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Effectiveness of Castration with Burdizzo, Calcium Chloride and Olive Oil in Sahel Bucks

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ABSTRACT

We evaluated the efficacy of castration with bilateral intratesticular injections of Calcium Chloride Dihydrate ($CaCl_2$) in ethanol, Olive Oil as well as Burdizzo technique in twenty Sahel bucks. The bucks were randomly distributed into four groups as A, B, C and D. Group A served as the control and were given bilateral intratesticular injection of 1.0 ml of normal saline. Bucks in group B were castrated with Burdizzo, while group C and D were given bilateral intra testicular injection of 1.0 ml of $CaCl_2$ and Olive oil, respectively. The sonograms and semen profiles of the bucks were evaluated. The diameters of the testicles and spermatic cords and the semen parameters were measured in real time after castration at days 1, 7, 14, 21, 28 and 60. The diameters in the Burdizzo, $CaCl_2$ and Olive oil castrated bucks significantly increased ($P < 0.05$) on days 7 and was decreased at day 60. Semen motility was absent in the bucks castrated with $Cacl_2$ after day 14. The concentration of spermatozoa also decreased significantly on day 7 in all the Burdizzo, $CaCl_2$ and Olive oil castrated goats. Sonogram of testes castrated with $Cacl_2$ showed discreet focal hyperechoic, surrounded by hypoechoic areas within the parenchyma due to the $CaCl_2$ deposition. The testes of bucks castrated with olive oil were characterized by a central hypoechoic area surrounded by clearly distinguishable hyperechoic areas within the parenchyma. It was concluded that intratesticular injection with Burdizzo, $CaCl_2$ and olive oil resulted in successful castration but $CaCl_2$ injection resulted in earlier azoospermia.

Keywords: Burdizzo; Castration; Calcium chloride; Goats; Olive oil; Sahel bucks

INTRODUCTION

Castration is a procedure to remove testes function by orchiectomy, damaging them irreparably or causing them to atrophy by stricture of the blood supply using physical or chemical methods (Currah *et al.;* 2009; Al-Asadi and Al-Kadi 2012; Rajkumar 2013). Castration involves the extirpation or suppression of gonadal function (Neto *et al.,* 2014). Following orchiectomy in goats, serum testosterone concentration significantly ($P < 0.01$) decreased from 6.1 ± 0.2 ng/mL on the day of castration to 0.6 ± 0.0 ng/mL 30 days later (Hasan *et al.,* 2016).

Animals are castrated to make them tractable, to prevent unwanted traits in breeding programs and in some disease conditions such as neoplasms of the testicles or irreversible testicular trauma like chronic orchitis (Stoffel *et al.,* 2009). Generally, castration decrease the frequency of sexual and aggressive behaviour, and it improves the handling in farms. Surgical castration is expensive and is not usually employed in ruminants except when indicated for pathological conditions (Molony *et al.,* 1995).

Burdizzo castration has been widely used (Stoffel *et al.,* 2009; Sawhney 2016). Although effective, failure of castration has been reported due to improper crushing of the spermatic cord (Stafford *et al.,* 2000). Chemical castration involves the injection of a sclerosing agent into testicular parenchyma to cause irreversible damage (Fordyce *et al.,* 1989; Leoci *et al.,* 2019). Various agents have been used for chemical castration in animals. They include Ferric chloride (Da *et al.,* 1982), Formalin (Ijaz *et al.* 2000; Bakir *et al.,* 2002; Al-Asadi and Al-Kadi 2012). Chlorhexidine (Mohammed and James 2013), Calcium chloride ($Cacl_2$) (Leoci *et al.,* 2014; Leoci *et al.,* 2019). Chemical castration has the advantage of achieving castration objective with a single, inexpensive and permanent technique without the need for any follow-up. It can also be

used in areas where cutaneous myiasis complicates surgical castrations, or reduce risk of haemorrhage and herniation (Jana and Samanta 2007).

On the other hand, chemical castration has been associated with some complications after intra-testicular injection. These complications include pain, pyrexia and even severe inflammation. However, Al-Asadi and Al-Kadi, (2012) reported that chemical castration with Cacl2 was more effective than external ligation of the spermatic cord in close castration (elastrator rubber band).

Calcium chloride can be dissolved in water, in alcohol or lidocaine solution (Jana and Samanta 2007). Calcium chloride effectively arrests spermatogensis, androgenesis and libido, with no toxicity and serious side effects (Webe and Barr 1984.). Several investigators have reported that a single, bilateral intra-testicular injection of $Cacl_2$ solution resulted in induction of permanent chemosterilization, including cessation of sperm production and decreased testosterone in male dogs (Jana and Samanta 2007).

