Comp Clin Pathol (2012) 21:315–320
DOI 10.1007/s00580-010-1098-y

ORIGINAL ARTICLE

Growth characteristics of fibroblasts isolated from the body and limb of the Caspian miniature horse and the effect of hydrocortisone in vitro

Omid Azari · Ladan Emadi · Seyed Mehdi Ghamsari · Mohammad Mehdi Dehghan · Mohammad Karamouzian

Received: 3 February 2010 / Accepted: 25 August 2010 / Published online: 4 September 2010

Abstract The present study was designed to determine the in vitro effects of hydrocortisone on the growth of fibroblasts isolated from both the neck and distal limb of the Caspian miniature horse. Three grammes of subcutaneous tissue was harvested from the neck and metacarpi of four Caspian miniature horses and tissue from each site was placed in a separate culture medium. After separation of fibroblasts using explant culture method, a concentration of 10,000 cells/ml was placed into each well of a 24-well plate. Hydrocortisone was added in three different concentrations (control group: without hydrocortisone, treatment groups A, 10; B, 100 and C, 300 μg/ml) into the culture medium containing fibroblasts, 4 days later the cells were counted. The effects of hydrocortisone on fibroblasts growth and viability were evaluated. Results show that the mean fibroblast growth rate from neck-derived fibroblasts was significantly greater than those derived from the limb. There were no significant differences in fibroblast growth rate between the control group and group A in both neck and limb-derived fibroblast ($P>0.05$), but groups B and C suppressed fibroblast growth significantly in comparison with their related control groups ($P<0.05$). Cell viability decreased significantly in treatment groups B and C in comparison with the control group in both neck and limb-derived fibroblasts. The mean percentage of fibroblast growth inhibition from the neck and limb significantly decreased in treatment groups B and C in comparison with their related control groups. The effect of hydrocortisone on cultured fibroblast growth was dose-dependant.

Keywords Hydrocortisone · Fibroblast culture · Neck · Limb · Caspian miniature horse

O. Azari (✉) · M. Karamouzian
Department of Clinical Sciences, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman,
Kerman, Iran
e-mail: omidazari@mail.uk.ac.ir

L. Emadi
Department of Basic Sciences, Faculty of Veterinary Medicine,
Shahid Bahonar University of Kerman,
Kerman, Iran

S. M. Ghamsari · M. M. Dehghan
Department of Clinical Sciences, Faculty of Veterinary Medicine,
University of Tehran,
Tehran, Iran

Introduction

Second-intention healing of lower limbs in horses is a slow and often complicated process (Bertone 1989; Caron 1992). Exuberant granulation tissue is the most common complication which consists primarily of developing blood vessels, fibroblasts and their protein products, which form the surrounding matrix (Fretz et al. 1983). Exuberant granulation tissue is unsightly, prone to abrasion and secondary infection and may produce mechanical restriction to normal movement (Bertone et al. 1985; Bertone 1989; Miller et al. 2000; Theort et al. 2001; Schwartz et al. 2002; Berry and Sullins 2003; Wilmink and Van Weeren 2005).

Several studies have reported that the wounds on the limb and body of ponies heal much faster and much favourably with less complication than the same wounds in horses (Wilmink and Van Weeren 2005). Some investigators believe that this variation between wound healing between horses and ponies is due to the difference in body size (Bertone 1989; Knottenbelt 2003) while others believe in the role of genetic discrepancy (Wilmink et al. 2003).

There is also a difference in quality and rate of formation of exuberant granulation tissue produced between trunk and limb wounds (Fretz et al. 1983; Bertone et al. 1985; Wilmink and Van Weeren 2005). In horses, wounds on the distal aspects of the limb produce more exuberant granulation tissue than trunk wounds. In comparison with limb wounds, trunk wounds heal more quickly with a faster rate of epithelialization, fewer complications and rarely produce exuberant granulation tissue (Jacobs et al. 1984; Knottonbelt 2003; Miller et al. 2000; Wilmink and Van Weeren 2004).

