Elsevier Editorial System(tm) for Animal Reproduction Science
Manuscript Draft

Manuscript Number: ANIREP-D-11-3050

Title: The effect of treatment with flunixin meglumine at different times relative to hCG administration on ovulation failure and luteal function in mares

Article Type: Research Paper

Keywords: Flunixin megluimine; prostaglandin inhibition; ovulation failure; luteal function; mare

Corresponding Author: Mr Juan Cuervo-Arango,

Corresponding Author's Institution: Facultad de Veterinaria Universidad Cardenal Herrera-CEU

First Author: Juan Cuervo-Arango

Order of Authors: Juan Cuervo-Arango

Abstract: Flunixin meglumine (FM), a prostaglandin synthetase inhibitor causes ovulatory failure in the mare. However the exact timing of FM treatment relative to the expected time of ovulation has not been determine nor has its effect on the luteal function of treated mares. Estrous mares with a follicle ≥ 32 mm were treated with 1.7 mg/kg b.w. of FM iv zero, 12, 24 and 36 h (n = 6), 24 and 36 h (n = 6), 28 and 36 h (n = 6), 24 h (n = 6) or 30 h (n = 6) after treatment with 1500 IU hCG. One group received no FM (control, n = 6). Progesterone concentration was determined using RIA. Mares treated with FM 0 to 36 h and 24 to 36 h had higher ($P < 0.05$) incidence of ovulatory failure (83 and 80%, respectively) than mares treated twice at 28 and 36 h, or once at 24 or at 30 h after hCG (16.7, 0 and 0%, respectively). The anovulatory follicles of FM treated mares luteinized and produce progesterone (> 2 ng/ml). The progesterone concentration was lower in mares treated with FM zero to 36 h and 24 to 36 h after hCG than in the rest of groups. In conclusion, FM administration to mares was effective in blocking ovulation only when the treatment began ≤ 24 h after hCG and was continued every 12 h until ≥ 36 h. In addition, the FM-induced anovulatory follicles underwent luteinization of follicular cells with active production of progesterone.

Suggested Reviewers:

The effect of treatment with flunixin meglumine at different times relative to hCG administration on ovulation failure and luteal function in mares

J. Cuervo-Arango*

Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad CEU-Cardenal Herrera, Valencia, Spain

Keywords: Flunixin megluimine; prostaglandin inhibition; ovulation failure; luteal function; mare

ABSTRACT

Flunixin meglumine (FM), a prostaglandin synthetase inhibitor causes ovulatory failure in the mare. However the exact timing of FM treatment relative to the expected time of ovulation has not been determine nor has its effect on the luteal function of treated mares. Estrous mares with a follicle ≥ 32 mm were treated with 1.7 mg/kg b.w. of FM iv zero, 12, 24 and 36 h (n = 6), 24 and 36 h (n = 6), 28 and 36 h (n = 6), 24 h (n = 6) or 30 h (n = 6) after treatment with 1500 IU hCG. One group received no FM (control, n = 6). Progesterone concentration was determined using RIA. Mares treated with FM 0 to 36 h and 24 to 36 h had higher ($P < 0.05$) incidence of ovulatory failure (83 and 80%, respectively) than mares treated twice at 28 and 36 h, or once at 24 or at 30 h after hCG (16.7, 0 and 0%, respectively). The anovulatory follicles of FM treated mares luteinized and produce progesterone (> 2 ng/ml). The progesterone concentration was lower in

* Corresponding author at: Dpto. Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad CEU-Cardenal Herrera, 46113 Moncada, Spain.
Email address: juan.cuervo@uch.ceu.es (Dr. J. Cuervo-Arango)

mares treated with FM zero to 36 h and 24 to 36 h after hCG than in the rest of groups. In conclusion, FM administration to mares was effective in blocking ovulation only when the treatment began ≤ 24 h after hCG and was continued every 12 h until ≥ 36 h. In addition, the FM-induced anovulatory follicles underwent luteinization of follicular cells with active production of progesterone.

1. Introduction

The ovulatory process culminates with the collapse of a preovulatory sized follicle with subsequent evacuation of fluid and oocyte release into the oviductal infundibulum. It is known for more than 30 years that prostaglandins (PGs) play an essential role during the process of follicular rupture (Armstrong, 1981; Murdoch et al., 1993). In the follicle, PGs are produced by the inducible cyclo-oxygenase isoform-2 (COX-2) also known as prostaglandin G/H synthase (PGHS) (Hedin et al., 1987).

