Vet.Med.J.,Giza. Vol.54, No.1. (2006):109-130.

MANAGEMENT OF EXUBERANT GRANULATING WOUNDS IN EQUINE DISTAL LIMB: NEW THERAPEUTIC ATTEMPTS.

EL-GHOUL, W. and SHAHEED, IMAN.*

Department of Surgery, Anesthesiology and Radiology and Department of Pathology*.

Faculty of Veterinary Medicine, Cairo University, Egypt

Received: 11. 10. 2005

Accepted: 20. 11. 20005

SUMMARY

The study was carried out on 20 horses and 6 donkeys that were presented with exuberant granulation tissue on the distal limbs and divided randomly into two groups according to the used dressing materials. Surgical excision was done and the dressing materials were carried on the wound dressing sheets (kaltostat or aquacel). The selection of the dressing material depends on the stage of healing and the nature of the granulating wounds. The tested dressing materials for group one are calcium-sodium alginate fiber (Kaltostat®), topical antibiotic spray (Tetravet® aerosal) and silver sulfadiazine cream (Dermazin®) and for group two are hydrofiber wound dressing (Aquacel®), fucidin® cream and pale sulfonated shale oils gel (Ichthol®). Fucicort® cream and zinc oxide cream (Zincosil®) were used in both groups. Histopathological examination was performed to samples from the exuberant granulation tissue. The results revealed that the use of kaltostat and aquacel as a primary wound dressing in combination with the selected topical dressings in both groups may lead to acceleration of the rate of healing of the equine distal limb wounds without the development of exuberant granulation tissue.

Key words: Exuberant granulating wound, proud flesh, equine distal limb.

INTRODUCTION

In equine practice, horses often suffer cutaneous wounds on the distal portion of the limb that often require expensive and prolonged treatment. These wounds are difficult or impossible to suture and often left to heal by second intention because of excessive skin tension, wound contamination, and tissue avulsion (Gomez et al, 2004; Wilmink and

Weeren, 2005 and Wilson, 2005).

Healing of equine distal limb wounds is frequently impaired by formation of excessive granulation tissue (hypergranulation or proud flesh) due to a longer preparatory phase of healing, slower rates and earlier cessation of wound contraction, and slower rates of epithelization (Bertone, 1989; Stashak, 1991; Schwartz et al, 2002 and Theoret, 2005).

Factors possibly involved in the production of exuberant granulation tissue in horses include infectious or foreign body responses, excessive motion or tension on the surrounding skin, reduced blood supply with resultant tissue hypoperfusion, large body size, wound location (distal limb wounds are predisposed), slow fibroblast growth, changes in the expression of transforming growth factor-beta and an imbalance of collagen synthesis, deposition and lysis (Bertone, 1989; Wilmink et al, 1999; Theoret, 2005 and Wilson, 2005).

Numerous treatments have been advocated for promotion of wound healing and prevention of hypergranulation tissue in horses, however, no approach has been universally effective (Bertone, 1989; Stashak, 1991 and Engelen et al, 2004). Many types of topical preparations, occlusive dressings and biological dressings have been tested in a variety of species in an effort to find medications that allow wounds to heal faster, without the development of exuberant granulation tissue and with a good cosmetic result. These preparations include medications, chemical agents, physical agents, nutrients and biomaterials (antimicrobials, hydroactive agents, biologic dressings, occlusive dressings, collagen, recombient equine growth factor hormone, corticosteroid, vitamins and a variety of micronutrients) (Bertone et al, 1989; Swaim, 1990; Steel et al, 1999; Svensjo et al, 2000; Dart et al, 2002/2005; Gomez et al, 2004; Brumbaugh, 2005; Sobol et al, 2005 and Wilson, 2005).

Researches into exuberant granulating wounds in horses and donkeys has mostly focused on superficial skin wounds, which are not representative of wounds commonly seen in clinical practice. Healing of open wounds of equine distal limbs is often problematic, especially exuberant granulation tissue formation. It is hypothesized that combination of more than therapeutic regimen would improve the healing. Thus the purpose of this study was to evaluate, in a randomized, controlled field trails, the effect of a combination of different topical wound preparations on the healing of exuberant granulating wounds of the distal equine limbs.

MATERIAL AND METHODS

Animals: The study was done on 20 horses and 6 donkeys suffering exuberant granulating wounds on distal parts of the limb and presented to the department of surgery, anesthesiology and radiology clinic, faculty of veterinary medicine, Cairo university. Horses ranged in weight from 250 to 600 Kg, and in age from one to 12 year; whereas donkeys ranged in weight from 150 to 250 Kg, and in age from 5 to 10 year.

Treatment trials of exuberant granulating wounds:

Surgical excision: The animals were premedicated with 80 (µg/kg of romifidine given intravenously over a period of approximately two minutes. Ten minutes later 0.4 mg/kg of diazepam was administered intravenously. Anesthesia was induced with 5 mg/kg IV thiopental sodium and maintained by infusion of a freshly prepared mixture of 30 mg romifidine, 1 g thiopental sodium and 25 g guaifenesin in 500 ml 5% dextrose (EL-Ghoul et al, 2004). Surgical excision of the exuberant granulation tissue was done and the dressing materials were carried on the wound dressing sheets (Kaltostat, Aquacel) and applied on the wound. The selection of the dressing material depends on the stage of healing and the nature of the granulating wounds.

Wound dressing: The animals were classified into two groups according to the evaluated wound dressing. In group one (10 horses and 3 donkeys), the wounds were treated by calcium-sodium alginate fiber wound dressing (Kaltostat®- ConvaTec, Princeton, United Kingdom); topical antibiotic spray (Tetravet® Aerosal Bomac Animal Health, Bomac laboratories LTD, Newzeland); silver sulfadiazine cream (Dermazin® Medical Union Pharm. Co Egypt); In group two (10 horses and 3 donkeys), the wounds were treated by hydrofiber wound dressing (Aquacel® ConvaTec, Princeton, United Kingdom); Fucidin® cream (Minapharm under license of Leo Pharmaceutical Ballerup-Denmark); and sulfonated shale oils gel (Ichthol® Ichthyol Co, Hamburg, Germany). In both groups Fucicort® cream (Minapharm Egypt under license of Leo pharmaceutical Products, Denmark) and zinc oxide cream (Zincosil® Misr Co. for Pharm. Ind., Egypt) were used.

Wound dressings were kept in place with bandage. After surgery, each horse received penicillin-streptomycin antibiotic (Streptopenicid® 20.000 IU/kg IM) for 5 days. Horses were observed daily for lameness, fever or bandage slippage. At dressing intervals, wounds were gently cleansed with sterile saline solution, redressed and rebandaged.

Assessment of wound healing:

Subjective observations: The wounds were assessed subjectively at the first day of treatment and, thereafter at each bandage change (weekly intervals), for the quantity and quality of granulation tissue, infection, wound retraction, contraction, presence of epithelization, wound exudates and severity of inflammation. The number of times excision of granulation tissue was required, and the time required for complete healing were determined (Wilmink et al, 1999).

