CSAC methodology emerged as the preferred method for recovering extremely small volumes of SF. Furthermore, the results of our pilot *in vitro* COMP analyses suggest that SF-based OA biomarker studies using mice are feasible, effective, and reliable.

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OESTROGEN DEFICIENCY, BUT NOT GLUCOCORTICOIDS ADMINISTRATION, INDUCES KNEE CARTILAGE LESIONS IN AN EXPERIMENTAL MODEL OF OSTEOPOROSIS IN RABBITS

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Background: The relationship between osteoporosis (OP) and osteoarthritis (OA) is complex and poorly understood. Recent studies suggest that OP could accelerate the progression of established OA, but the influence of OP in healthy cartilage is unknown. Moreover, the pathogenic role of systemic glucocorticoids on cartilage damage is not well defined neither.
Aim: To determine the influence of ovariectomy (OVX) either alone or combined with systemic glucocorticoids administration on healthy cartilage in an experimental model in rabbits.
Methods: Twenty female NZ white rabbits (10 months old; mean weight of 4.3 kg; r: 3.7-6.3) were randomly allocated in three different groups. Seven animals underwent bilateral OVX (OVX group). Low bone mineral content status was induced in 6 animals by OVX and subsequent parenteral methylprednisolone hemisuccinate (MPH) administration (1 mg/kg/d) for 4 weeks to assess the influence of OP on the homeostasis of normal cartilage (OP group). Seven animals were used as controls (Healthy group). To evaluate the bone mass variation, bone mineral density (BMD) was measured by dual energy X-ray absorptiometry (DXA) at both baseline and 6 weeks after OVX (Hologic® QDR-1000) in lumbar spine (L3-L4, LS), global knee (gK) and subchondral bone of the knee (sK). The histopathological cartilage damage at the end of the experimental interventions was evaluated in the medial femoral condyles following the Mankin's system.
Statistical analysis: The difference of the means of the cartilage damage and BMD between groups was calculated using the analysis of the variance (ANOVA). The "post hoc" comparison of the means was studied by the Tukey's test. Correlation between DXA and histological damage was done by Pearson' correlation test (SPSS, vs. 10.0).
Results: Baseline BMD values (mg/cm^2) did not show significant differences between groups in any of the anatomical regions analyzed (LS, gK and sK). However, BMD showed a significant decrease in OP rabbits when compared to both OVX and healthy rabbits ($p<0.05$) (Table 1). A significant negative correlation between BMD at LS and cartilage damage was also demonstrated ($p<0.05$), but no correlation could be established when BMD was determined at gK and sK.

Group	Mankin score	BMD-LS	BMD-gK	BMD-sK
HEALTHY	0.14 (0.14)	305±13	473±31	642±47
OVX	1.43 (0.30)*	268±37	426±25	578±70
OP (OVX+MPH)	2.42 (0.57)*§	232±40*§	362±86*§	490±97*#

*$p<0.05$ with respect to healthy group; §non significant differences between OVX and OP; #$p<0.05$ between OVX and OP. Results are expressed as mean ± SD.

Conclusions: Conclusions: Since isolated OVX induced statistically significant alterations in normal cartilage in our model, oestrogen deficiency might play a direct role in the ethiopathogenesis of OA. The fact that differences in cartilage damage using the Mankin score were not significant when OVX and OP rabbits were compared, suggests that systemic glucocorticoids do not play any pathogenic role in joint damage.

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EARLY CHANGES OF ARTICULAR CARTILAGE, SYNOVIAL MEMBRANE AND SUBCHONDRAL BONE IN AN EXPERIMENTAL MODEL OF GOAT OSTEOARTHRTITIS

