Effect of sodium cloprostenol and flunixin meglumine on luteolysis and the timing of birth in bitches

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At birth, the physiological role of prostaglandins in bitches is unclear. Bitches were treated before parturition with either saline, the prostaglandin analogue, sodium cloprostenol, or the prostaglandin synthetase inhibitor, flunixin meglumine. The animals were examined regularly to determine the onset of parturition and a series of blood samples were taken to define the hormonal profiles before, during and after birth. Animals treated with cloprostenol whelped earlier than did controls. In addition, the prostaglandin F₂₀ metabolite surge and decrease in plasma progesterone concentration and rectal temperature were earlier than in controls. Flunixin meglumine disrupted the normal 13,14-dihydro-15-keto prostaglandin F₂₀ profile but did not abolish prostaglandin synthesis completely or delay the onset of labour in treated animals. This study confirms that prostaglandins induce luteolysis and the onset of labour in the bitch. However, the partial inhibition of prostaglandin synthesis does not prevent parturition.

Introduction

The endocrinology of late gestation and parturition is poorly understood in bitches, although, as in other mammals, prostaglandins are thought to play a central role in the process of parturition (Smith and MacDonald, 1974; Concannon et al., 1977, 1989; Concannon, 1978; Chakraborthy, 1987; Hoffmann et al., 1996). In all mammals studied to date, there is an increase in prostaglandin F₂₀ (PGF₂₀) synthesis by the corpus luteum, placenta or endometrium at term (Challis and Lye, 1994; Liggins and Thorburn, 1994). It is well established that prostaglandins, especially prostaglandin PGF₂₀, play a major role in initiating labour and in the control of myometrial contractions. Changes in steroid hormones at term promote an increase in myometrial responsiveness to hormones such as prostaglandins and oxytocin and may also influence production of these compounds (Challis and Lye, 1994).

In human and veterinary medicine, exogenous prostaglandins are used extensively in the control of reproduction. PGF₂₀ and its synthetic analogues have been used in bitches to induce luteolysis but are not widely used to initiate parturition because of side effects associated with treatment. Cramp, diarrhoea, vomiting, salivation and accelerated respiration have all been reported when PGF₂₀ is given in high doses (Tsutsui et al., 1982, 1989; Ichijo et al., 1995; Iseki et al., 1995). While these adverse effects can be reduced using lower doses, repeated administration of PGF₂₀ for several days is then required to induce birth.

In bitches, there is a surge of prostaglandin in peripheral plasma at the time of birth. This prostaglandin surge is luteolytic and leads to a rapid decrease in progesterone concentration. During the surge, the concentration of the prostaglandin F₂₀ metabolite, 13,14-dihydro-15-keto-prostaglandin-F₂₀ (PGFM), in plasma increases to a peak value of about 2500 pg ml⁻¹ 12–24 h before birth (Concannon, 1989), then slowly decreases to basal concentrations 1–2 days after parturition.

Although prostaglandins induce uterine contractions and abortion, a clear physiological role of PGF₂₀ in parturition has not been shown in dogs. Hoffmann et al. (1996) treated four pregnant bitches with various doses of the prostaglandin synthetase inhibitor, indomethacin. The two animals receiving the highest doses maintained high progesterone and basal PGFM concentrations for 2 and 3 days later than controls, respectively, at which time the pups were delivered by Caesarean section.

This study was designed to investigate further the physiological role of PGF₂₀ in bitches at parturition by determining whether delivery can be induced by a continuous low infusion of the prostaglandin analogue, sodium cloprostenol, and, conversely, whether the prostaglandin synthetase inhibitor, flunixin meglumine, can inhibit parturition.

Materials and Methods

Animals

The 12 bitches used in this study were housed at the dog colony established at the University of Melbourne Veterinary Clinic in Werribee. Animals were maintained using the standard husbandry practices in this enclosure. The institutional animal ethics committees approved all
experiments. Three greyhounds and one crossbreed were allocated into each of three groups: a cloprostenol-treated, a flunixin-treated and a control group. The control animals served as controls for both treatments simultaneously. The mean body weights (mean ± SEM) of animals at 8 weeks of pregnancy were 29.0 ± 2.5 kg (cloprostenol), 30.1 ± 1.9 kg (flunixin) and 30.9 ± 3.1 kg (controls).

