The use of calcium chloride dihydrate in ethyl alcohol to nonsurgicallly sterilize adult male African pygmy goats (*Capra hircus*)

Angela Colagross-Schouten, DVM, BioConsult Veterinary Group

and Dina Allison, Relief Veterinary Services

Summary

Nonsurgical techniques such as the emasculatome clamp and elastrator band are frequently used to sterilize young male ruminants. Adult bucks present additional challenges due to rapid growth and resulting large testicular size. Surgical castration is currently the only option, but entails welfare considerations and increased expense. In an effort to develop a nonsurgical alternative for adult male goat sterilization, we evaluated the efficacy of intratesticular injection of calcium chloride dihydrate ($CaCl_2$) in ethyl alcohol to sterilize two African pygmy goats. $CaCl_2$ is a readily available, inexpensive compound that has been used to sterilize a number of animal species, but the optimal formulation has only recently been determined for dogs. Using this formulation, 4 ml was injected into each testis of the manually restrained but unanesthetized goats. The initial treatment did not have a lasting effect on testosterone or testicular size so a second identical injection was performed. Results were similar. Finally, a 10 ml solution was given and was effective at eliminating testosterone and shrinking the testicles due to widespread necrosis of the seminiferous tubular structure. However, both subjects evidenced ulceration of the scrotum following this dose and required subsequent treatment. This is the first study to evaluate serial injections of $CaCl_2$ in an effort to determine an effective dose. There appeared to be no residual effect of the repeated lower volume solutions, which is important for dose-finding when $CaCl_2$ may be used in a new species. The higher dose was effective at sterilizing the male goats, but not without complications.

Introduction

Nonsurgical male sterilization techniques have been evaluated as a means to avoid the potential health complications, expense, expertise and facilities required for surgical sterilization procedures. One of the most promising is calcium chloride dihydrate ($CaCl_2$), which has been utilized to chemically castrate a variety of species since 1977 [1]. Following intratesticular injection of $CaCl_2$, necrosis, fibrosis and degeneration of seminiferous tubules and Leydig cells occurs, reducing or eliminating the production of spermatozoa and testosterone [2]. $CaCl_2$ sterilization has the added benefits of being inexpensive, readily available and technically uncomplicated. More recent studies have focused mainly on companion animals and the potential use of $CaCl_2$ to nonsurgically sterilize stray dogs and cats [3-4]. The first long-term studies found that a 20% concentration of $CaCl_2$ in alcohol provided the most effective solution for permanent sterility in dogs with no side effects [5,6].

Goats present particular challenges regarding sterilization. Male goats achieve sexual maturity rapidly, with spermatogenesis occurring as early as 84 days [7]. Animals not needed for breeding programs are usually sterilized at a young age - as early as a few days old - to avoid the development of scent glands and undesirable reproductive behaviors. A number of methods of sterilization are available for young goats and sheep, including surgical castration, an emasculatome clamp (Burdizzo®) to rupture the spermatic cord, or an elastrator band which cuts off the blood supply to the testes. The latter two nonsurgical methods may not always be effective (e.g.[8]) and entail welfare concerns. Early sterilization also arrests urethral development, and urinary calculi can be more problematic due to the narrow urethra. Pygmy goats that were castrated at less than 6 months of age required surgery most often for obstructive urolithiasis [9]. However, sterilization of male goats using traditional nonsurgical techniques is more difficult at an older age due to the size of the testes. Surgical castration is usually considered the only viable option for mature bucks and rams. A nonsurgical alternative to sterilize older male goats could provide multiple benefits: avoid pain, potential complications and infection that may occur during surgical castration; and avoid developmental complications and welfare concerns potentially resulting from the use of emasculatome clamp and elastrator band on very young ruminants. It also allows a noninvasive method to castrate older males no longer used for breeding to make them acceptable as pets or to keep as a non-reproducing member of the herd.

$CaCl_2$ has been used previously as a sterilant for small ruminants using various concentrations and injection sites. Intraepididymal injection of 50% $CaCl_2$ in NaCl in rams produced an immediate reduction in sperm concentration but no effect on mounting behavior, since testosterone production was not affected [10]. Jana and colleagues [2] found a dose-dependent relationship between several concentrations of intratesticular $CaCl_2$ in saline and testicular weight and testosterone in small young adult male goats. The 40 mg/kg calcium chloride dose caused necrosis of the seminiferous tubules and interstitial Leydig cells, resulting in decreased sperm and testosterone production.

