EFFECT OF AN ORAL PROGESTIN ON THE ESTROUS CYCLE AND FERTILITY OF MARES

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Summary

On February 4, 14 of 28 seasonally anestrous mares were fed .044 mg per kilogram of body weight of an oral progestin, allyl trenbolone (17α-allyl-estratriene 4-9-11, 17β-ol-3-one) for 12 days. There was no difference (P>.05) between groups for the number of mares exhibiting estrus after treatment or follicular activity during or after treatment. After March 6, 18 mares that had not yet ovulated were used in a second trial. On day 3 of estrus, nine of 18 mares were assigned to be fed allyl trenbolone for 12 days. Estrus ceased in all mares within 3 days of treatment. Duration of post-treatment estrus, interval from end of treatment to ovulation and from estrus to ovulation were shorter (P<.01) for treated mares. Influence of stage of cycle at the onset of treatment was evaluated in a third trial. Mares (N = 25) were assigned to one of five groups: 1) controls artificially inseminated every other day (E/O) during estrus; 2) fed allyl trenbolone for 12 days beginning day 3 of estrus and bred E/O; and groups 3 to 5) fed allyl trenbolone for 12 days beginning day 3 of estrus or days 5 or 10 of diestrus. Mares in groups 3, 4 and 5 were given HCG (3,300 IU) on day 17 and inseminated on days 17 and 19. The interval from treatment to estrus was shorter (P<.05) for mares treated during diestrus vs those treated during estrus. Pregnancy rates were not different (P>.10) among groups after two cycles or between treated and controls after 1 cycle (60 and 60%) or 2 cycles (80 and 60%). In another study, 34 mares were exposed to 16 hr photoperiod. On January 25, 17 of the 34 mares were fed allyl trenbolone for 12 days. One-half of the mares in each group received 3,300 IU of HCG on day 2 of estrus. More (P<.05) treated mares exhibited estrus and ovulated within 12 days after treatment than controls. Pregnancy rates after 1 and 3 cycles were similar (P>.05). Progestin treatment effectively regulated estrual behavior early in the year, and synchronized estrus in cycling mares and mares previously exposed to artificial light. (Key Words: Estrus Control, Fertility, Mares, Oral Progestin.)

Introduction

Progesterone and progestins have been used for estrous control in cattle (Wiltbank and Kassen, 1968; Wiltbank et al., 1971; Woody and Abenes, 1975; Roche and Gosling, 1977), sheep (Hulet and Foote, 1967; Christenson, 1976) and swine (Polge, 1972; Webel, 1977). Daily intramuscular injection of progesterone for estrus and ovulation control of mares has produced variable results (Loy and Swan, 1966; Van Niekerk et al., 1973; Holtan et al., 1977). Holtan et al. (1977) reported favorable estrus synchronization and mare fertility following a progesterone-PGF2α-HCG treatment regimen. However, daily injections or progestin implants are impractical means of administering progestins to horses.

Synthetic oral progestins, used effectively for estrus control in several species, have proved much less effective in mares (Loy and Swan, 1966; Bowen, 1968; Hoppe et al., 1974). Webel (1975) reported on the use of an oral progestin, allyl trenbolone (17α-allyl-estratriene 4-9-11, 17β-ol-3-one) that was effective in controlling estrus in mares and was not detrimental to fertility. Using this same oral progestin, in combination with an artificial photoperi-
od, Palmer (1979) reported synchronization of ovulation in mares.

The purpose of these studies was to further evaluate the effect of allyl trenbolone on: a) induction of estrus in anestrous mares, b) control of the estrous period early in the breeding season, c) estrous synchronization and fertility and d) the effectiveness of this compound for estrous control when used in combination with artificial light.