Photographs were taken at case presentation to the clinic, immediately after wound excision and prior to reapplication of dressings at each bandage change.

Wound size was assessed by using two linear measurements: ``wound length`` (maximum length of the wound in any direction) and ``wound width`` (maximum width of the wound perpendicular to its length). These measurements were multiplied to estimate wound area (Engeleen et al, 2004).

Total rate of healing (cm2/day) =

$$\frac{\text{Wound size on beginning}}{\text{total number of days until the wound was healed}}$$

(Dart et al, 2002).

Healing time: The time between the excision of the exuberant granulation tissue and the day that each wound was covered with epithelium (Gomez et al, 2004).

Grading the amount of granulation tissue during the course of treatment: grade 1: below the skin edges; grade 2: level with the skin edges; grade 3: above but not overlapping the skin edges and grade 4: above and overlapping the skin edges (Dart et al, 2002). Granulation tissue was classified as exuberant (hypergranulation) if it protruded ≥ 3 mm above the skin surface at any point around the edge of the wound (Engelen et al., 2004).

Assessment of the healing process was classified to the following grades according to the degree of improvement and granulation tissue formation: Grade 0 (-): No improvement; grade 1(+): low degree improvement, with slow granulation tissue formation; grade 2(++): moderate degree improvement with moderate granulation tissue formation; grade 3(+++): high degree improvement with rapid granulation tissue formation and grade 4(++++): very high degree improvement with very rapid granulation tissue formation (Schwenzer and Gayko, 2003).

Histopathological examination: Tissue samples were taken from exuberant granulation tissue after surgical excision and during treatment. The samples were fixed in neutral buffer 10% formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5 µm and stained by Haematoxylin and Eosin (Bancroft et al., 1996). Some paraffin sections were stained with special stains as Gimesa for Polymorphnuclear leukocytes as eosinophils and neutrophils and for detection of bacterial colonies. Also Van Gieson and Masson Trichome stains were used for staining the connective tissue according to Drury et al (1979).

RESULTS

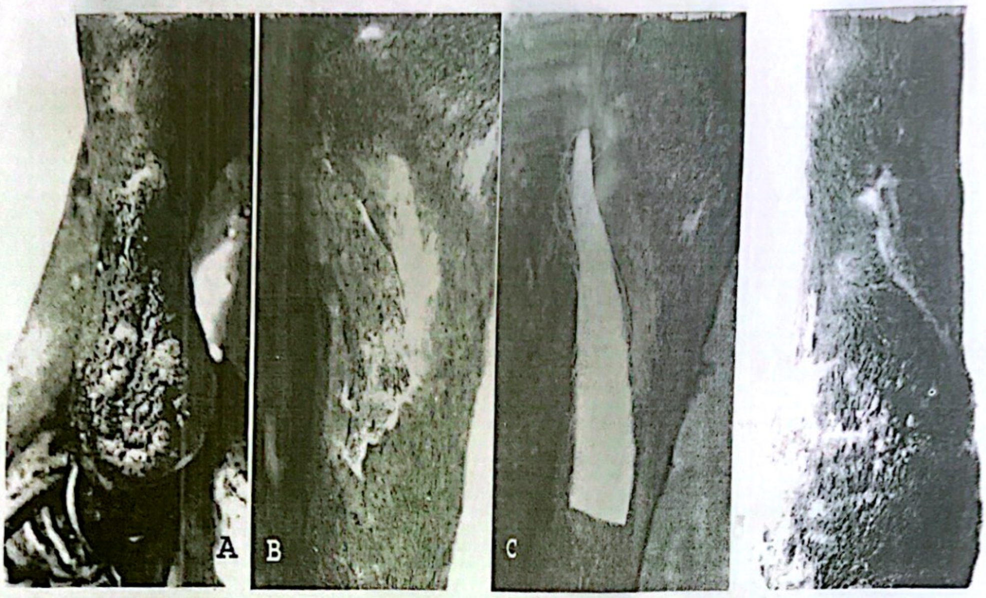
After surgical excision of the proud flesh, some horses manifested mild signs of discomfort (lameness, pain, inappetance) during the first bandage change. Thereafter, horses appeared comfortable, except for 2 horses that occasionally chewed the wraps over a wound that had developed excess exudates. Signs of mild inflammation (redness, swelling and discomfort) were observed immediately after wounding; however, these signs subsided within a week.

The results revealed that grade 4 granulation tissue required trimming once in 3 horses, twice in 4 horses, 3 times in 2 horses and 3 times in two donkeys during healing. The results revealed that the use of kaltostat and aquacel as a primary wound dressing in combination with the selected topical dressings lead to acceleration of the rate of healing of hypergranulating wounds without the development of exuberant granulation tissue at the equine distal limbs. Subjectively, application of kaltostat and aquacel as a primary dressing was found to absorb exudates, effect hemostasis and protect the wound from contamination. It allows trauma - free removal with no damage to newly formed granulation tissue, reduce wound pain and accelerate wound healing. It was observed that topical antibiotic / antiseptic application was noticed to control surface organisms in granulation bed. Dermazin was observed to limit spreading of necrosis, promote healing and control wound infection. Sulfonated shale oil gel was found to promote wound healing and reduce inflammatory process and control wound infection. Fucicort was found to slow the formation of granulation tissue without inhibiting epithelization and reduce itch of healing. Zincosil was found to enhance healing.

During the course of treatment the newly formed exuberant granulation tissue that protrudes slightly above the skin edges was effectively managed by application of a steroid antibiotic combination and pressure bandaging. The application of the steroid at this time, usually after 5 days, has little effect on wound healing and epithelization. The following cases were selected as a representative one to the healing process after excision of the hypergranulating tissue in this study.

