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EPIDERMAL WOUND HEALING IN PREPUBERTAL WEST AFRICAN DWARF (WAD) GOATS

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Abstract
There is dearth of information and data on wound healing assessment in prepubertal goats. Epidermal wounds were created on the trunk of all the goats. Each wound was measured daily using the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a vernier'scalliper.Blood was collected by jugular venipuncture using a sterile needle and syringe both for hematology and serum analyses using haematocrit and phot electric methods respectively. The wound contraction was significant on day 5 when compared with day 0, also significant on day 7 when compared with day 3 ($p<0.05$). It was observed that the wound healed on day 7. There was no remarkable difference in the blood and chemistry parameters as all fell within range for the specie. The consistency of epithelial response in the prepubertal goat demonstrates the ability of the epidermis for early re-epithelialization and proliferative activity just to ensure prompt healing.

Key words: prepubertal, wounds, epidermal, West African Dwarf goats

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Introduction

Wound healing is a complex process that involves the activation and synchronization of intracellular, intercellular and extracellular processes. It also involves coagulatory and inflammatory events, fibrous tissue accretion, deposition of collagen, epithelialization, wound contraction, tissue granulation and remodeling (Dorsett-Martin, 2004; Pessoa et al., 2004; Sultana et al., 2009). Wound healing processes activate local and systemic cells to restore tissue integrity through regeneration and scar formation, resulting in satisfactory repair of disrupted tissue (Forbes and Rosenthal, 2004).

Goats are considered as ideal animals to keep owing to their high ability to survive under severe conditions and their ability to produce high-quality meat and milk (Silanikove, 2010).Puberty in goats is characterized by the beginning of thereproductive activity which has great importance for thebreeding system. It allows defining management practices such as the separation of lots defined by sex, time of castration, early selection of animals for procreation, permitting greater effectiveness in the improvement of the herd (Pacheco et al., 2009).

Hematological, biochemical, and mineral profiles are important to be determined because they provide valuable information about the breed, sex and animals' health status (Madan et al.,2016).The physiological adaptation and the systemic relationship are widely determined using the hematological values (Shah et al., 2007).

Several studies have been conducted on epidermal wound healing in adult goats. However, there is dearth of information and data on wound healing assessment in prepubertal goats hence this study.

Materials and Methods

Experimental animals

Four 3-month old prepubertal West African Dwarf goats were put in stalls. The animals were housed in individual pens three weeks for stabilization before commencement of the experiment. Well-balanced diet consisting of concentrate, grass and cassava peels were fed to the animals and water provided adlibitum. The animals were dewormed with levamisole (10%) I/M at the dose rate of 10mg/kg body weight and also given penicillin-streptomycin preemptively to take care of possible bacterial infections.

Wound creation and measurement

Using a square stencil of dimension 1cm by 1cm, the portion of the epidermis to be surgically removed which is the right lateral side of the animal just ventral to the vertebrae column was marked using an ink marker. Three mg/kg of 2% lignocaine was used in caudal epidural block and local infiltration (inverted L-Block) to desensitize the skin in order to ensure complete desensitization of nerves that might escape epidural block and provide the required anaesthesia. Booster injections of up to one-half of the initial dose were administered as needed in order to ensure that the goats were pain-free during the skin excision procedure. Each marked portion was blocked individually before surgery was done. Epidermal wounds were created on the trunk of all the goats. A sharp sterilized scalpel was used and bleeding reduced by the use of pressure gauze and shortening of surgery duration. The full thickness of the skin within the incision was then carefully stripped away by sharp dissection from its underlying muscle. All excisions were made using a scalpel blade and forceps; with particular care taken that wound edges were sharply defined. Each wound was measured (in centimetre^2) daily using the length of the mid-horizontal and mid-vertical sides of the wound with the aid of a Verniercalliper. Error due to parallax was reduced by ensuring that wounds were measured under adequate illumination using the same blind observer all through the experiment. The length (L) and breadth (B) were then used to calculate the wound area in cm^2 (Olaifa and Fadason,2016).

