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Evaluation of Efficacy and Safety of Zinc Gluconate Associated with Dimethyl Sulphoxide for Sexually Mature Canine Males Chemical Neutering

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Contents

The aim of this research was to evaluate the efficacy of zinc gluconate associated with dimethyl sulphoxide (DMSO) for chemical neutering in canine males. Fifteen sexually mature male dogs were divided in two groups, named control and treated. An injection was administered to both testicles, at a concentration of 26.2 mg zinc gluconate per ml and 0.5% DMSO in the treated group (11 dogs). The control group was given injections of saline solution (four dogs). Clinical examination and blood collection for a haemogram were done both before and after drug injection. There were 12 spermograms performed to analyse sperm motility, sperm vigour, ejaculate volume, testicle size, pathology and sperm concentrations. Libido was also measured. An ultrasound examination and histopathology were performed at the end of the experiment. Dogs' libido after chemical injection was reduced by over 50%. The spermogram analysis showed final mean results of 14.54% for sperm motility, 0.72 of sperm vigour and 37 150 per million spermatozoa per millilitre, values considered below the necessary levels at which fertilization can occur. Ultrasound and histopathology analyses of testicles for the treated group revealed more intense injuries when compared with the control group, with compromised testicular parenchyma and a decrease of germ cell number leading to total atrophy, indicating that the treatment reduced the fertilizing potential of male dogs, promoting a possible subfertile status.

Introduction

According to the World Health Organization, canine population control is a necessary preventive public health action that has to be constantly performed at the municipality level as a complementary measure for canine zoonoses control, specifically with respect to rabies (World Health Organization 1992; World Society for the Protection Animal 1999).

Reports on canine population dynamics in Brazilian municipalities have shown a higher proportion of males in relation to females, with six to seven male dogs for each 10 animals (Dias 2001; Paranhos 2002; Soto 2003).

Of all contraceptive methods for canine male population control, surgical sterilization or orchiectomy is the most known and performed (Clevenger and Kass 2003; Gomes et al. 2003). Some resistance to this technique may come from owners who consider it incompatible with animal welfare and its behaviour alteration impact (Immegart and Threlfall 2000; Soto 2003).

Orchiectomy with chemical agents is also known as chemical orchiectomy and has been suggested as a safe, fast and low-cost alternative that can be used in a wide range of canine male populations, especially in poor regions where the problem of overpopulation may be more intense. Chemical neutering in animals is more useful than surgical methods because of its facility of use, low cost and minimal risk, as there is almost no need of post-intervention observation, and no infections, myiasis or other complications that may be seen with surgical neutering (Soerensen et al. 2007).

Among the available drugs for this purpose are chlorhexidine digluconate 3%, gossypol and zinc gluconate (Herath et al. 2004; Cedillo et al. 2006). Dimethyl sulphoxide (DMSO) is used as a vehicle, as it increases skin permeability, thereby facilitating absorption of drugs and other substances. Because of its biological activity related to cell membrane stability, Pineda and Hepler (1981) and Herath et al. (2004) used DMSO in association with different drugs to sterilize dogs.

The objective of this research was to evaluate the efficacy and safety of zinc gluconate associated with DMSO as the drug of choice for chemical neutering in male dogs for municipality canine population control.

Materials and Methods

This experiment was performed in the animal shelter 'Lar São Francisco de Assis', Ibiúna, SP, Brazil from February to October 2007. Initially 40 mature dogs were included in the experiment, and during the first 2 months, February and March, 15 mixed breed sexually mature males of over 1 year in age and weighing between 10 and 20 kg were selected, taking into consideration docility, for reasons of group management and ease in collecting semen.

Animals underwent clinical examinations and complete haemograms at the beginning and at the end of the experiment. Their libido was confirmed by putting them with females in oestrus. Dogs were trained for semen collection and complete spermograms were performed to classify them as fir for the experiment. Animals were randomly allocated to groups I and II, without a previously identified inclusion criterion.

Minimum spermogram reference values for dogs were considered to be: sperm motility superior to 70%, sperm count higher than 300×10^6/ml and sperm defects not more than 20%, in line with criteria proposed by Johnston et al. (2001). Only dogs with this minimum sperm quality were included in the experiment. Before injecting the chemical drug (treated group) and saline

solution (control group) these parameters were established to be similar between the groups.

