

Review Article

<https://doi.org/10.20546/ijcmas.2020.904.329>

## Chemical Castration in Animals: An Update

Gyan Singh<sup>1\*</sup>, Amit Kumar<sup>2</sup>, Ravi Dutt<sup>2</sup>, V. Arjun<sup>2</sup> and V. K. Jain<sup>1</sup>

<sup>1</sup>Department of Veterinary Clinical Complex, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Sciences, Haryana, India 125004

<sup>2</sup>Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary & Animal Sciences, Haryana, India 125004

\*Corresponding author

### ABSTRACT

#### Keywords

Buck, Bull,  
Chemical agents,  
Dog, Guinea pig,  
Rabbit, Ram, Rat,  
Population control

#### Article Info

Accepted:  
22 March 2020  
Available Online:  
10 April 2020

This review article deals with methods of population control in different animal species viz. Buck, Bull, Dog, Guinea Pig, Rabbit, Ram and Rat by using various chemical agents administered through different routes.

### Introduction

Sterilization of domestic animals is used as a means for controlling animal numbers, improving genetic gain by restricting gene transfer to genetically elite animals, modifying animal behavior and altering carcass composition in food animals. Stray animals population is a big challenge in India and stray cattle population has increased tremendously during last two-three decades.

However, reliable data about the number of calves, dogs, cat, swine and bulls roaming in the streets of villages, cities and metros are not available. Stray animals are responsible for spreading of communicable diseases.

Cross breeding programme has also contributed towards increasing in number of male animals and stray animals population. Methods to prevent unwanted pregnancies can be effective only if these animals have

responsible owners. In the absence of later, either serious charitable community efforts or legal compulsion can help in reducing stray animal population. Surgical techniques such as vasectomy and castration are both century old techniques available for the control of male fertility. However, they can't be used on mass scale in stray animals due to vast area and limit resources. It is also equally imperative for success of such a population control programme that the method used be affordable, reliable, safe and convenient with no or minimum post-operative care.

Non-surgical sterilization is one of the most popular and reliable methods of population control of stray animals through achieving male sterility. Use of such methods for prevention of unwanted pregnancies in stray animals can reduce the number of animals destined to become stray every year in India. Chemical sterilization is a suitable method for population control as it avoids spermatic granuloma formation which occurs during vasectomy (Schmidt and Morris, 1973). Chemical sterilization of cow bulls at mass scale may decrease the number of pregnant cows to the point of affecting population growth. Chemical sterilization may be used for preparation of teaser bull and more weight gain in calves. Many workers have reported chemical castration in different species *viz.* Buck, Bull, Dog, Guinea Pig, Rabbit, Ram and Rat etc. Mode of administration of these chemicals was either directly into testis or epididymis or administered via subcutaneously or oral route. Following is the information available on chemical castration of animals with the help of various chemicals.

### **Species wise scientific report on chemical castration**

#### **Calves and bulls**

Bierschwal and Ebert (1961) reported various sclerotising agents *viz.* T.T. oil, Iodine,

Phenyl mercuric nitrate and Silver nitrate for sterilization of bulls. Similarly they tried Dandron (sterilized mineral oil) as bilateral intra epididymal injection in bulls and revealed that the coiled duct of epididymis was completely destroyed and replaced with granulomatous tissue and observed complete azoospermia on day 21 days post treatment.

Koger (1978) injected 1.5 ml of 50% Calcium chloride into cauda epididymis of calves, cats, pig, goats and dogs and concluded that calcium chloride causes orchitis and testicular atrophy and can be used as rapid castration technique and observed surgical complications for population control programme. Gardner (1980) concluded that intra epididymal injection of 2.5 ml sclerotising agent of 3.6% formaldehyde in 90% ethanol in Aberdeen Angus bulls developed epididymitis after nine days of treatments and produced azoospermic ejaculate after 85 days of post treatment. Similarly, Pearson *et al.*, (1980) reported that ligation of epididymis with nylon had aspermia for several weeks in bull and also observed that intra epididymal injection of 2.5 or 5 ml of 5% Ethanolamine oleate had no effect on the ejaculate, whereas bulls treated with 5 ml of 3% Chlorhexidine gluconate produce azoospermic ejaculate within three weeks.

Feher *et al.*, (1985) compared the testosterone level by radioimmunoassay in blood sample taken on two successive days every three months and noticed that within 24 hours of chemical castration with tannic acid and zinc sulfate intra-testicular and surgical castration, there was more gradual fall in plasma testosterone level in 7-9 months old Holstein-Friesian bullock, that were castrated chemically, which was significant only after three months as compared to surgical castration where it fell down immediately and seasonal fluctuations were not statistically significant.

Hill *et al.*, (1985) designed an experiment for 196 days with eight male calves that were being suckled by their mothers. On first day, 40 calves were castrated chemically using chemi-cast (88 % lactic acid) and 40 calves castrated surgically and 20 calves in each group were implanted with steroid (200mg progesterone+20 mg estradiole benzoate) on days 1 and 121, respectively and reported that surgical castration with chemical castration resulted in significantly lower scrotal edema and increased rate of body weight gain at 28 days, whereas surgical castration did not significantly affect rate of body weight gain up to 196 days or weaning weight. On day 121, chemical castration was found to have destroyed both testes in 30 out of 40 calves. Implanted calves had slower rate of body weight gain than non-implanted calves.

Prince *et al.*, (1986) conducted an experiment on 56 Holstein Friesian male calves, which were divided in four group e.g. 1- castrated at 2 months of age, 2- no treatment given and left intact, 3- implanted subcutaneously with Zeranol (36 mg) at 100 days intervals starting from 2 months of age, 4- implanted as in group 3 but starting at 6 month of age and concluded that average live weight, daily weight gain and carcass weight were higher in group 4 whereas testes weight was more in group 2.

Garriz *et al.*, (1986) reported that chemical castration of Hereford bull calves of 70 days old with 99 kg body weight by intra - testicular injection of 3-12% Zinc tennate was more effective than surgical castration. There was no significant difference in carcass quality and weight or the weight of pistol cut, lion or leg between the groups.

Arshami and Rattle (1988) designed an experiment on eighteen yearling beef bulls to study the effects of Gossypol containing diets on spermatogenic tissue, Sertoli cells and

Leydig cells. Testicular tissues collected from bulls fed gossypol free diet and gossypol containing diet, and were compared histologically. After two months, half of the bulls that fed gossypol containing diet were kept on gossypol free diet for an additional two months to determine whether gossypol effects were reversible or irreversible and bulls showed improvement in histological changes indicating that gossypol induced effects were partially reversible.

The bulls fed with whole cotton seed and cotton seed meal had larger lumen, decreased wall thickness and a reduced number of cell layers in their seminiferous tubules. These changes indicated that gossypol had detrimental effects on spermatogenic tissue and associated cells. Breeding bulls had reduced fertility and had detrimental effect on their reproductive performance whereas intra testicular injection of lactic acid in Brahmin crosses calves was not suitable alternative to open surgical technique for castration (Spinelli, 1989).

Bagley *et al.*, (1989) conducted a study in three trial; in first trial, the cross bred calves were castrated at birth or at four months age with or without treatment with a Ralgro (zeranol) or Compudose (estradiol- 17 beta) implant and in second trial, calves were subjected to physical (banding), chemical castration at birth and surgical castration at four months and all the calves received a steroid implant at birth. In third trial, the effects of chemical castration at four months, with or without an implant at birth, on testicular growth were investigated. They reported that calves castrated at four months weighed slightly more at weaning than calves castrated at birth. Castration was easier and safer at birth although chemical castration with lactic acid was effective both at birth and at four months age while banding was better at birth than at four months and at four

months chemical castration was more convenient than surgical castration. Implants increased daily weight gain and body weight at weaning of castrated calves but implantation at birth reduced testicular development and front leg length and body weight at four months and at weaning.

Fordyce *et al.*, (1989) compared an intra-testicular injection of lactic acid in 58 Brahman cross calves with open surgical castration and reported that chemical castration appeared to be more painful than surgical castration whereas post-operative swelling and pain appeared similar for both operations and took three times longer duration than surgical castration. Scrotal necrosis occurred in 25% chemically castrated calves due to drug leakage from testes under high pressure of injection. Healing time for chemical castration was approximately twice that of surgical castrates. Five chemically castrated calves retained one testis whereas all five were rendered sterile and each calf maintained androgenesis. This led to secondary male behavior which caused management problems. It was concluded that lactic acid administration is not a suitable alternative to open surgical technique for castration.

