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Necrosuppurative Orchitis and Scrotal Necrotizing Dermatitis Following Intratesticular Administration of Zinc Gluconate Neutralized With Arginine (EsterilSol) in 2 Mixed-Breed Dogs

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Abstract

Intratesticular injection of EsterilSol (zinc gluconate neutralized with arginine) is a chemical sterilant for male dogs sometimes used in population control campaigns. Adverse reactions have been reported in 1% to 4% of treated dogs, but detailed histomorphologic descriptions are lacking. During a behavioral study conducted in the Chilean Patagonia in 2012, severe necrosuppurative orchitis and ulcerative dermatitis were observed in 2 of 36 (6%) dogs sterilized with EsterilSol according to the manufacturer's instructions. Reactions were noted on days 8 and 7 postinjection and required scrotal ablation on days 8 and 13, respectively; neither reaction was associated with the injection site. Although self-trauma following administration may have contributed, the cause of the adverse reactions is uncertain. EsterilSol is a relatively uncomplicated method to sterilize male dogs, but the occurrence of severe adverse reactions several days after administration emphasizes the need for the provision of long-term monitoring and veterinary care during sterilization campaigns using this product.

Keywords

adverse reaction, chemical castration, dog, EsterilSol, orchitis, testicular necrosis, zinc gluconate neutralized with arginine

Large uncontrolled populations of free-roaming dogs that cause human-animal conflict and endanger the welfare of domestic and wild animals are common in many regions of the world.[3] Intact males searching and competing for females in estrus are frequent victims of dogfight injuries and automobile accidents as they roam the streets unsupervised.[3] Free-roaming dogs also serve as reservoirs of zoonotic diseases of concern to the Food and Agriculture Organization of the United Nations and the World Organisation for Animal Health, such as rabies, cystic hydatidosis, leishmaniasis, leptospirosis, and trypanosomiasis.[5]

Massive surgical sterilization, one of the most commonly used and recommended population management methods worldwide,[3] can be difficult to implement in regions with economic and logistical restrictions or cultural objections to its practice.[4] In some Latin American countries, for instance, social prejudice against the surgical castration of male dogs can make the implementation of sterilization programs very difficult or focused primarily on female dogs.

Viable alternatives, such as chemical castration with the recently Food and Drug Administration (FDA)–approved EsterilSol (Zeuterin in the United States; Ark Sciences, New York, NY), a solution of zinc gluconate neutralized by arginine,[9] are being explored.[1,4] Unlike Zeuterin, which is currently approved only for dogs between 3 and 10 months of age, EsterilSol is licensed for any dog over 3 months old.[2] Although safety information for EsterilSol is available,[9] reports on histomorphology of the testicles following administration of identical or similar compounds (such as zinc gluconate with dimethyl sulfoxide or DMSO)[7] describe changes 5 to 7 months after administration[6,7] or include no images.[8] No detailed histopathological descriptions of cases where adverse reactions required surgical sterilization with scrotal ablation could be found. Here, we report 2 cases of adult male dogs that had severe adverse reactions after receiving an EsterilSol injection as per the manufacturer's specifications[2] by veterinarians certified in its application. The

