Preliminary study to evaluate the feasibility of chemical ablation of the seminal vesicles in the bull*  
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Abstract

Objective: To determine the feasibility of injecting four percent formaldehyde solution percutaneously into the seminal vesicles of bulls and the effectiveness of the formaldehyde solution in ablating the glands as a treatment for bulls affected with septic seminal vesiculitis.

Design: Randomized clinical trial

Animals: Eight two-year-old Angus bulls were randomly and equally divided into control and treatment groups. All bulls were satisfactory potential breeders based on evaluation of breeding soundness.

Procedure: One or both seminal vesicles of the treated bulls were injected percutaneously with a four percent formaldehyde solution to chemically ablate the gland. The seminal vesicles were evaluated ultrasonographically before and after injection; on day 30 the ejaculate of each bull was evaluated to determine the effect of treatment on semen quality. The seminal vesicles of treated and control bulls were removed when the bulls were slaughtered 60 days after treatment and examined histologically to determine the effect of treatment.

Results: Seventy-five percent (three of four) of the treated bulls and all four of the control bulls were classified as satisfactory potential breeders on day 30 of the study. One treated bull was classified as an unsatisfactory breeder because of abnormal sperm morphology, and another treated bull developed a pelvic abscess. At necropsy, six of seven infused seminal vesicles were enlarged to twice the size of the seminal vesicles of the control bulls and had fibrotic capsules. Vesicular parenchyma of six of the seven infused glands was necrotic. The parenchyma of the seventh infused gland contained an abscess. The ampulla, colliculus seminalis, and pelvic urethra were grossly and microscopically normal.

Conclusion: Four percent formaldehyde solution injected into the seminal vesicles may be effective in ablating the glands of bulls affected with septic seminal vesiculitis.

Keywords: Seminal vesiculitis, seminal vesicles, secondary sex glands, formaldehyde, sepsis, bovine

Introduction

The paired seminal vesicles of mature bulls are 2-4 cm wide, 10-15 cm long, and are located on the pelvic floor lateral to the ampullae and dorsal to the neck of the bladder. The size of the vesicles is age related. The glands are lobulated and secrete a clear fluid containing nutrients and buffers, which is discharged immediately before and during ejaculation through ducts that open into the urethra adjacent to the colliculus seminalis.  

The prevalence of infection of one or both of the seminal vesicles of bulls, based on clinical and abattoir evaluation of reproductive tracts, is reported to range from less than one percent to greater than nine percent and may be as high as 20 to 30 percent in small groups of closely confined yearling bulls. Septic seminal vesiculitis is predominately evident in peripubertal bulls and aged bulls. Peripubertal bulls confined in groups and fed high energy diets are more predisposed to developing the condition than are bulls reared in a range environment. Peripubertal bulls may spontaneously recover from septic seminal vesiculitis, but aged or chronically infected bulls rarely recover.  

Bulls affected with septic seminal vesiculitis are often classified as unsatisfactory potential breeders following breeding soundness examination. The semen of affected bulls may be grossly contaminated with exudate and blood, but often red blood cells and white blood cells are not seen grossly but can be detected microscopically. Abnormal concentrations of polymorphonuclear cells (PMNs),

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poor sperm motility, low fructose concentrations, and elevated seminal pH are characteristics of semen of bulls affected with vesiculitis. Semen of bulls with septic seminal vesiculitis freezes poorly and antibiotics used in extenders often do not significantly diminish the large number of bacteria in the ejaculate. Although chronic, unresponsive, septic seminal vesiculitis does not occur commonly in breeding bulls, the economic impact of this disease is considerable. The greatest economic loss associated with septic seminal vesiculitis occurs in bulls whose genetic value qualified them for inclusion in an artificial insemination program.4,5

