[EQ1]

The Effect of Cloprostenol on the Incidence of Multiple Ovulation and Anovulatory Hemorrhagic Follicles in Two Mares: A Case Report

Juan Cuervo-Arango, LV, MSc, CertVRep, MRCVS,[a,b] and John R. Newcombe, BVetMed, MRCVS[b]

ABSTRACT

Reproductive records of the entire lives of two mares with abnormally high incidence of multiple ovulation and hemorrhagic anovulatory follicles were analyzed retrospectively. Chi-square analysis was used to test statistically the effect of cloprostenol, a prostaglandin F2 analog, on the incidence of multiple ovulations, hemorrhagic anovulatory follicles, and its ultrasonographic appearance. A total of 319 estrous cycles during a 17-year period were analyzed. Cycles induced with cloprostenol were more likely ($P < .000$) to develop hemorrhagic anovulatory follicles than spontaneous cycles. The incidence of multiple ovulation was higher in induced cycles than in spontaneous cycles in one of the two mares.

Keywords: Mare; Induced cycle; Multiple ovulation; Anovulatory hemorrhagic follicle

INTRODUCTION

Cloprostenol (Estrumate; Intervet, Cambridge, UK) is a potent and commonly used prostaglandin F2 (PGF) analog in cattle and equine clinical reproduction. In the mare, PGF and its analogs are firmly established luteolysins for the shortening of individual estrus cycles, the treatment of mares with persistent diestrus, and the interruption of early gestation.

Although PGF is best known for its luteolytic effect, it also causes immediate release of luteinizing hormone (LH),[1] resulting in induction of ovulation (after treatment with fenprostalene) in the mare.[2] There is clinical and scientific evidence that administration of PGF may induce estrus or ovulation even in mares with baseline plasma progesterone concentration without a preceding luteolysis.[3,4] A recent study also showed a dose rate effect on the interval from treatment to ovulation in diestrous mares,[5] with larger doses of cloprostenol inducing ovulation faster than smaller doses. It has been shown that the ovulatory effect of PGF is not only local at the follicular level but also at the neurocrine hypothalamus–hypophysis axis in the central nervous system in horses[1] and cattle.[6] That study showed an immediate increase of follicle-stimulating hormone (FSH) and LH in peripheral blood and a later rise of gonadotropin-releasing hormone after administration of luprostiol, a PGF analog in transitional mares, resulting in continued elevation of blood concentrations of FSH and LH for several hours.[1]

Although the horse is regarded as a monovular species,[7] multiple ovulation is not uncommon. Multiple ovulation rate is associated with breed and is frequently reported to be higher in the Thoroughbred than in pony mares. Hormonal studies during the last 3 days before ovulation have reported lower FSH and higher estradiol concentrations in double as compared with single ovulators, whereas LH remained similar.[8]

A distinct cause of ovulatory failure observed in the mare is hemorrhage into the dominant follicle(s) with subsequent organization of follicular contents and, in most occasions, luteinization of the follicular wall without previous follicular collapse.[9] The pathogenesis of this condition is unknown. Its incidence has been linked to age of the mare[10] and more recently to the use of hormonal treatments for induction of estrus and ovulation.[11,12] Controlled studies have shown in detail hormonal profiles and Doppler ultrasonographic characteristics of the development of hemorrhagic anovulatory follicles in the mare.[13] The latter studies found only subtle differences in follicular wall vascularity between ovulatory and hemorrhagic follicles during the 3 days before ovulation/beginning of hemorrhage. Hormonal profiles on LH, FSH, and progesterone did not, however, reveal any significant difference.