Blood analysis

2.5ml of blood was collected by jugular venipuncture using a sterile needle and syringe both for hematology and serum analyses. The samples were collected in the morning when the animals were calm and the ambient temperature was low so as to reduce stress related consequences. Thereafter, the samples were immediately taken to the laboratory for analyses after proper storage in an ice pack.

The blood samples collected for hematology were evaluated for packed cell volume (PCV) using the haematocrit method (Jain and Schalm, 1986). Hemoglobin concentration was evaluated using the cyanomethaemoglobin method (Schalmet al.,1975). Red blood cell count was determined by the haematocytometry method (Jain and Schalm, 1986).Total white blood cell (WBC) counts and differential leucocyte counts were estimated according to Coles (1989). Serum urea and Creatinine levels was determined using photoelectric colorimeter (Coles, 1989). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using a colorimetric method (Reitman and Frankel 1957). The serum electrolyte levels were evaluated using flame photometry (Jones, 1995).

Histopathology- Appropriate sectional biopsies were taken from the wounds at days 0, 3, 5 and 7 under local anesthesia from the pre-pubertal goats. The wound biopsy were fixed in 10% neutral buffered formalin for routine histological processing and staining with haematoxylin and eosin for morphologic assessment.

Statistics –Data was summarized as mean and standard deviation, presented as chart and compared between days using ANOVA on Graphpad prism at 5% significance.

Results

Percentage wound contraction rate- The wound contraction was significant on day 5 when compared with day 0, also significant on day 7 when compared with day 3 ($p<0.05$). It was observed that the wound healed on day 7 (Figure 1).

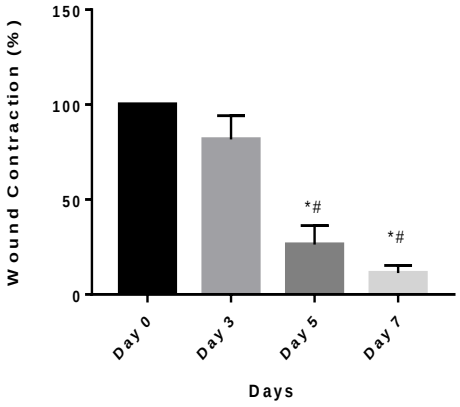


Figure 1: Percentage wound contraction rate in prepubertalWestAfrican dwarf (WAD) goats

*P<0.001 when compared with day 0

#P<0.001 when compared with day 3

Haematology– There was no remarkable difference in the blood parameters as they were within range for the specie, except the moderate neutrophilia. The packed cell volume PCV was 46.33±0.88%, red blood cell count was 9.50±0.38 (10^3/µL), haemoglobin concentration was 15.07±0.33 (g/dl), white blood cell count was 8.41±0.43 (10^3/µL), platelet count was 6.05±0.59 (10^5/µL), lymphocyte count was 2.69±0.12 (10^3/µL) and neutrophil counts was 5.66±1.31(10^3/µL).

Blood Chemistry -The blood chemistry falls within normal range, however, the estrogen level was more than the testosterone level (10.67±1.20 vs 3.23±0.18). The electrolytes concentration including sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), zinc (Zn^{2+}) and cupper(Cu^{2+}) are shown in figure 2).

