# Hemorrhagic anovulatory follicle in a tropical jenny

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**Summary**

An approximately nine-year-old tropical jenny was being routinely examined via transrectal ultrasonography as part of a follicular dynamics research study. On day 26 after her first documented ovulation, a 42.75-mm diameter presumptive preovulatory follicle developed an internal structure typical of a hemorrhagic anovulatory follicle. This ovarian structure remained ultrasonographically evident for 37 days. The interovulatory period of the cycle characterized by the hemorrhagic anovulatory follicle was shorter than the preceding cycle and two subsequent cycles that followed. This jenny continued to return to normal behavioral estrus each cycle. The progesterone concentrations measured during the cycle dominated by the hemorrhagic anovulatory follicle were numerically lower than those of the presumably normal ovulatory cycles of this jenny, but not statistically significant. The affected jenny was mated with a reproductively sound jack and became pregnant on the first attempt at the fourth recorded ovulation.

**Background**

There are no reports of hemorrhagic anovulatory follicle (HAF) development in tropical jennies and it is believed that this is the first documented case. A study published in 2008 reported one Catalonian jenny having similar hemorrhagic follicle patterns during two of 14 estrous cycles.[1] The follicles showed an interior fibrin network and reached 46- and 61-mm in size, similar to what was observed in this tropical jenny. These cases could provide further insight into the reproductive cycle of donkeys and the potential effects of HAF formation, if any. Controlling feral populations of donkeys is becoming a topic of interest in some tropical regions and in others, increased reproductive efficiency is desired. For both goals, a better understanding of reproductive pathophysiology of the species is required. These cases also serve as a comparison for other species in which hemorrhagic or luteinized anovulatory follicles occur, including women.[2]

**Case presentation**

A group of eight mature, cycling jennies was included in a trial designed to characterize ovarian follicular dynamics in tropical donkeys. Donkeys were housed in a dry paddock with unlimited access to water and local cane grass. Following protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Ross University School of Veterinary Medicine, the jennies were examined by transrectal ultrasonography using a 5-7.5 MHz linear probe daily at approximately the same time to track follicular development on both ovaries. Once a preovulatory follicle of 25- to 30-mm or larger was identified, they were examined by ultrasonography every six hours until ovulation. These jennies were followed through four cycles and subsequently removed from the study. The jenny in question had a 42.75-mm diameter follicle on day 26 after the previous ovulation when this follicle developed an internal structure typical of a HAF. This structure was monitored daily and ultrasound images of its progression are depicted in Figure 1. The presumptive HAF remained ultrasonographically evident for 37 days and ovulation during the third cycle occurred on the contralateral ovary after 19 days.

**Differential diagnosis**

The differential diagnoses considered in this case were a HAF, an ovulatory corpus luteum (CL) of anomalous structure, and ovarian hematoma. Based on this jenny's continued cyclicity, the progesterone assay data, and the information collected from similar cases in the literature, it is believed that this was a HAF.

**Treatment**

No treatment was administered to this jenny. She was allowed to cycle naturally without the influence of exogenous hormones or medical treatment while on this study.

**Outcome**

The length of this jenny's second estrous cycle with persistence of the HAF was shorter by six days relative to her first documented cycle on the study and the jenny continued to return to normal behavioral estrus. The third and fourth cycles were 23 days in length, four days longer than her second. These interovulatory periods are presented in the Table.

During the span of all four cycles, blood samples were collected on Mondays, Wednesdays, and Fridays to run serum progesterone assays. This jenny's fluctuations in progesterone levels were not statistically significant between cycles, but were seemingly lower during the cycle dominated by the HAF. A graph of the progesterone concentrations can be viewed in Figure 2. No other significant changes to this jenny's cycles were seen and no other jennies on this study had any similar ovarian abnormalities over the course of these four cycles.

Once the eight jennies in this study had been observed through four complete estrous cycles, they were bred using live cover by a reproductively sound jack. The jenny that had a HAF was successfully bred and maintained the pregnancy through 30 days.

