Risk Factors for the Development of Haemorrhagic Anovulatory Follicles in the Mare

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Contents

Haemorrhage into the dominant follicle during the reproductive season is a subtle but definitive cause of infertility in the mare population. This condition however can be of high relevance for an individual in which its incidence is abnormally high. Little is known about the nature and factors affecting the incidence of haemorrhagic anovulatory follicles (HAFs) in the mare. The objectives of the study were to define and characterize the ultrasonographic development and incidence of HAFs and to investigate possible risk factors influencing its occurrence. Detailed reproductive and ultrasound records of seven mares studied during their entire reproductive lives (>10 years and 612 oestrous cycles) were analysed retrospectively and computed into a statistical mixed model. Of all animal studied, two mares were found to have an unusually high incidence of HAFs of approximately 25%. Time of season and use of induction treatments (Cloprostenol) were found to influence its incidence. It appears that early-enhanced stimulatory effect of LH on an ovary with the presence of small and immature follicles might increase the risk of ovulatory failure of those follicles later in the cycle. Mares during the months of highest follicular activity (May to August) and after treatment with hormones to induce oestrus and ovulation are at greater risk to develop HAFs. The potential relevance of this study is two folds: clinical relevance for the practitioner to better understand this condition and so improve reproductive management of mares with abnormally high incidence; and to provide useful insights for researchers willing to further investigate the nature of this phenomenon.

Introduction

Failure to ovulate the dominant follicle and subsequent persistence of the anovulatory unruptured structure has been reported to occur in several domestic animal species and in women. The species in which this phenomenon has been demonstrated as naturally occurring are cows (Garverick 1997; Peter 2004), mares (Newcombe 1987; Ginther 1992; Ginther et al. 2007) and women (Marik and Hulka 1978; Katz 1988; Zhu 1989; Toda 1990). Research studies on methods of contraception and ovulatory process have also shown experimentally-induced unruptured follicles in rabbits (Grinwich et al. 1972; Salhab et al. 2003), rats (Armstrong and Grinwich 1972) and women (Killick and Elsein 1987). It seems that this phenomenon might occur naturally in all domestic species, however only in species like the cow and the mare in which follicular dynamics are easily and routinely followed ultrasonographically, allow sufficient number of observations to characterize this syndrome.

A distinct cause of ovulatory failure observed in the mare is haemorrhage of the dominant follicle(s) with subsequent organization of follicular contents and, in most occasions, luteinization of follicular wall without previous follicular collapse. This condition has been referred to in different ways: first reference to what seemed to be the same sort of follicle was back in the 1940s (Burkhardt 1948) where it was described as occurrence of large persistent follicles during the months of October to November. Later in the century they were given the name of 'autumn follicles' (Knudsen and Weiert 1961) as they were reported to occur more frequently at the end of the ovulatory season. Ginther (1979) was the first to describe the macroscopic features of this anovulatory follicle which was defined as blood-filled structures with the presence of luteal tissue in the surrounding wall and so termed 'haemorrhagic follicles'. With the advance in imaging techniques in the 1980's, several studies reported occurrence of this anovulatory condition in different population of mares (Ginther and Pierson 1984, 1989; Townson and Ginther 1988; Carnevale et al. 1989; Ginther 1992).

The fate of the haemorrhagic follicles will further classify them into luteinized or non-luteinized unruptured follicles depending on the degree of luteinization of granulosa cells and ability of secreting progesterone as evidenced by macroscopic and hormonal studies (McCue and Squires 2002). The latter study named them as 'persistent anovulatory follicles' and found that approximately 90% of them developed luteal tissue. Assessment of echodensity and thickness of granulosa layer of anovulatory follicles viewed on ultrasound can be used to differentiate the presence of luteal tissue. In fact, human studies based on ultrasound and histology evidence have reported the development and ultrasonographic appearance of luteinized follicles in a similar way to that described in mares (Coulam et al. 1982; Toda 1990). This condition in women was termed luteinized unruptured follicle (LUF) syndrome and resembles that seen in mares.

Most recently controlled studies have shown in detail hormonal profiles and Doppler ultrasonographic characteristics of the development of haemorrhagic anovulatory follicles (HAFs) in the mares (Ginther et al. 2006, 2007). The later studies found only subtle differences in follicular wall vascularity between ovulatory and haemorrhagic follicles during the 3 days prior to ovulation/beginning of haemorrhage. Hormonal profiles on LH, FSH and progesterone did not however reveal any significant difference.