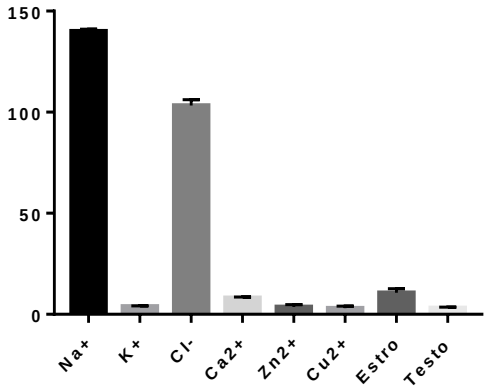
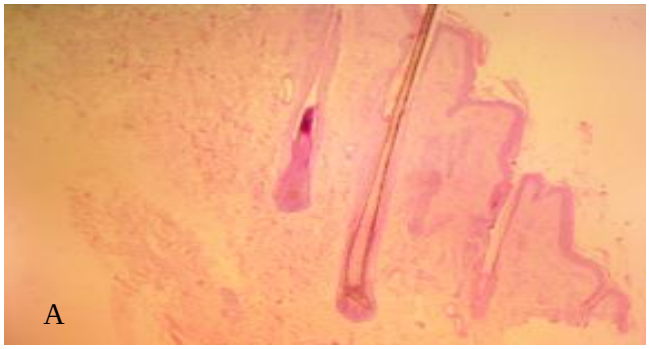
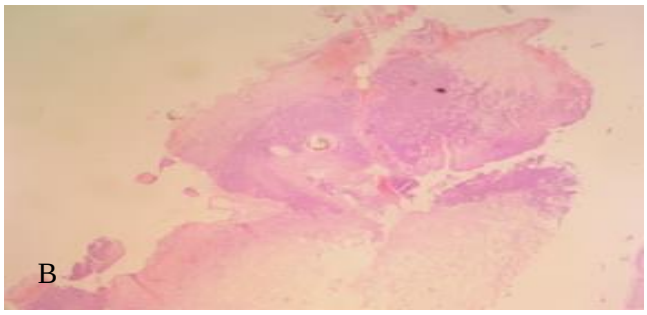


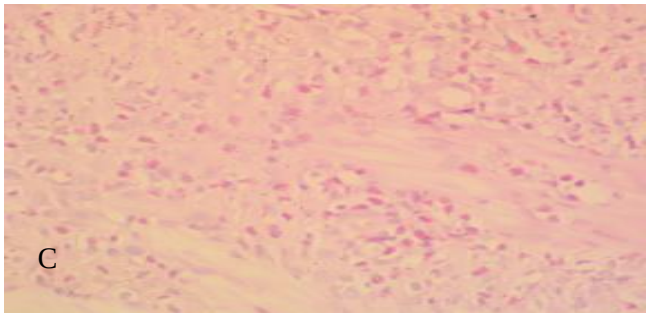
Figure 2: Electrolytes and hormonal concentrations in the wounded prepubertal West African dwarf (WAD) goats

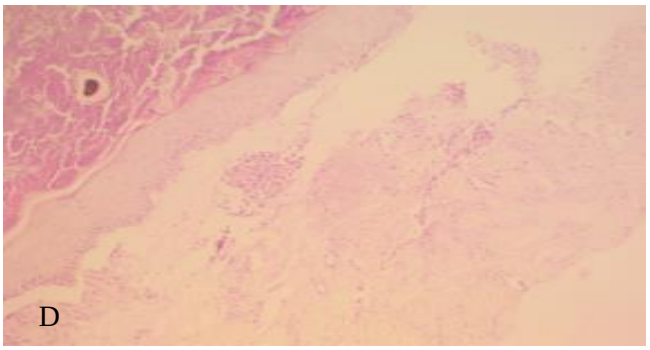
Histopathology of the prepubertal wound healing