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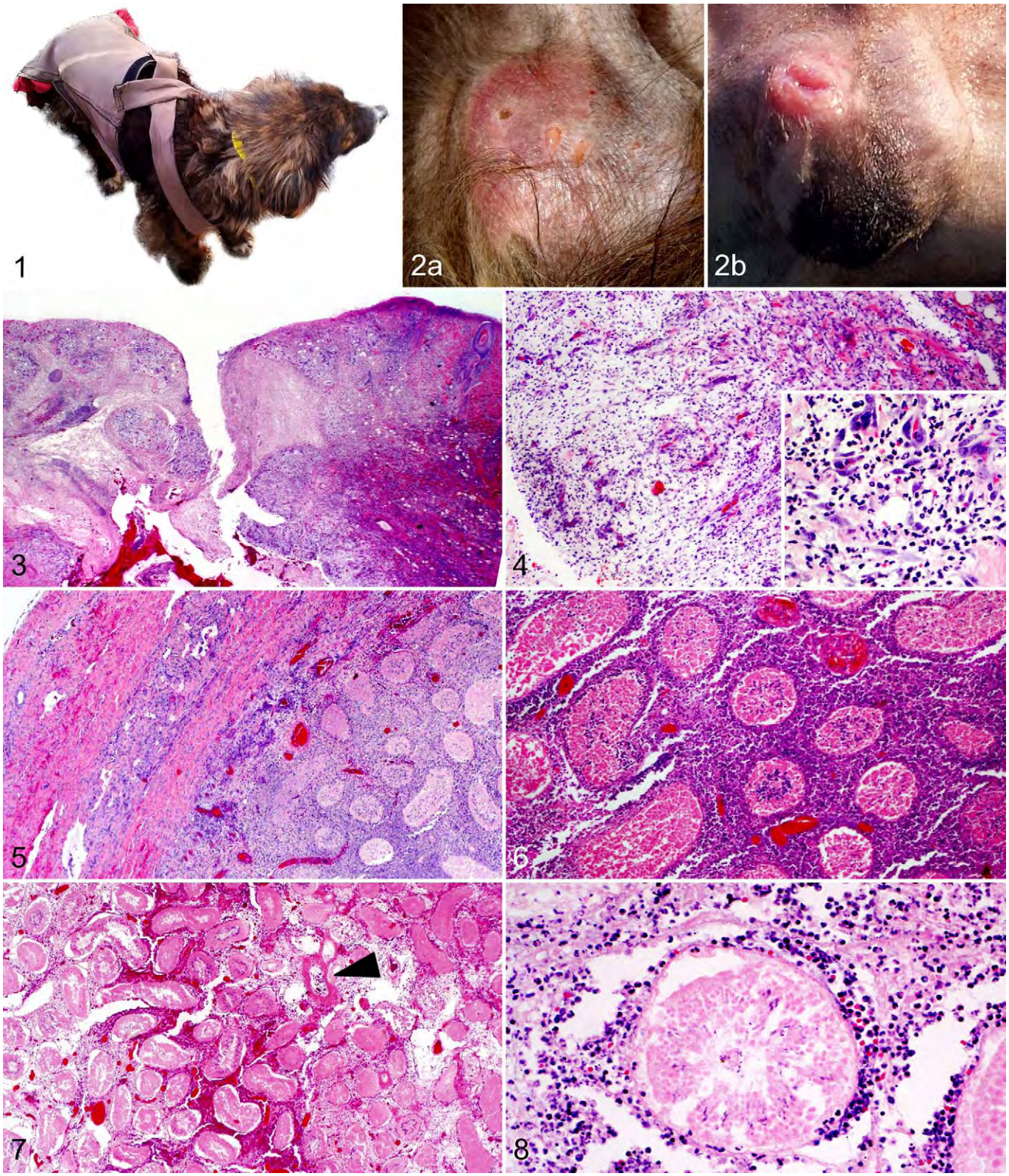

Figure 1. Dog No. 2. Purpose-made vest to prevent the dog from traumatizing its scrotal area after EsterilSol application. **Figure 2.** Scrotum, ventral view, cranial end toward the top of the image; dog No. 2. (a) Multiple small seeping ulcers 10 days after intratesticular administration of EsterilSol. (b) Scrotum shaved in preparation for ablation 13 days after administration of EsterilSol; a raised ulcer and a seeping tract are visible on the right testicle. **Figure 3.** Scrotal skin; dog No. 2. Necrotic skin and seeping tract flanked by inflammation, edema, hemorrhage, and granulation tissue. Hematoxylin and eosin (HE) stain. **Figure 4.** Scrotal skin; dog No. 2. Detail of the flank of the necrotic tract with maturing

dogs were sterilized as part of a male dog behavioral study undertaken by Veterinarians without Borders/Veterinarios sin Fronteras-Canada in Puerto Natales, a remote city in the Chilean Patagonia.

