CLINICAL AND PATHOLOGICAL FEATURES OF OSTEOARTHRITIS OF THE HIP JOINT IN ADULT GERMAN SHEPHERD DOGS IN KENYA

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FACULTY OF VETERINARY MEDICINE

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DECLARATION

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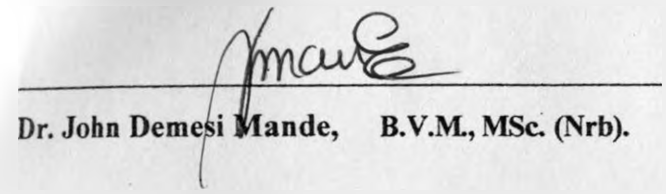

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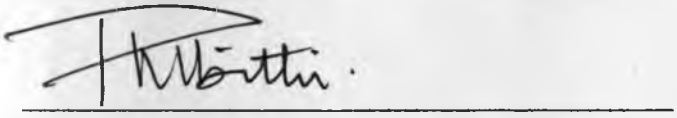

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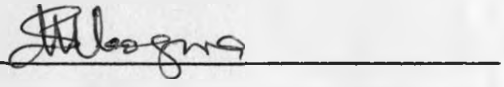

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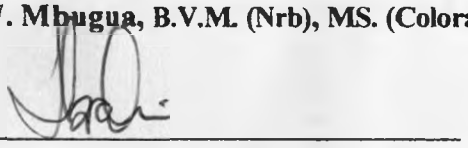

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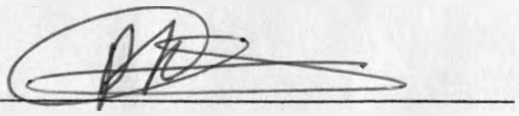

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DEDICATION

To my wife Beatrice Wangari Demesi and the family.

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TABLE OF CONTENTS

CHAPTER FOUR

CHAPTER FIVE

LIST OF TABLES

LIST OF FIGURES

APPENDICES

ABSTRACT

This study aimed at evaluating the clinical, radiographic, pathological, light and electron microscopic features of osteoarthritis of the hip joint in adult dogs in Kenya. Thirty-six adult German shepherd dogs were used [15 female (41.6 %) and 21 (58.3 %) male]. The mean weight of the animals was 27.3 kgs (range: 18.3 – 44.3 kgs). The mean age was 9.3 years (range: 5-17 years). History, visual inspection and clinical examination for lameness were used to classify the dogs as normal, with mild or severe osteoarthritis; assigned numbers 1, 2 and 3, respectively.

Dogs were classified as clinically normal when they exhibited normal conformation of the hindlimbs, normal gait and posture, good muscle cover of the hindlimbs and no clinical signs of hindlimb lameness. Dogs with mild lameness exhibited slight muscle atrophy, pain on flexion and extension of the hip joint, limited range of joint motion and mild lameness attributable to the hip joint. Dogs with severe hindlimb lameness had a history of prolonged lameness of the hindquarters, severe muscle atrophy and change in conformation of the hindlimbs, crepitus and pain on flexion and extension of the hip joints and decubital wounds on the paws of affected hindlimbs.

Standard pelvic ventrodorsal radiography was performed with the dogs under deep sedation or general anesthesia. The radiographs were evaluated based on subjective radiographic features of each joint and further classified into four broad categories. Grade 0 had C-shaped acetabulum, dorsal rim rounded and distinct femoral neck. Grade 1 had shallow acetabulum or marked dorsal rim attenuation, moderately osteophytic acetabular margin, rounded femoral head and minimal osteophytes on the femoral neck. Grade 2 had shallow acetabulum or marked dorsal rim attenuation, moderately osteophytic acetabular margin, flattened femoral head and shortened femoral neck with osteophytes. Grade 3 had flat acetabulum, severely osteophytic acetabular margin, markedly flattened or irregular femoral head and severely shortened femoral neck with osteophytes.

Thirty-two animals were euthanised by intravenous injection of pentobarbitone sodium (Euthatal[R] Rhone Meriux Ltd, Dublin). The muscles on the femur and pelvis were dissected and the femur disarticulated at the stifle joint. A band saw was used to cut the pubis, the ischium and the ilium to isolate the femoral head and acetabulum. The joint capsule was then incised, exposing the joint cavity. Sixty-four hip joints were evaluated for pathological changes.

The integrity of ligamentum capitis femoris was determined by visual inspection of each joint. The ligament was severed at its attachment to the fovea capitis and the acetabulum, and its volume (in milliliters) determined by a water displacement technique. The data was compared based on radiographic grades of osteoarthritis.

The color, relative thickness, texture and villous hypertrophy of synovial membrane were determined and recorded for each joint. Synovial membrane samples were collected, fixed in 10 % formalin solution and routinely processed. 5 μm thick sections were prepared and stained with hematoxylin and eosin and examined with a light microscope. Samples were classified according to previously described criteria.

Zero (0) score had normal tissue. Score one (1) had mild synovitis, which revealed focally thick synovium with plumper hypertrophied cells, sometimes producing a small-localized thickening (plaque) or villous extension. Score two (2) was defined as synovial proliferation which involved 50% to 75% of the surface examined. Villi were longer and more common, sometimes also lined by hyperplastic synovium. Capillary neovascularization and mild mononuclear cells infiltrates were sometimes observed in the adjacent collagenous stroma. Score three (3) had synovial proliferation involving the entire surface. Villous proliferation varied from numerous small structures to a mixture of small and large, stout villi. Lymphocyte infiltrates were heavier with focal aggregation in some cases. In score four (4), at times islands of cartilage from the eroded articular surface were embedded in the synovium.

Gross pathological changes on articular cartilage were used to classify the extent of hip osteoarthritis in the study. Slabs of cartilage from the femoral head and neck were cut using a band saw. The slabs were fixed in 10% formalin for 7-14 days and decalcified in 10 % nitric acid for 3-4 weeks. The decalcified slabs were trimmed and embedded in paraffin wax. Sections, 5 μm thick, were prepared and stained with either hematoxylin and eosin or Safranin-O-Fast Green. Twenty-two representative samples of articular cartilage sections were examined with a light microscope and graded according to previously described criteria.

Articular cartilage and synovial membrane samples collected from 4 dogs (8 joints) were immersed in 3 % glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) and fixed for 4 hours at 4 ° C. After the material was washed with buffer, it was postfixed in 1 % osmium tetroxide for one hour at 4 ° C and then soaked for one hour in 0.5 % uranyl acetate. The samples were dehydrated in a graded series of ethanol and embedded in plastic. Semi-thin sections were stained in toluidine blue and examined with a light microscope for orientation of special areas of interest to be obtained for ultrastructural study. Gray to silver thin sections were picked up on copper-coated 200 mesh grids and stained in 2 % uranyl acetate and lead citrate. Electron micrographs were taken with transmission electron microscope (JEOL 1010, GMBH, Germany). The morphology of chondrocytes and synoviocytes was qualitatively evaluated to outline their response in chronic synovitis and osteoarthritis.

Fourteen (38.9 %) dogs were clinically normal, while 22 (61.1 %) had either mild or severe hindlimb lameness attributable to the hip joint. Of the 22 animals with hindlimb lameness, six (16.7 %) had mild, while 16 (44.4 %) had severe and debilitating lameness that required euthanasia.

Thirty-seven (51.4 %) hip joints had normal radiographic features and were assigned grade 0. On the other hand, 11 (15.3 %) hip joints were graded 1, 8 (11.1 %) hip joints were graded 2, while 16 (22.2 %) hip joints were graded 3.

Ligamentum capitis femoris was intact in 46 (71.9 %) joints but was missing in 18 (28.1 %) of the 64 hip joints. The mean volume of ligamentum capitis femoris for grade 0 hip joints was 0.82 ± 0.3462 mls, while the mean for grade 1 hip joints was 0.65 ± 0.2544 mls. The mean volume of ligamentum capitis femoris for grade 2 hip joints was 0.31 ± 0.5551 mls, while all the hip joints with radiographic grade 3 had no intact ligamentum capitis femoris, with mean volume of 0 mls. There were significant differences ($p < 0.05$) between the mean volume of ligamentum capitis femoris among the four radiographic grades of osteoarthritis. There was an inverse correlation ($r = -0.75$) between the mean volume of ligamentum capitis femoris and the radiographic grades of osteoarthritis of the hip joints. The mean volume of ligamentum capitis femoris decreased (from 0.82 mls in normal hip joints) with increasing severity of radiographic grade of osteoarthritis (to 0 mls in severe osteoarthritis).

Synovial membrane from 27 normal hip joints (48.2 %) had normal size, color and no gross pathological changes. In contrast, synovial membrane from 29 osteoarthritic hip joints (51.8 %) had either synovial hyperplasia, gross thickening, discoloration and irregular shape and cartilaginous or bone transformation. From the severely inflamed samples, osteochondromatosis (joint mouse) was observed in 12 hip joints (21.4 %) either as free floating within the joint cavity or embedded within the synovial membrane. Nine hip joints had white colored, firm masses (the largest was 1.5 x 1.0 cm; the smaller masses were approximately 0.1 x 0.2 cm in diameter) within the joint cavity. Cartilage and bone tissue was demonstrated by light microscopic examination of a representative sample.

Twenty-two (34.4 %) of the 64 hip joints had normal anatomical appearance, while 11 (17.2 %) had gross pathological signs of mild osteoarthritis. Seven (10.9 %) hip joints had gross pathological signs of moderate osteoarthritis, while 24 (37.5 %) hip joints had gross pathological signs of severe osteoarthritis.

Electron microscopy of synovial tissue revealed extensive fibrosis and preponderance for electron dense deposits indicative of calcification, synoviocyte metaplasia, capillary vascularization and various stages of cell degeneration. Chondrocyte proliferation, degeneration, and eventual death were encountered in articular cartilage tissue. These observations were noted in clinically and radigraphically normal animals as well as in those with severe osteoarthritis. These changes could be related to age and severe synovitis associated with osteoarthritis.

This study has documented the clinical, radiographic and pathological features of naturally occurring hip dysplasia and osteoarthritis in adult dogs. The data is useful in predicting the likelihood of dogs to develop hip osteoarthritis and to determine the clinical severity of the disease. Clinical examination and ventro-dorsal pelvic radiography are none-invasive diagnostic aids for the determination of the existence and severity of this condition. Thus, the data is useful in judicial management and control of canine hip dysplasia and osteoarthritis. Further, the data could be used for future studies to determine the prevalence of hip osteoarthritis and the impact of current measures to control the incidence of canine hip dysplasia in Kenya.

CHAPTER ONE

1.0 INTRODUCTION AND OBJECTIVES

1.1 INTRODUCTION

Osteoarthritis is an important orthopedic disease in dogs. It is characterized by loss of articular cartilage, changes in subchondral bone architecture, capsulitis and synovitis (Olee, et al., 1999, Innes, et al., 2000a). The disease occurs most frequently in the large weight bearing joints of medium to large-sized dogs, but may affect any synovial joint. The best example of canine osteoarthritis is that occurring secondary to canine hip dysplasia [CHD] (Allan, 1998).

Joint laxity, trauma, age, heritability and genetic factors may modify the onset and progression of osteoarthritis and subsequent enzymatic degradation of cartilage and synovium. Non-genetic factors, including body size, growth rate, nutrition, dietary anion gap, in-utero endocrine influences and muscle mass are also involved. Despite these factors, the actual cause of canine hip dysplasia and osteoarthritis remains unknown (Lust, 1997). Weight and joint laxity were significant risk factors for 4 large breed dogs; German shepherd dogs, Golden Retrievers, Labrador Retrievers and Rottweilers. The risk of having osteoarthritis was 5 times for German shepherd dogs than the risk of the other 3 breeds combined (Smith, et al., 2001). Mayhew, et al. (2002) confirmed a contemporaneous association between a radiographic caudolateral curvilinear osteophyte on the femoral neck and osteoarthritis.

History and clinical examination facilitate tentative diagnosis of osteoarthritis. Although hip palpation techniques provide semiquantitative information on joint laxity and the likelihood of canine hip dysplasia, they have no clear and consistently reliable diagnostic or prognostic value in dogs (Adams, et al., 2000).

Due to difficulties in quantitation and standardisation, radiographic observations of osteoarthritis in canine hip dysplasia are almost invariably qualitative (Allan, 1998). Qualitative hip scores determined from the evaluation of standard ventrodorsal pelvic radiographs are commonly used. The subjective hip scores used by the Orthopaedic Foundation for Animals (OFA) is an example. However, quantitative measures of passive hip laxity such as Norberg Angle (NA) and Distractive Index (DI) derived from newer stress-radiographic diagnostic methods are used more commonly (Smith, 1997, Smith, et al., 1998). Standing radiography (Farese, et al., 1998), dynamic ultrasonography (Adams, et al., 2000) and computed tomography (Farese, et al., 1998) have been described in the diagnosis of CHD. Because CHD is polygenic, genetic mapping remains a challenge (Lust, et al., 2001b). Meomartino, et al. (2002) reported on morphometric assessment of the canine hip joint using the dorsal acetabular rim view and the centre-edge angle. Despite advances in diagnosis, the control and management of CHD continues to be a challenge for dog breeders and veterinarians worldwide (Kealy, et al., 1993, Smith, et al., 1995, Madsen, 1997). Furthermore, the necessary technical skills and costs of equipment make these advanced diagnostic tools inaccessible to clinicians in Kenya and East Africa. This has limited the capacity for accurate and timely diagnosis of osteoarthritis in small animals.

A semiquantitative radiographic method of postoperative evaluation of traumatic hip dislocation was described in young dogs (Evers, et al., 1997). Rasmussen, et al. (1998) used radiographic scoring criteria to evaluate triple pelvic osteotomy in CHD. There is paucity of information on the use of similar criteria in adult dogs.

Brinker, et al. (1990) reviewed the etiology, diagnosis and management of canine hip dysplasia. Early asymptomatic osteoarthritis in young Labrador Retrievers bred to produce canine hip dysplasia was described in Europe and America (Griesen, et al., 1982). Information on clinical signs, pathology, histology and electron microscopy of canine hip dysplasia and osteoarthritis in adults dogs, therefore, remains scanty.

The genetic and environmental factors causing canine hip dysplasia and osteoarthritis in Kenya are not necessarily similar (breeding protocols, nutrition and occupation of dogs) to those in Europe and America. No scientific information is available on canine hip dysplasia and osteoarthritis in adult dogs in Kenya. It is, therefore, necessary to determine the existence and severity of the clinical, radiographic, gross pathological and light microscopic changes attributable to osteoarthritis of hip joints in adult dogs in Kenya. The information would form a basis for a wider application of these parameters in the determination of the full extent of the disease in Kenya. The knowledge could be used by clinicians and breeders to advocate for development and adoption of diagnostic and breeding control measures, and contribute to improving the quality of life for adult dogs, by promoting good hip quality.

Light and electron microscopic studies would provide a deeper understanding of the pathophysiology of osteoarthritis and the biological basis of the healing of the synovial membrane and articular cartilage in canine hip dysplasia and osteoarthritis in adult dogs. This will facilitate rational and judicial decisions on prognosis and management of this debilitating condition in dogs in Kenya.

1.2 OBJECTIVES

The main objective of this study was to:

1. Evaluate the nature and severity of the clinical and radiographic features of osteoarthritis of the hip joint in adult German shepherd dogs in Kenya.

The minor objectives of this study are to

1. Evaluate the gross pathological changes in the synovial membrane and articular cartilage in osteoarthritis of the hip joint in adult German shepherd dogs in Kenya.

2. Evaluate the histological and histochemical changes in the synovial membrane and articular cartilage in osteoarthritis of the hip joint in adult German shepherd dogs in Kenya.

3. Evaluate electron microscopic changes in the synovial membrane and articular cartilage in osteoarthritis of the hip joint in adult German shepherd dogs in Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Background

The musculoskeletal system plays an important role in locomotion of animals. A dysfunctional musculoskeletal system often manifests clinically as lameness, pain and disability. One of the causes of lameness in small animals is osteoarthritis, an important orthopedic condition in man and animals (Bardet, 1995). Hip dysplasia is the most common cause of osteoarthritis in dogs, affecting mainly the large breed dogs (Allan, 1998).

