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# COMPARISON OF CHEMICAL AND SURGICAL VASECTOMY ON TESTICULAR ACTIVITY IN FREE-ROAMING HORSES (EQUUS CABALLUS)

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Abstract: Free-roaming horse (Equus caballus) management is a complex issue incorporating social, economic, emotional, political, and environmental factors. Currently, few proven field techniques exist for managing freeroaming horse population growth, which can reach 20-25% annually. Although there are several strategies available for sterilizing mares when managing free-roaming horse populations, surgical vasectomy is the only method used in the field for stallions. Some managers believe that surgically vasectomizing dominant stallions would have significant effects on reducing horse populations. However, sterilizing only dominant harem stallions results in a relatively modest reduction in population growth as substantial reproduction may occur even when 100% of the dominant harem stallions are sterilized if other males perform as little as 10% of the breeding. The overall goal of the current project was to evaluate the efficacy of a novel nonsurgical method for sterilizing freeroaming horses (chemical vasectomy). In September of 2013, stallions that had been previously surgically vasectomized (SURG, n = 25), previously chemically vasectomized (CHEM, n = 16), or untreated (CONT, n = 32) were captured and surgically castrated in preparation for adoption. When comparing both sterilization methods to CONT, serum testosterone and estrone sulfate concentrations did not differ (P > 0.05), suggesting that these methods for sterilizing free-roaming stallions would not disrupt herd social hierarchy. However, similar to the CONT, all CHEM stallions had sperm present within the vas deferens seminal fluid samples. CHEM stallions had more morphologically abnormal sperm than did CONT stallions but it is not known if this affected the actual fertility. Additional research is needed using alternative sclerosing agents for chemical vasectomy in free-roaming horse populations.

Key Words: Equus caballus, equid, spermatogenesis, stallions, sterilization, testosterone.

## INTRODUCTION

Free-roaming horse (*Equus caballus*) management is a complex issue incorporating social, economic, emotional, political, and environmental factors. Under the provisions of the Wild Free-Roaming Horses and Burros Act of 1971,<sup>4</sup> which applies only to lands administered by the Bureau of Land Management and the United States

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Forest Service, the government is required "... to protect and manage wild and free-roaming horses ..." and "... to achieve and maintain a thriving natural ecological balance on the public lands." However, high population growth rates of these horses have made this mandate a significant management challenge. Free-roaming horses are not normally vulnerable to native predators, and as such, their populations can increase by 20–25% annually. Overpopulation of free-roaming horses causes significant adverse impacts to the ecological integrity of the environment. These impacts have reduced the health and function of native habitats necessary to support native wild-life (e.g. pronghorn, Greater sage grouse). 13

Reducing the free-roaming horse population will likely result in substantial recovery and restoration of the ecological health and natural conditions. The Sheldon National Wildlife Refuge has identified reducing the free-roaming horse populations as the one of the most important management strategies necessary for achieving the wildlife refuge purposes and goals.<sup>36</sup> As there are no private cattle grazed on this public land, range deterioration can be solely attributed to

horses. The primary means used to reduce the number of horses has been live removal.37 Some (typically younger) horses have been adopted, but many have been removed to long-term holding on contract ranches.37 These removals are stressful for the horses and expensive for the government. The Government Accountability Office noted in 2008 that off-the-range holding costs would continue to absorb the majority of the Wild Horse and Burro Program budget, having increased from \$7 million in 2000 to \$21 million in 2007, with the expectation that costs would continue to increase rapidly.13 More recently it has been reported that captive wild horses will cost the United State over \$1 billion by 2030 unless management approaches change.11

Fertility control has long been recognized as a potentially useful approach in managing free-roaming horse populations because it could reduce or even eliminate the need to remove horses. 21.22 Research on fertility control in mares has been conducted for more than three decades, and several techniques have been successfully employed including hormone implants, 29 intrauterine devices, 17 ovariectomy, 3 and immunocontraception (e.g. porcine zona pellucida vaccine). 18,19 However, there has been significantly less research on sterilization methods for stallions. 9

