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## Comparative evaluation of sterilizing potential of intratesticular chemosterilants in male albino rats

Abdul Salam Ansari<sup>✉</sup>, Timanshi Chansoriya, Barkha Khilwani, Nirmal Kumar Lohiya

Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur – 302004, Rajasthan, India

## ABSTRACT

**Objective:** To investigate the sterilizing potential of zinc gluconate, calcium chloride (CaCl<sub>2</sub>) and cadmium chloride (CdCl<sub>2</sub>) following a single intratesticular administration in adult male rats.

**Methods:** 60 adult male Wistar albino rats (*Rattus norvegicus*) weighing 160-200 g and aged 5-6 months randomly received a single intratesticular injection of normal saline (Group A), zinc gluconate 13.3 mg/mL plus L-Arginine (Group B), 20% CaCl<sub>2</sub> (Group C), and CdCl<sub>2</sub> 0.5 mg/kg body weight plus ethylenediaminetetraacetic acid (EDTA) (Group D), respectively, along the entire route from the caudoventral aspect of each testis. They were euthanized up to 180 days to evaluate reproductive tract toxicology.

**Results:** The reproductive organ weights were markedly reduced, with testes severely atrophied in group B, pea-sized and stony hard in group C, and moderately reduced in group D. Azoospermia was evident in groups B and C, while sperm concentration was reduced to <1 million/mL with zero sperm motility in group D. Rats of groups B and C failed to show mounting and copulatory behaviour. A completely disorganized mesh of cellular elements was observed in the seminiferous tubules of group B, while pyknotic germ cell and arrest of spermatogenesis, exfoliated germ cells, occasional syncytial bodies and smaller Leydig cells were evident in groups C and D. Significantly reduced testosterone levels, increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, returned to normal after 90 days in group D.

**Conclusions:** Zinc gluconate and CaCl<sub>2</sub> injections severely affected the reproductive organs and libido and rats treated with CdCl<sub>2</sub> exhibited diminished spermatogenesis with normal libido. Thus, the need-based selection of intratesticular agents should consider their distinct effects on spermatogenesis, libido, and hormonal balance for achieving targeted sterilization outcomes.

**KEYWORDS:** Zinc gluconate; Calcium chloride; Cadmium chloride; Intratesticular chemosterilization; Azoospermia; Male rats; Reproductive toxicity; Canine population control

## 1. Introduction

According to the Dogster team the global dog population is estimated to be 900 million, approximately 75%-85% (747 million) of this number are free to roam and breed without restraint[1]. Notably, India is believed to harbor the largest number of stray dogs, approximately 30-70 million, accounts for 36% of the world's rabies deaths and their population is growing at an alarming rate of approximately 17% in recent years[2].

## Key Points

**Question:** Can a single intratesticular injection of zinc gluconate, calcium chloride (CaCl<sub>2</sub>) or cadmium chloride (CdCl<sub>2</sub>) induce effective sterilization?

**Findings:** Zinc gluconate and CaCl<sub>2</sub> caused severe testicular atrophy, azoospermia, disrupted testicular histology, loss of libido and significant alterations in testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels. CdCl<sub>2</sub> led to severely reduced sperm concentration and testicular damage, but sexual behaviour eventually recovered.

**Meaning:** Intratesticular administration of zinc gluconate or CaCl<sub>2</sub> can be used for complete sterilization, including loss of libido; whereas CdCl<sub>2</sub> may be useful when reduced fertility without affecting libido is desired, allowing for specific sterilization needs.

<sup>✉</sup>To whom correspondence may be addressed. E-mail: [abdulsansari@yahoo.com](mailto:abdulsansari@yahoo.com)

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This burgeoning street dog population poses myriad issues including being a primary source of over 95% of human rabies cases, transmitting zoonotic diseases such as *Echinococcosis* and *Leishmaniasis*, causing economic losses through livestock predation, competing with native species for resources, contributing to road traffic accidents, unprovoked biting incidents and exhibiting public nuisance behaviours such as noise, fouling and rummaging through garbage. Considering the tremendous ecological impact and public health hazard posed by the exploding street dog population, there is an urgent need of an effective solution for their population control[3]. Traditional culling methods face opposition due to ethical concerns and public resistance, while surgical sterilization techniques like spaying and neutering are considered reliable but they are encumbered by limitations including high costs, time intensiveness, specialized infrastructure, personnel requirements and postoperative complications[4].

Consequently, scientists have shifted their focus to non-surgical tools for humane and cost-effective dog population management. Various alternatives, including immunocontraceptives, hormone implants, chemical sterilants, and fertility inhibitors, have been developed for mass applications. Non-surgical methods offer advantages due to low cost, prompt effectiveness, easy administration, minimal infrastructure requirements, and improved social acceptability[5]. Immunocontraceptive vaccines, like the gonadotropin-releasing hormone (GnRH) vaccine, Gonacon, exhibit promise in inhibiting fertility with a favourable safety profile, despite requiring multiple injections for adequate immune titers[6]. Progestin drugs, such as melengestrol acetate, and Suprelorin, can chemically sterilize dogs but may entail significant side effects[7], while intra-epididymal injection of polymers like styrene maleic anhydride (SMA) also induces azoospermia but its usage remains limited[8]. Intratesticular injection of zinc gluconate and calcium chloride ( $\text{CaCl}_2$ ) solutions initially surfaced as potential methods for chemical sterilization. Zinc gluconate under trade names such as Neutersol<sup>®</sup>, Testoblock<sup>®</sup>, Zeuterin<sup>®</sup>, or EsterilSol<sup>®</sup>, gained commercial traction and received approval from the U.S. Food and Drug Administration (FDA) but was eventually withdrawn from the market. Various concentrations and doses of  $\text{CaCl}_2$  have undergone controlled and field studies in the U.S., Italy, and India; however, it is currently not approved by any regulatory agency. These methods induce testicular fibrosis and long-term infertility by damaging the seminiferous tubules but are also associated with severe side effects like testicular swelling, scrotal pain, necrotizing orchitis and scrotal ulceration[9]. Cadmium is one of the endocrine disruptors and is a well-known testicular toxicant. Cadmium chloride ( $\text{CdCl}_2$ ) injections have demonstrated potential as an effective chemosterilant by occluding the seminiferous tubules, leading to azoospermia within a relatively short period and interfering with the biochemical processes such as enzyme function and cell metabolism, thereby suppressing testicular

activity[10]. The toxic effects of heavy metals, particularly cadmium, can be significantly reduced by utilizing ethylenediaminetetraacetic acid (EDTA) as a chelating agent. With its hexadentate ligand properties, EDTA can form stable complexes with metal ions, effectively decreasing toxicity and facilitating the elimination of heavy metal ions from the body[11].

Although, intratesticular chemical sterilization has shown great promise in dogs, concerns remain regarding effective standardization of the dose, frequency, technique and delivery system to ensure safety and permanent infertility. Conceptually, an ideal chemical sterilizing agent must have no deleterious effects, easy to administer, have no specific target species, rapid and workable with a single intervention[12]. Hence, further research is required regarding appropriate dose regimens, efficacy, consistency, reversibility and mechanism of action of these chemosterilants to minimize the risk of adverse effects that need to be addressed through controlled research before they can be widely implemented in dog population management programs.

Therefore, this investigation aimed to provide a comparative assessment of zinc gluconate,  $\text{CaCl}_2$  and  $\text{CdCl}_2$  as chemosterilants when injected intratesticularly in male albino rats with their potential chelating agents for their sterilizing potential for a period of 180 days.

