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EFFECT OF CASTRATION ON HEALING OF FULL-THICKNESS CUTANEOUS WOUNDS IN BUCKS

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ABSTRACT
The purpose of the present study is to verify the effect of testosterone depletion on healing of full thickness surgical skin wounds in bucks depending on some parameters represented by a clinical follow-up, measurement of testosterone level and histopathological changes of the wounds. Twenty four adult local bucks weighing (25-30) kg were allocated to this trial. The bucks were assigned randomly and equally into two groups. The first group served as a control group, second group (castrated group) which was subjected to bilateral closed castration. Each buck was subjected to two full-thickness cutaneous wound about 10 cm length on the left lateral thoracic wall, then skin suture with interrupted horizontal pattern using silk (No.1). The castration group was wounding then sutured as mentioned in first group. Castration and wounding were performed under the effect of a sedative (xylazine hydrochloride 2%) and local anesthesia (lidocaine hydrochloride 2%). After follow-up, minor and transient secondary health problems were encountered few hours post-wounded. The animals of control group, had wound infection (two cases) and one cases in castrated group. Following castration there were swelling of the scrotal area, lameness with difficulty in walking, and hematoma at the operative site. Blood samples were collected via jugular vein puncture for hormonal assay (testosterone) prior to castration and then on days (3, 7, 14 and 21) post-castration using radioimmunoassay technique. Results reflected significant differences $P<0.05$ between animals of the castrated group among all studied periods. The lowest value (0.25 ±0.04 ng/ml) was recorded at 21 days post-castration. Biopsies $1cm^3$ were obtain from the wounds edges of both groups on 3, 7, 14 and 21 days post-wounded for microscopical evaluations (6 wounds/period). Microscopical finding at three days in control and treatment groups reflected presence of inflammatory zone. Furthermore, and with advancement of time there were granulation tissue and angiogenesis. At the end of experiment there were keratin layer, epidermis, dermis, hair follicle in addition to melanocytes. In conclusion the process of wound healing in castration group revealed better clinical and microscopic appearance in comparison to the control group and an accelerated rate of wound healing was readily apparent in the testosterone-deficient animals at day ten.

KEY WORDS: buck, skin, wound, castration.

INTRODUCTION

Wound healing is a complex process that involves the activation and synchronization of intercellular and extracellular processes. It also involves four phases *i.e*. coagulatory, inflammatory, proliferative events, remodeling and maturation (Eming *et al.,* 2014). Disruptions caused by tissue loss, inadequate blood flow, and secondary diseases complications can lead to chronic wounds that are difficult to manage (Martin and Leibovich, 2005). A clean, un-infected incision, surgically apposed with proper suture material produces the least amount of scar. Incisional wound alters the homeostatic state of the affected animal and trigger a sequence of events that constitutes four phases of wound healing (Braiman-Wiksman *et al.,* 2007; Nawaz and Bentley, 2011). In the acute inflammatory phase, the infiltrating phagocytes protect the wounded tissue from infection and remove tissue debris and necrosis (Li *et al.,* 2012). The proliferative phase is characterized by the formation of granulation tissue, synthesis and deposition of collagen fibers and matrix (Midwood *et al.,* 2004). The remodeling/maturation phase is characterized by progressive alignment of collagen bundles. Scar modification during this phase adds further to the restoration of wound tensile strength. The phases of wound healing are closely merged one into another without clear boundaries (Diegelmann and Evans, 2004). Surgical castration means removal of one testis (unilateral) or both (bilateral) is the commonest operative procedure routinely performed on food animals for economic purposes or for treatment of certain diseases affecting the testes (Kevin *et al*., 2015). Testosterone is a steroid hormone which is produced mainly from the testes (leydig cells) and in small amount from both adrenal gland and blood stream. It has both androgenic or masculinizing properties and anabolic properties (Mirando *et al*., 2007). Androgenic effects are presented to some degree in all anabolic steroids. Androgenic effects include development of male sex glands, determination of male hair growth pattern, increased libido, and it's important in spermatogenesis (Pelletier, 2013). The aim of the study is to evaluate the effect of castration on healing of full thickness cutaneous wounds in bucks based on clinical follow-up, assay of testosterone level and microscopic examination.

MATERIALS & METHODS

1. Experimental animals: Twenty-four apparently healthy adult bucks were used for the current research after ascertaining that they were free of any dermatological lesion. Bucks were aged from (1.5-2) years and weighing (25-30) kg. All bucks were maintained hygienically in animal shed of the Veterinary Medicine, University of Baghdad from January to May (2017).

