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CLINICAL EVALUATION OF EFFICACY AND SAFETY OF CALCIUM CHLORIDE ASSOCIATED WITH ETHYL ALCOHOL AND LIDOCAINE FOR NON-SURGICAL STERILIZATION OF SEXUALLY MATURE CANINE AND FELINE MALES.



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Non-Surgical Sterilization of Canine & Feline Males

Overpopulation of Dogs & Cats

There is a homeless small animal problem, not just here, but in every country on earth.

Free-roaming cats and dogs present a number of health threat to people and pets. Rabid dogs are the number one cause of rabies deaths in humans. Over 20,000 people die of rabies in India every year, many of them children, and the vast majority contract of the disease from dog bites.

It's estimated there are tens of millions of feral cats in US alone. In other countries, exploding populations of feral dogs are the problem – for example, there are an estimated 30 million unowned dogs in India.

Clearly, dog and cat overpopulation is a situation the world needs to get its arms around.




WHAT APPROACH SHOULD BE USED TO CONTROL THE STREET DOG & CAT OVER POPULATIONS?

Surgical Castration

The population control for male dogs and cats have been mainly accomplished through surgical Castration, i.e. **Orchidectomy**, however, it carries the **Risks of pain** to the pets and is expensive to perform on a large scale as well as requiring a skilled veterinarian with well equipped operating facilities.

- Presently a viable alternative to surgical Castration is being intensively investigated.




Non-Surgical Castration

- Hormonal treatment
Progestogens, Androgens, GnRH analogues (agonist/antagonist)
- Intra-testicular/intra-epididymal injection
- Immuno-contraception
- Ultrasound testicular ablation

Non-surgical castration in male dogs and cats:
Possibility, availability and humanity



Non-surgical Chemical Contraception

Suppress

- Reproductive function
- Sexual behaviour/Libido



- Easy to be administered in the field
- Suitable for mass-scale application
- Single dose–Reasonable cost
- Safe and effective - Permanent
- Quick onset

- Injection of sclerosing/necrotizing agents into the testes to induce aspermatogenic orchitis and tissue sclerosis resulting in infertility

The ideal chemical sterilizing agent:

- Arrests spermatogenesis – androgenesis - libido
- No toxic and untoward side effects

Intratesticular Injection

• Sclerosing agents

- Zinc gluconate (Levy et al., 2008)
- 20% hypertonic saline (Emir et al., 2008)
- Glycerol (Immegart et al., 2000)
- 1.5% chlorhexidinegluconate in 50% DMSO (Pineda et al., 1977)
- Calcium chloride (Jana and Samanta, 2002, 2005, 2006, 2007, 2011)



CaCl2 in various solutions and concentrations was tested as a necrotizing agent to induce chemo-sterilization in canine and feline males

1978: *Clinical Reports*

chlorhexidine and zinc gluconate, the most effective agents for sterilization of male dogs and cats.

calcium chloride castration

It is a simple and effective method for sterilizing male dogs and cats. The authors report that 100% of dogs and 100% of cats castrated with 1.5% chlorhexidine gluconate in 50% DMSO were sterile at 100 days post-operation.

2007:

Journal of Veterinary Research

Original research article

Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose-dependent study

Kulady Jee*, Pratik Kumar Samanta, et al.

2011:

Journal of Veterinary Research

RESEARCH ARTICLE

Clinical Evaluation of Non-surgical Sterilization of Male Cats with Single Intra-testicular Injection of Calcium Chloride

Kulady Jee* and Pratik K Samanta*

2011:

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RESEARCH ARTICLE

Clinical Evaluation of Non-surgical Sterilization of Male Cats with Single Intra-testicular Injection of Calcium Chloride

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Calcium Chloride Sterilization

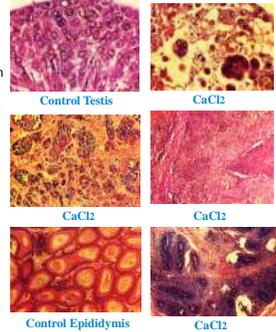
Calcium Chloride: Tissue Necrotizing Agent, Single bilateral intra-testicular injection of calcium chloride in different concentrations produced necrosis and atrophy (castration) in Rats, Goats, Dogs and Cats (Kogeret *al.*, **Modern Vet Prac.**(1976); Jana et al., **Vet. Res. Commun.**(2002); **Animal Reproduction Sci.** (2005); Jana & Samanta, **Contraception** (2006 & 2007); **BMC Veterinary Research**(2011).

Permanent Sterilization



Non-surgical Sterilization of male Rat with CaCl₂ solution

- Mature male Wistar albino rats weighing 130± 10 gm were selected. 0.1 ml of 10% CaCl₂ was injected per testis per 100 gm body weight.
- Coagulative necrosis in testicular parenchyma with multinucleated giant cell formation, vacuolization. Calcium deposition, tubular disruption and fibrous tissue deposition were evident in both testis and epididymis following CaCl₂ injection.
- Plasma Testosterone was decreased by 70% whereas, intra-testicular Testosterone was diminished by 80% compared to the control.
- Apparently, no stress response was elicited following 90 days of CaCl₂ treatment.
- No fertility performance was noted following 90 days after CaCl₂ treatment.