Before the experiment, all animals were de-wormed and received shots against distemper, leptospirosis, parvovirosis and rabies. Dogs of both groups were fed twice a day with the same commercial food containing 21% crude protein. The experiment was approved by an ethical committee constituted by veterinarians of the Center of Sanitary Surveillance and Zoonoses Control 'Tereza Rodrigues de Camargo' from Ibiúna Municipality, São Paulo State, Brazil.

Chemical drug and saline solution volumes injected into dogs' testicles

Two groups were constituted: group I or control had four dogs which were injected saline solution and group II or treated, had 11 dogs which were injected with 26.2 mg/ml zinc gluconate with 0.5% of DMSO.

Chemical and saline solution volumes varied according to testicle size, which was measured transversally (mm) considering the craniocaudal distance with the use of a digital paquimeters (São Paulo, SP, Brazil) according to Table 1. Chemical and saline solutions were administered once by direct injection to each testicle (right and left) without anaesthesia or sedative and using 0.3×13 mm needles. Each dog was positioned lying on its back and the needle was inserted into the dorsal cranial portion of the epididymis. After six spermogram evaluations, those dogs which still had over 50% of sperm motility received another chemical injection at the seventh evaluation.

Clinical evaluation and spermogram of dogs after chemical or saline solution injection

Testicle size and spermograms of all animals were evaluated at the point of chemical and saline solution injection and then every 15 days totalling 12 evaluations, completing two cycles of sperm production (Johnston et al. 2001). The spermogram evaluated sperm motility (%) and velocity of forward progression (0–5). Ejaculate volume was measured in millilitres (ml), the sperm count in million spermatozoa per millilitre and sperm pathology (%) was evaluated through wet preparation technique with saline formalin. Rectal temperature, cardiac and respiratory rhythms, and mucosa colouration were observed daily. Animal behaviour was evaluated for signs of pain and discomfort, licking at inoculation site, appetite and pain during palpation examination. In order to measure and classify these responses quantitatively, the method of Ridley and Hilson (1967) was used, reducing subjective 'information.

Table 1. Chemical drug and saline solution volumes in millilitres injected into dogs' testicles according to their size in millimetres

Testicular size (mm)	Volume injected (ml)
12–17	0.5
18–23	1.0
24–27	1.5
27 or more	2.0

Libido was evaluated four times during the experiment; first before chemical injection, second at 120 days after chemical injection, third at 135 days and fourth at 150 days. Libido was numerically quantified, with values from one for those animals which presented semi-erection and ejaculation; two for total erection and ejaculation and three for total erection, copula movement and ejaculation.

Ultrasound and histopathological analysis of testicles after chemical injection

The ultrasound examination was conducted on the left and right testicles of all dogs, using a Piemedical ultrasound (BC Maastricht, The Netherlands) with a 5 MHz dynamic B mode real-time transducer, from the control and treated groups at 5 and 6 months following chemical injection. Ultrasonographic examination was performed solely after the drug injection and is considered to be an accessory diagnostic tool for histopathological evaluation. Ultrasonographic lesions were semi-quantitatively graded, ranging from zero for the total absence of lesions; one for mild lesions; two for average lesions and three for severe lesions such as areas with higher echogenicity suggesting fibrotic alterations, gross heterogenic echogenicity and lack of the mediastinum band.

Seven months following chemical injection, all dogs from the control and treated groups were surgically castrated, with both testicles and epididymis submitted to histopathological processing and microscopic analysis. Observed lesions were graded through the same method used in the ultrasound examination to establish a correlation between both analyses. Total absence of spermatozoa in the epididymal duct lumen was considered a sign of successful neutering. Both ultrasound and histopathological examinations were blindfolded, therefore conducted without the examiner's knowledge regarding the condition 'control' or 'treated', thus decreasing operator bias.

Statistical analysis

Sperm motility, ejaculate volume, sperm cell number, sperm vigour, libido, ultrasound and histopathological testicular lesions, testicular size and number of abnormal sperm cells were compared between groups by the general linear model for repeated measures (Zar 1999). Significance level used was 5%.