Representative cases of group one:
Representative case No. 1: showed the healing sequences of the exuberant granulating wound after surgical excision: Exuberant granulation tissue over the planter surface of the pastern region in a 10 years old horse (planter and lateral view) (A&B); C: After surgical excision; D: one week after treatment; E: two weeks after treatment; F: four weeks after treatment; G: Six weeks after treatment; H: 7 weeks with Kaltostat application; I: 8 weeks after treatment; J: 9 weeks after treatment and K: 10 weeks after treatment

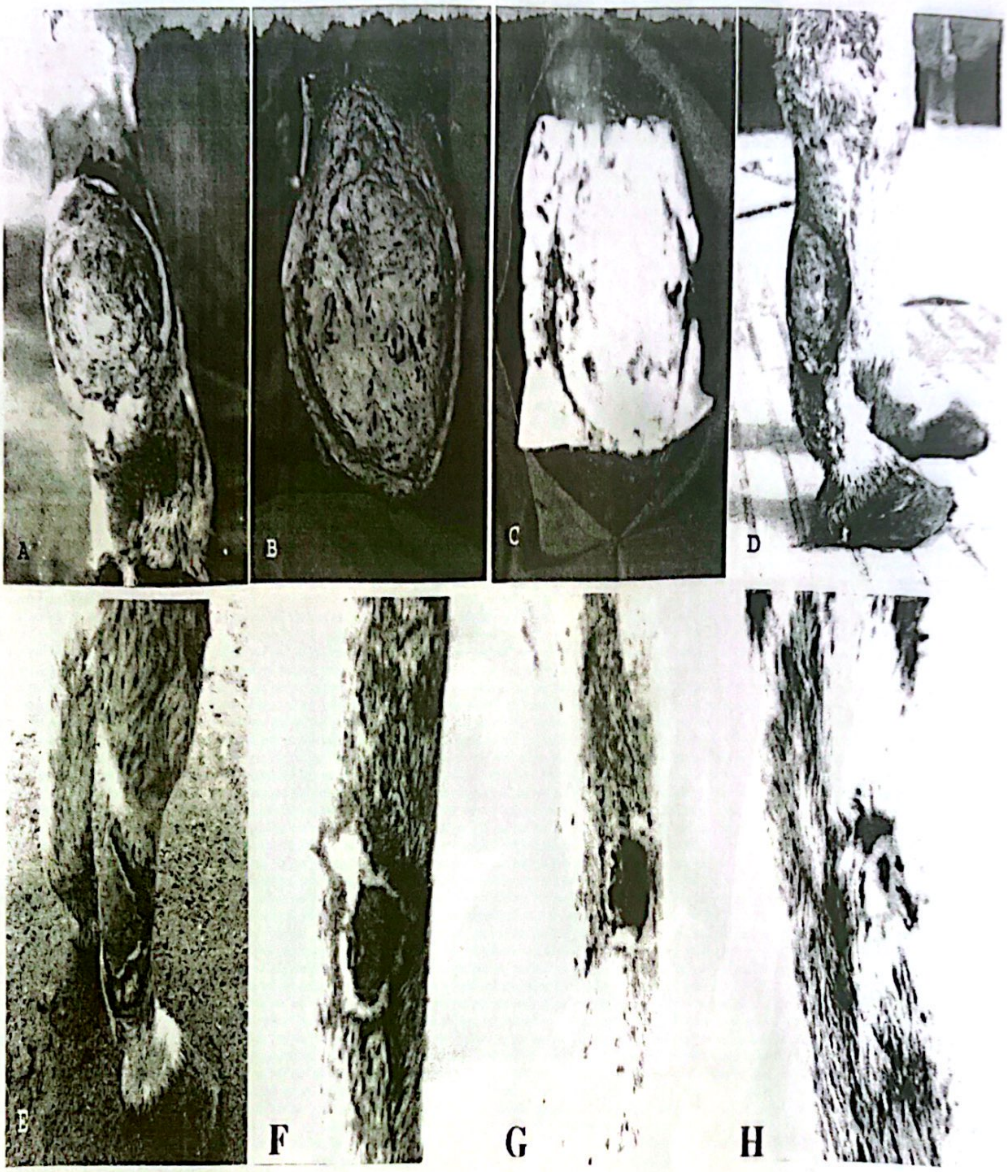
Representative case No. 2: showed the healing sequences of the exuberant granulating wound after surgical excision. A: Exuberant granulating wound over the dorsal surface of the metacarpal region in a 6 year old donkey; B: The wound after surgical excision. C: One week after treatment. D: Two weeks after treatment. E: Five weeks after treatment. F: Six weeks after treatment. G: Eight weeks after treatment. H: Nine weeks after treatment with complete healing.



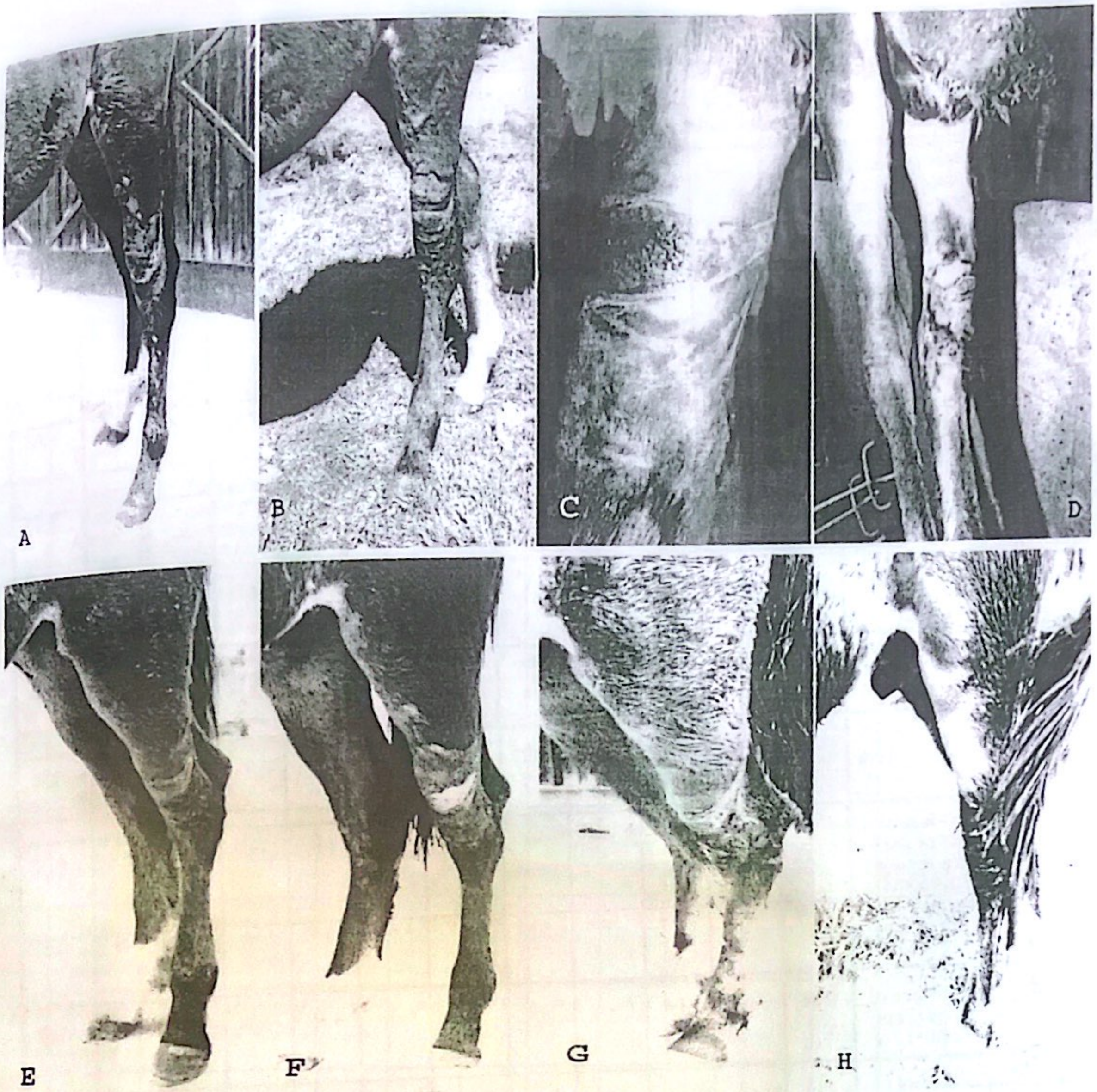
Representative case No. 3 showed the healing sequences of the exuberant granulating wound in one year old foal. A: hypergranulating wound before excision; B: two week after surgical excision; C: application of kaltostat with the dressing materials three weeks later. D: The wound four weeks after treatment.

