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RESEARCH ARTICLE

Effect of Chemical Castration Using High Osmolarity Solutions on Spermatological Parameters in Rats

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ABSTRACT

In study, the changes in spermatological parameters in rats subjected to chemical castration with high osmolarity solutions were investigated. In study, 8 male rats were used in each group. No chemicals were applied to rats in control group. Rats in second group were sterilized surgically. 0.9% physiological saline was administered to 3rd group, 0.9% physiological saline and 10% calcium chloride to 4th group, 20% mannitol to 5th group, 20% mannitol and 10% calcium chloride to 6th group, 20% dextrose to 7th group, 20% dextrose and 10% calcium chloride solutions were administered intratesticularly to 8th group at dose of 0.1 ml/100gr. Andrological findings compared to control group; it was determined that there was a significant difference in sperm motility and sperm density ($p<0.001$) in groups 3, 5, 7. However, it was observed that there was no difference in rate of abnormal spermatozoon. In groups 2, 4, 6, 8, motility, density, abnormal sperm count and epididymis weights could not be measured because sperm cells could not be collected. In addition, it was determined that the weights of epididymis and right cauda epididymis in groups 3, 5, 7 decreased compared to control ($p<0.05$), while in groups 2, 4, 6, 8, measurements could not be made because tissue samples could not be taken, and weights of testicles, vesicle seminalis and prostate decreased significantly ($p<0.001$) compared to control. In conclusion; it has been concluded that giving high osmolarity solutions in combination with calcium chloride will be much more effective in intratesticular chemical sterilization process.

Key Words: Calcium chloride, Chemical castration, Rat, Spermatological parameters.

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Ratlarda Yüksek Osmolariteli Solüsyonlar Kullanılarak Yapılan Kimyasal Kastrasyonun Spermatolojik Parametrelere Etkisi

ÖZ

Çalışmada, yüksek osmolariteli solüsyonlarla kimyasal kısırlaştırma oluşturulan ratlarda spermatolojik parametrelerin değişimi araştırılmıştır. Çalışmada, her grupta 8 erkek rat kullanıldı. Kontrol grubundaki ratlara herhangi bir kimyasal uygulanmadı. 2. gruptaki ratlar cerrahi olarak kısırlaştırıldı. 3. gruba % 0.9'luk serum fizyolojik, 4. gruba %0.9'luk serum fizyolojik ile %10 kalsiyum klorür, 5. gruba %20 mannitol, 6. gruba %20 mannitol ile %10 kalsiyum klorür, 7. gruba %20 dekstroz, 8. gruba %20 dekstroz ile %10 kalsiyum klorür solüsyonları 0.1ml/100 gr dozda intratestiküler uygulandı. Androlojik bulgular kontrol grubuyla kıyaslandığında; 3, 5, 7. gruplarda sperm motilitesi ve sperma yoğunluğunda ($p<0,001$) önemli derecede farklılık olduğu belirlendi. Ancak anormal spermatozoon oranında fark olmadığı görüldü. 2, 4, 6, 8. gruplarında ise sperm hücresi alınamadığından motilite, yoğunluk, anormal sperm sayısı ve epididimis ağırlıkları ölçümü yapılamadı. Ayrıca 3, 5, 7 gruplarında epididimis ve sağ kauda epididimis ağırlıklarının kontrole göre ($p<0,05$) düştüğü, 2, 4, 6, 8 gruplarında ise doku örneği alınamadığından ölçüm yapılamadığı ve testis, vezikula seminalis, prostat ağırlıklarının ise kontrole göre önemli derecede ($p<0,001$) düştüğü belirlendi. Sonuç olarak; testis içi yapılan kimyasal kısırlaştırma işleminde, yüksek osmolariteli solüsyonların kalsiyum klorür ile kombine olarak verilmesinin çok daha etkili olacağı kanaatine varılmıştır.

Anahtar Kelimeler: Kalsiyum klorür, Kimyasal kastrasyon, Rat, Spermatolojik parametreler.

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INTRODUCTION

In male animals, the termination of reproduction through the partial or complete removal of the testes from the body, or the irreversible or reversible cessation of sexual activity, is referred to as castration, sterilization, orchiectomy, vasectomy, neutering, or emasculation. Castration is commonly performed not only to eliminate reproductive capacity but also to prevent behavioral issues such as urination to mark territory indoors due to pheromone scent marking, or escaping to mate. It is also used to tame working animals, increase productivity in livestock, prevent animals with low fertility from being used as breeders, curb the spread of infectious diseases, and treat deep and complicated testicular injuries, tumors, or various genital disorders (Samsar, 1978; Doğan et al., 2015; Baran et al., 2016).

