

Using microCT (Skyscan, Belgium), trabecular bone volume fraction (BV/TV), trabecular thickness (TbTh) and subchondral plate thickness (PTh) were calculated for the epiphyseal part of the tibias. Next, the tibias were sectioned for histological analysis. The osteoarthritis cartilage histopathology scoring was used to evaluate the cartilage damage in medial and lateral compartment of the tibial epiphysis. For each compartment, a maximum score of 24 can be reached. In addition, the presence or absence of osteophytes was scored.

Results: *Cartilage and osteophytes:* Overall, the cartilage damage scores were fairly low, up to a score of 4. In $-/-$, ERbeta $-/-$, and ERalpha $-/-$ ERbeta $-/-$ mice, there was no difference in cartilage damage score when compared to wt mice.

In the ERalpha $-/-$ ERbeta $-/-$ mice, more and/or larger (seen in more histological sections) osteophytes were present compared with wt mice.

Subchondral plate thickness: In the ERalpha $-/-$ and ERbeta $-/-$ mice, no difference in subchondral plate thickness was observed when compared with wt mice. In the ERalpha $-/-$ ERbeta $-/-$ mice, the subchondral plate was thinner than in the wt mice, but only at the lateral side.

Epiphysis: In the WT and ERalpha $-/-$ mice, no significant differences were observed in trabecular BV/TV and TbTh. In contrast, in the ERbeta $-/-$ mice, trabecular BV/TV was 13% higher than in the wt, but there was no difference in TbTh. In the ERalpha $-/-$ ERbeta $-/-$ mice, trabecular BV/TV and TbTh were 11% lower than in the WT mice.

Conclusions: No cartilage changes were found in the ERalpha, ERbeta $-/-$, ERalpha $-/-$ ERbeta $-/-$ mice at 6 months of age. In the single knockout mice, deletion of one of the estrogen receptors may be compensated by the other estrogen receptor. However, in the double knockout mice, this is not the case. Increased cartilage damage was expected in the double knockout mice, as osteoarthritic cartilage changes were found in both rats and monkeys when estrogen signaling was inhibited by ovariectomy. The differences in epiphyseal trabecular bone phenotype did not influence the cartilage damage in the knockout mice. We did see more or larger osteophytes in the double knockout mice, indicating that there are some osteoarthritic developments. In the double knockout the lateral subchondral plate thickness was decreased, which is also observed in several osteoarthritic animal models. The increased osteophytosis and changed subchondral plate is an indication that at later age these mice likely develop more osteoarthritic changes.

68 AUTOLOGOUS PRGF IN THE OVINE ACL-TRANSECTED KNEE OSTEOARTHRITIS MODEL

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Purpose: We assessed the capacity of PRGF to impact OA progression in sheep following trauma to the joint caused by ACL rejection and cartilage defects performed on the medial condyle. This objective was first addressed by studying macroscopic cartilage changes in the femoropatellar and femorotibial compartments.

Methods: Latxa sheep ranging in age from 3 to 8 years (5.0 ± 1.9 years) and weighing 62 ± 9 kg (range: 47.8–74.4 kg) were used. A body condition score was evaluated by a senior veterinarian by estimating the level of muscling and fat [Editor1]deposition over and around the vertebrae in the loin region (scored from 1 to 5). The Neiker-Technalia Animal Care and Use Committee approved all procedures used. TGF- β levels were assessed in PRGF and synovial fluids by ELISA.

Sheep operations were performed bilaterally. Spinal anaesthesia was induced in the hind limbs with bupivacaine. A medial parapatellar incision was made and the patella was reflected laterally in order to expose the ACL, which was subsequently transected. Complete thickness cartilage defects were performed in the medial femoral condyles of both knees. Following the surgery, free movement was allowed.

Ultrasound-guided infiltrations were initiated three weeks after ACL transection. Sheep were treated with a series of six biweekly intra-articular injections of 4 ml of PRGF preparation and 4 ml of saline in the contralateral knee. PRGF and saline treatments were randomized to either the right or the left knee.

OA induction was followed-up by radiography. Sheep were sacrificed when OA features were evident on radiological images. This occurred at 36–38 weeks post-surgery, 16–18 weeks after the end of the treatment. Knees were macroscopically studied for the presence of osteophytes and signs of cartilage degeneration. Lateral and medial femur condyles, tibial plateaux, and retropatellar cartilage were inspected. The articular surfaces of the patella and tibial plateau were stained with diluted India

ink (1:5 v/v) to enhance detection of fibrillation. Specimens were photographed, and images were assessed using a digital program. The fibrillation area relative to the total area was calculated. ANOVA analysis was performed to assess the influence of treatment modality on macroscopic assessments. Age and body condition score were used as covariables.

