

Histopathologic changes in testes three days after administration of zinc gluconate neutralized with arginine as an intratesticular injection for contraception

Margaret V. Root Kustritz, Jennifer Brazzell, Jonna Swanson, Graham Brayshaw
College of Veterinary Medicine, University of Minnesota, St. Paul, MN

Abstract

Five prepuberal mixed breed dogs were treated with zinc gluconate neutralized with arginine as an intratesticular injection, which is approved by the Food and Drug Administration as a non-surgical sterilant for puppies aged 3-10 months with testicular width between 10 and 27 mm. The product was administered following manufacturer's recommendations. Three days later, all dogs underwent surgical castration and the testes, epididymes, and a portion of the spermatic cord submitted for histopathologic evaluation. Both testes from all five dogs showed variable degrees of liquefactive and coagulative necrosis with focal acute neutrophilic orchitis, hemorrhage, and edema. Percentage of the testis showing degenerative and necrotic changes averaged approximately 45.5% and ranged from 10-90% among the ten testes examined. Unaffected seminiferous tubules were lined by Sertoli cells and spermatogonia with rare elongated spermatids; this was compatible with the prepuberal life stage of the animals. Neutrophilic and lymphohistiocytic epididymitis with edema and vasculitis was present in six of the ten samples. Funiculitis (inflammation of the spermatic cord) was present in nine of the ten samples. This is the first report of testicular and epididymal histologic change within days of administration of the product and the only report documenting changes in the vas deferens at any point after administration of the product.

Key words: Castration, sterilization, non-surgical, zinc arginine, intratesticular

Introduction

Chemical castration may be preferred to surgical castration for logistical and societal reasons. Compounds used are easy to administer. It has the potential to be a less expensive option that may be suitable for large scale castration operations, especially in countries with feral dog populations that are difficult to control because of lack of availability of veterinarians and cultural or social impediments to culling for population control. Reported compounds have a high margin of safety. Finally, minimal observation of dogs is required after treatment.¹⁻⁵ In dogs, reported chemical castration techniques include injection of agents into the vas deferens or epididymis, where formation of fibrous occlusions precludes ejaculation of fluid from the testes and epididymes, and injection of agents into the testicular parenchyma, which may be associated with direct toxic effects on germ cells, inflammation and necrosis of testicular tissue, and eventual testicular atrophy or fibrosis. Products reported for injection in the vas deferens or epididymis in the dog include formaldehyde, formalin, chlorhexidine gluconate, chlorhexidine diacetate, methylcyanoacrylate, silver nitrate, ethanol, and potassium permagnate.⁶⁻¹⁰ Products reported for intratesticular injection include zinc gluconate neutralized with arginine and with or without DMSO or proprietary carriers, calcium chloride, glycerol, Freund's complete adjuvant, Bacillus Calmette Guerin (BCG), and dexamethasone.^{1,11-18}

The only product that has been approved by the Food and Drug Administration for use in the United States is 0.2 M zinc gluconate neutralized with L-arginine to a pH of 7.0, first approved under the brand name Neutersol® and more recently, as Zeuterin®. Zinc arginine effects contraception directly by inhibiting replication of germ cells and disrupting the Sertoli cell barrier and indirectly by inducing local immune and inflammatory responses and by affecting testosterone synthesis and metabolization.^{1,19-21} Presently the product is approved for dogs aged 3-10 months, with testicular width between 10 and 27 mm.²² The product should not be used if the dog does not have two descended testes, if the testes or epididymes are malformed or diseased, or if there is pre-existing scrotal irritation or dermatitis.