Animals can be sterilized using open or closed surgical procedures, depending on the species. In veterinary medicine, operative castration is one of the most frequently used methods to prevent reproduction. Although sterilization applies to both male and female animals, castrating males is considered more effective in preventing the fertilization of females. However, surgical castration in male animals may result in various negative effects. Since surgical castration, commonly applied to prevent reproduction and promote fattening in livestock, carries a high risk of complications, there is a growing need to develop alternative methods today (Turk and Ataman, 2016).

From traditional surgical sterilization to present-day practices, there has been a continuous effort to develop new methods to meet the growing need for effective sterilization. In male dogs and cats, non-surgical sterilization techniques such as immunocontraception, suppression of endogenous steroid hormone levels, intratesticular, intraepididymal, and intravas deferens chemical sterilant injections, reproductive toxins, and non-invasive mechanical methods are generally used (Baran et al., 2016).

Commonly used chemicals for chemical sterilization in domestic animals include calcium chloride, lactic acid, sodium chloride, chlorhexidine, formalin, zinc tannate, zinc gluconate, glycerol, glucose, ethanol, and silver nitrate (Başa and Canpolat, 2019). Today, chemical sterilization is increasingly replacing surgical sterilization. In chemical sterilization, drugs are administered directly into the testis and epididymis. These drugs are used intratesticularly to disrupt the structure of testicular tissue and induce atrophy. One of the advantages of intratesticular chemical sterilization is that it results in permanent loss of fertility. It also helps eliminate unwanted sexual behaviors. Chemical castration is considered one of the practical methods that can be performed with a single injection, making it both economical and cost-effective. These solutions can be produced on a large scale and easily applied in the field (Baran et al., 2016).

In recent years, research has continued to seek ideal chemical agents for effective sterilization. This study aims to achieve irreversible chemical sterilization using high-osmolality solutions that are inexpensive and easily accessible. Moreover, due to its ability to be applied to a large number of animals in a short period with minimal financial resources, chemical sterilization presents a strong alternative to surgical methods.

In our study, we aimed to investigate how spermatological parameters change in rats sterilized using low-cost, high-osmolality solutions without the need for surgical intervention. We believe that our research will contribute valuable data to the existing literature.

MATERIALS and METHODS

Research and Publication Ethics

This study was conducted by the decision of the Firat University Animal Experiments Local Ethics Committee dated 23/12/20200 and numbered 202/15, and was deemed appropriate.

Creation of Groups

The study was conducted on 64 Wistar male rats (5-6 months old, 350 g - 450 g) at the Firat University Experimental Animal Center. The rats were kept in plastic cages at 25 ± 2 °C, 55% ± 10% relative humidity, 12 hours of light and 12 hours of darkness, and daily feed (ready pellets) and water were given as ad libitum during the experiment. After a 15-day acclimation period, the animals were divided into 8 groups (8 rats in each group).

The first group was divided into groups as control group, 2nd surgical castration group, 3rd group as intratesticular 0.9% saline injection group, 4th group as intratesticular 0.9% saline and 10% calcium chloride injection group, 5th group as intratesticular 20% mannitol injection group, 6th group as intratesticular 20% mannitol and 10% calcium chloride injection group, 7th group as intratesticular 20% dextrose injection, 8th group as intratesticular 20% dextrose and 10% calcium chloride injection group (Table 1).

Table 1. Table Showing Subject Groups

Groups and Animal Numbers	Applications
Group 1 (8 subjects)	Control group
Group 2 (8 subjects)	Surgical castration
Group 3 (8 subjects)	Intratesticular 0.9% saline injection
Group 4 (8 subjects)	Intratesticular 0.9% saline injection with 10% calcium chloride
Group 5 (8 subjects)	Intratesticular 20% mannitol injection
Group 6 (8 subjects)	Intratesticular 20% mannitol injection with 10% calcium chloride
Group 7 (8 subjects)	Intratesticular 20% dextrose injection
Group 8 (8 subjects)	Intratesticular 20% dextrose injection with 10% calcium chloride

No procedure was applied to the rats in the 1st group used in the study. The rats in the second group of the

study were given general anesthesia with intraperitoneal 5 mg/kg xylazine (Xylazinbio 2%, Ivanovice na Hane, Czech Republic) and 90 mg/kg ketamine (Keta-Control, Istanbul, Turkey). After shaving and disinfecting the testicles, an incision was made in the scrotum and the testicles and spermatic cord were exposed. The spermatic cord was double ligated with 3-0 dexon thread and cut, and the testicles were removed. The scrotum was closed by suturing with 3-0 silk thread. The same procedures were repeated for the other testicle.

