Anim. Reprod., v.4, n.3/4, p.119-124, Jul./Dec. 2007

Evaluation of zinc gluconate, either associated or not to dimethyl sulfoxide, as contraceptive method for male dogs

F.R.M. Soto[1,4], W.G. Viana[2], A.J. Sousa[1], S.R. Pinheiro[2], G.B. Mucciolo[2], F.Y.M. Hosomi[2], S.S. Azevedo[3], R.A. Dias[2]

[1]Centro de Vigilância Sanitária e Controle de Zoonoses "Tereza Rodrigues de Camargo", Ibiúna, SP, Brazil.
[2]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, Brazil.
[3]Unidade Acadêmica de Medicina Veterinária, Universidade Federal de Campina Grande, Campus de Patos, PB, Brazil.

Abstract

The aim of this study was to evaluate the efficacy of zinc gluconate, either associated or not to dimethyl sulfoxide (DMSO), as a contraceptive method for the canine male population. Twenty-nine sexually mature male dogs were divided into five groups: Group I (control, saline solution); Group II (zinc gluconate 13.1 mg); Group III (zinc gluconate 26.2 mg); Group IV (zinc gluconate 13.1 mg and DMSO 0.5%); Group V (zinc gluconate 26.2 mg and DMSO 0.5%). Chemical injection was directly administered into the dorsal cranial portion of each testicle once. Animals were examined for testicular size and spermogram 15 days before chemical injection, on Day 0, and then every 15 days for 6 months. Pain and sensitivity were observed in one animal of Group III at the first day after treatment. Signs of eosinophilia were observed 120 days after drug injection in Groups II and III. Dogs did not become azoospermic, but it was observed that from the second collection on, Group V sperm cell number per mm^3 values were significantly lower than control group. Individual group analysis showed a significant cell motility decrease for Groups III, IV and V. Group V motility values were significantly lower than control group for collections 2, 3, 4, 5 and 8. Twelve months after chemical injection, two dogs of the control group and four from the group with more spermatic alterations (Group V) were surgically neutered and their testicles were examined through histopathological staining, revealing testicular degeneration, decreased number of germ cells, areas of atrophy, disruption of seminiferous tubule architecture, and loss of germ and Sertoli cells. The association of DMSO (0.5%) to zinc gluconate (26.2 mg) may be indicated as a contraceptive method for male dogs.

Keywords: dogs, zinc gluconate, dimethyl sulfoxide, contraceptive method.

Introduction

Canine overpopulation and stray dogs represent a worldwide problem, compromising public health and animal welfare. This problem has a negative influence on environmental hygiene and zoonoses (World Health Organization, 1992). In Brazil, reports on canine population dynamics show that males are a majority between 60 and 70% of total canine population, and females are between 30 and 40% of the total canine population (Dias, 2001; Paranhos, 2002; Soto, 2003). Educational interventions on responsible ownership, strict legislation and surgical neutering and adoption programs are methods to control canine overpopulation, minimize road kills and avoid the necessity of euthanasia (Nassar and Mosier, 1980; Larrieu *et al*., 1990; Rowan, 1994; Reichmann, 2000; Soto, 2003).

Of all contraceptive methods for canine male population control, surgical sterilization is the most known and performed (Clevenger and Kass, 2003; Gomes *et al*., 2003). Some restriction with the method might occur from owners who consider this technique not compatible to animal welfare (Immegart and Threlfall, 2000; Soto, 2003). Chemical orquiectomy with chemical agents has been suggested as a fast and low cost alternative that can be used in a wide range of canine population, especially in poor regions where the problem is more intense. Of all available drugs for this purpose, chlorhexidine digluconate 3%, gossipol and zinc gluconate are utilized in the chemical orquiectomy (Immegart and Threlfall, 2000; Cedillo *et al*., 2006).

Dimethyl sulfoxide (DMSO) is used as drug vehicle because it increases skin permeability, facilitating absorption. Due to its biological activity related to cell membrane stability, Pineda and Hepler (1981) and Herath *et al*. (2004) used DMSO as a potentiator for different drugs to sterilize dogs. However, as a consequence of this alteration in membrane permeability, this agent can induce cancer and other non desirable effects (Jacob and Herschler, 2003).

The objective of this study was to evaluate the efficacy of zinc gluconate, either associated or not to DMSO, as drug of choice as contraceptive method for the canine male population.