Results

Results have shown that all dogs from the control and treated groups presented regular clinical parameters for rectal temperature and cardiac and respiratory rhythms during 180 days after the drug or saline solution injections as the case may be. All dogs had normal appetite and pace. A mild inflammation was detected in the treated group's testicles after drug injection, which returned to normal within a week. No behaviour alteration or signs of discomfort were observed in dogs of both groups during the evaluation period. All dogs had normal parameters for the first haemogram. The

second examination at the end of the experiment detected mild eosinophilia in two animals of the treated group.

The control group animals presented a mean value of three for libido during the entire experiment for a total of four evaluations. For the treated group, before drug injection (first evaluation), the value was three, and then in all three following evaluations the mean value was 1.21. There was a statistical difference for the libido parameter between groups in all three evaluations after drug injection.

There was no alteration of mean values for the spermograms of the control group, which were: motility 88.54%, sperm vigour four, ejaculate volume 2.5 ml, sperm count of 359 million/ml and abnormal sperm cells of 10.22%. No alteration on the control group's testicular sizes was observed and the mean value was 22.75 mm during the experiment period. The treated group had a mean reduction of 4 mm of the animals' testicles at the last evaluation after chemical injection, (Table 2) however no statistical difference between the groups was observed in all 11 evaluations after chemical injection.

Mean results for the treated group's spermograms before and after chemical injection are presented in Table 2. The analysis of sperm motility showed a statistical difference between both groups in all 11 evaluations after chemical injection. After 3 months of treatment, three dogs still presented 60% of sperm motility and received one more chemical injection. At the next evaluation, sperm motility reduced to $< 20\%$.

There was a statistical difference between groups for sperm vigour in all 11 evaluations after chemical injection. Considering ejaculate volume, a statistical difference was detected between groups at the sixth, eighth and ninth collections after chemical injection. No statistical difference was observed for sperm cell number per cubic millimetre at the fourth and fifth collections, but at all others after drug injection, the difference between groups was significant. Testicle size evaluations showed no significant difference in all 11 evaluations after chemical injection. A statistical difference was observed for abnormal sperm cells between groups at the third collection after chemical injection.

Ultrasounds did not reveal lesions on control group testicles, which showed homogeneous ecotexture of testicular tissue with a well-defined mediastinal line, and a zero value was attributed to all testicles (right and left). In the treated group major lesions were detected on left and right testicles and consisted of: heterogeneous ecotexture, mild evidence of mediastinal line, presence of hipoecogenic areas and parenchyma heterogeneity. Lesions presented mean values of 1.65, with 1.50 for right testicles and 1.80 for the left ones at the first evaluation at 150 days after chemical injection, and a mean value of 1.80 at 180 days, with 1.65 for right testicles and 2.00 for the left ones, values close to two, which in this research was classified as an average lesion. There was a statistical difference between groups for this parameter at 150 and 180 days after chemical injection for both right and left testicles.

Histopathological examination of the control group animals showed smaller degrees of testicular lesions, with a 1.16 mean value, (1.33 for right and 1.00 for left testicles). All control group samples presented spermatozoa in the epididymis.

The treated group animals presented more severe lesions, with a 1.85 mean value (1.80 for right and 1.90 for left testicles). Observed findings included varying degrees of parenchyma testicular lesions, ranging from decrease of germ cell number (spermatogonia and spermatide cells) to total testicular atrophy (Fig. 1), loss of testicular architecture and fibrosis. Some samples also presented focal mononuclear inflammatory infiltrate at the testicular parenchyma and epididymis, neutrophilic infiltration and calcification foci (Fig. 2). Eighty per cent of right and left testicle samples had

Table 2. Mean results obtained for treated and control groups spermograms according to the period of evaluation

