Research Summaries

DAIRY III

Moderators: Dan Grooms and William Raphael

Systemic Effects of Peritoneal Instillation of a Polyethylene Polymer Based Obstetrical Lubricant in Cows

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Introduction

Incidence of dystocia in well managed herds has declined due to better selection of bulls that have proven 'calving ease' scores. However, obstetrical complications remain an important reason for veterinary services on many farms. Instillation of copious volumes of obstetrical lubricant is advocated to protect the delicate tissues of the cow's reproductive tract. Another important consideration is the need to distend the uterus such that additional space becomes available in which to manipulate the fetus. If attempts at manipulation are unsuccessful, then an experienced clinician may be able to resolve the problem by fetotomy. However, if a uterine laceration is present, or if a cesarean section becomes necessary, then the lubricant may enter the peritoneal cavity. This is especially likely during a standing flank cesarean section where complete exteriorization of the incised portion of the uterus does not always occur. A common obstetrical lubricant consists of a polyethylene polymer (PEP) in a dispersing agent base that, when mixed with water to the appropriate consistency, forms a 1-2% (w/v) solution. Effects of this PEP-based lubricant on the peritoneal cavity, and potential for toxicity, are not known. Thus, some surgeons express concern about prior use of large volumes of liquid obstetrical lubricant. However, the principal investigator counters that if copious volumes are not used, then far more cows will require surgery.

This project was designed to simulate accidental contamination of the peritoneal cavity during a cesarean section (approx. 1.0 liter), and to determine what effects, if any, a PEP-based lubricant may have on the bovine peritoneal cavity. The hypothesis was that a mild, transient peritonitis would result; that no fibrin deposition would occur; and that no systemic effects would occur. Peritonitis is a serious disease that, if not treated early and actively, can be fatal. The fibrin deposited on damaged or irritated tissues often results in adhesions which can lead to rumenal atony, intestinal blockage and abdominal pain. The proposal entailed serial abdominocentesis followed by euthanasia at two weeks to investigate the possibility of adhesions. The Ohio State University Institutional Laboratory Animal Care and Use Committee (ILACUC) required that a preliminary study be conducted on rodents.

Materials and Methods

RAT Experiment 1

Abdominocentesis and intraperitoneal injections were performed on anaesthetized rats (250-300gm). A 1-inch, 22-gauge IV catheter was inserted into the lower right quadrant of the abdomen, and 5 ml of warm normal saline was injected. The catheter was capped and left in place while the rat was gently rocked for one minute to ensure uniform distribution of saline throughout the abdominal cavity. A baseline peritoneal fluid sample was then collected by gravity flow into an EDTA vial. The PEP-based lubricant solution (2% w/v) was prepared to approximate the concentration that would be mixed in a nine liter bucket for use in a bovine dystocia. Assuming that one liter of lubricant may contaminate the peritoneal cavity of a cow (1,000 ml/500 kg) a 2 ml/kg volume of 2.0% (w/v) PEP-based lubricant was infused into the rat peritoneal cavity (n=6). Sterile water was used as a control (n=4). Rats were to be euthanized in a CO2 chamber at 72 hours, and a necropsy performed to evaluate gross and histologic evidence of peritonitis. Blood samples for CBC and chemistry were to be collected by direct cardiac stick immediately following euthanasia.

RAT Experiment 2

The commercial lubricant contains 25% (w/v) PEP and 75% (w/v) dispersing agent. The manufacturer responded to the preliminary findings by providing pure samples of the PEP and dispersing agent (sucrose). So-

lutions were prepared based on the component ratios (1:3), and the manufacturer's labeled mixing instructions (1.25% PEP-based lubricant solution). Rats were infused with 2ml/kg of either 0.31% (w/v) PEP solution, or a 0.94% (w/v) sucrose solution.