Vaginal cytology and plasma progesterone concentrations were used to determine the reproductive status of all bitches and the optimum time of mating. Ten of the animals used had natural oestrus. The other two were animals in which oestrus was induced by treatment of anoestrous greyhounds with the dopamine agonist, Dostinex (Cabergoline, 0.5 mg tablets; a quarter tablet each day for 7 days; Pharmacia Pty Ltd, North Ryde, NSW) (Arbeiter et al., 1988). Vaginal smears and blood samples were taken three times a week from all bitches during dioestrus until mating or artificial insemination. All bitches were mated or artificially inseminated 2–4 days after plasma progesterone concentrations had increased to between 2 and 4 ng ml⁻¹. Vaginal smears were taken each day after mating to detect the onset of dioestrus (day 1). Dioestrus begins at the end of standing heat (oestrus) and is characterized by an abrupt change in vaginal cytology (Holst and Pemister, 1974). Whelping dates are generally quoted as 57 days from the onset of dioestrus (Holst and Pemister, 1974). The pregnancy status of each animal was determined using ultrasonography around day 21. If fetuses were present, their viability was confirmed by the presence of a heartbeat. On day 45, animals were brought indoors and placed in whelping kennels. Blood samples (2.5 ml) were taken each day (12:00 h) from day 45 to day 50 and three times during day 51 (09:00, 14:00 and 20:00 h). Litter sizes were determined by X-ray examination at day 50.

At birth, animals were monitored for signs of dystocia. Animals unable to deliver consecutive pups over an 8–12 h period were given an infusion of calcium gluconate (5–10 ml of a 10% (w/v) solution, i.v.) and multiple oxytocin injections (each of 10 iu) to induce uterine contractions.

Induction of parturition using a PGF₂α analogue

Four animals were treated with the PGF₂α analogue, sodium cloprostenol (Jurox, Silverwater, NSW). Cloprostenol was delivered via an Alzet 2001 osmotic minipump in a continuous dose (3.5 μg kg⁻¹ day⁻¹) until birth. The four control animals received water filled minipumps. Pumps were inserted subcutaneously on day 52 at 09:00 h; 5 days before expected births using the technique described by Watts and Wright (1997). The pumps were removed 6–8 h after birth using similar procedures, or after a period of 8–12 h if cloprostenol-treated bitches were diagnosed with dystocia.

Inhibition of birth using a prostaglandin synthetase inhibitor

Four animals were injected twice a day with the prostaglandin synthetase inhibitor, flunixin meglumine (50 mg ml⁻¹; Finadyn; Schering–Plough Pty Ltd, Prahran, Victoria), at a dose of 0.25 ml per 22 kg body weight from day 54 until birth. This dose rate is the therapeutic dose used to treat dogs with endotoxic shock. The four control animals received saline treatments.

During the period of treatment, animals were observed for any possible adverse effects, such as salivation, vomiting, panting and diarrhoea. From 09:00 h on day 52, rectal temperatures were monitored at 4 h intervals to detect the characteristic decrease that occurs 24–36 h before parturition. After this decrease, the animals were watched more regularly for signs of uterine contractions and the expulsion of amniotic fluids. Pups were weighed twice a week from birth for 3 weeks to determine whether neonatal viability and growth rate were affected by treatment.

Sampling after treatment

Blood samples and rectal temperatures were taken at 4 h intervals from 09:00 h on day 52 until birth from cloprostenol-treated bitches. Blood samples and rectal temperatures were taken from control and flunixin treated animals at 4 h intervals from 09:00 h on day 52 for 48 h and then four times a day (at 03:00, 09:00, 15:00 and 21:00 h) until the completion of parturition. Daily samples (12:00 h) were taken from all animals for 3 days after parturition. All blood samples were taken from the cephalic or jugular vein using a syringe. Samples were placed in heparinized tubes and immediately centrifuged at 3000 r.p.m. for 10 min. After centrifugation, the plasma was separated from red cells and stored at −20°C until assayed for progesterone and PGFM.

Water intake (ml kg⁻¹) was measured each day (12:00 h) from day 45 until 3 days after parturition because cloprostenol can cause an increase in water intake (polydipsia) and urine output (polyuria) (Watts and Wright, 1997; J. Watts, unpublished). Polydipsia was defined as a water intake of >100 ml water kg⁻¹ day⁻¹.