The main relevance of this condition lies in the failure of collapse of the dominant follicle with consequently no release of the oocyte and therefore impossibility of fertilization and pregnancy unless it is accompanied by a

normal ovulation of another follicle. However, due to the low incidence of HAFs reported as low as 5–8% (Ginther and Pierson 1989; McCue and Squires 2002) the overall impact on fertility is low. Nevertheless it can be frustrating for the practitioner when dealing with pre-ovulation breeding (natural mating and AI with chilled semen) when the mare is bred on the basis of a normal dominant follicle which then fills with blood and luteinize without rupturing, leaving that cycle with no chance of conception. The relevance of this condition on fertility can increase dramatically in individuals that tend to have reoccurrence of HAFs in subsequent cycles (Ginther et al. 2006).

The mechanisms of development of HAFs in the mare remain unclear. It has been proposed that the incidence is higher during the autumn months and in mares aged > 20 years (Ginther et al. 2007).

To better understand this phenomenon in the equine species, this study focuses on risk factors affecting the development of HAFs. In order to do so, detailed reproductive ultrasonographic records of seven individual mares' breeding lives (> 10 years) were analysed retrospectively.

Materials and Methods

Animals

Clinical records from seven mares (aged from 12 to 26 years) were analysed for > 10 years (range 10–18 years) from 1990 to 2007 (a total of 612 oestrous cycles; Table 1). These mares were at least followed at 24 h intervals during the peri-ovulatory period but often three times a day. The mares were either resident at a veterinary clinic (used as donor and recipient mares for embryo transfer programme or other reproductive procedures). The mares were bred in the clinic and were mostly Irish Draft (mare details are shown in Table 1).

Records

Records were obtained from transrectal ultrasonographic examinations performed at least once daily during oestrus and up to three times daily as ovulation approached. The ultrasound equipment changed over the years, but was equipped always with a linear probe of 7.5 MHz. All observations were taken by the same operator. The use of hormonal treatments to induce oestrus and ovulation (Cloprostenol and/or hCG) was recorded in every case. The end points recorded were:

Ovulation

Detected as per rectal palpation and ultrasonography by the absence of the previously recorded follicle and the presence of a hypoechoic area within the same ovary as described by Newcombe (1996). Confirmation of ovulation was by the later presence of an echoic CL. The date of ovulation was recorded as the day in which it was first detected. An ovulation could be classified in two categories:

• Spontaneous: when no hormonal treatment had been given since the previous ovulation.
• PG-induced: when oestrous signs and ovulation followed the administration of a PGF analogue Cloprostenol (Estrumate®, Schering-Plough Animal Health Ltd, Welwyn Garden City, UK; 25 µg to 1 mg, subcutaneously). This hormonal treatment was used to lyse CL and/or luteinized follicles in order to induce oestrus. The variation in dose was due to parallel clinical trials involving different Cloprostenol doses. An ovulation was classified as PG-induced if the administration of Cloprostenol was followed by clinical signs of luteolysis (endometrial oedema, oestrus behaviour and/or cervix relaxation). The interval from Cloprostenol administration to ovulation could be short but was always < 11 days.

Haemorrhagic anovulatory follicle

Detected by transrectal ultrasonography as described in Ginther (1992). In brief, the previously fluid-filled follicle of anechoic echotexture fills with echogenic specks which 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 intra-follicular specks increase but still have a mobile/swirling appearance. The follicle diameter increases and eventually the contents acquire a static organized appearance (Fig. 1a–f). Luteinization of the follicle was assumed when follicular wall became highly echoic and 'thickened', the uterus acquired a

Table 1. Reproductive data of seven mares followed for ≥10 years

Mare	Breed	YOB	Period recorded	No. of foals	No. of cycles	Multiple ovulation rate (%)	Mean pre-ovulatory follicular diameter (mm)	Age at first HAF	Overall HAF incidence (%)	PG-induced cycles (%)	PG-induced HAF cycles (%)
1	TB	1988	91–07	5	162	24	33.8	5	25.3	61.1	97.6
2	ID	1988	90–07	1	158	47.2	34.9	3	24.7	67.7	84.6
3	SH	1982	91–07	8	53	10.5	52.8	19[a]	7.5	17	20
4	ID	1992	94–07	5	81	20.1	39.3	7	7.4	38.3	66.7
5	ID	1994	96–07	6	71	21.4	46.1	11	2.8	39.4	100
6	ID × WB	1996	98–07	6	37	2.9	48.5	6	5.4	27	50
7	ID	1993	96–07	7	50	22	44.8	–	0	36	–