The aim of this study was to use the optimal solution of 20% $CaCl_2$ in ethyl alcohol to sterilize adult male goats. This is also the first study of repeated intratesticular injections of $CaCl_2$ to determine the most effective dose.

Methods

Subjects

Two adult male African pygmy goats (*Capra aegagrus hircus)* with proven reproductive histories served as the subjects of the study. They were obtained from a local breeder. Goat 1 ("Roger") was 6.7 years and 54.4 kg while Goat 2 ("Tiny") was approximately 2.5 years and 31.7 kg when the study began. All experimental procedures were conducted with the approval of the Institutional Animal Care and Use Committee. The goats were housed in a sheltered stall and had access to a pasture, water *ad libitum*, pelleted feed, grass hay, and grazing opportunities. Standard preventive care (routine weighing, deworming, and hoof trimming) was provided.

Experimental Procedures

At week 0, baseline measures of testosterone and testicular size were completed and the goats received a bilateral intratesticular injection of 4 ml of $CaCl_2$ in ethyl alcohol in each testicle. At week 10, the subjects received a second bilateral intratesticular injection of 4 ml of $CaCl_2$ in alcohol. At week 25, a third bilateral intratesticular injection of 10 ml of $CaCl_2$ in alcohol was completed. Serum testosterone and testicular size were measured approximately every two weeks. At 30 weeks, testosterone was measured with human chorionic gonadotrophin (hCG) stimulation. The study was conducted from February to October, 2013 and concluded at 40 weeks, at which time the goats were surgically castrated and the testes were histologically evaluated. The male subjects were subsequently adopted out as pets.

Preparation and Injection of $CaCl_2$

The $CaCl_2$ was prepared at a compounding laboratory (Customceutical Compounding, Phoenix, AZ) under sterile conditions. The 20% weight by volume alcohol solution of $CaCl_2$ was prepared from 20 g of calcium chloride dihydrate powder USP brought to a final volume of 100 mL in 95% (190-proof) ethyl alcohol USP, mixed, and sterile-filled in 4mL vials.

For intratesticular injections, the goats were manually restrained and the injection site cleaned with alcohol. An 18-gauge 3.8 cm needle was inserted from the lateral aspect of the testis and gently pushed medially. The solution was slowly deposited as the needle was retracted. Without withdrawing the needle from the skin, the needle was then redirected cranially, towards the dorso-cranial pole and the procedure was repeated. Finally the needle was directed caudally towards the caudal pole and solution was expelled as the needle was withdrawn. The entire volume of solution was given before the needle was withdrawn from the testis to prevent seepage of the solution from the injection site. The procedure was repeated on the other testis. The entire procedure was completed in less than 5 minutes per animal (see Fig. 1).

Figure 1. Intratesticular injection of calcium chloride dihydrate. The adult male goat is manually restrained for this < 5 min procedure.

Testosterone and testicular size measurements

Reduction in testicular size below baseline measurement and elimination of testosterone production were considered criteria of effective chemical castration. Testosterone and testicular measurements were collected at approximately 2-week intervals. Additionally, photographs of the subjects' testes were taken at the time of each testicular measurement. The size of the left and right testicle's length, width and depth was measured with a 0-15.2 cm fractional dial caliper (Oshlun, Boulder City, NV) (Fig. 2). Testicular volume was calculated as 4/3Pi[(length/2)(width/2)(depth/2)] for each measurement. The average of right and left testicular volume was used to assess changes over time.

Figure 2. Calipers were used to measure the width and depth of the right and left testicle.

For measurement of testosterone levels, 3 ml of blood was drawn from the jugular vein. The blood serum was processed using radioimmunoassay procedures (Colorado State University, Reproductive Endocrine Laboratory, Fort Collins, CO).