In the mare, the hCG-induced preovulatory surge of LH induces the expression of COX-2 in granulosa cells (Sirois and Dore 1997). The amount of gen expression of COX-2 detected in equine granulosa cells increased gradually from 24 to 39 h after hCG treatment (Sirois and Dore 1997) with a marked increase in the expression from 30 to 33 h. The results of the latter study also showed that the assayed products of COX-2 (PGE and PGF) reached the maximum concentration in follicular fluid 36 h after hCG treatment, while its concentrations were undetectable at 24 h. Typically, an ovulatory dose of hCG (1500 to 3000 IU) administered to estrous mares with a follicle of ≥ 35 mm induces ovulation between 36 and 42 h after treatment in > 80% of animals (Harrison et al., 1991).

In a recent study (Cuervo-Arango and Domingo-Ortiz 2010), the treatment of mares with flunixin meglumine (FM), a non-selective COX inhibitor every 12 h from 0

to 48 h after hCG administration inhibited ovulation in 5 of 6 mares (83%). The developing anovulatory structures were assumed to be luteinized unruptured follicles (LUFs) because of the ultrasonographic appearance of a thickened follicular wall and the diestrous-like echotexture and tone of the uterus and cervix. However, progesterone concentration was not determined.

The objectives of this study were to determine the effect of FM administered at different times relative to hCG treatment on the ovulation rate and luteal function of mares. It was hypothesized that a single treatment of FM around the time of the expected rise in intrafollicular concentration of PGs would be sufficient to block ovulation and that the resultant FM-induced anovulatory structures would luteinize and produce progesterone. In order to test these hypotheses, 22 estrous mares were treated with hCG and FM at different times and frequencies. Follicular growth and ovulations were monitored by transrectal ultrasonography and the luteal function was evaluated by measuring plasma progesterone concentration by radioimmunoassay (RIA).

2. Materials and methods

2.1. Animals

Twenty-two mares were used for the experiment and handled according to the *Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching*. Mares were mixed breeds of large ponies and apparent pony–horse crosses aged 3 to 12 years old. The mares were weighed on a scale with a body weight ranging from 300 to 460 kg. The mares with a docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasound examinations (Ginther, 1995), were used during the period of August to September (summer in the northern hemisphere). The mares were kept under natural light in an open shelter and

outdoor paddock and were maintained on a mixture of alfalfa and grass hay, with access to water and trace mineralized salt. All mares remained healthy and in good body condition throughout the study. In all, 14 mares were studied for two consecutive estrous cycles while 8 of them were studied for a single cycle resulting in 36 estrous cycles monitored during the study.

2.2. Ultrasonography

Transrectal B-mode ultrasonographic examinations of the ovaries and measurement of follicles were performed daily using a real-time ultrasound scanner (Aloka SSD-900; AlokaAmerica, Wallingford, CT, USA) with a linear array 7.5-MHz transducer. Animals were scanned daily from day 12 after ovulation until they developed a follicle of ≥ 32 mm in diameter and acquired mild to moderate endometrial edema. Thereafter, the frequency of ultrasound examination was performed every 12 h. Twice daily examinations were continued until five days after hCG administration.

An ovulation was defined as the absence of the previously preovulatory sized follicle with evacuation of > 90% of follicular fluid (Ginther, 1995) and by the later presence of an echoic corpus luteum in the same ovary. When the follicle had not collapsed by the expected time of ovulation of 36 to 48 h after hCG treatment (Harrison et al., 1991), this was carefully studied for presence of echoic particles within the follicular antrum (hemorrhage) during ballottement of the ovary. Subsequent examinations of the same mare at 12 h intervals confirmed the development of an LUF as described previously (Cuervo-Arango and Domingo-Ortiz 2010) or the progressive regression or increase in diameter of the follicle until a subsequent ovulation. In the case of the diagnosis of an LUF, the ultrasound follow-up of this was continued until the organization or clotting of its follicular contents.

99

100 *2.3. Blood collection and progesterone determination*

101 Blood samples were taken from the jugular vein into heparinized 10 ml
102 vacutainer tubes. The tubes were immediately centrifuged during 10 min at 2000 ***g***.
103 Aliquots of plasma were then pipetted and transferred into 5 ml plastic tubes and frozen
104 to –20 ºC for later progesterone assay determination. Blood samples were taken at zero,
105 five and nine days after hCG administration. The plasma progesterone concentration
106 was determined using a solid-phase radioimmunoassay kit containing antibody-coated
107 tubes and ^{125}I-labeled progesterone (Coat-ACount Progesterone, Diagnostic Products
108 Corporation, Los Angeles, CA, USA) as described and validated for mare plasma
109 (Ginther et al., 2005). The intra-assay coefficient of variation and the sensitivity of the
110 assay were 6.2% and 0.02 ng/ml, respectively.

111

112 *2.4. Hormones and drugs*

113 The following drugs and hormones were used for the experiments: human
114 chorionic gonadotrophin (hCG) (10.000 IU/vial, Chorulon®, Intervet INC, Millsboro
115 19966 DE, USA) and flunixin meglumine (FM) (50 mg/ml, FluMeglumine®, Phoenix
116 Pharmaceutical INC, St Joseph 64507 MI, USA).