Olive oil is a fat obtained from olive seeds (*Olea lancifolia*). Oleuropein, the active principle of olive oil, is a phenolic compound with antioxidant, anti-inflammatory and antipyretic effect. (Visioli*et al.,* 2002). Olive oil also contains phenoortho-diphenols and various natural antioxidants, tocopherol (Vitamin E), flavones alcohols, sterols, chlorophyll, volatile aromatic substances (De Faveri *et al.,* 2008; Paiva *et al.,* 2011). Post-castration changes in testicular tissue can be monitored by external assessment (calliper measurements), histopathology (biopsies) or imaging (radiography and ultrasonography).

It was suggested that ultrasonography could permit non-invasive evaluation of the internal structure of the scrotum and testes for evaluating physio-pathologic conditions of goat testes or as a routine investigative method during breeding soundness and clinical examination (Sakthivel *et al.,* 2013; Olatunji-Akioye *et al.,* 2018). Furthermore, use of semen analysis in conjunction with sonography is potentially very useful in assessment of fertility status in animals (Gouletsou *et al.,* 2003). For these reasons, ultrasonography was applied in the current study to monitor the changes associated with different castration methods. In addition, it was reported that testes diameter was better accurately depicted with ultrasound compared with measurement of testes diameter with callipers (Andrade *et al.,* 2014).

Ibrahim *et al*. (2016) stated that of recent, researchers were interested in developing a method for chemical sterilization which may provide a better alternative to surgical castration which would effectively arrest spermatogenesis and androgenesis as well as libido while avoiding toxic or other side effects. Consequently, to find suitable and inexpensive alternative chemical methods of castration for Sahel bucks, this study was designed to determine the morphometric changes as well as echo-textural characteristics of the testes and key semen parameters of spermatozoa concentration and viability associated with Burdizzo, $Cacl_2$ and olive oil castration methods

MATERIALS AND METHODS

Experimental Animals

Twenty clinically healthy Sahel bucks aged 1.5-3 years weighing 15-20 kg body weight were used for the study. The animals were purchased from Maiduguri livestock market in Borno State, Nigeria. The animals were kept at the Large Animal Clinic, Veterinary Teaching Hospital, University of Maiduguri. They were allowed to acclimatize for two weeks before the commencement of the experiment.

Experimental Design

The bucks were randomly allocated into four groups A, B, C and D consisting of five animals each. The body weight was recorded and scrotal circumference of each testicle was measured using measurement tape prior to castration and on days 7, 14, 21 and 28 after castration.

Bucks in group A served as the control group and were administered intra-testicular injection of sterile water 1 mL (Unisal®). Group B were castrated using Burdizzo (Supervet®, CHIFA, Nowy, Tomysl, Poland). Group C received intra-testicular injection of 1 mL 20% calcium chloride dihydrate (Sigma®, London) (Jana *et al.* 2005). Group D bucks received an intra-testicular injection of 1mL Olive oil (Laser Virgin® olive oil Intl. UAE). After each castration method, 3 mLs of blood samples were drawn from the jugular vein into plain vacutainer tubes and serum harvested by centrifugation at 4000 g for 3 minutes in an electronic centrifuge (Centrifuge 800B®, Union Laboratories, England).

Ethical Statement

The experiment was conducted in accordance with international guiding principles for biomedical research involving animals (CIOMS, 1985).

Sonography of the Testes

Ultrasonograhic evaluation of the testes for all the groups A to D were carried out using an ultrasound scanner (Falco Vet 100® Pic Medical; Holland), connected to a linear array transducer with a frequency of 7.5 MHz (Sony Sao Paulo, Brazil). The bucks were placed on a recumbent position and the entire hair of each scrotal sac was shaved with razor blade. The shaved area was cleaned with 0.2% Chlorhexidine gluconate (Savlon®, Vervaadingdeur, Johnson and Johnson (pty) Ltd, London). Bucks were physically restrained by two assistants and the testes immobilized. The parenchyma echotexture of each testis was scanned in the sagittal, and transverse planes and diameters measured using the built-in callipers (Bukar *et al*., 2012; Sakthivel *et al.*, 2013; Olatunji-Akioye *et al.*, 2018).

Scanning of the testes and spermatic cords were similarly performed, were on days 7, 14, 21 and 28 post-castration.

Semen was collected using Bailey ejaculator® (Western Instrument Company, Denver, Colorado) prior to castration and on days 7, 14, 21 and 28. Each buck was restrained by two assistants and the ejaculator probe (lubricated with K-Y Jelly), inserted into the rectum of the buck and used until ejaculation according to manufacturer's instructions. The semen collected were observed and analysed immediately for the volume, progressive motility, livability and concentration. The same procedure was done for subsequent post-castration collections.

