OVARIAN FUNCTION IN CAPTIVE FERAL MARES

Authors: Plotka, E. D., Vevea, D. N., Eagle, T. C., Siniff, D. B., and Tester, J. R.

Source: Journal of Wildlife Diseases, 25(4) : 574-579

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-25.4.574

Journal of Wildlife Diseases, 25(4), 1989, pp. 574–579

OVARIAN FUNCTION IN CAPTIVE FERAL MARES

E. D. Plotka,[1] D. N. Vevea,[1] T. C. Eagle,[2] D. B. Siniff,[2] and J. R. Tester[2]

[1] Marshfield Medical Research Foundation, Inc., 510 North St. Joseph Avenue, Marshfield, Wisconsin 54449, USA

[2] Department of Ecology and Behavioral Biology, University of Minnesota, Minneapolis, Minnesota 55455, USA

ABSTRACT: Ovarian function was monitored for 33 mo in captive feral mares (*Equus caballus*) by following serum progesterone (P) levels. A P level >2.0 ng/ml was considered indicative of ovulation. Feral mares were seasonally polyestrus with the majority of animals ovulating between May and October. During the first year after capture, none of the mares ovulated during the anestrous season. However, in subsequent years, approximately 10% of mares ovulated during the months of November, January and February. P levels during the luteal phase of the cycle ranged from 2.0 to 21.0 ng/ml which were similar to levels in domestic breeds of mares. The pattern of P concentrations during pregnancy was also similar to the pattern in domestic mares. These data confirmed the seasonality of ovulation in feral mares but indicated that this seasonality was not as rigid as previously believed. Captive feral mares were similar to domestic breeds in the percentage of mares ovulating all year and in the P levels achieved during the estrous cycle and pregnancy.

Key words: Ovarian function, ovulation, serum progesterone, feral mares, *Equus caballus*, captive study.

INTRODUCTION

There are few studies on seasonal ovarian activity patterns in feral horses (*Equus caballus*). Previous studies indicate that feral horses are sharply seasonal with respect to breeding and foaling. Kirkpatrick and Turner (1983) reported that data from four feral mares suggested that behavioral estrus always occurred coincident with luteinizing hormone (LH) and estradiol peaks from April through July. However, behavioral estrus was occasionally observed from August through October, when LH peaks no longer occurred. These data were interpreted as suggesting that behavioral estrus is not a reliable indicator of ovarian cycling in feral mares (Kirkpatrick and Turner, 1983). More recently, Keiper and Houpt (1984) studied the reproductive rate of the free-ranging ponies on Assateague Island National Seashore (USA). They reported that 98% of foals were born between May and July. Less than 1% were born in August and September. Since gestation in the horse is approximately 11 mo, their data indicate that the reproductive season occurred between June and August.

The purpose of the present study was to determine the seasonal occurrence of ovulation in feral horses maintained in captivity over a 3 yr period. In much of the study, ovulation was measured indirectly by demonstrating an elevation in serum progesterone (P) concentrations indicative of the presence of a functional corpus luteum. In addition, we also report on P concentrations in blood serum during the luteal phase of the estrous cycle and during pregnancy.

MATERIALS AND METHODS

The 30 mares used in the study were captured in the spring of 1985 and after initial processing were confined at the Bureau of Land Management (BLM) Wild Horse Holding Facility in Lovelock, Nevada (USA; 40°11′N, 118°23′W). Mares were aged according to tooth wear (Ensminger, 1969) by BLM personnel and ranged between 4- and 9-yr-old. The reproductive tract of each mare was palpated per rectum to ensure each female was nonpregnant. Mares were housed with mares on another study in 30 × 30 m pens at a maximum density of 50 per pen. They were fed a ration of chopped alfalfa hay and straw twice daily, and allowed water ad libitum. From May through July 1986, they received 1.8 kg of pelleted supplement per day.

We collected blood from all mares at the beginning of the study in November 1985 and from a randomly chosen sample of five mares every 2 wk through 11 March 1986. From March through August 1986 blood was collected from

any mare that exhibited behavioral estrus 10 days after she exhibited estrus. Biweekly samples were collected from all mares from August through November 1986. Monthly samples were collected from November until March in both 1986 and 1987 and biweekly samples were collected from March through October during 1987 and March through July during 1988. On several occasions, a mare escaped the handling procedure and a sample was not collected. Thirteen mares died or were lost over the study period.