Traditionally, the prognosis of affected bulls for resolution of chronic septic seminal vesiculitis using antimicrobial therapy or surgical removal is guarded at best. Prolonged treatment using parenterally administered antimicrobial drugs such as oxytetracycline, penicillin, erythromycin, or gentamicin is usually ineffective.5 A recent study by Rovay et al. showed that intraglandular injection of ceftiofur or penicillin via the ischiorectal fossa appeared to be safe and effective in the treatment of seminal vesiculitis.6 This study also showed that a single tulathromycin treatment had a higher success rate (88%) than two tilmicosin treatments 72 hours apart (48%) when used to treat seminal vesiculitis in bulls.6 Surgical removal of the infected seminal vesicle is indicated when antimicrobial therapy fails. The vesicular gland is difficult to remove completely, however no matter which reported surgical approach (ie., the ischiorectal fossa, sub-rectal, or ventral pararectal approach) is used because of the gland’s close proximity to the urethra and the depth of the gland within the pelvic cavity.7,10 In one study the vesicular tissue remaining after vesiculectomy ranged from 2-3 cm long for the right seminal vesicle and from 3-7cm long for the left seminal vesicle. Infected remnants of the duct and gland remain a source of continued bacterial contamination of semen.7

Because bulls chronically affected with septic seminal vesiculitis respond poorly to traditional treatments, we investigated a method of chemically ablating the entire gland. A four percent formaldehyde solution infused into the parotid duct has been used successfully to involute the parotid salivary gland of horses.11 The goal of this study was to determine the feasibility of injecting the seminal vesicles of healthy bulls, percutaneously, with a four percent solution of formaldehyde and to determine the efficacy of this treatment in destroying the seminal vesicles as a potential treatment of bulls affected with chronic septic seminal vesiculitis.

Materials and methods

Bulls and procedure

Eight two-year old bulls determined to be satisfactory potential breeders, based on breeding soundness evaluations, were equally and randomly divided into treatment and control groups. Breeding soundness was evaluated according to the standards of the Society for Theriogenology and consisted of physical examination, determination of scrotal circumference, and evaluation of sperm motility and morphology. The eight bulls were considered healthy, based on physical examination, complete blood count, and a serum chemistry profile. Both seminal vesicles of three bulls and one seminal vesicle of one bull were injected percutaneously with a four percent aqueous solution of formaldehyde (40% aqueous solution of formaldehyde gas diluted with nine parts of water; 10% Formalin, Fischer Scientific Co., Suwanee, GA) to ablate the gland. The other four bulls served as controls. All bulls were housed at the Auburn University Bull Test Station, and Auburn University’s Institutional Animal Care and Use Committee approved the study.

With the bull restrained in a chute, routine caudal epidural anesthesia was administered using two percent lidocaine hydrochloride to minimize straining during the injection procedure; feces were manually evacuated from the rectum, and the perineal area was prepared for aseptic insertion of a hypodermic needle. A 16 gauge, 30 cm hypodermic needle was inserted through the skin percutaneously, 2-3 cm dorsal to the right or left tuber ischii and angled toward the ipsilateral seminal vesicle. Using a hand in the rectum to guide the needle and stabilize the gland, the shaft of the needle was directed into the body of the seminal vesicle. Placement of the needle within the seminal vesicle was confirmed by palpation per rectum and by distention of the gland after infusion of 15-40 mL of physiological saline.
solution. The seminal vesicle was then injected with 15-50 mL of a four percent solution of formaldehyde until the gland was completely distended. The procedure was then repeated for the contralateral vesicle (Figure 1).

The seminal vesicles were evaluated ultrasonographically prior to and immediately after injection of the gland. The rectal temperature of each bull was determined once daily for seven days following injection. On day 30 each bull received a second breeding soundness examination and its seminal vesicles were ultrasonographically examined to determine the effect of the formaldehyde solution on the seminal vesicles and the ejaculate. On day 60 the seminal vesicles and surrounding soft tissue structures of each bull were examined postmortem.

Histological evaluation

After fixing the seminal vesicles, ampulla, and colliculus seminalis in formalin, three samples of each tissue of interest were sectioned, processed routinely, and stained with hematoxylin and eosin. Histological sections were evaluated microscopically for alveolar density, the degree of inflammation, and parenchymal necrosis.

The reduction in alveolar density of infused vesicles was evaluated by comparing the number of alveoli present per 400X field in infused and control vesicles. The increase in diameter of the alveolar lumen was also evaluated to determine severity of alveolar loss and dilatation. Less than 25 percent reduction in alveolar density was considered mild, 25 to 50 percent reduction was considered moderate, and severe reduction in alveolar density was characterized as greater than 50 percent loss of alveolar density. The severity of loss or alveolar density was scored numerically (1 = normal, 2 = mild reduction, 3 = moderate reduction, 4 = severe reduction).

