

EFFECT OF ALTRENOGEST ON REPRODUCTIVE PERFORMANCE
AND LACTATION IN THE MARE

by

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CHAPTER I

INTRODUCTION

Foal heat usually occurs 6 to 13 d after foaling (Jainudeen and Hafez, 1980). Mares bred at foal heat tend to have lower conception rates and a higher incidence of abortion (Ginther, 1979). This may be due to the short interval between foaling and breeding at foal heat. The uterus needs time to repair itself adequately and return to its former condition by a process called uterine involution before another pregnancy can be established. Although uterine involution is rapid, repair may not be complete by the onset of foal heat. This might explain why mares bred at foal heat tend to have decreased fertility.

It would be of great benefit to the horse producer if the first estrous cycle coincided with completion of uterine involution. Control of the estrous cycle in most species is dependent upon the ability to alter the lifespan of the corpus luteum. Unfortunately, due to the long follicular phase in the mare, estrous control is more difficult. Therefore, it would be helpful to predict the time of ovulation with some degree of accuracy. This could reduce teasing and palpation, improve stallion utilization and possibly achieve higher pregnancy rates.

Altrenogest, an oral progestin, was shown to control successfully the estrous cycle and ovulation in the non-lactating mare (Squires et al., 1979; Allen et al., 1980; Turner et al., 1981; Squires et al., 1983). Few studies, however, have reported the effects of altrenogest on the reproductive performance of the postpartum mare. When compared to breeding during foal heat, altrenogest might be used to extend the postpartum interval to first estrus, thus allowing for more complete uterine involution and higher conception rates.

Early equine research has supplied a basic understanding of many physiological functions in horses. However, since milk from mares is not used commonly for human consumption in this country, limited research on equine milk production or composition has been reported. The majority of research concerning milk production in the mare has been performed by European researchers. Their interest in milk volume was related to the production of kumiss, an ancient fermented alcoholic drink derived from milk of horses.

Milk from the mare is the only source of nutrition for young growing foals. Horse producers have observed that some mares seem to be better suppliers of good quality milk than others. However, when selecting mares for breeding, this trait is considered rarely. A foal may

be of the very best bloodlines, but if it does not receive the necessary nourishment during early life, the full potential of the foal may never be realized.

Production and composition of milk continually change during lactation. There is a time period during lactation when the maximal nutritional requirements of the foal are no longer met by milk. Horse producers would benefit by knowing when peak lactation occurs to facilitate weaning, ensure maximal growth of the foal and help keep mares in good condition for rebreeding.

The objectives of this study were: (1) to examine the use of altrenogest in regulating the estrous cycle, follicular growth, concentrations of estradiol in serum, ovulation, uterine involution and conception rates in the postpartum mare, (2) to determine the effect of altrenogest on early milk production and composition in the postpartum mare.

CHAPTER II

LITERATURE REVIEW

Uterine Involution. The normal histology of the endometrium of the mare has been described by Andrews and McKenzie (1941), Brandt and Manning (1969), Ricketts (1975), and Kenney (1978). The endometrium of the postpartum mare is composed of three layers: endometrium, myometrium, and perimetrium. The endometrium is made up of surface epithelium and lamina propria. The luminal epithelium consists of cuboidal to tall columnar cells that vary in height during the estrous cycle. Surface epithelial cells usually are tallest during the latter part of estrus and the first 5 to 8 d post-estrus. The lamina propria is composed of two stromal cell layers: the stratum compactum and the stratum spongiosum. Uterine glands are derived from and connected to the surface epithelium via ducts that penetrate the stratum compactum and branch out into the stratum spongiosum.

Andrews and McKenzie (1941) reported that uterine epithelium of postpartum mares rarely had returned to normal within 10 d after foaling. Mares in which the first estrus occurred 20 to 29 d postpartum showed apparent endometrial regeneration by 13 to 25 d postpartum. Cell height of surface epithelium averaged

24.9 um on d 2 postpartum and 20.7 um on the first day of estrus.

Loy et al. (1975) observed considerable repair of the endometrium by d 5 postpartum, with minor additional repair between d 5 and 15. Tolksdorff et al. (1976) reported endometrial repair complete at about 6 wk postpartum, when evaluated by biochemical criteria.

Effect of Progestogens on the Estrous Cycle.

Progestogens have been used for estrous control in cattle (Roche and Gosling, 1977), sheep (Christenson, 1976) and swine (Webel, 1977 ; Kraeling et al., 1981). In the horse, daily injections of progesterone were shown to suppress estrus (Loy and Swan, 1966; Loy et al., 1975; Holtan et al., 1977; Loy et al., 1981).

Loy and Swan (1966) conducted a study to determine the effect of intramuscular injections of progesterone and 6a-methyl-17a-acetoxyprogesterone (MAP) in an oil solution on postpartum estrus and ovulation in the mare. Doses of 400 mg every other day or 100 mg/d inhibited estrus and ovulation, but not follicular development during treatment. Ovulation occurred 3 to 6 d following withdrawal of exogenous progesterone. Days to ovulation appeared to be dose-dependent.

Loy et al. (1975) showed that intramuscular injections of 100 mg/d given on d 1 through 10 and 100 mg or 200 mg/d given on d 5 through 14 blocked or delayed ovulation in the postpartum mare.

Effect of Oral Progestogens on the Estrous Cycle.