Materials and Methods

This experiment was performed in the animal shelter "Lar São Francisco de Assis", Ibiúna, SP, Brazil. Twenty-nine crossbred males aging from one to four years old and weighing between 10 and 20 kg were

[4]Corresponding author: chicosoto@ig.com.br
Phone: +55 (15) 3294-2223/3248-1880
Received: June 18, 2007
Accepted: November 27, 2007

selected. A clinical examination and hemogram was performed on all animals, and their libido was confirmed by placing them with females in estrus. Their previous medical records of reproductive problems or traumas were not available.

Chemical injection was administered by direct injection to the testicles without anesthesia or sedative. Each dog was positioned lying on its back, and the needle was inserted into the dorsal cranial portion of the testicle beside the epididymis.

Drug volume in milliliter varied according to testicular size in millimeter:

Testicular size	Chemical volume
12-17	0.3
18-23	0.7
24-27	1.0
27 or more	1.5

Chemical concentration was made as follows: Group I: 4 dogs (control, saline solution); Group II: 6 dogs (zinc gluconate 13.1 mg); Group III: 6 dogs (zinc gluconate 26.2 mg); Group IV: 6 dogs (zinc gluconate 13.1 mg and DMSO 0.5%); Group V: 7 dogs (zinc gluconate 26.2 mg and DMSO 0.5%).

All animals were examined for testicular size and spermogram was performed 15 days before chemical injection, on Day 0 and then every 15 days for 6 months. The spermogram analyzed sperm cell motility rate, ejaculate volume in milliliters, sperm cells per cubic milliliter and sperm morphology through formol saline conservation and Buker camera cell counting. Body temperature, cardiac and respiratory rhythms and mucosal color were taken daily on each animal. Animals were checked for signs of pain, licking at the injection site, appetite and libido. The method of Ridley and Hilson (1967) was used for value measurement and quantitative evaluation. Sperm cell number per mm^3, sperm cell morphology, sperm motility, ejaculate volume, testicular size and body weight were compared between groups for each collection through the Kruskal-Wallis test. As complement of the analyses a Mood test was used. The same parameters were compared separately between semen collections within each group with the Friedman test. For each parameter mean and standard error of the mean were calculated. Confidence intervals were the same standard means error. The significance level used was 5%. Twelve months after chemical injection, two dogs of the control group and four from the group which showed more spermatic alterations were surgically neutered and their testicles examined through hematoxilin-eosin histopathological staining.

Results

Results showed that all animals had no alteration on body temperature, cardiac and respiratory rhythms and mucosal color during 120 days after drug injection. Pain and sensitivity were observed only in one animal in Group III (1/6), and in 4.0% of total treated animals at the first day after treatment. No alterations on food consumption and libido were observed, and swelling was not measured.