Manufacturer recommendations for administration are the following. Withhold food for 12 hours prior to treatment to decrease risk of vomiting. Active dogs should be sedated to ensure they do not move during administration of the product. Although administration of the product will cause mild testicular distension, sedation is not required to control pain; the only afferent nerves associated with pain sensation

are located in the testicular capsule, not the testicular parenchyma.^{23,24} Place the dog in dorsal recumbency. Do not shave or clip the scrotum and clean it with a non-irritating disinfectant. Measure each testis separately using the calipers supplied by the manufacturer. Use three needles and two syringes for each dog. One needle is inserted into the product container and is left in place to permit drawing the product into the syringes. Once the correct volume is drawn up, a 28 gauge 1/2 -5/8" needle is attached to the syringe for administration of the product into the testis. The testis is held securely but without squeezing it. Palpate the head of the epididymis and insert the needle next to it, in the dorsal cranial portion of the testis. Insert the needle and administer the product slowly. If any resistance is felt, withdraw the needle and do not repeat the injection. The product should flow freely into the testis. Once administration is complete, hold the testis and syringe with compressed plunger in place for one minute, and then release everything at once, withdrawing the needle. Repeat with the other testis. Care must be taken not to introduce the compound into the scrotal skin or scrotal sac. After treatment, the dog must not be permitted to bite or lick at the scrotum and should not be housed on hard or wet surfaces.

Following injection, mild testicular swelling beginning 24 hours and peaking at about 48 hours after treatment is reported but with minimal pain response or change in behavior exhibited by treated dogs.²⁵ Reported decrease in testicular size is variable long-term. Rare negative side-effects reported include vomiting, biting or licking at the scrotum, scrotal ulceration or draining tracts, and very rarely, scrotal perforation.^{1,25,26}

Efficacy in one study was reported to be 98.7% (221/224 treated dogs) with 76.3% of those dogs having no seminal fluid collected at attempts six months after treatment (aspermia) and 22.3% having no spermatozoa in the ejaculate six months after treatment.²⁵ Overall, complete azoospermia was reported by three to six months after-treatment.^{25,27} The manufacturer recommends that treated dogs not be permitted near bitches in estrus for at least 60 days after injection.²² Change in serum testosterone is variable but generally is described as being decreased by about 50%; significance of this decline in regards to development of testosterone-dependent disease and exhibition of testosterone-dependent behavior is unknown.^{25,27}

This is a report documenting acute histopathologic changes in the testes, epididymes, and spermatic cords after administration of zinc arginine as a contraceptive agent administered by intratesticular injection in dogs.

Materials and methods

Five prepuberal dogs, aged 3-5 months and of mixed breeds, with weights ranging from 7.3 to 19.0 kg, that had been relinquished to a humane organization were treated with Zeuterin® as a training exercise for veterinarians and veterinary students to permit them to use this drug for population control. The intent was always that these dogs would undergo surgical castration at the humane society shortly after Zeuterin® treatment, as chemical castration is an uncommon procedure in this region and it was perceived that dogs with visible testes were less likely to be adopted despite having been treated with a chemical castration agent. All of the dogs were normal on physical examination and had been treated with dewormer and vaccinated. All were sedated with dexmedetomidine (500 mcg/M² IM) and continuously monitored by a veterinary student while under sedation. The intratesticular injection of Zeuterin® was performed according to manufacturer's directions as described previously. Sedation was reversed with atipamezole (equal volume as for dexmedetomidine IM) and dogs were treated with carprofen (4.4 mg/kg SQ) and once verified to be alert, were returned to the humane society. Three days after treatment with Zeuterin®, all dogs underwent surgical castration by an experienced veterinarian at that facility. After induction of anesthesia and primary surgical preparation of the pre-scrotal area, a mixture of lidocaine (2%, 0.5 ml/10 kg body weight) and bupivacaine (0.5%, 2.0 ml/10 kg body weight; volume split into two equal aliquots) was injected into the testes. A 22 gauge needle was inserted into the caudal pole of each testis and directed toward the spermatic cord and the fluid infused. Testes were surgically removed within five minutes of injection.

Testicles were submitted for histopathologic evaluation. All testes, epididymes, and spermatic cords were evaluated by one pathologist. Percentages of degenerative and necrotic change were estimated based on the two-dimensional histology image.