Osteoarthritis was described in human beings over 3,000 years ago. The first description of canine hip dysplasia was in the early 1930. Since then, the history, definition, etiology, pathophysiology and pathology of hip dysplasia and osteoarthritis have been described in dogs (Morgan, 1997, Lust, 1997). Conservative, medical and surgical procedures for management and control of canine hip dysplasia and osteoarthritis are the subject of ongoing debate and research (Brinker, et al., 1990, Johnston and Fox, 1997). The most common research questions relate to the etiopathogenesis and early accurate diagnosis of the condition for judicial decisions on breeding and management of clinical cases. In each region, variations in environmental, genetic, nutritional and occupational factors influence the clinical expression of the disease. An area focussed study on the clinical diagnosis and pathology of the disease is needed to aid in accurate diagnosis and management of the disease in a particular region.

2.1.1 Current status and challenges of osteoarthritis

Osteoarthritis of coxofemoral joints in dogs remains a major health problem for dog breeders, veterinarians and researchers. Degenerative changes develop in

joints of many aging dogs; however, the disease is often evident well before the geriatric period. Joint laxity is the most common risk factor for osteoarthritis in the coxofemoral joints (Smith, et al., 1995). The condition is most commonly associated with hip dysplasia and less frequently with other causes such as trauma or metabolic effects (Lust, 1997). Genetic predisposition and environmental factors are involved in the initiation of osteoarthritis. One theory pertaining to the pathogenesis of osteoarthritis is that excessive body weight leads to mechanical stress on the joints, thus promoting their degeneration. Excessive body weight has been documented as a risk factor for osteoarthritis development in human beings, guinea pigs, mice and dogs (Kealy, et al., 1997). The mechanical stress leads to the release of enzymes from cells in articular cartilage and synovial membrane. These enzymes digest the proteoglycan matrix of cartilage, leading to loss of mechanical integrity and subsequent pathology.

Medical and surgical therapies have limited effect in stopping or reversing these biochemical and mechanical alterations (Brinker, et al., 1990). Chondroprotective agents (e.g. polysulfated glycosaminoglycans and hyaluronic acid) are used in animals and humans in Europe and America (Huber, 1994). However, they are neither available nor affordable to clients in Kenya. Nonsteroidal anti-inflammatory drugs may alleviate the effect of osteoarthritis but they have toxic side effects in dogs (Johnston and Fox, 1997).

Veterinary clinicians, kennel clubs and dog breeders have developed measures for control of hip dysplasia, in all parts of the world. However, these efforts have been ineffective in reducing the incidence of this condition. No scientific reports are available on clinical, radiographic and pathological features of hip osteoarthritis in adult dogs in Kenya. If available, such information would aid in accurate diagnosis of canine hip dysplasia. Further, the information would support a more accurate prediction of the likelihood of dogs developing hip dysplasia and osteoarthritis. In addition, the information would assist in determining the

prognosis for medical and surgical therapy of osteoarthritis and in assessment of the impact of hip dysplasia control programs in dogs in Kenya.

2.1.2.0 Joints

2.1.2.1 Definition

A joint is formed where two or more bones are united by fibrous, cartilaginous or elastic tissue.

2.1.2.2 Classification of joints

Joints are classified on the basis of the type of motion that they allow. Three main groups are recognized: fibrous joints (immobile), cartilaginous joints (partially mobile) and synovial joints (freely mobile). Table 1 illustrates some of the joints in animals (Bennet and May, 1995).

2.2.0 Anatomy of synovial joints

Synovial joints are characterized by a wide range of low friction motion and the possession of a joint cavity, articular cartilage, synovial fluid, intra-articular and extra-articular ligaments and a joint capsule. The ends of the articulating bony surfaces are covered with articular cartilage, beneath which lies a thin plate of dense bone, known as the subchondral bone. The subchondral bone overlies a region of cancellous bone, interspersed with bone marrow. Arterial and venous trunks in the region of synovial joints provide blood supply to the capsule and the epiphyses of neighboring bones. The nerve supply to synovial joints includes proprioceptive fibers, pain fibers and sympathetic fibers with vasomotor function. They are derived from peripheral or muscular nerve trunks in the locality of the joint (Bennet, 1994). A schematic representation of the structures comprising a diarthrodial joint is illustrated in Figure 1A.

Table 1. Classification of joints.

MOBILITY	CLASS OF JOINT	SUBCLASS OF JOINT	EXAMPLES	NOTES
Freely mobile	**Diarthrosis** (synovial joints)	**Enarthrosis** (ball and socket)	Hip, shoulder	Allow flexion, extension, abduction, adduction, and rotation.
		Ellipsoidal	Radial-carpal	Similar to enarthrosis, but joint surfaces are ellipsoidal rather than spherical.
		Sellar (saddle-shaped)	Interphalangeal	Opposing surfaces are convex in one pane and concave in another, usually perpendicular plane.
		Condylarthrosis (condylar)	Stifle	Rounded prominences of one joint surface fit into depressions in the adjacent surface; often function as hinge joint.
		Ginglymus (hinge)	Elbow	Allow flexion, extension, and limited rotation.
		Trochoid (pivot)	Atlantoaxial	Primarily allow rotation.
		Arthrodial (plane)	Intercarpal, intertarsal	Flat-surfaced joints; allow slight degree of gliding.
Partially mobile	**Amphiarthrosis** (cartilaginous joints)	**Synchrondrosis**	Intervertebral discs, sternabrae, pelvic symphysis, mandibular symphysis.	Union by fibrocartilage and /or hyaline
Immobile	**Synarthrosis** (fibrous joint)	**Suture**	Suturae cranii	Fibrous joint with small amounts of intervening tissue; can be further classified by the shape of the apposing edges.
		Syndesmosis	Hyoid-petrous temporal bone	Fibrous joint with considerable intervening tissue.
		Gomphosis		Fibrous union between tooth and bone.

(Bennet and May, 1995).

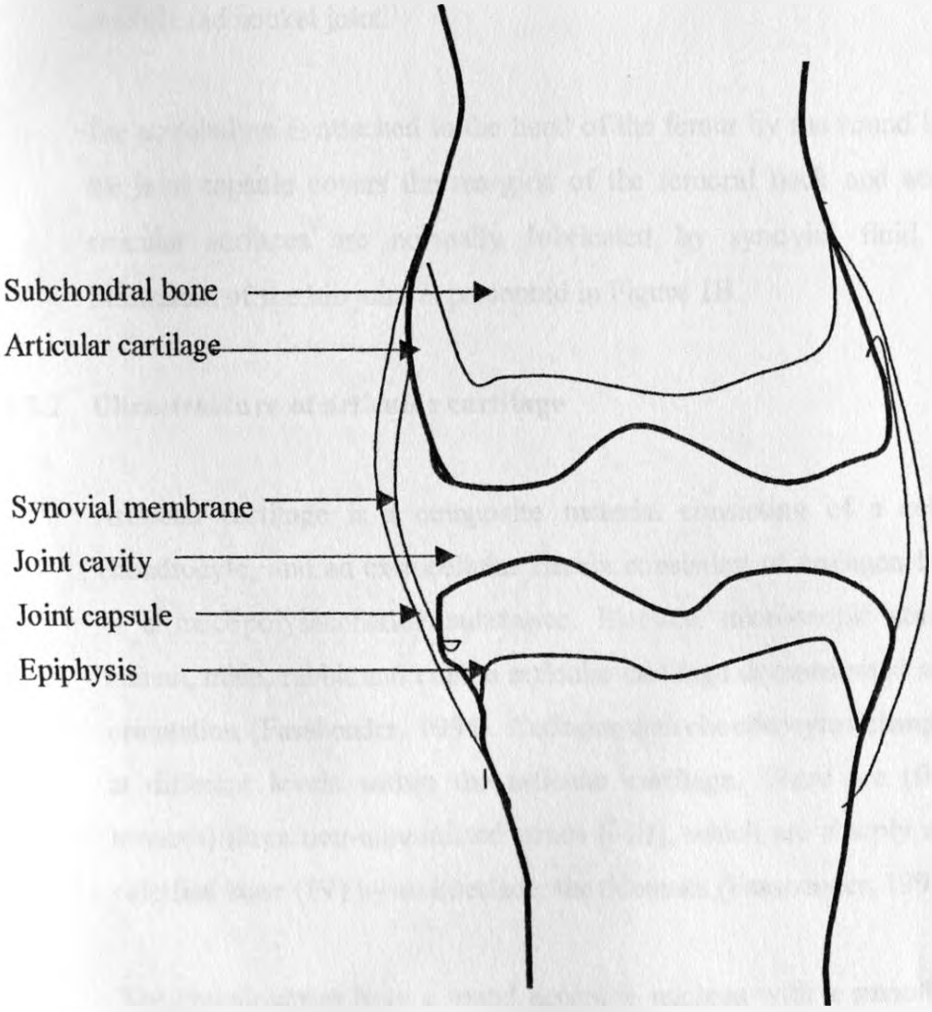

Figure 1 A. Schematic diagram of the structures comprising a diarthrodial joint.

2.2.1 Anatomy of the canine hip joint

The hip joint is formed by two articular surfaces, the acetabulum and the head of the femur. The acetabulum is formed by the fusion of three pelvic bones; the pubis, the ileum and the ischium, forming the socket. The roundish femoral head forms the ball. Smith, et al. (1990) described a conceptual outline of the hip joint as a ball and socket joint.

The acetabulum is attached to the head of the femur by the round ligament, while the joint capsule covers the margins of the femoral neck and acetabulum. The articular surfaces are normally lubricated by synovial fluid. A schematic illustration of the hip joint is presented in Figure 1B.

2.2.2 Ultrastructure of articular cartilage

Articular cartilage is a composite material consisting of a cellular part, the chondrocyte, and an extracellular matrix consisting of collagen fibers embedded in a mucopolysaccharide substance. Electron microscopic studies of normal human, mice, rabbit and canine articular cartilage demonstrated similar structural orientation (Fassbender, 1994). Collagen and chondrocytes change in orientation at different levels within the articular cartilage. There are (from the surface inwards) three non-mineralized zones (I-III), which are sharply separated from a calcified zone (IV) by an interface, the tidemark (Fassbender, 1994).

The chondrocytes have a round eccentric nucleus with a smooth membrane and frequently one nucleolus. The cell nucleus is surrounded by microfilament bundles arranged in parallel, which adjoin the nuclear membrane; this comprises a contractile structural protein. The cytoplasm contains numerous free ribosomes, abundant rough endoplasmic reticulum, mitochondria and the Golgi apparatus. Protoplasmic inclusions, mainly consisting of glycogen and lipid droplets (Fassbender, 1994).

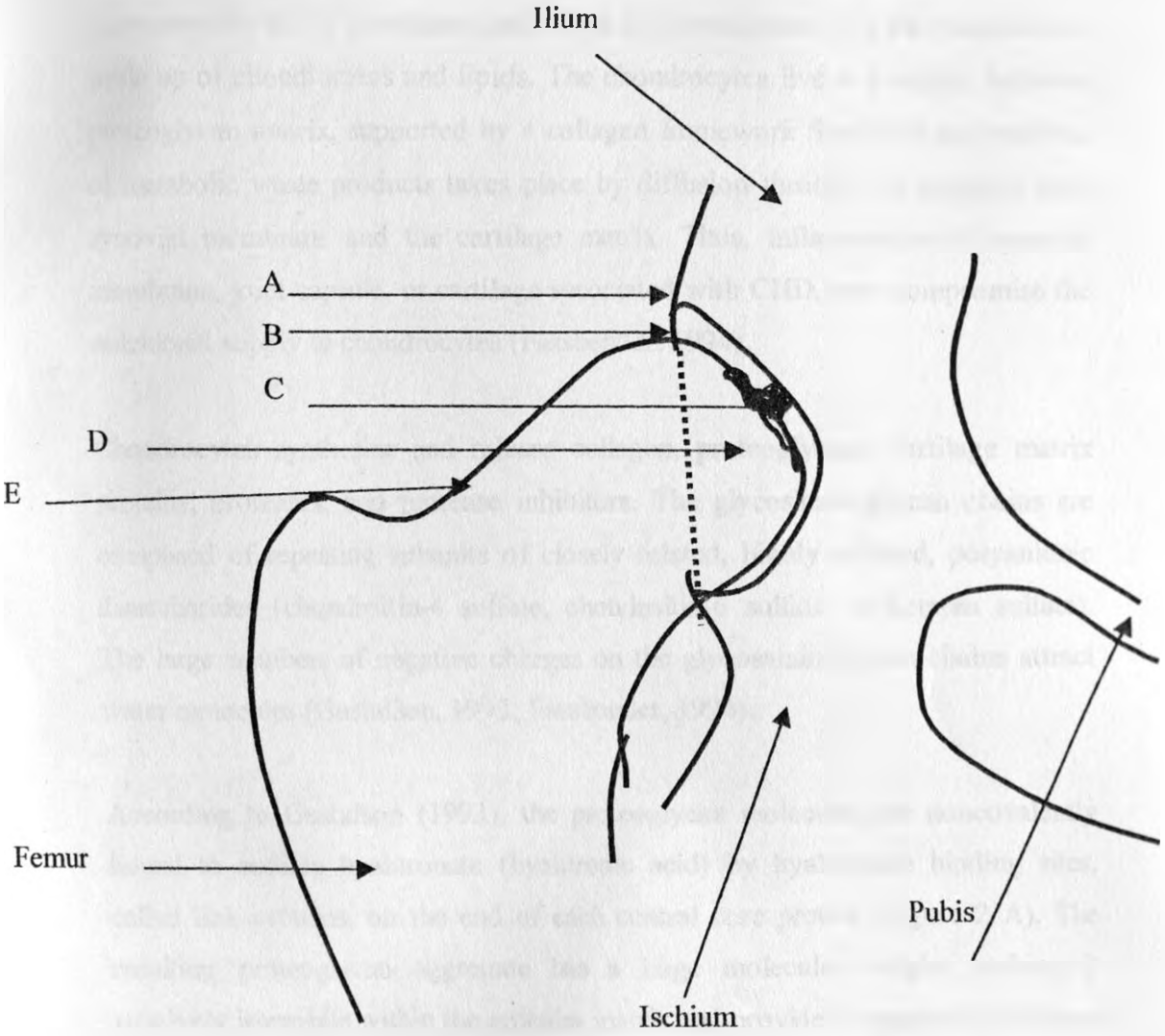

Figure 1 B. Schematic illustration of the canine hip joint.

A = Cranial acetabular rim.

B = Joint capsule.

C = Round ligament

D = Femoral head.

E = Femoral neck.

2.2.3 Chondrocytes, proteoglycans and collagen matrix

Hyaline cartilage is primarily (70 %) composed of water. Of the dry matter, approximately 60 % is collagen and 30 % is proteoglycan, and the remainder is made up of chondrocytes and lipids. The chondrocytes live in a highly hydrated proteoglycan matrix, supported by a collagen framework Synthesis and removal of metabolic waste products takes place by diffusion through the synovial fluid synovial membrane and the cartilage matrix. Thus, inflammation of synovial membrane, joint capsule, or cartilage associated with CHD, may compromise the nutritional supply to chondrocytes (Fassbender, 1994).

Chondrocytes synthesize and release collagen, proteoglycans; cartilage matrix proteins; proteases; and protease inhibitors. The glycosaminoglycan chains are composed of repeating subunits of closely related, highly sulfated, polyanionic disaccharides (chondroitin-4 sulfate, chondroitin-6 sulfate, or keratan sulfate). The large numbers of negative charges on the glycosaminoglycan chains attract water molecules (Gustafson, 1993, Fassbender, 1994).

According to Gustafson (1993), the proteoglycan molecules are noncovalently bound to sodium hyaluronate (hyaluronic acid) by hyaluronate binding sites, called link proteins, on the end of each central core protein (Figure 2 A). The resulting proteoglycan aggregate has a large molecular weight; making it relatively immobile within the articular matrix and provides compressive stiffness to cartilage (Gustafson, 1993). Delvin (1993) outlined a schematic representation of the formulae for some proteoglycans (Figure 2B)

Chondrocytes synthesize lysosomal enzymes for intracellular and extracellular use, which may attack proteoglycan or collagen molecules. On the other hand, synovial fluid contains natural enzyme inhibitors; their relatively large size often precludes their presence in the articular matrix (Gustafson, 1993).

