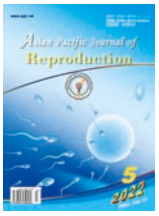

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Evaluation of intratesticular chlorhexidine gluconate for chemical contraception in dogs

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ABSTRACT

Objective: To investigate the contraceptive effect of intratesticular injection of chlorhexidine gluconate in dogs and compare it with that of zinc gluconate neutralized with arginine.

Methods: Twenty-four sexually mature male mongrel dogs were randomly divided by replicate into four groups (n=6 per group). Group Ⅰ received intra-testicular injection of 2 mL zinc gluconate (10 mg/mL) neutralized with arginine. Group Ⅱ received intratesticular injection of 2 mL chlorhexidine gluconate (5% w/v). Group Ⅲ received intratesticular injection of 2 mL chlorhexidine gluconate (4% w/v). Group Ⅳ did not receive any treatment and served as the control group. Testicular morphometry was conducted on day 0, 7, 15 and 30 after treatment. Semen was collected and evaluated on day 0 and 30. Data were analyzed using repeated measures analysis of variance.

Results: There was no difference in the mean values of various parameters between dogs treated with zinc gluconate and those treated with chlorhexidine gluconate at any of the time points. In dogs treated with zinc gluconate or chlorhexidine gluconate, there was a significant increase in the testicular morphometric parameters on day 7 followed by a significant reduction thereafter (day 15 and 30). In contrast, there was no change in any of the parameters in the control untreated dogs during the course of the study. Compared to the pre-treatment values, the mean scrotal circumference and the mean paired testicular volume and testicular weight on day 30 were significantly lower in the treated dogs. Semen samples collected on day 30 from treated dogs were found to be azoospermic, whereas no change in semen quality was observed in the control untreated dogs.

Conclusions: Intratesticular injection of chlorhexidine gluconate (5% w/v and 4% w/v) is equally as effective as zinc gluconate neutralized with arginine for chemical contraception in dogs.

KEYWORDS: Canine; Contraception; Zinc gluconate; Chlorhexidine gluconate; Morphometry

1. Introduction

Canine overpopulation continues to be a major problem worldwide. The global canine population estimates range between 700 million and 1 billion with free-roaming dogs constituting about 75% of that population[1,2]. This problem is of greater importance in developing countries such as India where a high density of free-roaming dogs has a significant negative impact on animal welfare, environment, and public health[3]. A vast majority of free-roaming dogs are in poor body condition with various health issues, the common ones being skin conditions and ectoparasitic infestations[4].

Significance

Chemical castration is a good potential alternative to surgical castration in dogs, especially in developing countries. Previous studies have investigated intratesticular injections of zinc gluconate and calcium chloride. Our study shows that intratesticular injection of chlorhexidine gluconate in dogs induces similar alterations in testicular structure and function to those caused by intratesticular injection of zinc gluconate. These findings suggest that intratesticular injection of chlorhexidine gluconate can be a potential method of chemical castration and contraception in dogs.

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Free-roaming dogs are a major source of human rabies in India. It is estimated that about 35% of the total global human deaths due to dog-transmitted rabies occurance in India[5]. The World Health Organization recommends that canine overpopulation control must be regularly performed at the municipality level as a supportive measure for controlling canine zoonoses[6].

Although surgical castration is an effective contraception method for dogs, its high cost and low accessibility are limiting factors, especially in low-income countries[7]. In recent years, there has been an increased interest in developing alternatives to surgical castration in dogs. Chemical castration using intratesticular injection of various agents is one of the approaches that have been a focus of active research in this area. Intratesticular injection of calcium chloride[7,8] and zinc gluconate[9-13] have been evaluated previously in dogs. Intratesticular calcium chloride at doses ranging from 5 to 20 mg per testis per kg body weight were associated with degenerative changes in testicular parenchyma and a significant reduction in testicular weight, epididymal sperm output, and plasma concentrations of testosterone 45 days after the injections[7]. Another study using calcium chloride concentrations ranging from 0% to 60% reported azoospermia in most of the treated dogs for up to 12 months after the injections[8]. Similarly, intratesticular injections of zinc gluconate have been shown to significantly affect testicular parameters and sperm production[9-13].

