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The effect of hormone treatments (hCG and cloprostenol) and season on the incidence of hemorrhagic anovulatory follicles in the mare: A field study

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Abstract

The association between use of hormone treatments to induce estrus and ovulation and the incidence of hemorrhagic anovulatory follicles (HAFs) was studied in a mixed population of mares during two breeding seasons in a commercial breeding clinic. Mares treated with cloprostenol (CLO) were more likely to develop HAFs than were mares with spontaneous cycles ($P < 0.001$) or those treated with human chorionic gonadotropin alone ($P = 0.08$). There was no significant effect of season on the incidence of HAFs. The mean (±SEM) interval from CLO treatment to beginning of HAF development was 6.1 ± 0.5 d. Age of mares with HAF cycles was not different (12 ± 1.3 yr; $P > 0.05$) from that of mares with ovulatory cycles (10.5 ± 1.5 yr).

Keywords: Anovulation; hCG; Induced cycle; Mare; Prostaglandin

1. Introduction

Ovulation failure in the mare is characterized by hemorrhage into the dominant preovulatory follicle(s), which fails to rupture or collapse with subsequent organization of its contents and in most occasions luteinization of the follicular wall. This type of anovulatory follicle in the mare has been referred to as autumn follicles [1], hemorrhagic follicles [2], persistent anovulatory follicles [3], and hemorrhagic anovulatory follicles (HAFs) [4], which seem to be the same structure. Some authors have suggested that HAFs in the mare share some similarities with other anovulatory conditions in other species such as luteinized unruptured follicle (LUF) in women [5] and ovarian cystic disease (OCD) in cattle [3].

The relevance of this anovulatory condition lies in the fact that mares with preovulatory follicles that fail to collapse will not conceive unless ovulation of a concurrent preovulatory follicle occurs in the same estrus. Although the overall incidence of hemorrhagic follicles in the mare population is relatively low, 5% to 8% [2,3], this condition can be very frustrating for the practitioner when mares are bred before ovulation because it appears impossible (using real mode B ultrasonography) to distinguish between one preovulatory follicle that will collapse and ovulate and another that will hemorrhage. It has been shown that mares with either type of preovulatory follicles share ultrasonographic follicular wall characteristics, uterine edema patterns, and reproductive hormonal profiles during the 3 d prior to ovulation/hemorrhage [4].

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Some mares, however, are reported to have an abnormally high incidence of hemorrhagic follicles as if they were some how predisposed to this anovulatory condition [5,6]. These mares often have a high reoccurrence rate in the same season. The reason for this individual predisposition to high HAF incidence appears to be an intrinsic high LH concentration [7].

Recent controlled hormonal studies have shown that early stages of follicular development preceding HAF formation are under the influence of higher LH peripheral concentration than that of developing follicles ending in normal ovulations [7,8]. In the experimental design of these studies, mares were short-cycled with prostaglandin on Day 10, and all existing follicles at the time were ablated transvaginally. Ginther and co-workers proposed therefore an association between the use of prostaglandin and the increased incidence of HAF (20% incidence in induced-waves compared with 2% in spontaneous waves). The authors, however, could not elucidate whether this increase in HAF incidence was due to the use of prostaglandin, follicular ablation, or a combination of both. A simultaneous retrospective study [5] with analysis of long-term reproductive and ultrasound records of seven mares, two of them with known high HAF incidence, found a clear association between the use of CLO and HAF development (around 90% of HAF cycles over the 20-yr period were induced with CLO). In the latter study, follicles were not ablated. Therefore, it seems that the use of prostaglandin alone to induce earlier luteolysis is able to increase the incidence of HAF in a clinical setting. Caution must be taken, however, when extrapolating the results of that study to the whole population of mares as it was based only in two mares with probably an intrinsic high luteinizing hormone (LH) concentration.

