

# Endocrine Responses in Cows Fed Ponderosa Pine Needles and the Effects of Stress, Corpus Luteum Regression, Progesterin, and Ketoprofen<sup>1,2</sup>

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**ABSTRACT:** Pregnant cows were fed pine needles (PN, 2 kg·cow<sup>-1</sup>·d<sup>-1</sup>) mixed with the diet to determine factors that affect abortion response. In Exp. 1, treatments were used to determine the effects of experimental stress and pelleting of pine needles. Pelleting needles and experimental stress delayed abortion response ( $P < .01$ ). Stress-induced delay was associated with abnormal patterns of progesterone and cortisol ( $P < .01$ ). In Exp. 2A and 2B, the role of the corpus luteum (CL) in abortion response to PN consumption was investigated by regressing the CL with prostaglandin F<sub>2α</sub>. Regression of the CL and PN feeding reduced interval to parturition, but the effect of PN feeding was less when the CL was regressed (PN × CL,  $P < .01$ ). The progesterone increase in response to experimental stress was decreased by CL

regression ( $P < .01$ ). In Exp. 3, melengestrol acetate (MGA) was fed (0, 2, or 4 mg·cow<sup>-1</sup>·d<sup>-1</sup>) in addition to PN. Parturition was blocked more effectively as dose of MGA increased ( $P = .075$ ), but only parturition was blocked rather than the effects of PN. In Exp. 4, CL regression was blocked by feeding ketoprofen. Ketoprofen delayed response to PN, but the effect was only temporary ( $P < .01$ ). Our conclusions are that 1) experimental stress delays abortion response to PN by increased concentrations of progesterone, 2) pelleting PN decreases their abortifacient activity, and 3) abortions caused by PN can be blocked by feeding a progesterin or a prostaglandin inhibitor, but these compounds do not block the primary abortifacient effects of PN.

Key Words: Cattle, *Pinus ponderosa*, Pine Needles, Abortion, Stress, Hormones

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## Introduction

Abortions can be caused by cows eating needles from Ponderosa pine trees (MacDonald, 1952; James et al., 1989) and are associated with abnormal profiles

of progesterone (Short et al., 1989). The mechanism is a profound decrease in blood flow to the uterus with the subsequent stress to the calf causing the normal cascade of events associated with parturition (Short et al., 1989; Christenson et al., 1992a,b). Abortion response to pine needles is consistent, but the interval to abortion is inconsistent (James et al., 1989; Christenson et al., 1992a; Short et al., 1992, 1994). Diet variables were not related to the variation in response (Short et al., 1994) except for conflicting data on heat and pelleting effects (Anderson and Lozano, 1977, 1979). Progesterone profiles also were inconsistent (unpublished data); response seemed to be related to degree of stress associated with blood sampling. If samples were taken by venipuncture, progesterone increased, but if samples were taken from jugular cannula in trained cows, progesterone concentrations were normal. We hypothesized that the progesterone response to pine-needle feeding reported earlier was an artifact caused by stress of bleeding. These experiments were conducted to test this hypothesis and to determine whether the response to pine needles could be blocked by a prostaglandin

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<sup>2</sup>We dedicate this paper to the memory of Ann Darling, who was our friend and colleague. Without her technical support, much of this research could not have been accomplished.

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inhibitor or a progestin, whether the corpus luteum (CL) is necessary for the response to pine needles, and whether pelleting pine needles affects their activity.

## Materials and Methods

General experimental protocols were as described by Short et al. (1992). Pine needles were collected from mature Ponderosa pine trees in Custer County, MT. Live trees were cut during the winter, and needles were collected by stripping them from branches at the time the trees were cut or by cutting branches and stripping needles after allowing them to dry. Air-dried needles were ground through a hammermill with a 2.25-cm screen and mixed (2 kg·cow<sup>-1</sup>·d<sup>-1</sup>) with the basal diet (coarsely ground hay; 9 kg·cow<sup>-1</sup>·d<sup>-1</sup>) at the time of feeding. Cows that aborted received a 20-mL injection of an antibiotic (Penstrep®, Durvet, Blue Springs, MO) each day for 3 d after calving to prevent complications from retained placenta (Short et al., 1992). The cattle were pregnant, multiparous Hereford or crossbred cows that weighed from 450 to 675 kg. Breeding dates were known, and feeding pine needles started at approximately 250 d of pregnancy with the 1st d of pine needle feeding designated as d 1. Statistical analyses were done with GLM procedures from SAS (1989). The ANOVA tested treatment effects with an animal error term, and repeated measures and any interactions with repeated measures were tested with an appropriate animal × repeated measure error term. Pooled standard errors were derived from appropriate error terms. Serum from blood samples was assayed for a prostaglandin F<sub>2α</sub> metabolite (13,14-dihydro-15-keto prostaglandin F<sub>2α</sub>, PGFM) with an RIA kit (no. SG-6006, Seragen Inc., Boston, MA) that was based on extracted samples, for progesterone with a solid-phase RIA kit (Diagnostic Products, Los Angeles, CA; Bellows et al., 1991), and for cortisol with an extracted-sample RIA (Short et al., 1989).