Chemisterisol® : The new nonsurgical single intra-testicular injectable sterilizing agent

CaCl₂ solution (Chemisterisol®) consists of:

1. Calcium chloride (dihydrate), the active ingredient
2. Ethyl alcohol
3. Lignocaine hydrochloride (Lidocaine)
4. Sodium chloride
5. Water for injection and other minor ingredients (Under patent)

Route of Administration:

Intra-testicular injection

Species:

Dogs & Cats (3 months and older)

Recommended Dose:

Single injection is given per testicle



Non-surgical Sterilization of Canine Males

Intra-testicular Injection of CaCl₂

The Dose of Chemisterisol

Testicular Width (mm)	Dose per testis (ml)
10-14mm.	0.25ml
15-18mm	0.5ml
19-22mm	0.8ml
23mm and above	1 ml



Procedure of Intra-testicular Injection

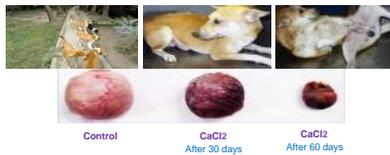
The needle (21/22 gauge) was directed from the ventral aspect of each testis approximately 0.5 cm from the epididymal tail towards the cranial aspect of that testis.

Necessary care should be taken to prevent the seepage of the solution from the injection site.

Clinical Evaluation following 60 days after CaCl₂ injection

- No changes in body weight, appetite, rectal temperature, heart and respiratory rates, and scrotal and inguinal integument.
- No changes in serum or saliva cortisol concentrations, serum prolactin, insulin, and fasting blood glucose, and complete blood haematology and blood urea nitrogen levels.
- No changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, calcium, urea, creatinine and total serum protein concentrations.

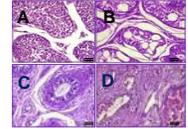
Comparative photographs of the Testicular Sizes following Chemisterisol injection



Comparative Testicular Histology following CaCl₂ injection

Light Microscopy

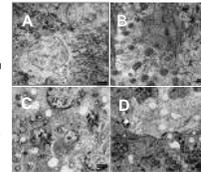
Necrosis and degeneration of Germ & Leydig cells along with vacuole formation and deposition of fibrous and hyaline tissue, leukocyte infiltration and tubular architecture derangement.



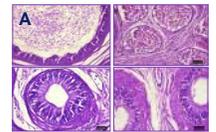
Photomicrographs of control (A) and 30 days (B), 45 days (C) & 60 days (D) after CaCl₂-treated dog testis (H & E; 40X)

Transmission Electron Microscopy

Degenerated Sertoli, germ and Leydig cells along with the presence of degenerating mitochondria with lack of matrix, myelin and lisosomal bodies, autophagosome and chromatin condensation as well as vacuole formation.

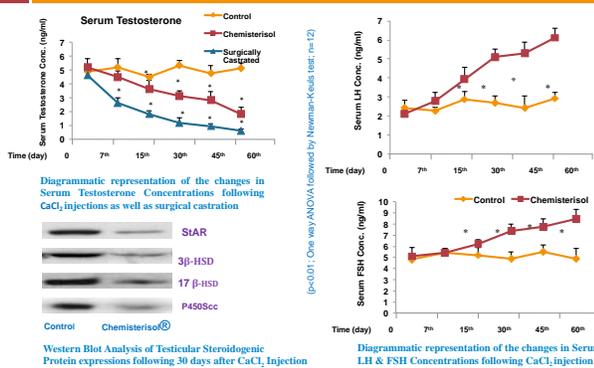


Transmission electron micrographs of Control (A & B) & 30 days after CaCl₂-treated (C & D) Dog Testis



Photomicrographs of control (A), 30 days (B) 45 (C) and 60 (D) days after CaCl₂ treated dog epididymis (H & E; 40X)

Hormonal assessment following CaCl₂ Injection

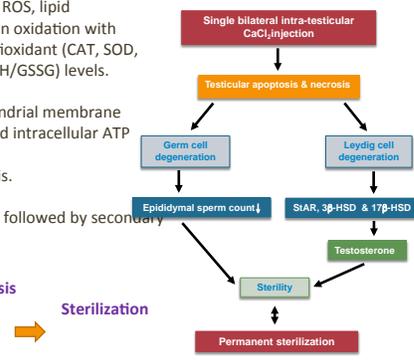


Mechanism of action at cellular level: following CaCl₂ injection

- Increased testicular ROS, lipid peroxidation, protein oxidation with reduced cellular antioxidant (CAT, SOD, GPx, GRd, GST & GSH/GSSG) levels.
- Decreased Mitochondrial membrane potential ($\Delta\psi_m$), and intracellular ATP levels in both testis and epididymis.
- Germ cell apoptosis followed by secondary Necrosis

Tissue/Cellular Necrosis (Testis/Epididymis)

Sterilization



Common short-term side effects

- Swelling of the testis (mild to moderate)
- Sensitivity to testicular palpation

