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Ultrasound confirmation of ovulation in mares: a normal corpus luteum or a hemorrhagic anovulatory follicle?

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Contents

The most common pathological anovulatory condition that occurs spontaneously during the breeding season in the mare is the hemorrhagic anovulatory follicle (HAF). A relatively high proportion of mares, soon after ovulation, develop a corpus hemorrhagicum (CH) with a central lacuna. This type of corpora lutea may resemble an HAF, which may complicate the accurate diagnosis of ovulation.. The main objective of this study was to compare the ultrasound data of mares examined frequently with HAFs and CHs to elucidate whether it is possible to distinguish them from each other. A total of 135 ovulating mares were classified according to the morphology of the corpus luteum (CL) in mares with: a solild CL, a CH with small or with large central cavities. Ultrasound characteristics of the development of 11 HAF and 13 CHs with a large central cavity were compared. The preovulatory follicular diameter of ovulatory mares was significantly correlated with the diameter of CH with large central cavities. The percentage of mares with post-ovulatory areas eligible to be mistaken with a CH was less than 25%. Although a predictive diagnosis of an HAF/CH can be made on the basis of several ultrasonographic endpoints, the only parameter that allows a definitive diagnosis is the thickness of the luteal border. This is < 3 mm in HAFs in contrast to > 5 mm in CHs. This only applies when the unidentified structure has non-organized contents.

Introduction

In equine practice, the accurate detection and timing of ovulation is important for the following reasons: a) to ensure that ovulation has occurred within the time window

considered adequate after pre-ovulation mating; b) to decide the optimum time for breeding with short-lived semen (frozen semen) for post-ovulation AI; c) to determine accurately the embryo age necessary for embryo flushing and recovery; d) to determine the number of ovulations in relation to the number of pre-existent follicles in order to manage any possible twinning properly; and e) to ensure the rupture and collapse of the follicle and so assume proper release of the oocyte in order to differentiate this from pathological anovulatory conditions.

The most common pathological anovulatory condition that occurs spontaneously during the breeding season in the mare is the hemorrhagic anovulatory follicle (HAF) (Ginther et al. 2007; Cuervo-Arango and Newcombe 2010). The main relevance of this condition lies in the failure of the dominant follicle to collapse. It is assumed that the oocyte cannot be released without follicular collapse and fluid evacuation. Therefore it is expected that in mares with HAFs and without normal concurrent ovulation, fertilization is not possible. The mating of 71 mares with solitary HAFs yielded no pregnancy (McCue and Squires 2002). It appears impossible that when using real-time B-mode ultrasonography to distinguish between a preovulatory follicle that will collapse normally and ovulate and another one that will hemorrhage.

For these reasons, it is clinically relevant to be able to diagnose accurately an ovulation and to distinguish it from an HAF. Proper diagnosis of ovulation may be easy to perform if the frequency between examinations is high (e.g. every 12 h) or when the collapse of the follicle results in a solid corpus luteum. A normal ovulation implies the complete collapse of the preovulatory follicle with evacuation of > 90% of fluid (Townson and Ginther 1987). Ultrasonographically, this is visualized as a hypoechoic and solid area in the ovary containing the previous preovulatory follicle during 12 to 15

h (Newcombe, 1996). This ovulatory area increases subsequently in echogenicity (Townson and Ginther 1989; Newcombe 1996).

However, a relatively high proportion of mares, soon after ovulation, develop a corpus hemorrhagicum (CH) with a central cavity or lacuna of varying dimensions containing fresh blood which later becomes organized (Townson and Ginther 1988; Newcombe 1996). Unfortunately, this type of corpora lutea may resemble an HAF (Ginther et al. 2007), which may complicate the accurate diagnosis of ovulation, especially when mares are not frequently examined for this purpose.

The main objective of this study was to compare the ultrasonographic records of mares examined frequently with HAFs and CHs to elucidate whether it is possible to differentiate the two entities when the mares are examined ultrasonographically at different intervals between examinations.

Materials and methods

Animals and ultrasound records

All ultrasound and reproductive data were obtained from mares resident to or visiting a private equine practice in the UK (northern hemisphere) during the breeding seasons of 2009 and 2010. These mares were of various breeds, including Irish Draught, Thoroughbred, Standardbred and Warmbloods. From all data available, ultrasound and reproductive records from a total of 135 mares with ovulatory cycles and 11 mares with HAF cycles were included in the study. Only one cycle per mare was used. The mares with ovulatory cycles had single ovulations and the exact time of ovulation was known within an interval of 8 h. In addition, the ultrasound examinations continued at least every 8 h until two days later (40 to 48 h post-ovulation).

The ultrasound records included diameters of the preovulatory follicle, the corpus luteum and the HAF at different times relative to ovulation / anovulation; and the appearance of the contents of the HAF's antrum and corpus hemorrhagicum's (CH) central lacuna. The ultrasound examinations were performed by the same operator with a portable scanner (Honda HS-2000V, Honda Electronics Ltd. San Jose, CA, USA) equipped with a 7.5-MHz linear-array transducer.