## 2. Methods

### 2.1. Animals

Sixty adult male Wistar albino rats (*Rattus norvegicus*) weighing 160-200 g and aged 5-6 months were used for the present study. The animals were maintained in the Departmental Experimental Animal Facility with a 12:12 h light and dark schedule in individual polypropylene cages (size 43 cm × 27 cm × 15 cm) and fed with rat pellet diet (Ashirwad Industries Limited, Chandigarh, India) and water *ad libitum*. Animals were maintained under perfect veterinary supervision in accordance with the Guidelines on the Regulation of Scientific Experiments on Animals[13].

### 2.2. Drug preparation

#### 2.2.1. Zinc gluconate

An aqueous solution was prepared by dissolving 273.402 mg of zinc gluconate and 104.52 mg of *L*-Arginine separately in 3 mL of distilled water. The zinc gluconate solution was made at a concentration of 13.3 mg/mL of 0.2 M solution[14]. To prepare the chelated working solution, 2500  $\mu\text{L}$  of zinc gluconate was mixed with 500  $\mu\text{L}$  of *L*-Arginine with adjustment of pH at 7.0.

#### 2.2.2. $\text{CaCl}_2$

The solution of  $\text{CaCl}_2$  (20%) was made by dissolving 0.6 g of  $\text{CaCl}_2$

in 3 mL of 0.9% NaCl; isotonic physiological saline solution. Before mixing with  $\text{CaCl}_2$ , the saline solution was neutralized to achieve the desired pH and chemical stability[15].

### 2.2.3. $\text{CdCl}_2$

$\text{CdCl}_2$  was prepared by dissolving 3 mg of  $\text{CdCl}_2$  in 3 mL of 1 M EDTA in a glass vial and the pH was adjusted to 7.0.

## 2.3. Experimental protocol

The fertile male rats were randomly allocated into four groups for the present investigation, containing 15 in each group. Group A: Vehicle injected control (0.9% NaCl; isotonic physiological saline); Group B: single intratesticular injection of zinc gluconate (13.3 mg/mL; chelated with *L*-Arginine) into each testis for a period of 180 days; Group C: single intratesticular injection of  $\text{CaCl}_2$  (20% in neutralized physiological saline) into each testis for a period of 180 days and Group D: single intratesticular injection of  $\text{CdCl}_2$  (0.5 mg/kg body weight) chelated with EDTA into left testis for a period of 180 days.

## 2.4. Injection procedure

Prior to the schedule of intratesticular injection procedure, access to food was withheld overnight and animals were then anaesthetized using sodium thiopentone (40 mg/kg body weight, *i.p.*; Thiosol Sodium, Neon Laboratories Ltd., Mumbai, Maharashtra, India). During injection procedure, the needle was directed from the caudoventral aspect of each testis approximately 0.5 cm from the epididymal tail towards the dorsocranial aspect of that testis. A total volume of 0.1 mL/testis was injected using a 0.5-mL U100 insulin syringe fitted with a 28-ga, 12-mm size needle. The dosage was determined to be ideal according to testicular width/body weight. The drug was carefully injected along the entire route by linear infiltration while withdrawing the needle from the proximal to distal end.

## 2.5. Assessment of key reproductive toxicity markers

Five animals, from each group were euthanized with an over dose of sodium pentathione (Thiosol Sodium, Neon Laboratories Ltd, Mumbai, India) at 30, 90 and 180 days after intratesticular injections and the following parameters were evaluated.

### 2.5.1. Morphological changes

Any alteration at the injection site of the testes and gross morphology of the scrotum were observed for initial two hours and daily following intratesticular injection. Mortality, morbidity, changes in skin, fur, mucous membrane and eye, tremors, salivation, diarrhea, convulsion, lethargy, animal behaviour, feeding pattern, changes in the level of motor activity, gait and posture, relativity to handling or sensory stimuli, grip strength were observed daily as phenotypic symptoms. Strange behaviours such as self mutilation

and walking backwards, were also recorded every day.

### 2.5.2. Body weight and reproductive organs weight

Initial body weight and weekly changes in body weight up to 180 days after chemosterilant administration were also recorded. After sacrifice, reproductive organs (testes, epididymides, vas deferens, seminal vesicle and ventral prostate) were dissected out, freed from fat and adherent tissues and weighed at the nearest milligram on an electronic balance.

### 2.5.3. Cauda epididymal sperm characteristics

The cauda epididymis was teased into small fragments in 1 mL of normal physiological saline to release spermatozoa and the clear fluid was analyzed for sperm concentration, motility, viability, and abnormalities[16].

### 2.5.4. Ultrastructure of spermatozoa

The spermatozoa collected at the time of sperm characterization were subjected to electron microscopy for examination of sperm morphology. The sperm pellets were initially fixed in 2.5% glutaraldehyde for 30 min and then washed thrice in phosphate buffer followed by distilled water. From the final suspension in distilled water, a thin film of spermatozoa was smeared on a clean glass slide piece, air dried in a desiccator, mounted on a scanning electron microscope (SEM) stub with silver paint, sputter coated at 350 Å with gold and was observed under SEM at 15 KV acceleration voltages (Model: Nova Nano FE-SEM 450).

### 2.5.5. Fertility test

To assess libido, mounting behaviour and fertility status, the control and injected animals were exposed to proven fertile female rats at 1:2 ratio a day prior to sacrifice at all intervals. The success of mating was confirmed by the appearance of a vaginal plug and spermatozoa in the vaginal smear in the successive morning, if any. Among the female rats paired with control and injected males, half of the animals underwent natural full-term pregnancies, while the remainder underwent cesarean for their implantation status a day or two prior to parturition under sodium thiopentone anesthesia. A midline incision was made to expose the uterine horns and ovaries, which were then removed and cleaned with normal saline to eliminate adherent fat and tissues. The gravid uterus, placenta and ovary weights were meticulously recorded. The total number of implantation sites or non-pregnant status was determined by isolating and staining non-gravid uteri with ammonium sulphide[17]. Corpora lutea, implantations and resorptions were quantified accordingly. Fetuses were extracted from opened uteri and individually submerged in 0.9% saline solution. Both deceased and viable fetuses were tallied, and their precise body weight, length and sex ratio were meticulously documented. Furthermore, detailed records of normally delivered litters, encompassing size, body weight, length, width, and sex ratio, were diligently maintained.

### 2.5.6. Histology of testis

After sacrifice at each interval, testis was immediately collected and fixed overnight in 4% paraformaldehyde. The testis of group C (CaCl<sub>2</sub>) was decalcified. They were dehydrated in a graded ethanol series, cleared in xylene, infiltrated and embedded in paraffin wax. Thin sections of 5 µm were cut, stained with hematoxylin and eosin (H & E) and observed under a light microscope of 200× magnification (Model: DM 1000, Leica, Wetzlar, Germany).

### 2.5.7. Hormone analyses

Blood samples were collected at the time of sacrifice by cardiac puncture, the collected blood was allowed to clot at room temperature and the serum was separated by centrifugation at 1388 g. The serum was used to assess leutenizing hormone (LH) and follicle stimulating hormone (FSH) levels using enzyme-linked immunosorbent assay (ELISA) kits from Autobio Diagnostic Co. Ltd. (Zhengzhou, China), with coefficients of variation (CVs) of 8% (intra-assay) and 10% (inter-assay) for both hormones. Then, the circulatory levels of serum testosterone were also assayed using ELISA kits (Cal biotech, Inc. El Cajon, CA) with intra- and inter-assay CVs of 6.62% and 8.91%, respectively.

### 2.6. Statistical analysis

The statistical analysis was made by using GraphPad Prism 9.5.1 software (GraphPad Software, Boston, USA). All data were tested for normality and found to conform to a normal distribution and one-way analysis of variance (ANOVA) was used for statistical comparison. Data were expressed as mean±standard deviation (mean±SD).  $P < 0.05$  was considered as statistically significant.