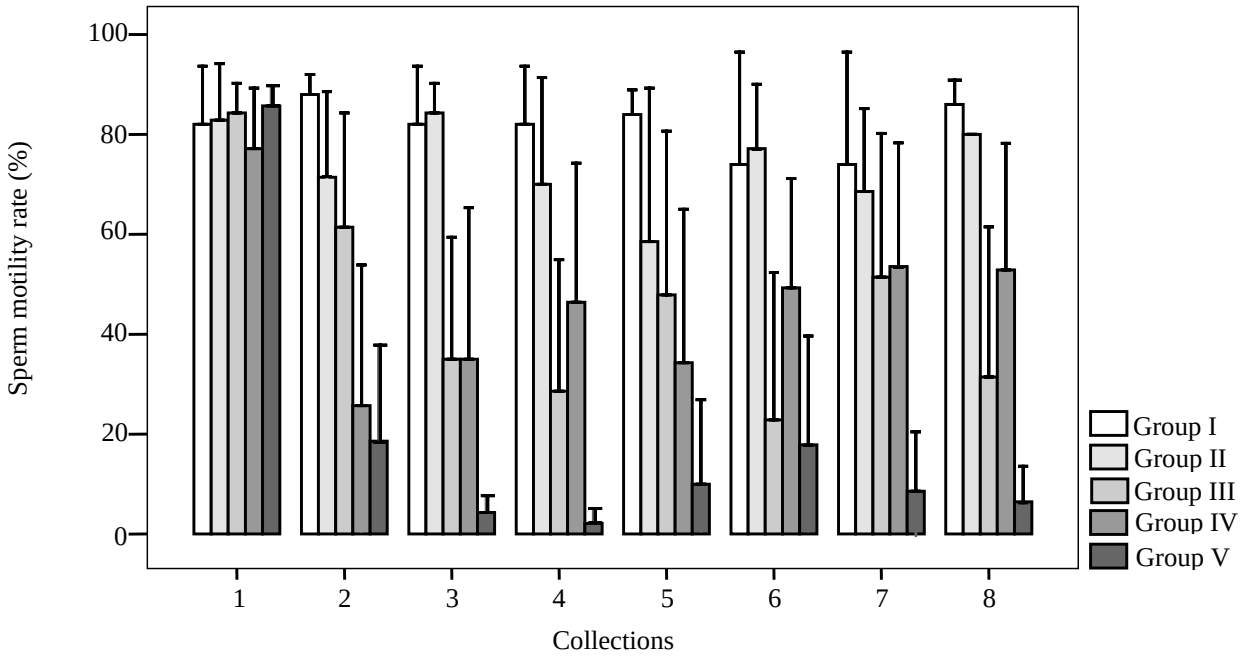

Figure 1. Mean of the sperm motility rate (%) according to group and collection day.

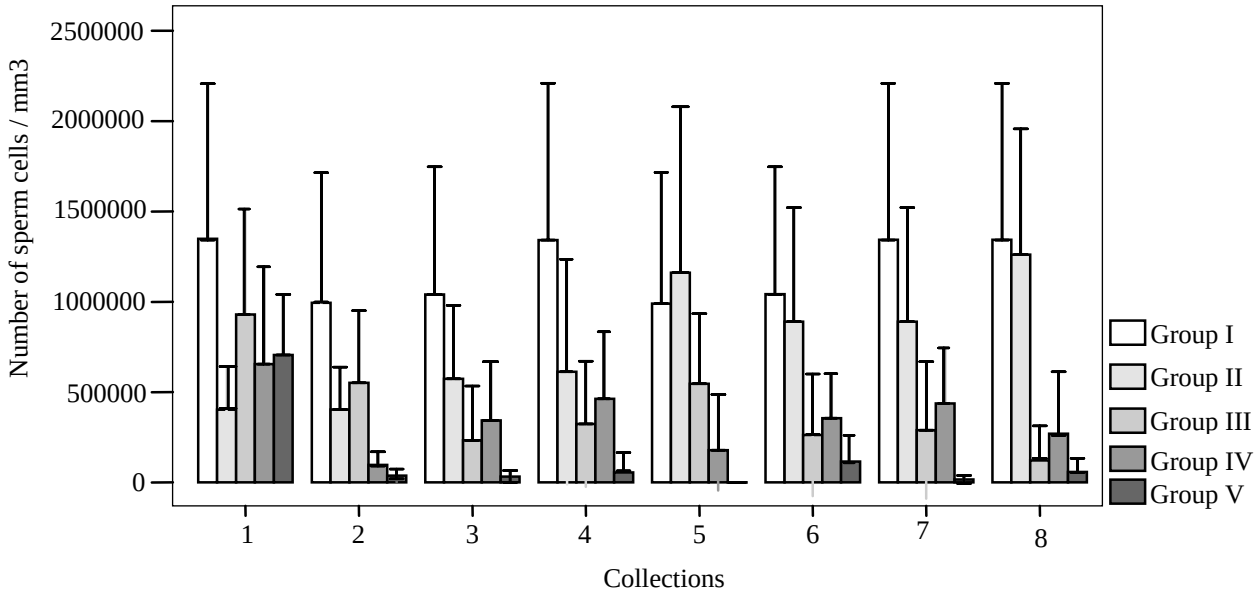

Figure 2. Mean of the number of sperm cells in cubic millimeters (mm^3) according to group and collection day.