Additional short-term complications seen only in field study

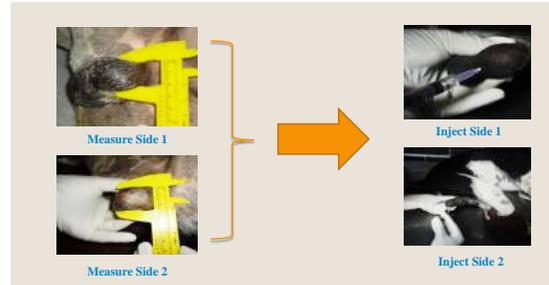
- Pain (mild to moderate)
- Lethargy
- Elevated rectal temperature (18-22%)
- Scaly or inflamed scrotal skin (6-8%)
- Progression to ulceration (3-4%)



If a separate needle is used for drawing up and injecting the solution, ulcer of the injection area should be avoided. The use of a non-steroidal anti-inflammatory drug (NSAID) was very effective to prevent distress that may be caused by post-injection swelling and that is the most common side effect.

No long-term adverse effects appeared during the course of the study.

Measurement and CaCl₂ injection



Six months following CaCl₂ injection



6 Months after CaCl₂ injection: Testicular size vs. Control

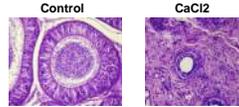


6 months following the CaCl₂ injection

Histology showed complete necrosis without presence of any germ/sperm cells, along with the appearance of fibrous/hyaline tissue in both testis and epididymis.



Serum testosterone was reduced by about 76% (Control, 8.52±0.21; CaCl₂ 2.05±0.14), along with loss of libido (sexual interest in oestrus female dog)



No significant differences with respect to clinical findings or any aspect of haematology, renal or hepatic functions or serum cortisol concentration.

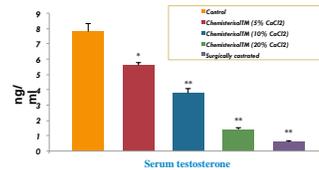
Non-surgical Sterilization of Feline Males

Adult feral cats weighing 2-3 kg, aged from 09 to 12 months.

Intra-testicular injections of 0.25 ml CaCl₂ solution containing different concentrations of calcium chloride were given in each testis.

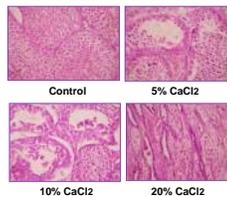
Testicular swelling was noted 24 hr. following injection. Ulceration was noted in 2% cases.

Sixty days following injection: Very low epididymal sperm count along with reduction of serum testosterone by at least 70% in 20% CaCl₂ dose.



Nonsurgical chemical contraception of feline males

Degeneration of seminiferous tubules and interstitial cells, presence of degenerated and coagulated germ cells, presence of giant cells with infiltration of leukocytes, derangement of tubular architecture with the appearance of fibrous tissue were evident in testicular histology following CaCl₂ injection.



Increased testicular lipid peroxidation, protein oxidation, with reduced antioxidants and mitochondrial membrane potential.

No significant differences with respect to clinical findings or any aspect of haematology, renal or hepatic functions or serum cortisol concentration.



Kitten sterilization with Calcium Chloride

Feral kittens, 5-6 months of age, 400-600 gm weight were selected for the study. 0.06-0.08 ml of CaCl₂ solution (calcium chloride, alcohol and lidocaine mixture) solution has been injected in each testis irrespective of body or testis weight.



Two months latter, there is a small tiny palpation of testis. More or less 40-56 % decrease of serum testosterone levels without any changes in body weight gain and or any metabolic toxicity were noted following CaCl₂ injection.

Testicular histology showed only presence of fibrous tissue without presence of any mature or immature germ cells or Leydig cells in CaCl₂ injected kittens.



Side effects: testicular swelling were noted in every kitten. Out of twelve, three kittens had testicular skin reactions

Surgical Castration vs. Chemisterisol® Injection

Surgical Castration	Chemisterisol® Injection
• Scientifically & socially accepted procedure	• New procedure
• Loss of masculinity and male aggressive behavior	• Loss of masculinity & male aggressive behavior
• Absence of testes visible from a distance	• Atrophy of testes visible from a distance
• Invasive procedure (surgical operation)	• Non-invasive procedure
• General anesthesia required	• No anesthesia required
• Post-operative care and management required	• Little post-operative care and management required
• Post-operative risks inherent in any surgical operation	• Apparently no severe adverse reactions
• Time consuming, complicated, and expensive	• Quick, simple and cost effective
• Challenging in large scale application	• Efficient in large scale application
• Skilled veterinarian with well equipped operating facilities required	• Any competent individual, anywhere can give the injection



Goals for Future Research

- Expand municipal field trials
- Identify formulation, dosage, and administration of calcium chloride to sterilize male puppies and kittens.



Conclusion

CALCIUM CHLORIDE SOLUTION (Chemisterisol®) is a safe and effective product for sterilization of male dogs and cats without doing surgery.

This chemical sterility method is shown to be easier and faster to administer and more cost effective than surgical castration.

Sedation is recommended for some cases and injection technique needs to be accurate and completed with care by a capable individual.



Acknowledgements

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