



ESTERILSOL™

(“ZINC NEUTERING”)



April 2012

Product Profile and Position Paper

Esterilsol™ is a non-surgical sterilant for male dogs delivered via intratesticular injection. The active ingredient is zinc gluconate neutralized by arginine. The formulation causes permanent infertility in one treatment. It is also known as “zinc neutering”.

ESTERILSOL™ (“zinc neutering”)

NON-SURGICAL STERILANT FOR MALE DOGS

INTRODUCTION

This product profile and position paper was created from a review of both published and unpublished literature, promotional information, and input from experienced users, representatives of the relevant organizations, and related experts. ACC&D did not conduct the clinical research on its own; the data quoted were obtained from the cited sources.

This formulation of the chemical compound of zinc gluconate neutralized by arginine was developed to chemically sterilize male dogs. The formulation was initially introduced in the U.S. under the name Neutersol® and developed by Pet Healthcare International. Esterilsol™ is the current name and Ark Sciences is the technology owner. This method of sterilization is also known as “zinc neutering” in the U.S. Esterilsol is derived from natural ingredients: zinc, glucose and arginine. It causes permanent infertility in a single treatment without removal of the testicles. Esterilsol offers savings of time, money, and space (both procedure and recovery space) for many veterinary clinics, animal shelters, and pet sterilization programs. Additionally, it provides an option for pet owners who prefer a non-invasive method of sterilization and for those whose pets cannot safely be anesthetized. Esterilsol may also be useful in settings in which surgery is particularly difficult or dangerous to provide. (Light sedation is recommended, but full anesthesia is not necessary, and there is no incision.)

MECHANISM OF ACTION

As with any medical intervention, safety and effectiveness depend upon proper administration. The exact mechanism of action is not known. The following is based on a description provided by Ark Sciences. The product should be administered as an intratesticular injection into the center of the testicle via the dorsal cranial portion of testicle, parallel to the longitudinal axis. After injection the compound diffuses in all directions from the center of the testis. In the concentration used, zinc gluconate acts as a spermicide and destroys spermatozoa in all stages of development and maturation. It results in permanent and irreversible fibrosis in the seminiferous tubules, rete testis and epididymis. This produces a reduction in the size and texture of the testicles and permanent sterilization. Testosterone production is reduced by 41-52%, and the endocrine feedback system remains intact. Zinc gluconate is absorbed and metabolized by the body within 72 hours after the injection.

ESTERILSOL OR “ZINC NEUTERING” IN THE U.S.

This formulation is approved by the U.S. Food and Drug Administration (FDA) for sterilization of male dogs from 3 to 10 months of age. The label allows for use “by or on the order of a licensed veterinarian”. While it was available in the U.S. under the name Neutersol it was used off-label in dogs over 10 months of age. Neutersol was introduced in the U.S. in 2003 by Addison Laboratories. In 2005, production and distribution were discontinued after a business divorce between Pet Healthcare and Addison Laboratories. Pet Healthcare International no longer has any rights to this technology. Ark Sciences, LLC, currently owns all rights and intellectual property. They have announced plans for a U.S. launch in 2012, focusing initially on the nonprofit sector. Through 2012, Ark Sciences is expected to offer all nonprofits a price of \$12.50 per ml. Ark Sciences reports that on average, 1ml will sterilize one dog (with a range of \$5 for the smallest dose to \$25 for the largest).

ESTERISOL INTERNATIONALLY

Ark Sciences introduced Esterilsol in Mexico in 2008 and began selling the product to private practice veterinarians, government programs, and non-governmental organizations (NGOs). In 2010 Esterilsol received regulatory approval in Bolivia, Panama, and Colombia. In Mexico, Colombia, Bolivia, and Panama, Esterilsol is approved for use in dogs three months and older. In Colombia it is also approved for use in cats. International distribution is temporarily on hold while regulatory agencies review paperwork from the new manufacturer. (See following paragraph.) Ark Sciences has announced plans to extend distribution to additional countries. In some countries in which Esterilsol is not approved by regulatory agencies, it has been used on a limited basis in field research projects with government permission.