117

118 *2.5. Experimental design*

119 In order to determine the effect of FM treatment to inhibit prostaglandin
120 production at different times relative to hCG on ovulation and luteal function, a total of
121 36 estrous cycles from 22 mares were studied. When the mares developed a follicle of ≥
122 32 mm in diameter after spontaneous return to estrus (presence of mild to moderate

endometrial edema), they were administered 1500 IU hCG iv and allocated randomly to one of 6 groups:

- Group 1 (CON): no further treatment was administered (n = 6);
- Group 2 (FM 0): mares were treated with 1.7 mg/kg b.w. of FM zero, 12, 24 and 36 h after hCG administration (n = 6);
- Group 3 (FM 24): mares were treated with 1.7 mg/kg b.w. of FM 24 and 36 h after hCG administration (n = 6);
- Group 4 (FM 28): mares were treated with 1.7 mg/kg b.w. of FM 28 and 36 h after hCG administration (n = 6);
- Group 5 (FM 24S): mares were treated with a single dose of 1.7 mg/kg b.w. of FM 24 h after hCG administration (n = 6);
- Group 6 (FM 30S): mares were treated with a single dose of 1.7 mg/kg b.w. of FM at 30 h after hCG administration (n = 6).

Mares that ovulated between 0 and 36 h were excluded from the study since the ovulatory cascade of these mares was assumed to have been triggered by an endogenous LH surge and not by the administration of exogenous hCG (Harrison et al., 1991).

Two mares entered a phase of prolonged diestrus characterized by maintenance of an ultrasonographically visible CL and the absence of endometrial edema ≥ 18 days post ovulation or LUF formation. One of the two mares was known to have a diestrous ovulation between five and 12 days after LUF formation. In these exceptional two cases, the mares received 50 µg of cloprostenol (250 µg/ml DL-cloprostenol, Estrumate®, Intervet INC, Millsboro 19966 DE, USA) subcutaneously. Both mares returned to estrus within two days of cloprostenol administration.

2.6. Statistical analysis

The end points analyzed for each group were: a) the fate of the follicle in term of ovulation occurrence or LUF evidence; and b) progesterone concentration zero days (just before hCG administration), 5 d and 9 d after hCG treatment. Frequency data (LUF incidence) were analyzed by Fisher's exact test. Numerical data (progesterone concentration) were tested by one-way ANOVA analysis. 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

Four estrous cycles were removed from the study since ovulations occurred between 12 and 36 h after hCG (CON: 1 cycle; FM 24: 1 cycle; FM 24S: 1 cycle and FM 30S: 1 cycle). All remaining mares from the control, FM 24S and FM 30S groups had normal ovulations between 36 and 48 h (Fig. 1). In contrast, mares from FM 0, FM 24 and FM 28 groups developed 5, 4 and 1 LUFs respectively. In addition, two mares ovulated > 48 h after hCG, one from the FM 24 and another from the FM 28 group, respectively.

The LUF incidences of mares from the FM 0 (83.3 %) and FM 24 (80%) groups were significantly higher than those from CON (0%), FM 24S (0%) and FM 30S (0%) groups. The LUF incidence of mares from the FM 28 group (16.7%) was lower than that of mares from FM 0 ($P = 0.08$) and FM 24 ($P < 0.05$) groups but was not different ($P > 0.05$) from that of CON, FM 24S and FM 30S groups (Table 1). All anovulatory follicles (LUFs) luteinized and produced progesterone (> 2 ng/ml) which gained access to plasma from at least five days after hCG treatment (three days after the expected time of ovulation). The mean progesterone concentration on the day of hCG treatment was

not significantly different amongst groups. However, the progesterone concentration five and nine days after hCG was lower in FM 0 and FM 24 cycles compared with the rest of groups. All follicular and progesterone data are shown in detail in Table 1.

The ultrasonographic characteristics of the LUFs resembled those reported previously after treatment with 2 mg/kg of FM 0 to 48 h after hCG treatment (Cuervo-Arango and Domingo-Ortiz 2010). They included the development of gradually increasing amounts of echoic specks floating freely into the follicular antrum and an increase in the thickness and echogenicity of the granulosa layer from 48 h after hCG treatment. The LUF diameter increased gradually from the moment of follicular haemorrhage (48 h after hCG). The follicular contents of LUFs organized eventually giving the appearance of a network of solid fibrin strands (Fig. 2).

4. Discussion

4.1. The effect of timing of FM relative to hCG on the ovulatory failure

From the results of a preliminary study in a small number of mares it was shown that a high dose of FM beginning at the time of hCG treatment and continued every 12 h until 48 h later successfully blocked ovulation in 83% of mares (Cuervo-Arango and Domingo-Ortiz 2010). Therefore, a similar dose was used in the present study to test the effect of the timing of FM treatment relative to the administration of hCG.