Currently, the only method used for sterilizing free-roaming stallions is surgical vasectomy. An advantage of vasectomy over surgical castration is the retention of circulating male sex hormones (testosterone) and consequently maintenance of natural stallion-like behaviors. In addition, the idea of using surgical castration for managing free-roaming horse populations is highly controversial with members of the public, who are concerned about changes in free-roaming horse social organization and behavior (2011 United States District Court case: American Wild Horse Preservation Campaign vs. Department of the Interior). According to the 2013 National Research Council's report, some believe that vasectomizing any one dominant stallion would have a greater effect on reproduction than sterilizing any one fertile female.21 Indeed, Asa (1999) reported that most surgically vasectomized stallions maintained their social hierarchical status, with harem turnover rates not different than those reported for intact stallions.2 However, sterilizing only dominant harem stallions results in a relatively modest reduction in population growth as substantial reproduction may occur even when 100% of the dominant harem stallions are sterilized if other males perform as little as 10% of the breeding.<sup>12</sup> In addition, although reduction in foaling occurs when dominant stallions are sterilized, instability of bands or breeding by subordinate males may limit the effectiveness of only sterilizing dominant stallions.<sup>31</sup>

Chemical vasectomy was developed as a method for nonsurgical sterilization in the dog25 and cat<sup>26</sup> in an effort to reduce overpopulation in these species. Although injection of sclerosing agents directly into the vas has also been reported,28 this method presented several difficulties because consistently penetrating the lumen of the vas was not easily achieved. Since the epididymal tubule is still coiled at the tail of the epididymis where it merges with the vas deferens, the authors hypothesized that sclerosing agents injected percutaneously into the tail of the epididymis would result in chemical vasectomy. In dogs, 0.6 ml of 1.5% chlorhexidine gluconate in 50% dimethylsulfoxide (DMSO) injected into the tail of the epididymis resulted in azoospermia by 35 days after treatment.25 In cats, 0.05 ml of 4.5% chlorhexidine digluconate injected into the tail of the epididymis resulted in either intact, nonmotile sperm or sperm with detached heads by 35 days after treatment.26

The overall goal of the current project was to determine if chemical vasectomy would be an effective method for sterilizing free-roaming stallions. The first hypothesis was that there was no difference in reproductive hormone concentrations between surgically (SURG) and chemically (CHEM) vasectomized stallions when compared to untreated controls (CONT). The second hypothesis was that both surgical and chemical vasectomy would induce sterilization through posttesticular occlusion.

# MATERIALS AND METHODS

### Study location

This study took place at the Sheldon National Wildlife Refuge (NWR, refuge) managed by the United States Fish and Wildlife Service (USFWS). Sheldon NWR is located in northwest Nevada along the southern Oregon border to the north and near the California border to the west (41°48′N, 119°14′W). Sheldon NWR covers approximately 232,964 ha of sagebrush-steppe habitat with elevations that range between 1,200–2,100 m. Average precipitation ranges between 18 and 33 cm. Plant communities are dominated by sagebrush (*Artemisia* spp.). Several tree species such as aspen

(Populus spp.), western juniper (Juniperus occidentalis), and curl-leaf mountain mahogany (Cerocarpus ledifolius) are found in scattered stands throughout Sheldon NWR.<sup>35</sup>

#### **Animals**

Stallions used in this study were part of a feral horse herd managed in accordance with the USFWS Comprehensive Conservation Plan for Sheldon NWR.<sup>34</sup> Horses were captured primarily by using a helicopter to herd them into a trap corral.

During the capture operations in 2008 and 2009, stallions were surgically vasectomized and then released back onto the refuge. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health, Pittsburgh, Pennsylvania 15205-9741, USA; 0.5 mg/lb), ketamine (Zoetis, Florham Park, New Jersey 07932, USA; 1 mg/lb), and succinyl choline (Hospira, Lake Forest, Illinois 60045, USA; 0.04 mg/lb) and placed on a hydraulic surgery table with the hind legs secured. The site was surgically prepped and the tail of the epididymis was identified by palpation. An incision was made over the tail of the epididymis exposing the vas deferens and the vas deferens was isolated distal to the epididymis. Two hemoclips were applied 3 cm apart and the vas deferens between the clips was excised. The procedure was repeated on the contralateral side. The skin incisions were left open for drainage and an intramuscular antibiotic (Excede®, Zoetis; 1.5 ml/ 100 lb) was administered.

