EFFECTS OF EQUIEMATE (ICI-81008) ON LEVELS OF LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE AND PROGESTERONE DURING THE ESTROUS CYCLE OF THE MARE

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SUMMARY

Jugular blood was collected at 6-hr intervals during estrus and at daily intervals during diestrus from 12 cycling mares. On day 8 of diestrus four mares were given saline (im) while the remaining eight mares were given Equimate (250 µg), a synthetic analog (ICI-81008) of PGF₂α. Following treatment, jugular blood was collected at 15-min intervals for 6 hr and then at 6-hr intervals until the end of the subsequent estrus. Blood was again collected at daily intervals until the end of the diestrus following treatment. Day of ovulation was estimated by palpation per rectum. Serum was analyzed for LH, FSH and progesterone by radioimmunoassay.

Injection of Equimate on day 8 of diestrus resulted in an immediate increase in levels of LH and FSH in serum. Concentrations of LH increased from 35.6 ± 8.2 ng/ml to 89.8 ± 25.2 ng/ml (P<.05) within 45 min after injection but had returned to baseline within 3 hours. Concentrations of FSH increased from 178 ± 34 ng/ml to 382 ± 86 ng/ml (P<.05) within 45 min after the injection and then returned to baseline over the next several hours. Concentrations of progesterone decreased to baseline (< 1 ng/ml) within 24 hr after Equimate and remained low until the end of the next estrus. Concentrations of LH and FSH during the estrus preceding Equimate and the estrus subsequent to Equimate did not differ (P>.05). Likewise, there was no difference (P>.05) between levels of progesterone during the diestrous periods of the cycles preceding, and subsequent to, Equimate. Similarly, concentrations of LH, FSH and progesterone were not different (P>.05) between mares treated with saline and those treated with Equimate during subsequent estrous cycles. Equimate appears to be an effective agent for reducing concentrations of progesterone in mares without interfering with the normal secretion of hormones during the subsequent estrous cycle.

(Key Words: Mares, LH, FSH, Progesterone, Prostaglandin Analog.)

INTRODUCTION

Prostaglandin F₂α (PGF₂α) is a potent luteolytic in mares (Douglas and Ginther, 1972, 1975; Noden et al., 1974; Douglas et al., 1976); however, treatment of mares with PGF₂α may cause sweating, diarrhea, increased heart and respiration rates and discomfort (Miller et al., 1976). Some synthetic analogs of PGF₂α may produce similar side effects in mares (Allen and Rowson, 1973; Allen and Cooper, 1975). Another synthetic analog of PGF₂α (Equimate, ICI-81008) has proven to be an effective luteolytic and is without adverse side effects at the optimal luteolytic dose of 250 µg per mare (Allen and Cooper, 1975).

The purpose of this investigation was to determine acute and chronic effects of Equimate on serum concentrations of progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in mares.

MATERIALS AND METHODS

Animals. Twelve nonlactating mares of mixed breeding, between the ages of 3 and 20 years and weighing 450 to 550 kg, were used. Mares were fed 4 kg of a balanced grain ration in individual feed bags and 7 to 9 kg of hay daily. Estrus was determined by daily teasing of each mare with a stallion as described by Back et al. (1974). Day of ovulation was estimated by daily examination of the ovaries per rectum. Mares were assigned to one of two treatment groups after May 1, but not until they had ex-
experienced a normal estrous cycle (at least 4 days of estrus followed by 8 continuous days during which estrus was not detected).

Jugular blood was collected from all mares at 6-hr intervals during estrus and at daily intervals during diestrus for two consecutive estrous cycles. On day 8 of diestrus of the first cycle four mares were given 5-ml sterile saline im (Group 1). The remaining eight mares were given 250 μg Equimate im on day 8 of diestrus (Group 2). After saline or Equimate, jugular blood was collected from all mares at 15-min intervals for 6 hr and then at 6-hr intervals until the end of the succeeding estrus.

Blood was allowed to clot at room temperature for 6 hr, refrigerated until the next morning and then centrifuged. Serum was collected and stored at −20°C.

**Hormonal Assays.** Concentrations of LH, FSH and progesterone in serum were determined by radioimmunoassay. Details of the radioimmunological procedures for LH (Nett et al., 1975) and progesterone (Niswender, 1973) have been described. Preparation of the antiserum to ovine FSH and radioiodination of the human FSH utilized for the equine FSH assay have been described (L'Hermite et al., 1972). Duplicate aliquots of 200 μl serum were assayed for FSH (cf. Appendix 1).

