Calcium chloride chemical castration in the rat: A possible solution for pocket pets?

A case study prepared by Parsemus Foundation, including observations provided by a pet rat's owner.

Background

Calcium chloride dihydrate ($CaCl_2$) has been studied as a nonsurgical neuter option in dogs, cats and other animals. The procedure is inexpensive and requires few physical resources, providing advantages over traditional castration which requires a surgical area and supplies. This makes calcium chloride potentially advantageous to pet owners with limited incomes and in low-resource environments.

The technique involves injection of the calcium chloride solution into each testicle while the animal is lightly sedated (to reduce movement). The chemical kills the tissue of the testicle resulting in sterility. Given this necrotizing effect, it is important that none of the solution escapes the testicular capsule onto healthy tissue. The testicles often swell initially, followed by significant shrinkage in size.

Calcium chloride chemosterilant has been studied in a number of species (see literature review at: https://www.parsemus.org/wp-content/uploads/2018/07/Calcium-Chloride-BIBLIOGRAPHY-_rev2018-07-18.pdf). Large-scale, controlled studies of $CaCl_2$ conducted by Jana (India) and Leoci (Italy) were very successful in producing azoospermia and significant reductions in testosterone, with few complications, in dogs. A solution of 20% $CaCl_2$ in ethyl alcohol was found to be an optimal solution for dog chemosterilization (Leoci et al., 2014 a, b). Following publication of this work, other researchers have continued to study alternative methods and different solutions of $CaCl_2$. During field use, some users experienced complications, possibly as a result of variations in injection technique, characteristics of the animal being treated, follow-up care, or other unknown variables. Less success has been experienced in animals with large testicles, as it is difficult to obtain adequate coverage of the $CaCl_2$ solution across a large testicular volume without leaking.

Calcium chloride for small animals

$CaCl_2$ may be especially suited to use in small mammals, including "pocket pets" such as guinea pigs, rats, and hamsters. These pets often reproduce quickly, and owners need to keep them segregated by sex or have them neutered if they do not want offspring. Surgically neutering a small pet may be cost-prohibitive to some owners, and the risk of anesthesia may also be a concern.

Calcium chloride has been used experimentally in rats. Drs. Jana and Samanta published two dose-finding studies using $CaCl_2$ in saline to sterilize 60 male albino rats in each project (Jana et al., 2002; Jana & Samanta, 2006). The researchers concluded that 10-20 mg/100g weight was sufficient to sterilize rats, measured by epididymal sperm count and mating studies. Additionally, they compared intratesticular to intraepdidymal injections of $CaCl_2$ in rats and the images below are from a presentation by Dr. Jana on this study in 2014. Fig. 1 shows the testicular injection technique and Fig. 2 illustrates the atrophy of the testicles over time.

Figure 1. The technique used to inject $CaCl_2$ in saline into the testicle of a rat as part of a study conducted in India (credit to Dr. Kuladip Jana)

Figure 2. Images illustrating the shrinking of the testicular tissues following injection of $CaCl_2$ in saline in a study on rats (credit to Dr. Kuladip Jana).

Another study of intratesticular injection of guinea pigs with 15 mg/100g weight of $CaCl_2$ in lidocaine, provided as one injection or three daily doses, resulted in significantly decreased testosterone and sperm count, but not azoospermia (Sen et al., 2017). No fertility assessment was completed. The difference from the earlier rat studies may be due to species differences or the diluent used with $CaCl_2$.

Finally, a large-scale study of intratesticular injection of 20% $CaCl_2$ in 0.5% dimethyl sulfoxide (DMSO) in 96 rats was recently completed by researchers in Brazil. At 100 days after the injection, rats were azoospermic, infertile, and evidenced testicular atrophy (Paranzini, 2019). Only one rat had complications.

No study has been conducted in small animals using the 20% $CaCl_2$ solution in ethyl alcohol. Thus, this information is provided to encourage additional research on the topic.

Case Study

The owner of an adult male pet rat, named Coffee, was interested in nonsurgical neuter using calcium chloride to eliminate the risk of pregnancy in a female rat. Basic details were collected on the procedure and outcome.

Methods:

Calcium chloride dihydrate topical solution 20% in 95% ethyl alcohol was obtained from an established and PCAB-accredited compounding pharmacy. The solution was sterilized by drawing solution into a Luer Lock syringe using an 18-gauge needle and filtering slowly through a syringe filter[1] into a sterile vial, avoiding undue pressure to prevent filter blow-out. 0.1 ml of filtered solution was drawn up into each of two commercially available insulin syringes[2] and set aside.

To reduce stress and eliminate movement, the rat was anesthetized prior to the procedure (Day 1). A premedication injection of butorphanol was followed by induction of gas anesthesia (isoflurane) using a gas chamber and maintenance using a nose cone. The testicular area was wiped clean. The veterinarian injected 0.1 ml of the solution into the caudoventral aspect of each testicle, with care taken not to allow any seepage of solution. Following the procedure, the rat was recovered. Pain medication (0.1-0.5 ml of Buprenorphine 0.3 mg/ml every 6-12 hrs) was provided for Day 1-3.