Figure 2 A. Schematic representation of proteoglycans and collagen matrix in articular cartilage (Adapted from Gustafson, 1993).

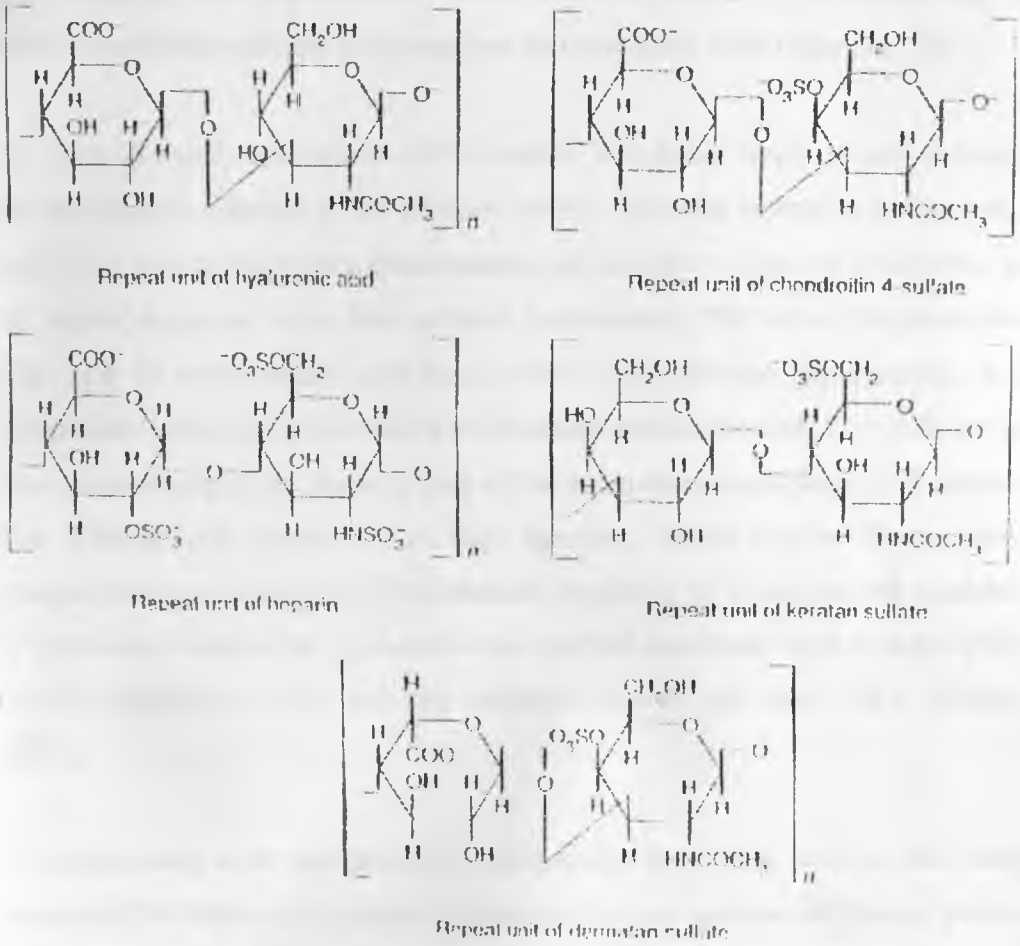

Figure 2 B. Major repeat units of glycosaminoglycan chains.
(Adapted from Delvin, 1995).

2.2.4 The joint capsule and synovial membrane

The normal joint capsule is composed of a thick peripheral fibrous layer, which is composed of fibrocytes and collagen fibers, and a thin inner layer, the synovial membrane (Morgan 1997). The synovial membrane is a vascular connective tissue, derived from mesenchyme, which lines the fibrous joint capsule and is reflected onto intra-articular ligaments and intra-articular bone (Morgan, 1997).

The synovial membrane consists of two layers. The lining layer, or intimal layer, lies immediately adjacent to the articular cavity. Synovial intimal or lining cells, one to four cells thick, form a discontinuous surface layer. They are designated as "A" cells (phagocytic cells that produce hyaluronate); "B" cells (fibroblast-like cells, rich in endoplasmic reticulum), which may produce glycoprotein; and intermediate cells, which have some of the characteristics of both. "A" cells are of bone marrow origin and may be part of the monocyte-macrophage cell system. The different cell types vary in their function, which include fibrogenesis, phagocytosis, and synthesis of hyaluronate, synthesis of cytokines and synthesis of proteases. Many of the products of the synovial membrane have a direct effect on the metabolism of the articular cartilage (Bennet and May, 1995, Morgan, 1997).

The supporting layer (subintimal or subsynovial layer) lies between the lining layer and the fibrous joint capsule. It contains varying amounts of fibrous, areolar, and adipose tissue. The supporting layer also carries nerves, lymphatics, elements of the reticuloendothelial system and a rich plexus of blood vessels. The plexus is a major source of nutrients for the synovial fluid (Bennet and May, 1995).

The outer layer is a heavy sheath that contributes to joint stability. It attaches to bone at its insertion at the margins of the joint and, thereby, encloses a segment of variable length within the joint cavity. This layer is well supplied by blood vessels and nerve endings. The synovial membrane is normally a very thin membrane,

barely visible to the naked eye. Tiny surface projections (villi) are normally present (Bennet and May, 1995).

The synovial membrane serves four important functions in the normal joint. These include a low-friction lining surface, the provision of lubricants, a contribution towards joint stability, and the transport of nutrients into the joint and metabolic waste out of the joint (Bennet and May, 1995).

2.3.0 Osteoarthritis

2.3.1 Definition

Osteoarthritis is a condition characterized by joint effusion, periarticular osteophyte formation, aberrant repair and eventual focal loss of articular cartilage, changes in subchondral bone architecture, capsulitis and synovitis. This condition is also referred to as degenerative joint disease (DJD). It is an important orthopedic disease in dogs and human beings (Olee, et al., 1999, Innes, et al., 2000a). Phylogenetically, osteoarthritis has been traced back to reptiles and amphibians, and is present in all mammalian species, except those that spend most of their lives inverted, such as bats and sloths (Hahn and Edwards, 1998).

2.3.2 Aetiology

Degenerative joint disease is the most common joint abnormality seen in small animal practice. It occurs most frequently in the large weight bearing joints, frequently the hip joint, of medium to large-sized dogs, but it may afflict any synovial joint. The next most frequent locations are the canine shoulder and stifle joints (Allan, 1998). Osteoarthritis in animals is generally secondary to traumatic, infectious, neoplastic, developmental, metabolic or immune–mediated disorders. In dogs osteoarthritis of the hip joint is very closely associated with canine hip dysplasia. The specific examples of conditions causing lameness in dogs have

been classified in detail (Bennet, 1994, Bardet, 1995). These include ligament, tendon and muscle disease associated with joint disease, including traumatic arthropathies, articular fractures, developmental disorders, congenital disorders, metabolic, dietary and endocrinologic arthropathies, arthropathies associated with inherited metabolic disorders. Others conditions include neoplastic and neoplastic-like arthropathies, and arthritis such as degenerative joint disease (either as osteoarthritis, traumatic arthritis). Inflammatory arthritis may be bacterial, fungal, tubercular, mycoplasmal, rickettsial, protozoal or viral. Inflammatory arthritis may also be immune–based or miscellaneous such as vaccination reactions or drug-induced, or crystal-induced arthritis. Degenerative spinal arthropathies include conditions such as spondylosis deformans and ankylosing hyperostosis, intervertebral disc disease osteochondrosis, osteoarthritis of the intervertebral joints and diseases of the lumbosacral junction.

Theories on the pathophysiology of osteoarthritis have been frequently linked with inflammation of the synovium, which further augments injury to articular cartilage (Gustafson, 1993).

2.3.3 Pathophysiology

Many theories exist concerning the role of trauma in the etiopathogenesis of degenerative joint disease. Some are oversimplifications and others are extrapolations from limited biochemical studies. Understanding the pathways leading to osteoarthritis aid in the choice of rational management of affected patients.

Traumatic injury may arise from a single or repeated joint use with a predisposing biomechanical abnormality (conformational defect). A single traumatic injury may result in direct cartilaginous, bone or ligamentous damage. Alternatively, "use trauma" may occur as a result of long-term stresses of excessive weight bearing associated with obesity or strenuous exercise. Trauma to articular

cartilage leads to chondrocyte damage, tearing of collagen fibrils, or disruption of the articular surface. Damaged chondrocytes have decreased proteoglycan synthesis, liberation of lysosomal enzymes and superoxide and hydroxyl radicals, resulting in cellular or matrix damage and release of cytokines (Gustafson, 1993).

Cytokines are potent polypeptides that mediate the intercellular signalling required for a co-ordinated immune response. The lymphokines are products of lymphocytes and participate in the processes of immune reaction, inflammation and haematopoiesis. Together with cytokines produced from monocytes, they have been designated interleukins, and those whose biological properties and amino acid sequences are known have been designated a number, so far, 12 have been identified (Table 2). Other cytokines are interferons and tumour necrosis factor (Williams, et al., 1994, Olee, et al., 1999, Fernandes, et al., 2002).

Interleukin-1 and prostaglandin–E_2 (PGE_2) both act to modify chondrocyte activity to become degradative to the matrix by releasing enzymes, which digest glycosaminoglycan, core protein or collagen. Trauma also results in intracellular release of lysosomal products directly causing cell death. With loss of collagen, cartilage loses its ability to resist tensile or shear forces and blisters develop, leading to superficial and deep fibrillation (Gustafson, 1993).

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Interleukin-18 (IL-18) is a new member of the IL-1 family of cytokines. It was originally identified as an IFN-γ inducing factor with functional similarities to interleukin-12 (IL-12) (Olee, et al., 1999).

Table 2. Outline of cytokine families.

Family	Examples of members
Interleukins	IL-1 –IL-12
Lymphokines	
Monokines	
Interferons	IFNα, IFNβ, IFNγ
Tumour necrosis factors	TNF-α, TNF-β
Colony-stimulating factors	GM-CSF
Other growth factors	TGF

(Williams, et al., 1994).

Two forms of IL-18 were detected in chondrocytes, the precursor with a molecular weight of 24 kDa and the mature form of 18kDa. The synthesis of both forms was increased by stimulation and processing of the precursor to the mature form as IL-1 converting enzyme. In chondrocytes, IL-18 inhibited cell proliferation and thus regulates the functions of TGF-α, one of the major growth factors for these cells (Olee, et al., 1999). These factors influence the pathogenesis of osteoarthritis in human beings.

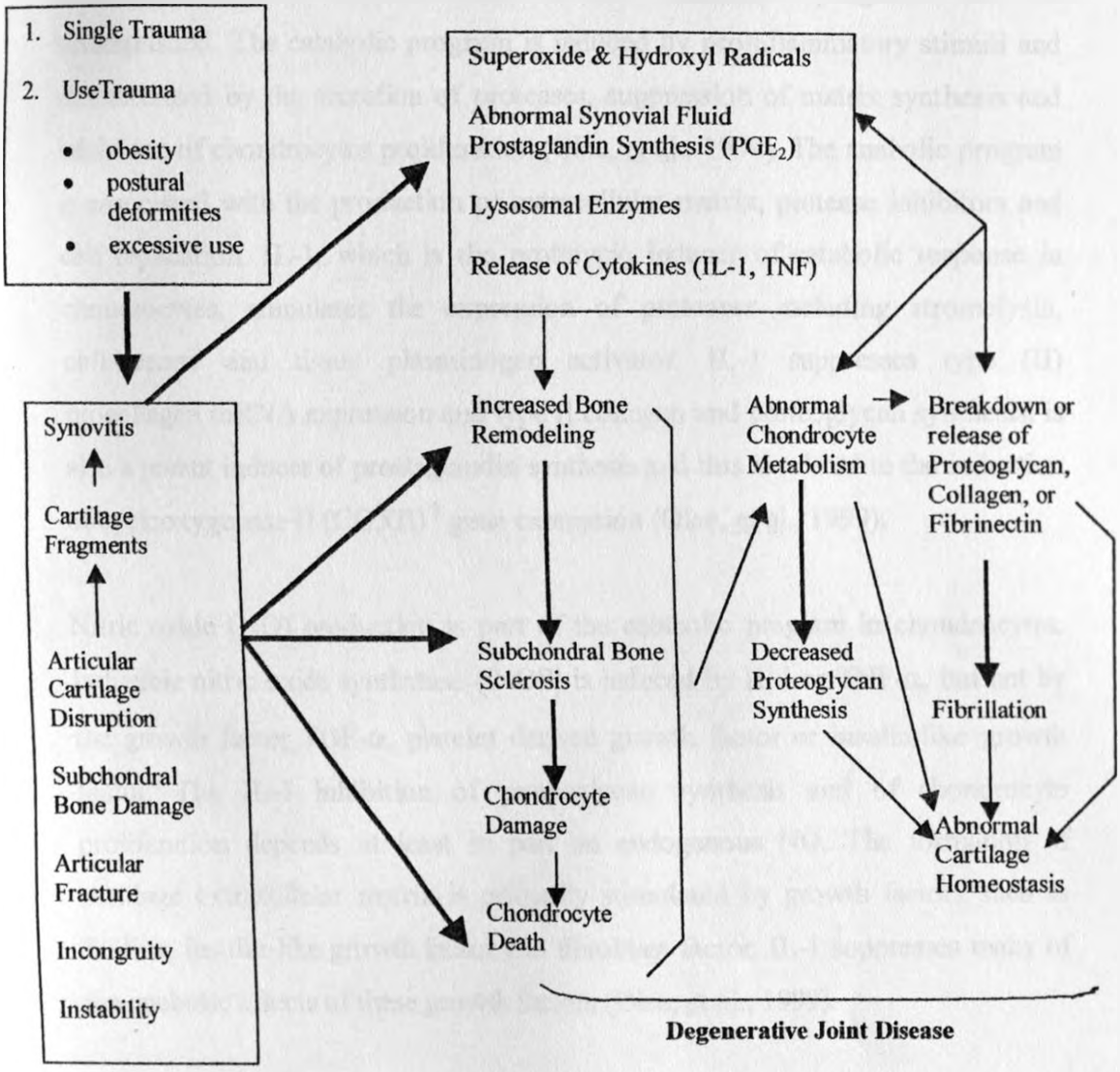
The latest developments on cytokines, growth factors and molecular mechanisms of the cell response, including modulation of gene expression profiling, were extensively reviewed in man (Goldring, 2000, Fukui, et al., 2001, Aigner and McKenna, 2002, Fernandes, et al., 2002). It is suggested that similar processes occur in canine osteoarthritis.

Chondrocytes in damaged cartilage attempt to repair the tissue by increasing their metabolism. They divide once or twice to form clumps of chondrocytes called clones. However, once significant disruption has occurred, repair is relatively ineffective. The relationship between trauma and the development of degenerative joint disease was outlined by Gustafson (1993) as presented in Figure 3.

Several theories exist regarding initiation of osteoarthritis. The mechanical theory is a hypothesis that repeated trauma may initiate sclerosis of subchondral bone, creating a less forgiving substructure for the articular cartilage on which it rests. This forces the cartilage to absorb increased concussive forces, leading to direct physical damage to the cartilage (Morris, et al., 1992).

The biochemical theory contends that a stimulus (perhaps trauma or inflammation, perhaps ageing coupled with a genetic propensity) induces production of a cytokine (tumour necrosis factor-α, IL-I) from synoviocytes or from chondrocytes, or a remote tissue that delivers the cytokine through the synovial fluid. Receptors on chondrocytes bind the cytokine and activate the cells, inducing production of

Figure 3. The relationship between traumatic injury and the development of degenerative joint disease.



(Gustafson, 1993).

prostaglandins and latent and active degradative enzymes, including stromelysin, collagenase, gelatinase and serine proteases (Morris, et al., 1992). These products further influence the functions of chondrocytes in the progression of the disease.