The bleeding procedure consisted of restraining the mare in a squeeze chute and puncturing the jugular vein with a 15 ga needle attached to a 35 ml syringe. Two to 5 ml of blood was immediately transferred to a vacuum tube containing Na EDTA for hematological analyses; the remainder was transferred to plain vacuum tubes and allowed to clot for 4 to 12 hr. The clotted blood tubes were then centrifuged, and the serum was decanted and frozen until assayed for levels of P.

From March through July of 1986, the mares were tested twice weekly for estrous activity (Ginther, 1979; Plotka et al., 1988b). Detection of estrus was accomplished by observing mares for behavioral patterns related to estrus (Ginther, 1979), including clitoral winking, frequent urination, and posturing. Groups of four to six mares were placed in a runway between two pens containing about 50 stallions each at the holding facility. The mares were allowed to stand for 20 to 30 min, then were observed for a period of 20 to 30 min and the incidence of pertinent behavior was recorded. Status of estrus (in estrus or not in estrus) was determined from frequency of the appropriate behaviors. Ten days following a mare's exhibition of estrous behavior, she was restrained in the squeeze chute, and a sample of blood was collected. Ovulation was considered to have occurred if blood samples had a level of P above 2 ng/ml (Plotka et al., 1988b).

Three mares were placed with a stallion in mid-July 1986. These animals were bled on the same schedule as the other mares until within 2 wk of parturition. In May 1988, a fertile stallion was placed with all mares to determine if all mares were fertile as part of the other study.

P levels were determined by radioimmunoassay as previously described (Plotka et al., 1975, 1988b). The extraction procedure has a sensitivity of 10 pg and a coefficient of variation of 17% at 10 pg. The commercial kit radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California 90045, USA) has a sensitivity of 100 pg, a coefficient of variation of 33% at sensitivity and a coefficient of variation of 13% at 2 ng. In general, the kit slightly underestimates values above 1.5 ng/ml. Serum cortisol levels were estimated by competitive protein binding assay as previously described (Plotka et al., 1988a).

TABLE 1. Percentage of feral mares ovulating per month throughout a 32 month period.

Month	Ovulated/ tested	Percent ovulating
November	0/30	0
December	0/5	0
January	0/5	0
February	0/5	0
March	4/29	14
April	11/24	46
May	15/23	65
June	15/24	62
July	17/23	74
August	3/4	75
September	12/22	54
October	13/22	50
November	5/19	25
December	0/19	0
January	1/19	5
February	1/18	6
March	2/17	12
April	2/16	12
May	8/15	53
June	7/15	47
July	14/15	93
August	10/12	83
September	8/14	57
October	10/16	62
November	2/17	12
December	4/17	24
January	1/17	6
February	2/17	12
March	2/17	12
April	6/17	31
May	13/15	87
June	7/7	100
July	4/4	100

Hormone levels are presented as mean ± standard error throughout. When more than one sample was collected in a month's time, the multiple values for each animal were averaged to give the best estimate of the hormone level for that month. For this reason, values of 2.0 ng/ml or higher were considered to be representative of luteal function and the animal was considered to have ovulated. Since it was impractical to observe breeding, data for pregnant mares are aligned from the last sample collected before parturition. Statistical tests, including analysis of variance for repeated measures, Student's *t*-test and chi-square analysis were calculated using the Number Cruncher statistical program written by Dr. Jerry Hintze (Kaysville, Utah 84037, USA).

TABLE 2. Estrus and ovulation in individual feral mares throughout the study period.

