

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

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Although uncommon, equine dystocia is considered to be an emergency situation with potentially fatal consequences for both the fetus and the mare. Limb and/or head and neck malpostures are the major cause of obstetrical difficulties. The foal's long extremities can make obstetrical complications especially difficult to correct. The mare's reproductive tract is extremely sensitive to manipulations, and development of scar tissue can have an adverse effect on future fertility. Instillation of copious volumes of obstetrical lubricant is advocated to not only protect these delicate tissues, but also to distend the uterus and thus provide additional space within which to manipulate the fetus. If attempts at mutation are not successful then an experienced clinician may be able to resolve the problem with a 1-2 cut fetotomy. However, if a uterine laceration is present, or if a cesarean section becomes necessary, then the lubricant may enter the peritoneal cavity. A common obstetrical lubricant consists of a polyethylene polymer (PEP)(25% w/w) in a dispersing agent base that, when mixed with water to the appropriate consistency, forms a 1-2% (w/v) solution. The effects of this lubricant on the peritoneal cavity, and potential for toxicity are not known. Thus, some surgeons express concern about the prior use of large volumes of liquid obstetrical lubricant. However, the principal investigator counters that if the volume is limited then far more mares will require surgery.

Equine surgeons continue to use assorted therapeutic agents for which anti-adhesive claims have been made. However, to date no substance has gained general acceptance (1). One such substance is carboxymethylcellulose sodium. A 1% solution of carboxymethylcellulose sodium has been used in human and equine abdominal surgery but its anti-adhesive efficacy has produced equivocal results (2, 3, 4). Pure carboxymethylcellulose sodium powder can be purchased for surgical use. The commercially prepared carboxymethylcellulose based veterinary lubricants are primarily intended to facilitate palpation per rectum. Some also contain propylene glycol. These 'palpation lubes' generally contain methyl and propyl parahydroxybenzoate as preservatives. Some veterinarians add other antiseptic solutions. Thus, these commercially prepared carboxymethylcellulose sodium formulations should not be assumed to be inert if they were to contaminate the peritoneal cavity.

This project was designed to simulate the accidental contamination of the peritoneal cavity during surgical manipulation (approx. 1.0 liter) during a cesarean section. The objective was to determine what effects, if any, a PEP-based lubricant may have on the equine 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. Equine peritonitis is a serious disease that, if not treated early and actively, can be fatal.

The fibrin that is deposited on damaged or irritated tissues often results in adhesions which can lead to abdominal pain and intestinal blockage. The proposal entailed serial abdominocentesis of horses; to be followed by euthanasia at 2 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.

RAT Experiment 1. Abdominocentesis and intra-peritoneal injections were performed on anaesthetized rats (250-300gm). A 1 inch, 22 guage IV catheter was inserted into the lower right quadrant of the abdomen and 5ml of warm normal saline was injected. The catheter was capped and left in place while the rat was gently rocked for 1 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 9 liter bucket for use in an equine dystocia. Assuming that 1 liter of lubricant may contaminate the peritoneal cavity of a mare (1,000ml/500kg) a 2ml/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.

Within 12hr (overnight) following injection of the 2.0% (w/v) PEP-based lubricant the 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 12hrs 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 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). Solutions 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. The PEP rats (n=2) displayed hindlimb weakness within 2 hr. Dark red urine was noted within 3 hr, and rats were euthanized at 5hrs post-infusion. At necropsy the findings were identical to those previously observed in the lubricant treatment rats. The sucrose rats remained bright and alert.

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, 4hr post-injection, and the remaining 2 rats at 5hr post-infusion. The sucrose rats were euthanized at 24hr (n=2) and 72hr (n=4). 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 1hr. At 2 and 3 hr post-infusion both PEP concentrations (3.1mg/ml; 1.5mg/ml) had caused a mild accumulation of eosinophilic granular material in the bladder, and the urine was slightly blood-tinged. At 4 and 5 hr 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. Elevated liver enzymes, BUN and creatinine are indicative of early hepatic and renal failure. However, 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 the total nucleated cell count was elevated.