In chondrocytes two qualitatively distinct functional programs can be distinguished. The catabolic program is induced by proinflammatory stimuli and characterized by the secretion of proteases, suppression of matrix synthesis and inhibition of chondrocytes proliferation (Olee, et al., 1999). The anabolic program is associated with the production of extracellular matrix, protease inhibitors and cell replication. IL-1, which is the prototypic inducer of catabolic response in chondrocytes, stimulates the expression of proteases including stromelysin, collagenase and tissue plasminogen activator. IL-1 suppresses type (II) procollagen mRNA expression and type II collagen and proteoglycan synthesis, is also a potent inducer of prostaglandin synthesis and this is related to the induction of cyclooxygenase II (COXII)[3] gene expression (Olee, et al., 1999).

Nitric oxide (NO) production is part of the catabolic program in chondrocytes. Inducible nitric oxide synthetase (iNOS) is induced by IL-I or TNF-α, but not by the growth factor TGF-α, platelet derived growth factor or insulin-like growth factor. The IL-1 inhibition of proteoglycan synthesis and of chondrocyte proliferation depends at least in part on endogenous NO. The formation of cartilage extracellular matrix is primarily stimulated by growth factors such as TGF-α, insulin-like growth factor and fibroblast factor. IL-1 suppresses many of the anabolic effects of these growth factors (Olee, et al., 1999).

In synoviocytes, IL-1 induces a similar spectrum of genes as in chondrocytes, and this includes a large number of cytokines, metalloproteinases and adhesion molecules. In contrast with chondrocytes, IL-1 stimulates the proliferation of synoviocytes and may contribute to pannus formation (Olee, et al., 1999).

IL-1 is secreted by stimulated macrophages (synovial type A cells or subintimal macrophages). IL-1 stimulates secretion of prostaglandins and neutral proteases from synovial fibroblasts and chondrocytes, thus increasing the degradation of proteoglycans from cartilage. The loss of proteoglycans from cartilage alters the hydraulic permeability of the cartilage. This interferes with joint lubrication, leading to further mechanically induced injury to cartilage (Gustafson, 1993).

The loss of proteoglycans, with subsequent inadequate lubrication of the articular surface, leads to disruption of collagen fibers on the surface of the articular cartilage. Affected areas of cartilage are yellow-brown and have a dull, slightly roughened appearance. As more proteoglycans are lost, the collagen fibers condense, and fraying of surface collagen fibres extend along the sides of the arcades as multiple vertical clefts (fibrillation) (Gustafson, 1993).

Fibrillation is accompanied by surface loss, overall thinning of articular cartilage, necrosis of some chondrocytes and attempted but ineffective, regenerative hyperplasia of chondrocytes. Loss of articular cartilage can become complete with exposure of subchondral bone. The continued rubbing of subchondral bone causes it to become dense, polished and ivory-like (eburnation). The process is illustrated in Figure 4 (Doige and Weisbrode, 1995).

Subchondral bone is dense in the area where the overlying cartilage is completely ulcerated. Degenerative changes in articular cartilage are often accompanied by the formation of periarticular osteophytes and by some degree of synovial inflammation and hyperplasia. Table 3 presents the pathological grades of osteoarthritis reported by Doige and Weisbrode (1995).

The synovial membrane commonly responds to injury by villous hypertrophy and hyperplasia, hypertrophy of lining cells, and pannus formation. Villous hypertrophy occurs with and without synovitis. The proportions of A and B cells in the synovium may change in various disease processes.

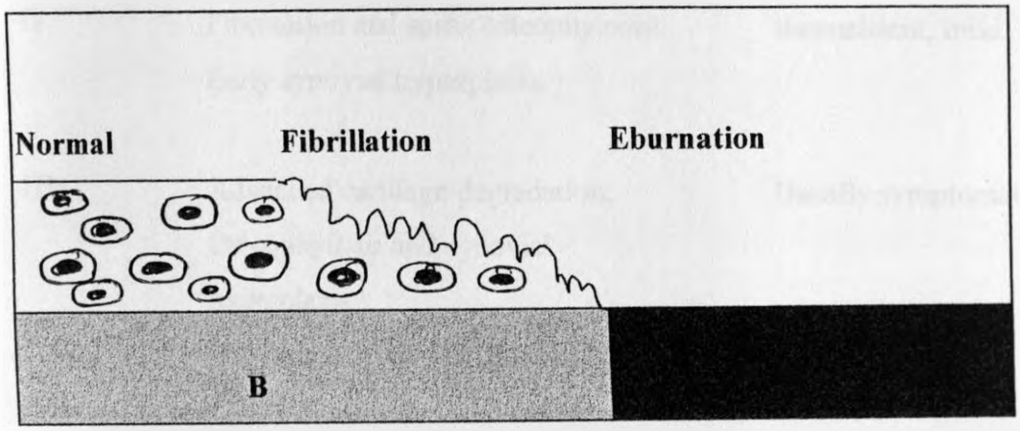


Figure 4. Structural changes that characterize fibrillation of articular cartilage and eburnation of subchondral bone. Subchondral bone (B) is dense (eburnated) in the area where the overlying cartilage is completely ulcerated (Adapted from Doige and Weisbrode, 1995).

Table 3. Pathological grades of osteoarthritis.

Grade	Articular changes	Clinical signs
I	Early, localized fibrillation / flaking.	None.
II	Fibrillation and some osteophytosis; Early synovial hyperplasia.	Inconsistent, mild.
III	Advanced cartilage degradation; Osteophytosis and synovial hyperplasia; + /- Exposure of subchondral bone.	Usually symptomatic.
IV	Exposed subchondral bone and eburnation; Subluxation, damage to intra-articular ligaments; extensive peri-articular fibrosis.	Severe.

(Doige and Weisbrode, 1995).

Fragments of articular cartilage may adhere to the synovium, surrounded by macrophages and giant cells. Larger pieces of detached cartilage can float freely and survive as joint mice that continue to be nurtured by synovial fluid (Bennet and May, 1995).

Inflammatory cells infiltration in the synovial membrane may impair fluid drainage from the joint, and joint fluid may lose some of its lubricating properties because hyaluronic acid may be degraded by the superoxide-generating systems. Chronic traumatic synovitis and capsulitis result in the release of cellular intermediaries, enzymes, and metabolites from synoviocytes and leukocytes. These compounds are deleterious to the articular cartilage (Bennet and May, 1995).

IL-1 and TNF-α released from the synoviocytes and monocytes not only potentiate synoviocytes, but also act on chondrocytes to stimulate release of degradative enzymes and prostaglandin-E_2. Prostaglandin-E_2 is also released from synoviocytes and act on chondrocytes to cause decreased matrix production and matrix degradation. Superoxide and hydroxyl radicals released by polymorphonuclear cells (PMNs) and synoviocytes destroy cell membranes of chondrocytes or synoviocytes, depolymerise hyaluronate, cleave core protein and damage collagen fibrils. Lysosomal enzymes released from the synoviocytes decrease the matrix content of proteoglycan (Bennet and May, 1995).

Synovitis also results in the production of an increased volume of synovial fluid with poor quality. Blood flow may be increased to the synovium and capsule, resulting in higher hydrostatic pressure. Inflamed synovium is a poor selective filter of plasma that causes production of synovial fluid with an increased total protein and fibrinogen content, enabling it to clot (Gustafson, 1993).

2.4.0 Canine hip dysplasia

2.4.1 Definition

Hip dysplasia is a literal translation from its Latin roots: "faulty development of the hip". Schnelle first described malformation of the coxofemoral joint in dogs, referred to as hip dysplasia, in 1937. It was termed "bilateral congenital subluxation of the coxofemoral joints" and was thought to be rare. However, the high prevalence of hip dysplasia in dogs was recognized in the 1950s (cited by Smith, 1997).

Henricson and others in 1966 defined the condition; 'Hip dysplasia is a varying degree of laxity of the hip joint permitting subluxation during early life, giving rise to varying degree of laxity during early life, giving rise to varying degree of shallow acetabulum and flattening of the femoral head, [and] finally, inevitably leading to osteoarthritis'. Thus, the connection between joint laxity at a young age and subsequent development of osteoarthritis was recognized early (Smith, 1997).

Hip dysplasia is an important orthopedic condition in human beings and most domesticated animals. Hippocrates first described it in man over 3,000 years ago (Hahn and Edwards, 1998). Since then the disease has been critically examined in both human and animals. Although our understanding of canine hip dysplasia has evolved over the years, progress has often been more a result of clinical empiricism than well-conceived scientific investigation. As a result, canine hip dysplasia and secondary osteoarthritis continue to be the source of considerable debate, controversy and frustration to veterinary clinicians, breeders and dog owners (Lust, 1997).

2.4.2 Aetiology and prevalence

Joint laxity and trauma are important predisposing factors towards initiation and progression of osteoarthritis and subsequent enzymatic degradation of the normal articular structure (Allan, 1998).

Age, heritability and genetic factors may modify the onset and progression, frequency and severity of clinical and radiographic expression of osteoarthritis in canine hip dysplasia. Environmental (non-genetic) factors, including body size, growth rate, nutrition, dietary anion gap, in-utero endocrine influences and muscle mass may also be involved. Although an interplay between many factors may trigger the condition, the actual cause of canine hip dysplasia remains unknown (Lust, 1997, Allan, 1998).

Dogs that develop hip dysplasia are born with apparently normal hip joints, but after the dogs have reached a few weeks of age, joint laxity can be demonstrated at necropsy (Kealy, et al., 1992). The disease frequently first appears in susceptible dogs when they are between 4 and 12 months old, although in some dogs, the disease is not evident radiographically until they are $\geq$ 24 months old (Smith, et al., 1995, Lust, 1997).

Studies of hip dysplasia genetics have indicated that the disease is polygenic and multifactorial, with estimates of heritability index in the range of 0.2 to 0.6. Phenotypic expression occurs along a continuum from normal to severely dysplastic animals (Swenson, et al., 1997). Phenotypic expression is recognized radiographically as femoral head subluxation. The end result of this self-perpetuating cycle of subluxation and remodeling is osteoarthritis (Kealy, et al., 1992).

2.4.3 Pathophysiology

Hip dysplasia and joint laxity in dogs may have a common aetiology. However, mechanical strength and atmospheric pressure, which depends on permeability of the joint capsule, may be associated with stability of the joint. Normally, the joint capsule acts as a barrier to accumulation of synovial fluid in a joint. Any increase in permeability of the joint capsule may create a leak in the barrier, which disturbs the vacuum effect. This leads to an accumulation of synovial fluid and allows for luxation of the joint (Madsen, 1997).

Degenerative changes appear concurrently in the articular cartilage and joint capsule of dogs with canine hip dysplasia. A decrease in the number of macrophage type-A cells is the first synovial change in dogs with hip dysplasia. As the degeneration accelerates, cells of an uncertain type replace necrotic synoviocytes. The changes in the synovium are characterized by proliferation of synoviocytes and hypertrophic villi, dilatation of venules packed with red blood cells, accumulation of interstitial fluid, infiltration by lymphocytes and mononuclear cells, and finally, development of fibrosis (Madsen, 1997).

Inflammatory changes associated with synovitis, such as synovial effusion and capsular edema, and leakage of proteins from the synovial vasculature, decrease the drainage from the joint. The accumulating fluid in the joint results in capsular distention and an increase in intracapsular pressure. Effusive synovitis and accumulation of synovial fluid are therefore early signs of canine hip dysplasia. In contrast, chronically inflamed joints yield less fluid than acutely inflamed joints (Feldman, 1995). Acute osteoarthritis, characterized by effusive synovitis and degenerative lesions on the surface of the articular cartilage, is the earliest alteration in immature dogs predisposed to canine hip dysplasia. As osteoarthritis progresses, capsular fibrosis, cartilage degeneration and bone deformation and remodeling develop. Eventually, osteoarthritic changes dominate (Madsen, 1997).

Due to these changes, studies of canine hip dysplasia are also studies of acute and chronic osteoarthritis. In fact, inflammatory, effusive and degenerative changes may obscure the dysplastic nature of the disease and make it impossible to distinguish between primary and secondary alterations. Inflammatory changes associated with osteoarthritis include activation of cytokines and proteolytic enzymes and accumulation of mononuclear cells in the joint. Liberation of enzymes and other active substances during degeneration of the joint capsule and articular cartilage will accelerate the inflammatory and degenerative processes. Finally the hemodynamic, inflammatory and degenerative alterations lead to a self-perpetuating cycle. Osteoarthritis becomes the most important disease in the dysplastic joints (Morgan, 1997).

The structural alterations common to canine hip dysplasia include a shallow acetabulum, joint subluxation, erosion of the articular cartilage and remodeling of the acetabulum and femoral head. Uniform, smooth layer of white to slightly grey-white cartilage covers the normal femoral head. An initial sign of canine hip dysplasia is slightly focal to multifocal dullness of the cartilage. As canine hip dysplasia progresses, more distinct surface irregularities and color changes develop. The cartilage becomes yellow or grey and eventually red or red-brown. This indicates extensive loss of articular cartilage and exposure of subchondral bone. Grossly detectable osteophytes, a sign of periosteal new bone proliferation, may be seen in advanced cases (Morgan, 1997).

Reactions of the deep layer of the synovial membrane to injury generally involve the fibrovascular stroma. The signs of the fibrous \ vascular stromal response include villous synovitis. Because the synovial membrane contains numerous nerves, excessive proliferation of synovial villi or increase in synovial fluid pressure will result in marked pain. Synoviocytes may respond to injury through hypertrophy, hyperplasia and rarely, necrosis. Hypertrophic synoviocytes appear more rounded than usual (Morgan, 1997).

2.4.4 Clinical signs

There are two recognizable clinical groups of dogs; young dogs between 4 and 12 months of age and animals over 15 months of age with chronic disease (Brinker, et al., 1990). Young dogs often show sudden onset of unilateral disease (occasionally bilateral). This is characterized by sudden reduction in activity associated with marked soreness of the hindlimbs. They will show sudden signs of difficulty in arising with decreased willingness to walk, run and climb stairs. The muscles of the pelvic and thigh areas are poorly developed. Most dogs will have a positive Ortolani sign. This is the click produced by the movement of the femoral head as it slips in and out of the acetabulum with adduction and proximal pressure applied to the femur followed by abduction (Brinker, et al., 1990).

The sudden onset of signs in young dogs is caused by occurrence of microfractures of the acetabular rims. When femoral heads are subluxated, the area of contact of the femoral head with the dorsal acetabulum is limited to the area between 10 o'clock and 2 o'clock, with an extreme buildup of stress in that area. This eventually overloads the acetabular rim, producing tissue fatigue, loss of tissue elasticity and contour, and eventual microfracture. Pain results from tension and tearing of nerves of the periosteum. Sharpey's fibers rupture, bleed and form osteophytes on the acetabulum and femoral neck. Osteophytes usually are not radiographically visible until 17 or 18 months of age, but may be seen as early as 12 months. The fractures heal by the time of skeletal maturity, the hip joint becomes more stable and pain is markedly decreased. Most dysplastic dogs between 12 and 14 months of age walk and run freely and are free of significant pain, despite the radiographic appearance of the joint (Brinker, et al., 1990).

Older dogs present a different clinical picture because they suffer from chronic degenerative joint disease and its associated pain. Lameness may be unilateral but is usually bilateral. The signs may have become apparent over a long period of time. Alternatively they may suddenly occur after brisk activity that results in a

tear or other injury of soft tissues of the abnormal joint. Most clinical signs result from prolonged degenerative changes within the joint (Brinker, et al., 1990).

There is lameness after prolonged or heavy exercise, a waddling gait. Often there is crepitus and restricted range of motion of the joint. The dog often prefers to sit rather than stand and arises slowly and with great difficulty. The thigh and pelvic muscles atrophy markedly. The greater trochanters become quite prominent and even more so if the hip is subluxated. Shoulder muscles hypertrophy because of the cranial weight shift and increased use of the forelimbs. The Ortolani sign is rarely present owing to the shallowness of the acetabulum and fibrosis of the joint capsule (Brinker, et al., 1990).