Cohen *et al.*, (1990) conducted a study to determine the degree of stress after castration of Holstein calves either surgically or chemically (Chem-cast;  $\alpha$  - hydroxy propionic acid) and main acute stress indicators (plasma cortisol, packed cell volume, glucose, protein, free fatty acid, creatinine, urea nitrogen and rectal temperature) were measured. The cortisol concentrations were significantly elevated at 3 and 6 hours after castration which were greater in surgical castrates (6hr after) than chemical castrates (3hr after) and it was concluded that acute stress was highest during surgical castration for 6-12 hours as compared

to chemical castration. The testosterone level decreased immediately in surgical castrates and remained at undetectable level after 63 days of castration but in chemically castrated, testosterone level declined immediately following chemi-cast injection, though there was gradual increase and long term maintenance of low level of testosterone but was greater than surgical castration. Palpation revealed severe testicular degeneration in chemical castrated males. The average daily weight gain did not differ between the entire males and surgical castrates or between entire males and chemical castrates, but average daily weight gain for chemical castrates was greater than surgical castrates.

Cohen *et al.*, (1991) conducted two experiments on male calves to find out the effects of multiple implants of Ralgro (zeranol, 36 mg) or steroid (200 mg progesterone + 20 mg estradiol), castration time (early or late) and castration methods (surgical or chemical) on testicular development and weight gain for 28 days following castration. Chemical castration was done by injecting 1.0-1.5 ml of chemi-cast ( $\alpha$  hydroxy propionic acid) into each testis and reported that Ralgro implanted bull calves had smaller scrotal circumference and testicular weight than non-implanted calves at early castration (59 day), late castration (157day) and at slaughter (459 day). Steroid had no effects on testis weight at early castration (40 day), but reduced testis weight at late castration (148 day). Average daily weight-gain during first 7 days after castration was less for surgically castrated than chemically castrated following early castration. Chemical castration increased scrotal circumference at 7, 14 and 28 days after castration though testicular atrophy was complete by 56 days. The average daily weight gain and plasma testosterone concentration were lower in surgical castrates than chemical castrates by  $\alpha$ -hydroxypropionic acid.

Comparative studies on chemical (Castate – Quin 14), surgical (Pen knife) and mechanical castration of cross bred bovine of 18 months old conducted by Sorensen *et al.*, (2001) and concluded that there was aspermia after 60 days of chemical and mechanical castration along with necrosis and partial necrosis in these two groups, respectively and loss of libido and service capacity in all the animals of three groups. The average daily weight gain was higher in chemically and surgically castrated animals as compared to mechanically castrates.

Singh *et al.*, (2005 and 2006) conducted an experiment on cow bulls by injecting 5 ml of 4.5 per cent chlorhexidine gluconate diluted with 50 % dimethyl sulfoxide into each cauda epididymis and reported that after 45 days of treatment, there were transient swelling of scrotum, extensive fibrous tissues proliferation, granuloma formation and sclerosis of cauda epididymis but spermatogenesis was normal in treated groups.

Canpolat *et al.*, (2006) studied effectiveness as well as compared chemical-castration potential of ethanol and calcium chloride by intra-testicular route. They injected 10ml of absolute ethanol or 10 ml of 30 % calcium chloride solution intra-testicularly in each testicle which were removed with the open surgical technique after 60 days for histopathologic evaluation. Testicular swelling was evident in both groups of bulls following injection and reached peak within 48 hours. While testicular volume decreased significantly in ethanol group after 3 weeks and no significant change occurred in calcium chloride treated group. The testicles underwent atrophy at the 60<sup>th</sup> day in ethanol group with no marked alteration in calcium chloride treated group. Though only 3 bulls were infertile in ethanol injected group whereas other bulls were fertile. The method is readily applicable with no major harms,

adverse effects and 50% success rate was considered satisfactory for its application particularly in large herds, where no chance of operation exists.

Neto *et al.*, (2014) evaluated the efficiency of intra-testicular injection (ITI) of hypertonic sodium chloride (NaCl; 20%) solution in male calf castration during the first weeks of life. Intra-testicular injection (ITI) induced coagulative necrosis of Leydig cells and seminiferous tubules leading to extensive testicular fibrosis. Testosterone secretion and testicular development were severely impaired in 12 month old animals, in which no testicular structure and sperm cells were observed during breeding soundness evaluation. In conclusion, ITI of hypertonic NaCl solution induces sterility and completely suppresses testosterone secretion when performed during the first 20 days of life.

Cavaleri *et al.*, (2015) determined the effects of intra-testicular administration of saline or one of the two doses of zinc acetate in *Bos indicus* bull calves of on semen quality and testicular changes. Zinc acetate treated group showed reduced number of progressively motile and morphologically normal sperm in comparison to the saline group.

Compared to saline treated controls, treatment with zinc acetate reduced the mean diameter of the testes after and total testicular weight at slaughter. Histological changes in testes of bulls treated with zinc acetate were characterized by germ cell depletion, vacuolation of Sertoli cells, interstitial fibrosis, epididymal duct atrophy with variable remnants of testicular tissue and degeneration.

Ball *et al.*, (2018) evaluated the effect of a zinc solution as an injectable castration method to bull calves pre-weaning. At weaning, there were no differences in growth, serum testosterone or scrotal thickness due to

the concentration of Zn solution used and the injectable castration method resulted in similar serum testosterone concentrations as in case of surgical castration.

### **Dogs and cats**

Vare and Bansal (1973) reported that atrophy and degenerative changes seen in seminiferous tubules during first four month after vasectomy but regeneration took place in most of the tubules after four months, though after six months all the seminiferous tubules appeared more or less normal. Only a few tubules underwent degeneration and replaced by fibrous tissue. The seminiferous tubules and intertubular stroma remained normal from seven to eighteen months. The Leydig cells remained unchanged after vasectomy.

Dixit and Lohiya (1975a) found that a single injection of 3 Chloro-1, 2 propaindiol at rate of 70 mg/kg body weight resulted in complete azoospermia by 33 days post treatment in dogs. Similarly Dixit *et al.*, (1975b) reported that a single intra testicular injection of Danazole at the rate of 200 mg in 0.5 ml of olive oil causes increase in size of testes, reduction in diameter of seminiferous tubules and azoospermic ejaculate after 25 days of post treatment in male dogs. Dixit *et al.*, (1978) reported that oral administration of *Menordica charantia L.* extract at the rate of 1.75 gm/day for 60 days causes atrophy of seminiferous tubules in dogs.

Dixit *et al.*, (1979) reported that intra testicular injection of Methallibure (ICI-33828), Dexamethasone, Metopiron, (SU-4885), Niridazole (33644 Ba) Alpha-chlorhydrin (U5897) and danazole resulted in sterility without altering metabolic activity, whereas spermatogenic arrest was observed mainly at spermatid stage in dogs. Pineda *et al.*, (1977) concluded that intra epididymal injection of Chlorhexidine

gluconate in 50% Dimethyl sulfoxide (DMSO) produce fastest azoospermia in all treated dogs. Murty and Sastry (1978a; 1978b) tried intra testicular injection of Cadmium chloride at a rate of 0.05 mg /kg body weight and observed atrophy along with fibrosis of seminiferous tubules within 50 days of post treatment in dogs.

Koger (1978) injected 1.5 ml of 50% Calcium chloride into cauda epididymis of cats as well as dogs and concluded that calcium chloride causes orchitis and testicular atrophy and can be used as rapid castration technique and observed surgical complications for population control programme.

Kostrya *et al.*, (1981) noticed that intra testicular injection of Sodium fluoride or Cadmium chloride in dogs produced dead spermatozoa upto 60 days post treatment. However, no spermatozoa were present at day 90 post treatment.

Pineda and Helper (1981) reported that intraepididymal injection of 0.5 ml or 1.0 ml of an aqueous solution of 3.0% chlorhexidine digluconate in 50% dimethylsulfoxide (DMSO) given into each tail of the epididymis of dogs resulted in azoospermic ejaculates by days 35 or 42 after treatment. The azoospermia induced by treatment was long-lasting and likely irreversible. Intraepididymal injection of 1.0 ml of an aqueous solution of 4.5% chlorhexidine digluconate, without DMSO, given into each tail of the epididymis of dogs resulted in azoospermic ejaculates by day 28 after treatment, but the permanence of the azoospermia was not determined. This method of intraepididymal injection of sclerotising agents appears safe and suitable for large-scale sterilization programs for controlling dog populations but Barnett (1984) noticed the effect of intra-epididymal injection of 4.5% Chlorhexidine gluconate on

dogs and found that the semen samples collected at four month after treatment were azoospermic.

Vickery *et al.*, (1985) reported that dogs injected with LH-RH agonist Nafrelin for 44 days resulted in decline in testosterone and LH, along with decrease in testicular size, sperm count and spermatogenesis was completely absent in all treated dogs. Gupta and dixit (1989) reported that palmitive hydroxide isolated from *Berberis chitna* root when administered to laboratory dogs resulted in suppression of spermatogenesis.