The objectives of this study were to investigate the effect of cloprostenol on (1) the incidence of multiple ovulation; (2) the incidence of hemorrhagic anovulatory follicles; and (3) the ultrasonographic appearance of the development of hemorrhagic anovulatory follicles. To do so, detailed clinical and ultrasonographic records of the entire reproductive lives of two mares known to have unusually high incidences

From the Royal Veterinary College, University of London, North Mymms, UK[a]; and Warren House Farm Equine Fertility Clinic, Brownhills, UK.[b]
Reprint requests: J. Cuervo-Arango, Warren House Farm, Barracks Lane, Brownhills, West Midlands WS8 6LS, United Kingdom.
0737-0806/$ - see front matter

doi:10.1016/j.jevs.2009.04.191

FLA 5.0 DTD ■ YJEVS888_proof ■ 22 May 2009 ■ 10:49 pm ■ ce 17

of multiple ovulation and hemorrhagic anovulatory follicles (HAFs) were analyzed.

MATERIALS AND METHODS

Animals

A Thoroughbred and an Irish Draught mare (both aged 20 years by the end of the study) were bred and lived at the same farm and were managed similarly. Reproductive records (n = 319 cycles recorded from a 17-year period) were obtained from regular ultrasound examinations (at least once daily during estrus and three times per day as they approached ovulation). Both mares tended to cycle all year round, and therefore data from all months were available. Relevant data for mares 1 and 2 are detailed in Table 1.

Experimental Design

For data analysis the experimental unit was the "cycle," which could be classified into different categories according to:

1. Use of induction treatment: any given cycle could be either (a) spontaneous: when no hormonal treatment was used from the previous ovulation; or (b) induced: when estrus and ovulation or HAF formation followed administration of cloprostenol during diestrus. Dose of cloprostenol used in the period recorded ranged from 25 to 1,000 µg because of parallel trials. The frequency of induced cycles was noted according to time of season and age of mare.

2. Whether there was HAF development: a cycle could be either "ovulatory" when no HAF accompanied normal ovulation(s) or "HAF" when the development of a hemorrhagic anovulatory follicle(s) occurred alone or accompanied by normal ovulation(s). Hemorrhagic anovulatory follicles (HAF) were diagnosed by ultrasound as described by Ginther.[9] In brief, the previously fluid-filled follicle of anechoic echotexture filled with echogenic specks that floated freely in the follicular fluid and swirled if balloted, and without any follicular collapse the granulosa layer becomes increasingly echodense and deeper (Fig. 1). Follicular luteinization was assumed when the follicular wall became hyperechoic and thickened, and the follicular contents organized without losing any contents or decreasing in follicular size (Fig. 1). The mare showed clinical signs coincident with those of the luteal phase (negative teasing behavior, absence of endometrial edema, and a tonic, closed cervix).

3. Number of ovulations and or HAFs per cycle. One cycle could have (a) a single ovulation; (b) multiple ovulations; (c) single ovulation and single HAF; (d) multiple ovulations and single HAF; (e) single HAF; (f) multiple HAFs; (g) single ovulation and multiple HAFs; or (h) multiple ovulations and multiple HAFs in the same cycle.

Table 1. Reproductive records of mares 1 and 2

Mare	Breed	YOB	Period Recorded	No of Foals	No of cycles	Mean Preovulatory Follicular Diameter (mm)	Age at 1st HAF	Overall HAF Incidence (%)	Overall Induced Cycles (%)	Induced HAF Cycles (%)
1	ID	1988	90-07	1	158	34.9	3	24.7	67.7	84.6
2	TB	1988	91-07	5	161	33.8	5	25.5	60.2	97.6

ID, Irish Draught; TB, Thoroughbred; YOB, year of birth; HAF, hemorrhagic anovulatory follicle.