RAT Experiment 3

Additional rats were divided into a 0.94% sucrose group (n=6); a 0.31% pure PEP group (n=6); and a 0.15% pure PEP group (n=6). One rat from each PEP group was euthanized at 1, 2, 3 and 4 hours post-injection, and the remaining two rats at 5 hours post-infusion. The sucrose rats were euthanized at 24 hours (n=2) and 72 hours (n=4).

COW Experiment

Based on the apparent - and unexpected - PEP toxicity that was evident in the rats, the Ohio State University ILACUC review approved a limited study in four cows. The omental sling must be pulled forward to permit surgical access to the gravid uterus during a cesarean section. Thus, any spilled lubricant would gain access to the entire peritoneal cavity, and not be contained within the omental sling. Merely introducing a large catheter into the peritoneal cavity risked depositing the solution inside the omental sling. Thus, the left paralumbar fossa was surgically prepared and the peritoneal cavity was visualized through a laparoscope. One liter of sterile water containing either 20 gm of PEP-based lubricant (2%), 10 gm PEP-based lubricant (1%), 5 gm PEP-based lubricant (0.5%), 2.5 gm PEP (0.25%), or 7.5 gm SUC (0.75%) was then infused between the body wall and omentum. Cows had been instrumented for an electrocardiogram, and a facial artery catheter was inserted for measurement of arterial blood pressure. Cows were to be serially monitored by physical examination, complete blood count, abdominocentesis, serum chemistry, hemostasis screen and urinalysis.

Results

RAT Experiment 1

Within 12 hours (overnight) following injection of the 2.0% (w/v) PEP-based lubricant, treatment rats were found dead, with blood-stained litter in their cages. Necropsy revealed dark, congested kidneys. The ureters and bladder were distended with dark red urine. The control rats were bright and alert, and at necropsy showed no evidence of peritonitis or pathology of the urinary tract. The peritoneal infusions were repeated using a 1.25% (w/v) PEP-based lubricant as per the manufacturer's mixing instructions. Two hours post-infusion, the treatment rats began urinating 'blood' and displayed progressive weakness that initially affected the hindlimbs. Within 12 hours of infusion all treatment rats (n=6) were dead, with blood-stained litter in their cages. Necropsy findings were identical to that observed in the 2.0% rats. Histologic examination of both treatment groups revealed identical lesions: marked accumulation of eosinophilic granular material in the bladder, kidneys and spleen.

RAT Experiment 2

The PEP rats (n=2) displayed hindlimb weakness within two hours. Dark red urine was noted within three hours, and rats were euthanized at five hours post-infusion. Necropsy findings were identical to those previously observed in the lubricant treatment rats. The sucrose rats remained bright and alert.

RAT Experiment 3

The sucrose rats had remained bright and alert, and no histologic or gross abnormalities were detected. No gross or histologic abnormalities were seen in the PEP rats at one hour. At two and three hours post-infusion, both PEP concentrations (3.1 mg/ml; 1.5 mg/ml) had caused a mild accumulation of eosinophilic granular material in the bladder, and the urine was slightly blood-tinged. At four and five hours post-infusion, the kidneys were dark and congested, and the ureters and bladder contained dark red urine. Both PEP concentrations had caused a marked deposition of eosinophilic granular material in the bladder, kidneys and red pulp of the spleen. Acute tubular degeneration and necrosis was apparent in kidney sections. Rats infused with PEP showed a significant elevation in several blood chemistry values (CK, ALT, AST, BUN, Creatinine and K+). The elevated CK may be indicative of muscle breakdown and could explain the progressive weakness, however a high hemolytic index can affect the analyzer's ability to accurately interpret this value. Elevated liver enzymes, BUN and creatinine are indicative of early hepatic and renal failure. It was suspected that the acute deaths may have been associated with hyperkalemia induced cardiac arrhythmia. The PCV of all PEP-infused rats was normal but total nucleated cell count was elevated.

