COMPARATIVE COMPUTER THOMOGRAPHIC (CT) ANGIOGRAPHIC STUDIES OF EXPERIEMTALLY-INDUCED OSTEOARTHRITIS (OA) OF THE KNEE JOINT IN SHEEP TREATED WITH PLATELETS-RICH PLASMA (PRP)

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ABSTRACT
Knee joint OA was induced after removing half of the medial minuscule of the joint. For twelve weeks after the operation at 10 days interval, 5 ml of PRP was injected three times in the right knee joint. The left knee joint was used as a control, and 5 ml of saline was injected three times.

Six weeks after the last application of PRP, angiograms of the two knee joints were performed, using spiral computed tomography (CT). The study was performed transversally from the middle of the femur to the middle of the tibia of the respective limb. The slides were 1.5 mm wide at 2 mm intervals. The structural changes in the two knee joints were studied and compared, as well as the degree of angiogenesis and the degree of recovery of the articular cartilage.

It was found that a. Saphena lateralis and medialis, and a. genus media are of the utmost importance, especially in ruminants, as they are responsible for the blood supply to the knee joints.

The use of PRP in sheep with experimentally induced OA of the knee joint has been shown to induce the formation of an additional vascular system in treated knee joints.

Key words: bone marrow (BM), osteoarthritis (OA), knee joint, computer tomography (CT), angiography, sheep.

Introduction
Osteoarthritis (OA) affects all joint elements. It is characterized by degeneration and loss of articular cartilage, remodeling of the subchondral bone, formation of bone tissue (osteophytes), tendon instability, weakening of periarticular muscles, meniscus rupture, formation of subchondral cysts, development of subchondral sclerosis, and in the final stages of disease development it is possible to monitor the development of synovitis (1).

A major role in the pathogenesis of OA is attributed to the growth of new blood vessels in the osteochondral compound and synovial membrane. By stimulating ossification of articular cartilage and transport of monocytes and macrophages into the synovial fluid, angiogenesis stimulates the formation of osteophytes and inflammation of the synovial membrane (2).

The main methods for detecting changes in osteoarthropathies are X-ray methods of investigation. The results obtained depend on the imaging methods used. X-ray examination can show osteoarthritis changes in bones, while those of the soft tissues have low diagnostic value. Moreover, radiographs are not show very well change and early pathological features of OA (3).

CT is a better method of conventional X-ray examination because it provides assessment of the changes in soft tissues and bones.

The aim of the present study was to investigate knee joint angiogenesis in experimentally induced OA and determine the effect of intra-articular administration of PRP on neoangiogenesis.
Materials and methods

The study sample consisted of six clinically healthy lambs weighing 22–28 kilograms.

Induction of OA

Medial access to right knee joint is gained in aseptic conditions following Anderson (1994). After arthrotomy, the removal of 1/2 of the medial meniscus (cranial part) is performed. The joint capsule is restored by discontinuous sutures with absorbable suture material 2/0 and subcutaneous tissue with a continuous mattress seam with absorbable suture material 2/0; skin with interrupted seam with unsorted 4/0 suture material. After a rest period of three weeks, all animals are forced to move on hard terrain of distance of 90 meters. During the rest of the time, the animals are allowed to move freely in an unlimited space.

Venous blood production and method of obtaining PRP in sheep

The animal is fixed in standing position and v. jugularis is prepared aseptically. 60 ml of venous blood is obtained from v. jugularis from each sheep was obtained in three 20 ml syringes, each of them contains 4 ml ACD-A (anticoagulant dextrose solution A) solution. The blood is distributed in 3 counts. Dr.PRP kit with a capacity of 20 ml disposable syringes. The syringes are placed in the centrifuge for first centrifugation at 2700 rpm for 5 min. This procedure divides the blood into its three major components: red blood cells, PRP and platelet-poor plasma (PPP). The resulting plasma is aspirated from the Dr.PRP kit with a 10 ml syringe. The separated plasma is divided into 3 pieces. 10 ml syringes, which are re-centrifuged. The second centrifugation is performed at 3500 rpm revolutions per minute for 15 min. The resulting supernatant is removed, leaving 2 ml of PRP in each syringe. The precipitate is mixed using a mixer (Vortex V-1, Biosan).