Although daily injections of progesterone have been shown to be effective in controlling the estrous cycle of the mare, they are undesirable under field conditions. Loy and Swan (1966) evaluated the use of orally active progestogens to control the equine estrous cycle. Mares were fed twice daily with synthetic progestogen, MAP and melengestrol acetate (MGA) in a soybean-meal supplement. Both MAP and MGA proved ineffective, however, in regulating the estrous cycle in the mare. Although daily injected levels of progesterone did inhibit cyclic activity in the mare that were comparable to effective doses in cattle, MAP had no effect on the estrous cycle when fed at levels approximately 10 times that of cattle (Zimbelman, 1963a). Similarly, MGA, reported to be 150 times more potent than MAP in ewes (Zimbelman, 1963b), also failed to inhibit estrus and ovulation. Webel (1975), however, reported successfully feeding horses and ponies a synthetic progestin 17-alpha-allyl-estratriene-4-9-11,17 beta-ol-3-one to control the time of estrus. Since then, others have reported the successful use of altrenogest for

estrous control in the mare (Palmer, 1979; Squires et al., 1979; Allen et al., 1980; Squires et al., 1983).

Effect of Altrenogest on the Estrous Cycle.

Altrenogest has been shown to be ineffective in inducing follicular activity, estrus and ovulation in the anestrus mare (Allen et al., 1980; Turner et al., 1981; Squires et al., 1983). However, it was effective in normalizing the estrous cycle and ovulation during the transitional period (Squires et al., 1979; Squires et al., 1983).

Squires et al. (1979) found no difference in follicular activity between mares fed .044 mg·kg body weight⁻¹·d⁻¹ of altrenogest for 12 d or within 12 d after treatment withdrawal. The mean interval from treatment withdrawal to estrus and ovulation was 4.8 and 9.8 d, respectively. Turner et al. (1981) reported follicles 20 to 25 mm ovulated 12.5 d following 15 d of altrenogest treatment. Follicles less than 20 mm were not altered in number or diameter by the progestin. Follicular activity for treated mares, however, was suppressed for follicles 20 mm or larger at the initiation of treatment despite elevation in FSH concentrations. Loy et al. (1975) injected progesterone (100 mg/d) from d 5 to 14 postpartum and found urinary estrogen concentrations highest between d 9 and 15 postpartum, only about one-half the estrogen

concentration in the control mares during postpartum estrus and ovulation. Squires et al. (1979) reported that mares exposed to 16 h light for 60 d, followed by a 12-d treatment with altrenogest, conceived sooner than control mares which received light only. Altrenogest, however, had no effect on pregnancy rates. Squires et al. (1983) reported that the mean interval from treatment withdrawal to estrus and ovulation was 8 and 25.1 d, respectively. Conception rates reportedly were higher in treated mares than controls.

Milk Production. Suckling intervals greatly influence milk yields in the mare. Foals of light horse breeds nurse from 60 to 70 times in a 24 h period (Neseni et al., 1958). Neseni et al. (1958) showed that the maximum, average and percent body weight for daily milk yields in light horses were 21.5 kg, 14.3 kg and 2.3%, respectively. Milk production was determined by separating the foal from the mare for 100 min, then weighing the foal before allowing the foal to nurse. After 20 min the foal was reweighed to determine the amount of milk consumed during that period.

Lihacev (1959) found that mares produced up to 23.2 kg/d. Cerepanova (1961) conducted studies on five heavy (455 to 605 kg) crossbred mares and determined milk yield in a manner similar to the method described by Neseni et

al. (1958). Foals were allowed to nurse every 2 h and were weighed before and after nursing. Mean daily yields of 15 to 18.6 kg were reported. Masloboev (1962) reported yields of 4.9 to 33.2 kg milk produced daily by Russian-bred mares 5 to 14 yr of age. He also observed that the faster milk was removed from the udder the greater the volume obtained and that milk yields were significantly reduced at feeding times. Another source of variation was noted by Meadows (1978) when mares were kept in stalls and milked for 3 d period. This lack of exercise and/or increase in stress due to confinement during the weigh-suckle-weigh method may have adversely affected milk yields.

Ashcraft and Tyznik (1976) obtained milk yield data every 2 h over a 48 h period and reported daily yields of 1.5 to 9.2 liters for Quarter Horse mares. Gibbs et al. (1982) measured milk production in 14 Quarter Horse mares during 150 d of lactation and reported that average daily milk yield ranged from 11.8 kg in early lactation to 9.8 kg in late lactation. Milk yield as percentage of body weight was 2.2, 2.3 and 2.2 on d 10, 30 and 45, respectively. Gibbs et al. (1982) also found average daytime milk yields of 5.4 kg were not significantly different from nighttime averages of 5.5 kg during a 12-h period.

Sigler et al. (1983) suggested that altrenogest may increase milk production of early lactating mares. Daily percentage milk yields averaged 2.88% of the body weight of the mares for the altrenogest-treated mares and 2.50% for control mares.

Oftedal et al. (1983) determined milk intake in five foals from deuterium oxide (D_2O) turnover to be 16, 15, 18 kg/d at 11, 25, and 39 days postpartum. Milk production was 3.1, 2.9 and 3.4% of the body weight of the mares at 11, 25 and 39 d postpartum. Milk samples were obtained by hand milking after oxytocin administration and while the foal nursed.

Neseni et al. (1958) found peak lactation in the mare to occur between 2 and 3 mo. Gibbs et al. (1982) observed the largest mean daily milk yield at d 30 postpartum during 150 d of lactation. Sigler et al. (1983) reported that milk yields tended to peak between d 8 and 15 postpartum.

Milk Composition. In addition to the quantity of milk produced, research has addressed the composition of milk. Early, notable research conducted on the composition of milk in mares was performed by Veith (1885). Veith collected 15 draught-type mares, 5 to 6 yr of age, at approximately 150 d of lactation. He hand milked mares at 1-h intervals and found little variation in milk

composition of the 15 mares. Veith (1885) found milk contained 1.09% fat, 1.89% protein, and 9.94% total solids. Turner (1952) reviewed research conducted from 1852 to 1947 and found average values to be around 1% for fat, 2% for protein, and 10% for total solids.