### 2.7. Ethics statement

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide Certificate No. UDZ/IAEC/IV/01 dated December 18, 2021.

## 3. Results

### 3.1. Morphological changes

There were no alterations in any phenotypic symptoms following intratesticular injection for 180 days. However, in groups B and C, animals exhibited high grade post-injection scrotal swelling which was resolved after 3 weeks of chemical administration. At 30 days of intratesticular injection, shrinkage of scrotum was seen which persisted throughout the experimental schedule. In contrast, animals in group D experienced only transient burning at the injection site and moderate scrotal swelling for 2-3 days post-procedure, with a normal scrotum after 30 days of injection. Morphological

examination of reproductive organs revealed significant loss of size and architecture, atrophied and almost regressed organs, which were difficult to recognize following the 30 days sacrifice schedule. After necropsy, initially locating the reproductive organs injected with zinc gluconate was challenging due to their drastically reduced size, while the testes injected with CaCl<sub>2</sub> became pea-sized and stony hard. In contrast, the testes of the animals treated with CdCl<sub>2</sub> also moderately decreased in size but maintained a normal appearance.

### 3.2. Body weight and reproductive organs weight

There were no discernible alterations noted in the body weight of any of the treated groups when contrasted with the control group treated with the vehicle. Throughout the experimental period, a drastic reduction ( $P < 0.001$ ) in the weight of all reproductive organs was observed in groups B and C compared to group A. In group D, the testis weight was markedly reduced ( $P < 0.001$ ) throughout experimental period, while the epididymis weight decreased significantly ( $P < 0.01$ ) after 30 and 90 days compared to group A. However, following 180 days of CdCl<sub>2</sub> injection (group D), the reduction in epididymal weight was found to be non-significant. Compared to group A, the weights of the vas deferens and seminal vesicles showed a non-significant decrease after 30 and 90 days of intratesticular injection in animals of group D, but found significantly decreased ( $P < 0.01$ ) at 180 days of the experimental schedule. A gradual decrement in the weight of the ventral prostate was noticed, which was found significantly different ( $P < 0.01$ ) at 90 and 180 days of experimental period (Table 1).

### 3.3. Cauda epididymal sperm characteristics

Azoospermia was evident in groups B and C, whereas in group D, the cauda epididymal sperm concentration was markedly inhibited (<1 million/mL), with zero sperm motility and a significant reduction in sperm viability. Additionally, there was an increase in the number of sperm abnormalities ( $P < 0.001$ ) observed in group D compared to group A (Table 2).

### 3.4. Ultrastructure of spermatozoa

The ultrastructure of spermatozoa obtained from the cauda epididymis of animals of the control group (group A), following 30, 90 and 180 days, showed a normal morphology with typical hook shaped head, mid-piece and long tail. The whole spermatozoa appeared intact with plasma membrane and organelles and the sperm acrosome exhibited a smooth configuration and covered with a thick plasma membrane (Figure 1A). The SEM observations of spermatozoa following 30 and 90 days of intratesticular injection of CdCl<sub>2</sub> (group D) revealed various anomalies. The plasma membrane and acrosomal membrane were disrupted in the anterior region of sperm head and constrictions in the mid region of sperm head

**Table 1.** Weight of reproductive organs (mg/100 g body weight) following intratesticular administration of zinc gluconate plus *L*-Arginine, calcium chloride ( $\text{CaCl}_2$ ), and cadmium chloride ( $\text{CdCl}_2$ ) plus ethylenediaminetetraacetic acid (EDTA) in male Wistar albino rats.

Parameters	Control			Zinc gluconate + <i>L</i> -Arginine			$\text{CaCl}_2$			$\text{CdCl}_2$ +EDTA		
	30 days	90 days	180 days	30 days	90 days	180 days	30 days	90 days	180 days	30 days	90 days	180 days
Testes	655.17±49.81	649.31±35.15	671.29±50.81	60.20±7.85**	69.32±5.56**	75.46±19.00**	181.38±73.43**	191.31±54.68**	186.67±54.68**	342.57±70.96**	388.83±50.09**	381.24±51.63**
Epididymides	277.55±20.13	286.58±18.31	268.69±38.87	55.47±38.28**	38.23±26.98**	42.55±18.24**	109.49±18.03**	99.51±10.37**	110.94±10.23**	187.03±52.67**	194.82±24.82**	207.97±49.12**
Vas deferens	190.46±37.33	201.70±76.27	199.29±22.58	95.07±63.02**	88.02±9.06**	76.58±25.19**	78.41±9.48**	83.68±13.37**	83.17±8.46**	118.01±41.45**	137.69±27.31**	129.67±38.22**
Seminal vesicle	369.60±22.23	378.75±35.62	380.41±27.00	102.51±54.99**	93.31±24.64**	108.42±51.40**	150.99±105.17**	110.69±32.76**	106.20±13.37**	214.04±42.39**	241.05±29.23**	203.20±17.80**
Ventral prostate	223.05±40.47	221.44±33.53	238.05±18.16	65.58±19.16**	99.77±15.35**	88.51±16.48**	113.39±53.01**	102.70±17.07**	140.97±28.29**	190.90±75.26**	182.99±25.49**	166.42±21.05**

Data are expressed as mean±SD; n=5. Compared to the control group at the same days: \* $P<0.01$ , \*\* $P<0.001$ .

**Table 2.** Cauda epididymal sperm characteristics following intratesticular administration of zinc gluconate plus *L*-Arginine, calcium chloride ( $\text{CaCl}_2$ ), and cadmium chloride ( $\text{CdCl}_2$ ) plus ethylenediaminetetraacetic acid (EDTA) in male Wistar albino rats.

Parameters	Control			Zinc gluconate + <i>L</i> -Arginine			$\text{CaCl}_2$			$\text{CdCl}_2$ +EDTA		
	30 days	90 days	180 days	30 days	90 days	180 days	30 days	90 days	180 days	30 days	90 days	180 days
Sperm concentration, million/mL	61.36±7.46	73.25±3.07	69.41±5.33	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	00.00±0.00	0.86±0.30*	0.45±0.38*	0.23±0.12*
Sperm motility, %	74.33±2.46	86.17±4.31	82.26±5.18	-	-	-	-	-	-	00.00±0.00*	00.00±0.00*	00.00±0.00*
Sperm viability, %	72.38±1.97	76.41±2.53	74.36±3.16	-	-	-	-	-	-	7.78±0.67*	3.89±0.25*	2.47±0.44*
Sperm abnormality, %	22.01±2.55	25.62±1.97	27.61±2.06	-	-	-	-	-	-	77.36±6.49*	82.61±3.05*	90.47±2.09*

Data are expressed as mean±SD; n=5. Compared to the control group at the same days: \* $P<0.001$ .

were observed. Abnormally bent head, headless tail and coiled tails due to cytoplasmic remnants were also seen. After 180 days of intratesticular injection, separation of the head from the tail was clearly observed in the majority of spermatozoa (Figure 1B-I).

