

HISTORY OF CALCIUM CHLORIDE INJECTABLE STERILIZATION IN MALE DOGS AND FIRST REPORT OF USE IN CATS

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Calcium chloride solution has an extensive publication history as an injectable sterilant in dogs and other large mammals. Here we present first published use in cats. In dogs, publications go back to 1978 (e.g. Kroger LM) and more recent studies by our team have provided quantitative data on efficacy, testosterone reduction, and measures of stress levels such as plasma concentrations of cortisol (Jana and Samanta 2007). With feral cat populations a continuing problem, we sought to explore its use in cats. Six cats per group were injected with 5%, 10%, or 20% calcium chloride (dihydrate) in saline solution with lignocaine hydrochloride local anesthetic, preservative, and several minor ingredients (Chemisterisol™), with 20% determined to be the optimal dose. At 8 weeks, cat testes were collected and showed complete testicular necrosis and replacement by fibrous tissue; serum testosterone was reduced at least 70%. Due to its permanent reduction of testosterone, Chemisterisol™ is in a different category than the injectable sterilant currently on the market (Freedom of Information Summary, Neutersol™, NADA 141-217). It should be used in cases where elimination or reduction of sex-based behavior is desired, such as for family pets and in street dog/feral cat programs; the FDA-approved injectable male sterilant, zinc gluconate neutralized by arginine (Neutersol™/Esterisil™), should be used when return of testosterone is desired for health reasons (such as in large purebred dogs susceptible to orthopedic disorders) or when the owner wishes to preserve behavior such as guarding. The primary practical advantage of Chemisterisol™ is ease of injection due to its less caustic nature, with little training required and complications avoided if any spilled solution is wiped away. The primary disadvantage is slow onset of action (4-6 weeks) and inter-individual variability in level of discomfort during injection, which we hope to address by calibrating injection volume more specifically to testicular volume rather than body weight. In our experience unspayed older dogs with larger testes tend to experience little discomfort during injection, while unspayed cats and small dogs exhibit some distress during the two-minute procedure; all animals regain normal behavior and exhibit no distress immediately upon procedure termination. In conclusion, Chemisterisol™ demonstrates potential for androgenesis-eliminating non-surgical sterilization of male cats in addition to its proven efficacy in dogs and larger animals.

Sterilization has long been recognized as the most effective means of controlling pet populations. Yet with the enormous number of owned and unowned cats and dogs in the U.S., the sterilization programs currently available are not enough. Spay/neuter requires anesthesia, a sterile surgical suite, a licensed veterinarian, recovery time, incision site observation, and more. In the U.S. alone, approximately 4.5 million animals were killed in shelters each year simply because they have no place to go. We are not reaching enough of the cats and dogs at greatest risk of contributing to those numbers. The situation in many other parts of the world is much more desperate. In dozens of under-developed nations, poverty is endemic. Veterinary access and financial resources are simply not at the needed levels for population control via surgical sterilization to be a real possibility. Municipal animal care and control systems are primitive or non-existent and surplus pets are often killed by archaic methods such as drowning and electrocution. Rabies is widespread and rabies control programs in many countries offer an existing infrastructure for delivery of non-surgical contraceptives, once they are available (Alliance for Contraception in Cats and Dogs, <http://www.aacc-d.org/About>).

To triumph over the above problems we have tried more than 10 years to evolve a new method of non-surgical contraception for pet population control. Recently, we have reported that single bilateral intra-testicular injection of calcium chloride solution results in induction of sterilization in male albino rats, goats and dogs through the germ cell apoptosis via- α -vis necrosis along with concomitant production of reactive oxygen species (ROS) in the testis as well as significant diminution in testosterone concentration without imposition of any general stress response, metabolic toxicity or any toxic and untoward side effects (Jana et al., 2002, 2004, 2006, 2007; Jana & Samanta, 2006, 2007).

In this study we have used a new sterilizing preparation in which calcium chloride (dihydrate) dissolved in normal saline solution with lignocaine hydrochloride, local anesthetic, a preservative, antibiotics and several minor ingredients (Chemisterisol™), to sterilize feral cats. This study was conducted in 2009 and is ongoing.

