Effects of Daily Administration of Estradiol-17β on Follicular Growth, Ovulation, and Plasma Hormones in Mares[1]

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ABSTRACT

Follicular growth and concentrations of circulating progesterone (P_4), FSH, and LH were measured in horse mares treated daily with estradiol-17β (E_2). Treatment of mares with 10 mg E_2 from Days 1 to 31 postovulation had no significant effect on luteal life span, while it significantly prolonged the length of estrus and the interovulatory interval (IOI), and suppressed follicular development and ovulation. E_2 treatment had no significant effect on baseline LH concentrations in the presence of a functional corpus luteum (CL); however, E_2 was able to stimulate LH secretion for up to 2 weeks after CL regression. Mares in the control group of this study exhibited a bimodal pattern of FSH secretion, while treatment of mares with E_2 altered the pattern of FSH secretion by suppressing FSH concentrations during early diestrus.

INTRODUCTION

Exogenous estrogens have been shown to alter the estrous cycle of numerous species. In species such as the cow and hamster, estrogen induces early regression of the CL when administered early in the cycle (Greenstein et al., 1958; Choudary and Greenwald, 1968a). In both guinea pigs and ewes, estrogens have been shown to be luteolytic (Choudary and Greenwald, 1968b; Stormshak et al., 1969) and antiluteolytic and/or luteotropic (Illingworth and Perry, 1973; Denamur et al., 1970). The different effects seem to be due to differences in the dose, length of treatment, and stage of the cycle in which the treatments were administered. Exogenous estrogens have been shown to prolong luteal function in rats, rabbits, and pigs (Nelson, 1935; Hammond and Robson, 1951; Nishikawa and Waide, 1958). Several investigators have suggested estrogen might be luteotropic or antiluteolytic in mares (Nishikawa, 1959; Berg and Ginther, 1978). In an experiment designed to test this hypothesis, estrogen had no significant effect on luteal life span in mares; however, it was found to prolong

the interovulatory interval (IOI) by inhibiting follicular growth and ovulation (Woodley et al., 1979). Because that experiment was conducted in the late breeding season (August and September) and evidence has been presented which shows seasonal or monthly changes in the pattern of gonadotropin secretion within the ovulatory season of mares (Turner et al., 1979), the present experiment was conducted to determine if estradiol administration has similar effects early in the breeding season (May and June) and to provide some information on the mechanism(s) by which estradiol inhibits follicular growth and ovulation.

MATERIALS AND METHODS

Thirteen horse mares of mixed breeding, 3–10 years old and weighing 300–500 kg, were teased daily to detect estrus, and their ovaries were palpated every 3rd day during diestrus and daily during estrus to monitor ovarian follicular growth and determine the day of ovulation. Mares were randomly assigned to one of three treatment groups: controls (n = 5, vehicle); low E_2 group (n = 4, 1.0 mg estradiol-17β/day); or high E_2 group (n = 4, 10.0 mg estradiol-17β/day). The vehicle consisted of 80% cottonseed oil, 20% benzyl benzoate. Injections were given i.m. starting Day 1 postovulation and continued until the next ovulation occurred or for 31 days if ovulation did not occur. Treatment termination on Day 31 was used to ensure a significant prolongation of the IOI in treated mares. Jugular blood samples were collected on Days 5, 10, 14, 16, and 18 postovulation and then daily until ovulation or Day 31 postovulation. Each mare was also bled on Day 5 postovulation of their post-treatment cycle. Plasma was separated and stored at

−20°C for subsequent hormone quantification. Plasma concentrations of progesterone, LH, and FSH were determined by radioimmunoassay as described (Hershman and Douglas, 1979; Whitmore et al., 1973; Freedman et al., 1979) and were validated for use in the author's laboratory. The sensitivities (smallest amount of hormone different from 0) of the respective assays were 0.125 ng P_4, 0.33 ng LH, and 0.33 ng FSH. Any samples below the sensitivities of the respective assays were arbitrarily assigned a value of 0. The equine LH used for standards and iodination had LH activity of 2.3 units per/mg as determined by the ovarian ascorbic acid depletion assay using NIH-LH-S1 as a reference (Braselton and McShan, 1970). The equine FSH used for standards and iodination had FSH activity of 90 units/mg expressed in terms of NIH-FSH-S1; 1 mg was considered to have 1 unit of FSH activity (Braselton and McShan, 1970).