Materials and Methods

General

Mares used in these experiments were of light-horse type, mixed-breeds, nonlactating, 3 to 20 years old and weighing 375 to 525 kilograms. Throughout the studies, mares were teased daily with one or more stallions to determine estrual behavior (Back et al., 1974). Follicular development and ovulation were determined by palpation of the mare's ovaries per rectum every third or fourth day unless a follicle > 30 mm was present, in which case palpations were performed daily. In those trials in which fertility was assessed, semen was collected and mares artificially inseminated according to procedures described by Pickett and Back (1973). Pregnancy diagnoses were by palpation per rectum at days 35 and 50 and a positive immunological pregnancy test. Analysis of variance was used to test treatment effects on pregnancy rates. Means were compared by Tukey's HSD test (Steel and Torrie, 1960).

Experiment 1a. Between January 10 and February 3, 1977, mares were teased daily to detect estrus. Mares exhibiting estrus > 4 days and having one or more follicles > 20 mm or ovulating were classified as cycling mares and were not used in this trial. On February 4, 28 anestrous mares were randomly assigned to an allyl trenbolone-treated or control group.

Experiment 1b. After March 6, 18 mares that had not exhibited an estrous period with ovulation were used in a second trial. On day 3 of estrus, mares were assigned to be fed grain containing allyl trenbolone or untreated grain. Duration of estrus for control mares was calculated from the first day of estrus to the last day of estrus following ovulation, regardless of whether or not estrus behavior was continuous during this period.

Experiment 1c. Once mares had exhibited their second post-treatment estrus after Experiment 1b and had experienced 8 days of diestrus, they became available for use in a third trial. However, no mares were assigned to this experiment prior to May 15 regardless of their status. As mares became available they were assigned to one of the following groups (N = 5/group): 1) controls — bred every other day during estrus beginning on day 2 or 3; 2) fed allyl trenbolone for 12 days beginning on day 3 of estrus and bred every other day during the post-treatment estrus; and groups 3 to 5) fed allyl trenbolone for 12 days beginning on either day 3 of estrus (group 3), day 5 of diestrus (group 4) or day 10 of diestrus (group 5). Mares in groups 3, 4 and 5 were given HCG (3,300 IU) intramuscularly on day 17 and were bred once on days 17 and 19 (day 0 = first day of allyl trenbolone treatment). All mares were inseminated with 500 × 10⁶ motile spermatozoa from one stallion for two consecutive estrous cycles or until pregnant, whichever came first.

Experiment 2. Beginning December 1, 1977,
34 mares were exposed to 16 hr of photoperiod per day until May 1 or 50 days of pregnancy. Mares were maintained in a band outside during the day and in individual box stalls at night. Artificial light was provided by a 200-watt incandescent bulb 4 m from the floor of each stall. On January 25, ovaries of each mare were palpated and follicular activity categorized as active (one or more follicles > 20 mm) or inactive (no follicles > 20 mm). Within ovarian groups, mares with active or inactive ovaries were assigned to either an allyl trenbolone treated or control group. Treatment began on January 26 or 27 and continued for 12 days. Within treatment groups, one-half of the mares received 3,300 IU of HCG on day 2 of estrus, and the other half received saline. All mares were inseminated with 200 x 10^6 motile spermatozoa from one stallion until pregnant or through three post-treatment estrous cycles. Mares were inseminated every other day during estrus beginning on day 2 or 3.

Results and Discussion

Experiment 1a. To determine the effect of an oral progestin on induction of estrus in anestrous mares, 14 of 28 mares received .044 mg of allyl trenbolone per kilogram body weight daily for 12 days. Two mares were in estrus when allyl trenbolone treatment was initiated. Signs of estrus of these two mares ceased within 2 days after initiation of treatment. There was no difference (P>.10) in follicular activity between treated and control mares during treatment or within 12 days after cessation of treatment. During the treatment period, five of 14 treated mares and three of 14 control mares had ovaries with one or more follicles > 20 millimeters. In addition, the number of mares displaying estrus within 12 days after treatment was similar (P>.10) for treated and control mares (10 and 8, respectively). None of the mares ovulated during the post-treatment estrus. The ovarian activity and sexual behavior for mares in this trial were similar to that reported previously for anestrous mares (Van Niekerk, 1967a,b; Ginther, 1974). Thus, progestin treatment alone, administered early in the breeding season, was not effective in stimulating follicular activity or inducing estrus accompanied by ovulation.