At day 0, the sections revealed normal skin with intact epidermis and adnexal structures in place. The various layers of the epidermis were fully and adequately represented. However various sections showed varying degrees of orthokeratotic hyperkeratosis but there was no significant epidermal defect(Plate A).At day 3, the sections showed extensive exudation of blood, plasma and inflammatory cellular debris which constituted a thick layer of crust on the surface of the epidermis. There was also a severe infiltration of the epidermis with intact and degenerate neutrophils (exocytosis), and enormous granulation tissue formation. The adjacent wound edges were hyperaemic with parakeratotic and activated keratinocytes containing hyperchromatic nuclei. The underlying dermis was overridden by organizing cellular debris and haphazard fibroblastic response. At day 5, there was retraction of granulation tissue, apposition of the wound edges over an underlying thin layer of epidermis. At day 7, there was marked contraction of the skin, and retraction of granulation tissue. There was dissolution of the necrotic and inflammatory debris on the surface of the dermis. There was complete replacement of the old epidermis with a new one (re-epithelialisation) and increased proliferation of the epidermal cells. The neutrophilic infiltration are now limited or focalin the superficial dermis, moderate eosinophilic infiltration and more fibroblastic activity with parallel orientation of collagen in the dermis.









Plates A-D showing histo-morphologic changes in the wounds of pre-pubertal WAD goats. A) Normal epidermal integrity and adnexal at Day 0 prior to wound formation. H&E x 100. B) Extensive exudation of blood, plasma, platelet and inflammatory cells on the surface of the apposed epidermis at Day 3. H&E x100. C) Complete apposition of the epidermis with marked contraction, resolution of the necrotic and inflammatory debri. H&E x100.D) High magnification of dermis in C showing more fibroblastic activity with parallel orientation of collagen. H&E x 400

Discussion

From the wound contraction rate result, the wound contracted to nearly zero on day 7 indicating that the wound healed before day 10. This is contrary to previous reports on epidermal wound healing in adult goats where the wound healed on day 15 (Olaifa and Akpan, 2017). It is possible that the prepubertal goats still maintain the fetal ability of faster wound healing. In response to tissue injury, the fetal dermis has the ability to regenerate a non-disrupted collagen matrix that is identical to that of the original tissue (Whitby and Ferguson, 1991; Beanes*et al.,* 2002). Several cells, cytokines, growth factors, stem cells, genes, and components of extracellular matrix are involved in the better fetal wound healing, which provide an important role in wound repair (Yagi *et al.,* 2016). The histologic findings in this work have demonstrated that wound response in the prepubertal goats recapitulated many of the morphologic events known to occur during cutaneous re-epithelialization.

The exudation of fluid including fibrin and other cellular components of blood may have set the pace for the early epidermal re-epithelialisation and proliferative activity. The presence of fibrin has found to accelerate keratinocyte activation and reduced the time of wound closure (Garlick 2003). The early closure of the wound at 7 days was of short duration when compared to our earlierl studies in adult goat (Olaifa and Akpan, 2017). This promotion of re-epithelialization may be associated with the *de novo* synthesis of soluble factors like α5 integrin, which is not expressed in mature epithelium and is known to be upregulated during wound response *in vivo* (Geer *et al* 2002) .

The high estrogen level could also account for faster wound healing in prepubertal animals. Because estrogen promotes wound healing by regulating a variety of genes associated with regeneration, matrix production, protease inhibition, epidermal function, and the genes primarily associated with inflammation (Hardman and Ashcroft, 2008). Estrogens accelerate wound healing and dampen the local inflammatory response (Ashcroft *et al.,* 2003b). It also promotes sequence of coordinated temporal and spatial events that prepare keratinocytes for new tissue formation. The "activated" keratinocyte thus undergoes a shift from a program of differentiation to one leading to directed and sustained migration and proliferation, which is then followed by stratification and differentiation (Garlick 2003).

This chronology of events in the prepubertal wound healing was very similar to those previously reported in earlier *in vivo* studies (Krawczyk, 1971,Garlick, 2003), thus commencing as an epithelial tongue by 48 hours after wounding and completely re-epithelialized within 72 h (Geer *et al* 2002). A well-stratified epithelium had reformed by7 days of biopsy which is quite similar to observations in the post 96 h wounds in epidermal organotypic cultures (Falanga*et al*., 2002).The consistency of epithelial response in the prepubertal goat demonstrates the ability of the epidermis for early re-epithelialization and proliferative activity just to ensure prompt healing.

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