Representative cases of group two:

Representative case No. 4 showed the healing sequences of the exuberant granulating wound after surgical excision. A: Exuberant granulating wound over the dorsal and medial surface of the metatarsal, fetlock and pastern region in a 8 year old horse, the wound reach to the metatarsal bone. B& C: one week after treatment. D: two weeks after treatment E: Three weeks after treatment. F: Four weeks after treatment. G: Five weeks after treatment. H: Seven weeks after treatment. I&J: Eight weeks after treatment. K&L: Nine weeks after treatment showed the complete healing



Representative case No. 5: A: Exuberant granulating wound over the palmar surface of the metacarpal region in a 9 year old donkey; B: the wound after surgical excision; C: Aquacel application with the used dressing materials. D: The healing process one week after treatment. E: Three weeks after treatment. F: Five weeks after treatment. G: Six weeks after treatment. H: Seven weeks after treatment with complete healing.



Representative case No. 6: showed the healing sequences of the exuberant granulating wound at the cranial aspect of the tarsus in 10 months old foal after surgical excision (A). B: The wound after one week. C: Two weeks after treatment. D: Three weeks after treatment. E: Four weeks after treatment. F: Five weeks after treatment. G: Six weeks after treatment. H: Seven weeks after treatment with complete healing and scar tissue formation.

Table 1: Assessment of Patients with hypergranulating wounds at the distal limb in group 1.

Case No.	Age. sex	Location, size and area of the wound	Assessment grades until to the treatment stop											
			1st Week	2nd Week	3 rd Week	4 th Week	5 th Week	6 th Week	7 th Week	8 th Week	9 th Week	10 th Week	Healing time (days)	Total healing rate (cm2/ Day)
1	10 years old horse	planter surface of the pastern region-7x12cm (84 cm^2)	++	++	++	++	++	++	++	++	++	++	105	0.8
2	8 years old horse	dorsal and medial surface of metatarsal fetlock and pastern region-5x10 cm (50 cm^2)	++	++	++	++	++	++	++	++	++	++	70	0.71
3	3 years old horse	Dorsal aspect of metatarsal region-4x10cm (40cm^2)	++	+++	++	++	++	++	++	++	++	-	63	0.63
4	one year old foal	lateral aspect of metatarsal region-3x8cm (24cm^2)	+++	+++	+++	+++	+++	+++	-	-	-	-	42	0.57
5	3 years old horse	palmar aspect of the metacarpal region-4x9cm (36cm^2)	+++	++	++	++	++	++	++	++	-	-	56	0.64
6	5 years old horse	palmar aspect of the pastern region -5x9cm (45cm^2)	++	++	++	++	++	++	++	++	++	++	70	0.64
7	1 year old foal	cranial aspect of the tarsus 4x11cm (44 cm^2)	++++	++++	++++	++++	+++	+++	++++	-	-	-	49	0.89
8	3 years old horse	palmo-lateral aspect of the pastern region 5x9cm(45cm^2)	++	++	+++	++	++	++	++	++	++	-	63	0.71
9	6 years old mare	Palmar pastern -6x11 cm (66cm^2)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	98	0.67
10	10 years old horse	Dorsal surface of carpus-6x12cm (72cm^2)	++	++	++	++	++	++	++	++	++	++	105	0.68
11	7 years old donkey	Cranial aspect of tarsus-4x9 cm (36cm^2)	++	++	++	++	++	++	++	-	-	-	49	0.73
12	9 years old donkey	palmar surface of metacarpal region -7x11cm (77cm^2)	++	++	++	++	++	++	++	++	++	++	105	0.73
13	6 years old donkey	dorsal surface of metacarpal region -5x10cm (50cm^2)	++	++	++	++	++	++	++	++	++	++	77	0.64

*+ low degree improvement, with slow granulation tissue formation; ++: moderate degree improvement with moderate granulation tissue formation; +++: high degree improvement with rapid granulation tissue formation; ++++: very high degree improvement with very rapid granulation tissue formation.

Table 2: Assessment of Patients with hypergranulating wounds at the distal limb in group 2.

Case No.	Age. sex	Location, size and area of the wound	Assessment grades until to the treatment stop											
			1st Week	2nd Week	3 rd Week	4 th Week	5 th Week	6 th Week	7 th Week	8 th Week	9 th Week	10 th Week	Healing time (days)	Total healing rate (cm2/ Day)
1	12 years old mare	planter pastern -6x12cm (72 cm^2)	++	++	++	++	++	++	++	++	++	++	98	0.73
2	8 years old horse	Dorsal metatarsal -7x12 cm (84 cm^2)	++	++	++	++	++	++	++	++	++	++	105	0.8
3	3 years old horse	Planter metatarsal -3x6 cm (18cm^2)	+++	+++	++	+++	++	-	-	-	-	-	35	0.51
4	11 years old horse	Dorsolateral surface of metatarsus -7x12cm (84cm^2)	++	++	++	++	++	++	++	++	++	++	112	0.75
5	4 years old horse	Dorsal pastern -5x9cm(45cm^2)	+++	++	++	+++	++	++	++	++	++	++	70	0.64
6	7 years old mare	-Lateral surface of metacarpal -4x8cm (32cm^2)	++	++	++	++	++	++	++	++	++	-	63	0.5
7	6 years old mare	Planter pastern -4x7cm (28 cm^2)	+++	+++	+++	++	++	++	++	-	-	-	56	0.5
8	5 years old horse	-Dorsal fetlock -5x11cm(55cm^2)	++	++	++	++	++	++	++	++	++	-	63	0.87
9	8 years old horse	-Palmar metacarpal -4x7cm (28cm^2)	++	++	++	++	++	-	-	-	-	-	35	0.8
10	10 years old horse	Dorsal metatarsal -6x10cm (60cm^2)	++	++	++	++	++	++	++	++	++	++	105	0.57
11	7 years old donkey	-Dorsal fetlock -5x8 cm (40cm^2)	++	++	++	++	++	++	++	++	++	++	84	0.47
12	5 years old donkey	Dorsal fetlock -5x8cm (40cm^2)	++	++	++	++	++	++	++	++	++	-	63	0.63
13	10 years old donkey	Palmar surface of fetlock -5x9cm (45cm^2)	++	++	++	++	++	++	+	+	+	+	91	0.49

*+ low degree improvement, with slow granulation tissue formation; ++: moderate degree improvement with moderate granulation tissue formation; +++: high degree improvement with rapid granulation tissue formation; ++++: very high degree improvement with very rapid granulation tissue formation.