COW Experiment

One cow served as its own control, initially receiving 7.5 g SUC intraperitoneally, and then 5 g PEP-based lubricant seven days later. Arterial blood pressure and ECG tracings remained normal for 24 hours. All physical parameters, serum chemistry, CBC and hemostasis screen remained normal in this cow over a four day period following SUC infusion.

Cows that received 5 gm, 10 gm or 20 gm of PEP-based lubricant, or 2.5gm of PEP intraperitoneally either died, or developed clinical signs severe enough to warrant euthanasia within three to six and one-half hours post-infusion. Cows became agitated, circled the

stall, head pressed, salivated and became dyspneic with open-mouth breathing. Cows became tachycardic and had injected mucous membranes. Systolic, diastolic and mean arterial blood pressure was elevated in all cows following peritoneal infusion of the PEP-based lubricant or the pure PEP solution. These remained elevated until death or euthanasia. Three cows were restrained for euthanasia, and two collapsed during restraint. One proceeded to have a convulsive seizure. The cows' serum fibrinogen was decreased, and one-stage prothrombin time was increased. Activated partial tissue thromboplastin time was elevated in the cow that died. Intraperitoneal infusion of PEP-based lubricant or pure PEP solution caused alteration in serum chemistry values. Cows became azotemic (creatinine -4.9 mg/dl), with an increased anion gap (25 mEq/l). The serum had a markedly elevated hemolytic index. Three cows had elevated creatinine kinase, but a high hemolytic index can affect the analyzer's ability to accurately interpret this value. No significant changes were noted in the complete blood counts. Urinalysis was positive for blood and protein.

Necropsy confirmed that the peritoneal infusate was into the peritoneal cavity in all four cows, and that no other structures were damaged. Histopathologic examination revealed no significant lesions, perhaps because the animals were euthanized before lesions could develop in the kidneys. The cause of the agitation, neural signs and respiratory distress cannot be explained by serum chemistry, complete blood counts, or necropsy findings. Both the 1H-NMR and 13C-NMR spectrums for the PEP-based lubricant powder, PEP powder and SUC powder demonstrated that the samples were very clean with no apparent impurities.

Significance

This PEP-based lubricant has proven to be safe and effective for intrauterine obstetrical application throughout many years of use in our veterinary hospital. However, results of this study demonstrate that peritoneal contamination with an amount as small as 1.25 gm PEP is toxic in cows. This equates to contamination of the peritoneal cavity with 1.0 liter of a 0.5% (w/v) solution of the commercial PEP-based lubricant. Veterinarians are advised to use caution if a cesarean section becomes necessary after this PEP-based lubricant has been infused into the uterus of a cow. In such cases it is especially important to prevent any spillage of lubricant into the peritoneal cavity. Human safety issues (powder aspiration) during preparation of the liquid PEP-based lubricant are currently being investigated in our laboratory.

Evaluation of a Rapid Test for NEFA in Bovine Serum

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Introduction

Excessive or prolonged periparturient negative energy balance (NEB) is an important issue for dairy producers, and may be associated with increased risk of clinical disease and impaired production and reproductive performance. Affected cows commonly have elevated circulating levels of non-esterified fatty acids (NEFA) prior to calving and increased beta-hydroxybutyrate (BHB) postpartum. Monitoring the incidence of subclinical ketosis postpartum has been the recommended method of surveillance for this problem. Prepartum, blood NEFA concentration may be used to detect cows at risk for problems with severe NEB. Serum NEFA greater than 0.4 mEq/L NEFA has been proposed to identify excessive prepartum NEB. Measuring NEFA has traditionally involved submission of serum to a diagnostic laboratory. The DVM NEFA test (Veterinary Diagnostics. Newburg, Wisconsin, USA) is a new, rapid, spectophotometry method to determine NEFA concentration in serum through light absorbance. The objective of this study was to determine the test characteristics of the DVM NEFA test and its usefulness as a method of identifying problems with NEB in prepartum dairy cows.

Materials and Methods

Primiparous and multiparous animals were enrolled between seven and four days prior to their ex-