After 10 minutes of 5mg/kg xylazine i.m injection to rats in study groups 3, 4, 5, 6, 7, 8, the scrotum was wiped with iodine solution and then 0.1 ml of solutions per 100 g body weight were injected into the testicle in multiple directions with a 27 gauge needle. For study groups 4, 6, 8; 1 gr. calcium chloride (Calcium Chloride Dihyrate 97%, Eschau, Germany) was sterilized at 160 ° C for two hours, then mixed separately with 10 milliliters of sterile 20% mannitol (ready-made medical product, Polifarma, Tekirdağ, Turkey), 20% dextrose (ready-made medical product, Polifarma, Tekirdağ, Turkey) and 0.9% physiological serum (ready-made medical product, Polifarma, Tekirdağ, Turkey) solutions to obtain the desired dose.

The testicles of all rats were evaluated daily for the first week and weekly thereafter for dermatitis, scrotal swelling, discharge, fistula and pain on palpation. In the surgical castration group (group 2), animals were administered 0.1 ml sultamicillin (Sulcid, Istanbul, Turkey) via gavage for 3 days, the operation area was cleaned with antiseptics every day for a week and wound care was performed.

The subjects were euthanized by decapitation at the end of the 60th day. In addition, after euthanasia, testicular tissues were taken from the subjects and spermatological examinations were performed.

Andrological Examinations

At the end of the study, animals in all groups were decapitated under xylazine/ketamine anesthesia. Reproductive organs such as testes, epididymis, seminal vesicles and ventral prostate were removed and cleaned of fatty tissues. Similarly, the right epididymis was used for sperm density determination, and the left epididymis was used for motility and abnormal spermatozoon determination.

Sperm Motility

The slide was placed on the heating table of the microscope and its temperature was allowed to reach 37 °C. After a few drops of Trisbuffer solution [Tris (hydroxymethyl) aminomethane 3.63 g, glucose 0.50 g, citric acid 1.99 g and distilled water 100 ml] were dropped onto the slide on the heating table, 3 microliters of spermatozoon containing spermatozoa taken from the left cauda epididymis were placed on this solution and mixed with the help of a coverslip. Then, motility was determined at 400x magnification. Motility was performed by examining 3 different fields. The average values of these 3 different fields were calculated as the % motility rate (Turk et al. 2008).

Sperm Density

The right cauda epididymis was taken and thoroughly broken into pieces in 1 ml physiological saline (0.9% NaCl) in a petri dish with a scalpel and scissors. Then the particles were thoroughly crushed with a forceps. It was left to incubate for 4 hours at room temperature so that all spermatozoa in the epididymal tissue passed into the liquid. Following the waiting period, the supernatant containing spermatozoa up to the 0.5 line of the red blood cell pipette was drawn from a solution containing 5 g sodium bicarbonate, 1 ml formalin, 25 mg eosin and 100 ml distilled water up to the 101 line. Approximately 10 µl of diluted supernatant was placed on both counting areas (total 400 small squares, 0.1 mm^3 volume) of the Thoma slide to which the coverslip had been previously attached. The Thoma slide was placed under the light microscope and waited for 5 minutes to ensure that the spermatozoa in the solution were distributed homogeneously throughout the area. Spermatozoa falling into all squares in both counting areas were counted at 200x magnification of light microscope. Sperm density in cauda epididymal tissue was calculated (Turk et al. 2008).

