

EVALUATION OF ANDROGENIZED MARES  
AS AN ESTRUS DETECTION AID

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ABSTRACT

Ten pony mares that had developed stallion-like sexual behavior as the result of anabolic steroid treatment (boldcnone undccylenate, 0.55 mg/kg intramuscularly (i.m.), once weekly for 12 injections) were evaluated for ability to aid in detecting estrus in cycling mares. In across-the-fence estrus detection trials, androgenized mares failed to elicit signs of estrus or diestrus. In 10-min pasture trials, in which each androgenized mare was placed in a group of 10 cycling mares (six of which were estrous), seven of the 10 androgenized mares elicited estrous behavior from one or two mares. Observations of the 10 androgenized mares among a pasture group of 21 cycling mares indicated that approximately one third of the mares in estrus could be identified on the basis of their response to androgenized mares.

**Key words:** mare, estrus detection, heterotypical behavior, anabolic steroid, stallion behavior

INTRODUCTION

Unlike the cow, ewe, or sow, the mare does not normally exhibit any male-type sexual behavior responses or sequences. However, under various abnormal steroid influences—including ovarian tumor<sup>1,2</sup> and administration of estrogen,<sup>3,4</sup> testosterone,<sup>5,6</sup> equine pituitary extract,<sup>7</sup> or, some anabolic steroid preparations<sup>8-10</sup>—mares display male-type sexual behavior. The striking completeness of stallion-like behavior of androgenized mares has led to suggestions for their use as stimulus animals to aid in detecting estrus in mares. Under many equine breeding management systems, androgenized mares would offer important advantages over stallions as an aid in detecting estrus. With no possibilities of accidental breeding, and infection or injury that may result from intromission, the androgenized mare could be allowed to mount mares. In addition, the androgenized mare might offer less threat of injury to mares and handlers than the stallion, thus facilitating pasture estrus detection.

Recently, we conducted a study<sup>a</sup> to evaluate the effects of two anabolic steroids, boldcnone undecylenate<sup>b</sup> and stanozolol,<sup>c</sup> on reproductive function in pony mares. All of the 10 mares treated with boldcnone undecylenate (0.55 mg/kg i.m., once weekly for 12 injections) in that study developed stallion-like behavior,

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<sup>b</sup>Equipoise, Squibb, Princeton, NJ.

<sup>c</sup>Winstrol-V, Winthrop Veterinary, New York, NY.

including herding, elimination/markings, olfactory investigation, flehmen, mounting, and thrusting responses.<sup>11</sup> These stallion-like responses were displayed in appropriate precopulatory and copulatory sequences. Within the pasture band, the androgenized mares seemed to assume the sociosexual role of a stallion, apparently being perceived as males by each other and the nonandrogenized herdmates. With some variation among individuals and over time in the intensity and frequency of stallion-like behavior, all boldenone-treated mares displayed a full complement of heterotypical responses and sequences. The stallion-like behavior emerged within 2 wk after treatments started, continued throughout the 12 wk of treatment, and remained until the study was terminated 10 wk after the treatments ended. The experiments and field observations reported in this paper were conducted within the context of this anabolic steroid study. They were designed to evaluate the ability of these androgenized intact pony mares to detect estrus in the nonandrogenized cycling mares in the same study.

## MATERIALS AND METHODS

### Subjects

The anabolic steroid study involved 33 mixed-breed pony mares, ages 3 to 16 yr, acquired at local auction. Each mare was assigned to one of three treatment groups: boldenone undecylenate (0.55 mg/kg i.m. once weekly for 12 injections), stanozolol (0.55 mg/kg i.m. once weekly for 12 injections) and saline control (equivalent volume and schedule). Initially, each treatment group comprised 11 mares; however, one boldenone-treated mare was lost to the study. The mares were maintained as a herd on 10 acres of pasture with shelter from December 1984 through June 1985. Supplemental hay, grain, and salt-mineral blocks were provided. For that project, mares were gathered into a holding pen and individually handled from three to seven times weekly for rectal palpation, blood sampling, and estrus detection with a pony stallion. In addition, the anabolic steroid project required occasional separation (10 to 30 min, approximately once weekly) of subgroups for observation and tests of sociosexual and dominance behavior. During Wk 12 of treatment, the following three experiments were conducted.

### Experiment 1

The purpose of Experiment 1 was to evaluate the potential of androgenized mares to aid in across-the-fence detection of estrus<sup>12</sup> by comparing estrus detection findings using an androgenized mare to those using a pony stallion. One androgenized mare that had been showing intense stallion-like behavior during the three observation periods in the week preceding this experiment was selected. The remaining 31 mares were each subjected to two estrus detection trials, one with the androgenized mare and one with a pony stallion. Each estrus detection trial consisted of placing a cycling mare in a small pen (4 m x 4 m, formed of 5-rail tubular steel farm gates) adjacent to a similar pen holding either the androgenized mare or the pony stallion. After 1 min, two experienced observers recorded independent determinations of "in estrus" or "not in estrus" based on responses of the two animals, as recommended for routine estrus detection on breeding farms.<sup>12</sup> Comments on the interaction of the two animals were also recorded. In addition, all tests were video recorded for later review. To control for possible effects of the order or time of day, the mares to be tested were separated into two groups of approximately equal size and placed in holding pens with hay and water provided. During the morning session, mares from one group were tested with the stallion;

then the remainder of the mares were tested with the androgenized mare. During the afternoon session, these mares were tested with the stallion; then the first group was tested with the androgenized mare. Within a group, mares were tested in the order in which they could be caught from the holding pen.