Experiment 1b. Nine of 18 mares were treated with allyl trenbolone in an attempt to regulate estrus early in the breeding season. Estrus behavior ceased within 3 days after initiation of treatment in nine of nine mares and suppression of estrus continued for the duration of treatment. However, the allyl trenbolone treatment did not inhibit ovulation since three treated mares ovulated during the 12-day treatment period compared to four controls. The reproductive behavior of the mares after the end of treatment is presented in table 1. The mean interval from allyl trenbolone treatment to estrus was 4.5 days. The duration of the post-treatment estrus, interval from the end of the treatment to ovulation and interval from the first day of estrus to ovulation were shorter (P<.01) for treated than control mares (table 1). All of the post-treatment estrous periods for the treated mares were associated with ovulation and were continuous as opposed to the split estrous periods (estrus interrupted by < 5 days diestrus) exhibited by six of the nine control mares. The occurrence of split estrous periods and anovulatory estrous periods has been reported to be fairly common early in the breeding season (Ginther, 1974). This type of sexual behavior was prevented by a short-term progestin treatment. Such a progestin treatment regimen would be a useful management tool, since the labor and number of inseminations required per pregnancy early in the year would be reduced. However, it should be noted that the treatment regimen did not hasten onset of the breeding season since the interval from January 1 to the first ovulation was similar for treated and control mares. The interval from January 1 to the first ovulatory estrus was negatively correlated with the duration of that estrus (r = -.90, r = -.91, and r = -.91).
treated and controls, respectively). Thus, the earlier a mare exhibited her initial estrus after January 1, the longer the duration of sexual receptivity. The size of the largest follicle on day 1 of the first ovulatory estrus was negatively correlated with the duration of that estrus ($r = -0.82$ and $r = -0.95$ for treated and control mares, respectively). These findings are in agreement with those of Ginther (1974).

The response to allyl trenbolone treatment was influenced by season. Mares treated with allyl trenbolone in March had a mean post-treatment duration of estrus of 17.5 days vs 56.4 days for controls; whereas, mares treated with progestin during April had a mean post-treatment duration of estrus of 7.0 days vs 14.2 days for controls. Further study is needed to determine the criteria best suited for selection of mares to be treated with progestins early in the breeding season. For example, follicular activity at the time of initiation of progestin treatment may be more important than time of year.

Experiment 1c. To minimize the necessity of estrus detection, the effectiveness of allyl trenbolone treatment for estrous synchronization was evaluated when treatment was initiated on either day 3 of estrus or days 5 or 10 of diestrus. Estrus behavior was suppressed within 2 days after initiation of treatment in the 10 mares in which treatment was begun during estrus. Of these 10 mares, seven ovulated during treatment. Ovulation occurred an average of 2.7 days after treatment was initiated. The reproductive behavior of these 25 mares is presented in table 2. The interval from treatment to estrus was shorter ($P < 0.05$) for mares treated during diestrus (2.8 and 3.0 days) vs those treated during estrus (4.8 days). In addition, the interval from treatment to ovulation was shorter ($P < 0.05$) for mares treated during diestrus (6.6 and 6.2 days) than for those treated during estrus and bred every other day (9.8 days). However, the difference is confounded because treated mares bred on appointment were given HCG, whereas treated mares bred every other day received no HCG. It was hypothesized that the longer interval to estrus after treatment, for mares treated during estrus, may have been due to an increased concentration of endogenous progesterone at the end of treatment. Although ovulation was detected in only seven of 10 mares treated during estrus, follicular luteinization may have occurred in the other three mares. Assuming a corpus luteum lifespan of 17 days, mares in this group may have had functional luteal tissue at the end of treatment. It is suspected that a longer duration of treatment such as 15 days would allow adequate time for the corpus luteum to regress. Webel (1975) reported an interval to estrus of 3.5 days for mares given the same progestin used in this study; however, the duration of allyl trenbolone treatment was 18 days.

The duration of the post-treatment estrus and interval from the first day of estrus to ovulation was similar ($P > 0.05$) among the five groups of mares. An injection of 3,300 IU of HCG was ineffective in shortening the duration of post-treatment estrus for mares treated with allyl trenbolone during diestrus because six of 15 mares had ovulated prior to HCG treatment.