A specific cause for the development of exuberant granulation tissue is unknown (Miller et al. 2000; Roques and Téot 2008). Mechanisms possibly involved in the horse include infections or foreign body response, excessive motion or tension on the surrounding skin, blood supply reduction with resultant hypoxia and imbalance of collagen synthesis, deposition and lysis (Bertone 1989; Miller et al. 2000; Theort et al. 2001; Berry and Sullins 2003; Wilmink and Van Weeren 2005; Hendrickson and Virgin 2005).

In vitro studies have shown that there are growth differences in fibroblasts isolated from the limbs in comparison with those isolated from the trunk of horses (Miller et al. 2000; Wilmink et al. 2001) and that there is a difference in fibroblast growth characteristics between horses and ponies (Miller et al. 2000; Wilmink et al. 2001).

Corticoids have been used in the treatment of keloid and hypertrophic scars since 1960, including hydrocortisone acetate, methylprednisolone, dexamethasone and triamcinolone. Corticosteroids affect fibroblast proliferation and production capabilities. Corticosteroids also inhibit the growth of fibroblasts and are responsible for the degeneration of fibroblasts. It has been demonstrated that there is an increase in the production of basic fibroblast growth factor and a decrease in the production of transforming growth factor-ß1 by human dermal fibroblasts, endogenous vascular endothelial factor and insulin-like growth factor-1 when induced by corticosteroids (Roques and Téot 2008).

Hydrocortisone is one of the most important glucocorticoids and it has extensive effects, such as anti-inflammatory, immunosuppressive, anti-mitotic and anti-growth effects on epidermal cells (Katzung 2001; Rang et al. 2003).

According to extensive research on the genetics and phenotype of Caspian horses, there is strong evidence that the Caspian horse is a horse and not a pony (Firouz 1978; Hatami-Monazah and Pandit 1979; Walser 1968). In this study, the growth characteristics of fibroblast isolated from the neck and distal aspects of the limb of Caspian miniature horse, as a unique race of equine species, were evaluated. Also the direct effects of hydrocortisone on the growth behaviour of fibroblasts from each of these areas were investigated.

Materials and methods

A total of four Caspian miniature horses (two males and two non-gravid females), ranging from 4 to 10 years of age were used in this study. All animals were healthy following a thorough clinical examination before the start of the experiment. Under general anaesthesia, a full-thickness skin incision was created in the mid part of the neck and lateral aspect of the mid-third of the left metacarpi using aseptic technique. Three grammes of dermal and subcutaneous tissues were harvested from both the neck and limb, minced separately and placed in separate 25-cm^2 ventilated flasks (Nunclon TM Cell Culture, No. 163371) containing RPMI-1640 culture medium (Roswell Park Memorial Institute, Sigma-Aldrich, Inc., St. Louis, MI, USA) in an incubator at 37°C in 5% CO_2. An explant culture method was used to separate the fibroblasts from the tissues. Fibroblasts were grown to confluence in RPMI supplemented with 10% foetal bovine serum (FBS), antimicrobial agents [penicillin-streptomycin (Bio Gene; Cat, AP 110), 100 IU/ml and 100 µg/ml, respectively], L-glutamine and bicarbonate buffer. The culture medium was replaced three times a week.

Once the fibroblast had reached the confluence stage cells were detached by trypsin treatment (Trypsin 1:250, T-4799, Sigma-Aldrich, Inc., St. Louis, MI, USA) with ethylenediaminetetraacetic acid (EDTA) (1 mM) which took approximately 3 min. The passage of neck and limb fibroblasts was then carried out and the cells were placed in a new ventilated flask containing 4.5 cc culture medium plus 0.5 cc FBS. Passage of cells was performed in a series of two during this study. When the cells grew and filled almost 90% of the flask floor, they were removed by trypsin with EDTA and then suspended in RPMI. After cell counting, a concentration of 10,000 cells/ml was placed into each well of a 24-well plate (Greiner Bio-One, Cellstar, No. 662160). At day 0, hydrocortisone (hydrocortisone-water soluble powder, 100 mg/gr, H0396, Sigma-Aldrich, Inc., St. Louis, MI, USA) was added to the culture medium of the treatment groups at three different concentrations.