Although various pain scales have been developed, none has gained widespread acceptance in veterinary medicine. The simple descriptive scale involves 4 or 5 degrees of severity (no evidence of pain, mild moderate, severe and very severe pain). The numerical rating scale may be produced by assigning numeric scores to the categories of standard descriptive scale or similar scale. The visual analogue scale is a simple scale, consisting of a straight-line usually 100 mm (horizontal or vertical) on paper, with a description of the limits of the scale written at each end. The University of Melbourne pain scale utilizes 6 categories (physiological data, response to palpation, activity, mental status, posture and vocalization). Heart rate, respiratory pattern, vocalization agitation and response to manipulation were used as criteria for scoring postoperative pain (Conzemius, et al., 1997, Holton, et al., 1998, Firth and Haldane, 1999, Grisneaux, et al., 1999). However, the assessment of adult dogs with chronic pain is not straightforward compared to dogs with acute pain, following experimental or surgical procedures.

Dew and Martin (1992) and Rasmussen, et al. (1998) used lameness, range of motion, joint stability, crepitus and muscle atrophy (mid-thigh circumference) in assessing the clinical function of dogs undergoing experimental joint surgery. Evers, et al. (1997) also used gait, degree of crepitus and range of motion as

criteria for postoperative evaluation of dogs that underwent treatment for hip joint dislocation. These criteria may not be consistent and reliable for assessing dogs with chronic pain, such as those with canine hip dysplasia.

Vasseur, et al. (1995) used a lameness grading system to assess response to treatment in dogs with osteoarthritis. Lameness, weightbearing, joint mobility, willingness to hold up contralateral limb and signs of pain were assigned a score ranging from 1-5 each and added to generate a cumulative score. In another report, osteoarthritis was categorized into three levels of severity mild, moderate or severe. This was based on clinical signs and incorporated radiographic features (Pfizer Animal Health, 1997, Appendix 1 B). It is difficult to clinically distinguish between mild and moderate severity of lameness attributable to chronic osteoarthritis of hip joints in adult dogs.

2.4.5 Diagnosis

History, clinical signs, radiography and ultrasonography are useful in diagnosis of canine hip dysplasia. Diagnostic methods of hip palpation (e.g. Ortolani, Bardens, and Barlow) provide semiquantitative information on joint laxity and the likelihood of dogs to suffer canine hip dysplasia. Although useful in diagnosing congenital hip dislocation in human neonates, hip palpation has no clear and consistently reliable diagnostic value in dogs (Smith, 1997). Confirmation of canine hip dysplasia is based on radiographic findings of subluxation of the hip joint or secondary osteoarthritis on extended pelvic ventrodorsal radiographs (Popovitch, et al., 1995).

Hip dysplasia is a developmental age-related disorder; it is not present at birth. A variable amount of time must elapse before radiographic changes are manifest. Once present these radiographic changes usually progress as the affected animal ages (Allan, 1998). The earliest recognizable change in the coxofemoral joints is joint laxity. This may be palpated or visualized radiographically. Subsequent radiographic changes are those of degenerative joint disease. The order of

subsequent changes is; perichondral osteophyte formation, remodeling of the femoral head and neck, remodeling of the acetabulum, sclerosis of subchondral bone of the femoral head and acetabulum (Allan, 1998).

As the degenerative phase advances, the femoral head loses its spheroidal shape and becomes flattened along its articular surface. The femoral neck becomes irregular, owing to the growth of a collar of perichondral osteophyte and becomes shallow. Increased bone opacity of the subchondral articular surfaces represents bone sclerosis and a response to cartilage thinning. A variable degree of coxofemoral subluxation is always present. Subchondral cyst formation is an infrequent manifestation of degenerative joint disease in small animals but may occasionally be observed (Allan, 1998).

Radiographic changes vary according to the stage of disease. The most readily recognizable change is osteophyte formation, which follows neovascularization of the chondrosynovial junction with resultant fibrocartilage formation. This fibrocartilage collar ossifies gradually with the formation of characteristic perichondral new bone (Allan, 1998). Continued attrition of the articular cartilage may be detected on radiographs obtained during weight bearing as thinning of the radioluscent joint space. Pathologic alterations of the subchondral bone shelf, including eburnation, compression and necrosis may be detected radiographically (Allan, 1998).

Affected joints exhibit decreased range of movement, which results in increased load of the diminished weight-bearing surface. The combination of increased load, diminished sub-chondral strength, and loss of shock-absorbing cartilage results in alterations in the shape of the sub-chondral bone table. This remodeling of the sub-chondral bone is complemented by the addition of peripheral new bone in the form of perichondral osteophytes. The altered shape of the osseous components of affected joints is readily identified radiographically (Allan, 1998).

Studies of large breed dogs have shown that the probability of developing osteoarthritis increases with age (Smith, et al., 2001). These adult dogs may have not undergone the standard extended pelvic radiographic examination as recommended early in life. Affected animals are unlikely to be detected by most dog owners until when it is too late. The average clinicians are unlikely to evaluate radiographs where radiographic changes are not obvious, as appropriately conducted by a panel of board certified radiologists. This necessitates the submission of ventrodorsal radiographs to a panel for a thorough scrutiny and grading. The owners should also be willing to submit their dogs for radiographic examination. Diagnostic expertise and equipment for accurate grading of hip joint laxity and osteoarthritis in dogs in Kenya require constant improvements.

Clinicians require a simple radiographic technique, probably an OFA-type, that will enable them evaluate the hip joint laxity and degenerative joint disease and assign a subjective score. This will enable them to quickly advice the clients on the severity of the changes, prognosis and possible management of the condition. Information on the use of these criteria in older dogs in Kenya is scanty. Such criteria would assist clinicians accurately evaluate the status of hips of German shepherd dogs submitted to them with hindlimb lameness. Previous studies (Lust and Summers, 1981, Griesen, et al., 1982) focussed on young dogs (average 11 months). These reports described early asymptomatic osteoarthritis in colonies of Labrador Retrievers, specifically bred to produce canine hip dysplasia. The international symposium on hip dysplasia and orthopedics in dogs, in 1996, dwelt on the diagnosis, prevention and treatment of canine hip dysplasia. Although several articles were subsequently published, a number of key questions remain unanswered (Lust, 1997). Hip dysplasia still remains one of the most common orthopedic disorders in dogs; a baffling and often, disheartening disease.

Hip joint laxity and secondary osteoarthritis in dogs may be measured using qualitative and quantitative radiographic methods. Kennels and veterinarians have

developed criteria for assessing this condition in most countries in Europe and America (Allan, 1998).

2.4.5.1 Qualitative methods

Various orthopedic procedures are employed in radiographic diagnosis of canine hip dysplasia. The most popular qualitative method for assessment of hip quality is the subjective hip scores according to the guidelines of the Orthopedic Foundation for Animals (OFA). The OFA formed a hip dysplasia registry when it established seven grades of variation in congruity of the femoral head and acetabulum (Brinker, et al., 1990).

The first three are considered as normal; **Excellent**-nearly perfect conformation; **Good**-normal conformation for age and breed; **Fair**-less than ideal but within normal radiographic limits; **Near normal**-a borderline category in which minor hip flattening of the femoral head often cannot be clearly assessed because of poor positioning during radiographic procedures (Brinker, et al., 1990).

Dysplastic animals fall into three categories; **Mild** - minimal deviation from normal with only slight flattening of the femoral head and minor subluxation. **Moderate** - obvious deviation from normal with evidence of a shallow acetabulum, flattened femoral head, poor joint congruency and in some cases subluxation with marked changes of the femoral head and neck. **Severe** - complete dislocation of the hip and severe flattening of the acetabulum and femoral head (Brinker, et al., 1990).

Dogs with moderate and severe grades are most likely to be clinically affected. Radiographic evaluation of hip dysplasia requires adequate relaxation for proper positioning in dorsal recumbency with the femurs extended parallel to each other and to the cassette and the patellae centered on the femoral condyles (Brinker, et al., 1990).

2.4.5.2.0 Quantitative methods

There are several radiographic techniques used to quantitate hip joint laxity in dogs.

2.4.5.2.1 The Norberg Angle [NA] and the British Scheme

The Norberg angle is a measure of hip joint subluxation that is obtained from the standard hip-extended radiographic projection. It is a component of the hip joint scoring schemes used in England and Sweden. The British Veterinary Association/Kennel Club published its guidelines on hip dysplasia scheme in 1994 (Appendix I A).

The Norberg Angle is determined on ventrodorsal pelvic radiographs, which are prepared for dogs that are at least one year old. General anaesthesia is required to facilitate safe restraint during radiography. The dog should be placed on its back with the pelvis in the middle of the cassette and the x-ray beam centred on the midline between the hips (i.e. the centring point should be at the level of the cranial edge of the pubis).

In order to avoid rotation, the head of the dog and body should be supported in a straight line by a cradle or by blocks at the thorax. Tilting of any part of the dog's body is likely to cause axial rotation of the pelvis and asymmetry of the hips. The hindlimbs should be fully extended and adducted so that the femora lie parallel to the film and to each other. The legs should be inwardly rotated so that the patellae lie central in the trochlear grooves. The radiographic film is exposed at 70 kV 3mA and routinely processed in the darkroom (Allan, 1998). The ventrodorsal pelvic radiographs are evaluated for various signs of degenerative joint disease as indicated in appendix 1A.

The Norberg angle is determined from ventrodorsal pelvic radiographic views. A transparent disk with concentric circles is helpful in determining the centre of the femoral head on the radiograph, and pencil marks made on the radiograph at this site. One line is drawn from the centre of the femoral head of one hip joint to the centre of the femoral head of the other hip joint. A line is drawn from the centre of the femoral head to the cranial effective acetabular rim and this angle determined. In the normal hip joint the cranial effective acetabular rim should be situated lateral to the 15° line. The hip joints are then scored based on the Norberg angle determined for each hip joint ad outlined in Figure 5 B.

Willis (1997) reviewed progress in canine hip dysplasia control in Britain using this scheme. In all the 6 breeds evaluated, there appeared to have been some selection to reduce total score. However, in all but one instance, the expected result was not achieved, with actual values being higher (i.e. worse) than those expected from theory. One explanation could be that the actual heritability is less than that calculated. Heritabilities could have been overestimated as a result of assortative mating or high inbreeding within populations. In Britain it has been established that not all-low scoring dogs transmit this trait to their offspring. In order to diminish the incidence of canine hip dysplasia in Britain, then as a first step, the kennel club must insist that in affected breeds, only progeny of scored parents may be registered. Although the scheme has been in use in Kenya since 1998, its impact on improvement of hip joint quality in dogs needs to be assessed.

This radiographic scheme, however, was not used for the study, as it requires specialized training for panelists to score radiographs. On the other hand most clinicians would more easily understand the criteria described by Rasmussen, et al (1998), if they had access to an X-ray machine.

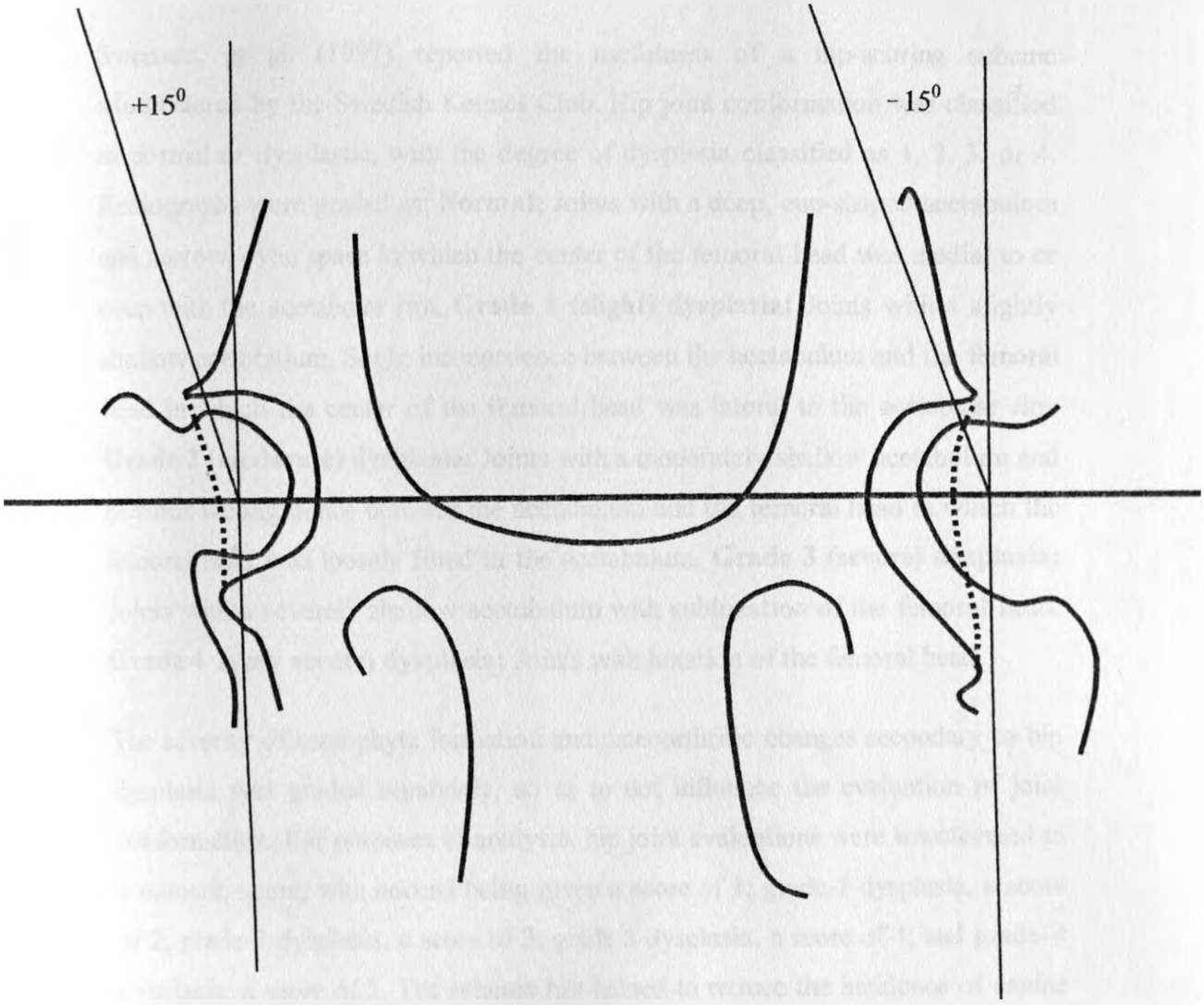


Figure 5 A. Schematic illustration of the Norberg Angle method of measuring canine hip joints.

Scoring	$0 = > +15^{O}$	$3 = 0^{O} - +5^{O}$	$6 = > -10^{O}$
	$1 = +10^{O} - +15^{O}$	$4 = 0^{O} - -5^{O}$	
	$2 = +5^{O} - +10^{O}$	$5 = -5^{O} - -10^{O}$	

2.4.5.2.2 Swedish Kennel Club Scheme

Swenson, et al. (1997) reported the usefulness of a hip-scoring scheme administered by the Swedish Kennel Club. Hip joint conformation was classified as normal or dysplastic, with the degree of dysplasia classified as 1, 2, 3, or 4. Radiographs were graded as; **Normal;** Joints with a deep, cup-shaped acetabulum and narrow, even space in which the center of the femoral head was medial to or even with the acetabular rim. **Grade 1 (slight) dysplasia;** Joints with a slightly shallow acetabulum. Slight incongruence between the acetabulum and the femoral head in which the center of the femoral head was lateral to the acetabular rim. **Grade 2 (moderate) dysplasia;** Joints with a moderately shallow acetabulum and obvious incongruence between the acetabulum and the femoral head in which the femoral head was loosely fitted in the acetabulum. **Grade 3 (severe) dysplasia;** Joints with a severely shallow acetabulum with subluxation of the femoral head. **Grade 4 (very severe) dysplasia;** Joints with luxation of the femoral head.

The severity of osteophyte formation and osteoarthritic changes secondary to hip dysplasia was graded separately, so as to not influence the evaluation of joint conformation. For purposes of analysis, hip joint evaluations were transformed to a numeric score, with normal being given a score of 1; grade-1 dysplasia, a score of 2; grade-2 dysplasia, a score of 3; grade 3 dysplasia, a score of 4; and grade–4 dysplasia, a score of 5. The scheme has helped to reduce the incidence of canine hip dysplasia in Sweden (Swenson, et al., 1997).

