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Changes in the Haematobiochemical Parameters in Experimental Stifle Osteoarthritis in Dogs

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Abstract: This study investigated the changes in the haematological and haematochemical parameters in osteoarthritis secondary to experimental Medial Patellar Luxation (MPL) along with single grove damage to the femoral condyles in the dog. MPL was surgically produced in the left stifle joint in 20 skeletally mature mixed small breed dogs (age 2 to 6 years, weight 2.8 to 9 kg) by placing purse string sutures around the parapatellar fibrocartilage and anchoring the patella with the fabellar ligament and by medial imbrication and lateral release. Physical and radiographic examinations of the experimental stifles were performed at every 1.5 months intervals to evaluate the position of the patella and development of osteoarthritis. The blood samples were collected prior to and 3, 6 and 9 months after the experimental induction of MPL and were subjected to Complete Blood Count (CBC) and Chemistry Screening (CS). In the CBC parameters, a significant difference in the platelet count (PLT) and in the CS parameters, a significant difference in the values of Alkaline Phosphatase (AP) were observed. The changes in the CBC and CS parameters can be impressive but cannot be the sole determinants of osteoarthritis in dogs.

Key words: Complete blood count, chemistry screening, osteoarthritis, medial patellar luxation, dog

INTRODUCTION

Osteoarthritis (OA) is a slow progressive disorder of synovial joints that affects about 20% of the canine population over 1 year of age. This joint disorder is characterized by a loss of balance between synthesis and degeneration of the articular cartilage constituents leading to subsequent erosion of joint cartilage, remodeling of the underlying bone, osteophyte formation and variable degrees of synovitis (Johnston, 1997; Hegemann *et al*., 2002). When clinical characteristics of OA (e.g., pain, loss of mobility and radiographic narrowing of the joint space) manifest, the actual changes in articular cartilage and subchondral bone have started long ago (Marijnissen *et al*., 2002; Matyas *et al*., 2004). To study early stages of OA *in vivo*, several animal models have been developed (Pond and Nuki, 1973; Troyer, 1982; Warskyj and Hukins, 1990; Lefkoe *et al*., 1993; Marijnissen *et al*., 2002). In our model, permanent instability in the stifle joint was induced by surgically made Medial Patellar Luxation (MPL) with single groove damage to the femoral condyles, which was followed by degenerative changes in cartilage and changes in synovial tissue that over the course of several months lead to canine OA, which resembles human clinical OA. Early diagnosis of OA is a major problem both in veterinary and human medicine because the diagnosis is routinely established on the basis of the clinical and radiographic changes that occur only in the later stages of the disease. The insidious onset and silent progression of OA not only obscure an early diagnosis, but also delay treatment that may help prevent further cartilage destruction and joint failure (Matyas *et al*., 2004). Hence, determinants/markers that can detect and monitor molecular events early in the pathogenesis of OA are of considerable interest as an expedient to distinguish preclinical diseases, to predict prognosis and to monitor the response to drug and therapy (Hegemann *et al*., 2002).

The complete blood count (CBC) and chemistry screening (CS) are the most commonly performed tests in health care because of the vast amount of data obtained through various components of these tests. The CBC and CS findings provide valuable diagnostic information about the haematobiochemical and other body systems, prognosis, response to treatment and recovery. The CBC consists of a series of tests that determine the number, variety, percentage, concentrations and quality of blood cells; whereas the CS provides information about the functional status of several organs/systems of the body. However, to the best of our knowledge till now there is

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paucity of literatures pertaining to the CBC and CS parameters in osteoarthritis. The purpose of this study is to investigate whether some of the CBC and/or CS parameters can be the determinant of early phages of canine osteoarthritis or not.

MATERIALS AND METHODS

Animals: This study was conducted in the Department of Surgery, College of Veterinary Medicine, Chonbuk National University, Republic of Korea during August, 2005 to May, 2006. Twenty skeletally mature healthy mixed small breed dogs of both sexes, age 2 to 6 years, weighing 2.8 to 9 kg were used in this experiment. They were free from infectious diseases, blood parasites and protozoa. Before engaging in the experiment they were kept in quarantine for 1 month, acclimated in cage confinement and treated with ivermectin (Ivomec®, Merck and Co., Inc., USA) 0.2 mg kg^{-1} SC for ecto - and endoparasites. They were housed in individual cages in the departmental animal shed. They were fed a standard commercial diet (Precept Adult®, Precept Co., USA) and had water *ad libitum*. The dogs were let out for exercise for 1 h daily.

