ORIGINAL RESEARCH

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Incidence, Endocrinology, Vascularity, and Morphology of Hemorrhagic Anovulatory Follicles in Mares

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ABSTRACT

The incidence of hemorrhagic anovulatory follicles (HAFs) is approximately 5% and 20% of estrous cycles during the early and late ovulatory season, respectively. The structures are more common in old mares (eg, >20 years), tend to occur repeatedly in individuals, and occur most frequently during the late follicular phase. In a recent study, the day of ovulation in controls and the first day of HAF formation, as indicated by cloudiness of follicular fluid, were defined as day 0. On day -1, future ovulating and HAF groups did not differ in follicle diameter or in the frequency of discrete gray-scale ultrasonic indicators of impending ovulation; however, in future HAFs, a greater percentage of the circumference of the follicle exhibited color-Doppler signals of blood flow. No differences were found between the two groups in systemic concentrations of progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) on days -4 to 2, but estradiol was elevated in the HAF group on day -3. The wall of the HAFs developed well-vascularized luteal tissue as indicated by echotexture and color Doppler signals and by the production of near normal levels of progesterone. In conclusion, HAFs formed from viable preovulatory follicles that did not differ from ovulatory follicles in diameter or gray-scale echotexture. Estradiol concentrations were elevated a few days before the failure of ovulation, and the wall of the follicle was more extensively vascularized on day -1.

Keywords: Anovulation; Estrogen; Hematoma; Hemorrhagic anovulatory follicles; Mares

INTRODUCTION

Extravasation of blood into follicles, ovulation sites, and corpora lutea is common in mares.[1,2] A hematoma that forms in the antrum in lieu of ovulation has been termed a hemorrhagic anovulatory follicle (HAF).[3,4] Initial descriptions of HAFs were based on transrectal ultrasonography[3] and on gross appearance after transection[5]; blood oozed from the hematoma of HAFs during sectioning. Structures similar in description also have been termed hemorrhagic follicles,[2,4,5] anovulatory hemorrhagic follicles,[6] and persistent anovulatory follicles.[7] What may have been the same structures were noted originally by transrectal palpation toward the end of the ovulatory season.[8] In a report on ovarian estrogen levels, structures with a similar gross appearance were termed autumn follicles.[9] The economic importance of HAFs as a breeding-management problem in mares has been noted[7,10,11] and reflects anovulation of a follicle after the mare has been bred.

A corpus luteum with a central blood clot is called a corpus hemorrhagicum (CH). In one study,[12] a CH with a blood clot that was greater than 10% of the cross-sectional area of the gland formed in 50% of 55 corpora lutea. After first detection at 20 hours after ovulation, the central areas increased in diameter until 72 hours.[13] Occasionally, however, blood may begin to fill the collapsed antrum immediately after ovulation (within 15 minutes) or after 75% of the antrum has evacuated.[2] Undisturbed function of CHs is indicated by unaltered progesterone production, echogenicity of the luteal tissue, and length of the interovulatory interval.[12-14] All CHs and most HAFs[2] have a peripheral wall of luteal tissue, as indicated by echotexture. A distinguishing feature between a CH and HAF is the clotting of blood immediately during extravasation into the evacuated antrum or developing corpus luteum versus delayed clotting when blood enters follicular fluid.

Structures that seem similar in echotextural description to HAFs in mares also have been detected during ultrasonic imaging in llamas[15] and women.[16] Therefore, study of HAF formation and the underlying mechanisms in mares may be of comparative importance for other species, including humans. In addition, the anovulation associated with HAF formation may be a useful natural model for studies on the mechanisms of ovulation and luteinization.

INCIDENCE

During a research study on echotexture of the follicular wall in ponies, HAFs formed during 3 of 56 (5%) estrous

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cycles early in the ovulatory season (March to May) and in 5 of 23 (23%) late in the season (September and October[17]). In earlier records from the same research herd, an incidence of 21% was found during September and October, and the structures were especially common (36%) in mares ≥20 years of age.[18] The incidence in 213 interovulatory intervals in Quarter Horses was 4.7%[4]; an HAF occurred without a companion ovulation or a second HAF during the late follicular phase (five mares), in conjunction with ovulation of another follicle (one mare) or during the luteal phase (three mares). An 8% incidence was found in a retrospective examination of reproduction records for 1845 estrous cycles of horses[7]; the incidence increased with age, and 44% of mares that formed an HAF repeated the formation during a subsequent estrous cycle. In a recent study of large ponies of mixed breeds,[19] six nonbred mares that had a mean of 3.5 HAFs during the preceding ovulatory season (HAF group) and six mares that had none (controls) were compared during the spring of the next season. The incidence in the HAF group during a total of 13 estrous cycles was a single (31%) or multiple (8%) HAFs during the late follicular phase, an HAF and an ovulation during the same follicular phase (8%), and single or multiple HAFs during the luteal phase (8%; total incidence, 54% of estrous cycles). Mares with no HAFs during the preceding season had none during 13 estrous cycles the next season.