No inflammatory cells present per 400X field were considered to be characteristic of normal vesicular alveolar tissue. Accumulation of one to five inflammatory cells (predominately lymphocytes and plasma cells) per 400X field was considered to be mild infiltration of the inter-alveolar connective tissue. Accumulation of five to ten inflammatory cells per 400X field was considered to be moderate infiltration, and accumulation of greater than ten inflammatory cells per 400X field was considered to be severe infiltration. The severity of infiltration of inflammatory cells was scored numerically (1 = normal, 2 = mild, 3 = moderate, 4 = severe).

Sparsely scattered, hyperchromatic, necrotic debris within the alveolar lumen was considered to be evidence of mild necrosis, and focal, dense accumulation of necrotic debris was considered to be evidence of moderate necrosis. Dense, homogenous accumulation of hyperchromatic debris was characterized as severe necrosis. The severity of the degree of accumulation of debris was scored numerically (1 = normal, 2 = mild, 3 = moderate, 4 = severe).

Statistical analysis

Data evaluated in this study included loss of alveolar density, the degree of infiltration of inflammatory cells, and the degree of accumulation of hyperchromatic debris. The severity of each variable was categorized as normal, mild, moderate, and severe, and ranked numerically. Analysis of variance was used to simultaneously contrast the effects of individual bulls, seminal vesicles (left vs. right), and grouping (control vs. infused) on each variable. Mean scores for each histologic variable were also compared between treated and control bulls using a Fischer’s PLSD. The differences were considered significant at P < 0.05. Eight bulls were the minimum number required to provide statistically significant information.

Results

One seminal vesicle of a treated bull was not successfully injected because the bull became unmanageable during the procedure. All injected bulls exhibited signs of abdominal pain (i.e., lying down and flank watching) within the first hour after infusion of the seminal vesicles. Signs of abdominal pain resolved and did not recur after one intravenously administered dose (1.1 mg/kg) of flunixin meglumine. No abnormalities were observed during daily physical examination. During
ultrasonographic examination of the seminal vesicles on day 30, the parenchyma of six of the seven infused seminal vesicles appeared to be dilated, compared to the parenchyma of the control bulls and the parenchyma of those glands before treatment. In the seventh infused seminal vesicle, a multiloculated, hypoechoic area containing hyperechoic foci replaced normal parenchyma.

Seventy-five percent (three of four) of the injected bulls appeared to be satisfactory potential breeders on day 30 of the study. One bull was classified as an unsatisfactory potential breeder because it had fewer than 70 percent morphologically normal sperm in the ejaculate.

At necropsy the ampulla, colliculus seminalis, and pelvic urethra of all bulls appeared grossly and histologically normal. The capsule of six of the seven infused seminal vesicles was thickened two to three times the thickness of the capsules of the seminal vesicles of the control bulls. On the cut surface of the same six infused glands, the parenchyma was replaced by multifocal, coalescing, black to dark-brown areas that were separated by thin, fibrous bands of connective tissue (Figure 2). The density of alveoli was moderately to severely decreased (Figure 3). Remaining alveoli were moderately to severely dilated and separated by intra-alveolar septa containing mild to moderate accumulations of lymphocytes and plasma cells, fewer macrophages, and rare neutrophils (Figure 4). The alveolar lumens contained abundant eosinophilic proteinic material and moderate accumulations of hyperchromatic debris indicating necrosis (Figure 5). Alveoli were lined by flattened to regenerative cuboidal epithelial cells. On the cut surface of the seventh infused seminal vesicle (the one that contained an abscess), the parenchyma was replaced by a multiloculated cavity filled with yellow to brown fluid. The capsule of this gland was not grossly different from the capsules of the seminal vesicles of the control bulls. The histologic appearance of this seminal vesicle was consistent with that of an abscess.

When histologically evaluating the alveolar density, the degree of inflammation, and parenchymal necrosis of each seminal vesicle, no significant differences were found within each group (control vs. infused) or between each gland (left vs. right). A significant difference was noted, however, between the control and infused groups (Table 1).

Discussion

The gross and histologic findings of this study showed that four percent formaldehyde solution damaged the seminal vesicle sufficiently to cause necrosis of the parenchyma but not complete fibrosis. There was evidence of epithelial regeneration based on histologic examination. There was a statistically significant difference in loss of alveolar density, degree of infiltration of inflammatory cells, and degree of accumulation of hyperchromatic debris between treated and control bulls based on histologic examination of seminal vesicles.

