Elsevier Editorial System(tm) for Theriogenology
Manuscript Draft

Manuscript Number:

Title: Ultrasound characteristics of experimentally-induced luteinized unruptured follicles (LUF) and naturally-occurring hemorrhagic anovulatory follicles (HAF) in the mare

Article Type: Original Research Article

Keywords: Mare; Ovulation failure; Hemorrhagic anovulatory follicle; Luteinized unruptured follicle; Ultrasound

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Abstract: The development of hemorrhagic anovulatory follicles (HAF) involves luteinization and hemorrhage of the follicle. This is observed on ultrasound as an increase in the echogenicity of the granulosa layer and formation of echoic particles in the antrum. The inhibition of prostaglandin synthesis with flunixin meglumine (FM) during the periovulatory period induces ovulatory failure with development of luteinized unruptured follicles (LUF). These two types of anovulatory follicles appear to share similar ultrasound features but they have not been compared critically. The following endpoints: follicle diameter, follicular contents score, interval from hCG administration to beginning of follicular hemorrhage, interval from hemorrhage to organization of follicular contents and cycle length, were studied and compared in mares with HAF ($n = 11$) and LUF ($n = 13$). The objective of this study was to elucidate whether these two unruptured follicles have a consistent clinical pattern of development and therefore can be considered as part of the same anovulatory syndrome. None of the endpoints analyzed differed significantly between HAF and LUF. However, there was a greater individual variation in some endpoints of HAF (interval from hCG to hemorrhage, follicular diameter at the administration of hCG and beginning of hemorrhage) than in LUF data. In conclusion, the HAF share a similar cascade of ultrasound characteristics with the experimentally-induced LUF. This finding may provide new insights in elucidating the pathogenesis of HAF. In addition, the experimental protocol of inducing LUF with flunixin meglumine seems a valid model to simulate the development of spontaneous HAF for research purposes.

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Ultrasound characteristics of experimentally-induced luteinized unruptured follicles (LUF) and naturally-occurring hemorrhagic anovulatory follicles (HAF) in the mare

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Abstract

The development of hemorrhagic anovulatory follicles (HAF) involves luteinization and hemorrhage of the follicle. This is observed on ultrasound as an increase in the echogenicity of the granulosa layer and formation of echoic particles in the antrum. The inhibition of prostaglandin synthesis with flunixin meglumine (FM) during the periovulatory period induces ovulatory failure with development of luteinized unruptured follicles (LUF). These two types of anovulatory follicles appear to share similar ultrasound features but they have not been compared critically. The following endpoints: follicle diameter, follicular contents score, interval from hCG administration to beginning of follicular hemorrhage, interval from hemorrhage to organization of follicular contents and cycle length, were studied and compared in mares with HAF (n = 11) and LUF (n = 13). The objective of this study was to elucidate whether these two unruptured follicles have a consistent clinical pattern of development

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1. Introduction

The term luteinized unruptured follicle (LUF) syndrome has been classically used in human medicine to refer to a common cause of infertility for women who fail to rupture and ovulate their preovulatory follicle despite secondary ovulatory changes such as luteinizing hormone (LH) peak, a rise of progesterone or the secretory transformation of the endometrium [1–5]. Instead of ovulating and forming a typical corpus luteum (CL), the anovulatory follicle increases in diameter, fills with echoic particles and eventually luteinizes with active production of progesterone [6–9]. The natural occurrence of spontaneous LUF is difficult to predict. In order to research this anovulatory syndrome, human scientists developed an experimental protocol that allows reliably the induction of LUF. This protocol consists of systemic administration of prostaglandin synthetase inhibitors [8,10]. The ultrasonographic characteristics of both spontaneous and experimentally-induced LUF are similar [8,9], which might indicate also similar pathogenic mechanisms.

In addition to the human scientific literature, the term LUF syndrome appears in several research studies performed with laboratory and domestic animal species such as the rat [11], guinea pig [12], rabbit [13], ewe [14], and baboon [15].

The term hemorrhagic anovulatory follicle (HAF) has been classically used in equine medicine to refer to the ovulatory failure of the preovulatory-sized follicle despite secondary typical signs associated with ovulation such as the preovulatory surge and LH peak, abrupt decrease in estradiol and a gradual increase in progesterone concentration [16,17], decreasing endometrial edema score [18,19], and normal length of the subsequent diestrous phase [17–19]. The equine HAF fails to rupture but increases in diameter. Subsequently, the HAF wall thickens and becomes highly echoic indicating active luteinization. Simultaneously, the follicular antrum fills with increasing amounts of echoic particles which move freely upon ballottement of the ovary. Eventually the HAF contents organize [17–23]. The reference to this type of anovulatory follicles as hemorrhagic originates from a macroscopic study [24] which demonstrated the presence of blood inside the follicle. The follicular hemorrhage later became clotted giving a jell-like appearance. The bleeding follicle was surrounded by a thick layer of what appeared to be luteal tissue. From that moment onwards, it has been assumed that the echoic particles within the follicular antrum of HAF observed on the ultrasound correspond with fresh blood entering the unruptured follicle.