In conclusion, the incidence of HAFs is approximately 5% and 20% during the early and late ovulatory season, respectively. The structures are more common in old mares, occur most frequently during the late follicular phase, and have approximately a 50% incidence of repeatability in individuals. The repeatability characteristic is an especially important clinical and economic aspect of the HAF syndrome. For example, a mare had an ovulation in the first two cycles of the year and thereafter formed an HAF during all of 10 cycles from March to November. Daily diameters of follicles and HAFs and concentrations of LH and progesterone for this mare are shown for 58 consecutive days (Fig. 1).

FOLLICLE MORPHOLOGY PRECEDING HAF FORMATION

The echotextural morphology of ovulatory-sized follicles that formed HAFs during the late follicular phase has been compared with those of a solitary follicle that ovulated.[19] An HAF that was considered appropriate for comparisons with controls was defined as a solitary HAF that developed during the late follicular phase without ovulation from another follicle during the phase and was not followed by HAF formation during the luteal phase. This was done so that hormonal comparisons between groups were not complicated by other structures. Both ovulation and the day of the beginning of HAF formation were designated day 0. The day of the beginning of HAF formation was assigned retrospectively as the first day of consecutive daily appearances of an excessive number of floating echoic specks imparting a cloudy appearance to the follicular fluid or the day before echoic strands or an echoic sheet appeared on the gray-scale image of the antrum. The gray-scale criteria for designation of the beginning of HAF formation were supported in retrospect by nonsignificant differences in LH concentrations on day 0 between mares that ovulated and mares that formed an HAF (Fig. 2).

Diameter of the preovulatory follicle on days -4 to -1 was not different between the two groups (Fig. 2). Discrete ultrasonic gray-scale characteristics of the preovulatory follicle that have been reported to indicate impending ovulation[20-23] were recorded as present or absent for both groups on days -2 and -1. The discrete end points were (1) decreased turgidity of the follicle under transducer pressure; (2) loss of spherical shape; (3) echoic spots floating in the antrum; (4) an apex or cone-shaped protrusion of the follicle (future area of ovulatory rupture); and (5) serration of granulosa. Serration of granulosa is indicated by an irregular or notched appearance of both surfaces of the granulosa (interface with the antrum and interface with the theca interna), especially on the side of the follicle opposite to the area of impending ovulation.[23,24] The number of discrete indicators for days -2 and -1 was compared between the two groups. The number in the controls was greater for day -1 (2.3 ± 0.6 indicators/mare) than for day -2 (0.1 ± 0.1), supporting a temporal relationship to impending ovulation. There was no difference between the controls (2.3 ± 0.6) and the HAF group (2.0 ± 0.7) on day -1. These results, as well as similar diameter on day -1, indicated that HAF formation involved entry of blood into a follicle that did not have altered structure, based on gray-scale ultrasonography. Furthermore, the follicle cells were viable, as indicated by luteinization of the wall of the HAF; luteinization was based on echotexture and approximately normal progesterone production. In conclusion, HAF formation occurred from a viable follicle and began on the day of expected ovulation; there was no indication that daily gray-scale ultrasonic imaging can be used to predict whether a follicle will ovulate or form an HAF.

Color-Doppler assessment of mural blood flow on day -1 was compared between follicles that ovulated and follicles that formed an HAF. The percentage of follicle wall with color Doppler signals on day -1 was greater for the HAF group (90% ± 4%) than for the controls (69 ± 7%; Fig. 3). The difference between the two groups in percentage of wall with color signals was attributable to vascularization of the follicle wall in the area of expected ovulation (apical area) in mares that formed an HAF but not in mares that ovulated. A difference in vascularity at the apex is compatible with nil or minimal hemorrhage during ovulation and massive hemorrhage during HAF formation. In this regard, prolonged antral evacuation (>3 hours) during ovulation and septation of the evacuating antrum is also associated with vascularization at the apical area on day -1.[24] HAFs and septated evacuations appear to have a common component in their pathogenesis, involving excessive vascularization of the apical area. Septated evacuation sites contained pockets of apparent follicular fluid while the

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