

December 2001

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PINE NEEDLE ABORTION – WHAT WE KNOW AS WELL AS
HOW TO PREDICT/PREVENT THE PROBLEM

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INTRODUCTION

Pinus ponderosa or western yellow pine (Pinnacle) is widely distributed in the U.S., but is most abundant in the Western U.S. and Western Canada. In the late 1920's cattle stockmen and ranchers first suspected that ingestion of needles and buds from ponderosa pine trees caused an abortion. Original claims of ponderosa pine needles (PPN) being an abortifacient were discounted because of the many other known causes of abortions such as infectious diseases (brucellosis, leptospirosis), phosphorus and Vitamin A deficiencies. It wasn't until 1952 that McDonald provided the first definitive evidence for a direct effect of a toxin in PPN needles in the induction of abortion in beef cows. In the years that followed, all of the extraneous causes of PPN abortion listed above were eliminated in a series of well-designed experimental studies. Premature parturition in cattle due to ingestion of PPN is now known to be a major economic problem in the Western U.S. and Western Canada, with losses as high as 50% in some herds (Lacey et al., 1988). Further, *Pinus ponderosa* is the only species of *Pinus* in the Western U.S. or Western Canada shown to cause abortion in cattle. Both green and dry needles appear to cause abortion, and bark and branch tips also appear to contain abortifacient principles. In the paper to follow, the factors mediating the susceptibility of pregnant cows to PPN-induced abortion, the physiologic changes precipitating the abortion and our current understanding of the causative factor (s) will be discussed. Also, a summary of our current abilities to predict/prevent PPN induced abortions will be presented.

SUSCEPTABILITY TO PONDEROSA PINE NEEDLE ABORTION

It was suggested by James et al. (1989) that in addition to the possible toxins on *Pinus ponderosa* needles, several other factors may be associated with and/or predispose an animal to abort following PPN consumption. These include: stage of gestation when PPN are ingested, condition of the animal when PPN are consumed (pregnant vs nonpregnant), physiologic differences between different animal species, and finally, the environmental stresses affecting consumption of PPN. These researchers found that daily consumption of PPN by late pregnant cows results in an abortion in from 3 days to 3 weeks, with most occurring 5-15 days. Short et al. (1987) examined if stage of gestation altered the susceptibility of beef cows to the abortifacient effects of PPN. In that study, groups of cows at 116, 167, 215, and 254 days of pregnancy were fed PPN for 3 weeks or until abortion. Three weeks of PPN feeding to the 116 day group had no effect on gestation length, while

38, 50, and 100% of the cows in the 167, 215, and 254 day groups, respectively, aborted. Furthermore, the interval from the initiation of PPN feeding to abortion was shorter in the 254 day group (5.4 days) as compared to the 215 day (8 days) and the 167 day (21 days) groups. No deleterious effects, including reproductive effects are observed when PPN were fed to nonpregnant cows, suggesting a specific effect on the gravid uterus. The toxin in PPN also appears to be species specific, since it's feeding to elk, sheep and goats had no effect on gestation length, while feeding them to cattle, and bison induced early parturition. Further, recent data (Short et al., 2001) suggests that the risk of abortion is increased if late pregnant cows, which are most sensitive to PPN-induced abortion, are exposed to extended periods of cold temperature.

PHYSIOLOGIC EFFECTS ASSOCIATED WITH PPN-INDUCED ABORTION

Although PPN abortion is the common name for this syndrome affecting cattle, it is in some ways misleading, since abortion is normally taken to signify expulsion of a dead or necrotic fetus. In contrast, late pregnant (≥ 250 days) cows consuming PPN give birth to weak calves, most of which can be saved if given immediate attention. A consistent observation at necropsy of cows sacrificed after experiencing PPN-induced abortion was a profound constriction of the caruncular arterial vasculature. A caruncular artery supplies maternal blood rich in oxygen and nutrients to each placentome. The placentome in an individual site of attachment between the placenta and the uterine wall, and is the site of fetal-maternal nutrient, oxygen and waste product exchange. In the cow there are 75-80 of these attachment sites. We speculated that if PPN contained a vasoactive agent which could constrict the caruncular artery, the fetus would be deprived of oxygen and nutrients, possibly causing the fetus itself to initiate an early parturition. Evidence for this concept is supported by visual observations which suggested that PPN fed to late pregnant cows exhibiting an early parturition, exhibited a normal sequence of maternal preparturient hormonal changes, udder edema, swelling of the genitalia, and relaxation of the pelvic ligaments.