During the capture operations in 2010 and 2011, stallions that were not previously surgically vasectomized were chemically vasectomized and then released back onto the refuge. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health; 0.4 mg/lb) and succinyl choline (Hospira; 0.4 mg/lb). The hind legs were secured and the site was surgically prepped. The tail of the epididymis was identified by palpation and injected with 10 ml of 1% chlorhexidine in 90% DMSO (v/v) using an 18-gauge needle and 20-ml syringe. The procedure was repeated for the contralateral side. Chlorhexidine with DMSO was selected as a sclerosing agent on the basis of previous sterilization successes with intraepididymal injections in dogs25 and cats.26 The volume of sclerosing agent was empirically derived from volumes successfully used in dogs and cats. Given that the tail of the epididymis is much larger in mature horses than in dogs and cats, larger volumes were administered. In September of 2013, a subset of the stallions that had previously been surgically (SURG, n = 25) or chemically

vasectomized (CHEM, n = 16) were again captured. In addition, stallions that had been previously untreated (CONT, n = 32) were also captured. All of these stallions were to be removed from the refuge, and as such were prepared for placement with adoption agents, which included a routine surgical castration. The surgical castration also permitted assessment of the efficacy of the previous treatments. Briefly, stallions were chemically restrained with xylazine (Bayer Animal Health; 0.4 mg/lb) and succinyl choline (Hospira; 0.04 mg/lb). The hind legs were secured and the site was surgically prepped. Lidocaine was locally infiltrated into the scrotal midline (20 ml) and into each spermatic cord (10 ml). A routine castration was then performed.

# Hormone analysis

Prior to castration, a jugular blood sample was collected for equine infectious anemia testing as part of the process for removing horses from the refuge. An aliquot of this sample was used to determine total serum testosterone concentrations. In a randomly selected subset of these samples (SURG, n = 9; CHEM, n = 14; CONT, n = 17), estrone sulfate concentrations were also analyzed. Total serum testosterone was measured using the enzyme immunoassay Immulite 1000 (#LKTW1, Siemens Medical Solutions Diagnostics, Los Angeles, California 90045-6900, USA) according to the manufacturer's instructions. Similar to results published by Illera et al.14 for stallions, our laboratory found close agreement between radioimmunoassay (RIA) and enzyme immunoassay (EIA) when measuring total serum testosterone concentrations (EIA =  $4.6331 + 0.6633 * RIA, R^2 =$ 0.8306, F(1, 38) = 186.3, n = 40, P < 0.001). Estrone sulfate was analyzed at the Cornell University Animal Health Diagnostic Center using a semiquantitative direct enzyme immunoassay with antiserum and conjugate purchased from University of California, Davis. The estrone sulfate assay has been previously validated for equine diagnostic applications.5,32

#### Seminal fluid evaluation

A sample of fluid from the vas deferens distal to the tail of the epididymis was collected immediately following castration for microscopic evaluation. For stallions that had been previously surgically vasectomized, the seminal fluid sample was collected from a portion of the remaining vas deferens distal to a hemoclip. A small drop of seminal fluid was placed on a microscope slide with a coverslip and evaluated using bright field light microscopy at ×200 magnification by a single observer (CMS). The presence or absence of sperm was recorded from almost all of the stallions (SURG, n = 19; CHEM, n = 16; CONT, n = 23). Following direct microscopic examination, a seminal fluid sample was prepared for sperm morphology evaluation. If no sperm were present in the initial direct examination, a sample of fluid for sperm morphology evaluation was collected from the tail of the epididymis (SURG, n = 10). The sample was then prepared as a smear, air dried, and stained with Hemacolor® (Harleco, EMD Chemical Inc., Gibbstown, New Jersey 08027, USA) using a 10-min incubation for each stain. Then, the sperm morphology was determined by a single observer (CMS) blinded to the treatments. Briefly, a minimum of 200 sperm were examined using bright field light microscopy at ×1000 magnification under oil immersion to determine the percentage of normal sperm. In addition, each type of abnormal sperm morphology was recorded as well as the number of times the abnormality occurred. Images of representative samples were digitally captured.