The aim of the current study was to investigate the effect of hormone treatments (CLO and hCG) on the incidence of hemorrhagic follicles in a large mixed population of mares. It was hypothesized that mares with induced cycles would be more likely to develop hemorrhagic follicles than would mares during spontaneous cycles. To test this hypothesis, reproductive records of a mixed population of mares during the years 2006 and 2007 were analyzed retrospectively.

2. Materials and methods

2.1. Animals and ultrasound examinations

Records from a total of 207 mares from a mixed population of Warmbloods, Irish Draught, and cross-breeds with 765 estrous cycles recorded were analyzed over the years 2006 and 2007 in the Northern Hemisphere. Forty percent of the mares (n = 83) had only one cycle included in the study. The remainder (n = 124) were followed for more than one cycle (range of 26 and median of 2) over the 2 yr. The mares were either resident at the clinic (used as recipients for embryo transfer program) or visiting mares for AI, ET, or other reproductive procedures. Mean and median age of the mares included in the study was 11 yr (range of 24 yr, ~~median of 11 yr~~).

Transrectal ultrasonographic examinations were performed by the same operator at least once daily and up to three times a day as ovulation approached. The ultrasound machine was equipped with a linear probe of 7.5 MHz.

2.2. Reproductive and ultrasound records

The use of hormonal treatments to induce estrus and ovulation (CLO and/or hCG) was recorded in every case. The end points recorded were ovulation, HAF, and endometrial edema.

Ovulation: Detected as per rectal palpation and ultrasonography by absence of the previously recorded follicle and presence of a hypoechoic area within the same ovary. Ovulation was confirmed by the later presence of an echoic corpus luteum (CL). The date of ovulation was recorded as the day in which it was first detected. An ovulation could be classified into four categories:

- Spontaneous: when no hormonal treatment had been given since the previous ovulation.
- CLO-induced: when luteolysis with signs of estrus and subsequent ovulation followed the administration of CLO (a PGF analogue) during diestrus (Estrumate; Intervet, Cambridge, UK). The dose varied from 250 to 500 μg given subcutaneously. However, only a reduced number of cycles were induced with more than 250 μg (<3%).
- hCG-induced: when the ovulation followed the subcutaneous administration of 1500 IU hCG (Chorulon; Intervet). The interval from hCG to ovulation was always within 96 h.
- CLO- and hCG-induced: when CLO-treated mares were given 1500 IU hCG while in estrus.

Hemorrhagic anovulatory follicle: HAF was detected by transrectal ultrasonography as described previously [5]. In brief, the previously fluid-filled follicle of anechoic echotexture fills with echogenic

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specks that float freely in the follicular fluid and swirl if balloted, and without follicular collapse the granulosa layer becomes increasingly echodense and deeper. The number and echodensity of the intrafollicular specks increase but still have a mobile/swirling appearance. The follicle diameter increases, and eventually the contents acquire a static organized appearance (Fig. 1). As with ovulating follicles, HAFs were regarded as occurring either spontaneously or induced after administration of CLO and/or hCG (as above). Intervals from induction treatment to HAFs were recorded (the interval from CLO to HAF development was less than