Injection of PRP into the knee joint

The areas of the knee joints were aseptically prepared. A puncture of both joints was performed with injection needle of 20G. The needle penetrates the middle of the triangle formed on the medial side of the patellar junction, the medial femoral condyle and the middle of the tibial plateau, and is directed toward the space between the medial and the lateral condyle. 5 ml of PRP is injected in the right knee joint. After injection, multiple flexion and extension is performed for 10 minutes to allow the injected PRP to be evenly distributed in the intraarticular space. 5 ml of saline solution is injected into the left knee joint; the method of application is the same as the one described above. These PRP injections were repeated three times over the next 10 days. The animals were euthanized six weeks after the third procedure. Samples for histological examination were taken from the medial femoral epicondyle and the tibial plateau.

CT angiography

The animals are placed in a chest lying position. Operational access to a. Femoralis is provided, followed by the introduction of an arterial catheter of 4 mm in width and 11 cm in length. An ULTRAVIST ® 370 769 mg/ml contrast is injected into the bloodstream at a rate of 0.5 ml per 1 second via an infusion pump. CT study was performed using a Picker® CT PQ 5000 helical CT. X-rays are directed in the transversal direction, starting from the middle of the femur to the middle of the tibia. The slices were taken at 2 mm. distance, the thickness of each cut was 1.5 mm. The research was done using the DICOM Viewer® computer software.
Results

The major blood vessels, namely the arterial and venous arteries, v. saphena lateralis and v. saphena medialis are monitored on all CT scans along the entire length of the knee joint and the two pelvic limbs of the studied sheep.

On the CT scan, distantly through the femoropathal joint, the following vessels are visualized: subcutaneous and cranial of v. saphena medialis – a. saphena, medial and in depth of them two other courts – a. genus descendens with the epithelium of the same vein, blood supplying the vastus medialis of the quadriceps femoral muscle, m. sartorius and the cranial part of m. semimembranosus, laterally and in depth of v. saphena lateralis (Figure 1), between m. gastrocnemius and m. biceps femoris – a. and v. caudalis femoris, blood-supplying m. semimembranosus, m. semitendinosus and m. biceps femoris. The listed arteries are separated by a. femoralis. A. and v. caudalis femoris in the popliteal region supply the two heads of m. gastrocnemius and the surface flipper and aa. surales (clones of a poplitea) support the blood supply of these muscles (Figures 2, 3, 4 and 5).

At the level of the condyles of the femoral, the separation of a. genus distalis lateralis and a. genus distalis medialis of the popliteal artery in the sheep is bilaterally established. Their continuations accompanied by the corresponding veins are identified as being caudally associated with the femorotipital joint. The same scan is observed a. saphena, medial and a. and v. caudalis femoris, laterally, again under m. biceps femoris. The same blood vessels with the same disposition but distal from the previous level are seen at a CT slice. Here we visualize the extreme branches of a. and v. genus distalis lateralis, which supply blood m. biceps femoris and m. popliteus and the lateral collateral connection; a. and v. genus distalis medialis supply blood to the caudal part of m. semimembranosus, m semitendinosus and m. gracilis and the medial collateral connection. The continuation of left and right concealed arteries and a. the genus media that supplies blood to corpus adiposum infrapatellare and the crossed knees joints in the sheep (Figures 2 and 4), whose blood supply is also aided by a. and v. genus descendens. This artery is identified at the level of the femorothybial joint between the femoral tendons and the popliteal artery. The knee veins are seen on CT scans from their onset to their infusion into the spinal vein. A. and v. genus media visualized are well on a CT scan.

Figure 1: CT slice through the femoropathal joint – Vsl – vena saphena lateralis; Acf – arteria caudalis femoris; Vp – vena poplitea; Ap – Arteria poplitea; Avgd – artery and vena genus descendens; As-isthria saphena; Vsm – vena saphena medialis
Figure 2: CT slice through the femortibial joint – Ap – arteria poplitea; Vp – vena poplitea; Acf – arteria caudalis femoris; Vcf – arteria caudalis femoris; Avcf – arteria and caudalis femoris vena; Asu – arteriae surales; Agdl – arteria of the genus distalis lateralis; Vgdl – the genus distalis lateralis; Agdm – arteria of the genus distalis medialis; Vgdm – vena genus distalis medialis; Agm – arteria genus media; Vgm – vena genus media; Vsm – vena saphena medialis; As – arteria saphena; Vsl – vena saphena lateralis