In a review article, Linton (1931) summarized milk composition studies and reported fat content ranged from 0.12 to 2.50%, with an average of 1.29% ; protein content ranged from 1.33 to 3.34%, with an average of 2.18% ; and total solids ranged from 7.47 to 11.20%, with an average of 9.78% in milk from several breeds. Mares ranged from 5 to 24 yr of age, and the stage of lactation varied from 4 to 270 d postpartum. Considerable variation in the percentages of the milk constituents were reported contrary to the findings of Veith's (1885).

Using seven Quarter Horse and three Arabian mares, Ullrey et al. (1966) hand-milked one teat while the foal was allowed to nurse the other. Milk samples were collected 15 to 30 min after parturition, then at 12 and 24 h, 2, 5 and 8 d, 3 and 5 wk, and at 2, 3 and 4 mo postpartum. Average values for fat, protein and total solids ranged from 1.3 to 2.4%, 1.8 to 2.7% and 10.0 to 11.5%, respectively, over a 4-mo lactation.

Gibbs et al. (1982) sampled 14 Quarter Horse mares at 10, 30, 45, 60, 90, 120 and 150 d postpartum and reported average percentages of fat, protein and total solids to vary from 1.0 to 1.6%, 1.8 to 2.7%, and 10.1 to 11.0%, respectively.

Sigler et al. (1983) fed 14 Quarter Horse mares altrenogest at $.04 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ for 15 d beginning on the first day postpartum. Percentage milk fat and protein did not differ between treated and control groups. Mean fat content was .84, .99, .81, and .67%, whereas protein averaged 2.98, 2.74, 2.37, and 2.21% on d 5, 9, 16 and 23 postpartum, respectively.

Oftedal et al. (1983) collected weekly milk samples averaging 500 ml from five lactating mares from 10 to 54 d postpartum. Average percentages of fat did not differ and ranged from 1.19 to 1.78%. Protein values averaged 2.64, 2.34, 2.08, 1.92, 1.96 and 1.83% on d 10 to 11, 17, 24 to 25, 31 to 33, 38 to 40 and 45-47 postpartum, respectively. Total solids averaged 11.6, 11.3, 10.6, 10.6, 10.6 and 10.4% on d 10 to 11, 17, 24 to 25, 31 to 33, 38 to 40 and 45 to 47 postpartum, respectively.

Schryver et al. (1986) reported total solid percentages for five Thoroughbred mares at 1, 4, 8 and 16 wk of lactation to average 12.0, 10.5, 10.0 and 10.2%, respectively.

Linton (1937) observed a decrease in milk fat percent with an increase in the intervals between milkings. The observed decrease in milk fat was contributed to the increase in the pressure within the udder. Linton (1937) concluded that long milking intervals would depress milk fat values, and milking intervals of 3 h or more significantly lowered milk fat percent.

Linton (1937) also observed recognizable changes in milk composition when mares were excited prior to milking. This seems reasonable since concentrations of epinephrine increase when animals become excited. Epinephrine directly blocks oxytocin from binding to myoepithelial cells. Oxytocin is responsible for the smooth muscle contractions of myoepithelial cells surrounding the outer surface of alveoli, resulting in milk let down (Tucker, 1985).

CHAPTER III

Summary

Twelve lactating Quarter Horse mares ranging from 5 to 24 yr of age were blocked by expected foaling date and age and assigned randomly to treated or control groups. Treated mares were fed altrenogest at $.044 \text{ mg}\cdot\text{kg body wt}^{-1}\cdot\text{d}^{-1}$ on d 1 through d 15 postpartum.

Mares in the altrenogest group were bred on the first estrus following treatment withdrawal. Mares in the control group were bred during the second postpartum estrus. Ovulation during estrus in which mares were bred occurred $26 \pm 1.0 \text{ d}$ postpartum or $11 \pm 1.1 \text{ d}$ after altrenogest withdrawal for treated mares ($n=6$), and $35.7 \pm 1.2 \text{ d}$ postpartum for control mares ($n=6$). Conception rates were similar ($4/6$ vs $6/6$) for control and altrenogest-treated mares.

No differences were found in uterine tone, uterine size or follicle size between treated and control groups.

Uterine cultures and biopsies collected on d 7 and 15 postpartum showed no difference between the groups in bacterial populations or endometrial height of epithelial cells.

Blood was collected 7, 11, 15, 19 and 23 d postpartum, and concentrations of estradiol-17B in serum were determined by radioimmunoassay. Mean estradiol

concentrations across days were $9.84 \pm .8$ and $11.55 \pm .6$ pg/ml for control and treated mares, respectively. Concentrations of serum estradiol were higher ($P < .05$) in the treated mares on d 23 postpartum.

The weigh-suckle-weigh method was used to determine daily milk yields. No difference was found between groups. Combining groups, mean daily milk yield throughout the 43 d lactation was 15.4 kg and averaged 3.1% of body weight. Milk yield tended to peak between d 22 and 29 postpartum. Milk composition did not differ between treatment groups on any collection day. After pooling groups, percentage fat, protein, and total solids averaged .9, 2.1 and 9.88%, respectively.

No difference was observed in foal growth between groups.

Introduction

Mares bred at foal heat tend to have reduced conception rates and higher incidence of abortion (Ginther, 1979; Loy, 1980). Andrews and McKenzie (1941) reported uterine epithelium of mares rarely returned to normal within 10 d postpartum. Loy et al. (1975), however, reported that considerable endometrial repair occurred by d 5 postpartum, with only minor additional repair after d 5.

Altrenogest, a synthetic oral progestin, has been used successfully to control the estrous cycle and ovulation in the nonlactating mare (Squires et al., 1979; Allen et al., 1980; Turner et al., 1981; Squires et al., 1983). Squires et al. (1983) reported that mares treated with altrenogest had suppressed follicle size during and shortly after treatment. Sigler et al. (1983) studied the effects of altrenogest on milk production and suggested an increase in early lactating mares treated with altrenogest. Limited data have been reported on the use of altrenogest during the postpartum period.