The samples from each site (neck and limb) were placed in 12 wells, separately. The wells were distributed into four groups (three wells in each), including control group without hydrocortisone and treatment groups A, B and C with 10, 100, 300 µg/ml hydrocortisone added in culture medium, respectively.

The fibroblasts were cultured in the wells for 4 days and were then detached with Trypsin in EDTA solution. After the addition of RPMI with FBS (10%) the cells were centrifuged (Eppendorf, centrifuge, 5810 R) at 10,000 rpm for 3 min and the fibroblasts counted and the mean number of fibroblasts per group recorded. The percentage viability of fibroblasts was determined by trypan blue exclusion.

Cell counting was performed using an inverted light microscope (Olympus IX 70) and standard haemocytometer.

In this study, the period of confluency stage was recorded and compared between the neck and limb-derived fibroblasts. Mean fibroblast growth rate and viability percentage were compared between several parameters:

I. The control groups for both neck and limb fibroblasts
II. The control and treatment groups for neck fibroblasts
III. The control and treatment groups for limb fibroblasts

Additionally, the percentage inhibition of fibroblasts growth for both neck and limb treatment groups was evaluated. Mean percentage inhibition of fibroblast growth for both neck and limb fibroblasts for all treatment groups were assessed regarding the fibroblast number of their control group as follows: Percentage inhibition fibroblast growth in treatment group x=[(fibroblast number of the relevant control group−fibroblast number of the treatment group x)/fibroblast number of the relevant control group] × 100 Statistical analysis was performed using one-way analysis of variance followed by post hoc, Tukey HSD test. The significance level was set at $P \leq 0.05$. The results were expressed as mean±SE.

Results

Pre-test culture period of fibroblasts Fibroblasts in all cultured tissues started to grow from the start of the study. The mean±SE time between culturing the tissue in ventilated flasks until fibroblast confluency (formation of big colonies of fibroblasts around the cultured tissues) was 13.50±0.64 days for neck derived fibroblasts and 17.25±0.96 days for limb-derived fibroblasts. There was a significant difference in the duration to reach confluency stage between neck and limb-derived fibroblasts ($P<0.05$).

After 4 days of cell proliferation in the culture medium, cell counting showed that the fibroblast growth rate was significantly higher in the control group of the neck (69,600±2,515) than that of the limb-derived (44,400±4,751) ($P<0.05$) (Fig. 1) fibroblast. The percentage viability of fibroblasts in the control groups of the neck (93.5±0.64) and limb-derived (87.32±2.30) fibroblast showed no significant difference ($P>0.05$) (Fig. 2).

Body-derived fibroblasts The results of this study showed that there was no significant difference in mean fibroblast growth rate between the control group and the A treatment group (56,200±1,355) ($P>0.05$), but the fibroblast number decreased significantly in the treatment groups B (47,600±1,840) and C (30,400±1,098) in comparison to the control group ($P<0.05$). There were significant differences between

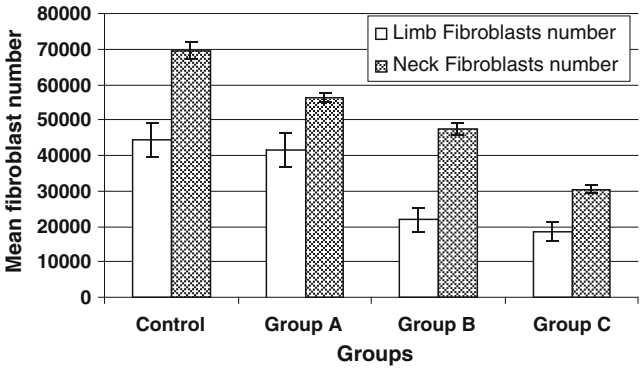

Fig. 1 Mean±SE fibroblast growth rate isolated from the neck and distal aspect of the limb of Caspian miniature horses that was cultured in media (control) and media with three different concentrations of hydrocortisone. *Group A* 10, *group B* 100 and *group C* 300 μg/ml

treatment groups A and C also between B and C ($P<0.05$). There were no significant differences in fibroblast number between treatment groups A and B ($P>0.05$) (Fig. 1).