It was found that the healing time of the examined cases in group one ranged from 42 to 105 days with an average of 72.1 days in horses and ranged from 49 to 98 days with an average of 74.6 days in donkeys. The total rate of healing ranged from 0.57 to 0.89 cm2/day with an average of 0.69 cm2/day in horses and ranged from 0.64 cm2/day to 0.73 cm2/day with an average of 0.7 cm2/day in donkeys. In group two the healing time ranged from 35 to 112 days with an average of 74.2 days in horses and ranged from 63 to 91 days with an average of 79.3 days in donkeys. The total rate of healing ranged from 0.5 to 0.87 cm2/day with an average of 0.66 cm2/day in horses and ranged from 0.47 cm2/day to 0.63 cm2/day with an average of 0.53 cm2/day in donkeys. There were no significant differences between horses and donkeys inside and between groups in the healing time or in the total rate of healing. It was noticed that young animals showed rapid total healing rate and short healing time than old animals (Table 1 and 2).

Histopathological examination of exuberant granulation tissue obtained from horses and donkeys after surgical excision revealed the presence of wavy bundles of mature collagen fibers accompanied with newly formed blood vessels (Fig 7). It lack epithelization but there were a large numbers of Polymorphnuclear leukocytes mixed with bacterial colonies (Fig 8) and fungal filament. Some cases showed the presence of multiple granuloms in-between the granulation tissue. Each granulome consists of large necrotic area surrounded by connective tissue capsule with very few numbers of mononuclear cells (Fig 9).

The histopathological examination of tissue samples obtained from the wound of horses and donkeys during the course of treatment revealed various stages of healing by second intension. Some cases showed the presence of large numbers of fibroblast cells surrounded with mucous like ground substance (Fig 10), scanty amount of collagen (Fig 11) in addition to clusters of angioblast cells which arranged to form newly formed blood vessels. Fibrin deposit were noticed in the periphery of the samples in most examined cases and in some cases it extended deeply as clefts in granulation tissues (Fig 12). Large numbers of polymorphnuclear leukocytes especially neutrophils accompanied with macrophages and lymphocytes were detected on the fibrin meshes. Some cases showed the beginning of the formation of exuberant granulation tissue. It appeared to extend to cover the border of epithelial lining while in other cases there were a large numbers of mononuclear cells aggregation above the covering epithelium (Figs 13&14). The exuberant granulation tissue showed signs of perivasculitis in which cellular aggregation of mononuclear were detected around newly formed blood vessels and in the dermis (Fig 15). Some cases, especially donkeys, showed the presence of large numbers of esinophils in-between the granulation tissue or around the newly formed blood vessels (Fig 16).

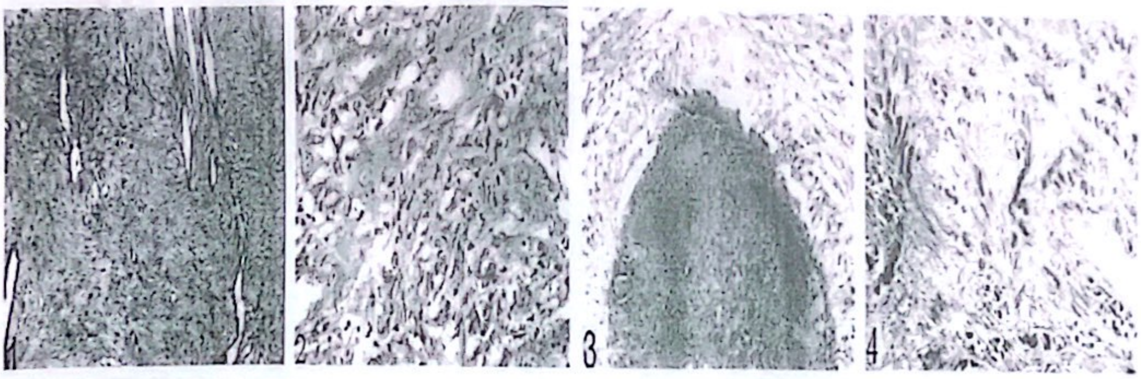


Fig. 7: Exuberant granulation tissue showing wavy bundles of mature collagen fibers and newly formed blood vessels (Masson trichrome X 33).

Fig. 8: Large numbers of polymorphnuclear cells in addition to colonies of bacteria (Giemsa stain X 132).

Fig. 9: Central necrotic area surrounded by fibrous connective tissue capsule (H&E X 66). Fig. 10: Scanty amount of collagen fibers (Van Gieson stain X 33).

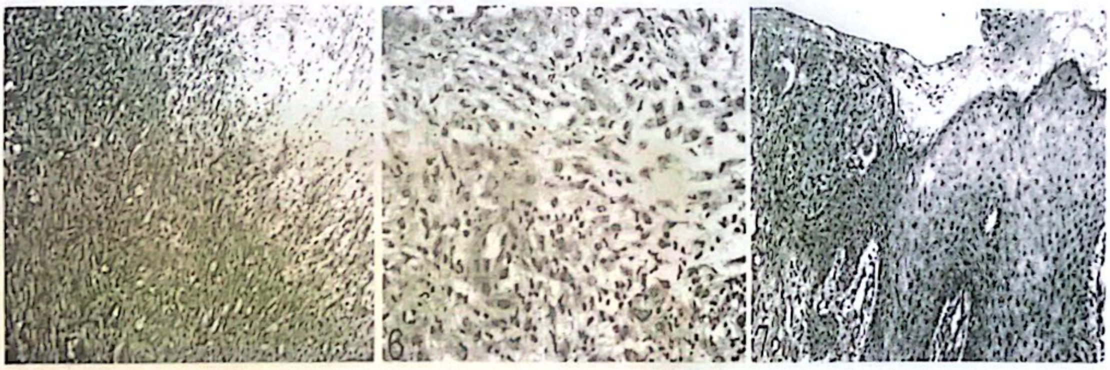


Fig. 11: Fibrin deposit at the periphery of the wound mixed with inflammatory cells (H&E X 66).

Fig. 12: Large numbers of fibroblast surrounded by mucous like ground substance (H&E X 33).

Fig. 13: Extension of granulation tissue that cover the neighbouring epithelium (H&E X 33).

