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Short Communication

Relationship Between Dose of Cloprostenol and Age of Corpus Luteum on the Luteolytic Response of Early Dioestrous Mares: A Field Study

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Contents

The objective of this study was to establish and characterize the relationship between the dose of cloprostenol (37.5, 250, 500 and 750 μg) and the age of the early corpus luteum (CL) (80, 88, 96, 104 and 112 h) on the luteolytic response of mares. Behavioural oestrus and ultrasonographic signs of return to oestrus were considered as the occurrence of full luteolysis. A total of 298 mares were divided into groups according to dose of cloprostenol and CL age. There was an effect of dose of cloprostenol ($p < 0.001$) and age of the CL at the time of treatment ($p < 0.001$) on the percentage of mares with full luteolysis. The efficacy of 37.5 μg of d-cloprostenol was similar to that of 250 μg of d,l-cloprostenol ($p > 0.05$), and that of 500–750 μg ($p > 0.05$). The higher dose groups (500 and 750 μg) induced full luteolysis more frequently than the lower dose groups (37.5 and 250 μg) 96–104 h post-ovulation. There was no effect of CL age or cloprostenol dose on the interovulatory interval ($p > 0.05$). In conclusion, the effect of cloprostenol on the percentage of mares undergoing full luteolysis is dose-dependent. However, this effect is only evident in mares with CLs aged between 96 and 104 h. There is no advantage of administering more than 500 μg of d,l-cloprostenol (Estrumate®), to obtain a higher percentage of mares with full luteolysis in mares with CLs aged 80–112 h.

Introduction

Prostaglandin $F_{2\alpha}$ (PGF) and its analogues are widely used in equine practice to shorten the oestrous cycle by inducing luteolysis. Cloprostenol, amongst other PGF analogues, is commonly used in practice. This synthetic compound is much more potent than the naturally occurring PGF (dinoprost), and whereas the manufacturer's recommended clinical dose is 5 mg for dinoprost (Lutalyse®; Pfizer), it is only 250 μg for cloprostenol (Estrumate®; Scering-Plough Animal Health Ltd). The recommended dosage is intended for mares with 'mature corpora lutea', i.e., five or more days old. However, it is known that a much lower dosage (less than a tenth of the recommended dose of PGF) is needed to induce full luteolysis in mares with older corpus luteums (CLs) (Handler et al. 2004; Barker et al. 2006). It has been shown that only 8.75 μg of cloprostenol (around 30 times less than the recommended dose) was effective in inducing full luteolysis in mares known to be in mid to late dioestrus (>10 days post-ovulation; Newcombe et al. 2008).

On the other hand, if PGF or cloprostenol is administered in early dioestrus (≤4 days post-ovulation) as a single bolus, partial luteolysis is achieved in most mares (Troedsson et al. 2001; Nie et al. 2003a,b; Bergfelt et al. 2006; Newcombe 2007; Tosi et al. 2008). Clinically, mares with partial luteolysis undergo a significant decrease in peripheral progesterone concentration within 24 h of treatment to approximately 1–2 ng⁄ml with a subsequent rebound in progesterone concentration (Bergfelt et al. 2006). In addition, the CL decreases in diameter to ultrasonographically undetectable size within 6 days of treatment, and the mare ovulates without showing oestrous signs (Bergfelt et al. 2006).

Repeated treatments with PGF or cloprostenol in early dioestrus (1–4 days post-ovulation) have been attempted (Mocklin et al. 2006; Holland and Pinto 2008; Rubio et al. 2008). In the latter studies, most mares underwent partial luteolysis with resurgence in progesterone concentration. In addition, protocols involving repeated treatments to control the oestrous cycle are not practical in field conditions owing to extra labour costs resultant from increased handling.