Information on milk production in the horse is limited. Gibbs et al. (1982) reported average daily yields ranged from 9.8 to 11.8 kg and 1.9 to 2.3% of the mare's body wt in a 150-d lactation. Sigler et al. (1983) reported average daily milk yields of 2.88 and 2.5% of body wt for altrenogest and control mares, respectively.

Ullrey et al. (1966) reported average values for fat, protein, and total solids ranged from 1.3 to 2.4, 1.8 to 2.7 and 10.0 to 11.5%, respectively. Gibbs et al. (1982) found average values of 1.3, 2.1 and 10.5% for fat, protein and total solids, respectively, for a 150 d lactation. Oftedal et al. (1983) reported fat, protein and total solids ranged from 1.19 to 1.78%, 1.83 to 2.64% and 10.4 to 11.6%, respectively, for a 54 d lactation.

The objectives of this study were to: 1) examine the use of altrenogest in controlling the estrous cycle, follicular growth, concentrations of estradiol in serum, ovulation, uterine involution and conception rates in the postpartum mare, 2) determine the effects of altrenogest on production and composition of milk in the postpartum mare and subsequent foal growth.

Materials and Methods

Twelve lactating Quarter Horse mares ranging from 5 to 24 yr of age were blocked by expected foaling date and age and then assigned randomly to two experimental groups. Treated mares (n=6) were fed altrenogest (allyl trenbolone or RU-2267; 17 β -hydroxy-17-(2-propenyl) estradiol, 4,9,11-trien-3-one) at .044 mg·kg body wt⁻¹·d⁻¹ on d 1 through 15 postpartum. Control mares (n=6) received similar rations with no altrenogest.

Mares were fed twice daily a concentrate mixture at a rate of 1 to 1.5% of body weight formulated to meet or exceed NRC (1978) requirements (table 1). Medium quality prairie hay was fed at approximately 1% of body wt. Mares were allowed ad libitum access to water throughout the trial. Mares were weighed weekly and amount of concentrate was adjusted to maintain constant body weight throughout the study.

TABLE 1. COMPOSITION OF CONCENTRATE DIET^a

Ingredients	Percent of Ration
Corn	40
Oats	35
Soybean meal	10.8
Dehydrated alfalfa pellets	6.4
Molasses	6.0
Dicalcium phosphate	.9
Limestone, ground	.4
Salt	.5

^aCalculated nutrients:

Crude protein, %	15.62
Digestible energy (mcal/kg)	3.45
Calcium, %	.57
Phosphorus, %	.55

During the milk collection periods, mares and foals were placed in 4.3 x 4.6 m pipe stalls on a dirt floor with straw bedding. At other times mares and foals were housed in 110 x 16 m dry lots. No more than four mares and foals were confined to a lot.

Estrus was detected by individual daily teasing with a stallion. Uterine tone was determined by rectal palpation and subjectively scored (1=Poor or 2=Fair or 3=Good or 4=Excellent) on d 1, 3, 5, 7, 9, 11, 13 and 15 postpartum. Uterine size, diameter of ovarian follicles and day of ovulation were determined by ultrasonic examination using the Technicare 210 DX (Johnson and Johnson Co., Englewood, Colorado). Measurements of both uterine horns were made approximately 5 cm lateral to the bifurcation of the uterus. Mares were examined every other day beginning on d 1 postpartum until day of ovulation in which bred. All mares were bred by artificial insemination to known fertile stallions. Mares in the control group were bred during their second postpartum estrus. Mares receiving altrenogest were bred on their first estrous period following altrenogest withdrawal. Pregnancy was determined ultrasonically on d 30 and 60 postpartum. Conception rates were evaluated by Chi-square analysis.

Bacterial cultures and biopsies of endometrium were collected on d 7 and 15 postpartum. Uterine cultures were performed using a disposable sterile guarded culture instrument (K₁-3000, Kalayjian Industries, Inc., Long Beach, California) as described by Brooks (1985). Bacterial populations were isolated and identified by Kansas State University Veterinary Diagnostic Laboratory.

The technique of endometrial biopsy in the mare, using a variety of instruments, has been described by Brandt and Manning (1969), Witherspoon et al. (1972), Kenney (1978) and Ricketts (1975). Rotating biopsy forceps (Edward Weck and Company, Inc., North Carolina) with a 60 cm working length and a jaw basket 3.5 mm by 10 mm were used to obtain tissue specimens. Endometrial specimens were fixed immediately in 10% neutral formalin. Biopsy samples were stained by Kansas State University Veterinary Diagnostic Laboratory for histological examination using hematoxylin and eosin (H&E) procedures (Glune, 1968). Epithelial cell heights were measured using an ocular micrometer at a magnification of 750. Ten representative areas were measured and averaged for each biopsy. The number of uterine glands present in ten random fields were counted and averaged at the same magnification.

Blood was collected from a jugular vein on d 7, 11, 15, 19 and 23 postpartum. Samples were refrigerated at 4° C and allowed to clot overnight, then centrifuged for 20 min at 3000 rpm. Sera were stored at -20° C until assayed. Serum concentrations of estradiol-17B were measured by radioimmunoassay (Skaggs et al. 1985). Ethel acetate was used to extract 6 ml serum per duplicate. Intra-assay coefficients of variation for two assays were 16.27 and 10.73%, with an average recovery of 71%. Inter-assay coefficients of variation could not be calculated due to different control samples between assays. Concentrations of estradiol-17B were corrected for extraction efficiency (recovery).

Milk yields were determined on d 8, 15, 22, 29 and 43 postpartum. The weigh-suckle-weigh method was used to determine daily milk production in all mares. Foals were held in adjacent pens so that mares and foals could see, smell, and touch each other, but nursing was prevented by a solid barrier. Foals and mares were separated at 0700 h for 3 h, then foals were weighed and allowed to nurse to satiety (about 20 min). Foals were reweighed, and milk consumed for that 3-h period was determined by the difference in weight before and after nursing. This procedure was repeated four times to determine milk production in a 12-h period and doubled to estimate total

daily milk yield. Incidences in which foals had urinated or deficated between pre- and post-nursing weighings were not included in calculations.

