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Different Castration Methods Induced Variations in Behavioural Responses, Scrotal Circumference and Testicular Histo-architecture in Red Sokoto Bucks

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ABSTRACT

In this study, we assessed the behavioural responses, scrotal circumference, and testicular histo-architecture of red Sokoto bucks after undergoing castration through Burdizzo, *in situ* spermatic cord ligation, and orchidectomy. Sixteen red Sokoto bucks aged 6 months to 1 year, weighing between 11-12 kg, were randomly assigned to four groups, each comprising four bucks: Group A (Burdizzo), B (*in situ* spermatic cord ligation), C (orchidectomy), and D (control). Following the castration procedures, the bucks were observed for behavioural changes, and scrotal circumference was measured. Testicular tissue sections were collected from bucks in Groups A and B at 3-, 6-, and 9-weeks post-castration, and processed for histological examination. The findings revealed diverse behavioural responses in Groups A, B, and C, including back arching, vocalization, teeth grinding, altered posture, changes in appetite, and dorsal lip curling. There was a significant ($p < 0.05$) progressive decrease in mean scrotal circumference in Groups A, B, and C. Histologically, the testes exhibited empty seminiferous tubules with fibrous architecture from three to nine weeks post-castration in Groups A and B. Castration via Burdizzo, *in-situ* spermatic cord ligation and orchidectomy induced distinct behavioural responses and changes in scrotal circumference in the red Sokoto bucks. The behavioural responses observed in the present study were noted to be mild in Group A, while Groups B and C presented moderate responses, thus, suggesting a varying degree of pain induction. This implies that Burdizzo castration induced less pain when compared to *in-situ* spermatic cord ligation and orchidectomy. Testicular histo-architecture was altered by Burdizzo and *in-situ* spermatic cord ligation in red Sokoto bucks. Therefore, the choice of castration method for red Sokoto bucks should be based on the specific characteristics of interest, availability of equipment, convenience, and technical efficiency.

Keywords: Behavioural responses; Castration; Scrotal circumference; Testicular histo-architecture; Red Sokoto bucks

INTRODUCTION

Goat production plays a vital role in global agriculture, providing valuable products and supporting the livelihoods of many (Nguyen *et al*., 2023). Goats are versatile animals that contribute to the livelihoods of millions of people worldwide (Devendra, 2013). Castration is a common practice in goat farming, especially for male goats that are not intended for breeding or specific purposes (Ahmed *et al*., 2023). The process involves the removal of the testicles, resulting in a male goat (buck) that is sterile and generally more manageable (Needham *et al*., 2017). Castration of goats is performed for several reasons including behavioural management, improvement of meat quality, prevention of uncontrolled breeding, ease of handling and reduction of odor amongst others (Ahmed *et al*., 2023). Castration can be achieved through surgical means, banding or Burdizzo clamp (Coetzee *et al*., 2010). The age of castration, hygiene and sterility, and pain management constitute the considerations for castration in goats (Underwood *et al*., 2015; Chen *et al*., 2022).

Goats, like many other animals, may exhibit various behavioural changes when experiencing pain (Ajuda *et al*., 2020). Thus, the recognition of these signs is essential for prompt intervention and mitigation (Steagall *et al*., 2021). Understanding the behavioural responses to pain in bucks is crucial for ensuring their welfare and implementing appropriate pain management strategies (Molony *et al*., 2002; Fonseca *et al*., 2023). Also, the observation and documentation of variations in post-operative behaviors would contribute to the refining and optimization of castration practices (Ajuda *et al*., 2020).

Scrotal circumference refers to the measurement around the widest part of the scrotum, encompassing both testicles, and it is usually expressed in centimeters or inches (Perumal, 2014). The monitoring and management of scrotal circumference in goats are essential components of successful goat breeding programs (Tirpan *et al*., 2020). Breeders and goat farmers use this measurement to make informed decisions about the selection of bucks,

contributing to improved reproductive performance and overall herd productivity (Alemayehu *et al*., 2021; Hussein *et al*., 2023). The impact of various castration methods on scrotal circumference in bucks remains an understudied area.

The testicular histoarchitecture in goats refers to the microscopic structure of the testes, and this is crucial for understanding the reproductive physiology of male goats (Gofur *et al*., 2023). The testes are responsible for the production of sperm and hormones essential for reproduction (O'Hara and Smith, 2015). Examination of the testicular histoarchitecture following different castration methods is crucial for evaluating the long-term effects on reproductive function (Martins-Santos *et al*., 2017; Hamed *et al*., 2023). This knowledge is vital for developing informed recommendations on the selection of castration methods in diverse goat farming scenarios.