The dose of the luteolytic agent and the age of the CL within the first 4 days of the oestrous cycle are likely to influence the proportion of mares that respond with partial or full luteolysis. However, to date, no dose-rate study that tests the effect of different doses administered to mares at different days in early dioestrus has been performed. The knowledge of this relationship between the dose of the luteolytic agent, the age of the CL and the proportion of mares with full luteolysis is relevant to equine reproduction. There may be clinical indications in which it is needed to bring a recently ovulated mare back in oestrus as soon as possible.

Over the last few years, data on reproductive clinical outcome of mares treated with different doses of cloprostenol within the first 4 days after ovulation have been collected in our clinic. The objective of this study was to establish and characterize the relationship between the dose of cloprostenol and the age of the early CL on the proportion of mares with full luteolysis. This was expressed as the percentage of mares that showed behavioural and ultrasonographic signs of return to oestrus following the luteolytic treatment. It was hypothesized that both the dose of cloprostenol and the age of the CL at the time of treatment would have a positive and significant relationship with the proportion of mares undergoing full luteolysis.

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Materials and Methods

Experimental design

This is a retrospective study involving reproductive data of 298 mares. The data were obtained during the breeding seasons (March–September) of 2006–2010. The mares were treated for clinical reasons at different times relative to the day of ovulation with either:

1. 37.5 μg of d-cloprostenol (0.5 ml sc of Genestran®; Forte Healthcare Ltd, Naul, Ireland) 80 h (n = 19), 88 h (n = 13), 96 h (n = 23), 104 h (n = 30) and 112 h (n = 20) post-ovulation.
2. 250 μg of d,l-cloprostenol (1 ml sc of Estrumate®; Scering-Plough Animal Health Ltd, Welwyn Garden City, UK) 80 h (n = 5), 88 h (n = 17), 96 h (n = 13), 104 h (n = 8) and 112 h (n = 4) post-ovulation.
3. 500 μg of d,l-cloprostenol (2 ml sc of Estrumate®) 80 h (n = 18), 88 h (n = 50), 96 h (n = 23), 104 h (n = 8) and 112 h (n = 4) post-ovulation.
4. 750 μg of d,l-cloprostenol (3 ml sc of Estrumate®) 80 h (n = 5), 88 h (n = 10), 96 h (n = 18), 104 h (n = 7) and 112 h (n = 3) post-ovulation.

The dose of cloprostenol is per mare, regardless of body weight.

Animals

The data were collected from a total of 298 mares, resident to or visiting a veterinary clinic in the UK (northern hemisphere) during the breeding seasons of 2006–2010. Most mares were Irish Draught and crosses between Irish Draught and Thoroughbred, with also some Standardbred, Thoroughbred and Warmblood mares. The age of mares varied from three to 25 years. Although the animals' weight was not measured, it was estimated to vary between 400 and 650 kg. All mares were enrolled in commercial AI or embryo transfer programs as donor or recipient mares. The mares were examined once daily during oestrus and three times a day around the ovulatory period. The mares were kept in outdoor paddocks during dioestrus and early oestrus. As ovulation was estimated to be approaching, the animals were brought into the clinic and kept in individual boxes.

Ultrasonography and detection of ovulation

Internal genitalia were examined by transrectal ultrasonography with an ultrasound scanner (Mindray DP-6600 Vet; Mindray Ltd) equipped with an 8-MHz linear-array transducer. Ovulation was detected as per rectal palpation and ultrasonography by the absence of the previously recorded follicle and the later presence of an initially hypoechoic area and subsequently a hyperechoic CL within the same ovary. All mares included in the study were examined every 8 h until ovulation was detected. Therefore, the age of the CL was known with a range of 8 h. Thus, the first time a mare was detected as having ovulated, the CL age was between 0 and 8 h. For the purpose of simplicity, only mares with single ovulations were included in the study. The age of the CL is expressed as the oldest possible time (e.g. a CL of 80 h, could be 72–80 h old).

