BEHAVIORAL, FOLLICULAR AND GONADOTROPIN CHANGES DURING THE ESTROUS CYCLE IN DONKEYS

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ABSTRACT

Sexual behavior, follicular development and ovulation, and concentrations of circulating gonadotropins during the estrous cycle were studied during the summer in 7 jennies. Mean behavioral estrous length was 6.4 ± 0.6 days (mean ± SEM, n=19; 5.6 ± 0.5 days preovulatory and 0.8 ± 0.2 days post-ovulatory). Mean diestrous length was 19.3 ± 0.6 days (n=14). Females in estrus typically showed posturing, mouth clapping, clitoral winking, urinating and tail raising. Mouth clapping began approximately one day sooner and lasted approximately one day longer than winking and tail raising, so that the total duration of clapping was significantly greater than for the other two signs. Follicular changes and concentrations of gonadotropins were determined for 14 estrous cycles (2 per jenny). The follicular end points [diameter of the largest follicle and number of large ($\geq$25 mm), medium (20-24 mm), and small follicles (<20 mm)] showed a significant day effect. The diameter of the largest follicle and the number of large follicles began to increase significantly 7 days prior to ovulation with a maximum value the day before ovulation. Medium follicles reached a maximum number 4 days prior to ovulation, and small follicles decreased significantly prior to ovulation. After ovulation, all follicular end points, except the number of small follicles, remained low for the next 12 days. Mean values of FSH were low during estrus and high during diestrus with 2 significant peaks, one 3 days and one 9 days after ovulation. In contrast, mean levels of LH were low during diestrus and high during estrus with a maximum value the day after ovulation. The LH profile showed a more prolonged gradual increase prior to ovulation, than that which has been reported for ponies and horses.

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INTRODUCTION

Only limited studies have been done on follicular development and sexual behavior in female donkeys (Equus asinus), and the concentrations of circulating hormones in the blood during the estrous cycle have not been characterized. The purpose of the present study was to obtain additional information on sexual behavior, follicular development and ovulation in jennies and to determine the plasma gonadotropin concentrations during the estrous cycle.

MATERIALS AND METHODS

Animals

Nineteen jennies and one jackass were maintained at the University of Wisconsin experimental farm from January to October, 1980. They were purchased in Wisconsin, and prior reproductive histories were unknown. The jennies were housed together and were given hay and grain during the winter and pasture and hay supplementation during the spring and summer. Seven jennies that exhibited regular estrous cycle patterns during the preceding months were selected for a detailed study of the characteristics of the estrous cycle during the summer (June 12-August 31). The jennies were 2 to 20 years of age as estimated by eruption and wear of the incisor teeth and weighed 192 to 238 kg. The breeds were unknown, but six jennies were dark brown and one was spotted. Since the brown color and external characteristics of some of the animals seemed similar to those found in mules, a chromosome study was done to confirm that the animals were jennies. Blood samples were taken from five brown and from the spotted jenny and leukocyte chromosome preparations were made (15). The chromosome number was 62 for all animals in agreement with the literature (4,23) confirming that the animals were donkeys.

Sexual behavior

Each jenny was teased individually every day by the jackass as described for mares (10). The jackass was led to the jenny from behind and was permitted to tease. Mounting was allowed and copulation was prevented by deflecting the penis. The teasing period lasted 1 to 2 minutes. Based on preliminary observations, the following behavioral signs were recorded: 1) mouth clapping (frequently opening and closing the mouth by vertical movements of the lower jaw accompanied by stretching and lowering of the head and holding the ears back), 2) winking (rhythmic eversion of the vulvar labiae with exposure of the clitoris), 3) raising tail (standing with tail raised at any time during the teasing period), 4) urinating (passage of urine and probably of genital tract fluids), 5) posturing or presenting (establishing a body position that is most accommodating to copulation including abducted rear legs, arched tail, tipped pelvis, and lowered perineal area), 6) tail down (holding tail down between hind legs when mounted), and 7) not interested (no positive or negative responses to the presence or teasing of the jackass). Moving, kicking, and tail switching were noted in the preliminary study as signs of non-receptivity, although it frequently happened that jennies initially kicked before establishing estrous symptoms. The holding down of the tail and lack of interest

were judged as intermediate signs. Clapping, tail raising, winking, and urinating were recorded as signs of estrous behavior. Clapping as the only sign or in combination with kicking, moving, or switching tail was attributed to a transitional stage into or out of the estrus.