# Histology

Left and right testicular tissue sections were collected from a subset of the stallions castrated (SURG, n = 19; CHEM, n = 16; CONT, n = 10). Tissues were fixed in 10% formalin and paraffin embedded. Sections were then cut at 4 µm and stained with hematoxylin and eosin. Briefly, randomly selected fields were examined using bright field light microscopy at ×200 magnification. Electronic images of representative samples were digitally captured. A single observer (RL) blinded to the treatments performed histomorphometry to evaluate effects of possible pressure distension following treatment. Two perpendicular diameter measurements across five seminiferous tubules with accompanying tubular basement membrane thickness were recorded for each tissue sample. In addition, a single observer (KMP) blinded to the treatments performed all of the Yoshida scoring (Table 1) to determine the effect on spermatogenesis.

# Statistical analyses

All data are reported as mean ± standard deviation (SD). The effect of treatment on the presence of sperm was evaluated using the Fisher exact test and performed using R (version 3.0.2). The effect of treatment on testosterone and

estrone sulfate concentration, sperm morphology, histomorphometry, and Yoshida scoring was compared using a one-way ANOVA with a Tukey-Kramer post hoc test. These statistical tests were performed using Graph Pad Prism (GraphPad Software, Inc., La Jolla, California 92037, USA). Significance was defined as P < 0.05.

# **RESULTS**

The hormone analysis showed no significant differences in testosterone or estrone sulfate concentrations when comparing either sterilization method (SURG or CHEM) to untreated (CONT) (Table 2). However, SURG stallions had significantly lower testosterone compared to CHEM stallions (P < 0.05). This difference was not evident when comparing estrone sulfate concentrations.

SURG stallions had no sperm present in the remaining section of the proximal vas deferens, whereas all of the CONT and CHEM stallions had sperm present within the vas deferens seminal fluid samples. The difference in presence of sperm between the CONT and CHEM compared to the SURG stallions was highly significant (P < 0.001).

For SURG stallions that had no sperm present in the remaining section of the proximal vas, a sample of fluid was collected from the tail of the epididymis. There were more morphologically normal sperm in the CONT stallions compared to SURG and CHEM stallions (Table 3, P <0.01). In addition, CHEM stallions had more morphologically normal sperm than did SURG stallions (P < 0.001). The majority of the defects recorded in all groups were detached heads, bent tails, and severely coiled tails (Table 3, Fig. 1). There were more detached heads in the SURG stallions compared to CHEM (P < 0.01) and CONT stallions (P < 0.001). However, there were more severely coiled tails in the CHEM stallions compared to SURG stallions (P < 0.05).

The histomorphometry results showed no significant difference in seminiferous tubule diameter (P=0.51) or basement membrane thickness (P=0.07) mediated by changes in intratesticular pressure following treatment (Table 4, Fig. 2). In addition, spermatogenesis as scored using the Yoshida method did not differ between treatment groups (SURG, 11.8  $\pm$  0.3; CHEM, 12.0  $\pm$  0.0; CONT, 12.0  $\pm$  0.0).

# DISCUSSION

Measurement of baseline total serum testosterone concentration is often used to assess normal

Table 1. The criteria used for scoring changes within the seminiferous epithelium of stallions that were surgically or chemically vasectomized, or untreated. Adapted from Yoshida et al. 1997.<sup>38</sup>

Score	Yoshida scoring method criteria
12	Many late spermatids or spermatozoa (≥10) present
11	Only a few late spermatids or spermatozoa (<10) present
10	No spermatozoa and no late spermatids, but many round spermatids (≥10) present
9	No spermatozoa and no late spermatids, but only a few round spermatids (<10) present
8	No spermatozoa and no spermatids, but many secondary spermatocytes (≥10) are present
7	No spermatozoa and no spermatids, but only a few secondary spermatocytes (<10) are present
6	No spermatozoa, no spermatids, no secondary spermatocytes, but many primary spermatocytes (≥10) present
5	No spermatozoa, no spermatids, no secondary spermatocytes, but only a few primary spermatocytes (<10)
4	No spermatozoa, no spermatids, no spermatocytes, many spermatogonia (>10) present
3	Only germ cells present are a few spermatogonia (<10) present
2	Absence of germ cells, but Sertoli cells are present
1	Total absence of cells in tubular section