**Experiment 1.** The first objective of this experiment was to determine the effects of a daily bleeding stress on response of cows to pine needle feeding and the progesterone and cortisol profiles associated with these effects. The second objective was to determine the effects of pelleting pine needles on abortifacient response. Fifty-two cows were assigned to one of seven treatments (Table 1). There were three blood sampling protocols: 1) bleeding started on the 1st d of pine needle feeding (d 0), 2) bleeding started 17 d before initiation of pine needle feeding (prebled), and 3) no bleeding. Cows that were prebled for 17 d were moved through a chute each morning, a blood sample drawn from the tail to acclimate these cows to being handled, and they were bled on a daily basis. These cows and those that were bled starting on the 1st d of pine needle feeding were fitted with a jugular cannula the

Table 1. Effect of bleeding stress, feeding pine needles (PN), and pelleting of pine needles on interval (d) to parturition in Exp. 1 (number of cows)<sup>a</sup>

Fed PN	Bled		Not bled	Pelleted PN
	Not prebled	Prebled		
No	28.6 (8) <sup>b</sup>	34.7 (6) <sup>b</sup>	29.6 (8) <sup>b</sup>	—
Yes	12.4 (7) <sup>c</sup>	7.9 (7) <sup>d</sup>	5.5 (8) <sup>e</sup>	15.2 (8) <sup>c</sup>

<sup>a</sup>Pooled SE = .90.

<sup>b,c,d,e</sup>Means with different superscripts differ ( $P < .01$ ).

day before pine needle feeding was started. Subsequent blood samples were taken through the jugular cannula. The three groups of cows that were not bled stayed in their respective pens and were not handled except for routine feeding and removal from pens as they calved. Half of the three bleeding stress groups were fed pine needles as explained earlier. An additional group of cows was fed pine needles from the same collection that had been ground a second time and pelleted through a commercial press to produce pellets 1.4 cm in diameter. Each treatment group was housed in a separate pen during the experimental period, and cows were removed as they calved.

Blood samples were taken daily at 0700 on d -16 through d 3 and d 18 through 2 d after calving. Blood samples were taken more frequently from d 4 through d 17 to determine whether there were any effects on pulsatile or daily hormone concentration patterns. Three blood samples were taken at 0700, 1100, and 1500 on d 5, 7, 9, 10, and 12 through 17. On d 4, 6, 8, and 11, blood samples were taken every 15 min for 1 h starting at 0700, 1100, and 1500. If a cow calved before d 18, she was bled once a day at 0700 on the day after calving and for two additional days. Serum from blood samples was assayed for progesterone and cortisol.

**Experiment 2A.** This experiment was conducted to determine whether regressing the CL with prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) before starting pine needle feeding would affect the response to pine needles. Forty-nine cows were assigned to a 2 × 2 factorial experiment (Table 2). The PGF<sub>2α</sub> (Lutalyse®, The Upjohn Company, Kalamazoo, MI) was injected (25 mg/d) on 239 and 240 d of gestation, and pine needle feeding was started on 253 d (d 0) of gestation. Blood samples were taken from the tail on d -9, -6, and -2 and then daily from d 0 until 3 d after calving. Serum from these samples was assayed for progesterone.

**Experiment 2B.** This experiment was similar to Exp. 2A (Table 2). Modifications were that PGF<sub>2α</sub> was injected once at 195 d of gestation, pine needle feeding started on d 250, and only one blood sample was taken 3 d after pine needle feeding started to minimize complications from bleeding stress. Twenty-eight pregnant cows were assigned to this study, but of 14 cows injected with PGF<sub>2α</sub>, six aborted before pine

Table 2. Effects of corpus luteum (CL) regression and pine needles (PN) on interval (d) to parturition and progesterone (P<sub>4</sub>) concentration in Exp. 2A and 2B (number of cows)

Trait and exp.	No PN		PN	
	CL present	CL regressed	CL present	CL regressed
Interval				
2A <sup>a</sup>	37.0(8)	20.4(8)	11.9(9)	15.5(8)
2B <sup>b</sup>	34.3(7)	22.8(4)	11.3(7)	12.3(4)
P <sub>4</sub> , ng/mL				
2A(d -9, -6, -2) <sup>c</sup>	7.1	2.9	8.3	3.4
2A(d 1 to 5) <sup>c</sup>	14.2	5.7	16.1	6.2
2B(d 3) <sup>d</sup>	5.4	1.8	3.7	2.4

<sup>a</sup>CL, PN, and CL × PN, *P* < .01, pooled SE = .94.