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Purpose: Aim of the study was to evaluate the early changes and chronological involvement of articular cartilage, synovial membrane and subchondral bone in an experimental model of goat osteoarthritis (OA).
Methods: OA was induced unilaterally in the right knee joint of nine goats by resection of the anterior cruciate ligament (ACL). Left knee has been considered as control. Three goats have been sacrificed at any time point, one, three and six months, respectively. Plain X-rays of knees have been performed prior to sacrifice. Microradiographic analysis has been carried out to assess volume of subchondral bone, thickness of cortical bone and trabeculae. Histology of synovial membrane was evaluated by ematossilin-eosin staining and the degree of involvement was ranked by using the score system proposed by *Pelletier JP et al*. Safranin staining and *Mankin HJ et al* scoring have been performed to assess cartilage changes.
Results: Analysis of cartilage has shown that ultrastructural changes and decrease of chondrocyte density were present already after one month with progressive reduction of proteoglycan staining which was more marked after six months in the right knee, and the differences with the left knee were statistically significant. After one month, synovial membrane showed a progressive increasing number of layers of the synovial lining and hyperplasia of synovial villi and the differences with the control knee were statistically significant. Also perivascular lymphomonocyte infiltrate was higher in the right knee that in the left knee. Morphometric analysis of subchondral bone detected significant differences between OA and control knee only after 6 months. Volume of cortical bone was increased but volume of trabecular bone was decreased. Also thickness of cortical bone was significantly increased after 6 months in the right knee, whereas no changes in trabeculae thickness was noted.
Conclusions: These findings provided evidence that, at least in our model of OA induced by ACL resection in goats, early signs of OA are detectable both in articular cartilage and synovial membrane. These changes may increase the production of procatabolic cytokines and other inflammatory mediators that could play a key role in amplifying the cartilage injury. Involvement of subchondral bone occurs later and always follows cartilage and synovial membrane changes.

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ORALLY ADMINISTERED COLLAGEN HYDROLYSATE HALTS THE PROGRESSION OF OSTEORATHRITIS IN STR/ort MICE

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Purpose: Experimental investigations on various chondrocyte

and cartilage explant models have demonstrated a clear stimulatory effect of Collagen Hydrolysate on chondrocyte metabolism and cartilage growth. The objective of this study was to investigate the efficacy of orally administered Collagen Hydrolysate on the development and progression of osteoarthritis (OA) in an appropriate animal model.
Methods: The inbred mouse strain STR/ort spontaneously develops osteoarthritic lesions of the knee joint by 35 weeks of age resembling human osteoarthritis. The efficacy of Collagen Hydrolysate was tested in a randomly assigned placebo-controlled animal study. In 6 month old male STR/ort mice 0.15 mg Collagen Hydrolysate or BSA/g body weight was orally administered once a day over a treatment period of 3 months. Thin sections of the knees were analyzed for osteoarthritic changes by haematoxylin-eosin staining. OA joint damage was assessed by a well-defined semi-quantitative histopathological score. Additionally, the progression of osteoarthritis in male mice at different ages was determined and the correlation between grade of OA and body weight was investigated. A total number of 48 male STR/ort mice were analyzed in this study.
Results: According to the literature the progression of the determined grade of OA in the non-treated STR/ort mice correlated with the aging of the animals. While female mice developed only mild forms of OA, 85% of the non-treated males showed extensive OA-like lesions at the end of the study. The oral administration of Collagen Collagen Hydrolysate over 3 months led to a pronounced decrease in cartilage tissue degeneration in the knee joints. The incidence of severe joint destruction was clearly reduced after Collagen Hydrolysate treatment and the determined grade of OA decreased statistically significantly in comparison to the untreated control animals.
Interestingly, a more detailed analysis of the data suggested a correlation between the determined grade of OA and the body weight of the STR/ort mice.
Conclusions: The results indicate that orally administered Collagen Hydrolysate was able to slow or even halt cartilage destruction in STR/ort mice. The data suggest that Collagen Hydrolysate may prevent the progression of joint degeneration in OA and could possibly be a potential disease-modifying agent for the treatment of degenerative joint diseases.