2. Experimental design: The bucks were allotted randomly into two equal groups as follow:

A. First (control) group contains (12) intact bucks, which were subjected to cutaneous wound only, then wounds were sutured.

B. Second (castrated) group contains (12) bucks, which were subjected to bilateral castration then skin wounded and finally sutured.

Technique of castration

Closed bilateral castration was performed on (12) bucks. These were done according to the technique used by Staffored *et al*. (2000). The castrated animals were left for 21days prior to skin wounding.

Technique of skin wounding

Prior to the operation, the bucks were deprived from food for 24 hours and water for 12 hours. The operative field was the left lateral thoracic wall which was prepared for aseptic surgical incisions. The animals were restrained in lateral position with the help of Xylazine 2% and anesthesia was accomplished with a linear sub-cutaneous infiltration of the intended incisional site with lidocaine hydrochloride 2%. Under aseptic conditions two linear equidistant full thickness skin incisions approximately (10 cm) in length were created on the left lateral thoracic wall (one parallel to another, ten cm apart) (thus the total wounds number were 48). Bleeding was arrested by routine manner. The skin wounds were restricted with interrupted horizontal mattress patterns with non-absorbable suture material (silk No. 1). Sterile bandages were fixed above the wound. A combination of systemic antibiotic containing penicillin and streptomycin was given IM, in a dose rate of 10.000 IU and 5mg/kg respectively for four consecutive days.

Parameters of the study

A. Clinical examination

Follow-up information was recorded from the day of surgical operation up to day 30 post-surgery to record any complications which may occur.

B. Testosterone measurement (ng/ml)

Blood samples were collected by jugular vein puncture from the castrated groups on 3, 7, 14 and 21 days post-castration. Then all sera were removed by centrifuging the blood samples at 3000 rpm for five minute and stored at -20C° until assayed. The serum testosterone level was determined by Enzyme-linked immuno- immunosorbent assay (ELISA) according to method mentioned by Rachmawati *et al*., (2013).

C. Microscopical examination

For this purpose, skin biopsies ($1cm^3$) were taken. The biopsies were collected from the wound areas (samples containing dermis and epidermis) of each experimental animal on days (3, 7, 14 and 21) (6 wounds/period) with standard surgical procedure. Sections (5-6) micron were prepared perpendicular to the original incision and were stained with hematoxylin and eosin (H & E) and Masson's trichrom stains then mounted with cover slip and examined under light microscope (Bancroft *et al*., 2013)

Statistical analysis

This was done by using Statistical Package for the Social Sciences (SPSS). In this study, all data were presented as Mean± SE. One way Analysis of Variance (ANOVA) was performed. In addition least significant differences (LSD) were used. The significance level was adopted at ($P<0.05$) (SAS, 2004).

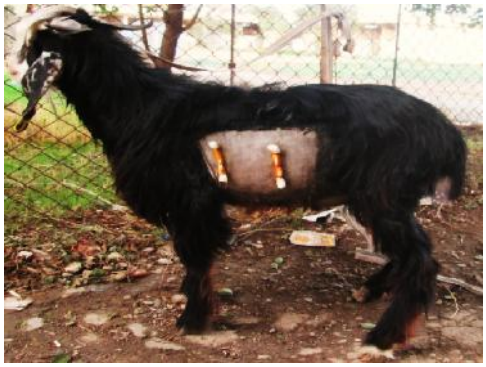

FIGURE 1. Shows the final appearance of the two skin wounds on the left lateral thoracic wall

RESULTS

1. Clinical observation of the cutaneous wounds

All bucks were followed-up clinically during the studied period to record any abnormalities. Thus there were certain secondary health problems encountered in both groups included two wound infections (abscess) were noticed in control group and one in castrated group. There were purulent discharge and dehiscence (opening of some skin stitches) in 5^{th} and 7^{th} days post-surgery. These abscesses were diminished completely after five days treatment in both groups. The wounds in the control group were slower and demanded about 2 weeks for complete healing. While in castrated group wound had a perfect wound edges approximation with completely healing in (8-10) days.

2. Complications post-castration:

In castrated group, some secondary complications were evacuated; these were illustrated in table (1).