Endpoints analyzed

According to the outcome of the preovulatory follicle, a mare could have an HAF, an ovulation with formation of a solid corpus luteum (solid CL) or an ovulation with formation of a corpus hemorrhagicum (CH). The following end points of these three types of cycle were calculated:

- Follicular diameter: referred to the diameter of the preovulatory follicle 8 h before ovulation or before the follicular antrum filled with echoic specks in the future HAF (Hour 0). This was 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.
- Ovulation: involved the collapse of the preovulatory follicle with loss of > 90% of fluid within the following 8 h examination.
- Hemorrhagic anovulatory follicle (HAF): involved the absence of ovulation, entry of echoic particles within the follicle antrum (Hour 0) and gradual increase in overall diameter and wall echogenicity. The HAF contents eventually organized and did not move freely upon ballottement of the ovary (Fig. 1). The overall HAF diameter was calculated from the outer surface of the wall by the same technique as for follicular diameter.

- Solid corpus luteum and corpus hemorrhagicum: a corpus hemorrhagicum had a central lacuna ≥ 5 mm in diameter within 48 h of ovulation (period during which the mare was examined). The lacuna contents appeared to be blood. This was observed initially as many echoic particles floating freely during ballottement of the ovary. Eventually the contents organized forming a network of fibrin strands (Fig. 2). The diameter of the lacuna increased gradually even after the beginning of the organization of contents. A solid corpus luteum had no central lacuna. On some occasions, the solid corpora lutea had a small amount of anechoic fluid (< 5 mm; apparently of follicular origin) which usually disappeared by the following examination. The CH and central lacuna diameters were obtained by the same technique as for HAFs.
- Organization of contents: The contents of the HAF or central lacuna of the CH were assumed to be blood. They appeared as many echoic particles (too numerous to count) floating freely during ballottement of the ovary. The term "organization" or "clotting" of contents referred to the time when at least one strand of fibrin developed in the central lacuna of the CH or HAF antrum. The strand appeared echoic on ultrasound and was firm and did not move upon ballottement of the ovary.
- Luteal border: corresponded to the luteal tissue of the HAF and CH. In the HAF, this was measured from the outer surface to the beginning of the HAF antrum. In the CH, it stretched from the outer surface of the CL to the beginning of the central lacuna. In both structures, the luteal border was more echoic than the antrum or central cavity, representing luteal tissue.

Experimental design

The percentage of ovulatory mares (n = 135) with a solid CL, CH with central lacuna between 5 and 25 mm and CH with lacuna ≥ 26 mm was calculated. The distinction between CHs with small and large lacunae was set at a diameter of 26 mm owing to the minimum diameter of naturally occurring HAFs observed in the present study. In addition, the relationship between the follicular preovulatory diameter and the future CL diameter was determined by correlation analysis.

In order to compare ultrasound events between mares with HAFs and CHs for a longer period, all HAF mares (n = 11) and a subset of CH mares with lacunae ≥ 26 mm (n = 13) were examined every 8 h until 96 h post-ovulation / anovulation. Furthermore, a final examination was performed 168 h post-ovulation / anovulation to determine the overall diameter after organization and shrinkage of lacuna and HAF contents.

Statistical analyses

All data were tested for normality. Data not normally distributed were ranked for later analysis. The last diameter recorded for the preovulatory follicle (8 h before ovulation) was correlated with the diameter of the CH or solid CL 48 h post-ovulation by Pearson's correlation test. In addition, the preovulatory follicular diameters of the future solid CLs, CHs with small and large lacunae were analyzed statistically by one way ANOVA.

The differences in HAF and CH lacuna diameters from Hour 0 to Hour 168 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).

A 2-sample t-test was used to test the difference in several endpoints analysed between HAFs and CHs: maximum diameter, Hour of maximum diameter relative to ovulation /

anovulation, Hour and diameter at which HAF and CH contents organized and the interval from the first evidence of blood to organization of contents.

Results

Correlation between preovulatory follicles and corpora lutea diameter

The 135 ovulating mares developed 43 solid CLs (31.9%) and 92 CHs with a central cavity (68.1%) or lacuna (Table 1). The central lacuna of CHs reached ≥ 26 mm in diameter in 34.8% of the mares (Table 1). The preovulatory follicular diameter of mares with future CHs with large lacunae (≥ 26 mm) was larger ($p = 0.04$) than those of mares with future solid CLs and CHs with small lacunae (Table 1). The mean diameter of solid CLs, CHs with small lacunae and CHs with large lacunae at 40 h post-ovulation differed significantly (25.7, 30.5 and 39.1 mm, respectively; $p < 0.001$). The diameter of CHs with large lacunae at 40 h post-ovulation was positively correlated ($r = 0.64$; $p = 0.001$) with the previous preovulatory diameter of its follicles (Fig. 3).