The percentage viability decreased significantly in treatment groups A (83±0.71), B (78.62±1.51) and C (72.50±1.04) in comparison with the control group ($P<0.05$). There was a significant difference between treatment groups A and C ($p<0.05$), but there were no significant differences in percentage viability between treatment groups A and B and also between B and C ($P>0.05$) (Fig. 2).

Limb-derived fibroblasts Statistical analysis indicated that there was no significant difference in mean fibroblast growth rate between the control group and group A (41,400±6,379) ($P>0.05$). Fibroblast growth in groups B (21,800±3,303) and C (18,400±2,671) was significantly suppressed in comparison with the control group and group A ($P<0.05$). There was no significant difference in fibroblast growth rate between groups B and C ($P>0.05$);

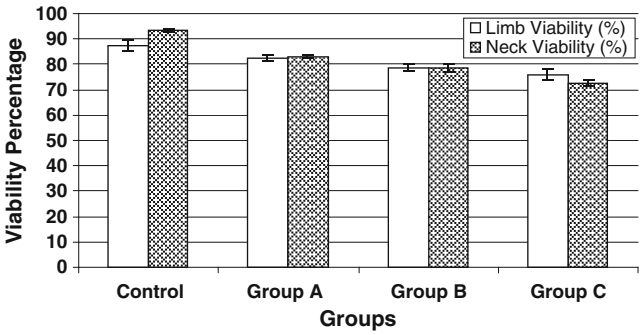

Fig. 2 Mean±SE viability percentage of fibroblasts isolated from the neck and distal aspect of the limb of Caspian miniature horses that were cultured in media (control) and media with three different concentrations of hydrocortisone. *Group A* 10, *group B* 100 and *group C* 300 μg/ml)

however, the number of cells in group B was a little higher C (Fig. 1).

There was no significant difference in percentage viability of limb fibroblasts between the control group and group A (82.38±1.14) ($P>0.05$). The mean percentage viability in group B (78.82±3.1) and C (75.62±2.15) was significantly less than the control group and group A ($P<0.05$). There was no significant difference between percentage viability in limb-derived fibroblasts between groups B and C ($P>0.05$) (Fig. 2).

Inhibition of fibroblast growth The results from this study indicate that an increase in the dose of hydrocortisone significantly decreases the mean percentage inhibition of cell growth in treatment groups in both neck and limb-derived fibroblasts ($P<0.05$).

Comparison of inhibition of cell growth between limb and neck-derived fibroblasts showed that in treatment group B, mean percentage inhibition of limb-derived fibroblast growth (49.165±2.61) was significantly more than that of the neck (31.60±1.75) ($P<0.05$). There were no significant differences in mean percentage inhibition of fibroblast growth between the limb (9.7±2.63) and neck (19.02±2.31) in treatment group A, also between the limb (59.03±1.90) and neck (55.995±2.82) in treatment group C ($P>0.05$) (Fig. 3).

Discussion

In equine medicine and practice, many studies have focused on the growth behaviour of distal limb fibroblasts since the formation of exuberant granulation tissue in the distal limb in horses is significantly higher than other sites of the body. Fibroblasts play a major role in the development of this complication (Silver 1982; Bertone 1989; Miller et al. 2000; Berry and Sullins 2003; Wilmink and Van Weeren 2004).

In several studies, local injection of different corticosteroids has been recommended to treat hypertrophic scar and keloids in human medicine (Roques and Téot 2008). Intra-lesional steroid injection has been shown to cause keloid regression in vivo, mainly by decreasing collagen and glycosaminoglycan synthesis, by reducing the inflammatory process in the wound, by decreasing fibroblast proliferation and by increasing hypoxia (Niessen et al. 1999; Roques and Téot 2008). Despite the side effects from these medications on wound healing, anecdotal evidence and controlled trials have been reported the positive effects of topical corticosteroids in the management of exuberant granulation tissue, especially in the distal limb wounds of horses (Bertone 1989; Miller et al. 2000; Dart et al. 2005).

