

1. INTRODUCTION

Castration is one of the most common surgical procedure in animals and in broad sense it is to make the animal sterile by any mean, which involve non-invasive, minimally invasive or invasive methods. Non-invasive and minimally invasive procedures result in ischemia with subsequent necrosis and atrophy of the testicles, whereas, invasive procedure is the removal of the testicles or orchidectomy/gonadectomy.

It is performed to decrease the aggressive behaviour, make the animal docile, easier to handle and also to prevent the unwanted mating and mounting activities or for certain testicular/inguinal pathologies (Edwards, 2008).

In meat producing animals, it is performed to improve meat quality and taste, feed efficiency, reduction in male aggressive behaviour (Anderson, 2007). Goats are castrated to prevent unwanted breeding resulting in genetic defect, unwanted pregnancies, enhance body weight gain and to reduce male associated odours and goaty smell from the meat (Merkel and Dawson, 2008).

Male goat has strong smell (buck odours) during the breeding season, due to the effect of the androgen hormones produced by special glands of skin, cornual gland area and nose. The taste of meat is also affected and differs from castrated animals (Van Lancker *et al.*, 2005). In cattle, male calves are castrated to make them docile and to improve their draught ability.

Many techniques are used for castration depending on the species of the animals, age, purpose of castration, testicular anatomy and surgeon's choice. The conventional techniques include physical methods which result in removal, irreversible damage or destruction of the testis such as application of elastrator bands or rubber rings, use of Burdizzo's clamp and surgical removal (by open, closed or modified closed techniques) (Coetzee *et al.*, 2010).

Chemosterilization includes injection of sclerosing or toxic agents like 3 per cent formalin in the testicular parenchyma (Al-Asadi and Al-Kadi, 2012). A variety of chemical agents and mixtures have been studied in ruminant such as lactic acid, silver nitrate, calcium chloride, ethanol and phenol, which are injected intratesticularly (Fordyce *et al.*, 1989 and Canpolat *et al.*, 2006).

In immunocastration, immunization against Gonadotrophin-Releasing Hormone (GnRH) or Luteinizing Hormone Releasing Hormone (LHRH) is performed to induce antibody against GnRH, resulting in decrease production of endogenous hormones (Ulker *et al.*, 2009).

Each one of these techniques has advantages and disadvantages. Recently many developments have appeared to simplify the technique and to perform comparatively less painful castration in animals.

In-situ spermatic cord ligation (Pinhole castration) has been described as a novel minimally invasive technique for male calf and buck sterilization, as the spermatic cord is ligated without incising or involving the scrotum and integumentary sensory nerves are avoided (Ponvijay, 2007 and Okwee-Acai *et al.*, 2008). The technique hence, provokes less pain or stress to the animal compared to conventional castration techniques such as the Burdizzo's, elastrator bands or the standard open knife castration (Okwee-Acai *et al.*, 2012).

Looking to the need for minimally invasive castration methods and scarcity of literature, the technique of Pinhole castration (*In-situ* spermatic cord ligation) needs to be evaluated. Further, this technique can be beneficial in field conditions when conventional tools are not available. Therefore, the present study was planned with the following objectives:

1. To evaluate the Pinhole castration technique in bucks and cattle calves.
2. To compare Pinhole castration technique with Burdizzo's method.

2. REVIEW OF LITERATURE

In the recent years, many efforts were made to identify reliable methods of pharmacologic and chemical sterilization. However, surgical methods have remained the mainstay in various species (Howe, 2006). In the present survey, Burdizzo's method of castration was widely adopted to castrate the ruminants. Thus, the main objective of this study was to compare the conventional Burdizzo's method with minimally invasive Pinhole technique and to identify the better, easy and field oriented castration procedure for ruminants.

2.1 Castration

Andersson and Linde-Forsberg (2002) in their study noticed increase in the bodyweight of 47 per cent of 122 castrated dogs. Although it might not only due to primary regulators of metabolism but also gonadal hormones had influenced bodyweight either directly by acting on centres in the brain that regulate satiety and activity, or indirectly by altering metabolism at the cellular level (Salmeri *et al.*, 1991).

Thuer *et al.* (2007) performed non-invasive castration in seventy calves following lidocaine hydrochloride injection into the spermatic cords and scrotal neck. They reported that serum cortisol concentrations returned to baseline values within one hour of castration, and remained at those levels for the remainder of the 72 hours sampling period.

Al-Asadi and Al-Kadi (2012) reported chemosterilization with injection of sclerosing or toxic agents like three per cent formalin in the testicular parenchyma leading to irreparable damage and loss of function of testis in bucks but it required longer procedural time and technical skills and almost twice the healing time compared with surgical castration.

2.1.1 Burdizzo's method

Kent *et al.* (1993) compared different castration techniques in lambs and observed that closing the Burdizzo's clamp over the spermatic cord for atleast 10 seconds was enough for adequate castration (Anderson, 2007). Then the procedure was repeated approximately 1 cm below the previously

crushed line. So, excessive damage to the vas may be prevented by reducing the time of the Burdizzo's crushing the plexus.

Stafford *et al.* (2000) reported failures in calves castrated using Burdizzo's clamp by farmers due to inadequate crushing of spermatic cord (Ponvijay, 2007).

Stafford and Mellor (2005) concluded that the faulty Burdizzo's procedures may cause excessive crushing, leading to wound and subsequently maggots infestation, suppuration, necrosis and gangrene or even irreparable damage to urethra. Few complications may prove fatal.

Burdizzo's procedure required costly instrument whereas, Pinhole castration technique of percutaneous spermatic cord ligation was a simple, minimally invasive, less painful and field applicable alternative technique of castration in rams. Therefore, it could be adopted by field veterinarians of developing nations as many of their units lack Burdizzo's emasculotome (Fazili *et al.*, 2009).

2.1.2 Pinhole technique

Nickel *et al.* (1973) and Jana *et al.* (2005) observed that standard surgical castration procedure took thrice longer time than a Pinhole procedure in goats. Surgical castration required a lot of skill and experience while Pinhole technique was simple as the spermatic cord was easily accessible and could be manipulated within the scrotum and was also very easy to ligate inside the skin.

Mann and Constatinescu (1998) and Fazili *et al.* (2009) even without using antibiotics after Pinhole castration in stray dogs did not detected any infection, when the scrotal neck area was properly prepared and aseptic measures were strictly followed during procedure. Use of chlorhexidine instead of povidone-iodine for scrotal disinfection also minimized the chances of scrotal dermatitis.

Following experimental torsion and ligation of the spermatic cord in rats, Bergh *et al.* (2001) concluded that acute ischemia and coagulative necrosis resulted was responsible for irreversible testicular dysfunction because acute testicular ischemia for as little as five minutes was sufficient to produce

irreversible damage to the germinal epithelium through apoptosis. They hypothesized that ligature pressure for 12-48 hours could lead to permanent sterility in larger animals.

Brenda (2002) and Okwee-Acai *et al.* (2008) observed surgical castration four times costlier than the Pinhole method in bucks. It was advantageous, as required mild sedation and a surgical needle loaded with non absorbable suture material to ligate the spermatic cord within the scrotal skin.

Tibary and Van Metre (2004) concluded that Pinhole procedure should be performed in mature bucks instead of surgical castration because achieving proper anaesthesia and haemostasis was difficult due to large size of testicular cord at that age.

Fazili *et al.* (2009) did not record any castration failure in 12 Pinhole castrated rams due to sufficient tightness of the suture around the spermatic cord resulting in effective ischemia. To ensure complete compression of the spermatic artery, double ligation at two places bilaterally could also be practiced.

Ramadan *et al.* (2014) compared prescrotal castration technique (closed method) with the Pinhole technique as an economic and noninvasive method but it was more stressful, needed more professional experience, required longer postoperative management of twenty eight days and failed to induce necrosis of the testis of dogs *in situ*. The prescrotal castration technique proved advantageous than pinhole technique in dogs as it was less painful, effective, simple and require only ten days postoperative care for male dog sterilization.

2.2 Clinical observations and evaluation

2.2.1 Rectal temperature, heart rate and respiration rate

Smith *et al.* (1999) on observation of clinical response to stress and postoperative pain in cats, noticed no significant differences in physiological parameters such as heart rate, respiration rate and rectal temperature after surgery and at different time intervals. So, these variables cannot be considered useful in the recognition of postoperative pain.

Comparison of surgical sterilization with laparoscopic sterilization was done by Mahalingam *et al.* (2009) in 10 dogs and they observed normal

respiration in all animals throughout the observation period of one week. Significant decrease in respiration rate and heart rate immediately after the operation was observed in both groups. The mean rectal temperature remained within the normal range even after the surgery.

Okwee-Acai *et al.* (2012) in surgically castrated pups, noted a marked rise in mean rectal temperature on second day which dropped to near pre-treatment values by the 4th post operative day. Whereas, the rectal temperature of Pinhole castrated pups, returned to reference values after the 6th post castration day.

Abid and Al-Baghdady (2013) encouraged to adopt Pinhole technique as, it was simple, less painful, economic and could be performed when the animal was in standing position, lateral recumbency, vertical holding or restrained on its rump. Only a small quantity of local anaesthetic was required, further it needed no specialized equipments or skilled persons.

2.2.2 Inflammatory signs

Kent *et al.* (1995) observed discomfort, acute pain and inflammation following Burdizzo's castration in lambs for first three hours of crushing the spermatic cord, if the procedure was performed without anaesthesia.

Stafford *et al.* (2002) reported that surgical castration resulted in significantly increased level of plasma cortisol concentration indicating stress with pain to the animal. However, such increased levels were not observed with Pinhole castration. They concluded that Pinhole castration is a novel, minimally invasive and less stressful procedure.

Thuer *et al.* (2007) preferred Burdizzo's method because they observed significant abnormal posturing in rubber ring castrated calves for first week after application and exhibited signs of pain on scrotal palpation for 4 weeks longer than those castrated using Burdizzo's clamps.

Okwee-Acai *et al.* (2008) reported maximum swelling in the *insitu* ligated caprine testis on day 2 post castration that subsequently reduced below to the pre-ligation value after observation period of one month.

Fazili *et al.* (2009) reported Pinhole as less painful procedure, where the signs of acute pain disappeared half an hour after application of ligation and no chronic pain was further recorded during the 30 days of observation by touching or palpating the scrotum, while Burdizzo's castration induced higher levels of acute pain.

Majority of rams castrated with Pinhole technique by Fazili *et al.* (2009) were observed with mild oedema on palpation of scrotal area and slight discomfort during first week of ligation that subsided gradually.

2.3 Hematological estimations

Murata (1997) reported significant increase in circulating white blood cells and neutrophils while significant reduction of lymphocytes following Burdizzo's castration in 3 to 4 months old bull calves. These values returned to baseline by seven days after castration. These changes were likely due to reactive leukocytosis and stress.