General comparisons between endpoints of CHs and HAFs

The overall mean diameter of HAFs 0 to 168 h post-ovulation was higher ($p < 0.001$) than that of central lacunae from CHs with large cavities (Fig. 4). In both groups, the diameter of HAFs and central lacunae changed over time ($p < 0.001$). There was a significant effect of group by hour interaction on the diameter of HAFs and CHs central lacuna. This effect resulted from a delay in the development of the central lacuna relative to Hour 0 compared with the HAF formation and from an earlier (64 h vs 96 h post-ovulation) beginning of gradual decrease in diameter of the central lacunae compared with that of HAFs (Fig. 4).

The first evidence of ultrasonographic development of central lacunae in CHs relative to Hour 0 varied greatly from 8 to 32 h post-ovulation (Table 2). The proportion of CH central lacunae and HAFs with organized contents (presence of solid fibrin strands) changed over time (Table 2). By Hour 56, all CH central lacunae contents had organized. On the other hand, HAF contents had clotted by Hour 72 (Tables 2 and 3). The luteal border of HAFs was significantly thinner that the border of CHs before organization of contents. Although still significant, this difference became smaller once the contents of both structures had organized (Table 3).

The rest of endpoints analysed are shown in Table 3. Overall, there was a great individual variation in the data obtained from both HAF and CH mares. This is indicated by the large range of data points for most parameters analysed. It is worth noting the shorter interval between the first ultrasonographic evidence of blood and the clotting of contents for CHs compared with HAFs (10.4 h and 51.8 h, respectively; $p < 0.001$). Furthermore, the mean diameter of HAFs and CH central lacunae at which the contents first developed fibrin strands differed significantly (59.8 mm and 25.3 mm, respectively).

Direct comparison between HAFs and CHs at different intervals between examinations

- Daily examination: in this scenario the possible youngest and oldest age for HAFs and CHs would be 0 and 24 h, respectively. At this stage, neither HAFs nor CH would have organized contents. In addition, a percentage of future CHs would not have yet a visible central lacuna (46.1 to 76.9% of ovulating mares; Table 2). In the latter scenario (collapsed follicle without fluid), there would not be possible misdiagnoses. The two most accurate criteria that could be used to distinguish between both structures are a)

the thickness of luteal border: in HAFs, the luteal border was always < 3 mm in thickness, while CHs with central lacunae had a luteal border of ≥ 5 mm in thickness (Table 3); and b) the difference in diameter between the previous preovulatory follicle and the newly developed HAF or CH: the HAFs had a similar or larger diameter than the preovulatory follicle while the CHs was significantly smaller than the preovulatory follicle.

- **Every other day examinations:** the CH and HAF possible ages range between 0 and 48 h. At this stage, a percentage of CHs may have developed a central lacuna (46.1 to 100%), which would have organized contents in 0 to 92.3% of ovulating mares (Table 2). This percentage would increase along with time. As for HAFs, the antrum would have organized contents in 0 to 45.5% of times. An organized HAF would indicate an age of ≥ 32 h. When a structure with non-organized fluid is present, the same diagnostic criteria as above apply to differentiate between an HAF and CH. If the unknown structure has organized contents, the best two criteria to distinguish between an HAF and a CH are a) the luteal border thickness, which would be usually, but not always smaller in HAFs than in CHs (Table 3); and b) the difference between the diameters of the preovulatory follicle and the future organized HAF or CH. In the HAF group, this difference is higher (21.5 ± 3.7 mm; range of 3 to 35 mm; $p < 0.001$) than that (4.9 ± 0.9 mm; range of ─ 4 to 8 mm) in the CH group.

- **Intervals between examinations longer than two days:** at this stage, all ultrasonographic appearances of CHs and HAFs are possible: a) a recently collapsed follicle or a solid CL without fresh blood would undoubtedly indicate follicular rupture and normal ovulation; b) a circular structure with various amounts of fresh blood moving freely upon ballottement of the ovary could indicate either a CH or an HAF. If the luteal border is regular and thinner than 3 mm, it can be ascertained that ovulation

did not occur; and finally c) a circular structure with a central network composed of echoic and firm fibrin strands could represent either an organized HAF or CH. In the case of HAFs, the luteal border would be usually thinner than 5 mm, and the overall diameter considerably larger (around 20 mm) than the previous preovulatory follicle.

