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Follicular aspiration during transition to advance the onset of cyclicity

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Summary

In an attempt to shorten the period of transition after seasonal anestrus and hasten the onset of cyclicity in mares, ultrasound guided transvaginal follicular aspiration was performed on mares when a follicle >35 mm was present. Mares that underwent follicular aspiration had serum progesterone > 1 ng/ml sooner than untreated controls (80.5 ± 7.3 d after January 1 vs. 104.3 ± 8.8 d, mean ± SE; P = 0.024). Nine of 11 treatment mares formed luteal tissue within 8 d after the first aspiration. The remaining two mares formed luteal tissue after a second aspiration procedure. When an aspiration procedure resulted in the formation of luteal tissue, progesterone rose to >1 ng/ml within one week (6.7 ± 0.9 d).

Keywords: transition, ovary, ovulation, estrous cycle, corpus luteum, mare

Follikelaspiration zur Beschleunigung des Einsetzens der zyklischen ovariellen Aktivität während der Übergangszyklen im Frühjahr

Bei dem Versuch, die Übergangsperiode nach dem Winteranöstrus zu verkürzen und den Eintritt in den saisonalen Zyklus zu beschleunigen, wurde unter Ultraschallkontrolle eine transvaginale Follikelaspiration bei Stuten durchgeführt, deren Follikel eine Größe von >35mm aufwiesen. Der Serumprogesteronspiegel der Stuten, bei denen eine Follikelaspiration durchgeführt wurde, erreichte schneller einen Wert von >1ng/ml als unbehandelte Kontrolltiere (80,5 ± 7,3 d nach dem 1. Januar im Vergleich zu 104,3 ± 8,8 d ± SE; P = 0,024). Bei 9 der 11 behandelten Stuten war bereits 8 Tage nach der ersten Aspiration Gelbkörpergewebe nachweisbar. Die übrigen zwei Tiere wiesen dieses erst nach einer zweiten Aspiration auf. In den Fällen, in denen die Aspiration zu der Ausbildung von Gelbkörpergewebe geführt hat, stieg der Serumprogesteronspiegel innerhalb von einer Woche (6,7 ± 0,9 d) auf >1ng/ml.

Schlüsselwörter: Reproduktion, Übergangszyklus, Ovar, Ovulation, Gelbkörper, Zyklus

Introduction

Because many horse breed associations arbitrarily impose an artificial birthday of January 1 in the Northern hemisphere, horse breeders desire to get mares pregnant early in the year. However, mares are seasonal long-day breeders, and are usually in anestrus during the winter months. Before resuming normal estrous cycles, mares go through transition, a period of anovulatory estrual activity. During transition, estrous periods are irregular and ovulation unpredictable, frustrating reproductive management on the stud farm.

Researchers have investigated many ways to advance the onset of cyclicity and shorten transition in order to expedite getting mares pregnant earlier in the year. Manipulating the photoperiod by providing artificial light to simulate increased day length earlier in the year is one of the most successful methods to advance cyclicity, however mares still go through a period of transition, and the added photoperiod must be begun around December 1. Attempts to induce ovulation during transition by pharmacological means have not been routinely successful. Daily injections of progesterone in oil failed to advance the mean date of first ovulation (*Alexander* and *Irvine* 1991). Use of deslorelin in late transitional mares advanced the date of first ovulation by approximately two weeks, but repeated injections were needed (*McKinnon* et al. 1996).

Follicular aspiration, used for research and assisted reproductive techniques, has resulted in apparent luteinization of the aspirated follicular structure when performed on cyclic mares during the breeding season (*Hinrichs* et al. 1991). If follicular aspiration during transition could do likewise, it is anticipated that the resulting luteal structure could be lysed with prostaglandin, thereby initiating cyclicity. *Bogh* et al. (2000) reported that in a small group of transitional mares that had undergone repeated aspiration of all follicles > 8 mm, all mares ovulated within 12 days of the last aspiration. Although there were no control mares with which to compare, it appeared that the onset of cyclicity had been advanced somewhat. We hypothesized that by aspirating the dominant follicle during the late transitional period, we could induce formation of luteal tissue and thereby hasten the onset of cyclicity. Our specific objective was to perform follicular aspiration and compare the date of first rise in progesterone between treated and control mares.