Abnormal Sperm Rate

A few drops of Tris buffer solution were dropped onto a clean, dry and pre-warmed (37°C) slide, then a small drop of suspension taken from the left cauda epididymis and a few drops of Eosin-Nigrosin (1.67 g eosin, 10 g nigrosin and 2.9 g sodium citrate for 100 ml distilled water) dye mixture were dropped onto it and mixed with another slide to make it homogeneous. Then, thin smears were taken from this Tris spermatozoon suspension-dye mixture and allowed to dry in air. The dried smears were examined at 400x magnification of light microscope. A total of 200 spermatozoa were examined in a smear and the total abnormal spermatozoon rate was expressed as a percentage (Turk et al. 2008).

d) Weights of Testis and Surrounding Tissues

At the end of the study, the testes and surrounding tissues were removed and separated from each other by removing the fatty tissues. Testis, epididymis, right cauda epididymis, seminal vesicle and prostate tissues were weighed individually on a precision scale and recorded (in grams).

Statistical Analyses

In our study, statistical significance between the groups was evaluated using IBM SPSS Version 22.0 (IBM Corp. Armonk, NY, U.S.A) software for spermatological parameters (sperm motility, sperm density, abnormal spermatozoon rate, testicular and surrounding tissue weights). Shapiro-Wilk normality test was used to determine whether the raw values of all measured parameters showed normal distribution

It was determined that the values of all parameters showed normal distribution.
According to the results of this test, one-way analysis of variance (ANOVA) was used to evaluate group differences and post hoc Duncan test was used to compare binary groups. The findings obtained in the study were expressed as mean and standard error. $P<0.05$ values were accepted as statistically significant.

RESULTS

Clinical Findings

No symptoms of dermatitis, scrotal swelling, discharge, fistula or pain on palpation were observed in the animals. In the animals that underwent intratesticular application, although the testicles were large and hard in volume on the first days, they were not at a level that affected the vital well-being of the animals. No complications occurred in the wounds of the animals in the surgical castration group, and complete recovery was observed at the end of the study.

Andrological Findings

The changes observed in spermatological parameters after the applications are shown in Table 2.

Sperm Motility

Sperm motility rates were found to be significantly lower in the groups that received intratesticular saline, dextrose and mannitol compared to the control group ($p<0.001$) (Table 2).
The decrease in motility was found to be more pronounced in the mannitol group compared to the other groups ($p<0.001$) (Table 2).
Intratesticular physiological saline+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, sperm motility could not be evaluated because there were no cells in the tissue sample (Table 2).

Sperm Density

In intratesticular physiological saline, dextrose and mannitol groups, sperm density was found to be significantly lower compared to the control group ($p<0.001$) (Table 2).
In intratesticular physiological saline+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, sperm density could not be evaluated because there were no cells in the tissue sample (epididymis and ductus deferens that did not contain sperm) (Table 2).

Abnormal Spermatozoa Rate

No statistically significant change was found in abnormal sperm rates in intratesticular physiological saline, dextrose and mannitol groups compared to the control group (Table 2).
Since there were no cells in the tissue samples in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups, the abnormal spermatozoon rate could not be evaluated (Table 2).

Weights of Testis and Surrounding Tissues

When the epididymis and right cauda epididymis weights were compared with the control group, they decreased in the groups that were administered intratesticular physiological serum, dextrose and mannitol ($p<0.05$). The decrease in the right cauda epididymis in the group that was administered intratesticular dextrose was found to be insignificant when compared with the control group (Table 2).
When the testis and seminal vesicle weights were compared with the control group, they were found to be significantly lower in all groups ($p<0.001$) (Table 2).
It was determined that the decrease in the testis and seminal vesicle weights was more pronounced in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$ and mannitol+$CaCl_2$ groups compared to the other groups (Table 2).
In addition, it was observed that the decrease in testicular weight was more pronounced in the intratesticular dextrose+$CaCl_2$ group ($p<0.001$) (Table 2).
In prostate weight, no difference was observed between the control group and the groups administered intratesticular physiological serum, dextrose, and mannitol, while a significant decrease was observed in the intratesticular physiological serum+$CaCl_2$, dextrose+$CaCl_2$, and mannitol+$CaCl_2$ groups compared to the other groups ($p<0.001$) (Table 2).

Table 2. Table Showing Sperm Motility, Sperm Density, Abnormal Sperm Rate Percentages, Testis, Epididymis, Right Cauda Epididymis, Seminal Vesicle and Prostate Weights in Chemically Castrated Rats.