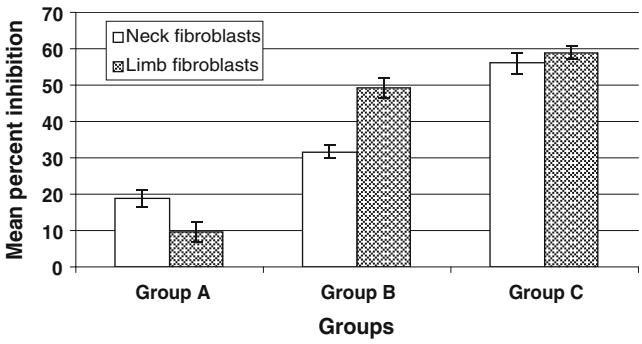

Fig. 3 Mean±SE percent inhibition of fibroblast growth of the neck and distal aspect of limb of Caspian miniature horses that cultured in media with three different concentrations of hydrocortisone. *Group A* 10, *group B* 100 and *group C* 300 μg/ml)

In recent years, many in vitro studies have investigated the effects of corticosteroids on the growth characteristics of fibroblasts in cell culture medium. The results of these studies revealed that effects of corticosteroids on fibroblasts depend on the type of cell and dose of agents. As it has been suggested, proliferative response to hydrocortisone shows cell-type specificity as well as a steroid's molecular specificity. The response appears to be mediated by the high affinity of glucocorticoid to its binding site (Rosner and Cristofalo 1979).

Several in vitro studies have demonstrated that there are significant differences in the inherent growth of harvested fibroblasts from the limb and body of horses in cell culture medium (Miller et al. 2000; Cochrane et al. 2003; Wilmink et al. 2001). The present study confirmed that there was a significant difference in growth characteristics between fibroblasts isolated from the neck and limb of Caspian miniature horses, as fibroblast growth from the neck was significantly greater than those harvested from the limb. It has been suggested that fibroblast growth from the trunk was greater than the limb of horses (Miller et al. 2000; Wilmink et al. 2001), but there was no significant difference in the growth rates between cells derived from the trunk and limb of ponies (Miller et al. 2000). The viability of fibroblasts is reported to show no difference between the limb and body (Wilmink et al. 2001). In the present study there was no significant difference in percentage viability between the neck and limb in the Caspian miniature horse either.

Fibroblast growth characteristics are different between the horse and pony. It has been stated that fibroblasts growth rate for the horse limb was significantly less than the pony limb (Miller et al. 2000; Wilmink et al. 2001). According to extensive research on the genetics and phenotype of Caspian miniature horses, there is strong evidence to confirm that the Caspian horse is a horse and not a pony (Firouz 1978; Hatami-Monazah and Pandit 1979). Therefore, wound healing problems can involve the Caspian miniature horse as well as the large breeds of horses. It has been revealed that there is no significant difference in growth characteristics of fibroblasts isolated

from the distal limb of thoroughbred and Caspian miniature horses (Azari et al. 2007).

In Miller's study, the limb fibroblasts were harvested from the metacarpus region and the body fibroblast harvested from the flank site of horses and ponies (Miller et al. 2000), but in Wilmink's research, the limb fibroblasts were isolated from the metatarsus region and body fibroblasts isolated from the hindquarter of horses and ponies (Wilmink et al. 2001). In the current study, limb and body fibroblasts were harvested from metacarpus and neck region of Caspian miniature horses, respectively. Some differences in fibroblast growth behaviour between Miller's, Wilmink's and our studies may exist due to the anatomical alteration for fibroblast isolation or because of the genetic differences between the experimented animals.