Based on the preliminary observations, estrus was defined as clapping plus at least one of the following: 1) winking or 2) raising tail at any time during the teasing period. Observations which did not meet these criteria were defined as non-estrus. Diestrus was defined as the period between two successive behavioral estrous periods. The interovulatory interval was the number of days between 2 successive ovulations. Split estrus was defined as going out of estrus for one day and returning to estrus again during what was apparently the same estrous period.

Data for the 3 behavioral signs that were used to define estrus (clapping, winking, tail raising) were analyzed for differences in the lengths of occurrence. Data for the duration of each behavioral sign were analyzed for the total days observed, the days prior to ovulation, and the days after ovulation. Data for the length of occurrence of the 3 behavioral signs were compared for each of these periods by a factorial analysis of variance from which animal variation was removed. Estrous sequence was included as a factor in the analysis. If there was a significant difference in the lengths of the behavioral signs, means were compared by Duncan's multiple range test (22).

Follicular and gonadotropin concentration changes during the estrous cycle

After the behavior test, rectal palpation was performed daily to follow follicular development and ovulation. The size, number, and the location of the follicles were estimated. Ovulation was defined as the absence of a large follicle ($\geq$25 mm) which was present the previous day. Follicular end points were: diameter of the largest follicle, number of small follicles (<20 mm), number of medium follicles (20-24 mm), and number of large follicles ($\geq$25 mm).

Blood samples were collected daily to characterize the gonadotropin profiles during the estrous cycle. Samples were collected by jugular venipuncture and plasma was stored at -20°C. Plasma samples were assayed using double-antibody radioimmunoassays previously validated in this laboratory for equine LH (28) and equine FSH (8). Serial dilutions of jenny diestrous and estrous plasma were tested for parallelism with mare plasma and equine standards. Significant deviations from parallelism were not detected.

For the purpose of statistical analyses, the estrous cycles were normalized to the day of ovulation (day 0). Since the mean interovulatory interval was 25 days, a period encompassing 12 days before and 12 days after ovulation was used for two consecutive ovulations for each jenny. In one jenny, in which a double ovulation occurred, the second ovulation was used as day 0 in the analysis, since estrus did not cease until after the second ovulation. The follicular and gonadotropin data were analyzed by a split-plot in

time analysis of variance. If this analysis indicated a significant value for the appropriate main effects or interactions, comparisons among means were done with a least significant difference (lsd) procedure (22).

RESULTS

Sexual behavior

All estrous periods (n=19) which were observed were accompanied by an ovulation. Mean length of estrus was 6.4 ± 0.6 days (mean ± SEM; 5.6 ± 0.5 days before and 0.8 ± 0.2 days after ovulation; Table I). Time of ovulation was significantly closer to the end of estrus than to the beginning. Mean diestrous length (n=14) was 19.3 ± 0.6 days, and mean interovulatory interval was 24.9 ± 0.7 days (Table I).

TABLE I. CHARACTERISTICS OF THE ESTROUS CYCLE IN JENNIES

End point	No.	Mean number of days ± SEM		
		Total	Before ovulation	After ovulation
Interovulatory interval	11	24.9 ± 0.7	-	-
Length of diestrus	14	19.3 ± 0.6	-	-
Length of estrus	19	6.4 ± 0.6	5.6 ± 0.5	0.8 ± 0.2
Duration of estrous signs				
Clapping	19	8.3^{a} ± 0.6	6.7 ± 0.5	1.6^{a} ± 0.3
Winking	19	6.0^{b} ± 0.6	5.3 ± 0.5	0.7^{b} ± 0.2
Tail raising	19	6.2^{b} ± 0.6	5.5 ± 0.5	0.7^{b} ± 0.2

^{a,b} Means with different superscripts in the same column are different ($P<0.05$).

There was no significant effect of estrous sequence for any of the three signs of estrus (clapping, winking, tail raising), and data were combined (19 estrous periods in 7 jennies). Mean values for length of occurrence of the 3 estrous signs are shown in Table I. Mouth clapping began approximately one day sooner and lasted approximately one day longer than winking and tail raising, so that the total duration of clapping was significantly greater than for the other two signs. Split estrus occurred two times in two different jennies. In both cases the estrus was interrupted for only one day.