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AMBIVALENT PROPERTIES OF HYALURONATES IN EXPERIMENTAL INDUCED OSTEOARTHRITIS RAT KNEE

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Purpose: This experimental study was undertaken to determine whether viscosupplementation with intra-articular (i.a.) low- or high-molecular-weight HA injections influences both chondral and synovial lesions in rats with surgically-induced OA knee.
Methods: Male Wistar rats underwent anterior cruciate ligament transection (ACLT). Rats were divided into 5 groups: naive group (n=10), sham group (n=10), ACLT and saline i.a. injection group (n=10), ACLT + Synvisc® (high molecular weight, HMW) i.a. injection group (n=10), ACLT + Hyalgan® (low molecular weight, LMW) i.a. injection group (n=10). Intra-articular injections of sterile saline or HAs were performed on D7, D14, and D21 after ACLT. Animals were killed on D28 for histological assessment. Grading of chondral lesions was performed according to Mankin's score. Rooney's score was used to assess concomitant synovitis. Concomitant immunostainings of activated Caspase 3 and Hsp70 was also performed.
Results: Articular damages (D28) were significantly reduced in HAs-treated knee joints *vs* control joints: 17.55 for HMW and 19.2 for LMW vs 31.3 for ACLT control rats ($p<0.05$). In the cartilage of ACLT+HAs treated groups, articular surface presented minor fibrillations. A significant increase of histological score of synovial membrane was noted in both ACLT+HAs groups (HMW, $p<0.05$; LMW, $p<0.05$) *versus* matched ACLT+saline group. Both HAs-treated groups exerted an inflamed synovial membrane. A basal expression of caspase 3 (6,7%) was observed in the sham group whereas it was significantly increased in ACLT control rats (23.9%). In contrast, apoptotic events significantly decreased in both HAs groups. Additionally, basal expression of Hsp 70 was quite similar in sham and ACLT groups. In contrast, Hsp70 increased significantly in both HA groups.
Conclusions: Although previous works showed that hylan could be responsible for synovial granulomatous inflammation in the clinics, our pilot preliminary study conducted in the ACLT-induced rat OA knee suggests that HAs may exert ambivalent properties on articular structures, simultaneously exerting chondroprotective properties and promoting synovitis

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ORAL HYALURONIC ACID ADMINISTRATION IMPROVES OSTEOCHONDROSIS CLINICAL SYMPTOMS AND SLIGHTLY INCREASES INTRA-ARTICULAR CONCENTRATION OF HYALURONIC ACID IN A HORSE MODEL: A PILOT SURVEY

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Purpose: The intra-articular content of hyaluronic acid (HA) generally declines in inflammatory arthritis (Carro & Blaya, 2002; Takahasi et al, 2004). Intra-articular administration of HA has been used historically, but less information is available about the effectiveness of the oral route. The objective of this study was to determine the effect of the oral administration of an HA concentrate (Hyal-Joint™) on synovial fluid quality and on the clinical condition of horses with osteochondrosis (OCD).
Methods: The horse was used as an animal model because allows the obtaining of high amounts of synovial fluid at different time points. Twelve horses with a radiographic diagnose of OCD were randomly divided in two groups and assigned to receive orally 250mg of Hyal-Joint (HJ) or placebo during 60 days in a blinded randomized controlled clinical pilot trial. At the end of the treatment (d60) and 30 days after finalization (d90) a sample of synovial fluid was extracted from each animal to analyse HA concentration. The degree of synovial effusion measured with ultrasonographic evaluation and the degree of lameness according to AAEP scale were also evaluated.
Results: On day 0 no differences on intra-articular HA concentration were detected among groups (353 ± 45 vs. 301 ± 137 μg/L for HJ and placebo groups respectively). However during the experimental period intra-articular HA concentration increased numerically in the HJ group but decreased in the placebo group, resulting in differences among groups on day 60 (384 ± 42 vs. 209 ± 104 μg/L; P=0.07) and on day 90 (424 ± 89 vs. 286 ± 119 μg/L; P=0.05) which tended to reach statistical significance. Increases of the intra-articular HA concentration in HJ treated horses were associated on d90 with numerical improvements on the synovial effusion scale (1.25 vs. 2.00 points for treated and control groups respectively) and on the degree of lameness (0 vs. 1.5 degrees for treated and control groups respectively), although differences among groups failed to reach statistical significance due to the reduced number of animals.