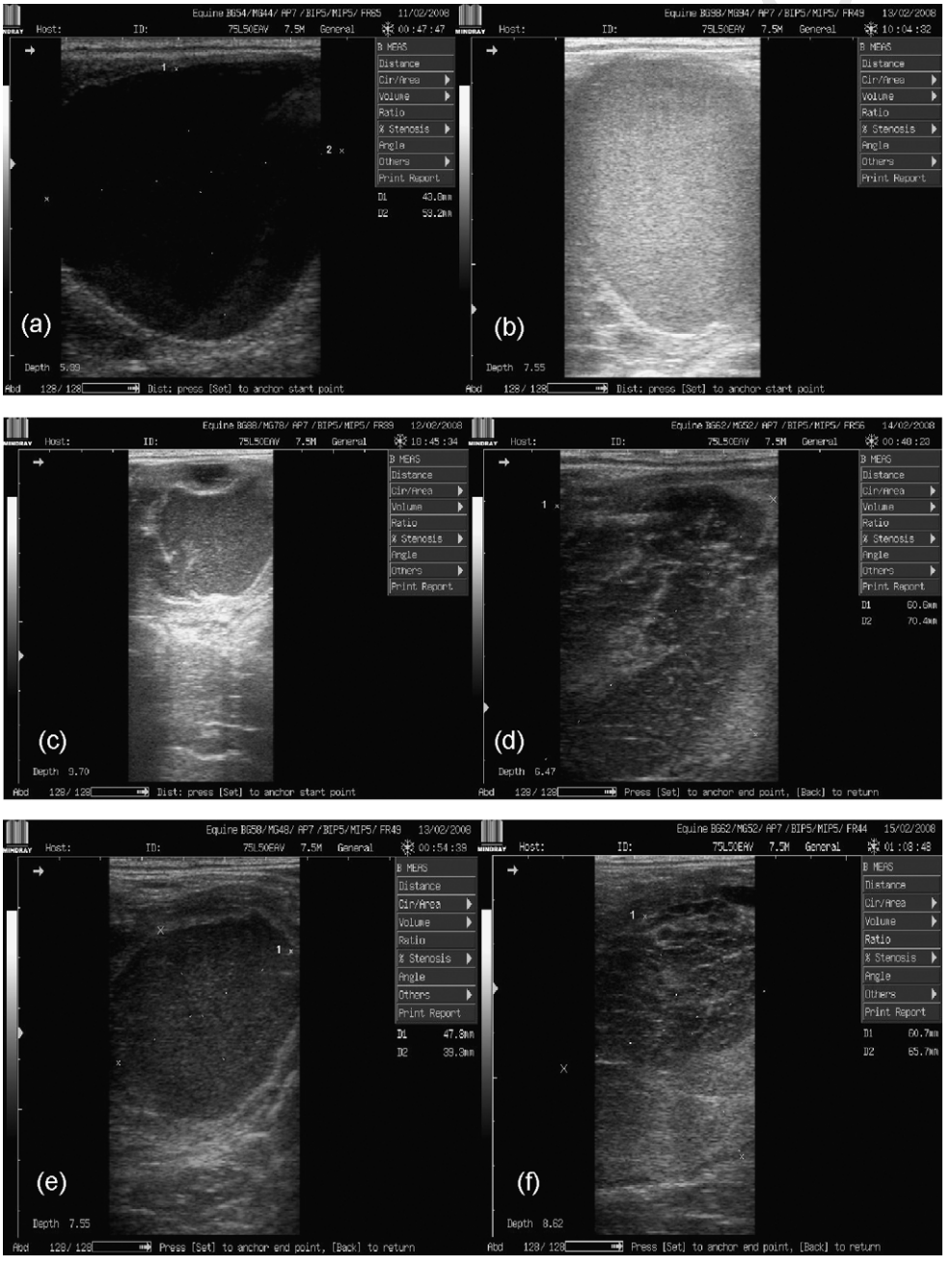

Fig. 1. Chronologic order of sonogram series of (A) a preovulatory follicle (a) that hemorrhaged and subsequently luteinized without lost of follicular fluid. (B, C) Follicle fills with blood (hyperechoic specks) and follicular wall thickens and becomes hyperechoic. (D) Increase in number of hyperechoic specks as a result of further hemorrhage. (E, F) Organized follicular contents and luteal tissue become more apparent (lower part of pictures), and the overall diameter of the unruptured follicle increases to more than 60 mm.

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12 d in all cases). Some HAFs occurred during the same cycle of a normal ovulation. For data analysis, the date of HAF was estimated on the day the follicle filled with echodense specks (day of expected ovulation: Day 0).

Endometrial edema: Degree of endometrial folding was subjectively assessed by transrectal ultrasonography in both ovulatory and HAF cycles (only those with a solitary HAF). Increasing scores of 0.5 were given to the uterus from 0 (no endometrial folding coincident with diestrus-like echotexture) to 3 (maximum endometrial folding).