### 3.5. Outcomes of fertility test

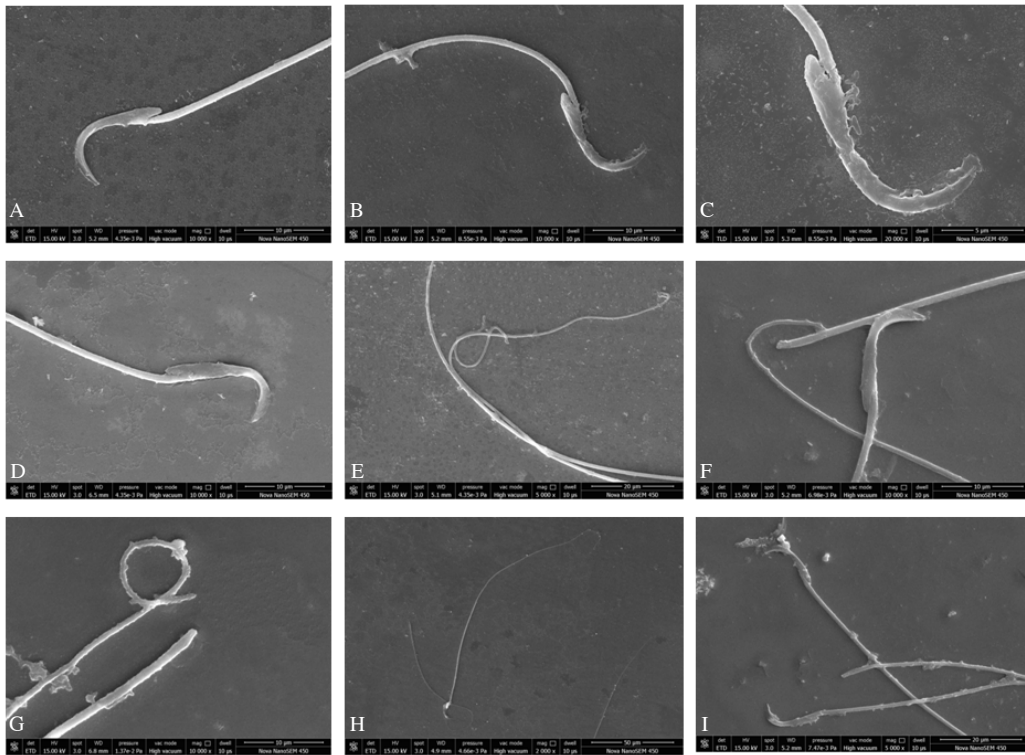
After 30, 90 and 180 days of chemosterilant administration, all animals in groups B and C were found to be completely sterile and failed to exhibit mounting and copulatory behaviour, showing no libido. However, animals in group D were also infertile but displayed normal libido.

During pre-natal development, pregnancy in cesarean females mated with group A (vehicle injected control) males following 30, 90 and 180 days of intratesticular injection, *viz.*, weights of gravid uterus, ovary and placenta and number of corpora lutea, corpora albicans, pre- and post-implantation loss, number of implantations or number of fetuses, body weight, body length and body width of fetuses were found within normal range. Females mated with animals of groups B, C and D was not pregnant.

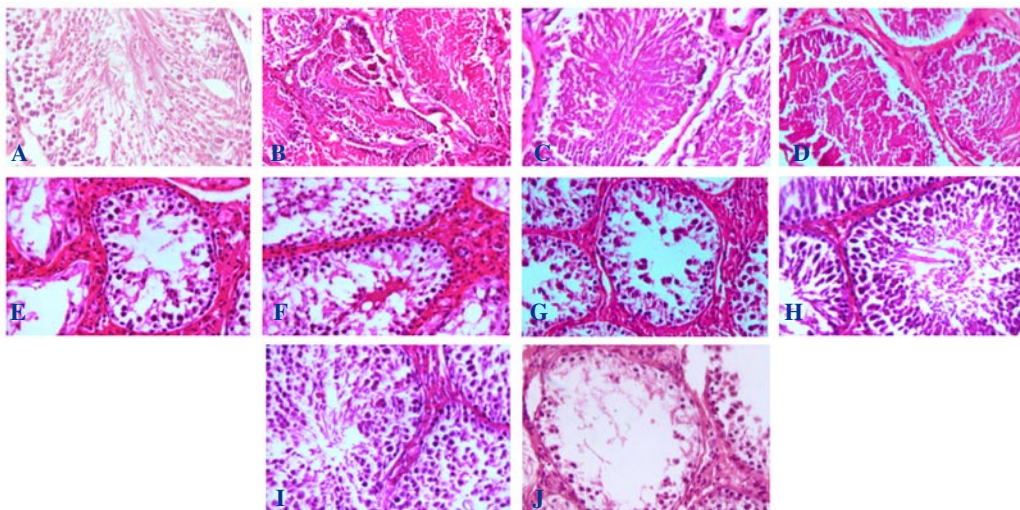
During post-natal development, after full term delivery of mated females with male rats of group A (vehicle injected control) following 30, 90 and 180 days of intratesticular injection, the observation on offspring, *viz.*, litter size, body weight, body length, body width and sex ratio was found within normal range. Normal external and skeletal morphology was noticed in  $F_1$  progeny delivered from cesarean and full-term females mated with group A (vehicle injected control). All mated females were found to be non-pregnant and therefore  $F_1$  progeny was not sired by animals of groups B, C and D.

### 3.6. Outcomes of histology of testis

The histology of testis of animals of group A showed round or oval seminiferous tubules with basal lamina and germinal epithelium. The epithelium contains Sertoli cells and germ cells, showing different stages of spermatogenesis. The spermatogonial and immature cells of the germ line rest upon the basement membrane. The tubular lumen contained mature spermatozoa. The round or oval Leydig cells with prominent nuclei were observed in the interstitial spaces between seminiferous tubules. Testicular histoarchitecture following intratesticular injection of zinc gluconate in group B revealed complete necrotic seminiferous tubules, resulting in unidentifiable germ cells and Sertoli cells. The Leydig cells in the interstitial spaces were completely disorganized with infiltration of leucocytes. In  $\text{CaCl}_2$  injected (group C) testis, calcium deposits were observed in both testicles. Following 30, 90 and 180 days of injection, multinucleated giant cells were occasionally visible; smaller seminiferous tubules containing only a few pyknotic germ cells and Sertoli cells were also observed. Spermatogenesis was arrested at the secondary spermatocyte stage, the lumen contained erupted germ



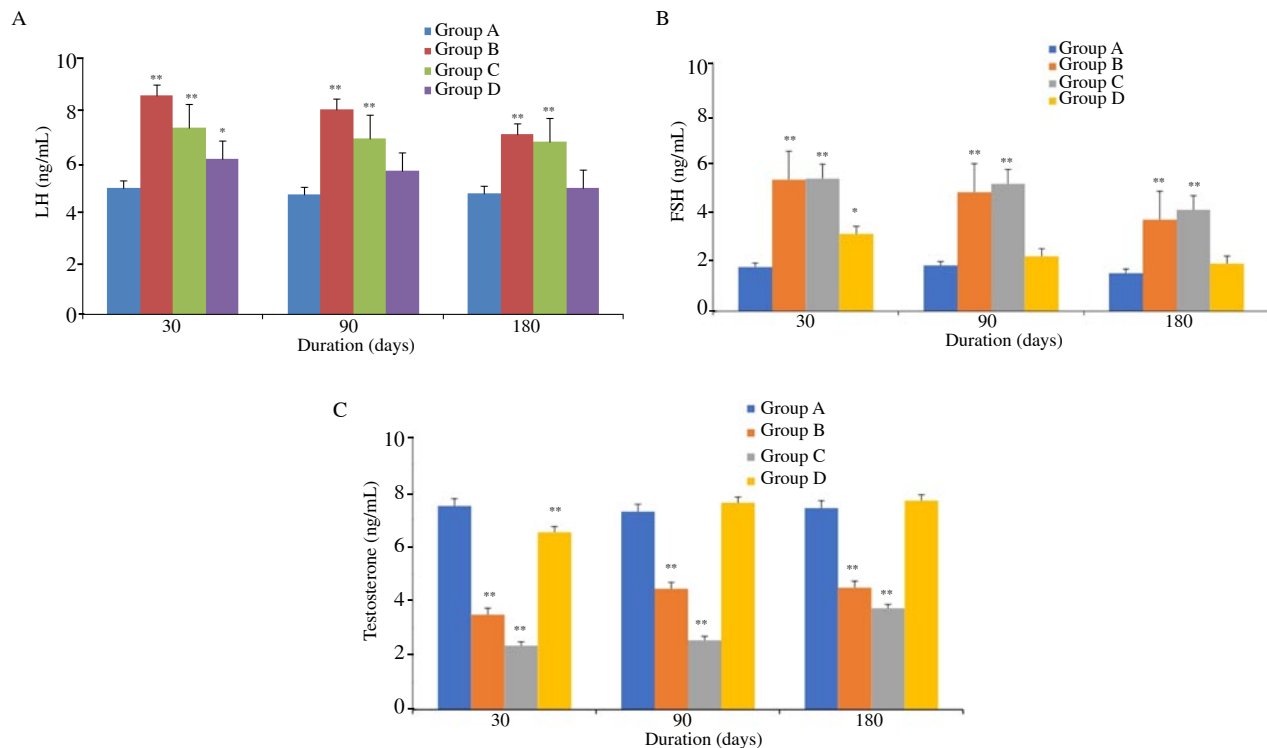
**Figure 1.** Scanning electron microscopy of cauda epididymal spermatozoa following 30, 90 and 180 days of intratesticular administration of cadmium chloride ( $\text{CdCl}_2$ ) plus ethylenediaminetetraacetic acid (EDTA) in male Wistar albino rats. A: Micrograph of the spermatozoa of the control group (group A) showing the distinct normal morphology of spermatozoa; B-I: Micrographs of spermatozoa following 30, 90 and 180 days treatment showing different abnormalities like damaged acrosome and ruptured membrane in head region, nuclear membrane degeneration, degenerated plasma membrane of tail, coiled tail and head tail-separation in group D ( $\text{CdCl}_2$  plus EDTA).



