SEASONAL OVARIAN FUNCTION IN FERAL MARES: Seasonal Patterns of LH, Progestins and Estrogens in Feral Mares

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SUMMARY

The reproductive physiology of feral mares in North America is largely unstudied, thus it was of interest to examine seasonal ovarian function in these animals. Four adult mares of proven fertility from the Pryor Mountain National Wild Horse Range (Montana) were captured, and maintained in a corral on alfalfa hay. After 3 months acclimation, blood was collected every 3 days for 13 months (Sept. 1978-Sept. 1979). A teaser stallion was kept in an adjacent corral and the mares were tested for behavioral estrus. Plasma LH and total estrogens (E) were measured by RIA and progesterone (P) by CPB assay. In the captive mares average basal plasma LH levels (ng/ml) were greater from Apr. through July (8.1+/-0.5) than from Nov. through Jan. (2.2+/-0.2). A total of 21 LH peaks occurred between Apr. 13 and Aug. 31 among the four mares. Many peaks exceeded 20X basal levels, and a trend of higher LH levels in each succeeding peak was observed. Basal to peak LH increases were greater than those reported for domestic mares. In all instances except one, LH peaks were associated with P levels of 0.5 ng/ml or less and with concomitant elevations of E (peak average=43.1+/-12.1 pg/ml). Basal P levels from Apr. through July (1.5+/-1.2 ng/ml) did not differ significantly from levels for Oct. through Jan. (1.1+/-0.7 ng/ml), nor did basal E levels differ significantly between these two time periods (8.4+/-3.2 pg/ml versus 12.9+/-4.6 pg/ml respectively). Behavioral estrus always occurred with LH and E peaks from Apr. through July. However, behavioral estrus was occasionally observed from Aug. through Oct., when LH peaks no longer occurred, suggesting that behavioral estrus is not a reliable indicator of ovarian cycling in feral mares.

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INTRODUCTION

Presently, an estimated 85,000 feral horses inhabit public lands in ten western states, up from an estimated 15,000 in 1972. These horses are protected under feral laws and as a result population increases have been unchecked. The horses are of mixed or in some cases unknown ancestry and little is known about their basic reproductive biology. Although the reproductive patterns of feral horses may not differ from domestic horses, some herds have been free-roaming for over 200 years in extremely harsh environments and the possibility of adaptive changes must be considered. In order to better understand feral mare reproductive patterns and their control, the present study was done to determine seasonal changes in plasma LH, progesterone and total estrogens in captive feral mares.

METHODS AND MATERIALS

Three mares from the Pryor Mountain Range were captured in May of 1978, and transported to Billings, Montana (latitude 45° N). A fourth Pryor Mountain mare was acquired in November, 1978. Three of the mares (Tiger Lily, age 5; Columbine, age 7; Dahlia, age 15) were of proven fertility, and the fertility of one (Sunflower, age 4) was unknown. The mares were confined to a corral approximately 10,000 m^2 which was adjacent to a corral confining a domestic quarterhorse stallion. Each mare was fed 9 kg of alfalfa hay per day (half in the morning and half in late afternoon). In an effort to approximate natural conditions, no shelter was provided except for the left side of a small building. Free-running water was available only during periods when deep snow was absent. Under natural conditions, there are no free-water sources during freezing weather and the feral animals regularly eat snow.

Blood Collection

After a 3-month training period to acclimate the animals, blood collections were begun in September, 1978. Each

horse was blindfolded with blood collected from the free-standing unrestrained animal by jugular venipuncture in 10 cc heparinized Vacutainers every three days through September 19, 1979. The blood was centrifuged immediately at the corral and the plasma was frozen and stored at -20°C.

Behavioral Assessment

Estrus behavior in the captive mares was evaluated as described by Ginther (1974) after bringing the mares into close proximity with the domestic teaser stallion. The behaviors of (1) raising the tail, (2) urinating during teasing, and (3) winking the clitoris were taken collectively to indicate behavioral estrus. No attempt was made to use a more refined scale or to quantify the intensity of estrus behavior.