Progesterone assay

Plasma progesterone concentrations were determined without extraction using a commercially available coated tube radioimmunoassay (Coat-A-Count No Extraction Progesterone Assay, DPC, Biomediq, Doncaster, Vic), validated for use in dogs (Srikandakumar et al., 1986). The assay sensitivity was 0.06 ± 0.02 ng ml⁻¹ plasma. The interassay coefficients of variation (CV) for four quality control plasma pools containing 1.6, 3.3, 6.5, and 12.8 ng ml⁻¹ were 17.6, 7.5, 7.78 and 4.4%, respectively (n = 6 assays). The intra-assay CV was <20% over the range of 0.26 ± 0.1 to 40.6 ± 5.1 ng ml⁻¹.

PGFM assay

PGFM concentrations were measured using a direct plasma assay similar to that described for tammar wallaby plasma (Lewis et al., 1986), and validated for dogs. The PGFM antiserum is highly specific (Lewis et al., 1986; Renfree et al., 1994). The intra-assay CVs for two quality control plasma pools containing 433.2 and 1636.2 pg ml⁻¹ were 15.5 and 10.9%, respectively (n = 12 assays). The interassay CVs

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Table 1. Effect of treatment on the timing of birth, birth weight, delivery and growth rate (mean ± SEM) in bitches

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Time to birth after cloprostenol treatment on day 52 (h)</th>
<th>Time to birth after flunixin injections on day 54 (h)</th>
<th>Number of pups</th>
<th>Delivery rate (pups h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloprostenol</td>
<td>31.5</td>
<td>-</td>
<td>8</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>55.0</td>
<td>-</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>47.0</td>
<td>-</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>57.0</td>
<td>-</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>47.6 ± 5.8</td>
<td>-</td>
<td>8.5 ± 0.3</td>
<td>0.4³</td>
</tr>
<tr>
<td>Control</td>
<td>190.0</td>
<td>142.0</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>148.0</td>
<td>100.0</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>174.0</td>
<td>126.0</td>
<td>7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>136.0</td>
<td>88.0</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>162.0 ± 12.3</td>
<td>114.0 ± 12.3</td>
<td>6.3 ± 0.8</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Flunixin</td>
<td>-</td>
<td>-</td>
<td>102.0</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>127.0</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>132.0</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>132.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>120.3 ± 9.3a</td>
<td>2.5 ± 0.2</td>
</tr>
</tbody>
</table>

*Abbreviation: *cloprostenol.

For the same quality control plasma pools were 19.2 and 9.9%, respectively. The recovery of three known amounts of PGFM added to male dog plasma was within ±13% of the values expected. The assay sensitivity was 39.0 ± 6.8 pg ml⁻¹ plasma. Standard curves in male dog plasma gave a displacement curve parallel to that obtained with standards in buffer. The immunoactivity of plasma extract had the same mobility as authentic PGFM when separated by TLC on a silica gel plate using a solvent phase of 40% (v/v) ethyl acetate, 15% (v/v) trimethyl pentane, 35% (v/v) distilled H₂O, 10% (v/v) acetic acid. A male greyhound treated with cloprostenol over 2 days showed no change in plasma PGFM, indicating that cloprostenol and its metabolite(s) are not detected by the assay at the doses used (Fig. 1).

Fig. 1. Cloprostenol alone does not increase assayed 13,14-dihydro-15-keto-prostaglandin F₂α (PGFM) in a male greyhound treated with cloprostenol (■) for 2 days.

for the same quality control plasma pools were 19.2 and 9.9%, respectively. The recovery of three known amounts of PGFM added to male dog plasma was within ±13% of the values expected. The assay sensitivity was 39.0 ± 6.8 pg ml⁻¹ plasma. Standard curves in male dog plasma gave a displacement curve parallel to that obtained with standards in buffer. The immunoactivity of plasma extract had the same mobility as authentic PGFM when separated by TLC on a silica gel plate using a solvent phase of 40% (v/v) ethyl acetate, 15% (v/v) trimethyl pentane, 35% (v/v) distilled H₂O, 10% (v/v) acetic acid. A male greyhound treated with cloprostenol over 2 days showed no change in plasma PGFM, indicating that cloprostenol and its metabolite(s) are not detected by the assay at the doses used (Fig. 1).

