

## Comparative evaluation of cutaneous wound healing in castrated and uncastrated black bengal goats

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### Abstract

Castration leads to testosterone depletion, which may impact the efficacy of cutaneous wound healing. The present study aims to compare cutaneous wound healing in castrated and uncastrated goats, assessing morphological, hemato-biochemical, and histopathological parameters. Ten apparently healthy goats, who have body weights of 15 to 20 kg and ages ranging from 2 to 3 years, were used in this experiment. A total of 40 full-thickness incised wounds were made by giving a 1-inch-long linear incision on either side of the vertebral column, and studies were conducted in two groups: uncastrated (group A) and castrated (group B). Morphological characters with morphometric data were recorded at different day intervals. On days 3rd, 7th, and 15th post-wound creation, blood and biopsies were taken for hemato-biochemical and histopathological assessment. The wound healing days were monitored for 18 days, and morphological and morphometric data indicated that wound healing occurred faster in the animals of Group B (15 days) compared to those in Group A (18 days), as confirmed by histopathological findings of biopsy samples. The average wound contraction rate in Group B was significantly higher ( $P < 0.05$ ) than that in Group A. During this study, the values of hemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, total protein, and albumin were altered at different time intervals, but these changes were not statistically significant in both groups. These findings suggest that castration positively influences wound healing efficiency by optimizing the inflammatory phase and promoting faster tissue regeneration.

**Keywords:** Castration; Wound Healing; Histology; Hematology; Goats.

### 1. Introduction

Castration, a routine surgical practice in male goats, is the process of removing or inactivating the testes. This procedure is performed for various health, behavioral, and economic benefits, ensuring better farm productivity, improved meat quality, and easier herd management [1]. Castrated goats (wethers) exhibit better carcass composition, as they tend to deposit more fat and less muscle and the meat of wethers is tender, less musky, and has better marbling, making it more desirable in the market than intact males [2,3,4]. However, the procedure of castration causes tissue damage, inflammatory response, physiological stress, inhibition of immunological function, and decline in performance to varied degrees, which requires an effective wound healing process to prevent complications such as infection, delayed healing, and excessive inflammation [5,6]. In small ruminants like the Black Bengal goat (*Capra hircus*), a breed widely reared for its superior meat quality and adaptability, post-castration wound healing can significantly influence animal welfare and productivity and healing may differ from that of uncastrated animals due to hormonal variations [7,8].

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Wound healing is a complex biological process that involves three key phases: inflammation, proliferation, and remodeling of tissues to restore skin integrity after injury [9,10]. For optimal wound healing, rapid hemostasis, followed by controlled inflammation, differentiation, proliferation, and migration of mesenchymal cells to the wound site are crucial for ensuring the strength of the healing tissue [11,12]. In livestock, efficient wound healing is essential for ensuring animal welfare, growth performance, reproductive health, and economic sustainability [7]. Among the factors influencing wound healing, hormonal status, especially testosterone levels, plays a crucial role in regulating inflammatory response, collagen synthesis, angiogenesis, and tissue regeneration [13,14]. However, the impact of castration on cutaneous wound healing is not fully understood.

Testosterone, a key androgenic hormone, plays a vital role in enhancing fibroblast activation, angiogenesis, and extracellular matrix remodeling. Its immunosuppressive effects may prolong inflammation and delay re-epithelialization [15, 5]. In goats, testosterone levels vary between castrated and uncastrated males, potentially influencing wound healing dynamics [4].

Understanding the precise role of testosterone in cutaneous wound healing is essential for optimizing post-operative management in goats, particularly in those undergoing surgical procedures such as castration. The extent to which castration positively or negatively affects wound healing in goats remains a topic of debate, requiring further investigation. Therefore, comparing wound healing between castrated and uncastrated Black Bengal goats is essential to understanding the physiological and clinicopathological variations that may affect surgical outcomes. Despite the routine practice of castration in Black Bengal goats, limited research exists on how castration influences wound healing efficiency. Therefore, the present study has been commenced to evaluate the efficacy of wound healing in castrated and uncastrated Black Bengal goats based on morphological, haemato-biochemical and histopathological assessments.