In 2011, Ark Sciences moved production from Argentina to a manufacturing facility in the U.S. It has not yet been announced what the vial size will be from the new manufacturer. (Previously, both a 2ml and 20ml vial were produced.) The cost of Esterilsol varies by country; cost is likely to be different for private practice veterinarians than for government agencies and NGOs. International pricing information is not currently available.

ACC&D Field Studies

ACC&D has supported several Esterilsol field projects and studies. The purpose of the grants has been to help organizations extend reach of their population control programs and to gather data to increase learning about field use of the product. More information on our grant-supported programs is available at www.acc-d.org/EsterilsolGrants.

Country	Esterilsol Distributor
Mexico	Ark Sciences Mexico
Colombia	Gabrica S.A.
Panama	Suplidora Internacional
Bolivia	Mathiesen Group

USE AND EFFECTIVENESS

Esterilsol is administered via an injection to each testicle with either a 28 gauge, 3/4 inch or a 30 gauge, 1/2 inch needle, depending on the dose determined for the individual dog. Ark Sciences plans to distribute the needles with the product. (These needle sizes are not readily available through veterinary suppliers.) Dosage is determined by measuring each testicle with a caliper provided with the product. The correct dose is indicated on the caliper and is based on the maximum width of each testicle. Esterilsol is labeled for use in dogs with an individual testicular width of 10-27mm; some dogs' testicles will not fall into this range. Ark Sciences recommends light sedation to ensure that the dog holds still during the injection. (Anesthesia is not necessary. Reversible sedation is commonly used so that dogs are awake and alert in as little as 15-20 minutes after the Esterilsol injection/zinc neutering.) Most programs with which ACC&D is familiar routinely sedate dogs (to varying degrees, depending on the dog and the situation) prior to Esterilsol injection. Experienced practitioners report that the process of measuring the testicular width (to determine dose), preparing the injections, and administering the injection into each testicle takes two to five minutes.

Following proper administration protocol is critical to reduce the risk of injection site reactions (see "Complications" below).

In the clinical trial results presented to the U.S. Food and Drug Administration (FDA), this formulation was found to cause permanent sterility in 99.6% of treated dogs. Pre-pubescent males never become fertile. Post-pubescent males become infertile in less than 30 days after treatment. Ark Sciences recommends use of a non-steroidal anti-inflammatory drug (NSAID) to prevent discomfort which may be caused by post-injection swelling. (Scrotal pain was the most common local reaction observed in the FDA-reviewed study, reported in 6.3% of dogs). Most programs with which ACC&D is familiar report consistently using NSAIDs.

Upon hearing that administration is through an intratesticular injection, many people express concern about pain caused by the injection itself. However, in studies reviewed and accepted by the FDA (in which 76% of dogs were not sedated), only 2.5% of dogs showed discomfort by moving or vocalizing. The other 97.5% did not show any reaction to the injection.

Following injection, the testicles atrophy over a period of time ranging from weeks to months, resulting in a reduction in testicular size and changes in shape or texture. These changes may or may not be symmetrical.

Esterilsol does not reduce testosterone to the same concentration as surgical castration does, and its effects on hormone-

dependent diseases and behaviors have not been established. A lesser impact on testosterone production is considered a drawback by some pet owners who seek reproductive behavioral changes commonly associated with surgical castration. However, other pet owners prefer a limited impact on testosterone, citing health and behavior risks associated with surgical castration. It is important to note that testosterone levels range widely among dogs.