Serum testosterone is known to vary diurnally and seasonally and is responsive to environmental and social factors. An alternative method of measuring testosterone is to use human chorionic gonadotrophin (hCG) to stimulate testosterone production from the testes, an indication of functional Leydig cells [11]. After the final $CaCl_2$ injection at week 30, hCG was given to the goats to stimulate gonadic testosterone. A single dose of 2,500 IU of veterinary grade hCG ("Chorulon", Intervet/Merck) was injected IM and 10 mL of blood was drawn at pre-injection, one and four hours post-injection. Blood was analyzed as described above.

Histology

Testes were fixed in formalin and 10 representative sections (six from Goat 1 and four from Goat 2) were prepared. Routine paraffin light microscopic sections cut at 4-6 microns were stained with hematoxylin and eosin and examined with a Zeiss Axioskop light microscope at 20X-400X (Necropsy Services Group, Davis, CA).

Monitoring and Healthcare

Animals were observed daily by trained technicians for appetite, activity and general health and behavior. Following the intratesticular injections, animals were administered 1-2 mg/kg flunixin meglumine IM or 0.005 mg/kg buprenorphine SC. Flunixin meglumine was continued once daily for 48 hours post-injection. A physical examination and evaluation of the condition of the subjects' testicles was completed at 1, 2, 7, 14, 28, 42 and 56 days after each treatment by the veterinarian. Any adverse conditions, including swelling, discomfort, or ulceration, were treated accordingly by the veterinarian.

Results

The goats' weight remained consistent throughout the 10 months of the study (Goat 1 = 41.87 kg, SD = 2.71 kg; Goat 2 = 32.27 kg, SD = 2.52 kg) with no loss of appetite following procedures. The animals tolerated the intratesticular injections well and manual restraint was adequate to complete the treatment. Following the first injection of 4 ml of $CaCl_2$ in each testicle at week 0, swelling of the testes was recorded but returned to baseline volume six and eight weeks later for Goat 2 and Goat 1, respectively (see Fig. 3). Testes were firm upon palpation at two weeks post injection. At six weeks post injection, Goat 1 had a small necrotic area on the caudal end of the left testis which resolved without intervention (see Fig. 4). Non-hCG-stimulated serum testosterone levels showed a decrease two weeks following the first injection, but had a high degree of variability during the ten week post injection period (Post injection 1: Goat 1 mean = 3.81, SD = 2.2; Goat 2 mean = 3.71, SD = 1.1) (Fig. 3).