Results

Both testes from all five dogs showed variable degrees of liquefactive and coagulative necrosis with focal acute neutrophilic orchitis, hemorrhage, and edema (Figures 1-4). Percentage of the testis showing degenerative and necrotic changes averaged approximately 45.5% and ranged from 10-90% among the ten testes examined. Unaffected seminiferous tubules were lined by Sertoli cells and spermatogonia with rare elongated spermatids; this was compatible with the prepuberal life stage of the animals.²⁸ Neutrophilic and lymphohistiocytic epididymitis with edema and vasculitis was present in six of the ten samples.

Funiculitis (inflammation of the spermatic cord) was present in nine of the ten samples. Funiculitis was regionally most extensive in the portion of the spermatic cord directly abutting associated tissues and was therefore most likely an extension of inflammation in the associated testis and epididymis. Inflammation in the spermatic cord varied between neutrophilic and lymphohistiocytic with varying degrees of necrosis, hemorrhage, and edema. Severity was commensurate with that seen in the associated epididymis and testis. Percentage of the cord involved could not be estimated as amount of spermatic cord submitted varied among dogs.

Discussion

The dogs in this study had been relinquished to a humane organization and had no permanent owner at the time of castration so a decision was made to perform surgical castration as that was perceived to be associated with greater likelihood of those dogs being readily adopted. All five dogs were adopted from the humane organization within three days of surgery; four of the five were adopted within one day of surgery.

Chemical castration was demonstrated to be well accepted by owners in two studies, both of which were completed in the Galapagos Islands. In the first, owners were permitted to choose if they wanted the dogs chemically castrated when pets were microchipped. Twenty-three participants were questioned three to seven days after treatment and the majority considered the method to be good or excellent.²⁹ In the second, pet owners were permitted to choose between surgical and chemical castration of their animals. One hundred three of 161 dogs (64%) underwent chemical castration and the technique was reported to be well accepted by the community.⁴

Dogs in this study were treated with an injectable anti-inflammatory drug at the time of treatment, at the recommendation of the manufacturer and the veterinarians leading the training. This therapy is believed to minimize the dog biting and licking at the scrotum after therapy and potentially causing scrotal injury. While it may be perceived that this would preclude desired inflammatory change within the testis, this has not been demonstrated to be true. In one study comparing groups of dogs treated with zinc gluconate by intratesticular injection either with or without simultaneous administration of an anti-inflammatory drug, treatment was equally effective at inducing azoospermia in the two groups.¹⁹

Histologic changes within the testis reported here are appropriate for short-term exposure to the compound. All testes were injected with lidocaine and bupivacaine immediately prior to castration but there was insufficient time for changes specific to exposure to these drugs to be evident in testicular tissue. Histology of testes after administration of zinc gluconate with L-arginine previously has been reported only after prolonged exposure of the testicular tissue to the compound. At 60-75 days after treatment, coagulation necrosis, fibrosis, and atrophy of seminiferous tubules was reported.³ At five months, reported changes included decreased number of germinal cells, lack of elongated spermatids in the seminiferous tubules, and seminiferous tubules lined only with vacuolated Sertoli cells, and degenerative changes in the testicular parenchyma.³⁰ At one year after treatment, there was increase in connective tissue especially around the rete testis, and decreased diameter of the rete testis.²⁷ By two years after treatment, there was severe atrophy of the testis with decreased thickness of the basement

membrane, decreased height of the germinal epithelium, and decreased diameter of the seminiferous tubules.²⁵

Histology of the epididymis after intratesticular injection with zinc gluconate with L-arginine is more rarely reported and histology of the vas deferens after this treatment has not been reported. In this study, acute inflammation of the epididymis and spermatic cord was observed. Other studies report lack of spermatozoa in the coils of the epididymis, decreased height of columnar epithelium, and increase in fibrous tissue one year after treatment and severe atrophy of the epididymis two years after treatment.^{25,27}

In a survey of veterinarians in Nigeria, 67.4% of those surveyed expressed an interest in alternatives to surgical castration but only 9.7% had attempted any alternative.³¹ The authors are unaware of a similar survey of veterinarians in the United States. Publications documenting research in non-surgical contraceptives have increased in number six-fold since the 1980s.³² It is hoped that information such as that presented here will help veterinarians better understand the use of these products.