Chemical Castration (Chemo-sterilization)

- Injections of steroid hormones i.e. androgen (Matsumoto, 1988), progestagens (Swierloff et al., 1992), anabolic steroids (Dhar & Setty, 1990) and anti-androgens (Dhar & Setty, 1990).
- GnRH agonists (Trembley et al., 1984; Dube et al., 1987)
- Antibodies against gonadotropins and GnRH (Gonzalez et al., 1989; Dowsett et al., 1991)
- Intratesticular injection of glycerol (Wiebe & Barr, 1984), Lactic Acid (Fordyce et al., 1989), Cadmium chloride (Parizek, 1960) BCG (Naz & Talwar, 1981), Formaldehyde (Marcy et al., 1985), Danazol (Diaz et al., 1975) & α -hydroxy-propionic acid (Cohen et al., 1990)

However, these treatments did not consistently result in sterility.

Calcium Chloride Castration:
 Calcium Chloride: Tissue necrotizing agent
 Single bilateral intra-testicular injection of calcium chloride in different concentrations produced necrosis and atrophy (castration) in Calves, Pigs, Lambs, Goats, Dogs and Cats (Koger et al., 1979), rats, goats, and dogs (Jana et al., 2002, 2005; Jana & Samanta, 2006, 2007).

Animal selection, care and management:

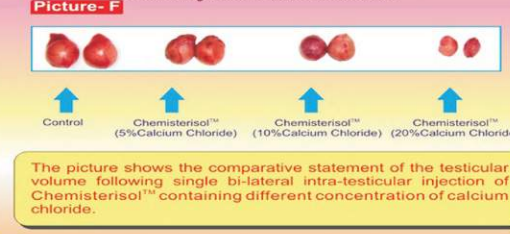
Clinically healthy male adult cats (n = 24) weighing 2.5 kg (range 2-3 kg) respectively, aged from 09 to 12 months with normal libido and serving ability in the breeding season were acclimatized initially for one month in the animal house. They were routinely dewormed and vaccinated prior to arrival in the animal housing area. The animal house had artificial lighting and controlled temperatures (20°C, ranging from 18 to 22°C). Cats were housed in indoor runs and received food and water ad libitum. The Animal Ethics Committee of the Institute approved the protocol of this experiment. All the animals were maintained and all the experiments were performed following "National Guidelines on the Proper Care and Use of Animals in Laboratory Research (Indian Science Academy, New Delhi, India).

Composition of Chemisterisol™
 The new non-surgical single intra-testicular injectable sterilizing agent Chemisterisol™ is composed of Calcium chloride (dihydrate), the active ingredient, Lignocaine hydrochloride, Sodium chloride, Ethyl alcohol, Antibiotics, Preservative and several other minor ingredients.

Intra-testicular Injection of Chemisterisol™ and routine surgical castration:
 Intra-testicular injections of Chemisterisol™ containing different concentrations of calcium chloride were given by using 2 ml syringe (single use). 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. The solution was carefully deposited along the entire route by linear infiltration while withdrawing the needle from proximal to distal end. Necessary care should be taken to prevent the seepage of the solution from the injection site. All the animals were kept for 60 days under routine clinical observations. Parallel surgical castrations were done in another group of animals according to the standard procedure.



Testicular impact following 60 days after intratesticular injections of increasing concentrations of Chemisterisol™



The picture shows the comparative statement of the testicular volume following single bi-lateral intra-testicular injection of Chemisterisol™ containing different concentration of calcium chloride.