Days chosen for statistical examination of hormone concentrations were based on previous studies (for review see Ginther, 1979). Emphasis was placed on quantitating concentrations of hormones on days of the cycle on which marked changes in concentrations of each of the circulating hormones have been shown to occur. Data were examined by analysis of variance and split-plot analysis of variance when appropriate. Bonferroni's T procedure was utilized to determine significant differences among means (Gill, 1978).

RESULTS

Luteal function was not affected (P>0.05) by daily E_2 administration; mean plasma P_4 concentrations dropped to estrous values of <1 ng/ml at the expected time (Days 14−20) and were associated with the beginning of estrous behavior for mares in each of the three treatment groups (Table 1).

The mean length of estrus was longer (P<0.05) for mares in the high E_2 group compared with mares in the control and low E_2 groups. Mares in the control and low E_2 groups ceased estrous behavior by 2 days postovulation (Days 21−25), while mares in the high E_2 group did not ovulate but remained in estrus until 2 or 3 days after their last E_2 injection (Day 31). This period of prolonged estrus was followed by typical diestrous behavior for the next 13 to 16 days and a second estrus which was accompanied by ovulation ∿21 days after the last E_2 injection. This was reflected by a prolonged IOI (P<0.05, Table 1).

The mean diameter of the largest palpable ovarian follicle during the treatment period was smaller (P<0.05) for mares in the high E_2 group compared with the mean diameter for the largest follicle for mares in the control and low estradiol groups (Table 1).

Plasma Hormone Concentrations

E_2 administration had no significant effect on plasma P_4 concentrations (Fig. 1). Mean P_4 concentrations for mares in all three groups averaged between 3 and 4 ng/ml on Day 5 postovulation, increased to between 6 and 10 ng/ml by Day 10 post-ovulation, and then dropped to estrous concentrations of below 1 ng/ml by Day 20 postovulation and remained low during estrus. Mean P_4 concentrations rose to between 3 and 4 ng/ml by Day 5 postovulation of the post-treatment cycle, indicating the establishment of new CL in mares in the control and low E_2 groups; however, mares in the high E_2 group, which did not ovulate, continued to have low P_4 concentrations between 0.5 and 1.5 ng/ml until the end of treatment (Day 31 postovulation).

Plasma LH concentrations followed their expected pattern in mares from all three groups up to the day of ovulation and are depicted in Fig. 2. Mares in the high E_2 group did not ovulate, and thus day of ovulation values are represented by LH concentrations on Day 23 postovulation of the treatment cycle [the use of Day 23 as an expected ovulation date was based on the mean IOI for mares receiving 0 or 1 mg E_2/day under similar conditions in a previous experiment (Woodley et al., 1979)]. Five days later (Day 28 postovulation), these mares had still not ovulated, had low P_4 concentrations, and significantly higher LH concentrations compared with mares in the control or low E_2 groups which had ovulated 5 days earlier and in which LH levels had declined to early luteal phase levels (1−3 ng/ml).

Examination of plasma FSH concentrations which are shown in Fig. 3 revealed that the high E_2 treatment beginning on Day 1 postovulation resulted in significantly lower FSH concentrations on the 5th day of treatment as compared with concentrations in the control mares, while mares in the low E_2 group had concentrations between those in the control and high E_2 groups. On Day 10 postovulation, FSH concentrations were at low basal levels for mares in all three groups except for one mare in the low estradiol group which appeared to have elevated concentrations. By Day 14 postovulation, mean FSH concentrations were between 6 and 9 ng/ml with no significant differences among the three groups. During midestrus (Day 4), mean FSH concentrations were between 2.5 and 6 ng/ml, and although not statistically significant,

TABLE 1. Effect of daily injections of estradiol-17β on the length (days) of various aspects of the estrous cycle and on the mean diameter (mm) of the largest palpable follicle during the treatment period in mares (mean ± SEM).