References

1. Alliance for contraception in cats and dogs: Contraception and fertility control in dogs and cats. eBook. Available at www.acc-d.org/ACCD%20symposia. pp 50-55. Accessed 06-16-14.
2. Soerensen B, Silva PJS, Christhavo FG: [Advantages of chemical castration compared with the traditional method of surgical castration]. *Univ Marilia* 2007;1:15-20.
3. Tepsumethanon V, Wilde H, Hemachudha T: Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. *J Med Assoc Thai* 2005;88:686-689.
4. Levy JK, Crawford PC, Appel LD, et al: Comparison of intratesticular injection of zinc gluconate versus surgical castration to sterilize male dogs. *Am J Vet Res* 2008;69:140-143.
5. Oliveira ECS, Moura MRP, de Sa MJC, et al: Permanent contraception of dogs induced with intratesticular injection of a zinc gluconate-based solution. *Theriogenology* 2012;77:1056-1063.
6. Pineda MH, Reimers TJ, Faulkner LC, et al: Azoospermia in dogs induced by injection of sclerosing agents into the caudae of the epididymides. *Am J Vet Res* 1977;38:831-838.
7. Perez MEG, Ramirez RMP, Lacroix CE, et al: [Sterilization in the dog by injection of methylcyanoacrylate in the tail of the epididymis]. *Vet Mex* 1994;25:261-265.
8. Barnett BD: Chemical vasectomy of domestic dogs in the Galapagos Islands. *Theriogenology* 1985;23:499-509.
9. Dixit VP, Lohiya NK, Aryam M, et al: The effects of chemical occlusion of vas deferens on the testicular function of dog: a preliminary study. *Acta Eur Fertil* 1975;6:348-353.
10. Pineda MH, Hepler DI: Chemical vasectomy in dogs. Long-term study. *Theriogenology* 1981;16:1-11.
11. Soto FRM, Viana WG, Mucciolo GCB, et al: Evaluation of efficacy and safety of zinc gluconate associated with dimethyl sulfoxide for sexually mature canine males chemical neutering. *Reprod Domest Anim* 2009;44:927-931.
12. Jana K, Samanta PK: Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose-dependent study. *Contraception* 2007;75:390-400.
13. Alliance for contraception in cats and dogs: ACC&D statement and recommendations regarding calcium chloride injection for chemical castration. Available at www.acc-d.org/ACCD%20docs/ACCD-RecommCalcChlor2.pdf. Accessed 06-17-14.
14. Immergart HM, Threlfall WR: Evaluation of intratesticular injection of glycerol for non-surgical sterilization of dogs. *Am J Vet Res* 2000;61:544-549.
15. Dixit VP: Chemical sterilization of male dogs (*Canis familiaris*) after single intratesticular administration of methalibure (ICI-33828), dexamethasone, metopron (SU-4885, Ciba), niradazole (33644-Ba, Ciba), alpha-chlorohydrin (U-5897) and danazol. *Indian J Exp Biol* 1979;17:937-940.
16. Talwar GP, Naz RK: Immunological control of male fertility. *Arch Androl* 1981;7:177-185.
17. Naz RK, Talwar GP: Immunological sterilization of male dogs by BCG. *Intl J Androl* 1981;4:111-128.
18. Talwar GP, Naz RK, Das C, et al: A practicable immunological approach to block spermatogenesis without loss of androgens. *Proc Natl Acad Sci USA* 1979;76:5882-5885.
19. Oliveira ECS, Muller PM, Silva FLM, et al: Oral administration of an anti-inflammatory drug does not compromise the efficacy of intratesticular injection of zinc gluconate as a contraceptive for dogs. *Anim Reprod Sci* 2012;132:207-212.
20. Fahim MS, Wang M, Sutcu MF, et al: Zinc arginine, a 5-alpha-reductase inhibitor, reduces rat ventral prostate weight and DNA without affecting testicular functions. *Andrologia* 1993;25:369-375.
21. Kellokumpu S, Rajaniemi H: Effect of zinc on the uptake of human chorionic gonadotropin (hCG) in rat testis and testosterone response in vivo. *Biol Reprod* 1981;24:298-305.
22. Zeuterin® package insert. Available at www.zeuterin.com/images/pdf/zeuterinCMCPackageInsertFinal.pdf. Accessed 06-16-14.
23. Christensen GC: The urogenital apparatus. In: Evans HE, Christensen GC, editors. *Miller's anatomy of the dog*. Philadelphia: WB Saunders; 1979. p. 544-601.