Mares 1–7. Breed (TB = Thoroughbred; ID = Irish Draught; SH = Shire; WB = Warmblood).
[a]The reproductive period 84–90 in mare 3 is missing and therefore the age of first HAF might not be accurate.
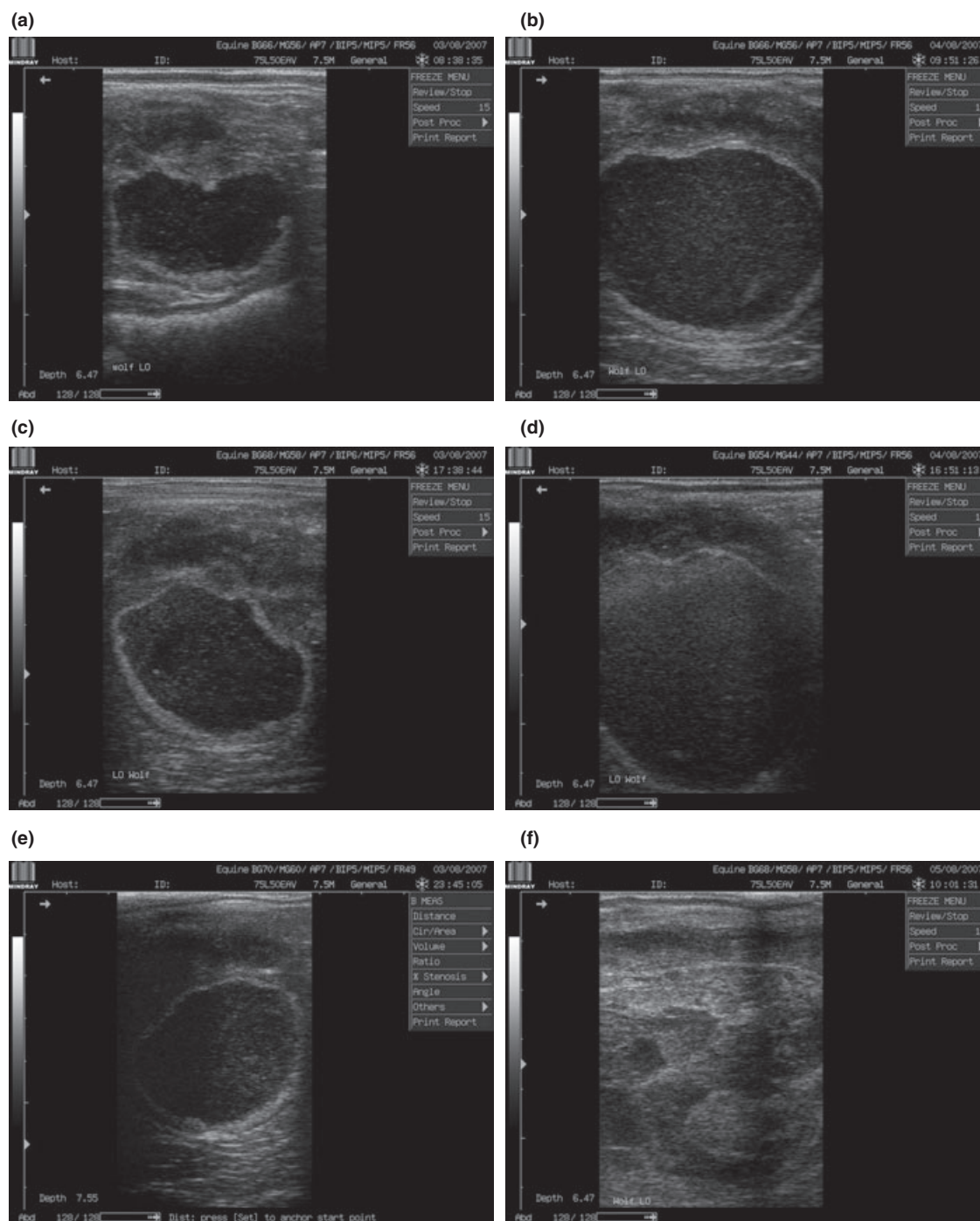YOB = year of birth.