**Discussion**

Little is known about the mechanisms affecting the incidence of HAFs in equids.[2,3] As previously mentioned, this phenomenon has been documented in a Catalonian donkey, but all other information found on HAF development in equids was for mares.[1] Formation of HAFs in pony mares was reported to be accompanied by elevated plasma estradiol for a few days before expected ovulation, but no significant differences in systemic progesterone, luteinizing hormone (LH), or follicle stimulating hormone (FSH) levels were seen. [2,4,5] There was also greater vascularity and thickening of the follicle within a few days before expected ovulation associated with the conversion of the viable follicle into a HAF.[3-5] The ultrasonographic appearance of the structure in question more closely resembled a HAF than an ovulatory structure, in part, because of the thickness of the luteal border. In mares, the luteal border of an anovulatory structure with central lacunae usually exceeds 5-mm.[5,6] Although measurements were not taken of the border of the HAF in this jenny, thickening of the wall is visible in Figure 1 as the follicle progressed and developed the fibrin network. No other jenny in this study had a CL with morphology similar to that described here. Ovarian hematoma was ruled out because the central part of the ovarian structure had an irregular echogenicity in contrast to more uniform echogenicity of most hematomata, the affected ovary was not enlarged, and the progesterone profile was not consistent with diagnosis of a hematoma.[4]

Another study in 2007 determined that HAFs are more common in older mares (>20 years).[2,4,7] This research also defined that a distinguishing feature between a corpus hemorrhagicum (CH) and HAF is the clotting of blood immediately during extravasation into the evacuated antrum versus delayed clotting when blood enters follicular fluid, respectively.[4,5,7] Hemorrhage into the evacuated follicle has been described as scattered free-floating echogenic spots within the follicular fluid, which could explain the follicular appearance viewed on ultrasound near ovulation and before the fibrin network appeared in the jenny of interest in this case.[3]

Other factors that may contribute to incidence of HAFs is time of season and the use of prostaglandin to induce ovulation. In one study from 2008, only one out of seven mares studied over ten years had no recorded HAF.[2] The other six ranged from 2.8% to as high as 25.3% incidence.[2] Use of prostaglandin, especially in higher doses during months of high follicular activity, increased the likelihood of induction and recurrence of a HAF.[2,3] The mechanisms by which prostaglandin increases the likelihood of developing HAFs is still unclear, but it may be related to LH influence after administration when only small immature follicles are present on the ovaries.[2] Neither the season nor the use of prostaglandin to control cyclicity were explored in this study, but all jennies were cycling and were not treated with prostaglandin for the duration of the project.

Ultimately, pregnancy is often the end goal for owners and veterinarians that are regularly tracking follicular development in donkeys or mares. The most relevant information found with respect to

HAF formation is that failure of ovulation or collapse of the dominant follicle can inhibit release of the oocyte. If this occurs, pregnancy cannot be achieved unless it is accompanied by normal ovulation of another follicle.[2,3] The jenny in question was not bred on the HAF cycle, so it cannot be determined if she would or would not have become pregnant. However, once the HAF regressed, she was successfully bred and produced a pregnancy that was detected through day 30.

**Learning points**

- The estrous cycle following development and persistence of a HAF in this tropical jenny was decreased by several days, but it did not appear to have any other significant effects on behavioral estrus or her ability to continue cycling during and after its regression. This contradicts previous findings in the literature regarding mares that had significantly prolonged inter-ovulatory periods, but the ability to continue cycling is consistent.[3]
- Serum progesterone levels were not significantly altered by the presence of the HAF in this case, but it cannot be determined at this time if preovulatory plasma levels of estradiol correlate with the production of a HAF as has been shown in the mare.[4,5,7] The lower progesterone levels measured in the HAF cycle mentioned above, while not statistically significant, may have allowed for earlier dominance of another follicle on the contralateral ovary.
- While the formation of a HAF was only documented during one cycle for this tropical jenny, more research would need to be done to determine if recurrence would occur within this individual. This has been documented in a Catalonian donkey and in mares.[1,2,4,7]
- A persistent HAF does not appear to impair the ability of this tropical jenny to become pregnant following its regression, even without hormone treatment or other therapy. Based on evidence described in the mare, it is hypothesized that the jenny would likely not have conceived if she had been bred on the cycles when the HAF was present.[2,3]