TABLE 1. The post-surgical complications happened in castrated bucks

Complication type	numbers of affected animals	Response to treatment
Scrotal swelling	12	+
Hematoma	2	+
Lameness	4	+
Wound infection	1	+

Scrotal swellings were evident in all castrated bucks shortly after recovery from operation. It reached its peak at 2-3 days post-surgery This phenomenon was receded spontaneously on the 4th days gradually and completely diminished at 6th day post-surgery. Hematomas were seen in two bucks as clotted blood accumulated in the scrotum at the 3th day post-surgery. They removed by gentile pressure and the bucks retained to its normal condition. Lameness, is a common complication following castration. It took place in four bucks soon after recovery from analgesia. It treated with pain suppuration (metalgin) in a dose of 5 mg/kg IM for three days, in addition to the antibiotics (both drugs used for all experimental animals as a post-operative care). Wound infection, showed in one bucks of castrated group in 5th day post-surgery. The wound was treated as mention in treatment of wound infection in control group. All previously mentioned complications were minor, transient and respond to the treatment. In addition all bucks survived till the end of the study.

3. Serum testosterone concentration:

The value of testosterone concentration prior to castration was (5.27 ±0.48 ng/ml). In contrast the values were significantly differed P≤ 0·05 among animals related to castrated group. It dropped sharply with the advancement of experimental period and reached to its lowest value (0.25 ±0.04 ng/ml) at the end of experiment (21 days) post-castration (table- 2).

TABLE 2. Mean values of serum testosterone concentration (ng/ml) in castrated group at different studied periods.

Times (days)					
Zero	3	7	14	21	LSD
	M ±SE	M ±SE	M ±SE	M ±SE	
5.27 ±0.48	0.98 ±0.033	0.70 ±0.05	0.45 ±0.02	0.25 ± 0.04	0.35
A	B	BC	CD	D	

Zero time= prior to castration.
Different capital letters denote differences P≤ 0·05 among periods in castrated groups.

4. Microscopical findings

A. Three days:

In control group and at this time there were inflammatory zone contain inflammatory cells mainly neutrophils and macrophage in addition to the dermis, thick fibrin deposit, degenerated collagen bundles and hair follicle (Fig. 2). In castrated group at the same period, section reflected epithelium of epidermis, dermis, dermal papillae, fibrin clot, degenerated collagen bundles which stained green with special stain (Masson trichrom stain) in addition to hemorrhage (Fig. 3).

B. Seven days:

In control group, section revealed fibrin clot, inflammatory zone, granulation tissue beside blood vessels (Fig. 4). In castrated group, section stained with Masson trichrom stain, there were keratin layer, epithelium and mature collagen bundles which took the green color and hair follicles (Fig. 5).

C. Fourteen days: In control group and at this moment, section stain with H&E reflected the presence of epithelium covered by keratin layer in addition to organized granulation tissue (Fig. 6). In castrated group, section stained with Masson trichrom stain revealed keratin layer, epithelium, dermal bud, dermal papilla and collagen bundle which stained in (green color) (Fig. 7).

D. Twenty-one days:

In control group, the main findings at this time were the presence of keratin layer, epithelium, organized granulation tissue, hair follicle and sebaceous gland (Fig. 8). In castrated group, there were thin layer of keratin, epithelium, mature granulation tissue of the dermis and blood vessels (Fig. 9). On Masson trichrom stain, there were thick keratin layer, epithelium, mature granulation tissue of dermis and blood vessels (Fig. 10).

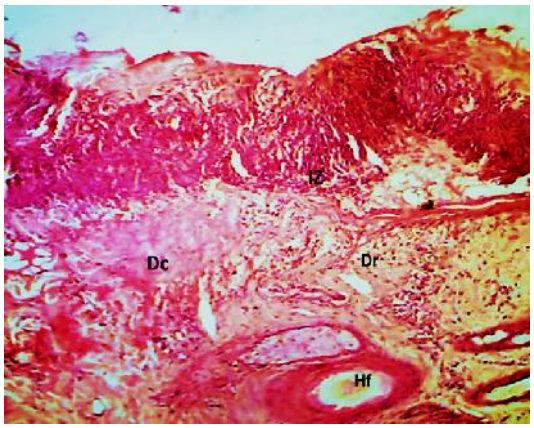

FIGURE 2. Histopathological changes of skin on 3 days control group shows, inflammatory zone (Iz) dermis (Dr), degenerated collagen bundles (Dc), and hair follicle (Hf) (H&E.,10X).