Fig. 1. Sonogram series of haemorrhagic anovulatory follicle (HAF) development in mare 1 taken from day 0 (day of expected ovulation) to day 2 at 8 h intervals approx. (a) day 0: follicle fills with echodense specks and acquires irregular shape. (b) +8 h and (c) +16 h: granulosa cells layer becomes more echodense and thickened and intrafollicular specks increase in number and echodensity. (d) +24 h and (e) +32 h: luteinized follicle increases in diameter. (f) +48 h: intrafollicular contents organize and become static in appearance. Progesterone levels for day 0 (a) = 2.2 ng/ml; day 1 (d) = 4.0 ng/ml and day 2 (f) = 4.3 ng/ml. Uterine oedema scores for day −3, −2, −1, 0, 1, 2 were 2, 1.5, 0.5, 0, 0 and 0 respectively

dioestrus-like echotexture, on manual examination *per vaginam*, the cervix contracted and increased in tone as following normal ovulation. Additionally the mares then experienced what appeared to be a normal dioestrus period and in most instances did not return to oestrus for at least 2 weeks unless this dioestrus was foreshortened by prostaglandin.

When a luteinized follicle was concurrent with a normal ovulation and development of a CL, then a clinical diagnosis of luteinization was dependent on the progressive ultrasonic changes in the follicle as described previously. Follicles that haemorrhaged but showed no clinical or ultrasonic signs of luteinization were excluded from the study. These types of follicles followed the

same course and appearance of follicles that subsequently luteinized but failed to develop a hyperechoic 'thickened' neither follicular wall nor an organized cavity. In the absence of a concurrent normal ovulation, the mare remained in a clinical non-luteal phase.

As with ovulating follicles, HAFs were classified as occurring either spontaneously, or induced after administration of Cloprostenol (as above). Intervals from induction treatment to HAF were recorded (the interval from Cloprostenol to HAF development was less than 12 days in all cases). Some HAF 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 as in Fig. 1a).

Endometrial oedema

Degree of endometrial folding was subjectively assessed by transrectal ultrasonography. Increasing scores of 0.5 were given to the uterus from zero (no endometrial folding coincident with dioestrus-like echotexture) to three (maximum endometrial folding).

Follicular diameter

Largest follicular diameter at the time of Cloprostenol administration and immediate pre-ovulatory follicular diameters were measured and recorded as shown previously (Cuervo-Arango and Newcombe 2008).

Study design

For data analysis the experimental unit used was the 'cycle' which could be either 'ovulatory' (when no HAF developed during one inter-ovulatory period) or 'haemorrhagic' (when single or multiple HAFs whether accompanied by ovulation(s) or on its own developed and luteinized during an inter-ovulatory period).

Statistical analysis of the data was performed by using a mixed model, with three fixed factors:

- Induction treatment (spontaneous or induced with Cloprostenol).
- Time of season (winter: December to March; early: April to July; or late in the ovulatory season: August to November).
- Age (young: 2–8; middle age: 9–14 and old: >15 years old).

And corresponding two-way/three-way interactions using the procedure MIXED of SAS® (SAS System, Release 8.2, SAS Institute Inc., Cary, NC, USA, 1999). In addition, because of the great range in Cloprostenol doses used for induction treatment, the mean Cloprostenol dose and largest follicular diameter at the time of treatment for both HAF and non-HAF induced cycles were tested for difference by Mann–Whitney non-parametric test and two-sample t-test, respectively.

Results

Only one out of the seven mares studied over 10 years had no recorded HAF. Four mares had between 2.8%

and 7.5% of HAF incidence and the remaining two had an incidence as high as 24.7% and 25.3% (Table 1). The recurrence rate after the first HAF cycle in the same year was 0% (mean age of first HAF cycle 6.44 ± 1.3 years). However, the recurrence rate of HAF at some oestrous cycle during the lifetime was 100% in all mares. Due to the low number of HAF cycles in mares 3–6, the analysis of risk factors for HAF development was performed only in data from mares 1 and 2.

The two mares with highest incidence (mares 1 and 2) were more likely to have HAF cycles after induction with Cloprostenol (p < 0.000) than in spontaneous cycles (Table 1). There was a significant effect of Cloprostenol dose on HAF incidence (p = 0.004): the Cloprostenol dose used in non-HAF cycles averaged 254.5 ± 21.5 μg compared with 375 ± 35 μg in HAF cycles (Fig. 2). The mean largest follicular diameter at the time of Cloprostenol (PG) administration in HAF cycles (15.6 ± 1.4 mm) was non-significantly smaller than in non-HAF cycles (18.8 ± 1.9 mm) (p > 0.05). Interval from PG administration to ovulation/beginning of HAF was not different (7.1 ± 0.24 and 7.7 ± 0.26 days, respectively). Effect of induction with hCG on HAF was not estimated due to the low number of cycles induced with this hormone (4% of all oestrous cycles).