Figure - 1, 2, 3 & 4 Non Surgical Pet Contraception



Figure - 5, 6, 7 & 8 Non Surgical Pet Contraception

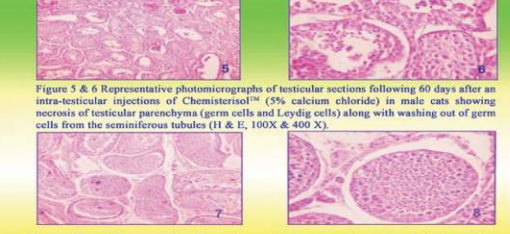


Figure - 7 & 8 Representative photomicrographs of a testicular sections following an intra-testicular injection of Chemisterisol™ (10% calcium chloride) in male cats showing a high degree of coagulative necrosis in seminiferous tubules and interstitial spaces (H & E, 100X & 400 X).

Figure - 9, 10, 11 & 12 Non Surgical Pet Contraception

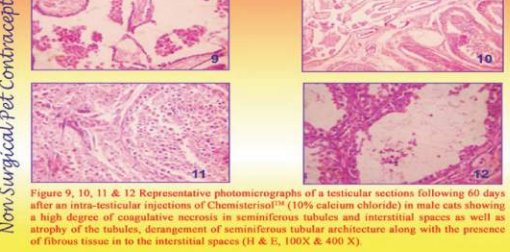


Figure -13, 14, 15 & 16 Non Surgical Pet Contraception

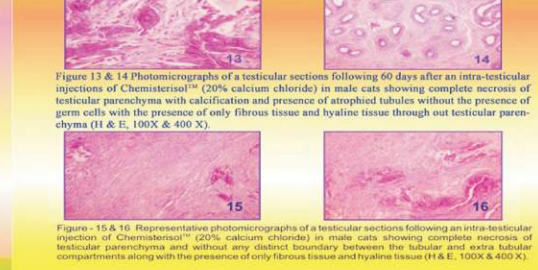


Figure - 15 & 16 Representative photomicrographs of a testicular sections following an intra-testicular injection of Chemisterisol™ (20% calcium chloride) in male cats showing complete necrosis of testicular parenchyma and without any distinct boundary between the tubular and extra tubular compartments along with the presence of only fibrous tissue and hyaline tissue (H & E, 100X & 400 X).

Figure - 17 & 18 Non Surgical Pet Contraception

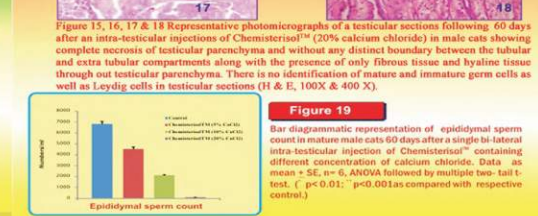


Figure 19 Bar diagrammatic representation of epididymal sperm count

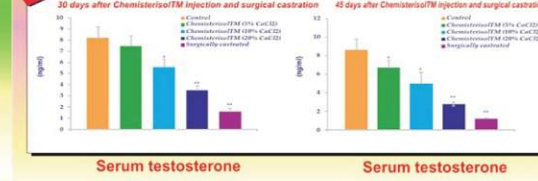


Figure 20 Serum Testosterone at 30 & 45 days after Chemisterisol™ injections

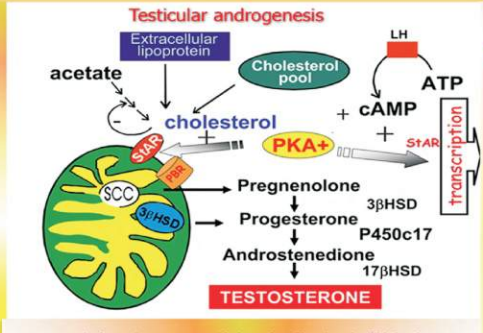
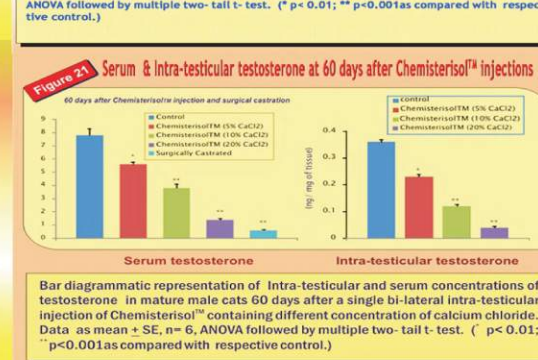