Discussion

Occurrence and physiology of fluid-filled luteal glands

About two thirds (68.1%) of the ovulating mares included in the present study developed corpora hemorrhagica (CHs) with varying amounts of blood within their central cavities. The proportion of mares with CHs described in the literature agrees with the results of the present study. In two combined studies, Ginther and co-worker found 68.2% (15/22) of mares with CLs with a central clot (Townson and Ginther 1988; Townson and Ginther 1989). In a larger study, Newcombe (1997) reported that 62.4% (118/189) of ovulations developed a fluid-filled central lacunae 42 to 72 h post-ovulation. The reported three studies and the current study also agreed in the great individual variation in terms of timing of central lacuna development relative to ovulation and in the size of the central cavity. This great variation appears to be due to differences in the timing and degree of intraluteal hemorrhage amongst mares. Furthermore, whether a mare develops a solid CL or CH after ovulation seems to occur by chance (Pierson and Ginther 1985). In approximately half of mares with sequential ovulations, a CH formed after one ovulation but not after the other ovulation (Pierson and Ginther 1985). Similarly, in approximately half of the mares that double ovulated, one ovulation developed a CH and the other ovulation a solid CL (Pierson and Ginther 1985).

The results of the current study added new information about the relationship between the size of the preovulatory follicle and the final diameter of the CH. This is indicated by the positive and significant correlation between the preovulatory follicular diameter and the CH diameter 40 h post-ovulation. A logical explanation for this correlation could be that a large collapsed follicle would have a larger surface and therefore allow a greater expansion of the newly developing CH as a result of active hemorrhage.

The contents of the central cavity remained non-organized for a short period of time (between one and two 8 h-examination intervals). Such a short interval from beginning of hemorrhage to clotting of contents is not surprising since the central cavity of the CH appears to be composed mainly of blood and little, if any, residual follicular fluid. The absence or small amount of follicular fluid, which is rich in a heparin-like substance with anticoagulant properties (Stangroom and Weevers 1962), allows a more rapid fibrinization of the CH contents. The first evidence of clotting was observed as the formation of a solid and echoic strand of fibrin within the CH central cavity. Despite the early organization of the lacuna contents, the size of the central cavity continued to increase to larger diameters. This occurred apparently from further intraluteal hemorrhage, since the ultrasonographic appearance of the CHs with growing lacunae combined the presence of solid strands and echoic particles moving freely upon ballottement of the ovary. The visualisation of these echoic particles is compatible with the presence of fresh blood (Ginther 1992).

In contrast to the developmental characteristics of the CH central lacuna, the HAF contents remained in a fluid stage for much longer (32 to 72 h), owing to the higher proportion of follicular fluid in the HAF antrum relative to fresh blood. Furthermore, once the HAF contents organize, the overall diameter reaches a plateau and soon after that, the size of the HAF begins to decrease.

Accuracy of using different ultrasonographic endpoints to distinguish an HAF from a CH

If frequent examinations are possible (every 8 to 12 h), the best way to assure that a mare has ovulated is to visualize ultrasonographically the absence of the previously recorded preovulatory follicle. The expected ovulatory site will appear as a hypoechoic area with little or no presence of anechoic follicular fluid (Pierson and Ginther 1985; Newcombe 1996). This indicates the rupture and collapse of the follicle with evacuation of > 90% of follicular fluid and assumes the completion of the process of oocyte release. However, if a longer interval between examination elapses, the presence of a newly formed central cavity with fresh blood within the CH may complicate the accurate diagnosis of follicular collapse. This hypothetical scenario would only occur in less than 23% of the cases. In the remaining proportion of mares (approximately 75%), the accurate diagnosis of ovulation would be possible even if the frequency between two examinations is delayed for 40 to 48 h. This proportion of mares is expected to have a solid CL (with no central blood clot) or a CH with a central lacuna smaller than the possible smallest HAF (< 27 mm in diameter).

Unfortunately, the great variation in the maximum diameter of HAFs (42 to 75 mm) overlaps with the maximum CH diameter (35 to 54 mm). And therefore, this overlapping could technically mean a source of error for the practitioner if the criteria for the diagnosis of an HAF/CH are only based on the overall HAF or CH diameter. According to the results of this study there appears to be two ultrasonographic parameters upon which an accurate and definitive diagnosis of an HAF/CH can be based: the luteal border thickness and the difference in diameter between the preovulatory follicle and the future HAF/CH.

The luteal border of HAF is thinner than that of CHs. The thinner border and therefore the smaller quantity of luteal tissue of HAFs may result from its lack of follicular collapse. The lower concentrations of progesterone in HAF mares involved a reduced vascularisation of developing luteal tissue in unruptured follicles compared with that of corpora lutea which originate from collapsed follicles (Cuervo-Arango et al. 2011). The luteal border thickness is particularly useful in distinguishing between an HAF and CH when their contents remain non-organized.

The greater overall HAF diameter compared with the CH's results from the lack of fluid loss in unruptured follicles in addition to the new blood entry that expands the intrafolicular volume of fluid within the HAF. As discussed earlier, the difference between the preovulatory follicle and the unidentified structure may aid the diagnosis of an HAF, especially at early stages of formation.

