ELSEVIER

ESTROUS CYCLE CHARACTERISTICS AND RESPONSE TO ESTRUS SYNCHRONIZATION IN MAMMOTH ASSES (EQUUS ASINUS AMERICANUS)

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ABSTRACT

Breeding records from a herd of mammoth asses (Equus asinus americanus) maintained on pasture in southeast Texas from 1990 to 1998 were reviewed. Jennies were pasture or hand mated, and estrus was either observed while the jennies were on pasture or when exposed to a jack after being penned. Eighty-one estrus periods and 43 diestrus intervals were recorded in 33 jennies over 4 seasons of the year (January-March, April-June, July-September, and October-December). Estrous cycle length and the duration of estrus were similar among seasons. Over all seasons, estrous cycle length was 23.3 ± 2.6 d, duration of estrus was 5.9 ± 2.1 d, and diestrus length was 17.4 ± 2.6 d (mean ± SD). During these same 9 yr, 58 injections of $PGF_2\alpha$ (5 mg, im) were administered to 38 jennies without regard to stage of estrous cycle. Seventy-six percent (44/58) of the jennies showed signs of estrus after $PGF_2\alpha$ treatment, with an interval to estrus of 4.4 ± 1.6 d and a duration of estrus of 5.6 ± 1.7 d.

Two estrus synchronization schemes were also assessed. Trial 1 was performed in October to November 1996, and Trial 2 was performed in February to March 1998. In Trial 1 (Group PE+PGF, n = 10), each jenny was injected intramuscularly once daily for 10 d with 150 mg progesterone and 10 mg estradiol-17β in sesame oil, and $PGF_2\alpha$ (10 mg) was injected intramuscularly on the last day of treatment. In Trial 2 (Group PGF-2X, n = 11), each jenny was injected intramuscularly twice, 16 d apart, with 10 mg $PGF_2\alpha$. All Group PE+PGF jennies responded to treatment. One jenny in Group PGF-2X did not respond to either injection of $PGF_2\alpha$, while 2 jennies responded to the first but not the second $PGF_2\alpha$ injection (8 of 11 jennies returned to estrus and ovulated after the second $PGF_2\alpha$ injection). Duration of estrus was 6.8 ± 1.9 d for Group PE + PGF and 7.1 ± 1.8 d for Group PGF-2X jennies. Interval to estrus and interval to ovulation following the last treatment were 9.0 ± 0.9 d and 14.5 ± 1.7 d, respectively, in Group PE+PGF jennies, and 4.5 ± 0.9 d and 10.4 ± 1.8 d, respectively, for Group PGF-2X jennies. In summary, estrous cycle characteristics of mammoth asses are similar to those reported for standard jennies, and estrus synchronization schemes used in horses are effective in mammoth asses.

Key words: mammoth ass, estrous cycle, estrus synchronization, prostaglandin, progesterone

INTRODUCTION

The mare (Equus caballus) has a relatively long period of estrus, with ovulation occurring variably from 1 to 10 d after the beginning of sexual receptivity (4). In comparison to the mare, few studies have been reported regarding the estrous cycle of the jenny (Equus asinus). In one study of 20 estrous cycles in 13 jennies, the duration of estrus varied from 3 to 13 d, and the duration of diestrus varied from 14 to 21 d (10). In another study of 48 estrous cycles in 6 jennies, duration of the estrous cycle was 25.9 ± 2.7 d, with estrus lasting 7.9 ± 2.5 d (6). There is general agreement that while the duration of estrus is similar between jennies and mares, the duration of diestrus is longer in jennies (5,6,10,15). Ginther et al. (5) also found the estrous cycle of jennies to be less affected by season than that of either ponies or horses, with a high percentage of jennies cycling throughout the year. Henry et al. (6) reported a 40% seasonal anestrus rate in jennies in a year-round study of 10 cyclic jennies. Most of the studies reported in the literature apparently refer to standard jennies. In the United States, the breeding of large, imported asses has produced a unique large ass that has come to be recognized as a distinct breed called the American Mammoth Jack or American Mammoth Jackstock, and has been given the scientific name of Equus asinus americanus (1). The Majorca, Maltese, Andalusian, Catalonian, and Poitou were the primary breeds contributing to the development of the American Mammoth Jack (8). Little is known regarding differences in the estrous cycle of mammoth asses.