	Control	SF	Dextrose	Mannitol	SF+$CaCl_2$	Dex+$CaCl_2$	Man+$CaCl_2$	p
Sperm Motility (%)	70,0±5,34^{a}	38,75±7,66^{b}	48,75±4,79^{b}	20,0±5,0^{c}				p<0,001
Sperm Density (million/cauda)	93,87±6,19^{a}	62,12±2,26^{b}	72,75±7,82^{b}	60,37±2,0^{b}				p<0,001
Abnormal Sperm Rate (%)	2,63±0,38	3,25±0,37	3,25±0,25	2,87±0,40				-
Testis (gr)	1,50±0,04^{a}	1,16±0,05^{b}	1,23±0,03^{b}	1,21±0,04^{b}	0,68±0,07^{cd}	0,55±0,04^{d}	0,75±0,08^{c}	p<0,001
Epididymis (gr)	0,63±0,04^{a}	0,49±0,02^{b}	0,52±0,02^{b}	0,56±0,05^{b}				p<0,05
Right Cauda Epididymis (gr)	0,22±0,01^{a}	0,18±0,01^{b}	0,20±0,01^{ab}	0,19±0,01^{b}				p<0,05
Seminal Vesicle (gr)	1,14±0,11^{a}	0,77±0,17^{bc}	1,03±0,15^{ab}	0,58±0,04^{c}	0,18±0,03^{d}	0,20±0,01^{d}	0,23±0,02^{d}	p<0,001
Prostate (gr)	0,46±0,05^{a}	0,38±0,07^{a}	0,45±0,07^{a}	0,43±0,03^{a}	0,08±0,01^{b}	0,08±0,01^{b}	0,08±0,01^{b}	p<0,001

a, b, c, d: Values with different superscripts are statistically different from each other

DISCUSSION

Castration is inevitable for taming work animals, increasing productivity in livestock farming, not using animals with low fertility as breeding stock, preventing the spread of infectious diseases, deep and complicated wounds of the testicles, tumoral cases and various genital organ diseases (Bakır et al. 2006).

There are many methods for sterilization such as surgical castration. In surgical castration; excessive cost, difficulty in postoperative care, use of antibiotics and various postoperative complications create problems in terms of animal welfare (Jana and Samanta 2007; Hassan and Fromsa 2017).

Another alternative method for sterilization is the use of agents that block LH, FSH and GNRH hormones, although it causes testosterone levels and sperm production to decrease to a level that will cause infertility, since the duration of effect is limited and studies show that the ability to fertilize can be restored, the application must be repeated and the suspicion that repeated applications may have side effects make such methods disadvantageous (Bowen 2008; Çevik et al. 2019; Driancourt and Briggs 2020). Intratesticular chemical sterilization has the advantage of creating permanent sterility, having little to no postoperative care, preventing negative sexual behaviors, not causing side effects in target organs, achieving high success when applied by experienced personnel, easy storage and transportation of the chemical agents to be used, and being less costly (Baran et al. 2016). However, it should not be forgotten that animals can be fertile during this period due to the reserve sperm in the epididymis until the 60th day following intratesticular applications and pregnancies can occur after mating (Turk and Ataman, 2016).

In the study conducted by Canpolat et al. (2006a) on intratesticular chemical castration in cattle, no symptoms other than hardness were observed in the testicles after the application of $CaCl_2$. In the study where intratesticular chemical castration was performed on goats, mild swelling was observed in the testicles after intratesticular $CaCl_2$ application. This swelling gradually decreased. The animals continued their lives in good health throughout the study (Jana et al. 2005).

In a study conducted on dogs, swelling was observed in the testicles after intratesticular $CaCl_2$ application and it was observed that the swelling decreased after two days (Canpolat et al. 2006b). In dogs to which $CaCl_2$ was applied, no symptoms were observed except for a 3-4-day testicular hardness (Jana et al. 2005; Leoci et al. 2014a; Leoci et al. 2019). In cats, no undesirable situation was observed except for mild tension in the palpation of the testicles after intratesticular $CaCl_2$ application (Coetzee et al. 2010). In dogs, degeneration was observed in the testicular tubules after intratesticular formalin application (Bakır et al. 2006). Canpolat et al. (2016) showed widespread and significant degenerative changes in the seminiferous tubules of the testicles in the histopathological examination of the testicles on the 60th day after intratesticular application of 20% sodium chloride in dogs.