<sup>b</sup>CL, PN, and CL × PN, *P* < .01, pooled SE = 2.0.

<sup>c</sup>d -9, -6, -2 = period (P) 1; d 1 to 5 = period 2; CL, P, P × CL, *P* < .01, pooled SE = .35.

<sup>d</sup>CL, *P* < .05, pooled SE = .40.

needle feeding began and were dropped from the study. The PGF<sub>2α</sub> was injected earlier in this experiment to allow more time for progesterone production to be switched from the CL to the placenta. Serum from blood samples was assayed for progesterone.

**Experiment 3.** Progesterone decreases dramatically in the days before a pine needle-induced abortion (Short et al., 1989). This experiment was conducted to determine whether this decrease in progesterone is an integral part of the process. Melengestrol acetate (MGA<sup>®</sup>, The Upjohn Company) was fed to provide a progestin source. Forty-seven cows were assigned to one of six treatments summarized in Table 3. Four treatments were arranged in a 2 × 2 factorial with pine needle feeding and MGA as the two factors. Two additional groups were used to give a dose titration for MGA. The cows were acclimated to experimental stress by moving them through a bleeding chute each day for 2 wk before the start of the experiment. Each cow was touched and handled, the tail raised, but no blood samples were taken. Pine needles and MGA were fed for 14 d starting at 250 ± 3 d of gestation. In this experiment, feeding of the basal diet was changed to include .9 kg·cow<sup>-1</sup>·d<sup>-1</sup> of ground corn, and hay was decreased to 8 kg·cow<sup>-1</sup>·d<sup>-1</sup>. This diet was started at the time of the first PGF<sub>2α</sub> injection. The MGA (1, 2, or 4 mg·cow<sup>-1</sup>·d<sup>-1</sup>) was mixed with the ground corn, which was fed separately before the hay portion of the

diet. Blood samples were taken from the tail once a day (starting with the 1st d of pine needle feeding) each morning before feeding until 2 d after calving. Serum from blood samples was assayed for progesterone.

**Experiment 4.** A release of PGF<sub>2α</sub> from the uterus causes regression of the CL during pine needle-induced and normal parturition (unpublished PGFM data from Short et al., 1992). The objective of this experiment was to determine whether the PGF<sub>2α</sub> signal could be blocked during a pine needle-induced parturition. This was tested by feeding a prostaglandin inhibitor (ketoprofen, Kubota et al., 1979; Powell and Cochrane, 1982; Ueno et al., 1987) during the time of pine needle feeding. Fifteen cows were assigned to one of three treatments (Table 4). One cow went off feed during the experiment and was dropped from the study. The experimental diet was changed as in Exp. 3 to include 1.8 kg·cow<sup>-1</sup>·d<sup>-1</sup> of ground corn, which was fed twice daily (0600 and 1800) to each cow. Cows were acclimated to experimental stress for 2 wk as in Exp. 3. Pine needles were fed for 9 d beginning on d 260 ± 3 d of gestation. Ketoprofen was mixed with the ground corn and fed at the rate of 4 g·cow<sup>-1</sup>·feeding<sup>-1</sup> starting 1 d before initiation of pine needle feeding and continued through the last day of pine needle feeding. Cows in the ketoprofen group were individually fed the corn portion of the diet. Blood samples were taken from a

Table 3. Effect of feeding pine needles (PN) and(or) melengestrol acetate (MGA) on interval (d) to parturition in Exp. 3 (number of cows)<sup>a</sup>

Fed PN	MGA, mg·cow <sup>-1</sup> ·d <sup>-1</sup>			
	0	1	2	4
No	35.0(7)	—	—	31.9(8)
Yes	14.4(8)	15.5(8)	19.0(8)	22.5(8)

<sup>a</sup>Pooled SE = 1.17; PN × MGA, *P* < .05; linear effect of MGA, *P* = .075.

Table 4. Effect of ketoprofen (KP) and pine needles (PN) on interval (d) to parturition in Exp. 4 (number of cows)<sup>a</sup>

Fed PN	No KP	KP
No	24.0(5) <sup>b</sup>	—
Yes	7.2(5) <sup>c</sup>	17.5(4) <sup>d</sup>

<sup>a</sup>Pooled SE = 1.14.

<sup>b,c,d</sup>Means with different superscripts differ (*P* < .01).

jugular cannula once a day before the morning feeding starting on the 1st d of ketoprofen feeding and continued until d 2 after calving. On d 2 and 4, blood samples were taken every 2 h from 0600 to 1800. Serum from blood samples was assayed for progesterone and cortisol.