Hormone Measurement

Plasma LH was assayed by the double antibody radioimmunoassay described by Niswender, *et al.* (1978). The antibody was GDN15 antiovine LH antibody, and the equine reference standard used in all assays was LER-1138-1 (potency=0.27 NIH-LH-SI). The percent equine LH bound was 29.5%+/-2; the 50% intercept was 17.7 ng ligand/ml; the slope for the standard curve was -2.11 and the correlation coefficient of accuracy was r=0.987. Since plasma LH has not been previously measured in feral horses, it was necessary to validate the assay in these animals. Domestic equine LH standard curves showed identical slopes to serially diluted plasma from peak estrus feral mares when assayed using the GDN15 anti-LH antibody. PMSG standards were also assayed using GDN15 antibody and showed 35% cross reactivity compared to LH. Measurement of domestic equine LH standards and feral horse plasma described above using (1) GDN15 and (2) an anti-PMSG antibody (supplied by Dr. M. Garcia) validated for LH revealed no difference in slopes between assay (1) and assay (2). These data suggest that the GDN15 double antibody radioimmunoassay measures immunoreactive LH in feral horse plasma which is not assayably different from LH in the domestic horse. Cross reactivity with PMSG in this assay must be considered in data evaluation.

Total plasma progestin concentrations were measured by the competitive-protein-binding assay of Johansson (1969) with several modifications. Petroleum ether (boiling point=30-60°C) was used to selectively extract progestins from plasma. Dog plasma was used as a source of binding protein and Florisil 60-100 mesh was used to separate bound from unbound steroid. Recovery, measured by internal standard, was 96.3+/-5.2%. The coefficient of variation for precision was 8.4% for 12 duplicate determinations assayed on different days. Distilled water blanks, in 12 duplicate assays, yielded a value of 0.13+/-0.04 ng. Accuracy was determined by measuring known amounts of progesterone in water blanks. The correlation coefficient as determined by linear regression analysis gave a value of r=0.981.

Total estrogens were measured by radioimmunoassay, described by Lindner, *et al.* (1972), using ovine antiserum prepared against estradiol-17 beta-succinyl-bovine serum albumin. The lyophilized antiserum was rehydrated in phosphate buffer (pH=7.0) and labelling was carried out with 6,7-^{3}H estradiol-17β. The specific activity was 40-60 Ci/mM and the total mass of tracer was 35-55 pg. The antiserum was diluted to give 50% binding. To aliquots of mare plasma ranging in size from 0.5 to 2.0 ml, 700-1000 cpm of 6,7-^{3}H estradiol-17β was added to determine procedural losses. Estrogens were extracted as described by Nett, *et al.* (1973). To each tube, 0.1 ml of phosphate buffer and 0.05 ml of labelled antiserum were added, and incubation was carried out for 24 hours at 4°C. Separation of free from bound antigen was achieved by selective absorption of the bound with activated destran-coated charcoal and centrifugation (1200 g x 15 min) at 4°C. The supernatant was decanted into 5.0 ml of scintillation cocktail (PPO-POPOP) and counted on a Beckman model LS-100C scintillation system for five minutes. The cross reactivity of the antiserum is shown in Table 1. Recovery of estradiol after extraction was 89.5+/-3.5%. The coefficient of variation for precision was 10.8% for 15 duplicate determinations carried out on different days. The interassay and intra-assay variation was 12.7% and 8.6% respectively. Accuracy was determined by measuring known amount of estradiol-17β. For 15 duplicate samples a linear regression analysis yielded a correlation coefficient of r=+0.99. Ten distilled water blanks gave an average value of 2.7+/-2.1 pf. The lowest value for measurable estradiol standards which differed significantly from the blank value (P less than 0.05) was 5.0 pg/ml.