Corpus luteum age groups

According to the age of the CL at the time of treatment, the mares were divided into five different CL-age groups. The age groups were the following: 80, 88, 96, 104 and 112 h post-ovulation. The cut points for the youngest and oldest groups were estimated based on preliminary results: mares with CLs younger than 80 h were known to hardly respond with full luteolysis to any of the luteolytic treatments attempted with different doses. On the other hand, it was known that most mares (treated even with small luteolytic doses) responded when the CL age was more than 112 h.

Cloprostenol dose

All mares were treated by subcutaneous administration with one of the four different doses of cloprostenol. The three largest dose groups were 250, 500 and 750 μg of d,l-cloprostenol (250 μg/ml of Estrumate®, Scering-Plough Animal Health Ltd). Therefore, the final volume of product of Estrumate® was equivalent to 1, 2 and 3 ml for 250, 500 and 750 μg of d,l-cloprostenol, respectively. The smallest dose group was 37.5 μg of d-cloprostenol, equivalent to 0.5 ml of Genestran® (75 μg/ml of Genestran®; Forte Healthcare Ltd). According to the data sheet information of Genestran®, the recommended dose of d-cloprostenol for mares with a 'mature CL' is 22.5–37.5 μg. The reduction in dose compared with the racemic form (d,l-cloprostenol) is based on the higher potency of the active dextrorotatory isoform (d-cloprostenol) resultant from the removal of the inactive isoform, l-cloprostenol (Kral 1988). The reason to include this different compound was to produce data available for comparison purposes between d,l- and d-cloprostenol and so to demonstrate the manufacturer's claim that d-cloprostenol is approximately 3.3 times as potent as d,l-cloprostenol.

Classification and diagnosis of clinical luteolysis

Once the mares were treated with cloprostenol, they were examined again 5 days later and classified into three groups (full luteolysis, partial luteolysis or no response), according to the results obtained from the clinical examination. The clinical examination involved the following parameters: assessment of the tone, patency and oedema of the cervix by manual examination *per vaginam*; ultrasound examination of the uterus and ovaries including estimation of the endometrial oedema score (Fig. 1) and measurement of the diameters of the CL; and observation of the mare's behaviour in front of a teaser stallion. The mare was examined subsequently at least once daily until the next ovulation occurred. The following criteria had to be met to classify a mare as having:

1. Full luteolysis: Five days after treatment, a significant reduction in the size (< 20 mm in diameter) or complete ultrasonographic disappearance of