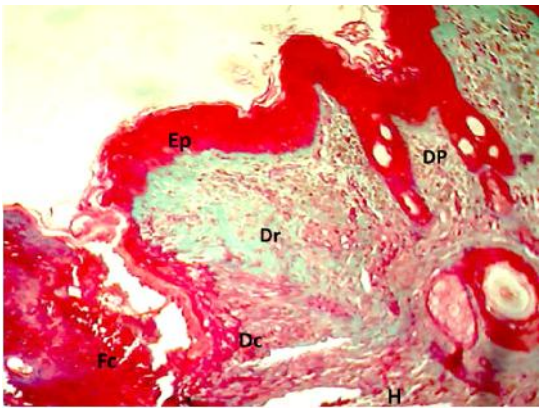

FIGURE 3. Histopathological changes of skin on 3 days castration group shows, epithelium of epidermis (Ep), Dermis (D), Dermal papillae (Dp), fibrin clot (Fc), degenerated collagen bundles (Dc), hemorrhage (H) (Masson trichrom stain 40X).

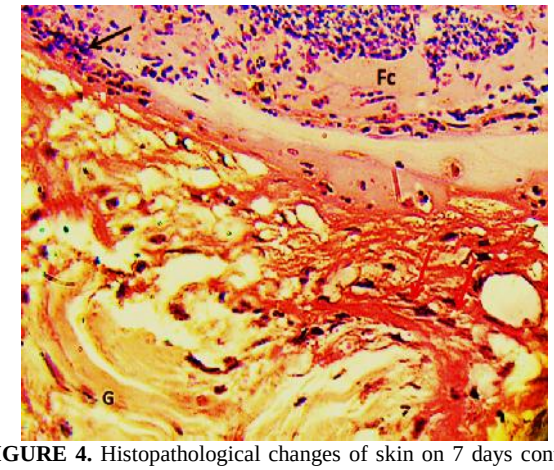

FIGURE 4. Histopathological changes of skin on 7 days control group shows, fibrin clot (Fc), inflammatory zone (black arrow), granulation tissue (G) and fibroblasts (Red arrows) (H&E., 40X).

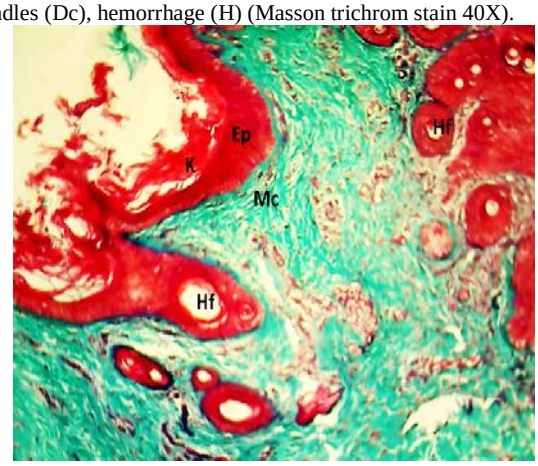

FIGURE 5. Histopathological changes of skin on 7 days castration group shows, keratin layer (K), Epithelium (Ep) and mature collagen bundles (Mc) and hair follicles (Hf). (Masson trichrom stain, 20X).

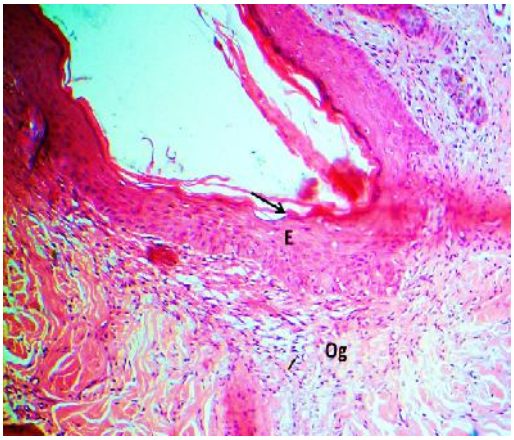

FIGURE 6: Histopathological changes of skin on 14 days control group shows, epithelium (E) covered by keratin layer (arrow) and organized granulation tissue (Og) (H&E., 10X).

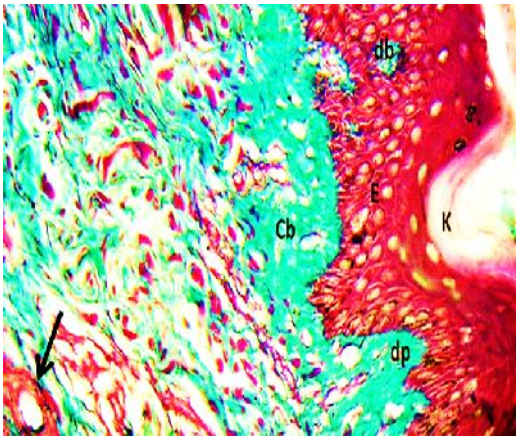

FIGURE 7. Histopathological changes of skin on 14 days castration group shows, keratin layer (K), epithelium (E), dermal bud (db), dermal papilla (dp) and collagen bundles (Cb) (Masson trichrome, 40X).

