SAFETY OF ALTRENOGEST IN PREGNANT MARES
AND ON HEALTH AND DEVELOPMENT OF OFFSPRING

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SUMMARY

Fifty-one light-horse mares were utilized to evaluate the safety of an oral progestin, altrenogest, administered throughout gestation on: gestation length, embryonic and fetal loss, periparturient events, health and development of offspring, and future reproductive capabilities of the mares. Pregnancies were established by inseminating mares with $250 \times 10^6$ progressively motile spermatozoa from the same stallion every other day throughout estrus or by non-surgical transfer of embryos. Mares were randomly assigned to 1 of 2 treatments upon confirmation of pregnancy on day 20: 1) controls, 2 ml of neobee oil orally per 44.5 kg of body weight; and 2) treated, 2 ml of altrenogest dissolved in neobee oil at a concentration of 2.2 mg/ml orally per 44.5 kg of body weight. Treatments were administered daily from day 20 to 320 of gestation.

There were no significant differences between treatment groups for duration of gestation, placental weight, time to placental expulsion and incidence of retained placental membranes. Number of female foals born from altrenogest treated mares (14 of 23) was greater ($P < .05$) than the number from untreated control mares (4 of 16). Female foals born from altrenogest treated mares had larger clitoris ($P < .05$) than those from control mares. Times to sternal recumbency, standing and nursing were similar for the 2 groups ($P > .05$). Body weight and height at withers, heart girth circumference and length and width of cannon were measured at time of birth and at 2, 4, 6, 8, 12 and 16 weeks of age. Measurements did not differ ($P > .05$) between treated and control foals for any development parameters.

Beginning on day 20 postpartum, mares were teased daily. During estrus, mares were inseminated every other day with $250 \times 10^6$ motile spermatozoa. Teasing and/or insemination was continued for 2 cycles or until mares were 35 days pregnant. The number of mares pregnant after 1 cycle and after 2 cycles of insemination was similar ($P > .05$) for treated and control mares. Nineteen of 21 treated mares and 15 of 16 control mares were pregnant after 2 cycles of insemination. Number of cycles per pregnancy was similar ($P > .05$) for treated and control mares (1.37 vs 1.13) as was number of days mares exhibited estrus (6.30 vs 6.13). Number of inseminations per cycle did not differ ($P > .05$) between treated and control mares (2.92 vs 3.00). In summary, there was no effect of treatment with altrenogest from day 20 to 320 of gestation on periparturient events, viability and growth of offspring and subsequent reproductive performance of mares.

INTRODUCTION

Pregnancy maintenance in mares is largely dependent on adequate production and secretion of progesterone. Initially, the primary corpus luteum and subsequently secondary corpora lutea are solely responsible for production of progesterone. Based on measurements of progesterone in the uterine vein and successful maintenance of pregnancy after ovariectomy, it would appear that the placenta becomes a source of progesterone at approximately 60 to 80 days of gestation. Inadequacies in production or secretion of progesterone due to failure of luteal function has been suggested as a cause of early embryonic death (EED) and termination of pregnancy.

Pregnant mares are frequently given injections of progesterone in an attempt to prevent abortion. Mares ovariectomized on day 25 of gestation maintained preg-
nancies when injected intramuscularly with 100 mg of progestosterone in oil daily. Pregnancy was terminated 2-6 days after treatment ended. Both injectable progestosterone and a synthetic progestogen, altrenogest, effectively maintained pregnancy in mares ovariectomized on day 34 or 35 of gestation. Intramuscular injections of 1000 mg of progestosterone in propylene glycol every 4 days were required to maintain endogenous levels sufficient to maintain pregnancy. Unfortunately this is an impractical and inconvenient way of administering progestosterone to mares, primarily due to local inflammatory reactions at the injection site. Oral administration of a progestin would seem to be more desirable.

Altrenogest, an orally active progestin, has been used effectively for suppression of estrous behavior and pregnancy maintenance. Use of ovariectomized progestosterone treated mares as recipients for embryo transfer has recently been reported. Thirty-five day pregnancy rates for ovariectomized progestin-treated recipients (28 of 40, 70%) were similar to those of synchronized intact mares (16 of 20, 80%).

Despite the apparent widespread use of altrenogest for pregnancy maintenance, adequate studies have not been performed to determine safety with long term supplementation. This experiment was designed to determine effect of altrenogest therapy throughout pregnancy on gestation length, pregnancy loss, periparturient events, further reproductive capabilities of mares, and overall health and development of their offspring.