It was reported that rats tolerated intratesticular $CaCl_2$ well, and there was no restlessness, fever or swelling in the testicles (Jana and Samanta 2006). In a different study, it was stated that no complications were observed after intratesticular $CaCl_2$ application, except fever in the first 3 days. In the studies; it was understood that age, number of applications, and doses of the determined chemicals are the parameters that play an important role in changing testosterone levels (Pařızek 1960; Jana et al. 2005; Canpolat et al. 2006b).

In our preliminary study, a solution was created by dissolving 2g $CaCl_2$ in 10ml physiological serum (%20 $CaCl_2$) and applied intratesticularly. After the application, death was observed in some animals and generally severe necrosis and fistula formation in the testicles.

It was observed that the groups formed with 1g $CaCl_2$ (%10 $CaCl_2$) and other high osmolarity solution applied groups in our study tolerated the solutions well, parallel to the literature. In all groups that received intratesticular application, tension and slight swelling were observed in the testicles in the first days, and the swelling gradually decreased after 48 hours. In the daily checks of the animals, no complications such as restlessness, loss of appetite, fever, inflammation in the testicles, fistula and ulceration were observed. While no undesirable conditions were observed in the surgical castration group, it was observed that wound healing occurred at the end of 2 weeks.

During intratesticular application, the needle tip was directed into the testicle in a cauda-ventral direction, approximately half a centimeter away from the tail of the epididymis, and the injection was made along the determined line. In our study, intratesticular application was made in multiple directions into the testicle, aiming for complete sterilization by completely penetrating the testicular tissue with chemicals. In order to prevent drug leakage into the spermatic cord and surrounding tissues, the appropriate syringe and syringe tip for the animal structure were carefully selected. Care was taken to prevent leakage, and it was thought that it would be more appropriate to wait for a while and terminate the application after the chemical solutions were completely injected into the tissue.

Albino mice were administered cetremide at doses of 5, 10, 15, 20 mg/100 gr. Histopathological examination 30 days after the application revealed degeneration in the testicular tissue tubules and apoptosis in germ cells (Fesseha and Negash 2020).

In another study, it was revealed that intratesticular application of 20% salt solution to Sprague-Dawley rats caused deterioration in the DNA structure of the testis, histopathological examination revealed significant degeneration in the seminiferous tubules, and apoptosis occurred in the testicular cells. It was reported that similar results were obtained between the surgical castration group and the 20% salt solution applied groups (Kwak and Lee 2013).

In another study conducted on rats, 20% salt solution was applied intratesticularly and a significant decrease in testosterone levels was shown after the application. It is thought that 20% salt solution intratesticular application may be an alternative to surgical castration (Dursun 2005). In another study conducted in recent years, 20% mannitol and 20% sodium chloride were applied intratesticularly to rats, and according to spermatological and histopathological results, 20% mannitol application was thought to be an alternative to surgical castration (Maadi et al. 2021).

In our study; 20% dextrose, 20% mannitol solution and 0.9% physiological serum solutions were tried and the aim was to find the best alternative to surgical castration.

Ali et al. (1991) applied a mixture of 25%, 50% and 70% $CaCl_2$ in sterile water intratesticularly to donkeys in their study on donkeys. Considering the measurements of testicular volumes taken on certain days and sperm vitality rates, they predicted that 70% $CaCl_2$ causes infertility in animals. Ibrahim et al. (2016) concluded that although necrosis occurred in the testicular tubules and Sertoli cells were destroyed after intratesticular application of 20% $CaCl_2$ dissolved in pure alcohol in donkeys, since no significant decrease was observed in testosterone levels in donkeys administered 20% $CaCl_2$ dissolved in pure alcohol; this method was ineffective in sterilization in the applied animals.

Our study revealed that there is a connection between the volumetric size of the testicles and the amount of $CaCl_2$ applied, that repeated applications will not be necessary if the correct dose is found, and that the age of the animals, testicular size, weight and the solutions used to dissolve $CaCl_2$ are important criteria for the effectiveness of sterilization in applications with $CaCl_2$. Zeuterin® (Ark Sciences, New York, USA) solution is approved by the FDA in the United States for chemical castration in male dogs. It contains 0.2 M zinc gluconate (13.1 mg zinc/ml) neutralized with 0.2 M zinc arginine (pH 7.0). The same active substance is available for use in both cats and dogs in Latin American countries under the name Esterilsol (Oliveria et al. 2012). Injections were made using calipers for each testicle for dogs of all ages, and the amount of zinc gluconate solution was calculated intratesticularly based on these measurements. Baran et al. applied 0.2 ml of $CaCl_2$ solution into each testicle at different concentrations of 0% (n=1 cat), 5% (n=1 cat), 10% (n=1 cat), and 20% (n=1 cat). Male cats treated with 5% and 10% $CaCl_2$ were oligospermic (<20 million sperm/ml), cats treated with 0% $CaCl_2$ had normal ejaculate (>20 million sperm/ml), while no sperm was found in the ejaculate of a male cat treated with 20% $CaCl_2$, and histological evaluation showed degeneration and calcification in the seminiferous tubules and fibrosis in the interstitial cells on the 60th day after application (Baran et al. 2010).