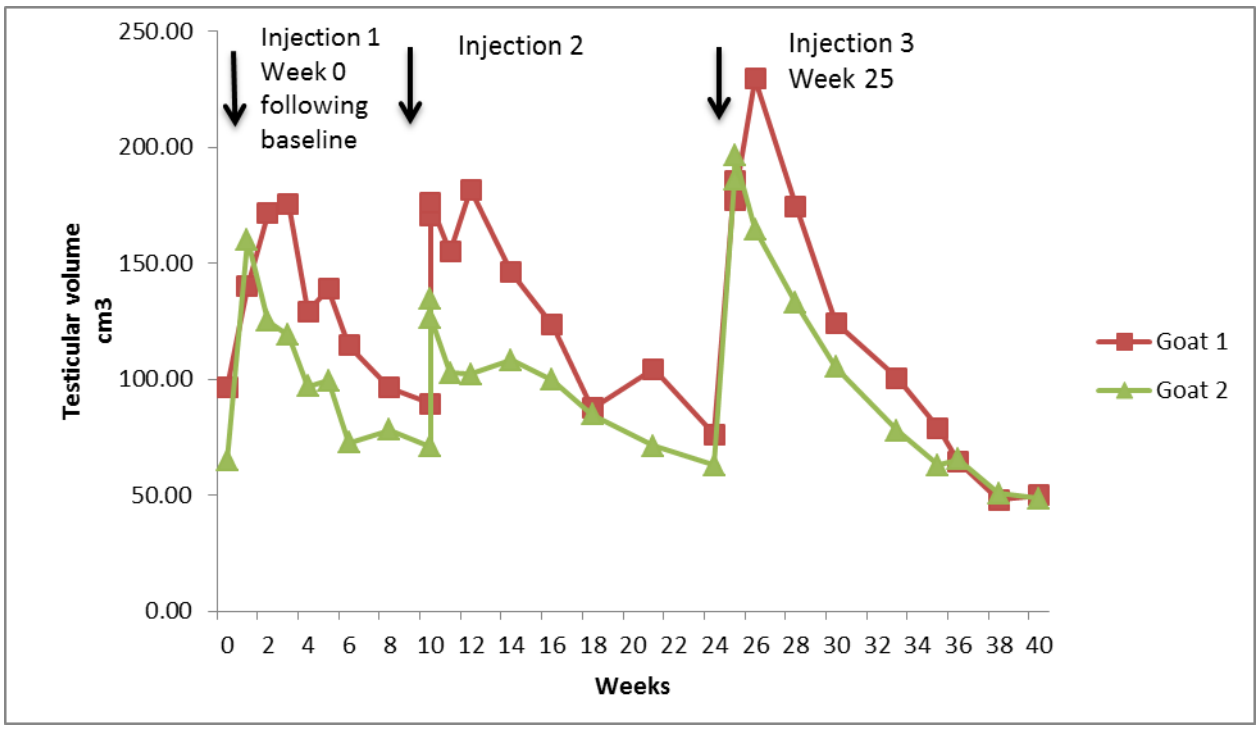

Figure 3. Testicular volume for the two male goats following each injection of $CaCl_2$. Injection dates are indicated by arrows. Testicular volume dropped below baseline levels at week 35 after the third injection of 10 ml $CaCl_2$.

Figure 4. Scrotum of Goat 1 at 6.5 weeks after injection 1. A small necrotic area resolved without intervention.

Due to the lack of evidence of a durable effect from the first injection, a second $CaCl_2$ injection at week 10 was completed using the same dosage per testicle (4 ml). Within one week, both testes were firm and had significant swelling. Testicular size slowly decreased over the next seven weeks but did not drop below original baseline measurements (Fig. 3). The shape of the testicles became flattened and bi-lobed. Some minor hair loss from the flanks was noted at four weeks post injection 2. Serum testosterone levels decreased and then stabilized from week 16-24 (Goat 1 = 3.44, SD = 0.44; Goat 2 = 1.40, SD = 0.28) (Fig 5). Although Goat 2's testosterone level was reduced by 68% of the baseline value, levels for both goats remained in the normal range.