Freshmen *et al.*, (1990) concluded that oral administration of 50 mg Methyl testosterone per day for 90 days resulted in decreased in number of spermatozoa, concentration of LH, FSH and testosterone in male grey hounds. However, all these parameter returned to normal 90 days after cessation of treatment. Gupta *et al.*, (1990) concluded that oral administration of Dihydro-palmitive, Berberin and Protapine alkaloid resulted in inhibition of spermatogenesis.

Fahim *et al.*, (1993) observed azoospermia for 90 days in male dogs after treatment with intra epididymal injection of 0.5 ml of Zinc arginine. Histological examination of the epididymis and ductus deferens revealed formation of sperm granuloma, reduction in the diameter of the tubules and absence of spermatozoa on the lumen of epididymal tubules.

Inaba *et al.*, (1996) reported reversible method of birth control in male dogs with single injection of prolonged released micro-encapsulated leuprolide acetate (GnRH agonist) at the dose rate of 1 mg/kg body weight, subcutaneously.

This treatment resulted in azoospermia within 8 weeks post treatment. However complete

recovery of spermatogenesis and testosterone level occurred at 20 and 11 weeks respectively after the injection.

Immergant and Threlfall (2000) tried intra testicular injection as a non-surgical sterilizer of male dogs and observed that intra testicular injection of 70% glycerol solution did not cause azoospermia in dogs. Lal (2002) reported that intra epididymal injection of 0.5 ml of 4.5% Chlorhexidine gluconate caused occlusion of cauda epididymis after 35 days post treatment without affecting the spermatogenesis in dogs.

Oliveira *et al.*, (2007) administered zinc gluconate (Testoblock®) to adult male dogs intra testicularly. Transmission electron microscopy post 5 months of injection revealed degenerated Sertoli and Leydig cells, hyperplastic and hypertrophic smooth endoplasmic reticulum and numerous Golgi apparati in cytoplasm of elongated spermatids and lysis of acrosomal vesicles of round spermatids in Golgi phase. Histological examination of treated groups revealed seminiferous tubules in majority lined only by vacuolated Sertoli cells and authors suggested that changes are irreversible and zinc-based solution (Testoblock®) is effective as a chemical sterilant for dogs.

Jana and Samantha (2007) administered single bilateral intra-testicular injection of calcium chloride (CaCl<sub>2</sub>) at the doses of 5, 10, 15 or 20 mg per testis per kg body weight and carried out sterilization after 45 days. Histomorphological measures of testes showed total necrosis of testicular tissue at 45 days after an injection of either 10 or 15 or 20 mg CaCl<sub>2</sub> along with fibrosis and hyalinization in seminiferous tubules and interstitial spaces. Infiltration of leucocytes was also observed with 10 or 15mg dose. Disintegration of germ cell arrangement in seminiferous tubules and washing out of germ

cells from the tubules were noted with 5mg dose. Relative organ weight, epididymal sperm count, plasma and intratesticular concentrations of testosterone, testicular activities of D5, 3 $\alpha$ -hydroxysteroid dehydrogenase (D5, 3 $\alpha$ -HSD), 17 $\alpha$ -hydroxysteroid dehydrogenase (17 $\alpha$ -HSD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and superoxide dismutase (SOD) and testicular contents of glutathione (GSH) and glutathione disulphide (GSSG) and the ratio of GSH/GSSG, all were declined in each of the calcium chloride treated groups in comparison to the control group. Increase occurred in testicular malondialdehyde (MDA) and plasma concentrations of LH and FSH with each of the treatments by comparison with the control group. Their findings suggested that an intra-testicular injection of CaCl<sub>2</sub> at specified doses could be a suitable method of sterilization in preference to surgical castration of dogs.

Soto *et al.*, (2007) studied efficacy of zinc gluconate, either associated or not to dimethyl sulfoxide (DMSO), as a contraceptive method for the canine male population. After treatment, dogs did not become azoospermic, but it was observed that from the second collection onwards sperm cell number per mm<sup>3</sup> were significantly lower in DMSO (0.5%) and zinc gluconate (26.2 mg) treated group than control group. In DMSO (0.5%) and zinc gluconate (26.2 mg) treated groups, sperm motility values were significantly lower than control group in successive collections. Twelve months after chemical injection, two dogs of the control group and four from the treatment group were surgically neutered and their testicles were examined through histopathological staining, revealing testicular degeneration, decreased number of germ cells, areas of atrophy, disruption of seminiferous tubule architecture and loss of germ and Sertoli cells. In conclusion, association of DMSO (0.5%) to zinc

gluconate (26.2 mg) may be indicated as a contraceptive method for male dogs.

Chatterjee *et al.*, (2009) studied efficacy of CaCl<sub>2</sub> intra-testicular injection for control of stray dog population. Epididymal sperm counts decreased in a dose-dependent manner, with 0.31x10<sup>3</sup> sperm per ml of suspension in the animals receiving the highest dose vs 12.41x10<sup>6</sup> sperm per ml in the control animals. Serum testosterone levels also decreased in a dose dependent manner, with levels about 2ng/ml in dogs receiving the highest dose vs 8.5ng/ml in the control animals (76% reduction). Histologic studies of the testis observed a complete disruption of the tubular architecture, degeneration of interstitial Leydig cells, and absence of germ cells at the highest dose.

Baran *et al.*, (2010) studied efficacy of CaCl<sub>2</sub> intra-testicular injection for control of cat's population. In the cats treated with CaCl<sub>2</sub>, decreased numbers of live and motile sperm were observed in the semen. The scrotum was swollen or testis reported to be sore in all cats post-injection. Based on histological findings, the highest dose (40mg) was considered suitable for effective chemical sterilization in cats. Jana and samantha (2011) studied efficacy of CaCl<sub>2</sub> intra-testicular injection for controlling cat population. Six cats per group were injected with 5, 10 or 20% calcium chloride dihydrate in saline solution with lignocaine hydrochloride, a local anaesthetic. At the 60<sup>th</sup> day post-injection, cat testis were collected and showed complete testicular necrosis and replacement by fibrous tissue; very low sperm counts; and reduction of serum testosterone by at least 70% in 20% dose. Androgenic enzyme activities and their expressions were also reduced in all the treated groups along with intra-testicular testosterone concentration was also low. Increased testicular lipid peroxidation, with reduced antioxidants and mitochondrial



membrane potential, were evident following calcium chloride treatments. However, there were no apparent changes in serum concentrations of cortisol, fasting blood sugar level, blood urea nitrogen, packed cell volume, or total serum protein following calcium chloride injection, suggesting that this method of sterilization is not associated with any general stress response. In conclusion, calcium chloride solution demonstrates potential for androgenesis-eliminating nonsurgical sterilization of male cats in addition to its proven efficacy in dogs and other mammals.

Leoci *et al.*, (2012) conducted study for dose determination of Calcium chloride dihydrate in 40 dogs of mixed breed, divided in four equal groups, respectively injected with 10, 20, 30 and 60% calcium chloride in saline solution. Semen evaluation was performed by CASA (Computer Assisted Sperm Analysis) system at months 2, 6, and 12. This study revealed a dose-dependent relationship when CaCl<sub>2</sub> in saline was used. 10 and 20% concentrations maintained sterility past 6 months in some but not in all dogs. The maximum response in contraception was noted at 30 and 60% concentrations, but the higher concentrations had higher risk of abscess-fistulization. In conclusion, calcium chloride in saline has considerable effectiveness; it is not reliably effective at doses that are free from risk of adverse events. Based on the dose-determination study, 20% concentration of CaCl<sub>2</sub> has the best chance at effectiveness without adverse events.

Leoci *et al.*, (2014a) conducted a 12 month dose response study in dogs to identify the formulation of CaCl<sub>2</sub> in saline offering the best combination of safety and efficacy. All dogs were azoospermic at 2 and 6 months post injection and all doses significantly lowered semen volume, total sperm count,

testosterone levels and testicular size compared to the control group. At 12 months after injection, serum testosterone concentrations showed a dose dependent decrease; the serum testosterone concentration in the 60% CaCl<sub>2</sub> group was 83% lower than that of the control group. All dogs treated with 30% or 60% CaCl<sub>2</sub> were azoospermic at 12 months, but 2 dogs that received 20% CaCl<sub>2</sub> and 6 dogs that received 10% CaCl<sub>2</sub> regained some sperm production by 12 months after injection. Though the higher doses resulted in more durable azoospermia, they were also associated with a higher risk of complications. All dogs experienced slight increases in firmness of testes on palpation from 24 hours to days 3-7 after injection. Dogs receiving 10 or 20% CaCl<sub>2</sub> showed no agitation, fever, or marked inflammatory swelling of the testes. However, some dogs treated with 30 or 60% CaCl<sub>2</sub> showed signs of discomfort and licked the injection site. Two dogs that received 30% CaCl<sub>2</sub> and 6 dogs that received 60% CaCl<sub>2</sub> developed abscesses and underwent surgical castration. In conclusion intra-testicular CaCl<sub>2</sub> injection is a promising sterilization agent, and that a 20% solution of CaCl<sub>2</sub> was the most effective dose not associated with a risk of serious complications.