Follicular and gonadotropin concentration changes during the estrous cycle

The changes in follicular end points and gonadotropins during the period from 12 days before to 12 days after ovulation are shown in Figure 1. There were no significant effects due to period (ovulation 1 versus ovulation 2) for any of the follicular end points except for the number of medium follicles ($P<0.001$), nor was there a period effect for either gonadotropin. There were also no significant interactions between period and day for any end point. Therefore, the 14 periods were combined to examine the overall day effects. There was a day effect for FSH ($P<0.003$), LH ($P<0.0001$), diameter of the largest follicle ($P<0.0001$), and the number of large follicles ($P<0.0001$), medium follicles ($P<0.0001$), and small follicles ($P<0.0001$).

From day -12 to day -8, the mean diameter of the largest follicle was less than 20 mm (16.2-19.6 mm). The first significant increase occurred 7 days prior to ovulation. The mean diameter continued to increase significantly thereafter and reached a maximum of 36 mm on day -1 (range 30-40 mm). Following ovulation the mean diameter of the largest follicle remained less than 15 mm for the next 12 days (range 12-14.8 mm).

The mean number of large follicles ($\geq$25 mm) was low (<0.4) until 7 days prior to ovulation (Fig. 1) and began to increase significantly by day -6. However, the number of large follicles did not exceed a mean value of 1.2 and the changes recorded followed a pattern similar to the changes recorded for the diameter of the largest follicle.

No medium follicles were detected from day -12 to day -8. Mean number of medium follicles began to increase significantly 7 days prior to ovulation, reached a maximum value (0.64) on day -4, declined to lower values on the day of ovulation (0.14), and decreased to 0 again 2 days after ovulation.

Mean number of small follicles decreased significantly prior to ovulation (Fig. 1). The number was significantly less on day -4 than on day -8 and declined progressively reaching the minimal values on day 0 and day 1 after ovulation. The mean number of small follicles began to increase at day 2 and by day 7 the number was significantly higher than on day 1 (2.8 versus 1.9).

Double ovulation occurred in only one estrous period of one jenny; each ovary ovulated once at a 2-day interval. No difference was found between the left or the right ovaries in frequency of ovulation (9 versus 10).

Mean concentrations of FSH were relatively high (>11.0 ng/ml) on day -12 to day -10. Concentrations decreased thereafter so that the day -6 value (8.8 ng/ml) was significantly lower than on day -10 (11.7 ng/ml). The minimum mean value was reached three days before ovulation (7.4 ng/ml). Concentrations began to increase progressively one day prior to ovulation and by day 3 after ovulation mean FSH concentrations were significantly higher than on the day of ovulation (12.1 ng/ml

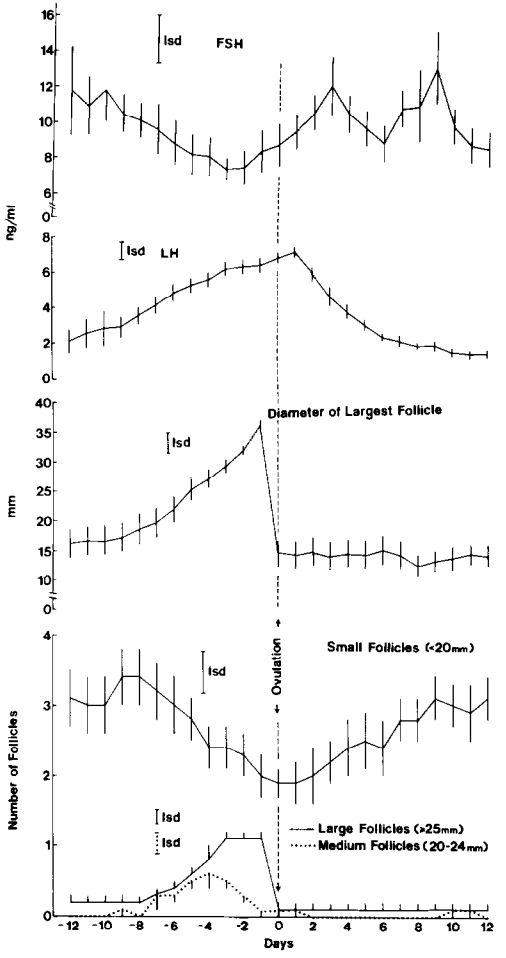

Figure 1. Mean (± SEM) daily plasma gonadotropin concentrations and numbers and diameters of follicles for 12 days prior to and 12 days after ovulation in 14 estrous cycles (2 per jenny). The lsd bars represent the magnitude of least significant differences ($P<0.05$).

versus 8.8 ng/ml). After a decline from day 3 to day 6 (8.9 ng/ml), FSH concentrations again increased so that the mean concentration at day 9 (13.1 ng/ml) was significantly higher than on day 6. Mean concentrations decreased after day 9; the concentration at day 10 (9.7 ng/ml) was significantly lower than on day 9.