The results of a previous study (Sirois and Dore 1997) showed clearly a gradual increase in the expression of COX-2 in granulosa cells from 24 to 39 h after hCG treatment in mares. This increase in enzyme expression paralleled a similar increase in its products (PGE and PGF) within the follicular fluid and so PGF concentration changed gradually from basal levels at 0 h (0.7 ng/ml) to peak levels of 10 ng/ml

approximately at 36 h to decrease again to 7 ng/ml approximately at 39 h after hCG administration.

The results showed no difference in the ability of a high dose of FM in blocking follicular collapse and ovulation when treatment began either at 0 or 24 h and was continued twice daily until 36 h after hCG administration. In contrast, when the beginning of the prostaglandin synthetase inhibitor was delayed beyond 28 h post-hCG, most follicles ovulated as expected. From these results it can be concluded that, for ovulation to be blocked, an intravenous administration of FM to inhibit follicular production of prostaglandins must be performed no later than 24 h after the administration of an ovulatory dose of hCG. This observation seems conflicting if compared with the results reported by Sirois and Dore (1997) in which the significant increase in PGE and PGF production in follicular fluid occurred between 33 and 36 h after hCG treatment. This discrepancy could be explained by a delay between the intravenous administration of FM and the moment in which the COX-2 inhibitor was able to block effectively the production of prostaglandins.

The clinical response to an intravenous administration of 1 mg/kg FM measured in a model of induced arthritis (using stride length and skin temperature to measure the response) was maximal from 2 h after treatment and lasted for 10 h (Toutain et al., 1994). However, the pharmakodynamics and pharmacokinetics of FM in equine follicular fluid have not been determined. These may be different from those in plasma.

A single dose administration of FM at the critical point of 24 h after hCG treatment also failed to inhibit ovulation in all treated mares. This finding is relevant for clinical practice of equine reproduction, since clinical therapy of FM is typically administered to mares only once daily for pain relief amongst other indications. Therefore daily treatment with FM, even at higher doses than recommended by the

manufacturer's data sheet, is unlikely to block ovulation. The equine plasma half-life of FM ranges from 1.6 to 2.5 h (Chay et al., 1982), but sufficient concentration to maintain a maximal clinical response remains in plasma for up to 10 h at an initial single dose of 1 mg/kg (Toutain et al., 1994) and for 16 h with some remaining effect for up to 24 h at a dose of 2 mg/kg. The clinical effect of FM therefore is not directly proportional to plasma concentration as confirmed by the fact that a single administration of 1.1 mg/kg of FM was able to reduce the concentration of PGE in an inflammatory exudate for about 24 h (Higgins et al., 1986). However, the rate of passage and permanence of FM in follicular fluid is unknown and therefore a single administration of FM may not be sufficient to provide the minimum COX-2 inhibitory concentration of FM in the follicular fluid for long enough to block the production of prostaglandins and ovulation.

In addition, the minimum amount of PGF required to initiate the enzymatic cascade of extracellular matrix degradation leading to follicular wall breakdown is unknown and hence it could also account for the discrepancy with the results of Sirois and Dore (1997). Perhaps very small amounts, probably below assay sensitivity, might be already present in the follicular fluid between 24 and 30 h post hCG and be sufficient to trigger the ovulatory cascade in spite of the later inhibition of further production of prostaglandins by exogenous FM. Furthermore, differences in individual response to hCG or in the endogenous LH levels as a result of the effect of seasons (Turner et al. 1979) may account, at least in part, for the discrepancy in the timing of prostaglandin inhibition required to block ovulation.

Two mares had delayed ovulations (e.i. longer than the expected interval of 36 to 48 h after hCG). This could be attributed to the development of antibodies against hCG after repeated treatments (Siddiqui et al., 2009), since the two mares with delayed ovulations had been treated for the third time in that season. On the other hand, it could

be speculated that the FM treatment protocol in these mares did not block but delayed the expected interval between hCG administration and ovulation. Further studies involving shorter ultrasound examination intervals to detect ovulation and larger number of mares would be needed in order to critically study the effect of FM on the interval to ovulation.

