Evaluation of a novel minimally invasive pinhole castration technique compared with open surgical castration in bulls

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Abstract

This study compared a minimally invasive pinhole (keyhole) castration technique with the conventional open surgical method in 24 Holstein-Friesian crossbred bulls (18–24 months; n = 12 per group). Animals were randomly allocated into two groups. Group 1 underwent pinhole castration, while Group 2 received open surgical castration. The pinhole technique resulted in a significantly shorter surgical duration (10.63 ± 2.26 min) compared to the open method (36.00 ± 5.80 min; $P < 0.05$). Postoperative complications were fewer and less severe in Group 1, with only minor suture loosening and mild suppuration, whereas Group 2 showed a higher incidence of oedema, wound dehiscence, and pus formation ($P < 0.05$). Serum testosterone concentrations declined significantly in both groups following castration. Although testosterone suppression was more rapid and profound in the open surgical group, the pinhole group demonstrated a progressive decline associated with marked testicular regression. Baseline testosterone concentrations were comparable between groups. Scrotal circumference decreased significantly over time in both groups, with a more pronounced reduction observed following open castration. Most bulls resumed normal activity within 48 h postoperatively, with faster functional recovery observed in the pinhole group. In conclusion, pinhole castration represents a faster, less invasive, and welfare-oriented alternative to open surgical castration in adult bulls. Despite a relatively gradual endocrine suppression, the technique achieves adequate testicular regression with fewer complications, making it a practical and low-cost option for field conditions.

Testosterone suppression, scrotal circumference, postoperative recovery, minimally invasive surgery

Castration is a common surgical procedure in animals aimed at inducing sterility. It is routinely performed to reduce aggressive behaviour, make animals more docile and easier to handle, prevent unwanted mating and mounting activities, and to address certain testicular or inguinal pathologies (Edwards 2008). In meat-producing animals, castration is also employed to enhance carcass muscle characteristics, improve meat quality traits such as tenderness and fat composition, and minimize aggressive behaviour in males (Skele et al. 2024).

Castration methods vary depending on animal species, age, production objectives, testicular anatomy, and surgeon preference (Coetzee et al. 2010). Broadly, castration techniques may be classified as hormonal, chemical, or physical. Physical methods can be further subdivided into non-invasive, minimally invasive, and invasive techniques. In general, these methods aim to either surgically remove the testes, irreversibly damage testicular tissue, or restrict blood supply to induce ischaemia and subsequent testicular atrophy (Stafford and Mellor 2005; Currah et al. 2009). Non-invasive and minimally invasive techniques,

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such as Burdizzo castration and elastrator banding, induce testicular ischaemia without scrotal incision, whereas invasive techniques involve surgical orchidectomy through scrotal incisions (Sawhney 2016). Although conventional open surgical castration is effective, it is frequently associated with postoperative complications including haemorrhage, oedema, infection, delayed wound healing, and prolonged recovery, particularly in large ruminants. These complications adversely affect animal welfare and increase management costs under field conditions. The pinhole castration technique, also known as *in situ* spermatic cord ligation, has been proposed as a minimally invasive alternative to conventional methods. This technique involves percutaneous ligation of the spermatic cord without exteriorization or excision of the testis, thereby avoiding extensive scrotal incisions and reducing tissue trauma. Previous studies have demonstrated the applicability of pinhole castration in dogs, goats, calves, and bucks, reporting advantages such as reduced surgical time, minimal postoperative complications, and lower cost (Ponvijay 2007; Okwee-Acai et al. 2008, 2012; Sawhney 2016).

However, despite these encouraging findings, systematic evaluation of the pinhole castration technique in adult bulls remains limited. Adult cattle present distinct anatomical and management challenges compared to small ruminants and calves, including larger spermatic cords, increased scrotal mass, and higher risk of postoperative complications. Moreover, comparative data on postoperative recovery, testicular regression, and endocrine suppression following pinhole castration in adult bulls are scarce. Minimization of pain, stress, and postoperative morbidity is a critical component of welfare-oriented livestock management. Techniques that reduce surgical trauma while maintaining effectiveness are particularly valuable in resource-limited and field settings. Therefore, the present study was undertaken to evaluate the effectiveness, safety, and practical applicability of the pinhole castration technique in adult bulls by comparing it with conventional open surgical castration. The study specifically assessed surgical duration, postoperative complications, changes in serum testosterone concentration, scrotal circumference regression, and postoperative recovery, with the objective of determining the suitability of the pinhole technique as a field-friendly alternative for cattle castration.