Earley and Crowe (2002) concluded that, surgical castration results in increased haptoglobin and decreased gamma-interferon production. Haptoglobin exerted a suppressive effect on lymphocyte function, and reduction of gamma-interferon production result in suppression of the immune system's cell-mediated immunity and response to antigens. So, their study indicated that castration-associated leukocyte depression may be limited or eliminated by pre-surgical administration of a local anesthetic and a systemic analgesic.

Mohammad *et al.* (2008) observed significant elevation in mean total leucocyte count one day after surgery in eight surgically castrated lambs, which continued to be elevated throughout the study period of seven days. Total neutrophils was reported to be increased significantly on day 3 post-castration while lymphocytes significantly decreased. Both values returned to normal on 7th day post castration. The percentages of monocytes, eosinophils and basophils in the blood did not change significantly during the observation period.

2.4 External testicular morphology

Ijaz *et al.* (2000) noticed significant reduction in the mean weight, length, width and circumference of ligated testes at 30th postoperative day when

compared to control goats. Similar findings were also observed in pigs, lambs and calves following chemical castration.

Ponvijay (2007) in Pinhole castrated calves, after the first week, typically between 15 to 20 days, observed progressive reduction of approximately 50 per cent when compared to normal size of testis as evaluated by manual palpation.

Okwee-Acai *et al.* (2008) performed Pinhole castration in bucks and reported significant increase in mean scrotal circumference from 21.3 cm before castration to 25.3 cm on 2nd day after castration. However, mean scrotal circumference subsequently reduced significantly from 22.3 (day 7) to 19.8 cm (day 14).

Munahi and Abid (2011) compared spermatic cord ligation and spermatic cord torsion in buck. On macroscopic examination of castrated testicle, they further observed significant decrease in length and girth of testicular mass, which was attributed to stoppage of blood supply to the testicular tissue leading to atrophy of testicular parenchyma.

Baba *et al.* (2012) performed Pinhole castration in twelve stray dogs and recorded that mean scrotal circumference and mean testicular volume were found maximum and minimum on day 3 and 28 post castration respectively. Significantly higher values on day 3, 7, 14, 21 and significantly lower values on day 28 were obtained in comparison to intact dogs, respectively. The mean testicular volume revealed 40.57 per cent reduction of the control value. It was calculated by using the standard formula (Length × Width × Height × 0.71).

2.5 Ultrasonographic findings

Ganem *et al.* (1999), Quane and Kidney (2000) and Ragheb and Higgins (2002) observed testicular microliths on scrotal ultrasonography of humans and concluded it to be a rare entity in which there were numerous punctate calcifications of less than 3 mm within the testicular parenchyma. These calcifications resulted from degeneration of the cells lining the seminiferous tubules. The ultrasonographic appearance using a high-frequency transducer

depicted multiple diffuse echogenic foci with no acoustic shadowing. It had been associated with testicular atrophy, infertility, torsion, or testicular neoplasms.

Paltiel *et al.* (2002) concluded that ultrasound method of testicular volume measurement was more accurate and precise than orchidometry in dogs. The formula of $L \times W \times H \times 0.71$ provides a superior estimate of testicular volume and should be used in clinical practice.

2.6 Histopathological examination

Fazili *et al.* (2009) performed histological examination of the castrated testis of rams after six months of cord ligation. They observed the seminiferous tubules of these atrophic testis were devoid of spermatogonial cells and were either filled with eosinophilic debris or revealed calcification of the necrotic epithelium. The interstitial connective tissue was highly thickened. Areas of coagulative necrosis with infiltration of inflammatory cells at the periphery were also noticed.

On histological assessment of testicular residues in lambs and calves after Burdizzo's castration, Stoffel *et al.* (2009) observed severe necrosis and complete destruction of testicular tissue due to ischemia after ligation of spermatic cord with no evidence of spermatogenesis and viable leydig cells.

Abu-Ahmed *et al.* (2012) after twelve days of vascular occlusion in dogs confirmed replacement of seminiferous tubules by an 'amorphous mass' and absence of basement membrane. Other undesirable findings were interstitial edema and suppurative inflammation that could be due to infection during spermatic cord ligation.

Based on histopathological examination of the sections from the remnants of the testes in 12 Pinhole castrated dogs, Baba *et al.* (2012) observed degeneration of spermatogonial cells, hyalinization, thickening of the interstitium and atrophy of seminiferous tubules on 28th postoperative day.

Abid and Al-Baghdady (2013) performed Pinhole castration in bucks and on histological examination concluded massive destruction of cellular structures of the castrated testis. The seminiferous tubules were seen less in number and its lining cells appeared suffering from coagulative necrosis with

desquamation in the lumen of the tubules resulting in complete hyalinization of tubules with no signs of spermatogenesis.

Ramadan *et al.* (2014) reported that the testicular ischemia for upto four hours in dogs resulted in ceasation of spermatogenesis and damage to Sertoli and Leydig cells (Smith, 1955). While ten hours or more of sustained ischemia results in elimination of all Leydig cells and replacement of the testicular elements by fibrous connective tissue (Dixit, 1977).

2.7 Complications

In the survey by Stafford *et al.* (2000), reported potential complications associated with castration. These included hemorrhage, excessive swelling or edema, infection, poor wound healing, failure of castration and even death. Use of the Burdizzo's clamp may be associated with a higher failure rate mostly caused by operator's error. Risk of hemorrhage was greater after surgical castration.

Okwee-Acai *et al.* (2013) performed mass Pinhole castration in 278 male dogs and reported complications in 23 (8.30%) dogs after 2-3 days of castration. The common complications were painful swelling of the testes and dullness of the treated animals. Two dogs (0.72%) were reported to have continued mating even three months after castration, and observed with unilateral testicular atrophy. Histopathological examination of the non atrophic testes revealed the tissue architecture typical of a viable testis. Generally, the frequency of complications increased with the age of the dog at the time of castration.

3. MATERIAL AND METHODS

3.1 Location and place of work

The research work was carried out in the Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Complex (TVCC), Amanala Goat Farm, Composite Livestock Farm, Adhartal, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U.) and Dayodaya Pashu Samvardhan avam Paryavaran Kendra (Gowshala) Tilwara, Jabalpur (M.P.).

3.2 Meteorological data and features of place

Jabalpur is situated at 23.17° latitude and 79.57° East longitudes at 410.87 mean sea level in the southern part of second agro-climatic zone, including Satpura Plateau and Kymore hills. It has a tropical climate having average rainfall of 1241 mm.

3.3 Study period

The study was performed for a period of nine months from August, 2015 to April, 2015.

3.4 Animals

The study was conducted on twelve bucks aged 3-6 months and twelve cattle calves of 6 months to 1 year age presented for elective castration.

3.5 Experimental design

The animals included in the study were randomly divided into two equal groups of twelve each (Table 01).

3.5.1 Group I (n=12)

This group comprised of twelve bucks of age 3-6 months. It was further divided randomly into two subgroups (IA and IB) each comprised of six animals. In group IA, animals were castrated by standard Burdizzo's method while in group IB, Pinhole technique of castration was adopted.

3.5.2 Group II (n=12)

This group comprised of twelve cattle calves aged 6 months to 1 year. It was further divided randomly into two subgroups (IIA and IIB) each

comprised of six animals. In group IIA, animals were castrated by standard Burdizzo's method while in group IIB, Pinhole technique of castration was adopted.

Table 01: Treatment design

Treatment group	No. of animals	Castration procedure
Group-I (Bucks)	IA (n=6)	Burdizzo's method
	IB (n=6)	Pinhole technique
Group-II (Cattle calves)	IIA (n=6)	Burdizzo's method
	IIB (n=6)	Pinhole technique

3.6 Instrumentation

3.6.1 Ultrasound machine

The ultrasonographic examination was done by using real time B-mode, gray scale, ultrasound machine of Philips¹ Healthcare model HD7 XE with linear transducer (for bucks) or by Aloka² 500 SSD (portable machine for calves) with convex transducer (Plate 01).

Coupling media was applied on skin surface to ensure an intimate contact between the transducer and body surface. The images on the monitor were freezed, different measurements were recorded with the help of inbuilt caliper and saved. Interpretations of these images were made as described by Nyland and Mattoon (1995).

3.6.2 Bard Max core biopsy instrument

Automatic spring loaded Bard Max³ core biopsy gun (Plate 02) having needle size of 16G X 16 cm for calves or 18G X 16 cm for bucks with sample notch of 1.9 cm was used for tissue core biopsy of castrated testicle on 30th postoperative day.

1. Philips Healthcare model HD7 XE- Philips India Limited

2. Aloka 500 SSD- Hitachi Aloka

3. Bard Max- Bard Max core biopsy gun, Raksha Enterprises, New Delhi.

3.6.3 Burdizzo's castrator

Separate Burdizzo's castrator for bucks and calves (Plate 03) were used for castration as per the standard protocol.

3.6.4 Vernier calipers

The testicular measurements (length, width and thickness) were noted by Vernier callipers⁴ (Plate 04) and testicular volume was calculated by formulae ($L \times W \times T \times 0.71$).

3.6.5 Measuring tape

Scrotal circumference was measured using commercially available measuring tape (Plate 05).

3.7 Sterilization

All biopsy instruments were sterilized by placing them in a closed formaline vapourizing chamber for 24 hours. The instruments were removed from formaline chamber before biopsy, soaked in 1.5 per cent chlorhexidine solution⁵ for 30 minutes and then rinsed with sterile distilled water and wiped with dry sterile gauge.

The Burdizzo's castrator was disinfected using 5% Povidone iodine and 1% metronidazole solution⁶ before each application.

Surgical straight traumatic needle and black braided silk No. 1 or 2 were sterilized by autoclaving.

3.8 Castration procedure

3.8.1 Animal Preparation

Animal was restrained in lateral recumbency and scrotal area was prepared for aseptic procedure prior to surgery by shaving hair followed by thorough scrubbing and application of 5% povidone iodine and 1% metronidazole solution⁶ at the proposed surgical site.

4. Vernier callipers- Mitutoyo Vernier callipers, Japan

5. Savlon- Johnson and Johnson, Bangalore

6. Metronidazole 1% and Povidone iodine 5%- Metricare I.U. (30 ml)- Sarabhai Zydus Animal Health, Ahmedabad, India

3.8.2 Anaesthesia

Animals prepared for elective castration were sedated with Inj. Triflupromazine hydrochloride⁷ @ 0.2mg/kg intramuscularly followed by Local anaesthetic (Inj. Lignocaine hydrochloride⁸ 2% solution) infiltration at the neck of scrotum (Plate 06).

3.8.3 Castration procedure

Group - IA and IIA

In all the animals of these groups, the Burdizzo's castration procedure was performed under suitable anaesthesia as explained by Anderson (2007), each spermatic cord was hold and ensured that it was not slipped and clamped by Burdizzo's castrator for atleast 10 seconds. This procedure was then repeated with approximately 1 cm below the previously crushed line of the cord (Plate 07 and Plate 08).