**MATERIALS AND METHODS**

**General**

Fifty-one light-horse mares were utilized. Mares selected were 3 to 15 years of age, and weighed between 386 and 500 kg. Pregnancies were established by artificially inseminating mares with 250 x 10^6 progressively motile spermatozoa every other day throughout estrus or by non-surgical transfer of embryos (N=24) or by ovulation ultrasonography and randomly assigned to 1 of 2 groups: 1) controls, 2 ml of neobee oil (carrier for altrenogest) orally per 44.5 kg body weight; 2) treated, 2 ml of altrenogest dissolved in neobee oil at a concentration of 2.2 mg/ml orally per 44.5 kg body weight: this was the recommended dose for estrous suppression. Treatments were administered daily from day 20 to 320 of gestation. Originally, 27 treated and 24 control mares were assigned to this study using a table of random numbers. However, the number of animals available for evaluation changed over the course of the study. Early embryonic death in 5 control and 2 altrenogest treated mares occurred between 30 and 60 days. Two control mares were excluded from the trial, due to maintenance of twin pregnancies and 1 control mare died of an undetermined cause. Abortions occurred in 2 altrenogest treated mares on days 165 and 250 of gestation. One abortion was apparently due to a traumatic kick, while the cause of the second was unknown. After these losses, total number of mares per treatment group was 23 treated and 16 control.

All mares were maintained in a single pasture and fed 12 kg of alfalfa and 2 kg of grain mixture daily. Water and trace mineralized salt were available ad libitum. Mares were weighed monthly and dosages were adjusted. Ultrasound was used to assess viability of the conceptus on day 25, 30, 35, 40, 50, 60, 80, 100 and 120 of gestation. Parturition was performed monthly thereafter to assess pregnancy status.

**Periparturient events**

Beginning approximately 1 month prior to scheduled foaling, each mare was observed for signs of impending parturition. Those mares estimated to be within one week of foaling were placed in 4.9 x 3.7 m foaling stall and closely observed. After parturition, mares and foals were examined and initial assessments made regarding general health. Assessment of foal health was determined by: 1) hair coat - normal or short; 2) attitude - aggressive, slow, dull/sleepy or comatose; 3) maturity - mature (gestation length ≥ 320 days and normal foal), premature (gestational length <320 days) or dismature (gestational length ≥320 days and abnormal foal). Data collected included sex of foal, time from birth to: 1) sternal recumbency, 2) standing, 3) nursing, and 4) placental expulsion. Weight of placenta and incidence of membrane retention were also recorded. Placental membranes were classified as retained if not expelled within 3 hours after foaling. Body weight and height at withers, heart girth circumference and length and width of cannon bone were also measured at time of birth and at 2, 4, 6, 8, 12 and 16 weeks of age. Progestin-treated and control mares were compared to determine if treatment had an effect on duration of pregnancy. Clitoris of fillies were measured at birth and subsequent measurements were taken at 1, 4, 6, 10, 12, 15, 18 and 21 months of age. The width and length (mm) of each clitoris was measured twice by 2 technicians. The average clitoral area (mm^2) was used for statistical analysis. Comparisons were made between recipient mares and natural mothers to determine effects of embryo transfer on gestational length, sex ratio and placental weight. Time from birth to: 1) sternal recumbency, 2) standing, and 3) nursing; and birth weight and height, heart girth circumference and length and width of cannon bone were also compared between foals from embryo recipients and natural dams.

An outbreak of foal septicemia occurred at the Equine Reproduction Laboratory in some foals in both groups and 6 foals died as a result of identified microorganisms and were not available for progressive measurements. In addition, 1 control mare delivered a stillborn foal. Foal losses...
were similar (P>.05) between treated (N=4) and control groups (N=3). Number of live foals per group after these losses was 19 treated foals and 13 control foals. Because of the time involved with foal observation and neonatal care, some data points were not collected.

Reproductive Soundness

Mares and foals were maintained in 3 pastures and grouped according to age of foal. Beginning 20 days postpartum, mares were teased daily until they had been inseminated 2 cycles or were 35 days pregnant. Mares were artificially inseminated with 250 x 10⁶ progressively motile spermatozoa from 1 stallion beginning on day 2 or 3 of estrus and continuing every other day until cessation of estrus. Data collected were: 1) number of cycles per pregnancy; 2) number of inseminations per cycle; 3) duration of estrus for cycle 1 and 2; 4) pregnancy rate per cycle; and 5) overall pregnancy rate for treated and control mares.

Statistical Analysis

Chi-square analyses were used to compare: 1) number of foals from treated and control mares; 2) number of male and female foals from treated and control mares and embryo transfer dams versus natural mothers; and 3) number of treated and control mares pregnant per cycle and after 2 cycles. Quantitative data were analyzed by two-sample t-tests to compare differences between treatment groups.

RESULTS AND DISCUSSION

Duration of gestation was similar (P>.05) between treated and control mares (324.7 vs 326.9 days). Treatment of mares with altrenogest during gestation had no effect on placental weight, expulsion of placenta or incidence of retained placental membranes (Table 1).