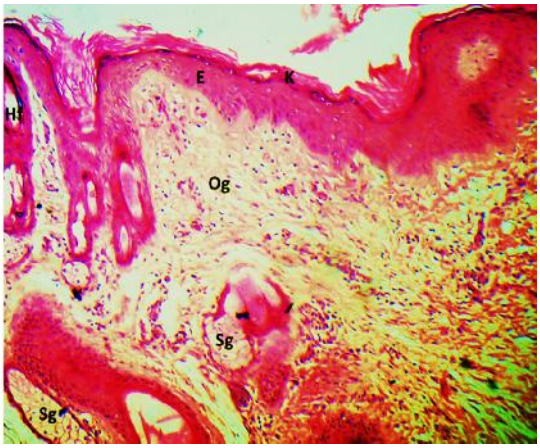

FIGURE 8.Histopathological changes of skin on 21 days control group shows, keratin layer (K), epithelium (E), organized granulation tissue (Og) hair follicle (Hf) and sebaceous gland (Sg) (H&E., 20X).

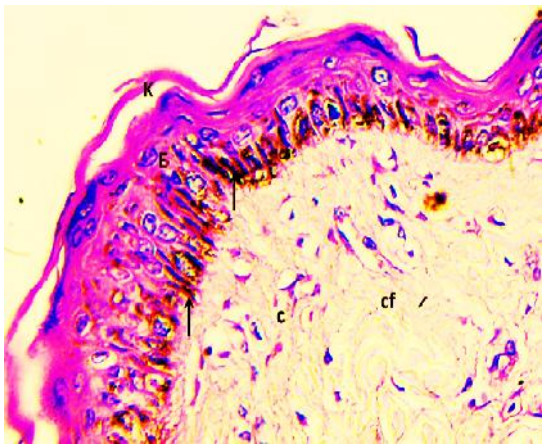

FIGURE 9. Histopathological changes of skin on 21 days castration group shows, keratin layer (K), epithelium (E), capillaries (c) collagen fibers (Cf) and melanocytes (arrows) (H&E., 40X).

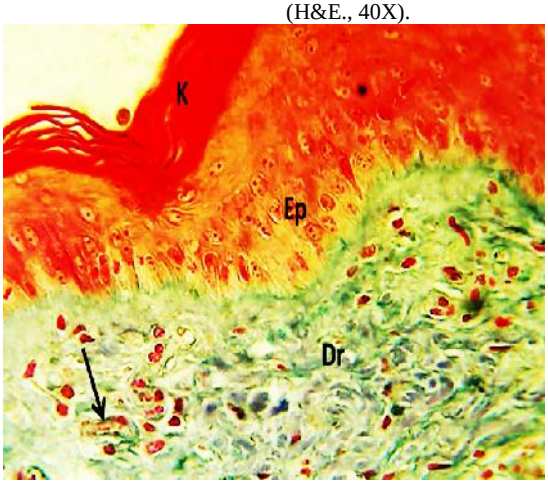

FIGURE10. Histopathological changes of skin on 21 days castration group shows, thick keratin layer (K), epidermis (Ep), mature granulation tissue of dermis (Dr), blood vessels (arrow) (Masson trichrom stain, 40X).

DISCUSSION

1. Clinical observation: There were certain minor non-specific secondary health problems encountered in both groups, these include:

A. Wound infection: Three localized and superficial wounds infection were happened in both groups. Two were seen in control group and one in castrated group. This is a common complication with most surgical operations including castration and may become clinically evident as early as a few days post-operatively. We can ascribe this problem to bacterial infection. Similar finding was recorded by Al-Asadi and Al-Kadi (2008) in bucks. In an experimental study, Eugster *et al.* (2004) has been described wound infection as a complication of 0.8% to 18.1% of small animal surgical procedures with significant variation associated with surgery type.

Baba *et al.* (2012) referred that inadequate drainage, contamination; excessive swelling and poor surgical technique increase the occurrence of postoperative wound infection. Adin (2011) indicated that a sepsis represented by proper preparation of the scrotal and inguinal areas is essential with different castration techniques because introducing of infection into the scrotal cavity would result in severe complications.

B. Scrotal swelling: Scrotal swelling happened in all bucks from day of castration and reached its peak at 3rd to 4th day then receded gradually and totally disappeared at 6th day. Swelling may be attributed to inflammation evocated from the techniques used in this experimental trial. Swelling is one of the cardinal signs of inflammation. Signs of swelling of the scrotum had been reported in many previous researches concerning castration (Melches *et al.*, 2007; Naoman and Taha, 2010).