We expected infusion of four percent formaldehyde solution to cause complete fibrosis of the parenchyma of the seminal vesicles. Incomplete fibrosis could have resulted from dilution of the four percent formaldehyde solution with the physiologic saline solution that was infused into the glands to ascertain correct placement of the needle prior to infusion of the formaldehyde solution. Transrectal ultrasonography may aide needle placement within the vesicular gland, allowing for a more uniform filling of each gland and avoiding potential dilution of the four percent formaldehyde in future studies. The difference in the volumes of the formaldehyde solution injected into each seminal vesicle may have varied because of the individual size of each gland, or because varying volumes of physiological saline solution were required to distend the glands sufficiently to confirm needle placement. Accurate placement of the needle into these normal seminal vesicles was difficult, but the glands of bulls with septic seminal vesiculitis are usually enlarged, and placing a needle into an enlarged gland may be easier than placing a needle into a normal seminal vesicle. The amount of inflammatory cell accumulation that occurred after four percent formaldehyde infusion may have been attenuated by the administration of flunixin meglumine even though each bull only received one dose. Flunixin meglumine is a cyclooxygenase inhibitor resulting in the suppression of prostaglandin and leukotriene production, both of which activate leukocytes and enhance adhesion molecule expression. If these pathways were inhibited or reduced, the inflammatory response that occurred secondary to the formaldehyde injection would most likely be reduced significantly. Another significant factor that may have played a role in the amount of
fibrosis noted in the treated seminal vesicles could be the timeline of the study. The seminal vesicles were collected 60 days after injection of formaldehyde. If the bulls were euthanized at a later date to allow for complete resolution of the inflammatory and fibroplastic processes, the degree of ablation or fibrosis may actually be more significant than noted in the current study. Another option to expedite the fibroplastic process within each treated seminal vesicle would be to increase the number of injections of four percent formaldehyde. This would most likely need to be accomplished under ultrasonographic guidance since the vesicles would probably not distend with saline infusion due to the amount of capsular thickening noted grossly at necropsy.

The infusion of the seminal vesicles with a four percent formaldehyde solution was not without complications. All injected bulls exhibited signs of abdominal pain within the first hour after infusion of the seminal vesicles. The authors would suggest treating bulls with flunixin meglumine prior to undergoing this procedure in the future based on how well each bull responded in the current study to one dose of this medication. Also, one of the bulls was classified at 30 days after infusion as an unsatisfactory potential breeder because of poor sperm morphology, which might have been caused by non-septic inflammation of the seminal vesicles resulting from injection of formaldehyde solution. Abnormalities in sperm morphology, however, most commonly originate in the testis or epididymis, and the cause of the poor sperm morphology in this bull may not have been the formaldehyde treatment. It is also possible that more of the bulls from the treated group might have had to be classified as unsatisfactory potential breeders if breeding soundness examinations had been performed later or if the duration of the study had been extended. Based on the active inflammatory process noted grossly and histologically at the completion of the study, the impact on these bulls as potential breeders might have been more significant had the fibroplastic process within the treated vesicles come to completion. Finally, a multiloculated abscess replaced the parenchyma of one of the infused seminal vesicles of a second bull. Although the contents of the gland were not cultured to identify a microorganism, the seminal vesicle was possibly inoculated with pathogenic bacteria during the injection or from a contaminated genitourinary tract.

A solution of one to four percent formaldehyde infused into the bladder has been used successfully to treat people suffering from acute hemorrhagic cystitis secondary to cyclophosphamide therapy, radiation therapy, or an infiltrating bladder tumor. In one study, 14 of 16 patients with hemorrhagic cystitis were treated successfully by infusing formaldehyde solution into the bladder, and in another study, 31 of 35 similarly affected people were treated successfully. Recently, Lojanapiwat et al. showed that formaldehyde-soaked pledgets were more effective in controlling hemorrhage secondary to radiation cystitis than intravesicle instillation of formaldehyde and with fewer complications. Hemorrhagic radiation-induced proctitis has also been successfully treated with four percent formaldehyde-soaked gauze applied topically to the affected rectal mucosa.