During cycle 1, there was a difference ($P < 0.10$) in pregnancy rates among the five groups of mares (table 3). Pregnancy rates were lower ($P < 0.05$) for mares treated initially on day 10 of diestrus and bred on appointment than for those treated initially on day 3 of estrus and bred on appointment. The low overall pregnancy rates during cycle 1 for mares treated during diestrus (30%) was due to the fact that 60% of these mares were not inseminated until after ovulation. There were no differences ($P > 0.10$) in pregnancy rates among the groups after being inseminated for two cycles (60, 80, 100, 80 and 60%, respectively). In addition, there were no differences ($P > 0.10$) in pregnancy rates between treated and control mares after one cycle (60 and 60%) or after two cycles (80 and 60%). Pregnancy rates for mares bred every other day and mares bred on appointment were similar ($P > 0.05$) after one cycle (70 and 53%) and after two cycles (70 and 80%). However, additional experiments utilizing larger numbers of mares are needed before recommendations can be made regarding the optimal insemination frequency after progestin treatment. In another experiment conducted in our laboratory, Voss et al. (1979) demonstrated that after PGF$_{2\alpha}$ synchronization, pregnancy rates were lower ($P < 0.10$) for mares inseminated only once vs those inseminated daily, but not for mares inseminated every other day. In addition, daily insemination appeared to be of some advantage since 64.7% of the mares became pregnant during cycle 1 compared to 38.9% for those inseminated every other day. Pregnancy rate per cycle, number of cycles per pregnancy and
TABLE 2. EFFECT OF DAY OF INITIATION OF PROGESTIN TREATMENT ON ESTRUS (EXPERIMENT 1c)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Bred E/O day&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bred on appointment&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Initiation of progestin treatment (day)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrus</td>
<td>Diestrus</td>
</tr>
<tr>
<td>Interval to estrus (days)</td>
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<td></td>
<td>4.8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Interval to ovulation (days)</td>
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<td></td>
<td>9.8&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Estrus duration (days)</td>
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<td>6.6</td>
</tr>
<tr>
<td>Day of ovulation&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>5.2</td>
<td>9.0</td>
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</tbody>
</table>

<sup>a</sup>Inseminated every other day during estrus beginning on day 2 or 3.  
<sup>b</sup>Given HCG on day 17 and inseminated once on days 17 and 19 (day 0 = first day of progestin treatment).  
<sup>c</sup>Not calculated since mares were started on treatment as they became available and not all at the same time.  
<sup>d</sup>Interval from first day of estrus to ovulation.  
<sup>e</sup><sup>f</sup>Within rows, means with different superscripts are different (P<.05).

On the first day of allyl trenbolone treatment (January 26 or 27) 10 of 17 mares in the treated group were in estrus. Estrous behavior ceased in all mares by the third day of treatment and continued to be inhibited for the duration of the treatment period. The mean interval from the end of allyl trenbolone treatment to estrus was 3.4 days. This observation is similar to the results of Experiment 1 and previous studies (Weibel, 1975). The number of treated mares exhibiting their first day of estrus within 12 days after treatment (17 of 17) was greater (P<.05) than for control mares (seven of 17). The duration of the post-treatment estrus for number of inseminations per cycle were similar (P>.05) among the five groups of mares.

**Experiment 2.** It was concluded from Experiment 1a that treatment of anestrous mares with allyl trenbolone alone was ineffective in the induction of an ovulatory estrus. Thus, in 1977, 34 mares were exposed to a 16 hr photoperiod beginning December 1. Subsequently one-half of the mares were treated with allyl trenbolone for 12 days. All mares were exposed to artificial light since it has been amply demonstrated (Koosistra and Ginther, 1975) that a 16 hr photoperiod was effective in induction of estrus in anestrous mares.