## Results

*Experiment 1.* Feeding pine needles (PN) decreased the interval to parturition in all groups (PN,  $P < .01$ ), but that decrease was not uniform across treatments (Table 1, treatment  $\times$  PN,  $P < .01$ ). There was a consistent decrease in interval as bleeding stress decreased (PN-not prebled vs PN-prebled vs PN-not bled; linear,  $P < .05$ ). Pelleted needles caused an effect, but this effect was not as great as with unpelleted needles (within nonbled, pelleted PN vs PN vs control,  $P < .01$ ).

Progesterone and cortisol concentration profiles from serum samples collected three times daily or every 15 min showed no effect of treatment within day (all treatment-time interactions,  $P > .25$ ). All subsequent analyses included only the 0700 blood samples.

Progesterone concentrations are shown in Figure 1. Progesterone was erratic during the prebleeding period. Concentrations in all groups increased when cannula sampling was started at the beginning of pine needle feeding and subsequently decreased, but the decrease was more rapid in those cows fed pine needles, especially those that had been prebled (day<sup>2</sup> and day<sup>2</sup>  $\times$  treatment,  $P < .01$ ). These profiles are consistent with the effects on interval to parturition.

Cortisol concentrations are summarized by day of treatment in Figure 2. Cortisol was erratic and decreased during the prebleeding period, indicating that there was stress and cows were acclimating during this period. However, when the method of sampling was changed there was some increase, but this increase was not as great as that which occurred in the groups that were not prebled (treatment  $\times$  day<sup>2</sup>,  $P < .01$ ). To understand these effects better, the data also were summarized by day from calving (Figure 2). The prebled, pine-needle-fed group had greater concentrations, apparently caused by earlier calving, and all groups showed a typical increase preceding parturition (treatment and day<sup>2</sup>,  $P < .05$ ). Progesterone and cortisol concentrations were correlated ( $r = .224$ ,  $P < .01$ ,  $n = 2,281$ ) based on data from all samples.

*Experiments 2A and 2B.* The effects of CL regression and pine needle feeding on interval to parturition were similar in both experiments (Table 2). Both treatments reduced the interval to parturition (CL and PN,  $P < .01$ ), but the effects were not uniform (CL  $\times$  PN,  $P < .01$ ). Injecting PGF<sub>2 $\alpha$</sub>  to regress the CL reduced progesterone concentrations in both experiments. In Exp. 2A, progesterone concentrations in-

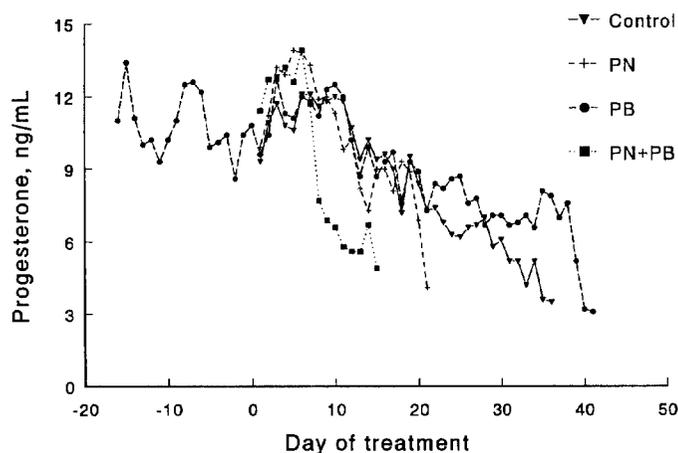


Figure 1. Effects of feeding pine needles (PN) and prebleeding (PB) on progesterone concentrations during treatment, Exp. 1 (SE = .095; day<sup>2</sup>, day<sup>2</sup>  $\times$  treatment,  $P < .01$ ).

creased when sampling frequency increased at the beginning of pine needle feeding (period 1 vs period 2,  $P < .01$ ), and that effect was less when the CL had been regressed (CL  $\times$  period,  $P < .01$ ). Progesterone concentrations in Exp. 2A were plotted against day from calving (Figure 3). Concentrations decreased markedly as parturition approached, and this decrease was greater for control and pine needle-fed cows because they started at greater concentrations (CL  $\times$  PN,  $P < .01$ ).

*Experiment 3.* Feeding MGA delayed the effect of pine needles, the duration of delay being progressively greater as the amount of MGA was increased linearly (LIN; Table 3, PN  $\times$  MGA,  $P < .05$ ; LIN-MGA,  $P = .075$ ). However, the effect was only to delay parturition and not to prevent the effects of pine needles because the pine needle-fed cows calved as soon as MGA feeding was discontinued after 21 d. This result is understandable in view of the progesterone concentration response (Figure 4). All cows showed an initial increase in progesterone concentrations for 5 to 10 d followed by a decrease; that decrease was more pronounced in pine needle-fed cows (day<sup>2</sup>  $\times$  treatment,  $P < .01$ ). The progesterone decrease caused by pine needles was not prevented by feeding MGA; the MGA only prevented parturition if the dose was great enough. The MGA alone also caused a decrease in progesterone, but that decrease was not as great as when MGA was fed with pine needles. In cows fed MGA but not pine needles, parturition did not occur immediately after withdrawal of MGA, as it did when pine needles were fed with the MGA. When the progesterone concentration data were summarized by day from calving, there were also effects of MGA and pine needle feeding (Figure 4, treatment  $\times$  day<sup>2</sup>,  $P < .01$ ). The decrease in progesterone concentration was greatest in treatments with the shortest interval to