There was an effect of time within year (p = 0.004): higher during early (April to July) and late in the ovulatory season (August to November) than in the winter months, with HAF incidences of 31%, 28.1% and 11.7%, respectively (Fig. 3). Incidence of HAF amongst different age groups was not different (p > 0.05): 23.5%, 25.7% and 26.1% for the young, middle age and old periods, respectively (Fig. 4). However, mares 1 and 2 had only one HAF cycle (2.4% incidence) during the first 3 years of their reproductive lives (2–4, 41 oestrous cycles).
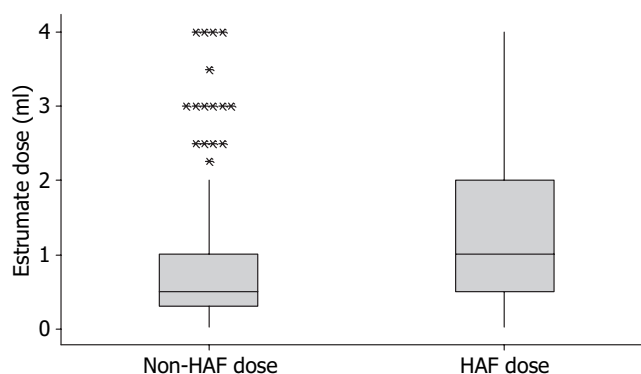
Fig. 2. Box plot distribution of cloprostenol dose in HAF (n = 73) and non-HAF induced cycles (n = 133) of mares 1 and 2. The dose of cloprostenol is represented as ml of Estrumate® (250 μg cloprostenol/ml). The asterisks show unusually small number of observations. The box for each dose-group is divided by the three horizontal lines: the upper line represents the third quartile (75% of the data are less or equal to that value), the middle line is the median (50% of the data are less or equal to that value) and the lower line represents the first quartile (25% of the data are less or equal to that value). Whiskers represent the dose range for each group. Medians for HAF and non-HAF cloprostenol-induced cycles (1.0 and 0.5 ml respectively) were significantly different (p = 0.004)

Fig. 3. Effect of month on HAF incidence. The values above each bar represent the total number of oestrous cycles recorded per month over the reproductive lives of mares 1 and 2. Bars show HAF incidence (%) sorted by month. There was significant effect within group of months (different filling pattern), Dec–Mar lower than Apr–Jul and Aug–Nov, $p = 0.004$

Fig. 4. Effect of age on HAF incidence. Bars represent the percentage of HAF cycles for mares 1 and 2 of all oestrous cycles during each year of their reproductive life. Values on top of each bar represent the total number of cycles recorded in each year. Incidence of HAF is not different amongst age-groups (different filling pattern; $p > 0.05$)

The likelihood of having two consecutive HAF cycles was 31.5%. If the cycle consecutive to HAF was induced with PG, the probability increased to 40%, whereas if no induction treatment was used, a new HAF cycle developed only in 9% of the times.

The uterine oedema patterns of HAF cycles without concurrent ovulations (n = 34 HAF cycles) were compared with those of non-HAF cycles (n = 30 chosen randomly); no significant difference ($p > 0.05$) at any observation time between the two types of cycles was found.

Mare 1 and 2 had a total of 80 HAFs of which 40% had solitary HAF(s) only without concurrent ovulation whereas the remaining 60% had normal ovulation(s) during the same cycle. Details of different types of HAF cycles are shown in Table 2.

Discussion

This study intended to investigate the risk factors associated with the development of HAFs in the mare. This anovulatory condition is difficult to research, in part due to the low overall incidence in a given population of mares and also because of the little knowledge of its nature. The present study has allowed the possibility to investigate in depth the occurrence of this phenomenon mainly by long-term analysis of the ovarian activity of two mares with abnormally high HAF incidence ($>24\%$) over all their entire reproductive life.

Possible mechanisms of ovulatory failure

The most obvious factor affecting HAF incidence of this study was the use of Cloprostenol to induce the following oestrus. Yet the mechanisms by which administration of prostaglandin during the luteal phase increases the likelihood of developing HAFs remains unclear.

It is known that the LH surge initiates a cascade of proteolytic activity (Robker et al. 2000) driven by enzymes such as matrix metalloproteinases (MMPs) and plasminogen activators (PA)/plasmin which is required in the tissue remodelling accompanying the ovulatory process (Smith et al. 2002). The same pre-ovulatory LH surge induces prostaglandin synthesis by equine granulosa cells (Sirois and Dore 1997) which has been proven in numerous species to be essential for ovulation to occur since administrations of prostaglandin inhibitors, given either intra-follicular or systemically, caused luteinization of follicles without previous rupture and oocyte release (Armstrong and Grinwich 1972; Grinwich et al. 1972; Killick and Elsein 1987; Salhab et al. 2003). It remains still undefined but it is hypothesized that prostanoids may regulate various MMPs and PA (Li et al. 2006).

