Lactational anestrus in Caribbean donkeys
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Summary
Lactational anestrus is common in many species, but not in equids. This case report documents an unusual finding of eight donkeys (from a group of 25) on the Caribbean island of St. Kitts that remained anestrus after foaling during the physiological breeding season. Twenty-five feral female donkeys in advanced pregnancy were acquired. No breeding history was known and all pregnancies were monitored via weekly transabdominal ultrasonography. All jennies foaled without complications and weekly transrectal ultrasonographic examinations were conducted to monitor uterine involution and cyclicity. Four foals died from septicemia within the first week and all of their dams soon re-established ovarian cyclicity. Thirteen jennies had a foal heat with overt signs of estrus within 2 weeks after foaling, then returned to normal cyclicity. However, the remaining eight jennies did not ovulate or display signs of estrus until their foals were weaned, ~4 months later. Following weaning, all eight jennies promptly returned to normal cyclicity. That these eight jennies returned to normal cyclicity following weaning supported our diagnosis of lactational anestrus during the physiological breeding season, a phenomenon apparently never reported in donkeys.

Keywords: Donkey, jenny, lactation, anestrus, Caribbean

Case presentation

History
Twenty-five feral female donkeys were obtained from the island of Nevis and transported to the sister island of St. Kitts for a research study, approved by the Ross University School of Veterinary Medicine Animal Care and Use Committee. Jennies were estimated to be 3 to 13 years of age, with body condition scores of 4 to 6. All jennies were confirmed to be in late gestation via transabdominal ultrasonography, and all subsequently foaled without complications between March and July (spring/summer) 2016. Jennies and foals were monitored daily after foaling for nursing and general health. Four foals died from septicemia within their first week, although their dams had no apparent health issues. Beginning 1 week after foaling, transrectal ultrasonography was done once weekly. Uterine horn diameter and endometrial edema, scored on a scale of 0 to 3, were evaluated. Ovaries were monitored for ovarian follicular and corpora lutea development. Jennies were teased twice weekly using jacks to detect estrus behavior. Estrus behavior was graded as follows: 0 rejects the presence of the jack; 1 indifferent to the jack’s presence; 2 performs mouthing behavior only; 3 performs mouthing behavior, backs up to the jack, squatting, urination and clitoral winking. Once weekly for 20 weeks, starting 5 weeks before weaning, blood samples were collected from each jenny by jugular venipuncture. Serum was separated within 3 hours after collection and stored at -80°C.

Within 1 month after foaling, 17 jennies had displayed behavioral signs of estrus and had ultrasonographic evidence of ovarian follicular development, uterine horn and endometrial edema, ovulation and CL formation. However, the remaining eight jennies (32%), all with foals, had no behavioral signs of estrus, and based on transrectal ultrasonographic examinations, smaller uterine horns, no evidence of ovulation and no CL.

Serum samples were thawed and analyzed with a commercial enzyme immunoassay for quantitative determination of progesterone (Arbor Assay, K025-H5; Ann Arbor, MI, USA). Validation was done to assess possible matrix effects when this assay was used for donkey serum. Pools of serum were prepared from blood samples collected from jennies during estrus, diestrus and early pregnancy. Each pool was subsequently diluted in a serial manner (1:1, 1:2, 1:4, 1:8, 1:16, and 1:32) to determine parallelism with the reference standard curve. Four of six dilutions were within the linear range of the
standard curve, with a CV of 26%, indicative of parallelism.\textsuperscript{11} Thereafter, the assay was conducted in accordance with the manufacture’s protocol, using 50 µL of sample diluted 1:16 (estrous cycle samples) or 1:32 (pregnancy samples) with assay buffer. Samples with progesterone concentrations outside the range of the reference standard were further diluted and re-analyzed as necessary. Within-assay CV and sensitivity were 17% and 0.05 ng/mL, respectively.