Leoci *et al.*, (2014b) conducted a study to evaluate the long-term efficacy of 2 formulations of 20% CaCl<sub>2</sub>; 20% CaCl<sub>2</sub> in 95% ethanol and 20% CaCl<sub>2</sub> in 1% lidocaine. All dogs injected with CaCl<sub>2</sub> had decreased sperm counts relative to controls at 2, 6, and 12 months after injection. All dogs injected with CaCl<sub>2</sub> in alcohol were azoospermic at 12 months, compared to 81% of those injected with CaCl<sub>2</sub> in lidocaine. At 2 and 6 months after injection, serum testosterone levels decreased in all dogs injected with CaCl<sub>2</sub> relative to baseline and relative to the control group. At 12 months after injection, the mean serum testosterone of dogs treated with CaCl<sub>2</sub>

in alcohol remained significantly decreased (63.6% lower than the mean baseline level at time zero), while average levels increased back to baseline levels in dogs treated with CaCl<sub>2</sub> in lidocaine. In conclusion a single, bilateral intra-testicular injection of 20% CaCl<sub>2</sub> in alcohol represents an optimal method for sterilizing male dogs, producing azoospermia and significantly reducing serum testosterone for at least 12 months with few inflammatory reactions or other side effects.

Canpolat *et al.*, (2016) in their study argued that sodium chloride application in young male dogs was an effective non-operative method of sterilization. This study evaluated intra-testicular injection of sodium chloride for chemical castration in 12 dogs both adult and non-adult. The sodium chloride at 20% concentration per testis was injected intratesticularly until tension occurs and the testicles were removed with the open surgical technique about 30 days for histopathologic evaluation. Testicular swelling was evident in both group dogs following injection and reached peak within 48 hours. While testicular volume decreased significantly in both group after 2-4 weeks. The testicles underwent atrophy at the 30<sup>th</sup> day in non-adult group with no marked alteration in adult group. It was concluded that intra testicular injections of sodium chloride at 20% concentration administration may not be accepted as a suitable alternative to the open surgical technique for castration in adult dogs but could be used as a preferable treatment option for non-adult dogs.

Ansari *et al.*, (2017) administered Talsur, containing zinc tannate, developed by the National Institute of Immunology (NII) of India in 1988 in a street-dog control program in which 22% of treated dogs developed complications and excessive scrotal swelling which lead to discontinuation of the formulation.

Paksoy (2018) administered vinyl cyclohexenediepoxy intraperitoneally for 8 days to adult male dogs @ 80 and 320 mg/ kg and it caused down regulation of caspase 8 and 9 at the level of apoptosis. The down regulation of caspase 8 at the gene level shows that the chemical used damages the testicular tissue.

Chaudhary *et al.*, (2018) administered zinc arginine solution @ 0.2 ml into each cauda epididymis percutaneously into pre-pubertal dogs and surgically removed testis and epididymis from two animals for histopathological studies on day 10, 20, 30, 40, 50, 60, 70, 80 and 90 respectively and reported complete degeneration of epididymal tubules, fibrous tissue presence and loss of structural details in most of the tubules on day 20 and 30 after injection.

### **Guinea pig and swine**

Sen *et al.*, (2017) investigated the intra testicular use of calcium chloride (CaCl<sub>2</sub>) and 4-vinylcyclohexene 1, 2-monoepoxide (VCM) injections as a side effect-free alternative method for the control of reproduction in guinea pigs. Fifty male guinea pigs were randomly assigned to five groups. In all groups, the chemical agents were injected into both the testes in 1% lidocaine hydrochloride. While Groups I, II and III were administered with a single dose (0.25 ml) of sterile physiological saline, 15 mg/100 g CaCl<sub>2</sub>, and 240 mg/kg VCM, respectively, Group IV and V received a daily dose of 15 mg/100 g CaCl<sub>2</sub>, and 240 mg/kg VCM for 3 days, respectively. The epididymal sperm count decreased in all treatment groups. Excluding 2 animals, Group V displayed azoospermia. When compared to the control group, Group V displayed the highest blood prolactin and lowest testosterone levels and Group III showed the highest testosterone level. Histopathological examination revealed no intoxication finding. Chemical castration

with VCM may be a good alternative to surgical castration as it enables mass sterilization without postoperative risks in guinea pig.

Bierschwal and Ebert (1961) reported various sclerotising agents *viz.* T.T. oil, Iodine, Phenyl mercuric nitrate and Silver nitrate for sterilization of boars. Similarly, they tried Dandron (sterilized mineral oil) as bilateral intra epididymal injection in boars and reported that the coiled duct of epididymis was completely destroyed and replaced with granulomatous tissue and there was complete azoospermia on day 21 post treatment. Freeman *et al.*, (1973) reported that various chemical agents *viz.* 95% Ethanol, 10% Silver nitrate and 3.6% formaldehyde produce complete blockage of vas deferens after injection in the same.

Benkov *et al.*, (1975) injected 10 and 15 mg cadmium chloride intra testicularly for chemical castration in boars weighing 30 and 60 kg respectively and found daily gain during fattening and slaughter weight were significantly higher in surgically castrated pigs as compared to chemically castrated pigs and higher lean percentage of the carcass & thinner back fat in chemically castrated pigs as compare to surgically castrated boars. Besides, there was 6% higher daily weight gain and better food conversion in pigs chemically castrated at 30 kg body weight as compared to those at 60 kg body weight. Koger (1978) injected 1.5 ml of 50% Calcium chloride into cauda epididymis of boars and concluded that calcium chloride caused orchitis and testicular atrophy.

Lipatnikov (1980) reported chemical castration in boars by intra testicular injection of 1.5- 2.0 ml of potassium permanganate (0.5 gm/200 ml) in 17% acetic acid into each testis at 20 - 40 days of age. The testes of treated boar weighed 5-6 gm at 6 months of age. In

this trial, 13 boar were castrated chemically, 12 boar castrated surgically and 12 were kept non-castrated. The average daily weight gain was higher in chemically castrated as compared to surgically castrated boar.

Kang *et al.*, (1993) tried 1 or 2 ml of intra testicular injection of (0.1, 0.5, 1.0 or 5% (12 parts silver nitrate to 88 parts lactic acid) Silver nitrate solution with or without lactic acid in boars and observed complete testicular atrophy within 14 and 21 days after injection of 1 ml and 2 ml of 5% solution with or without lactic acid whereas lower concentration of silver nitrate were not effective, which were confirmed by autopsy of boars at the age of 170 days.

Giri *et al.*, (2002) conducted a studied to develop a suitable technology of chemical castration in boars and twenty piglets were divided into two groups (n = 10), one group castrated surgically and other group castrated chemically (0.25 gm potassium permanganate + 17 ml glacial acetic acid +83 ml distil water) in each testis and concluded that chemical castration in boars is simple, more economical and easier to adopt than the surgical castration and found that average daily weight gain was significantly higher in chemically castrated pigs. The seminiferous tubules were devoid of spermatozoa due to its delicate structure and high sensitivity to irritants.

### **Buck and ram**

Bull *et al.*, (1972) reported that intra testicular injection of Cadmium chloride in rams resulted in sterility, due to permanent damage to testes. Koger (1978) injected 1.5 ml of 50% Calcium chloride into cauda epididymis of bucks and concluded that calcium chloride caused orchitis and testicular atrophy and can be used as rapid castration technique for population control programme. Plant *et al.*,

(1979) attempted non-surgical sterilization of rams using injection of 2.5 ml of 3.6% formaldehyde in 90% ethanol into the cauda epididymis. No live spermatozoa were observed in ejaculate collected on 35, 57, 91 and 196 days post treatment.

Similarly chemical castration in rams with intra epididymal injection of 10% formalin and Calcium chloride solution tried by Torrel *et al.*, (1979) and observed that formalin treated lamb showed no pain. Whereas Calcium chloride caused severe discomfort. Formalin treated lamb's testicle was fibrotic and Calcium chloride treated animal showed moderate to severe sloughing. Sclerosis was observed in epididymis 55 days post treatment and suggested that chemical castration is better than surgical castration. Similarly, the blockage of sperm transport in rams by bilateral intra epididymal injection of 3 ml 50% solution of Calcium chloride was also reported by Bowman *et al.*, (1978). It was observed that there was no sperm motility 12 days post treatment while libido of the treated animal was not affected.