Figure 22 Androgenic enzymes at 60 days after Chemisterisol™ injections

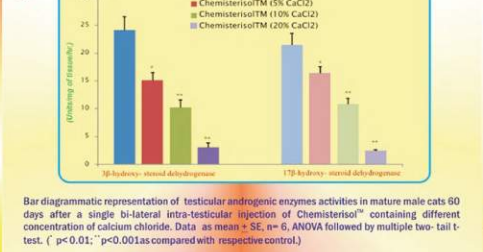


Figure 23 Steroidogenic Acute Regulatory Protein: STAR

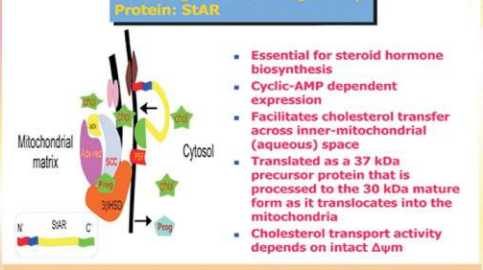


Figure 23 Western blot of androgenic proteins at 60 days after Chemisterisol™ injections

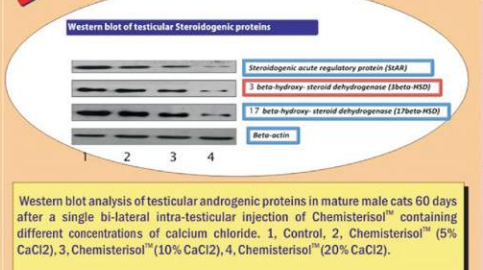


Figure 24 Stress measures at 5 & 10 days after Chemisterisol™ injections & surgical Castration

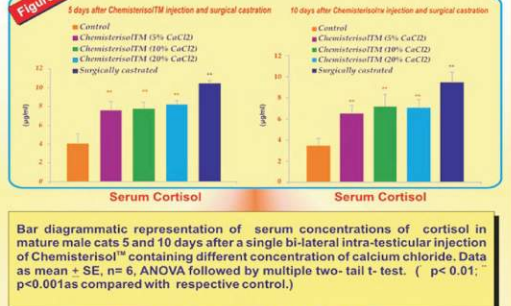


Figure 25 Stress measures at 15 & 30 days after Chemisterisol™ injections & surgical Castrations

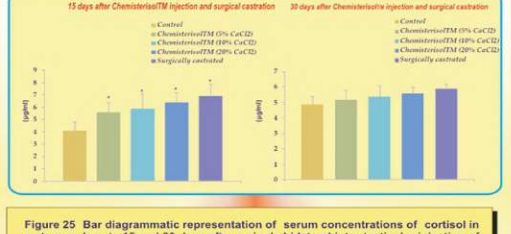
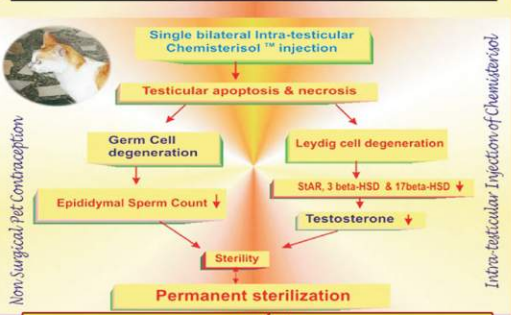


Figure 25 Bar diagrammatic representation of serum concentrations of cortisol in mature male cats 15 and 30 days after a single bi-lateral intra-testicular injection of Chemisterisol™ containing different concentration of calcium chloride. Data as mean ± SE, n=6, ANOVA followed by multiple two-tail t-test. (p < 0.01; **p < 0.001 as compared with respective control.)



Conclusion

A single bi-lateral intra-testicular injection of Chemisterisol™ is effective, economical, safe, easy to perform and free from any untoward side effects and causes sterilization in cats. It provides a simple alternative method to surgical castration.

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