Materials and Methods

Ethics approval

All animal procedures were approved by the Institutional Animal Ethics Committee of Indian Council of Agricultural Research-Indian Veterinary Research Institute (ICAR-IVRI), Izatnagar, Bareilly, Uttar Pradesh, India.

Animals and experimental design

The study was conducted on twenty-four Holstein-Friesian crossbred bulls (18–20 months of age; mean body weight ≈ 520 kg) maintained at the University Farm, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh, India. Animals were selected based on normal clinical health status, absence of scrotal or testicular abnormalities, and normal external genital conformation following a thorough preoperative clinical examination. Animals were randomly allocated into two experimental groups (n = 12 each) using a simple randomization method. Baseline characteristics including age, body weight, and scrotal circumference did not differ significantly between groups ($P > 0.05$), ensuring comparability prior to intervention. Bulls were fasted for 36 h prior to surgery, with free access to water.

Anaesthesia, analgesia, and preoperative medication

Sedation was achieved using xylazine (Xylaxin®, Indian Immunologicals Ltd., Hyderabad, India) administered intramuscularly at 0.1 mg/kg body weight to ensure adequate restraint and sedation. Analgesia was provided using meloxicam (Melonex®, Intas Pharmaceuticals Ltd., Ahmedabad, India) at 0.2 mg/kg body weight intramuscularly. Additionally, 2% lignocaine hydrochloride (Xylocaine® 2%; AstraZeneca Pharma India Ltd., Bengaluru, India) was infiltrated subcutaneously at the base of the scrotum to provide local anaesthesia. Preoperative antibiotic prophylaxis was administered using enrofloxacin (Enrocin®, Intas Pharmaceuticals Ltd.), at 10 mg/kg body weight intramuscularly.

Baseline measurements

Scrotal circumference and testicular length were measured using a flexible measuring tape prior to surgery to establish baseline reproductive parameters. These measurements were used for subsequent comparison to assess post-castration regression (Nev et al. 2024). The surgical site was prepared by clipping the scrotal hair and disinfecting the area using povidone-iodine solution under aseptic conditions.

Pinhole castration technique (Group 1)

Pinhole castration was performed using a standardized *in situ* spermatic cord ligation technique. A small (~1 cm) cranial midline scrotal incision was made to access the subcutaneous space. Blunt dissection was carried out using artery forceps to identify the spermatic cord. The artery forceps were then passed subcutaneously between the medial aspects of both spermatic cords, and the tip was exteriorized through a second small caudal scrotal incision. A non-absorbable vetafil suture (polymerized caprolactam, size 2) was grasped with the forceps and looped percutaneously around the spermatic cord. Two circumferential loops were placed around each spermatic cord—one medial and one lateral—to ensure uniform compression. The ligature was tightened manually until complete occlusion of cord pulsation was confirmed by palpation, indicating interruption of testicular blood supply. The same procedure was repeated for the contralateral testis. Both scrotal incisions were cleaned aseptically and closed using single interrupted non-absorbable polyamide sutures (size 1). The total duration of the surgical procedure was recorded for each animal (Plate I, Fig. 1).

Open surgical castration technique (Group 2)

Open surgical castration was performed on a separate group of twelve bulls following the same preoperative preparation, sedation, analgesia, and antibiotic protocol as described above. A vertical scrotal incision approximately 3–4 cm in length was made over each testis. The tunica vaginalis was incised, and the testis along with the spermatic cord was exteriorized. The spermatic cord was ligated using two layers of absorbable chromic catgut suture (No. 2), and the testis was excised distal to the ligature. The scrotal wound was thoroughly lavaged with antiseptic solution and left open to heal by second intention to facilitate drainage, which is standard practice in open castration procedures. The duration of surgery was recorded.

Postoperative care and monitoring

Postoperatively, animals in both groups received antibiotics and analgesics for five consecutive days. Bulls were monitored daily for general health status, appetite, posture, locomotion, and signs of surgical site complications such as swelling, haemorrhage, wound discharge, or infection. Postoperative complications were recorded descriptively as present or absent. Severity grading and standardized pain or behavioural scoring systems were not employed and are acknowledged as limitations of the study. Scrotal circumference was measured on Day 0 (pre-castration), Day 45, and Day 90 postoperatively. Serum testosterone concentrations were assessed on Day 0, Day 15, and Day 30 following castration to evaluate endocrine changes and confirm testicular regression.

