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Intratesticular injection of a zinc-based solution for contraception of domestic cats: A randomized clinical trial of efficacy and safety

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ABSTRACT

It has been reported that a commercial zinc gluconate preparation disrupts spermatogenesis and apparently causes permanent sterilization in male dogs, but there is little information regarding similar approaches in the male cat. The objective of this study was to evaluate zinc gluconate as a permanent contraceptive for domestic male cats. Sixteen sexually mature mixed breed cats were allocated at random, by replicate, into two groups and given a single injection into each testis of either isotonic saline or zinc gluconate, respectively. Clinical and reproductive parameters were assessed immediately before injection and after 60 and 120 days.

On day 120 the testis size of treated cats was decreased ($P < 0.05$). Azoospermia occurred in 8/11 (73%) cats, and penile spines were decreased in 6/11 (55%) and absent in 4/11 (36%) cats, and there were substantial reductions in male behavior. However, plasma testosterone concentrations (single samples collected at each assessment) were not significantly different between treated and control cats at any time point. Although additional studies are warranted, intratesticular injection of zinc gluconate might have potential as a permanent contraceptive for cats.

Introduction

There are very few reports regarding chemical sterilization of male cats although intra-epididymal injection of 4.5% chlorhexidine digluconate caused azoospermia or severe oligospermia (Pineda and Dooley, 1984); intratesticular injection of calcium chloride has been shown to have potential for permanent contraception (Jana and Samanta, 2011), and preliminary studies of an intratesticular injection of zinc gluconate have been very promising (E.C.S. Oliveira et al., unpublished data). The objective of the present study was to investigate the effects of a single injection of intratesticular zinc gluconate as a permanent contraceptive method for cats.

Materials and methods

Test compound

The test compound was a proprietary zinc gluconate solution for intratesticular injection (Testoblock, BioRelease Technologies LCC). The product contained 0.2 M zinc gluconate (13.1 mg zinc/mL), which was pH-neutralized in a physiological vehicle.

Cats

Sixteen intact shorthair male cats from two private colonies, approximately 9–12 months old and 2.0–4.5 kg bodyweight, were used. The number of animals was based on differences between groups in a previous study in dogs (Wang, 2002), as well as logistics and available funding. The study was approved by the Animal Experimentation Ethics Committee of Federal Rural University of Pernambuco (Protocol 008/2010). All cat owners were given detailed information and signed a research consent form.

The work was undertaken between January and November in Recife, PE, Brazil (8°04′South; 33°55′West). At this location, there is approximately 12 h of light per day and a mean temperature of 26.4 °C. There were no clinical abnormalities in any of the cats based on a physical examination, hematology and clinical chemistry. All cats produced ejaculates with ⩾80% progressively motile sperm; sperm counts were within normal limits, and all males displayed sexual interest in an estrous queen.

Cats were randomly allocated (in replicates of three; all assignments were made by the primary investigator) into two groups, controls ($n = 5$) and treated ($n = 11$), and each cat was given a single injection into each testis of either isotonic saline or zinc gluconate, respectively. Group assignments were known to investigators conducting animal-based procedures, but were not known to cat owners or to persons assessing hematology, serum chemistry, or plasma testosterone concentrations. All cats remained at their owners' residence throughout the study and all animal-based procedures were done there.

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Anesthesia

Cats were anesthetized for intratesticular injections and semen collection. Access to food and water was withheld for 12 h and 7 h, respectively, before anesthesia, which was induced with an injection of xylazine (0.5 mg/kg, IM; Rompun 2%, Bayer) followed by ketamine (5.0 mg/kg, IM; Ketalar, Pfizer). If needed, additional anesthetic drugs (approximately 25% of the original dose of each product) were subsequently given.

Intratesticular injection

Anesthetized cats were restrained in a supine position and the scrotum was cleaned (10% povidone iodine, SLF). The width of each testis was measured with calipers, and a single injection of isotonic saline or zinc gluconate was given into each testis (1 mL of solution for every 27 mm of testis width; adapted from Wang, 2002). The volume injected per testis ranged from 0.44 to 0.51 mL. Injections were performed using a 0.5 mL U100 insulin syringe with a 28 G, 12 mm needle (a separate needle was used for each testis). The injection was given in the cranial area of the testis, lateral to the caput epididymis (near the ductuli efferentes), with the needle parallel to the long axis of the testis.

Clinical and laboratory assessments

General attitude, bodyweight, ability to walk, scrotal changes, and rectal temperature were evaluated on days 0 (immediately before treatment), 60, and 120. Concurrently, testis width was measured (with calipers), the penis was examined for the presence of testosterone-dependent spines, and blood samples were collected (saphenous venipuncture) for complete hematology, hepatic and renal function (alanine and aspartine aminotransferase, urea and creatinine), and plasma testosterone concentrations. The latter were measured using radioimmunoassay kits (Coat-A-Count, Diagnostic Products Corporation), previously validated for domestic cats (Levy et al., 2004).

Semen collection and evaluation

On days 0 (pre-treatment), 60, and 120, all cats were anesthetized (with xylazine/ketamine, as previously described) and semen was collected with an electroejaculator (Eletrojet, Eletrovet) connected to a 12 V source. A rectal probe (10.0 × 0.8 cm) with two stainless steel electrodes was used and the procedure was performed as described by Wildt et al. (1983). Semen was collected into plastic tubes and immediately evaluated (by the same person). Total sperm count was assessed on a scale of 0–3 (0, azoospermia; 3, apparently normal sperm count) and progressively motile sperm (increments of 10%) were evaluated subjectively by light microscopy (400×). Following ejaculation, urine was collected (by cystocentesis) and evaluated for sperm (retroejaculation).