Group - IB and IIB

In all the animals of these groups, Pinhole castration was performed under suitable anaesthesia as per the procedure described by Ponvijay (2007). After pulling the spermatic cord towards the lateral side of scrotal skin, threaded suture needle (black braided silk⁹ no. 1 or 2) was passed in a caudal to cranial direction adjacent to medial aspect of spermatic cord, leaving the suture in place. The needle was reintroduced (in reverse direction) through the previously made skin holes adjacent to the lateral margin of the released spermatic cord. The knot was tied after completing loop around the cord at the caudal aspect. Both suture strands were transacted 3.0mm from the skin. Thereafter, the needle holes were only visible immediately after submerging the knot inside the scrotal skin (Plate 09 and Plate 10).

3.9 Post operative care

The surgical area was dressed with 5 % povidone iodine and 1%

7. Injection triflupromazine hydrochloride 2%-Siquil, Zydus Animal Health Limited, Anand, Gujarat

8. Injection Xylocaine 2%- AstraZeneca Pharma India Limited, R.R. District

9. Black Braided silk No.1 or 2- Ethicon, Johnson and Johnson LTD, Aurangabad

metronidazole solution daily for three consecutive post operative days. Injection Amoxycillin sodium and Sulbactam sodium combination¹⁰ was administered @ 10 mg/Kg body weight intramuscularly twice daily and injection Meloxicam¹¹ was administered @ 0.3mg/Kg body weight intramuscularly once in a day for 3 days after castration.

3.10 Parameters of the study

3.10.1 Anamnesis

Complete history of the case was recorded including age and health status of the animal.

3.10.2 Clinical observations and evaluation

Rectal temperature (°F), heart rate (beats/minute) and respiration rate (breaths/minute) were recorded prior to the procedure and 30 minutes after castration. Inflammatory signs were recorded on the same day after castration, on 7th and 30th post castration days and were graded as per the method adopted by Bhowmick (2014).

Table 02: Grading score card for exudation, pain and swelling

Exudation, pain, swelling	Grade	Score
Nil	-	0
Mild	+	1
Moderate	++	2
Marked	+++	3

3.10.3 External morphology

Measurement of scrotal circumference by measuring tape, testicular length, width and thickness by Vernier calipers (Plate 11 and Plate 12) and volume by formulae ($L \times W \times T \times 0.71$) were carried out before castration and on 7th and 30th post castration days.

10. Inj. amoxicillin + sulbactam- Inj. Amoxirum forte- Virbac Animal Health India.

11. Inj. Melonex 0.5%- Intas Pharmaceuticals Limited, Ahmedabad

3.10.4 Ultrasonographic examination

Ultrasonography was performed for echotexture, width and thickness of the testicle were measured, before and on day 30th after castration.

3.10.5 Haematological estimations

Five millilitres of venous blood was collected aseptically from the jugular vein and transferred to vacutainer containing EDTA. Evaluation of the following parameters was carried out before and on 7th and 30th post-castration days.

- Haemoglobin (g/dl)
- Total leukocyte count ($\times 10^3/\mu\text{l}$)
- Differential leukocyte count(%)

The haematological parameters were estimated manually as described by Chauhan (2003).

3.10.6 Histological studies

For histological observations three biopsy tissue samples from each group were collected by Bard Max Core biopsy instrument on 30th day post-castration (Plate 13).

Immediately after collection, the samples were fixed in 10 per cent buffered formalin for minimum 72hrs. These fixed tissue samples were processed in acetone-benzene sequence, embedded and blocked in paraffin wax 50-60°C melting point (Lillie and Fullmer, 1976). Five to six micron (μm) thick sections were cut with the help of rotatory microtome (spencer), mounted on clean albuminized glass slides and stained with Haematoxylin and Eosin for general histomorphological structures and Van Gieson's stain for collagen fibres as per standard protocol given by Drury and Wallington (1980).

3.10.7 Complications:

Complications encountered during the study period were noted.

3.11 Statistical analysis

The quantitative data was analyzed by one-way ANOVA to compare the means value within a group and at corresponding intervals among different groups as described by Snedecor and Cochran (1994).

4. RESULTS

The study was conducted on 12 apparently healthy bucks and 12 cattle calves, which were divided into two equal groups. One group of animals (n=6) subjected to Burdizzo's method of castration whereas other group (n=6) of animals were subjected to Pinhole technique of castration in both the species.

4.1 Anamnesis

History of each animal was taken for normal feeding, watering, defaecation, urination, any previous illness or specific injury to external genitalia of the animal. Irrespective of management and breed, animals which were about to attain puberty were selected. The study included 12 apparently healthy bucks aged 3-6 months for group I and 12 cattle calves of age 6 months to 1 year for group II. All the animals of both the groups were maintained by the owner on traditional food including roughage with concentrate and were managed by themselves. Animal with any congenital genital abnormality were excluded from the study.

4.2 Clinical observations and evaluation

4.2.1 Rectal temperature, heart rate and respiration rate

The mean values for rectal temperature (°F) of bucks observed were within the normal range in both the groups IA and IB at both the intervals (Table 03). The temperature range in group IA (Burdizzo's method) was recorded with mean value of 102.56 ± 0.14 and 101.80 ± 0.17 °F before and 30 minutes after the castration, respectively. In group IB (Pinhole technique), the mean values were recorded 102.73 ± 0.26 and 101.95 ± 0.17 °F before and 30 minutes after castration, respectively. The values did not differ significantly ($p > 0.05$) within and between both the groups. None of the animal showed abnormal values of temperature at both the intervals.

The mean heart rate (beats/minute) for both the groups IA and IB were within the normal range at interval of 30 minutes (Table 04). The mean values for heart rate were observed 75.17 ± 0.91 before and 73.67 ± 0.91 beats/minute after 30 minutes of castration in bucks of group IA, whereas in group IB mean heart rate were recorded 75.50 ± 0.88 and 73.33 ± 0.88

beats/minute respectively. Mean heart rate decreased non-significantly ($p>0.05$) after the procedure in both the groups and did not differ significantly ($p>0.05$) between the groups.

The mean values of respiration rate (breaths/minute) in all bucks of group IA and IB remained within the normal range throughout the observation period and none of the animal showed any abnormal value (Table 05). The mean value of respiration rate recorded in group IA before castration was 34.66 ± 1.76 and 28.66 ± 0.98 breaths/minute recorded 30 minutes post castration. In group IB, 36.16 ± 1.42 breaths/minute before castration and 28.33 ± 2.09 breaths/minute after 30 minutes of castration was recorded respectively. A significant decrease ($p<0.05$) in respiration rate after 30 minutes of castration was observed in both the groups. No significant difference ($p>0.05$) was observed in respiration rate when compared between the groups.

The mean values for rectal temperature ($^{\circ}\text{F}$) in calves were observed within the normal range in both the groups IIA (Burdizzo's method) and IIB (Pinhole technique) at the interval of 30 minutes (Table 03). The mean rectal temperature recorded in group IIA was 102.10 ± 0.17 before castration and $101.33\pm0.14^{\circ}\text{F}$ after 30 minutes of castration. In group IIB, the mean mean rectal temperature was recorded respectively as 101.90 ± 0.16 before castration and $101.40\pm0.19^{\circ}\text{F}$ post-castration. The values did not differ significantly ($p>0.05$) between the groups.

The mean heart rate (beats/minute) recorded in the group IIA in calves was 68.00 ± 2.25 before castration and 63.33 ± 2.52 beats/minute 30 minutes after castration were (Table 04). Whereas in group IIB, mean heart rate was recorded 74.00 ± 1.77 and 69.00 ± 1.52 beats/minute before and 30 minutes after castration respectively. Mean heart rate was within the normal range at both the time intervals and non-significant decrease ($p>0.05$) 30 minutes of castration was noticed in both the groups and did not differ significantly ($p>0.05$) between the groups.

The mean values of respiration rate (breaths/minute) in all the calves in group II remained within the normal range throughout the observation

period of 30 minutes (Table 05). The mean value of respiration rate observed in group IIA was 27.50 ± 1.33 and 22.83 ± 1.32 breaths/minute recorded before and 30 minutes after castration respectively. Whereas in group IIB, mean respiratory rate was 30.83 ± 1.04 and 26.33 ± 0.80 breaths/minute recorded respectively. A significant decrease ($p < 0.05$) in respiration rate 30 minutes post-castration was observed in both groups. No significant difference ($p > 0.05$) was observed in respiration rate when compared between the groups at different time intervals.

4.2.2 Inflammatory signs

On the same day after castration, moderate pain on scrotal palpation was noticed in all the bucks of group IA castrated with Burdizzo's method (Table 06). However, with Pinhole technique mild pain was observed (group IB). On 7th and 30th post-castration days, no symptoms of pain were noticed in any of the animal in both the groups.

The calves on the same day after castration with Burdizzo's method (group IIA) exhibited moderate pain on scrotal palpation (Table 06). While, mild degree of pain was noticed in the animals with Pinhole technique (group IIB). At 7th and 30th post-castration days, no pain was detected in any of the calf.

In bucks, castrated with Burdizzo's method (group IA) moderate swelling (Table 07) on scrotal palpation was noticed. However, it was mild swelling in animals castrated with Pinhole technique (group IB). On observation of animals at 7th post-castration day, mild swelling in Burdizzo's group (group IA) and moderate swelling in Pinhole group (group IB) on scrotal palpation was observed. However, none of the animal had scrotal swelling on 30th day of castration.

After castration, moderate swelling (Table 07) on scrotal palpation was noticed in all the calves castrated with Burdizzo's method (group IIA). However, with Pinhole technique the scrotal swelling was of milder degree (group IIB). Observation of animals on 7th post-castration day, mild swelling in group IIA and moderate swelling in group IIB on scrotal palpation was recorded. On day 30th, the scrotal swelling had subsided completely.

No exudation was found at the scrotal area, in any of the species (bucks or calves) castrated with either methods, on 30 days of observation.

4.3 Haematological estimations

Five milliliters of blood was collected aseptically from external jugular vein in EDTA coated vacutainer prior to surgery and on day 7th and day 30th after surgery for estimation of haemoglobin (g/dl), total leucocyte count ($\times 10^3$ / μ l) and differential leukocyte count (%) to evaluate the health status of animals and to correlate with the inflammatory or degenerative changes.

4.3.1 Haemoglobin

The mean haemoglobin concentration for all the bucks in groups IA and IB were within normal range at each time interval (Table 08) and did not differ significantly ($p > 0.05$) within or between the groups.