A primary goal of reproductive management of the equid is to efficiently maximize production of live foals. To efficiently utilize the breeding male, management strives to breed the equid female near the time of ovulation. Hormonal treatment to regulate estrus and ovulation is helpful for scheduling breeding at desired times. Hormones commonly administered to mares to synchronize estrus include $PGF_2\alpha$, progesterone or progestin, and progesterone plus estradiol-17β. Prostaglandin $F_2\alpha$ administration shortens the lifespan of the corpus luteum and thus induces estrus (3,12). Administration of progesterone/progestin artificially prolongs the luteal phase of the estrous cycle and, when it is withdrawn, allows for estrus to begin (9,13,14). Daily injections of both progesterone (to artificially prolong the luteal phase) and estradiol (to suppress follicular growth) has been advocated to provide better synchrony in the onset of estrus and ovulation among mares in a group (9,16). A combination of progesterone, estradiol and prostaglandin has been shown to decrease the incidence of silent or shortened estrous behavior and to reduce the variation in days to ovulation compared with mares treated with either progesterone + prostaglandin or progesterone + estradiol (2).

The primary objective of this study was to provide information on the estrous cycle of the mammoth ass. We also evaluated responses of mammoth asses to single injections of prostaglandin $F_2\alpha$ (given without regard to stage of the estrous cycle) to induce estrus for breeding, and to 2 estrus synchronization schemes - either progesterone and estradiol administered for 10 d with $PGF_2\alpha$ administered on the last day of treatment, or 2 injections of $PGF_2\alpha$ administered 16 d apart.

MATERIALS AND METHODS

Breeding records from 1990 to 1998 were reviewed from a herd of mammoth asses (Equus asinus americanus) on a farm in southeast Texas. The jennies were maintained on pasture and were fed supplemental hay and grain to maintain body condition. Jacks were maintained in paddocks and fed similar diets. The herd under study was known to have a high incidence of multiple ovulations and twin births. Most nonpregnant jennies in this herd had a history of regular estrous cycles throughout the year. Jennies were pasture or hand-mated, and estrus was either observed while the animals were on pasture or when exposed to a jack upon being penned (7). Jennies were not examined by transrectal palpation or ultrasound to confirm ovulation during these estrous cycles. Duration of estrus was often recorded without the duration of diestrus (i.e., many estrous periods were recorded from observed matings and were thus followed by conception with failure to return to estrus). Likewise, some diestrus periods were calculated between successive estrus periods when only the last day or two of the estrus preceeding breeding and the first day or two of the estrus following breeding were recorded. Additionally, many times estrus was induced for mating by administering $PGF_2\alpha$. Since the durations of estrus and diestrus were not always recorded, means were calculated only from complete data in which $PGF_2\alpha$ was not used to induce estrus. This resulted in recordings of 81 complete estrus periods and 43 complete diestrus periods in 33 jennies (not treated with $PGF_2\alpha$) over 4 seasons of the year (January-March, April-June, July-September, and October-December). Estrous cycle data were compared among seasons by analysis of variance. Finally, 58 injections of $PGF_2\alpha$ (5 mg, im) were administered, without regard to stage of the estrous cycle, to 38 jennies during these same years. Mean interval to estrus and duration of estrus following $PGF_2\alpha$ treatment were calculated, and comparisons among seasons were made by analysis of variance.