2.3. Experimental design and statistical analysis

For data analysis, the experimental unit used was the ''cycle,'' which could be either ''ovulatory'' (when no HAF developed during one interovulatory period) or ''hemorrhagic'' (when single or multiple HAFs whether accompanied by ovulation[s] or on its own developed during an interovulatory period). The association between induction treatment and incidence of hemorrhagic follicles was analyzed by chi-square test as well as the effect of year (2006 and 2007) and season (winter, December to March; early in the ovulatory season, April to July; and late in the ovulatory season, August to November). Differences in age and edema pattern between mares with HAFs and with ovulatory cycles were analyzed by two-sample *t*-test and Mann-Whitney nonparametric test, respectively. Effect of CLO dose on HAF incidence was analyzed by Fisher's exact test.

3. Results

The overall incidence of HAFs was 4.9% (38 of 765 estrous cycles for years 2006 and 2007). Cycles induced with CLO had a higher incidence of hemorrhagic anovulatory follicles (22 HAF cycles out of 263; 8.4%) than that of those induced with both hCG and CLO (9 HAF cycles out of 118; 7.6%; $P > 0.05$), hCG (5 HAF cycles out of 135; 3.7%; $P = 0.08$), or of spontaneous cycles (2 HAF cycles out of 249; 0.8%; $P < 0.001$). There was no effect of CLO dose on the incidence of HAF ($P > 0.05$). Incidence of HAF cycles in mares treated only with hCG tended to be ($P = 0.054$) higher than that of spontaneous cycles. The effect of hormone treatment was not different between years ($P > 0.05$). In CLO-induced HAF cycles, the mean interval from treatment to the day of expected ovulation was 6.1 ± 0.5 d, whereas in HAF cycles induced with hCG, the mean was 2.0 ± 0.3 d. The HAF reoccurrence rate in a consecutive cycle (n = 26 cycles) and following year (n = 5 mares) was 4% and 60%, respectively (Table 1).

The incidence of cycles with solitary HAF(s) was 68% (26 of 38 HAF cycles), whereas the incidence of those accompanied by concurrent normal ovulation(s) was 32% (12 of 38). The two spontaneous noninduced HAF cycles had no concurrent ovulation(s).

There was no significant effect of season on HAF incidence ($P > 0.05$). When data from both years were pooled together, the incidence of HAF was 2.4%, 5.6%, and 3.9% for winter and early and late in the ovulatory season, respectively. Hemorrhagic anovulatory follicle incidences per month and year are shown in Table 2. The mean age of mares that developed HAF cycles was 12 ± 1.3 yr (youngest 2 yr old and oldest 26 yr old). This was not different ($P > 0.05$) from mares with normal cycles (mean age, 10.5 ± 1.5 yr). All HAF cycles (n = 8) of mares aged 10 or younger were induced with CLO.

The uterine edema pattern of HAF cycles without concurrent ovulations was compared with those of ovulatory cycles. No significant difference ($P > 0.05$) in uterine edema pattern at any observation time between the two types of cycles was found.

Table 1
Incidence of HAFs in relation to type of cycle.

Year	Cycle type	Sp	CLO	hCG	CLO + hCG
2006	HAF	0	10	3	4
	Non-HAF	124	128	55	38
2007	HAF	2	12	2	5
	Non-HAF	123	113	75	71
	Total cycles	249	263	135	118
2006–2007	HAF cycles	2	22	5	9
	HAF incidence (%)	0.8[a]	8.4[b]	3.7[b*]	7.6[b]

Sp, spontaneous noninduced; CLO, cloprostenol induced; hCG, hCG induced; CLO + hCG, estrus induced with cloprostenol followed by hCG administration, all for the period 2006–2007.
[a,b]Within a row, different superscripts indicate significant difference: $P < 0.001$; [a,b*]$P = 0.054$.

Table 2
Effect of month on HAF incidence.