In group IA, the haemoglobin concentration ranged from 9.8-11.4, 9.8-11.4 and 10.6-12.0 g/dl with mean values 10.66 ± 0.26 , 10.80 ± 0.27 and 11.13 ± 0.27 g/dl before castration and on 7th and 30th post castration days respectively. Whereas in group IB, the haemoglobin concentration ranged from 9.6-12.0, 9.4-11.8 and 10.0-11.8 g/dl with mean values 10.93 ± 0.35 , 10.86 ± 0.34 and 10.33 ± 0.29 g/dl observed before and after castration on 7th and 30th days respectively.

In calves of group IIA, the haemoglobin concentration ranged from 9.8-11.8, 9.8-11.2 and 10.4-11.6 g/dl with mean values of 10.66 ± 0.34 , 10.53 ± 0.22 and 10.86 ± 0.16 g/dl respectively, before castration and on 7th and 30th post castration days (Table 08). In group IIB, the haemoglobin concentration ranged from 9.6-11.4, 9.8-11.4 and 10.0-11.8 g/dl with mean values 10.53 ± 0.25 , 10.53 ± 0.34 and 10.76 ± 0.24 g/dl before castration and on 7th and 30th post castration days respectively. The mean haemoglobin concentration for all the calves in groups IIA and IIB were within normal range at each time interval and did not differ significantly ($p > 0.05$) within or between the groups.

4.3.2 Total leukocyte count

The mean total leucocyte count ($\times 10^3$ cells/ μ l) for groups IA and IB was within normal range at each time interval (Table 09) in bucks, but significant

increase ($p < 0.05$) in this count was noted on 7th day within the group when compared with the pre-castration values. These mean values returned to normal on 30th post-castration day. No significant ($p > 0.05$) deviation was noted between both the groups at different observation periods. In group IA the mean values of total leucocyte count before castration was 8.15 ± 0.36 and after castration observed were 9.68 ± 0.67 and 7.90 ± 0.21 ($\times 10^3$ cells/ μ l) on 7th and 30th day respectively. Whereas, in group IB, these values were 7.45 ± 0.29 , 9.38 ± 0.56 and 7.18 ± 0.37 before castration and on 7th and 30th post castration day respectively.

The mean total leucocyte count in calves for the groups IIA and IIB were within normal range at each time interval (Table 09) but significant increase ($p < 0.05$) was observed on 7th post castration day within the group and no significant ($p > 0.05$) deviation was noted between both the groups. In group IIA, the mean values of total leucocyte count were 7.76 ± 0.35 before castration, 8.88 ± 0.30 on 7th day and 7.13 ± 0.25 ($\times 10^3$ cells/ μ l) on 30th post castration day respectively. In group IIB, these values were depicted as 6.80 ± 0.20 , 8.61 ± 0.27 and 6.88 ± 0.19 before and on 7th and 30th post-castration day respectively. The mean values returned to normal on 30th post castration day.

4.3.3 Differential leukocyte count

In group IA and IB the mean values for differential leukocyte count were observed to be within normal range in bucks. The values did not differ significantly ($p > 0.05$) within or between the groups at different time intervals.

There was non-significant increase ($p > 0.05$) in the neutrophil count (%) from 36.00 ± 2.30 to 41.36 ± 1.24 in group IA and 36.83 ± 1.66 to 41.00 ± 1.21 in group IB from pre-castration period to 7th post-castration day respectively (Table 10). The lymphocytes count (%) showed non-significant decrease ($p > 0.05$) from 58.66 ± 2.48 to 55.16 ± 2.57 in group IA and 58.00 ± 1.63 to 55.16 ± 1.13 in group IB from pre-castration period to 7th post-castration day respectively (Table 11). These changes were transient and total neutrophil and lymphocyte count returned to normal pre-castration values on day 30th. The mean values for total neutrophil count were 36.16 ± 1.24 and 37.16 ± 1.40 , lymphocyte count were 59.16 ± 1.40 and 58.66 ± 1.28 in group IA and IB respectively on 30th post-

castration day. Total monocytes, eosinophils and basophils count did not show any significant ($p>0.05$) change throughout the study period of 30 days and remained within the normal range in both the group IA and IB (Table 12, Table 13 and Table 14).

In group IIA and IIB the mean values for differential leukocyte count were observed to be within normal range in calves. The values did not differ significantly ($p>0.05$) within or between the groups at different time intervals.

There was non-significant increase ($p>0.05$) in mean neutrophil count (%) from 30.66 ± 2.60 to 35.67 ± 2.67 in group IIA and 33.16 ± 1.77 to 35.00 ± 2.94 in group IIB from pre-castration period to 7th post-castration day respectively (Table 10). Mean lymphocytes (%) showed non-significant decrease ($p>0.05$) from 63.83 ± 2.54 to 60.66 ± 3.01 in group IIA and 61.16 ± 2.16 to 61.00 ± 2.78 from pre-castration period to 7th post-castration day respectively (Table 11). Total neutrophil and lymphocyte count returned to normal pre-castration values on day 30 of castration. In group IIA and IIB, the mean values for total neutrophil count were 29.00 ± 2.52 and 30.83 ± 1.04 , lymphocyte count were 64.00 ± 2.82 and 62.50 ± 1.38 (%) respectively on 30th post-castration day. Total eosinophils and basophils (Table 12 and Table 13) did not show any significant ($p>0.05$) change throughout the study period of 30 days and remained within the normal range in both the groups. Total monocyte remained within the normal range in both the groups but showed significant increase ($p<0.05$) on 30th day of castration, when compared to pre-castration and 7th day values (Table 14). In group IIA these values were 1.50 ± 0.42 , 1.33 ± 0.42 and 4.00 ± 0.73 , in group IIB 1.33 ± 0.49 , 1.33 ± 0.42 and 3.66 ± 0.55 observed at pre-castration period, on 7th and 30th post-castration day respectively.

4.4 External morphology

4.4.1 Scrotal circumference

The mean scrotal circumference (cm) for bucks in group IA, were observed to be 15.96 ± 0.83 , 17.36 ± 0.78 and 14.33 ± 0.77 cm before and on 7th and 30th post-castration days respectively (Table 15). That is, non-significant ($p>0.05$) increase from pre-castration period to 7th post-castration day in group IA

was noticed. Significant ($p<0.05$) decrease from pre-castration period and 7th post-castration day was noticed with 30th post-castration day value. In group IB, the mean scrotal circumference (Table 15) showed significant ($p<0.05$) increase from pre-castration period to 7th post-castration day. Thereafter, significant ($p<0.05$) decrease from pre-castration and 7th post-castration day was noticed on 30th day. Mean value were found 18.33 ± 0.54 , 20.41 ± 0.56 and 16.55 ± 0.55 cm before and on 7th and 30th day after castration respectively. When compared between both the groups, significant ($p<0.05$) difference in mean scrotal circumference was noted on 7th and 30th post-castration day.

The mean scrotal circumference in group IIA calves, showed non-significant ($p>0.05$) increase from pre-castration period to 7th post-castration day. Significant ($p<0.05$) decrease from both these periods was noticed with 30th post-castration value (Table 15). Mean value were found 21.18 ± 1.62 , 23.17 ± 1.67 and 17.20 ± 1.48 cm before and on 7th and 30th day after castration respectively. Significant ($p<0.05$) difference was found on comparison of both the groups. In group IIB, there was significant increase ($p>0.05$) in mean scrotal circumference from pre-castration period to 7th post-castration day. Significant ($p<0.05$) decrease from pre-castration and 7th post-castration value was noticed with 30th post-castration value. Mean values were found 19.75 ± 1.44 , 22.50 ± 1.61 and 16.80 ± 1.27 cm before castration and on 7th and 30th day after castration respectively.

4.4.2 Testicular measurements

The mean testicular measurements (length, width and thickness) of both the right and left testicle of group IA revealed non-significant ($p>0.05$) increase from pre-castration to 7th day post-castration day. Thereafter it significantly ($p<0.05$) decrease on 30th day of castration. Mean value of length (Table 16) were found 5.20 ± 0.19 , 5.24 ± 0.22 and 4.42 ± 0.21 cm, width (Table 17) 3.18 ± 0.16 , 3.17 ± 0.15 and 2.75 ± 0.19 cm, thickness (Table 18) was 3.40 ± 0.17 , 3.66 ± 0.20 and 2.71 ± 0.25 on pre-castration period, 7th and 30th post-castration day respectively. But significant ($p<0.05$) difference on 7th postoperative day was noted between Burdizzo's and Pinhole castrated bucks in group IA and IB

respectively, i.e., there was significant ($p<0.05$) increase in testicular measurements on 7th post castration day in group IB. The mean testicular measurements (length, width and thickness) of both the right and left testicle of bucks in group IB showed significant ($p<0.05$) increase from pre-castration period to 7th day and thereafter significant ($p<0.05$) decrease to 30th post-castration day. Mean value of length were found 5.86 ± 0.12 , 6.38 ± 0.19 and 4.85 ± 0.12 cm, width 3.80 ± 0.07 , 4.18 ± 0.07 and 3.25 ± 0.06 cm, thickness was 3.91 ± 0.07 , 4.33 ± 0.08 and 3.36 ± 0.08 before and on 7th and 30th post-castration days respectively.

The mean testicular measurements of calves (length, width and thickness) of both the right and left testicle of group IIA showed non-significant ($p>0.05$) increase from pre-castration to 7th post-castration day and there was significant ($p<0.05$) decrease from pre-castration and 7th day to 30th post-castration day. Mean value of length (Table 16) were found 7.35 ± 0.25 , 7.46 ± 0.31 and 5.85 ± 0.17 cm, width (Table 17) 3.37 ± 0.14 , 3.52 ± 0.17 and 2.86 ± 0.14 cm, thickness (Table 18) was 3.75 ± 0.09 , 3.91 ± 0.09 and 3.08 ± 0.09 before and after castration on 7th and 30th day after castration respectively. The mean testicular measurements (length, width and thickness) of both the right and left testicle of bucks in group IIB, revealed significant ($p<0.05$) decrease from pre-castration and 7th day to 30th post-castration day. But significant ($p<0.05$) increase from pre-castration value to 7th post-castration value was noticed. Mean value of length were found 7.73 ± 0.36 , 8.09 ± 0.38 and 5.94 ± 0.23 cm, width 3.40 ± 0.15 , 3.87 ± 0.19 and 2.99 ± 0.13 cm, thickness was 3.57 ± 0.14 , 3.82 ± 0.15 and 3.07 ± 0.13 cm before and on 7th and 30th day after castration respectively. When compared between both the groups, significant difference ($p<0.05$) was noted on comparison of testicular length, width and thickness on 7th post-castration day, which significantly increased ($p<0.05$) in the group IIB.

4.4.3 Testicular volume

The mean testicular volume (length x width x thickness x 0.71) of both the right and left testicle of group IA bucks (Burdizzo's method) revealed non-significant ($p>0.05$) increase from pre-castration value to 7th day and thereafter significant ($p<0.05$) decrease to 30th post-castration day (Table 19).