TABLE 1

Cross Reaction of Total Estrogen Antiserum with Various Steroids

Steroid	Percent Cross Reaction at 50% Displacement
17 beta-estradiol	100
Estrone	50
17 alpha-estradiol	0.1
Estriol	15
Equilin	5
Equilenin	7

(Corticosterone, cholesterol, progesterone, 17-hydroxyprogesterone, cortisol, deoxycortisol, deoxycorticosterone and testosterone all reacted at 0.05% or less.)

RESULTS

The 13-month profiles of plasma LH, progesterone and total estrogen concentrations for the four mares are shown in Figure 1. Peak concentrations of LH which were 12 to 25 times greater than average baseline levels commenced on April 13 and ended August 31; 16 of the 21 LH surges were associated with ovulation. The duration of LH elevation above baseline averaged 7.2 days. Ovulatory LH peaks were associated with progesterone levels of 0.5 ng/ml or less and peak total estrogen concentrations of 43.1+/-12.1 pg/ml. LH peaks were followed by increased plasma progesterone concentrations which remained elevated for 6-9 days (peak average=7.9+/-0.5 ng/ml). Average basal plasma LH levels were significantly greater (P less

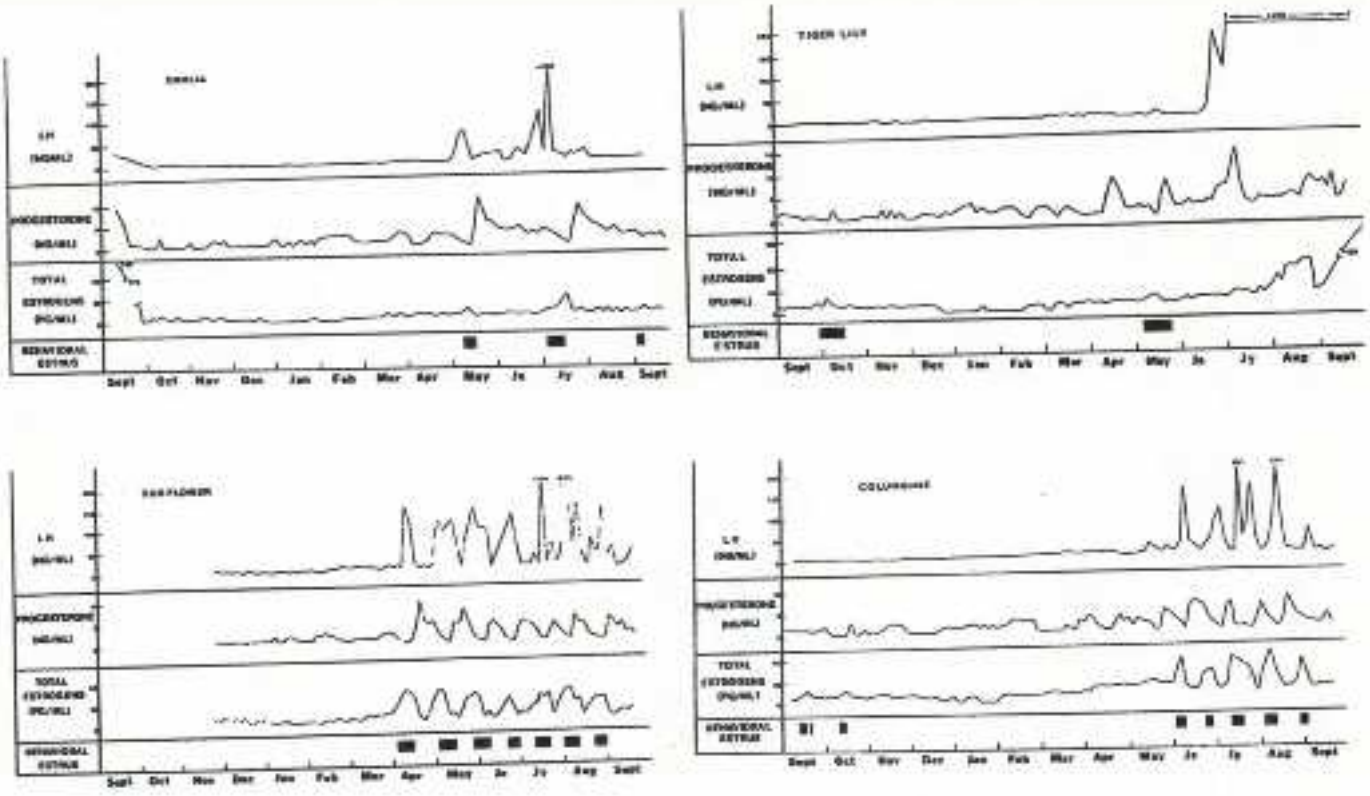

Figure 1. Seasonal patterns of plasma LH, progesterone, and total estrogens in four feral mares.

than 0.05) from April through July (8.1+/-0.05 ng/ml) than from October through January (2.2+/-0.2 ng/ml). Basal plasma progesterone levels from April through July (1.5+/-1.2 ng/Ml) did not differ significantly (P less than 0.05) from levels for October through