ABSTRACT

One Catahoula Leopard Cowdog and three German Shepherd dogs, previously trained to detect odors characteristic of estrus in cows, were used in three experiments to determine when estrus-related odor first appears, how long it persists, and when it disappears in relation to time of estrus. Samples were swabs of reproductive tract fluids taken from vaginas of cycling cows on the day of estrus, at diestrus (8 to 10 days after estrus), and at daily intervals before and after estrus. Estrous odor emerges slowly during the 3 days before estrus, reaches a definite peak in intensity on the day of estrus, and disappears within 1 day thereafter.

INTRODUCTION

In a previous study, dogs were trained to detect a characteristic odor in the cow’s reproductive tract at estrus (1). The dogs reliably identified the odor on swabs taken from vaginas of estrous cows; they also identified estrous cows directly. For these findings to have any application in solving the difficult problem of detection of estrus in cattle, the time-course of occurrence of the odors in relation to estrus and ovulation must be established. Our experiments were to determine when the odor first appears during the proestrus period and how long it persists after the end of estrus.

MATERIALS AND METHODS

Test Samples

Vaginal fluids were obtained from dairy cows in the Beltsville herd as in (1). Briefly, samples were taken by swabbing deep into the vagina with sterile cotton balls on sterile forceps. The cotton remained in the tract for about 15 s, and the vestibule and clitoris were swabbed with the cotton as it was removed. Multiple samples were taken at approximately 24-h intervals from individual cows for several days before estrus (samples E-1, E-2, etc., to designate the number of days before estrus), on the day of estrus (E samples), and for several days after estrus (samples E+1, E+2, etc.). Diestrous (DE) samples were obtained from the same cows 8 to 10 days after estrus. Samples from individual cows were placed in sterile glass jars as collected and frozen at −15 to −20°C until used. The frozen samples were thawed to room temperature shortly before use. After thawing, the samples were transferred into different sterile jars with perforated metal caps to facilitate handling and to minimize odor cross-contamination during sessions of testing. The perforated caps were replaced with clean ones after each dog was worked.

Dogs

Six dogs were purchased for training to detect odors associated with estrus in cattle; none had any prior experience with estrus-related odors from cows or with any other olfactory discrimination tasks. Four of the six represented working stock dogs (3 Catahoula Leopard Cowdogs and 1 Catahoula Leopard-Border Collie cross). The other two were Italian...
Greyhounds small enough to be hand-held in working (adult weight about 5 kg). Olfactory discrimination work with a hand-held Italian Greyhound was successful (2). Unfortunately, however, the two Italian Greyhounds purchased for these studies could not be trained to discriminate reliably between E and DE samples. The same was true for the Catahoula Leopard-Border Collie cross. Distractability and erratic motivation, rather than inability to discriminate between the two types of samples, appeared to be the major problems for all three dogs.

Three of the four dogs were adult German Shepherds (1 male, 2 spayed females) that had been used in the previous estrus detection study (1). The fourth dog was one of the three Catahoula Leopard Cowdogs mentioned above, an adult female. Techniques of differential operant reward conditioning were used to train the dogs to discriminate between E and DE samples. During training, samples were presented to the dogs on a “discrimination board” device that was 10.8 cm high × 11.5 cm wide × 244 cm long. The surface of the board contained three equally spaced holes that held the samples 90 cm apart. A training trial consisted of a single confrontation of an individual dog with a decision-making situation involving simultaneous presentation of one E and one (sometimes 2) DE samples. The dogs were trained to respond by sitting down immediately adjacent to the E sample. The dogs were reinforced with praise and small pieces of food for correctly detecting and responding to the E sample. Incorrect choices and failures to respond (passes) were not reinforced. The positions of the E and DE samples were changed randomly between trials.

Procedures

Results from a preliminary study with the discrimination board and samples from various days before, during, and after estrus suggested that odors characteristic of estrus began to appear on days E-2 and E-1. These findings were not definitive, however, because the dogs were permitted to make repeat comparisons among samples (four were presented at a time) before responding on each trial. The opportunities to make comparative judgments may have resulted in the dogs responding to samples associated with the strongest estrus-related odors (presumably the E samples) and withholding responses to samples taken before or after estrus that may have contained estrus-related odors to a lesser degree. In view of this potential source of confounding, a procedure that eliminated opportunities for comparisons among samples was adopted for all subsequent experiments as described below.