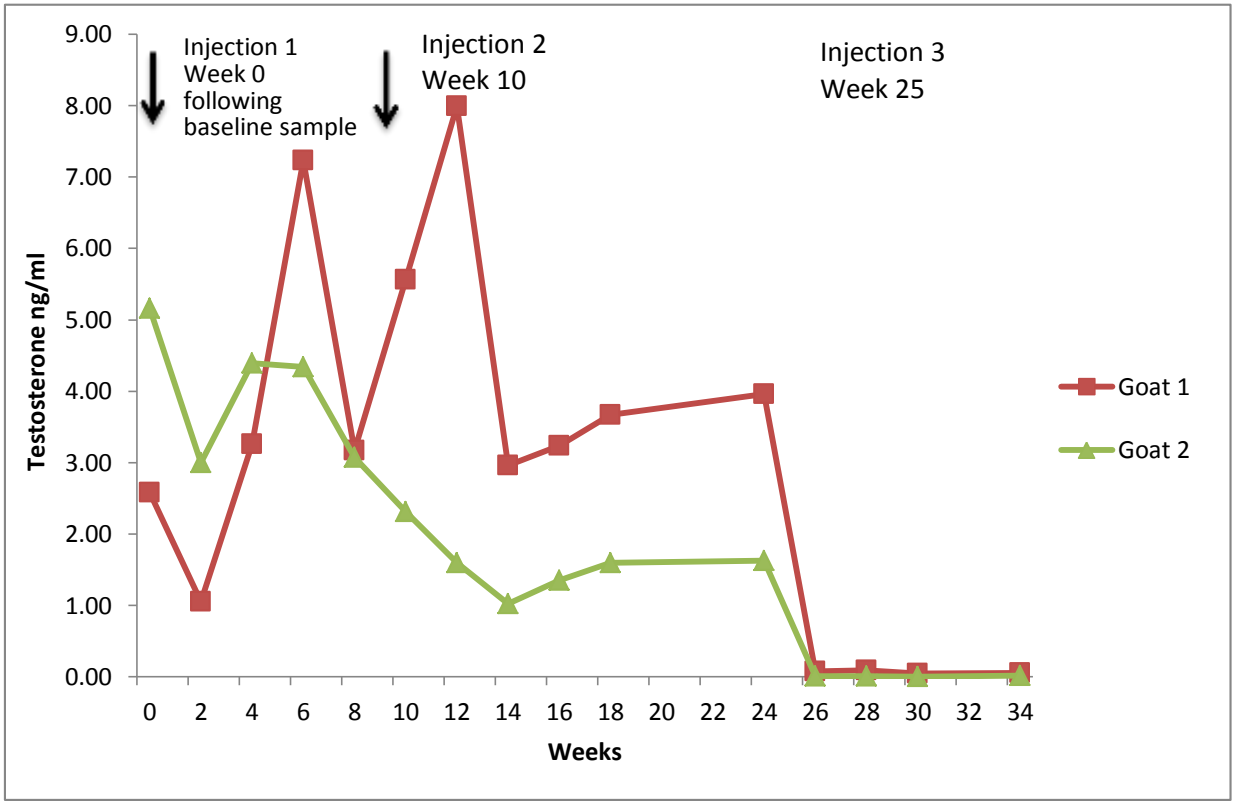

Figure 5. Serum testosterone levels over time. Injection dates are indicated by arrows. Goat 1's testosterone level was initially highly variable. Both subjects' testosterone dropped below 0.1 ng/ml following the third injection of $CaCl_2$.

Due to the lack of efficacy of the previous two injections to reduce testicular size and eliminate testosterone, a third injection of $CaCl_2$ solution was given at week 25 at a higher volume of 10 ml per testicle. The treatment was initially well tolerated by the male goats, but skin necrosis was observed on the scrotum at week 27 on both goats. At six weeks following the final injection (week 31), Goat 1 developed a full-thickness skin ulcer on the scrotum and was treated with 5 days of antibiotics (oxytetracycline (LA-200) at 20 mg/kg subcutaneously once/day) and two days of analgesics (flunixin megulmine at 1 mg/kg intramuscularly once/day). Lesions did not develop any signs of infection and slowly contracted in size over approximately 4 weeks. Wound-Kote™ spray (Farnam, active ingredients acriflavin and isopropyl alcohol) was used to cover any open areas and promote healing.

The testes swelled to approximately three times the volume of baseline measures following the third injection and gradually decreased in size over the next 16 weeks. At study conclusion (week 40), the testicular volume was 50% less than the baseline measurement for Goat 1 and 22% less for Goat 2 (week 0 vs. week 40: Goat 1: 96.4 cm^3 vs. 47.9 cm^3; Goat 2: 64.9 cm^3 vs. 50.9 cm^3). At two weeks following the third injection, Goat 1 and Goat 2's unstimulated testosterone levels had dropped 97.8% and 99.3% from baseline, respectively. The hCG stimulation test was then conducted at week 30, confirming the dramatic drop in testosterone production from Leydig cells. Testosterone levels for Goat 1 were 0.04624 ng/mL and 0.027 ng/m/dL and for Goat 2 were 0.005 ng/mL and 0.001 ng/m at 1 hour and 4 hours post hCG stimulation.

Histological evaluation of the testes following castration indicated that both subjects' testes were morphologically similar and showed severe, diffuse necrosis, leaving few normal structures to allow for spermatogenesis (Fig. 6). Specifically, the sections included patchy, regional, acute necrosis/infarction of testicular parenchyma in which the seminiferous tubular structures remained recognizable but were nonviable as evidenced by uniform eosinophilic staining of all components (spermatozoa, Sertoli cells).