Incidence of a similar condition described in humans (luteinized unruptured follicle syndrome, LUF) is increased in women who have undergone super-ovulation programmes with Clomiphene (a steroid receptor inhibitor which increases circulating concentrations of FSH and LH) (Zhu 1989). On the basis of that link and studies that showed the effect of LH/hCG on metabolism of intra-follicular $PGF_{2\alpha}$ and PGE_2 (Channing

Table 2. Different presentations of HAF cycles in mares 1–6

	1 HAF	2 HAFs	>2 HAFs	1 HAF + 1 ov	2 HAFs + 1 ov	1 HAF + 2 ov	2 HAFs + 2 ov	Overall
Mares 1–2	17	14	3	33	6	6	1	80
	20%	16%	4%	43%	7.5%	7.5%	1.4%	100 (%)
Mares 3–6	8	3	0	3	1	0	0	15
	53%	20%	0%	20%	7%	0%	0%	100 (%)

Upper values represent number of cycles with only one (HAF) or more (HAFs) haemorrhagic anovulatory follicles with (+ ov) or without concurrent ovulation(s). The lower value shows percentage of cycles from the total of each row.

1973), human researchers have hypothesized that if LH/hCG stimulation of a follicle occurs too early, premature luteinization can result with a consequent increased PGE_2 synthesis, decrease in $PGF_{2\alpha}$ synthesis and inhibition of follicular rupture (Coulam et al. 1982).

Allen (1979) showed that pregnant pony mares under the constant effect of eCG presented ovaries with haemorrhagic follicles which luteinized subsequently. This effect of eCG on the ovaries from day 50 to 100 approximately has also been shown in Thoroughbred and Irish Draught mares (Newcombe, personal communication 2007). The continuous stimulatory effect of eCG LH-like activity on small follicles (Urwin and Allen 1982) could be inducing haemorrhage and luteinization of follicles in a similar way that HAF occurs in cyclic mares.

Due to the low number of HAF cycles in mares 3–6, the following analysis of risk factors for HAF development was performed only in data from mares 1 and 2.

Risk factors
Multiple ovulators
It is worth noting that the mares with highest HAF incidence (mares 1 and 2) were also the ones with highest multiple ovulation rate (Table 1). These mares often had triple ovulations and when a follicle(s) haemorrhaged, it was accompanied in the majority of cases by one or two ovulations (Table 2). This increase in number of follicles passed the point of follicular deviation in HAF cycles was significantly higher than in mares with lower HAF rate (Table 2). Perhaps these mares had an intrinsic higher gonadotrophin circulating concentration in early stages of follicular development. Hormonal studies during the early stages of follicular development remain to be done to elucidate this enigma.

Month
There is the belief that HAF cycles tend to occur during the autumn (Burkhardt 1948; Ginther et al. 2006) and have on that basis been termed 'autumn follicles' (Knudsen and Weiert 1961). Although the highest incidence of HAF cycles occurred during the month of November, the low number of cycles in that month may have precluded it from being statistically significant. It also could be argued that the higher HAF incidence experienced at the end of the season might be due to the fact that reproductively 'normal mares' become pregnant early in the season whereas the problematic mares are left to the end. Apart from that peak during November, it was observed that the majority of HAF cycles concentrated around the months of maximal follicular activity (May to August) coincident with the highest circulating LH monthly mean values (Turner et al. 1979).

Age
There was no significant effect of age on HAF incidence. It appeared to be though a protective effect against HAF development during the first few youngest years of age

(only one HAF in mares 1 and 2 over the 41 oestrous cycles during the age period 2–4; Fig. 4). Research on effect of aging on follicular dynamics and gonadotrophin concentrations tell us that middle age mares (15–19 years) have higher circulating FSH than young mares (5–7 years) while LH remains similar. In older mares (>20 years old) both FSH and LH are raised (Carnevale et al. 1993). Interestingly, the only mare older than 20 years (mare 3) in the present study had her first HAF cycle recorded at 19 years old. In the following 7 years, aged 20–26%, 26.1% of her cycles (n = 21) developed HAFs spontaneously (without use of induction treatments). Not surprisingly this mare had the highest spontaneous HAF rate of all the mares studied.