To investigate this possibility, we collected individual placentomes from late pregnant (≥ 250 days of gestation) cows at a commercial slaughter facility by dissecting them from for the uterine wall (Christenson et al., 1992a). We then placed the placentome in a specially build chamber, and placed a tube in the caruncular artery so that we could infuse materials extracted from PPN and measure increases in intraluminal pressure as the artery contracted. When we perfused blood plasma collected from PPN-fed late pregnant cows which ultimately calved prematurely, through the caruncular artery, we observed a profound and sustained contraction. Caruncular arterial contraction failed to occur following perfusion of blood plasma from late pregnant control-fed (No PPN) cows. These data suggested the presence of a potent vasoconstrictor in PPN. To confirm a PPN-induced reduction in blood flow to the gravid uterus, we next measured uterine blood flow from day 250 to parturition in PPN and control-fed cows (Christenson et al., 1992b). This was accomplished by placing an electromagnetic flow meter around the main uterine artery, and exteriorizing the device on the flank, placed it in a pouch which was glued to the cow. We could then simply stanchion the cows daily and "plug" the device into a machine which gave us the amount of blood flowing through the uterine artery in milliliters/minute. We observed that the consumption of PPN resulted in a marked and progressive decline ($> 75\%$) in uterine blood flow over an 8

day feeding period (Figure 1), followed by the premature delivery of a weak but viable calf on day 258 of gestation. This premature birth was associated in every case by the presence of a retained placenta. We were able to preserve reproductive fitness of all the cows with antibiotics and to save all calves via administration of colostrum via stomach tube, as they were unable to stand for some period. Control-fed cows exhibited no change in uterine blood flow from day 250 to parturition which occurred on about day 280.

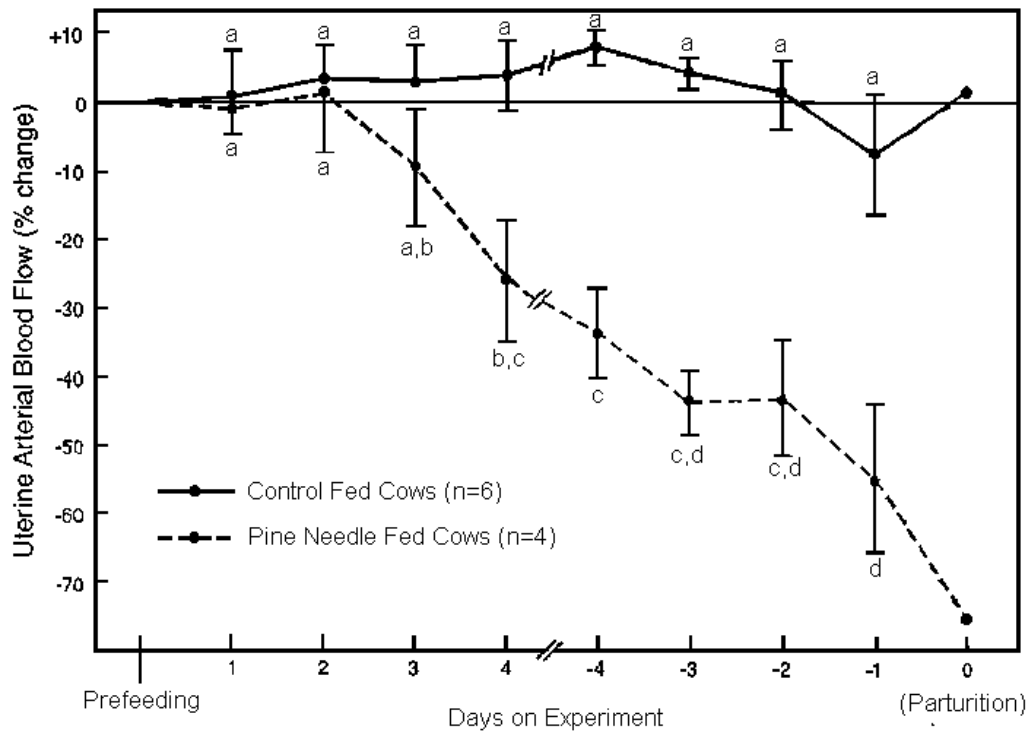


Figure 1. Blood flow to the gravid uterine horn (percentage change from the pre-feeding value) for control-fed cows and *Pinus ponderosa* needle-fed cows that calved early during the 4 days after initiation of the diets (day 250 of gestation) and the 4 days preceding parturition. ^{a,b,c,d}Means within treatments with different superscripts differ ($P < .05$). Each mean SEM represents values from six control-fed cows or four pine needle-fed cows.