Anaesthesia, surgery and postoperative care: After premedicated with atropine sulphate (Atropine Sulfate Inj®, Dai Han Pharm. Co. Ltd., Korea) 0.05 mg kg^{-1} SC, the anaesthesia was induced using thiopentone sodium (Thionyl Inj®, Dai Han Pharm. Co. Ltd., Korea) 25 mg kg^{-1} IV and maintained with enflurane and oxygen delivered through a cuffed endotracheal tube. Cephalexin (Methilexin Inj®, Union Korea Pharm. Co. Ltd., Korea) 25 mg kg^{-1} IV was administered at the time of induction.

In all the animals, left stifle arthrotomy was performed using a standard parapatellar approach. The incision was made through the fascia lata just lateral to the patellar ligament. Care was taken to prevent bleeding and soft tissue damage as much as possible. The joint was thoroughly explored to observe the condition of the cruciate ligaments and menisci. A single damage to the weight bearing area on the medial and lateral condyles were done using the sharp end of a Kirschner wire. MPL was produced by placing purse string sutures around the parapatellar fibrocartilage and anchoring the patella with the fabellar ligament using monofilament non-absorbable sterile nylon suture. Medial retinacular reinforcement (imbrication) and lateral release were also performed to interfere the neutral tracking of the patella. The wound was closed in a routinely manner.

The postoperative treatment was given with cephalexin (Methilexin Inj®, Union Korea Pharm. Co. Ltd., Korea) 25 mg kg^{-1} IV, every 8 h, for 5 days and dexamethasone (Dexamethasone Inj®, Daewon Pharm. Co. Ltd., Korea) 0.2 mg kg^{-1} IV, every 6 h for 3 days. The external stitches were removed after 1 week.

Physical and radiographic examinations: Postoperatively physical and radiographic examinations were performed at every one and half month intervals. The heart rate, respiration and temperature were recorded. The gait, posture, limb function and joint motion were observed. The postoperative radiographs were assessed to ensure a permanent luxation of the patella outside the trochlear groove and to evaluate the development of radiographic signs of osteoarthritis.

Collection and analysis of blood samples: The blood samples were collected prior to and after 3, 6 and 9 months of the experimental induction of MPL and were subjected to a Complete Blood Count (CBC) which includes Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC), Hemoglobin (Hb), Packed Cell Volume (PCV), Platelet Count (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red cell Distribution Width (RDW), Mean Platelet Volume (MPV) and Differential Leukocyte Count (DLC) and Chemistry Screening (CS) which includes Alkaline Phosphatase (AP), Gamma Glutamyl Transferase (GGT), Aspartate Transferase (AST), Alanine Transferase (ALT), amylase, Blood Urea Nitrogen (BUN), glucose, Phosphorus (P), Calcium (Ca), albumin, cholesterol, uric acid, Creatine Kinase (CK), creatinine, Total Bilirubin (TBL), Total Protein (TP), globulin, BUN/creatinine ratio and Albumin/Globulin (A/G) ratio.

Five milliliter of blood samples from each animal were collected using a 5 mL disposable syringe attached with a 22 gauge needle through jugular venipuncture; half of the samples were transferred to sterile screw-capped tubes containing EDTA 2K for CBC and the other halves were transferred to the tubes without anticoagulant, left undisturbed in the room temperature for coagulation of blood, centrifuged at 1500 g for 15 min, the supernatant serum was collected and transferred to Eppendorf tubes for CS.

The CBC was analyzed using an automatic hematology analyzer (Scil Vet abc®, Scil Animal Care Company, USA). Briefly, the EDTA-blood sample tubes were placed on a roller mixer and rolled at 33 rpm and 16 mm amplitude (rise and fall) for proper mixing. The sample identity was entered in the automatic hematology analyzer and once the sample needle was down it was put into the blood samples until it touched the bottom of the

tube and then the tube was slightly back off the needle. Once the start button was pressed, 20 μL of the blood sample was automatically taken in by the sample needle. The results were available for read by 90 sec on the LCD and were received as printed form as well. The hematology analyzer counts RBC, WBC and PLT by electric impendence and assays Hb by spectrophotometry.