LOW RESOLUTION FIG

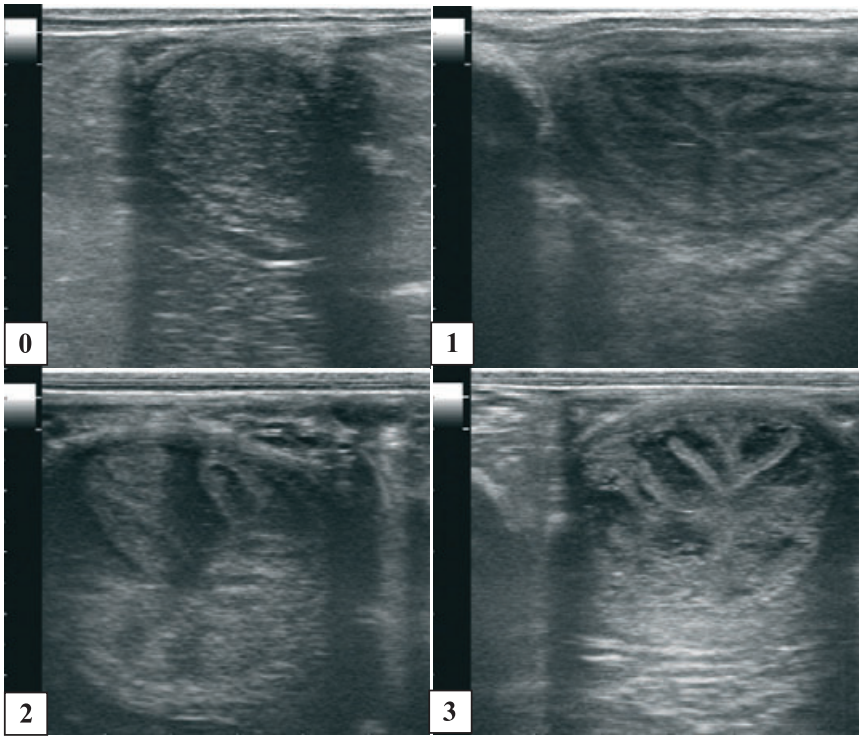

Fig. 1. Endometrial oedema scoring system. Score of 0: absence of endometrial folding (dioestrous-like echotexture). Scores of 1–3: increasing prominence of endometrial folds (oestrous-like echotexture)

the CL and endometrial oedema score of ≥ 1 (0 = no endometrial oedema; 3 = maximal endometrial oedema) were observed. If little or no endometrial oedema was present, the palpation of a relaxed and open cervix along with positive oestrous signs to teasing (tail raising, eversion of clitoris and urination) was indicative of full luteolysis. The interovulatory interval (IOI = the time period between two successive ovulations) was 8–18 days.

2 Partial luteolysis: Five days after treatment, a significant reduction in the size (< 20 mm in diameter) or apparent ultrasonographic disappearance of the CL, endometrial oedema score of 0, palpation of a tight cervix with increased tone and negative signs to teasing (tail movement sideways and kicking) were observed, and typically an IOI of 8–18 days.

3 No response: Five days after treatment, a slight or normal gradual reduction in the CL diameter (but ≥20 mm), endometrial oedema score of 0, palpation of a tight cervix with increased tone and negative oestrous signs to teasing (tail movement sideways and kicking) was observed and an IOI of > 18 days, typically of 20–23 days.

Statistical analyses

For statistical analysis of categorical data, the luteolytic response after cloprostenol treatment was simplified as presence or absence of full luteolysis. Therefore, the absence of full luteolysis included mares with partial and no responses. For simplicity, the percentage of mares for each group with no response, full or partial luteolysis is presented in Fig. 2 but was not analysed statistically. Frequency data (presence or absence of full luteolysis) were analysed by binary logistic regression. The regression model included two factors, age of CL and dose of cloprostenol, with five (CL ages) and four (cloprostenol doses) different levels, respectively. Within each factor, the difference in the response rate was measured by chi-square test. Sequential data (IOI)

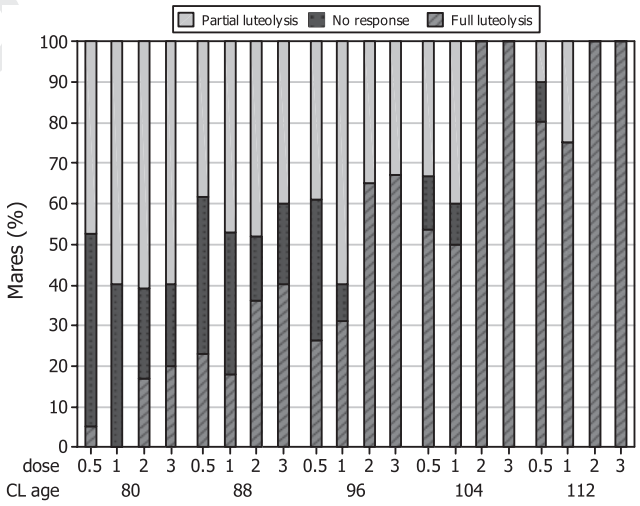

Fig. 2. Distribution of different luteolytic responses (full, partial or no response) in mares following treatment with different cloprostenol doses at 80, 88, 96, 104 and 112 h post-ovulation. The cloprostenol doses were 0.5 ml of Genestran® (37.5 μg d-cloprostenol) and 1, 2 and 3 ml of Estrumate® (250, 500 and 750 μg d,l-cloprostenol, respectively). Statistical analysis was not performed

were analysed by a general linear model of variance. The model included two crossed factors (CL age and cloprostenol dose) with no covariates. If an effect of any factor was found ($p < 0.05$) on the IOI, a Tukey's test was used to analyse any significant difference amongst levels. Frequency data are expressed as percentage (%) and sequential data as mean ± SEM.