		Evaluation made from 1st to 12th					
Mean parameters	Group	1st[1]	2nd[2]	3rd to 5th[3]	6th to 8th[3]	9 th to 11th[3]	12th[4]
Sperm motility (%)	I	87.50[a]	87.50[a]	87.50[a]	90.00[a]	89.27[a]	87.50[a]
	II	87.27[a]	40.00[b]	29.09[b]	24.54[b]	26.21[b]	14.54[b]
Sperm vigour	I	4.00[a]	4.00[a]	4.00[a]	4.00[a]	4.00[a]	4.00[a]
	II	4.00[a]	1.45[b]	1.18[b]	1.15[b]	1.06[b]	0.72[b]
Volume of ejaculate (ml)	I	3.25[a]	2.62[a]	2.41[a]	1.83[a]	2.96[a]	3.00[a]
	II	2.00[a]	1.30[a]	1.20[a]	1.02[b]	0.95[b]	1.31[a]
Sperm cell number	I	477.32[a]	479.19[a]	294.04[a]	358.23[a]	345.68[a]	360.15[a]
	II	398.73[a]	84.19[b]	133.83[b]	71.42[b]	36.21[b]	37.15[b]
Abnormal sperm cell number	I	14.00[a]	9.50[a]	9.00[a]	12.00[a]	10.08[a]	6.00[a]
	II	8.80[a]	19.60[a]	21.04[b]	15.80[a]	18.54[a]	20.00[a]
Testicle size (mm)	I	22.75[a]	22.75[a]	22.75[a]	22.75[a]	22.75[a]	22.75[a]
	II	24.90[a]	22.09[a]	20.30[a]	20.27[a]	20.27[a]	20.90[a]

Parameters: sperm motility in percentage (%), sperm vigour, ejaculate volume (ml), sperm cells in thousands per million spermatozoa per millilitre, number of abnormal sperm cells for group of 100 cells and testicular size (mm).

[1]Mean results before chemical injection.

[2]Mean results after 15 days of chemical injection.

[3]Mean results after three subsequent evaluations.

[4]Mean results at the last evaluation.

[a,b]For each parameter and period of evaluation, different letters indicate statistical difference between groups ($p < 0.05$).

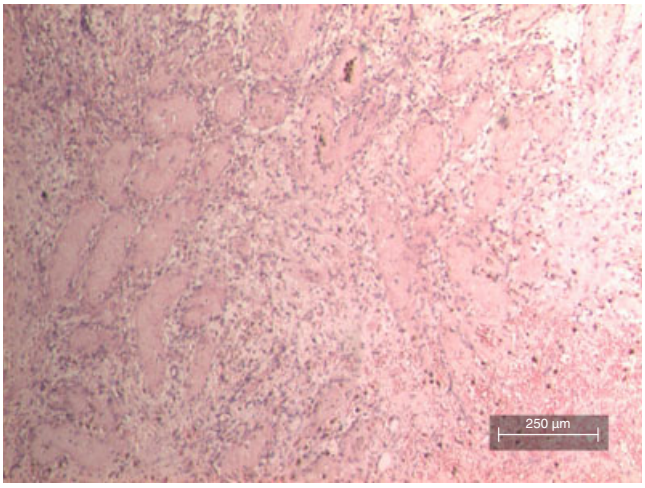

Fig. 1. Testicular atrophy, treated group

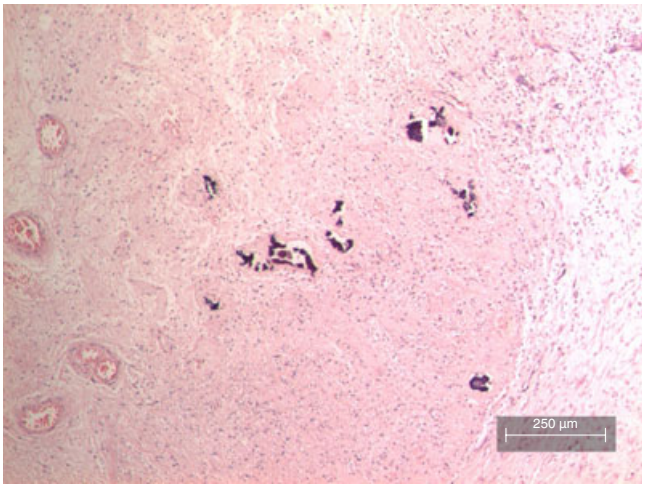

Fig. 2. Testicular calcification, treated group

reduced or no spermatozoa content in the epididymis. Although no statistical difference had been observed between groups at the histopathological examination for the right testicles, the value obtained for the left testicles ($p = 0.054$) was near statistical significance.