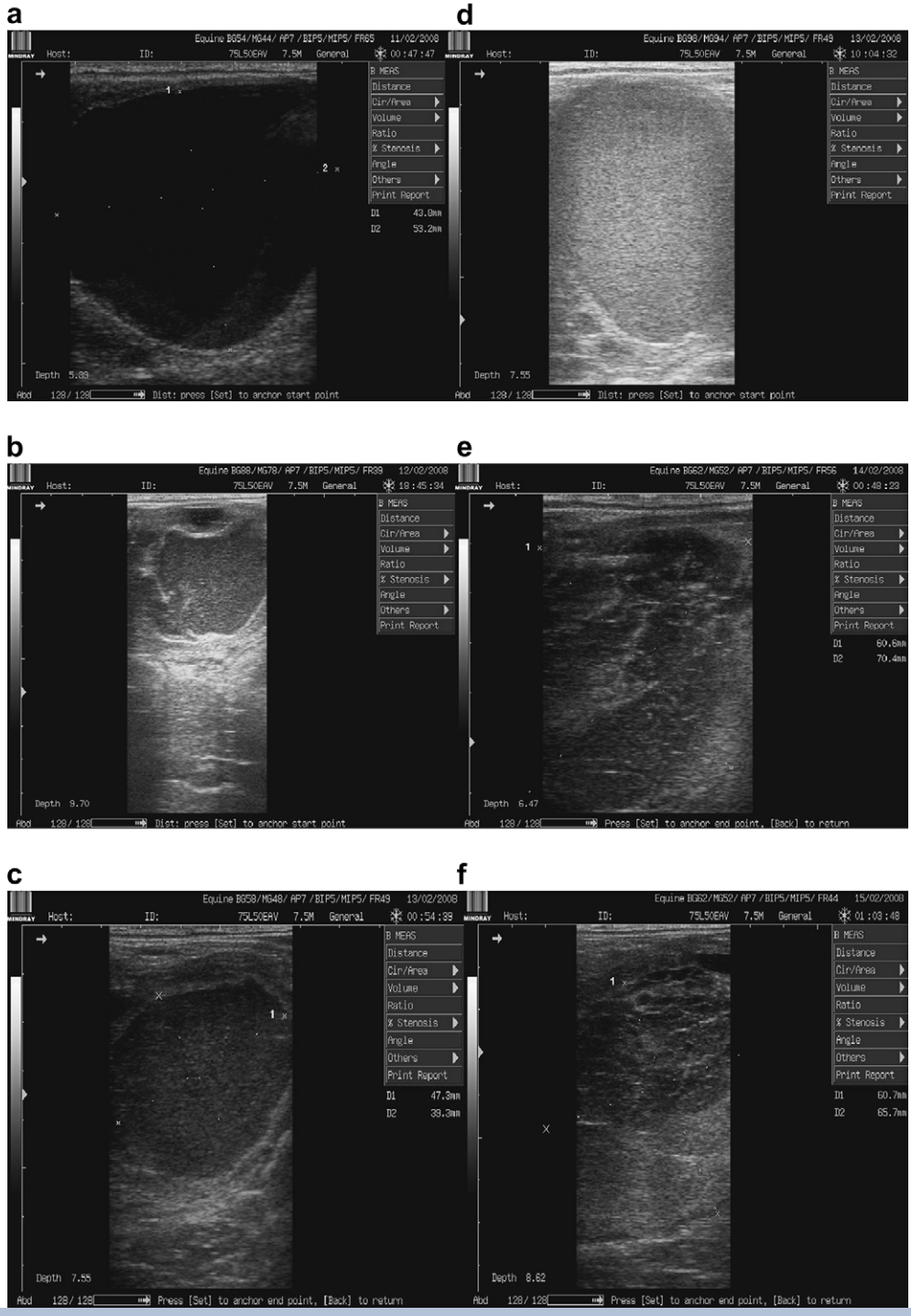

Figure 1. Chronological order of a sonogram series of a preovulatory follicle (a) that hemorrhaged and subsequently luteinised without loss of follicular fluid; (b) and (c) follicle fills with blood (hyperechoic specks), and follicular wall thickens and becomes hyperechoic; (d) increase in number of hyperechoic specks as a result of further hemorrhage; (e) and (f) organized follicular contents, luteal tissue becomes more apparent (lower part of pictures), and the overall diameter of the unruptured follicle increases to over 60 mm. Picture (b) is considered as hour 0 (beginning of hemorrhage); (a) − 42 hours; (c): + 6 hours; (d): + 16 hours; (e) + 30 hours; (f) + 54 hours.