The results of this study demonstrated that hydrocortisone decreases neck and limb fibroblast growth in cell culture medium and this effect is dose-dependant. The addition of 10 μg/ml of hydrocortisone in cell culture medium was ineffective; however, addition of 100 and 300 μg/ml of hydrocortisone suppressed fibroblasts growth. In our previous in vitro study, we demonstrated that hydrocortisone at 100 and 300 μg/ml in culture medium suppresses the fibroblast growth isolated from distal aspects of the limb of thoroughbred and Caspian horses (Azari et al. 2006).

In an experimental study, the effects of hydrocortisone on primary culture of dermal fibroblast from neonate mice were evaluated. The results showed that hydrocortisone produced a dose-dependant inhibition of DNA synthesis in these cells (Verbruggen and Salmon 1980). Hydrocortisone decreases the rate of collagen synthesis and the amount of type 1, 3 and 5 collagen mRNA in normal skin and normal scar fibroblasts (Roques and Téot 2008). The inhibition of fibroblast proliferation by corticosteroids may be dose-dependant and may not be observed at lower concentrations as declared by tissue culture studies (Roques and Téot 2008). Miller also evaluated the effect of triamcinolone as corticosteroids agent on fibroblast growth rate of horses and ponies. He stated that triamcinolone suppressed limb and trunk fibroblast growth significantly in horses and ponies (Miller et al. 2000).

Based on the results of the present study, it seems that inhibitory effects of hydrocortisone on the growth rate of fibroblasts isolated from the limb are more than fibroblasts isolated from the neck. As explained before, the cell proliferative response to hydrocortisone depends on cell-type specificity as well as a steroid's molecular specificity (Rosner and Cristofalo 1979).

The results of this study demonstrated that the addition of hydrocortisone in cell culture medium decreases the viability of limb and neck fibroblast. This effect of hydrocortisone was also dose-dependant. Glucocorticides decrease adhesiveness of cells to culture plate and causes cell death in culture medium (Jung-Testase and Baulieu 1984; 1985).

According to the present study, there is a significant difference in fibroblast growth rate between fibroblasts isolated from distal limb and neck of Caspian miniature horses. Hydrocortisone suppressed the growth of fibroblasts which were isolated from the neck and distal limb of Caspian miniature horses, so that this effect on the limb fibroblasts is more than that of neck fibroblasts. Hydrocortisone also decreases the viability of the fibroblasts of the limb and neck. The effects of hydrocortisone on the fibroblasts growth and viability are dose-dependant in both neck and distal limb sites.

References

Azari O, Ghamsari SM, Dehghan MM, Aghcheloo MR (2006) Effects of hydrocortisone on the growth of cultured equine fibroblasts isolated from distal aspects of the limb. Iran J Vet Surg 1:7–13

Azari O, Ghamsari SM, Dehghan MM, Aghcheloo MR (2007) Growth characteristics of isolated fibroblasts from the distal limb of thoroughbred horses and Caspian miniature horses. Iran J Vet Res 62:169–176

Berry DB, Sullins KE (2003) Effects of topical application of antimicrobials and bandaging on healing and granulation tissue formation in wounds of the limb in horses. AJVR 64:88–92

Bertone AL (1989) Management of exuberant granulation tissue. Vet Clin North Am Equine Pract 5:551–562

Bertone AL, Sullins KE, Stashak TS, Norrdin RW (1985) Effect of wound location and the use of topical collagen gel on exuberant granulation tissue formation and wound healing in the horse and pony. Am J Vet Res 46:1438–1444

Caron JP (1992) Management of special wounds. In: Auer JA (ed) Equine surgery. Saunders, Philadelphia, pp 232–244

Cochrane CA, Pain R, Knottenbelt DC (2003) In-vitro wound contraction in the horse: differences between body and limb wounds. Wounds 15:175–181

Dart AJ, Dowling BA, Smith CL (2005) Topical treatments in equine wound management. Vet Clin Equine 21:77–89

Fretz PB, Martin GSM, Jacobs KA, Mcilwraith CW (1983) Treatment of exuberant granulation tissue in the horse: evaluation of four methods. Vet Surg 12:137–140