**Statistical Analyses.** Effects of Equimate on parameters of the estrous cycle were analyzed by Student's t-test. Differences in hormonal concentrations were determined by Dunnet's procedure (Steel and Torrie, 1960).

**RESULTS**

**Radioimmunoassay of FSH.** Inhibition curves produced by equine sera, saline extracts of equine pituitary glands and partially purified equine FSH (LER-1138-1) were parallel (figure 1). This indicates that the compounds present in these biological samples responsible for inhibiting the binding of radioiodinated human FSH to the antiserum have identical immunogenic sites. Varying quantities of exogenous equine FSH added to sera could be measured quantitatively (figure 2), thus indicating that other components of equine sera do not interfere with the quantification of FSH. Equine FSH (LER-1138-1) and the compound in extracts of equine pituitary glands that inhibits binding of radioiodinated human FSH to the antiserum migrated similarly (figure 3) during electrophoresis through 5 × 65 mm polyacrylamide gels (7.5% polyacrylamide, pH 8.9). A single peak of immunoreactive FSH was observed following the electrophoretic separation and this was distinct from immunoreactive LH. This indicates that adenohypophyseal hormones with electrophoretic properties different than FSH do not cross react in this assay system.

**Effect of Treatment on Duration of Estrous Cycles.** The effects of saline or Equimate on various parameters of the estrous cycle are listed in table 1. Time from saline or Equimate to onset of estrus was 5.5 ± .5 days (range = 4

![Figure 1](image-url)  
**Figure 1.** Inhibition curves produced by equine sera, a crude extract of equine pituitary (1 mg fresh tissue equivalent per ml of physiological saline) and a partially purified equine FSH (LER-1138-1 = .4 units NIH-FSH-S7 per mg).

![Figure 2](image-url)  
**Figure 2.** Measurement of varying quantities of equine pituitary extract that had been added to equine sera. Determinations were made in triplicate.
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Figure 3. Polyacrylamide gel electrophoresis of 500 µg equine pituitary extract (5 x 65 mm gels, 7.5% polyacrylamide, pH 8.9). After electrophoresis the gels were divided into 3 mm segments. The hormones present in each segment was allowed to diffuse in 2 ml of phosphate-buffered saline containing 0.1% gelatin and 200 µl aliquots of the buffer were analyzed for LH and FSH by radioimmunoassay.

to 6 days) and 4.4 ± 1.0 days (range = 2 to 8 days), respectively. Three mares given Equimate returned to estrus within 48 hr, whereas the other mares did not return to estrus until 4 to 8 days after treatment. One mare treated with Equimate did not ovulate until 18 days after treatment. This mare did not return to estrus after the post-treatment ovulation. Therefore, data from this mare have been excluded from analyses.

Ovulation occurred 13.3 ± 1.5 days after treatment in mares given saline and 9.4 ± 1.3 days after treatment in mares given Equimate (P < 0.05). In both groups ovulation occurred 2.0 ± 0.3 days prior to the end of estrus. None of the side effects reported to occur after injection of PGF₂α were noted in any of the mares receiving Equimate.

Hormonal Response to Treatment. During the estrous cycle prior to treatment, LH increased gradually from onset of estrus reaching a maximal concentration 14.5 ± 8.3 hr prior to the end of estrus (range = 48 hr prior to 60 hr after estrus). Concentrations of LH declined gradually after estrus but did not reach baseline until the fourth or fifth day of diestrus. There was no effect within 4 days after saline on concentrations of LH (figure 4). In contrast, Equimate increased LH from 35.6 ± 8.2 ng/ml to 89.8 ± 25.2 ng/ml within 45 min (P < 0.05) but concentrations of LH decreased to baseline within three hr (figure 5). After three hr LH did not increase again until progesterone had decreased to baseline (figure 6).

Maximum concentrations of LH during estrus did not differ (P > 0.05) between mares given saline and mares given Equimate. Furthermore, there was no difference (P > 0.05) in maximum concentrations of LH between the first and second estrous cycle in either group of mares (figures 4, 6, 7, 8).