Hand milkings provided samples for analysis of fat, protein and total solids in milk on d 9, 16, 23, 30 and 44 postpartum. Mares and foals were separated at 0700 h for 3 h. Following separation, foals were allowed to nurse one teat while the other was hand-milked as completely as possible into a 1000-ml plastic beaker. Milk samples were stored in a refrigerator at 4° C. This procedure was repeated with the separation of mare and foal at 1600 h for 3 h followed by the hand milking of the alternate teat at 1900 h. Total fat, protein and solids were determined by the Kansas Dairy Herd Improvement Laboratory using the Multi-spec M milk analyzer.

Weight, height at the withers, heart girth and cannon bone length on foals were measured on d 1, 7, 14, 21, 28, 35 and 41 postpartum.

All data were analyzed by analysis of variance using the Statistical Analysis System (SAS, 1979) to determine sources of variation. The model accounted for variation due to treatment (altrenogest), stage of lactation (day), horse within treatment and interaction of treatment and day.

Results and Discussion

Estrus and ovulation. Postpartum interval to estrus and ovulation are shown in table 2. Results of this experiment indicate that altrenogest can be used successfully in the mare to shorten postpartum interval to breeding compared to breeding on the second postpartum estrus.

The number of days to ovulation following treatment withdrawal was in close agreement with other research using nonlactating mares. Turner et al. (1981) reported that estrus occurred in altrenogest-treated mares 4.8 d and ovulated 12.5 d following a 15-d treatment period. Squires et al. (1983) also reported treated mares displayed estrus 8 d and ovulated 10.1 d postpartum following a 15 d treatment with altrenogest. The data in the present study suggests little difference between intervals to estrus or ovulation in lactating mares as opposed to non-lactating mares in response to altrenogest.

Conception rates. There was no difference in conception rates between treatment groups (table 3). It should be noted that the mare that aborted between d 30 and 60 in the control group had a condition score of one (Henneke et al., 1983) at 60 d, which may have been the cause for the loss. It has been suggested that weight loss and associated stress may alter progesterone and

TABLE 2. MEAN^a POSTPARTUM INTERVAL TO ESTRUS AND OVULATION IN CONTROL AND ALTRENOGEST-TREATED^b MARES

Item	Control	Altrenogest
Days postpartum to first estrus	9.8 ± .9	20.4 ± 1.0
Days to estrus after treatment withdrawal	-----	5.4 ± 1.0
Length of first estrus (d)	3.7 ± .6	6.2 ± .9
Days postpartum to first ovulation	13.7 ± 1.3	26.0 ± 1.0
Days to ovulation after treatment withdrawal	-----	11.0 ± .1
Days postpartum to second estrus	31.2 ± 1.8	-----
Length of second estrus (d)	5.2 ± .7	-----
Days postpartum to second ovulation	35.7 ± 1.2	-----

^aLeast squares means ± standard error.

^bFed at .044 mg·kg body wt⁻¹·d⁻¹ beginning on d 1 postpartum and continuing through d 15 postpartum.

TABLE 3. FIRST SERVICE PREGNANCY RATE IN CONTROL AND ALTRENOGEST-TREATED^a MARES

Day post-ovulation	Control	Altrenogest
30	(5/6) 83%	(6/6) 100%
60	(4/6) 67%	(6/6) 100%

^aFed at $.044 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ beginning on d 1 postpartum and continuing through d 15 postpartum.

glucocorticoid levels resulting in reproductive dysfunction (Henneke et al., 1984; Fines, 1985).

Uterine tone. Changes in uterine tone or diameter between the altrenogest and control groups were not different on any measured day. Combining treatment groups, uterine diameter averaged 9.3, 7.0 and 6.2 cm on d 3, 9 and 15, respectively. Berg and Ginther (1978) reported no increase in uterine tone for nonpregnant, ovarian intact mares injected with 100 mg/d progesterone for 10 or 50 d post-ovulation.

Diameter of ovarian follicles. There was no difference in mean diameter of the largest preovulatory follicle for altrenogest (23.8 mm) and control (25.7 mm) mares.

Uterine cultures. Microorganisms isolated from uterine cultures for each mare on d 7 and 15 postpartum are described in table 4 for control and treatment groups, respectively. Altrenogest had no apparent effect on the presence or type of organisms isolated from uterine cultures. Culture and biopsy data were not collected from one mare in the control group due to a vaginal tear that occurred during foaling.

TABLE 4. BACTERIA^a ISOLATED FROM UTERINE CULTURES IN CONTROL AND ALTRENOGEST-TREATED^b MARES

Mare Identification		Day 7		Day 15	
Number		Postpartum		Postpartum	
Control	Altrenogest	Control	Altrenogest	Control	Altrenogest
3	1	----	----	AS	----
4	2	----	----	----	----
6	5	BS	----	B	----
8	7	----	S,B,A	----	----
11	9	S,AS,BS	----	E	BS
---	10	----	S,BS	----	S,BS

- ^a A = Acinetobater
AS = Alpha - Streptococcus sp.
B = Bacillus sp.
BS = Beta - Streptococcus sp.
E = Escherichia coli
S = Staphylococcus aureus

^bFed at .044 mg·kg body wt⁻¹·d⁻¹ beginning on d 1 postpartum and continuing through d 15 postpartum.