Results

The percentages of mares with full luteolysis for each cloprostenol dose and CL age groups are shown in Table 1. There was an effect of dose of cloprostenol ($p < 0.001$) and age of the CL at the time of treatment ($p < 0.001$) in the percentage of mares with full luteolysis. The two lowest doses (250 μg of d,l-cloprostenol and 37.5 μg of d-cloprostenol) yielded a similar percentage ($p > 0.05$) of mares with full luteolysis at any of the CL age groups tested. Likewise, the two highest doses (500 and 750 μg of d,l-cloprostenol) did not produce any difference ($p > 0.05$) in terms of percentage of mares with full luteolysis after treatment 80–112 h post-ovulation.

The difference in the percentage of mares with full luteolysis between the high (500 and 750 μg) and low (37.5 and 250 μg) doses was significant at 96 and 104 h post-ovulation (Fig. 3). Within the two groups with highest doses (pooled data from 500 to 750 μg), the mares treated at 80 and 88 h post-ovulation had no significant difference in the percentage of full luteolysis (17.4% and 36.7%, respectively; $p > 0.05$), but there was a difference between mares treated at 88, 96 and 104 h post-ovulation (36.7%, 65.8% and 100% of mares with full luteolysis, respectively; $p < 0.01$; Fig. 3). Similarly, the two lowest doses (pooled data from 37.5 to 250 μg) yielded a similar percentage of mares with full luteolysis at 80 and 88 h post-ovulation ($p > 0.05$), but the difference increased gradually at 96, 104 and 112 h post-ovulation (27.8%, 52.6% and 79.2%, respectively; $p < 0.01$, Fig. 3).

Table 1. Effect of cloprostenol dose and corpus luteum (CL) age on the percentage of mares with full luteolysis

	Mares with full luteolysis (%)			
	d-cloprostenol		d,l-cloprostenol	
CL age (h)	37.5 μg	250 μg	500 μg	750 μg
80	1/19	0/5	3/18	1/5
	5.2%	0%	16.7%	20.0%
88	3/13	3/17	18/50	4/10
	23.1%	17.6%	36.0%	40.0%
96	6/23	4/13	15/23	12/18
	26.1%[a]	30.8%[a]	65.2%[b]	66.7%[b]
104	16/30	4/8	8/8	7/7
	53.3%[a]	50.0%[a]	100%[b]	100%[b]
112	16/20	3/4	4/4	3/3
	80%	75.0%	100%	100%

d-cloprostenol (Genestran®); d,l-cloprostenol (Estrumate®).
Within row, different letters indicate significant difference ($p < 0.05$) in the percentage of mares with full luteolysis amongst dose groups.