Results: Body condition scoring ranged from 2.25 to 3.0 (2.73 ± 0.27). Most sheep ($n=9$) were able to stand up 1–3 hours after surgery. PRGF is a plasma preparation containing a moderate number of platelets, $448 \times 10^6 \pm 194 \times 10^6$ platelets/cc, platelet volume was 4.8 ± 0.3 fl. The levels of TGF- β 1 in the PRGF were $13,240 \pm 5,650$ ng/ml as assessed by ELISA. Platelet number correlated significantly with TGF- β 1, Pearson correlation = 0.9079, $p < 0.001$. Intra-articular TGF- β at the time of sacrifice was 1983 pg/ml (CI: 1561–2406) in the treated joint and 1513 pg/ml (CI: 1127–1898) in the saline group, $p = 0.1015$. Body condition score, but not age, significantly influenced the intra-articular levels of TGF- β 1, $p = 0.0306$. Transection of the ACL and full thickness defects in the medial condyle resulted in the development of lesions in the lateral femoral condyle, meniscus, tibial plateau, and patella. Osteophytes were located mainly on the patellar trochlea ridge. Changes in the tibial plateau are shown in Table 1. The percentage of patella fibrillation was 10.1% (CI: 5.95%–14.24%) for the saline group and 4.3% (0.17%–8.46%) for the PRGF group, $p = 0.053$. Age significantly influenced patella fibrillation, $p = 0.036$.

Conclusions: This study suggests that there may be a therapeutic benefit associated with intra-articular injection of PRGF following traumatic injury to the knee.

69 DOES LITOVET, A HERBAL REMEDY MADE FROM ROSA CANINA, ACT AS AN ANTI-INFLAMMATORY AGENT IN HORSES EXPOSED TO STRENUOUS EXERCISE – A RANDOMIZED, PLACEBO-CONTROLLED, PARALLEL, DOUBLE-BLIND STUDY ON THE IMMUNE SYSTEM OF HORSES, THEIR WORKING CAPACITY AND BEHAVIOUR

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Purpose: A standardised powder prepared from rose-hip (*Rosa canina*), has shown anti-inflammatory properties and improved the flexibility of the hip and knee joints and reduced pain in humans suffering from the early as well as the late stages of osteoarthritis. Recently a galactolipid named GOPO with anti-inflammatory properties was isolated from this rose-hip powder. The present study aimed to investigate if LitoVet, a rose-hip powder produced by HybenVital, especially for animals, would affect the immune system, working capacity and behaviour of horses exposed to strenuous exercise.

Methods: Seventy-four horses, all trotters, which often develop osteoarthritis early in their life, due to their intensive training program and their performance in an oval track, were included in a double blind, placebo controlled trial. The horses were randomized in blocks of three of which two were given LitoVet (210g daily as a dry powder added to their food) and one was given the same amount of placebo powder with a similar taste, odour and colour. Both groups of horses were then treated for a three months period. The anti-inflammatory capacity was estimated as chemotaxis of peripheral blood neutrophils using a Boyden chamber, the anti-oxidative capacity by using chemiluminescence and by simply measuring the vitamin C content of serum. The working capacity of the horses was estimated by counting the seconds used to run 1000 meter and the behaviour of the horses was evaluated on questionnaires which was answered by the staff daily taking care of the horses (data given are mean \pm sd).

Results: During LitoVet treatment neutrophil chemotaxis declined from 30.4 ± 14.0 to 9.0 ± 13.5 indicating an enhanced anti-inflammatory activity ($p < 0.004$). No change was observed during placebo. LitoVet also significantly improved the antioxidant capacity when estimated as chemiluminescence ($p < 0.05$) and the vitamin C content of serum ($p < 0.05$). Horses on LitoVet also shortened their time to run 1000 meter as shown by a mean decline of 1.1 ± 1.5 second ($p < 0.02$). The placebo group slightly increased their time to run the same distance. In agreement with the changes described above it was reported from questionnaires, that the horses benefit from LitoVet treatment ($p < 0.05$) and that horses on LitoVet were more lithe after strenuous exercise than horses treated with placebo ($p < 0.05$).