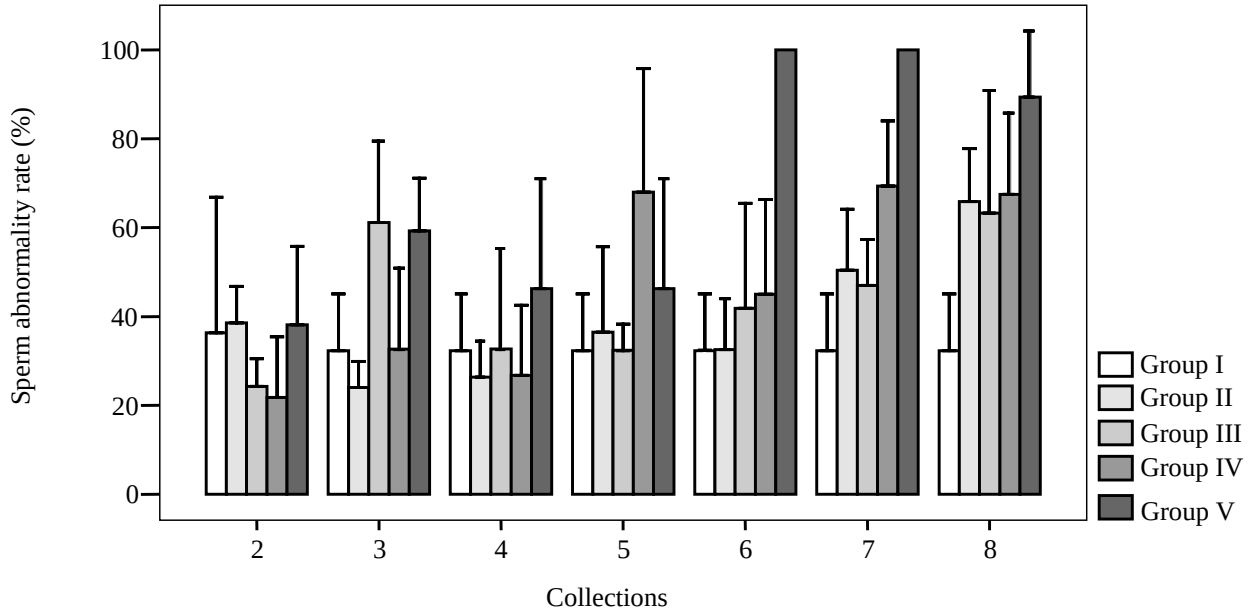

Figure 3. Mean of the sperm abnormality rate (%) according to group and collection day.

All dogs had normal parameters for the first erythrogram and leucogram. However, 120 days after drug injection, Groups II and III presented signs of eosinophilia. There was no difference in testicular sizes among the groups for each evaluation ($P > 0.05$). When groups were individually analyzed, a decrease was observed in testicular size for Groups III and IV ($P < 0.01$ and $P < 0.002$, respectively). Results for sperm motility rate, cell number per mm^3, sperm abnormality rate and semen volume are shown in Fig. 1 to 4.

Dogs did not become azoospermic, but it was observed that sperm cell number per mm^3 from the second collection on for Group V values were significantly lower than control group ($P < 0.05$). The analysis of Group II showed an increase in sperm cell number per mm^3 ($P < 0.004$), while the same parameter decreased for Groups IV and V ($P < 0.01$ and $P < 0.001$, respectively).

When comparing all five groups for sperm abnormal morphology, only at the third collection values of Group V were significantly higher than Group I ($P < 0.006$). Groups II and III had an increase of sperm abnormal morphology during the experiment ($P < 0.006$ and $P < 0.02$, respectively).

In relation to sperm cell motility, Group V values were significantly lower than Group I ($P < 0.05$) for collections 2, 3, 4, 5 and 8. Individual group analysis showed a significant decrease in motility for Groups III, IV and V ($P < 0.05$). There was no difference for ejaculate volumes among groups or when analysis was made for each group individually ($P > 0.05$). There was no statistical difference in animal weight among groups.