4.2. Effect of FM and anovulation on the luteal function

The presence of plasma progesterone above 2 ng/ml in mares with anovulatory follicles confirmed the luteinization of the unruptured follicular wall. This is in agreement with the result of other study in which LUFs were experimentally produced with indomethacin, another non-selective COX inhibitor, in women (Killick and Elstein 1987). Luteal function, measured by the ability of producing progesterone, appeared not to be affected by any of the FM protocol treatments as long as there was a collapse of an ovulatory follicle with evacuation of the follicular fluid. There was only a reduction in the progesterone concentration in mares from the groups with higher incidence of LUFs from 5 to 9 days after hCG treatment. The luteal tissue of these mares was competent but produced significantly less amounts of progesterone at least on the days measured. It appears that the lack of follicular collapse affected somehow the development of full productive luteal cells. It is not surprising since during the development of the corpus luteum in ovulatory mares, within 24 h of ovulation, the microscopic appearance of the equine early corpus luteum shows folds of stromal tissue beginning to grow into the luteinizing tissue accompanied by proliferating capillaries which provide the required nutrients and growth factors for continued development of luteal cells (Van Niekerk et al., 1975; Watson and Sertich 1990). Therefore, if the formation of new blood vessels within the body of the corpus luteum is impeded by the lack of follicular collapse in

LUFs, then the development of luteal cells might not be complete and so their ability to secrete progesterone.

4.3. The use of FM as a possible contraceptive method

The experimental production of FM-induced LUFs has been proposed as a possible method of contraception in the mare (Cuervo-Arango and Domingo-Ortiz 2010). In equine assisted reproductive technologies, a possible indication for contraception is oocyte transfer. One or several oocytes obtained from a valuable donor mare can be transferred surgically into the oviducts of a healthy recipient mare whose oocyte has been previously removed by ovum-pick (Carnevale, 2004). A proposed alternative to the reported technique could be a contraceptive method. The oocyte release from the follicle is avoided by inhibiting follicular collapse while the granulosa cells still luteinize and produce sufficient progesterone required to maintain a successful pregnancy. Clinical trials with oocyte recipient mares need to be carried out to test this hypothesis. In human reproduction, the transfer of embryos was successfully performed into women who had LUF cycles. This indicates an adequate maintenance of pregnancy by progesterone production from this type of unruptured follicles (Wang et al., 2008). Although the start of FM treatment at a dose of 1.7 mg/kg either at 0 or 24 h after hCG administration was equally effective in inducing LUFs, a protocol starting at 0 h and continue every 12 h until signs of anovulation (hemorrhage of antrum and luteinization of wall) is recommended by the author. With this protocol, mares that have already initiated the spontaneous LH surge before hCG treatment would be more likely to develop an LUF than if FM treatment is delay 24 h further. Nevertheless, a draw back of this method is that not all mares seem to respond to FM therapy. In addition, some mares may ovulate before the expected interval after hCG treatment. Further research

297 studies testing the specific reasons why some mares ovulate despite COX-2 inhibitory
298 therapy should be carried out to improve this proposed contraceptive method.
299 In conclusion, FM administration to mares was effective in blocking ovulation
300 only when the treatment began ≤ 24 h after hCG and was continued every 12 h until ≥
301 36 h. In addition, the FM-induced anovulatory follicles underwent luteinization of
302 follicular cells with active production of progesterone. Finally, mares treated with FM
303 during the periovulatory period that ovulated produced similar concentrations of
304 progesterone than ovulatory untreated controls.
305
306 **Acknowledgments**
307 The author thanks Dr. M.A. Beg and Dr. O.J. Ginther for assistance with
308 progesterone assay determination.
309 This study was supported by the Grant "2010 Beca de Movilidad CEU-Santander"
310 awarded to J. Cuervo-Arango by the "Universidad CEU-Cardenal Herrera", Moncada,
311 Spain in collaboration with "Banco Santander".
312
313 **References**
314 Armstrong, D.T., 1981. Prostaglandins and follicular functions J. Reprod. Fertil. 62,
315 283–291.
316 Carnevale, E.M., 2004. Oocyte transfer and gamete intra-fallopian transfer in the mare.
317 Anim. Reprod. Sci. 82-83, 617–624.
318 Chay, S., Woods, W.E., Nugent, T., 1982. The pharmacology of nonsteroidal anti-
319 inflammatory drugs in the horse: flunixin meglumine (Banamine). Equine Pract. 4,
320 16–23.