Addressing the knowledge gap in the behavioural responses, scrotal circumference changes, and testicular histoarchitecture of male goats undergoing different castration methods is essential for advancing both animal welfare and agricultural practices. Therefore, in this study, we assessed the behavioural responses, scrotal circumference, and testicular histo-architecture of Sokoto red bucks after undergoing castration through Burdizzo, *in situ* spermatic cord ligation, and orchidectomy.

MATERIALS AND METHODS

Location of the Study

This study was conducted in the Large Animal Surgery Unit, Department of Veterinary Surgery and Radiology, Ahmadu Bello University (A.B.U.) Zaria, Kaduna State, Nigeria.

Ethical Statement

The approval for the use of bucks in this study was granted by the Ahmadu Bello University Committee on Animal Care and Use (ABUCAUC/2022/047), A.B.U. Zaria.

Experimental Animals

This study involved sixteen (16) Sokoto red bucks, aged between 6 months and 1 year, with weights ranging from 11 to 12 kg. The bucks were sourced from Giwa market, Giwa Local Government Area, Kaduna State, and were housed in the small ruminant pens at the Department of Veterinary Surgery and Radiology, A.B.U. Zaria. Prior to arrival of the bucks, these pens were thoroughly cleaned, disinfected, and treated with insecticide. After their arrival, the bucks were stabilized and allowed a two-week acclimatization period. They had continuous access to feed and water, with the provided feed consisting of groundnut hay, bean husks, and maize offal.

Grouping of Bucks

After the acclimatization period, the bucks were randomly allocated into four groups, each comprising four animals (Groups A, B, C, and D). Castration methods differed among the groups: Bucks in Group A underwent castration using Burdizzo, B were subjected to castration via *in situ* spermatic cord ligation, C were castrated using orchidectomy, while Group D served as the control and were not castrated.

Procedure for Burdizzo Castration

This was performed using the Burdizzo castrator (Agri Health castrator; Agri Health Ltd, Ireland) as described by Olaifa and Opara, (2011). After proper restraint of each buck, the hind limbs were spread apart, and the scrotal area was exposed to the surgeon. Castration was achieved by applying the Burdizzo laterally onto the scrotal neck. The first finger and thumb were used to hold the cord laterally in the scrotal neck, while the second hand slowly directed the position of the jaws until they were about 8-10 mm apart to grip the skin and cord firmly. The surgeon ordered and maintained rapid closure for 15-30 seconds while ensuring that the cord was properly crushed.

Procedure for *in-situ* Spermatic Cord Ligation

In-situ spermatic cord ligation was carried out following aseptic preparation of the skin enveloping the spermatic cord (Ponvijay,2007). Each buck was restrained on the surgical table in lateral recumbency, and local anaesthesia was achieved through a linear subcutaneous infiltration of 1 mL of 2% Lidocaine HCl on each lateral aspect of the scrotum. Non-absorbable suture material (Nylon size 2/0), (Anhui Kangning Industries, China) was used for a double external trans-fixing ligation of the entire spermatic cord, 2 cm apart. The procedure was repeated on the other cord.

Procedure for Orchidectomy

Orchidectomy was performed as described by Malbrue and Zorilla (2018) with modifications. The scrotal area was shaved, and scrubbed with soap and water, and chlorhexidine (Saro Life Care Limited, Nigeria) was applied to disinfect. The buck was sedated intramuscularly with 0.05 mg/kg Xylazine (Bioveta, Komenskeho, Czech Republic). A linear subcutaneous infiltration of 2% lidocaine HCl (Afirst life science Ltd, India) was achieved at 4mg/kg on the lateral aspect of the scrotal sac and was used to achieve local anaesthesia. Each goat was restrained on the surgical cradle in dorsal recumbency. The scrotum was then grasped, and a vertical incision through skin and fascia at the lateral part of the scrotum was made. This allowed the exteriorization of one of the testicles which were stripped off the vaginal tunic with a gauze sponge. The spermatic cord was ligated in three places with an absorbable suture ligature (Chromic catgut size 2/0, Anhui Kangning Industries, China), which was covered by a vaginal tunic. The spermatic cord was cut 1 cm below the ligature and the stump was checked for bleeding. Similarly, the opposite testicle was removed.

Observation of Behavioural Responses

The bucks were observed for behavioural responses which were indicative of pain (Fonseca *et al*., 2023). The indicators used were back arching, vocalization, teeth grinding, posture, change in appetite, and dorsal lip curling. The number of bucks displaying each response in each group were counted, and the grade were as follow; 0 – no response, 1 buck displaying the response – mild, 2 bucks displaying the response – moderate, 3 bucks displaying the response – severe, and 4 bucks displaying the response – very severe.