Statistical analysis

Pretreatment means of progesterone, PGFM, rectal temperatures and water intake were analysed using a two sample t test assuming unequal variances. Mean values within animals before, during and after treatment were tested with repeated measures analysis of variance (ANOVA). Birth weights are affected by litter size and so were also tested with analysis of covariance. The growth rate differences between treatment and control groups were tested using repeated means ANOVA analysis.

The time from the start of treatment to the time of the decrease of progesterone concentrations to half was ‘Time ½’. The time of the decrease of progesterone concentrations to < 2.0 ng ml⁻¹, which is characteristically seen 12–24 h before the onset of labour, was ‘Time < 2.0 ng ml⁻¹’. These parameters estimate the rapidity of luteolysis induced by cloprostenol treatment.

The interval from treatment to peak PGFM values in each bitch was used as a response variable to analyse the effect of treatment on PGFM profiles. The areas under the PGFM curves of flunixin-treated and control animals were determined to assess the effect of treatment on PGF₂α production.

Results

Timing of birth, birth weight, delivery and growth rate

First delivery in cloprostenol treated animals occurred on day 54.4 ± 0.2, significantly earlier than in controls which delivered on day 59.1 ± 0.5 (P ≤ 0.001; Table 1; Fig. 2a). However, three of these four treated animals only delivered 1–2 pups within 8–12 h, and so were given treatment for dystocia. The oxytocin injections and calcium infusion produced immediate results with each bitch completing parturition. All control animals whelped without any complications. There were no differences in the mean birth weights (P ≥ 0.24; Fig. 2a) or growth rates (P ≥ 0.11; Fig. 2b) of young from treated and control animals. The only cloprostenol-treated animal that completed parturition
Cloprostenol-treated dogs. Ultrasonography showed that fetuses were still viable and so they were delivered by Caesarean section. Owing to the trauma associated with delivery and the litter size in this animal (13 pups), pups were killed after surgery. This animal was not used in the statistical analysis because parturition was never initiated.

**Progestrone**

Plasma progesterone concentrations in cloprostenol-treated animals decreased markedly (P < 0.0001) within 24 h of treatment (Table 2; Figs 3 and 4). The plasma progesterone concentrations of controls did not change over the corresponding period (P ≥ 0.80). Progesterone concentrations among cloprostenol-treated animals halved (5.0 ± 1.0 h versus 120.0 ± 10.4 h; P ≤ 0.007) and decreased to < 2.0 ng ml⁻¹ (22.0 ± 14.3 h versus 133.5 ± 10.8 h; P ≤ 0.008) more rapidly than they did in controls.

In contrast, flunixin had no effect on plasma progesterone concentrations within 24 h of treatment (P ≥ 0.47; Table 2; Figs 3 and 4) nor did vehicle injections (P ≥ 0.05) in controls over the corresponding period. However, flunixin subsequently delayed the start of the progesterone decrease (time ½ 106.5 ± 7.9 h versus 72.0 ± 10.4 h; P < 0.04), although there was no difference in the time progesterone concentrations decreased to < 2.0 ng ml⁻¹ between flunixin-treated and control animals (104.0 ± 10.6 h versus 85.5 ± 10.8 h; P ≥ 0.28). The removal of pups by Caesarean section from the fourth flunixin-treated bitch led to a rapid increase in progesterone concentrations back to about half of the pretreatment concentrations (Figs 3i and 4i).

**PGFM**

The mean pretreatment plasma PGFM concentration of cloprostenol-treated animals (462.0 ± 46.9 pg ml⁻¹) was not different from that in controls (397.3 ± 12.5 pg ml⁻¹) before minipump treatment (P ≥ 0.24; Fig. 4). After cloprostenol treatment, an immediate surge in PGFM concentrations occurred reaching peak values significantly earlier than the parturient PGFM peak of controls (17.0 ± 5.3 h versus 148.5 ± 9.9 h, P < 0.0001). No difference in peak PGFM values was observed between cloprostenol-treated and control animals (2000 ± 168.3 pg ml⁻¹ versus 2025 ± 77.7 pg ml⁻¹; P ≥ 0.56). Oxytocin administration to animals treated for dystocia was quickly followed by PGFM surges (Fig. 4a-c).