In conclusion, the preovulatory follicular diameter of ovulatory mares is significantly correlated with the diameter of corpora hemorrhagica with large central cavities (≥ 26 mm in diameter). The percentage of mares with post-ovulatory areas eligible to be mistaken with a CH is less than 25%. Although a predictive diagnosis of an HAF/CH can be made on the basis of several ultrasonographic endpoints, the only parameter that allows a definitive diagnosis is the thickness of the luteal border. This is < 3 mm in HAFs in contrast to > 5 mm in CHs. This diagnostic criterion only applies when the unidentified structure has non-organized contents. If the contents are organized, the luteal border can still be used as a diagnostic criterion though its accuracy is reduced.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

J.R. Newcombe collected the data and J. Cuervo-Arango design the experimental protocol and wrote the manuscript up.

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Table 1. Proportion of mares with different luteal morphologies

	n	%	POF [A] (mm)	Diam [B] 40 h (mm)	Corr POF-Diam CH
Solid CL	43	31.9	41.7 ± 0.8[a]	25.7 ± 0.6[a]	0.37
Lac 5-25	60	44.4	42.0 ± 0.7[a]	30.5 ± 0.6[b]	0.39
Lac ≥ 26	32	23.7	44.7 ± 1.1[b]	39.1 ± 1.1[c]	0.64
P-value			0.04	0.001	

A: Preovulatory follicular diameter (POF) 8 h before ovulation of mares with solid corpora lutea (CL), with corpora hemorrhagica (CH) and central lacuna of 5 to 25 mm and ≥ 26 mm in diameter.
B: Diameter of CLs and CHs (outer ring) 40 to 48 h post-ovulation.
C: Pearson's correlation coefficient (r) between the POF and the CL/CH diameter 40 to 48 h post-ovulation.

Table 2. Ultrasonographic events of HAF and CH lacuna contents

Hours post-ovulation/anovulation (h)	% of CH with visible lacuna	Diameter of lacuna (mm)	% of CH with clotted lacuna	% of HAF with clotted contents
0	46.1% (6/13)	3.5 ±0.5	0.0% (0/6)	0.0% (0/11)
8	30.1% (4/13)	13.2±1.2	0.0% (0/4)	0.0% (0/11)
16	53.8% (7/13)	17.6±2.3	2.3% (1/13)	0.0% (0/11)
24	76.9% (10/13)	21.3±3.6	23.1% (3/13)	0.0% (0/11)
32	100% (13/13)	24.8±3.4	61.5% (8/13)	27.3% (3/11)
40	100% (13/13)	30.1±3.0	84.6% (11/13)	36.4% (4/11)
48	100% (13/13)	34.1±2.1	92.3% (12/13)	45.4% (5/11)
56	100% (13/13)	36.8±1.7	100% (13/13)	63.6% (7/11)
64	100% (13/13)	36.8±2.1	100% (13/13)	81.8% (9/11)
72	100% (13/13)	35.3±2.1	100% (13/13)	100% (11/11)

Table 3. Ultrasonographic endpoints of HAF and CH with central lacunae ≥ 26 mm in diameter

	n	Max [A] diam (mm)	Luteal border [B] (mm)	Luteal border [C] (mm)	Hour of [D] max diam (h)	Diam at [E] clotting (mm)	Hour of [F] clotting (h)	Interval [G] hemorrhage-clotting (h)
HAF	11	61.0±3.4	2.1±0.1	4.3±0.3	74.2±4.5	59.8±3.3	51.8±4.8	51.8±4.8
(range)		42-75	1.5-2.5	3.0-5.5	60-96	40-73	32-72	32-72
CH	13	47.5±1.5	7.9±1.9	6.5±1.3	61.8±5.5	25.3±2.1	39.6±3.7	10.4±1.2
(range)		35-54	5.0-11.0	4.5-9.0	40-96	17-28	16-56	8-16
P-value		0.003	0.001	0.03	NS	0.001	0.01	0.001

A: Overall maximum diameter (outer ring) of hemorrhagic anovulatory follicles (HAF) and corpus hemorrhagicum (CH) with a central lacuna ≥ 26 mm in diameter.
B: Luteal border of HAF and CH with non-organized (fresh blood moving freely upon ballottement of the ovary) contents at its maximum diameter.
C: Luteal border of HAF and CH with organized contents at its maximum diameter.
D: Hour relative to Hour 0 (hour of ovulation / anovulation) at which the HAF and CH reached the maximum diameter.
E: Diameter of HAF and CH central lacuna at which the contents organized
F: Hour relative to Hour 0 at which the contents of HAF and CH central lacuna organized
G: Interval in hours between from the first evidence of fresh blood in HAF and CH to the moment of organization of contents.