For CS analysis 90 μL serum was drawn from the Eppendorf tubes using the automatic pipettor/dilutor. The serum was then dispensed into a test rotor (VET-16 Veterinary Test Rotor®, Hemagen Diagnostics, Inc., USA) and the rotor cap was snapped into place. The test rotor was loaded in the automatic chemistry analyzer (Hemagen Analyst®, Hemagen Diagnostics, Inc., USA) and a complete result was received in a printed form in about 10 min.

Statistical analysis: The data obtained in the present study were analyzed using ANOVA and student's t-test and $p<0.05$ or less was considered as statistically significant. The data are presented as mean±Standard Deviation (SD).

RESULTS

Physical and radiographic examinations: The vital signs; heart rate, respiration and temperature were within the reference range throughout the experimental period (data not presented). Evaluation of the postoperative radiographs did not show any remarkable changes up to 6 months but revealed a permanent medial luxation of the patella. However, radiographs obtained on 9 months postoperatively revealed the clear evidences of osteoarthritis which included osteophytosis, soft tissue

Table 1: Changes in the complete blood count (CBC) values in experimental osteoarthritis in dogs

Parameters		Control (Mean±SD)	3 months (Mean±SD)	6 months (Mean±SD)	9 months (Mean±SD)
TLC (10^3 mm^{-3})		10.70±2.42	10.32±2.33	10.00±3.09	10.08±1.36
TEC (10^6 mm^{-3})		7.27±1.05	8.36±0.56*	7.95±0.47	7.93±1.04
Hb (g dL^{-1})		15.18±2.28	17.24±0.62*	17.20±0.99*	17.02±1.89
PCV (%)		46.64±6.99	53.20±3.11*	53.02±3.83*	50.70±5.82
PLT (10^3 mm^{-3})		399.38±103.31	587.80±229.21*	515.20±170.02	505.40±101.50*
MCV (μm^3)		64.38±2.77	63.78±5.21	67.20±2.49	64.40±4.22
MCH (pg)		20.88±0.90	20.64±0.67	21.78±0.75	21.54±1.54
MCHC (g dL^{-1})		36.69±12.03	32.46±1.78	33.62±1.69	32.46±0.45
RDW (%)		13.04±1.38	13.64±0.91	14.36±0.32	13.12±1.77
MPV (μm^3)		9.50±0.70	8.88±0.84	8.88±0.84	9.84±0.87
DLC	L (10^3 mm^{-3})	1.39±0.43	1.72±0.99	1.72±0.99	1.74±1.09
	M (10^3 mm^{-3})	0.59±0.20	0.48±0.20	0.48±0.20	0.46±0.18
	Gr (10^3 mm^{-3})	8.30±2.30	7.80±2.11	7.80±2.11	7.88±1.33

* Statistically significant $p<0.05$ or less, SD-Standard deviation, TLC- Total Leukocyte Count, TEC- Total Erythrocyte Count, Hb-Hemoglobin, PCV-Packed Cell Volume, PLT- Platelet count, MCV- Mean Corpuscular Volume, MCH- Mean Corpuscular Hemoglobin, MCHC- Mean Corpuscular Hemoglobin Concentration, RDW- Red Cell Distribution Width, MPV- Mean Platelet Volume, DLC- Differential Leukocyte Count, L- Lymphocyte, M- Monocyte and Gr-Granulocyte (Neutrophil, Eosinophil, Basophil)

Table 2: Changes in the blood chemistry screening (CS) values in experimental osteoarthritis in dogs