Mean values were found 42.57 ± 5.32 , 42.35 ± 5.86 and 26.80 ± 5.79 cm³ before, on 7th and 30th day after castration respectively. There was 37.04 per cent (mean) reduction of testicular volume on 30th post-castration day when compared from the pre-castration value (Plate 14). Whereas, in group IB, the bucks castrated with Pinhole technique show mean reduction of 39.01 per cent testicular volume on 30th post castration day (Plate 15). The mean testicular volume of both the testicle of group IB revealed significant ($p < 0.05$) increase from pre-castration period to 7th post-castration day. Significant ($p < 0.05$) decrease from pre-castration period and 7th post-castration day was noticed with 30th post-castration day value. Mean value were 62.77 ± 3.36 , 83.05 ± 4.69 and 38.28 ± 2.32 cm³ before and on 7th and 30th day after castration respectively. But significant ($p < 0.05$) difference in volume was depicted between 7th post-castration values of group IA and IB, i.e., there was significant ($p < 0.05$) increase in testicular volume in group IB.

The mean testicular volume (length x width x thickness x 0.71) of both the right and left testicle of calves in group IIA (Burdizzo's method) showed non-significant ($p > 0.05$) increase on 7th day when compared with pre-castration values. But significant ($p < 0.05$) decrease from pre-castration and 7th post-castration day values was noticed with 30th post-castration value (Table 19). Mean values were found 68.26 ± 6.88 , 75.55 ± 8.25 and 37.84 ± 4.00 cm³ before and after castration on day 7th and 30th respectively. There was 44.56 per cent (mean) reduction of testicular volume on 30th postoperative day when compared from the pre-castration value (Plate 16). However, significant ($p < 0.05$) difference was depicted between groups IIA and IIB on 7th post-castration day. In group IIB, the calves castrated with Pinhole technique showed mean reduction of 42.18 per cent (Plate 17) of testicular volume on 30th post-castration day on comparison with pre-castration value. The mean testicular volume of both the testicle showed significant ($p < 0.05$) increase from pre-castration period to 7th post-castration day. Thereafter significant ($p < 0.05$) was noticed on 30th post-castration value with pre-castration and 7th post-castration values. Mean values were observed

70.48±8.74, 88.61±11.05 and 40.75±4.78 cm³ before and on 7th and 30th day after castration respectively.

4.5 Ultrasonographic findings

In all examinations preoperatively, the echotexture of testes of bucks were discerned by the presence of very homogenous, granular pattern, a thin hyperechoic capsule and a hyperechoic central line called the mediastinum testis, a landmark for their identification during ultrasonography. No significant differences in echogenicity were detected on the ultrasonographic appearance of the testes among the studied animals of both the groups (Plate 18 and Plate 19). In group IA, mean value of width (Table 20) and thickness (Table 21) measured preoperatively in Burdizzo's method were respectively 2.73±0.16 and 2.80±0.20 cm. Whereas, in group IB (Pinhole technique) these mean values were 3.41±0.09 and 3.39±0.09 cm respectively.

On day 30, the castrated buck testis appeared to be smaller than the normal testis seen preoperatively. In longitudinal/frontal images, the parenchyma of testicle was slightly less echogenic with hyperechoic spots/testicular microliths were also depicted, which were may be small punctate calcifications with no acoustic shadowing and presumably representing the mediastinum testis, with a thick echogenic tunica albugenia (Plate 18). These findings were more evident in the Pinhole castrated group (Plate 19). The testicular parenchyma was not homogeneous but mottled in appearance. The epididymal tail was also atrophied but more echogenic than normal. In group IA, width and thickness measured were 2.44±0.18 and 2.56±0.24cm and in group IB, 2.97±0.11 and 2.97±0.10 cm at 30th postoperative day respectively (Table 20 and Table 21).

In bucks, the mean testicular measurements (width and thickness) of both the right and left testicle in group IA (Burdizzo's group) showed significant ($p<0.05$) decrease from 0 day to 30th postoperative day. In group IB (Pinhole technique), these mean testicular measurements showed significant ($p<0.05$) decrease from 0 day to 30th postoperative day. No significant ($p>0.05$) difference

was observed when compared different time intervals between both the groups IA and IB.

In calves, the echotexture of testes preoperatively were discerned by the presence of very homogenous, granular pattern, a thin hyperechoic capsule and a hyperechoic central line called the mediastinum testis, a landmark for their identification during ultrasonography. No significant differences in echogenicity were detected on the ultrasonographic appearance of the testes among the studied animals of both the groups (Plate 20 and Plate 21). In group IIA (Burdizzo's method), mean value of width and thickness measured preoperatively were respectively 3.26 ± 0.15 and 3.53 ± 0.13 cm (Table 20 and Table 21). Whereas, in group IIB (Pinhole technique) these mean values were 3.22 ± 0.16 and 3.36 ± 0.15 cm respectively.

On day 30, the castrated testis appeared to be smaller than the normal testis seen preoperatively. In longitudinal/frontal images, the parenchyma of testicle was slightly less echogenic with hyperechoic spots/testicular microliths were also depicted, which were may be small punctate calcifications with no acoustic shadowing and presumably representing the mediastinum testis, with a thick echogenic tunica albugenia. The testicular parenchyma was not homogeneous but was mottled in appearance. The epididymal tail was also atrophied but more echogenic than normal (Plate 20). In the Pinhole castrated calves these findings were more evident (Plate 21). In group IIA, width and thickness measured were 2.76 ± 0.14 and 2.97 ± 0.09 cm and in group IIB, 2.84 ± 0.12 and 2.84 ± 0.12 cm at 30th postoperative day respectively (Table 20 and Table 21).

In group IIA Burdizzo's castrated calves, the mean testicular width of both the right and left testicle showed significant ($p < 0.05$) decrease from 0 day to 30th postoperative day and significant ($p < 0.05$) decrease in mean testicular thickness was also observed. In group IIB, the mean testicular width and thickness of both the right and left testicle showed significant ($p < 0.05$) decrease from 0 day to 30th postoperative day. Ultrasonographic findings showed non-

significant ($p>0.05$) difference in reduction of width and thickness at different time intervals between the groups IIA and IIB.

4.6 Histological findings

In bucks, histological examination of the Burdizzo's and Pinhole castrated testicle on 30th day, revealed atrophy of seminiferous tubules with desquamation and obliteration by cell debris in the lumen. Destruction of testicular elements and spermatogonial cells with presence of dead spermatozoa in the central portion of some of the seminiferous tubules were evident. These spermatozoa were more in number in Burdizzo's castrated testicles (Plate 22).

Microscopic examination under high magnification revealed, no evidence of Leydig or Sertoli cells in the castrated testicular parenchyma of bucks in both the groups, but in the Pinhole technique vacuolations at the basal part with eosinophilic cellular remnants in the seminiferous tubules was evident (Plate 23).

The intertubular spaces showed the presence of collagen bundles, which were pronounced in Pinhole technique in comparison to Burdizzo's method (Plate 24 and Plate 25).

In calves, microscopic examination of seminiferous tubules on 30th post-Burdizzo's castrated testicle showed, obliteration of lumen with cellular debris. However, in some of the tubules the remnants of the spermatogenic cells were also observed. Under high magnification, few cells at basal region of seminiferous tubules showed the presence of picnotic nuclei (Plate 26).

In Pinhole castrated testicle of calves, the seminiferous tubules appeared hyalinized with cell debris completely filling the lumen. Under high magnification, nuclei of spermatogenic cells were not evident on the 30th postcastration day (Plate 27).

The collagen bundles in between the seminiferous tubules were more in Pinhole castrated calves (Plate 28 and 29).

4.7 Complications

On day 30 after castration, one buck castrated by Burdizzo's method (group IA) showed right testicular atrophy. However, there was increase in left testicular dimensions as observed ultrasonographically and manually using Vernier calipers. No signs of hydrocele or swelling were observed. There was no

decline in male characteristic features even after 30 days of castration, while other animals of the same group displayed diminished male nuisance behaviour (Plate 30). Animal was again castrated with Burdizzo's method and after 30 days, showed atrophy of the same testicle.

In group IB, clinical examination of one buck castrated by Pinhole technique, on 30th postcastration day revealed, unilateral atrophy of right testicle. However, there was no change in size of left testicle. Ultrasonography of the same testicle showed severe hydrocele with fibrin deposits in between layers of tunica vaginalis (Plate 31). Degenerative changes in form of microliths were also evident. There was reduction in male characteristic features. Animal was then sold by the owner.

Table 03: Mean rectal temperature (°F) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 hour	102.56±0.14	102.80±0.26	102.10±0.17	101.90±0.16
30 min	101.80±0.17	101.95±0.17	101.33±0.14	101.40±0.19

Table 04: Mean heart rate (beats/min) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 hour	75.17±0.91	75.50±0.88	68.00±2.25	74.00±1.77
30 min	73.67±0.91	73.33±0.88	63.33±2.52	69.00±1.52

Table 05: Mean respiration rate (breaths/min) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 hour	34.66 ^a ±1.76	36.16 ^a ±1.42	27.50 ^a ±1.33	30.83 ^a ±1.04
30 min	28.66 ^b ±0.98	28.33 ^b ±2.09	22.83 ^b ±1.32	26.33 ^b ±0.80

a,b- Values within and between the same treatment group with different superscript differed significantly (p<0.05) at different time intervals

Table 06: Degree of pain by arbitrary score card in both the groups at different time intervals

Time interval	Group I				Group II			
	IA		IB		IIA		IIB	
	Grade	Score	Grade	Score	Grade	Score	Grade	Score
0 day	Moderate (++)	2	Mild (+)	1	Moderate (++)	2	Mild (+)	1
7 day	Nil (-)	0	Nil (-)	0	Nil (-)	0	Nil (-)	0
30 day	Nil (-)	0	Nil (-)	0	Nil (-)	0	Nil (-)	0

Table 07: Degree of inflammation by arbitrary score card in both the groups at different time intervals

Time interval	Group I				Group II			
	IA		IB		IIA		IIB	
	Grade	Score	Grade	Score	Grade	Score	Grade	Score
0 day	Moderate (++)	2	Mild (+)	1	Moderate (++)	2	Mild (+)	1
7 day	Mild (+)	1	Moderate (++)	2	Mild (+)	1	Moderate (++)	2
30 day	Nil (-)	0	Nil	0	Nil (-)	0	Nil	0

Table 08: Mean haemoglobin (g/dl) of animals of both the groups at intervals different

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	10.66±0.26	10.93±0.35	10.66±0.34	10.53±0.25
7 day	10.80±0.27	10.86±0.34	10.53±0.22	10.53±0.22
30 day	11.13±0.27	10.33±0.29	10.86±0.16	10.76±0.24