Blood collection and testosterone assay

Approximately 5 ml of blood was collected aseptically from the jugular vein of each animal between 08:00 and 09:00 h to minimize the effect of diurnal variation in hormone concentrations. Blood samples were allowed to clot at room temperature, and serum was separated by centrifugation at 1000 × *g* for 10 min. The harvested serum was stored at −20 °C until further analysis. Serum testosterone concentration was estimated using a bovine-specific competitive enzyme-linked immunosorbent assay (ELISA) kit (GENLISA® Bovine Testosterone ELISA kit, Krishgen Biosystems, India), following the manufacturer's instructions. Absorbance was measured using a microplate reader, and testosterone concentrations were calculated from the standard curve provided with the kit.

Statistical analysis

Statistical analysis was performed using SPSS software (Version 14.0). Data distribution was assessed for normality prior to analysis. Between-group comparisons were performed using independent-samples *t*-tests, while within-group temporal changes were analysed using repeated-measures analysis of variance (ANOVA). Results are presented as mean ± standard deviation (SD). A value of $P < 0.05$ was considered significant.

Results

The mean duration of surgery differed significantly between the two groups. Group 1 (pinhole castration) required a significantly shorter surgical time (10.63 ± 2.26 min) compared to Group 2 (open surgical castration), which required 36.00 ± 5.80 min ($P < 0.05$) (Table 1, Fig. 2). Postoperative complications were observed in both groups;

however, the incidence and severity of complications were markedly lower in Group 1. In the pinhole castration group, two animals exhibited minor suture loosening and one animal showed mild pus formation, all of which resolved with routine postoperative management. No cases of oedema or wound dehiscence were observed in Group 1. In contrast, Group 2 exhibited a significantly higher frequency of postoperative complications ($P < 0.05$), including oedema in three animals, wound dehiscence in two animals, and pus accumulation in one animal (Table 2, Fig. 3).

Table 1. Duration of surgery (in minutes, mean ± SD) between pinhole and open surgical castration in bulls.

Group	Duration of surgery
Group 1 (pinhole castration)	10.63 ± 2.26^{a}
Group 2 (open surgical castration)	36.00 ± 5.80^{b}

Different lowercase superscripts indicate a significant difference ($P < 0.05$).

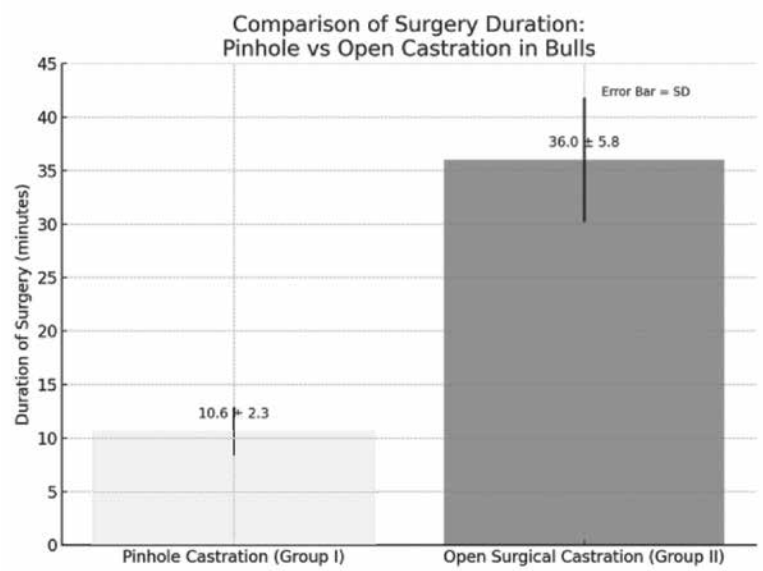

Fig. 2. Comparison of mean surgery duration between pinhole and open surgical castration in bulls.

Table 2. Postoperative complications in pinhole vs. open surgical castration.