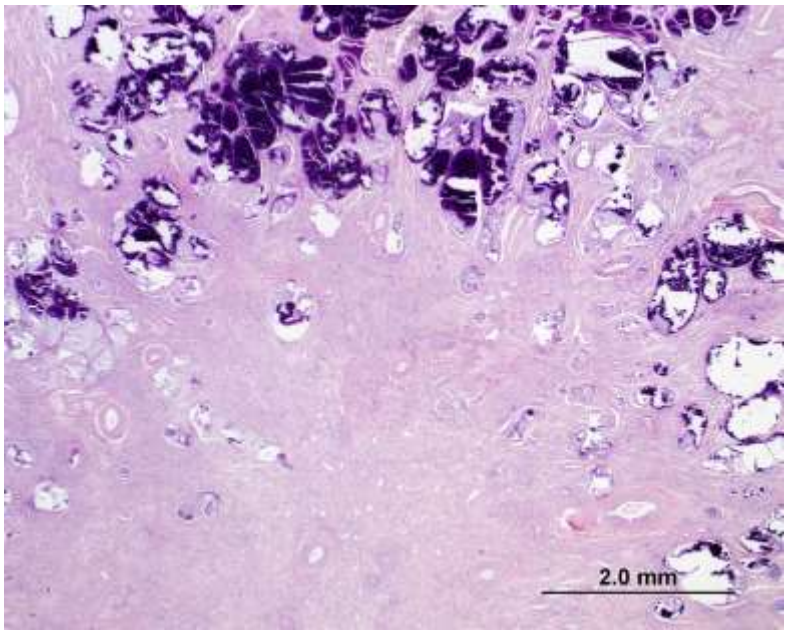

Figure 6. Histology of testicular tissue from Goat 1. Few residual seminiferous tubules are visible at the periphery of the section, most containing mineralized debris. The architecture at the center of the section in replaced by fibrosis subsequent to necrosis of testicular parenchyma.

The epididymis was devoid of spermatozoa in all testicles (Fig 7). Interstitial cells were not identified and vascular wall necrosis was present in areas of apparent infarction and necrosis of all components. Arteriosclerosis of testicular arteries was observed throughout examined sections. Evaluation indicated chronic tissue injury with multifocal, severe, granulomatous inflammation with random mineralization of seminiferous tubular structures and basement membranes with scarring (Fig 8). In one goat (Goat 1),

the inflammatory and fibrotic process penetrated the tunica vaginalis and extended into the tunica dartos.

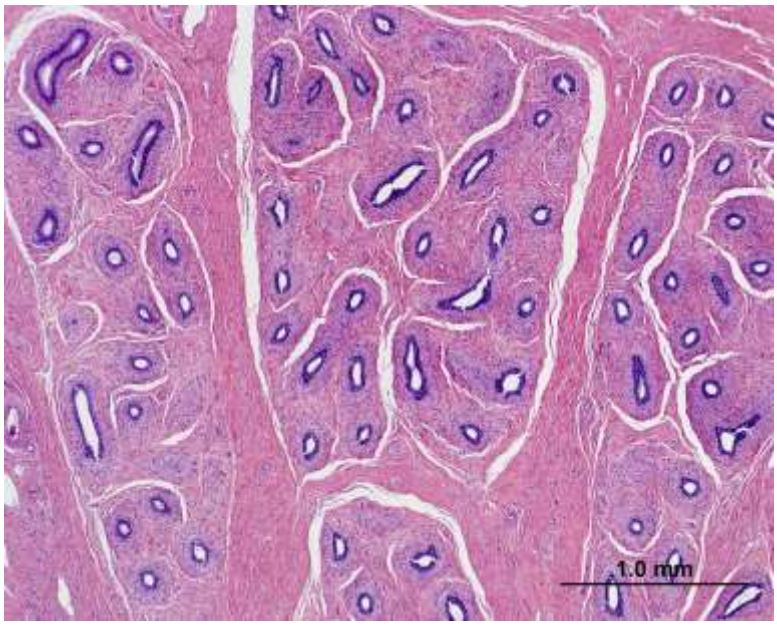

Figure 7. Histology of testicular tissue from Goat 1. A section from the tubules of the epididymus showing a complete lack of spermatocytes.