In regard to behavior, one should remember that while surgical castration has been promoted as a treatment for behavior problems, it has only been shown to be linked to decreases in indoor urine marking, roaming, sexual mounting, and dog-to-dog aggression around females in estrus, and does not always decrease these behaviors. Studies report inconsistent findings on how castration status correlates to aggressive behavior toward humans. Currently, Veterinarians Without Borders—Canada is collaborating with dog population control and dog behavior experts to conduct a study comparing observed behavior changes after sterilization in dogs surgically castrated with those sterilized with Esterilsol. ACC&D is supporting this study and will share results as they become available. However, additional studies will be needed to provide a more complete picture of what behavior changes may be expected with either sterilization method in different populations of dogs.

In regard to health, surgically castrated dogs have been found to have lower risk for some health problems but higher risk for others. There is no conclusive evidence that surgically castrating male dogs makes them healthier overall. While more is known about the non-reproductive effects of surgical castration than sterilization with Esterilsol, further study on both methods is merited.

Dogs sterilized with either Esterilsol or surgical castration may continue to engage in mating behavior. Therefore, neither method of sterilization should be expected to prevent the spread of transmissible venereal tumors (TVT). (Male dogs surgically castrated prior to puberty display significantly less mating behavior than those castrated as adults. Comparable data on Esterilsol is not available.)

As with surgically spayed female dogs, visual confirmation of sterility may be difficult in Esterilsol-treated dogs. Although the testicles do atrophy over time, the decrease in size is variable. Palpation of the testicles may reveal an abnormal contour and/or texture resulting from scar tissue. (However, other conditions, such as the zoonotic disease *Brucella canis*, also cause atrophy and scarring of the testes.) Other methods of identifying dogs as having been sterilized with Esterilsol should be utilized. Ark Sciences provides uniquely numbered ID tags, and recommends a “Z” tattoo on the abdomen near the inner thigh. Alternately, or in addition, a microchip may be used; microchip companies will include information on neuter status in a dog’s record if this information is provided when pet owners register their pet’s microchip. Past and current programs with which ACC&D is familiar have used tattoos indicating sterilization status on the inside of the ear, inner thigh, abdomen, or pre-scrotal area, either alone or in addition to microchips.

COMPLICATIONS

During FDA-required Target Animal Safety work, this formulation of zinc gluconate caused no deaths and was determined to be safe. One of its greatest appeals is that it does not require general anesthesia or a surgical incision, completely removing the risk factors (the most serious of which is death) associated with surgery. (One U.S. study of anesthesia-related deaths found the risk for dogs to be 1 in 1,849.) However, any medical intervention presents risks.

Complications reported with Esterilsol use include—but are not limited to—the following:

- **Testicular swelling and pain.** Mild to moderate testicular swelling is to be expected and peaks 24-48 hours post-injection. Follow-up consultation with the administering veterinarian or another veterinarian familiar with the product is indicated for dogs displaying severe swelling. Mild to moderate pain is also to be expected and peaks 48-72 hours post-injection. Ark Sciences recommends administration of an injectable non-steroidal anti-inflammatory drug (NSAID) at the time of treatment to minimize post-injection discomfort.
- **Vomiting.** In one study, 12 out of 270 dogs (4.4%) vomited one to four times within four hours post-injection. Ark Sciences notes that vomiting is more likely to occur if xylazine is used as a sedative. Fasting for 12 hours before

the injection is recommended to decrease the chance of vomiting.

- **Injection site reactions.** In some cases, adverse reactions may occur at the injection site. Reactions range from scaly scrotal skin to ulceration and (rarely) tissue necrosis requiring scrotal ablation (surgical removal of the scrotal tissue and contents). Adverse reactions are believed to be primarily due to imprecise injection technique or failing to follow the recommended administration protocol in other ways (e.g. incorrect needle size, use of product from a vial that was first punctured more than six hours prior). Serious reactions may occur when a dog chews or licks the scrotum due to irritation caused by a mild reaction. Following injection technique and all administration protocol precisely and follow-up care is critical to prevent adverse reactions.