Table 09: Mean total leucocyte count ($\times 10^3/\mu\text{l}$) of animals of both groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	8.15 ^a ±0.36	7.45 ^a ±0.29	7.76 ^a ±0.35	6.80 ^a ±0.20
7 day	9.68 ^b ±0.67	9.38 ^b ±0.56	8.88 ^b ±0.30	8.61 ^b ±0.27
30 day	7.90 ^a ±0.21	7.18 ^a ±0.37	7.13 ^a ±0.25	6.88 ^a ±0.19

a,b- Values within and between the same treatment group with different superscript differed significantly ($p < 0.05$) at different time intervals

Table 10: Mean neutrophil count (%) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	36.00±2.30	36.83±1.66	30.66±2.60	33.16±1.77
7 day	41.36±2.45	41.00±1.21	35.67±2.67	35.00±2.94
30 day	36.16±1.24	37.16±1.40	29.00±2.52	30.83±1.04

Table 11: Mean lymphocyte count (%) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	58.66±2.48	58.00±1.63	63.83±2.54	61.16±2.16
7 day	55.16±2.57	55.16±1.13	60.66±3.01	61.00±2.78
30 day	59.16±1.40	58.66±1.28	64.00±2.82	62.50±1.38

Table 12: Mean monocyte count (%) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	1.50±0.42	1.33±0.55	1.50 ^a ±0.42	1.33 ^a ±0.49
7 day	1.33±0.42	1.33±0.42	1.33 ^a ±0.42	1.33 ^a ±0.42
30 day	1.33±0.49	1.16±0.30	4.00 ^b ±0.73	3.66 ^b ±0.55

a,b- Values within and between the same treatment group with different superscript differed significantly ($p < 0.05$) at different time intervals

Table 13: Mean eosinophil count (%) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	3.66±0.55	3.66±0.55	3.83±0.47	4.16±0.47
7 day	2.16±0.47	2.50±0.42	3.00±0.57	2.50±0.42
30 day	3.33±0.61	3.16±0.47	3.50±0.61	3.00±0.51

Table 14: Mean basophil count (%) of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	IIB
0 day	0.16±0.16	0.16±0.16	0.16±0.16	0.16±0.16
7 day	0	0.16±0.16	0	0.16±0.16
30 day	0	0	0	0

Table 15: Mean scrotal circumference of animals of both the groups at different intervals

Time interval	Group I		Group II	
	I A	I B	II A	II B
0 day	15.96 ^a ±0.83	18.33 ^a ±0.54	19.75 ^a ±1.44	21.18 ^a ±1.62
7 day	17.36 ^a ±0.78	20.41 ^b ±0.56	22.50 ^a ±1.61	23.17 ^b ±1.67
30 day	14.33 ^b ±0.77	16.55 ^c ±0.55	16.80 ^b ±1.27	17.20 ^c ±1.48

a,b- Values within and between the same treatment group with different superscript differed significantly ($p<0.05$) at different time intervals

Table 16: Mean testicular length (cm) using Vernier calipers in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	5.18±0.28	5.21±0.28	5.20 ^a ±0.19	5.88±0.17	5.85±0.17	5.86 ^a ±0.12	7.31±0.38	7.38±0.37	7.35 ^a ±0.25	7.63±0.49	7.83±0.56	7.73 ^a ±0.36
7 day	5.26±0.32	5.21±0.33	5.24 ^a ±0.22	6.43±0.21	6.33±0.15	6.38 ^b ±0.19	7.36±0.46	7.56±0.45	7.46 ^a ±0.31	8.10±0.59	8.08±0.55	8.09 ^b ±0.38
30 day	4.66±0.40	4.18±0.21	4.42 ^b ±0.23	4.85±0.17	4.86±0.18	4.85 ^c ±0.12	5.86±0.26	5.85±0.26	5.85 ^b ±0.17	5.86±0.32	6.01±0.37	5.94 ^c ±0.23

a,b- Values within and between the same treatment group with different superscript differed significantly (p<0.05) at different time intervals

Table 17: Mean testicular width (cm) using Vernier calipers in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	3.16±0.22	3.18±0.22	3.17 ^a ±0.15	3.80±0.13	3.81±0.07	3.80 ^a ±0.07	3.40±0.23	3.35±0.20	3.37 ^a ±0.14	3.41±0.23	3.38±0.23	3.40 ^a ±0.15
7 day	3.16±0.24	3.20±0.25	3.18 ^a ±0.16	4.26±0.12	4.10±0.08	4.18 ^b ±0.07	3.55±0.28	3.50±0.22	3.52 ^b ±0.17	3.71±0.28	4.03±0.31	3.87 ^b ±0.19
30 day	2.78±0.30	2.71±0.27	2.75 ^b ±0.19	3.21±0.10	3.30±0.09	3.25 ^c ±0.06	2.86±0.22	2.86±0.22	2.86 ^c ±0.14	3.05±0.19	2.93±0.19	2.99 ^c ±0.13

a,b- Values within and between the same treatment group with different superscript differed significantly (p<0.05) at different time intervals

Table 18: Mean testicular thickness (cm) using Vernier calipers in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	3.36±0.26	3.43±0.26	3.40 ^a ±0.17	3.95±0.13	3.88±0.08	3.91 ^a ±0.07	3.80±0.10	3.71±0.15	3.75 ^a ±0.09	3.63±0.12	3.51±0.28	3.57 ^a ±0.14
7 day	3.30±0.32	3.43±0.26	3.66 ^a ±0.20	4.45±0.14	4.21±0.08	4.33 ^b ±0.08	3.90±0.08	3.93±0.17	3.91 ^a ±0.09	3.71±0.16	3.93±0.26	3.82 ^b ±0.15
30 day	2.91±0.44	2.51±0.25	2.71 ^b ±0.25	3.30±0.13	3.43±0.10	3.36 ^c ±0.08	3.10±0.12	3.06±0.15	3.08 ^b ±0.09	3.18±0.17	2.96±0.21	3.07 ^c ±0.13

a,b- Values within and between the same treatment group with different superscript differed significantly ($p<0.05$) at different time intervals

Table 19: Mean testicular volume (cm³) using Vernier calipers in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	41.91±9.15	42.80±8.22	42.35 ^a ±5.86	63.61±5.86	61.93±3.90	62.77 ^a ±3.36	69.25±10.22	67.28±10.17	68.26 ^a ±6.88	69.84±10.90	71.13±14.73	70.48 ^a ±8.74
7 day	42.39±8.13	42.75±7.65	42.57 ^a ±5.32	87.96±8.13	78.13±4.58	83.05 ^b ±4.69	74.82±11.99	76.29±12.48	75.55 ^a ±8.25	80.04±9.77	97.17±20.32	88.61 ^b ±11.05
30 day	32.11±10.96	21.49±4.04	26.80 ^b ±5.79	37.16±3.56	39.41±3.25	38.28 ^c ±2.32	38.10±5.88	37.57±5.98	37.84 ^b ±4.00	42.20±7.00	39.31±7.12	40.75 ^c ±4.78

a,b- Values within and between the same treatment group with different superscript differed significantly ($p<0.05$) at different time intervals

Table 20: Mean testicular width (cm) using ultrasonography in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	2.75±0.24	2.70±0.23	2.73 ^a ±0.16	3.44±0.15	3.37±0.11	3.41 ^a ±0.09	3.29±0.23	3.22±0.21	3.26 ^a ±0.15	3.18±0.25	3.27±0.24	3.22 ^a ±0.16
30 day	2.60±0.31	2.28±0.19	2.44 ^b ±0.18	2.91±0.16	3.02±0.18	2.97 ^b ±0.11	2.75±0.22	2.77±0.21	2.76 ^b ±0.14	2.92±0.20	2.77±0.17	2.84 ^b ±0.12

a,b- Values within and between the same treatment group with different superscript differed significantly (p<0.05) at different time intervals

Table 21: Mean testicular thickness (cm) using ultrasonography in both the groups at different time intervals

Time interval	Group I						Group II					
	IA			IB			IIA			IIB		
	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean	Left	Right	Mean
0 day	2.86±0.25	2.73±0.33	2.80 ^a ±0.20	3.46±0.10	3.32±0.15	3.39 ^a ±0.09	3.65±0.11	3.41±0.25	3.53 ^a ±0.13	3.34±0.20	3.37±0.26	3.36 ^a ±0.15
30 day	2.75±0.42	2.36±0.26	2.56 ^b ±0.24	2.90±0.14	3.04±0.15	2.97 ^b ±0.10	2.98±0.12	2.96±0.160	2.97 ^b ±0.09	2.82±0.13	2.86±0.21	2.84 ^b ±0.12

a,b- Values within and between the same treatment group with different superscript differed significantly (p<0.05) at different time intervals

5. DISCUSSION

Various procedures for sterilization of ruminants had been studied for years in veterinary practice. Yet, there are many complications associated with castration such as high degree of acute or chronic pain response, haemorrhage, excessive swelling or edema, infection, poor wound healing and failure of castration. Even higher complications including necrosis, gangrene and death continue to plague veterinary practitioners (Stafford *et al.*, 2000 and Stafford and Mellor, 2005). The procedure of Pinhole castration has been suggested by many authors for male calf and goat sterilization (Ponvijay, 2007; Abid and Al-Baghdady, 2013 and Okwee-Acai *et al.*, 2008). This minimally invasive Pinhole technique was reported to be superior substitute to conventional methods which combines the advantages of avoiding the integumentary sensory nerves, incising or involving the scrotum, hence, provokes less pain or stress to the animal and an effective, quicker, simpler and cheaper alternative under field conditions (Okwee-Acai *et al.*, 2012).

5.1 Anamnesis

Oehme (2012) concluded that castration should be performed before the onset of puberty in ruminants. Castration of very young animal may inhibit the development of penis and urethra so, dispose the wether to urinary obstruction from urolithiasis. Therefore, 3-6 months bucks and 6months to 1year aged calves were selected for the present research work.

In present study, the goats were maintained by the marginal owners on small scale and provided proper care and attention for the health of individual animal by themselves. However, the calves were raised at farm under good feeding and managerial practices. Therefore, the animals selected for the study were apparently healthy. Animals with congenital reproductive anomalies were excluded from the study. The animal presented weak was dewormed, nutritionally supplemented and castrated, when found healthy. Thus, no disparity was observed in the animals under study.

5.2 Clinical observations and evaluation

The Burdizzo's castration procedure was performed as explained by Anderson (2007). Each spermatic cord was crushed for atleast 10 seconds then repeated approximately 1 cm below the previously crushed line. The faulty procedures may cause excessive crushing, leading to wound and subsequently maggots infestation, suppuration, necrosis and gangrene or even irreparable damage to urethra. Few complications may be fatal (Stafford and Mellor, 2005). However, no such complications were observed following Burdizzo's castration in the animals of the present study. Only one animal showed unilateral failure of castration, which might be due to faulty procedure. The ultrasonographic examination revealed the echogenicity typical of a viable testicle.