Mean LH concentrations were minimal ($\leq$3 ng/ml) on day -12 before ovulation and concentrations increased thereafter reaching a significant increase by day -8 (3.5 ng/ml) and a maximum value at day 1 after ovulation (7.0 ng/ml). After the peak value on day 1, LH levels declined over the next 5 days reaching concentrations below 3 ng/ml. From day 7 to day 12 after ovulation, mean LH concentrations remained low (<3 ng/ml).

DISCUSSION

In this study, estrus lasted 6.4 days and diestrus 19.3 days for a total estrous cycle length of 25.7 days. The length of the interovulatory interval (24.9 ± 0.7 days) is similar to that reported (14) for ponies (25.0 ± 0.6), but longer than for horses (22.7 ± 0.7). These results in donkeys were similar to previous reports (5,13,16), although Nishikawa (16) found a mean cycle length of only 22.8 ± 0.1 days (n=46).

Typical estrous behavior of jennies included mouth clapping, posturing, tail raising, urinating, and winking. Mouth clapping was a peculiar sign of jennies in estrus and has been reported previously (5,13,18). For horses and ponies the clapping behavior has been described only for young males and fillies directed towards an adult male (7,27). Similar clapping action has been described in zebras during estrus (1,2,12,24). In this study, clapping occurred consistently; there was no behavioral determination in which winking or posturing occurred in the absence of clapping. Furthermore, on the average, clapping began a day sooner and lasted a day longer than the other signs of estrus, and during individual determinations clapping was frequently the first sign to appear. Clapping was an especially useful indicator, because it appeared consistently, often with only minimal teasing, was readily observed, and often could be detected by sound, alone. In previous studies (5,16), however, clapping was not always observed and a few jennies showed no clapping or other overt estrous signs even though a mature follicle was detected (covert estrus). The other estrous signs were similar to those described in horses and ponies (11). However, one study (18) reported the absence of winking in jennies in estrus. The reason for these differences in observation is not known. Tail raising was associated mostly with estrous behavior, but occasionally was seen also during diestrus.

Split estrus occurred twice (n=19) and was characterized by one day of non-estrous symptoms within one estrous period. Homosexuality was observed on 2 and 3 consecutive days during estrus in two jennies, respectively. This seems frequent when compared with horses and ponies where it is reported to be rare (3,11,21), although critical species comparisons have not been done.

During the preovulatory period a decrease in number of small follicles (<20 mm) was accompanied by an increase in the number of medium (20-24 mm) and large (≥25 mm) follicles. Apparently, on the average, 7 days prior to ovulation a small follicle started to grow and became a medium follicle (20-24 mm) resulting in a decreased number of small and increased number of medium follicles. The medium follicle continued to grow and became a large follicle (≥25 mm) at about 4 days before ovulation. The large follicle became the ovulatory follicle and a maximum mean diameter of 36 mm was recorded the day before the ovulation. This mean preovulatory diameter was similar to that described by Nishikawa (18). In ponies, the diameter of the largest follicle also increased progressively over the 7 days before ovulation although an initial increase was noted several days earlier (11). The development of a large preovulatory-sized follicle during diestrus, as has been reported for horse mares (11), was not detected in jennies.

This study, as well as a previous study (17), was too limited to allow conclusions on possible differences in frequency of ovulation between right and left ovaries. In horses, ovulation occurs with a slightly greater frequency from the left ovary (11). In ponies, no significant differences were found between left and right ovaries in the occurrence of ovulation (9,11). A double ovulation was found in one of 19 ovulations (5.3%). Nishikawa (17) found polyovulation in 7 of 22 jennies (31.8%) and concluded that polyovulation was prevalent. It should be mentioned that in horses the reported incidence of double ovulation also ranges from rare to common (11). In one report (26) in horses (n=28) the incidence of multiple ovulation was 43%, and it was suggested that this may have been due to a period of high energy feeding. More study will be necessary to evaluate the prevalence of polyovulation and its determining factors in jennies.