Firouz L (1978) The Caspian miniature horse in Iran. In: The international Caspian stud book vol 2. Countrywide livestock Ltd. Haltwhistle, England pp 11–16

Jacobs KA, Leach DH, Fretz PB, Townsend HGG (1984) Comparative aspects of the healing of excisional wounds on the leg and body of horses. Vet Surg 13:83–90

Jung-Testase I, Baulieu EE (1984) Anti-steroid action in cultured L-929 mouse fibroblasts. J Steroid Biochem 20:301–306

Jung-Testase I, Baulieu EE (1985) Effects of steroid hormones and antihormones in cultured cells. Exp Clin Endocrinol 86:151–164

Hatami-Monazah M, Pandit RV (1979) A cytogenic study of the Caspian pony. J Reprod Fert 57:331–333

Hendrickson D, Virgin J (2005) Factors that effect equine wound repair. Vet Clin Equine 21:33–34

Katzung BG (2001) Basic and clinical pharmacology. A Lange medical book, 8th edn. McGraw-Hill, New York, pp 660–671

Knottenbelt DC (2003) Handbook of equine wound management. Elsevier Science. Saunders, London, pp 5–23

Miller BC, Wilson DA, Kreeger KKG, JM AEH, Ganjam VK (2000) Growth characteristics of fibroblasts isolated from the trunk and distal aspects of the limb of horses and ponies. Vet Surg 29:1–7

Niessen FB, Spauwen P, Schalwijk J, Kon M (1999) On the nature of hypertrophic scars and keloids: a review. Plast Reconstr Surg 104:1435–1458

Rang HP, Ritter JM, Moore PK (2003) Pharmacology. Elsevier science 4th ed. Churchill Livingstone, New York, pp 411–418

Rosner BA, Cristofalo VJ (1979) Hydrocortisone: a specific modulator of in vitro cell proliferation and aging. Mech Aging Dev 9:458–496

Roques C, Téot L (2008) The use of corticosteroids to treat keloids: a review. Int J lower extrem wounds 7:137–145

Schwartz AJ, Wilson DA, Ganjam KKG, VK SY, Weber KT, Zhang J (2002) Factors regulating collagen synthesis and degradation during second-intention healing of wound in the thoracic region and the distal aspect of the fore limb of horses. Am J Vet Res 63:1564–1570

Silver IA (1982) Basic physiology of wound healing in the horse. Equine Vet J 14:7–15

Theort CL, Barber SM, Moyona TN, Gordon GR (2001) Expression of TGFβ1β3 and basic fibroblast factor in full thickness skin wound of equine limb and thorax. Vet Surg 30:269–277

Verbruggen LA, Salmon DS (1980) Glucocorticoid receptors and inhibition of neonatal mouse dermal fibroblast growth in primary culture. Arch Dermatol Res 269:111–126

Walser G (1968) Age and places of horse breeding. In: Herzfeld E (ed) The Persian empire: studies in geography and ethnography of the ancient near east. Franz Steiner Verlag, Wiesbaden, pp 1–34

Wilmink JM, Van Weeren PR (2004) Differences in wound contraction between horses and ponies; application of research results to the clinical approach of equine wounds. Clin Tech Equine Pract 3:123–133

Wilmink JM, Van Weeren PR (2005) Second-intention wound repair in the horse and pony and management of exuberant granulation tissue. Vet Clin North Am Equine Pract 21:15–35

Wilmink JM, Nederbragt H, Van Weeren PR, Stolk PW, Barneveld A (2001) Differences in wound contraction between horses and ponies; the in vitro contraction capacity of fibroblasts. Equine Vet J 33:499–505

Wilmink JM, Veenman JN, Van Den Boom R, Rutten VP, Niewold TA, Broekhuisen DJM, Lees R, Armstrong S, Van Weeren PR, Barneveld A (2003) Differences in polymorphonucleocyte function and local inflammatory response between horses and ponies. Equine Vet J 35:561–569