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## 2. Materials and Methods

### 2.1. Experimental Animals

This experiment involved ten apparently healthy goats, each weighing between 15 and 20 kg and aged between 2 and 3 years. The animals were housed in an animal shed under standard conditions and continuous veterinary supervision. Before the study the goats were kept in quarantine for two weeks and all the experimental goats were dewormed with anthelmintics (Endex®, Novartis pharmaceuticals, Bangladesh, Ltd) and vaccinated with tetanus toxoid and Peste des Petits Ruminants (PPR) (P.P.R vaccine@ LRI, Bangladesh).

### 2.2. Experimental Design

Forty full-thickness surgical incisions were made on both sides of the vertebral column in goats. The animals were then categorized into two groups, Group A and Group B, with five goats in each group. Group A: Surgical wounds in non-castrated goats; Group B: Surgical wounds in castrated goats.

### 2.3. Presurgical Patient Preparation and Wound Creation

Clinical examination was performed to ensure the animal is healthy and free from infections and infestations. The goat was positioned in lateral recumbency. The surgical sites on both sides of the vertebral column were clipped, shaved, and cleaned with soap water before creating incised wounds. Following this, 70% ethanol and Povidone Iodine were applied to maintain aseptic conditions during surgery. Local anesthetic, 2% lidocaine hydrochloride (Jasocaine®, Jayson Pharmaceuticals, Bangladesh) was infiltrated with linear block in the sites of the incisions. Then the full thickness cutaneous wound was made by giving a 1-inch-long linear incision on either side of vertebral column. The skin was carefully separated from the underlying tissues using blunt dissection and all surgical wounds were then closed with nylon sutures in a simple interrupted pattern.

### 2.4. Post-operative Care

Antibiotics, antihistamines, and anti-inflammatory drugs were not used to prevent any impact on the healing process. Observations and follow-up data were documented from the day of surgery until the 18th day post-operation. Turpentine oil was applied around the wound as fly repellent. The suture was removed on day 7 of post operation. These animals were maintained carefully by checking health status day to day inspection.

## 2.5. Monitoring of Wounds and Calculation of Wound Contraction

The healing process was monitored by recording morphological characteristics such as swelling, pain sensation, and pus formation. Wound contraction was measured using digital slide calipers on the following days: Day 3 (D3), Day 6 (D6), Day 9 (D9), Day 12 (D12), Day 15 (D15), and Day 18 (D18).

The wound size reduction was assessed every alternate day, and the percentage of wound contraction was calculated using the following formula: % wound contraction = {wound area day 0 – wound area day (n)} / wound area day 0 × 100. [n = 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, and 18<sup>th</sup>].

## 2.6. Collection of Blood Samples for Hematobiochemical Examination

Peripheral blood samples were obtained from the jugular vein of the experimental goats on days 0, 3, 7, and 15 after the operation. Blood samples collected with anticoagulant (EDTA) were analyzed for the assessment of Packed Cell Volume (PCV), Hemoglobin (Hb), Total Erythrocyte Count (TEC) and Total Leukocyte Count (TLC). Hematological examinations were performed by using automatic Veterinary hematology analyzer (DYMIND®, DF56VET, Japan). Blood samples collected in clot activator tube were centrifuged at 3000 rpm for 10 minutes for serum separation. Then the supernatant was collected in an Eppendorf tube using micropipette for biochemical analysis of Total protein and Albumin. These biochemical tests were performed by semiautomatic blood chemistry analyzer (Clindia® SA-20, Belgium) using commercial diagnostic kits (Linear Chemicals SL®, Spain).

## 2.7. Collection and Processing of Tissue Samples

On days 3 (D3), 7 (D7), and 15 (D15) following wounding, full-thickness cutaneous tissues, including the underlying muscle layers, were surgically excised by all experimental groups. These tissue samples were then fixed in 10% formalin at room temperature for 24 hours. Following fixation, the tissues underwent paraffin embedding after a dehydration process using graded alcohol and xylene for clearing. Tissue blocks were sliced into 4 µm thick sections using a microtome (Histoline®, USA), and the sections were stained with hematoxylin and eosin. The stained slides were examined under a photographic light microscope (Micros®, Austria) to assess histopathological changes in the tissue.

## 2.8. Statistical Analysis

The data were presented as Mean ± SEM (Standard Error of Mean). One-way ANOVA (Analysis of Variance) and an independent sample t-test were used to compare the data both within and between groups. These analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 20.0. The probability value of P < 0.05 was considered statistically significant.