PGFM concentrations were higher in flunixin-treated animals before treatment than they were in controls (787.3 ± 86.9 pg ml⁻¹ versus 401.75 ± 25.7 pg ml⁻¹; P ≤ 0.03) but decreased significantly after the start of flunixin treatment (Fig. 4; P ≤ 0.005). Although both flunixin-treated and control animals had PGFM peak values at around the same time after treatment (122.3 ± 12.9 h versus 100.5 ± 9.9 h; P ≥ 0.23), the PGFM profiles differed greatly (Fig. 4). In control animals, PGFM concentrations rose gently 72 h before parturition but, in flunixin-treated animals, PGFM concentrations did not start to increase until 24 h before birth (Fig. 5). Peak concentrations of PGFM were higher (P ≤ 0.005) in flunixin-treated animals (2700 ± 35.4 pg ml⁻¹) than they were in controls (2025 ± 77.7 pg ml⁻¹). The removal of pups from one bitch led to a marked decrease in PGFM concentration (Fig. 4i) coincident with an increase in progesterone.

**Rectal temperatures**

There was no difference in mean pretreatment rectal temperature between cloprostenol-treated and control animals (P ≥ 0.13; Table 3; Fig. 3). Rectal temperature profiles from all animals showed a circadian rhythm. However, the temperature changes caused by parturition were more marked than the circadian effect. The time taken to reach minimum rectal temperatures from the onset of treatment was shorter in cloprostenol-treated animals than it was in...
controls ($P < 0.0001$). The interval from minimum rectal temperature to birth was not different in cloprostenol-treated animals and controls ($P \geq 0.18$). Postpartum rectal temperatures were higher than the mean pretreatment rectal temperatures observed in both cloprostenol-treated ($P \leq 0.01$) and control animals ($P < 0.0001$).

There was no difference in mean pretreatment rectal temperatures ($P \geq 0.90$) or in the time minimum rectal temperatures were reached relative to treatment between flunixin-treated and control animals ($P \geq 0.93$; Table 3; Fig. 3). The interval from minimum rectal temperature to birth was not different between flunixin-treated animals and controls ($P \geq 0.69$). Increased postpartum rectal temperatures were observed in controls ($P \leq 0.006$). In contrast, increased postpartum rectal temperatures were not observed in flunixin-treated animals ($P \geq 0.14$). After the pups were removed from one bitch, there was an increase in rectal temperatures that paralleled an increase in progesterone concentration (Fig. 3).

**Water intake**

The water intake of cloprostenol-treated animals was similar to that of controls before minipump insertion ($P \geq 0.29$; Fig. 6), but water intake rose markedly with the start of cloprostenol treatment ($P \leq 0.0001$). In contrast, flunixin did not affect water intake ($P \geq 0.47$; Fig. 6).

**Discussion**

The PGF$_{2\alpha}$ analogue, cloprostenol, in a relatively low dose induced birth near term in pregnant bitches. However, PGF$_{2\alpha}$ is clearly not the only hormone needed for successful parturition since three of four cloprostenol-treated bitches required medical intervention to complete whelping.

Cloprostenol-induced labour was similar in several ways to that of natural parturition. The similarities included a decrease in rectal temperatures, a rapid decrease in progesterone concentration, a surge of plasma PGFM, expulsion of fetuses and lactation. The decrease in rectal temperature at the time of luteolysis is the result of progesterone withdrawal (Concannon and Hansel, 1977). The progesterone concentrations around the time of birth (<2.0 ng ml$^{-1}$) were similar to those reported in earlier studies (Concannon et al., 1977; Concannon, 1978). Cloprostenol caused an increase in plasma PGFM concentration, presumably through stimulation of endogenous release of PGF$_{2\alpha}$.

Indeed, the only difference observed between control and cloprostenol-treated animals was in the timing of the hormonal events and labour, which were all significantly advanced by treatment, and the difficult labour in treated bitches.

Although the cause of the dystocia was unclear, it is similar to the effects seen after administration of oxytocin receptor inhibitors, which prolongs labour in rats and guinea-pigs but does not prevent it (Antonijevic et al., 1995; Schellenberg, 1995). The profile of PGFM in control animals indicates that, in normal birth, prostaglandin concentration is increased only for a short period. PGF$_{2\alpha}$ can downregulate its own receptor (Vaananen et al., 1998). The prolonged continuous infusion of cloprostenol may have downregulated prostaglandin receptors, resulting in the failure of entire litters to be delivered in three cloprostenol animals. This hypothesis is strengthened by the success observed in the one cloprostenol-treated animal that whelped without complications since, in this case, labour was initiated shortly after infusion started and completed within 47 h. The other cloprostenol-treated bitches diagnosed with dystocia did not initiate delivery until after 47 h, indicating that the onset of dystocia may be time-dependent. Starting cloprostenol treatment closer to term, infusing for a brief period or using a lower dose, may avoid dystocia.