	Estradiol-17β (mg)		
Endpoint	0 (Controls)	1.0	10.0
	Days		
Luteal lifespan			
Day that plasma P_4 became <1 ng/ml	15.5[a] ± 1.0	16.0[a] ± 0	17.8[a] ± 1.8
Interestrous interval	15.2[a] ± 1.0	16.0[a] ± .6	17.5[a] ± 1.4
Estrus	6.6[a] ± 1.2	7.2[a] ± 1.2	15.5[b] ± 2.5
Interovulatory interval	21.2[a] ± 1.1	22.8[a] ± 1.6	52.2[b] ± 2.5
	Diameter of largest follicle (mm)		
	36.0[a] ± 3.1	33.0[a] ± 3.5	15.0[b] ± 3.5

[a,b]Means within a row with a different superscript are different (P<0.05).

FSH concentrations appeared lower in the control mares (<3 ng/ml) compared with those in the high E_2 group (5–7 ng/ml).

In fact, mares in the high E_2 group had stable FSH concentrations between 4 and 7 ng/ml during the entire prolonged period of estrus in which follicular growth and ovulation were suppressed. By Day 5 of the post-treatment cycle or Day 28 postovulation for mares in the high E_2 group, mean FSH concentrations were between 4 and 8 ng/ml, with no significant differences among the three treatment groups.

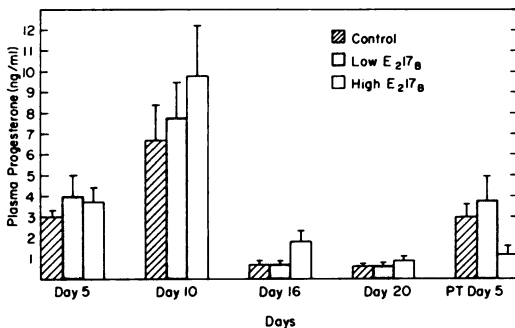When FSH concentrations were examined within treatment groups over the days studied,

FIG. 1. Plasma P_4 concentrations (ng/ml ± SEM) in mares injected daily with 0 mg (controls), 1.0 mg (low $E_2$17β), or 10 mg (high $E_2$17β) estradiol-17β. Days 5, 10, 16, and 20 are days after the pretreatment ovulation. Post-treatment (PT) Day 5 is the 5th day after the first ovulation during the treatment period for controls and low E_2 groups. Since mares in the high E_2 group did not ovulate during the treatment period, Day 28 from the pretreatment ovulation was used as PT Day 5 for this group. There were no differences (P>0.05) among groups within each of the 5 days studied.

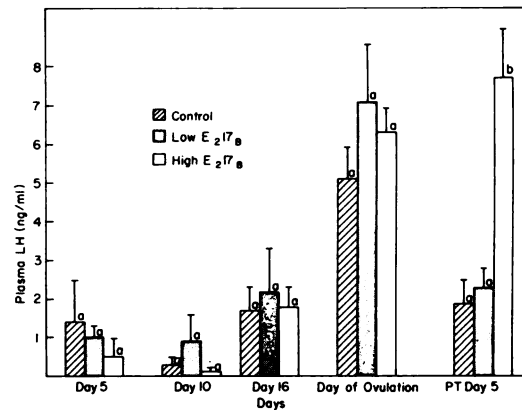FIG. 2. Plasma LH concentrations (ng/ml ± SEM) in mares injected daily with 0 mg (controls), 1.0 mg (low $E_2$17β), or 10.0 mg (high $E_2$17β) estradiol-17β. Days 5, 10, and 16 are days after the pretreatment ovulation, and day of ovulation is the day mares ovulated during the treatment period for controls and low $E_2$17β groups. Since mares in the high E_2 group did not ovulate during the treatment, Day 23 from the pretreatment ovulation was used as day of ovulation for this group. Post-treatment (PT) Day 5 is the 5th day after the first ovulation during the treatment period for controls and low $E_2$17β groups, while Day 28 from the pretreatment ovulation was used as PT Day 5 for the high $E_2$17β group. Superscript letters denote differences (P<0.05) among groups within each of the 5 days studied.