Materials and Methods

In January, mares documented to be in anestrus were randomly assigned to either the treatment or control group. The control mares (n = 6) were monitored by palpation and ultrasonography, per rectum, twice weekly, until ovulation was detected. The treatment mares (n=11) were monitored in the same manner until a follicle >35 mm was identified. At that time, the follicular fluid was evacuated by transvaginal ultrasound guided aspiration. Aspiration was performed with the mare standing in stocks, under sedation (detomidine, 20.0 ug/kg, iv, and butorphanol, 0.1 mg/kg, iv). A transvaginal curved linear array 5 MHz transducer (Sonovet 600, Medison Inc) equipped with a 60 cm, 17 g needle, was used for the follicular aspiration. After aspiration, the ovaries were monitored by ultrasound every two to four days for presumptive luteal tissue formation as indicated by the appearance of a hyperechoic structure at the site where the follicle had been located. At each examination throughout the study, blood was obtained and plasma stored at -20 C for subsequent progesterone determination. To confirm that normal luteal tissue was present and cyclicity had begun, PGF (10 mg, im) was administered 9 to 14 days after aspiration to cause luteolysis and induce estrus, followed by hCG (2500 IU, iv) administration to induce ovulation when a follicle >35 mm was present during the induced estrus.

Concentrations of progesterone were determined with commercially available reagents (Diagnostic Systems Laboratory, Webster TX, USA) that were previously validated for horse plasma by personnel in the Louisiana State University Department of Animal Sciences. Intra- and interassay coefficients of variation and assay sensitivities were 6%, 8%, and 0.1 ng/ml.

Results

The time from January 1 to the first rise in serum progesterone was 23.8 days earlier for the treated mares than for the control mares (80.5 ± 7.3 and 104.3 ± 8.8, mean ± SE, for treatment and control groups, respectively; P = 0.024). The time from detection of a follicle > 35 mm to progesterone > 1 ng/ml was shorter for aspirated mares (10.0 ± 2.4 and 34.7 ± 7.4 days for treatment and control mares, respectively; P = 0.008). Of the 11 treatment mares, nine formed luteal tissue within eight days after the first aspiration. Two mares required a second aspiration procedure before developing luteal tissue. When an aspiration procedure resulted in the formation of luteal tissue, progesterone rose to >1 ng/ml in 6.7 ± 0.9 days. Subsequent PGF administration resulted in progesterone declining to <1 ng/ml within three days. Administration of hCG during the ensuing estrus induced ovulation in all mares.

Discussion

Previous reports indicated that aspiration of a follicle during estrus could result in the formation of luteal tissue (*Hinrichs* et al. 1991). *Alvarenga* et al. (1999) aspirated all follicles > 10 mm from the ovaries of transitional mares once a follicle > 30 mm was detected, and administered hCG concurrently. Aspiration resulted in luteinization in only three of eight mares within seven days. In the present study, only the dominant follicle was aspirated. Because the transitional period is characterized by waves of follicular growth and regression, it was hypothesized that by waiting until the follicle reached at least 35 mm in diameter that luteinization would be more likely to result. In fact, 9 of 11 mares responded to the first aspiration, usually within less than a week. Interestingly, although not used as a criteria for treatment in this study, each mare that underwent follicular aspiration had ultrasonographic evidence of uterine edema (score of > 2 on a scale of 0 to 4) at the time of aspiration. However, *Watson* et al. (2003) have reported that uterine edema is not a reliable indicator of follicular competence or ovulation.

The exact time period from aspiration to serum progesterone could not be ascertained in our study because mares were only examined every two to four days after treatment. Nevertheless, an increase in progesterone, indicating the presence of functional luteal tissue, was observed on the average within a week after aspiration. Furthermore, the onset of cyclicity was confirmed because each mare responded to subsequent prostaglandin injection with luteolysis, estrous behavior and ovulation. Because the average estrous cycle is approximately 21 to 22 days long, advancing the date of first rise in progesterone by over 3 weeks would provide an additional breeding opportunity during the breeding season. Results of this study indicate that follicular aspiration of a follicle >35 mm during late transition may be a means to advance the onset of cyclicity in mares.

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