



Evaluation of Healing Intervals of Incisional Skin Wounds of Goats Closed with Three Suture Patterns

ABUBAKAR, A.A.¹, ADAMU, U.², ADEYANJU, J.B.³, KENE, R.O.C.¹, SONFADA, M.L.⁴, YAKUBU, A.S.¹, SAHABI, S.M.⁵, UMAR, A.A.⁴, IBRAHIM, H.M.⁶, SA'IDU, B.⁶ and BELLO, A.⁴

¹Department of Veterinary Surgery and Radiology, Usmanu Danfodiyo University, Sokoto, ²Department of Theriogenology and Animal Production, Usmanu Danfodiyo University, Sokoto, ³Department of Veterinary Surgery and Radiology, University of Ilorin, Ilorin, ⁴Department of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto, ⁵Department of Histopathology, Usmanu Danfodiyo University Teaching Hospital, Sokoto, ⁶Department of Veterinary Physiology and Biochemistry, Usmanu Danfodiyo University, Sokoto. *Corresponding Author: babaj32002@gmail.com, +2348036659884

SUMMARY

The aim of this study was to compare the healing intervals among simple interrupted (SI), ford interlocking (FI) and subcuticular (SC) suture patterns in goats. We hypothesized that these common suture patterns used for closure of incisional skin wounds may have effect on the healing interval. To test this hypothesis, two parameters (subjective healing interval and histologic objective healing interval) were used to investigate the healing interval of the three suture patterns. Our findings showed that, there was significant differences ($P < 0.05$) in subjective healing interval between subcuticular (SC) with ford interlocking (FI), but there was no significant difference between subcuticular with simple interrupted patterns. There were also no significant differences between ford-interlocking and simple interrupted. Histologic findings at seventh day post surgery revealed low polymorphonuclear leukocytes (PMNL) infiltrations and early fibroblast, collagen fibers and epidermal keratinization in the subcuticular group in comparison with the two other groups. At fourteen day post-surgery, there was marked

reduction of inflammatory infiltrates in the subcuticular group when compare with the two other groups, while the collagen fiber density and epidermal keratinization increased in the subcuticular group. At twenty first-day post-surgery, there were no inflammatory cells in subcuticular group, while collagen density was higher, and the orientation of the collagen fibers were horizontal, suggestive of faster healing in comparison with the simple interrupted and ford interlocking groups. It was concluded as measured by subjective healing interval and histologic objective healing interval that surgical skin-wound closed by subcuticular suture pattern alone healed faster than simple interrupted and ford interlocking suture patterns reinforced after subcuticular closure, on the other hand simple interrupted pattern healed faster than ford interlocking.

Key words: Incisional wound healing, histology, goat, suture patterns, healing interval

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). This process occurs via activation of local and systemic cells to restore tissue integrity through regeneration and scar formation, and often these cumulative sequential processes result in satisfactory repair of disrupted tissue (Dovi *et al.*, 2003; Forbes and Rosenthal, 2014). Disruptions caused by tissue loss, inadequate blood flow, and secondary diseases complications can lead to chronic wounds that are difficult to manage (Gal *et al.*, 2006; Guo and DiPietro, 2010).

A clean, uninfected incision, surgically apposed, produces the least amount of epithelial and connective tissue cell death and limits the extent of epithelial basement membrane disruption. Incisional wound alter the homeostatic state of the affected animal and trigger a sequence of events that constitutes three phases of wound healing (Vidinsky *et al.*, 2006, Braiman-Wiksman *et al.*, 2007). In the acute inflammatory phase, the infiltrating phagocytes protect the wounded tissue from infection and remove tissue debris and necrosis (Martin and Leibovich, 2005; Braiman-Wiksman *et al.*, 2007; Guo and DiPietro, 2010; Li *et al.*, 2012). The proliferative phase is characterized by the formation of granulation tissue, which is mainly composed of fibroblast, and newly formed blood vessels. Synthesis and deposition of collagen and matrix are also active during this phase (Midwood *et al.*, 2004; Martin and Leibovich, 2005; Azari *et al.*, 2008). The maturation phase is also called the remodeling phase, is characterized by fibroplasias and progressive alignment of collagen bundles. Scar modification during this phase adds further to the restoration of wound

tensile strength (Stadelman *et al.*, 1998; de la Torre and Sholar, 2006; Midwood *et al.*, 2004; Lorenz and Longaker, 2003). Although the process involves intense and complex cell to cell, cell to matrix and cell to environment interaction, the phases of wound healing are closely merged one into another without clear boundaries (Diegelmann and Evans, 2004; Braiman-Wiksman *et al.*, 2007; Eming *et al.*, 2007).