Our investigation of the time-course of appearance and disappearance of estrus-related odors involved three experiments. Experiment 1 focused on the proestrus period. For this study, vaginal swabs were obtained on 5 days (E, E-1, E-2, E-3, DE) from each of 10 cycling cows. On days of testing, jars containing the thawed samples were placed into plastic beakers recessed in holes in the tops of small wooden boxes (each about 20.0 cm long × 11.5 cm wide × 10.0 cm high); a separate box was used for each sample. Ten boxes, each containing one sample, were arranged in a straight line at about 1.0-m intervals. The five samples from one cow were placed in the first five boxes and those from a second cow in the second five boxes. The sequence of samples was random for each cow. Two trials were conducted with each dog and each set of samples. A trial consisted of the investigation of each of the 10 samples in order by one of the dogs. Working on leash, the dog was required to proceed systematically from the first to the last box, examining each sample in turn. No opportunities to compare samples were allowed; instead, the dog had to examine the samples one at a time and respond or not respond to each individually. Reversals in direction of travel were not permitted. Responses to E samples were rewarded with food and praise; responses to E-1, E-2, and E-3 samples were recorded but not reinforced; and responses to DE samples were negatively reinforced with a mild verbal reprimand.

After two trials with samples from the first two cows, samples from two other cows were placed into the boxes. The process was repeated until the samples from all 10 cows had been examined. Hence, during the course of the 10 trials of each daily session, each dog had two exposures to sets of within-cow samples from 10 cows.

There were fifteen sessions of testing over 4 wk in Experiment 1. The first six sessions were indoors in a laboratory. Because the dogs responded to only 46% of the estrous samples,
the studies were moved outdoors in an attempt to improve the dogs’ performance. The first outdoor session involved samples carried over (refrigerated overnight) from the last indoor session. The dogs performed well in the first outdoor session with repeat samples, and the experiment was continued in the outdoor setting, each set of samples being used for two consecutive days. Repeat samples were refrigerated between sessions.

In Experiment 2, investigation of the proestrus period was extended to days E-4 and E-5, and in Experiment 3 the investigation was extended to a 3-day interval after estrus (E+1 to E+3). The studies were outdoors, and procedures were generally similar to those of Experiment 1 with specific exceptions noted under Results. Because of the large number of samples involved, we were not able to study the periods before and after estrus in one experiment.

**RESULTS**

**Experiment 1**

The results, averaged over dogs, are in Table 1. The dogs responded to the estrous samples better outdoors than indoors. The response to samples from days E-1, E-2, and E-3 was similar for indoor and outdoor sessions, about 20%. The dogs responded to the DE sample 4 to 8% of the time.

Of particular interest was the comparison of the results of the first and second weeks of testing. The average response to E-1, E-2, and E-3 samples declined from approximately 31% during the 1st wk to about 9% during the 2nd. Responses to DE samples also declined from 8% to 3%. Finally, response as a function of day of cycle did not differ for fresh and repeat samples.

**Experiment 2**

Because of no difference in rate of response to samples from days E-1 to E-3 in Experiment 1, samples from days E-4 and E-5 were tested in Experiment 2. Because of limited availability of samples, Experiment 2 involved comparisons between cows. Procedures were similar to those of Experiment 1 except that each trial involved two estrous samples ($E_1$ and $E_2$), one DE sample, and one sample from each of days E-5, E-4, E-3, E-2, and E-1. Each of the 8 samples came from a different cow. The 4 dogs participated in 10 trials per day (8 sample boxes per trial) for 5 days.

Table 2 summarizes the results of Experiment 2 in terms of mean percentage of responses (averaged over dogs and sessions) as a function of estrous cycle day. The table also shows the ranges in daily means over all dogs during the 5 days of the experiment. Response to DE, E-5, and E-4 samples were negligible, responses to E-3, E-2, and E-1 samples were slightly higher, and responses to E samples were high.

**Experiment 3**

In Experiment 3, the time-course of disap-

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**TABLE 1. Results of studies of within-cow vaginal swab samples taken on the day of estrus (E), on days 1 to 3 before estrus (E-1 to E-3), and at diestrus (DE).**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Number of sessions</th>
<th>Total trials</th>
<th>E-3</th>
<th>E-2</th>
<th>E-1</th>
<th>E</th>
<th>DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoors</td>
<td>6</td>
<td>240</td>
<td>15</td>
<td>15</td>
<td>22</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>Outdoors-overall</td>
<td>9</td>
<td>360</td>
<td>22</td>
<td>19</td>
<td>23</td>
<td>87</td>
<td>5</td>
</tr>
<tr>
<td>Outdoors-fresh samples</td>
<td>4</td>
<td>160</td>
<td>20</td>
<td>19</td>
<td>23</td>
<td>86</td>
<td>5</td>
</tr>
<tr>
<td>Outdoors-repeat samples</td>
<td>5</td>
<td>200</td>
<td>23</td>
<td>18</td>
<td>23</td>
<td>89</td>
<td>4</td>
</tr>
<tr>
<td>Outdoors-first week</td>
<td>5</td>
<td>200</td>
<td>33</td>
<td>26</td>
<td>35</td>
<td>86</td>
<td>8</td>
</tr>
<tr>
<td>Outdoors-second week</td>
<td>4</td>
<td>160</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>89</td>
<td>3</td>
</tr>
</tbody>
</table>

TABLE 2. Results of studies of between-cow vaginal swab samples taken on the day of estrus (E), on days 1 to 5 before estrus (E-1 to E-5), and at diestrus (DE).a