Mean concentrations of FSH were highest during diestrus, low during estrus, and began to increase near the time of ovulation. The pattern of decreasing FSH secretion beginning approximately 10 days before ovulation is similar to patterns reported in horses and ponies (6,14). Following ovulation, significant peaks occurred on day 3 and on day 9. Furthermore, there seemed to be a third surge of FSH on approximately day 15 (equivalent to day -10). Based on the least significant difference, the value at day -10 (11.8 ng/ml) was greater than at day 12 (8.2 ng/ml; Fig. 1). However, study of a possible third peak at day 15 was beclouded by the discontinuity of data between day 12 and day 15. The relatively high value at day -12 was due primarily to one sample as indicated by the large standard error. In summary, these data indicate that on the average 3 surges of FSH occurred following ovulation at 6-day intervals with peaks on days 6, 9, and 15 followed by a prolonged depression beginning at the peak of the third surge (day 15 or day -10) and encompassing the length of estrus. This overall hypothesis will require critical testing. A bimodal curve for FSH during the estrous cycle in horse mares has been reported (6). However, in horse mares the second surge occurred later in the diestrous period with the peak value 10 to 13 days before the next ovulation. In another study (14) in ponies, elevated levels of FSH were found from day 3 to day 15 after ovulation, but a bimodal pattern was not observed. Seasonal effects likely accounted for the divergent results in these 2 studies since a

later study found that 2 surges occurred early in the ovulatory season, whereas only one occurred late in the ovulatory season (25). Further study is necessary to determine if similar seasonal effects exist in jennies.

Mean LH concentrations were low during mid-diestrus and elevated during estrus with a maximum value the day after ovulation (Fig. 1). Circulating levels of LH were significantly elevated above diestrus concentrations for almost one half of the estrous cycle. Apparently, the LH increase prior to ovulation is more prolonged and gradual than what has been reported (14) for horses and ponies (Fig. 2). In all three (donkey, horse, pony), however, the concentrations reached a maximum the day after ovulation and then declined over the next 5 days. The jenny, like the horse and pony mare, shows an expanded LH surge in contrast to the short preovulatory peak in other mammalian species (cows, ewes, sows and rats; 19,20).

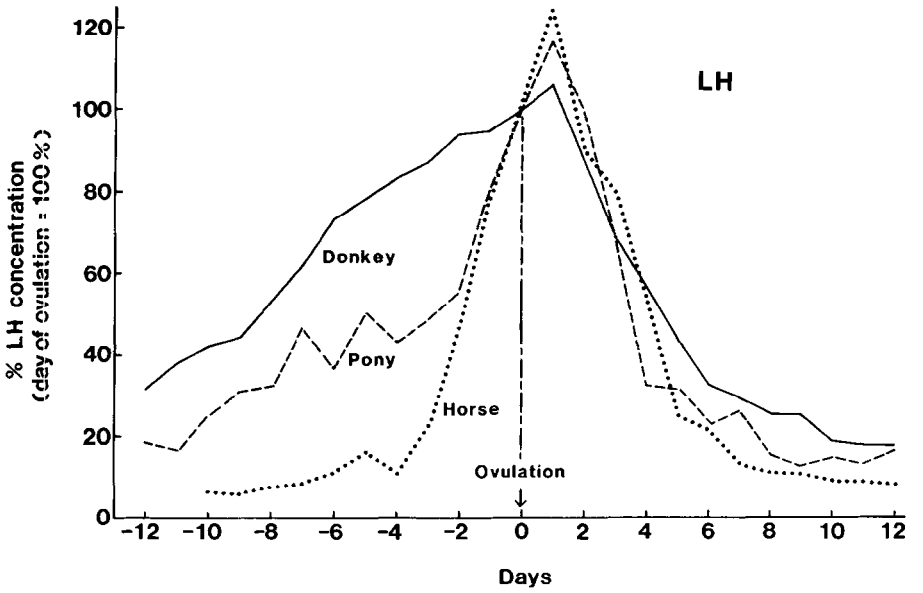

Figure 2. Mean circulating LH concentrations in the horse, pony, and donkey during the estrous cycle. The mean concentration on the day of ovulation (day 0) was assigned the value of 100% and the other data are expressed as a percentage of this value. The data for horses and ponies are from previous work in this laboratory (14).

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