Fourteen mammoth jennies were also used in 2 estrus synchronization trials. Seven jennies were used in both trials. Three additional jennies were used in Trial 1 (n = 10), while 4 additional jennies were used in Trial 2 (n = 11). Trial 1 was conducted in October to November 1996, while Trial 2 was conducted in February to March 1998. In Trial 1, 150 mg progesterone and 10 mg estradiol-17β in sesame oil were injected intramuscularly once daily for 10 consecutive days. On the last day of progesterone/estradiol treatment, 10 mg $PGF_2\alpha$ were injected intramuscularly. In Trial 2, 10 mg $PGF_2\alpha$ were injected intramuscularly twice, 16 d apart. To detect estrus after prostaglandin administration in both trials, jennies were haltered, tied and exposed to a jack. Signs used to confirm estrus included mouth clapping, posturing for the jack, lifting the tail, eversion of the clitoris, and allowing the jack to mount (7). For both trials, transrectal ultrasonographic examinations were performed to monitor follicular activity at the beginning and end of treatments. Beginning 4 d after the last treatment, the ovaries of jennies were scanned every other day until a follicle 30 mm in diameter was detected. Thereafter, examinations were performed daily to detect ovulation. When multiple ovulations occurred, the day the first ovulation was detected was used to calculate the interval to ovulation. Since no controls were included in either estrus synchronization trial, no statistical comparisons were made.

RESULTS

Eighty-one estrus periods and 43 diestrus periods were recorded in 33 jennies over 4 seasons of the year (January-March, April-June, July-September, and October-December). Duration of estrus, length of diestrus, and length of the estrous cycle are presented in Table 1. Estrous cycle length and duration of estrus were similar among seasons ($P > 0.10$). Diestrus length was longer in October to December than in January to March or July to September ($P < 0.05$). Over all periods, estrous cycle length was 23.3 ± 2.6 d, duration of estrus was 5.9 ± 2.1 d, and diestrus length was 17.4 ± 2.6 d (mean ± SD).

Table 1. Mean (± SD) duration of estrus, length of diestrus, and length of estrous cycle in 33 mammoth asses

Season	n	Duration of estrus (days)	n	Diestrus length (days)	n	Estrous cycle length (days)
Jan-Mar	22	6.4 ± 2.1	11	16.1 ± 3.5[a]	9	23.3 ± 3.2
Apr-Jun	24	5.3 ± 1.8	16	17.8 ± 2.0[a,b]	15	23.0 ± 2.8
July-Sept	4	7.0 ± 1.0	3	15.0 ± 1.0[a]	3	22.0 ± 1.0
Oct-Dec	31	5.8 ± 2.4	13	18.6 ± 1.9[b]	12	24.1 ± 2.2
All	81	5.9 ± 2.1	43	17.4 ± 2.6	39	23.3 ± 2.6

[a,b]Means with different superscripts are significantly different ($P < 0.05$).

To induce estrus for breeding, 58 injections of $PGF_2\alpha$ (5 mg, im) were administered to 38 jennies without regard of stage of the estrous cycle. Seventy-six percent (44/58) of the jennies showed signs of estrus after $PGF_2\alpha$ treatment, with an interval to estrus of 4.4 ± 1.6 d and a duration of estrus of 5.6 ± 1.7 d (Table 2). Interval to estrus following $PGF_2\alpha$ administration was longer in July to September than in October to December or April to June ($P < 0.05$).

Table 2. Mean (± SD) interval to estrus and duration of estrus in 38 mammoth asses after intramuscular administration of 5 mg $PGF_2\alpha$ given without regard to stage of estrous cycle[a]

Season	n	Interval to estrus (days)	n	Duration of Estrus (days)
Jan-Mar	6	5.2 ± 1.3[bc]	1	6.0
Apr-Jun	26	4.2 ± 1.6[b]	18	5.2 ± 1.1
July-Sept	7	6.7 ± 1.2[c]	1	5.0
Oct-Dec	12	4.0 ± 1.5[b]	10	6.3 ± 2.4
All	44	4.4 ± 1.6	30	5.6 ± 1.7

[a]76% (44/58) of jennies exhibited estrus after injection of $PGF_2\alpha$.
[b,c]Means with different superscripts are significantly different ($P < 0.05$).