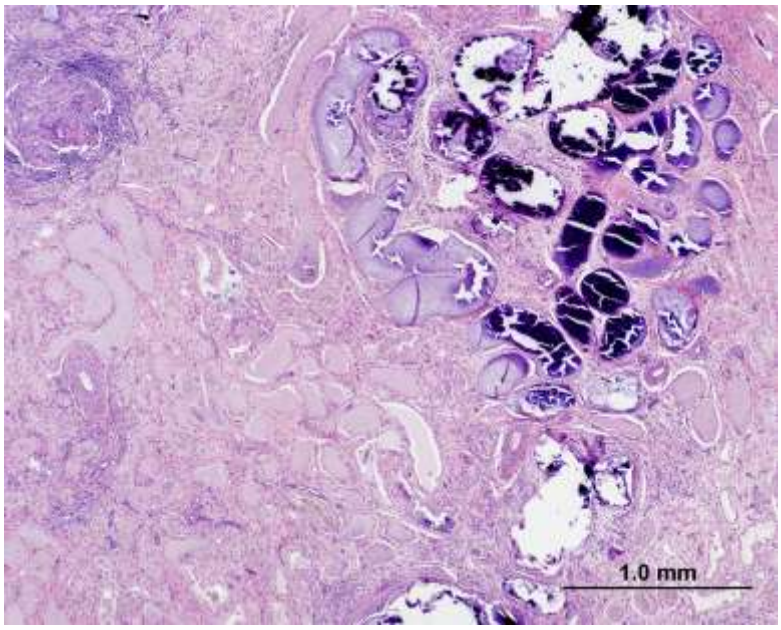

Figure 8. Histology of testicular tissue from Goat 1. Residual seminiferous tubules contain necrotic and unrecognizable debris. Some areas have undergone mineralization and the interstitium is distorted by chronic (lymphocyte and macrophage) inflammation. Spermatogenesis is absent.

Discussion

Calcium chloride was used as a chemical sterilant in an effort to develop a nonsurgical alternative for older male ruminants. The results provide preliminary evidence for the efficacy of this sterilization method, as measured by a decrease in testicular volume, elimination of testosterone, and diffuse necrosis of testicular tissue. While an effective sterilizing dose of $CaCl_2$ was determined, it was not without adverse reactions. The initial dose of 4 ml of 20% $CaCl_2$ in alcohol was inadequate to affect testicular size or eliminate testosterone and a repeated dosage was similarly inadequate. It was not until a 10 ml volume was given that clear indication of testicular atrophy and loss of gonadic testosterone (as measured in serum and by hCG stimulation) was achieved. Histological evaluation confirmed that the goats were sterile and the testes were unlikely to ever recover functionality. The testicular tissue evidenced chronic granulomatous inflammation and subsequent fibrosis resulting from necrosis of the seminiferous tubular structure.

The 10 ml dose of $CaCl_2$ did result in ulceration of the scrotal skin of the goats, which required treatment, and histology indicated that the fibrotic process had penetrated the testicular membrane in one subject. This may have been the result of leakage from the injection site into the scrotum due to back pressure generated by the larger volume injection. The 10 ml volume included 2000 mg of $CaCl_2$ and was a significantly higher dose than reported previously for goats. Jana and colleagues [2] found that in 8 kg Black Bengal goats, a dose of 320 mg $CaCl^2$ significantly decreased testicular weight and testosterone as compared to controls 30 days after injection and subsequent histology revealed complete necrosis of the seminiferous tubules and interstitial Leydig cells. In the present study, two intratesticular injections of 4 ml each (which contained 800 mg $CaCl_2$) did not sterilize the goats and the effective dose we identified was almost seven times greater than previously reported. The difference in effective dose could have been due to differences in testicular size, weight, age, breed or diluent (alcohol vs. lignocaine hydrochloride). It is also possible that the final dose used in our study was excessive and a slightly lower final dosage of $CaCl_2$ would have been effective without the side effects, but further study would be required to make a final determination.

To our knowledge, this is the first study to inject $CaCl_2$ in a serial manner, and we found no ill effect of the procedure in the goats at lower doses. This finding is important, as effective doses of the $CaCl_2$ sterilant may vary between individuals and breeds, potentially requiring repeated injections to achieve complete sterilization. Interestingly, our results infer that repeated injections of a sub-optimal dose did not have a reliably additive effect. Though the testosterone level of the smaller goat was lower after the second injection as compared to baseline, testosterone levels remained within physiological range and testes volume for both goats returned to baseline after each 4 ml dose.

