Increased Retention of Spermatozoa in the Reproductive Tract of Estrous Rabbits after Administration of Prostaglandin F$_{2\alpha}$ Immediately before Insemination

H. W. Hawk and B. S. Cooper


The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://www.journalofanimalscience.org/content/49/1/154
INCREASED RETENTION OF SPERMATOZOA IN THE REPRODUCTIVE TRACT OF ESTROUS RABBITS AFTER ADMINISTRATION OF PROSTAGLANDIN F2α IMMEDIATELY BEFORE INSEMINATION

H. W. Hawk and B. S. Cooper

U. S. Department of Agriculture
Beltsville, MD 20705

Summary

Two experiments were conducted to determine whether prostaglandin F2α (PGF2α) administered to does immediately before natural or artificial insemination would increase the retention of sperm in the reproductive tract. The PGF2α was injected intramuscularly at 200 μg/kg of body weight. In the first experiment, 16 estrous does, eight injected intramuscularly with PG2α, were mated naturally and necropsied 3 hr later. In the second experiment, of 2 x 2 design, 32 estrous does, 16 injected with PGF2α, were inseminated artificially with either 34 or 102 million sperm and necropsied 3 hr later.

After necropsy, each segment of the reproductive tract was flushed and sperm were counted. In each experiment, the PGF2α treatment increased by at least threefold the number of sperm recovered from the oviducts, uterus, cervixes and vagina, as well as the total number recovered from the reproductive tract. The PGF2α treatment reduced the normal loss of sperm from the reproductive tract during the first 3 hr after insemination.

(Key Words: Rabbits, Sperm transport, Prostaglandin F2α.)

Introduction

In attempts to improve fertility or to improve sperm transport in the female reproductive tract, several researchers have added prostaglandin to semen used for artificial insemination or have administered prostaglandin to females near the time of insemination. Mandl (1972) added prostaglandin E1 (PGE1) and Sanchez-Algaba and Melgar (1976) added prostaglandin F2α (PGF2α) to rabbit semen and recovered more sperm from the uterus and oviducts of treated than of untreated does 2 to 4 hr after insemination. Spilman et al. (1973) used ovum fertilization as a measure of sperm transport and obtained higher fertilization rates by adding PGF2α to semen or injecting PGF2α into does but not by adding or injecting PGE1 or PGE2. Conversely, Chang et al. (1974) obtained higher fertilization rates by adding PGE1 or PGE2 to diluted rabbit semen but not by adding PGF2α. Edquist et al. (1975) and Gustafsson et al. (1977) added PGE1 or PGF2α to the inseminate or injected PGF2α at the time of insemination of ewes and recovered more sperm from the oviducts of treated than untreated ewes 16 or 24 hr after insemination. Dimov and Georgiev (1977) added PGE2 and PGF2α to the inseminate and increased the lambing rate of ewes by about 15%. Although results have been somewhat inconsistent, apparently one or more of the prostaglandins can sometimes improve sperm transport in the female reproductive tract.

In the studies cited above, the general assumption was that prostaglandin improved sperm transport to the oviducts by stimulating contractility of the female reproductive tract. However, results of recent work in this laboratory suggested that increased retention of sperm in the reproductive tract might also be a factor in sperm transport. Treating does with estradiol immediately before insemination resulted in the recovery of more sperm from...
each segment of the reproductive tract—the vagina, cervix, uterus and oviducts—2.5 hr later (Hawk and Cooper, 1978). Apparently, estradiol reduced the normally rapid loss of sperm from the tract; thus, the presence of more sperm in the vagina and cervix might by itself have resulted in the transport of more sperm to the uterus and oviducts.

In none of the previous work with prostaglandins has the total number of sperm been quantified in the entire female reproductive tract. In the present study, two experiments were conducted to determine whether exogenous PGF$_2$$\alpha$ would increase the retention of sperm in the reproductive tract of does after natural mating or artificial insemination.

Materials and Methods

Rabbits were New Zealand Whites weighing about 4 kg each. Eight bucks, selected for high quality semen, were used in rotation for mating or for collection of semen. No buck was used more than three times weekly.