**References**


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## Figures and Tables

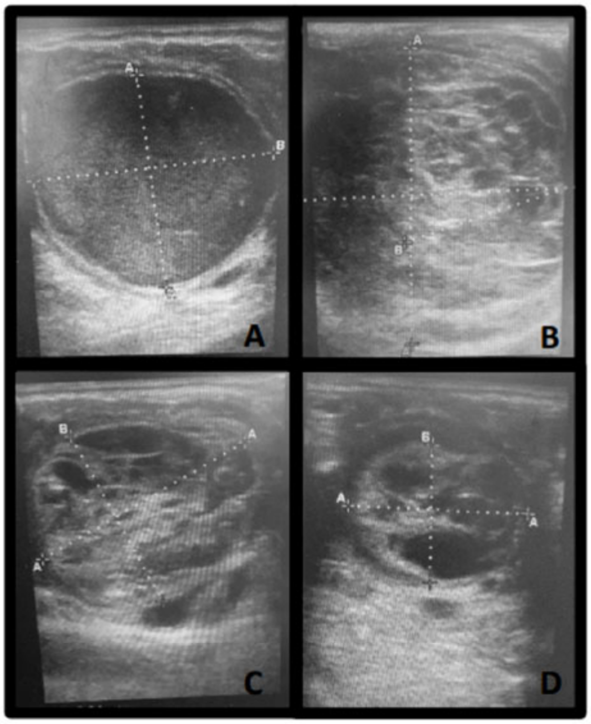


**Figure 1.** ***Ultrasonographic progression of the HAF.*** Various stages of development of the HAF are depicted. (A) One day prior to ovulation and formation of the HAF, the preovulatory follicle (49.5x44.5-mm) presented with an echogenic pattern within the follicular fluid. (B) Day 0 of the cycle of interest was characterized by a prominent fibrin network and the largest diameter of the HAF (68.6x61.3-mm). (C) Various patterns of the fibrin network were visible with the persistence of the HAF (47.7x39.4-mm). (D) The decrease in size of the HAF was accompanied with growth and dominance of another follicle on the contralateral ovary (36.2x27.1-mm).

| **Estrous Cycle** | **Length of Cycle (days)** |
|---|---|
| 1 | 25 |
| 2 (HAF) | 19 |
| 3 | 23 |
| 4 | 23 |

**Table.** Interovulatory periods. The length, in days, of each of the four estrous cycles that were documented during this study.

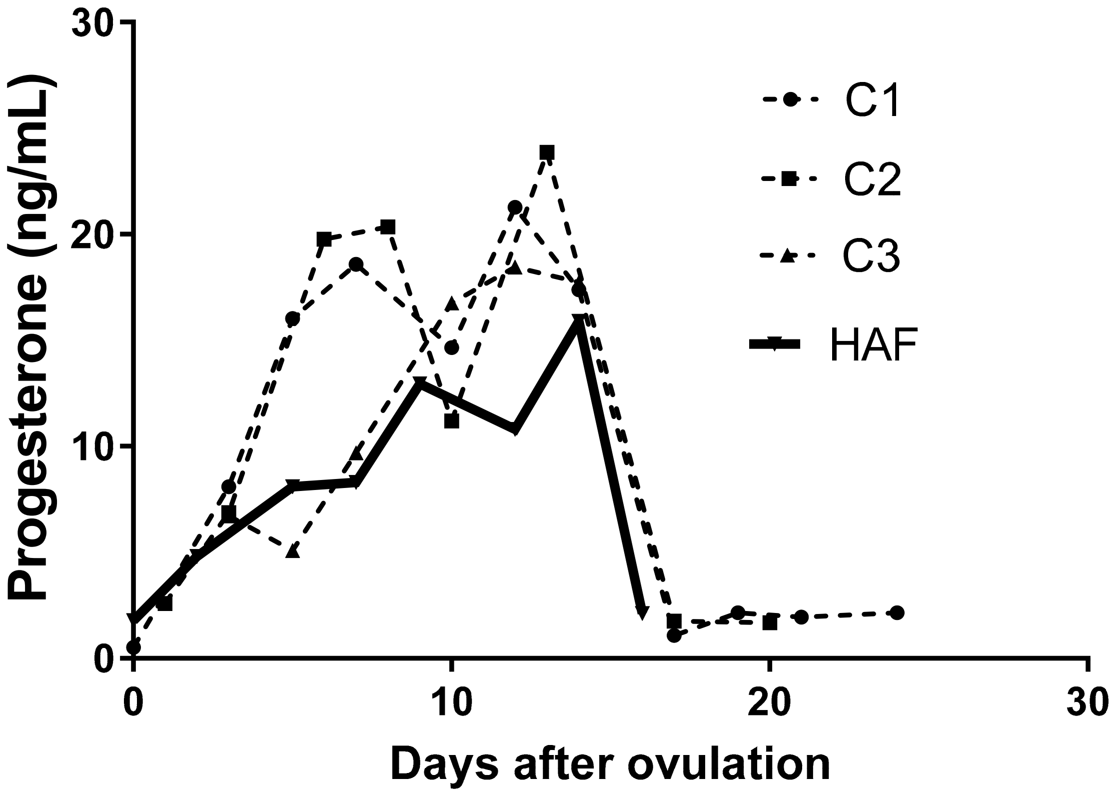


**Figure 2.** Graph of progesterone concentrations by estrous cycle. Each of the serum progesterone concentrations for samples collected over the course of this study are graphed based on the estrous cycle and days after ovulation. C1, C2, and C3 correspond to the first, second, and fourth cycles that were normal and not dominated by the presence of the HAF. HAF (the solid line) graphs progesterone concentrations during the cycle dominated by the HAF.