Like in human LUF, the occurrence of equine spontaneous HAF is difficult to predict, which greatly hinders the research of its possible pathogenic mechanisms and therapeutic options. Just recently, the experimental protocol to induce LUF in women with non-steroidal anti-inflammatory drugs (NSAIDs) developed by human gynecologists [8] was successfully attempted in mares [25]. On this occasion, the systemic administration of flunixin meglumine (FM), a prostaglandin synthetase inhibitor commonly used in equine medicine, blocked ovulation during the expected periovulatory period in 83% of treated mares [25]. The authors concluded that the resultant FM-induced underwent ultrasound signs of luteinization and therefore they were termed LUF. The treatment with FM not only provoked anovulation with luteinization of the follicle but also entry of many echoic specks within the LUF antrum [25]. This resembled the echoic particles observed in spontaneous HAF, which are originated from follicular hemorrhage. During a second series of experiments, it was shown that FM-induced equine LUF shared a similar profile of reproductive hormones to that reported for spontaneous HAF [26].

The objective of the current study was to compare directly the ultrasound records during the development of naturally-occurring HAF with those of FM-induced LUF. If the cascades of ultrasonographic events that take place during the development of both types of anovulatory follicles have a consistent pattern, then it could be thought of a similar pathophysiology leading to anovulation.

2. Materials and methods

2.1. Animals and ultrasound records

The records from mares with FM-induced LUF were obtained from two original studies [25,26]. In these two studies, five [25] and eight [26] mares were treated with higher than recommended doses of FM to experimentally induce LUF every 12 h beginning at the time of hCG administration when the mares were in estrus with a follicle ≥ 32 mm. Ultrasound examinations were continued every 12 h for nine days after hCG administration. The first evidence of echoic particles within the follicular antrum of LUF occurred at 48 h after hCG. This moment was designated as Hour 0.

The data from mares with naturally-occurring HAF were obtained from the ultrasound and reproductive records of mares resident to or visiting a private equine practice during the 2009 and 2010 breeding seasons in the UK, northern hemisphere. The characteristics of HAF have been defined and described previously [16-18]. From these data, mares with HAF were chosen based on the following criteria: a) the cycle with an HAF was not accompanied by either an ovulation or another HAF; b) the mares had been treated with 1500 IU of hCG (Chorulon, Intervet, Cambridge, UK) before the first evidence of intrafollicular presence of echoic specks (Hour 0); c) the mares had been examined at 8 h intervals from before the first evidence of echoic specks within the follicular antrum (Hour 0) until the organization of HAF contents (clotting of blood) and continued thereafter every 24 h for at least seven days after Hour 0. In addition, the next periovulatory period had to be monitored to detect the accurate time of ovulation or beginning of a HAF formation within 24 h. The ultrasound examinations were preformed by the same operator with a portable scanner (Honda HS-2000V, Honda Electronics Co., Ltd, USA) equipped with a 7.5-MHz transrectal probe. For each mare a series of ultrasound pictures were frozen and saved in the scanner machine for latter analysis. Overall, eleven mares with eleven solitary HAF cycles were obtained. These mares were five to 20 years old and of various breeds Warmblood (n = 3), Irish Draught (n = 4), Thoroughbred (n = 2) and Standardbred (n = 2). For reproductive management reasons (clinical programs of AI or embryo transfer) these mares had been treated with cloprostenol (250µg/ml Estrumate®, Intervet, Cambridge, UK) during the previous diestrus and hCG (n = 7) or with hCG alone (n = 4) when the mares were in estrus. All these criteria had to be met in order to match ultrasound records of mares with FM-induced LUF so that direct comparisons were possible.

2.2. Endpoints analyzed

The following endpoints of mares with LUF and HAF cycles were characterized and compared:

- Follicular diameter: the diameter of follicles from the time of hCG administration and later HAF and LUF were measured with the electronic callipers of the ultrasound machine by average of two measurements taken at right angles from the follicular antrum when the diameter was maximum. Within this section, three further endpoints were taken into account: a) the minimum diameter at which the unruptured follicle was identified in first place with intrafollicular echoic specks (Hour 0); b) the maximum diameter of the HAF or LUF achieved at any point of its life span; and c) the diameter of the unruptured follicle when its contents were first identified as organized; that is, when the follicular contents no longer moved freely upon ballottement of the ovary (“diameter at clotting”).
- Partial collapse: a follicle was classified as having partial collapse when the diameter of the HAF or LUF decreased by ≥ 5 mm between Hour 0 and Hour 24 but without complete loss of follicular fluid (the partially collapsed follicles remained with a diameter of at least 60% of the initial diameter at Hour 0). The reduction in diameter was assumed to be a partial loss of follicular fluid [23].
- Follicular contents score: the contents of HAF and LUF were carefully studied during ballottement of the ovaries containing the unruptured follicles. The starting point of follicular hemorrhage (Hour 0) was designated as the first

ultrasound examination when the future HAF or LUF presented more than five intrafollicular echoic specks that moved freely within the follicular antrum during ballottement of the ovary. At this point, the follicular specks were often small in size and easy to count. The unruptured follicle was scored according to the appearance of its follicular contents (**Fig. 1**): a score of 0 was given when the follicle had anechoic fluid with no echoic speck within the follicular antrum; a score of 1 was given when the follicle had a countable number of echoic specks floating freely within the antrum; a score of 2 was given to follicles with a moderate to heavy presence of echoic specks (too numerous to count) but that still moved freely within the antrum; a score of 3 was given to follicles with heavy presence of echoic speck which moved freely but which had started to form solid clots or strands of fibrin. A score of 3 was also given to follicles with heavy presence of echoic specks moving freely with no strands but with the formation of echoic sediments in the bottom of the follicle which swirled if balloted (appearance of a white sheet). Finally, a score of 4 was given to unruptured follicles whose contents had organized and no longer moved freely (either quivered or remained static) during ballottement of the ovary. According to the appearance of the organized HAF or LUF (score 4), the unruptured follicle was classified into three different morphologies (**Fig. 2**): a) a solid appearance was defined for unruptured follicles whose contents presented a solid and homogenous mass of echoic tissue which remained firm during ballottement of the ovary (**Fig. 2–A1**); b) a cob-web like appearance was allocated to unruptured follicles whose contents organized forming a network of tissue connected by strands of fibrin which firstly quivered and later became firm during ballottement of the ovary (**Fig. 2–B1**); c) a “mixed” appearance was used for unruptured follicles whose contents organized forming separate sections: a solid and homogenous echoic mass adjacent to a cavity with either strands or echoic particles moving freely within the cavity (**Fig. 2–C1**). The cavity could be located either in the periphery or central part of the antrum.