In a study conducted by Leoci et al. (2014b), they applied $CaCl_2$ solutions prepared with 0.9% physiological saline solution to dogs at doses of 10%, 20%, 30%, and 60% intratesticularly. They showed that sperm motility was close to zero and testosterone levels decreased significantly in the treatment groups. It was concluded that the 20% dose did not cause any complications, but different solvents should be used to increase the sterilization power and duration of effectiveness.

In our study, physiological saline, high osmolarity dextrose, and mannitol solutions were used, and a significant decrease in sperm motility was observed compared to the control groups. It was understood that the decrease was most pronounced in the mannitol group. Again, when sperm density was evaluated, it was determined that there was a significant decrease in the physiological saline, dextrose, and mannitol groups compared to the control groups. In the combined groups with $CaCl_2$, since sperm samples could not be taken (i.e. epididymis and ductus deferens that do not contain sperm), sperm motility and sperm density could not be evaluated. Again, when the groups were evaluated in terms of abnormal spermatozoon rate, no significant difference was found in the physiological serum, dextrose and mannitol groups compared to the control group. Since not even a single sperm cell could be found in the combined groups with $CaCl_2$, an evaluation could not be made in terms of abnormal spermatozoon rate.

Since the testis and surrounding tissues atrophied in the $CaCl_2$+dextrose, $CaCl_2$+mannitol and $CaCl_2$+physiological serum combined groups, even weights could not be taken for andrological examination. In fact, it was found that the testis and surrounding tissues in the $CaCl_2$+dextrose group were the most significant group in terms of shrinkage among the groups. In another study, after intratesticular application of $CaCl_2$ in various doses (ranging from 2.5mg/100g to 20mg/100g) to albino rats, it was determined that the valid and ideal dose was 10mg/100g and 20mg/100g (Jana et al. 2002).

In high doses of $CaCl_2$, fistula structures may form on the testis due to destruction of surrounding tissues and scrotum. In the presented study, it is thought that the combination of 10% $CaCl_2$ with suitable solvents, especially in the $CaCl_2$+dextrose group of our study, can increase the significant decrease in testicular weight, the effectiveness of $CaCl_2$ at low doses, and that sterilization can be achieved without damaging the surrounding tissues of the testis.

In recent studies; the search for an ideal sterilization with chemical drugs continues. In our study, high osmolarity mannitol and dextrose were used to find a chemical that could solve this problem. As a result; In this study, it was tried to show that an ideal chemical castration can be done with easily available and low-cost high osmolarity solutions.

In addition, in the $CaCl_2$ combined groups, the testicles and surrounding tissues atrophied, therefore sperm production completely stopped. It was observed that the group with the most positive results among the combined groups was the $CaCl_2$+Dextrose group, and it is predicted that effective castration can be done by using small amounts of $CaCl_2$.

CONCLUSION

The most important aspect of our study; It was concluded that cats and dogs, whose populations are rapidly increasing in our country, can be castrated with easily accessible chemicals without wasting time with a simple injection, complications can be minimized with appropriate doses, and it is possible to perform irreversible castration with a single dose application in terms of animal welfare. We believe that the results of our study will provide a new perspective and create a resource while chemical castration research is being conducted.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: ŞÖK, İC and HA contributed to the project idea, design and execution of the study. ŞÖK, İC and HA contributed to the acquisition of data. ŞÖK, İC and HA analysed the data. ŞÖK, İC and HA drafted and wrote the manuscript. ŞÖK, İC and HA reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This study was carried out at Firat University Research Animals Application Center. This research was approved by Fırat University Animal Experiments Local Ethics Committee, (With the decision of the ethics committee dated 23/12/2020 and numbered 2020/15).

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