**Figure 2.** Histoarchitecture of the testis following 30, 90 and 180 days of intratesticular administration of zinc gluconate plus *L*-Arginine, calcium chloride ( $\text{CaCl}_2$ ), and cadmium chloride ( $\text{CdCl}_2$ ) plus ethylenediaminetetraacetic acid (EDTA) in male Wistar albino rats (H & E; magnification:  $\times 200$ ). A shows different stages of spermatogenesis in group A (control); B, C and D show complete necrosis of seminiferous tubules and completely disorganized cellular elements in group B (zinc gluconate plus *L*-Arginine) for 30, 90, 180 days, respectively; E, F, and G show severe tubular degeneration, pyknosis of germ cells, vacuolation, eruption and increased fibroblast elements in group C ( $\text{CaCl}_2$ ) for 30, 90, 180 days, respectively; H, I and J show degenerated spermatogenic cells, pyknotic nuclei, vacuolation and erupted germ cells in group D ( $\text{CdCl}_2$  plus EDTA) for 30, 90, 180 days, respectively.

cells and a thickening of lamina propria with increased fibroblast like elements in the interstitium was observed. Sparse Leydig cells were also present in the interstitium. In group D, following 30 days of intratesticular injection of  $\text{CdCl}_2$ , disorganized seminiferous epithelium with pyknotic germ cells and Sertoli cells were seen.

Seminiferous tubules contain erupted germ cells and widening of interstitium with shrinkage in size of Leydig cells with increased fibrotic elements was also seen. The infiltration of leucocytes in the interstitium was a characteristic feature of this group. Regeneration and normal appearance of Leydig cells were found. Following 90



**Figure 3.** Circulatory levels of leutenizing hormone (LH) (A), follicle stimulating hormone (FSH) (B) and testosterone (C) following intratesticular administration of zinc gluconate plus *L*-Arginine, calcium chloride ( $\text{CaCl}_2$ ), and cadmium chloride ( $\text{CdCl}_2$ ) plus ethylenediaminetetraacetic acid (EDTA) in male Wistar albino rats. \* $P < 0.01$ ; \*\* $P < 0.001$ : compared to the control group. Group A (the control group) receives 0.9% NaCl, isotonic physiological saline; Group B receives zinc gluconate plus *L*-Arginine; Group C receives  $\text{CaCl}_2$ ; Group D receives  $\text{CdCl}_2$  plus EDTA.

and 180 days of injection, the histoarchitecture of seminiferous tubules was more or less similar to that observed following 30 days of injection. However, increased vacuolization in the seminiferous tubules was observed (Figure 2).

### 3.7. Outcomes of hormone analyses

The LH and FSH levels were significantly elevated ( $P < 0.001$ ) in groups B and C compared to group A during the entire treatment schedule. Compared to group A, both LH and FSH levels in group D also increased significantly ( $P < 0.01$ ) at 30 days post-injection but normalized at 90 and 180 days, aligning with vehicle-injected controls. The circulatory levels of testosterone were markedly declined ( $P < 0.001$ ) in groups B and C in comparison to group A all through the experimental schedules. After 30 days of intratesticular injection in group D, the testosterone levels were significantly decreased ( $P < 0.01$ ) compared to group A, while following 90 and 180 days, the concentration of testosterone levels was returned to normal when compared with group A (Figure 3).

## 4. Discussion

Chemical sterilization is the most emerging method of male contraception for controlling domestic and stray animal populations.

Over the last several decades, researchers have investigated distinct chemical castration methods in different animal models to explore non-invasive approaches for managing their overpopulation and to ascertain their potential as a non-invasive means of contraception. Present research endeavours to compare the contraceptive efficacy of three chemical sterilizing methods in male rats. It juxtaposes FDA-endorsed formulation - zinc gluconate and calcium chloride, against cadmium chloride, an emergent contender for male sterilization, examining seminal and other parameters post intra-testicular administration. The findings of the present study could advance reproductive control strategies, considering both investigative and practical implications for mitigating overpopulation dynamics among animal cohorts, with ethical considerations regarding animal welfare and long-term ramifications[9]. Zinc gluconate and  $\text{CaCl}_2$  have undergone protracted investigations as chemical sterilants administered intratesticularly. Their mechanisms of action involve the induction of male infertility through testicular coagulation and necrosis, culminating in cellular demise[18]. Zinc gluconate has been removed from the market and  $\text{CaCl}_2$  has not garnered the approval from any regulatory agency for the intended purpose. Moreover,  $\text{CdCl}_2$  has been explored as a male contraceptive due to its antifertility effects, inducing testicular toxicity resulting in seminiferous tubule occlusion and Leydig cell damage[19]. However, co-administration with the chelating agent-EDTA can mitigate its toxicity by binding and neutralizing cadmium ions, preventing generalized tissue damage. Other chelating agents such as

dimercaptosuccinic acid (DMSA), 2,3-dimercapto-1-propanesulfonic acid (DMPS), diethylenetriaminepentaacetic acid (DTPA), and British anti-Lewisite (BAL) facilitate the urinary excretion of heavy metals like cadmium but EDTA is commonly preferred because of its availability, affordability and safety, as it can form stable complexes with cadmium to reduce free ion release and subsequent toxicity. The rationale lies in the mechanism of chelation therapy, where chelating agents effectively mobilize toxic metals mainly into urine, forming stable complexes that shield biological targets by reducing local toxicity[11]. Intratesticular injection stands out as a method where the chemosterilant is administered directly to the target organ, ensuring a heightened local concentration while minimizing systemic exposure. This mechanism contributes to the prompt and dependable antifertility outcomes attained with comparatively modest doses that seem to lack discernible clinical toxicity.