Induction treatments
The present results suggest strong evidence of a link between HAF and use of induction treatments (PGF analogue, especially when high doses are used >300 μg) which have been proven to induce both a direct release of LH in the horse (Jöchle et al. 1987) and indirect increase in LH as the negative feed back of progesterone is released after PGF-induced luteolysis. On the basis of the latter study it could be hypothesized that the increased LH concentration following induction with PGF analogue could interfere with intra-follicular metabolism of prostanoids and proteolytic enzymes in an immature follicle. It would however have to be a slow process as the mean interval from Cloprostenol administration to HAF development was 7.7 ± 0.26 days. However, it seems that this effect is rather synergistic in mares that are somehow predisposed to develop HAFs. Preliminary results of hormonal controlled studies have also shown a link between HAF and use of prostaglandin in the mare (Ginther et al. 2008) which supports this theory.

Clinical relevance and conclusions
There is little a veterinarian can do after detecting a follicle which develops haemorrhage and subsequent luteinization (especially in mares that have been already mated) but make arrangements for the next cycle to breed again. It is important to bear in mind that some follicles before undergoing follicular collapse during the ovulatory process can look exactly like a follicle about to haemorrhage (Fig. 1a,b), however unlike HAF, the rupturing follicle will collapse completely giving the appearance of a hypoechoic area for at least 12–15 h, after which they may fill quickly with blood to resemble a HAF in early stages. It is therefore important for this purpose to examine mares at least twice daily to avoid mistaking a normal ovulation/corpus haemorrhagicum for a HAF.

Assuming the fact that the mare cannot conceive following haemorrhage into the follicle, most practitioners would try to induce the following oestrus with prostaglandin. On the basis of our results and especially if the mare is known to have high percentage of HAFs cycles, the next cycle preferably should not be induced. In the case of necessity of using induction

treatment, it is recommended to administrate the lowest luteolytic dose of PGF (e.g. 25–125 µg Cloprostenol/0.1–0.5 ml Estrumate) to minimize likelihood of recurrence. It is worth highlighting that mares 1 and 2 were more than four times more likely to develop consecutively a new HAF after Cloprostenol induction than in spontaneous cycles. These two mares had a fairly good fertility in non-HAF cycles (and HAF cycles with concurrent normal ovulations), therefore it is still possible to get this type of mares pregnant as long as they have a ovulation from a ruptured follicle.

In conclusion this study has provided some useful data for the practitioner in understanding this anovulatory condition in the mare and new insights for prospective researchers involving hormonal studies to elucidate the proposed effect of $PGF_2\alpha$ on the pathogenesis of HAF development.

Author contributions

JC contributed to the study design, writing up, some data collection and statistics; JRN contributed to data collection, study design and writing up.

References

Allen WE, 1979: Abnormalities in the oestrous cycle of the mare. Vet Rec 104, 166–167.

Armstrong DT, Grinwich DL, 1972: Blockade of spontaneous and LH-induced ovulation in rats by indomethacin, an inhibitor of prostaglandin synthesis. Prostaglandins 1, 21.

Burkhardt J, 1948: Some clinical problems of horse breeding. Vet Rec 60, 243–248.

Carnevale EM, Squires EL, McKinnon AO, Harrison LA, 1989: Effect of human chorionic gonadotropin on time to ovulation and luteal function in transitional mares. J Equine Vet Sci 9, 27–29.

Carnevale EM, Bergfelt DR, Ginther OJ, 1993: Aging effect on follicular activity and concentrations of FSH, LH and progesterone in mares. Anim Reprod Sci 31, 287–299.

Channing CP, 1973: The interrelationship of prostaglandins, cyclic 3′-5′-AMP and ovarian function. Res Prostaglandins 2, 1.

Coulam CB, Hill LM, Breckle R, 1982: Ultrasonic evidence for luteinization of unruptured preovulatory follicles. Fertil Steril 37, 524–529.

Cuervo-Arango J, Newcombe JR, 2008: Repeatability of preovulatory follicular diameter and uterine edema pattern in two consecutive cycles and how they are influenced by ovulation inductors. Theriogenology 69, 681–687.

Garverick HA, 1997: Ovarian follicular cysts in dairy cows. J Dairy Sci 80, 995–1004.

Ginther OJ, 1979: Reproductive Biology of the Mare: Basic and Applied Aspects, 1st edn. Equiservices, Cross Plain, WI, p. 413.

Ginther OJ, 1992: Haemorrhagic follicles. In: Reproductive Biology of the Mare: Basic and Applied Aspects, 2nd edn. Equiservices, Cross Plain, Wisconsin, pp. 224–226.