Data obtained were analyzed using One Way Analysis of Variance ANOVA. GraphPad Prism® Version 4.0 Software was employed for the data analysis. Analysis was considered as significant at $P<0.05$.

RESULTS

The left testicular diameter (Table 1) was not significantly different ($P < 0.05$) in the control group throughout the 60-day scanning period. However, the diameters in the Burdizzo, $Cacl_2$ and Olive oil castrated groups significantly increased ($P < 0.05$) from21.38±6.90, 19.26±8.27 and 25.96±5.46 respectively to 27.00±7.26, 22.28±8.23 and 29.44±0.85 respectively on days 7 and was decreased at day 60 to 23.46±16.14, 23.36±14.18 and 24.38±14.23, respectively.

Similarly, right testicular diameter (Table 1) was not significantly different ($P < 0.05$) in the control group during t he 60-day scanning period. However, the diameters (mm) in the Burdizzo, $Cacl_2$ and Olive oil castrated groups significantly increased ($P < 0.05$) 22.70±5.90, 20.90±8.81and 23.48±2.66 respectively to 27.48±6.47, 26.94±7.71and 27.02±4.49 respectively on days 7 and was decreased at day 60 to 23.86±14.40, 23.20±14.23 and 25.36±14.26 respectively (Table 1). The mean spermatic cord diameter of the left before castration with Burdizzo, $Cacl_2$ and Olive oil were 5.62±2.27, 6.98±2.09 and 5.30±2.43 respectively. These values significantly increased ($P < 0.05$) to 11.74±5.84, 7.06±2.19 and 6.02±2.31respectively on day 7. At day 60, the mean diameters had decreased to 4.92±3.05, 5.02±3.04 and 5.70±3.44, respectively (Table 2).

The values of right spermatic cord diameter (mm) before castration with Burdizzo, $Cacl_2$ and Olive oil also significantly increased ($P < 0.05$) from 5.76±1.35, 5.98±1.12 and 6.32±1.05 respectively. These values significantly increased ($P < 0.05$) to 9.86±3.14, 7.90±1.54 and 5.78±1.09 respectively on day 7. At day 60, the mean diameters had decreased to 5.12±2.87, 4.60 ± 2.58 and 5.90±3.51, respectively (Table 2).

Table 1: Diameter of the left and right testicles (mm) in Sahel bucks castrated with Burdizzo, $CaCl_2$ and olive oil

Castration Method		Testicular diameter (mm) in days after castration					
		1	7	14	21	28	60
Control	Left	28.84±7.06	29.02±3.94^{a,b}	26.98±4.06	26.12±2.76	27.16±2.55	21.86±12.26
	Right	21.98±9.26	22.28±8.10	21.98±9.61	22.74±9.87	22.46±8.10^{a}	18.26±14.56
Burdizzo	Left	21.38±6.90	27.00±7.26	27.84±8.52	28.14±6.03	28.54±7.80	23.46±16.14
	Right	22.70±5.90	27.48±6.47	28.18±8.05	28.62±5.16	27.78±4.67	23.86±14.40
$CaCl_2$	Left	19.26±8.27	19.26±8.27^{b,c}	31.64±6.34	34.82±4.34	34.86±4.83	23.36±14.18
	Right	20.90±8.81	20.90±8.81	35.92±3.16	31.68±3.14	32.32±7.56^{b}	23.20±14.23
Olive oil	Left	25.96±5.46	29.44±0.85^{c}	33.52±3.89	32.12±3.21	32.44±4.56	24.38±14.23
	Right	23.48±2.66	27.02±4.49	34.86±4.80	34.24±1.40	33.04±3.62^{c}	25.36±14.26

^{a,b,c} Values with different superscripts within columns differ significantly ($P < 0.05$)

Table 2: Diameter of the left and right spermatic cord (mm) in Sahel bucks castrated with Burdizzo, $CaCl_2$ and olive oil

Castration Method		Spermatic cords diameter (mm) in days after castration					
		1	7	14	21	28	60
Control	Left	6.30±1.45	8.56±2.96	6.18±1.46	5.90±1.41^{a,b}	5.96±0.58	5.96±3.80
	Right	5.82±1.99	9.00±2.34^{a,b}	6.06±1.54^{a}	6.66±1.85	4.98±0.67^{a}	4.82±3.18
Burdizzo	Left	5.62±2.27	11.74±5.84^{a,b}	7.68±3.05	7.78±1.61	6.10±2.04	4.92±3.05
	Right	5.76±1.35	9.86±3.14^{b,c}	8.04±2.10	7.26±1.93	5.58±1.61	5.12±2.87
$CaCl_2$	Left	6.98±2.09	6.98±2.09	7.12±2.29	8.98±1.83^{b,c}	6.52±0.81	5.02±3.04
	Right	5.98±1.12	5.98±1.12^{c}	8.46±1.81	8.00±2.21	7.04±0.77^{b}	4.60±2.58
Olive oil	Left	5.30±2.43	6.02±2.31^{b,c}	7.12±0.94	6.60±1.27^{c}	6.22±0.58	5.70±3.44
	Right	6.32±1.05	5.78±1.09^{d}	8.74±1.85^{b}	6.84±2.53	6.80±1.06^{c}	5.90±3.51