In experiment 1, 16 estrous does were used in eight pairs. Each doe was mated to two bucks within 10 minutes. Bucks were used so that over the course of the experiment each buck was mated to equal numbers of control and treated does. Variation among days and bucks in numbers of sperm deposited in the does was assumed to be distributed randomly between control and treated does. Immediately before mating, one doe of each pair was injected intramuscularly with 200 $\mu$g of PGF$_2$$\alpha$/kg of body weight, or about 800 $\mu$g per doe. The prostaglandin was used as tromethamine salt from The Upjohn Company; the micrograms of PGF$_2$$\alpha$ noted above were measured as free PGF$_2$$\alpha$. Control rabbits were injected with saline solution.

In Experiment 2, does were checked for estrus by the use of vasectomized bucks, but mating was prevented to avoid the deposition of seminal fluids in the vagina. In a $2 \times 2$ factorial experiment with eight replicates, 32 estrous does were assigned to be inseminated artificially with an average of either 34 or 102 million sperm. One half of the does of each insemination group were treated with PGF$_2$$\alpha$ as in Experiment 1. The does that received 102 million sperm were inseminated with .2 ml of freshly collected semen. Does that received 34 million sperm were inseminated with .2 ml of diluted fresh semen (.067 ml of semen and .133 ml of .9% NaCl solution).

Three hours after mating or insemination, the does were sacrificed by an overdose of pentobarbital injected into the marginal ear vein. The entire reproductive tract, including the vulva, was removed, and the oviducts, uterine horns, cervices and vaginas were flushed or washed with saline solution (.9% NaCl). The procedures and efficiency of sperm recovery have been described (Hawk and Cooper, 1978). Briefly, each oviduct was flushed with 6 ml of saline solution and each uterine horn with 20 milliliters. The cervices and vaginas were cut into strips and soaked overnight in saline solution. The flask was then shaken to remove adhering sperm from the tissues. Aliquots of the flushings or washings of the tract were counted at $\times$ 200 in a hemocytometer under a phase contrast microscope.

Individual sperm counts were transformed to log$_{10}$ and analyzed statistically by $t$ test (Experiment 1) or by analysis of variance (Experiment 2). Sperm counts were converted to logs for statistical analysis to normalize the distribution. Arithmetic means and standard errors are given in the tables.

Results

Experiment 1. More sperm were recovered from each segment of the reproductive tract of PGF$_2$$\alpha$-treated does than of control does (table 1). The magnitude of the differences in numbers of sperm between control and treated does averaged more than 10-fold for the oviducts and uterus and about threefold for the cervix and vagina. Because most of the sperm recovered from each reproductive tract came from the cervix and vagina, the number of sperm recovered from the entire tract also averaged about threefold greater for PGF$_2$$\alpha$-treated does than for control does.

Experiment 2. This experiment was conducted to determine that the results of Experiment 1 were not caused by some effect of the PGF$_2$$\alpha$, administered immediately before mating, on the buck-doe interaction at mating, such as the ejaculation of more sperm into the treated does. In Experiment 2, known numbers of sperm were deposited in the anterior vagina by artificial insemination. Again, more sperm were recovered from each segment of the reproductive tract of PGF$_2$$\alpha$-treated does than of control does (table 2). The effect of PGF$_2$$\alpha$ treatment was apparent when the inseminate
TABLE 1. EFFECT OF EXOGENOUS PROSTAGLANDIN F₂α ON NUMBERS OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 3 HR AFTER MATING

<table>
<thead>
<tr>
<th>Group</th>
<th>Sperm recovered</th>
<th>Oviducts</th>
<th>Uterus</th>
<th>Cervices</th>
<th>Vagina</th>
<th>Entire tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>10²</td>
<td>73 ± 27</td>
<td>13 ± 3</td>
<td>33 ± 7</td>
<td>46 ± 9</td>
<td></td>
</tr>
<tr>
<td>2. PGF₂α</td>
<td>37 ± 11</td>
<td>1040 ± 188</td>
<td>36 ± 7</td>
<td>93 ± 24</td>
<td>130 ± 27</td>
<td></td>
</tr>
</tbody>
</table>

(Arithmetic means ± SE)

<table>
<thead>
<tr>
<th>(10²)b</th>
<th>(10³)b</th>
<th>(10⁶)b</th>
<th>(10⁶)b</th>
<th>(10⁶)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>1 ± 1</td>
<td>149 ± 39</td>
<td>5 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>2. PGF₂α</td>
<td>38 ± 12</td>
<td>328 ± 214</td>
<td>13 ± 4</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(Probabilitiesc)</th>
<th>&lt;.001</th>
<th>&lt;.001</th>
<th>&lt;.01</th>
<th>&lt;.05</th>
<th>.01</th>
</tr>
</thead>
</table>

a Eight does per group.
b Multiplier for each mean.
c Probabilities by t test of differences between means for control and PGF₂α-treated does.

