Non-Surgical Sterilization of Canine & Feline Males

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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.

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 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/necrotising agents into the testes to induce aspermatogenic orchi's 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)

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

**1978:**

**2007:**

**2011:**

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


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, vascularisation. Calcium deposition, tubular disruption and fibrous tissue deposition were evident in both testes 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**

<table>
<thead>
<tr>
<th>Testicular Width (mm)</th>
<th>Dose per testis (ml)</th>
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<tbody>
<tr>
<td>10-14mm.</td>
<td>0.25ml</td>
</tr>
<tr>
<td>15-18mm.</td>
<td>0.5ml</td>
</tr>
<tr>
<td>19-22mm.</td>
<td>0.8ml</td>
</tr>
<tr>
<td>23mm and above</td>
<td>1ml</td>
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**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 levels.
- No changes in serum or saliva cortisol concentrations, serum prolactin, insulin, and sodium, potassium, calcium, urea, creatinine and total serum protein levels.
- No changes in serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, calcium, urea, creatinine and total serum protein levels.

Comparative photographs of the Testicular Sizes following Chemisterisol injection

Control  CaCl₂ After 30 days  CaCl₂ After 60 days

Hormonal assessment following CaCl₂ Injection

Diagrammatic representation of the changes in Serum Testosterone concentration following CaCl₂ injection

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 (Δψm), and intracellular ATP levels in both testis and epididymis.
- Germ cell apoptosis followed by secondary Necrosis
- Tissue/Cellular Necrosis (Testis/Epididymis) → Sterilization

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.

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

Dog Testis (A & B) & 30 days after CaCl₂ injection

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

Photomicrographs of control (A) and treated (B) 45 days after CaCl₂ treated (B) epididymis (C & D; H & E)

Photomicrograph of control (A) and 30 days after CaCl₂ treated (B) epididymis (C & D; H & E)

Single bilateral intra-testicular injection

Preliminary sperm count

Germ cell degeneration

Leydig cell degeneration

Epididymis space emptying

Sterility

Sterilization

Permanent sterilization

ACC&D 5th International Symposium on Non-Surgical Contraceptive Methods of Pet Population Control
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

Comparative photographs of the testicular sizes following 6 months after CaCl₂ injection.
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.

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

This chemical sterilization 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.

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Thank You!