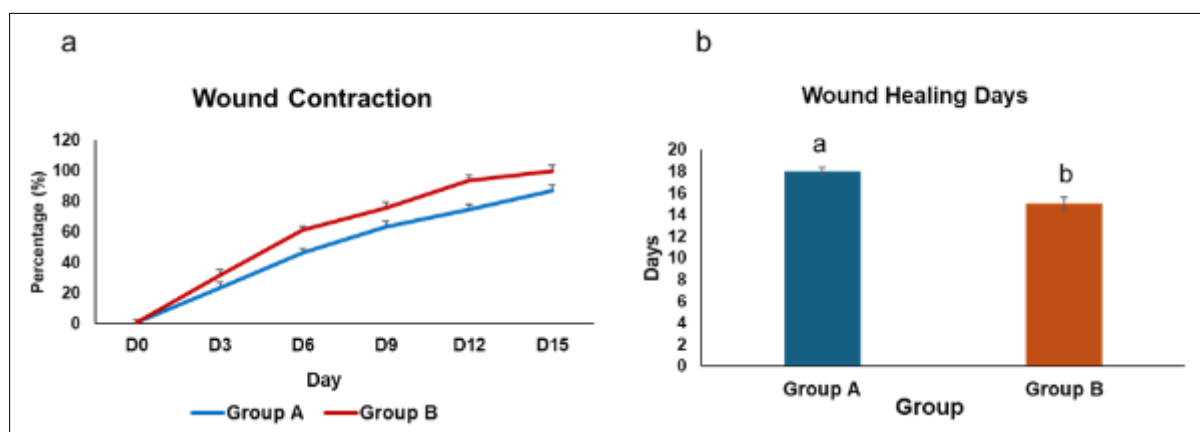
# 3. Results

## 3.1. Macroscopic Evaluation and Wound Contraction Measurement

There were marked differences in exudation, pain sensation and crust development between group A (non-castrated) and group B (castrated) at different observation time points. In this study, the pain sensation and exudation were less in group B compared to group A. At various observation time points, significant differences were noted between group A (non-castrated) and group B (castrated) in terms of exudation, pain sensation, and crust development. Crust formation, an indicator of wound healing, occurred more quickly in group B, leading to faster wound healing, whereas group A exhibited delayed healing, as illustrated in Figure 1. In comparison to group A, the average rate of wound contraction in group B was statistically significant (P < 0.05) at each indicated time point. The wound contraction rate was recorded 99.76% in group B and 86.78% in group A on day 15 (Figure 2a). Complete healing time was recorded on day 15 in group B whereas it took around 18 days in group A (Figure 2b). In this study, wounds healed up promptly in group B than other groups of wounds.



**Figure 1** Progress of wound healing and macroscopic evaluation of wound contraction process in both the groups at different experimental days