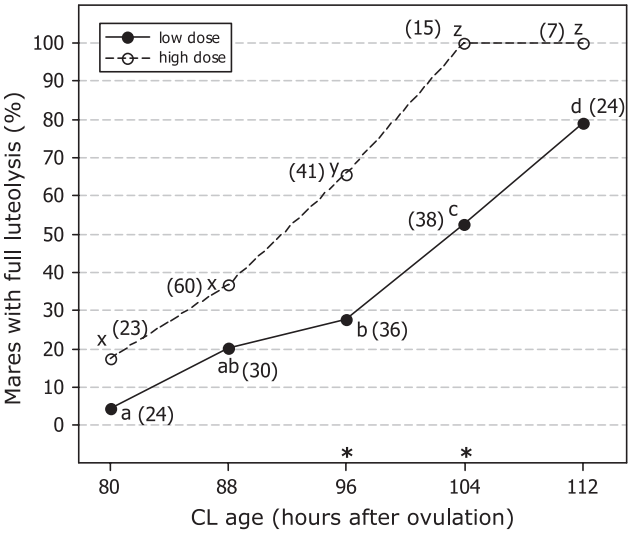

Fig. 3. Percentage of mares with full luteolysis after treatment with cloprostenol at different times post-ovulation. The low-dose group consists of pooled data from the 250 μg d,l-cloprostenol and 37.5 μg d-cloprostenol groups; the high-dose groups consist of pooled data from the 500 and 750 μg d,l-cloprostenol groups. Within each dose group, different letters indicate significant difference ($p < 0.05$) in the percentage of mares with full luteolysis amongst CL ages. *$p < 0.05$, percentage of mares with full luteolysis between dose groups at a given CL age group

The proportion of mares for each group with partial luteolysis, full luteolysis and no response to the cloprostenol treatment is shown in Fig. 2. The mares with earliest CLs and treated with lowest doses appeared to have the greatest proportion of no responses.

There was no effect of CL age or cloprostenol dose on the IOI ($p > 0.05$; Table 2).

Discussion

Effect of CL age on luteolytic response

Luteal PGF receptors have been reported at various stages of dioestrus (Kimball and Wyngarden 1977; Vernon et al. 1979), but they have not been reported in mares with CLs <5 days old. The results of this study and those of others (Troedsson et al. 2001; Nie et al. 2003a,b; Bergfelt et al. 2006; Tosi et al. 2008) showed indirect evidence that the early CLs must have PGF receptors as exogenous luteolytic treatment caused a decrease or a delay in the rise of peripheral progesterone concentration in mares with early CLs. Furthermore, there is direct evidence of luteal PGF receptors in cows with CLs <4 days old (Tsai and Wiltbank 1998).

It is not surprising that the percentage of mares that respond to cloprostenol treatment with full luteolysis increases along with the age of the CL. However, it is worth noting that a difference of only 8 h (i.e. from 96 to 104 h post-ovulation) was sufficient to increase significantly the percentage of mares undergoing full luteolysis regardless of the cloprostenol dose. In terms of responsiveness to an exogenous luteolytic drug, the age of the CL becomes critical only during a narrow

Table 2. Effect of cloprostenol and corpus luteum (CL) age on the interovulatory interval (IOI)

	IOI (days)			
	d-cloprostenol		d,l-cloprostenol	
CL age (h)	37.5 μg	250 μg	500 μg	750 μg
80	(19)	(5)	(18)	(5)
	16.9 ± 1.5	18.4 ± 2.5	16.1 ± 1.5	15.0 ± 1.9
88	(13)	(17)	(50)	(10)
	18.7 ± 1.4	17.4 ± 1.1	15.4 ± 0.7	15.9 ± 1.7
96	(23)	(13)	(23)	(18)
	17.9 ± 1.0	14.8 ± 0.9	14.5 ± 0.6	14.9 ± 0.8
104	(30)	(8)	(8)	(7)
	15.4 ± 1.1	13.9 ± 0.8	13.6 ± 1.2	13.9 ± 0.5
112	(20)	(4)	(4)	(3)
	13.3 ± 1.1	13.2 ± 0.6	14.2 ± 1.3	12.7 ± 1.2

The number of mares for each group is shown in brackets.

window of time. Before 96 h post-ovulation, relatively few mares responded fully to the treatment of cloprostenol, even when the dose was three times higher than the manufacturer's recommended dose. Between 96 and 104 h post-ovulation, the number of full responses increased significantly, and this increase was dose dependant. From 104 h onwards, most mares responded with full luteolysis to the cloprostenol treatment, regardless of the dose.