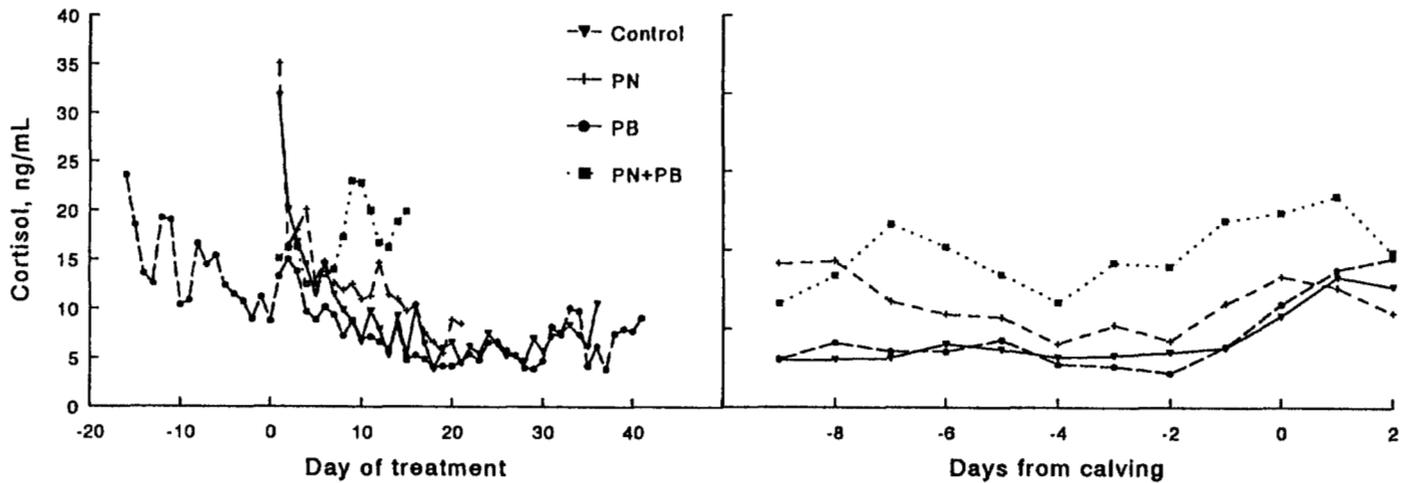


Figure 2. Effects of feeding pine needles (PN) and prebleeding (PB) on cortisol concentrations during treatment (day of treatment, SE = .20; treatment,  $P < .05$ ; treatment  $\times$  day, treatment  $\times$  day<sup>2</sup>,  $P < .01$ ) and around calving (days from calving, SE = .28; treatment,  $P < .01$ ), Exp. 1.

parturition (PN and low-dose MGA); the smallest decrease was in the treatment with a delayed effect of pine needles (high-dose MGA + PN).

*Experiment 4.* Unpublished data from Short et al. (1992) reveal that PGFM concentrations increase with PN-induced parturition. Concentrations of PGFM increased in PN-induced calvings in a manner very similar to that of controls except that concentrations remained greater after calving (PN  $\times$  d,  $P < .01$ , Figure 5). Feeding PN at different stages of pregnancy increased PGFM concentrations, and this increase became greater as stage of pregnancy increased (stage, stage  $\times$  day,  $P < .01$ , Figure 6). Induced

parturition response also increased as stage of pregnancy increased (Short et al., 1992).

Feeding ketoprofen (**KP**, prostaglandin synthesis inhibitor) delayed the effects of pine needles, but just as with MGA in the previous experiment, the effects lasted only as long as ketoprofen was being fed (Table 4, control vs PN and PN+KP, PN vs PN+KP,  $P < .01$ ). Progesterone concentration profiles were affected by treatment (Figure 7, day<sup>2</sup>  $\times$  treatment,  $P < .01$ ), partly because PN-fed cows calved much earlier and had a rapid decrease, but also because PN-fed cows had greater concentrations during the first few days of feeding. An analysis of intensive bleeding data on d 2 and 4 revealed that PN-fed cows had greater concentrations of progesterone (control vs PN and PN+KP,  $P < .05$ ) and that there were no treatment interactions with day (2 vs 4) or time (0, 2, 4, 6, 8, 10, or 12 h). Ketoprofen-fed cows had a delayed decrease in progesterone concentration. Cortisol concentrations were erratic during the treatment period and decreased as the cows became acclimated to the experimental stress (Figure 8, day and day<sup>2</sup>,  $P < .01$ ). The correlation between cortisol and progesterone concentrations was .12 ( $n = 375$ ,  $P < .05$ ).