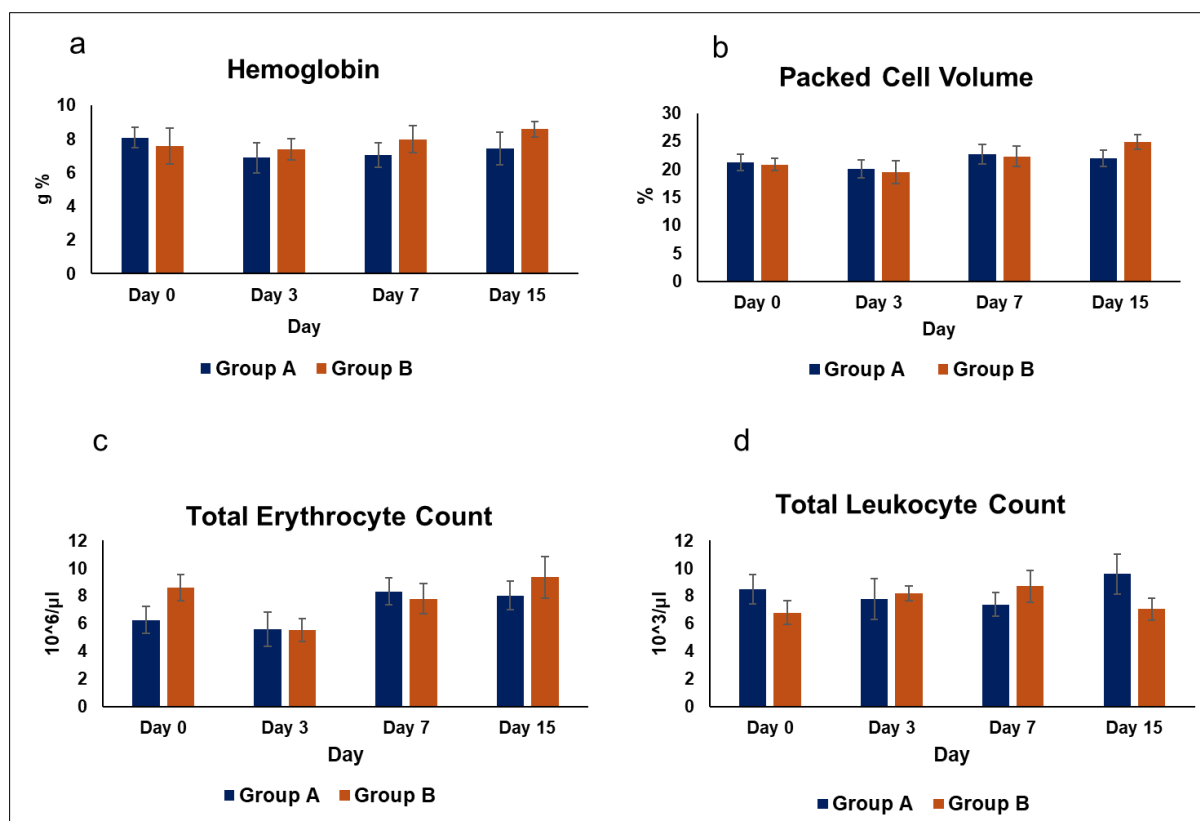


**Figure 2** Morphometric assessment of wound. (a) Percentage of wound contraction. (b) Complete healing time on days (Mean value). (a,b) indicates significant difference at 5% level

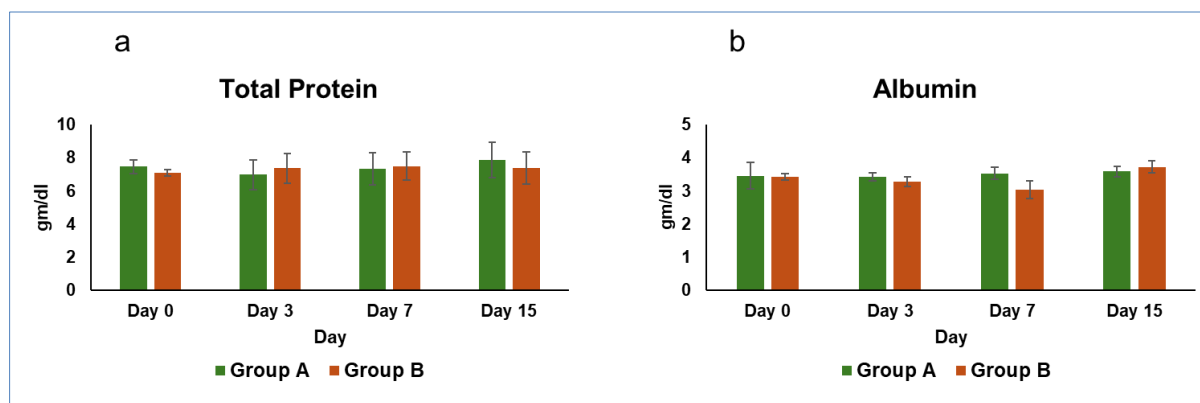
### 3.2. Haemato-biochemical Findings

In this study we assessed some haemato-biochemical parameters to observe the alterations of those parameters during the progress of wound healing. The value of hemoglobin and packed cell volume were altered throughout the study period, but these alterations were not statistically significant ( $P > 0.05$ ) (Figure 3a, b). Whereas the value of total erythrocyte count was varied significantly ( $P < 0.05$ ) on day 3 in the animals of group B as compared with other days in same group (Figure 3c). However, the changes observed between the experimental groups on different days intervals were not statistically significant. However, there was a difference in the total leukocyte count between the animals in groups A and B during the experimental days, although the differences were not considerably significant ( $P > 0.05$ ) (Figure 3d).

During this study, the value of total protein and albumin were altered on different time intervals, but changes were not statistically significant ( $P > 0.05$ ) in all the experimental groups (Figure 4a, b).