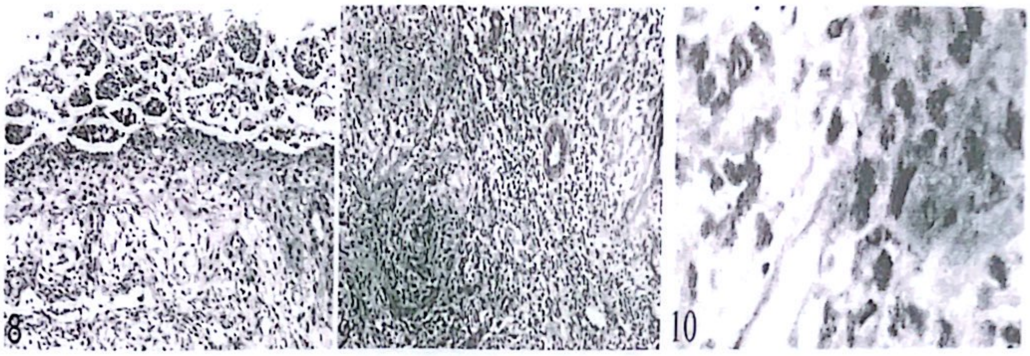


Fig. 14: Inflammatory cells above the epithelial covering (H&E X 66).
Fig. 15: Mononuclear inflammatory cells around the blood vessels (H&E X 33).
Fig. 16: Large numbers of esinophils inbetween the granulation tissue (Giemsa stain X 132).

DISCUSSION

Equine chronic wounds are a major problem for the horse-owning community, and are frequently responsible for restriction of performance and loss of commercial value. Wounds of the equine distal limb, with large tissue deficits, are associated with species specific problem with the formation of excessive exuberant granulation tissue. Theoretical factors that may be involved in impeding of optimal wound healing and the formation of exuberant granulation tissue include excessive movement of the healing tissue, lack of soft tissue covering, contamination, excessive tension and reduced blood supply to the distal extremities (Stashak, 1991and Wilmink et al, 1999).

In this study exuberant granulation tissue was surgically excised to be level with the skin edges. Treatment of proud flesh may be done by chemical cautery, cryosurgery and surgical resection. The surgical method is generally preferred because it is easy to perform, preserves the epithelial margin, removes much of the unwanted granulated tissue and leaves healthy tissue underneath. Caustics and astringents effectively remove and prevent the formation of granulation tissue by destroying it chemically. Unfortunately, they are not selective in their destructive effect and will destroy the migrating epithelial cells. The end results is often prolonged healing and excessive scarring when compared to surgical resection (Bertone, 1989 and Stashak, 1991).

The selected dressings used in this study were calcium-sodium alginate fiber (Kaltostat®), hydrofiber wound dressing (Aquacel®) as a primary wound dressing and topical antibiotic spray (Tetravet® aerosal), Fucicort® cream, fucidin (Fucidin®), silver sulfadiazine cream (Dermazin®), zinc oxide cream (Zincosil®) and pale sulfonated shale oil gel (Ichthol®) as a secondary dressing in combination. Dressing selection is determined by the stage of wound healing, wound type description, characteristics and bacterial profile (Liptak, 1997). The principal reasons for applying a dressing are to produce rapid and cosmetically acceptable healing, remove odour, reduce pain, prevent or combat infection, control exudates and cause minimum distress to the patient. As no one dressing is suitable for the management of all types of wounds, and few are ideally suited for the treatment of a single wound during all stages of the healing cycle, successful wound management depends upon a flexible approach to the selection and use of products based upon an understanding of the healing process combined with a knowledge of the properties of the various dressings available. Ideal dressing should maintain a moist wound environment, control exudates, allow for gaseous exchange, maintain optimum pH of wound environment, provide an effective barrier to micro-organisms, require infrequent changing, provide thermal insulation and should not introduce toxins or fibrer into wound and cause no trauma on removal (Bowler et al, 1999 and Dart et al, 2005).

The results revealed that the use of calcium-sodium alginate fiber (Kaltostat®) and hydrofiber (Aquacel®) as a primary wound dressing lead to acceleration of wound healing. This may be due to that Kaltostat® absorb the wound exudates to form a non-adherent gel and moist environment which accelerate reepithelialization, inflammatory reaction and angiogenesis, promote hemostasis and reduce wound pain and infection rate (Hajek and Sedlarik, 1992; Mohr et al, 1999; Suzuki et al, 1999 and Williams, 1999). Also it was recorded that Aquacel® creates a soft gel which maintains a moist environment that supports the healing process and aids in the removal of unnecessary material from the wound (autolytic debridement), without damaging newly formed tissue. It was hypothesized that a moist environment would accelerate wound healing process by faster contraction and development of granulation tissue, decrease necrosis and better quality newly regenerated epidermis (Bowler et al, 1999 and Svensjo et al, 2000).

Kaltostat and aquacel were used as a primary wound dressing and drug-delivery system to the secondary dressing used in this study. This drug-delivery system permit the release of bacteriostatic/bactericidal concentrations of the antibacterial for a period of time needed to treat surgical infections that may initiate or accelerate healing of chronic wounds. Also it decrease the frequency of dressing changes and increase the potentiating effect of secondary dressing (Segal et al, 1998; Thomas, 2000 and Ziegler et al, 2003).

Topical antibiotic was found to prevent wound infection and control surface organisms in the granulation bed. Tetravet® spray is composed of oxytetracycline Hcl, gentian violet and propellant hydrocarbon. Fucidin® composed of sodium fusidate which exerts powerful antibacterial activity against a number of gram-positive organisms. The therapeutic efficacy of topically applied fucidine is due to partly to the pronounced antibacterial activity of fucidin against the organisms responsible for skin infections, and partly to the unique ability of this antibiotic to penetrate intact skin (Werner and Russell 1999). Antimicrobial medication and topical antibacterial agents should be considered an adjunct to the general care of wounds intended to promote healing (Swaim, 1990; Caron, 1991 and Brumbaugh, 2005).

In group one the use of silver sulfadiazine cream (Dermazin®) as a local chemotherapeutic limits the spreading of necrosis into deeper layers and promotes healing of wounds. This may be because of sulphadiazine penetrates into granulating wounds and it has a broad-spectrum antibacterial action, low toxicity and minimal tissue reaction (Fox, 1983 and Bocchiotti and Robotti, 1990). Ulcers treated with silver sulfadiazine cream responded more rapidly, with one-third showing lower bacterial level (Kucan et al, 1981 and Lansdown et al, 2005). Silver coated dressings provide a moist environment for the healing of wound combined with an effective antimicrobial agent and this significantly accelerates healing (Olson et al, 2000). When silver sulfadiazine and hydrocortisone are used in combination, wound healing progresses at a more rapid rate (Altman et al, 1990).

The use of pale sulfonated shale oil gel (Ichtho®) in group two was noticed to promote the healing of wounds which may be due to its antimicrobial, antimycotic and anti-inflammatory effect and through its proliferation-promoting action as well as generation of wound healing factor (Gayko et al, 2000 and Martens and Gayko, 2002).