The nature and mechanism of incisional wound healing has been and continues to be of interest to clinicians and wound healing researchers (Koch *et al.*, 2000; Wang *et al.*, 2003). An estimated 50 million elective surgical operations are performed around the world each year in both human and animals (do Nascimento *et al.*, 2004; Branski *et al.*, 2009). To shorten the time required for incisional wound to heal is not only relevant to reducing postoperative pain and impairment as well as convalescence but is also cost-effective. Therefore, it is very important to understand the nature, mechanism, and the process of incisional wound healing before choosing any suture pattern to close the incision, as it may affect the healing process and interval. We hypothesized that various common suture patterns used for closure of incisional skin wounds may have effect on the healing interval.

MATERIALS and METHODS

Animals and Experimental Design

Fifteen ($n=15$) apparently healthy goats free of any dermatological lesion, five males and ten females, average age and weight 15.85 ± 6.71 months, 16.41 ± 3.75 kilograms (Mean \pm SD) respectively were used for this investigations. The animals were conditioned for 3 months during which they were evaluated and stabilized for the study. They were maintained on daily ration comprising wheat bran, beans husks, groundnut and bean's hay. The animals were randomized divided into three groups [simple interrupted (SI), ford interlocking (FI) and subcuticular (SC) groups]; with each group containing five goats.

Surgical Procedure

The left flank region of each goat was prepared for aseptic surgery by clipping the hair around the surgical site. The site was scrubbed with Purit[®] antiseptic solution (0.3% chlorhexidine gluconate and 3% Cetrimide, Saro LifeCare Limited, Lagos, Nigeria) and methylated spirit (Binji Global Pharmaceutical Company, Sokoto, Nigeria).

The animals were mildly sedated using Xylazine 20[®] (2% Xylazine HCl, Kepro Holland) at 0.025mgkg⁻¹ intramuscularly and 0.06% Atropine sulphate (Laborate Pharmaceuticals India) at 0.05mgkg⁻¹ intramuscularly to reduce salivation and balance heart rates. Local anaesthesia was achieved following sedation with plain lidocaine (2% lignocaine hydrochloride, Sahib Singh Agencies, Mumbai, India), using inverted "L" technique as describe by Taylor, (1991). Animals were placed on right lateral recumbency, and draped as for routine flank

laparotomy (Freeman, 2003). Uniform dorsoventral standard 12 cm vertical skin incision was made on the flank from the epidermis into subcutaneous layer as in laparotomy incision as described by Freeman (2003), the incision was made 3cm away from the last thoracic rib. The incision was routinely closed from within outward. The subcutaneous layer in all groups was closed with Becton[®] chromic catgut size 3/0 (Anhui Kangning Industrial Groups, China) using simple continuous subcutaneous tissue closure. The skins in SI and FI were further closed using simple interrupted (SI) and ford-interlocking (FI) suture patterns respectively with Agary[®] Nylon size 0 (Agary Pharmaceutical Ltd, Xinghuai, China) (Figure 1). Post-surgery, the surgical site was dressed with sterile gauze bandage, adhesive plaster and a paediatric vest ((ABC fashion, Fujian China)). Sutures were removed 7 day post surgery in groups SI and FI groups.