The present study was conducted to evaluate the contraceptive effect of intratesticular injection of chlorhexidine gluconate (5% w/v and 4% w/v) in male dogs and compare it with that of zinc gluconate neutralized with arginine.

2. Materials and methods

2.1. Animals

A total of 24 apparently healthy, 3- to 5-year-old intact stray male mongrel dogs (*Canis lupus familiaris*) were used in the present study. The dogs were obtained from the local community and returned after completion of the study period. All the dogs were examined and were found to be free of any general or reproductive disorders. They were given a balanced diet and had access to clean drinking water *ad libitum*.

2.2. Materials

Chlorhexidine gluconate was purchased from Sigma-Aldrich Chemicals Private Limited (Bangalore, Karnataka, India). Zinc gluconate and arginine were purchased from HIMEDIA Laboratories (Thane West, Maharashtra, India).

2.3. Experimental design

The dogs were randomly divided by replicate into four groups (n=6 per group). Group Ⅰ received intra-testicular injection of 2 mL zinc gluconate (10 mg/mL) neutralized with arginine. Group Ⅱ received intratesticular injection of 2 mL chlorhexidine gluconate (5% w/v). Group Ⅲ received intratesticular injection of 2 mL chlorhexidine gluconate (4% w/v). The dose rates of zinc gluconate and chlorhexidine gluconate were based on previous pilot studies. Group Ⅳ did not receive any treatment (control).

2.4. Intratesticular injection

Prior to the intratesticular injections, the dogs were kept off-feed for 6 to 12 h and administered atropine sulphate (0.025 mg/kg body weight, Kaptrop, Iskon Remedies) and xylazine (1 mg/kg body weight, Xylaxin, Indian Immunologicals Limited) through intramuscular route. After sedation, the dogs were restrained in dorsal recumbency followed by cleaning of their testicular region using an antiseptic solution (10% povidone iodine solution, Unidine, Unichem laboratories limited). The intratesticular injections were performed using 26-gauge needles and sterile syringes.

2.5. Testicular morphometry

Testicular morphometry was conducted on days 0, 7, 15 and 30 post-treatments. The study duration was chosen based on the longest feasible duration to keep the dogs under observation in our settings. Testicular length (TL), width (TW) and thickness were measured using digital Vernier calipers in centimeter. Scrotal circumference (SC) was measured using a silk thread and commercially available measuring tape. The scrotum along with the testes was encircled using a silk thread and the corresponding length was measured on the measuring tape. Paired testicular volume was calculated using the formula: Paired volume of testes (cm^3) = 0.0396 × average TL × $(SC)^2$. Total testicular weight was calculated from the formula[14]: Total testicular weight (g) = 0.5533 × TL × $(TW)^2$.

2.6. Semen collection and examination

Semen samples were collected on day 0 and day 30 using the standard digital manipulation technique and examined under the microscope (Leica DM1000 LED, Leica Microsystems, Kurla West, Mumbai, India) for spermatozoal presence and motility at 100-400× magnification.

2.7. Statistical analysis

Data were analyzed using commercial statistical software (SAS version 9.4, SAS Institute Inc.). Normality of the data was tested using the Shapiro-Wilk test and data transformation was performed before the analysis for parameters that were not normally distributed. The effect of treatment on the testicular parameters was evaluated for statistical significance by repeated measures analysis of variance. *Post hoc* comparisons were performed using Duncan multiple

range test. The results are presented as mean±standard deviation (mean±SD). Differences were considered significant at $P<0.05$.

2.8. Ethics statement

Animal handling and clinical procedures used in the study were approved by the Institutional Ethical Committee (Approval No. 782/DI/NDVSU/2018 Dated 01/09/2018).