^{a,b,c,d} Values with different superscripts within columns differ significantly($P < 0.05$)

Semen was no longer ejaculated from the bucks castrated with $CaCl_2$ by day 14. However, semen was collected from all the other groups (Table 3). The colour of semen in bucks castrated with olive oil appeared yellow-straw and different from the other groups.

Semen motility was absent in the bucks castrated with $CaCl_2$ after day 14 whilst motility was observed in the Burdizzo and Olive oil castrated groups until day 28 (Table 4).

The concentration of spermatozoa also decreased significantly on day 7 in all the Burdizzo, $CaCl_2$ and Olive oil castrated goat (Table 5).

By day 60, all the spermatozoa seen in the semen of $CaCl_2$ and olive oil castrated goats were dead. Only a few live spermatozoa (2.00±4.47) were seen in the smear of some Burdizzo castrated bucks (Table 6).

Table 3: Semen volume (mL) in Sahel bucks castrated using Burdizzo, $CaCl_2$, and olive oil

Castration Method	Semen collection in days after castration					
	1	7	14	21	28	60
Control	0.64±0.48	0.72±0.50	$0.62±0.22^{a,b,x}$	0.36±0.41	0.36±0.30	0.30±0.67
Burdizzo	0.50±0.34	1.04±0.58	$0.60±0.42^{b,c,x}$	0.43±0.40	0.49±0.62	$0.29±0.62^{a,x}$
$CaCl_2$	0.50±0.34	0.48±0.46	$0.00±0.00^{c,d,x}$	$0.00±0.00^{a,x}$	$0.00±0.00^{a,x}$	$0.00±0.00^{b,x}$
Olive oil	0.48±0.29	0.48±0.46	$1.16±0.47^{d,x}$	$0.86±0.41^{b,x}$	$1.08±0.53^{b}$	0.24±0.53

^{a,b,c,d} Values with different superscripts within columns differ significantly($P < 0.05$)

Table 4: Semen progressive motility (%) in Sahel bucks castrated using Burdizzo, $CaCl_2$, and olive oil

Castration Method	Semen collection in days after castration					
	1	7	14	21	28	60
Control	0.66±0.20	0.38±0.36	$0.46±0.27^{a,b}$	0.38±0.24	0.20±0.33	0.46±0.35
Burdizzo	0.46±0.42	0.12±0.26	0.37±0.44	0.34±0.46	0.34±0.42	0.00±0.00
$CaCl_2$	0.28±0.23	0.06±0.13	$0.00±0.00^{b,c}$	0.00±0.00	0.00±0.00	0.00±0.00
Olive oil	0.58±0.34	0.08±0.17	0.10±0.12	0.16±0.23	0.26±0.32	0.00±0.00

^{a,b,c,d} Values with different superscripts within columns differ significantly($P < 0.05$)

Table 5: Semen concentration ($x10^9$/µl) in Sahel bucks castrated using Burdizzo, $CaCl_2$, and olive oil

Castration Method	Semen collection in days after castration					
	1	7	14	21	28	60
Control	$48.0±34.5^{ab}$	$23.0±24.0^{ab}$	$15.0±17.2^{a}$	28.0±33.3	76.0±14.5	71.0±25.5
Burdizzo	$60.0±16.4^{bc}$	$44.0±54.0^{bc}$	$70.0±11.0^{b}$	$20.0±44.5^{a}$	17.0±26.5	0.00±0.00
$CaCl_2$	$94.0±64.5^{cd}$	$28.0±29.7^{cd}$	$0.00±0.00^{c}$	$0.00±0.00^{b}$	00.0±00.0	0.00±0.00
Olive oil	$41.0±95.9^{d}$	$41.0±30.5^{d}$	60.0±10.0	$33.0±34.0^{c}$	17.0±31.2	0.00±0.00

^{a,b,c,d} Values with different superscripts within columns differ significantly($P < 0.05$)

Table 6: Live total (%) of spermatozoa in Sahel bucks castrated using Burdizzo, $CaCl_2$, and olive oil