Results:

Behavioral and physical observations were provided by the owner. He had a normal appetite throughout the period.
Days 1-2: Stayed in his nest following the procedure and the next day.
Day 3: Started coming out of his nest
Day 6: Active, normal behavior
Day 10: No evidence of skin irritation
Day 14: Some slight skin irritation, possibly shrinkage of testicle
Day 16: No skin irritation, pictures taken with evidence of swelling compared to adult offspring (see Fig. 3)
Day 32: Testicles are visibly shrunken (see Fig. 4 left)
Day 41: Only remnant of testicle left (see Fig. 4 right)

[1] Corning 431212 Regenerated Cellulose Sterile Syringe Filter with Female Luer Lock Inlet/Male Luer Slip Fitting Outlet, 4mm Diameter, 0.2µm Pore Size
[2] BD 1 mL, 12.7 mm, 30G

Figure 3. Day 16 images of Coffee on left and his son Oliver on right as normal comparison

Figure 4. Day 32 (left) and Day 41 (right) images of Coffee, illustrating reduction in testicular size

Testing Coffee's fertility was discussed, but testosterone tests were deemed to require too much blood and mating studies were not conducted due to the chance that he was still fertile. The owner decided to house Coffee with a compatible younger male rather than risking an unwanted litter in the future.

Summary:

The owner of the pet rat Coffee was pleased with the outcome of using $CaCl_2$ chemical sterilant and that the procedure was quick, generally non-traumatic, less expensive than surgery, and did not put the rat at surgical risk. The veterinarian was pleased with the procedure's simplicity and that no complications were encountered given that it was her first $CaCl_2$ injection procedure.

The use of calcium chloride in alcohol was simple to administer in the rat and effectively reduced the size of the testicles to small remnants. This finding agrees with previous studies in rats which showed that calcium chloride in saline caused necrosis of testicular tissue and fibrosis (Jana, 2002, 2006).

While no evaluation was conducted to confirm sterility in this case study, it is likely that Coffee was no longer fertile given the condition of his testicles following treatment. The veterinarian expressed confidence that the testicular remnants would not support fertility. In research studies using various solutions, a decrease in testicular size following injection of $CaCl_2$ has been accompanied by infertility/azoospermia and decreased testosterone in rats (Jana et al., 2002; Jana & Samanta, 2006; Paranzini, 2019), dogs (Leoci et al., 2014 a,b), and cats (Jana & Samanta, 2011; Paranzini et al., 2017). Previous research has indicated that treated rats no longer exhibited sexual behavior (Jana & Samanta, 2006) and dogs were no longer interests in females in heat (Leoci et al., 2014 b).

Calcium chloride has been used as a chemosterilant for several animal species. Given its successful use in large experimental rat studies, and the experience outlined here for a pet rat, further research on $CaCl_2$ as an inexpensive and nonsurgical sterilization option for rats and other pocket pets is warranted.

References:

Jana K, Samanta PK, Ghosh D. (2002) Dose-dependent response to an intratesticular injection of calcium chloride for induction of chemosterilization in adult albino rats. Vet Res Comm. 26(8):651-673.

Jana K, Samanta PK. (2006) Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization in adult albino rats. Contraception 73:289– 300

Jana K, Samanta PK. (2011) Clinical evaluation of non-surgical sterilization of male cats with single intra-testicular injection of calcium chloride. BMC Veterinary Research, 7:39.

Leoci R, Aiudi G, Silvestre F, Lissner E, Marino F, Lacalandra G. (2014 a) A dose-finding, long-term study on the use of calcium chloride in saline solution as a method of nonsurgical sterilization in dogs: evaluation of the most effective concentration with the lower risk. Acta Veterinaria Scandinavica, 56:63

Leoci R, Aiudi G, Silvestre F, Lissner E, Lacalandra G. (2014b) Alcohol diluent provides the optimal formulation for calcium chloride non-surgical sterilization in dogs. Acta Veterinaria Scandinavica, 56:62.

Paranzini CS. (2019) Effects of calcium chloride with DMSO injection on testicular function and fertility in rats. 120 p. Thesis (PhD Degree in Animal Biotechnology) – Faculdade de Medicina Veterinária e Zootecnia, Campus Botucatu, Universidade Estadual Paulista.

Paranzini CS, Sousa AK, Cardoso GS, Perencin FM, Trautwein LGC, Bracarense APFRL, Martins MIM. (2017) Effects of chemical castration using 20% CaCl2 with 0.5% DMSO in tomcats: Evaluation of inflammatory reaction by infrared thermography and effectiveness of treatment. Theriogenology, 106:253-258.

Sen CC, Yumusak N, Faundez R, Temamogullari F, Taskin A. (2017) Evaluation of intra-testicular injections of calcium chloride and 4-vinylcyclohexene 1, 2 monoepoxide for chemical sterilization in guinea pigs. Polish J Vet Sci, 20(2):251-260.