Parameters	Control (Mean±SD)	3 months (Mean±SD)	6 months (Mean±SD)	9 months (Mean±SD)
AP (U L^{-1})	53.80±6.57	53.00±24.54	63.20±49.29*	97.75±32.57*
GGT (U L^{-1})	5.38±2.45	4.60±1.52	3.60±2.19	7.00±1.22
AST (U L^{-1})	28.38±9.21	14.20±4.92	14.00±5.66	25.4±16.41
ALT (U L^{-1})	29.00±10.47	38.80±33.16	42.00±38.83	28.5±8.50
Amylase (U L^{-1})	678.25±148.77	671.00±97.01	628.8±131.95	633.8±187.55
BUN (mg dL^{-1})	21.48±7.27	21.02±5.25	21.04±4.55	18.2±5.77
Glucose (mg dL^{-1})	118.13±19.47	123.60±14.57	124.00±17.20	144.6±31.60
P (mg dL^{-1})	5.76±0.93	5.32±1.15	5.22±1.31	5.54±1.20
Ca (mg dL^{-1})	12.09±0.71	12.06±1.59	11.92±1.31	12.26±1.09
Albumin (g dL^{-1})	3.31±0.23	3.48±0.29	3.22±0.22	3.52±0.22
Cholesterol (mg/dL^{-1})	209.00±36.21	241.40±32.97	240.4±34.63	275.00±82.41
Uric acid (mg dL^{-1})	1.12±0.58	1.60±0.80	1.5±0.84	2.50±1.12
CK (U L^{-1})	169.5±49.40	102.00±37.48	101.2±40.73	210.40±454.23
Creatinine (mg dL^{-1})	0.23±0.05	0.21±0.12	0.26±0.06	0.38±0.08
TBL (mg dL^{-1})	0.11±0.05	0.16±0.10	0.15±0.08	0.27±0.29
TP g (dL^{-1})	6.34±0.43	6.56±0.83	6.62±0.86	6.78±0.19
Globulin (g dL^{-1})	3.03±0.30	3.38±0.58	3.42±0.75	3.25±0.25
BUN/Creatinine	94.23±48.65	90.23±54.86	84.23±38.55	48.84±14.71
A/G	1.11±0.14	1.04±0.27	0.98±0.24	1.08±0.13

* Statistically significant $p<0.05$ or less, SD-Standard deviation, AP-Alkaline phosphatase, GGT-Gamma Glutamyl Transferase, AST- Aspartate transferase, ALT-Alanine transferase, BUN- Blood Urea Nitrogen, P- Phosphorus, Ca- Calcium, CK- Creatine Kinase, TBL- Total bilirubin, TP- Total Protein, A/G-Albumin/Globulin ratio

thickening, narrowing of the joint spaces and subchondral sclerosis (Fig. 1). The physical examination of the animals 12 weeks postoperatively revealed stiffness of gait, lameness, decreased range of motion and palpable crepitus along with some degrees of joint swelling and pain. The animals were found reluctant to move and use the operated limbs (left).

Changes in the complete blood count (CBC) values: No significant changes were observed in the values of the TLC, MCV, MCH, MCHC, MPV and RDW throughout the experimental period. The preoperative control values of TEC significantly increased ($p<0.05$) on 3 moths, but no significant differences were observed when compared with those obtained on 6 and 9 months. A significant increase ($p<0.05$) in the control values of Hb were noticed when compared with those obtained on 3 and 6 moths. A slight increase in the PCV values during the experiment was observed, but this was not statistically significant. The preoperative control values of PLT were found to increase significantly ($p<0.05$) in comparison to the values obtained on 3, 6 and 9 months. There were no significant changes observed in the DLC, however a slight increase (does not exceed the normal range) in the preoperative control values of lymphocyte count was noticed during the experimental period. The changes in the CBC values during different experimental periods are shown in the Table 1.

Changes in the blood chemistry screening (CS) values: The control values of the AP were increased during the experimental period and this increase in the values was statistically significant ($p<0.05$) when compared with the values obtained on 6 and 9 months. No statistically significant changes were observed in the values of GGT, AST, ALT, amylase, BUN, glucose, P, Ca, albumin, cholesterol, uric acid, CK, creatinine, TBL, TP, Globulin, BUN/creatinine ratio and albumin/globulin ratio throughout the experimental period. The changes in the CS values during different experimental periods are presented in the Table 2.