In most studies dealing with exogenous luteolysis in mares with early CLs, the frequency between examinations to detect ovulation has been 24 h (Troedsson et al. 2001; Nie et al. 2003a,b; Bergfelt et al. 2006). Bergfelt et al. (2006) administered 10 mg of dinoprost on day 3 of the oestrous cycle (day 0 = day of ovulation; mares examined every 24 h); therefore, the day 3 CLs could have been 72–96 h old. The 10-mg dinoprost treatment induced full luteolysis in 25% of treated mares (4/16). The authors concluded that the difference in the type of luteolytic response (full vs partial) could be attributed to different ovulation times within day 0 (Bergfelt et al. 2006). Indeed, mares that responded fully had greater concentration of progesterone before treatment than mares that responded partially. A higher concentration of peripheral progesterone is a signs of CL maturity (older age; Ginther 1992).

In the present study, the recommended dose of d,l-cloprostenol (250 μg) induced complete luteolysis in 20% (7/35) of mares with CLs of 72–96 h (from groups 80, 88 and 96 h). When these mares are classified into three CL-age groups (8 h-groups), the percentage of mares with full luteolysis increases from 0% to 17% and to 30% for mares with CLs of 72–80, 80–88 and 88–96 h old, respectively. The results of this study provide evidence on the relevance of accurately determining the time of ovulation. This is especially important to design an experiment to test the efficacy of a luteolytic drug, so that a homogenous group of mares with similar CL ages can be obtained.

Effect of cloprostenol dose on the luteolytic response of early CLs

The dose-rate effect of cloprostenol on luteolysis was only evident in mares with CLs of 96–104 h old and between 250 and 500 μg of d,l-cloprostenol. However, an extra 50% of the compound (750 μg) apparently did not induce a higher percentage of mares with full luteolysis. A reason for the lack of effect could be that the extra 50% of cloprostenol was not sufficient to induce an increase in the percentage of mares with full luteolysis. On the other hand, there could be a threshold after which the association between dose and full luteolysis is no longer positive. This is also the case of mares with CLs younger than 96 h old. In these mares, the effect of cloprostenol on full luteolysis is not dose dependant.

There was a similar percentage of mares with full luteolysis after treatment with either 250 μg of d,l-cloprostenol or 37.5 μg of d-cloprostenol at any of the CL-age groups tested. At least in this compound (Genestran®), the removal of the levorotatory isomer made it over six times more potent than the racemic cloprostenol. This is double the potency claimed by some of the d-cloprostenol manufacturers (Genestran® and Dalmazin®).

Effect of dose of cloprostenol and CL age on the IOI

The results of the current study did not show any effect of either dose of cloprostenol or CL age on the IOI. This is not surprising as mares with partial and full luteolysis are expected to have a similar IOI (Bergfelt et al. 2006). Although not significant, mares treated with lower doses of cloprostenol and with earlier CLs tended to have longer IOIs than mares treated with higher doses and with older CLs. This tendency could account for a higher overall percentage of mares with no luteolytic responses (IOI > 18 days) in the 80–96 h CL age groups and 37.5- and 250-μg dose groups.

In conclusion, the effect of cloprostenol on the percentage of mares undergoing full luteolysis is dose-dependent. However, this effect is only evident in mares with CLs aged between 96 and 104 h. There is no advantage of administering more than 500 μg of d,l-cloprostenol (Estrumate®) to obtain a higher percentage of mares with full luteolysis. The efficacy of 37.5 μg of d-cloprostenol is equivalent to 250 μg d,l-cloprostenol in terms of inducing full luteolysis. The IOI of treated mares is not affected by either the cloprostenol dose or CL age in spite of differences amongst dose and age groups in the percentage of mares with full luteolyis.

Conflicts of interest

None of the authors have any conflict of interest to declare.

Author contributions

J. Newcombe performed data collection and experimental design, and J. Cuervo-Arango performed the statistical analyses and wrote the manuscript.

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