Uterine biopsies. Uterine epithelium was found intact with no histological differences in uterine gland numbers or epithelial cell height. Uterine gland number per field averaged $3.0 \pm .3$ and $2.8 \pm .2$ on d 7 for treated and control mares, respectively. On d 15 mean uterine gland number was $4.2 \pm .5$ and $3.6 \pm .6$ for treated and control mares, respectively. Mean epithelial cell heights (table 5) ranged from 19.1 to 28.1 μm , and 28.2 to 40.1 μm on d 7 and 15 postpartum, respectively. Endometrial epithelium ranged from cuboidal to columnar in shape. Cell heights were similar to those of Andrews and McKenzie (1941) who reported average cell height of surface epithelium to be 24.9 μm on d 2 postpartum and 20.7 μm on the first day of estrus.

Estradiol concentrations. Total average concentrations of estradiol did not differ significantly between groups across the measured days. Pooled estradiol concentrations were 10.5, 12.3, 10.3, 9.7 and 10.6 on d 7, 11, 15, 19 and 23 postpartum, respectively. Mean estradiol concentrations for control and treatment groups averaged across days were 9.8 pg/ml and 11.6 pg/ml, respectively. The altrenogest group tended to have higher concentrations ($P < .2$) on d 15 and 19, and higher concentrations ($P < .05$) on d 23 postpartum (table 6). This may be related to the presence of estrogen active follicles at this time.

TABLE 5. MEAN^a ENDOMETRIAL EPITHELIAL CELL HEIGHT (μm) IN CONTROL AND ALTRENOGEST-TREATED^b MARES.

Day Postpartum	Control	Altrenogest
7	22.2 \pm 1.4	24.8 \pm 2.3
15	32.7 \pm 1.6	32.4 \pm 2.0

^aLeast squares means \pm standard error.

^bFed at .044 mg·kg body wt⁻¹·d⁻¹ beginning on the d 1 postpartum and continuing through d 15 postpartum.

TABLE 6. MEAN^a ESTRADIOL-17 β CONCENTRATIONS (pg/ml)
IN CONTROL AND ALTRENOGEST-TREATED^b MARES

Day postpartum	Control	Altrenogest
7	11.5 \pm 1.9	9.4 \pm 1.5
11	12.1 \pm 1.4	12.5 \pm 3.2
15	8.4 \pm 1.5	12.3 \pm 2.4
19	8.6 \pm 1.2	10.9 \pm 1.0
23	8.6 \pm 1.2	12.7 \pm 1.1 ^c

^aLeast squares means \pm standard error.

^bFed at .044 mg·kg body wt⁻¹·d⁻¹ beginning on d 1 postpartum and continuing through d 15 postpartum.

^cDifferent from control (P<.05).

Treated mares on d 23 tended to be in their first postpartum estrus, while control mares were in mid-diestrus at this time (table 2). This allowed a longer exposure to higher levels of estradiol in the altrenogest-treated mares. Highest estradiol concentration on d 11 is in agreement with Nett et al. (1975) who reported peaks between d 8 and 12. Nett et al. (1973) found that estrogen concentrations (expressed as estrone equivalent) during estrus ranged from 25 to 50 pg/ml with a subsequent drop to 5 to 15 pg/ml. Basal levels of estradiol during diestrus have been reported to average 20 pg/ml with an increase to 141 pg/ml 1 to 3 d before ovulation (Pattison et al., 1974). Noden et al. (1975) reported estradiol concentrations of 4.3 and 11.5 pg/ml for mid-diestrus and 1 d before ovulation. Nett et al. (1975) reported estrogen peaks in serum ranged from 22 to 56 pg/ml between d 8 and 12 postpartum.

Daily Milk Production. There was no difference in milk production between groups. Combining treatment groups, mean daily milk yield throughout the 43 d lactation was 15.4 kg representing 3.1% of the mare's body weight (table 7). Milk production on d 8, 15, 22, 29 and 43 postpartum averaged 13.6, 15.2, 16.6, 16.9 and 14.8 kg/d, respectively. These values are higher than those reported by Gibbs et al. (1982) of 11.4, 11.8 and 11.3

TABLE 7. MILK PRODUCTION VALUES BY QUARTER HORSE MARES DURING EARLY LACTATION (kg).

Day of lactation	Range	X \pm S.E.	% of body wt
8	8.6 - 16.8	13.6 \pm .78	2.7
15	12.3 - 17.7	15.2 \pm .43	3.1
22	11.4 - 20.9	16.6 \pm .98	3.3
29	13.2 - 22.7	16.9 \pm .81	3.4
43	9.7 - 19.2	14.8 \pm .84	3.0

kg/d for d 10, 30 and 45 postpartum, respectively. Milk yield expressed as a percentage of the mare's body weight was 2.7, 3.1, 3.3, 3.4 and 3.0 % on d 8, 15, 22, 29 and 43 postpartum, respectively. These values are higher than those reported by Neseni et al. (1958), 2.3%; Gibbs et al. (1982), 2.1%; and Sigler et al. (1983), 2.7%.

Milk yield appeared to peak ($P=.07$) between d 22 and 29 postpartum. Six of the mares had their largest yields on d 29, and four on d 22 postpartum. Similarly, Gibbs et al. (1982) reported the largest daily milk yield of 11.8 kg and 2.3% of body weight on d 30 postpartum.

The mares were of similar weight and body condition at foaling and were fed to maintain this state during lactation. Body weight and condition of the mares had no significant ($P>.10$) influence on milk production.

Milk Composition. Altrenogest had no effect on measured milk components. Milk composition did not differ between groups on any collection day. After pooling groups, percentage fat, protein and total solids were analyzed to determine the normal variation in the composition of mares' milk over the 44-d lactation. This information may be useful in determining when milk may no longer adequately meet the nutrient requirements of the growing foal.

Average percentage fat, range and standard deviation by day of lactation are presented in table 8. Percentage fat averaged .90%, ranged from .53 to 1.59% and did not change during the measured lactational period. Percentages tended to be lower than those reported by Ullrey et al. (1966), Gibbs et al. (1982), and Oftedal et al. (1983).