Table 4 Relationship between the diameter of preovulatory follicles and CH /HAF

	n	POF [A] (mm)	Diam fluid [B] contents (mm)	Difference [C] (mm)
HAF (range)	11	38.5 ± 1.8 (26/49)	39.2±2.2 (27/49)	0.7 ± 1.8 (−11/8)
CH (range)	13	42.5 ± 1.5 (34/51)	19.9 ± 1.4 (10/28)	−22.5 ± 1.2 (−30/−15)
P-value		0.03	0.001	0.001

A: preovulatory diameter of follicles 8 h before ovulation or the formation of hemorrhagic anovulatory follicles (HAF).
B: HAF minimum diameter and CH lacuna maximum diameter at which its contents remain non-organized.
C: Difference between the diameter of A and B.
All CH data are from CHs with lacunae > 26 mm in diameter.

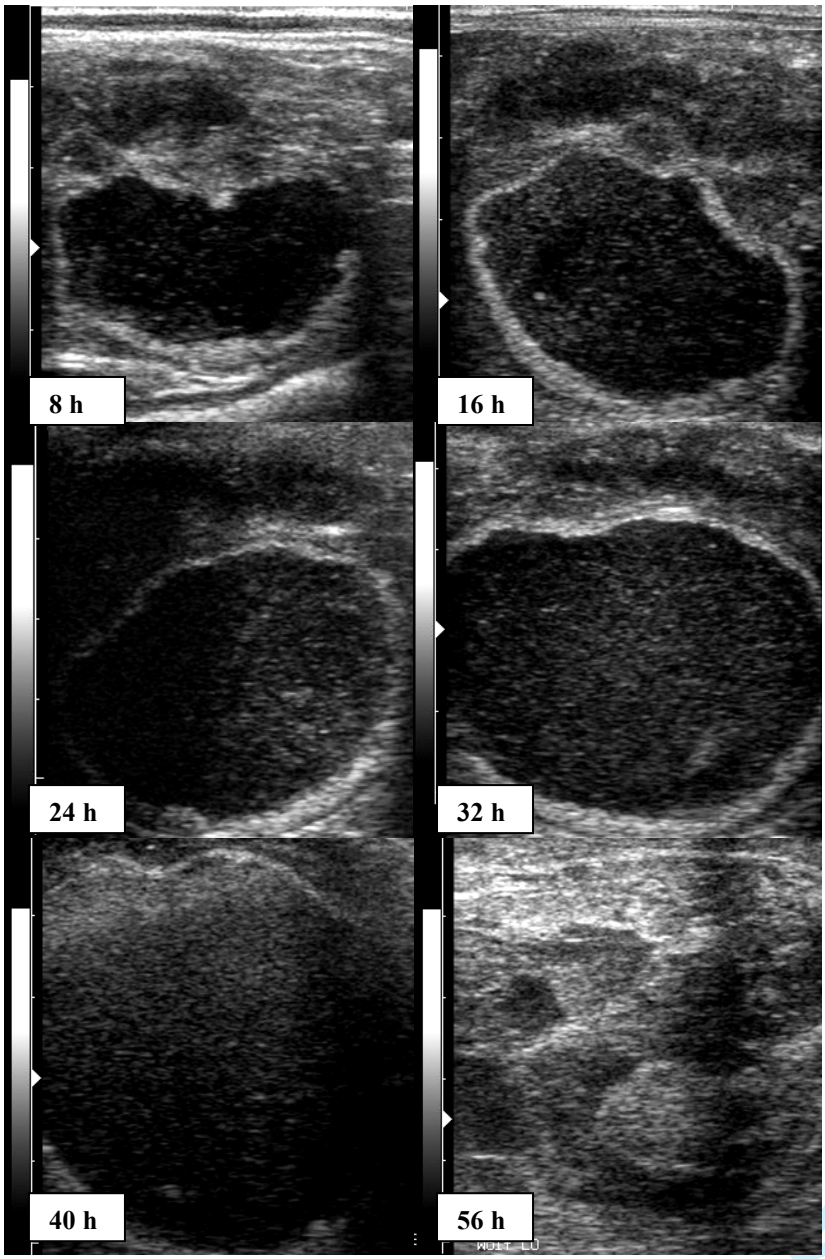

Fig. 1.