Castration Method	Semen collection in days after castration					
	1	7	14	21	28	60
Control	74.8±13.9	$62.0±35.6^{ab}$	$49.0±31.5^{a}$	31.6±36.9	36.0±39.1	43.00±6.70
Burdizzo	57.0±38.4	$14.0±19.4^{bc}$	$0.00±0.00^{b}$	26.0±37.1	18.4±34.8	2.00±4.47
$CaCl_2$	55.2±32.1	$5.20±8.67^{cd}$	$0.00±0.00^{c}$	0.00±0.00	0.00±0.00	0.00±0.00
Olive oil	67.8±38.0	$0.00±0.00^{d}$	$1.80±2.68^{d}$	21.6±28.7	24.0±35.6	0.00±0.00

^{a,b,c,d} Values with different superscripts within columns differ significantly($P < 0.05$)

Ultrasonography

The sonogram of the normal uncastrated testes showed low to moderate echogenicity of the parenchyma with echogenic vaginal tunic (Figure 1). In the Burdizzo castrated bucks (Figure 2), sonogram of testes showed low to moderate echogenicity. The echogenicity increased on days 14 and 21.

Sonogram of testes castrated with $CaCl_2$ (Figure 3) showed discreet focal hyperechoic areas within the parenchyma due to the $CaCl_2$ deposition. The hyperechoic areas were surrounded by hypoechoic areas. These hyperechoic areas were increased in their echogenicity and could be found on days 14, 21 and 28.

The sonogram of testes chemically castrated with olive oil (Figure 4). A central hypoechoic area surrounded by clearly distinguishable hyperechoic areas within the parenchyma. These hypoechoic areas persisted until day 28 but were gradually decreased in diameter. At day 60, these hypoechoic areas were not present.

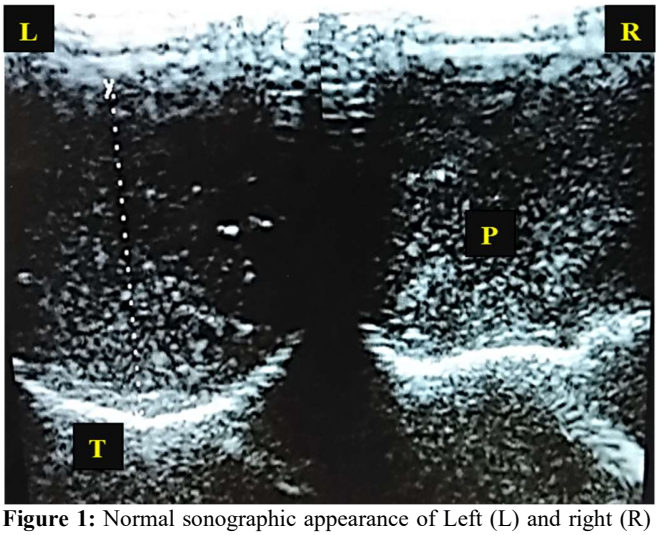

Figure 1: Normal sonographic appearance of Left (L) and right (R) testes in a Sahel buck. Note the hyperechoic *Tunica vaginalis* (T) (arrows) and moderately echogenic parenchyma (P).

DISCUSSION

The diameters of the left and right testicles in uncastrated bucks did not change throughout the duration of the current study. There was no significant difference between the left and right testes in the current study as was similarly reported previously (Sakthivel *et al.,* 2013).

However, the diameters in the Burdizzo, $CaCl_2$ and Olive oil castrated groups significantly increased ($P < 0.05$) from day 7 and was decreased at day 60 to 23. Swelling of the testes following castration with Burdizzo or chemical methods, which later subsided after a few days have been reported previously (Jana *et al.,* 2005; Al-Asadi and Al-Kadi 2012; Mohammed and James 2013). The resolution of the post castration inflammation and associated swelling indicates healing of damaged tissue and cessation of spermatogenesis provided that the castration was successful. Testosterone production is usually affected as was previously reported that testosterone concentration was significantly decreased from 6.1 ng/ml on the day of castration to 0.6 ng/ml 30 days later (Al-Asadi and Al-Kadi 2012; Hasan *et al.* 2016). As was observed for the testes, the diameters of the left and right spermatic cords also increased after castration with Burdizzo, $CaCl_2$ and Olive oil but were decreased by day 28 after castration.

In the current study, compared with semen from uncastrated bucks, the Burdizzo, $CaCl_2$ and Olive oil castrated bucks had reduced volume and quality of semen from on day 7 after castration. In the $CaCl_2$ castrated bucks, semen could not be successfully ejaculated from day 7 after castration. However, semen was successfully collected in the Control, Burdizzo and Olive Oil treated groups although with decreased spermatozoa concentration. This Azoospermia and Aspermia was probably due to the impairment of Sertoli cell and seminiferous tubule function. Similar findings were reported by Al-Asadi and Al-Kadi (2012) in surgically castrated as well as Formalin (Chemically) castrated bucks.