The Pinhole technique was performed without involving the scrotum, as per the procedure described by Ponvijay (2007). Faulty technique may lead to failure of castration, hydrocele or haemorrhage and suppuration but, it is least complicated. In the present study, only one testicle on ultrasonographic examination revealed degenerative changes with hydrocele, which might have occurred due to trauma to the scrotal structures or insufficient pressure of ligature at the spermatic cord.

5.2.1 Rectal temperature, heart rate and respiration rate

Rectal temperature, heart rate and respiration rate recorded at both the intervals fluctuated within the normal range in all animals of both the groups.

Mean rectal temperature dropped non-significantly ($p>0.05$), 30 minutes after the procedure in both the species which may be due to the post anaesthetic effects of triflupromazine on basal metabolic rate (Tranquilli *et al.*, 2007). Thereafter, from the 1st postcastration day no significant increase in rectal temperature was recorded due to administration of analgesics. It is in corroboration with findings of Pang *et al.* (2006) who did not observed any increase in rectal temperature when carprofen was administered to bulls during castration.

Mean heart rate decreased non-significantly ($p>0.05$), 30 minutes after castration in both the groups. These findings were in accordance with those

of Lees and Meradith (1983), who reported that the systemic effects of phenothiazines on cardiovascular system may cause vasodilation and fall in blood pressure but heart rate remains unchanged.

Significant ($p < 0.05$) decrease in respiration rate 30 minutes after the castration procedures was observed in both the groups. It is in corroboration with Parry *et al.* (1982) and Hall and Clarke (1991) who explained that the clinical doses of triflupromazine make the sedated animals to breath slowly but tidal volume remained unchanged. While, Stafford *et al.* (2003), Stilwell *et al.* (2010) and Pieler *et al.* (2013), also found reduced respiration rates, indicating a transient reduction in vagal tone in calves due to postoperative stress after castration.

Contrary to above findings, Smith *et al.* (1999) concluded that heart rate, respiration rate, and rectal temperature did not change significantly after surgery and these variables cannot be considered useful in the recognition of postoperative pain. Paul (2012) opined that neuropeptide SP in the blood samples was the actual stress marker in identifying pain associated with castration in bulls.

5.2.2 Inflammatory signs

On the day of castration, moderate pain on scrotal palpation was noticed in all the bucks and calves castrated with Burdizzo's method, which might be due to crushing of the scrotal skin and acute inflammation. Whereas, in Pinhole technique mild pain was observed as the sensory nerves of scrotal skin were avoided. It is in accordance with the findings of Okwee-Acai *et al.* (2008) and Okwee-Acai *et al.* (2012) in bucks. On observation of animals on 7th and 30th postoperative days, none of the animals displayed symptoms of pain. Similar findings were also observed by Fazili *et al.* (2009) who reported Pinhole technique to be less painful in rams during the same period. Molony *et al.* (1993) and Kent *et al.* (1995) observed disappearance of signs of acute pain half an hour after application of ligation and no chronic pain was further observed during the 30 days observation period on touching or palpation of scrotum, while Burdizzo's castration induced higher level of acute pain.

In bucks and calves on the day of castration, scrotal palpation revealed moderate swelling with Burdizzo's method due to acute injury and subsequent inflammation caused by crushing of the scrotal skin, spermatic cord and associated structures. However, with Pinhole technique it was noticed mild because only needle prick holes were induced on scrotal skin during ligation of the cord and thus excessive trauma was prevented. On 7th post castration day, mild swelling was observed on scrotal palpation in animals castrated with Burdizzo's method, whereas in Pinhole castrated animals, the swelling increased from mild to moderate degree. These observations were in accordance with the findings of Fazili *et al.* (2009) in rams. However, on 30th day no animal revealed scrotal swelling, which was consistent with findings of Ijaz *et al.* (2000) in lambs. Okwee-Acai *et al.* (2008) reported maximum swelling in the ligated caprine testis on day 2 post castration that subsequently reduced below to the pre-ligation value after 30 days. It was in corroboration with Baba *et al.* (2012) who also reported significantly higher swelling on day 3, 7, 14 and 21 which significantly lowered on day 28 post castration in dogs.

During the observation period of 30 days, no exudation was noticed at the scrotal area, in either of the species (bucks or calves) castrated with both methods because proper aseptic preparation of surgical site and antibiotic therapy was provided to all the animals. Similarly, Fazili *et al.* (2009) had also not observed infection without using antibiotics in Pinhole castrated rams following aseptic preparation of the surgical site (Mann and Constatinescu, 1998).

5.3 Haematological estimations

The mean haemoglobin concentration (g/dl) was observed to be within normal range in both the groups at all the time intervals in bucks and calves. There was no significant difference ($p>0.05$) between both the techniques of castration, suggesting that there was no deterioration of the general body condition in any animal castrated by either of the procedure and all the animals remained healthy throughout the observation period. Earley and Crowe (2002) concluded normal haemoglobin levels as the indicator of the animal's health during their observations in castrated calves.

In bucks and calves, the mean total leukocyte count for both the groups were observed within the normal range at each time interval, but significant increase ($p < 0.05$) was noted on 7th day post castration. The altered leucogram could be the result of endogenous glucocorticoid release in response to tissue trauma and inflammation (Latimer, 1995). This change occurred concurrently with increase in neutrophil count suggesting acute inflammatory condition caused by castration. Neutrophils are suggested as the first line of defence and therefore, their number increased after the onset of inflammation (Coles, 1974; Schlam *et al.*, 1975 and Sastry, 1989). Paladino *et al.* (2010) reported maximum leucocytosis in blunt injuries compared to the other major injuries in humans.

The mean neutrophil and lymphocyte count on 7th postoperative day revealed only transient changes and the values returned to normal on day 30 after castration. The monocyte, eosinophil and basophil count in the blood did not change significantly during the study period in bucks. Similar findings were reported by Mohammad *et al.* (2008) in lambs and Mahalingam *et al.* (2009) in dogs during their study period of 7 days. Duncan *et al.* (1994) corroborated this finding and stated that these changes are likely due to reactive leukocytosis and stress. Earley and Crowe (2002) conclude that castration resulted in increased haptoglobin values exerting a suppressive effect on lymphocytes. Moreover, since it occurred in both the groups, it may be unrelated with the castration procedures.

Calves castrated by both the techniques, revealed significant increase ($p < 0.05$) in mean monocytes on 30th post castration day, which may be due to the role of monocytes in chronic inflammatory reactions, including granulomatous inflammation (Meuret *et al.*, 1975).

5.4 External morphology

5.4.1 Scrotal circumference

In Burdizzo's castrated animals of group IA and IIA, non-significant ($p > 0.05$) increase in scrotal circumference was noticed on 7th post-castration day when compared with pre-castration value. There was significant ($p < 0.05$)

decrease in scrotal circumference on 30th day when compared with pre-castration and 7th post-castration day values. It is in accordance with Thuer *et al.* (2007) who observed that the Burdizzo's castrated calves exhibited only acute inflammation and pain as compared to other methods.

In group IB and IIB, the mean scrotal circumference in Pinhole castrated animals was significantly ($p<0.05$) higher from pre-castration value to 7th post-castration value. Thereafter, scrotal circumference significantly decreased ($p<0.05$) from pre-castration and 7th post-castration period to 30th post-castration day. Similarly, maximum inflammation on 3rd postoperative day and thereafter a decreasing trend was observed by Okwee-Acai *et al.* (2008) and Fazili *et al.* (2009) in small ruminants and Baba *et al.* (2012) in dogs.

On 30th postcastration day, the significant decrease in scrotal circumference in all the groups may be due to alteration in testicular parenchyma and resultant testicular atrophy. It is in corroboration with the findings of Olar *et al.* (1983) who also observed relationship of altered testicular parenchyma with scrotal circumference in dogs.

Woodall and Johnstone (1988) concluded that the testicular size including physical measurement of the testicular length, breadth and height is reflection through the scrotum. Thus, it may be proposed that the scrotal circumference is directly related to the testicular width and thickness, but unrelated to its length because it was a measure of transverse section through the scrotal skin. Hence, the increase or decrease in these parameters is reflected same to the scrotal circumference.

5.4.2 Testicular measurements (length, width and thickness)

There was non-significant ($p>0.05$) increase in testicular dimensions on 7th post castration day in group IA and IIA (Burdizzo's method). It might be due to mild inflammation on 7th day after castration. Whereas, there was significant ($p<0.05$) increase in group IB and IIB (Pinhole group), which might be due to altered blood supply resulting in moderate inflammation of testicles and associated structures.

On 30th post castration day, there was significant ($p<0.05$) decrease in testicular dimensions in both the groups. It is in accordance with the findings of Slauson and Cooper (2002), in bucks. They concluded that occlusion of the arterial supply or the venous drainage of the testis, it results in inevitable ischaemic or coagulative necrosis of the testicular tissue. On the basis of above facts ischemia produced by both the method might have resulted in testicular atrophy.

5.4.3 Testicular volume

The mean testicular volume was calculated by the formula (length x width x thickness x 0.71) as described by Baba *et al.* (2012) and Paltiel *et al.* (2002) in dogs.

Approximately 70–80% of testicular mass consists of seminiferous tubules thus testicular volume is largely a reflection of spermatogenesis (Setchell and Brooks, 1988) and require active blood supply. Therefore, the resultant ischemia from any procedure might have lead to testicular degeneration, atrophy and reduction in testicular volume.

The mean testicular volume of both the right and left testicle of group IA and IIA (Burdizzo's method) revealed non-significant ($p>0.05$) increase from 0 day to 7th day and thereafter significant ($p<0.05$) decrease to 30th post-castration day. There was nearly 40 per cent (mean) reduction of testicular volume on 30th post-castration day in bucks and calves when compared from the pre-castration value. It is in corroboration with the findings of Baba *et al.* (2012) who reported 40.57 per cent reduction in testicular volume on 28th day in dogs.

Whereas, in group IB and IIB, the bucks and calves castrated with Pinhole technique also showed nearly 40 percent reduction of mean testicular volume on 30th day after castration. Ponvijay (2007) also reported it to be approximately 50 per cent in two months older calves on 21st postcastration day. This difference might be due to the age factor of the selected animals.

Thus both the procedures of castration were observed equal in reducing the testicular volume on 30th post-castration day.

5.5 Ultrasonographic findings

On ultrasonogram, the echotexture of buck and calves testis, were discerned by the presence of very homogenous, granular pattern, a thin hyperechoic capsule and a linear hyperechoic structure in the central long axis called the mediastinum testis, a landmark for their identification during ultrasonography. These findings were similar to those of Pugh *et al.* (2005) in dogs and Ahmad *et al.* (1999) in small ruminants.

On day 30, the castrated testis of both the species, appeared to be smaller than the normal testis seen before castration. Kim and Lipshultz (1996) concluded that ultrasonography played an important role in the evaluation of testicular size, atrophy and degeneration based on parenchymal echogenicity in humans.