The periodical application of fucicort® cream in both groups was found to slow the formation of granulation tissue without inhibiting epithelization. This may be due to that fucicort combines the potent topical antibacterial action of fusidic acid with the anti-inflammatory and antipruritic effect of betamethasone. Corticosteroid-antibiotic agents were effective at reducing neovascularization, although dexamethasone- based products were more effective at reducing extracellular matrix deposition (Sobol et al, 2005). Anti-inflammatory drugs are important to good wound healing; they reduce the thickness of the wound, allow better blood flow, reduce the itch of healing and thus reduce the need for chewing the wound. A combination of anti-inflammatory, antibacterial, anti-fungal and drying agents was formulated for slow healing wounds and is ideal for the treatment of wounds on the horse limbs. Similarly, Wilson (2005) observed that exuberant granulation tissue formation can be readily controlled by the periodic application of steroids which works well on immature granulation tissue and the bed will usually flatten. Continual use of steroids should be avoided since delayed epithelization may occur.

The results proved that the use of zinc oxide cream (Zincosil®) in both groups enhancing the healing of the hypergranulating chronic wounds which may be due to its protective, astringent and soothing action of its components. Zinc is an essential co-factor for the activity of many metalloproteinases enzymes including collagenases that are important in the breakdown of connective tissue collagen. Many of these enzymes play key óles in extra-cellular matrix remodeling, wound iealing, connective tissue repair, inflammation, ınd cell proliferation. Zinc also has mild antibac-erial action, may promote debridement, facili-ates biochemical reactions that mediate the ;rowth and remodeling of tissue, and maintain the noisture solution at the desired acidic pH (Agren :t al, 1993). It was observed that zinc oxide incor-porated in gauze enhanced healing of chronic wounds and hydrocolloid dressing with zinc oxide inhibiting bacterial growth (Tarnow et al, 1994).

The results showed that the healing time and the total rate of healing of distal limb exuberant gran-ulating wounds were varied between the two ex-amined groups. There were no significant differ-ences between horses and donkeys inside and between groups. This variation in the healing pro-cess may be due to the changes in the size of the wound and the physical condition of the animal (Hendrickson and Virgin, 2005).

Bandages are included in the regimen of wound management in all cases in this study and was no-ticed to help in debriding and cleansing the wound, prevent oedema, absorb exudate, enhance epithelialization, limiting the motion in the area of the wound, speed the healing process, allowing skin to grow over the granulation tissue which greatly reduces the occurrence of proud flesh. Similar observations were recorded by many au-thors (McGlennon, 1988, Lidsay, 1989; Swaim, 1990; Stashak, 1991 and Gomez and Hanson, 2005).

Histopathological finding of the excised exuber-ant granulating tissue in both groups revealed contamination of the wound by colonies of bacte-ria and fungus filaments, perivasculitis and ne-crotic areas. These are considered as irritating and ischemic factors help in the formation of exuber-ant granulation tissue. Also collagen is chemotac-tic to cellular elements of healing such as granu-locytes, macrophages and fibroblast cells which can explain the large numbers of inflammatory cells detected (Vegad, 1995).

Wound healing is a complex but orderly phenom-enon involving a numbers of processes which in-clude migration and proliferation of both epithe-lial and connective tissue cells, synthesis of extra cellular matrix, protein remodeling of connective tissue and parenchymal component and collageni-zation and acquisition of wound strength (Sta-shak, 1991).

It was obvious from histopathological examina-tion that exuberant granulation tissue that formed during the course of treatment in both groups dis-play all stages of healing processes. The presence of high numbers of polymorphnuclear leukocytes and mononuclear cells noticed in the present study is considered as defense mechanism against microorganisms. In chronic wounds as in the present study leukocytes can be attracted by medi-ators produced by macrophages. Leukocytes can prolong the inflammatory reaction by releasing mediators and toxic radicals. Polymorphnuclear leukocytes remained for longer period in the present study may possibly due to the size and depth of the wound. The persistence involvement of leukocytes may lead to cell destruction and altered composition of extracellular matrix with subsequent failure of epithelization (Cotran et al., 1994 and Wilmink et al., 1999). The chronic nature of equine wounds may be due to that leukocytes produce few mediators and weak inflammatory response which inhibits wound contration and give rise to formation of exuberant granulation tissue (Wilmink and Weeren, 2005).

Fibrin deposition acts as haemostatic barrier and provides a structural frame for repair. Latter on it has to be removed because it can inhibit the migration of fibroblast and epithelial cells. If it is not disappeared like in the present study it was probably related to less effective inflammatory response (Dinev and Dzhurov, 1987 and Wilmink and Weeren, 2005). An excessive production of collagen can explain the exuberant granulation tissue observed in the present study during the wound healing (Vegad, 1995).

In conclusion it can be said that the use of kaltostat and aquacel as a primary wound dressing in combination with the selected topical dressings in both groups may lead to acceleration of the rate of healing of equine distal limb wounds without the development of exuberant granulation tissue.

REFERENCES:

Agren, M., Franzen, L. and Chvapil, M. (1993): Effects on wound healing of zinc oxide in a hydrocolloid dressing. J Am Acad Dermatol, 29:221.

Altman, M., Suleskey, C., Delisle, R. and DeVelasco, M. (1990): Silver sulfadiazine and hydrocortisone cream 1% in the management of phenol matricectomy. J Am Podiatr Med Assoc.;80(10):545-7.

Bancroft, J., Stevens, A. and Turner, D. (1996): Theory and practice of histological technique. 4th ed. Churchill Livigstone and NewYork.

Bertone, A. (1989): Management of exuberant granulation tissue. Vet Clin North Am Equine Pract; 5(3):551-562.

Bocchiotti, G. and Robotti, E. (1990): The use of a topical antibacterial agent (silver sulfadiazine) on soft-tissue wounds. Minerva Chir. 15;45(9):677-81.

Bowler, P., Jones, S., Davies, B. and Coyle, E. (1999): Infection control properties of some wound dressings. J Wound Care.;8(10):499-502.

Brumbaugh, G. (2005): Use of antimicrobials in wound management. Vet Clin North Am Equine Pract.;21 (1):63-75.

Caron, J. (1991): Management of superficial wounds. In: Equine surgery; Auer, J.. W. B. Saunders Company.

Cotran, S., Kumar, V. and Robbins, S. (1994): Cellular growth and differentiation: normal regulation and adaptation, inflammation and repair. In: Schoen F.J. (Ed), Robins pathologic basis of the disease, 5th Ed, pp 35-92. W.B Saunders Co., Philadelphia.

Dart, A., Cries, L., Jeffcott, L., Hodgson, D. and Rose, R. (2002): Effects of 25% propylene glycol hydrogel (Solugel) on second intention wound healing in horses. Veterinary Surgery, 31:309-313.