	1985		1986												1987								
ID	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S
66	–	0	0	0	–	–	+	–	–	0	+	+	–	–	–[b]								
67	–	0	0	0	–	–	+	+	+	–	+	–	–	–	–	–	–	–	–	+	+	+	0
68	–	0	0	0	–	–	+	×	×	0	+	–	–	–	–	–	–						
69	–	0	0	0	–	+	×	×	×	0	+	–											
70	–	–	–	–	–																		
71	–	–	–	–	–	+	+	+	+	–	–	+	[	..... Pregnant/Lactating								]	+
72	–	0	0	0	–	–	×	+	+	0	+	–	–	–	–	–	–	–	–	–	–	–	–
73	–	0	0	0	+																		
74	–	0	0	0	–	+	+	+															
75	–	0	0	0	–	–	×	+	×	0	+	+	–	–	–	–	–	–	+	–	–	–	–
76	–	0	0	0	–	–	×	×	×	0	+	–	–	–	–	–	–	–	–	–	+	0	+
77	–	0	0	0	–	–	×	×	×	0	–	–	–	–	–	–	–	–	–	+	+	+	+
78	–	0	0	0	+																		
79	–	0	0	0	–																		
80	–	0	0	0	–	–	+	+	+	0	+	+	–	–	–	–	–	–	+	–	+	+	
81	–																						
82	–	–	–	–	–	+	+	–	+	+	–	+	[	..... Pregnant/Lactating									]
83	–	0	0	0	–	+	+	+	+	0	+	+	–	–	–	–	+	+	+	–	+	+	+
84	–	0	0	0	–	–	+	×	×	0	–	–	–	–	–	–	–	–	–	+	+	0	–
85	–	0	0	0	–	–	+	×	+	0	–	+	–	–	–	–	–	–	+	–	+	–	+
86	–	0	0	0	–	–	×	×	×	0	+	–	–	–	–	–	–	–	+	–	+	0	+
87	–	0	0	0	–	–	×	×	+	0	–	+	+	–	–	–	–	–	+	+	+	+	–
88	–	–	–	–	–	+	+	+	–	+	[	..... Pregnant/Lactating											]
89	–	0	0	0	–	+	+	+	×	0	–	+	–	–	–	–							
90	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	–
91	–	0	0	0	–	–	×	×	+	0	–	+	+	–	–	–	–	–	+	+	+	0	–
92	–	0	0	0	–	–	×	×	×	0	–	+	+	–	–	–	–	–	+	–	+	+	+
93	–	0	0	0	+	+	+	+	+	0	+	+	+	–	–	–	–	–	–	–	+	+	+
94	–	0	0	0	+																		
95	–	0	0	0	–	+	+	+	+	0	–	–	–	–	–	–	–	–	–	+	+	+	–

[a] Months beginning with November

[b] The symbols have the following meanings: +, positive evidence of luteal activity; –, no evidence of elevated P levels indicative of ovulation; ×, no estrous activity observed; 0, animal not tested; P, pregnant; and blanks after the last symbol mean the mare was lost to followup.

RESULTS

The incidence of ovulation is presented in Table 1. None of the mares sampled had elevated serum P concentrations between the start of the study in November 1985 and the next breeding season. Ovulation started in March of the first year with most of the animals ovulating by May. Greater than 50% of the mares continued to ovulate into October and five mares (25%) ovulated in November. One mare (6%) ovulated in January and February 1987 and two mares (12%) ovulated in March 1987. The majority of animals did not ovulate until May 1987 (Table 1). The second breeding season continued through October. Anestrus for the majority of animals occurred from November through March. However, one, two and two mares, respectively, ovulated in January, February and March of the third anestrous season (Table 2). Two of the mares ovulated during both winters.

P levels in open mares ranged from 0.1ng/ml to 21.0 ng/ml. P concentrations in the three pregnant mares ranged from 2.4 ng/ml to 87.8 ng/ml (Table 3) and averaged 8.4 ± 1.6 ng/ml. One value was >2 standard deviations higher than the rest of the values. Without this value, P

TABLE 2. Continued.

1987			1988								
O	N	D	J	F	M	A	M	J	J	A	S*
0	–	–	–	–	–	–	+	P			
+	–	–	–	–	–	–	+	+	+	+	P
–	–	–	–	–	–	–	+	+	P		
+	–	–	–	–	–	–	+	+	+	P	
–	–	–	–	–	–	–	–	+	P		
+	–	+	–	–	–	–	–	+	+	P	
+	+	–	–	+	+	+	+	P			
–	–	–	–	–	–	–	+	+	+	+	
+	+	–	–	–	–	–	+	P			
+	–	–	–	–	–	–	+	P			
+	–	+	–	–	–	+	P				
–	–	–	–	–	–	–	+	P			
–	–	–	–	+	+	+	+	P			
+	–	–	–	–	–	+	+	P			
+	–	+	+	–	–	–	+	+	P		
+	–	+	–	–	–	+	+	P			
–	–	–	–	–	–	+	P				

concentration averaged 7.1 ± 0.8 ng/ml during pregnancy.