Complication	Group 1 (pinhole castration)	Group 2 (open surgical castration)
Suture loosening	2	0
Pus formation	1	1
Oedema	0	3
Wound dehiscence	0	2

Data are presented as number of animals affected. Complications were recorded descriptively without severity grading.

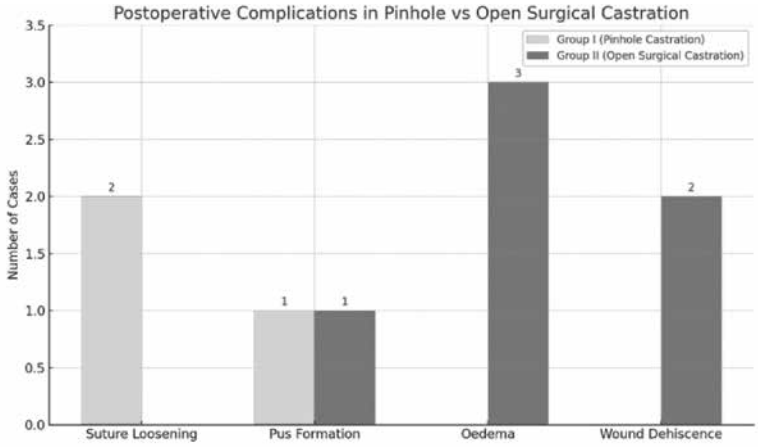

Fig. 3. Comparison of postoperative complications between pinhole and open surgical castration in bulls.

Table 3. Comparison of serum testosterone concentrations (ng/ml) between pinhole (Group 1) and open surgical (Group 2) castration methods in bulls at different time intervals.

Group	Pre-castration (Day 0)	Post-castration (Day 15)	Post-castration (Day 30)
Group 1	6.45 ± 3.80^{a}	2.70 ± 1.10^{a}	1.05 ± 0.80^{a}
Group 2	6.88 ± 3.30^{a}	0.70 ± 0.20^{b}	0.20 ± 0.55^{b}

Values are expressed as mean ± SD. Different lowercase superscripts within a column indicate a significant difference ($P < 0.05$).

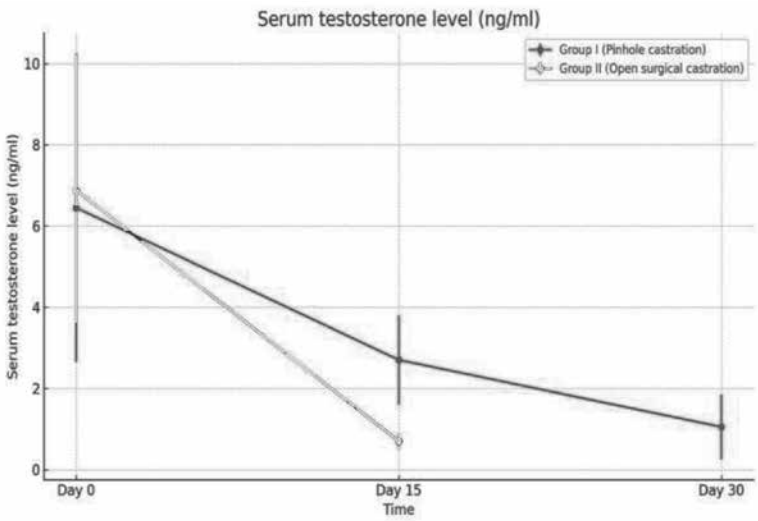

Fig. 4. Time-dependent changes in serum testosterone concentrations (ng/ml) following pinhole and open surgical castration in bulls.

Serum testosterone concentrations showed a significant decline following castration in both groups (Table 3, Fig. 4). Baseline (Day 0) testosterone concentrations were comparable between Group 1 (6.45 ± 3.8 ng/ml) and Group 2 (6.88 ± 3.3 ng/ml) ($P > 0.05$). By Day 15 post castration, testosterone concentrations declined to 2.7 ± 1.1 ng/ml in Group 1 and 0.7 ± 0.2 ng/ml in Group 2, with a significantly greater reduction observed in Group 2 ($P < 0.05$). This declining trend continued on Day 30, with mean testosterone concentrations of 1.05 ± 0.8 ng/ml in Group 1 and 0.20 ± 0.55 ng/ml in Group 2.