321 Cuervo-Arango, J., Domingo-Ortiz, R., 2010. Systemic treatment with high dose of
322 flunixin-meglumine is able to block ovulation in mares by inducing hemorrhage
323 and luteinization of follicles. Theriogenology
324 doi:10.1016/j.theriogenology.2010.10.011.
325 Ginther, O. J., 1995. In: Ultrasonic Imaging and Animal Reproduction: Book 2, Horses.
326 Equiservices Publishing: Cross Plains, WI.
327 Ginther, O.J., Beg, M.A., Gastal, E.L., Gastal, M.O., Baerwald, A.R., Pierson, R.A.,
328 2005. Systemic concentrations of hormones during development of follicular
329 waves in mares and women: a comparative study. Reproduction 130, 379–388.
330 Harrison, L.A., Squires, E.L., McKinnon, A.O. (1991) Comparison of hCG, Buserelin
331 and Luprostiol for induction of ovulation in cycling mares. J. Equine Vet. Sci. 11,
332 163–166.
333 Hedin, L., Gaddy-Kurten, D., Kurten, R., DeWitt, D.L., Smith, W.L., Richards, J.S.,
334 1987. Prostaglandin endoperoxide synthase in rat ovarian follicles: content,
335 cellular distribution, and evidence for hormonal induction preceding ovulation.
336 Endocrinol. 121, 722–731.
337 Higgins, A.J., Lees, P., Taylor, J.B.O., Ewins, C.P., 1986. Flunixin meglumine:
338 quantitative determination in and effects on composition of equine inflammatory
339 exudate. Brit. Vet. J. 142, 163–169.
340 Killick, S., Elstein, M., 1987. Pharmacologic production of luteinized unruptured
341 follicles by prostaglandin synthetase inhibitors. Fertil. Steril. 47, 773–777.
342 Murdoch, W.J., Hansen, T.R., Mcpherson, L.A., 1993. A review–role of eicosanoids in
343 vertebrate ovulation. Prostaglandins 46, 85–115.

344 Siddiqui, M.A., Gastal, E.L., Gastal, M.O., Beg, M.A., Ginther ,O.J., 2009. Effect of
345 hCG in the Presence of hCG Antibodies on the Follicle, Hormone Concentrations,
346 and Oocyte in Mares. Reprod. Domest. Anim. 44, 474–479.
347 Sirois, J., Dore, M. 1997. The late induction of prostaglandin G/H synthase-2 in equine
348 preovulatory follicles supports its role as a determinant of the ovulatory process.
349 Endocrinol. 138, 4427–4434.
350 Toutain, P.L., Autefage, A., Legrand, C., Alvinerie, M., 1994. Plasma concentrations
351 and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse:
352 pharmacokinetic/pharmacodynamic modelling. J. vet. Pharmacol. Therap. 17,
353 459–469.
354 Turner, D.D., Garcia, M.C., Ginther, O.J., 1979. Follicular and gonadotropic changes
355 throughout the year in pony mares. Amer. J. Vet. Res. 40, 1694–1700.
356 Van Niekerk, C.H., Morgenthal, J.C., Gerneke, W.H., 1975. Relationship between the
357 morphology of and progesterone production by the corpus luteum of the mare. J.
358 Reprod. Fertil. Suppl. 23, 171–175.
359 Wang, L., Qiao, J., Liu, P., Lian, Y., 2008. Effect of luteinized unruptured follicle
360 cycles on clinical outcomes of frozen thawed embryo transfer in Chinese women.
361 J. Assist. Reprod. Genet. 25, 229–233.
362 Watson, E.D., Sertich, P.L., 1990. Secretion of prostaglandins and progesterone by cells
363 from corpora lutea of mares. J. Reprod. Fertil. 88, 223–229.

Table 1

Fate of preovulatory follicles and luteal function of mares treated with flunixin meglumine at different times relative to hCG administration.

group	n	Fx (h)	Ov (n)	LUFs (n)	LUF (%)	> 48 h	P4 day 0	P4 Day 5	P4 day 9
CON	5	-	5	0	0.0[a]	0	0.3± 0.2[a]	6.1±1.2[a]	11.3±1.5[a]
FM 0	6	0-12-24-36	1	5	83.3[b*]	0	0.2±0.1[a]	2.9±0.9[b]	5.3±0.9[b]
FM 24	5	24-36	0	4	80.0[b]	1	0.1±0.1[a]	2.4±0.8[b]	6.1±0.5[b]
FM 28	6	28-36	4	1	16.7[a]*	1	0.2±0.1[a]	5.2±1.4[a]	10.7±1.5[a]
FM 24S	5	24	5	0	0.0[a]	0	0.3±0.1[a]	6.4±0.8[a]	11.7±1.3[a]
FM 30S	5	30	5	0	0.0[a]	0	0.2±0.1[a]	5.9±1.1[a]	10.9±1.8[a]

Flunixin meglumine (FM) mares received 1.7 mg FM/kg b.w. at different times relative to hCG (0 h) treatment: CON: control group with no FM treatment; Fx: frequency of FM treatments (h) relative to hCG (0 h); Ov: number of ovulatory follicles; LUFs: number of luteinized unruptured follicles; LUF %: percentage of mares that developed LUFs after hCG treatment; > 48 h: number of follicles that ovulated more than 48 h after hCG treatment; P4 day 0, 5 and 9: mean ± SEM progesterone concentration for each group just before, five and nine days after hCG administration. Within column, different letters indicate significant difference ($P < 0.05$). Difference in LUF % between group FM 0 and FM 28 approached significance ($P = 0.08$ [ab*]).