C. Hematoma: Hematomas were seen in two bucks as clotted blood accumulated in the scrotum at the 3th day post-surgery. This may be due to technical error *i.e.* improper hemostasis during surgical operation or poor drainage. It is not serious and treated manually with success result. Scrotal hematomas were recorded in previous study concerning castration (Al-Asadi and Al-Zaidi, 2010).

D. Lameness: Lameness is a common signs following castration. It happened in four bucks soon after recovery from analgesia. Lameness was sub-sided in a shorter time and may be attributed to pain elicited from the operation. Many procedures can subject animal to such conditions, castration is one of them. This procedure has a profound effect on the animal behavior including increased rate of restlessness and lying time. These signs noticed in some

castrated bucks in present study. This is in accordance with the findings of Thorntan and Waterman, (1999) in lambs; Robertson *et al.,* (2007) in calves.

2. Testosterone assay: All castrated bucks had serum testosterone concentrations that were significantly declined $P<0.05$ than control. This observation may result from removal of the testes which is considered as the main source of testosterone production. This outcome was in accordance with previous study demonstrating the effect of luteinizing hormone-releasing hormone (LH-RH) immunization on testicular steroid genesis in bulls (Aissat *et al*., 2008).

3. Microscopical finding

Microscopical finding of the wound in current study were similar to many researches correlated with wound healing (Lindley *et al.,* 2016; Pereira and Bartolo 2016; Olaifa and Adeyemi 2017). The control group at three days revealed zone of inflammatory cells mainly neutrophils infiltration in the incisional site, this may occur due to fibrin clot and platelet factor that attract the inflammatory cells and this idea was in agreement with (Kim *et al.,* 2008) who demonstrated that fibrin clot helps in leukocytes migration as well as platelets adhere to it and secrete factors.

The lesions at three days post-incision in castrated animals characterized by regeneration of epithelial cells with polymorph-nuclear cells infiltration, these lesions may indicated beginning of cellular phase of wound healing which involves several types of cells working together to mount an inflammatory response, synthesize granulation tissue, and restore the epithelial layer. The presence of macrophage in the lesion at day three in the current study may indicated that the healing process in this group was faster than those in control group due to the fact that macrophages play essential role in regeneration and repair process and this idea was in agreement with observation of certain authors (Gilliver *et al.*, 2007; Gundra *et al.*, 2014), who showed that one of the macrophage's roles is to phagocytize bacteria and damaged tissue and they also debride damaged tissue by releasing proteases .and has a role in regeneration and are essential for wound healing. The microscopical of control group at seven days post-wounding, revealed immature granulation tissue such result may indicated that the healing process reached to early proliferative phase which is characterized by angiogenesis, collagen deposition produce by fibroblasts which grow and form a new (ECM) by excreting collagen and fibronectin, this results come in line with Pastar *et al.* (2014).

In castrated group at the same period, we recorded mature granulation tissue regeneration in addition to keratin layer, these stage may be indicated the late stage of proliferative phase, this evidence was supported by Singer and Clark (1999) who demonstrated that the process of wound healing is divided into two major phases: the early phase and the cellular phase: The early phase, which begins immediately following skin injury, involves cascading molecular and cellular events leading to hemostasis and formation of an early ECM that provides structural staging for cellular attachment and subsequent cellular proliferation. The cellular phase involves several types of cells working together to mount an inflammatory response, synthesize granulation tissue, and restore the epithelial layer.

At day 14 post incision, the control group showed completed epithelization with granulation tissue that characterized by collagen fibers deposition, and less angiogenesis this result may be indicated that the healing process reached late proliferative stage. The proliferative phase in the present study was noticed in castrated group at day 7 and at day 14 post incision in control group, these results may be attributed to the influence of testosterone on angiogenic factors, this finding was supported by Franck-Lissbrant *et al.* (1998) who found that testosterone stimulates angiogenesis and vascular re-growth in castrated animals.

The microscopical picture in castrated group and at 14 days in our study reflected the presence of angiogenesis with fibroblast this was in agreed with Haase (2003) who noticed that fibroblasts begin entering the wound site two to five days after wounding as the inflammatory phase is ending, and their numbers peak at one to two weeks post-wounding. Putnins *et al.* (1999) stated that by the end of the first week, fibroblasts are the main cells in the wound. Fibroplasia ends two to four weeks after wounding.