### Experiment 2

The purpose of Experiment 2 was to evaluate the ability of each of the 10 androgenized mares to aid in detecting estrus in an across-the-fence estrus detection arrangement. The 10 androgenized mares and two control mares, one known to be estrous and another known to be diestrous based on daily exposure to a pony stallion, were removed from the herd and placed in separate holding pens. Estrus detection trials were conducted as described in Experiment 1. Each androgenized mare served as a stallion-like stimulus for sequential 1-min estrus detection trials for each stimulus mare. The order of presentation of the estrous and diestrous stimulus mares to the androgenized mares was alternated. The androgenized mares were selected in the order in which they could be caught from the holding pen.

### Experiment 3

The purpose of Experiment 3 was to evaluate the ability of androgenized mares to aid in detecting estrus when placed among mares in a paddock. Eleven cycling mares from the project (control group) were separated from the pasture herd and placed in a small paddock (8 m x 30 m). The 10 androgenized mares were also separated from the herd and placed in a holding pen. In the order in which they could be caught from the holding pen, each androgenized mare was placed for a period of 10 min in the pen with the cycling mares. Based on the behavioral interactions of each animal, two experienced observers independently made determinations of "in estrus," "not in estrus," or "unable to determine" for each cycling mare. Trials were video recorded. Immediately following Experiment 3, the cycling mares were individually checked for estrus with a pony stallion to determine "true" condition for comparison with results of Experiment 3.

### Field Observations

To evaluate field sexual behavior of these androgenized mares, a series of four weekly 30-min focal-animal observation sessions of the pasture herd were conducted during the second through fifth week after treatment had stopped. The frequency and temporal relationship of all male-type and female-type sexual responses and the identity of the individuals involved were recorded.

## RESULTS

### Experiment 1

In the across-the-fence estrus detection trials, 13 mares exhibited estrus in response to the stallion. In response to the androgenized mare, only one mare exhibited estrus. Review of observers' comments and the video record indicated that the remaining 30 mares had no interaction with the androgenized mare that indicated estrus or diestrus. Among the 18 mares that were determined to be non-estrous with the stallion, eight mares exhibited diestrous behavior, and only seven mares failed to interact.

## Experiment 2

Eight of the 10 androgenized mares failed to elicit any behavioral reaction from either mare. Of the remaining two androgenized mares, one elicited estrous behavior from the estrous mare but had no interaction with the diestrous mare, while the other elicited diestrous behavior from the diestrous mare and had no interaction with the estrous mare.

## Experiment 3

Estrus detection with the pony stallion indicated that six of the 11 cycling mares were in estrus on the day of Experiment 3. In the simulated pasture estrus detection arrangement, four of the 10 androgenized mares elicited estrous behavior from one mare, and three other androgenized mares elicited estrous behavior from two mares during the 10-min trial. All other cycling mares were recorded as "unable to determine," in all cases because of no interaction with the androgenized mare. Five of the androgenized mares mounted cycling mares, with a range of two to five mounts in 10 min.

## Field Observations

On each observation day, approximately 12 of the 21 nonandrogenized mares in the pasture herd were in estrus (based on individual estrus detection with a stallion). A mean of 36% of the estrous mares were identified on the basis of behavioral interaction with any of the 10 androgenized mares. In addition, on two of the four observation days, two and three mares that had not shown estrous behavior when exposed to the stallion exhibited clear estrous response to an androgenized mare. A mean of 8.75 androgenized mares showed male-type behavior and a mean of two androgenized mares mounted during the 30-min observations.

## DISCUSSION

Results of the across-the-fence estrus detection trials of Experiments 1 and 2 indicated that an androgenized mare would not be a useful substitute for a stallion. Most interactions were characterized as indifferent. Therefore, these androgenized mares failed to elicit estrous as well as nonestrous responses. A clear indication of diestrus is often useful information when following the reproductive cycle of mares.

Results of paddock estrus detection trials of Experiment 3, together with subsequent field observations, suggested to us that androgenized mares may be most useful for pasture estrus detection. Although all mares in estrus were not identified during the 10-min trials, results seem comparable to what has been found under similar situations with stallions. During trials in which each of three pony stallions was introduced into a group of 10 mares,<sup>d</sup> about one third of the mares in estrus exhibited estrous behavior and approximately one fourth were mounted during the first 10 min. In another study of pasture breeding,<sup>13</sup> pony stallions introduced into groups of 20 mares for 3-h sessions every 2 d bred an average of 3.7 times per session. Comparison of the behavior of stallions in these two studies with behavior of these androgenized mares suggests that mounting may be more frequent among androgenized mares than stallions. In a report of the effects of testosterone propionate treatment on sexual behavior of four intact mares,<sup>5</sup> it was noted that

<sup>d</sup> McDonnell, unpublished observations.

treated mares, when placed in a small pen with an estrous mare, mounted more frequently than did a stallion under the same conditions. This difference may be related to a mare's inability to ejaculate and resultant absence of a postejaculatory refractory period.