Dog No. 1 was a 10-year-old, 25-kg mixed-breed dog that developed scrotal erythema and constant licking 8 days after administration of 1 ml of EsterilSol per 27-mm-wide testicle; upon examination, a deep seeping tract was observed on the right caudal side of the scrotum, and the left testicle felt completely adherent to the overlying soft tissues and skin. Ablation was subsequently performed on day 8 postinjection. Dog No. 2 was a 3-year-old, tricolored 8-kg mixed-breed dog that received a 0.7-ml injection of EsterilSol in the 21-mm-wide right and 20-mm-wide left testicles. Seven days after administration, the dog began licking its scrotum constantly despite having received anti-inflammatory therapy and being kept in a purpose-made vest to prevent access to the area up to day 5 after EsterilSol treatment (Fig. 1). Anti-inflammatory therapy was resumed and the condition improved initially but worsened on day 10 after administration when several small seeping ulcers developed on the scrotum (Fig. 2a). Ablation was performed on day 13 following administration (Fig. 2b).

The testicles of both dogs and affected scrotal skin were fixed in 10% formalin and sent to the Atlantic Veterinary College, Prince Edward Island, Canada, for histologic processing and examination. Histological lesions were bilateral and similar in both dogs, varying only in chronicity. The testicles had undergone severe coagulative to liquefactive necrosis, with massive infiltration of neutrophils, most of which were degenerated, along with hemorrhage and/or fibrin deposition (Figs. 5–8). The lesions extended outward from the testicular parenchyma into the tunica albuginea, tunica vaginalis, and in areas corresponding to open tracts noted grossly, merged with the overlying scrotal skin. Vascular degeneration and necrosis, along with scattered thrombosis, were present in the testicle and overlying scrotum (Fig. 7). Seeping tracts were flanked by numerous neutrophils, severe fibrinous edema, occasional foamy macrophages, and rare eosinophils (Fig. 3). In dog No. 1, in which ablation was performed soon after clinical signs were reported, granulation tissue was loose and disorganized (immature, not shown), while in dog No. 2, in which clinical treatment had been attempted before ablation, there was a mixture of immature and organized (mature) granulation tissue (Fig. 4). In neither case were the seeping tracts located at the site of EsterilSol administration. The epididymides were empty of spermatozoa but had no significant lesions.

Based on reports of successful intratesticular injection of various forms of zinc gluconate (with arginine,[4,6,9] DMSO,[7] or unspecified buffers[9]), sterilization seems to result from coagulation necrosis[9] followed by massive fibrous (scar) tissue formation accompanied by atrophy or degeneration of the seminiferous tubules detectable 5[7] to 7[8] months after administration. Sterility, evidenced by aspermia (no semen ejaculate), azoospermia (no spermatozoa in ejaculate), necrospermia (dead or motionless spermatozoa in ejaculate), or oligospermia (sperm concentration below 20 million/ml), is reported between 26 days[9] and 4 months[10] postinjection, with some reversion to fertility in less than 1% of cases at 6 months postinjection.[9] In the present cases, orchiectomies were performed 8 and 13 days after administration, and histological changes were characterized by necrosis and inflammation, with granulation tissue formation along seeping tracts.