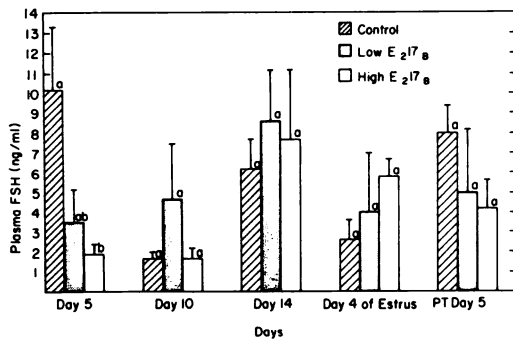FIG. 3. Plasma FSH concentrations (ng/ml ± SEM) in mares injected daily with 0 mg (controls), 1.0 mg (low $E_2 17\beta$), or 10.0 mg (high $E_2 17\beta$) estradiol-17β. Days 5, 10, and 14 are days after the pretreatment ovulation, and Day 4 of estrus is the 4th day of the first estrus during the treatment period. Post-treatment (PT) Day 5 is the 5th day after the first ovulation during the treatment period for controls and low $E_2 17\beta$ groups. Since mares in the high $E_2 17\beta$ group did not ovulate during the treatment period, Day 28 from the pretreatment ovulation was used as PT Day 5 for this group. Superscript letters denote differences (P<0.05) among groups within each of the 5 days studied.

Previous studies have suggested that diethylstilbestrol (DES) was able to prolong CL life span when administered during mid-diestrus (Nishikawa, 1959; Berg and Ginther, 1978). It may be that due to its longer duration of action DES administered once daily is capable of maintaining luteal function. Further studies with multiple E_2 injections, E_2 implants, or longer acting estrogens should be conducted. It should be mentioned that in preliminary experiments daily administrations of E_2 in doses of 0.1, 1, or 10 mg/day beginning on Day 7, 10, or 14 postovulation had no effect on luteal life span based on interestrous and interovulatory intervals (n = 2 or 3/group), which suggests that treatment beginning later in the cycle also was ineffective in maintaining luteal function.

The increased length of estrus (Table 1) for mares in the high E_2 group is most likely due to the effect of E_2 in the presence of basal P_4 concentrations. Previous reports have demonstrated that a single injection of 0.5 mg of E_2 will induce estrous behavior in ovariectomized mares (Hillman and Loy, 1975). All mares in
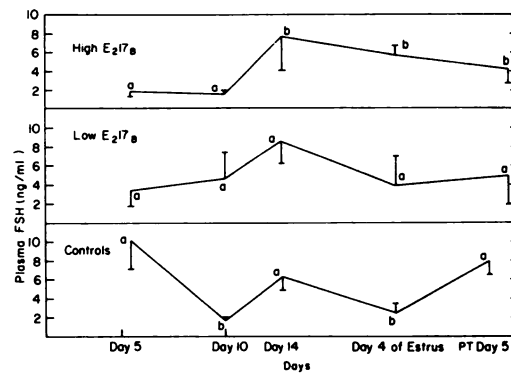
three different patterns were observed as shown in Fig. 4. Mares in the control group displayed a bimodal FSH pattern during the estrus cycle, one peak during early diestrus and a second one during late diestrus, while levels were consistently low during mid-diestrus and estrus. FSH concentrations were higher (P<0.05) on Days 5 and 14 postovulation compared with concentrations on Day 10 postovulation and Day 4 of estrus. Mares in the low E_2 group had extremely variable FSH concentrations with only one apparent peak during late diestrus, which was not significantly different from all of the other days measured. Mares in the high E_2 group seemed to exhibit a prolonged, slowly declining unimodal pattern of FSH secretion with concentrations on Days 5 and 10 postovulation being lower (P<0.05) than concentrations on Day 14 postovulation, Day 4 of estrus, and Day 28 postovulation of their treatment cycle.

DISCUSSION

Daily administration of 10 mg E_2 to mares beginning Day 1 postovulation did not significantly affect luteal function (Table 1). These results are in agreement with those of Woodley et al. (1979), and show that exogenous E_2 in doses up to 10 mg/day was unable to act as a luteotropic or antiluteolytic signal in the mare.