DISCUSSION

Ovulation was considered to have occurred when the serum P concentration exceeded 2.0 ng/ml. Since mares have a 21–22 day estrous cycle with approximately 15 days of luteal function and a 6 day estrous phase (Ginther, 1979), a 14 day sampling interval would allow the greatest chance of catching one of two samples in the estrous phase (P < 2.0 ng/ml) and other in the luteal phase (P > 2.0 ng/ml). Collecting a sample at the end of the estrous phase where the P concentration had not yet risen above 2.0 ng/ml followed by collecting a sample at the end of the luteal phase when the P concentration had dropped below 2.0 ng/ml would lead one to falsely conclude that the mare had not ovulated. However, the chance of this occurring is less than 10% (2/21). More likely, two successive samples would be collected during the luteal phase. This could occur in eight of 21 or 38% of samples. Two successive samples with elevated progesterone concentrations could also occur if the mare had an extended luteal phase. Stabenfeldt and Hughes (1987) reported that extended luteal phases can occur in as many as 25% of estrous cycles. Since the persistent luteal activity reportedly lasts up to 60 days (Stabenfeldt and Hughes, 1987), an extended luteal phase would be detected by three successive P concentrations being over 2.0 ng/ml. Overall, this sampling interval may overestimate the number of ovulations in a month but would be a slightly conservative estimate of overall ovarian function.

Our data confirm the seasonality of estrous cycles in feral horses. The incidence of ovulation was low during the months of November through March and peaked during the months of April through July. A small percentage of horses continued to evidence of ovulation through the anestrous season. Kirkpatrick and Turner (1983) reported that ovulation in feral mares was confined to the months of April through

TABLE 3. Progesterone concentrations in blood serum from pregnant feral mares.

Month	Mean	Standard error
0	0.17	0.1
1	6.07	1.2
2	5.83	0.8
3	9.73	3.6
4	32.23	26.8
5	15.30	9.4
6	9.07	1.9
7	4.93	0.8
8	4.2	1.4
9	4.57	1.4
10	7.00	0.5
11	12.05	0.9
12	1.3	1.0

July. Behavioral estrus was occasionally observed from August through October but they (Kirkpatrick and Turner, 1983) suggested that these were not accompanied by ovulation. Our data on P levels, presumably an accurate indicator of luteal function, indicate that feral horses from Nevada have a slightly longer estrous season than the horses of the Pryor Mountains of Montana.

Horses in the Carson National Forest of New Mexico (USA) showed seasonal foaling patterns (Nelson, 1978). However, Nelson (1980) witnessed mating activity throughout the year. Berger (1983) also observed year-round mating activity among horses in the Granite Range of Nevada (USA). In both of these studies, foaling was seasonal, from March to August, despite the occurrence of year-round mating. Alternately, Welsh (1975) reported both breeding and foaling throughout the entire year among horses from Sable Island, Nova Scotia (Canada).

Kirkpatrick and Turner (1986) suggested that mating activity observed in months other than April to August was the result of anovulatory behavioral estrus. This suggestion was supported by their previous paper (Kirkpatrick and Turner, 1983) reporting that they observed estrous cycles with "proven ovulation" occurring exclusively from April to August and by a report by Nelson (1980) who observed mating activity through the year. Our data suggest that a low percentage of animals do exhibit ovulation throughout the year. It may be that maintaining animals on a high plane of nutrition extends the breeding season from March through October and allows a few animals to ovulate from November through February. Ginther (1979) reported that winter ovulations, based on rectal palpation, can occur in as high as 20% of domestic mares, but are rare in ponies.

The lack of any evidence of luteal function during the first anestrous season after capture is similar to the pattern seen by Kirkpatrick and Turner (1983) and suggests that the initiation of routine handling of feral mares can alter estrous cycles by inducing stress. However, serum cortisol levels in these animals were not elevated and thus tend to negate this idea. Another possibility is that the recently captured animals were in poorer condition (Plotka et al., 1988a) and that, as stated above, condition may affect the length of the estrous season. After the first year, the estrous season began in March and extended into November.