Summarily, the results found in the present study were convincingly demonstrated that a single intratesticular injection has the ability to induce consistent and prolonged contraception in male rats. At 180 days schedule, infertility was persistent with all three of the drugs that were evaluated and extensive damage to the testicular tissue and irreversible azoospermia were observed. Based on comprehensive clinical, hematological, biochemical and histological evaluations, there was evidence of severe clinical toxicity or adverse consequences associated with zinc gluconate and  $\text{CaCl}_2$ [9]. However,  $\text{CdCl}_2$  with EDTA chelating agent could show desired effects with some partial toxicological effects[11]. In this study, effects of zinc gluconate and  $\text{CaCl}_2$  were observed to be rather immediate with one hundred percent of infertility being induced during the first month after treatment. Even though  $\text{CdCl}_2$  had a more gradual onset, its effects remained until the end of the trial on day 180. When compared to the other chemosterilants that were studied,  $\text{CdCl}_2$  appeared to provide the best possible combination of efficacy, safety and intensity. The data offer compelling evidence that the use of intratesticular chemical sterilization for the purpose of non-surgical male fertility control in dogs and other animals is feasible.

In the current investigation, intratesticular administration of zinc gluconate and  $\text{CaCl}_2$  elicited massive scrotal swelling due to testicular edema, culminating in complete testicular necrosis within 24 to 48 hours post-injection, which subsequently resolved over a 3-week period. Conversely, animals injected with  $\text{CdCl}_2$  experienced scrotal burning sensations, though these sensations subsided within 2-3 days. Previous research has documented the pronounced necrotic effects induced by zinc gluconate, leading to the replacement of seminiferous tubules with fibrous scar tissue. This process impairs both sperm production and transport, with fibrosis onset occurring 2-3 days following zinc gluconate administration and progressing over the course of several weeks[20]. Previously studied doses of zinc gluconate can exacerbate the extent of coagulative necrosis within the seminiferous tubules. This form of irreversible cell death manifests within a 24-hour timeframe and advances over several days, ultimately encompassing the entire depth of the seminiferous

epithelium. Concurrently, interstitial edema occurs alongside the loss of germ cells, with Sertoli cells engaging in the phagocytosis of cellular debris as part of the necrotic process[21].  $\text{CaCl}_2$  induces rapid coagulative necrosis in testicular tissue within hours of injection, progressing irreversibly over several days. This necrosis is followed by interstitial fibrosis and hormonal shifts that impair spermatogenesis. Azoospermia can ensue within 1-2 months due to extensive tissue damage and the replacement of seminiferous tubules with scar tissue. Additionally, necrotic and fibrotic changes disrupt essential paracrine signaling, which is crucial for sperm production, among Sertoli, Leydig, and germ cells[22]. Intratesticular administration of  $\text{CdCl}_2$  triggers leukocyte infiltration and cytokine production, leading to testicular degeneration and edema. Cadmium also induces endothelial damage and micro vascular thrombosis, restricting testicular blood flow and fostering hypoxic injury[23]. Even at low doses, cadmium has been observed to elicit prolonged infertility by compromising the functions of Sertoli and Leydig cells[24].

In the present study, complete sterility was achieved in 100% of the rats treated with zinc gluconate,  $\text{CaCl}_2$  and  $\text{CdCl}_2$ . Successful mating was noted in the control and  $\text{CdCl}_2$  treated rats, as evidenced by vaginal plugs, but no trace of mating was noticed in zinc gluconate and  $\text{CaCl}_2$  treatment groups due to the loss of libido. However, none of the cohabited females conceived to any treated males and pregnancy was recorded only in cohabited females of the control group. Earlier studies have also reported definitive sterilizing effects of these metallic solutions following single bilateral intratesticular injection[14,25]. Interestingly, single unilateral intratesticular injection of  $\text{CdCl}_2$  induced permanent sterility in 100% rats even at a very low dose of 0.5 mg/kg body weight. Testicular degeneration without any regeneration was conspicuous even after 180 days[26].

The sperm concentration, motility, viability and morphology provide vital information regarding testicular damage following exposure to reproductive toxicants[27]. In the present study, azoospermia was achieved in zinc gluconate and  $\text{CaCl}_2$  treated rats while few spermatozoa were still evident in  $\text{CdCl}_2$  treated rats. Dearth of advanced germ cells in the damaged seminiferous tubules could account for the depletion of sperm reserve in the cauda epididymis. Other studies have also reported dose and duration dependent decrease in cauda epididymal sperm concentration after chemosterilization using various formulations of zinc and  $\text{CaCl}_2$ [28]. However, in  $\text{CdCl}_2$  injected rats, non-motile spermatozoa were observed on day 30. Additionally, there was a significant decrease in initial viability, which persisted from day 30 onwards, leading to the presence of only dead spermatozoa in this group by day 180. Furthermore, an increase in spermatozoa abnormality was noted progressively throughout the experimental procedure. Earlier researchers have also reported a significant decline in sperm motility and viability following the administration of cadmium and other chemicals[29]. Ultrastructural observations of spermatozoa revealed intense pathological changes in the acrosome, nucleus, mitochondria and tail region of epididymal spermatozoa in cadmium

chloride injected rats. Disruption of the head cap and swollen or rudimentary acrosome were conspicuous in treated rats. Decapitation was also frequently noticed with the extrusion of abnormal chromatin material. The mid piece and principal piece also exhibited structural disorganization. Excessive loss and degeneration of germ cells could be responsible for release of defective spermatids which subsequently impart structural anomalies in the maturing epididymal spermatozoa[30].

The weights of the testis and other accessory organs were drastically reduced in all treated rats in this study[31]. The atrophic changes were discernible right from day 30 and continued till the end of the experiment. The degenerative changes were less pronounced in the CdCl<sub>2</sub> treated rats followed by CaCl<sub>2</sub> and zinc gluconate. Earlier reports on intratesticular administration of chemical sterilants revealed the marked regression of reproductive organs[26,32]. The depletion of germ cells is known to cause atrophy of epididymis and accessory sex glands due to the decreased availability of testosterone. Histoarchitectural studies of testis of zinc gluconate and CaCl<sub>2</sub> injected groups reported the severe necrosis of germ cells in present study. The complete coagulation necrosis destroys the normal microanatomy of seminiferous tubules and interstitium, germ cells are lost and Sertoli cells are damaged. By the end of experiment, all seminiferous tubules were completely fibrous leaving no scope for regeneration in these groups. The degenerative changes observed in this study corroborates well with earlier reports on testicular damage induced by heavy metal toxicity[5,32] and their mechanism of treatment can also be supported by various studies. The blood-testis barrier separates the ad luminal compartment of the seminiferous tubules from the interstitial space and systemic circulation. It is formed by tight junctions between Sertoli cells. Zinc gluconate administration leads to structural disorganization of Sertoli cell tight junctions, causing increased permeability of the blood-testis barrier. This allows immune cells and antibodies to access the ad luminal compartment which can trigger an autoimmune response against sperm cells[33]. Zinc gluconate administration results in increased germ cell apoptosis, particularly affecting spermatocytes and spermatids. This is likely due to increased oxidative stress and disruption of normal zinc homeostasis[34]. Calcium ions interact with phospholipid components of cell membranes, altering their permeability. This allows intracellular contents to leak out. High calcium concentrations denature proteins and cause them to aggregate into insoluble precipitates that coagulate in the cytoplasm[35]. Calcium overload in cells disrupts adenosine triphosphate (ATP) production *via* effects on mitochondria and due to lack of ATP; this prevents energy-dependent cell processes[36]. The intratesticular administration of CaCl<sub>2</sub> results in precipitation of intravascular proteins which can increase vascular permeability and impair local blood flow, ultimately leading to ischemia as a result of obstruction by the coagulated blood[37]. After unilateral intratesticular administration of CdCl<sub>2</sub>, histological observations revealed a disorganized seminiferous epithelium, pyknosis and germ cell eruption in our study. Cadmium targets Sertoli cells by

disrupting Sertoli cell tight junctions and the cytoskeleton, impairing their ability to support developing germ cells. This causes sloughing of immature germ cells which accumulate and obstruct the tubular lumen[38]. CdCl<sub>2</sub> administration damages Leydig cells in the testicular interstitium. This disrupts the production of testosterone which is essential for spermatogenesis. Cadmium also induces reactive oxygen species (ROS) production which damages sperm membrane lipids, proteins and DNA. This contributes to increased germ cell apoptosis and necrosis[24]. The imbalance between endogenous and exogenous ROS following Cr and As intoxication can trigger lipid peroxidation, activate antioxidant defenses and potentially cause oxidative damage to tissues[39–41].

