efficacy of conventional versus innovative therapies for treating skin wounds in veterinary medicine.

M Patruno[1], C Gomiero[1], Martinello T[1], A Perazzi[2], F Gemignani[2], GM DeBenedictis[2], S Ferro[1], M Zuin[3], E Martines[3], L Cordaro[3], P Brun[4], L Maccatrozzo[1], SY Broeckx[5], JH Spaas[5], K Chiers[6], Iacopetti I[2]
[1]Department of Comparative Biomedicine and Food Science, University of Padua, Italy; [2]Department of Animal Medicine, Production and Health, University of Padua, Italy, [3]Consorzio RFX, Padua, Italy, [4]Department of Molecular Medicine, University of Padua, Italy, [5]Global Stem Cell Technology-ANACURA group, Evergem, Belgium, [6]Department of Pathology, Bacteriology and Poultry Diseases, University of Gent, Belgium.

INTRODUCTION: The skin is the largest organ of mammals. The loss of skin integrity may induce important dysfunctions or even death. For superficial wounds, the endogenous healing mechanisms in combination with traditional wound care are sufficient to achieve functional repair. In contrast, in larger wounds, like third and fourth degree burns, chronic wound or deep ulcers it is difficult to obtain the *restitutio ad integrum* and fibrosis and/or scar tissue develops[1,2]. The aim of this study was to verify the efficacy of conventional and innovative topic treatments on skin regeneration, induced experimentally in sheep. To achieve this goal different types of investigations (clinical, molecular, histological, immunohistochemical) were performed.

METHODS: Six skin lesions (4x4cm) were surgically created on the back of six healthy adult sheep; every single wound was destined, in a randomized way, to one of the following treatments: Acemannan gel, Manuka Honey, hyaluronic acid, Plasma[3] (ionized gas), allogeneic mesenchymal stem cells isolated from peripheral blood (PB-MSCs). The sixth wound was the placebo. Biopsies were collected with a surgical punch (0,6x0,6 cm) at time T0, T15 and T40 days. Lesions were clinically evaluated considering the presence and color of wound fluid, the state of hydration, the wound surface/surroundings and other parameters. Histological examinations considered crust formation, re-epithelization and epidermal thickness, dermis edema, extension of granulation tissue, acute and chronic inflammation. Immunohistochemistry for evaluation of inflammation, vascularization and cell proliferation was performed using CD3, CD20, MHCII, von Willebrand factor (vWF) and KI67 antibodies. Furthermore, Real time-PCR investigated genes as Vascular endothelial growth factors (VEGF), Transforming growth factor beta 1(TGFβ1), Vimentin (VIM), Collagen 1α1 (Col1α1) and hair Keratin (hKER).

RESULTS: Clinically, the lesions treated with plasma healed more rapidly respect to other treatments and a reduced bacterial load was observed. At T7 wounds treated with stem cells and plasma were less macerated than lesions treated with other therapies**.** At T15 the wounds treated with hyaluronic acid showed a normal state of hydration while lesions treated with Manuka Honey exhibited a normal hydration from the third week only (Acemannan gel at fourth week). From the second week onwards all wounds did not show presence of fluid and exhibited a dry and clean secondary layer. All lesions, excluded wounds treated with acemannan gel, presented a red (hyaluronic acid and plasma) and dark red (Manuka Honey, PB-MSCs) granulation tissue starting from the first week. Molecular analysis showed a correspondence between clinical and molecular/histologic results. For instance, VEGF mRNA expression confirms angiogenetic events observed at histological level while TGF-β, CD3 and CD20 mRNA/protein expression indicated the presence/absence of inflammation in the used treatments.

DISCUSSION & CONCLUSIONS: Innovative therapies led to surprising results regarding regeneration of mammalian skin. Indeed, on the basis of clinical analysis, wounds treated with plasma and MSC healed more rapidly. Further examinations are ongoing in order to elucidate possible mechanisms explaining these differences.

REFERENCES: [1]S.Y. Broeckx, S. Maes, T. Martinello, et al (2014) *Equine epidermis: a source of epithelial-like stem/progenitor cells with in vitro and in vivo regenerative capacities* Stem Cells Dev, pp 1134-48. [2]J.H. Spaas, C. Gomiero, S.Y. Broeckx, et al (2016) *Wound healing markers after autologous and allogeneic epithelial-like stem cell treatment* Cytotherapy 2016 (in press). [3]E. Martines, M. Zuin, R. Cavazzana, et al. (2009) *A novel plasma source for sterilization of living tissues*, New J. Phys. 11, 115014.