- Interval from hCG administration to hemorrhage: the interval in hours from the time when the mare was administered hCG to the Hour 0 (first evidence of intrafollicular echoic specks).
- Interval from follicular hemorrhage to clotting: This was defined as the interval from the Hour 0 to the moment at which the follicular contents had organized or clotted. The frequency between examinations was every eight and 12 h for HAF and LUF cycles, respectively.
- Cycle length: The interval in days from the Hour 0 to the next periovulatory period designated by the moment when an ovulation or another HAF occurred. The Hour 0 and the next periovulatory period had to be clearly separated by a period of luteolysis indicated by significant reduction in HAF or LUF diameter followed by the growth of preovulatory sized follicle and presence of an estrous-like echotexture of the uterus with prominence of endometrial folds. Mares with diestrous ovulations or development of a new HAF during diestrus (within 14 days after Hour 0) were excluded from the analysis of "cycle length".

2.3. Experimental design

In order to compare follicular diameters and contents between mares with HAF and LUF, the data from both types of anovulatory cycles were compared at 24 h intervals relative to Hour 0 (first evidence of intrafollicular echoic specks). For other endpoints analyses (minimum and maximum diameter, diameter at clotting, interval from hemorrhage to clotting, and cycle length), the data were compared, relative to Hour 0,witht frequencies between examinations of eight and 12 h for HAF and LUF cycles, respectively.

2.4. Statistical analyses

Sequential data for follicular diameter and contents were analyzed by the SAS MIXED procedure with a repeated statement to account for autocorrelation between sequential observations (Version 9.2; SAS Institute, Cary NC, USA) after testing the data for normality of distribution. Data not normally distributed were ranked (follicular contents score). If an effect of group (HAF and FM-induced LUF) or an interaction of group and hour was significant, data were examined further by an unpaired Student's *t*-test within each hour, whereas a difference between hours within a group was examined by a Student's paired *t*-test. The differences in the mean cycle length, maximum and minimum diameter, and interval from Hour 0 to organization of follicular contents and diameter at clotting between HAF and LUF cycles were analyzed by 2 sample t-test. Frequency data (percentage of HAF and LUF with partial collapse or with different type of ultrasonographic morphology after organization of contents) were analyzed by Fisher's exact test. A probability of $P \leq 0.05$ indicated that a difference was significant and probabilities between $P > 0.05$ and $P \leq 0.1$ indicated that a difference approached significance. Data are given as mean ± SEM, unless stated otherwise.

3. Results

There was no effect of group (HAF vs LUF) or group by hour interaction on the diameter and follicular contents score of both types of unruptured follicles ($P > 0.05$; **Fig. 1**). In HAF and LUF, the follicle diameter increased with time ($P < 0.001$; **Fig. 1**, lower panel) until Hour 72, and began to decrease after Hour 96. In spontaneous HAF, the first significant increase ($P < 0.05$) in diameter from the previous examination point occurred between Hour 24 and 48 (**Fig. 1**, lower panel). In LUF, however, this occurred 24 hours earlier (from Hour 0 to Hour 24).

The intrafollicular hemorrhage in HAF and LUF began at Hour 0. The amount of echoic specks increased gradually in HAF and LUF at a similar rate ($P > 0.05$) as evidenced by their contents score (**Fig. 1**, upper panel). The follicular contents of all mares (HAF and LUF) had organized (score 4; **Fig. 1**, upper panel) by Hour 96. The appearance of HAF and LUF with organized contents included all three types of morphologies (cobweb-like, solid and mixed morphologies; **Fig. 3**). The most common appearance of spontaneous organized HAF was the cobweb-like (54.5%; 6/11), followed by the mixed and solid morphologies (27.3% and 18.2%, respectively). In contrast, the most common appearance of organized LUF was a solid mass of echoic tissue (46.1%, 6/13), followed by the cobweb-like and mixed morphologies (30.8% and 23.1%, respectively). The proportion of HAF and LUF with solid or cobweb-like morphologies differed significantly. The proportion of HAF and LUF with organized contents of a mixed morphology was not different ($P > 0.05$).

Table 1 summarizes the rest of endpoints analyzed: follicle diameter at the moment of hCG administration, interval from hCG treatment to Hour 0 (beginning of intrafollicular specks formation), minimum and maximum diameter of HAF and LUF with presence of specks moving freely, diameter at which follicle contents organized, interval from Hour 0 to organization of contents and cycle length. None of these endpoints differed significantly between HAF and LUF. However, a greater individual variation in the spontaneous HAF group was noted. This is shown by the greater SEM in some of the HAF data (**Table 1**). In the case of the interval from hCG administration to Hour 0 (beginning of follicular hemorrhage), all LUF mares had an interval of 48 h. In contrast, three, six and 2 HAF mares had an interval of < 36 h, 48 h, and > 48 h, respectively.