2.4.5.2.3 Measurement of percentage of femoral head coverage

The percentage of femoral head coverage was assessed (Rasmussen, et al., 1998) on preoperative and 6 weeks postoperative radiographs following triple pelvic osteotomy. A line was drawn from the fovea capitis to the greater trochanter, and measurements were taken along this line to standardize measurements between dogs (Figure 5 B).

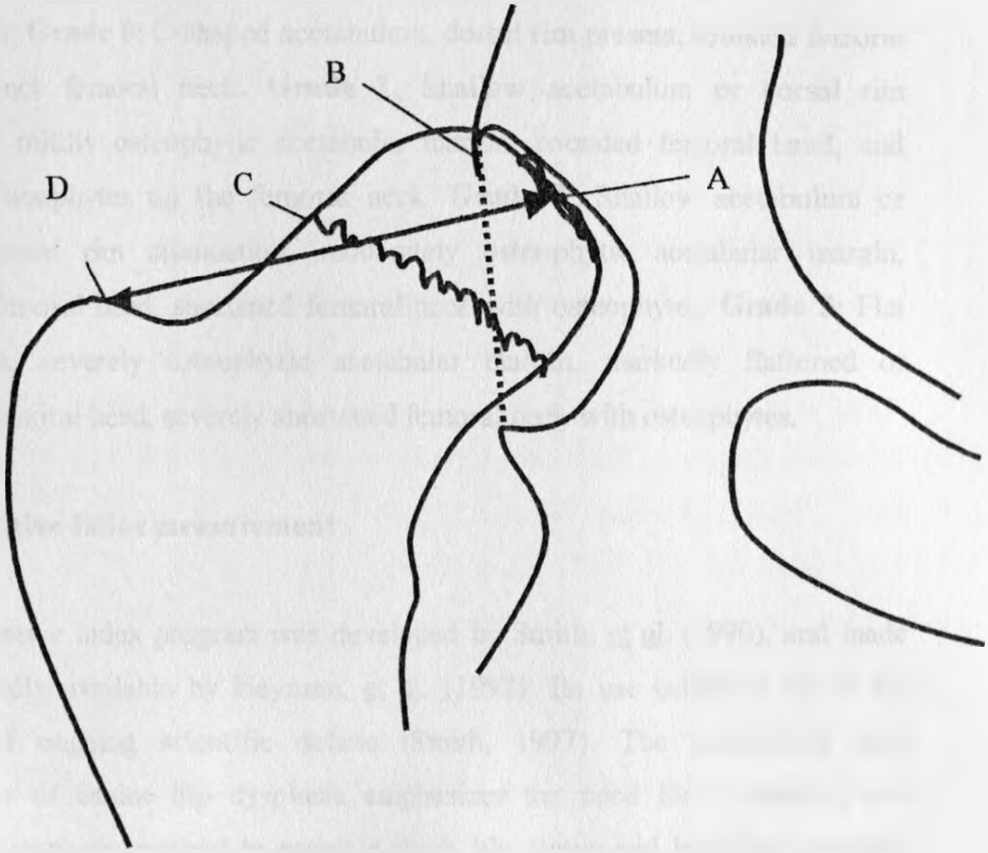


Figure 5 B. Measurement of percentage of femoral head coverage.

A = fovea capitis; B = dorsal acetabular rim; C = physeal scar;
D = greater trochanter. Bold line from A to D = standard measurement line; distance between A and B divided by distance between A and C = femoral head coverage (adapted from Rasmussen, et al., 1998).

2.4.5.2.4 A criteria for grading severity of degenerative joint disease

Rasmussen, et al. (1998) evaluated the preoperative and postoperative status of the coxofemoral joints of dogs prior to and after performing triple pelvic osteotomy in dogs. The criteria for grading the severity of degenerative joint disease was; **Grade 0**; C-shaped acetabulum, dorsal rim present, rounded femoral head, distinct femoral neck. **Grade 1**; Shallow acetabulum or dorsal rim attenuated, mildly osteophytic acetabular margin, rounded femoral head, and minimal osteophytes on the femoral neck. **Grade 2**; Shallow acetabulum or marked dorsal rim attenuation, moderately osteophytic acetabular margin, flattened femoral head, shortened femoral neck with osteophytes. **Grade 3**; Flat acetabulum, severely osteophytic acetabular margin, markedly flattened or irregular femoral head, severely shortened femoral neck with osteophytes.

2.4.5.2.5 Distractive Index measurement

The distractive index program was developed by Smith, et al. (1990), and made commercially available by Heyman, et al. (1993). Its use continues to be the subject of ongoing scientific debate (Smith, 1997). The persistently high prevalence of canine hip dysplasia emphasizes the need for a sensitive and specific diagnostic method to assess a dog's hip status and breeding potential. Moreover, for optimal value, this assessment should occur earlier in a dog's life than the currently recommended age of 24 months (Smith, et al., 1990).

Smith, et al. (1990), described the original protocol for measurement of the distractive index. The procedure requires that dogs be under general anaesthesia or heavy sedation. Stress radiography is done with the hips in compression and distraction positions. To radiograph the hips in the compression view, the dog is positioned in dorsal recumbency on the radiographic table, and weights placed immediately lateral to its hips. By grasping the hocks, the knees are positioned at a distance sufficient to avoid radiographic superimposition of the knees on the

hips, but not wider than the normal knee spacing during the stance phase of weightbearing. The hips are seated in their most congruent position by a small compressive force imposed on the hips via an assistant pushing the weights together. Alternatively, in small dogs, the individual holding the hocks manually seats the hip joints by externally rotating the tibias while keeping the knees in flexion.

For radiography of the hips in the distraction view, the dog is maintained in dorsal recumbency, A custom-designed adjustable table distractor device is placed between the legs, and an assistant firmly presses it down onto the pelvis. While grasping the hocks, the examiner pushes the knees together, using the device as a fulcrum to impose a lateral distractive force bars was set at approximately the interacetabular distance. This spacing allows for the proper stance –phase distance between the knees during force application of the distraction procedure. Even and firm downward force on the distractor helps to maintain pelvic positioning while manipulation is performed. Distraction is maintained for a short time (approximately 1 to 2 seconds), sufficient to permit exposure of the radiographic film. Circle gauges are used to determine the compression index (CI) and the distraction index (DI). The index is calculated by dividing the measured distance between the femoral head centre and acetabular centre (d) by the radius (r) of the femoral head (I = d / r). This yields a unitless variable that ranges from 0 to approximately 1. A hip having an index of 0 is tightly compressed in its congruent position, and a hip having an index of 1 has little to no acetabular coverage of the femoral head (i.e., joint luxation) (Smith, et al., 1990, Smith, 1997).

Passive hip laxity, as quantitated by distractive index, was a significant risk factor for degenerative joint disease, irrespective of the age at evaluation (4, 12 or 24 months) of dogs representing 13 breeds. The strength of the correlation increased with the age of the dog. In contrast, the Norberg angle, a measure of hip laxity on the standard hip extended radiograph, was not found to be a significant risk factor for degenerative joint disease (Smith, et al., 1995).

Leighton (1997) reviewed genetic progress in improving hip quality in German shepherd dogs and Labrador Retrievers in a colony of dogs. A 9-point quality scale was adapted to evaluate hip quality;

1. Hip dysplasia with severe degenerative joint disease (DJD),
2. Hip dysplasia with moderate DJD.
3. Hip dysplasia with mild DJD.
4. Hip dysplasia with no DJD.
5. Borderline.
6. Near normal.
7. Less than ideal, but within normal limits.
8. Normal for age and breed.
9. Excellent for age and breed.

In less than 5 generations the percentage of hip dysplasia was decreased from 55 to 24 % in the German shepherd dogs and from 30 to 10 % in the Labrador Retrievers. The hip extended position and a modified OFA were used in the evaluation procedure. The results provide some moderately strong evidence that a major gene for hip quality may be segregating. However, it must be emphasized that only planned experiments using specific crosses and segregation analyses can definitively prove the existence of a major gene (Leighton, 1997).

The distractive index is the most accurate method for early diagnosis of hip dysplasia in puppies. Allan (1998) compared the DI method with the other methods such as the subjective hip scores. A distractive index < 0.4 at 4 months of age correctly predicted normal hips in 88 % of the cases and a distractive index > 0.4 correctly predicted hip dysplasia in 57 % (Lust, et al., 1993). Adams, et al. (1998) evaluated (using palpation, OFA method, DI and Norberg angle measurements) hip laxity in 4 breeds of dogs. Distractive Index and Norberg angle measurements at 6-10 and 16-18 weeks were the most reliable predictors of hip dysplasia, with DI being more reliable. This radiographic technique is restricted to America; it requires custom-made equipment and special training

(Allan, 1998). Its marketing has provoked considerable controversy, prompting the Orthopedic Foundation for Animals to comment the subject on its website.

The Orthopedic Foundation for Animals and distractive index data are not representative of the general population of dogs. This is because the programs are voluntary, most dogs are in pet homes and are not radiographed. In addition, not all radiographs of radiographed dogs are submitted for evaluation by either program (Smith, 1997). This is probably the case for dogs in Kenya. Not all dogs are radiographed and submitted for evaluation. This makes it difficult to determine the full extent of the condition in dogs in Kenya.

2.4.5.2.6 Dorsolateral subluxation of the femoral head test

A recently invented diagnostic method for canine hip dysplasia (Farese, et al., 1998) was tested in controlled studies and found to be accurate at 8-months of age. The dorsolateral subluxation of femoral heads (DLS) test was devised to evaluate displacement of femoral heads from acetabula when a hip is radiographed in a load-bearing position. In two published reports (Farese, et al., 1998, Farese, et al., 1999) dysplastic joints had a DLS score of 40 % or less, whereas normal hip joints had scores greater than 60 %.

Lust, et al. (2001a) examined dogs at 8-months of age and related the DLS score to the appearance of osteoarthritis at a later age. The presence of hip osteoarthritis is an outcome assessment of the dysplastic trait. Eight months was the preferred age since the DLS score was stable by age 8-months, and 8-months might be early enough for decision making for many breeding programs. The likelihood ratios for the presence of osteoarthritis for three categories varied by a factor of 40 (Lust, et al., 2001a).

When the DLS score was compared with the distractive index at 8-months of age to predict hip osteoarthritis, less than 40 % of the variation in DLS score was

accounted for by DI; $r^2 = 0.36$. A logistical multiple regression analysis suggested that the DLS score had a better odds ratio for osteoarthritic joints to normal joints by a factor greater than two. The DLS score at 8-months of age was a good predictor of hip osteoarthritis and is useful for programs selecting for either hip dysplastic or normal dogs (Lust, et al., 2001b).

There is a proliferation of radiographic techniques for early diagnosis of canine hip dysplasia and hence timely decisions regarding breeding puppies. This trend indicates the importance of this genetic and multifactorial condition in dogs, worldwide.

Postmortem examination of joints for osteoarthritic changes is not routinely performed by most veterinary clinicians. Available literature describes studies on osteoarthritis in general and that secondary to canine hip dysplasia in young dogs in Europe and America (Doige and Weisbrode, 1995, Morgan, 1997).

Useful information may be obtained on changes in degenerating synovial membrane and articular cartilage. Interpretation of pathological findings, however, must also consider the age and clinical history. These findings would confirm the clinical and radiographic diagnosis of osteoarthritis. Visual inspection for colour, texture and morphology of the articular surfaces and evidence of osteophyte formation are useful features. The colour, texture and presence of villous hypertrophy of synovial membrane help to determine the severity of synovitis. Synovial fluid evaluation is useful in the distinction between infectious and non-infectious joint disease (Feldman, 1995).

The integrity and volume of ligamentum capitis femoris can be used to determine pathological alterations in the hip joint. The volume was estimated using the water displacement technique as previously described (Lust and Summers, 1981, Burton-Wurster, 1999). Histological evaluation of synovial membrane and articular cartilage has been described in details (Lust and Summers, 1981). Light

microscopy of articular cartilage and synovial membrane is not routinely performed. Histologic-histochemical assessment of articular cartilage following staining with Safranin-O or Toluidine Blue was reported in dogs (Dew and Martin, 1992).

Griesen, et al. (1982) reported light and electron microscopy of early degenerative changes of articular cartilage and synovium in hip joints of dogs with spontaneous joint disease. Synovium from joints with normal articular cartilage contained 33 % type A and 64 % type B cells. The first evidence of articular cartilage degeneration was loss of the surface amorphous layer and disruption of collagen fibrils.

Transmission and scanning electron microscopic studies of normal and inflamed synovium and articular cartilage were reported in dogs (Griesen, et al., 1982) and man (Aigner, et al., 2001, Lucchinetti, et al., 2002). Recent reports on electron microscopic studies of synovium and articular cartilage from adult dogs with canine hip dysplasia and osteoarthritis were not found in literature.

The loss of histochemical proteoglycan staining reflects the damage at the molecular level, whereas the supramolecular matrix destruction leads to fissuring and finally to the loss of cartilage. Chondrocytes react by increasing matrix synthesis, proliferating and changing their cellular phenotype. Gene expression mapping in situ and gene expression profiling allows characterization of the osteoarthritic cellular phenotype, a key determinant for understanding and manipulating the osteoarthritic disease process reviewed in man (Aigner and McKenna, 2002). However, the necessary technical expertise, and equipment, render these techniques inaccessible to researchers in developing countries.

2.4.6.0 Treatment and management

Treatment of animals with osteoarthritis can be considered in three stages; lifestyle adjustments, pharmacologic support and surgical treatment.

2.4.6.1 Lifestyle adjustments

In many dogs with clinical osteoarthritis, significant improvements can be made by lifestyle adjustments alone. Before initiating lifestyle changes, it is essential to establish therapeutic goals that are acceptable to the animal, the owner and the clinician. The ultimate aims of therapy will be influenced by many factors. These include the major presenting clinical problem, the type of dog and its usage, the requirements of the owner, the age and size of the dog, the presence of intercurrent disease or drug therapy that may interfere with the use of anti-inflammatory drugs and any financial constraints (Bennet and May, 1995).

Weight reduction is the single most important lifestyle adjustment for overweight osteoarthritic animals. Continual use of an osteoarthritic joint in a way that causes pain decreases the quality of life. However, the muscles and ligaments surrounding joints serve important protective functions (Bennet and May, 1995). In overweight dogs with clinical and radiographic signs of lameness due to hip osteoarthritis, weight reduction alone resulted in a substantial improvement in clinical lameness (Impellizeri, et al., 2000).

Controlled physical therapy, increasing muscle tone through exercise, is of proven benefit in the management of osteoarthritis in man. It is obviously impractical to instigate complex isometric exercise programs for dogs with osteoarthritis, but moderate exercise regimen should be maintained. Excessive level of exercise may be revised downward with progressive worsening of the osteoarthritis. It is important to reduce caloric intake in line with reductions in exercise levels to maintain an optimum body weight (Bennet and May, 1995).

2.4.6.2 Medical management

Drugs are important in the management of osteoarthritis, but they should be used judiciously and in combination with lifestyle adjustments. Many osteoarthritic patients have only transient or intermittent joint pain, and relatively few suffer severe unrelenting pain requiring permanent drug therapy. However, most dogs with clinical osteoarthritis require drug therapy (Johnston and Fox, 1997).

The goal of medical management for osteoarthritis is to improve quality of life by controlling pain, increasing mobility, preventing continued degradation of affected joints, and providing support to reparative processes. This is most successfully achieved by balanced weight and exercise control programs, and drug treatment. There are four categories of drugs; agents intended for use in human beings, including over-the-counter (OTC) drugs, veterinary nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, nutraceuticals and glycosaminoglycans (Johnston and Fox, 1997).

The most commonly recommended drugs for medical treatment of osteoarthritis in dogs are NSAIDs. These agents typically are antipyretic, analgesic and anti-inflammatory, but not all NSAIDs have a lot of these effects. The analgesic dose of most NSAIDs is about half the anti-inflammatory dose, for reasons that are not completely understood. Nonsteroidal anti-inflammatory drugs suppress synthesis of prostaglandins by inhibiting transformation of arachidonic acid to prostaglandins via blockage of the enzyme cyclooxygenase (COX). The NSAIDs also exert an inhibitory effect on neutrophil activation that is independent of COX inhibitory effect on neutrophils. Some NSAIDs have only limited anti-inflammatory activity, possibly because by blocking only the COX pathway of the arachidonic cascade, they allow precursors to enter the lipoxygenase pathway and still cause considerable inflammation (Johnston and Fox, 1997).