24. Davis JR, Langford GA, Kirby PJ: The testicular capsule. In: Johnson WR, Gomes WR, editors. The testis. New York: Academic Press Inc, 1970. p. 285-293.
25. Technology Transfer Inc. Freedom of information summary: Neutersol® injectable solution for dogs. Available at www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm118024.pdf. Accessed 06-06-14.
26. Forzan MJ, Garde E, Perez GE, et al: Necrosuppurative orchitis and scrotal necrotizing dermatitis following intratesticular administration of zinc gluconate neutralized with arginine (EsterilSol) in 2 mixed-breed dogs. *Vet Pathol* 2014;51:820-823.
27. Fahim MS, Wang M, Sutcu MF, et al: Sterilization of dogs with intraepididymal injection of zinc arginine. *Contraception* 1993;47:107-122.
28. Frame SR, Hurtt ME, Green JW: Testicular maturation in prepubertal New Zealand White rabbits. *Vet Pathol* 1994;31:541-545.
29. Soto FRM, Bittencourt DO, Neves AM: [Experiment with using chemical sterilant with microchips for male dogs in the city of Redencao da Serra-State of Sao Paulo]. *Rev Cienc Vet* 2011;7:16-23.
30. Oliveira ECS, Moura MR, Silva VA, et al: Intratesticular injection of a zinc-based solution as a conceptive for dogs. *Theriogenology* 2007;68:137-145.
31. Ayisat AT: Approaches to canine castration in Nigeria [abstract]. *Clin Therio* 2013;5:388.
32. Massei G, Miller LA: Nonsurgical fertility control for managing free-roaming dog populations: a review of products and criteria for field applications. *Theriogenology* 2013; 80:829-838.

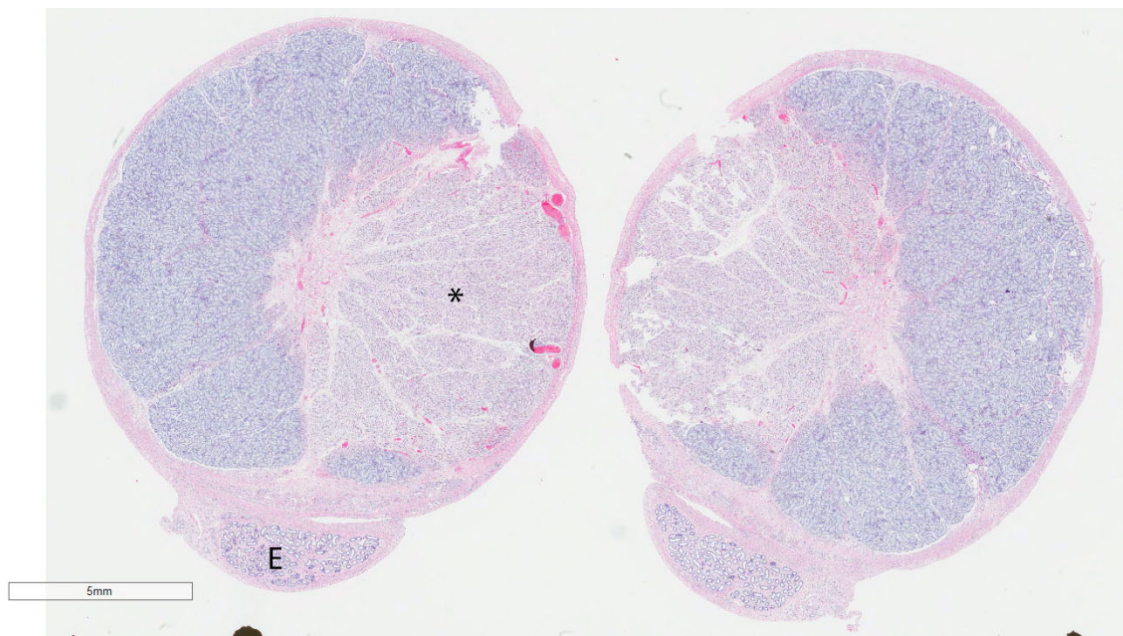


Figure 1: Cross section of a Zeuterin™ injected testis with a portion of the epididymis (E). Approximately 40% of the seminiferous tubules have undergone coagulative necrosis (*); 0.4x magnification, hematoxylin and eosin.