Measurement of Scrotal Circumference

The scrotal circumference was measured, using a measuring tape, 24 hours before castration in groups A and B, and then weekly up to nine weeks. Both testicles were manipulated by downward massage so that they were completely within and against the lowest point in the scrotum, lying side by side, and with no evidence of wrinkling of the scrotum. The testes were then held firmly in the scrotum with one hand (usually the measurement is right-handed). The other (usually left) hand is moved from the side and above to encircle the scrotal neck gathering up any loose scrotum and finally holding both testes firmly into the lower scrotum. The thumb of the hand holding the neck of the scrotum was not allowed to cause any pressure on the middle of the scrotum. Then the tape was looped around the testes and placed at the level judged to have the largest circumference. The tape was drawn firmly in contact with the entire circumference to cause moderate indentation of the scrotum.

Collection of Testes for Histology

Testes were surgically removed from bucks in groups A and B at weeks 3, 6 and 9 post-castrations. Thereafter, testicular sections were collected in 10% buffered neutral formalin and processed for histological examination (Luna, 1968).

Data Analyses

Data were presented in a table, chart, and photomicrographs. Scrotal circumferences were expressed as mean and standard error of the mean (mean ± SEM) and subjected to students t-test. Graph pad prism version 5.0 (San Diego California, USA) was used for the analysis. Values of $p \leq 0.05$ were considered significant.

RESULTS

Behavioural responses

There was moderate back arching only at 0- and 4-hours post-castration in Groups A, B and C, and this was not further observed for the five days observation period. There was moderate vocalization at 0- and 4-hours post-castration in Groups B and C, and none for the five days observation period. It was observed that Group A did not show any signs of vocalization within the period under observation (Table 1).

All the castrated groups showed mild teeth grinding at 4 hours post-castration but none from day 1 to 5 of observation. There was moderate altered posture in Group C at 0 hour, 4 hours, and a mild altered posture from day 1 to 5 (standing and lying down, feet stamping). In Group B, no abnormal posture was observed at 0 hour, but a mild altered posture was observed at 4 hours and day one to day five. There was a mild change in posture at 0 hour and 4 hours and none observed from day 1 to 5 in Group A (Table 1).

There was moderate change in appetite at 0 to 4 hours and none from day 1 to 5 in Group C; no change in appetite at 0 hours, but a mild change at 4 hours and no change from days 1 to 5 in Group B; a mild change in appetite at 0 and 4 hours post-castration, and none from days 1 to 5 in Group A. Mild dorsal lip curling was exhibited by animals across all the groups at 0 and 4 hours post-castration and none from days 1 to 5 of observation (Table 1).

Table 1: Behavioural responses of Sokoto red bucks before and after bilateral castration using Burdizzo method, *in situ* spermatic cord ligation and orchidectomy.

Group	Procedure	Back arching	Vocalization	Teeth grinding	Posture	Change in appetite	Dorsal lip curling
A	Burdizzo castration	0 H -moderate	0 H -moderate	0 H - None	0 H - mild	0 H - mild	0 H - mild
		4 H -moderate	4 H -moderate	4 H - mild	4 H - mild	4 H - mild	4 H -moderate
		Day 1-5 -none	Day 1-5 –none	Day 1-5 – none	Day1-5 - none	Day 1-5 - none	Day 1-5 -none
B	*In situ* Spermatic	0 H -moderate	0 H - none	0 H - none	0 H -moderate	0 H - none	0 H - none
	cord ligation	4 H -moderate	4 H - none	4 H - mild	4 H -moderate	4 H - mild	4 H - mild
		Day 1-5 -none	Day 1-5 -none	Day 1-5 -none	Day 1-5 -mild	Day 1-5 - none	Day 1-5 - none
C	Orchidectomy	0 H -moderate	0 H -moderate	0 H - none	0 H -moderate	0 H - mild	0 H - mild
		4 H -moderate	4 H -moderate	4 H - mild	4 H -moderate	4 H - mild	4 H - mild
		Day 1-5 -none	Day 1-5 - none	Day 1-5 – moderate	Day 1-5 - mild	Day 1-5 - none	Day 1-5 - none
D	None	0 H - none	0 H - none	0 H - none	0 H - none	0 H - none	0 H - none
		4 H - none	4 H - none	4 H - none	4 H - none	4 H - none	4 H - none
		Day 1-5 - none	Day 1-5 - none	Day 1-5 - none	Day 1-5 - none	Day 1-5 - none	Day 1-5 - none