The rate of injection-site reactions requiring follow-up treatment ranged from 0.7% to 3.9% in early studies. Incidence decreased substantially as better practices (including administering a light sedative to provide chemical restraint during injection and using separate needles for drawing up and injecting the solution) were implemented after the initial introduction. Reports from a large field trial in Mexico showed an average complication rate of 1.2%. In Peru in 2010, 249 dogs were treated and only three adverse reactions (1.2%) were reported. However, in Colombia and the Dominican Republic in 2010, higher rates of injection site reactions were reported. (The number of dogs in these two projects was too small for a percentage of reactions to be statistically reliable.)

It is important that dogs have appropriate after-care with access to a veterinarian if any problems arise. Ark Sciences' Esterilsol Client Information Sheet recommends that dogs be prevented from licking or biting the scrotum, that their exercise be limited to leash walks for several days, and that they not be kept on hard or wet surfaces. (Of 270 dogs that participated in the clinical trial reviewed by the FDA, two developed serious scrotal ulcerations. In both cases these reactions were associated with the dogs chewing the scrotum in the days following their injection. Ark Sciences recommends use of an Elizabethan collar for dogs observed licking or chewing the scrotum.) Dog caregivers are asked to contact the administering veterinarian if they see "any redness, discharge, or broken skin in the scrotal area". After sedation, dogs are less able to regulate their body temperature and should be kept in a comfortable environment (usually inside) for the first night after treatment. Because of the need to control and monitor dogs post-injection, use on dogs that do not have owners or caregivers that can and will comply with guidelines may result in more frequent and more severe adverse reactions.

Esterilsol does not contain any preservatives. It is anticipated that a vial will hold enough product to treat more than one dog, on average. Ark Sciences recommends that any unused product be discarded within six hours after the first puncture of the vial to prevent potential product contamination, which could lead to adverse reactions.

It is possible that environment, individual animal physiology and health status, and/or other factors might also influence the rate of adverse reactions.

- **Long-term effects.** In the past decade (and on a more limited basis for the past several decades), thousands of male dogs have been successfully neutered with this zinc gluconate formulation with no reports of any long-term detrimental effects.
- **Other side effects.** These include other local reactions as well as neutrophilia, anorexia, diarrhea and leukocytosis. Package insert should be referenced for details.

(A description and discussion of adverse reactions observed in the FDA-reviewed clinical trial are available through the Freedom of Information Act. The report describing the FDA-reviewed studies is available on ACC&D's website at www.acc-d.org/Esterilsol.)

CONCLUSION

Esterilsol is a safe, effective method for sterilizing male dogs without surgery. Though sedation is recommended and administration protocol requires precision and care, Esterilsol may offer savings in cost, time and facility requirements, helping animal welfare organizations sterilize more animals and/or redirect resources to other lifesaving projects. It also presents an option for pet owners who are opposed to castration or surgery, or whose pets cannot safely undergo anesthesia. It is safer for use in dogs with owners or caregivers that can monitor the dogs in the days following treatment. Esterilsol has regulatory approval in the U.S., Mexico, Colombia, Panama, and Bolivia and is expected to be commercially available in these countries in 2012. Ark Sciences has announced future plans to introduce Esterilsol in other countries.

RECOMMENDATIONS AND DISCUSSION

ACC&D believes that Esterilsol can be a meaningful tool for organizations to reach further in their sterilization programs. Esterilsol may offer significant cost benefits over surgical castration. Space and time for recovering dogs from anesthesia is a limiting factor for many organizations, and the shorter recovery time required for Esterilsol-treated dogs may provide a significant advantage to such organizations, allowing them to sterilize more dogs. Savings in cost, time, and space will vary depending on an organization's infrastructure and the efficiencies achieved with surgical sterilization.