Scrotal circumference decreased progressively over time in both groups (Table 4, Fig. 5). At baseline (Day 0), mean scrotal circumference was 31.42 ± 3.1 cm in Group 1 and 33.6 ± 2.16 cm in Group 2, with no significant difference between groups ($P > 0.05$). By Day 45 post castration, scrotal circumference decreased to 24.76 ± 1.8 cm in Group 1 and 11.13 ± 1.22 cm in Group 2. This reduction was further accentuated by Day 90, with measurements of 15.20 ± 1.14 cm in Group 1 and 6.12 ± 1.67 cm in Group 2, indicating a more rapid and pronounced scrotal regression following open surgical castration.

Most animals in both groups resumed normal posture, feeding, and locomotion within 48 h following surgery. However, animals in the pinhole castration group demonstrated earlier return to normal activity and fewer postoperative management requirements compared to the open surgical group.

Table 4. Changes in scrotal circumference over time in bulls undergoing pinhole (Group 1) and open surgical (Group 2) castration.

Group	Pre-castration (Day 0)	Post-castration (Day 45)	Post-castration (Day 90)
Group 1	31.42 ± 3.10[a]	24.76 ± 1.80[a]	15.20 ± 1.14[a]
Group 2	33.60 ± 2.16[a]	11.13 ± 1.22[b]	6.12 ± 1.67[b]

Values are expressed as mean ± SD. Different lowercase superscripts within a column indicate a significant difference ($P < 0.05$).

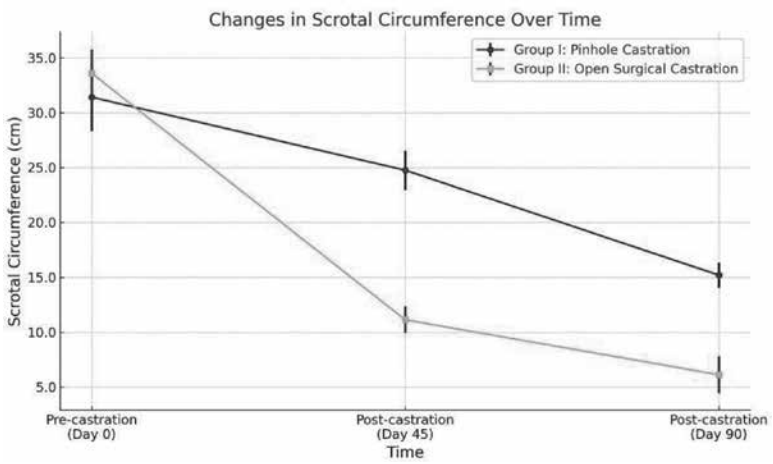

Fig. 5. Time-dependent changes in scrotal circumference following pinhole and open surgical castration.

Discussion

The present study demonstrates that pinhole castration is associated with a significantly shorter surgical duration and a lower incidence of postoperative complications compared to conventional open surgical castration in adult bulls. These advantages are primarily attributable to the minimally invasive nature of the pinhole technique, which avoids extensive scrotal incisions, reduces tissue trauma, and minimizes exposure of the surgical site to environmental contamination.

The markedly reduced operative time observed in the pinhole castration group is consistent with earlier reports in small ruminants and dogs, where *in situ* spermatic cord ligation required substantially less surgical manipulation than open orchidectomy (Nickel et al. 1973; Jana et al. 2005; Sawhney 2016). Shorter surgical duration is clinically relevant in large ruminants, as it reduces restraint time, stress, and anaesthetic exposure, thereby improving procedural safety and animal welfare.

Postoperative complications were significantly fewer and less severe following pinhole castration. The absence of extensive scrotal wounds and exteriorization of testicular tissue likely contributed to the reduced incidence of oedema, wound dehiscence, and suppuration observed in the pinhole group. Similar reductions in postoperative morbidity following pinhole castration have been reported in bucks, rams, and dogs (Okwee-Acai et al. 2008; Fazili et al. 2009; Okwee-Acai et al. 2013). In contrast, open surgical castration has been consistently associated with higher rates of haemorrhage, infection, and delayed healing due to open wound management and greater tissue disruption (Stafford et al. 2000; Stafford and Mellor 2005).