January (1.1+/-0.7 ng/ml), nor did basal total estrogen levels differ significantly (P greater than 0.05) between these two time periods (8.4+/-3.2 pg/ml versus 12.9+/-4.6 pg/ml, respectively). Basal values are defined as any value not involved (i.e., increasing or decreasing) in a hormone surge associated with physiological estrus. An illustration of two successive estrus cycles in Sunflower is shown on an expanded scale in Figure 2.

Behavioral estrus accompanied all LH peaks but was also seen twice in Columbine, once in Tiger Lily and once in Dahlia during diestrus in the fall. Behavioral estrus which accompanied LH peaks (Table 2) ranged in length from six to ten days with a mean of 8.4+/-1.1 days. All behavioral estrus during the ovulatory season was associated with elevated total plasma estrogens. Four periods of behavioral estrus noted in the fall, which were not accompanied by LH surges were associated with an increase in estrogen concentration. Diestrus in Columbine and Sunflower ranged in length from 9 to 15 days with a mean of 12.5+/-1.9. Dahlia had a 53 day diestrus and Tiger Lily became pregnant following her first ovulation.

The precipitous drop of plasma hormones in Dahlia in September 1978 preceded an aborted foal (September 24)(Figure 1). The fetus had a crown-rump length of 41 cm, which dates conception at approximately mid-March, 1978, and the age of the aborted fetus at

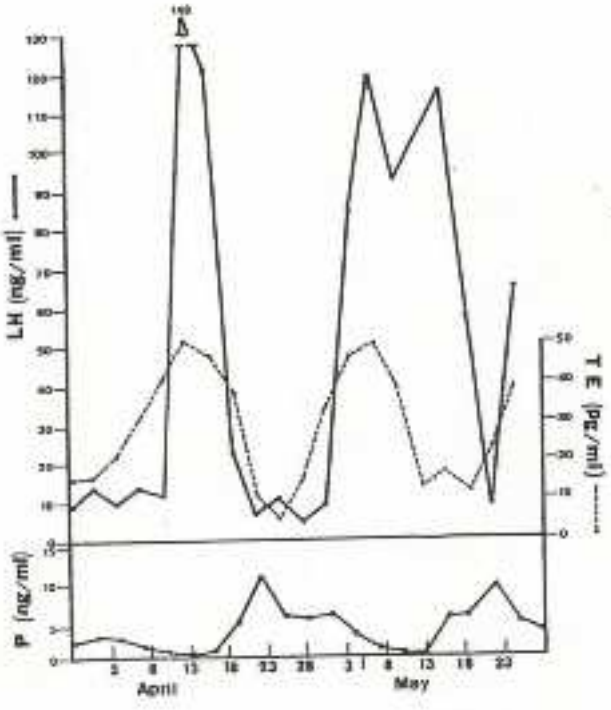

Figure 2. Two successive estrus cycles in the mare Sunflower.