CAUSATIVE FACTOR (S) IN PPN- INDUCED EARLY PARTURITION IN LATE PREGNANT BEEF COWS

It is well known that when a late pregnant cow is exposed to a life threatening situation, such as an attack by a hungry predator, one of the first responses to the stress is that blood flow to the gravid uterus is severely reduced, due to the release of epinephrine (adrenaline) from the

adrenal medulla. This is vitally important, as the gravid uterus commands approximately 25% of the female's cardiac output. The extra blood resulting from this decline in uterine blood flow will be shunted to the skeletal muscles so that the cow can attempt to run from the predator. Once the threat is over, however, blood flow to the gravid uterus returns to normal within 5-10 minutes, even though epinephrine levels in the blood stream may remain elevated for up to 30 minutes. This rapid return to normal uterine flows is required to prevent the death of the fetus from lack of oxygen, and was facilitated by placental production of a hormone which dilates uterine blood vessels. After some effort, we managed to isolate and identify this vasodilatory hormone which turned out to be an estrogen metabolite called catechol estrogen. This hormone functioned by limiting calcium uptake by uterine arterial smooth muscle cells in the placentomes, thus relaxing these cells and preventing their prolonged contraction. The conceptus uses catechol estrogens to remove the ability of the cow to severely limit the flow of blood to the placentomes except for brief periods during emergency situations. Catechol estrogens only have local effects on the caruncular vascular bed because they are very rapidly inactivated after they enter the blood stream of the cow.

Next, we utilized the isolated perfused placentome system from late pregnant beef cows to test the fractionated components of PPN for contractile activity. Upon bioassay guided fractionation on PPN (i.e. bovine placentome), a novel class of vasoactive lipids was identified. These lipids, which are contained in the cutin waxes of the needles, contained all the vasoconstrictor activity in PPN which were dominated by the presence of alkanediols with myristic and/or lauric acids. Next, it was decided to evaluate the abortifacient activity of PPN components in the late pregnant guinea pig (Ford et al., 1999). The guinea pig was chosen as a relevant model for the cow because of its relatively long gestation length (~ 70 days), its large precocious offspring, and its fermentation-type (cecal) digestive system. Further, we had previously shown that the late pregnant guinea pig (>50 days of gestation) was susceptible to the abortifacient effects of PPN ingestion. First, we exhaustively extracted PPN with methylene chloride, which we had previously shown would remove all of their vasoactive lipids and abortifacient activity. Next, we added 1-12-dodecanedioyl-dimyristate (14-12-14) back to these methylene chloride extracted PPN. This was the most potent member of the novel vasoactive lipids in constricting the caruncular artery of the isolated perfused bovine placentome. Late pregnant guinea pigs consuming methylene chloride extracted PPN laced with 14-12-14 experienced premature parturition of small piglets exhibiting reduced viability, and had a high incidence of retained placentae.

Further research demonstrated that the vasoactive lipids in PPN inhibited catechol estrogens from preventing calcium uptake by caruncular arterial smooth muscle cells, resulting in a progressive and profound decrease in caruncular arterial diameter (Ford et al., 1992). This decrease in caruncular arterial diameter resulted in progressive hypoxia and nutrient deprivation of the fetal calf, ultimately resulting in a fetal initiated premature parturition.

CURRENT CONCEPTS IN HOW TO PREDICT/PREVENT PPN-INDUCED PREMATURE PARTURITION

To date, there is no clear cut method of predicting or preventing PPN-induced abortion in late pregnant beef cows outside of physically isolating cows from exposure. We have shown in previous studies that high-protein diets may increase the risk of these losses and that losses cannot be decreased by straw, mineral, salt, or bentonite (chemical binder) supplementation. Exposure to any source of pine needles, whether they are fresh, dry, or on fallen trees, should be avoided. There is strong evidence, however, that season of calving may have a significant impact on the risk of PPN-induced abortion. Recently Short et al. (2001) presented data that suggested that low temperatures are one of the primary risk factors increasing PPN consumption. Thus, while nothing can be done at present to alter the sensitivity of late pregnant cows to PPN-induced abortion once they have consumed the needles, exposing them to low environmental temperatures during this period can increase the risk of abortion by increasing consumption. These researchers recommended that for ranges containing *Pinus ponderosa*, calving should be scheduled in summer or fall rather than winter or spring. Further, they suggested that locations with weather patterns similar to Miles City, MT, have the highest risk of PPN-induced abortions when calving times are from January 15 through May 1.

Research is continuing to find an effective way of preventing the vasoactive lipids in PPN from entering the circulation of the pregnant cow. Interestingly, the only species of ruminant that we have found that can consume PPN and exhibit no plasma vasoactivity in the isolated perfused bovine placentome is the elk. It has not been determined if there is a lack of absorption or these vasoactive lipids across the gut, or whether they are metabolized to inactive compounds once they are absorbed (i.e. liver?). We plan to continue our work with the elk to determine how this species is able eliminate both the vasoactivity and abortifacient activity of PPN. It is hoped that because we now understand the physiologic basis of PPN-induced early parturition in the cow, and the compounds involved, we will find a method of preventing this highly significant economic problem for cattle producers.

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