Protein for all mares averaged 2.1% and ranged from 1.71 to 2.77% (table 9). Protein content agrees with data of Ullrey et al. (1966) and Gibbs et al. (1982) who both reported ranges of 1.8 to 2.7%. There was a trend toward higher protein content in the treated group on d 8 ($P>.14$), 15 ($P>.07$) and 22 ($P>.16$) postpartum. Protein content was highest in this study on d 8 and decreased as lactation advanced. Gibbs et al. (1982) reported highest milk on d 10 postpartum.

Total solids averaged 9.88% and ranged from 9.31 to 10.47% (table 10). These data agree with those of Veith (1885) and studies reviewed by Linton (1931) and Turner (1952). However, percentages were lower than values reported by Linton (1931), Linton (1937), Ullrey et al. (1966), Gibbs et al. (1982) and Oftedal et al. (1985).

Contrary to earlier research, there was no significant change in total solids between the collection days. Linton (1937) reported an increase from 1 to 16 wk, while Gibbs et al. (1982) and Oftedal et al. (1983) found

TABLE 8. MILK FAT AT DIFFERENT STAGES OF LACTATION IN MARES

Day of lactation	% Fat	
	Range	X \pm S.E.
9	.62 - 1.13	.92 \pm .05
16	.58 - 1.30	.93 \pm .06
23	.56 - 1.23	.86 \pm .06
30	.53 - 1.59	.95 \pm .09
44	.61 - 1.04	.83 \pm .05

TABLE 9. MILK PROTEIN AT DIFFERENT STAGES OF LACTATION IN MARES

Day of lactation	% PROTEIN	
	Range	X \pm S.D.
9	2.02 - 2.77	2.44 \pm .06
16	1.82 - 2.74	2.22 \pm .06
23	1.76 - 2.31	2.05 \pm .04
30	1.74 - 2.20	2.00 \pm .04
44	1.71 - 2.10	1.89 \pm .04

a,b,c,d Means within column with different superscripts differ (P<.05)

TABLE 10. TOTAL MILK SOLIDS AT DIFFERENT STAGES OF LACTATION IN MARES

Day of lactation	TOTAL SOLIDS	
	Range	X \pm S.E.
9	9.31 - 10.27	9.95 \pm .1
16	9.49 - 10.47	9.90 \pm .08
23	9.32 - 10.19	9.87 \pm .07
30	9.42 - 10.16	9.90 \pm .06
44	9.61 - 10.09	9.88 \pm .05

mean total solids to be highest on d 10 postpartum but decreasing as lactation advanced.

Foal growth. Weight, height, heart girth and cannon length did not differ between treated and control groups (table 11). Average weight of all foals on d 1, 7, 14, 21, 28, 35 and 42 postpartum were 50.6 ± 1.6 , 64.5 ± 1.7 , 76.2 ± 2.3 , 85.0 ± 2.4 , 94.1 ± 2.9 , 101.0 ± 2.6 and 107.7 ± 2.6 kg, respectively. Average wither height of all foals at the beginning of the trial was $95.6 \pm .9$ and $108.0 \pm .8$ cm at the end of the trial. Heartgirth averaged 85.5 ± 1.0 and $108.1 \pm .8$ cm on d 1 and 42 postpartum, respectively. Cannon bone length averaged $15.0 \pm .2$ and $16.1 \pm .3$ cm on d 1 and 42, respectively. Average daily gains (ADG) calculated for each week were not different for foals from altrenogest-treated and control mares for any week except the first week postpartum when ADG for foals from treated mares were higher ($P < .03$) than foals from control mares (2.3 vs 1.7 kg/d). ADG from d 1 to 42 was 1.4 and 1.3 kg/d for treated and control groups, respectively.

Conclusion. Results of this research indicate that altrenogest can be used to delay the onset of the first estrus in the postpartum mare while allowing more time for uterine involution. Altrenogest had no apparent effect on conception rates, uterine condition or estradiol

TABLE 11. GROWTH RATES OF NURSING FOALS FROM CONTROL AND ALTRENOGEST-TREATED^a MARES

Parameter	Day 1 Postpartum		Day 42 Postpartum		Mean Increase \pm S.E.	
	Control	Altrenogest	Control	Altrenogest	Control	Altrenogest
Body weight (kg)	50.3	50.8	105.9	109.4	55.6 \pm 2.9	58.5 \pm 3.2
Wither height (cm)	96.7	94.5	108.6	107.6	11.8 \pm 1.0	13.1 \pm 1.4
Heart girth (cm)	86.8	84.9	108.2	106.5	21.6 \pm 1.6	22.1 \pm 1.7
Cannon length (cm)	14.7	15.3	15.9	16.2	1.2 \pm .5	.9 \pm .5

^aFed at .044 mg·kg body wt⁻¹, d⁻¹ beginning on d 1 postpartum and continuing through d 15 postpartum.

production.

Milk production, composition and subsequent foal growth did not appear to be affected by altrenogest treatment. The results of this experiment indicated milk yield tended to be highest around d 29 postpartum. Knowledge of the lactational curve will provide valuable information to the horse producer for broodmare and foal management.

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APPENDICES

APPENDIX TABLE 1. MEAN^a MARE WEIGHTS
AND STANDARD DEVIATIONS (kg)

Mare	Kg
1	497.5 ± 4.5
2	468.6 ± 2.1
3	472.2 ± 1.5
4	509.5 ± 1.2
5	589.6 ± 2.3
6	496.5 ± 4.0
7	559.1 ± 3.9
8	491.3 ± 4.8
9	577.9 ± .7
10	444.9 ± 1.7
11	435.8 ± 2.3
12	466.0 ± 1.1

^aLeast squares means ± standard error.