TABLE 2

Frequency and Length of Behavioral Estrus and Diestrus During the Breeding Season

Horse	Age	# of Estrus Cycles	Mean Length (days) of Behavioral Estrus	Mean Length (days) of Diestrus
Tiger Lily[1]	5	1	6	-
Columbine	7	6	8.5 +/-1.0 (S.D.)	12.6 +/-1.5 (S.D.)
Dahlia	15	2	8.5 +/-0.7 (S.D.)	53
Sunflower	4	7	8.33 +/-1.4 (S.D.)	12.5 +/-2.2 (S.D.)

[1]became pregnant following first ovulation

somewhere between 150 and 200 days. The apparent drop in LH probably primarily reflects a decrease in PMSG which cross-reacted with the LH antiserum. There was no behavioral estrus following the abortion and cause(s) of the abortion is not known.

During a period of physiological estrus in Tiger Lily in mid-May 1979, a stallion broke into the mare's corral. The incident occurred at night and the stallion was assumed to have spent about six hours with the estrus mare. Thirty-six to 41 days later, gonadotropin concentrations abruptly rose to greater than 200 ng/ml and remained there until exactly 120 days post-breeding, after which they began to decline until the end of the study at 126 days. Following the assumed breeding, progesterone never returned to basal levels and increased slowly but steadily for the next 120 days. During the final four weeks of the study (days 98-126 post-breeding) plasma progesterone levels averaged 6.4+/-1.8 ng/ml. At approximately 65 days post-breeding, total estrogen concentrations began to increase sharply, culminating at 80 pg/ml at 126 days. In accordance with federal regulations, the mares were returned to the Pryor Mountains immediately following the study, and in early June 1980 Tiger Lily was identified on the horse range accompanied by a foal.

DISCUSSION

Endocrine data indicates that the feral mares are seasonal breeders. The ovulatory season of the feral mares begins at roughly the same time of the year reported for domestic mares (Kenney, *et al.*, 1975). The four captive feral mares in this study represent a small sample size but it is nonetheless significant that none of the four mares ovulated after August 31. This is in contrast to studies reporting significant numbers of domestic mares ovulating through the fall and into the early winter (Ginther, 1974; Van Niekerk, 1967; Satoh and Hoshi, 1932; Palmer, 1978). Collectively these studies report that the percent of the mares ovulating in the fall and winter ranges from 60% in September to approximately 20% in December. The ovulatory season of ponies ends sooner than in domestic horses and more closely approximates the ovulatory season of the feral horses (Ginther, 1974; Satoh and Hoshi, 1932; Wesson and Ginther, 1981). Palmer (1978) found no ovarian activity in winter among Welsh ponies compared to 66% in saddle-type mares. In one study of semi-wild Korean ponies (Satoh and Hoshi, 1932), all mares ceased ovulating by October 1. Ginther (1979) speculates that it was the primitive nature of these animals which resulted in a sooner and more abrupt end to the ovulatory season. The Pryor Mountain horses are thought to represent the most primitive types in the U.S. (Hall, 1972) and striking differences in the age at which sexual maturity is attained have been reported. Since the feral horses have inhabited the Pryor Mountain region for at least 200 years (there is speculation they may have been there even longer, Wyman, 1945), it is possible that natural selection has led to a population of mares with a more well defined ovulatory season than domestic mares. This ovulatory season, in contrast to the longer and more variable breeding season reported for domestic mares, would limit foaling to the period most favorable for survival. Since the origin of America's feral horses is diverse, care must be taken not to attribute this physiological difference to all feral mares. Also, Palmer (1978) has shown that seasonal reproductive patterns of domestic mares will differ from year to year and this initial study of feral mares encompasses only one year. Osborne (1966), Sharp and Ginther (1975), Kooistra and Ginther (1975), and Freedman, *et al.* (1979) have all demonstrated the stimulating effects of lengthening photoperiods upon the initiation and maintenance of ovarian activity in mares. The peak breeding activity of the feral mares coincides with the summer solstice in June.