**Figure 3** Changes of some hematological parameters (a) Hemoglobin, (b) Packed cell volume (c) Total erythrocyte count (TEC), (d) Total leukocyte count (TLC) in response to experimental groups on different days intervals of post-wounding in goats

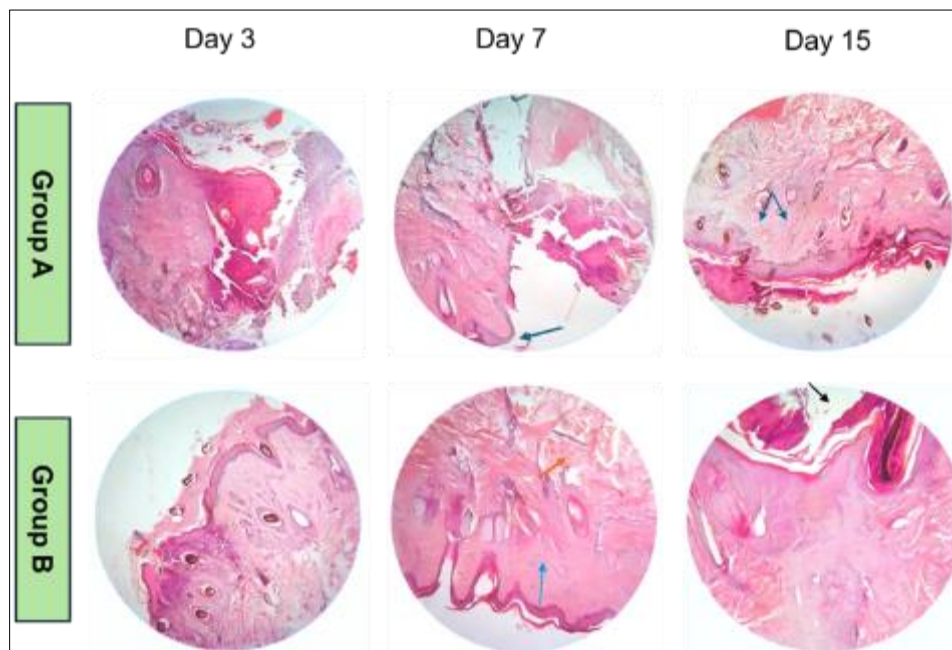


**Figure 4** Changes of serum total proteins (a) and albumin (b) in response to experimental groups on different days intervals of post-wounding in goats

### 3.3. Histopathological Findings

Normal healing requires presence of the reactive cells, proliferation of fibrous connective tissue, regeneration of epidermis, and accumulation of exudates. To address these parameters, samples were collected on day 3, day 7 and day 15 post-operatively. Microscopical examination showed the presence of reactive cells in all experimental groups in Day 3 which indicates the onset of inflammation. However, massive number of inflammatory cells along with capillary bud proliferation was found in castrated group at day 3 (Figure 5).

On day 7, the numbers of reactive cells decreased markedly, and the area was devoid of tissue debris in group A and group B. Fibroblast proliferation was marked in wounds of castrated group. Loosely packed collagen fiber found in tissue samples of both groups. On day 15, the fully formed keratin layer was presence which was thicker in group B than in group A. Angiogenesis and proliferation of collagen fibers was observed in all groups but more pronounced in group B. A greater proliferation of hair follicles was observed in castrated animals (group B) (Figure 5).



**Figure 5** Hematoxylin and eosin-stained based histopathological analysis of the skin wound tissues on day 3, day 7 and day 15 post surgery in group A and group B. (magnification x10)

#### 4. Discussion

Wound healing is a complex process through which the skin or other tissues regenerate after injury [16]. healing process can occur through primary intention, where the wound is closed with sutures, or through secondary intention, where the wound is allowed to heal naturally. In secondary intention, the healing involves the regeneration of connective tissue and the regrowth of epithelium to repair the damaged area [17,18]. This study evaluates the efficacy of cutaneous wound healing in castrated and uncastrated goats and observed the healing process in castrated goats occurred significantly earlier ( $P < 0.05$ ) than in uncastrated goats, as evidenced by morphological changes, wound contraction, and histopathological alterations. Similar findings also observed by Hameed [13], Olaifa [4], Kar et al. [19]. They reported that castrated goats may exhibit faster wound contraction rates and reduced inflammation due to lower testosterone levels, which can contribute to a more controlled healing process. However, conflicting findings suggest that uncastrated goats may experience enhanced tissue regeneration due to the anabolic effects of testosterone on fibroblast activity [2]. Nevertheless, the wound healing process may differ from that of uncastrated animals due to hormonal variations, immune response, and tissue regeneration capacity [4]. According to Fisher et al. [20], animals castrated shortly after birth tend to have greater tissue swelling and display more signs of pain. However, their incisions heal more rapidly, and their weight gain is less compromised. This observation aligns with the current findings. In this study, swelling, pain sensation, and pus formation were observed postoperatively in the animals of group A and B, and prompt healing response was noticed in group B which agrees the findings of Sultana et al. [21], Orchy et al. [22]. Increased inflammatory cell infiltration indicates that the inflammatory phase and early wound healing activities are being promoted.