HORSE

Based on the apparent - and unexpected - PEP toxicity that was evident in the rats, the Ohio State University ILACUC review panel approved a limited study in 4 horses. The left paralumbar fossa was surgically prepared, and a 22 fr. thoracic drain was inserted into the peritoneal cavity. One liter of sterile water containing either 20gm of PEP-based lubricant (2%), 10 gm PEP-based lubricant (1%), 2.5 gm PEP (0.25%), or 7.5 gm SUC (0.75%) was infused into the peritoneal cavity in each horse. Horses were instrumented for an electrocardiogram, and with a facial artery catheter for measurement of arterial blood pressure. Horses were serially monitored by physical examination, complete blood count, abdominocentesis, serum chemistry, hemostasis screen, and urinalysis.

One horse served as its own control - initially receiving 7.5g SUC intraperitoneally, and then 2.5g PEP seven days later. The horse developed a suppurative peritonitis at 24 hrs post-SUC infusion, but the peritoneal fluid values had returned to baseline by 96 hours. Arterial blood pressure and ECG tracings remained normal for 24 hours. All physical parameters, serum chemistry, CBC, and hemostasis screen remained normal in this horse over the seven day period following SUC infusion.

Horses that received 10 gm or 20 gm of PEP-based lubricant, or 2.5gm of PEP intraperitoneally either died (n=2) or developed clinical signs severe enough to warrant euthanasia (n=2) between 3 minutes and 15 hours post-infusion. Horses became agitated, pawed, circled the stall, head pressed, star-gazed, and demonstrated evidence of abdominal discomfort. These signs were only transiently responsive to medication with flunixin meglumine or xylazine. Horses were euthanized if signs of pain recurred after two doses of analgesics. Horses became tachycardic and had injected mucous membranes. Systolic, diastolic and mean arterial blood pressure was elevated in all horses following

peritoneal infusion of the PEP-based lubricant or the pure PEP solution. These remained elevated until death or euthanasia. Serum fibrinogen, one stage prothrombin time, and activated partial tissue thromboplastin times were unaffected by intraperitoneal infusion of PEP-based lubricant or pure PEP solution. The serum remained negative for the presence of fibrinogen degradation products at 1:5 and 1:25 in all horses. Intraperitoneal infusion of PEP-based lubricant or pure PEP solution caused alteration in serum chemistry values. Horses became azotemic (creatinine -15.2mg/dl) and hyperosmolar (293 mOsm/kg), with an increased anion gap (39 mEq/l). Total protein, albumin, and globulins were decreased. No significant changes were noted in the complete blood counts. The serum had a markedly elevated hemolytic index. Urine was green to black in color, and urinalysis was positive for blood and protein.

Histopathologic examination revealed no significant lesions in the heart, lung, liver, spleen, intestinal tract, central nervous system (cortex, mesencephalon, myelencephalon, and cerebrum) or skeletal muscle. The renal tubules were diffusely distended with large amounts of eosinophilic granular material and lesser amounts of amorphous eosinophilic material. Widespread minimal acute tubular epithelial cell degeneration was present. Lymphatics in the diaphragm of the horse that died acutely (3 min) were distended by amorphous eosinophilic material, and all viscera were diffusely congested.

The presence of pink serum, black urine, and eosinophilic material in the renal tubules are indicative of hemolysis. Specific tests were not performed to distinguish hemoglobinuria from myoglobinuria. The presence of a normal creatinine kinase and the absence of skeletal muscle lesions on histopathology are suggestive of hemoglobinuria. There is no satisfactory explanation for the one peracute death (2% PEP-based lubricant w/v) observed. Necropsy confirmed that the peritoneal infusate was into the peritoneal cavity in all four horses, and that no other structures were damaged. The cause of the colic, agitation, and neural signs can not be explained by serum chemistry or 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.

This PEP-based lubricant has proven to be safe and effective for intra-uterine obstetrical application throughout many years of use in our veterinary hospital. However, the results of this study demonstrate that peritoneal contamination with an amount as small as 2.5 gm pure PEP powder is toxic in horses. This equates to contamination of the peritoneal cavity with 1.0 liter of a 1% (w/v) solution of the commercial PEP-based lubricant. The presence of a uterine laceration can be confirmed by abdominocentesis (5, 6). If this PEP-based lubricant has been infused into the uterus and then a caesarean section becomes necessary, extreme caution should be exercised to prevent any spillage into the peritoneal cavity. Human safety issues (powder aspiration) during preparation of the liquid lubricant are currently being investigated in our laboratory.

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