testicular endocrine function in stallions.1 However, the diagnostic usefulness of a single baseline sample is limited because of the extreme fluctuating nature of testosterone concentrations in circulation. In the horse, serum testosterone levels can range from 0-120 ng/dl over a 12-hour period.<sup>34</sup> Due to this unpredictability, it is often recommended that a more accurate measurement of the testicular function is an hCG-stimulation response test.7 The field conditions in this study and the wild nature of the subjects made an hCGstimulation response test impractical. Therefore, to determine with the utmost confidence that the sterilization method under evaluation had no effect on reproductive endocrine status, a baseline estrone sulfate concentration was also measured. Estrone sulfate is the most abundant plasma estrogen in the stallion.30 The concentration of estrone sulfate in the plasma of mature stallions is nearly 100-fold greater than that of testosterone.8 In fact, estrone sulfate is believed to be involved in the maintenance of libido in stallions, since it was demonstrated that administration of exogenous estradiol without testosterone to geldings could restore libido.33 In the current study, serum testosterone concentrations from CHEM stallions did not differ from CONT stallions. However, there was an unexplainable reduction in testosterone in the SURG stallions compared to the CHEM stallions. Because of the unpredictability in baseline testosterone measurements and the importance of estrogens in the maintenance of stallion libido, baseline estrone sulfate concentrations may be a more reliable way to determine whether testicular endocrine tissue is still functioning normally. In the current study, estrone sulfate concentrations did not differ between

Pressure damage can occur within the seminiferous tubule postsurgical vasectomy because sperm production continues without interruption even though there is no outlet for release.<sup>23</sup> However, effects on the seminiferous epithelium following surgical vasectomy appear to differ between species. In rats, changes in testicular histology including loss of spermatogenic cells and vacuole appearance become evident within 1 mo after surgical vasectomy.<sup>6</sup> This is in contrast to rhesus monkeys, where there were no histological changes seen in the testes or the epididymides of

**Table 2.** Serum estrone sulfate and total testosterone concentrations (mean  $\pm$  SD) in stallions following sterilization treatment in Nevada, USA, in 2013.

	Treatment group				
Hormone	Surgical vasectomy	Chemical vasectomy	Untreated		
Estrone sulfate (ng/ml) Testosterone (ng/dl)	$70.95 \pm 15.59 \ (n = 9)$ $19.7 \pm 10.70^{a} \ (n = 25)$	$66.94 \pm 20.37 (n = 14)$ $38.68 \pm 30.05^{a} (n = 16)$	$68.05 \pm 23.29 \ (n = 17)$ $27.17 \pm 26.56 \ (n = 32)$		

<sup>&</sup>lt;sup>a</sup> Values in the same row with this superscript differ (P < 0.05).

Table 3.	Sperm	morphology	results	(mean	$\pm$	SD)	ın	stallions	following	sterilization	treatment i	n Nevada,	
USA, in 20	13.												

	Surgical vasectomy $(n = 10)$	Chemical vasectomy $(n = 16)$	Untreated $(n = 23)$
Total normal (%)	$31.8 \pm 13.3^{a}$	$48.0\pm9.9^{\rm ab}$	$59.7 \pm 6.6^{ab}$
Detached head (%)	$48.8 \pm 24.7^{ab}$	$27.9 \pm 11.1^{b}$	$23.2\pm6.7^{\rm a}$
Bent tail (%)	$16.0 \pm 12.3$	$16.50 \pm 10.2$	$11.6 \pm 5.3$
Severely coiled tail (%)	$3.6 \pm 3.0^{\circ}$	$7.2 \pm 3.4^{\circ}$	$4.7 \pm 3.4$
Bent midpiece (%)	$0.0\pm0.0$	$0.1 \pm 0.2$	$0.2\pm0.9$
Abnormal acrosome (%)	$0.0\pm0.2$	$0.0\pm0.0$	$0.0\pm0.0$
Microcephalia (%)	$0.0\pm0.0$	$0.0\pm0.0$	$0.2\pm1.1$

<sup>&</sup>lt;sup>a</sup> Values in the same row with this superscript differ (P < 0.001).