Figure 3: CT slice through the proximal tibia – Ap – arteria poplitea; Vp – vena poplitea; Acf – arteria caudalis femoris; Vcf – arteria caudalis femoris; Vsl – vena saphena lateralis
Figure 4: CT slice through the femoral joint – Ap – arteria poplitea; Vp – vena poplitea; Acf – arteria caudalis femoris; Vcf – arteria caudalis femoris; Avcf – arteria and caudalis femoris vena; Asu – arteriae surales; Agdl – arteria of the genus distalis lateralis; Vgdl – the genus distalis lateralis; Agdm – arteria of the genus distalis medialis; Vgdm – vena genus distalis medialis; Agm – arteria genus media; Vgm – vena genus media; Vsm – vena saphena medialis; As – arteria saphena; Vsl – vena saphena lateralis

Figure 5: CT slice through the medial head of the tibia – Ap – arteria poplitea; Vp – vena poplitea; Acf – arteria caudalis femoris; Vcf – arteria caudalis femoris; Vsl – vena saphena lateralis

The CT examined knee joints of sheep are absent (Figure 6, 7) and with contrast (Fig 8, 9), after induced OA and after treatment with PRP in their right knee. When measuring the density of femoral and tibial lumps by HU and comparison between the left and right legs (Figures 6, 7, 8, 9), the following results were obtained (Table 1).

Table 1: The density of femoral and tibial lumps

<table>
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<th>Without contrast HU</th>
<th>With contrast HU</th>
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<tr>
<td>Left medial condyle of the femur</td>
<td>-205 to 1059</td>
<td>91 to 1500</td>
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<tr>
<td>Left medial condyle of the tibia</td>
<td>-2 to 710</td>
<td>62 to 1214</td>
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<tr>
<td>Right medial condyle of the femur</td>
<td>-22 to 813</td>
<td>240 to 1033</td>
</tr>
<tr>
<td>Right medial condyle of the tibia</td>
<td>-11 to 818</td>
<td>163 to 954</td>
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Reduced density of the medial femoral condyle and medial condyle of the right leg tibia are observed, compared to those on the left on CT scan both with contrast and without contrast (Fig. 6, 7, 8, 9). This reduced density may be due to the sustained angiogenesis process in the PRP-treated right knee of the sheep. The medial condyles of the left femur and tibia are of higher density due to the increased angiogenesis which is evidence of a more advanced OA.

Figure 6: CT without contrast of the condyles of the left and right femurs; the arrows identify the areas of the condyles where HU has been measured

Figure 7: CT slice without contrast of the condyles of the left and right tibia; the arrows identify the areas of the condyles where HU have been measured
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Figure 8: CT slice with contrast of the condyles of the left and right femur; the arrows identify the areas of the condyles where HU have been measured

Figure 9: CT slice with contrast of the condyles of the left and right tibia; the arrows identify the areas of the condyles where HU have been measured

Discussion

CT arthrography is recommended as a reliable and accurate imaging diagnostic method for diagnosing various joint disorders (4).

Vandeweerd J.-M. et al., (2013) investigated with CT anatomical location and severity of cartilage defects in the stifle joint within a population of adult ewes (5).
CT did not identify any signs of subchondral bone (SB) sclerosis or osteolysis. Osteophytes were found in only two animals. CT identified SB sclerosis only in one 11-year-old sheep. Unlike Vandeweerden J.-M. et al., (2013), we used contrast angiography to track the development of induced OA and the therapeutic effect of PRP. We demonstrated that the joints not treated with PRP, were found to have a more developed angiogenesis, an indication of developing an arthrosis process.

OA has long been recognized as a "wear and tear" disease; it is now considered a much more complex disease of low grade inflammation induced by inflammatory mediators released by cartilage, bone, and synovium (6).

Angiogenesis contributes to synovial inflammation and promotes the destruction of cartilage and bone (7, 8). Furthermore, increased vessels and accompanying sensory nerves into the osteochondral, synovium, and meniscus are a cause of chronic pain in the OA (9, 10).

**Conclusion**

- Increased angiogenesis in the left limb is indicative of development of an arthrosis process.
- Angiogenesis in the right leg is the same as in healthy animals, indicating the therapeutic effect of the applied PRP.

**References**