The present study demonstrated significantly reduced testosterone levels in all treated groups, accompanied by compensatory increases in LH and FSH, reflecting negative feedback from the hypothalamic-pituitary-gonadal (HPG) axis due to diminished androgen levels. Intratesticular administration of chemicals disrupted HPG axis function by suppressing GnRH pulses and impairing LH/FSH release, further aggravating testosterone deficiency. Additionally, direct cytotoxic effects on Leydig and Sertoli cells, along with associated necrosis and fibrosis, disrupted the paracrine signaling crucial for spermatogenesis. This hormonal disruption inhibited meiosis and sperm maturation[42]. The most pronounced effects were observed in the zinc gluconate and CaCl<sub>2</sub> groups, with elevated FSH levels indicating compromised Sertoli cell function. In contrast, CdCl<sub>2</sub> caused milder hormonal alterations, likely due to lower cytotoxicity to Leydig cells. Persistent hormonal imbalances up to 180 days highlight the long-term endocrine impact of zinc gluconate and CaCl<sub>2</sub>.

The combined impact of these mechanisms is the cessation of spermatogenesis, which is likely to achieve a state of azoo/oligospermia. The intratesticular administration of chemosterilants causes seminiferous tubule disruption, Leydig cell injury, oxidative stress, inflammation and vascular damage that conspire to arrest sperm production and maturation. The net result is a complete blockade or substantive reduction in sperm output coupled with testosterone deficiency that induces infertility.

The present study has several limitations. Despite demonstrating effective chemical sterilization in male rats, both zinc gluconate and CaCl<sub>2</sub> caused severe local reactions including scrotal swelling and irreversible testicular necrosis. Additionally, loss of libido and reduced testosterone levels were observed, which impacts animal behaviour, raising ethical concerns. Although CdCl<sub>2</sub> combined with EDTA appeared less toxic, it still induced testicular degeneration and partial systemic effects. Regulatory challenges also exist, as none of these agents are currently approved for this application in any animals. The results are species-specific and may not be directly extrapolated to other species. Further research is necessary to assess long-term safety and cross-species efficacy. While the 180-day study duration yielded significant findings but it does not address potential lifelong consequences such as systemic toxicity, altered behaviour or chronic reproductive tract pathology. Moreover, the irreversible

nature of the induced sterility may not be suitable in contexts where temporary contraception is desired.

In conclusion, the comparative study of intratesticular injections of zinc gluconate, CaCl<sub>2</sub> and CdCl<sub>2</sub> in male rats for a period of 180 days revealed that zinc gluconate and CaCl<sub>2</sub> injections severely affected the reproductive organs and libido and CdCl<sub>2</sub> exhibited diminished spermatogenesis with normal libido. Thus, any intratesticularly administered agent may be used as per requirements for the purpose of sterilization. Further studies on long-term reversibility, optimal dosage, delivery systems and field trials in stray dogs can help identify the most suitable candidate of chemosterilant for humane and effective fertility control.

### Conflict of interest statement

All authors declare no significant competing interests.

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### Authors' contributions

Abdul Salam Ansari designed the study. Timanshi Chansoriya and Barkha Khilwani conducted the experiment, analyzed data and drafted the manuscript. Abdul Salam Ansari and Nirmal Kumar Lohiya finally approved the final version to be published. All authors read and approved the final manuscript.

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### References

- [1] HYPERLINK "http://www.dogster.com" www.dogster.com. (2025). *How many dogs are there? US & worldwide statistics 2025*. Dogster/Pangolia Pte. Ltd. [Online] Available from: <https://www.dogster.com/statistics/how-many-dogs-are-there-statistics> [Accessed 20th February 2025].
- [2] National Geographic. *The new cool: Adopting street dogs is gaining popularity in India* (2020). [Online] Available from: <https://www.nationalgeographic.com/animals/2020/04/street-dogs-indies-india-pets/>. [Accessed 27th February 2025].
- [3] Hughes J, Macdonald DW. A review of the interactions between free-roaming domestic dogs and wildlife. *Biol Conserv* 2013; **157**: 341-351. doi: 10.1016/j.biocon.2012.07.005.
- [4] Abdulkarim A, Khan MAKBG, Aklilu E. Stray animal population control: Methods, public health concern, ethics, and animal welfare issues. *World Vet J* 2021; **11**(3): 319-326. doi: 10.54203/scil.2021.wvj44.
- [5] Kumar R, Soni N, Kumar S, Pandey AK. Chemical control of fertility in male dogs: A review. *Int J Curr Microbiol Appl Sci* 2018; **7**(6): 1760-1773. doi: 10.20546/ijcmas.2018.707.209.
- [6] Chang AM, Chen CC, Lee JW, Hou DL, Huang HH, Ke GM. Effects of a novel recombinant gonadotropin-releasing hormone-1 vaccine on the reproductive function of mixed-breed dogs (*Canis familiaris*) in Taiwan. *Vaccine* 2023; **41**(13): 2214-2223. doi: 10.1016/j.vaccine.2023.02.061.
- [7] Mevel V, Berthevas C, Briand-Amirat L, Dordas-Perpinya M, Nguyen F, Bruyas JF. Effect of anti-GnRH vaccine on testicular tissues in stallions. *J Equine Vet Sci* 2023; **125**: 104600. doi: 10.1016/j.jevs.2023.104600.
- [8] Ochoa JS, Favre RN, García MF, Stornelli MC, Sangache WC, Rearte R, et al. Immunocontraception of male domestic cats using GnRH vaccine Improvac. *Theriogenology* 2023; **198**: 211-216. doi: 10.1016/j.theriogenology.2022.12.020.
- [9] Alliance for Contraception in Cats & Dogs (ACC&D). (2022). *What's available now for dogs and cats?* [Online] Available from: <https://www.acc-d.org/products> [Accessed 12th January 2025].
- [10] Novak S, Yakobson B, Sorek S, Morgan L, Tal S, Nivy R, et al. Short term safety, immunogenicity, and reproductive effects of combined vaccination with anti-GnRH (Gonacon) and rabies vaccines in female feral cats. *Front Vet Sci* 2021; **8**: 650291. doi: 10.3389/fvets.2021.650291.
- [11] Flora SJ, Pachauri V. Chelation in metal intoxication. *Int J Environ Res Public Health* 2010; **7**(7): 2745-2788. doi: 10.3390/ijerph7072745.
- [12] Asa CS. Contraception in dogs and cats. *Vet Clin North Am Small Anim Pract* 2018; **48**(4): 733-742. doi: 10.1016/j.cvsm.2018.02.014.
- [13] CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). *Guidelines on the regulation of scientific experiments on animals*. Ministry of Environment and Forests, Animal Welfare Division, Government of India. 2010. [Online] Available from: [https://ccsea.gov.in/Content/54\\_1\\_ACTS,RULESANDGUIDELINES](https://ccsea.gov.in/Content/54_1_ACTS,RULESANDGUIDELINES) [Accessed 28th January 2025].
- [14] Tepsu Methanon V, Wilde H, Hemachudha T. Intratesticular injection of a balanced zinc solution for permanent sterilization of dogs. *J Med Assoc Thai* 2005; **88**(5): 686-689. doi: 10.1016/0264-410x(91)90186-a.
- [15] Jana K, Samanta PK. Sterilization of male stray dogs with a