There was no partially collapsed follicle in mares with FM-induced LUF. In contrast, 36.4% (4/11) of spontaneous HAF had a marked reduction in HAF diameter between Hour 0 and 24 (from 38 to 27 mm, from 41 to 30 mm, from 39 to 26 and from 48 to 43 mm, for the four mares with partially collapsed HAF between Hour 0 and 24, respectively). The difference in the percentage of follicles with partial collapse between HAF and LUF was significant (P = 0.03).

4. Discussion

The results of this study show that the ultrasound features of FM-induced LUF are similar to those observed in naturally-occurring HAF. Nonetheless, this similarity in ultrasound characteristics only demonstrates a common cascade of events leading to ovulatory failure, but not necessarily a similar etiology. A temporary cessation of follicular growth precedes the first signs of anovulation, which resembles that observed in preovulatory follicles 36 to 48 hours before ovulation. The cessation in follicular growth has been associated with the beginning of the preovulatory LH surge and the subsequent shift in production from estradiol to progesterone by granulosa cells in ovulatory follicles [27], FM-induced LUF [26], and spontaneous HAF [17]. The shift in steroid production by the granulosa cells can be triggered by either the spontaneous or hCG-induced LH surge [28].

In HAF and LUF the cessation of follicular growth is followed by follicular hemorrhage without ovulation. The entry of blood increases gradually along with the diameter. After the first sign of hemorrhage, the increase in diameter of HAF is delayed by 24 h compared with LUF. This can be explained by the partial loss of follicular fluid (and overall diameter) in some HAF between Hour 0 and 24, which likely accounts for the overall delay in growth of the HAF group. During the growing phase, both types of anovulatory follicles maintain its contents in a fluid stage (fresh blood mixed with follicular fluid) owing to the anticoagulant properties of equine follicular fluid [29]. Once the amount of blood apparently exceeds that of fluid, the contents of HAF and LUF organize. At this stage, the follicular hemorrhage appears to stop since there is no further increase in diameter or evidence of newly blood entry.

The size of HAF and LUF began to decrease after Hour 96, probably due to contraction of clotted fibrin. In most mares, although with a significant reduction in diameter, the remnants of HAF and LUF were still clearly visible at the beginning of the next estrus (15 to 16 days post-anovulation). The development of endometrial edema and subsequent ovulation indicated the end of the cycle. The length of the cycle was not different in HAF and LUF mares: 21.5 and 22.2 days, respectively. A cycle length of approximately 21 to 22 days is similar to that described for ovulatory cycles [30]. This confirms an adequate luteolytic mechanism after HAF and LUF formation.

The interval from follicular hemorrhage (Hour 0) to organization of contents (blood clotting) averaged 55 and 52 h in the LUF and HAF groups, respectively. However, within each group there was a marked individual variation with some intervals of as little as 32 h to as much as 84 h. This great variation may be attributed to differences in the amount of blood that gained access to the follicular antrum affecting the overall proportion of blood (fibrinogen)/follicular fluid (heparin-like substance).

The mares with experimentally-induced LUF were administered hCG for the purpose of normalizing LUF and control cycles to the beginning of the LH surge, so that comparisons between hormone profiles and follicle data were possible [26]. In these mares treated with a prostaglandin synthetase inhibitor, the first evidence of LUF formation was observed between 36 and 48 h after hCG administration. This is also the expected interval from treatment to ovulation when hCG is administered to estrous mares [31] as long as they are free from antibodies against hCG [32]. In the present study, only mares treated with hCG were included in the HAF group, so that data on the interval from hCG treatment to the beginning of follicular hemorrhage (Hour 0) could be compared between HAF and LUF groups. Although, the interval from hCG to Hour 0 in HAF and LUF groups was similar (46.1 and 48 h, respectively), this varied from 16 to 96 h in HAF mares.

On the authors' opinion the marked variation in the interval from hCG to Hour 0 in the HAF group may be explained by two reasons. Firstly, the mares that showed evidence of HAF formation < 36 h ($n = 3$) after hCG treatment may have initiated the LH surge spontaneously prior to hCG administration. In clinical practice it is not unusual to have mares ovulated before the expected interval of 36 h, especially during the summer months and/or when hCG treatment is administered to mares with follicles ≥ 40 mm in diameter. In the current study, several HAF mares were administered hCG when they have a follicle > 40 mm. On the contrary, in the experimental controlled studies of LUF mares, the hCG was administered to mares with follicles ≥ 32 mm and never larger than 38 mm. Secondly, the HAF mares with an interval from hCG to follicular hemorrhage > 48 h ($n = 2$), may have had presence of hCG antibodies and therefore the treatment was incapable of inducing the LH surge [32]. The data of the HAF group were obtained from mares enrolled in a clinical AI or embryo transfer programs in which hCG is administered on regular basis.

In a large field study [19], the treatment with hCG alone was not associated with a significant increase in the incidence of HAF formation. In the latter study, the interval from hCG treatment to HAF formation was also 48 h. On the other hand, if PGF2α or its analogues (PGF) are used to induce estrus, the incidence of HAF cycles is increased [18–22] even though the PGF treatment is administered early in the cycle, around a week before the beginning of HAF formation [19]. Therefore it seems that the pathogenic mechanisms that lead to follicular hemorrhage and ovulatory failure in the future HAF occur long before the clinical signs of anovulation become evident, probably during early stages of follicular development. The definite etiology of naturally-occurring HAF is unknown. However, the evidence that the blockade of prostaglandin production by flunixin meglumine induces a similar type of anovulatory follicles might indicate a common pathogenic mechanism leading to anovulation.