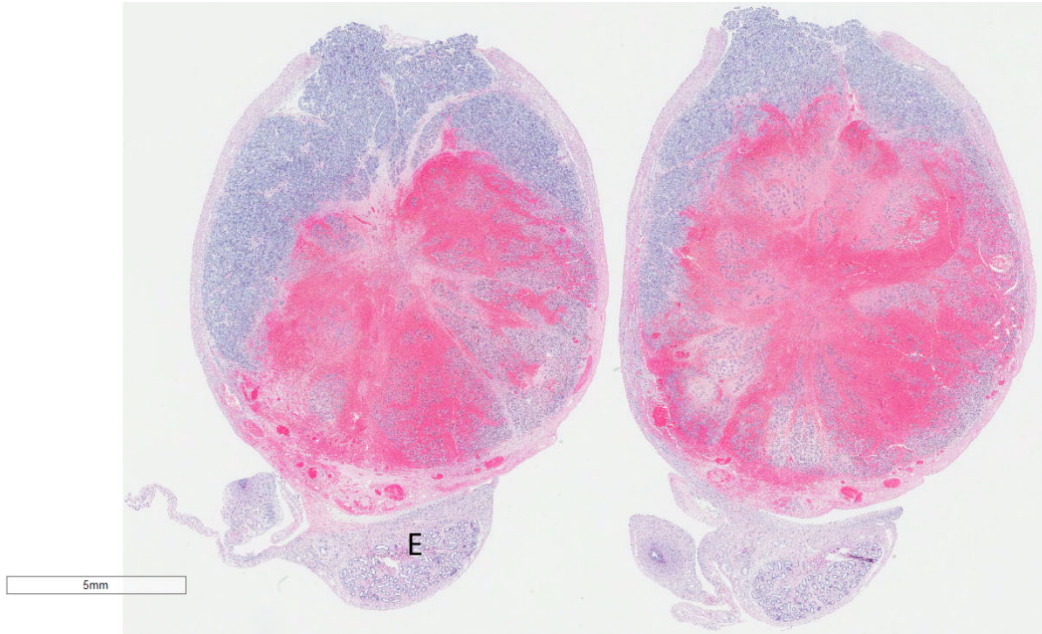


Figure 2: Cross section of a Zeuterin™ injected testis with a portion of the epididymis (E). There is a focus of liquefactive necrosis and hemorrhage occupying approximately 60% of the testicular parenchyma. 0.4x magnification, hematoxylin and eosin.