Serum P levels in these animals were of the same magnitude as in domestic breeds of horses. Smith et al. (1970), Plotka et al. (1972, 1975), and Stabenfeldt et al. (1972) all reported P levels during the estrous cycle to peak around 10 to 15 ng/ml. Levels during estrus average <0.2 ng/ml. P levels during the months of March and September through November were similar to levels during the peak breeding season, suggesting that luteal function was not different between the two periods.

ACKNOWLEDGMENTS

This research was supported by Bureau of Land Management contract AA852-RP5-27 and the Marshfield Medical Research Foundation. We are grateful to Bureau of Land Management personnel of the Carson City, Nevada District and to the personnel of Nevada Nile Ranch who provided help in handling and bleeding of the horses. Cheryl Asa provided expert advice on the procedure for detecting estrus in the mares. Our thanks also go to the following for valuable technical assistance: G. C. Schmoller and F. Zarembka helped perform hormone assays, Ann Zweber performed the estrous testing and Doreen Luepke provided editorial assistance.

LITERATURE CITED

BERGER, J. 1983. Induced abortion and social factors in wild horses. Nature (London) 303: 59–61.

ENSMINGER, M. E. 1969. Horses and horsemanship, 4th ed. The Interstate, Danville, Illinois, 546 pp.

GINTHER, O. J. 1979. Reproductive biology of the mare, basic and applied aspects. McNaughton and Gunn, Incorporated, Ann Arbor, Michigan, 411 pp.

KEIPER, R., AND K. HOUPT. 1984. Reproduction in feral horses: An eight-year study. American Journal of Veterinary Research 45: 991–995.

KIRKPATRICK, J. F., AND J. W. TURNER, JR. 1983. Seasonal ovarian function in feral mares: Season-

al patterns of LH, progestins and estrogens in feral mares. Equine Veterinary Science 3: 113–118.

———, AND ———. 1986. Comparative reproductive biology of North American feral horses. Equine Veterinary Science 6: 224.

NELSON, K. J. 1978. On the question of male-limited population growth in feral horses (*Equus caballus*). M.S. Thesis. New Mexico State University, Las Cruces, New Mexico, 68 pp.

———. 1980. Sterilization of dominant males will not limit feral horse population. United States Drug Administration Forest Service Paper RM-116, Washington, D.C., 7 pp.

PLOTKA, E. D., T. C. EAGLE, S. J. GAULKE, J. R. TESTER, AND D. B. SINIFF. 1988a. Hematologic and blood chemical characteristics of feral horses from three management areas. Journal of Wildlife Diseases 24: 231–239.

———, ———, D. N. VEVEA, A. L. KOLLER, D. B. SINIFF, J. R. TESTER, AND U. S. SEAL. 1988b. Effects of hormone implants on estrus and ovulation in feral mares. Journal of Wildlife Diseases 24: 507–514.

———, C. W. FOLEY, D. M. WITHERSPOON, AND G. C. SCHMOLLER. 1975. Periovulatory changes in peripheral plasma progesterone and estrogen concentration in the mare. American Journal of Veterinary Research 36: 1359–1362.

———, D. M. WITHERSPOON, AND C. W. FOLEY. 1972. Luteal function in the mare as reflected by progesterone concentrations in peripheral blood plasma. American Journal of Veterinary Research 33: 917–920.

SMITH, I. D., J. M. BASSETT, AND T. WILLIAMS. 1970. Progesterone concentrations in the peripheral plasma of the mare during the oestrous cycle. Journal of Endocrinology 47: 523–524.

STABENFELDT, G. H., AND J. P. HUGHES. 1987. Clinical aspects of reproductive endocrinology in the horse. Compendium on Continuing Education for the Practicing Veterinarian 9: 678–684.

———, ———, AND J. W. EVANS. 1972. Ovarian activity during the estrous cycle of the mare. Endocrinology 90: 1379–1384.

WELSH, D. A. 1975. Population, behavioral, and grazing ecology of the horses of Sable Island, Nova Scotia. Ph.D. Thesis. Dalhousie University, Halifax, Nova Scotia, 378 pp.

Received for publication 13 February 1989.