Previous use of various PGF$_{2\alpha}$ analogues to induce parturition in the bitch have been accompanied by a multitude of side effects (Concannon and Hansel, 1977; Socolowski and Geng, 1977; Tsutsui et al., 1982, 1989; Lein, 1986; Ichijo et al., 1995; Iseki et al., 1995). Almost all side effects were eliminated by using a low, continuous dose via a minipump. Only polydipsia, previously documented using cloprostenol as an abortifacient (Watts and Wright, 1997; J. Watts unpublished), was observed. The polydipsia was most likely due to renal effects of PGF$_{2\alpha}$ (Zook and Strandhoy, 1981) and reflects the fact that this systematically administered dose of cloprostenol was pharmacological since endogenous prostaglandin had no effect on water intake in controls. Prostaglandins affect kidney function by inhibiting vasopressin-mediated water reabsorption, which results in the formation of isotonic urine (Anderson et al., 1975; Zook and Strandhoy, 1981). There was no decrease in water intake in flunixin-treated animals, although prostaglandin inhibitors, such as indomethacin and meclofenamate, are reported to enhance the effect of vasopressin in the kidney (Anderson et al., 1975).

Treatment with flunixin initially suppressed the synthesis

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**Table 2.** Plasma progesterone concentrations (ng ml$^{-1}$) in bitches treated on (a) day 52 with sodium cloprostenol (mean ± SEM) or (b) day 54 with flunixin meglumine (mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Pretreatment progesterone concentration (ng ml$^{-1}$)</th>
<th>Progesterone concentration (ng ml$^{-1}$) 24 h after treatment$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h before treatment</td>
<td></td>
</tr>
<tr>
<td>(a) Cloprostenol</td>
<td>$12.4 \pm 3.2$</td>
<td>$1.0 \pm 0.4$</td>
</tr>
<tr>
<td>Control</td>
<td>$12.6 \pm 2.3$</td>
<td>$12.0 \pm 1.8$</td>
</tr>
<tr>
<td>(b) Flunixin</td>
<td>$8.0 \pm 0.9$</td>
<td>$6.8 \pm 1.1$</td>
</tr>
<tr>
<td>Control</td>
<td>$10.0 \pm 2.0$</td>
<td>$11.2 \pm 2.5$</td>
</tr>
</tbody>
</table>

$^a$Value represents a single plasma sample 24 h after onset of treatment.
Fig. 3. Effect of treatment on plasma progesterone concentrations (●) and rectal temperatures (△) of (a-d) cloprostenol-treated bitches, (e-h) control bitches, and (i-l) flunixin-treated bitches. The decreases in both plasma progesterone concentrations and rectal temperatures were significantly advanced by cloprostenol treatment (■) compared with control saline infusions (□). In contrast, flunixin injections (♦) had no effect on progesterone concentrations and rectal temperatures compared with control saline injections (♦). Birth (▼); Caesarean section (◇).
Fig. 4. Effect of treatment on plasma progesterone (●) and 13,14-dihydro-15-keto prostaglandin F₂₀ (PGFM) (○) concentrations on (a–d) cloprostenol-treated bitches, (e–h) control bitches, and (i–l) flunixin-treated bitches. A decrease in plasma progesterone concentrations and a surge in PGFM were significantly advanced by cloprostenol treatment (■) relative to saline infusion (□). In contrast, flunixin injections (▼) had no effect on progesterone concentrations compared with control saline injections (▼), but did disrupt the normal synthesis of PGFM. Oxytocin injection to treat dystocia (arrows); birth (▼); Caesarean section (◇).
of PGFM in the 24 h before the start of delivery. Prostaglandin synthetase inhibitors, such as indomethacin, do not completely abolish prostaglandin synthesis (Renfree et al., 1994; Hoffmann et al., 1996). In addition, prostaglandin synthetase inhibitors, such as flunixin and phenylbutazolidine, fail to block PGF<sub>2α</sub> synthesis in horses (Archbald et al., 1983; Daels et al., 1995) and cattle (Odensvik et al., 1991; Odensvik and Gustafsson, 1994). It is possible that the repeated administration of higher doses of flunixin inhibit the secretion of PGF<sub>2α</sub> but adverse effects and toxicity then become an issue. Hoffmann et al. (1996) only partially suppressed the PGFM concentrations of two bitches using indomethacin. Similarly, in the present study, a Caesarean was performed to deliver the litter of one flunixin-treated animal. The apparent extension of gestation in these animals indicate that the suppression of prostaglandin synthesis may interfere with the normal course of gestation, partially inhibiting luteolysis and prolonging the secretion of prostagorne, implying that prostaglandins are associated with events facilitating birth in bitches.