0 H = 0 hour, 4 H = 4 hours post-castration

Scrotal Circumference

Pre-castration, there was no statistically significant ($p > 0.05$) difference in the mean scrotal circumference in Groups A (20.00 ± 0.91 cm) and B (21.75 ± 0.85 cm) (Table 2). From 1 to 6 weeks post-castration, the mean scrotal circumference was significantly ($p < 0.05$) higher in Groups B (20.50 ± 0.87 cm; 13.00 ± 0.29 cm) compared to A (18.00 ± 0.41 cm; 12.00 ± 0.60 cm). This was followed by no significant ($p > 0.05$) differences in mean scrotal circumference of castrated goats from 7 to 9 weeks post-castration. However, the mean scrotal circumference showed progressive statistically significant ($p < 0.05$) decrease in Groups A and B from 1 to 9 weeks post-castration (Figure 1).

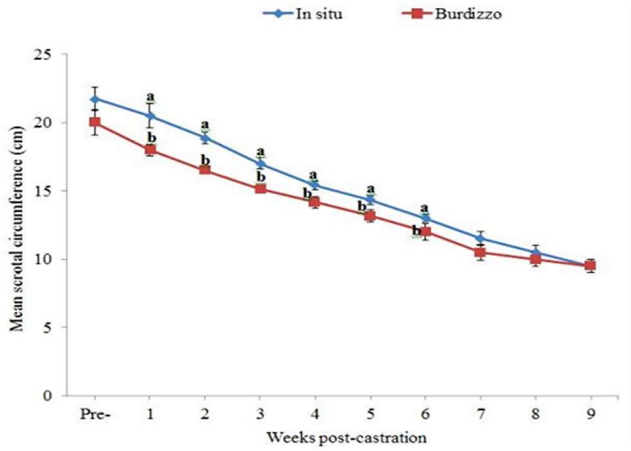

Figure 1: Mean scrotal circumference (cm) of red Sokoto bucks before and after bilateral castration using burdizzo method (Group A) and *in situ* spermatic cord ligation (Group B). Values with different alphabets in the same week differ significantly at $p < 0.05$.

Outcome of Histological Examination

The testes of bucks from control group showed intact histo-architecture and seminiferous tubules containing spermatozoa (Figure 2). In Groups A and B, the testes showed marked interstitial fibrosis, and shrinkage of seminiferous tubules at three, six and nine weeks (Figures 2 and 3).

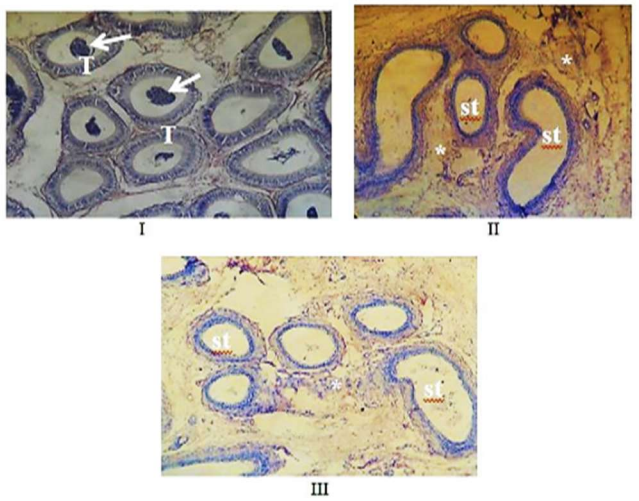

Figure 2: Photomicrographs of testicular sections from control (I), Burdizzo- (II) and *in situ* spermatic cord ligation-castrated (III) Sokoto red bucks at three weeks post-castration. Note intact seminiferous tubules (T) and spermatozoa (arrows) in I, and marked interstitial fibrosis (asterisks), and shrinkage of seminiferous tubules (st) in II and III. H & E x 200.