Prostaglandins are produced by the inducible enzyme cycloogygenase-2 (COX-2) in the equine granulosa cells in response to a spontaneous or hCG-induced LH surge [33]. The concentration of its products, PGF and PGE, increases gradually in the follicular fluid from 30 h after treatment of mares with hCG [33]. Although not the only factor, prostaglandins play an essential role in the activation of several matrix-metalloproteinases and other collagenases involved in the degradation of extracellular matrix in the follicle wall leading to ovulation [34–36].

Elevated LH concentration during early stages of follicular development has been associated with increased incidence of HAF in mares [33], LUF in women [9,37] and rats [11]. Overexposure of granulosa cells to LH at early stages of follicular development could interfere with the metabolisms of prostaglandins in the future preovulatory follicle of women [4,5]. In that way, the use of GnRH antagonists to reduce LH concentration have been advocated for the treatment of women with a history of LUF recurrence [5].

It is worth noting the lack of partial collapses in the LUF group. The production of prostaglandins was probably inhibited completely in the whole follicle wall owing to the high dose of flunixin meglumine (150% of the recommended dose) leaving no chance for digestion of partial areas of the follicular wall of LUF. It is reasonable to think that in natural-occurring HAF, there might be a whole spectrum of possibilities including the total/partial absence of the “key factor” responsible for the degradation of the follicle wall. In that regard, a preovulatory follicle of an estrous mare during the ovulatory season may result in: a) a normal ovulation with a rapid evacuation of follicular fluid (the majority of mares), usually within 90 seconds [30]; b) an ovulation with a more slowly fluid evacuation, so called “septated evacuation” [38] which may take up to several hours to complete the follicular collapse with total loss of fluid; c) a partial collapse, with loss of some fluid, refill with blood and eventually development into a growing full HAF [23]. In order to differentiate accurately these last two modalities, frequent examinations of the ovaries may be required; and finally, d) an HAF with no follicular fluid loss.

Cloprostenol, a PGF analogue, has been administered to mares in the attempt to reverse the anovulatory effect of COX-2 inhibitors without success [39]. However the simultaneous administration of cloprostenol and FM resulted in the formation of atypical LUF with minimal follicular hemorrhage [39].

In conclusion, the hemorrhagic anovulatory follicles share a similar cascade of ultrasound characteristics with the experimentally-induced luteinized unruptured follicles. This finding may provide new insights in elucidating the pathogenesis of this naturally-occurring anovulatory condition in mares. In addition, the experimental protocol of inducing LUF with flunixin meglumine seems a valid model to simulate the development of spontaneous HAF for research purposes.

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Tables

Table 1
Ultrasound and cycle characteristics of spontaneous hemorrhagic anovulatory follicles and flunixin meglumine-induced luteinized unruptured follicles in mares.

Endpoints	n	Diam at hCG (mm)[a]	Interval hCG to Hour 0 (h)[b]	Min diam (mm)[c]	Max diam (mm)[d]	Diam at clotting (mm)[e]	Interval Hour 0 to clotting (h)[f]	Cycle length (days)[g]
FM-LUF	13	34.9 ± 0.5 32–38	48.0 48–48	38.8 ± 1.1 34–40	61.2 ± 1.6 50–69	56.5 ± 2.6 40–68	55.4 ± 4.4 36–84	22.2 ± 0.6 19–25
HAF	11	35.3 ± 1.4 29–46	46.1 ± 5.8 16–96	38.7 ± 2.6 30–50	61.0 ± 3.4 42–75	59.8 ± 3.3 40–75	51.8 ± 4.8 32–72	21.5 ± 0.7 18–24
P value		NS	-	NS	NS	NS	NS	NS

[a] Hemorrhagic anovulatory follicle (HAF) and flunixin meglumine-indiced luteinized unruptured follicle (FM-LUF) diameters at the time of administration of 1500 IU of hCG
[b] Hours from the time of hCG administration to the first evidence of intrafollicular hemorrhage (Hour 0)
[c] Minimum diameter of HAF and LUF with non-organized follicular contents
[d] Maximum diameter of HAF and LUF with non-organized follicular contents
[e] Diameter of HAF and LUF at the first evidence of organization of follicular contents
[f] Hours from the beginning of hemorrhage to organization of follicular contents
[g] Days from the beginning of HAF and LUF formation (Hour 0) to the next ovulation or HAF formation
NS: not significant.