3. Results

3.1. Testicular morphometric parameters

The mean values of various testicular parameters (testicular length, width, thickness, volume, and weight) and scrotal circumference are presented in Tables 1 to 6. There was no difference in the mean values of different parameters between dogs treated with zinc gluconate and those treated with chlorhexidine gluconate at any of the time points. In dogs treated with zinc gluconate or chlorhexidine gluconate (groups Ⅰ, Ⅱ, and Ⅲ), there was a significant increase in the testicular morphometric parameters initially (by day 7) that was followed by a significant reduction thereafter (days 15 and 30). In contrast, there was no change in any of the parameters in the untreated dogs (group Ⅳ) during the course of the study. Compared to the pre-treatment values, the mean scrotal circumference and the mean paired testicular volume and testicular weight on day 30 were significantly lower in the treated dogs (Tables 4 to 6).

3.2. Semen analysis

A subjective microscopic analysis of the semen samples collected on day 0 (before treatment) revealed fairly normal concentration of motile spermatozoa in all the dogs used in this study. However, the semen samples collected on day 30 from treated dogs (Groups Ⅰ, Ⅱ, and Ⅲ) were found to be azoospermic. No change in semen quality was observed in the untreated controls (Group Ⅳ).

Table 1. Mean testicular length (cm) before and after treatment in dogs.

Days	Left testicle				Right testicle			
	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	3.59 ± 0.93^{Ba}	3.65 ± 0.49^{Ba}	3.43 ± 0.27^{Ba}	3.37 ± 0.27^{Aa}	3.53 ± 0.81^{Ba}	3.69 ± 0.47^{Ba}	3.50 ± 0.44^{Ba}	3.29 ± 0.24^{Aa}
Day 7	4.40 ± 0.78^{Aa}	4.26 ± 1.08^{Aa}	4.07 ± 0.42^{Aa}	3.37 ± 0.27^{Ab}	4.33 ± 0.69^{Aa}	4.28 ± 0.49^{Aa}	4.08 ± 0.27^{Aa}	3.31 ± 0.24^{Ab}
Day 15	3.62 ± 0.66^{Ba}	3.73 ± 0.49^{Ba}	3.64 ± 0.37^{Ba}	3.38 ± 0.27^{Aa}	3.55 ± 0.78^{Ba}	3.82 ± 0.64^{Ba}	3.76 ± 0.34^{Ba}	3.32 ± 0.24^{Aa}
Day 30	3.00 ± 0.34^{Ca}	3.14 ± 0.51^{Ba}	3.01 ± 0.39^{Ba}	3.36 ± 0.24^{Aa}	3.14 ± 0.73^{Ba}	3.22 ± 0.49^{Ba}	3.13 ± 0.42^{Ba}	3.31 ± 0.24^{Aa}

Data are expressed as mean±SD. Different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$). Group Ⅰ receives intra-testicular injection of 2 mL zinc gluconate (10 mg/mL) neutralized with arginine. Group Ⅱ receives intratesticular injection of 2 mL chlorhexidine gluconate (5% w/v). Group Ⅲ receives intratesticular injection of 2 mL chlorhexidine gluconate (4% w/v). Group Ⅳ does not receive any treatment and serves as the control group.

Table 2. Mean testicular width (cm) before and after treatment in dogs.

Days	Left testicle				Right testicle			
	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	2.14 ± 0.32^{Ba}	2.10 ± 0.39^{Ba}	2.07 ± 0.34^{Ba}	1.96 ± 0.12^{Aa}	2.13 ± 0.32^{Ba}	2.08 ± 0.34^{Ba}	2.08 ± 0.39^{Ba}	1.95 ± 0.10^{Aa}
Day 7	3.04 ± 0.73^{Aa}	2.73 ± 0.44^{Aa}	2.67 ± 0.39^{Aa}	1.97 ± 0.12^{Ab}	3.06 ± 0.83^{Aa}	2.89 ± 0.32^{Aa}	2.63 ± 0.27^{Aa}	1.96 ± 0.10^{Ab}
Day 15	2.15 ± 0.29^{Ba}	2.32 ± 0.44^{Ba}	2.18 ± 0.64^{Ba}	1.96 ± 0.12^{Aa}	2.13 ± 0.27^{Ba}	2.35 ± 0.44^{Ba}	2.41 ± 0.56^{Ba}	1.96 ± 0.10^{Aa}
Day 30	1.85 ± 0.27^{Ba}	1.84 ± 0.37^{Ba}	1.92 ± 0.27^{Ba}	1.96 ± 0.12^{Aa}	1.81 ± 0.27^{Ba}	1.93 ± 0.22^{Ba}	2.03 ± 0.42^{Ba}	1.95 ± 0.10^{Aa}