Behavior

Libido was assessed on days 0 (pre-treatment), 30, 60 and 120. Each male cat was individually allowed access (for 30 min) to a female cat in spontaneous estrus (confirmed by behavior) from the same private colony. It was subjectively assessed (by a trained observer from the research team, who was responsible for all of these) on a scale of 0–3, where 0 represented no interest, 3 represented rapid interest and mounting, and 1 and 2 were gradations between these extremes. In addition, owners completed a standardized questionnaire (regarding aggressive behavior, mounting, roaming, urine marking, etc.) and were interviewed by the research team throughout the experiment. Note that for assessment of sexual interest, mounting behavior was considered the cardinal sign.

Statistical analyses

A mixed models ANOVA (for repeated measures) was used to determine the effects of group, time, and their interaction, on bodyweight, testis width and plasma testosterone concentrations. For testis width, the average of the left and right measurements was used in the statistical analysis. Testosterone concentrations were highly variable and not normally distributed; therefore, a log10 transformation was done before analysis. Furthermore, for testosterone concentrations, due to some missing values (lost samples), statistical analysis was restricted to days 60 and 120. For main effects and interactions, $P < 0.05$ was considered significant. A Bonferroni test was used to locate significant differences. All analyses were done with Statistical Analysis System (SAS) software (SAS Institute).

Results

Following treatment, rectal temperature remained normal. Owners reported no biting or licking of the scrotum or testes, but they did report transient testicular swelling (1 day after injection) in all cats receiving zinc gluconate but in none of the cats receiving saline. However, there was no apparent scrotal or testicular pain or tenderness, except for one treated cat, with apparent discomfort (reduced activity and feed intake) 2–3 h after injection. This cat was given sodium dipyrone (25 mg/kg orally three times per day, for 2 days) and the animal recovered uneventfully.

In both groups, hematology and clinical chemistry were consistently within normal reference ranges for domestic cats (ÓBrien et al., 1998). For bodyweight, neither main effect nor their interaction was significant (data not shown).

Mean ± standard error (SEM) testis width on days 0, 60, and 120 was 10.5 ± 0.26, 10.5 ± 0.92, and 10.5 ± 1.03 mm in control cats, and 12.4 ± 0.18, 8.7 ± 0.62, and 7.9 ± 0.65 mm in treated cats (group x time interaction, $P < 0.005$). Testis width of treated cats decreased over days 0–120 ($P < 0.05$). Furthermore, testis width was smaller ($P < 0.05$) in treated cats compared to control cats on days 60 and 120. However, testis width did not change over time in control cats ($P > 0.05$; Fig. 1).

On day 120, testosterone-dependent penile spines were prominent in control cats, but were absent in 4/11 (36%) and decreased in 6/11 (55%) treated cats (Fig. 2A). One cat still had well-developed penile spines (Fig. 2B).

On day 60, 10/11 treated cats (91%) were azoospermic and the remaining cat had reductions in both sperm count and sperm motility. On day 120, eight cats (73%) were azoospermic, one with well developed penile-spines had necrospermia, and two had sperm in their ejaculates (scale 1/3 or 2/3), with reduced motility. Urine collected by cystocentesis after eletroejaculation did not contain sperm on day 60, although a few non-motile sperm were present in 2/10 cats at day 120. In contrast, cats in the control group consistently had excellent semen quality (normal sperm counts and progressive motility) throughout the study. Furthermore, 3/5 cats in the control group (60%) had evidence of retrograde sperm flow. Regarding plasma testosterone concentration, there were no significant differences for group or time, nor was there a significant group by time interaction. On days 60 and 120, owners reported that treated cats had reduced aggression, roaming, mounting and urine marking (spraying), whereas these activities persisted in control cats.

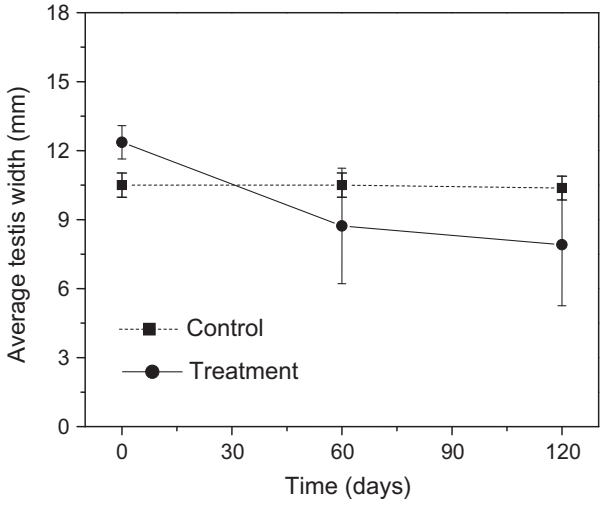

Fig. 1. Testis width (mean ± SEM) of cats following intratesticular injection of either isotonic saline (control, $n = 5$) or a zinc gluconate (treated $n = 11$) according to the period of evaluation. Treated cats had a reduction ($P < 0.05$) in testis width from day 60 to the end of the study.

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