Nonsteroidal anti-inflammatory drugs are extremely valuable and generally well tolerated agents for treatment of osteoarthritis and musculoskeletal disorders. However, they are potentially lethal irritants of the gastrointestinal mucosa and renal system, can cause bleeding tendencies by preventing thromboxane A formation in platelets (which decreases platelet adhesion) and may inhibit enzyme activity required for chondrocyte replication and biosynthesis of proteoglycans within articular cartilage (Johnston and Fox, 1997).

With increasing attention to pain relief within the veterinary profession has come a parallel increase in the use of NSAIDs, as well as a concomitant rise in the prevalence of NSAID-induced toxicoses (Johnston and Fox, 1997). Because many NSAIDs are available over-the -counter, they are often mistakenly assumed to be for use in dogs. Drugs that are suitable for use in dogs and available in Kenya include paracematol, phenyl butazone and ibuprofen. These drugs are not used for a prolonged duration of administration (i.e. more than 14 days) because of their potential to cause gastrointestinal irritation.

In fact, there are few NSAIDs approved for use in dogs and some of these are potentially toxic. Extrapolation of human use to use in dogs should be done cautiously because of possible differences in volume of distribution, therapeutic concentrations or safety margin. Furthermore, there are differences between breeds of dogs in the effects induced by NSAID (Johnston and Fox, 1997).

Johnston and Fox (1997) reviewed the mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis (Figure 6). Many of the inflammatory products believed to be important in development of osteoarthritis are released as a result of cell membrane damage and subsequent arachidonic acid metabolism, and these products are involved in the production of pain (Johnston and Fox, 1997).

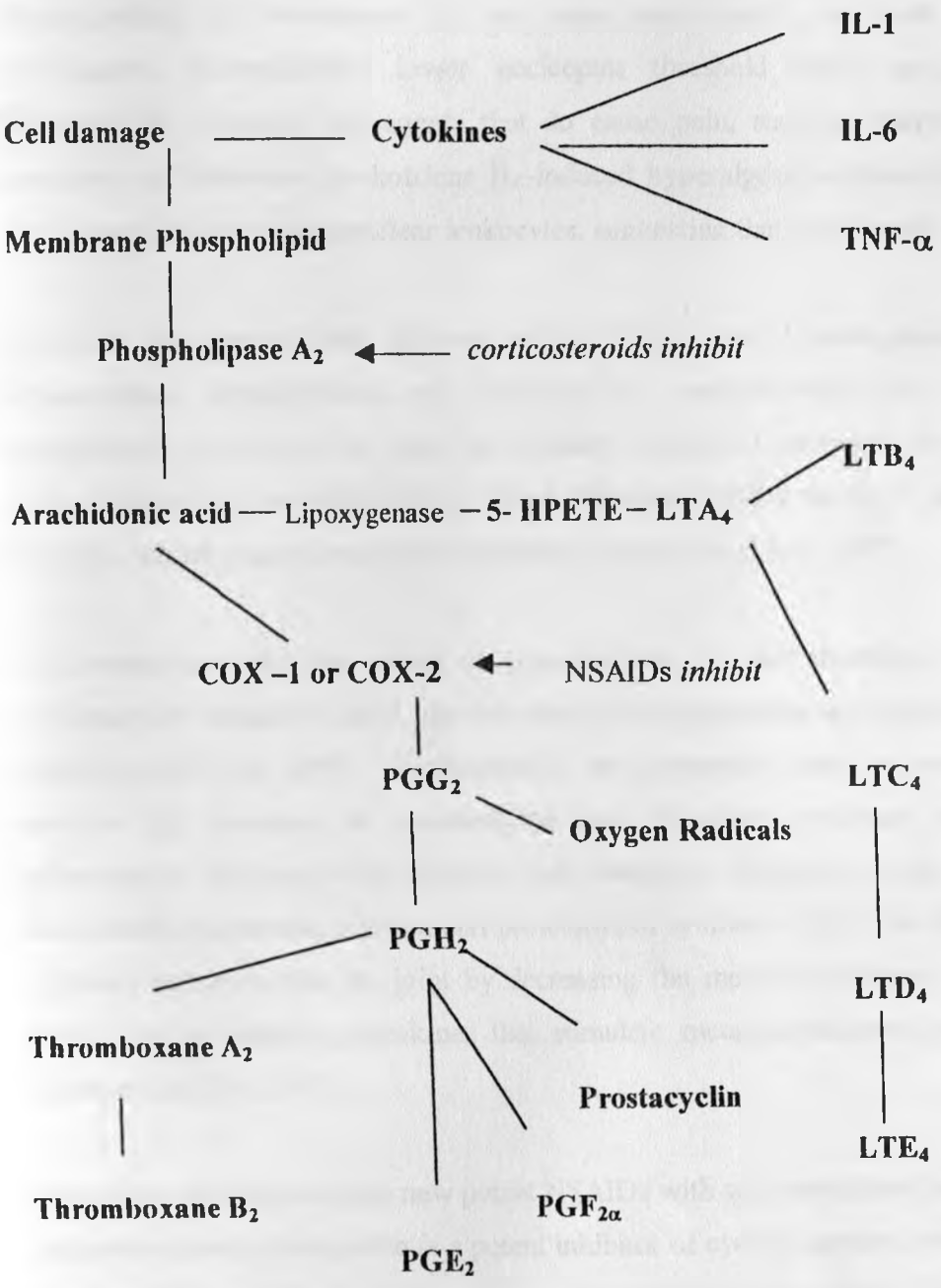


Figure 6. Metabolic pathways involved in the production of proinflammatory mediators following cell damage (Johnston and Fox, 1997).

IL – Interleukin; **TNF-α** -Tumuor Necrosis Factor- α; **COX** - cyclooxygenase;

5 HPETE 5-hydroperoxyeicosatetraenoic acid;

LT - Leukotrienes;

PG - Prostaglandins.

Prostaglandins and leukotrienes do not cause pain directly, but both cause hyperalgesia. Prostaglandins lower nociceptor threshold levels and, thus potentiate the effects of the agents that do cause pain, such as bradykinnin, serotonin and histamine. Leukotriene B_4–induced hyperalgesia is dependent on the presence of polymorphonuclear leukocytes, suggesting that a neutrophil

product is the cause of pain. Because of the critical role of prostaglandins in inflammation, administration of NSAIDs or corticosteroids to inhibit prostaglandin production has been the primary method of treatment for many years. Nonsteroidal anti-inflammatory drugs effectively inhibit the COX pathway and, thus, inhibit production of prostaglandins (Johnston and Fox, 1997).

Corticosteroids inhibit the action of phospholipase A_2 and therefore, inhibit production of arachidonic acid, the precursor of prostaglandin and leukotrienes (Johnston and Fox, 1997). Corticosteroids are frequently used in veterinary medicine for treatment of osteoarthritis and effectively decrease synovial inflammation. However, when used at high dosages or frequently, these agents can potentially decrease collagen and proteoglycan synthesis. They also decrease catabolic activity within the joint by decreasing the metalloproteinases activity directly or by inhibiting cytokines that stimulate metalloproteinases synthesis (Johnston and Fox, 1997).

Ketoprofen and carprofen are new potent NSAIDs with well-established analgesic properties in dogs. Ketoprofen is a potent inhibitor of cyclooxygenase, with some in vitro inhibitory effect on lipoxygenase and synthesis of bradykinnin. Therefore, it inhibits synthesis and release of prostaglandins, and to some extent synthesis of leukotrienes, leading to a peripheral analgesic effect common to most NSAIDs. Ketoprofen also provides analgesic effects at the central level (Johnston and Fox, 1997). Vasseur, et al. (1995) reported on a randomized, controlled trial of the efficacy of carprofen, a nosteroidal anti-inflammatory drug, in the treatment of osteoarthritis in seventy dogs. Grisneaux, et al. (1999) compared the efficacy of

analgesia provided by ketoprofen and carprofen and the nature and importance of their adverse effects when administered prior to orthopedic surgery in dogs. Carprofen inhibits cyclooxygenase (prostaglandin synthetase), blocking production of prostaglandin and provides analgesic, anti-inflammatory and antipyretic effects. Longer-term studies and crossover comparisons with other drugs are necessary.

Bargai (1999) described a clinical trial with cartoflex (a product made from natural collagen type II) as a treatment against limitations caused by degenerative joint disease due to hip dysplasia in dogs in Israel. Although the clinical signs of lameness disappeared completely in most of the dogs, there was no improvement in the severity of osteoarthritis on radiographic examination.

Polysulfated glycosaminoglycan (PSGAG) has been used for treatment of osteoarthritis in people in Europe and equine patients in North America since early 1980s. PSGAG inhibits the loss of glycosaminoglycan induced by enzymes, most notably metalloproteinase, scavenges free radicals, blocks the inflammatory complement cascade and stimulates the metabolism of synoviocytes. In addition, PSGAG blocks interleukin-1 mediated loss of glycosaminoglycan in cartilage tissue culture explants (Gustafson, 1993). Although PSGAG has been used in dogs (Huber, 1994, Todhunter and Lust, 1994, Innes, et al., 2000b) and horses (Phillips, 1994) in other parts of the world, the product is not available in Kenya. Similarly, hyaluronic acid is not commonly used in dogs because of its high cost (Bennet and May, 1995).

2.4.6.3.0 Surgical management

Surgical therapy may be considered if non-surgical therapy is no longer effective or in prolonged disability. The ultimate goal of the surgical therapies is to improve the quality of life for the patient (Brinker, et al., 1990).

2.4.6.3.1 Triple pelvic osteotomy

In young patients with minimal evidence of arthritis, a stabilizing reconstruction of the hip joint is recommended. This involves reconstructing congruency and stopping the subluxation and laxity that lead to severe arthritis, a technique referred to as triple pelvic osteotomy. It is a reliable and predictable mode of treatment in those cases which meet the case selection criteria. The objectives for this technique are to; increase the amount of acetabular coverage over the femoral head by rotating the acetabular portion of the pelvis; maintain the normal architecture and congruency of the femoral head and acetabulum; and prevent the development of osteoarthritis (Olmstead, 1994).

2.4.6.3.2 Femoral head and neck excision arthroplasty

This salvage procedure can be performed in dogs of all ages. The objectives are to remove the femoral head and neck, eliminate painful contact points in the joint, and allow a fibrous tissue joint to replace the ball and socket joint. A cranial approach or a ventral approach is used to perform osteotomy of the femoral neck by cutting the bone from the lateral-most edge of the trochanteric fossa to a point just dorsal to the lesser trochanter (Brinker, et al., 1990).

2.4.6.3.3 Pectineal myotomy

This procedure can be done on dogs of all ages. Performance of this procedure does not exclude attempting other procedures, should this be unsuccessful. This procedure does not alter the progression or intensity of changes in the joint caused by hip dysplasia, but may palliate joint pain. The objectives are to remove the belly of the pectineus muscle bilaterally and to alter the muscle pull on the hip, thus changing the articular contact points. It is only useful when performed on very young animals (Brinker, et al., 1990).

2.4.6.3.4 Intertrochanteric osteotomy

Patient selection is important and qualifications include; a marked increase in the angle of anteversion and / or inclination; minimal degenerative changes in the joint, and an age close to skeletal maturity (6-8 months). The objectives are to decrease the angles of inclination and anteversion (Olmstead, 1994).

Evers, et al. (1997) reported a retrospective study to determine whether intertrochanteric osteotomy can prevent the progression of osteoarthritis in dysplastic hip joints. Lameness was scored according to a grading system. A scoring system was also developed to assess radiographically evident osteoarthritis on a ventrodorsal projection of the coxofemoral joints on excision. It was concluded that intertrochanteric osteotomy does not prevent progression of osteoarthritis in the dysplastic hip. Knowledge of the long-term effects of intertrochanteric osteotomy is essential for surgeons trying to achieve improvements in dogs with hip dysplasia.

2.4.6.3.5 Total hip replacement

Total hip replacements in the dog have become well established over the past 20 years as an effective method for treating disabling hip conditions. A fixed head prosthesis has been the mainstay during most of this period. The procedure provides an artificial femoral head and artificial acetabular cup. It demands a high degree of technical proficiency and strict adherence to good aseptic and surgical techniques. Referral to an experienced specialist is recommended (Olmstead, 1995).

The growth plate must be closed before this procedure can be performed; thus, the animal must be at least 9 months of age. There is no upper age limit, but older animals should be evaluated for systemic disease. Depending on the size of the femur and the depth of the acetabular cup, the minimum weight of the animal is

13-18 kgs. The objectives are to replace the degenerative coxofemoral joint with a high-density polyethylene cup and a cobalt, chrome or titanium component. The acetabulum is replaced with a plastic cup prosthesis (Brinker, et al., 1990).

The aim is to provide a mechanically sound, pain-free joint that will last the dog's life. Over 95 % of dogs treated with this procedure have satisfactory function if established techniques are followed. Increased muscle mass, extended exercise tolerance and improved hip motion are commonly observed. Although osteoarthritis usually is present in both hips, 80 % of dogs receive sufficient relief that the other hip does not need to be replaced. The limb with the hip replacement becomes dominant, thus reducing the unoperated limb's weight-bearing load (Brinker, et al., 1990). Studies suggest that total hip replacement is more effective in returning large dogs to full functional weight bearing (Olmstead, 1995).

2.4.6.3.6 Juvenile pubic symphysiodesis

The pubic symphyseal growth plate contributes significantly to the development of the pelvis. Mathews, et al. (1996) first described pubic symphysiodesis (PS) in guinea pigs, in which the pubic symphysis was destroyed by electrocautery. PS resulted in significant narrowing and shortening of the pubic bones, and outward rotation of the acetabula.

Swainson, et al. (2000) reported the effect of pubic symphysiodesis on pelvic development in the skeletally immature greyhound. Specific measurements included acetabular ventroversion, Norberg angle, lateral center-edge angle and pelvic inlet dimension and hip distraction indices. Pubic symphysiodesis at 4 month of age using a stapling device failed. Pubic symphysiodesis using hand made staples was successful at 5 months of age and did not result in any clinically significant intraoperative or postoperative complications. Pubic symphysiodesis decreases pelvic canal size, increases acetabular ventroversion and does not appear to have any clinically significant complications. Pubic symphysiodesis

performed in skeletally immature dogs with hip dysplasia may provide an effect similar to a triple pelvic osteotomy and warrants further investigation (Swainson, et al., 2000).

Dueland, et al. (2001) described the long-term effects of juvenile pubic symphysiodesis (JPS) in dysplastic puppies. JPS resulted in significant ventrolateral acetabular rotation, increased hip coverage and diminished hip laxity, normal pain-free gait and insignificantly reduced pelvic size. Dysplastic hips in young dogs were significantly improved in JPS. These new surgical techniques are not frequently performed in Kenya. The required technical expertise, the costs and availability of orthopedic equipment (prostheses) make them not easily applicable in Kenya. However, some surgeons perform femoral head and neck resection as a salvage procedure for canine hip dysplasia and osteoarthritis in Kenya.

2.4.7 Control programs

Radiologic screening of coxofemoral joint conformation has been performed extensively in several countries around the world, in order to reduce its incidence. Veterinary clinicians have shared this responsibility with kennel clubs and breed clubs (British Veterinary Association / Kennel Club, 1994, Leighton, 1997).

Despite considerable research and the application of screening programs during the past three decades, the incidence of canine hip dysplasia remains disturbingly high. Mild canine hip dysplasia is underdiagnosed by subjective evaluation of adult dogs, while moderate canine hip dysplasia is underdiagnosed by subjective assessment of immature dogs (Smith, 1997, Adams, 2000).

Although radiographic, dynamic ultrasonographic and computed tomography techniques have been described, the distractive index is considered a highly predictive indicator of hip laxity in dogs (Adams, et al., 1998, Farese, et al., 1998,

Farese, et al., 1999, Adams, 2000, Adams, et al., 2000, Lust, et al., 2001b). Most of these techniques require that a puppy be at least one year of age, while some require that the animals be two years.