Based on data from the present study, it appears that nutrition does not play a major role in determining the onset and length of the ovulatory season in the feral mares. Each mare was given 9 kg of alfalfa hay daily and approximately 500 gms of grain (corn-oats-molasses mixture on bleeding days). This regimen resulted in relatively stable weights over the 13 month study period. It was assumed that the captive mare's plane of nutrition was equal to or greater than that on the two ranges for two reasons: (1) on the Pryor Mountain range, January through March are extremely lean months nutritionally and are characterized by significant weight losses and mortality, and (2) Hall (1972) has documented the selection of winter forage among the Pryor horses and few vegetation types have substantial nutritive value. In spite of the assumed improvement in nutrition in the captive mares, their ovulatory season was not different from that of mares on the range, based on the appearance of the foals.

The length of behavioral estrus and diestrus among these mares revealed several irregularities. On two occasions diestrus lasted for only nine days (Columbine and Sunflower). This was significantly shorter than the average length of diestrus (about 12.5 days for both mares). In both cases progesterone levels dropped below 1.0 ng/ml by day nine, but the cause(s) are not known. One possible cause for the shortened diestrus could have been endometritis which can result in premature luteolysis. Another possible explanation is that tests of behavioral estrus were carried out only on the days blood was collected. Thus, by testing for behavior estrus every three days, some error in the length of diestrus is probable, however, in each case the diestrus was accompanied by the appropriate endocrine profile for the three hormones studied.

Dahlia, the 15-year old mare, went into a prolonged diestrus which lasted 53 days, despite the fact that progesterone dropped below the 1.0 ng/ml level on several occasions. She did not show the classic progesterone levels seen in cases of persistent corpora lutea. The cause(s) for this prolonged diestrus remain unknown.

Endocrinologically, ovulation in the feral mares was qualitatively similar to ovulation in domestic mares. Since blood samples were taken every three days, the precise daily relationships of LH, progesterone, and total estrogens to one another were impossible to determine, but the general temporal relationships are clear. Quantitatively both estrus and diestrus levels of total estrogens are similar to those reported by Nett, *et al.* (1973) and Plotka, *et al.* (1975). Although seasonal variations for urinary estrogens have been reported previously (Hillman and Loy (1975) no descriptions of seasonal plasma estrogens were available. Seasonal changes in total estrogens in the feral mares appear to result only from estrogen surges during estrus and not from elevated basal levels.

Plasma progesterone concentrations during the ovulatory season compared favorable with those reported by Stabenfeldt, *et al.* (1975) for domestic mares and by Sharp and Black (1973) for ponies. Basal plasma LH levels were similar among the feral mares, and the average basal values were significantly greater from April through July (breeding season) than from November through January. In domestic mares quantitatively similar LH concentrations and seasonal patterns have been reported (Garcia, *et al.*, 1979). The durations of the LH elevations were similar among the feral mares and this average (7.2 days) was comparable to durations reported for domestic mares (Geschwind, *et al.*; 1975: Noden, *et al.*, 1975; Evans and Irvine, 1975). In the feral mares peak LH levels were much greater in some cycles than in others, with an increasing trend as the cycling season progressed. Evans, *et al.*,(1979) has reported episodic LH secretion during LH peaks in some domestic mares. It was not possible to determine if this occurred in feral mares due to long intervals between samples. Many LH peaks exceeded 20X basal levels, which was much greater than the 5 to 10 fold increases reported in domestic mares (Pattison, *et al.*, 1972; Whitmore, *et al.*, 1973; Geschwind, *et al.*, 1975; Snyder, *et al.*, 1979).

This difference is probably not due to feral-domestic differences in the immunoreactivity of the LH released. With both the GKN15 and the anti-PMSG antibodies, domestic LH standard curves showed parallelism to LH dilution curves in plasma from peak estrus feral mares. The possibility that intrinsic differences exist between feral and domestic mares in the regulation of the hypothalamic-hypophysical-ovarian axis deserves investigation.

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