The number of female foals born from mares treated with altrenogest (14 of 23) was greater (P<.05) than the number from untreated control mares (4 out of 16). The reason for the apparent alteration of sex ratio was most likely due to small numbers and chance alone, since treatment started after pregnancy had been established. Females from treated animals had larger clitori at each measurement period indicating that altrenogest had some virilizing effect

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groupa</th>
<th>Sternal recumbency (min)</th>
<th>Birth to stand (min)</th>
<th>Birth to nurse (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altrenerg (N=3)</td>
<td>6.4±3.5</td>
<td>44.1±2.8</td>
<td>77.1±4.9</td>
<td></td>
</tr>
<tr>
<td>Control (N=9)</td>
<td>5.0±1.1</td>
<td>38.3±2.8</td>
<td>75.6±4.3</td>
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</tr>
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</table>
| *Number of observations per mean ranged from 11 to 12 for the treated group and N=9 for controls.*

There was no effect of type of dam on birth weight or height, heart girth and cannon length, however, cannon width was smaller (P<.05) in embryo transfer foals versus natural mothers. The significance of this finding was unknown. Presented in table 5 are measurements taken on foals in treated and control groups at birth and at weeks 2, 4, 6, 8, 12, and 16.

Weight changes over the time period were similar (P>.05) between treated and control animals. Similarly, changes in height at withers and heart girth measurements were not different (P>.05) between treated and controls at each of the measurement periods. In addition, width and length of cannon increased similarly (P>.05) in both groups.
throughout the measurement period. Thus it appeared, based on data collected at time of birth, and measurements collected over a 16-week period, that there was no effect of treatment of pregnant mares with altrenogest on parturient events or viability and growth of offspring.

As a result of mares entering seasonal anestrus, 2 treated mares were not inseminated and were omitted from the analyses. The number of mares pregnant after 1 cycle and after 2 cycles of insemination was similar (P>.05) for mares in the 2 groups. Nineteen of 21 treated mares and 15 of 16 control mares were confirmed pregnant on day 15 and after 2 cycles of insemination was similar (P>.05) for mares in treated (1.37) and control (1.13) groups as was duration of estrus (6.30 vs 6.13 days). Number of inseminations per cycle was not different (P>.05) between treated and control mares (2.92 vs 3.00). Based on these data, reproductive efficiency was not affected in mares previously treated with altrenogest. Fifty-five to 65% pregnancy rate on first cycle is considered normal reproductive efficiency. In this study, the pregnancy rate on first cycle was 76.2% for treated mares and 93.8% for control mares.

In summary, there was no effect of treatment with altrenogest from day 20 to 320 of gestation on parturient events, viability and growth of offspring and subsequent reproductive performance of mares.

### Table 4. Effect of type of dam on birth weight and height, heart girth and length of cannon (mean±S.E.)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Birth weight (kg)</th>
<th>Birth height (cm)</th>
<th>Heart girth (cm)</th>
<th>Cannon length (cm)</th>
<th>Cannon width (cm)</th>
</tr>
</thead>
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<tr>
<td>Type of dam</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural*</td>
<td>38.2±1.5</td>
<td>92.8±1.4</td>
<td>75.2±1.1</td>
<td>19.9±3.0</td>
<td>2.9±0.04</td>
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<tr>
<td>Embryo transfer*</td>
<td>38.2±1.4</td>
<td>92.1±1.2</td>
<td>74.7±9.2</td>
<td>19.9±3.0</td>
<td>2.7±0.03</td>
</tr>
</tbody>
</table>

*Number of observations ranged from 23 to 24. 
*Number of observations was N=13.

### Table 5. Effect of administering altrenogest during pregnancy on weight, height, heart girth, cannon length and width of foals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Heart girth (cm)</th>
<th>Cannon length (cm)</th>
<th>Cannon width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (wk)</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>38.4±3.8</td>
<td>91.4±8.3</td>
<td>94.3±5.6</td>
<td>76.0±11.3</td>
<td>20.2±3.0</td>
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<tr>
<td>2</td>
<td>60.6±6.2</td>
<td>98.9±9.9</td>
<td>99.7±9.6</td>
<td>88.1±11.3</td>
<td>21.3±3.0</td>
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<tr>
<td>4</td>
<td>77.3±7.7</td>
<td>104.6±10.5</td>
<td>105.7±10.0</td>
<td>95.6±11.3</td>
<td>22.3±3.0</td>
</tr>
<tr>
<td>6</td>
<td>88.5±9.0</td>
<td>108.1±10.9</td>
<td>109.9±10.0</td>
<td>100.0±11.3</td>
<td>22.8±3.0</td>
</tr>
<tr>
<td>8</td>
<td>98.7±9.9</td>
<td>111.3±11.3</td>
<td>112.7±10.4</td>
<td>104.3±10.5</td>
<td>23.1±3.1</td>
</tr>
<tr>
<td>12</td>
<td>124.1±12.8</td>
<td>114.6±10.8</td>
<td>116.0±11.9</td>
<td>111.9±11.3</td>
<td>23.4±3.4</td>
</tr>
<tr>
<td>16</td>
<td>147.9±15.2</td>
<td>118.4±11.8</td>
<td>118.1±11.8</td>
<td>118.6±11.8</td>
<td>23.6±3.8</td>
</tr>
</tbody>
</table>

*Altrenogest treated - N=15 to 19. 
*Control mares - N=12.

### REFERENCES