Ginther OJ, Pierson RA, 1984: Ultrasonic evaluation of the reproductive tract of the mare: ovaries. J Equine Vet Sci 4, 11–16.

Ginther OJ, Pierson RA, 1989: Regular and irregular characteristics of ovulation and the interovulatory interval in mares. J Equine Vet Sci 9, 4–12.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2006: Conversion of a viable preovulatory follicle into a haemorrhagic anovulatory follicle in mares. Anim Reprod 3, 29–40.

Ginther OJ, Gastal EL, Gastal MO, Beg MA, 2007: Incidence, endocrinology, vascularity, and morphology of haemorrhagic anovulatory follicles in mares. J Equine Vet Sci 27, 130–139.

Ginther OJ, Jacob JC, Gastal MO, Gastal EL, Beg MA, 2008: Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. Anim Reprod Sci 106, 181–187.

Grinwich DL, Kennedy TG, Armstrong DT, 1972: Dissociation of ovulatory and steroidogenic actions of luteinising hormone in rabbits with indomethacin, an inhibitor of prostaglandin biosynthesis. Prostaglandins 1, 89.

Jöchle W, Irvine CHG, Alexander SL, Newby TJ, 1987: Release of LH, FSH and GnRH into pituitary venous blood in mares treated with a PGF analogue, luprostiol, during the transition period. J Reprod Fertil Suppl 35, 261–267.

Katz E, 1988: The luteinized unruptured follicle and other ovulatory dysfunctions. Fertil Steril 50, 839–850.

Killick S, Elsein M, 1987: Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. Fertil Steril 47, 773–777.

Knudsen O, Weiert V, 1961: Ovarian oestrogen levels in the non-pregnant mare: relationship to histological appearance of the uterus and to clinical status. J Reprod Fertil 2, 130–137.

Li Q, Jimenez-Krassel F, Kobayashi Y, Ireland J, Smith GW, 2006: Effect of intrafollicular indomethacin injection on gonadotropin surge-induced expression of select extracellular matrix degrading enzymes and their inhibitors in bovine preovulatory follicles. Reproduction 131, 533–543.

Marik J, Hulka J, 1978: Luteinized unruptured follicle syndrome: a subtle cause of infertility. Fertil Steril 29, 270.

McCue PM, Squires EL, 2002: Persistent anovulatory follicles in the mare. Theriogenology 58, 541–543.

Newcombe JR, 1987: Investigation of equine ovarian cyclical changes by ultrasound scanning and rectal palpation. Br J Radiol 60, 628.

Newcombe JR, 1996: Ultrasonography of ovulation and development of the corpus luteum in the mare; a personal view. Equine Vet Educ 8, 47–58.

Peter AT, 2004: An update on cystic ovarian degeneration in cattle. Reprod Domest Anim 39, 1–7.

Robker RL, Russell DL, Yoshioka S, Sharma SC, Lydon JP, O'Malley BW, Espey LL, Richards JS, 2000: Ovulation: a multi-gene, multistep process. Steroids 65, 559–570.

Salhab AS, Amro BI, Shomaf MS, 2003: Further investigation on meloxicam contraceptivity in female rabbits: luteinizing unruptured follicles, a microscopic evidence. Contraception 67, 485–489.

Sirois J, Dore M, 1997: The late induction of prostaglandin G/H synthase-2 in equine preovulatory follicles supports its role as a determinant of the ovulatory process. Endocrinology 138, 4427–4434.

Smith MF, Ricke WA, Bakke LJ, Dow MP, Smith GW, 2002: Ovarian tissue remodelling: role of matrix metalloproteinases and their inhibitors. Mol Cell Endocrinol 191, 45–56.

Toda T, 1990: Ultrasonographical study on luteinized unruptured follicle. Nippon Sanka Fujinka Gakkai Zasshi 42, 1195.

Townson DA, Ginther OJ, 1988: The development of fluid-filled luteal glands in mares. Anim Reprod Sci **17,** 155–163.

Turner DD, Garcia MC, Ginther OJ, 1979: Follicular and gonadotropic changes throughout the year in pony mares. Am J Vet Res **40,** 1694–1700.

Urwin VE, Allen WR, 1982: Pituitary and chorionic gonadotrophic control of ovarian function during early pregnancy in equids. J Reprod Fertil Suppl **32,** 371–381.

Zhu Q, 1989: Luteinized unruptured follicle syndrome. Chung Hua Fu Chan Ko Tsa Chih **24,** 22.

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