In contrast to LH, concentrations of FSH in all mares remained low, or tended to decrease, during the first few days of estrus and then increased to a maximum 2.7 ± 9.6 hr after the end of estrus (range = 30 hr prior to the end of estrus to 60 hr after estrus). In addition to an increase in FSH near the end of estrus, FSH also increased during diestrus in 16 of the 20 complete estrous cycles observed in this study. The increased levels of FSH during diestrus persisted for 1 to 3 days, were of similar magnitude to the FSH peak observed near the end of estrus, but did not occur at any particular time relative to the onset, or end, of diestrus in individual mares. Since the data in figures 4, 5, 7 and 8 are plotted relative to time of ovulation and since the mid-diestrous rise in FSH did not occur at a specific time relative to ovulation it is obscured. Data presented in figure 9 are normalized to the mid-diestrous rise in FSH. Occurrence of the mid-diestrous rise in FSH rela-

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<th>Table 1. Effect of Equimate on the Estrous Cycle</th>
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<sup>a,b</sup>Values within a column having different superscripts differ significantly (P < 0.05). Each value represents the mean ± standard error.
Figure 4. Effect of injection of saline on day 8 of diestrus on concentrations of progesterone, LH and FSH in mares. The data are normalized to the day of ovulation (n=4).

Saline given on day 8 of diestrus had no measurable effect on levels of FSH. However, 250 μg Equimate given on day 8 of diestrus stimulated release of FSH with concentrations increasing from 178 ± 34 ng/ml to 382 ± 86 ng/ml (P<.05) within 45 min after injection (figure 5). Concentration of FSH returned to baseline over the next several hours and did not increase again until near the end of the induced estrus (figures 6, 7). As with LH, maximum concentrations of FSH were not different (P>.05) during the first and second estrous periods nor between mares given saline or Equimate.

Concentrations of progesterone in the peripheral circulation were basal during estrus (<.5 ng/ml), began to increase near the end of estrus and reached a plateau by day 5 of diestrus. Concentrations of progesterone did not return to baseline until 4.5 ± .5 days after saline (figure 4). In contrast (figure 6), concentrations of progesterone had returned to baseline by 1.2 ± .2 days after Equimate (P<.001). Even though time from Equimate until concentr-
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Figure 7. Concentrations of progesterone, LH and FSH in sera of mares during the estrous cycle following injection of saline on day 8 of diestrus during the previous cycle. Data are normalized to the day of ovulation. (n=4).

Pattern of secretion and concentrations of progesterone during the estrous cycle following Equimate were similar to those observed in control mares. There was no difference (P>.05) in concentrations of progesterone during the diestrous periods of the first and second estrous cycle nor between groups of mares.

Discussion

The fact that an effective luteolytic dose of Equimate in mares is without adverse side effects (Allen and Cooper, 1975) makes it a particularly attractive compound to use for treatment of prolonged diestrus or for estrous synchronization. The lack of side effects after treatment of mares with Equimate is probably a result of its being 50 times less effective in stimulating smooth muscle activity than PGF₂α (Dukes et al., 1974). The dosage of Equimate used in this study was certainly effective in inducing luteolysis as evidenced by the rapid decrease in serum levels of progesterone. Five

Figure 8. Concentrations of progesterone, LH and FSH in sera of mares during the estrous cycle induced by injection of Equimate on day 8 of diestrus during the previous cycle. Data are normalized to the day of ovulation. (n=7).

Figure 9. Concentrations of FSH in serum of mares during diestrus (n=16). Data are normalized to the maximum concentration of FSH observed during diestrus. This occurred between days 4 and 11 of diestrus in individual mares.
of eight mares given Equimate on day 8 of diestrous did not return to estrus until 4 to 8 days after treatment. Examination of the ovaries per rectum indicated that there was a lack of follicular growth in each of these five mares.

Van Rensburg and Van Niekerk (1968) suggested that follicles mature at approximately 10-day intervals in mares. Hence, mature follicles may be present on the ovaries of mares at mid-diestrus, some of which ovulate but the luteal phase may be present on the ovaries of mares at 10-day intervals. This may have been the case in the five mares treated with Equimate that did not return to estrus for 4 to 8 days after treatment.

The effect of Equimate on serum levels of progesterone was similar to that reported previously (Allen et al., 1974). In addition, the fact that the estrous cycle following induction of luteolysis by Equimate appeared to be normal with respect to levels of LH, FSH and progesterone in serum was not surprising since similar data have been reported after induction of luteolysis in mares using PGF$_2$\alpha (Oxender et al., 1975).

