COITAL BEHAVIOR IN DOGS, IV. EFFECTS OF PROGESTERONE IN THE BITCH*

BY FRANK A. BEACH AND ARIEL MERARI

UNIVERSITY OF CALIFORNIA, BERKELEY

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For a number of years it has been the practice in our laboratory to prepare female dogs for mating tests with males by injecting them with estradiol benzoate.1 The standard treatment has consisted of giving two intramuscular injections spaced 48 hours apart. Each injection contains 0.38 mg/kg of hormone. Females usually are ready to accept the male 2–3 days after the second injection and tend to remain in behavioral estrus for approximately 10–14 days. The behavior shown by bitches in induced estrus is qualitatively similar to that exhibited by females during natural heat, but in certain respects it is quantitatively inferior. When the same males are tested with a female when she is in natural estrus and again when she is in induced estrus, several differences are apparent. Many males are slower to initiate sexual contact with the bitch during induced estrus. More mounts may be needed in order to achieve insertion, the rate of mounting often is slower, the percentage of tests in which complete mating is achieved is lower, and the duration of the genital lock or tie tends to be shorter.

Recently we had occasion to examine the effects of a different form of estrogen on the sexual interaction of male and female dogs. Instead of estradiol benzoate we used estradiol cypionate (ECP),2 which had been reported by Hart3 to induce estrus in the spayed bitch. Although we followed the treatment schedule recommended by Hart, and although our beagles were derived from the same stock as his, our experimental animals did not develop a full complement of estrous behavior. In particular, they did not appear to arouse any intense or sustained interest in the sexually experienced males with which they were tested. When a third injection of ECP failed to remedy this situation, we administered a single injection of progesterone to each bitch. The results of this treatment form the substance of this report.

Three spayed female beagles were given two intramuscular injections of 1 mg of estradiol cypionate, with an interval of 48 hours between injections. Three days after the second injection, an additional 1 mg was injected. Six days after the last estrogen treatment, each female received a subcutaneous injection containing 5 mg of progesterone.

Three male beagles with extensive mating experience were used to test the females for sexual receptivity. Daily tests began immediately after the second estrogen injection and continued for the next 17 days. On the twelfth day of the experiment, the tests were begun four hours after the injection of progesterone.

A test consisted of placing a male and female in a fenced paddock 25 × 25 ft and recording the timing and frequency of a standardized list of responses by each animal. If the male failed to show mounting and thrusting within the first 3 minutes, the test was terminated forthwith. If one mount lasting 10 seconds or longer, or two or more shorter mounts occurred within the first 3 minutes, the test was extended to a total of 5 minutes.
Several different masculine responses were scored, but the only one to be dealt with in this report is the mount-and-thrust response in which the male mounts the female from the rear, clasps her hindquarters between his forelegs, and executes thrusting movements of his pelvic region. Note was made of the female's response to each mount by the male. She was scored as accepting all mounts that were spontaneously terminated by the male. A mount was counted as having been rejected if the female dislodged the male or if she barked, snarled, snapped at, or bit the male and thus prevented completion of the mount.

Test records also included the frequency of a variety of positive reactions shown by females toward males. A "positive social response" (PSR) was defined as "any act by one dog that clearly is oriented to a second individual and that has the effect of initiating or prolonging nonantagonistic interaction between the members of the pair." In the present study, PSR scores were given for the following types of response on the part of the female: attempt to mount the male, rear mount with or without thrusting, nuzzling the male with the head, clasping the male's neck, striking the male with a forepaw, "prancing" in front of the male with forelegs flexed and head oriented toward him, and "presenting" to the male. In executing the presentation response, the female places herself directly in front of the male's head and orients her hindquarters to him with her tail laterally or vertically deviated. Upon occasion she may back up toward the male so that her vulva comes into contact with his muzzle. The female's presentation frequently is followed by a mounting response on the part of the male.

In addition to examining the interaction between males and females, we investigated the response of males to the female's vaginal secretions. Vaginal discharge was collected by swabbing the inside of the vaginal opening with a ball of cotton approximately 1 in. in diameter. Control stimuli were provided by swabbing the vaginae of spayed females that had not been treated with hormone.

Immediately prior to testing, one experimental and one control ball were applied to the genitalia of the appropriate types of females and then tacked to small plywood plaques. The two plaques were fastened to the floor of the testing room at locations 8 ft apart. The testing room was 10 feet square, and the floor and lower walls were painted with nonadsorbing material so that they could be hosed down periodically.

Males were released in the testing room one at a time and their behavior was observed for a period of three minutes. When a dog held his nose within approximately 1 in. of a cotton ball, he was scored as "sniffing" the stimulus. When he brought his tongue, lips, and/or teeth in contact with the ball, he was scored as "chewing and licking" the ball. Test scores consisted of the total amount of time spent sniffing or chewing and licking the experimental and the control stimuli.