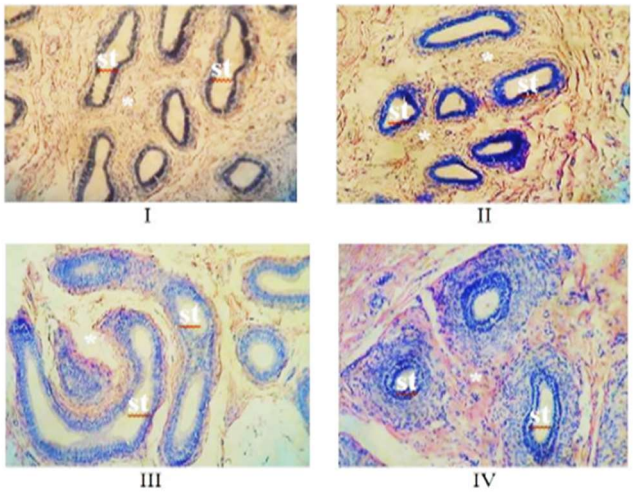

Figure 3: Photomicrographs of testicular sections from Burdizzo- and *in situ* spermatic cord ligation-castrated Sokoto red bucks at six (I and II respectively) and nine (III and IV respectively) weeks post-castration showing marked interstitial fibrosis (asterisks), and shrinkage of seminiferous tubules (st). H & E x 200.

DISCUSSION

The behavioural responses demonstrated by the castrated Sokoto red bucks in this study appear to be closely associated with the potential discomfort resulting from the performed procedures, as indicated by Brusin *et al*. (2022). Building upon the comprehensive investigation into pain-associated signs in goats conducted by Besson (1997), a myriad of indicators such as vocalization, abnormal standing posture, teeth grinding, tail swishing, altered facial expressions, decreased body weight or milk production, reluctance to move, reduced appetite, diminished grazing, kicking or stamping of feet, restlessness, head turning, limping, and depression were considered. Furthermore, Roughan and Flecknell (2003) suggested anorexia, vocalization, and a dull, depressed attitude as hallmark signs of pain in ruminants. Notably, the behavioural responses observed in the present study were noted to be mild in Group A, while Groups B and C presented moderate responses, thus, suggesting a varying degree of pain induction. This implies that Burdizzo castration might have induced less pain when compared to *in situ* spermatic cord ligation and orchidectomy. This variance could be attributed to the less invasive nature of Burdizzo castration, causing minimal disruption to tissues when compared to the other methods under consideration.

Moreover, the progressive decrease in scrotal circumference observed in Groups A and B, in contrast to their respective pre-castration values, raises intriguing possibilities of testicular atrophy. This potential atrophy could be a consequence of the reduced blood and nutrient supply to the testes, stemming from the ligation (*in situ* spermatic cord ligation) and crushing (Burdizzo) of the spermatic cord, as suggested by Cohen *et al*. (1985). This decrease might have subsequently led to ischaemic necrosis (Miller and Zachary, 2017) of the testes, fibrous tissue deposition, shrinkage, and,

consequently, a decrease in scrotal circumference. This nuanced exploration of the physiological changes following castration sheds light on the intricate interplay between surgical methods and subsequent outcomes.

On histology, the testes of bucks in Groups A and B exhibited intriguing characteristics, with degenerated and empty seminiferous tubules displaying a fibrous architecture at three, six-, and nine-weeks post-castration. These histological alterations accentuate the decreased blood and nutrient supplies to the testes due to the ligation (*in situ* spermatic cord ligation) and crushing (Burdizzo) of the spermatic cord (Cohen *et al*., 1985). The induced ischaemic necrosis might have triggered a cascade of events, including necrosis of the germinal epithelium, complete absence of spermatogenesis, and the replacement of interstitial tissues by fibrous connective tissue. Interestingly, these findings parallel those reported by Ramadan *et al*. (2014), who investigated pinhole castration by *in situ* spermatic cord ligation in dogs. Therefore, the nuanced examination of the testicular histo-architecture post-castration in this study implies that castration was effectively achieved through *in situ* spermatic cord ligation and the Burdizzo method, thus, offering valuable insights into the intricacies of the employed techniques and their consequences on testicular structure and function over time.

CONCLUSION

Castration via Burdizzo, *in-situ* spermatic cord ligation, and orchidectomy induced distinct behavioural responses and changes in scrotal circumference in the red Sokoto bucks, the behavioural responses observed in the present study were noted to be mild in Group A, while Groups B and C presented moderate responses, thus, suggesting a varying degree of pain induction. This implies that Burdizzo castration induced less pain when compared to *in-situ* spermatic cord ligation and orchidectomy. Also, testicular histo-architecture was altered by Burdizzo and *in-situ* spermatic cord ligation in red Sokoto bucks. Therefore, the choice of castration method for red Sokoto bucks should be based on the specific characteristics of interest, availability of equipment, convenience and technical efficiency.

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Conflict of Interest

The authors have no conflict of Interest to declare.

Authors' Contribution

NTO, OO and AJT were involved in carrying out the Surgical procedures and writing of the manuscript. OO analysed the data. All authors reviewed, read, and approved the manuscript.

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