ACC&D recognizes that sterilizing males alone may have limited impact on population size unless a very high rate is achieved. Female reproduction must be controlled to achieve population stability or reduction. However, birth control is part of a comprehensive approach to managing dog populations and improving the welfare of animals in any community. We understand that population control programs around the world provide sterilization for both females and males as part of their work teaching responsible pet ownership and improving the health of animals in the communities they serve. And for individual population control programs, the ability to spend less time and money on male dog sterilization may free up resources to sterilize more female dogs. It is also important to note that there is very little understanding of the population dynamics of free-roaming dogs and cats (more data is available on wildlife populations, but this may or may not apply to dogs and cats), so the impact of sterilizing males has not been conclusively established.

ACC&D realizes that administration protocol and injection technique are critical to reduce the incidence of injection site reactions. Ark Sciences currently requires all practitioners to receive hands-on training, a practice ACC&D supports. We urge all practitioners to follow administration protocol exactly. As with surgical castration, systems should be put in place to help dog owners and caregivers provide after-care for treated dogs and to receive immediate follow-up care for any adverse reactions that occur. Minor reactions can become severe reactions if prompt care is not sought or received. Dog owners should adhere to all follow-up care instructions carefully and contact their veterinarian with any questions or concerns. Use in dogs that do not have owners or caregivers who can provide the recommended monitoring and care post-treatment (as may be the case for unowned or loosely-owned dogs) may result in a higher rate of and more severe adverse reactions. Organizations that are able to follow up directly with dogs in their home (or, for unowned dogs, their home territory) for several days post-injection may be able to use Esterilsol on these dogs more safely.

Most pet owners, veterinarians, and animal welfare organizations are accustomed to the risks and benefits of surgical sterilization. While many are enthusiastic about the concept of alternative non-surgical methods of sterilization, new approaches can be met with caution and some skepticism. ACC&D feels strongly that any new sterilization method should

What about other species?

While it was available in the U.S., this formulation was used off-label to sterilize other species including cats, bears, wolves, goats and other livestock. As noted above, approval for use in cats has been received in Colombia. Ark Sciences reports that they are seeking regulatory approval for use of Esterilsol in cats in the U.S. and one or more other countries. However, no published or otherwise publically-available studies establish dosage, safety, or efficacy in cats (or any species other than dogs).

Esterilsol™ presents a less invasive neutering method for cats. Time savings may differ compared to dogs because surgically castrating cats is a simpler and faster procedure than surgically castrating dogs. The impact of Esterilsol on cat behavior (e.g. urine marking, vocalizing, fighting with other cats) has not been established. Data on what behavior changes can be expected will help guide pet owners, veterinarians, animal welfare organizations, and those managing feral and free-roaming cat populations.

be introduced with careful thought and adequate resources for education so that potential users can determine the best use of new non-surgical methods. ACC&D encourages veterinarians, animal welfare organizations, and dog caregivers/owners to consider new sterilization methods with an open mind, to explore the characteristics of any method, and to consider how alternate methods might help them achieve their goals.

As with any medical intervention, ACC&D encourages all pet owners, veterinarians, and organizations to consider the associated risks and benefits and to engage in open discussion with other stakeholders (i.e. family members, practice partners, organizational staff/volunteers) about potential use.

When treating un-owned or loosely owned free-roaming dog populations as part of TNR (Trap/Neuter/Return) or ABC (Animal Birth Control) programs, both surgery and Esterilsol treatment present some risks for the individual animal, especially when dogs are released soon after the procedure with limited or no supervision. Organizations need to weigh the risks and benefits for both individual animals and the population as a whole. With Esterilsol, the potential with for mild adverse injection site reactions to progress to serious reactions in dogs that are not properly supervised should be considered carefully. ACC&D recommends Esterilsol use be limited to dogs with owners/caregivers that can provide appropriate care.

Additional information is available at www.acc-d.org/Esterilsol and www.acc-d.org/EsterilsolGrants. Questions and comments can be directed to info@acc-d.org. Ark Sciences' web address is www.arksciences.com.

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