<table>
<thead>
<tr>
<th>Comparison</th>
<th>E1</th>
<th>E2</th>
<th>E1+E2b</th>
<th>E-1</th>
<th>E-2</th>
<th>E-3</th>
<th>E-4</th>
<th>E-5</th>
<th>DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>80</td>
<td>96</td>
<td>88</td>
<td>7</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Range</td>
<td>Highland</td>
<td>98</td>
<td>100</td>
<td>99</td>
<td>15</td>
<td>18</td>
<td>12</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Low</td>
<td>50</td>
<td>85</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

aData: Daily means over all dogs for five days of testing (tests were done on five days over a period of eight days). bCombined response to E1 and E2 samples, which were estrous samples from different cows.

The appearance of the estrous odor after estrus was addressed. The procedure was identical to that of Experiment 2 except that Experiment 3 involved samples within cows from the first 3 days after estrus (E+1, E+2, E+3); E and DE samples were included as before. The samples were obtained from the same 10 cows that were sampled for Experiment 1.

Table 3, which summarizes the results of Experiment 3, indicates that the estrous odor essentially had disappeared by the 1st day after estrus. Overall, the dogs responded 2.4% of the time to samples from day E+1, 1.8% to samples from day E+2, and 2.8% to samples from day E+3. These responses were not different from the 4% for DE samples but contrasted markedly with the 94% response to E samples.

DISCUSSION

Results of this study confirm and extend those of earlier studies that indicate a distinctive odor in reproductive tract fluids of estrous cows (1). The results of Experiment 1 suggest that odors characteristic of estrus may appear as many as 3 days before estrus, although reduced relative to that on the day of estrus. Overall, the rate of response was greater to E-1, E-2, and E-3 samples than to E-4, E-5, and DE samples, but markedly less than that to E samples. The negligible response to E-4 and E-5 samples in Experiment 2 indicates that estrus-related odors probably are not present to any significant degree on days E-4 and E-5.

The results of Experiment 3 were clear cut; the response dropped from an average of 94% for samples taken on day E to 2.4% for samples taken on day E+1. For samples from days E+1 to E+3 response averaged 2.3%, not different from the 4% for DE samples. The combined results of the present experiments thus suggest that odors characteristic of estrus in cows emerge slowly during the 3 days before estrus, reach a definite peak in intensity on the day of estrus, and disappear within 1 day thereafter.

The rapid emergence and disappearance of estrus-related odors before and after estrus suggested by the present data must be regarded with some reservation because the dogs may have progressively acquired the ability to differentiate between samples on the basis of subtle differences in odor intensity or quality. This possibility arises because the dogs were rewarded for responding to samples taken on day E but were not reinforced for responding to samples taken on days E-1 to E-5 or days E+1 to E+3. This procedure was necessary to prevent deterioration of the dogs' olfactory discrimination performance, but it may have contributed to the learning of subtle discrimina-

TABLE 3. Results of studies of within-cow vaginal swab samples taken on the day of estrus (E), on days 1 to 3 after estrus (E+1 to E+3), and at diestrus (DE).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>% Responses by estrous cycle day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
</tr>
<tr>
<td>Overall</td>
<td>94</td>
</tr>
<tr>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Highland</td>
<td>98</td>
</tr>
<tr>
<td>Low</td>
<td>89</td>
</tr>
</tbody>
</table>

aData: Daily means over all dogs for 10 days of testing (10 days over a period of 16 days).
tions in odor intensity or quality. The ability to make such discriminations could have affected the apparent pattern of emergence and disappearance of estrus-related odors before and after estrus. The systematic decrease in response to E-1, E-2, E-3 and DE samples with successive experiments is consistent with this possibility and may have reflected the gradual development of enhanced powers of discrimination over sessions of testing; that is, the dogs' responses to E-1, E-2, and E-3 samples declined from an overall mean of 31% during the 1st wk of Experiment 1 outdoors to 9% during the 2nd and then to 7% in Experiment 2. Similarly, responses to DE samples declined from 8% to 3% and ultimately to 1%. In contrast, there were no progressive trends in the high rates of responding to E samples either within or over successive experiments. The learning of subtle differences in odor intensity or quality would have represented an extremely challenging task in these experiments because the dogs never were allowed to compare samples before responding.

Dogs can be trained to discriminate samples of bovine reproductive tract fluids taken on the day of estrus from those obtained 1 day before or 1 day after estrus. Further studies are planned to investigate the source, quantity, and chemical composition of the odorous substance.

ACKNOWLEDGMENTS

The authors are indebted to James Polonis and Donald Alexander of the Southwest Research Institute and Deborah Wetzel of the Reproduction Laboratory, Animal Science Institute, Beltsville Agricultural Research Center, for their invaluable technical assistance. Thanks also are due to Douglas Bolt and Harold Hawk of the Reproduction Laboratory for helpful discussions and suggestions.

REFERENCES