vasectomized monkeys when examined 6 mo following surgery.<sup>24</sup> The results appear to be even more confounding in rabbits, where vasectomy results in damage to spermatogenesis within 10 days following surgery, but the effects on spermatogenesis were less severe at 6 and 12 mo

following surgery.<sup>20</sup> In the stallions of the present study, the results based on the histologic evaluation performed more than 24 mo following any treatment indicate there were no long-term effects on spermatogenesis (P = 0.95), seminiferous tubule diameter (P = 0.51), or basement mem-

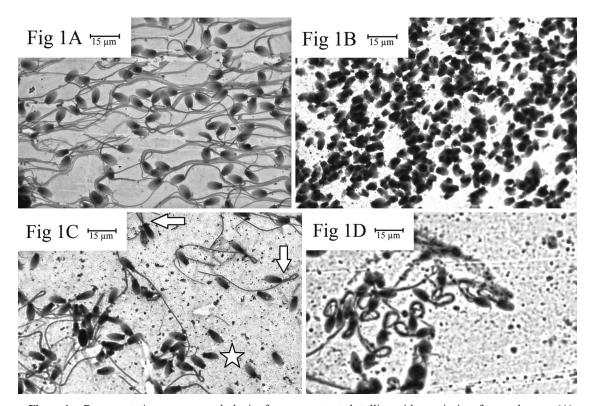


Figure 1. Representative sperm morphologies from an untreated stallion with a majority of normal sperm (A), from a surgically vasectomized stallion with almost all sperm having detached heads (B), from a chemically vasectomized stallion with several bent tails (arrows) and detached heads (star) (C), and from a chemically vasectomized stallion with a high percentage of sperm with severely coiled tails (arrow) (D). Sperm were stained with commercial modified Romanowsky stain. Photomicrographs were digitally captured at ×1000 magnification under oil immersion.

<sup>&</sup>lt;sup>b</sup> Values in the same row with this superscript differ (P < 0.01).

 $<sup>^{\</sup>circ}$  Values in the same row with this superscript differ (P < 0.05).

**Table 4.** Seminiferous tubule diameter and basement membrane thickness (mean  $\pm$  SD) from testicular tissue samples of stallions following sterilization treatment in Nevada, USA, in 2013.

Histomorphometry	Surgical vasectomy $(n = 19)$	Chemical vasectomy $(n = 16)$	Untreated $(n = 10)$
Seminiferous tubule diameter (μm) Basement membrane thickness (μm)	$192.25 \pm 15.37 \\ 3.12 \pm 0.53$	$194.69 \pm 18.81 \\ 2.83 \pm 0.16$	$201.535 \pm 28.81 \\ 3.18 \pm 0.53$

Values in the same row did not differ significantly (P > 0.05).

brane thickness (P=0.07). It is unknown if either the surgical or the chemical vasectomy caused acute pressure damage within the testes (under 1 mo) as the stallions were not evaluated at this time point. However, based on the histology performed several months following either treatment, there was no evidence of irreparable effects on the seminiferous epithelium.

We anticipated that similar to what was reported previously in dogs<sup>25</sup> and cats,<sup>26</sup> percutaneous injection of a sclerosing agent into the tail of the

epididymis would cause irritation of the tubular and intertubular tissues, resulting in sclerosis and occlusion, thereby preventing passage of sperm from the epididymides to the vasa deferentia. The finding of significant numbers of intact, motile sperm in the vas following chemical vasectomy may indicate recanalization of a previous obturating tissue reaction. Because the stallions in the current study were chemically vasectomized in 2010 and 2011 and seminal fluid samples were only collected once at the end of the study, it is not