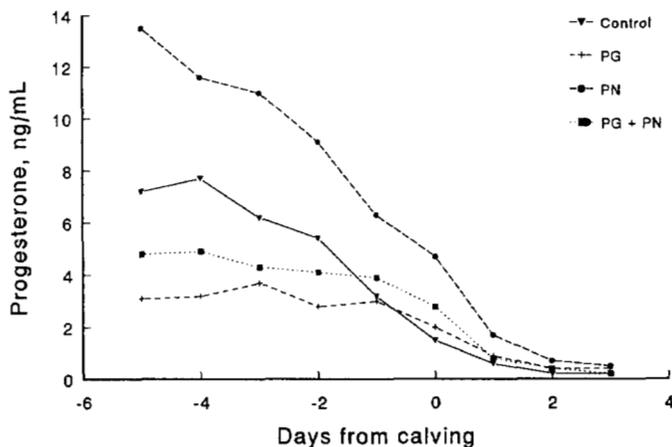


Figure 3. Effects of feeding pine needles (PN) and prostaglandin F<sub>2 $\alpha$</sub>  treatment (PG) on progesterone concentrations around calving, Exp. 2A (SE = .099; day, day  $\times$  PG, day  $\times$  PN, day<sup>2</sup>  $\times$  PG,  $P < .01$ ; day  $\times$  PG  $\times$  PN,  $P < .05$ ).

## Discussion

Feeding pine needles consistently induced parturition in these experiments, as has been reported previously (MacDonald, 1952; James et al., 1989; Short et al., 1992). Parturition was induced regardless of whether the CL was present (Exp. 2A and 2B), but the interval was prolonged by experimental stress (Exp. 1), feeding a progestin (Exp. 3), or feeding a prostaglandin synthesis inhibitor (Exp. 4). The effect

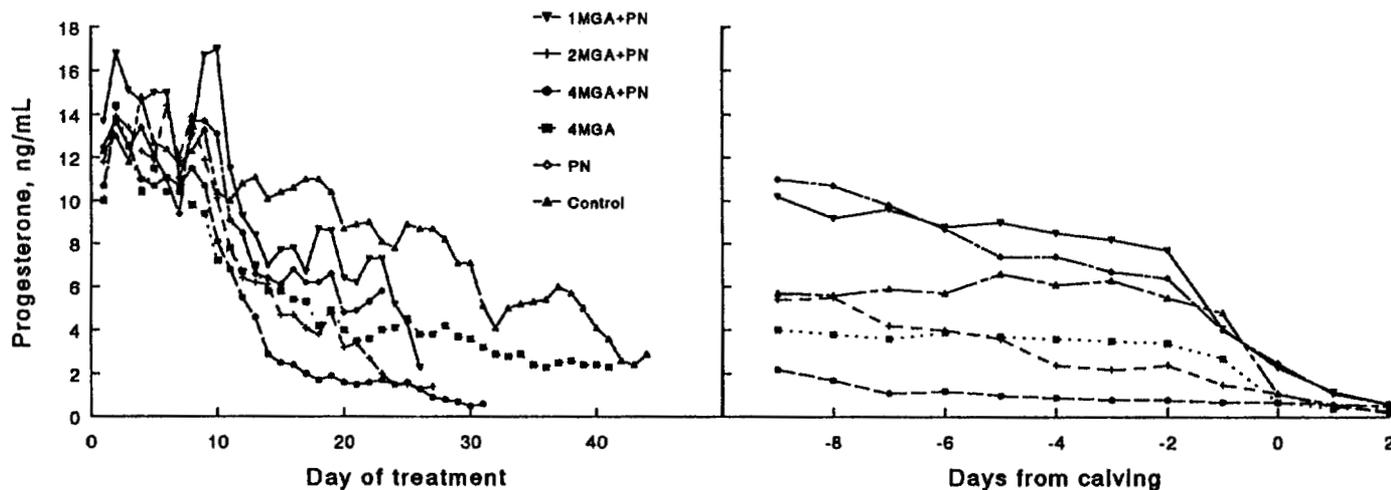


Figure 4. Effects of feeding pine needles (PN) and melengesterol acetate (MGA; 1, 2, or 4 mg·cow<sup>-1</sup>·d<sup>-1</sup>) on progesterone concentrations after treatment (day of treatment, SE = .07; day, day<sup>2</sup>, day × treatment, day<sup>2</sup> × treatment,  $P < .01$ ) and around calving (day of calving, SE = .084; day, day<sup>2</sup>, day × treatment, day<sup>2</sup> × treatment,  $P < .01$ ), Exp. 3.