The microscopical pictures of the present study at day 21 post incision showed that the control group expressed complete healing process but the healing process in the castrated group was better than those in the control group, these result may indicate that the maturation phase is started early in the castrated group. The complete healing of the incision in the castrated group at this time associated with normal epidermal layer and dermal appendages such as hair follicles and sweat glands which was clear in the microscopical sections and similar findings were noticed by Jahoda, and Reynolds, (2001) and Miller *et al.* (1998) in pigs, on another hand, Ashcroft and Mills (2002) referred that testosterone deficiency accelerated skin wound healing and the presence of testosterone may inhibit growth factor and cellular migration.

During remodeling/maturation phase, type III collagen, which is prevalent during proliferation, is replaced by type I collagen (Diegelmann, 2003). The onset of the maturation phase may vary extensively, depending on the size of the wound and whether it was initially closed or left open, ranging from approximately 3 days to 3 weeks (Finnson *et al.*, 2013; Shiro *et al.*, 2017). The maturation phase can last for a year or longer, similarly depending on wound type. As the phase progresses, the tensile strength of the wound increases (Jimi *et al.*, 2017).

REFERENCES

Adin, C.A. (2011) Complications of orchiectomy in animals. Vet. Clin. Small Anim. 41: 1023–1039.

Aissat, D., Sosa, J.M., Avila, D.M., Bertrand, K.P. and Reeves, J.J. (2008). Endocrine, growth and carcass characteristics of bull immunized against LH-RH fusion protein. J. Anim. Sci., 80:2209-2213.

AL-Asadi, R.N. and AL-Kadi, K. K. (2008). Castration of the bucks via comparative study of using single and double ligation of the spermatic cord. MSc. Thesis ,

College of Veterinary Medicine- University of Baghdad-Baghdad-Iraq.

Al-Asadi, R.N. and Al-Zaidi, A.A. (2010). Chemical Castration of Rams Via Intra-testicular Injection of Sodium Chloride or Acetic Acid. MSc. Thesis- College of Veterinary Medicine- University of Baghdad- Baghdad-Iraq.

Ashcroft, G. S. and Mills, S.J. (2002). Androgen receptor-mediated inhibition of cutaneous wound healing. J. Clin. Invest.,110:615–624.

Baba, M. A., Fazili, M. R., Athar, H., Mir, M. S.. and Khan, I. (2012). Pinhole castration technique: An alternative to orchiectomy in stray dogs. Anim. Reprod. Sci., Pp: 113–118.

Bancroft, J.D., Layton, c. and Suvarna, S.K. (2013). Bancroft's Theory and Practice of Histological Techniques. 7th ed, Churchill Livingstone, Elsevier. P: 151.

Braiman- Wilesma, L., Solomonir, I. and Tennenbum, T. (2007). Novel Insights into Wound Healing Sequence of Events. J. Toxicol. Pathol., 34: 767-779.

Diegelmann, R. (2003). Analysis of collagen synthesis. Methods Mol. Med., 78: 349-353.

Diegelmann, R.F. and Evans, M.C. (2004). Wound healing: an overview of acute, fibrotic and delayed healing. Front. Biosci., 9:283-289.

Eming, S.A., Martin, P. and Tomic-Canic, M. (2014) Wound repair and regeneration: mechanisms, signaling, and translation. Sci. Transl. Med., 6: 265-266.

Eugster, S., Schawalder, P. and Gaschen F. (2004) A prospective study of postoperative surgical site infections in dogs and cats.Vet. Surg., 33(5):542–50.

Finnson, K.W., McLean, S., di Guglielmo, G.M. and Philip, A. (2013) Dynamics of Transforming Growth Factor Beta Signaling in Wound Healing and Scarring. Adv. Wound Care, 2, 195–214.

Franck-Lissbrant I., Haggstrom, S. and Bergh, A. (1998) Testosterone stimulates angiogenesis and vascular regrowth in castrated adult rats. Endocrinology, 139: Pp:451–456.

Gilliver, S.C., Ashworth, J.J. and Ashcroft, G.S. (2007) The hormonal regulation of cutaneous wound healing. Clin. Dermatol., 25: 56-62.

Gundra, U.M., Girgis, N.M., Kurtz, Z.D., Wiens, K.E., Basu-Roy, U. and Mansukhani, A. (2014) Alternatively activated macrophages derived from monocytes and tissue macrophages are phenotypically and functionally distinct. Blood, 123: 110–122.

Jahoda, C.A. and Reynolds, A.J. (2001) Hair follicle dermal sheath cells: unsung participants in wound healing. Lancet. 358:1445–1448.

Jimi, S., de Francesco, F., Ferraro, G.A., Riccio, M. and Hara, S.A. (2017) Novel Skin Splint for Accurately Mapping Dermal Remodeling and Epithelialization During Wound Healing. J. Cell Physiol., 232, 1225–1232.