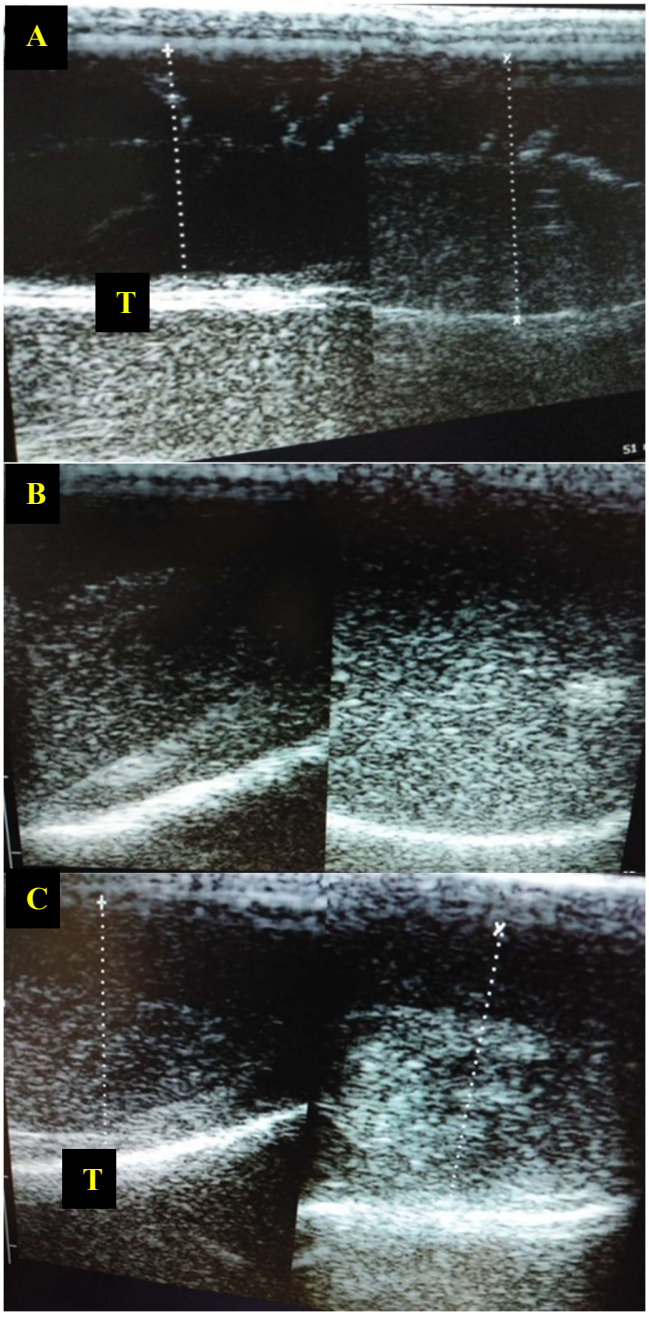

Figure 2: Sonogram of testes castrated with Burdizzo on day 7 (A) showing hypoechoic parenchyma and *Tunica vaginalis* (T). By day 14, the testes showed moderate echogenicity (B). The sonographic appearance of the testes by day 21 showed increased echogenicity within the parenchyma.