Prostaglandins have been reported to stimulate release of anterior pituitary hormones from a variety of species (see review of Hafs, 1975; Noden et al., 1977). PGE$_2$ and PGF$_2$\alpha seem to be the most potent naturally occurring prostaglandins in stimulating release of gonadotropins. Warberg et al. (1976) concluded that prostaglandins have a cis double bond in the 5,6 position of the carboxyl side chain, and an 11-hydroxy group and a trans double bond in the 13,14 position of the alkyl side chain were the most potent stimulators of LH release in vitro. Thus, Equimate has all of the essential structural characteristics to be a potent stimulator of LH release (Dukes et al., 1974). The release of LH induced by exogenous PGE$_2$ (Eskay et al., 1975) and PGF$_2$\alpha (Haynes et al., 1977) appears to be mediated, at least in part, by release of gonadotropin-releasing hormone from the hypothalamus. Hence, if Equimate induces release of GnRH from the hypothalamus it is not surprising that a rise in both LH and FSH ensues since exogenous GnRH stimulates release of both LH and FSH in mares (Evans and Irvine, 1976).

Considering the magnitude of the LH and FSH peaks that occur following injection of a luteolytic dose of Equimate into mares it seems unlikely that they are of any physiological significance. However, release of gonadotropins after injection of a luteolytic dose of prostaglandin F$_2$\alpha may be responsible for the increase in concentrations of progesterone prior to the initiation of luteolysis in mares (Noden et al., 1977). It is also possible that the increase in progesterone after prostaglandin F$_2$\alpha was due to an increase in heart rate and blood pressure (i.e., more blood flowing through the corpus luteum) rather than an increase in gonadotropins. If this was the cause of the increased progesterone noted by Noden et al. (1977) then a similar increase would not have been expected following Equimate since it does not appear to increase heart rate or blood pressure.

**APPENDIX I**

Preparation of Antisera. Antiserum #620 was raised in a rabbit in response to an immunization with ovine FSH (LER-866-3, 26.2 x NIH-FSH-S1). Details of the immunization procedure and collection of antiserum are described elsewhere (Midgley et al., 1970).

Radioiodination. Five μg purified human FSH (LER-1801-3, 160 x NIH-FSH-S1) in 50 μl .5M phosphate buffer (pH 7.0) were radioiodinated for 30 sec with 1 μCi Na$_{125}$I (Amersham/Searle Corp., Arlington Heights, IL) using 30 μg of chloramine-T dissolved in 15 μl of .05M phosphate buffer (pH 7.0). Radioiodination was stopped by adding 60 μg of sodium metabisulfite in 30 μl of .05M phosphate buffer to the reaction mixture. Contents of the reaction vial were then subjected to electrophoresis for 90 min in 7.5% polyacrylamide gel (5 x 65 mm columns) with a constant current of 4 mA/gel. The buffer for electrophoresis (pH 8.9) contained 4 mM EDTA, .012M borate and .075M Tris. After electrophoresis the polyacrylamide gels are cut into 3 mm segments and the segments were counted to determine the location of $^{125}$I-human FSH. Those segments
containing the $^{125}$I-human FSH were added to 2 ml of phosphate-buffered saline (.01M phosphate, .14M sodium chloride, pH 7.0) containing 1% gelatin (PBS-gel) so that the $^{125}$I-human FSH would diffuse out of the polyacrylamide gel. One-hundred $\mu$L ($\approx 40,000$ cpm) $^{125}$I-human FSH was added to each assay tube. In the absence of inhibiting hormone $\approx 25\%$ of the $^{125}$I-human FSH was bound to antisemum.

Radioimmunoassay. Disposable glass tubes $(12 \times 75$ mm) were used for radioimmunoassay. Varying amounts of sample or standard were placed in each tube and the volume was adjusted to 500 $\mu$L with PBS-gel. Anti-ovine FSH serum and serum from non-immunized rabbits (NRS) were added and mixed. Anti-ovine FSH serum was further diluted with 1:400 NRS to give the final working solution (1:1200) and 200 $\mu$L were added to each assay tube. After incubation for 24 hr at 4 C 100 $\mu$L $^{125}$I-human FSH was added to each tube, the contents of the tubes were mixed and incubation was continued for an additional 24 hours. At the end of the second 24-hr incubation 200 $\mu$L sheep antirabbit gamma globulin (diluted in PBS-EDTA) to effect maximum precipitation of the antibody bound hormone was added to precipitate the FSH-anti-FSH complexes. After 72 hr of further incubation 3 ml PBS (4 C) were added to each assay tube. After incubation for 30 min. The supernatant was decanted and the radioactivity in the precipitate was counted.

**LITERATURE CITED**