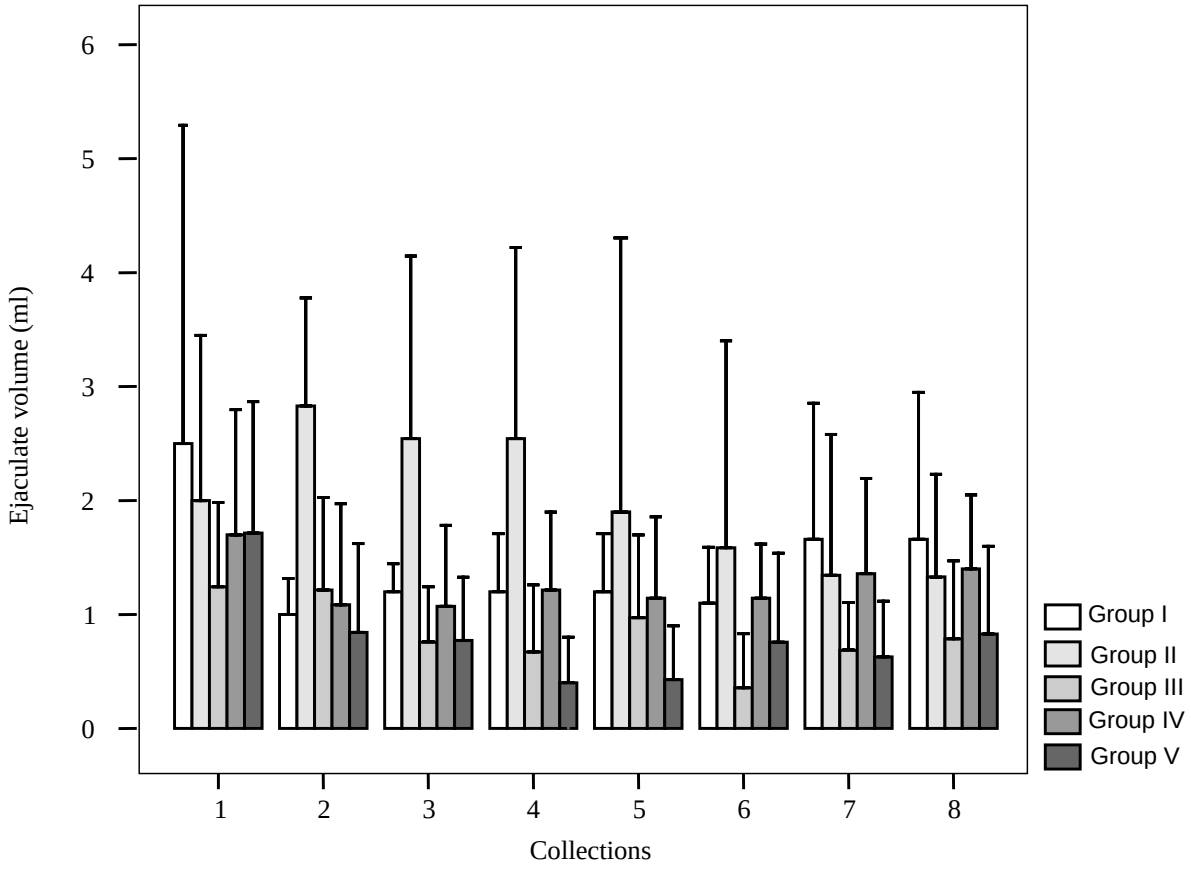

Figure 4. Mean of the ejaculate volume in milliliters (ml) according to group and collection day.

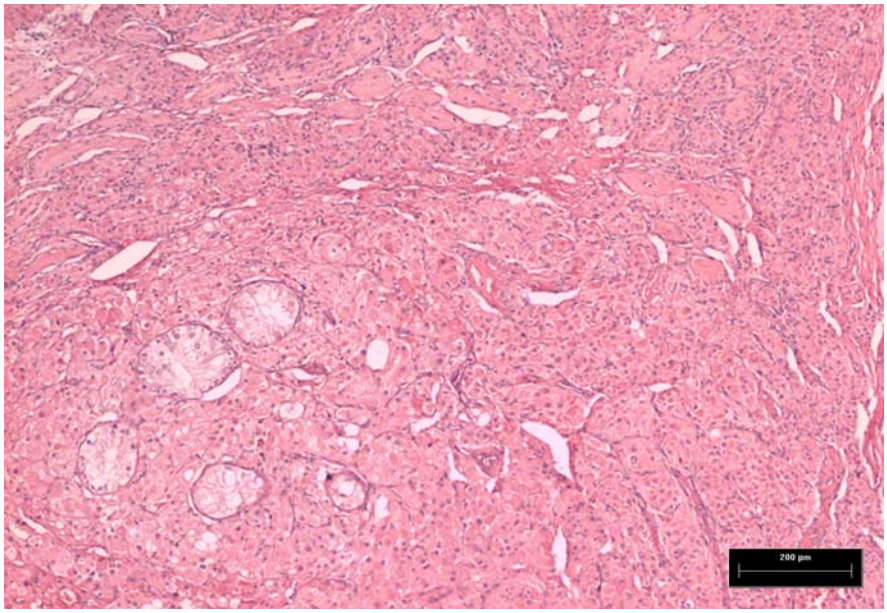

Figure 5. Histopathological photomicrography showing severe testicular atrophy in treated animal. Bar scale: 200μm.