Severe adverse reactions to intratesticular injection of zinc gluconate neutralized with arginine, originally registered as Neutersol (Addison Biological Laboratory, Fayette, MO), have been reported.[1,4,9] The compound is known to cause severe damage if it comes in contact with nontarget tissues.[4] During prelicensing trials, studies on Beagle dogs reported variable occurrence of necrotizing reactions: 38% (5/13), 3% (1/30), and 25% (3/12).[9] Necrotizing orchitis and ulcerative dermatitis, many of which required scrotal ablation, were observed in 1% (3/255)[9], 3% (78/3000),[1] and 4% (4/103)[4] of treated dogs during sterilization campaigns using Neutersol, yielding an overall prevalence of 2.5% (85/3358). The present report concerns 2 of 36 (6%) dogs treated with EsterilSol. Although the percentage appears higher than expected based on the above-mentioned reports, it actually fits within what is probabilistically expected: when a mathematical model[10] runs a similar campaign (involving 36 dogs) 100,000 times with 2.5% of dogs developing adverse reactions, the simulation indicates that in 1 of every 4 campaigns, 2 or more dogs will develop adverse reactions.

Reported reactions are more commonly, but not exclusively, observed in dogs that receive the upper end of the recommended dose (0.8–1.0 ml of solution per testicle).[4] Although some authors speculate that the reactions are due to an improper injection technique,[4] all studies report cases that followed the manufacturer's instructions for application (such as using 1 needle to draw the solution and a different one to inject it into the tissue) and often describe lesions that developed several inches away from the injection site (dorsocranial portion of the testicle). In the present 2 cases, the application protocol was strictly followed, and lesions developed away from the injection site: as a single seeping tract in the caudal surface of the scrotum in dog No. 1 and as several randomly scattered seeping ulcers and then a single deep necrotic tract in dog No. 2.

Figure 4. (continued) granulation tissue and inflammation. Inset: predominantly neutrophilic inflammation. HE stain. **Figure 5.** Testicle; dog No. 2. Necrotic seminiferous tubules surrounded by severe interstitial inflammation that extends to the tunica albuginea vaginalis. HE stain. **Figure 6.** Testicle; dog No. 2. Necrotic seminiferous tubules with complete destruction of lining Sertoli cells and seminiferous epithelium, with residual spermatids in some tubules; severe inflammation is accompanied by loss of Leydig cells. HE stain. **Figure 7.** Testicle; dog No. 2. Widespread necrosis of seminiferous tubules with multifocal interstitial hemorrhage and vascular necrosis (arrowhead). HE stain. **Figure 8.** Testicle; dog No. 2. Detail of necrotic seminiferous tubule containing a few degenerated spermatids and surrounded by infiltrating inflammatory cells (mostly degenerated neutrophils) and necrotic debris. HE stain.

Ulceration associated with improper injection of EsterilSol occurs within 2 to 3 days postinjection,[2] yet the time lapse between administration of zinc gluconate neutralized with arginine in any of its registered presentations (Neutersol, EsterilSol, or Zeuterin) and the development of severe necrotizing reactions is extremely variable, from a couple of days to 3 months.[9] This variability in onset and the evidence of severe necrosis and inflammation in the testicular tissue of dogs with and without clinically evident adverse reactions suggest that the compound remains highly irritating for at least several weeks. Since the necrotizing reactions are not necessarily associated with the injection site, it must be possible for the compound to escape the confines of the tunica albuginea/vaginalis independent of a needle tract. Whether this is associated with mild to moderate trauma to the area is uncertain, but it is recommended to ensure that the dogs do not lick or bite the scrotum for at least 7 days postinjection.[9]

Adverse reactions could, alternatively, be due to drug-induced vascular injury. The possibility of EsterilSol causing toxic vasculitis, resulting from direct damage to the endothelium and/or tunica media, was considered. However, since no extra-testicular vascular changes were evidenced in either case, the vascular degeneration and necrosis observed are likely the result of local irritation/inflammation.

The use of chemical sterilization is of growing interest for government and nongovernmental organizations associated with management of free-roaming and stray dog populations.[1,4] EsterilSol is an appealing choice for situations when the public is unlikely to opt for surgical castration, since it is FDA approved and its application requires little equipment. The occurrence of severe adverse reactions at unpredictable times after administration, however, emphasizes the need for the provision of long-term monitoring and veterinary care during sterilization campaigns.

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