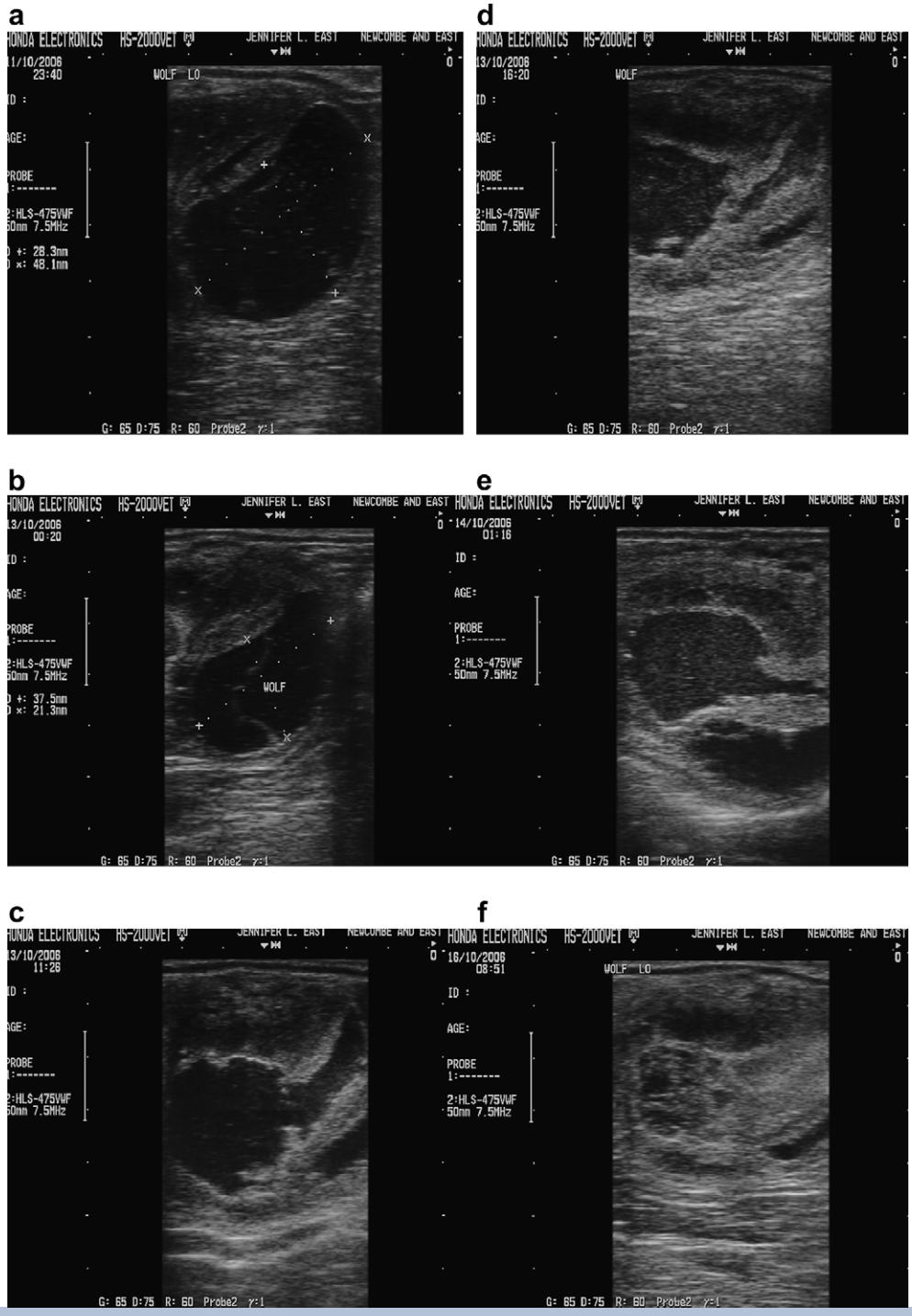

Figure 2. Chronological order of a sonogram series of a follicle that collapsed partially with substantial loss of follicular fluid with subsequent hemorrhage and luteinization; (a) preovulatory-sized follicle with some degree of hemorrhage (hyperechoic specks); (b) reduction in follicular diameter as a result of partial loss of follicular fluid; (c) and (d) the partially collapsed follicle is further divided in follicular compartments, follicular wall thickens and becomes hyperechoic as a result of luteinization; (e) hemorrhage becomes more apparent; (f) follicular contents organize acquiring static appearance. (a) Hour 0 (beginning of hemorrhage); (b) + 24 hours; (c) + 35 hours; (d) + 40 hours; (e) + 49 hours; (f) + 105 hours.

Table 2. Effect of cloprostenol on the incidence of hemorrhagic anovulatory follicles (HAFs)

Mare	Type of Cycle	No of Ovulatory Cycles	No of HAF Cycles	*P*-Value
1	Spontaneous	45	6 11.8%	.009
	Induced	74	33 30. 1%	
2	Spontaneous	63	1 1.6%	<.000
	Induced	57	40 41.21%	
1 + 2	Spontaneous	108	7 6.1%	<.000
	Induced	131	73 35.8%	

4. Type of HAF ultrasonographic appearance. Hemorrhagic anovulatory follicles were classified into partially collapsed hemorrhagic follicles (Fig. 2) or noncollapsed HAFs as described. In partially collapsed HAFs, the preovulatory-sized follicle appeared to be collapsing with loss of some follicular fluid, subsequently acquiring an irregular shape, but instead of completing collapse, the partially collapsed follicle filled with hyperechoic specks and then followed the same events as described, but the subsequent size and shape either remained in the partial collapse stage with limited further growth or hemorrhage continued until the follicle had refilled, assuming the appearance of an organizing noncollapsed HAF.

Statistical Analysis

Chi-square test was used to analyze the effect of induction with cloprostenol on the incidence of HAF cycles, incidence of multiple ovulations, and ultrasonographic appearance of HAFs.

RESULTS