Kevin, H., Dale, R.Z. and Daren, C. (2015) Effects of delayed castration on beef cattle production and carcass traits. J. Anim. Sci., 44(2): 1-7.

Kim, M.H., Liu, W., Borjesson, D.L., Curry, F.R., Miller, L.S., Isseroff, R.R. and Simon, S.I. (2008) Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. J. Investig. Dermatol., 128, 1812–1820.

Li, Y.B., Shi, Y., Zhang, W. and Wen, Z. (2012) Roles of Macrophages and Neutrophils During Regeneration. J.B.C., 287(30): 25353–25360.

Lindley, L.E., Stojadinovic, O., Pastar, I., Tomic-Canic, M. (2016) Biology and Biomarkers for Wound Healing. Plast. Reconstr. Surg.,138: 18−28.

Martin, P. and Leibovich, S.J. (2005) Inflammatory Cells during Wound Repair. Trends Cell Biol., 15(11): 599-607.

Melches, S., Mellema, S.C., Doherr.; M.G., Wechsler, B. and Steiner, A. (2007) Castration of lambs: A welfare comparison of different castration techniques in lambs over 10 weeks of age. Vet. J., 173 (3): 554-563.

Midwood, K.S., Williams, L.V. and Schwarzbauer, J.E. (2004) Tissue repair and the dynamics of the extracellular matrix. Int. J. Biochem. Cell Biol., 36:1031−1037.

Miller, S.J., Burke, E.M., Rader, M.D. and Lavker, RM. (1998) Re-epithelialization of porcine skin by the sweat apparatus. J. Invest. Dermatol., 110, 13–19.

Mirando, M.A., Hoagland, T.A. and Riesen, J.W. (2007) The influence of unilateral castration on testicular morphology and function in adult rams. Biol. of reprod., 41 (5): 798-806.

Naoman, U.T. and Taha, M.B. (2010) Effect of hemi-castration on testicular growth and seminal characteristics of Iraqi male goats. Iraqi J. of Vet. Sci., 24:71-74.

Nawaz, Z. and Bentley, G. (2011):Surgical Incisions and Principles of Wound Healing. Surgery, 29 (2): 59-62.

Olaifa, A.K. and Adeyemi, I.M. (2017) Epidermal Wound Contraction Rates on Different Parts of the Body of West African Dwarf Goats. J. of Vet. Med. and Surgery. 1 (1): 7-13.

Pastar, I., Stojadinovic, O., Yin, N.C., Ramirez, H., Nusbaum, A.G., Sawaya, A., Patel, S.B., Khalid, L., Isseroff, R.R. and Tomic-Canic, M. (2014)

Epithelialization in wound healing: a comprehensive review. Adv. Wound. Care, 3: 445−454.

Pelletier, G. (2013) Localization of androgen and estrogen receptors in primate tissues. Histol. Histopathol., 15:1261–1270.

Pereira, R.F., Bartolo, P.J. (2016) Traditional therapies for skin wound healing. Adv. wound care, 5: 208−229.

Putnins, E.E., Firth, J.D., Lohachitranont, A., Uitto, V.J. and Larjava, H. (1999) Keratinocyte growth factor (KGF) promotes keratinocyte cell attachment and migration on collagen and fibronectin. Cell Commun. Adhes., 1,7(3):211–221.

Rachmawati, L., Ismaya, and Astuti, P. (2013) Level of testosterone, libido, and sperm quality of Cross-Breed bucks. Anim. Produc., 15(2):76-82.

Robertson, I.S., Kent, J.E. and Molony, V.K. (2007) Effect of different methods of castration on behavior and plasma cortisol in calves of three ages. Res. Vet. Sci., 56 (1): 8-17.

SAS (2004) SAS/STAT Users Guide for Personal Computers. Release 7.0. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).

Shiro, J., Michele, R., Shuuji, H. and Hiroyuki, O. (2017) Acceleration Mechanisms of Skin Wound Healing. Int. J. Mol. Sci., 18: 1-14

Singer, A.J. and Clark, R. (1999) Cutaneous wound healing. New Engl J Med., 2 (10):738–46.

Stafford, K.J., Mellor, D.J. and McMeekan, C.M. (2000) A survey of the methods used by framers to castrate calves in New Zealand. N.Z. Vet. J., 48:16-19.

Thorntan, P.D. and Waterman, A.E. (1999) Quantification of pain and distress response to castration in young lambs. Res. Vet. Sci., 66: 107-118.