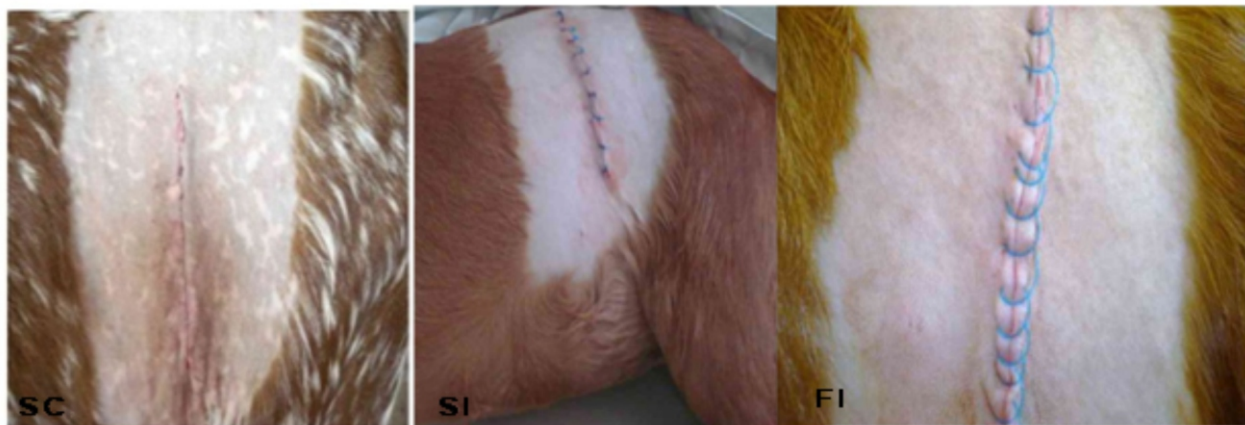


Fig 1: Closure of the three suture patterns, subcuticular (SC), simple interrupted (SI) and ford interlocking (FI)

Healing Interval

The healing intervals of the three suture patterns were monitored and evaluated subjectively and objectively according to Cooke *et al.*, (2003). Subjective evaluation was carried out by physical observation and taking note of number of days in which the surgical wound healed completely. Objective evaluation of healing

intervals was assessed by taking skin excisional biopsy perpendicular to incision line at, 7, 14 and 21 day post surgery; three (2cm) perpendicular length incision line was excised from all the animals under mild sedation and local anaesthetic block as describe earlier under surgical procedures. Semi quantitative analysis of the histologic cellular infiltrates of the three

groups was assessed as described by Gal *et al.*, (2008); Vidinsky *et al.*, (2006).

Histologic Tissue Preparation

Skin biopsy samples collected were fixed in 10% buffered formalin overnight for routine histological processing. Briefly the tissues were dehydrated in a series of increasing concentration of alcohol and embedded in paraffin according to standard procedure (Duttmeyer, 2011) and tissue sections (5 μ m) cut using microtome (Reichert-Jung Microtome 205 Supercut, Arnsberg, Germany). The sections were then mounted on grease free microscope slides and dried. After deparaffinization with xylene and rehydration with series of decreasing alcohol concentrations, the slides were stained with Masson's trichrome and mounted with cover slip.

Semi Quantitative Evaluation

Semi-quantitative method was used for objective evaluation of histological sections. The microscopic structures and changes (epithelization, polymorphonuclear leukocytes (PMNL), tissue macrophages, fibroblasts, neoangiogenesis and new collagen fibers) were evaluated. The degree of cells appearance per microscopic field of view was scored ({-}

absent, {+1} mild, {+2} mild to moderate, {+3} moderate, and {+4} severe) according to Gal *et al.*, (2006); Vidinsky *et al.*, 2006. The slides were viewed with Motic[®] stereo-microscope (AE30 trinocular inverted microscope, fitted with moticam 580; Motic Asia, Hong Kong) via USB cable attached with PC.

Statistical Analysis

Data obtained from subjective healing interval were determine to be normally distributed and were expressed as Means \pm SD. One-way analysis of variance (ANOVA) was used to compare statistical significant differences among the three suture patterns using GraphPad Software Inc. USA. *P* value was consider significant when *P*<0.05.

RESULTS

Subjective Healing Interval

One-way analysis of variance showed significant difference between the subcuticular and the ford-interlocking groups (*P*<0.05). The subcuticular group had the least healing interval (Table I), indicating faster healing time when compared with other groups. However, there was no significant difference between subcuticular with simple interrupted groups, and ford-interlocking with simple interrupted groups (*P*>0.05) (Table I).