In longitudinal/frontal images, the parenchyma of testicle was slightly less echogenic with hyperechoic spots/testicular microliths were also depicted, which were may be small punctate calcifications with no acoustic shadowing and presumably representing the mediastinum testis. It is in corroboration with the findings of Ganem *et al.* (1999) and Quane (2000) in humans who concluded that the testicular microliths were punctate calcifications, echogenic with no acoustic shadowing and associated with testicular atrophy, infertility, torsion or testicular neoplasms.

The testicular parenchyma was not homogeneous but was mottled in appearance, with a thick echogenic tunica albugenia.. The epididymal tail was also atrophied but more echogenic than normal. Similar findings were also observed by Ahmad *et al.* (1991) and Ahmad and Noakes (1996) in small ruminants. This altered echogenicity may be due to ischemia, fibrosis or even necrosis of testicles.

All these findings were more evident in the Pinhole castrated group when compared to the Burdizzo's method in both the species revealing Pinhole technique as a superior procedure, due to greater degenerative process.

Significant reduction ($p < 0.05$) in testicular width and thickness on 30th postcastration day was measured using ultrasonography in both the

techniques and species, which was in accordance with the findings of Ahmad *et al.* (1999) who also observed reductions in testicular parameters in bucks.

5.6 Histological findings

In bucks, histological examination of the Burdizzo's and Pinhole castrated testicles on 30th postoperative day, revealed atrophy of seminiferous tubules with desquamation and obliteration by cell debris in the lumen. Similar findings were observed by Abid and Al-Baghdady (2013) in bucks. Destruction of testicular elements and spermatogonial cells (Fazili *et al.*, 2009) with presence of dead spermatozoa in the central portion of few seminiferous tubules were evident. These spermatozoa were more in Burdizzo's castrated animals. It might be due phagocytic activity and the degeneration process still continuing in the Burdizzo's group. Ramadan *et al.* (2014); Stoffel *et al.* (2009) and Smith (1955), also found no evidence of Leydig or Sertoli cells in the testicular parenchyma of the castrated testicle of bucks. In the Pinhole technique besides eosinophilic cellular remnants, vacuolations at the basal part of the seminiferous tubules were also observed.

The intertubular spaces showed the presence of collagen bundles. Similar findings were also observed by Ramadan *et al.* (2014) in dogs. The collagen bundles in between the seminiferous tubules were more in Pinhole technique. So, it showed that the activity of fibroblast cells and degree of degeneration was more active in Pinhole technique. These findings were in accordance with Birbrair *et al.* (2014) and Mitra *et al.* (2015) who concluded that the fibrosis is formation of excess fibrous connective tissue in an organ under reparative or reactive process which can obliterate the architecture and function of the underlying organ or tissue. Thus, the presence of more connective tissue or collagen in the intertubular spaces in Pinhole Technique indicates faster degeneration of testicular elements than Burdizzo's method.

In Burdizzo's castrated calves, spermatogenic cells were also observed in few of the tubules and high magnification revealed, picnotic nuclei of cells at basal region of seminiferous tubules, when compared with Pinhole castrated testicle on 30th postoperative day.

In calves, the seminiferous tubules appeared hyalinized with cell debris completely filling the lumen. Under high magnification, no evidence of spermatogenic cells were observed on 30th post castration day. It is similar to the findings of Markey *et al.* (1995) in rams. Therefore in calves castrated by Pinhole technique faster degree of degeneration was evident.

5.7 Complications

The bucks castrated by Burdizzo's method showed unilateral testicular atrophy without decline in male characteristic features. It is also confirmed on ultrasonographic examination. So, it can be concluded that castration failure may have occurred due to faulty technique. Similar findings were also observed by Ponvijay (2007) and Stafford *et al.* (2000) who reported failures in calves castrated using Burdizzo's clamp due to inadequate crushing of the spermatic cord.

The Pinhole castrated buck, after 30 post-castration day revealed, unilateral atrophy of right testicle. However, on ultrasonographic examination there was no change in size of left testicle but degenerative changes in form of microliths and severe hydrocele with fibrin deposits in between layers of tunica vaginalis of left testicle were also observed. Reduction in male characteristic features were evident. This might be due to unnoticed trauma to the spermatic cord and associated structures during ligation or insufficient ligature pressure as the knot may have slipped off few hours after ligation, still leading to degeneration. It is in consonance with Bree and Hoang (1996) and Seigel (1997) in humans who observed hydrocele as anechoic fluid between the layers of the tunica vaginalis on ultrasonographic examination of testicles associated with trauma, testicular torsion, neoplasms or with congenital abnormalities. Bergh *et al.* (2001) concluded that the ligation pressure for 12-48 hours could lead to permanent sterility in larger animals. Thus, degenerative changes were observed.

According to findings of the present study, it can be summarised that the Burdizzo's procedure elicits higher degree of acute pain and inflammation in both the species, because whole of the scrotal structures were crushed during the procedure. On gross examination, non-significant ($p>0.05$) increase in

testicular measurements and finally about 40 per cent atrophy on 30th day was observed.

In Pinhole technique, acute inflammatory signs were of lesser degree but there was significant ($p < 0.05$) increase in testicular measurements on 7th day. However, testicular atrophy was also nearly 40 per cent. On ultrasonographic examination of Pinhole castrated testicle revealed rapid degeneration, fibrosis or punctuate calcifications. Histological observations hyalinization, vacuolations and collagen fibres were more pronounced as compared to Burdizzo's castrated group.

6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Various non-invasive, minimally invasive and invasive methods have been evaluated to castrate the ruminants. Each technique has its own merits and demerits. Recently, many developments have taken place to simplify the technique and to perform ethical and comparatively less painful castration in animals. Presently, Burdizzo's method is widely accepted to castrate the ruminants, which require skill, equipments and considered as painful and inhumane procedure. Therefore, the present study was planned with the objective to evaluate the minimally invasive Pinhole technique and to compare it with the conventional Burdizzo's method in bucks and cattle calves.

The study was conducted on 12 apparently healthy bucks aged 3-6 months and 12 cattle calves of 6 months to 1 year age divided equally in two groups, subjected to Burdizzo's (group IA and IIA) and Pinhole castration (group IB and IIB). This study was carried out for nine months.

Clinical observation, evaluation and haematological estimations were conducted at pre-castration period. Measurement of scrotal circumference was carried out by measuring tape, testicular length, width and thickness by Vernier calipers and volume by formulae ($L \times W \times T \times 0.71$). Ultrasonography of the testicles was performed for echotexture and measurement of width and thickness. In both the species, the echotexture was homogenous, granular with thin hyperechoic capsule. A linear hyperechoic structure in the central long axis called the mediastinum testis was also observed.

Then, the scrotal area was prepared for aseptic procedure. Animals were sedated with Inj. triflupromazine hydrochloride @ 0.2mg/kg I/M followed by infiltration of 2% lignocaine hydrochloride at the neck of scrotum.

Castration in group IA (bucks) and IIA (calves) was performed with the standard Burdizzo's procedure. Each spermatic cord was held and clamped by Burdizzo's castrator for 10 seconds. This procedure was then repeated approximately 1 cm below the previously crushed line of the cord.

In group IB and IIB, the animals were castrated with the Pinhole technique. After pulling the spermatic cord towards the lateral side of scrotal skin, threaded suture needle (black braided silk no. 1 or 2) was passed adjacent to medial aspect of spermatic cord, leaving the suture in place. The needle was reintroduced through the previously made skin holes from the lateral margin of the released spermatic cord. The knot was tied at the caudal aspect, transacted 3 mm and scrotal skin was lifted to submerge the knot inside.

After 30 minutes of completing the castration procedure, rectal temperature, heart rate and respiration rate were again recorded and the animals were observed for inflammatory signs. In both the species, there was non-significant ($p>0.05$) decrease in rectal temperature and heart rate whereas, respiration rate decreased significantly ($p<0.05$). Moderate pain on scrotal palpation was noticed with Burdizzo's method, whereas, in Pinhole technique mild pain was observed. Swelling was moderate with Burdizzo's method and was mild with Pinhole technique.

On 7th day after castration, no signs of pain were noticed. Mild swelling in animals castrated with Burdizzo's method was observed while it was of moderate degree in Pinhole castrated animals. On haematological examination, neutrophils and lymphocytes showed transient inflammatory changes. In Burdizzo's castrated group, there was non-significant ($p>0.05$) increase in scrotal circumference, testicular length, width, thickness and volume, whereas, in Pinhole castrated animals, these external morphological parameters showed significant ($p<0.05$) increase.

On examinations at 30th day, pain, swelling or exudation were not noticed in any castrated animal. Haematological parameters returned to pre castration range, however significant ($p<0.05$) increase in monocytes of calves was observed. There was significant decrease ($p<0.05$) in external morphological parameters with testicular volume reduction of 37.04% and 39.01% in bucks, 44.56% and 42.18% in calves castrated by Burdizzo's and Pinhole methods respectively. Ultrasonography of the castrated testicle revealed atrophy and mottled echotexture of parenchyma, which was slightly less echogenic with

hyperechoic spots/testicular microliths (small punctate calcifications with no acoustic shadowing) and with thick echogenic tunica albugenia.

Castrated testicular samples were collected by Bard Max core biopsy instrument for histological evaluations on 30th post castration day. In both the species, it revealed atrophy with desquamation and obliteration by cell debris in the lumen of seminiferous tubules. Leydig or Sertoli cells were not observed. Higher evidence of dead spermatozoa in the central portion of few seminiferous tubules in Burdizzo's castrated buck testicle was noticed. In the Pinhole technique vacuolations at the basal part with eosinophilic cellular remnants in the seminiferous tubules were observed. Intertubular collagen bundles were thicker in Pinhole castrated testicles. In calves, more hyalinization with picnotic nuclei at basal region of few seminiferous tubules were also observed in Burdizzo's group when compared with Pinhole.

Complications recorded were unilateral failure of castration in one buck castrated by Burdizzo's method while in other buck, castrated by Pinhole technique, the ultrasonogram showed unilateral hydrocele with degenerative changes in testicular parenchyma.

On the basis of physical measurements, ultrasonographic and histological findings, it can be concluded that the Pinhole technique was satisfactory in both the species as it was effective, less painful, field oriented, required no instrumentation, ethical and observed with less complications.

Pinhole technique may also be regarded superior as greater degree of testicular degeneration was observed in both the species.

6.2 Conclusions

On the basis of observations made during the present study, it may be concluded that:

1. The Pinhole technique may be regarded as effective, less painful, field oriented, required no instrumentation, ethical and observed with less complications.
2. Pinhole technique may also be regarded superior as greater degree of testicular degeneration was observed in both the species.

6.3 Suggestions for further work

1. Pinhole technique can be evaluated for dog and equine castration.
2. Teasure bulls can be raised using Pinhole technique.

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