Hematological parameters serve as effective indicators of an animal's physiological health and evaluating them is essential for assessing how animals respond to various physiological stressors [23]. In this study there were no significant changes observed in the value of Hemoglobin and Packed cell volume (PCV) between the group A and group B, which is contrast to the findings of Olaifa [4], Kelani et al. [24]. They reported that the decreased hemoglobin level after post wounding in castrated animals is associated with suppression of erythropoiesis. In this study, TLC (total leucocyte count) and TEC (total erythrocyte count) level altered throughout the experiment in both groups but the changes of only TEC level on day 3 were statistically significant ( $P < 0.05$ ) in the animals of group B which is similar to

the findings of Samaddar et al. [25]. High levels of TEC are observed in conditions such as hemoconcentration due to burns, cholera etc., chronic heart disease, emphysema and polycythemia [24,26]. TEC level decrease in old age, pregnancy, group of diseases classified under anemias [27].

Serum total protein consists of albumin and globulins, which are essential for cellular function, osmotic balance, and tissue regeneration. Total protein (TP) and serum albumin are attributed to increased synthesis of amino acids and protein in the remodeling phase of wound healing and these biochemical markers play crucial roles in tissue repair and immune response. In this study, no significant changes were observed in the total protein (TP) and albumin levels in the animals of both group A and group B. The total protein levels remained within the reference range for goats (5.45–7.4 gm/dl) [25]. Castrated goats showed a temporary dip in total protein and albumin levels due to metabolic stress but later recovered as healing progressed, suggesting that castration may not significantly impact these parameters [8] which is similar to our research finding.

The present study demonstrated that cutaneous wound healing is significantly faster in castrated goats compared to uncastrated goats as evidenced by histological findings. This difference can be attributed to hormonal influences, particularly androgens, which are known to affect immune response, collagen deposition, and epithelialization. Castrated goats exhibit reduced testosterone levels, which may enhance the inflammatory response, facilitating faster wound debridement and transition to the proliferative phase. A study by Gilliver et al. [7] reported that androgen deprivation accelerates wound closure by increasing keratinocyte migration and proliferation. The present study exhibited enhanced re-epithelialization in castrated goats that are observed in histological sections which agree with the findings of Gilliver et al. [28]. Collagen synthesis and deposition are crucial for wound strength and integrity [22,29]. Histological analysis in the present study showed that castrated goats had higher collagen deposition and better angiogenesis than uncastrated goats. This finding aligns with previous research demonstrating that androgens negatively regulate fibroblast function and extracellular matrix production [30,21]. Angiogenesis in castrated goats compared to uncastrated goats, as evidenced by the higher density of new capillaries within the wound bed. This finding aligns with previous research indicating that testosterone inhibits vascular endothelial growth factor (VEGF), which is a critical regulator of angiogenesis [31, 18]. The enhanced neovascularization in castrated goats likely contributed to faster granulation tissue formation and epithelialization, as seen in the present study.

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## 5. Conclusions

In conclusion, these findings suggest that castration positively influences wound healing efficiency by optimizing the inflammatory phase and promoting faster tissue regeneration. The absence of testosterone in castrated goats appears to result in a more regulated inflammatory response and enhanced epithelialization, leading to quicker wound closure and improved tissue remodeling. These findings emphasize the need for enhanced post-operative management in uncastrated goats, including nutritional support and wound care protocols, to optimize healing outcomes. Further research is recommended to explore the mechanisms underlying testosterone's role in wound healing and to investigate nutritional or therapeutic interventions that could enhance wound recovery in uncastrated goats.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors have no conflict of interest to declare.

### *Statement of ethical approval*

This animal experiment was conducted with the approval of the Animal Experimental Ethics Committee (AEEC) (Approval number: AWEEC/BAU/2023(13)), following the guidelines and recommendations set by the Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. The received date of permission was 14/10/2023.

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