Figure

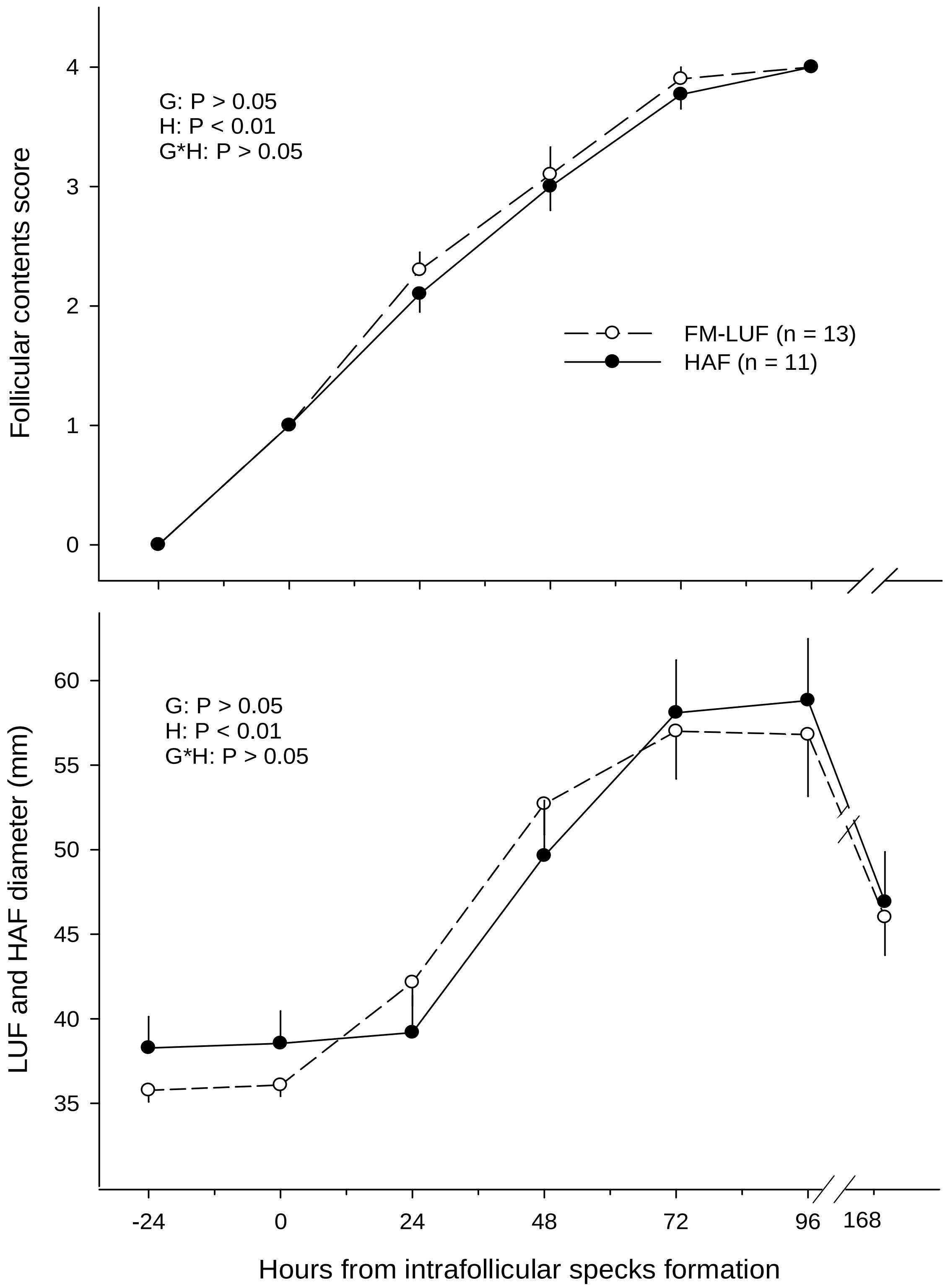
G: P > 0.05
H: P < 0.01
G*H: P > 0.05
Follicular contents score
0
1
2
3
4
FM-LUF (n = 13)
HAF (n = 11)
G: P > 0.05
H: P < 0.01
G*H: P > 0.05
LUF and HAF diameter (mm)
35
40
45
50
55
60
-24
0
24
48
72
96
168
Hours from intrafollicular specks formation

Fig. 1.