Complications of formaldehyde therapy for the treatment of people affected by hemorrhagic cystitis included ureterovesical obstruction accompanied by hydronephrosis or anuria, or both, papillary necrosis, urethral fibrosis, and fatal intraperitoneal extravasation. Complications associated with formaldehyde therapy for hemorrhagic proctitis included non-occlusive stenosis of the rectum, rectosigmoidal necrosis and rectovaginal fistula. In one study, one patient out of 20 treated with formaldehyde for hemorrhagic proctitis developed rectosigmoidal necrosis resulting in the patient requiring an intestinal resection. In the same study, two patients developed a rectovaginal fistula. Based upon the previously mentioned studies, complications with formaldehyde therapy in humans for hemorrhagic cystitis and proctitis are uncommon, but are significant if they are encountered.

Four percent formaldehyde solution has been used successfully to involute the equine parotid salivary gland. Horses whose salivary gland was infused with formaldehyde solution did not have significant anorexia and moved more freely, and reacted less to palpation of the gland compared to horses whose salivary gland was infused with silver nitrate or two percent chlorhexidine. In that study a solution of formaldehyde eliminated salivary secretions in as few as eight days. Histologic study of the parotid glands performed 30 days after infusion showed that formaldehyde infused glands scored the lowest for necrosis and supplicative inflammation and highest for glandular atrophy.
Regarding the regulatory status of using four percent formaldehyde to treat seminal vesiculitis in food producing animals, the authors contacted the United States Food and Drug Administration’s Center for Veterinary Medicine. Under the Animal Medicinal Drug Use Clarification Act of 1994 and their guidelines, this drug meets the requirements for extra-label use. When the Food Animal Residue Avoidance and Depletion Program was contacted regarding withdrawal times for the use of formaldehyde in cattle, they were unable to determine any published recommendations. So before this procedure could be recommended in the clinical setting, the authors recognize the need to establish accurate withdrawal times for formaldehyde.

**Conclusion**

Based on the results of our study we believe that the infusion of four percent formaldehyde solution into a seminal vesicle, performed percutaneously using a hypodermic needle, is feasible and resulted in a significant inflammatory response and necrosis of the gland parenchyma. The effects of four percent formaldehyde infusion on a bull’s overall breeding performance needs further evaluation based on the findings in the current study. This study provides a basis for further investigation of the efficacy of formaldehyde solution in ablating an infected seminal vesicle of bulls with naturally-occurring or experimentally-induced septic seminal vesiculitis.

**Acknowledgement**

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**References**

Table 1. The mean score (± standard deviation) of each histologic category evaluated in this study to determine the effects of formaldehyde injection in the bull seminal vesicle. There was a statistically significant difference between control and treated groups (P < 0.05). No significant differences were found within each group (control vs. infused) or between each gland (left vs. right). (Score for histologic categories: 1 = normal, 2 = mild, 3 = moderate, 4 = severe)

<table>
<thead>
<tr>
<th>Seminal Vesicle</th>
<th>Alveolar Density</th>
<th>Degree of Inflammation</th>
<th>Parenchymal Necrosis</th>
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</thead>
<tbody>
<tr>
<td>Left, control</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
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<tr>
<td>Left, treated</td>
<td>2.67 ± 1.07</td>
<td>2.42 ± 1.25</td>
<td>2.58 ± 1.09</td>
</tr>
<tr>
<td>Right, control</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>Right, treated</td>
<td>2.92 ± 0.52</td>
<td>2.33 ± 0.67</td>
<td>2.50 ± 0.52</td>
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Figure 1: This diagram shows how the seminal vesicles were injected via the pararectal fossa using a 16 gauge 30 cm needle. These are lateral and dorsal projections of the procedure and associated anatomy. Sketch was provided by Dr. Phil D. Garrett, Retired Associate Professor, College of Veterinary Medicine, Auburn University.
Figure 2: This figure shows gross changes in the seminal vesicle associated with infusion of formaldehyde at postmortem 60 days after infusion.

Figure 3: This figure shows histologic evidence of loss of alveolar density in formaldehyde-infused seminal vesicles. H & E stain (400X field)
Figure 4: This figure shows histologic evidence of white blood cell infiltration in formaldehyde-infused seminal vesicles. H & E stain (400X field)

Figure 5: Histologic evidence of hyperchromatic debris consistent with necrosis in formaldehyde-infused seminal vesicles is shown in this figure. H & E stain (400X field)