TABLE I: SUBJECTIVE HEALING INTERVAL OF THE THREE SUTURE PATTERNS UNDER INVESTIGATION IN DAYS (MEAN \pm SD)

Groups	<i>n</i> =15	Healing interval (Days)
SC	5	13.00 \pm 0.82 ^a
FI	5	15.25 \pm 0.96 ^b
SI	5	14.25 \pm 0.50 ^{ab}

ab: Mean on the same row with different superscript are significantly different (*P*<0.05)

Objective Healing Interval

Histologic sectioning at 7 day post surgery revealed higher inflammatory cell density in SI group when compared with FI and SC groups. The predominant cells were neutrophils accounting for 75%, 85% and 90% of the inflammatory cells in SC, FI and SI group

respectively (Table II). SC group had higher granulation tissues, collagen fibers, fibroblasts, and keratinized cells at the wound surface when compared with group FI and SI (Figure 2).

TABLE II: SEMI QUANTITATIVE MICROSCOPIC FEATURES OF THE THREE SUTURE PATTERNS AT DAY 7

Parameters	SC (%)	FI (%)	SI (%)
Inflammatory cells density	+	++	+++
Inflammatory cells types	PMN (75%) Lymph (25%)	PMN (85%) Lymph (15%)	PMN (90%) Lymph (10%)
Granulation tissue formation	++++	+++	++
Collagen fiber density	++++	+++	+++
Fibroblast density	++++	++	+++
Keratinization @ wound surface	+++	++	++

Key: PMN= polymorphonuclear; lymph= lymphocyte; + mild; ++ mild to moderate; +++ moderate;

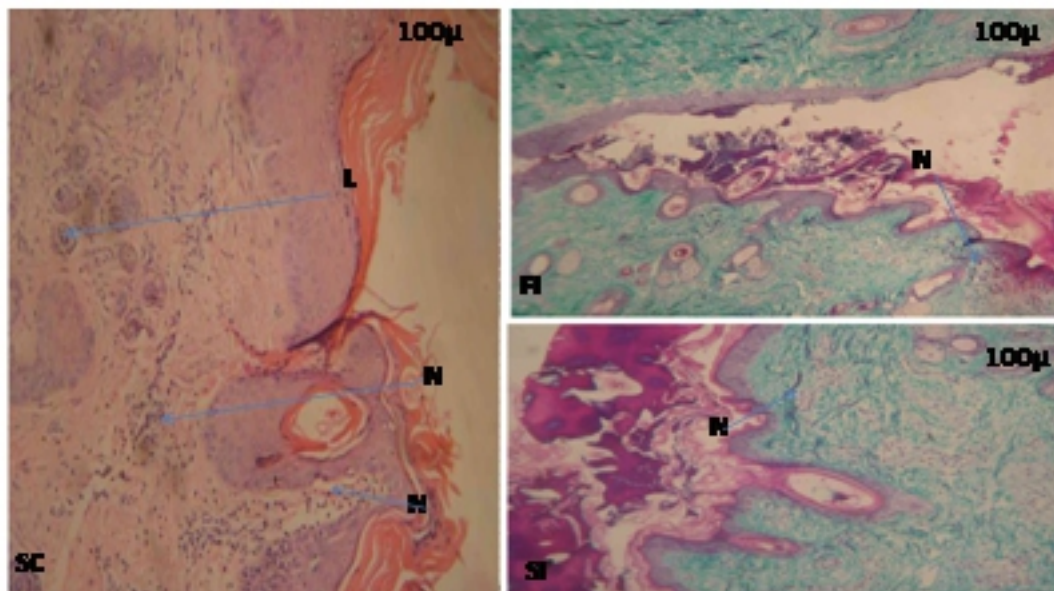


Fig 2: At day 7 post-surgery, Arrow indicating neutrophilic infiltration ,with marked reepithelization in SC group compared to FI and SI, the inflammatory cells at papillary dermis in SC were dominated by lymphocytes (L), while FI and SI were dominated with neutrophils (N). Slides were stained with with with Masson's trichrome stain: X200 objective magnification, scale bar 100µ.

At day 14 post-surgery, there was mild infiltration of inflammatory cells in SC group when compared with FI and SI groups (Table III). The density of collagen fibers, fibroblast and keratinized cells at the epidermal border were higher in SC group, then follow by SI in comparison with FI group.