Results of the 2 estrus synchronization trials are presented in Table 3. All Group PE+PGF jennies responded to treatment by displaying estrus and ovulating. One jenny in Group PGF-2X did not display estrus after either injection of prostaglandin, while 2 jennies displayed estrus after the first but not the second injection of prostaglandin (8 of 11 jennies returned to estrus and ovulated after the second prostaglandin injection). Duration of estrus appeared similar between Group PE + PGF and Group PGF-2X jennies. Both the interval to estrus and interval to ovulation following the last treatment appeared to be greater in Group PE+PGF jennies than in Group PGF-2X jennies.

Table 3. Mean (± SD) duration of estrus, interval to onset of estrus, and interval to ovulation in mammoth jennies after treatment with either 150 mg progesterone and 10 mg estradiol-17β in sesame oil injected intramuscularly daily for 10 days with 10 mg $PGF_2\alpha$ injected intramuscularly on Day 10 (PE + PGF), or 2 intramuscular injections of 10 mg $PGF_2\alpha$ administered 16 days apart (PGF-2X)

	Group PE + PGF	Group PGF-2X[a]
n	11	10
Percentage of jennies in estrus	100% (10/10)	73% (8/11)
Interval to onset of estrus (days)	9.0 ± 0.9	4.5 ± 0.9
Duration of estrus (days)	6.8 ± 1.9	7.1 ± 1.8
Interval to first ovulation (days)	14.5 ± 1.7	10.4 ± 1.8
Percentage of jennies with multiple ovulations	70% (7/10)	50% (4/8)
Diameter of largest follicle at onset of treatment (mm)	19.7 ± 12.9	21.3 ± 9.1
Diameter of largest follicle on day of last treatment (mm)	16.5 ± 4.7	21.5 ± 5.8

[a]One jenny did not display estrus after either injection of prostaglandin, and 2 jennies displayed estrus after the first but not the second prostaglandin injection (8 of 11 jennies returned to estrus and ovulated after the second prostaglandin injection)

Multiple ovulations were detected on the same day in 8 jennies, and over a 2-d period in 3 jennies. Two jennies had 3 ovulations (all in the PE + PGF Group), and 8 jennies had double ovulations. No jenny ovulated after signs of estrus ceased. Six of seven (86%) jennies with single ovulations ceased showing signs of estrus 0 to 1 d after ovulation was detected, with the remaining jenny exhibiting estrus until 2 d after ovulation was detected. Nine of eleven (82%) jennies with multiple ovulations remained in estrus 0 to 1 d after the last ovulation was detected, with the 2 remaining jennies exhibiting estrus for 2 d after the last ovulation was detected.

DISCUSSION

Duration of estrus in the group of mammoth jennies in our study was similar to that previously reported for standard breeds of jennies (5,10,15) but was shorter than the 7.9 ± 2.5 d reported by Henry et al (6). It is possible that pasture observations of estrus were less accurate than those determined by individual teasing, thus contributing to a shorter recorded duration of estrus in the mammoth asses. The finding of a longer duration of estrus in the individually-teased

jennies during the estrus synchronization trials would support this observation. All but 1 mammoth jenny in the estrus synchronization trials responded to treatment by returning to estrus, indicating they were still having estrous cycles during October to November and February to March. This was similar to the previous finding that the estrous cycle of the standard jenny is less affected by season than that of horses or ponies (5). In that study (5) 64% of standard jennies ovulated in December and 82 to 100% ovulated during the other months. Henry et al. (6) also reported a low incidence (40%) of seasonal anestrus in standard jennies.

Ginther et al. (5) reported that the duration of estrus tended to be shorter in standard jennies in May to October than in November to April. Perhaps the longer estrus periods during the winter accounted for the failure to demonstrate greater synchrony in ovulations in the PE + PGF-treated jennies, since mean follicular diameter was only 0.5 mm less at the end of treatment than in PGF-2X-treated jennies. Seasonal effects on follicular size and length of estrus might have limited variability in the interval to ovulation in PGF-2X-treated jennies as only 2 had follicles ≥ 25 mm in diameter at the time of the second prostaglandin injection. It would be interesting to examine the same mammoth jennies during summer months to see if more variation in follicular size would be present after the second prostaglandin injection.