In sheep, pigs and many other species, the placenta and uterine tissues are the major sources of prostaglandin (Challis and Lye, 1994). The source of prostaglandin in whelping bitches has not been investigated. Non-pregnant hysterectomized bitches exhibit normal ovarian function with no permanent change in the secretion of progesterone or oestrogen (Baker et al., 1980; Olson et al., 1984; Okkens et al., 1985; Hoffmann et al., 1996), indicating that the uterus of nonpregnant animals does not influence ovarian function or release a luteolytic factor. However, in the present study, the removal of the placenta from one bitch resulted in an immediate, rapid decrease in plasma PGFM and an increase in progesterone concentrations. This finding indicates that luteolysis at term is due to the luteolytic effects of PGF<sub>2α</sub> produced by the placenta.

The present study confirms that prostaglandins induce luteolysis and the onset of labour in bitches. However, since pharmacological doses of cloprostenol failed to induce a normal labour in most cases, it is clear that the prepartum prostaglandin surge is only one component of a coordinated system initiating birth. A lower dose or shorter treatment may allow the normal progress of delivery by initiating endogenous production of PGF<sub>2α</sub> while avoiding the side effects. The failure of flunixin to delay birth indicates a prepartum mechanism of endogenous PGF production that is only poorly responsive to this prostaglandin-H synthase.

Table 3. Rectal temperatures in bitches treated on (a) day 52 with sodium cloprostenol (mean ± SEM) or (b) on day 54 with flunixin meglumine (mean ± SEM)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Pretreatment temperature (°C)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time of minimum temperature (h)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Time from minimum temperature to birth (h)</th>
<th>Postpartum temperature (°C)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Cloprostenol</td>
<td>38.4 ± 0.1</td>
<td>19.0 ± 5.3</td>
<td>26.6 ± 2.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>39.5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>38.5 ± 0.1</td>
<td>136.5 ± 9.0</td>
<td>17.5 ± 5.1</td>
<td>39.1 ± 0.1</td>
</tr>
<tr>
<td>(b) Flunixin</td>
<td>38.2 ± 0.1</td>
<td>110.3 ± 13.5</td>
<td>22.33 ± 9.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>39.3 ± 0.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>38.1 ± 0.1</td>
<td>112.5 ± 19.8</td>
<td>17.5 ± 5.1</td>
<td>39.1 ± 0.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean rectal temperature 24 h before treatment.
<sup>b</sup>Time minimum temperature was reached from treatment.
<sup>c</sup>Three of four cloprostenol-treated animals failed to complete parturition without human intervention. Data represent one cloprostenol-treated animal.
<sup>d</sup>A caesarean section was performed on bitch 405. Data represent the three flunixin-treated animals that gave birth.
<sup>e</sup>Mean of daily rectal temperature measurements for 3 days after birth.

Fig. 5. Effect of flunixin on PGFM production in bitches. Area under the 13,14-dihydro-15-keto prostaglandin F<sub>2α</sub> (PGFM) curve for control (□) and flunixin-treated (■) bitches over the period 48–24 h before birth and 24–0 h before birth.

Fig. 6. Effect of treatment with cloprostenol (■) and flunixin (▼) on water intake in bitches. Water intake in cloprostenol-treated animals was increased markedly during treatment. In contrast, flunixin had no effect on water intake during treatment compared with controls (▼).
inhibitor. Increasing the dose and administration of flunixin or the use of a more potent alternative inhibitor of prostaglandins may be needed to prevent parturition.

The authors thank F. Davidson and S. Henderson for their assistance with all aspects of animal handling and husbandry during these experiments. A. Duns, C. Borchers and S. Williams provided advice on radioimmunoassays and C. Beck performed ultra-sonographic examinations. The authors are also grateful to Dr B. Patton of Jurox, Australia, for the gift of the sodium cloprostenol.

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