Figure

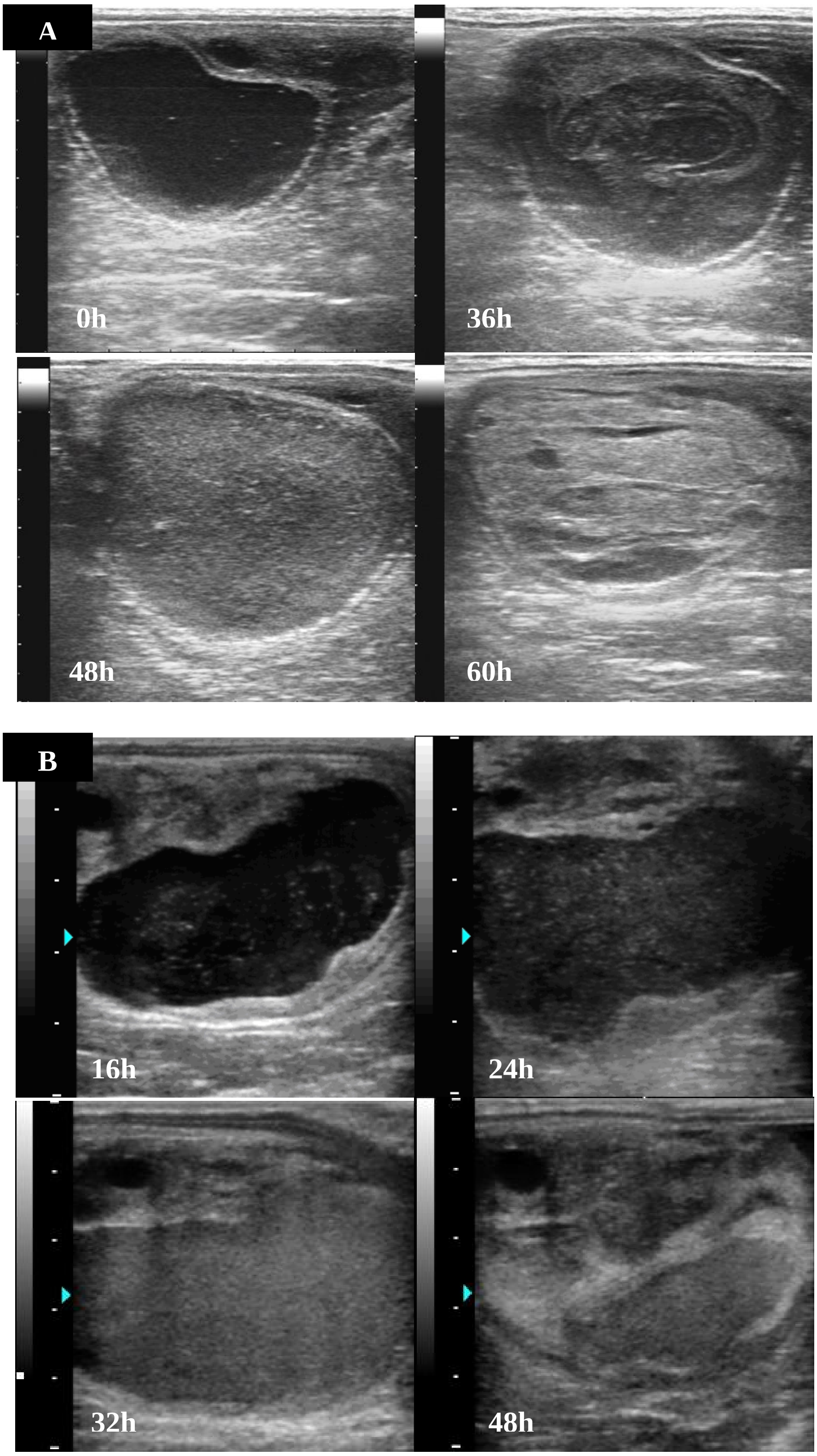

Fig. 2

Figure

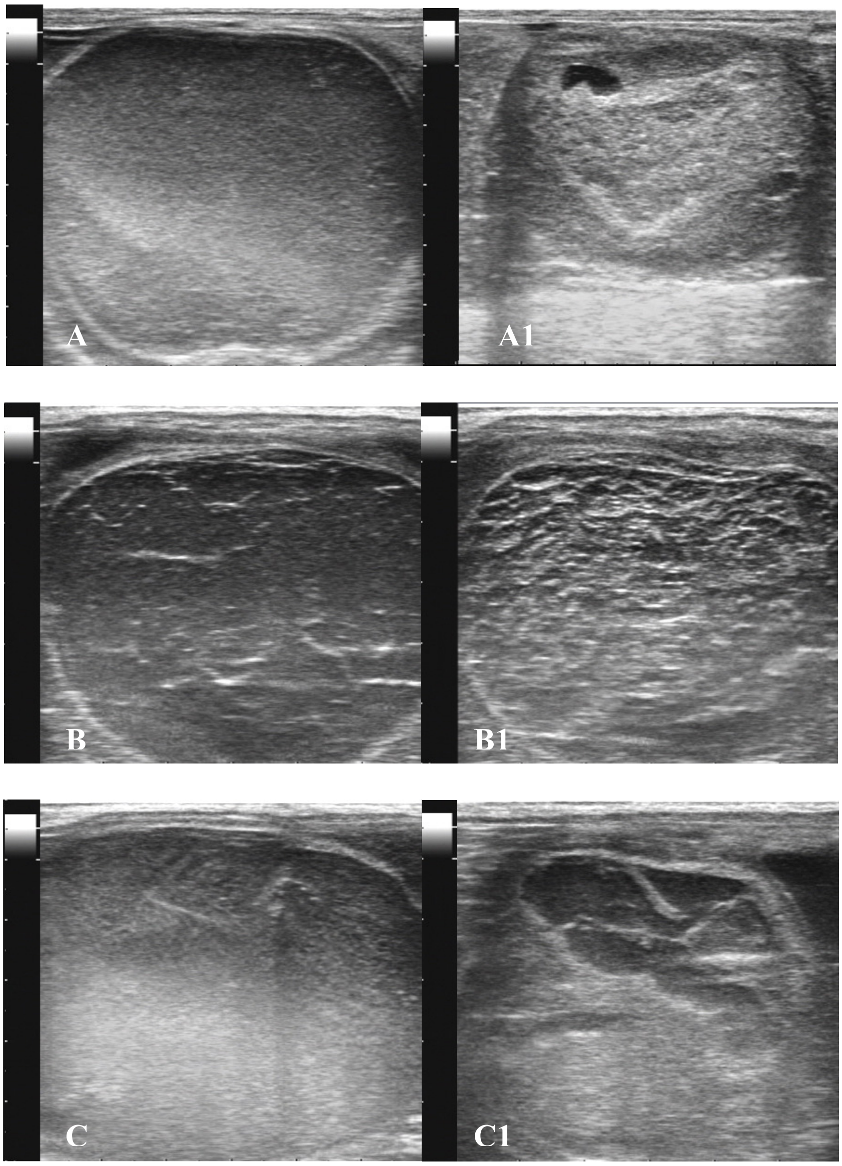

Fig. 3

Figure

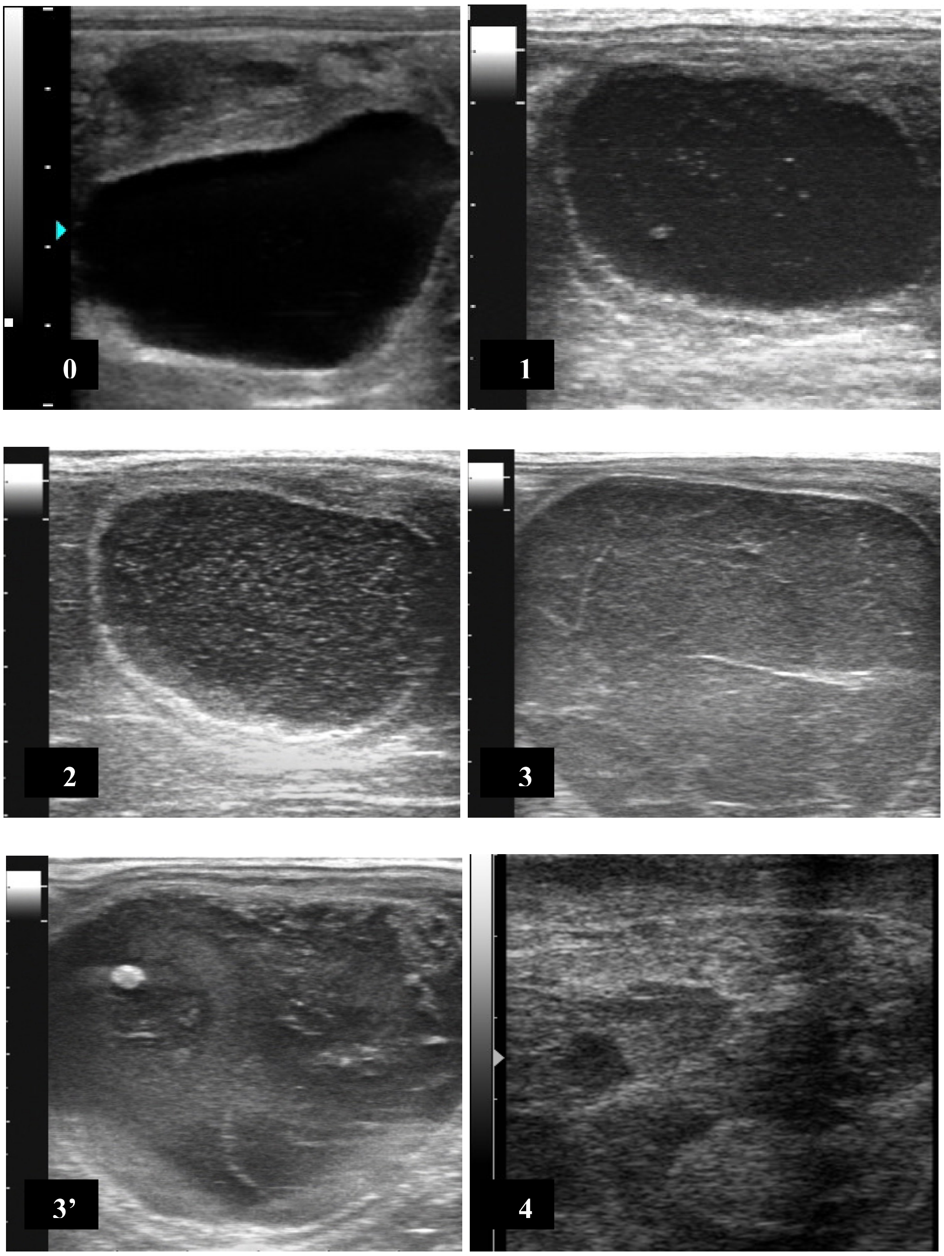

Fig. 4.

Figure

Fig. 1. Mean (± SEM) follicular content score (upper panel) and diameter (lower panel) of mares with spontaneous hemorrhagic anovulatory follicles (HAF; n = 11) and flunixin meglumine-induced luteinized unruptured follicles (FM-LUF; n = 13) -24 to 168 h from the beginning of intrafollicular specks formation (Hour 0, beginning of hemorrhage). The contents score vary from 0 (anechoic fluid with no specks) to 4 (clotted blood). Probabilities for main effects of group (G) and hour (H) and the group-by-hour interaction (G*H) are shown.

Fig. 2. Representative B-mode ultrasonograms of two mares with a flunixin meglumine-induced luteinized unruptured follicle (A) 0 h to 60 h from beginning of follicular hemorrhage (0 h) and a spontaneous hemorrhagic anovulatory follicle (B) 16 h to 48 h from beginning of hemorrhage (0 h).

Fig. 3. Representative B-mode ultrasonograms of three mares with luteinized unruptured follicles (LUF). The LUF contents of Mares A, B and C passed from a fluid stage (A, B and C) to organized structures with a solid morphology (A1), a cobweb-like morphology (B1) and a mixed morphology (C1), respectively. Note that the organized LUF with a cobweb-like morphology (B1) maintained its previous diameter. This is in contrast to the significant reduction in diameter after organization of contents in LUF with solid (A1) and mixed (C1) morphologies.

Fig. 4. B-mode ultrasonograms representative of the scoring system for follicular contents of luteinized unruptured follicles (LUF) and hemorrhagic anovulatory follicles (HAF). The scores vary from 0 (anechoic fluid), 1 (slight presence of echoic specks), 2 (moderate amount of echoic specks, too numerous to count), 3 (massive amount of echoic specks with formation of fibrin strands), 3’ (massive amount of echoic specks with formation of sediments at the bottom of the follicle) and 4 (when the contents are organized and no longer move freely upon ballottement of the ovary).