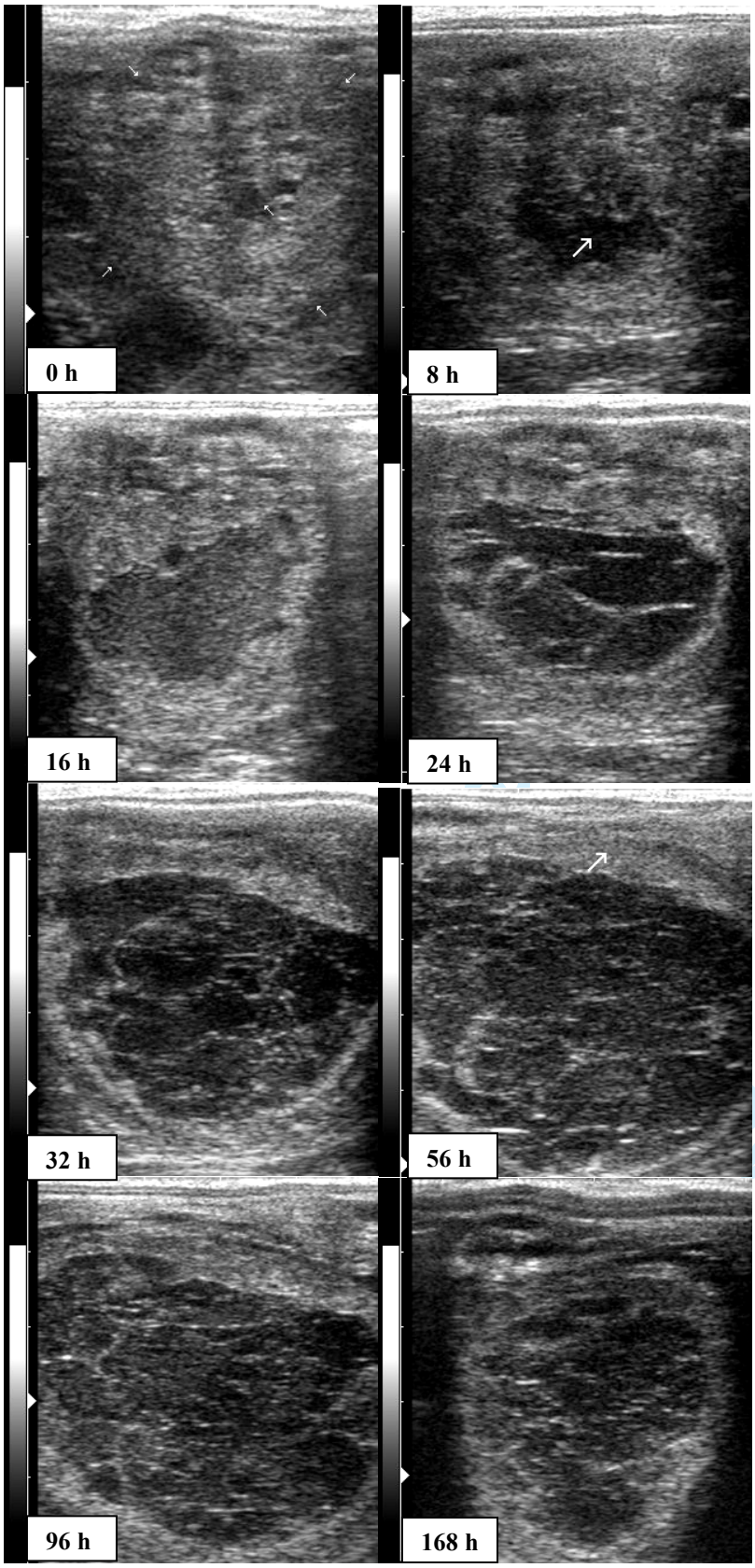

Fig. 2.