While the farm owner had previously achieved a good success rate using only 5 mg $PGF_2\alpha$, we chose to use 10 mg $PGF_2\alpha$ since it is the recommended dose for horses (4). We also selected a 16-d interval between prostaglandin injections for the PGF-2X-treated jennies because of their longer reported diestrus length compared to mares, yet 2 jennies responding to the first prostaglandin injection did not respond to the second prostaglandin injection. While jennies were only teased and ovulations were not confirmed following the first prostaglandin injection, these 2 jennies were the last to go out of estrus prior to the second injection (4 and 5 d prior to the second prostaglandin injection). Therefore, we assumed that the failure of these 2 jennies to respond to the second prostaglandin injection was due to insensitivity of the newly forming corpora lutea to prostaglandin. Six of the 8 jennies responding to PGF-2X treatment went out of estrus 6 to 10 d prior to the second prostaglandin injection. One jenny responding to PGF-2X treatment was in estrus with an ultrasonographically detectable corpus hemorrhagicum and a 35 mm diameter follicle on the day of the first prostaglandin injection. This jenny was out of estrus the next day and returned to estrus 5 d after the second prostaglandin injection. The remaining PGF-2X-treated jenny did not show estrus until 6 d after the second prostaglandin injection. Perhaps a longer interval between the 2 injections of prostaglandin (i.e., more than 16 d) would be more effective in synchronizing estrus in mammoth jennies.

The incidence of multiple ovulations for standard jennies has been reported to range from 5.3 to 37.2% (5,6,10,11,15). The incidence of double ovulations in this herd of mammoth jennies was higher than that reported for standard jennies. Whether this is due to a herd effect or is typical for mammoth jennies is not known. The incidence of double ovulations in the horse has been found to be affected by breed, with larger breeds of horses having a higher incidence than smaller breeds of horses or ponies (4). Ginther (4) also found that multiple ovulations are characterized by significant repeatability within individual mares, and suggested that the condition may be heritable. The 4 jennies with multiple ovulations in the second trial also had multiple ovulations in the first trial, and the remaining 3 jennies with multiple ovulations in the first trial

either were not used in the second trial or did not respond to the PGF-2X treatment by displaying estrus and ovulating.

While caution must be exercised in comparing the 2 estrus synchronization treatments because untreated controls were not included, and the 2 trials were conducted at different times, the percentage of jennies responding to treatment appeared to be higher in the PE + PGF-treated jennies. However, synchrony in both the onset of estrus and the interval to ovulation did not appear to be improved in PE + PGF- compared with PGF-2X-treated jennies. This was unexpected because we had previously found that in mares the addition of estradiol and prostaglandin to a progesterone regimen increased synchronization efficacy by reducing variation in days to ovulation (2). Since mean follicle size at end of treatment appeared to be smaller in PE-PGF- than in PGF-2X-treated jennies, suppression of follicle size may have occurred. However, whether this was due to a seasonal effect or to steroid suppression of follicle size cannot be determined since untreated controls were not included, and the 2 trials were not conducted at the same time. Perhaps repeating the experiment in summer, when the duration of estrus is reported to be shorter in jennies, rather than in late fall/winter, when duration of estrus is longer, would have altered this finding. Additionally, treatment in summer might have also resulted in less synchrony in the PGF-2X- treated jennies due to expected larger variation in follicle size at the time of treatment.

In summary, estrous cycle characteristics of mammoth jennies are similar to those reported for standard jennies, and both regimens for estrus synchronization produced satisfactory clinical responses in mammoth jennies. Since synchrony in the interval to onset of estrus or ovulation was not improved with PE + PGF treatment, the additional time and expense of this regimen may not be justified when compared with the PGF-2X treatment.

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