Dart, A., Dowling, B. and Smith, C. (2005): Topical treatments in equine wound management. Vet Clin North Am Equine Pract.;21(1):77-89.

Dinev, D. and Dzhurov, A. (1987): Histogenesis of granulation tissue in wound healing by second intention in horses and cattle. Vet Med Nauki.;24(1):72-9.

Drury, R., Wallington, E. and Cameron, R. (1979): Carleton's Histological technique. 4th ed. Oxford University Press, London, New York and Toronto.

EL-Ghoul, W., Zabady, M. and Saleh, I. (2004):Total intra-

Scanned with CamScanner

venous anaesthesia in donkeys (EQUUS ASINUS): comparison of anaesthetic and cardiorespiratory effects of four anaesthetic drug combinations. Vet Med. J. Giza, 52 (1):69-87.

Engelen, M., Besche, B., Lefay, M., Hare, J. and Vlaminck, K. (2004): Effects of ketanserin on hypergranulation tissue formation, infection, and healing of equine lower limb wounds. Can. Vet. J.; 45:144-149.

Fox, C. (1983): Topical therapy and the development of silver sulfadiazine. Surg Gynecol Obstet.;157(1):82-8.

Gayko, G. (2001): Sulfonated shale oils in wound healing. Veterinary Wound Healing Association 5th Congress, Hannover, Germany.

Gayko, G., Cholcha, W. and Kietzmanm, M. (2000): Antiinflammatory, antibacterial and antimycotic effects of dark sulfonated shale oil (Ichthammol). Ber. Munch. Tierarztl. Wschr., 113:368-373.

Gomez, J. and Hanson, R. (2005): Use of dressings and bandages in equine wound management. Vet Clin North Am Equine Pract.;21(1):91-104.

Gomez, J., Schumacher, J., Lauten, S., Sartin, E., Hathcock, T. and Swaim, S. (2004): Effect of 3 biologic dressings on healing of cutaneous wounds on the limbs of horses. The Canadian Journal of Veterinary Research; 68:49-55.

Hajek, M. and Sedlarik, K. (1992): Advantages of alginate bandages for coverage of extensive and poorly healing wounds. Rozhl Chir.;71(3-4):152-4.

Hendrickson, D. and Virgin, J. (2005): Factors that affect equine wound repair. Vet Clin North Am Equine Pract.;21(1):33-44.

Kucan, J., Robson, M., Heggers, J. and Ko, F. (1981): Comparison of silver sulfadiazine, povidone-iodine and physiologic saline in the treatment of chronic pressure ulcers. J Am Geriatr Soc.;29(5):232-5.

Lansdown, A., Williams, A., Chandler, S. and Benfield, S. (2005): Silver absorption and antibacterial efficacy of silver dressings. J Wound Care.;14(4):155-60.

Lindsay, W. (1989): Equine bandaging techniques. Vet Clin North Am Equine Pract.;5(3):513-38.

Liptak, J. (1997): An overview of the topical management of wounds. Aust Vet J.;75(6):408-13.

Martens, J. and Gayko, G. (2002): Application of sulfonated shale oils in horses under special consideration of a new pale sulfonated shale oil-report on clinical experience. Der Praktische Tierarzt, 83:532-537.

McGlennon, N. (1988): The role of bandaging in the management of open wounds. Vet Rec, 122:630-633.

Mohr, V., Spelter, H., Schmidt, J. and Zirngibl, H. (1999): Wound dressings in chronic wounds. Zbl Chir.;124 Suppl 1:56-64.

Olson, M., Wright, J., Lam, K. and Burrell, R. (2000): Healing of porcine donor sites covered with silver -coated dressings. Eur J Surg, 166(6):486-9.

Schwenzer, K. and Gayko, G. (2003): Einsatz sulfonierter Schieferole beim Pferd unter besonderer Berucksichtigung eines neuen, hellen sulfonierten Schieferols-Klinischer Erfahrungsbericht zur Wundheilung. Der Praktische Tierarzt, 84:290-299.

Segal, H., Hunt, B. and Gilding, K. (1998): The effects of alginate and non-alginate wound dressings on blood coagulation and platelet activation. J Biomater Appl.;12 (3):249-57.

Sobol, S., Keswani, S, Parvadia, J., Crombleholme, T. and Potsic, W. (2005): Effect of corticosteroid-antibiotic agents on granulation tissue in a murine model. Arch Otolaryngol Head Neck Surg.;131(4):330-5.

Stashak, S. (1991): Equine wound management. Lea & Febiger. Philadelphia -London.

Suzuki, Y., Tanihara, M., Nishimura, Y., Suzuki, K, Yamawaki, Y., Kudo, H., Kakimaru, Y. and Shimizu, Y. (1999): In vivo evaluation of a novel alginate dressing. J Biomed Mater Res.;48(4):522-7.

Svensjo, T., Pomahac, B., Yao, F., Slama, J. and Eriksson, E. (2000): Accelerated healing of full-thickness skin wounds in a wet environment. Plast reconstr Surg; 106:602-12.

Swaim, S. (1990): Bandages and topical agents. Vet Clin North Am Small Anim Pract.;20(1):47-65.

Tarnow, P., Agren, M., Steenfos, H. and Jansson, J. (1994): Topical zinc oxide treatment increases endogenous gene expression of insulin-like growth factor-1 in granulation tissue from porcine wounds. Scand J Plast Reconstr Surg Hand Surg.;28(4):255-9.

Theoret, C. (2005): The pathophysiology of wound repair. Vet Clin North Am Equine Pract.;21(1):1-13.

Thomas, S. (2000): Alginate dressings in surgery and wound management -Part 1. J Wound Care.;9(2):56-60.

Vegad, J. (1995): Textbook of Veterinary general Pathology. 1st ed. Vikas publishing house. New York.

Werner, A. and Russell, A. (1999): Mupirocin, fusidic acid and bacitracin: activity, action and clinical uses of three topical antibiotics. Veterinary Dermatology, 10 (3):225-240.

Williams, C. (1999): Algosteril calcium alginate dressing for moderate/high exudate. Br J Nurs. 11-24;8(5):313-7.

Wilmink, J., Stolk, P., Weeren, P. and Barneveld, A. (1999): Differences in second-intention wound healing between horses and ponies: macroscopic aspects. Equine Vet. J.; 31:53-60.

Wilmink, J. and Weeren, P. (2005): Second-intention repair in the horse and pony and management of exuberant granulation tissue. Vet Clin North Am Equine Pract. Apr;21(1):15-32.

Wilson, D. (2005): Principles of early wound management. Vet Clin North Am Equine Pract.;21(1):45-62.

Ziegler, U., Schmidt, K., Keller, H. and Thiede, A. (2003): Treatment of chronic wounds with an alginate dressing containing calcium zinc and manganese. Fortschr Med Orig. ;121(1):19-26..