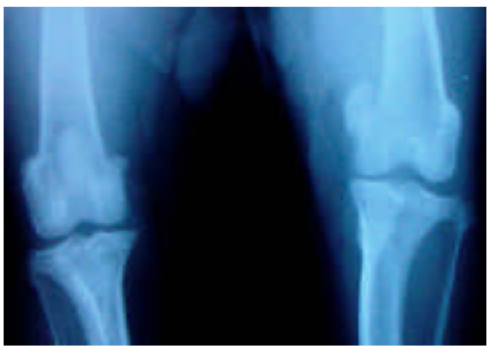

Fig. 1: Craniocaudal radiograph of the stifle joints 9 months after the surgical induction of MPL. The right stifle revealed a normal state of the joint with the normal position of the patella, whereas the left stifle (experimental) revealed medially luxated patella along with the radiographic changes of osteoarthritis; osteophytosis, soft tissue thickening, narrowing of the joint spaces and subchondral sclerosis

DISCUSSION

Osteoarthritis is a slowly progressive disease; the initiating events of its pathogenesis are obscure (Johnston, 1997). The characteristic clinical signs of osteoarthritis; joint pain, limitation of movement, effusion and variable degrees of local inflammation were noticed by 12 weeks of the surgical induction of MPL with single groove damage to the femoral condyles. These finding are in agreement with the previous reports on the animal models of osteoarthritis (Pond and Nuki, 1973; Troyer, 1982; Warskyj and Hukins, 1990; Lefkoe *et al*., 1993; Marijnissen *et al*., 2002). The early stages of osteoarthritis (OA) are difficult to diagnose on radiography. Radiographic changes include joint capsular distension, osteophytosis, soft tissue thickening, narrowing of the joint spaces and subchondral sclerosis which occur only at the later stages of OA (Marijnissen *et al*., 2002; Matyas *et al*., 2004). In this study, we also did not notice any remarkable radiographic changes in the early stages of the disease. However, the radiographic changes were evidenced after 9 months of the surgical induction of MPL. These findings are in agreement with the reported literatures (Marijnissen *et al*., 2002; Matyas *et al*., 2004).

The changes in the CBC values observed in our study are almost similar to those reported in degenerative joint disease in cattle (Van Pelt, 1975). The increase in the preoperative control values of TEC on 3 months postoperatively may be due to better care and management of the experimental animals rather than being a determinant of osteoarthritis. The increase in the control values of Hb and PCV are due to the increase in the TEC values. Though there was a slight increase in the preoperative control values of lymphocyte count during the experiment, it did not exceed the normal range. Van Pelt (1975) also reported an increase in lymphocyte count in arthitic cattle. Anyway, this increase in the lymphocyte count might be due to body defense against some other conditions irrelevant to osteoarthritis. The platelets are tiny cells produced by the bone marrow to help blood clotting in the event of a cut or scrape. A high number

might be seen in people with cancer, a blood disease, or rheumatoid arthritis. The increase in the PLT in this experiment is thought to be related with the progression of osteoarthritis but the mechanism is not clear.

The alkaline phosphatase is an enzyme found in many tissues, but the most important sites are the bone, liver, bile ducts and gut. AP is a nonspecific phosphomonoesterase that hydrolyze phosphate monoesters and is found attached to the plasma membranes where extensive transport takes place, indicating that AP is involved in fundamental biological processes (Kim and Wyckoff, 1990; Padmini *et al*., 2004). Since it is found localized in the plasma membrane of the osteoblastic cells, its role in bone mineralization is justified (Rodan and Rodan, 1983; Padmini *et al*., 2004). A high level of AP in the blood may indicate bone, liver or bile duct disease (Sodicoff, 1995). It has been reported in literature that diseases causing bone remodeling in adults cause elevations of AP (Sodicoff, 1995; Jacques *et al*., 2002). Osteoarthritic subchondral bone exhibits an altered metabolism when compared to normal bone based on the levels of proinflammatory mediators produced (Hilal *et al*., 1998; Lavigne *et al*., 2005). Modifications in AP and osteocalcin levels in OA subchondral bone have already been described implying that osteoblasts from this pathology have a higher metabolic profile (Lavigne *et al*., 2005). These cells, however, are laying down less mineralized matrix which results in an impaired functional quality of osteoarthritic bone compared to normal individuals (Dean, 1991; Li and Aspden, 1997; Lavigne *et al*., 2005). The increase in the values of AP in this study is thought to be due to the increased subchondral bone metabolism in osteoarthritis, correlating the observation to decreased bone mineralization and ossification and an increased bone resorption.