Figure

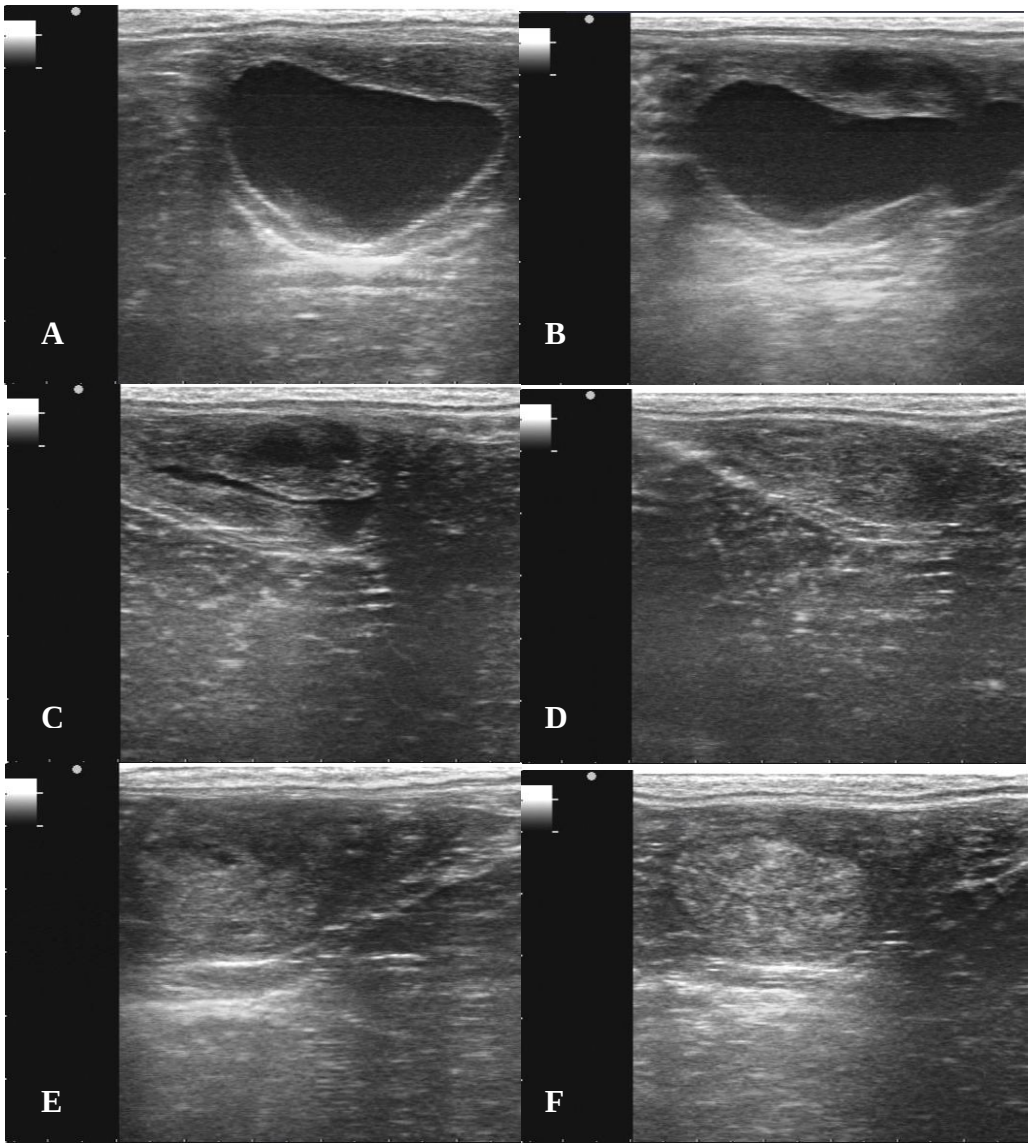

Fig. 1

Figure

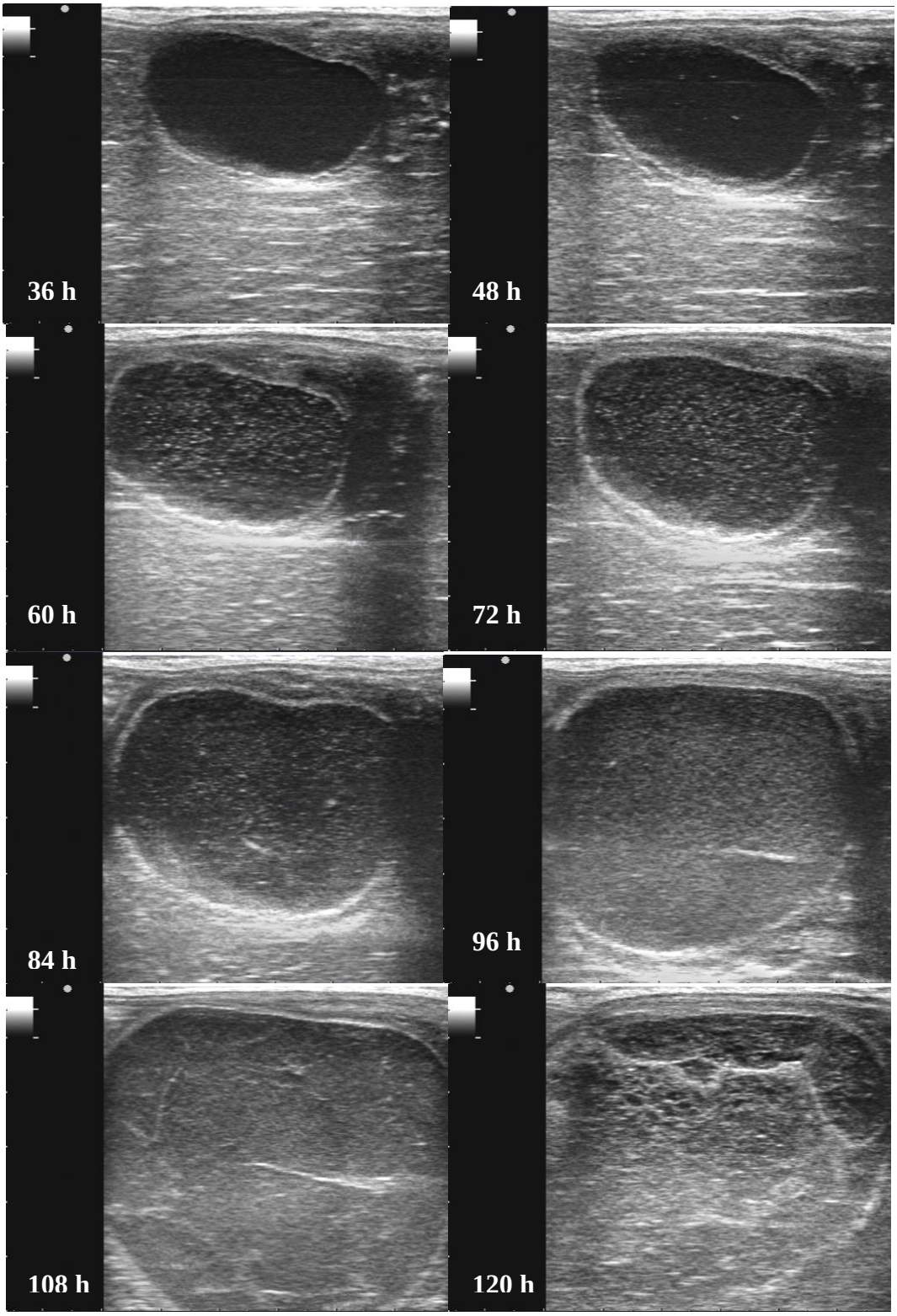

Fig.2

Figure

Fig. 1. Sonogram series of a control mare during the follicular collapse and early corpus luteum formation. The mare was administered 1500 IU hCG when the follicle was 35 mm in diameter (0 h). A) + 37 h: irregular follicular shape pointing towards the apex part of the follicular circumference; note the prominence of the anechoic band and the increased echogenicity of the granulosa layer. B) 10 min later after image A was taken: note the formation of a follicular compartment. C) two minutes later after image B was taken: most of the follicular fluid evacuation has been completed. D) about 20 sec later after image C was taken: follicular collapse and fluid evacuation has been completed. E) + 59 h: 22 h post-ovulation hypoechoic ovulatory area. F) + 84 h: a 46 h old hyperechoic well defined and solid corpus luteum.

Fig. 2. Sonogram series of a luteinized unruptured follicle (LUF) of a mare from group FM 0 h from. The mare was administered 1.7 mg/kg of flunixin meglumine zero, 12, 24 and 36 h after hCG administration (0 h). Images were taken approximately at 12 h intervals. 36 h) echoic-free preovulatory follicle. 48 h) slight amount of echoic specks within the follicular antrum; 60 h) substantial amount of echoic specks indicative of moderate follicular hemorrhage. 72 h) slightly greater amount of echoic specks; note the increase in thickness of the granulosa layer indicative of luteal tissue development. 84 h) significant increase in follicular diameter as a result of further hemorrhage.96 h) further increase in follicular diameter and hemorrhage; note the formation of one solid fibrin strand in the middle of the follicular antrum. 108 h) LUF at its maximum diameter and degree of hemorrhage; note the development of a network of fibrin strands within the follicular antrum that quiver upon ballottement. 120 h) organization of all follicular contents; note the cobweb-like appearance. The progesterone plasma concentration in this mare five days (120 h) after hCG administration was 3.45 ng/ml.