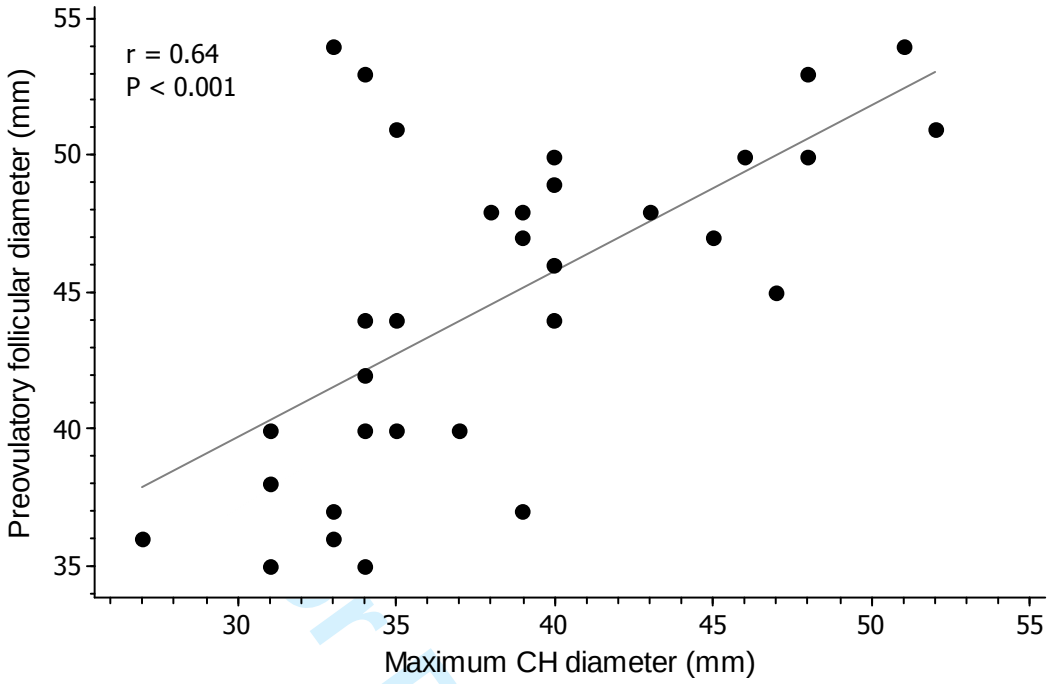

Fig. 3

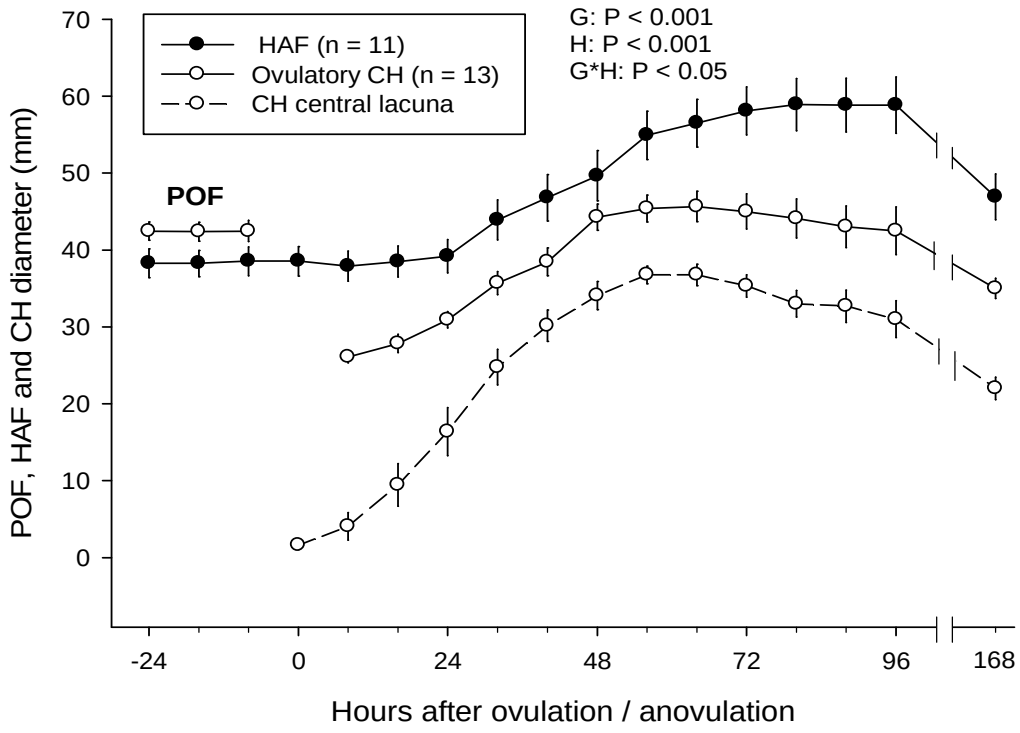

Fig. 4

Fig. 1. Representative B-mode ultrasonograms of a mare that failed to ovulate and developed a hemorrhagic anovulatory follicle (HAF).

Fig. 2. Representative B-mode ultrasonograms of a mare with an ovulation and subsequent corpus hemorrhagicum development with a growing central lacuna 0 (ovulation) to 168 h post-ovulation. Note in pictures “0 h” and “8 h” the presence of residual follicular fluid mostly anechoic. In picture “16 h” the central lacuna is composed of many echoic particles moving freely during ballottement of the ovary. This appearance is compatible with fresh blood. In picture “24 h”, the central lacuna contents begin to organize: a solid and echoic fibrin strand can be observed across the center of the lacuna.

Fig. 3. Scatter plot diameters of preovulatory follicles and diameters of corpora hemorrhagica (outer ring) with central lacunae ≥ 26 mm 40 to 48 h post-ovulation (n = 32). The Pearson’s correlation coefficient was significant ($p < 0.001$) and indicated a positive correlation ($r = 0.64$) between the POF and the CH diameters two days later.

Fig. 4. Mean diameter ± SEM of preovulatory follicles, hemorrhagic anovulatory follciles (n = 11), corpora hemorrhagica (n = 13) and central lacunae (n = 13) – 24 to 168 h post-ovulation/anovulation. Only mares with CHs and central lacunae ≥ 26 mm in diameter were included. The effect of group (HAF *vs*. central lacuna of CHs), effect of Hour and effect of group by hour interaction on the diameter of HAF and central lacuna was significant (G: $p < 0.001$; H: $p < 0.001$; G*H: $p < 0.05$, respectively).