The larger inseminate (102 million sperm) resulted in the recovery of more sperm from the vagina, uterus and entire tract than did the smaller inseminate (34 million sperm; table 2). Interactions between the number of sperm in the inseminate and PGF₂α treatment did not approach statistical significance.

TABLE 2. EFFECT OF EXOGENOUS PROSTAGLANDIN F₂α ON NUMBERS OF SPERM RECOVERED FROM THE REPRODUCTIVE TRACT OF DOES 3 HR AFTER ARTIFICIAL INSEMINATION

<table>
<thead>
<tr>
<th>No. of sperm per inseminate and group</th>
<th>Sperm recovered</th>
<th>Oviducts</th>
<th>Uterus</th>
<th>Cervices</th>
<th>Vagina</th>
<th>Entire tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>34 × 10⁶</td>
<td>(10²)b</td>
<td>(10³)b</td>
<td>(10⁶)b</td>
<td>(10⁶)b</td>
<td>(10⁶)b</td>
<td></td>
</tr>
<tr>
<td>1. Control</td>
<td>14 ± 4</td>
<td>119 ± 39</td>
<td>5 ± 2</td>
<td>2 ± 1</td>
<td>7 ± 3</td>
<td></td>
</tr>
<tr>
<td>2. PGF₂α</td>
<td>38 ± 12</td>
<td>328 ± 214</td>
<td>13 ± 4</td>
<td>8 ± 3</td>
<td>21 ± 6</td>
<td></td>
</tr>
<tr>
<td>102 × 10⁶</td>
<td>12 ± 4</td>
<td>228 ± 76</td>
<td>10 ± 4</td>
<td>8 ± 3</td>
<td>18 ± 6</td>
<td></td>
</tr>
<tr>
<td>3. Control</td>
<td>70 ± 11</td>
<td>919 ± 237</td>
<td>38 ± 14</td>
<td>14 ± 3</td>
<td>53 ± 16</td>
<td></td>
</tr>
</tbody>
</table>

(In Probabilitiesc)

| Inseminate | (Probabilitiesc) | .17 | <.025 | .09 | <.01 | <.05 |
|PGF₂α | <.001 | <.02 | <.02 | <.05 | <.02 |

a Eight does per group.
b Multiplier for each mean.
c Probabilities by F test of differences between main effect means.
inseminated with 34 million sperm, 18% and 55%, respectively; for control and PGF2α-treated does inseminated with 102 million sperm, 16% and 48%.

**Discussion**

Treating does with PGF2α immediately before natural or artificial insemination consistently increased the number of sperm recovered from the reproductive tract 3 hr later. After mating or insemination of rabbits, most sperm are normally lost by drainage or expulsion to the exterior within minutes (Morton and Glover, 1974). Treating does with PGF2α apparently caused some kind of response that reduced or delayed the loss of sperm from the reproductive tract and resulted in the retention of greater numbers of sperm in the tract. Perhaps PGF2α caused constriction of the vulvo-vaginal junction or caused other changes in contractility of the reproductive tract that resulted in functional occlusions to reduce the loss of sperm.

The PGF2α-induced increase in the number of sperm in the uterus and oviducts might have resulted from a PGF2α-induced increase in contractility of the reproductive tract (Kirton et al., 1974), which presumably occurred in these does, or from the reduced loss of sperm from the reproductive tract, which increased the number of sperm available in the vagina and cervix for transport anteriorly.

The effects of PGF2α in the present experiments were similar to the effects of exogenous estradiol in increasing sperm numbers throughout the reproductive tract of the doe (Hawk and Cooper, 1978). Because estradiol is known to stimulate the production of prostaglandin in uteri of animals of several species (Kirton, 1975), the estradiol administered to rabbits may have caused the production of prostaglandin, which in turn activated mechanisms that increased the retention of sperm in the reproductive tract.

**Literature Cited**