A total of 319 cycles were recorded from mares 1 and 2, of which 204 (63.9%) were induced with cloprostenol. Percentage of induced cycles did not differ among years or months within a year ($P > .05$). Cycles induced with cloprostenol were more likely to develop HAFs than spontaneous cycles ($P < 0.000$; Table 2); this was the case for both mare 1 ($P = .009$) and mare 2 ($P < .000$). Overall HAF incidence was 25.1%. When analyzed by mare, mare 1 had a higher multiple ovulation rate (62.1%, $P = .005$) in induced cycles than in spontaneous cycles (35.6%). However, this was not the case in mare 2 (29.8% vs 25.4%; $P = .59$; Table 3). There was a greater incidence of cycles with multiple dominant follicles (including those ending in both ovulation and HAF) after induction with cloprostenol in both mares 1 and 2 ($P < .000$ and $P = .004$, respectively; Table 4). The different types of cycles observed according to follicle number is shown in Table 3. The incidence of partially collapsed HAFs (19%) was lower than that of noncollapsed HAFs ($P < .01$) and was not associated with the use of cloprostenol ($P > .05$).

DISCUSSION

The aim of the current study was to investigate the effect of cloprostenol (a PGF analog) on the incidence of hemorrhagic anovulatory follicles and multiple ovulation. In addition, the association of cloprostenol and ultrasonic appearance of HAF development was studied. This was possible by analyzing retrospectively reproductive records of two mares known to have a high incidence of HAFs and multiple ovulations.

Results of this study showed a clear association between the use of cloprostenol to induce estrus and the incidence of cycles with both development of HAFs and multiple dominant follicles. The association between cloprostenol and incidence of multiple ovulations is not that clear, and the results can lead to confusion because mare 2 had similar multiple ovulation rate in both spontaneous and induced cycles. However, this mare, like mare 1, had a high incidence of induced cycles with multiple dominant follicles that developed into either normal ovulations or HAFs. If we included these types of cycles with those with multiple ovulations, then the association between the use of cloprostenol and the incidence of multiple ovulation would become even more obvious.