of pine needles was independent of the CL, but if CL regression was blocked or a progestin replacement used, parturition response was delayed. The primary effect on uterine blood flow apparently was not altered because with either blockage of CL regression (ketoprofen) or progestin replacement (MGA), parturition occurred as soon as treatment stopped. Other data that support this conclusion were obtained from a preliminary experiment (Short et al., unpublished data) in which MGA was fed for longer periods. In that experiment, parturition was delayed beyond the period of pine needle feeding, and the calves died in utero, apparently because of problems caused by the decreased uterine blood flow. Because of that effect,

treatments to delay parturition without maintaining blood flow to the uterus would not be a practical tool for preventing pine needle-induced abortions.

In cattle, the placenta is capable of producing progesterone in late pregnancy but normally does not unless the CL is regressed (Conley and Ford, 1987). Forcing the placenta to be more metabolically active in steroid production by regressing the CL, as in Exp. 2A and 2B, did not provide protection against the effects of pine needles. There was a small increase in progesterone concentration in CL-regressed cows in response to stress, and it is not known what the source and mechanism is. It likely would not involve an adrenergic response in the uterus because the  $\alpha_2$

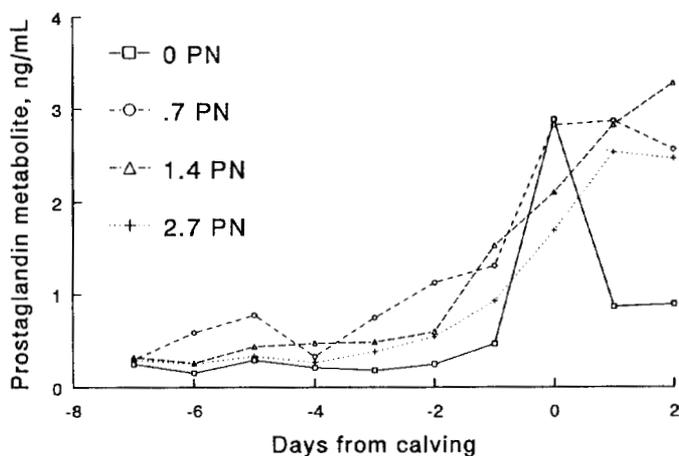


Figure 5. Effect of level of pine needle (PN) feeding (0, .7, 1.4, or 2.7 kg·cow<sup>-1</sup>·d<sup>-1</sup>) on prostaglandin F<sub>2α</sub> metabolite profiles around calving, Exp. 4.

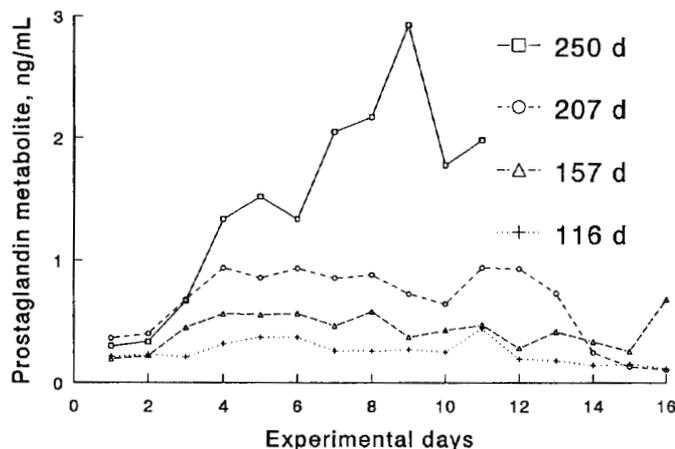


Figure 6. Effect of stage of pregnancy (116, 157, 207, or 250 d) on prostaglandin F<sub>2α</sub> metabolite profiles after the start of pine needle feeding, Exp. 4.

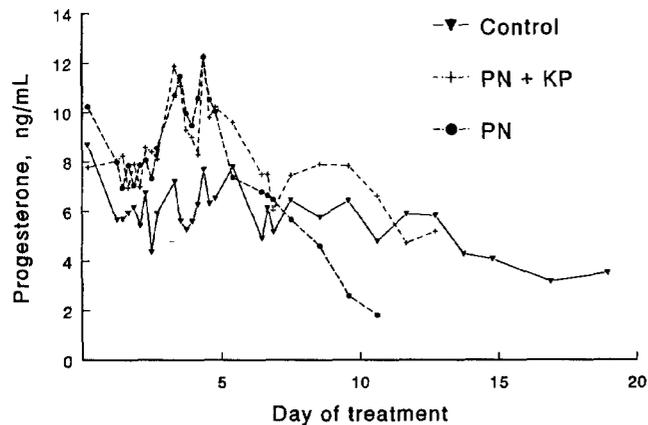


Figure 7. Effects of feeding pine needles (PN) and ketoprofen (KP) on progesterone concentrations during treatment, Exp. 4 (SE = .106; day<sup>2</sup>, day<sup>2</sup> × treatment,  $P < .01$ ).

adrenergic sensitivity, which may mediate changes in uterine arterial diameter, in placentomes disappears in late pregnancy (Sauer et al., 1989).