Ijaz *et al.*, (2000) studied that the effects of intra testicular injection of neutral buffered formalin on seminiferous tubules in Awassi lamb. Animals injected with 1 ml of 10% formalin in right testis showed decrease in weight and size of testis, increase in vascularity and seminiferous filled with connective tissue. It was found that formalin causes reduction in size and weight of the testes by destroying seminiferous tubules. Therefore, formalin had potential to be considered for chemical castration.

Awal *et al.*, (2004) examined the effects of 10% formalin on testicular tissue of six healthy pre-pubertal male black Bengal goats (21days old) and evaluated the potentiality of formalin for chemical castration and revealed that testicular atrophy appeared two weeks

after exposure to formalin. At thirty days, the mean weight, length and width of testes were significantly decreased and histological changes were not uniform. A distinct wrinkling of tunica albuginea was observed and peripheral seminiferous tubules were more affected. Marked fibrosis in inter-tubular spaces was also observed. It is suggested that 10% formalin can be used for early castration in black Bengal goats.

Kadi and Asadi (2012) evaluated the effectiveness of intra-testicular injection of formalin and external ligation of the spermatic cord in inducing sterilization in bucks. Twelve adult bucks were divided randomly into three groups equally. The first group served as a control and was injected 5 of ml distilled water intra-testicularly, while the second group was injected with 5ml of 3% formalin and in the third group silk was used for external ligation of spermatic cord. Blood samples were collected from all bucks prior and post to treatment to assay serum testosterone level. In addition, semen samples were collected via artificial vagina to evaluate some semen parameters.

Clinical follow-up of animals revealed no any secondary complications in control group, while treatment group showed certain minor complications such as testicular swelling, hydrocele, scrotal ulcer and lameness which disappeared in a short time. Results indicated that testosterone levels significantly declined ( $P<0.05$ ) in the second and third groups and reached to  $0.30\pm 0.05$  and  $1.66\pm 0.25$  ng/ml, respectively at the end of experiment.

All semen parameters were significantly  $P<0.05$  decreased on days 21 and 28 in treatment groups, in which there was only seminal plasma, asthenozoospermia and azoospermia. All the animals were subjected to traditional castration on 30 and 60 days

post-injection or ligation to harvested testicular biopsies for histopathological examination. Testicular sections revealed depletion of Sertoli cells and interstitial fibrosis which replaced Leydig cells. These changes were more severe in chemical group when compared with ligation group. This means that both techniques reflected their ability to impair or stop the testicular functions with superiority of chemical castration.

Jana *et al.*, (2005) described the induction of chemosterilization in three groups each of six adult male Black Bengal goats at 30 days after a single bilateral intratesticular injection of a calcium chloride solution at the doses of 10, 20 or 40 mg/kg body weight/testis, in 2 ml of normal saline. Another one group of animals received only 2 ml of normal saline per testis as a control. The induction of chemosterilization was measured using relative testicular weight as well as histomorphological parameters including seminiferous tubular architecture and germ cell association in seminiferous tubules along with morphology of the interstitial space. Biochemical markers included activities of testicular 5, 3-hydroxysteroid dehydrogenase (5, 3-HSD), 17-hydroxysteroid dehydrogenase (17-HSD), catalase, glutathione peroxidase (GPx), glutathione S-transferase (GST) and superoxide dismutase (SOD) as well as monitoring the level of testicular thiobarbituric acid reactive substances (TBARS), conjugated dienes and reduced glutathione (GSH) content along with plasma concentrations of testosterone, LH and FSH.

Histomorphological measures of testes showed total necrosis of testicular tissue along with fibrosis in seminiferous tubules and interstitial spaces on 30 days after an injection of either 20 or 40 mg CaCl<sub>2</sub>. Infiltration of leucocytes was observed with

the 40 mg dose. Disintegration of germ cell arrangement in seminiferous tubules and washing out of germ cells from the tubules were noted with the 10 mg dose. Relative organ weights, plasma concentrations of testosterone, testicular activities of 5, 3-HSD, 17-HSD, catalase, GPx, GST, and SOD and testicular contents of GSH all were declined. Increase was reported in testicular TBARS, conjugated dienes and plasma concentrations of LH and FSH with each of the treatments by comparison with the control group.

Plasma concentrations of cortisol and fasting blood sugar level as well as packed cell volume (PCV) and total plasma protein were recorded to monitor the changes of chronic stress in the experimental animals. Changes in these parameters were not significant. An intratesticular injection of calcium chloride at specified doses could be a suitable method of sterilization in preference to surgical castration of bucks.

Mohammad and James (2013) performed chemical castration in six apparently healthy Borno white bucks weighing 15±1.6 kg and aged 1.3±0.3 years. Two and half (2.5) ml Purit<sup>®</sup> (chlorhexidine gluconate 0.3% B.P W/V and cetrimide 3.0% B.P W/V CAPL Lagos) were injected bilaterally into the cauda of each epididymis following sedation with xylazine hydrochloride. The pre-study scrotal circumference was 20.1±1.5cm; Significant decrease in scrotal circumference (P<0.01) 15.8±1.6cm occurred 35 days post injection. There was no significant difference (P>0.05) between pre-injection semen volume 0.6±2.1ml and 24 hour post injection semen volume 0.3±1.4cm. Subsequently, only few drops could be collected and from day 20 post injection, no semen ejaculate could be collected from all the six bucks. Azoospermia was noted from day 16 post injection with 0% motile cells, 95% dead cells and 25-60% abnormal sperm cells.

## Donkey

Ibrahim *et al.*, (2016) studied clinical efficacy of chemical castration with 20 % calcium chloride dissolved in absolute ethanol in comparison with surgical castration in donkeys. Chemical castration with intra testicular calcium chloride versus surgical castration failed to reduce serum concentrations of testosterone throughout the whole duration of the study; however it induced orchitis that was evident by focal necrotic areas in seminiferous tubules, cellular infiltration of neutrophils, proliferative inter-tubular fibrosis with a compensatory proliferation of Leydig cells. In conclusion intra-testicular calcium chloride can't be considered an effective method for chemical castration in donkeys.

## Rats

Kar *et al.*, (1961) reported that single subcutaneous injection of CdCl<sub>2</sub> causes acute degenerative changes in the testis of rats. The seminiferous epithelium is permanently destroyed but the interstitial elements regenerate after an initial phase of disorganization. The animals became sterile as early as 24 hours after administration of the salt.

Ericsson (1970) use a substance U - 5897 (3-chloro- 1,2- propainediol) at a dose rate 35 mg/kg body weight daily orally or 45mg/kg body weight orally as a single dose and reported that U-5897 cause local ischaemia that lead to epithelial desquamation resulting in blockage of epididymis, sperm blockage in ductuli efferentes and testicular swelling due to fluid accumulation which cause pressure degeneration of germinal epithelium, formation of spermatoceles, sperm granuloma and fibrosis but did not affect the functioning of leydig cells. Turner (1971) conducted an experiment using  $\alpha$ -chlorhydrin

at 8 mg/kg body weight on twenty male rats of the sprague dawley strain which were divided into four groups. Group 1- as control, group 2- injected with 8 mg/kg body weight of  $\alpha$ -chlorhydrin, group 3- placed a ligature around lower half of epididymis, group 4 – ligated epididymis along with daily injections of  $\alpha$ -chlorhydrin at 8 mg/kg body weight and reported that male rats lost fertility within 5 days of start of treatment and regained fertility within 1 week of withdrawal of the drug. It was supposed that drug acted on head of the epididymis and affect maturation and motility of spermatozoa.

Akbarsha *et al.*, (1990) fed 20 mg dry powder of dry leaves of *Andrographis paniculata* (Nees) in 1 ml of 2% gum acacia for 60 days to male albino rats and observed inhibition of spermatogenesis, degenerative changes in the seminiferous tubules, regression of Leydig cells and degenerative changes in the epididymis, seminal vesicle, prostate and coagulating gland. Size, weight and fluid content of accessory sex gland reduced and accumulation of glycogen and cholesterol in testis whereas increased activities of lactate dehydrogenase and alkaline phosphatase therefore suggested antispermatogenic and androgenic effects of the plant.

Ouerghi *et al.*, (1992) studied the individual and combined influences of chemical castration with Buserelin, a gonadotrophin releasing hormone (GnRH) agonist and adrenalectomy on energy balance in rats. The rats were weighed during treatment on every second day and total food consumed was measured. After 14 days, the rats were killed and analyzed for protein, fat and energy content. Body weight, protein, fat and energy gain were more in Buserelin treated than saline infused rats. They concluded that adrenalectomy can significantly attenuate the influence of castration on energy gain, providing evidence that adrenal and ovaries

are interacting in the regulation of energy balance. Jana *et al.*, (2002) used intratesticular injection of  $\text{CaCl}_2$  dissolved in normal saline for sterilisation of rats. Thirty days following injection, plasma testosterone concentrations and epididymal sperm counts were reduced in a dose dependent manner in all groups except the group in which 2.5%  $\text{CaCl}_2$  was injected. At the highest 2 doses, histological studies revealed a dose-dependent increase in necrosis of the germinal epithelium of the semiferous tubules and Leydig cells. The authors concluded that the 10% and 20% doses were optimal effective doses for induction of chemosterilization.