Group V histopathologic analyses showed testicular degeneration and decreased number of germ cells. Several areas presented intense atrophy (Fig. 5), with disruption of seminiferous tubule architecture, and loss of germ and Sertoli cells. Focal areas of intratubular calcification and vasiculitis were observed. It was also verified an epididymal impair with evident loss of tubular architecture and diminished epididymal tubules intraluminal content.

Histopathologic results for Group I revealed less severity, extension and degree of tissue damage than the other groups testicles examined.

Discussion

Sensitivity reactions and pain were observed in only one dog from Group III at the first post injection day. These results are in agreement to similar studies. Tepsumethanon *et al*. (2005) did not observe behavior alteration or signs of pain in 5 five dogs, and Cedillo *et al*. (2006) found that only 3.1% of zinc gluconate injected dogs had scrotal ulcers or fistulas.

All animals showed signs of eosinophily at the last hemogram, which can be explained by the role of these cells increasing inflammatory response and causing tissue damage (Jones *et al*., 2000).

Fahim *et al*. (1993) described zinc gluconate as a non mutagenic and non carcinogenic product after one year evaluation in dogs. In the present study, no tumor cells were observed, however DMSO is considered as a possible carcinogenic agent (Jacob and Herschler, 2003) and this chemical must be studied in a longer term period before it can be considered totally safe.

In the second collection, Group V sperm number was significantly lower than control group. The same situation was observed for Groups IV and V, suggesting that zinc gluconate 26.2 mg and DMSO 0.5% association is a promising method for chemical neutering. Similar studies have indicated azoospermy in animals from 26 days after chemical injection, thus considering them infertile and zinc gluconate efficient for chemical neutering (Fahim *et al*., 1993; Tepsumethanon *et al*., 2005; Cedillo *et al*., 2006).

Group V sperm motility was significantly lower than the control group for all post inoculation collections, except six and seven. Groups III, IV and V presented a significant decrease of this same parameter. Spermogram minimal reference values for dogs to consider a possibility of fecundation is 85% motility, sperm number 0.3 x 10^9/ml and normal cells number at least 80% (Morrow, 1988), thus, Group V spermogram values after drug injection are below necessary for fecundation to occur. Fahim *et al.* (1993) worked with zinc gluconate and also observed diminishment of sperm motility in 50 dogs at 90 days post injection.

When comparing sperm morphology results, Groups II and III showed an increase in abnormalities, although there was no difference observed between these groups and the control. Only at the third collection it was verified a significant increase between Group V and control.

Histopathologic findings are characteristic of mild to moderate trauma, since immune responses are suppressed in immunologically privileged sites such as testicles and they are susceptible to immune response when there is an antigenic contact such as control injection (Jones *et al*., 2000).

Group V histopathologic analysis suggested spermatogenesis impair, as did spermogram results. These findings are in agreement with data described by Fahim *et al*. (1993). Previous history for testicular lesions in our experimental animals is unknown and may have influenced results.

Considering animal weight, testicular size, ejaculate volume and libido, no alterations were observed, agreeing with other studies (Fahim *et al*., 1993; Tepsumethanon *et al*., 2005; Cedillo *et al*., 2006).

Other chemicals used for orquiectomy, such as chlorhexidine gluconate 1.5%, formalin suspension 3.3% and glycerol induced azoospermy and oligospermy after five to seven days post injection (Pineda *et al*., 1977). Fahim *et al*. (1993) injected zinc gluconate at the epididymal tail and obtained considerable lesions in sperm cells, reduced motility, and semen volume.