The procedure was technically uncomplicated to perform and the goats were simply manually restrained without need for anesthesia. This is an improvement over other sterilization procedures. Pain response is a welfare concern when elastrator banding and emasculatome clamping methods are used for young ruminants, and surgically castrating lambs older than 10 weeks is also not recommended, as they evidenced the most negative response including pain and weight loss [12]. By contrast, in the current study, weight and appetite were unaffected by $CaCl_2$ injection.

Conclusions

In conclusion, intratesticular injection of $CaCl_2$ in ethyl alcohol provides an alternative to surgical castration for adult male goats. A 4 ml dose, though well tolerated, was ineffective in a larger goat and insufficient in a smaller goat; but a 10 ml dose was effective at eliminating testosterone, reducing testicular size and causing diffuse necrosis of the testes in both goats. At the high dose, ulceration of the scrotum was evidenced in both subjects, requiring medical intervention. However, both animals were otherwise clinically stable. Further study may be required to determine if there is a lower effective dose that avoids side effects.

Acknowledgements

The authors thank Bill Spangler, DVM, PhD, DACVP for providing pathology services and histology photographs, and to the dedicated animal care staff for their assistance and vigilance. Elaine Lissner and Linda Brent provided editorial assistance with the manuscript. This project was financially supported by Parsemus Foundation, Berkeley, CA, USA.

References

1. Koger, L.M., 1977. Calcium chloride, practical necrotizing agent. Bovine Pract. 12, 118-119.

2. Jana, K., Samanta, P.K., Ghosh, D. 2005. Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization of male Black Bengal goats (Capra hircus): a dose-dependent study. Anim. Reprod. Sci. 86, 89-108.

3. Jana, K., Samanta, P.K. 2007. Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose-dependent study. Contraception. 75, 390-400.

4. Jana, K., Samanta, P.K. 2011. Clinical evaluation of non-surgical sterilization of male cats with single intra-testicular injection of calcium chloride. BMC Vet. Res. 7, 39.

5. Leoci, R., Aiudi, G., Silvestre, F., Lissner, E.A., Marino, F., Lacalandra, G.M. 2014. 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 lowest risk. Acta Vet. Scand. 56, 63.

6. Leoci, R., Aiudi, G., Silvestre, F., Lissner, E.A., Lacalandra, G.M. 2014. Alcohol diluent provides the optimal formulation for calcium chloride non-surgical sterilization in dogs. Acta Vet. Scand. 56, 62.

7. Abebe, G. 2010. Reproduction in sheep and goats., in: Yami, A., Merkel, A.C., (Eds.), Sheep and Goat Production Handbook for Ethiopia. Ethiopia Sheep and Goat Productivity Improvement Plan, pp. 60-79.

8. Stoffel, M.H., von Rotz, A., Kocher, M., Merkli, M., Boesch, D., Steiner, A. 2009. Histological assessment of testicular residues in lambs and calves after Burdizzo castration. Vet. Rec. 164, 523-527.

9. Ewoldt, J.M., Anderson, D.E., Meisner, M.D., Saville, W.J. 2006. Short- and long-term outcome and factors predicting survival after surgical tube cystotomy for treatment of obstructive urolithiasis in small ruminants. Vet. Surg. 35, 417-422.

10. Bowman, T.A., Senger, P.L., Koger, L.M., Gaskins, C.T., Hillers, J.K. 1978. Blockage of sperm transport using intraepididymal calcium chloride injections in the ram. J. Anim. Sci. 46, 1063-1065.

11. Falvo, R.E., Buhl, A.E., Reimers, T.J., Foxcroft, G.R., Dunn, M.H., Dziuk, P.J. 1975. Diurnal fluctuations of testosterone and LH in the ram: effect of hCG and gonadatrophin releasing hormone. J. Reprod. Fertil. 42, 503-510.

12. Melches, S., Mellema, S.C., Doherr, M.G., Wechsler, B., Steiner, A. 2007. Castration of lambs: a welfare comparison of different castration techniques in lambs over 10 weeks of age. Vet. J. 554-563.