

# Abstract

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## 1. Introduction

Historically, the use of stallion stimuli in lieu of a live stallion for estrus detection in mares has received limited research attention, and has yielded conflicting conclusions. Veeckman and Odberg (1978), reported greater than 95% agreement with live stallion results for both estrus and diestrus determinations using (a) a two-minute playback of stallion vocalizations (recorded while a stallion was interacting with mares for estrus detection), (b) tactile stimulation (manual rubbing of the flank and external genitalia), or (c) both vocalizations and tactile stimulation in combination. In contrast, Asa (1986) described poor effectiveness of recorded stallion vocalization for estrus detection in mares in a research program, indicating that with repeated exposure to recorded vocalizations, mares' interest and response diminished. McCall (1991) evaluated the on-farm practicality of using stallion vocalization playback. Using a three-minute sequence of teasing vocalization samples recorded from multiple stallions presented to mares restrained in teasing stocks, McCall reported unsatisfactory results.

It is common to observe situations in which mares display signs of estrus or diestrus in response to the sounds of stallions that are out of sight or the scents of stallions when the stallion itself is not present (McDonnell, unpublished observations). The purpose of the present study was to re-visit the effectiveness of using auditory and olfactory stallion stimuli in the absence of live stallions for detection of estrus, with goals that included understanding the discrepancies in published results and refining a method for on-farm use. In our experience, when a mare hears sounds of a stallion as if he were in close interactive contact, yet she can see that there is no stallion nearby, the mare typically appears distracted or anxious as if confused by the contextual incongruity. Close confinement seems to increase the distraction and anxiety. This apparent "confusion while confined" appears to increase non-receptive behavior especially, which appears to delay or supplant display of estrus. Therefore, one of our aims was to devise a more contextually congruent presentation of the stallion sexual vocalizations that could be presented to the mare in a comfortable environment. Our approach was to select vocalizations characteristic of a stallion while approaching from a distance that could logically be "out of sight" for a mare at rest in a familiar box stall. An additional aim was to record sounds from a stallion that was especially vocal when teasing, and whose vocalizations had been believed by experienced breeding farm staff to be particularly effective at eliciting a sexual response from mares. Pickerel et al. (1993), in a two-choice stallion paradigm, found a positive correlation between the number of stallion vocalizations per minute and mare preference for that stallion. Therefore, an additional goal was to include a large number of vocalizations per minute on the soundtrack. A final aim was to explore the feasibility and potential benefit of adding olfactory stimulation, which is known anecdotally to elicit estrous responses in some mares and is known to affect sexual response in other species (Izard, 1983). To the best of our knowledge, the use of stallion scent in lieu of a live stallion as an aid in estrus detection has not been formally investigated.

#### 2. Materials and methods

Eleven horse mares from resident teaching and embryo transfer recipient herds at a veterinary school reproduc-

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tion clinic were exposed to auditory with and without olfactory stimuli obtained from a stallion on a Monday-Wednesday-Friday schedule on 12 occasions (trials) over a four-week period, with the aim of following each mare through at least one ovarian cycle. Auditory stimuli alone or with added olfactory stimuli obtained from a stallion were presented on alternating occasions resulting in six trials with each condition. The auditory stimulus was a threeminute playback of a hi-fidelity recording of vocalizations recorded as a stallion approached a mare from a distance. The recording was made from the mares' perspective, such that playback simulated a live stallion approaching from out of view of the mare at rest in a familiar stall. The olfactory stimulus was a preputial smegma sample from the same stallion, placed on a clean disposable gauze sponge, and diffused into the mare's stall using a household electric fan mounted on the stall front. Each three-minute estrus detection trial was videotaped for subsequent review to record signs and intensities of estrous, diestrous, and ambivalent behaviors. Frequency as well as latency to the first occurrence was recorded for each of six specific receptive and six non-receptive responses. Confirmation of ovarian status for comparison of results of this auditory and olfactory stimuli method with live-stallion estrus detection methods was based on clinical records of daily teasing with a live stallion along a teasing rail and chute system following a standard protocol, transrectal palpation and ultrasonography. RIA serum progesterone levels were obtained as needed to confirm ovarian status.

#### 3. Results

During the four-week study period, seven of the 11 mares exhibited one estrous period/ovulation and the remaining four exhibited two estrous periods/ovulations, for a total of 15 estrous periods/ovulations. For 12 of these 15 ovulations, estrus was detected with the Monday-Wednesday-Friday olfactory and auditory estrus detection protocol, within zero to 10 days before ovulation (mean 4.4 days, SD 2.7). For each of the four mares that exhibited two estrous periods/ovulations, the response to the olfactory and auditory protocol was similar for the two estrous periods/ovulations. For the total of 130 trials (nine mares each on 12 occasions and two mares each on 11 occasions) estrus was observed in 28. Of these 28, 13 pairs of trials of the same mare with and without olfactory stimuli (within 48 h during the same estrous period) were compared and the mare's estrous signs ranked as more or less demonstrative based on quantitative data. For 12 of the 13 pairs, estrus was more demonstrative when the olfactory stimulus was added. For the one remaining pair, estrus was equally demonstrative with and without the olfactory stimulation. We identified 36 instances in which information was available from estrus detection with the live stallion within 24 h of an auditory and olfactory protocol trial, and for which a determination of ovarian status at the time of each estrus detection was certain. For 27 of these 36 paired estrus detections, the live stallion and auditory and olfactory trial results were in agreement as to the overall determination of weak or strong estrus or diestrus, or

ambivalent behavior. For the remaining nine cases, the two methods were in disagreement. For eight of the nine cases of disagreement, the overall call made for the auditory and olfactory method was more consistent with ovarian status. In only the one remaining case, the overall determination based on exposure to the live stallion was more consistent with ovarian status (Fisher's exact test, P < 0.05). The overall mean number of responses per auditory and olfactory trial for the eleven mares remained similar across the 12 trial days, suggesting no habituation to that method. We conclude that auditory stimuli in the form of recorded stallion vocalizations presented to mares in a contextually congruent manner in this study was at least as effective as, and in some instances more accurate than, the standard daily teasing with a live stallion as done at this teaching facility.

#### 4. Discussion

These results indicate reasonable efficiency in detecting estrus in mares using stallion vocalization playback with and without added stallion scent as presented in this study on a minimal schedule of three times weekly. For 80% of the ovulations that occurred, estrus was detected with the stallion auditory and olfactory stimuli method, on average more than four days before ovulation. This interval should be sufficient for most farms to organize veterinary care and/or breeding arrangements as needed. By design we employed a conservative approach in which the observer remained blind to the results of earlier estrus detection sessions, evaluating each new session independently. After completion of the study, it was clearly evident that comparison of day-to-day changes for an individual mare, as would be recommended for routine on-farm practice, in many instances revealed trends and changes in behavior that would have been useful for predicting pre-ovulatory status. In practice, results would be expected to improve if information about each mare's reproductive status garnered from transrectal palpation and ultrasonography, reproductive history and day-to-day trends in behavior were considered collectively.

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## Conflict of interest

None.

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