APPENDIX TABLE 2. INDIVIDUAL AND MEAN DAILY MILK YIELDS IN MARES (kg)

Mare	Day of Lactation					\bar{X}
	8	15	22	29	43	
1	13.2	15.5	17.3	15.9	17.3	15.8
2	15.0	15.5	15.9	13.7	15.4	15.1
3	12.7	15.0	14.5	13.2	18.2	14.7
4	14.6	14.1	12.3	15.9	11.8	13.7
5	16.4	14.6	22.7	22.7	16.8	18.6
6	16.8	17.7	20.9	—	12.3	16.9
7	8.6	16.8	18.6	15.5	9.7	13.8
8	15.5	15.4	15.0	18.2	13.6	15.5
9	15.5	12.3	16.4	17.7	14.5	15.3
10	10.0	16.4	11.4	15.9	13.2	13.4
11	10.9	14.1	17.7	18.2	15.9	15.4
12	14.6	15.0	16.8	19.1	19.2	16.9
\bar{X}	13.6	15.2	16.6	16.9	14.8	15.4

APPENDIX TABLE 3. INDIVIDUAL AND MEAN DAILY
MILK FAT PERCENTAGES IN MARES

Mare	Day of Lactation					\bar{X}
	9	16	23	30	44	
1	.62	.58	.56	.96	.65	.67
2	.97	1.17	.81	.64	.61	.84
3	.71	.60	.71	.62	.64	.66
4	.96	.75	.64	.53	.79	.73
5	1.25	.92	.94	.97	1.04	1.02
6	1.13	.88	.69	.74	.81	.85
7	.82	.93	1.01	1.00	.78	.91
8	.74	.84	.89	1.59	.80	.97
9	.89	1.30	1.23	.93	.96	1.06
10	.97	1.18	.88	.92	.92	.97
11	1.00	.94	.92	1.07	.96	.98
12	1.02	1.12	1.08	1.37	1.02	1.12
\bar{X}	.92	.93	.86	.95	.83	.90

APPENDIX TABLE 4. INDIVIDUAL AND MEAN DAILY MILK PROTEIN PERCENTAGES IN MARES

Mare	Day of Lactation					\bar{X}
	9	16	23	30	44	
1	2.71	2.74	2.29	2.02	1.97	2.35
2	2.15	2.05	1.88	1.95	1.71	1.95
3	2.33	2.13	1.90	1.95	1.85	2.03
4	2.34	2.17	2.06	1.83	1.93	2.07
5	2.56	2.11	2.20	1.99	1.92	2.16
6	2.51	2.12	2.06	2.03	1.92	2.13
7	2.77	2.48	2.31	2.12	2.10	2.36
8	2.39	2.22	2.02	2.17	1.94	2.15
9	2.45	2.15	2.00	1.95	1.72	2.05
10	2.51	2.56	2.03	2.00	2.08	2.24
11	2.48	2.10	2.11	2.20	1.84	2.15
12	2.02	1.82	1.76	1.74	1.71	1.81
\bar{X}	2.44	2.22	2.05	2.00	1.89	2.12

APPENDIX TABLE 5. INDIVIDUAL AND MEAN DAILY
TOTAL MILK SOLID PERCENTAGES IN MARES

Mare	Day of Lactation					\bar{X}
	9	16	23	30	44	
1	9.87	9.81	10.19	10.04	9.88	9.96
2	10.27	10.47	9.89	10.00	9.82	10.09
3	10.14	10.05	9.98	9.91	9.62	9.94
4	10.02	9.90	9.75	9.75	9.61	9.81
5	9.35	9.49	9.32	9.42	9.75	9.47
6	9.31	9.55	9.62	9.69	9.82	9.60
7	9.59	9.66	9.72	9.85	9.92	9.75
8	9.61	9.92	9.88	10.16	10.04	9.92
9	9.91	10.08	10.17	10.06	10.06	10.06
10	10.04	9.96	9.99	10.08	10.09	10.03
11	10.09	10.04	10.08	9.97	10.01	10.04
12	10.05	9.92	9.87	9.92	9.94	9.94
\bar{X}	9.85	9.90	9.87	9.90	9.88	9.88

EFFECT OF ALTRENOGEST ON REPRODUCTIVE PERFORMANCE
AND LACTATION IN THE MARE

by

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ABSTRACT

Twelve lactating Quarter Horse mares ranging from 5 to 24 yr of age were blocked by expected foaling date and age and assigned randomly to treated or control groups. Treated mares were fed altrenogest at .044 mg·kg body wt⁻¹·d⁻¹ on d 1 through d 15 postpartum.

Mares in the altrenogest group were bred on the first estrus following treatment withdrawal. Mares in the control group were bred during the second postpartum estrus. Ovulation during estrus in which mares were bred occurred 26 ± 1.0 d postpartum or 11 ± 1.1 d after altrenogest withdrawal for treated mares (n=6), and 35.7 ± 1.2 d postpartum for control mares (n=6). Conception rates were similar (4/6 vs 6/6) for control and altrenogest-treated mares.

No differences were found in uterine tone, uterine size or follicle size between treated and control groups.

Uterine cultures and biopsies collected on d 7 and 15 postpartum showed no difference between the groups in bacterial populations, uterine gland number or endometrial height of epithelial cells.

Blood was collected 7, 11, 15, 19 and 23 d postpartum, and concentrations of estradiol-17B in serum were determined by radioimmunoassay. Mean estradiol concentrations across days were $9.84 \pm .8$ pg/ml and $11.55 \pm .6$

pg/ml for control and treated mares, respectively. Concentrations of serum estradiol were higher ($P < .05$) in the treated mares on d 23 postpartum.

The weigh-suckle-weigh method was used to determine daily milk yields. No difference was found between groups. Combining groups, mean daily milk yield throughout the 43 d lactation was 15.4 kg and averaged 3.1% of body weight. Milk yield tended to peak between d 22 and 29 postpartum. Milk composition did not differ between treatment groups on any collection day. After pooling groups, percentage fat, protein, and total solids averaged .9, 2.1 and 9.88%, respectively. Foal growth did not appear to be affected by altrenogest treatment.