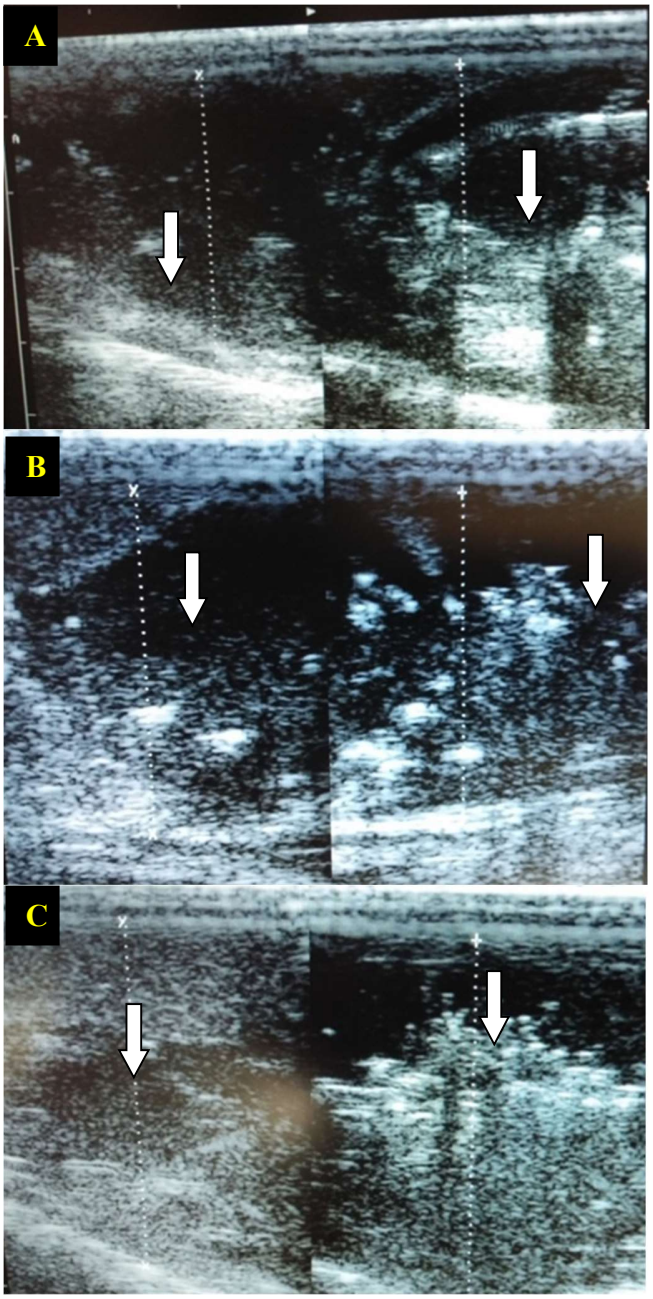

Figure 3: Sonogram of testes castrated with $CaCl_2$ on day 7 (A) showing hyperechoic areas within the parenchyma (arrows). By day 14, the hyperechoic areas on the testes (arrows) were increased (B). The hyperechoic areas (arrows) were present at day 21 (C).

The semen volume of the olive oil castrated bucks was not significantly decreased as the $CaCl_2$ group but the appearance changed to yellow-straw color. This suggests that no significant damage occurred to testes function in olive oil castrated bucks compared with the other castration methods studied. The yellowish tinge of the ejaculate could be attributed to the olive oil injection getting into the ejaculate through the direct injection of the olive oil into the testis parenchyma.