The results are summarized in Table 1 and in Figure 1. The number of positive social responses exhibited by females increased considerably after the administration of estradiol cypionate, but did not seem to be affected by the addition of progesterone. The estrogen effect began to decline within two weeks after the third injection of ECP. The tendency of females to present to males was increased somewhat by estrogen treatment, and the addition of progesterone
markedly facilitated this response. This effect was detectable within 4 hours following the injection of progesterone and reached a peak 48 hours later, after which time it declined steadily and was greatly decreased 96 hours after treatment. Some hormonal effects on presentation responses were still detectable two weeks after the last ECP injection.

The males' interest in females as measured by the frequency of mount-and-thrust responses increased steadily during the four days following the first injection and the two days following the third injection of ECP. During the next three days mount frequency declined, but four hours after the injection of progesterone, a dramatic rise in this activity occurred. Figure 1B shows that the increased mounting by males was not due to an abrupt change in the responsiveness of females. Feminine tendencies to reject the male's mounts declined rapidly under the influence of estrogen and reached their lowest point four days before progesterone was administered. The frequency of rejection remained low until 48 hours after progesterone treatment and then increased to a level approximately equal to that obtaining prior to the third estrogen injection.

The sudden increase in masculine activity following administration of progesterone to the females was due at least in part to a change in the vaginal secretions. This effect is revealed in the table and in Figure 1C. Prior to the administration of progesterone, cotton balls impregnated with the vaginal discharge from estrogen-treated females were investigated for longer periods of time than were balls that had been applied to the vaginas of anestrous females. However, when estrogen-primed bitches were injected with progesterone, the stimulus value of their vaginal secretions was greatly enhanced. Four hours after progesterone treatment, the total investigation time devoted to cotton balls bearing vaginal secretions from experimental females was increased above the highest level attained during the period of estrogen treatment. In contrast, the amount of time spent in the investigation of cotton balls carrying anestrous secretions was unchanged.

Prior to the administration of progesterone, males only sniffed the experimental

<table>
<thead>
<tr>
<th>Expl. stage</th>
<th>Behavior of Female</th>
<th>Behavior of Male</th>
<th>Response to Vaginal Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days after 2nd estrogen injection</td>
<td>Total PSR</td>
<td>Presentations</td>
<td>Ments</td>
</tr>
<tr>
<td>3.20</td>
<td>0</td>
<td>49.53</td>
<td>16.10</td>
</tr>
<tr>
<td>5 days after 3rd estrogen injection</td>
<td>103.50</td>
<td>19.64</td>
<td>6.64</td>
</tr>
<tr>
<td>6 days after injection of progesterone</td>
<td>101.73</td>
<td>47.50</td>
<td>27.70</td>
</tr>
<tr>
<td>7–10 days after injection of progesterone</td>
<td>57.90</td>
<td>14.13</td>
<td>46.53</td>
</tr>
</tbody>
</table>

* Mean values were calculated according to the formula: total response frequency / minutes of observation × 100.

† PSR = positive social responses.
and control stimulus balls. After progesterone had been injected, the cotton balls used to swab the vaginae of experimental females were chewed and licked by all males. The effects of progesterone on response to the cotton balls were markedly reduced by the sixth day after treatment, but some influence of hormones on sniffing behavior was still evident four days later.

When ovarian hormones first became commercially available, numerous investigators observed that sexual receptivity could be induced in ovariectomized rats, mice, and guinea pigs by injecting large amounts of estrogen; and for a number of years thereafter, this was the standard method of preparing females for mating tests with males. Somewhat later it was discovered that behavioral estrus could be induced more reliably by pretreatment with much smaller amounts of estrogen followed by a single injection of progesterone.5, 6

Results presented in this report indicate that a similar effect may be obtained in the dog. Past success in producing a receptive condition by administration of estrogen alone may well have been due to the massive doses employed. Furthermore, there is reason to suspect that even when females treated with large amounts of estrogen do become receptive, they are not as stimulating to the male nor as capable of a full estrous response as are females in natural estrus.

According to our findings, the effects of progesterone administered after several injections of estrogen consist primarily of increasing the female's excita-
tory value for the male. At least a part of this increase probably is due to a change in the character of the vaginal secretions, which serve as an important source of stimulation for the male.

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1 Schwarz, M., and F. A. Beach, Am. Psychol., 9, 467 (1954).

2 Estradiol cypionate was generously supplied by Dr. James H. Sokolowski of The Upjohn Co., Kalamazoo, Michigan.


5 Boling, J. L., and R. J. Blandau, Endocrinology, 25, 359 (1939).