Serum testosterone concentrations declined significantly in both groups following castration; however, the magnitude and rate of hormonal suppression differed between techniques. Open surgical castration resulted in a rapid and profound decline in testosterone concentrations due to immediate removal of testicular tissue. In contrast, pinhole castration produced a more gradual reduction in serum testosterone, which may be explained by delayed ischaemic degeneration of Leydig cells following spermatic cord ligation. Experimental and clinical studies have shown that interruption of testicular blood supply leads to progressive testicular ischaemia, seminiferous tubular collapse, and eventual Leydig cell degeneration rather than immediate endocrine cessation (Bergh et al. 2001; Fazili et al. 2009; Baba et al. 2012).

Despite the relatively slower hormonal decline, progressive scrotal regression observed in the pinhole group indicates effective testicular atrophy, supporting the functional efficacy of the technique. Similar patterns of delayed but sustained testicular regression following *in situ* spermatic cord ligation have been reported in goats and dogs (Munahi and Abid 2011; Baba et al. 2012). From a clinical perspective, gradual endocrine suppression may be acceptable in production systems where immediate elimination of androgenic effects is not critical. Behavioural observations indicated earlier return to normal activity and reduced postoperative discomfort in the pinhole castration group, although formal pain scoring and standardized behavioural assessments were not employed. Previous studies have documented reduced pain responses and stress indicators following minimally invasive castration techniques compared to conventional methods (Stafford and Mellor 2005; Okwee-Acai et al. 2012). These findings suggest a potential welfare advantage of pinhole castration, particularly under field conditions.

The present study has certain limitations that should be acknowledged. The sample size was relatively small (n = 12 per group), the postoperative observation period was limited to 90 days, and long-term fertility outcomes, histopathological evaluation of testicular degeneration, and standardized pain or behavioural scoring were not performed. Additionally, serum testosterone was monitored only up to 30 days post castration. Future

studies incorporating larger sample sizes, longer follow-up periods, endocrine profiling over extended durations, and objective welfare assessment tools would further strengthen the evidence base for this technique.

Overall, the findings of the present study support pinhole castration as a practical, economical, and welfare-oriented alternative to open surgical castration in adult bulls, particularly in resource-limited settings where simplicity, reduced complication rates, and rapid recovery are of paramount importance.

In conclusion, pinhole castration is a simple, economical, and minimally invasive alternative to conventional open surgical castration in adult bulls. The technique offers significant advantages in terms of reduced surgical duration, lower incidence of postoperative complications, faster functional recovery, and improved animal welfare. Although endocrine suppression following pinhole castration is more gradual compared to open orchidectomy, the technique results in progressive and adequate testicular regression, indicating effective long-term sterilization potential. Given its low cost, reduced requirement for surgical expertise, and suitability for field conditions, pinhole castration represents a practical option for large-scale cattle management, particularly in resource-limited settings.

Further investigations incorporating longer follow-up periods, fertility assessment, histopathological evaluation, and objective pain scoring are recommended to comprehensively evaluate the long-term outcomes of this technique.

Conflict of interest

The authors declare no conflict of interest.

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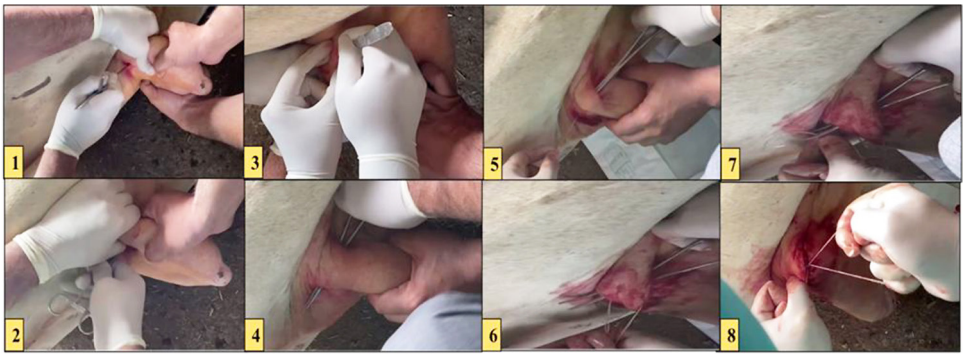

Fig. 1. Pinhole castration in a bull. The procedure was performed via two small scrotal incisions to ligate the spermatic cord percutaneously using double Vetafil loops. The cord was ligated securely around both medial and lateral aspects, and incisions were closed with polyamide sutures.