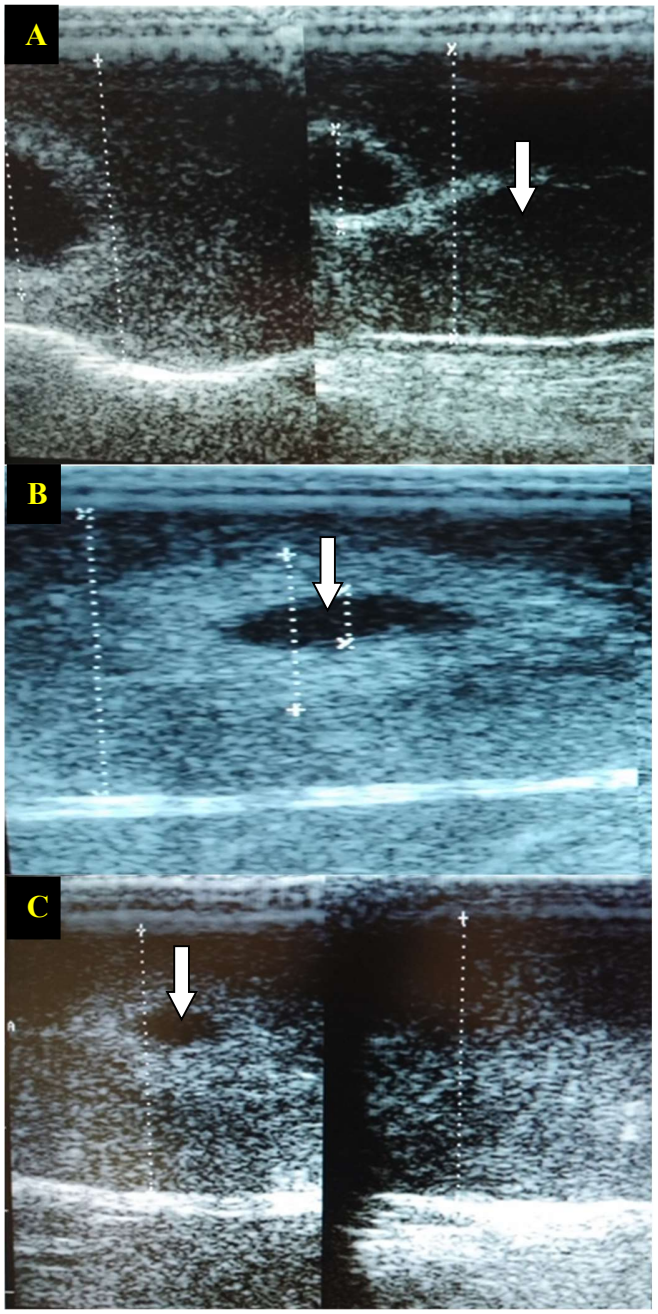

Figure 4: Sonogram of testes chemically castrated with olive oil on day 7 (A) showing a central hypoechoic area (arrows) surrounded by clearly distinguishable hyperechoic areas within the parenchyma (Arrows). By day 14 (B), and day 21 (C) (arrows), the hyperechoic areas were still present but noticeably smaller surrounded by less hyperechoic areas (arrows).

The mass motility, concentration and viability of bucks in the Burdizzo castrated bucks did not decrease significantly. This finding suggests that failure of castration had occurred. This failure has been reported previously in lambs and calves (Stafford *et al.,* 2000).

The progressive forward spermatozoa motility was markedly decreased on day 7 in the Burdizzo, $CaCl_2$ and Olive oil castrated groups compared with the control group. Among the groups, the spermatozoa were non motile earlier in the $CaCl_2$ castrated group at day 14. However, at 60 days after castration, the Burdizzo and olive oil castrated groups had non motile spermatozoa.

The concentration of spermatozoa also decreased significantly on day 7 in all the Burdizzo, $CaCl_2$ and Olive oil castrated goats. Thereafter, the least motility was observed in the $CaCl_2$ castrated group.

The proportion of live spermatozoa also decreased significantly on day 7 in all the Burdizzo, $CaCl_2$ and Olive oil castrated goats. Thereafter, the least motility was observed in the $CaCl_2$ castrated group. Interestingly, by day 60, all the spermatozoa seen in the semen of $CaCl_2$ and olive oil castrated goats were dead. A few live spermatozoa were seen in the smear of some Burdizzo castrated bucks.

The changes observed in semen quality such as decreased ejaculate volume, azoospermia, poor spermatozoa motility, high proportion of dead cells, varying semen colour, are indications of successful castration (Jana *et al.,* 2005; Sawhney 2016). In the current study, azoospermia was observed from day 14 post castration as well as other semen indices signifying non fertility. The indices were observed earlier in the $CaCl_2$ castrated bucks suggesting that it was the most effective chemical castration method. This agrees with previous reports where $CaCl_2$ was evaluated in bucks (Jana *et al.,* 2005; Al-Asadi and Al-Kadi 2012), dogs (Jana and Samanta 2007; Leoci *et al.,* 2014; Leoci *et al.* 2019), guinea pigs (Sen *et al.,* 2017). A detailed review of the use of $CaCl_2$ based solutions, their effectiveness and some factors that could improve castration responses had been published (Cavalieri 2017).

The sonogram of the normal uncastrated testes showed low to moderate but homogenous echogenicity of the parenchyma with hyperechogenic vaginal tunic. This echotexture mirrors the findings in (Sakthivel *et al.,* 2013; Olatunji-Akioye *et al.,* 2018) and rams (Gouletsou *et al.,* 2003; Andrade *et al.,* 2014). In the Burdizzo castrated bucks, sonogram of testes showed low to moderate parenchyma. The echogenicity increased on days 14 and 21.

Sonogram of testes castrated with $CaCl_2$ showed discreet focal hyperechoic areas within the parenchyma. The hyperechoic areas were surrounded by anechoic areas. A recent study by Leoci et al. (2019), stated that these anechoic areas corresponded to areas of tissue damage caused by the $CaCl_2$ injected. These hyperechoic areas increased and persisted in their echogenicity, probably due to the sclerosed parenchyma tissues.

These hypoechoic areas persisted until day 28 and are likely the non-absorbed olive oil deposited within the parenchyma tissue.

A single bilateral intratesticular injection of $CaCl_2$ resulted in aspermia and azoospermia within 14 days of injection. Burdizzo castration was effective but was associated with failure to completely crush the spermatic cord in some bucks. Semen volume was not affected by castration with olive oil but the spermatozoa concentration and viability were completely absent by day 60 after castration.

In conclusion, intratesticular injection with $CaCl_2$ injection in Sahel bucks resulted in aspermia and azoospermia. Thus, $CaCl_2$ solution provided the best castration outcomes compared with Burdizzo and olive oil. It is recommended that methods be developed to mitigate the complications such as swelling and pain.

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Conflict of Interest

The authors do not have any conflict of interest to declare.

Authors Contribution

AM and BMM designed and supervised the work. DL performed the experiments, analysed the data and wrote the draft manuscript. All authors have read and approved the final manuscript.

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