Blood is the body fluid that is routinely examined in clinical pathology. The routine blood and urine analyses can identify certain inflammatory arthritis and metabolic defects that cause joint pain and pathology but, as yet, reveal little about the OA process. Blood and urine measurement probably reflect more systemic metabolism. Thus blood and urine are likely to be more reflective of systemic changes unless the OA load is sufficient to be detectable and recognizable (Matyas *et al*., 2004). However, some of the CBC (e.g., platelet count) and CS parameters (e.g., alkaline phosphatase) can be impressive but cannot be the sole determinant for the diagnosis of early stages of osteoarthritis in the dog.

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REFERENCES

Dean, D.D., 1991. Proteinase-mediated cartilage degradation in osteoarthritis. Semin. Arthritis Rheum., 20: 2-11.

Hegemann, N., B. Kohn, L. Brunnberg and M.F. Schmidt, 2002. Biomarkers of joint tissue metabolism in canine osteoarthritic and arthritic joint disorders. Osteoarthr. Cartil., 10: 714-721.

Hilal, G., J. Martel-Pelletier, J.P. Pelletier, P. Ranger and D. Lajeunesse, 1998. Osteoblast-like cells from human subchondral osteoarthritic bone demonstrate an altered phenotype *in vitro*: Possible role in subchondral bone sclerosis. Arthritis Rheum., 41: 891-899.

Jacques, D., L. Cauzinille, B. Bouvy and G. Dupre, 2002. A retrospective study of 40 dogs with polyarthritis. Vet. Surg., 31: 428-434.

Johnston, S.A., 1997. Osteoarthritis: Joint anatomy, physiology and pathobiology. Vet. Clin. North Am. Small Anim. Pract., 27: 699-723.

Kim, E.K. and H.W.W. Yckoff, 1990. Structure and function of alkaline phosphatases. Clin. Chim. Acta, 186: 175-187.

Lavigne, P., M . Benderdour, D. Lajeunesse, P. Reboul, Q. Shi, J.P. Pelletier, J. Martel-Pelletier and J.C. Fernandes, 2005. Subchondral and trabecular bone metabolism regulation in canine experimental knee osteoarthritis. Osteoarthr. Cartil., 13: 310-317.

Lefkoe, T.P., P.G. Trafton, M.G. Ehrlich, W.R. Walsh, D.T. Dennehy and H.J. Barrach, 1993. An experimental model of femoral condylar defect leading to osteoarthrosis. J. Orthop. Trauma, 7: 458- 467.

Li, B. and R.M. Aspden, 1997. Mechanical and material properties of the subchondral bone plate from the femoral head of patients with osteoarthritis or osteoporosis. Ann. Rheum. Dis., 56: 147-154.

Marijnissen, A.C.A., P.M. Van Roermund, J.M. TeKoppele, J.W.J. Bijlsma and F.P.J.G. Lafeber, 2002. The canine groove model, compared with the ACLT model of osteoarthritis, Osteoarthr. Cartil., 10: 145-155.

Matyas, J.R., L. Atley, M. Ionescu, D.R. Eyre and A.R. Poole, 2004. Analysis of Cartilage Biomarkers in the Early Phases of Canine Experimental Osteoarthritis. Arthritis Rheum., 50: 543-552.

Padmini, A., B. Ushasree, B. Ravi and N. Pratibha, 2004. Association of alkaline phosphatase phenotypes with arthritides. Indian J. Hum. Genet., 10: 5-8.

Pond, M.J. and G. Nuki, 1973. Experimentally induced osteoarthritis in the dog. Ann. Rheum. Dis., 32: 387- 388.

Rodan, G.A. and S.B. Rodan, 1983. Expression of the Osteoblastic Phenotype. In: Bone and Minerals Research. Peck, W.A. (Ed.), Elsevier Science Publication Annual Z. Amsterdam, pp: 244-245.

Sodicoff, C.H., 1995. Laboratory Profiles of Small Animal Diseases, A Guide to Laboratory Dsiagnosis. 2nd edn., Mosby-Year Book, Inc., St Louis, pp: 3-20.

Troyer, H., 1982. Experimental models of osteoarthritis: A review. Semin. Arthritis Rheum., 11: 362-374.

Van Pelt, R.W., 1975. Tarsal degenerative joint disease in cattle: Blood and synovial fluid changes. Am. J. Vet. Res., 36: 1009-1014.

Warskyj, M. and D.W. Hukins, 1990. Animal models for osteoarthritis-ensuring experimental validity. Br. J. Rheumatol., 29: 219-221.