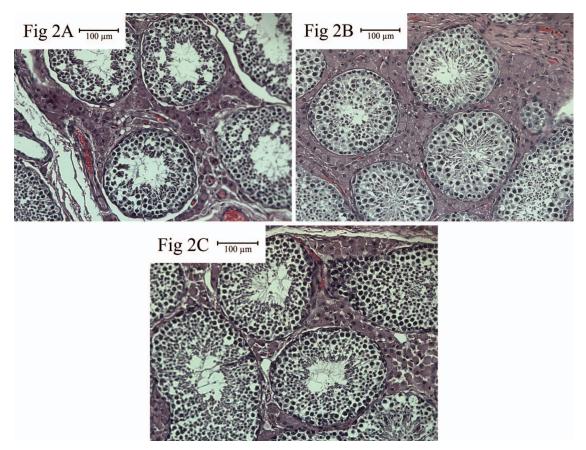
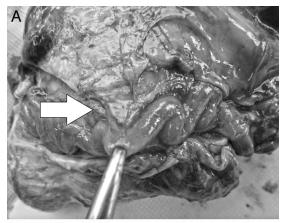


Figure 2. Representative cross sections of the seminiferous epithelium from stallions that were surgically vasectomized (A), chemically vasectomized (B), or untreated (C). Paraffin-embedded testicular biopsy sections (4  $\mu$ m) were stained with hematoxylin and eosin. Photomicrographs were digitally captured at ×200 magnification.

possible to determine whether or not temporary azoospermia may have resulted from the injection but then recovered over time. A similar study performed in dogs using either 3.0% chlorhexidine digluconate in 50% DMSO or 4.5% chlorhexidine digluconate without DMSO injected into the tail of the epididymis caused azoospermic ejaculates by within 35 or 42 days after treatment.27 Based upon the histopathology, the authors of this canine study assumed the treatment would be irreversible but they did not do any long-term evaluations so it is possible that the effects were reversible as well. It is of interest to note that in cats, little or no adhesions were present between the scrotum and tail of the epididymides in males that became azoospermic following a similar treatment.26 In the current study, there was evidence at the time of castration of adhesions and scar tissue surrounding the epididymis and vas deferens in CHEM stallions (Fig. 3). This is suggestive of an increased amount of seepage of chlorhexidine into the vaginal cavity of the scrotum following treatment and may indicate a smaller amount injected (e.g. 5 ml) would have improved efficacy as seen in cats.<sup>26</sup> Other than seepage, another explanation for treatment failure and the formation of the adhesions within the scrotal vaginal cavity would be that DMSO carried the chlorhexidine outside the epididymal luminal walls, calling into question the use of DMSO for chemical vasectomy. Even though CHEM was not effective as a sterilant, it did have long-lasting effects on fertility as illustrated by a significant increase in sperm morphologic abnormalities. However, fertility, in terms of foals produced by each stallion following treatment, was not assessed in this study.

Free-roaming horse populations can be managed with surgical vasectomy but it is time consuming and expensive. Additional research is needed on alternative methods for nonsurgical sterilization in free-roaming horse populations. In dogs, zinc arginine injected into the tail of the epididymis also causes infertility.10 This application has not yet been tried in horses. A compound that has not yet been tested in the vas deferens of any species is polidocanol. Polidocanol is a powerful sclerosing agent most commonly used in treatment of varicose veins. In baboons, polidocanol administered into the uterine lumen induces oviductal occlusion causing sterility in females.16 In stallions, polidocanol could be injected into the epididymis, which might result in a permanent occlusion similar to what is seen in the oviducts of baboons.



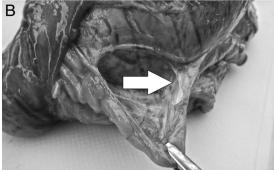


Figure 3. Gross images digitally captured at the time of castration from chemically vasectomized stallions. Note the presence of adhesions and scar tissue near the tail of the epididymis and vas deferens (arrows), presumably resulting from irritation and inflammation induced following injection of the chemical sterilant into this location (A-B).

# **CONCLUSION**

In conclusion, only surgical vasectomy induced sterilization of free-roaming horses. Chemical vasectomy with 10 ml of 1% chlorhexidine diluted in 90% DMSO was not an effective method for sterilizing free-roaming stallions. Increasing the concentration of chlorhexidine, eliminating the use of DMSO, and/or decreasing the volume injected may improve the efficacy of this technique. Neither surgical nor chemical vasectomy significantly affected serum estrone sulfate concentrations, which are less unpredictable than baseline testosterone measurements for evaluating testicular endocrine function. Additional research is needed before chemical vasectomy can be a reliable sterilization method in freeroaming horse populations.

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