Year		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	Total cycles	8	22	41	76	111	157	135	90	62	40	12	11
2006–2007	HAF cycles	0	1	1	2	6	12	7	4	2	1	2	0
	HAF incidence (%)	0	4.5	2.4	2.6	5.4	7.6	5.2	4.4	3.2	2.5	16.7	0

The interovulatory interval of two normal cycles with a HAF cycle in between (without additional ovulations or use of luteolytic treatments) was 40 ± 4 d, whereas the interval from beginning of HAF development to the next spontaneous ovulation was 21.6 ± 1.3 d.

4. Discussion

The results of the current study strongly suggest that induction of estrus with CLO substantially increases the likelihood of developing HAFs.

This study confirms the results obtained by Ginther and co-workers [7]. In addition, the theory that HAF incidence is increased by use of prostaglandin alone and not by follicular ablation is supported. When prostaglandin-induced luteolysis occurs, progesterone concentration drops rapidly, and apart from the immediate rise in gonadotropins induced by it [9], the removal of the negative feedback of progesterone on LH allows LH to rise early during the beginning of follicular development, thus reaching higher LH concentration before follicular deviation [7]. It has been hypothesized that the LH surge could interfere with intrafollicular metabolism of prostanoids and proteolytic enzymes necessary for the process of ovulation and follicular collapse if it occurs during the development of immature follicles [10].

Perhaps follicular ablation at the time of luteolysis, although not essential for HAF development, could be synergistic as all new follicles would have to develop from pre-antral stages while LH concentration is already high. We found previously [5] an association between HAF formation and use of high doses of CLO. In our practice, higher doses of CLO (500 to 1000 μg) are given early in diestrus (3 to 4 d postovulation) to cause full luteolysis compared with lower doses (125 to 250 μg) later in the cycle. Although not analyzed critically, this correlation between higher doses and higher incidence of HAF could be due to the fact that by inducing luteolysis earlier in diestrus (Day 3 to 4), the subsequent rise in LH occurs at earlier stages of follicular wave development in contrast with luteolysis later in the cycle when follicle development is more advanced. In this study, there was no effect of CLO dose, however high doses were only used in very few mares, which may preclude finding a significant difference. Further research on the effect of treating mares with prostaglandin at different postovulation times remains to be done.

The effect of hCG on HAF incidence is not completely clear. Mares treated with hCG were more likely to develop HAF than were mares during spontaneous cycles. Although the difference approached significance (P = 0.054), the number of HAF cycles was too small to investigate some other risk factors related to the hCG regimen. It is true, however, that studies reporting HAF had often used hCG to induce ovulation [11,12].

Uterine edema score during the few days prior to ovulation/hemorrhage was not different. This is not surprising as follicles that are destined to hemorrhage secrete similar amounts of estradiol as ovulatory follicles [6].

Although evidence in the literature shows that aged mares are more likely to develop HAFs [3], it is not rare for young mares (3 to 5 yr old) to develop them. It is worth noting though that all HAF cycles from young mares (<10 yr) had been treated with CLO.

The season did not appear to have an effect on the incidence of HAFs, contrary to the belief that they are more common in the fall [1,13]. It seems that the HAF incidence in November was significantly higher than that during the rest of the months (Table 2). However, the high incidence (16%) could be biased by the low number of cycles at that month (n = 12) and by the fact that reproductively ‘‘abnormal mares’’ are more difficult to get in foal and therefore are more likely to be cycling later in the season. In fact, the 16% HAF incidence was the result of two consecutive HAF cycles from the same mare, which had developed more HAFs previously in the same year. Mares with high HAF reoccurrence rate, so-called repeaters, are thought to have an intrinsic high LH concentration, which perhaps is constantly elevated all year round [7]. Apart from November, the months with highest HAF incidence were May, June, and July, which are also the months with highest mean LH concentration in the mare population [14].

In conclusion, mares treated with prostaglandin alone are at increased risk of developing HAFs.

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This knowledge may be useful for practitioners so that the use of prostaglandin to short cycle can be avoided in mares predisposed to develop HAFs as well as for researchers to provide new insights to further understand the pathogenesis of the development of hemorrhagic anovulatory follicles in the mare.

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