Doses of 26.2 mg zinc gluconate associated to DMSO 0.5% seem to be efficient in eliminating sperm. However, additional studies must be performed with a larger animal number and for a longer observation period in order to verify total security and absence of fertility recovery. Future studies should consider age, weight, breed, and previous medical history to minimize bias, which might have influenced our results. The main goal of this study was considered achieved since it was possible to identify the most efficient dosage to be used as canine male contraceptive drug.

References

Cedillo VG, Vargas PF, Monroy OV. 2006. Results of the massive sterilization project using gluconate neutralized with arginine in male dogs living in the States of Hidalgo, Mexico and Puebla. *In*: Proceedings of the XVII Rabies in the Americas (RITA), 2006, Brasília, DF, Brazil. Brasília: Editora do Ministério da Saúde. pp.15-20.

Clevenger J, Kass PH. 2003. Determinants of adoption and euthanasia of shelter dogs spayed neutered in the University of California Veterinary student surge: program compared to other shelter dogs. *J Vet Med Educ*, 30:372-378.

Dias RA. 2001. *Emprego de sistemas de informação geográfica (SIG) no controle da raiva canina*. São Paulo, SP: Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Dissertation. 97pp.

Fahim MS, Wang MF, Suctu Z, Fahim RS. 1993. Sterilization of dogs with intra-epididimal injection of zinc arginine. *Contraception*, 1:107-122.

Gomes HL, Almeida FM, Paranhos TN, Garcia CR, Nunes PFV, Neto MH, Cardoso SMS. 2003. Avaliação de riscos à saúde e intervenção local associadas ao convívio com cães e gatos, Jardim Paraná, Brasilândia. *Rev Educ Cont CRMV-SP*, 6:83-92.

Herath CB, Jin W, Watanabe G, Arai K, Suzuki AK, Taya K. 2004. Adverse effects of environmental toxicants, octylphenol and bisphenol A, on male reproductive functions in pubertal rats. *Endocrine*, 25:163-172.

Immegart HM, Threlfall WR. 2000. Evaluation of intratesticular injection of glicerol for nonsurgical sterilization of dogs. *Am J Vet Res*, 61:544-549.

Jacob SW, Herschler R. Pharmacology of DMSO. 2003. Available in: http://www.dmso.org/articles/information/herschler.htm#34. Accessed in December 4th, 2006.

Jones CL, Hunt RD, King NW. 2000. *Patologia Veterinária*. São Paulo: Manole. 1415pp.

Larrieu E, Alvarez T, Cavagion L, Herrasti A. 1990. Dinâmica de la poblacion canina de General Pico, Argentina em el período 1986/1990. *Vet Arg*, 9:242-247.

Morrow DA. 1988. *Current Therapy in Theriogenology*. Philadelphia: WB Saunders.

Nassar R, Mosier JE. 1980. Canine population dynamics: a study of the Manhattan, Kansas, canine population. *J Am Vet Méd Assoc*, 41:1798-1803.

Paranhos TN. 2002. *Estudo das populações canina e felina em domícilio, município de São Paulo*. São Paulo, SP: Faculdade de Saúde Pública, Universidade de São Paulo. Dissertation. 83pp.

Pineda MH, Hepler DI. 1981. Chemical vasectomy in dogs: long-term study. *Theriogenology*, 16:1-11.

Pineda MH, Reimers TJ, Faulkner LC, Hopwood ML, Seidel GEJ. 1977. Azoospermia in dogs induced by injection of sclerosing agents in the caudae of the epididymides. *Am J Vet Res*, 38:831-838.

Reichmann MLAB. 2000. *Controle de Populações de Animais de Estimação*. São Paulo: Instituto Pasteur. 44pp.

Ridley DS, **Hilson GRF**. 1967. A logarithmic index of bacilli in biopsies. I. Method. *Int J Lepr*, 35:184-186.

Rowan NA. 1994. Shelters and pet overpopulation: a statistical black hole. *Anthrozoös*, 5:140-143.

Soto FRM. 2003. *Dinâmica populacional canina no Município de Ibiúna- SP: estudo retrospectivo de 1998 a 2002 referente a animais recolhidos, eutanasiados e adotados*. São Paulo, SP: Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Dissertation. 100pp.

Tepsumethanon V, Wilde H, Hemachudha T. 2005. Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. *J Med Assoc Thai*, 88:686-689.

World Health Organization. Expert committee on rabies. 1992. *8th Report*. Geneva: WHO, 1992. 87pp. (Technical report series)