Experimental stress not only delayed parturition, it also was associated with increased concentrations of progesterone (Exp. 1, 2A, 3, and 4) and cortisol (Exp. 1 and 4). These increases were primarily associated with stress from conducting the experiment or changes in experimental protocols and not with the feeding of pine needles. However, in Exp. 4 there was some indication that pine needle feeding induced an increase in progesterone concentration, which agrees with data reported earlier (Short et al., 1989). It seems likely that the major effects on progesterone concentrations in these experiments are due to indirect effects of stress and not due to pine needle feeding. The effect seen in Exp. 4 and our previous report may be an artifact due to differential stress between control and pine needle-fed cows. Although not specifically observed in these studies, there is considerable variation in how readily cows will consume a diet containing pine needles. Initially, some cows will reduce feed intake considerably and(or) take much longer to consume the daily ration; this could elicit a stress response and would be reflected in progesterone concentrations. Other unpublished data (Staigmilller and Short) support our conclusion that pine needles do not affect progesterone concentrations directly. In this study, cows were cannulated, trained, and then fed pine needles. There was no increase in progesterone detected during the experimental period in either control or pine needle-fed cows.

The mechanism of action and source of progesterone associated with the stress effect is not known. From Exp. 2A and 2B, we can conclude that the CL is a major source of the progesterone because the stress response was less in cows in which the CL had been regressed. Research has shown that biogenic amines

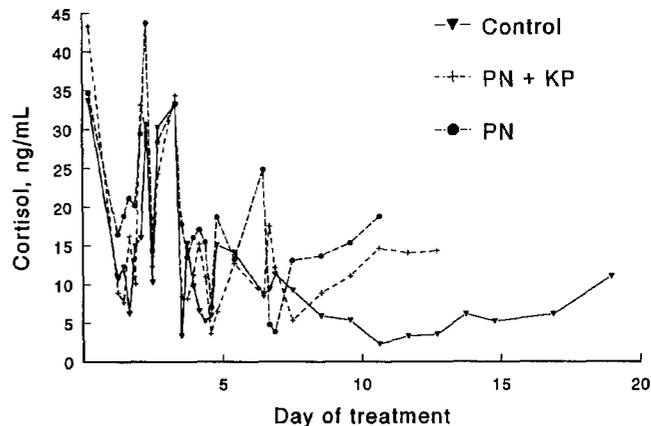


Figure 8. Effects of feeding pine needles (PN) and ketoprofen (KP) on cortisol concentrations during treatment, Exp. 4 (SE = .569; day, day<sup>2</sup>,  $P < .01$ ).

will induce an increase in progesterone secretion by the CL during the estrous cycle (Battista et al., 1987; Skarzynski and Kotwica, 1993; Pesta et al., 1994) and that  $\beta$ -adrenergic receptors exist in the CL. Biogenic amines were not measured in these studies, but from the cortisol data we can conclude that stress conditions did exist. If the CL of pregnancy reacts similarly to the CL during the estrous cycle, it is possible that biogenic amines released during periods of stress caused an increase in progesterone secretion from the CL.

Pelleting was a convenient way to feed pine needles because they were more readily consumed by cows than when ground needles were mixed with hay. However, there was a decreased response with pelleted needles. Thus, pelleting should not be used in experiments with pine needle-induced abortions. Other workers have reported a destruction of the abortifacient effects with pelleting (Anderson and Lozano, 1979). Pelleting may destroy the activity by heat and(or) pressure or by allowing a physical separation and removal of the plant parts containing the active agent(s) during the grinding and pelleting process. Research results on the effects of heat are inconsistent with activity being reported to be either heat-stable or heat-labile (Anderson and Lozano, 1977, 1979). Panter et al. (1990) showed that activity differed with different plant parts, so it may be possible that there is a loss of needle components that are more active during grinding and pelleting.

### Implications

Abortions in cattle caused by cows consuming needles from Ponderosa pine trees can be a serious problem. We have shown that the endocrine response to these needles is similar to that of a normal parturition and that experimental stress can alter

that response. Effects of pine needles are independent of the corpus luteum and are specific for the uterus. Parturition can be delayed with a progestin or a prostaglandin inhibitor, but those treatments did not prevent the primary effect on decreased blood flow to the uterus. These results help to understand pine needle-induced abortions but do not provide practical solutions.

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