligated vagina of the ewe: disappearance and breakage (Hawk and Conley, 1971). It seems likely that many sperm that were not recovered from the vagina were “broken” before they “disappeared.”

The mean number of sperm cells that “disappeared” from the vagina varied between 74 and 314 million for the main groups listed in table 1. The figures represent the difference between the number deposited in the vagina and the total number recovered. Most sperm cells that “disappeared” were probably lost within the vagina. In previous studies on the fate of sperm cells in ewes with ligated vulvovaginal junctions, relatively few sperm cells were recovered from the cervix, uterine horns or oviducts (Hawk and Conley, 1971; Hawk, 1972), and it seems likely that no more than a very small proportion of the sperm cells that disappeared passed through the oviducts and into the body cavity. Most sperm cells that disappeared from the vagina were probably phagocytized; nearly all of the sperm cells disappearing from ligated uterine horns were found inside of leukocytes (Brinsfield, Clark and Hawk, 1971).

The mediator of sperm cell “breakage” in the female reproductive tract is not known. Chang (1956) suggested that enzymatic reactions might be involved in sperm breakage in the rabbit uterus.

When semen was deposited in the ligated uterus of the ewe, estradiol intensified sperm breakage and progesterone suppressed it (Hawk and Cooper, 1971). Whether natural ovarian hormones influence sperm breaking mechanisms in the vagina is not known.

The local application of progestagen stimulated spermicidal actions to a greater degree than did the administration of progestagen by other routes. The intravaginal route was the only one which increased sperm disappearance significantly. Also, this route apparently intensified sperm breakage to a greater degree than did the others, as suggested by a higher proportion of tailless heads.

From the standpoint of responsiveness of spermicidal mechanisms, it appears that the sperm-breaking mechanism may be more sensitive than the sperm-disappearing one; the sperm-breaking mechanisms responded to progestagen administered by each route, while the sperm-disappearing mechanism responded only to local intravaginal treatment.

All treated and control ewes in the study were in heat when inseminated, and thus free from the estrus-suppressing effect of exogenous or endogenous progestagen. However, ewes in estrus after progestagen treatment have, in addition to intensified sperm cell breakage in the vagina, increased sperm cell loss from the reproductive tract (Hawk and Conley, 1971) and poor sperm transport to the oviducts (Quinlivan and Robinson, 1967, 1969; Hawk and Conley, 1972). Thus progestagen-treated ewes come into estrus while the progestagen is still exerting detrimental influences, directly or indirectly, on several functions of the reproductive tract associated with sperm survival and transport.

Literature Cited