Discussion

Considering the dogs' clinical parameters after drug injection, zinc gluconate associated with DMSO has been shown to be a safe product, with experiment subjects having a regular rectal temperature, cardiac and respiratory rhythms and food consumption during all 180 days of the experiment. The absence of behavioural alterations and signs of discomfort in the treated group of dogs corroborates to the drug's safety. These results are in agreement with similar studies such as the one described by Tepsumethanon et al.(2005) who did not observe behavioural alterations or signs of pain in five dogs.

The mild inflammatory condition, which was detected after chemical injection, evolved to normality within a week; similar result was also observed in other studies such as the one of Cedillo et al. (2006) who found that only 3.1% of dogs injected with zinc gluconate neutralized with arginine had scrotal ulcers or fistulas and the others had a mild inflammatory condition. Discrete eosinophilia detected in two animals at the end of experiment was probably associated with the important eosinophilic function of increasing inflammatory response, which is the consequence of chemical injection and followed by tissue injury or parasitism (Jones et al. 2000).

The influence of the chemical injection on libido reduction was evident, which before chemical injection had a value equal to three and in the following three evaluations the mean value was 1.21, a reduction of over 50%. This result is important for owned dogs because male libido is inconvenient for the majority of owners (Soto et al. 2007a,b).

Spermogram mean results before and after chemical injection are presented in Table 2, and they corroborate to the drug efficacy as a chemical sterilant, with mean final results of 14.54% sperm motility, sperm vigour 0.72 and sperm cells of 37 150 per million spermatozoa per millilitre. These parameters were reduced by 83.3%, 82% and 90.6% respectively. The product was able to alter sperm quality in 72% of dogs with one injection and 100% with two injections, which indicates its practical and efficient use for male canine chemical neutering and to control canine population growth. These results indicated that treatment reduced the fertilizing potential of male dogs, promoting perhaps a subfertile status. Similar researches have indicated azoospermy in animals from 26 days after chemical injection, thus considering zinc gluconate efficient for chemical neutering (Tepsumethanon et al. 2005; Cedillo et al. 2006). Fahim et al. (1993) injected zinc gluconate for canine chemical neutering and observed reduced sperm motility in 50 dogs after 90 days of chemical injection.

Ultrasound and histopathological examination of the treated group testicles corroborated to the drug efficacy in the induction of more severe lesions when compared with the control group, which was sufficient to establish a permanent sterility condition showing testicular parenchyma lesions, varying from a decrease of germ cell number up to total testicular atrophy (Fig. 1). These injuries are similar to those detected by Oliveira et al. (2007) who injected zinc gluconate into testicles of sexually mature male dogs and observed degeneration, testicular atrophy and Leydig cell necrosis, which suggests an irreversible sterility condition. The control group's mild lesions observed by histopathology were probably associated with the fact that the animals received a needle puncture in their testicles for the saline solution injection. This procedure is sufficient to explain the observed histopathological lesions which occur in mild-to-moderate trauma, as 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 a control injection (Jones et al. 2000). The association between zinc gluconate and DMSO successfully increased chemical drug diffusion in testicular parenchyma. Sperm quality in a DMSO plus zinc gluconate group was

significantly altered in relation to a group without DMSO (Soto et al. 2007a,b). The presence of a large quantity of sperm fluid in the epididymis detected by histopathological examination reinforces the statement that even under a mild trauma condition, spermatogenesis was not jeopardized. Different results were observed for the treated group, with 80% of the samples containing reduced or no sperm fluid in the epididymis, which shows the efficacy of this drug for chemical neutering.

Conclusions

- Zinc gluconate associated with DMSO was demonstrated to be a safe drug from a clinical point of view, with an absence of behaviour alterations and discomfort of the treated group of dogs.
- The histological lesions detected in testicles were compatible with permanent sterilization.
- Testicular treatment with zinc gluconate and DMSO reduced the fertilizing potential of male dogs, promoting perhaps a subfertile status. This indicates its practical and efficient use for chemical neutering in canine males.

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