Previous reports[11,12] have hypothesized that the link between induced cycles and development of HAFs can be explained by the fact after administration of PGF and subsequent lysis of CL and decline in progesterone concentrations, LH levels are increased for longer and from earlier stages of follicular development.[11] Mean interval from administration of cloprostenol and beginning of HAF development was reported to be 7.7 days.[12] It appears that enhanced LH concentration during early

Table 3. Effect of cloprostenol on the type of cycle (according to number of ovulations and HAFs)

Mare	Induction with Cloprostenol / Type of Cycle	Single ov	Twin ov	Triplet ov	Single HAF	Twin HAFs	Triplet HAFs	Single ov / Single HAF	Single ov / Twin HAFs	Twin HAFs / Single ov	Twin HAFs / Twin ov	Triplet HAFs / Single ov
1	Induced	28	38	8	5	5		14	4	3	1	1
		26%	28%	7.5%	4.7%	4.7%		13.1%	3.7%	2.8%	0.9 %	0.9 %
	Spontaneous	29	14	2	2	2		1	1			
		56.9%	27.4%	3.9%	3.9%	3.9%		2 %	2%			
2	Induced	40	16	1	10	7	2	17	1	3		
		41%	16.5%	1 %	10.3%	7.2%	2.1%	17.5%	1%	3.1%		
	Spontaneous	47	15	1				1				
		73%	23%	1.5%				1.5%				

stages of follicular growth at least in humans could induce a premature luteinization of the follicular wall with subsequent increased prostaglandin E_2 synthesis, decrease in $PGF_2\alpha$ synthesis, and inhibition of follicular rupture.[14] Clinical observations on the use of superovulation treatments with equine pituitary extract reveal a lower number of ovulations/number of preovulatory follicles ratio than expected. Perhaps luteinization of these preovulatory follicles before follicular collapse might account for, at least in part, this lack of agreement.

With regard to the increased incidence of multiple ovulation/dominant follicles after induction with cloprostenol, the mechanisms by which this phenomenon is observed are not completely clear. Previous studies on gonadotropin concentrations in single and double ovulating mares[8] did not find any specific cause that could explain this enigma. Ginther's group only found a lower FSH concentration in cycles with double ovulation; nevertheless, the reason for this difference was supposedly a consequence of having twin follicles (with increased estradiol) rather than a cause. Hormonal measurements in the latter study were done, however, in the last 3 days before ovulation, long past the point of follicular deviation.[15] Simultaneous research in the same laboratory[11] showed that although the number of waves with two dominant follicles was not different between spontaneous and induced cycles (43% vs 33%, respectively), the number of twin dominant follicles ending in ovulation was significantly higher in $PGF_2\alpha$-induced cycles (86%) than in spontaneous cycles (0%). The LH concentration in the induced group was greater (as compared with the spontaneous group) from 7 to 2 days before deviation, after which it reached a plateau; LH continued to be greater on days 3 and 2 before ovulation but not thereafter. In addition, the induced group had an HAF incidence of 24% in contrast to the 0% in the spontaneous group. The results of this excellent study are in agreement with ours and seem to strengthen the previously proposed theory on HAF development.

Use of induction treatment appeared not to influence the ultrasonographic appearance of HAFs, and the incidence of partially collapsed HAFs was not affected by the treatment. It is not known whether this different appearance and formation of HAFs has any relevance in terms of mechanisms of development, but the clinical relevance seems to be the same because one mare with single partially collapsed HAF was mated on two occasions without a subsequent pregnancy.