Data are expressed as mean±SD. Mean values bearing different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$).

Table 3. Mean testicular thickness (cm) before and after treatment in dogs.

Days	Left testicle				Right testicle			
	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	2.30 ± 0.32^{Ba}	2.24 ± 0.37^{Ba}	2.23 ± 0.49^{Ba}	2.07 ± 0.15^{Aa}	2.29 ± 0.29^{Ba}	2.24 ± 0.29^{Ba}	2.22 ± 0.34^{Ba}	2.06 ± 0.10^{Aa}
Day 7	2.75 ± 0.37^{Aa}	2.94 ± 0.47^{Aa}	2.87 ± 0.42^{Aa}	2.07 ± 0.15^{Ab}	2.87 ± 0.34^{Aa}	2.87 ± 0.42^{Aa}	2.77 ± 0.29^{Aa}	2.06 ± 0.10^{Ab}
Day 15	2.23 ± 0.34^{Ba}	2.29 ± 0.37^{Ba}	2.61 ± 0.64^{Ba}	2.08 ± 0.15^{Aa}	2.18 ± 0.32^{Ba}	2.43 ± 0.32^{Ba}	2.53 ± 0.56^{Ba}	2.06 ± 0.10^{Aa}
Day 30	1.82 ± 0.37^{Ca}	1.94 ± 0.34^{Ba}	2.13 ± 0.47^{Ba}	2.07 ± 0.15^{Aa}	1.85 ± 0.29^{Ca}	2.05 ± 0.24^{Ba}	2.11 ± 0.39^{Ba}	2.06 ± 0.10^{Aa}

Data are expressed as mean±SD. Mean values bearing different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$).

Table 4. Mean scrotal circumference (inches) before and after treatment in dogs.

Days	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	5.37 ± 0.91^{Ba}	5.15 ± 0.96^{Ba}	5.13 ± 0.64^{Ba}	4.90 ± 0.22^{Aa}
Day 7	6.72 ± 0.78^{Aa}	6.62 ± 0.96^{Aa}	6.57 ± 0.66^{Aa}	4.93 ± 0.24^{Ab}
Day 15	5.32 ± 0.81^{Ba}	5.30 ± 0.96^{Ba}	5.15 ± 0.56^{Ba}	4.98 ± 0.17^{Aa}
Day 30	4.35 ± 0.88^{Ca}	4.33 ± 0.83^{Ca}	4.10 ± 0.47^{Ca}	4.97 ± 0.17^{Aa}

Data are expressed as mean±SD. Mean values bearing different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$).

Table 5. Mean paired volume of the testes (cm^3) before and after treatment in dogs.

Days	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	4.45 ± 3.04^{Ba}	3.98 ± 2.06^{Ba}	3.72 ± 1.18^{Ba}	3.17 ± 0.49^{Aa}
Day 7	8.11 ± 3.53^{Aa}	7.68 ± 2.79^{Aa}	7.08 ± 1.76^{Aa}	3.23 ± 0.54^{Ab}
Day 15	4.27 ± 2.40^{Ba}	4.43 ± 2.16^{Ba}	3.95 ± 0.98^{Ba}	3.29 ± 0.44^{Aa}
Day 30	2.49 ± 1.54^{Ca}	2.50 ± 1.13^{Ca}	2.08 ± 0.61^{Ca}	3.42 ± 0.39^{Aa}

Data are expressed as mean±SD. Mean values bearing different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$).

Table 6. Mean total testicular weight (g) before and after treatment in dogs.