Similarly Omari *et al.*, (2005) used eighty adult male Sprague Dawley rats and were injected with one dose (50 micron L) of different concentration of formalin solution (2.5, 5 and 10%) intra-testicularly for chemical castration. Weight, food and water consumptions were monitored. All the rats maintained in a controlled atmosphere 21 °C less than 12 hours light and 12 hours darkness schedule. Para formaldehyde (Prilled 95% obtained from Aldrich Chemical Company, Milwaukee - WI 53233, USA), was dissolved in water and different concentrations were prepared. After two months of treatment, rats were kept for ten days for mating with virgin untreated females to evaluate mating capability of treated rats and observed that injection did not affect behavior of the treated rats, but size and weight of testes were reduced. This study showed that a single intra testicular injection of formalin solution has adverse effect on the reproductive system of male rats.

Hayes *et al.*, (2006) reported that atrazine is a potent endocrine disruptor that may be used as chemical castrate and feminizes male amphibians and suggested that atrazine induces gonadal malformation resulting from the depletion of androgens and production of

estrogen, perhaps subsequent to the induction of aromatase by atrazine, a mechanism established in rodents and humans.

Emir *et al.*, (2008) reported that testicular tissue ablation could be done by administering hypertonic saline solution in a rat model. A total of 40 male Wistar rats were divided into orchietomy (n = 20) and experimental groups. In the experimental group, 20% (n = 20) hypertonic saline solution was injected into the rat testes. Blood was taken prior to, 1 day, and 30 days after the intervention for testosterone determination.

Testicles were surgically removed for pathologic examination. Skin infection, necrosis, and testicular abscess were not detected in any rat. Histopathological examination revealed necrosis in almost all areas of the testicle. The comparison of 0, day 1 and day 30 measurements of total testosterone did not reveal any statistically significant difference between the control and hypertonic saline groups at any of the three time points (Mann-Whitney U-test,  $P > 0.05$ ). Intra-testicular hypertonic saline injection seems to be an alternative method in the future to its rivals such as orchietomy and medical castration.

Kwak and Lee (2017) compared the effects of Orchietomy (ORX) and of hypertonic saline bilateral intra testicular injection (BITI) on the androgen-sensitive tissues such as pituitary and hypothalamus. Serum testosterone levels of Orchietomized animals and hypertonic saline BITI animals (SAL) after 4 weeks of the manipulations exhibited significantly drops as compared with the levels of intact animals (Intact: ORX: SAL=7.74±1.31;1.34±0.19;1.28±0.18ng/ml,  $p<0.05$ ). In conclusion, hypertonic saline BITI method had equivalent efficacy of testosterone depletion to surgical castration in rats.

Jayusman *et al.*, (2018) reported that there were limited data on the effects of degarelix, a newer series of potent and long acting GnRH antagonist on bone. Eighteen male Sprague-Dawley rats were randomly divided into sham (SHAM), orchidectomized (ORX), and degarelix-induced (DGX) groups. Chemical castration was performed by subcutaneous degarelix injection (2 mg/kg) at the scapular region. The rats were scanned for baseline bone mineral area (BMA), bone mineral content (BMC) and bone mineral density (BMD) using dual-energy x-ray absorptiometry (DXA).

Following six weeks of experimental period, BMA, BMC, and BMD were measured again with DXA and blood was collected for testosterone and bone biomarkers (osteocalcin and C-terminal of type I collagen crosslink (CTX-1)) measurements. The rats were euthanized and femora were dissected for bone biomechanical strength analysis. Bilateral orchidectomy and degarelix administration significantly lowered serum testosterone level, decreased whole body BMC, femoral BMA, femoral BMC, and femoral BMD ( $P < 0.05$ ) compared with the SHAM group. However, no significant changes were observed in bone biochemical markers and bone mechanical strength in all experimental groups. In conclusion, degarelix administration had comparable effects on bone as bilateral orchidectomy.

### **Rabbit**

Paufler and Foote (1969) conducted an experiment on rabbits which were divided into five groups, group 1- contained ten animals as control, group 2 - five animals given one injection of 0.2 mg/kg body weight of triethylenemelamine (TEM), group 3 had two animals received double the amount of TEM as compared to group second, group 4 contained five animals which received five

weekly injections of 0.4mg TEM/kg body weight, group 5 had five animals injected with one injection of 0.05/kg body weight m-mole CdCl<sub>2</sub> and reported that TEM inhibit the spermatogonial division. Aspermia occur in 8 weeks and 4 weeks in CdCl<sub>2</sub> treated group, though changes were reversible in all the treated groups.

Majeed (2011) determined the efficacy and safety of intratesticular injection of ethanol or formalin as a novel method for chemosterilization of rabbits. Histopathological examination revealed atrophy of the seminiferous tubules, degeneration or vacuolation of Sertoli cells and the Leydig cells replaced by fibrous connective tissue. The tail of epididymis exhibited narrowing of the epididymal lumen. The severity and distribution of the lesions were more pronounced in formalin group, although both agents had the ability to induce sterilization in rabbits.

### **Monkey**

Kar *et al.*, (1965) reported that single local injection of ferrous sulphate or ferric chloride causes total destruction of the testis of adult rhesus monkeys. Histochemically, the injected iron is found to be localized in the tunica propria of the tubules and in the interstitium; it accumulates in the mitochondrial and the supernatant fractions almost in equal amounts. It seems that iron causes a generalized damage to the testis through properties common to other heavy metallic ions.

From the work available in the literature, it appeared that various chemicals have been used to castrate the animals that directly affect spermatogenesis. Most of these chemical agents used for castration appeared effective in achieving the goals, however such procedures are not free from side effects



including pain and swelling of the organs of the animal, though these side effects subside with the passage of time. Still, further research in this area is needed to arrive at a definite conclusion.

## References

- Akbarsha, M. A., Manivannan, B., Shahul, H. K. and Vijayan, B. (1990). Antifertility effect of *Andrographis paniculata* (Nees) in male albino rat. *Ind. J. Exp. Biol.* 28: 421- 426.
- Al- Asadi and Al-Kadi, R. (2012). Non-Surgical Castration in Bucks A Comparative Study between Chemical Castration and External Ligation of the Spermatic Cord. *Al-Qadisiyah Journal of Veterinary Medicine Sciences.* 11(1): 92-102.
- Ansari, A.S., Badar, A. and Lohiya, N.K. (2017). Fertility Control Modalities in Animals: An Overview. *BAO J. Vet. Sci.* 1(1): 004.
- Arshami, J. and Rattle, J. L. (1988). Effects of diets containing gossypol on spermatogenic tissues of young bulls. *Theriogenol.* 30(3): 507- 515.
- Awal, M. A., Rahman, M. M., Das, S. K., Siddiki, N. H., Kurohmaru, M., Bibin, A. B., Hayashi, Y. (2004). Formaline affects the male reproduction black Bengal goats during prepubertal stage even at low concentration. *J. Medical. Sci. Pak.* 4(1): 84- 89.
- Bagley, C. P., Feazel, Y. I., Morrison, D. G. and Saxton, A. M. (1989). Castrating and implanting suckling male beef calves. *Bulletin Louisiana Agricultural Experiment Station.* 809: 26
- Ball, J. J., Kegley, E. B., Lawrence, T. E., Roberts, S. L., Powell, J. G. and Richeson, J. T. (2018). Zinc Injection as a Novel Castration Method in Beef Bulls: Effects on Carcass Traits and Consumer Acceptability. *J. Anim. Sci.* 96(3): 890–891.
- Baran, A., Ozdas, A., Gulcubuk, A., Hamzaoglu, A. I., Tonguc, M. (2010). Pilot study: intra testicular injection induces sterility in male cats. Poster presented at the ACC and D conference, 2010.
- Barnett, B. D. (1984). Chemical vasectomy of domestic dogs in the Galapagos Islands. *Theriogenol.* 23: 499-509.
- Benkov, M., Vasilev, T. S., Stefanova, S. and Rachev, R. (1975). Comparison of fattening performance of pigs castrated by cadmium chloride injection. *Zhivotnov dni— Nauki,* 12(4): 65-69.
- Bierschwal, C. J. and Ebert, E. P. (1961). Clinical application of a scierothe-therapeutic agent. *Vet. Med.* 56: 323-332.
- Bowman, T. A., Senger, P. L., Koger, L. M., Gaskins, C. T. and Hiller, J. K. (1978). Blockage of sperm transport using intra epididymal calcium chloride injection in rams. *J. anim. Sci.* 46(4): 1063-1065.
- Bull, L., Faulkner, L.C. and Fateih, N. M. (1972). Spermatic vascular ligation and intra-testicular cadmium injection in the ram. *Int. Surg.* 57: 813.
- Canpolat, I., Gur, S., Gunay, C., Bulut, S. and Eroksuz, H. (2006). An evaluation of the outcome of bull castration by intra testicular injection of ethanol and calcium chloride. *Revue de Medicine Veterinary.* 157: 420-425.
- Canpolat, I., Karabulut, E. and Eroksuz, Y. (2016). Chemical Castration of Adult and non-Adult Male Dogs with Sodium Chloride Solution. *IOSR Journal of Agriculture and Veterinary Science.* 9(12/1): 09-11.
- Cavalieri, J., Wang, M. and Johnson, L. (2015). Chemical sterilization of *Bos indicus* bull calves following intra testicular injection of zinc acetate: Effects on semen quality and testicular changes. *Anim. Reprod. Sci.* 156: 23–33.
- Chatterjee, K., Monjur, K., De, D., Mallick, C. and Ghosh, D. (2009). Induction of chemosterilization by single intra testicular calcium chloride injection in stray dogs. *Res. J. Anim. Vet. Sci.* 4: 22-29.
- Chaudhary, R. (2006). Effects of intra-epididymal injection of Zinc Arginine on epididymis and spermatogenesis in pre-pubertal dogs. M.V.Sc. Thesis submitted to C.C.S. Haryana Agricultural University, Hisar, Haryana (India).
- Chaudhary, R., Sharma, A.K., Mehra, U.S., Dutt, R., Kumar, P. and Yadav, R. (2018). Histopathological studies on intra epididymal zinc arginine administration in