In all eight jennies with lactational anestrus, serum progesterone concentrations were < 4.0 ng/ml from 5 weeks before weaning to 1 week after weaning (Figure 1), in stark contrast to the remaining jennies with physiologic postpartum estrous cycles (Figure 2). However, beginning 1 week after weaning, progesterone concentrations in subsequent estrous cycles began to fluctuate, similar to jennies with regular postpartum estrous cycles (Figure 3). Serum progesterone concentrations were consistent with behaviors in response to teasing and ovarian follicular development, as monitored ultrasonographically.

**Outcome**

All eight jennies (100%) affected by lactational anestrus ovulated 1 to 2 weeks after their foals were weaned and thereafter continued to cycle similar to their herd mates.

**Discussion**

The influence of housing and nutrition on this condition was questionable. All jennies were housed together in an outdoor pasture, received the same diet of pasture supplemented with sugar cane grass twice daily and \textit{ad libitum} access to a fresh clean water source. All were in similar body condition (body condition scores of 4 to 6), with no apparent loss of body condition after foaling. In contrast, three jennies increased at least by one body condition score grade during the time of observation. Therefore, it is unlikely that the lack of estrus in these eight jennies was related to nutrition, as widely documented in many species.\textsuperscript{3-6}

St. Kitts is on the 17\textsuperscript{th} parallel north, where daylight only varies by approximately 2 hours throughout the year. Daylight affects the reproductive cycle in mares, although donkeys cycle throughout the year, even in more northern latitudes.\textsuperscript{7-10} Weekly monitoring of estrus behavior as well as uterine and ovarian structures throughout the winter demonstrated no change in average interovulatory interval in herd mates. Therefore, shortened exposure to daylight was an unlikely cause of anestrus.

In contrast to the mare, relatively higher serum progesterone concentrations observed at all stages of the cycle agreed with earlier reports.\textsuperscript{11,12} As noticed, progesterone concentrations can be suprabasal at ovulation.\textsuperscript{11} Although postpartum mares can have ovarian inactivity for known and unknown reasons,\textsuperscript{13} in the current study, the most likely cause was lactation, as suggested for mares.\textsuperscript{13} Although horses are seasonally polyestrous, the majority of donkeys ovulate year-round, even at temperate latitudes (43\textdegree N), although the duration of estrus and interovulatory interval fluctuate due to seasonality.\textsuperscript{13} Conversely, horse mares are sensitive to seasonal changes in daylength, even in temperate climates such as southern Mexico (latitude 15-22\textdegree N).\textsuperscript{14} Almost all horse mares ovulate soon after foaling, with a very small minority entering an anovulatory period, most often associated with foaling in the nonovulatory season.\textsuperscript{15}

Although the failure to return to normal estrus following parturition has been attributed to factors such as shortened daylight hours, disease or nutritional deficits in equids, all seem unlikely in our population. Based on daily monitoring plus weekly transrectal ultrasound examinations, there were no indications that ovarian abnormalities or disease caused anestrus. Additionally, all jennies received the same nutrition and were in a similar body condition, making a nutritional disorder doubtful. Lactating mares with a foal at foot commonly do not demonstrate estrus behaviors due to the presence of the foal.\textsuperscript{15} However, in this case, weekly transrectal ultrasonography detected no ovarian activity, making it unlikely that these jennies experienced silent estrus. That each jenny began cycling normally 1 to 2 weeks after weaning supported our diagnosis that eight of the 25 donkeys entered a true lactational anestrus during the physiological breeding season.
Learning points

- Eight of 25 donkeys in the Caribbean experienced lactational anestrus
- Cyclicity did not return until foals were weaned
- Lactational anestrus is not common in equids

References

**Figure 1.** Weekly serum progesterone concentrations in eight jennies with lactational anestrus.

**Figure 2.** Weekly serum progesterone concentrations in 13 normally cycling jennies.

**Figure 3.** Average serum progesterone concentrations of lactational anestrus and normally cycling jennies.