In conclusion, the use of cloprostenol, a PGF analog, increased significantly the likelihood of developing HAFs and inducing cycles with multiple dominant follicles and ovulations in two mares with abnormally high intrinsic HAF and multiple ovulation incidences. The use of cloprostenol is widespread in equine practice; however, the incidence of HAF is relatively low. Nevertheless, the results

Table 4. Effect of cloprostenol on the incidence of multiple dominant follicles

Mare	Induction with Cloprostenol	Single Follicle Cycles	Multiple Follicles Cycles	*P* Value
1	Induced	33	74 69.2%	<.000
	Spontaneous	31	20 39.2%	
2	Induced	50	47 48.4%	.004
	Spontaneous	47	17 26.6%	
1 + 2	Induced	83	121	<.000
	Spontaneous	78	37	

of this case report indicate that in this type of mare with intrinsic high HAF incidence, which are often a nightmare for the practitioner, so called "repeater" mares,[13] treatment with cloprostenol to short-cycle may increase significantly the likelihood of developing an anovulatory follicle in the subsequent estrus with no chance of conception.[12]

REFERENCES

1. Jochle W, Irvine CHG, Alexander SL, Newby TJ. Release of LH, FSH and GnRH into pituitary venous blood in mares treated with a PGF analogue, luprostiol, during the transition period. J Reprod Fertil [Suppl] 1987;35:261–267.
2. Savage NC, Liptrap RM. Induction of ovulation in cyclic mares by administration of a synthetic prostaglandin, fenprostalene, during oestrus. J Reprod Fertil [Suppl] 1987;35:239–243.
3. Lamond DR, Buell JR, Stevenson WS. Efficacy of a prostaglandin analogue in reproduction in the anoestrus mare. Theriogenology 1975;3:77–85.
4. Reiner UR, Jochle W. Effectiveness of a novel prostaglandin analogue in postpartum mares and mares with early season anoestrus. Zuchthyygiene 1981;16:110–115.
5. Newcombe JR, Jochle W, Cuervo-Arango J. Effect of dose of cloprostenol on the interval to ovulation in dioestrous mares: a retrospective study. J Equine Vet Sci 2008. In Press. JES773. [Q2]
6. Hafs HD, Louis TM, Stellflug JN, Convey EM, Britt JH. Blood LH after PGF2a in dioestrous and ovariectomised cattle. Prostaglandins 1975;10:1001–1009.
7. Ginther OJ, Beg MA, Bergfelt DR, Donadeu FX, Kot K. Follicle selection in monovular species. Biol Reprod 2001;65:638–642.
8. Ginther OJ, Gastal EL, Rodriguez BL, Gastal MO, Beg MA. Follicle diameters and hormone concentrations in the development of single versus double ovulations in mares. Theriogenology 2008; 69:583–590.
9. Ginther OJ. Haemorrhagic follicles. In: Reproductive biology of the mare: basic and applied aspects, 2nd ed. Cross Plain, WI: Equiservices; 1992:224–226.
10. McCue PM, Squires EL. Persistent anovulatory follicles in the mare. Theriogenology 2002;58:541–543.
11. Ginther OJ, Jacob JC, Gastal MO, Gastal El, Beg MA. Follicle and systemic hormone interrelationships during spontaneous and ablation-induced ovulatory waves in mares. Anim Reprod Sci 2008; 106:181–187.
12. Cuervo-Arango J, Newcombe JR. Risk factors for the development of haemorrhagic anovulatory follicles (HAFs) in the mare. Reprod Dom Anim 2008 [In Press]. [Q3]
13. Ginther OJ, Gastal EL, Gastal MO, Beg MA. Incidence, endocrinology, vascularity, and morphology of haemorrhagic anovulatory follicles in mares. J Equine Vet Sci 2007;27:130–139.
14. Coulam CB, Hill LM, Breckle R. Ultrasonic evidence for luteinization of unruptured preovulatory follicles. Fertil Steril 1982;37: 524–529.
15. Ginther OJ, Beg MA, Donadeu FX, Bergfelt DR. Mechanism of follicle deviation in monovular farm species. Anim Reprod Sci 2003;78:239–257.