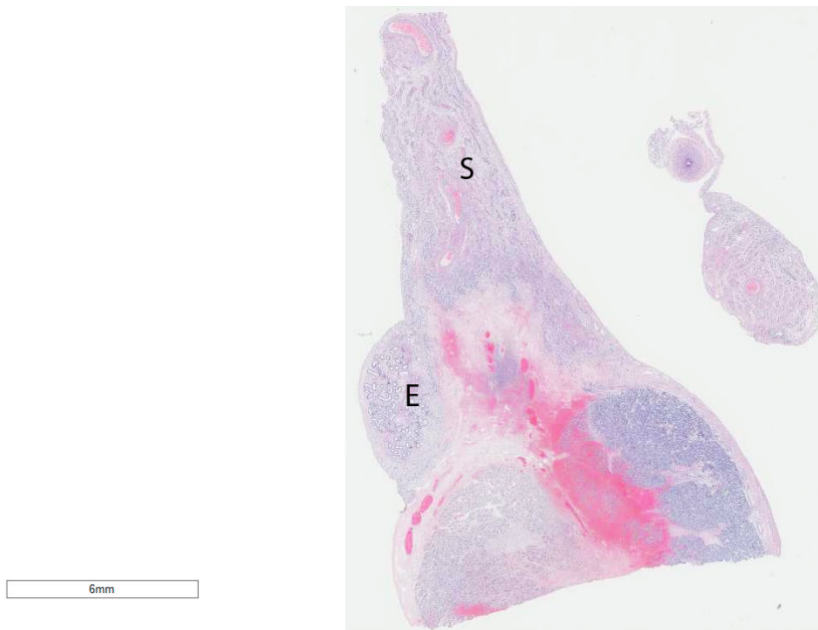


Figure 3: Longitudinal section of a Zeuterin™ injected testis including the epididymis (E) and spermatic cord (S). A linear area of liquefactive necrosis and hemorrhage extends from the testis through the tunica albuginea into the epididymis and spermatic cord. 0.4x magnification, hematoxylin and eosin.

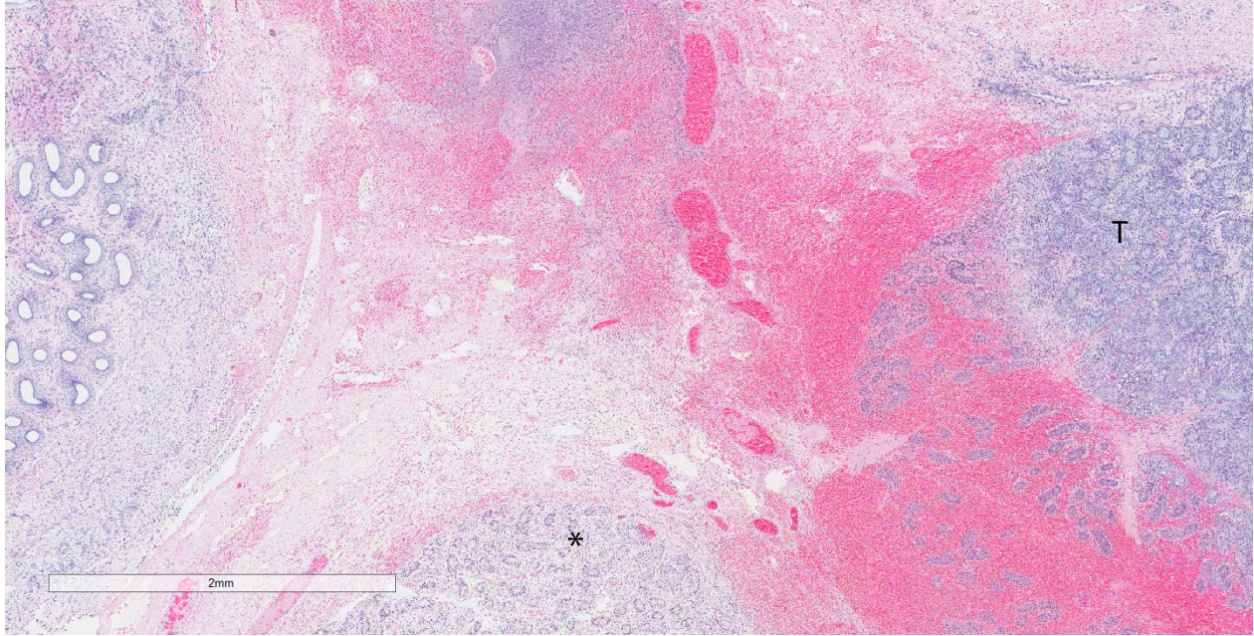


Figure 4: Higher magnification of Figure 3 showing the area of liquefactive necrosis and hemorrhage extending from the testis between an area of coagulative necrosis (*) and normal seminiferous tubules (T) outward into the interstitium of the spermatic cord and epididymis. Normal epididymis is visible on the left. 2x magnification, hematoxylin and eosin.

(Editor's note: Photographs in this manuscript are available in color in the online edition of Clinical Theriogenology.)