- pre-pubertal dogs. *Int.J.Curr.Microbiol. App.Sci.* 7(05): 569-581.
- Chaudhary, R., Sharma, A. K., Mehra, U. S., Dutt, R., Kumar, P. and Yadav, R. (2018). Histopathological Studies on Intra Epididymal Zinc Arginine Administration in Pre-Pubertal Dogs. *Int. J. Curr. Microbiol. App. Sci.* 7(5):569-581.
- Clermont, Y. and Leblond, C. P. (1955). Spermatogenesis of men, monkey, ram and other mammals as shown by the periodic acid Schiff technique. *Am. J. anat.* 96:229-253.
- Cohen, R. D. H., King, B. D., Janzen E. D., Hunter, P. S. W. (1991). Efficacy of chemical castration and effects of age at castration and implant regime on growth rate, testicular measurements and testosterone level of beef calves. *Canadian J. Anim. Sci.* 71(1):1-11.
- Cohen, R. D. H., King, B. D., Janzen, E. D. and Thomas, L. R. (1990). Efficacy and stress of chemical versus surgical castration of cattle. *Canadian J. Anim. Sci.* 70(4): 1063-1072.
- Dixit, V. P. and Lohiya, N. K. (1975a). Chemical sterilization effects of single high dose of 3-chloro-1, 2-propanediol on the testes and epididymis of dog. *Acta Europaea-Fertilitatis.* 6:57-62.
- Dixit, V. P., Khanna, P. and Bhargava, S. K. (1978). Effects of *Momordica Charantia* L. fruit extract on the testicular function of dog. *Plant Medica.* 34(3): 280-286.
- Dixit, V. P., Lohiya, N. K., Arya, M. and Agrawal, M. (1975b). Chemical sterilization of male dogs after a single intratesticular injection of "danazoole". *Folia Biologica Poland.* 23(3): 305-310.
- Emir, L., Dadalı, M., Sunay, M., Erol, D., Çaydere, M. and Üstün, H. (2008). Chemical castration with intratesticular injection of 20% hypertonic saline: A minimally invasive method. *Urologic Oncology: Seminars and Original Investigations,* 26(4): 392-396.
- Ericsson, R. J. (1970). Male antifertility compounds: U-5897 as a rat chemostrilant. *J. Reprod. Fert.* 22: 213-222
- Evans, H. E. (1993). *Millers Anatomy of the Dog.* 3<sup>rd</sup> Edition. W.B. Saunder Company. 508.
- Fahim, M. S., Wang, M., Suteu, M. P., Fahim, Z. and Youngquist, R. S. (1993). Sterilization of dogs with intraepididymal injection of zinc arginine. *Contraception Stoneham.* 47(1): 107-122.
- Feher, T., Bodrogi, L. and Makray, S. (1985). Dynamics of serum testosterone levels in bulls. *Magyar Allatorvosok Lapja.* 40(1): 13-16.
- Fordyce, G., Hodge, P. B., Beaman, N. J., Laing, A. R., Campero, C. and Shepherd, R. K. (1989). An evaluation of calf's castration by intra testicular injection of a lactic acid solution. *Australian Vet. J.* 66(9): 272-276.
- Freeman, C. and Caffey, D. S. (1973). Sterility in male animals induced by injection of chemical agents into the vas deferens. *Fertil. Steril.* 24: 884-890.
- Freshmen, J. L., Olson, P. N., Amann, R. P., Carison, E. D., Twedt, D.C. and Bowen, R. A. (1990). The effects of methyl testosterone on reproductive function in male grey hounds. *Theriogenology.* 33(5):1057-1073.
- Gardner, A. L. (1980). Sclerosing sterilization in bulls. *New South Wales Veterinary Proceeding.* 16: 60-61.
- Garriz, C., Billaudots, A., Gallinger, M., Parodi, J., Ludden, B., Gil, A. and Marcellia, M. (1986). Chemical castration of Hereford calves. Effect on carcass composition and quality. *Revista Argentina-da Production Animal.* 6: 627-636.
- Giri, S. C., Yadav, B. P. S. and Panda, S. K. (2002). Chemical castration in pigs. *Indian J. Anim. Sci.* 72(6): 451-453.
- Gupta, R. S. and Dixit, V. P. (1989). Testicular cell population dynamics following palmitine hydroxide treatment in male dogs. *J. Erithnopharmacoloty.* 25(2): 151-157.
- Gupta, R. S., Dixit, V. P. and Dobhal, M. P. (1990). Antifertility studies of isoquinoline alkaloid with special emphasis on structure activity relationship. *Fitoterapia* 61(1): 67-71.
- Hayes, T. B., Stuart, A. A., Mendoza, M., Collons, A., Noriega, N., Vonk, A., Johnston, G., Lui, R. and Kapodzo, D. (2006). Characterization induced gonadal malformation in African clawed frogs

- (*xenopus laevis*) and comparison with effects of an androgen antagonist (cyproterone acetate) and exogenous estrogen (17- beta-estradiol): Support for the demasculinization/feminization hypothesis. *Environ. Health Perspectives*. 114(1): 134-141.
- Hill, G. M., Neville, W. E. J., Richardson, K. L., Utley, P. R. and Stewart, R. L. (1985). Castration method and progesterone estradiol implant effects on growth rate of suckling calves. *J. Dairy Sci*. 68(11): 3059-3061.
- Ibrahim, A., Ali, M. M., Abou-Khalil, N. S. and Ali, M. F. (2016). Evaluation of chemical castration with calcium chloride versus surgical castration in donkeys: testosterone as an end point marker. *BMC Veterinary Research*, 12(1): 46
- Ijaz, A., Abalkhail, A. A. and Khamas, W. A. H. (2000). Effect of intratesticular injection of formalin on seminiferous tubules in Awassi lambs. *Pakistan Vet. J*. 20(3): 129-134.
- Immergart, H. M. and Threlfall, W. R. (2000). Evaluation of intratesticular injection of gossypol for non surgical sterilization of dogs. *American J. Vet. Res*. 61(5): 544-549
- Inaba, T., Umehara, T., Mori, J., Torn, R., Tamada, H. and Swada, T. (1996). Reversible suppression of pituitary-testicular function by a sustained release formulation of a GnRh agonist (*Leuprolide acetate*) in dogs. *Theriogenol*. 46: 671-677.
- Jana and Samanta (2011). Clinical Evaluation of Nonsurgical Sterilization of Male Cats with Single Intra-testicular Injection of Calcium Chloride. *BMC Veterinary Research*. 7:39.
- Jana, K., and Samanta, P. K. (2007). Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose dependent study. *Contraception* 75(5): 390-400.
- Jana, K., Samanta, P. K. and 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(1-2): 89-108.
- Jana, K., Samanta, P.K. and 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
- Jayusman, P. A., Mohamed, I. N., Shuid, A. N. (2018). The Effects of Chemical Castration with Degarelix on Bone Turn over: Densitometric and Biomechanics Bone Properties of Male Rats. *Int J Endocrinol Metab*. 16(3): in press
- Kang, Y. S., Park, C. S. and Chung, H. S. (1993). Chemical castration by intratesticular injection of silver nitrate solution in pigs. *Korean J. Anim. Sci*. 35(6): 463- 469.
- Kar, A. B. (1961). Chemical sterilization of male rhesus monkeys. *Endocrino*. 69(6):1116–1119.
- Kar, A. B., Kamboj, V., Goswami, A. (1965). Sterilization of male rhesus monkeys by iron salts. *J. Reprod. Fertil*. 9:115–117
- Koger, L. M. (1977). Calcium chloride-Practical necrotising agent. *Bovine Practitioner*. 12:118-119.
- Koger, L. M. (1978). Calcium chloride castration. *Modern Veterinary Practice*. 59: 119-121.
- Kostyra, J., Krzyzanowski, J. and Ziolo, T. (1981). Sterilization of dogs using intratesticular injection of chemical compounds. *Medycyna Weterynaryjna*. 37(9): 551- 554.
- Kwak, B. K. and Lee, S. H. (2017). Evaluation of Newly Developed Chemical Castration Method: Changes in Hormone Gene Expression of Hypothalamic-Pituitary Axis. *Development and Reproduction*. 21(3): 307-315.
- Lal, B. (2002). Studies on effects of Chlorhexidine gluconate on epididymis and spermatogenesis in dogs. M.V.Sc. Thesis, Haryana Agricultural University, Hisar (Haryana), India.
- Leoci R, Aiudi G, Silvestre F, Lissner E, Lacalandra G. ( 2014a). Alcohol diluent provides the optimal formulation for calcium chloride non-surgical sterilization in dogs. *Acta Veterinaria Scandinavica*. 56:62
- Leoci R. (2012). Calcium chloride dihydrate nonsurgical sterilization in 81 dogs: Dose, formulation, and best practices implications for maximal effectiveness and minimal complications, from the first large study