- single intratesticular injection of calcium chloride: A dose-dependent study. *Contraception* 2007; **75**(5): 390-400. doi:10.1016/j.contraception.2007.01.022.
- [16] World Health Organization. *WHO laboratory manual for the examination and processing of human semen (5th ed.)*. Geneva, Switzerland: WHO Press; 2010. <https://iris.who.int/handle/10665/44261>.
- [17] Derelanko MJ, Auletta CS. *Handbook of toxicology*. 3rd ed. Boca Raton: CRC Press; 2014. doi: 10.1201/b16632.
- [18] Nakagawa Y, Maeda A, Takahashi T, Kaneoka Y. Gastric necrosis because of ingestion of calcium chloride. *ACG Case Rep J* 2020; **7**(8): e00446. doi: 10.14309/crj.0000000000000446.
- [19] Zhang Q, Zou P, Zhan H, Zhang M, Zhang L, Ge RS, et al. Dihydroipoamide dehydrogenase and cAMP are associated with cadmium-mediated Leydig cell damage. *Toxicol Lett* 2011; **205**(2): 183-189. doi: 10.1016/j.toxlet.2011.06.003.
- [20] Moreno D, Sobarzo CM, Lustig L, Peña MGR, Guazzone VA. Effect of ketotifen fumarate on experimental autoimmune orchitis and torsion of the spermatic cord. *Asian J Androl* 2020; **22**(1): 112-117. doi: 10.4103/aja.aja\_30\_19.
- [21] Shojaeepour S, Dabiri S, Dabiri B, Imani M, Abadi MFS, Hashemi F. Histopathological findings of testicular tissue following cadmium toxicity in rats. *Iranian J Pathol* 2021; **16**(4): 348. doi: 10.30699/ijp.2021.130581.2443.
- [22] Alkandari MH, Zini A. Medical management of non-obstructive azoospermia: A systematic review. *Arab J Urol* 2021; **19**(3): 215-220. doi: 10.1080/2090598X.2021.1956233.
- [23] Azenabor A, Ekun AO, Akinloye O. Impact of inflammation on male reproductive tract. *J Reprod Infertil* 2015; **16**(3): 123-129.
- [24] Ali W, Ma Y, Zhu J, Zou H, Liu Z. Mechanisms of cadmium-induced testicular injury: A risk to male fertility. *Cells* 2022; **11**(22): 3601. doi: 10.3390/cells11223601.
- [25] Leoci R, Aiudi G, Silvestre F, Lissner EA, Marino F, Lacalandra GM. 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* 2014; **56**: 1-8. doi: 10.1186/s13028-014-0063-1.
- [26] Nad P, Massanyi P, Skalicka M, Korenekova B, Cigankova V, Almasiova V. The effect of cadmium in combination with zinc and selenium on ovarian structure in Japanese quails. *J Environ Sci Heal A* 2007; **42**(13): 2017-2022. doi: 10.1080/10934520701629716.
- [27] Creasy D, Bube A, Rijk ED, Kandori H, Kuwahara M, Masson R, et al. Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol Pathol* 2012; **40**(6 Suppl): 40S-121S. doi: 10.1177/0192623312454337.
- [28] Soto FRM, Viana WG, Mucciolo GCB, Hosomi FYM, Vannucchi CI, Mazzei, et al. Evaluation of efficacy and safety of zinc gluconate associated with dimethyl sulphoxide for sexually mature canine males chemical neutering. *Reprod Domest Anim* 2009; **44**(6): 927-931. doi: 10.1111/j.1439-0531.2008.01119.x.
- [29] Qadori YT, Al-shaikh MN. Effects of high and low dose of cadmium chloride on male reproductive system in mice. *J Fac Med Baghdad* 2012; **54**(1): 110-114. doi: 10.32007/jfacmedbagdad.541787.
- [30] Adamkovicova M, Toman R, Martiniakova M, Omelka R, Babosova R, Krajcovicova V, et al. Sperm motility and morphology changes in rats exposed to cadmium and diazinon. *Reprod Biol Endocrinol* 2016; **14**(42): 1-7. doi: 10.1186/s12958-016-0177-6.
- [31] Karmakar SN, Das SK. Chemosterilization induced by intratesticular injection of calcium chloride (CaCl<sub>2</sub>) - A tool for population control. *Int J Pharm Chem Biol Sci* 2017; **7**(1): 25-35.
- [32] Oliveira ECS, Moura MRP, de Sá MJC, Junior VAS, Kastelic JP, Douglas RH, et al. Permanent contraception of dogs induced with intratesticular injection of a zinc gluconate-based solution. *Theriogenology* 2012; **77**(6): 1056-1063. doi: 10.1016/j.theriogenology.2011.10.008.
- [33] Kang K, Ma YD, Liu SQ, Huang RW, Chen JJ, An LL, et al. SARS-CoV-2 structural proteins modulated blood-testis barrier-related proteins through autophagy in the primary Sertoli cells. *Viruses* 2023; **15**(6): 1272. doi: 10.3390/v15061272.
- [34] Farzaneh M, Mokhtari S, Moraveji SF, Sayahpour FA, Masoudi NS, Javadi A, et al. *In vitro* investigation of zinc oxide nanoparticle toxic effects in spermatogonial cells at the molecular level. *Chem Biol Interact* 2022; **351**: 109687. doi: 10.1016/j.cbi.2021.109687.
- [35] Sciacca MF, La Rosa C, Milardi D. Amyloid-mediated mechanisms of membrane disruption. *Biophysica* 2021; **1**(2): 137-156. doi: 10.3390/biophysica1020011.
- [36] Walkon LL, Strubbe-Rivera JO, Bazil JN. Calcium overload and mitochondrial metabolism. *Biomolecules* 2022; **12**(12): 1891. doi: 10.3390/biom12121891.
- [37] Bigdelou P, Vahedi A, Kiosidou E, Farnoud AM. Loss of membrane asymmetry alters the interactions of erythrocytes with engineered silica nanoparticles. *Biointerphases* 2020; **15**(4): 041001. doi: 10.1116/6.0000246.
- [38] Jensen CFS, Wang D, Mamsen LS, Giwercman A, Jørgensen N, Fode M, et al. Sertoli and germ cells within atrophic seminiferous tubules of men with non-obstructive azoospermia. *Front Endocrinol (Lausanne)* 2022; **13**: 825904. doi: 10.3389/fendo.2022.825904.
- [39] Singh G, Thaker R, Sharma A, Parmar D. Therapeutic effects of biochanin A, phloretin, and epigallocatechin-3-gallate in reducing oxidative stress in arsenic-intoxicated mice. *Environ Sci Pollut Res* 2021; **28**: 20517-20536. doi:10.1007/s11356-020-11740-w.
- [40] Tripathi S, Parmar D, Raval S, Mishra R, Singh G. Attenuation of chromium (VI) and arsenic (III)-induced oxidative stress and hepatic apoptosis by phloretin, biochanin-A, and coenzyme Q10 via activation of SIRT1/Nrf2/HO-1/NQO1 signaling. *J Biochem Mol Toxicol* 2024; **38**(9): e23817. doi: 10.1002/jbt.23817.
- [41] Tripathi S, Kharkwal G, Mishra R, Singh G. Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling in heavy metals-induced oxidative stress. *Heliyon* 2024; **10**(18): e37545. doi: 10.1016/j.heliyon.2024.e37545.
- [42] Plunk EC, Richards SM. Endocrine-disrupting air pollutants and their effects on the hypothalamus-pituitary-gonadal axis. *Int J Mol Sci* 2020; **21**(23): 9191. doi: 10.3390/ijms21239191.