TABLE III: SEMI QUANTITATIVE MICROSCOPIC FEATURES OF THE THREE SUTURE PATTERNS AT DAY 14

Parameters	SC (%)	FI (%)	SI (%)
Inflammatory cells density	+	++	++
Inflammatory cells types	Lymph (100%)	Lymph (95%) PMN (5%)	Lymph (95%) PMN (5%)
Granulation tissue formation	-/+	+	+
Collagen fiber density	+++	++	++
Fibroblast density	++	+	+
Keratinization @ wound surface	++	+	+

Key: *PMN*= polymorphonuclear; *lymph*= lymphocyte; -/+ very mild; + mild; ++ mild to moderate;

At 21day post-surgery, histologic sectioning revealed no inflammatory cells in SC and SI groups, with very mild lymphocytic cells in FI group. Collagen fibers density was higher in SC group when compared with FI and SI groups (Table IV). The orientations of the collagen

fibers are mostly horizontal in SC group, while vertical orientation predominates in FI group and to a lesser extent in SI group. Epidermal keratinization was higher in SC group in comparison with FI and SI groups.

TABLE IV: SEMI QUANTITATIVE MICROSCOPIC FEATURES OF THE THREE SUTURE PATTERNS AT DAY 21

Parameters	SC (%)	FI (%)	SI (%)
Inflammatory cells density	-	-/+	+
Inflammatory cells types	Lymph (100%)	Lymph (100%)	Lymph (90%)
Granulation tissue formation	-	-/+	-
Collagen fibers density	++++	+++	+++
Orientation of collagen fibers	horizontal	vertical	vertical
Color of collagen fibers	dark blue	light blue	light blue
Fibroblast density	-	+	+
Keratinization @ wound surface	++	+	+

Key: *PMN*= polymorphonuclear; *lymph*= lymphocyte; -/+ very mild; + mild; ++ mild to moderate; +++ moderate; +++++ severe

DISCUSSION

Surgical wound healing is a complex biological process that involves the function of a variety of cell types. The manner by which a distinct cell population modulates the function of another cell population has been of great interest. Normal wound healing is characterized by a transient inflammatory response, while chronic wounds often contain a non-resolving inflammatory response for considerable period of time (Wetzler *et al.*, 2000; Pierce, 2001).

The shorter subjective healing interval

recorded in SC group could be as a result of less tissue manipulation during intradermal apposition of the subcutaneous layer of the skin with surgical instruments. In the SC group, it is only subcutaneous layer of the skin was closed in comparison with the two other suture patterns under investigation in which the skin was closed aside subcutaneous closure. Nawaz and Bentley (2011); Armitage and Lockwood (2011); Yagmur *et al.* (2011) reported that excessive manipulation of the surgical site with traumatic surgical instruments may delay surgical wound healing. The long subjective

healing interval recorded in simple interrupted and ford interlocking patterns may be attributed to tissue reaction in response of additional suture materials used for skin closure. It was reported that, the excessive use of suture material could likely elicit tissue response and hence delayed healing (Hare *et al.*, 2002; Abubakar *et al.*, 2012). However, Koliyadan (2004); Kudur *et al.* (2009); Chupeco *et al.* (2013) reported that subcuticular closure alone may not be sufficient in a tension area where there is less skin elasticity, as the wound edges may not be apposed completely, hence complications may arise that will hinder healing processing.

The presence of low polymorphonuclear leukocytes (PMNL) infiltrates recorded in SC group at day 7 post surgery is a strong indication of early healing as reported by many researchers (Crowe *et al.*, 2000; Dovi *et al.*, 2003; Gal *et al.*, 2006; Vidnsky *et al.*, 2006; Guo and DiPietro, 2010), that inflammatory infiltrates should be transient in responsive wound closure. While early dominant appearance of fibroblast and keratinization at wound margin in the SC group is also a good indication that early bridging of the surgical incision gap must have started taking place.

CONCLUSION

It was concluded that surgical skin-wound closed by subcuticular suture pattern alone without additional skin sutures healed faster than subcuticular sutures re-enforced with either simple interrupted or ford interlocking suture patterns. On the other hand subcuticular re-enforced with simple interrupted pattern healed faster than ford interlocking. Therefore single layered simple continuous intradermal skin closure could be sufficient enough to facilitate complete normal skin closure. This can only be achieved if the surgical site is dressed appropriately to avoid possible contamination.

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