Days	Left testicle				Right testicle			
	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Day 0	9.98 ± 6.32^{Ba}	9.54 ± 5.12^{Ba}	8.41 ± 2.72^{Ba}	7.20 ± 1.57^{Aa}	9.47 ± 4.97^{Ba}	9.30 ± 4.29^{Ba}	8.87 ± 3.43^{Ba}	6.95 ± 1.27^{Aa}
Day 7	23.54 ± 12.05^{Aa}	18.49 ± 8.25^{Aa}	16.74 ± 5.34^{Aa}	7.29 ± 1.54^{Ab}	24.17 ± 14.60^{Aa}	20.36 ± 7.05^{Aa}	15.87 ± 3.58^{Aa}	7.05 ± 1.25^{Ab}
Day 15	9.76 ± 4.83^{Ba}	11.87 ± 5.58^{Ba}	10.64 ± 6.05^{Ba}	7.26 ± 1.54^{Aa}	9.37 ± 4.12^{Ba}	12.61 ± 5.93^{Ba}	12.99 ± 7.62^{Ba}	7.11 ± 1.32^{Aa}
Day 30	5.88 ± 2.35^{Ca}	6.40 ± 2.94^{Ca}	6.39 ± 2.52^{Ca}	7.25 ± 1.52^{Aa}	6.09 ± 3.21^{Ca}	6.86 ± 2.35^{Ca}	7.63 ± 3.99^{Ca}	7.04 ± 1.25^{Aa}

Data are expressed as mean±SD. Mean values bearing different superscripts in columns (A, B, C) or rows (a, b) differ significantly ($P<0.05$).

4. Discussion

The present study demonstrates that intratesticular injection of 4%-5% chlorhexidine gluconate in dogs results in similar changes in testicular size and semen quality to those induced by intratesticular treatment with zinc gluconate. The reduction in testicular size after intratesticular injection of zinc gluconate is in agreement with the findings of previous studies[12,13]. The changes in scrotal size after intratesticular injection of zinc gluconate have been attributed to degenerative changes and atrophy of the testicular tissue induced by the chemical agent[11,13,15]. Although it is likely that the changes in testicular morphometry observed in dogs treated with chlorhexidine gluconate involve similar mechanisms, further studies are required for confirmation. The initial transient increase in testicular morphometric parameters in the treated dogs may be attributed to mild inflammation, resulting in vasodilation and edema. This is followed by testicular degeneration that leads to a reduction in testicular size thereafter.

The effect of intratesticular zinc gluconate injection on sperm quality observed in this study was similar to the findings of Vannucchi *et al*[13] who reported a significant reduction in spermatozoal motility and count post treatment with zinc gluconate. Azoospermia following treatment with zinc gluconate was also reported by Oliviera *et al*[12]. A significant reduction in plasma testosterone was reported but hematological and serum biochemistry parameters remained within the normal range[12].

An apparent limitation of the current study was that semen quality was analyzed using a subjective approach. Further studies are warranted to confirm the effects of chlorhexidine gluconate on semen quality, preferably involving objective evaluation of spermatozoal concentration, motility, and morphology. The effects of this treatment on serum biochemical parameters and hormones are also worth investigating.

In conclusion, the findings of the present study suggest that intratesticular injection of chlorhexidine gluconate in dogs induce similar alterations in testicular structure and function to those caused by intratesticular injection of zinc gluconate. Based on these findings, intratesticular injection of chlorhexidine gluconate can be a potential method of chemical castration and contraception in dogs. Further studies are required to confirm these findings and investigate the long-term effects of intratesticular chlorhexidine gluconate injections in dogs.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

Aeknath Virendra conducted the experiments, collected the data, and wrote the first draft of the manuscript. Om Prakash Shrivastava, Satya Nidhi Shukla, Manish Kumar Shukla, and Nitin Kumar Bajaj assisted with the study design and supervised the research. Afroza Khanam and Firdous Ahmad Khan assisted with interpretation of the results and prepared the final version of the manuscript. All authors read and approved the final version.

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