- outside of India. Presented at the 1st International Conference on Dog Population Management, York, UK, Sept. 6, 2012
- Leoci, R., Aiudi, G., Silvestre, F., Lissner, E., Marino, F., Lacalandra, G. (2014b). 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(1): 63
- Lipatinikov, V. F. (1980). Chemical castration of boars. *Sel skokhozyaistvennaya Biologiya*. 15(1): 130-134.
- Majeed, A. A. (2011). Chemical Castration of Rabbits via Intratesticular Injection of Ethanol or Formalin. *The Iraqi Journal of Veterinary Medicine*. 35(1): 10-15.
- Mohammed, A. and James, F. (2013). Chemical castration by a single bilateral intra-testicular injection of chlorhexidine gluconate and cetrimide in bucks. *Sokoto Journal of Veterinary Sciences*. 11(1): 62-65
- Murty, T. A. and Sastry, G. A. (1978a). The effects of cadmium chloride injection on the histopathology of the testis and the prostate in dog. I. Intratesticular procedure. *Indian Vet. J.* 55(1): 368-371.
- Murty, T. A. and Sastry, G. A. (1978b). Effect of cadmium chloride (CdCl<sub>2</sub>) injection on the histopathology of the testis and the prostate in dogs II. Subcutaneous procedure. *Indian Vet. J.* 5(10): 815-818.
- Neto, O. A., Gasperin, B. G., Rovani, M. T., Ilha, G. F., Nóbrega, J. E., Mondadori, R. G., Antoniazzi, A. Q. (2014). Intratesticular hypertonic sodium chloride solution treatment as a method of chemical castration in cattle. *Theriogenol.* 82(7): 1007-1011.
- Oliveira, E. C., Moura, M. R., Silva, V. A. Jr., Peixoto, C. A., Saraiva, K. L., de Sá M.J., Douglas, R. H. and de Pinho Marques, A. Jr. (2007). Intratesticular injection of a zinc-based solution as a contraceptive for dogs. *Theriogenol.* 68(2): 137-45.
- Omari, Al. O., Weal, K., El- Beteiha, A. (2005). Assesment of fertility and mating capabilities in adult male rats after single intra testicular of formalin solution. *J. Anim. Vet. Advances*. 4(6): 615- 623.
- Ouerghi, D., Rivest, S. and Richard, D. (1992). Adrenalectomy attenuates the effect of chemical castration on energy balance in rats. *J. Nutrit.* 122(2): 369-373.
- Paksoy, Z. (2018). The effects of 4-vinylcyclohexene diepoxide on gene expression in dog testes. *Inter. J. Vet. Sci.* 7(2): 60-64.
- Paufler, S. K. and Foote, R. H. (1969). Effect of triethylenemelamine (TEM) and cadmium chloride on spermatogenesis in rabbits. *J. Reprod. Fertil.* 19: 309-319.
- Pearson, H., Arthur, G. H., Rosevink, B. and kakati, B. (1980). Ligation and sclerosis of the epididymis in the bull. *Vet. Rec.* 20(9): 285-287.
- Pineda, M. H. and Helper, D. L. (1981). Chemical vasectomy in dogs. Long term study. *Theriogenol.* 16(1): 1-11.
- Pineda, M. H. Remers, T. J. and Faulkner, L. C. (1977). Azoospermia in dogs induced by injection of sclerosing agents into the cauda of the epididymides. *Am. J. Vet. Res.* 38: 831-838.
- Plant, J. W., Seaman, J. T. and Jackoxljevic, D. (1979). Non-surgical sterilization of rams using a sclerosing agent. *Australian Vet. J.* 55(6): 263-264
- Prince, L., Quirike, J. F. and Roche, J. F. (1986). Chemical castration of bulls. *Anim. Prod.* 76-77.
- Schmidt, S. S and Morris, R. R. (1973). Spermatic granuloma: The complication of vasectomy. *Fertil. Steril.* 24: 941- 947.
- Sen, C. C., Yumusak, N., Faundez, R., Temamogullari, F. and Taskin, A. (2017). Evaluation of intra-testicular injections of calcium chloride and 4-vinylcyclohexene 1, 2-monoepoxide for chemical sterilization in guinea pigs. *Polish Journal of Veterinary Sciences*. 20(2): 251-260.
- Singh, G., Sharma A. K. and Chandolia, R. K. (2006). Effects of Chlorhexidine gluconate on Cauda Epididymis and Spermatogenesis of Cow Bulls. *Ind. J. Vet. Res.*, 15(2): 42-48.
- Singh, G., Sharma, A. K. and Chandolia, R. K. (2005). Effects of Chemical Castration in

- Cow Bulls. National Seminar on Conservation, Reproduction and Production held on 5-7 March, 2005, organized by college of veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, p. 271.
- Sorensen, B., Soutello, R.V.G.de., Gomes, C.R.de. A., Garcia, R., Marini, A., Fonzar, J. F., Paes, F., Rasteiro, V. S., Souza, Filho, W. F. de., Tozi, C. R., Gasparelli junior, A. G., Correa, B. G., Moitiho, M. P., de, A. (2001). Chemical, surgical and mechanical castration of bovine. *Ciencias Agrarias e da Saude*. 1(2): 7-10.
- Soto, F., Viana, W., Sousa, A., Pinheiro, S., Mucciolo, G., Hosomi, F. and Azevedo, S. (2007). Evaluation of zinc gluconate, either associated or not to dimethyl sulfoxide, as contraceptive method for male dog. *Anim. Reprod*. 4(3/4): 119-124.
- Spinelli, J. (1989). Chemical castration of cow. *Veterinaria Argentina*. 6: 540-541.
- Torrel, D. T., Duella, B. D. and Durant, R. H. B. (1979). Sterilization of lamb by chemical sclerosing of epididymis formaldehyde and calcium. *J. Anim. Sci*. 49: 204.
- Turner, M. A. (1971). Effects of  $\alpha$ -chlorhydrin upon the fertility of spermatozoa of the cauda epididymis of the rat. *J. Reprod. Fertil*. 24: 267-269.
- Vare, A. M and Bansal, P. C. (1973). Change in the canine testes after bilateral vasectomy. *Fertil. Steri*. 24: 793-797.
- Vickery, B. H., McRai, G. I., Briones, W. V., Roberts, B. B., Worden, A. C., Schanbacher, B. D. and Falvo, R. E. (1985). Dose response studies on male reproductive parameters in dogs with nafarelin acetate, a potent LHRH agonist. *J. Androl*. 6(1): 53-60.

**How to cite this article:**

Gyan Singh, Amit Kumar, Ravi Dutt, V. Arjun and Jain, V. K. 2020. Chemical Castration in Animals: An Update. *Int.J.Curr.Microbiol.App.Sci*. 9(04): 2787-2807.  
doi: <https://doi.org/10.20546/ijemas.2020.904.329>