TABLE 3. EFFECT OF PROGESTIN TREATMENT ON PREGNANCY RATES IN MARES (EXPERIMENT 1c)

<p>| Cycle | Bred E/O day&lt;sup&gt;a&lt;/sup&gt; | Bred on appointment&lt;sup&gt;b&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th></th>
<th>No. of mares</th>
<th>Preg. (%)</th>
<th>No. of mares</th>
<th>Preg. (%)</th>
<th>No. of mares</th>
<th>Preg. (%)</th>
<th>No. of mares</th>
<th>Preg. (%)</th>
<th>No. of mares</th>
<th>Preg. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>60&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5</td>
<td>80&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>40&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>5</td>
<td>20&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
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<td>...</td>
<td>3</td>
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<td>5</td>
<td>100</td>
<td>5</td>
<td>80</td>
<td>5</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>a</sup>Inseminated every other day during estrus beginning on day 2 or 3.  
<sup>b</sup>Given HCG on day 17 and inseminated once on days 17 and 19.  
<sup>c</sup><sup>d</sup>Within rows, means with different superscripts are different (P<.10).
15 of 17 mares treated with allyl trenbolone was 6.8 days. However, two of the treated mares exhibited extended post-treatment estrous periods (28 and 42 days, respectively). Three of the control mares exhibited their first day of estrus on January 6 and continued to exhibit estrus for 54, 73 and 74 days, respectively. In order to make a conservative estimate of the post-treatment duration of estrus for control mares, the last day of the treatment period was used as day 1 of estrus for these three mares. Based on these calculations the mean post-treatment estrus duration for the 17 control mares was 11.8 days.

Administration of HCG on day 2 of estrus shortened (P<.05) the duration of estrus (5.2 vs 9.7 days). The interval from the last day of allyl trenbolone treatment to ovulation was shorter (P<.05) for treated (8.1 days) than control (15.1 days) mares. In addition, more (P<.01) treated mares ovulated within 12 days after treatment than controls (13 vs 8). It was concluded that treatment with allyl trenbolone in combination with an artificial photoperiod was effective in synchronizing estrus and ovulation and in establishing a "normal" estrus early in the year.

The effect of treatment with allyl trenbolone on pregnancy rate is presented in table 4. Pregnancy rates after cycle 1 and after three cycles were similar (P>.05) for treated and control mares. Since the control mares had to have exhibited at least 5 days of diestrus (prior to February 1) before they became eligible for insemination, mares exhibiting extended estrous periods were not bred during that estrus. Thus, the number of inseminations per cycle during cycle 1, 2, 3 and overall was similar (P>.05) between groups of mares. Mean number of inseminations per cycle for treated and control mares was: cycle 1, 2.8, 3.2; cycle 2, 3.4, 4.8; cycle 3, 3.9, 3.5, respectively. Other measures of reproductive performance, such as total number of cycles mares were bred and number of cycles mares were bred per pregnancy, were similar (P>.05) for both groups. However, the mean date of conception for treated mares (February 23) was earlier (P<.05) than for controls (March 5). The administration of HCG during cycle 1 did not affect (P>.05) pregnancy rates during cycles 1, 2, 3 or overall. It has been shown that when HCG was given during cycle 1, pregnancy rates of treated mares during cycles 2 or 3 were increased (Voss et al., 1974). In experiments conducted by Voss et al. (1974), inseminations began on the first cycle after May 1, consequently, season may have accounted for the difference between these studies.

From these studies we concluded that a short-term treatment of allyl trenbolone was effective in: 1) controlling the long, erratic estrous periods frequently encountered at the onset of the breeding season, and 2) synchronization of estrus in normally cycling mares or in mares previously exposed to an artificial photoperiod. In addition, the allyl trenbolone had no detrimental effect on fertility. Thus, this progestin treatment regimen can be useful to effectively manage the estrous cycle of the mare.

**Table 4. Pregnancy Rate (%) After Progestin Treatment (Experiment 2)**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58.8</td>
<td>64.7</td>
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<tr>
<td>2</td>
<td>42.8</td>
<td>50.0</td>
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<tr>
<td>3</td>
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<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>76.5</td>
<td>88.2</td>
</tr>
</tbody>
</table>

aN = 17.

**Literature Cited**


Koosistra, L. H. and O. J. Ginther. 1975. Effect of