The androgenized mare may be most useful in pasture estrus detection if a system for marking, such as is used with cattle and sheep, were to be developed. For example, marking devices attached to the androgenized mare's forelegs above the knees could be employed to label mares that are mounted. Conceivably, such a system might distinguish mares that stand for mounting from those that move away.

Several important questions concerning the use of androgenized mares as an aid in estrus detection remain to be answered. First, our experimental population of mares does not represent what would be a practical grouping of mares. Under more practical conditions, of perhaps one or two androgenized mares among 10 to 20 mares in a pasture, very different behavior might occur, with more or less satisfactory results. In field observations, we saw considerable chasing and herding of mares. Such behavior would be undesirable in most brood mare bands. Again, this behavior may have been the result of the competitive presence of so many male-type animals and may not have occurred if fewer androgenized mares were present. If a single androgenized mare also exhibited such undesirable aggressive and herding behavior, periodic introduction of an androgenized mare into a pasture might be a useful alternative to continuous exposure.

Pasture estrus detection with a mare would offer some farms many advantages over existing methods. In addition to those advantages mentioned in the introduction, we found that under field conditions identifying mares in estrus was facilitated by a fuller range of responses indicating estrus. Mares were able to exhibit approach and following behavior as described by 'Asa.<sup>4</sup> As a result, mares did not have to interact directly with the male-type stimulus animal to signal estrus. The androgenized mares and mares in estrus tended to form a sexually active group that was easily recognized. Sexual interaction of a pair appeared to elicit estrous behavior in other mares.

In summary, the androgenized mare did not appear to be an acceptable substitute for a stallion in across-the-fence individual estrus detection. The pasture performance of androgenized mares under these experimental conditions suggest further study to determine performance under various farm conditions and usefulness of a marking system.

#### REFERENCES

1. Fretz, P.B. Behavioral virilization in a brood mare. *Appl. Anim. Ethol.* 3:277-280 (1977).
2. Stabenfeldt, G.H., Hughes, J.P., Kennedy, P., Mcagher, D.M. and Neely, D.P. Clinical findings, pathological changes and endocrinological secretory patterns in mares with ovarian tumours. *J. Reprod. Fertil. Suppl.* 27:277-285 (1979).
3. Azzie, M.A.J. Some clinical observations of the effect of an implant of oestradiol benzoate in brood mares. *J. Reprod. Fertil. Suppl.* 23:303-306 (1975).
4. Nishikawa, Y. *Studies on Reproduction in Horses*. Shiba Tamuracho Minatoku, Tokyo 1959, pp. 93-100.

5. Withrow, J. M., Sargent, G.F., Scheffrahn, N.S. and Kesler, D.J. Induction of male sex behavior in pony mares with testosterone propionate. *Theriogenology* 20:485-490 (1983).
6. Cougouille-Gauffreteau, B. Effects of androgen treatment on social and sexual behavior in mares. *Appl. Anim. Behav. Sci.* 13:175 abstr. (1984).
7. Woods, G.L. and Ginther, O.J. Induction of multiple ovulations during the ovulatory season in mares. *Theriogenology* 20: 347-355 (1983).
8. Maher, J.M., Squires, E.L., Voss, J.L. and Shidcler, R.K. Effect of anabolic steroids on reproductive function of young mares. *J. Am. Vet. Med. Assoc.* 183:5 19-524 (1983).
9. Squires, E.L., Voss, J.L., Maher, J.M. and Shidcler, R.K. Fertility of young mares after long-term anabolic steroid treatment. *J. Am. Vet. Med. Assoc.* 186:583-587 (1985).
10. Turner, J.E. and Irvine, C.H.G. Effect of prolonged administration of anabolic and androgenic steroids on reproductive function in the mare. *J. Reprod. Fertil. Suppl.* 32:213-218 (1982).
11. Waring, G.H. **Horse Behavior**. Park Ridge, New Jersey: Noyes Publications, 1983, pp. 107-119.
12. Ginther, O.J. **Reproductive Biology of the Mare: Basic and Applied Aspects**. Equiservices, Cross Plains, WI. 1979, pp. 75-82.
13. Ginther, O.J., Scraba, S.T. and Nuti, L.C. Pregnancy rates and sexual behavior under pasture breeding conditions in mares. *Theriogenology* 20: 333-345 (1983).
14. Asa, C.S., Goldfoot, D.A. and Ginther, O.J. Socio-sexual behavior and the ovulatory cycle of ponies (*Equus caballus*) observed in harem groups. *Horm. Behav.* 13: 49-65 (1979).