FIG. 4. Plasma FSH concentrations (ng/ml ± SEM) in mares injected daily with 0 mg (controls), 1.0 mg (low $E_2 17\beta$), or 10.0 mg (high $E_2 17\beta$) estradiol-17β. Days 5, 10, and 14 are days after the pretreatment ovulation, and Day 4 of estrus is the 4th day of the first estrus during the treatment period. Post-treatment (PT) Day 5 is the 5th day after the first ovulation during the treatment period for control and low $E_2 17\beta$ groups. Since mares in the high $E_2 17\beta$ group did not ovulate during the treatment period, Day 28 from the pretreatment ovulation was used as PT Day 5 for this group. Superscript letters denote differences (P<0.05) within the different treatment groups. For controls, Days 5, 14, and PT Day 5 vs Day 10 and Day 4 of estrus. In the high E_2 group, Days 5 and 10 vs Day 14, Day 4 of estrus, and PT Day 5.

the high E_2 group ceased estrous behavior by the 3rd day after the last E_2 injection.

The effect of E_2 on LH secretion in this study (Fig. 2) is in agreement with a previous study (Garcia and Ginther, 1978) which showed that E_2 is stimulatory and progesterone is inhibitory to LH secretion in the mare. Also, it appears that E_2 is able to stimulate LH secretion for up to 2 weeks in mares with low progesterone concentrations.

It seems that the reason mares in the high E_2 group did not ovulate during treatment was because the high E_2 treatment suppressed follicular growth throughout the treatment period (Table 1). This finding is in agreement with previously published studies using estradiol-17β (Woodley et al., 1979) or DES (Berg and Ginther, 1978). Daily estrogen administration has also been shown to result in a reduction of follicular development in heifers and ewes (Greenstein et al., 1958; Piper and Foote, 1968). In comparative studies, daily treatment of mares, cows, goats, and sows with DES (3—10 mg) also suppressed follicular growth (Nishikawa, 1959). It is interesting that decreased follicular growth in the present study was associated with suppression of FSH concentrations during early diestrus at a time when FSH was elevated in the control mares and an early wave of follicular growth is thought to occur (Pineda et al., 1973). This suggests that FSH secretion during early diestrus and/or the wave of follicular growth that accompanies it may have a preparative role in the follicle destined for ovulation. Nishikawa (1959) was able to induce similar suppression of follicular growth in mares treated with DES and was able to overcome this suppression with exogenous administration of pregnant mare serum gonadotropin (PMSG) for 10 or 11 days in two mares. Treatment with PMSG commenced after the last DES injection, and no contemporary controls were included in his experiment. This suggests that DES suppressed follicular growth without altering follicular gonadotropin receptors. However, further studies are needed to elucidate this effect. It should be noted in the present study that E_2 treatment was continued through the time of expected follicular growth and ovulation, and it is possible that E_2 treatment during this period prevented ovarian follicles from responding to FSH and LH.

The bimodal pattern of FSH secretion in the control mares is in agreement with previous studies conducted early in the breeding season

(Evans and Irvine, 1975). Recently, it has been reported that this bimodal pattern during the early breeding season changes to a unimodal pattern of secretion during the late breeding season (August-October; Turner et al., 1979). However, it seems likely that seasonal changes in the FSH secretory pattern are not a contributing factor in this experiment because E_2 administration is equally effective in suppressing follicular growth in the late breeding season (Woodley et al., 1979).

The result of this study confirms previous findings that daily E_2 administration is a potent inhibitor of follicular growth in mares (Woodley et al., 1979). It also shows that this treatment is equally effective in the early and late breeding season. And last, it shows that mares in the control group exhibited a bimodal pattern of FSH secretion while the high E_2 treatment altered the pattern of secretion with suppression of FSH concentrations during early diestrus. Studies are currently being conducted to determine if estrogen is involved in the physiological regulation of FSH secretion in the mare or if the suppression of FSH observed in this paper is a pharmacologic action of estrogen.

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