The concept of heritability of the condition contributes to the challenge. Heritability is defined as an estimate of how much environmental factors play in the expression of the inherited genes (Leighton, 1997).

This may be shown simply as;

$$H_2 = \frac{V_{genetics}}{V_{genetics} + V_{environment}}$$

H_2 = heritability index.

$V_{genetics}$ = variance due to genetics.

$V_{environment}$ = variance due to environment.

The higher heritability index means that environmental considerations are not as important as genetic elements. Leighton (1997) published a detailed review on the genetics of canine hip dysplasia. Wood, et al. (2000) studied the heritability of canine hip dysplasia and its components. Regression models showed strong positive relationships between offspring and parental hip scores and emphasized the need for both sires and dams; particularly dams to have zero or small hip scores. Reed, et al. (2000) reported that hip conformation scores, assigned by the Orthopedic Foundation for Animals as a criterion for breeding selection, have moderate heritability in dogs and selection of breeding stock with better hip conformation scores will increase the percentage of progeny with phenotypically normal hip joint conformation. This confirmed the value of selective breeding using the radiographic screening procedure.

Ohlerth, et al. (2001) described estimation of genetic population variables for six radiographic criteria of hip dysplasia in a colony of Labrador Retrievers. Canine hip dysplasia is heritable to a moderate degree, and signs of subluxation revealed the highest heritability estimates. Leppanen and Saloniemi (1999) concluded that control programs had not resulted in fast progress. Selecting against hip dysplasia cannot be expected to be very effective when based only on mass selection on phenotypic observations. Predictive breeding values based on progeny testing would probably give better results. Leppanen, et al. (2000) also reported the factors affecting hip dysplasia in German Shepherd dogs in Finland.

In contrast, the prevalence of hip dysplasia has decreased for several other nationwide screening programs. This was observed mainly in well-controlled, small population of dogs bred for specific purposes (Smith, 1997, Swenson, et al., 1997, Leighton, 1997, Todhunter, et al., 1999). Bliss, et al. (2002) reported quantitative genetics of traits associated with hip dysplasia in a canine pedigree constructed by mating dysplastic Labrador Retrievers with unaffected Greyhounds. A statistical model was designed to test the effects of Labrador Retriever and Greyhound alleles on age at detection of femoral capital epiphyseal ossification, 8-month distraction index and 8-month dorsolateral subluxation score. This pedigree should be useful for identification of quantitative trait loci underlying the dysplastic phenotype. The genetic quality of normal dogs, with reference to hip dysplasia, depends on the prevalence of dysplasia in the population from which they are recruited. Consequently, a decrease in prevalence of dysplasia in the family, line, or domestic population or even in the breed, increases the average quality of normal dogs in that breed.

Skeletal development in the dog results from an interaction of genetic, environmental and nutritional influences (Richardson, 1995). Long-term clinical experience indicates that commercial dog foods available in Kenya do not have sufficient minerals and require mineral and vitamins supplementation. In addition, dogs fed on red meat diets have been reported to develop osteoporosis and other

orthopedic problems. Schoenmakers, et al. (2000) reported that dogs fed on high calcium diet without a proportionally high phosphorus intake became hypercalcemic, hypophosphataemic, and had severe disturbances in skeletal development and mineralization, typical of rickets.

Nutrition is a major environmental factor; excess energy consumption increases the frequency and severity of hip dysplasia in genetically predisposed dogs (Kealy, et al. 1997). There was greater frequency and severity of osteoarthritis in ad libitum-fed group of dogs, while limited feeding had a beneficial inference on the phenotypic expression of hip dysplasia (Kealy, et al., 1992). Surgical procedures including pectineal myotomy, triple pelvic osteotomy and juvenile pubic symphysiodesis should be further studied to determine their effectiveness in controlling canine hip dysplasia and osteoarthritis in developing countries.

Screening for the phenotypic features of canine hip dysplasia is the only known control program practiced in Kenya for the last years. The Kenya Veterinary Board / East African Kennel Club Hip Dysplasia Scheme guidelines (1998) were adapted from the British Veterinary Association / Kennel Club guidelines. The availability of this service in Kenya has reduced the delays and expenses previously incurred when radiographs were submitted for evaluation in Britain.

While this programme focuses on preventing breeding of genetically predisposed dogs, it does not address the effects of the level of mineral content of diets for dogs in developing countries. High levels of nutrition have been shown (Keally, et al. 1997) to influence the incidence of orthopedic joint diseases including canine hip dysplasia. While the Kenya Veterinary Board / East African Kennel Club has established a hip dysplasia scheme, this service is accessible only to a few conscientious breeders. Even then, no systematic advice is provided to these breeders and dog owners on the risks of oversupplementation and overnutrition of puppies of large breed dogs in Kenya. These issues will continue to limit the effectiveness of the control programme that is practiced in this country. Although

the prevalence of the condition has not been determined, it is necessary to establish the clinical and pathological features of the disease in the country. The information would further improve diagnostic skill and also assist in the provision of accurate prognosis in the management and control of canine hip dysplasia in Kenya.

The results are used to advice whether to breed from such dogs or not. However, many dogs are allowed to breed without regard for genetic quality. This is due to the lack of awareness on the genetic and nutritional factors that predispose large breed dogs towards being afflicted by orthopaedic joint diseases such as canine hip dysplasia.

The genetic, nutritional, environmental and occupational factors contributing to canine hip dysplasia in Kenya are different from those in Europe and America. This study aimed to establish baseline diagnostic and prognostic features of the condition in Kenya. This would sharpen existing diagnostic skill, develop useful local resource material for teaching and generate additional scientific information. The data would facilitate wider cross-sectional studies of the disease in Kenya.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 The study animals

3.1.1 Location

The study was conducted at the Faculty of Veterinary Medicine, University of Nairobi and the International Livestock Research Institute (ILRI), Kenya. The geographical features are tropical Savannah, at an altitude of 1662 ft above sea level. The larger part of the Nairobi City is developed with most inhabitants either residing in the urban or peri-urban parts of the city. Most households keep dogs as pet, guide or guard dogs.

3.1.2 Selection, admission and accommodation

German shepherd dogs were recruited into the study for consistency and the fact that they are known to be more susceptible to canine hip dysplasia and osteoarthritis. A total of 36 adult German shepherd dogs (or crosses) with history and clinical signs of hind limb lameness were used. Twenty one (58.3 %) of the dogs were male while 15 (41.7 %) were female. The average weight was 27.3 kgs. The estimated age (based on dentition, history and available records) of the animals ranged between 4 and 17 years, with a mean of 9.25 years (Appendix II-III). Older dogs were selected because the probability of developing osteoarthritis increases with age (Smith, et al., 2002) and owners were more willing to give consent for humane disposal of old and severely lame dogs (for postmortem evaluation) than puppies which could be managed by medical or surgical therapy when affected.

The study animals were obtained from veterinary clinics around Nairobi. The veterinarians had been informed about the objectives of the study and gave prior consent, before their animals were recruited into the study. Patients submitted to the University Veterinary Clinic for humane disposal for various reasons; were also included with prior consent from the owners. The dogs were from the military and police forces, private security firms, Dog Pound of the Nairobi City Council, referrals by private practitioners or clients at the Small Animal Clinic, University of Nairobi.

The dogs were admitted (on different dates) according to routine University of Nairobi veterinary clinic protocol for admission of patients. Dogs were housed in kennels at the Small Animal Clinic. Food consisted of a daily meal of commercially prepared cereal (Besbix^{R} Procta & Allan, Kenya), and a mixture of bone and meat. Water was provided ad libitum.

3.2.0 Medical history and clinical examination of the study animals

Medical history was obtained from the dog owners and the available medical records. Animals with normal joint function were also included in the study for comparison with the ones that were ill. Animals presented with signs of hindlimb lameness were also studied. All the dogs were subjected to general physical examination and orthopedic examination for hindlimb lameness.

Orthopedic examination involved observation of dogs for general body condition, conformation, limb position when standing, musculoskeletal symmetry, and any outward signs of trauma. Specific abnormalities of gait such as altered stride length, limited range of voluntary joint movement, and abnormal limb movement were noted. The response to digital palpation, flexion and extension of the hip joints was noted as previously described (Bardet, 1995, Kelly, 1995). The degree of lameness was categorized as normal, mild or severe and assigned a numerical grade 1, 2 or 3, respectively according to a modified criteria for lameness

osteoarthritis (Appendix I C). The clinical category for the study animals was recorded (Appendix II-III). Photographs were taken to illustrate normal posture and varying degree of lameness attributable to the hip joints of the study animals.

3.3.0 Radiographic examination of hip joints of the study animals

Standard pelvic extended ventrodorsal radiography of the thirty-six dogs was performed. This was done while the animals were under general anesthesia, as previously described (Allan, 1998). Acetylpromazine (Tranquilin^{R} injectable solution, Acepromazine Maleate diluted to a concentration of 2 mg / ml) was administered intravenously at a dose rate of 0.04 mg per kg body weight for premedication. General anesthesia was induced using intravenous thiopental sodium (Thiopentone injection, BP 500 mg Rotex, Medica GMBH, Germany 50 mg /ml at 25 mg per kg) or intravenous pentobarbitone sodium (60 mg/ml at 25 mg per kg, Sagatal^{R} Rhone Meriux, Essex). Alternatively animals were given intravenous xylazine hydrochloride (20 mg/ml at 2 mg per kg, Agrar^{R} Holland) and ketamine hydrochloride (50 mg/ml at 10 mg per kg, Agrar^{R} Holland) combination.

For each dog, the radiographic exposures were made (at 65 kV, 3 mA for 6 seconds and while the cassette was placed under the burky table) and routinely processed (Allan, 1998). Based on the radiographic findings, the 72 joints were graded according to criteria on the severity of degenerative joint disease previously described by Rasmussen, et al. (1998) and illustrated in Table 4. The scores were recorded for analysis as given in Appendix IV and V. The radiographic features were described. Radiographs were also photographed. Although the distractive index and Norberg angle measurements are more accurate tools for assessing hip joint laxity, these methods require technical equipment (custom-made devices) and skill generally not available in Kenya. The use of radiographic scores represents a more clinically applicable diagnostic tool for most clinicians in developing countries, such as Kenya.

Table 4. Radiographic grading system for assessing the degree of coxofemoral osteoarthritis and canine hip dysplasia.

Radiographic grade	Radiographic features
Grade O	C-shaped acetabulum, dorsal rim rounded distinct femoral neck.
Grade 1	Shallow acetabulum or marked dorsal rim attenuation, moderately osteophytic acetabular margin, rounded femoral head, minimal osteophytes on the femoral neck.
Grade 2	Shallow acetabulum, or marked dorsal rim attenuation, moderately osteophytic acetabular margin, flattened femoral head, shortened femoral neck with osteophytes.
Grade 3	Flat acetabulum, severely osteophytic acetabular margin, marked flattened or irregular femoral head, severely shortened femoral neck with osteophytes.

(Rasmussen, et al., 1998).

3.4.0 Postmortem examination of the hip joints

Thirty-two of the dogs were euthanised by intravenous administration of pentobarbitone sodium (200 mg / ml; Euthatal^{R} Rhone Meriux, Dublin) and then exsanguinated to reduce the amount of blood in the carcass. The area of the hip joints was skinned to expose the pelvic muscles and bones. The femur was disarticulated at the stifle joint. The hip joints were harvested by excising the semitendinosus, semimembrinosus and quadriceps femoris muscles from the femur and the gluteal muscles from the pubis, ischium and ileum.

Cutting the pubis, ilium and ischium using a band saw isolated the hip joint. Excess connective tissue was excised from the hip joint using a scapel blade. Incising the joint capsule with a scapel blade exposed the joint cavity and articular surface.

3.4.1 Measurement of the volume of ligamentum capitis femoris

The presence and integrity of ligamentum capitis femoris was determined by visual inspection of exposed hip joints. The volume of ligamentum capitis femoris was determined for each joint using a previously described water displacement technique (Lust and Summers, 1981, Burton-Wurster, et al., 1999).

The ligament was excised by dissecting its origin at the fovea capitis and its attachment to the acetabular fossa. A 10 ml-calibrated (glass) cylinder was filled with water and the initial reading obtained and recorded. The ligamentum capitis femoris was then picked with thumb forceps and immersed in the water contained in the cylinder. A final reading of the water level in milliliters was obtained and recorded. The volume of ligamentum capitis femoris was calculated by subtracting the initial reading of the meniscus from the final reading of the meniscus. To facilitate comparison of the data, a reading of zero was assigned to any joint in which the ligament was not present.

The volume of ligamentum capitis femoris was compared for all joints and recorded as shown in Appendix IV. The volume of ligamentum capitis femoris was grouped on the basis of radiographic scores and the mean for these groups compared.

3.4.2 Pathological changes in synovial membrane and joint cavities

The gross pathology of synovial membrane from the opened hip joints was described based on color, surface integrity, contour, consistency and texture. The presence of villous hypertrophy, cartilaginous and bone tissue were determined by visual inspection and digital palpation for each joint.

3.4.3 Pathological evaluation of degradation of articular cartilage

The gross pathology of articular cartilage of the 64 hip joints was examined by visual inspection. The presence and extent of articular cartilage defects (such as periarticular osteophytes, flaking, fibrillation or eburnation of subchondral bone) were determined by visual inspection of articular surfaces of the femoral head and acetabulum. Articular cartilage from the femoral head and acetabulum of 12 representative hip joints was immersed in 10 % formalin to preserve the gross pathological features.

3.4.4.0 Light microscopic evaluation of osteoarthritis

3.4.4.1 Histological changes in synovial membrane and joint mice

Of the 64 hip joints examined for gross pathological changes, 24 representative joints were examined histologically to evaluate the status of synovial membrane. These represented a sample of the various grades of osteoarthritis determined by radiography and gross pathology.

Sections of joint capsule (with synovium) were taken from the cranio-ventral (rostral) and cranio-dorsal areas of the femoral neck and fixed in 10% buffered formalin solution for 7-14 days. The tissues were trimmed, dehydrated in serial dilutions of alcohol and embedded in paraffin wax. Sections, 5 μm thick, were prepared using a microtome, mounted onto microscope slides by water floatation and routinely stained with hematoxylin and eosin. Evaluation of the synovium was performed using a light microscope, on coded sections without knowledge of other joint parameters. Specimens were scored according to the method described by Griesen, et al. (1982) and shown in Table 5.

Further studies using formalin fixed tissue from adult dogs with severe osteoarthritis of the hip joint, were conducted to determine the basis for the synovial thickening and formation of firm joint masses. A sample of the joint masses was fixed in 10 % formalin solution for 2 weeks and decalcified in 10 % nitric acid for 3 weeks. The sample was appropriately trimmed, dehydrated in alcohol and embedded in paraffin wax. 5 μm thick sections were prepared using a microtome and routinely stained with hematoxylin and eosin for histological examination. The other joint masses were preserved in 10 % formalin solution.

3.4.4.2 Histologic and histochemical grading of articular cartilage degradation

Slabs of articular cartilage of the femoral head and neck from 22 representative hip joints were prepared using a band saw. The slabs were fixed in 10 % formalin solution for 7-14 days. They were then decalcified in 10 % nitric acid for 2-3 weeks and routinely prepared for histological examination. Examples of such slabs are illustrated in Figure 7.

Table 5. Criteria for histological grading of changes in synovial membrane of hip joints in adult dogs.

Histological score	Histological features
Score 0	Normal tissue.
Score 1	Mild synovitis, which revealed focally thick synovium with plumper hypertrophied cells, sometimes producing a small-localized thickening (plaque) or villous extension.
Score 2	Synovial proliferation which involved 50% to 75% of the surface examined. Villi were longer and more common, sometimes also lined by hyperplastic synovium. Capillary neovascularization and mild mononuclear infiltrates were sometimes observed in the adjacent collagenous stroma.
Score 3	Synovial proliferation involved the entire surface. Villous proliferation varied from numerous small structures to a mixture of small and large, stout villi. Lymphocyte infiltrates were heavier with focal aggregation in some cases.
Score 4	At times islands of cartilage from the eroded articular surface were embedded in the synovium.

(Griesen, et al., 1982).

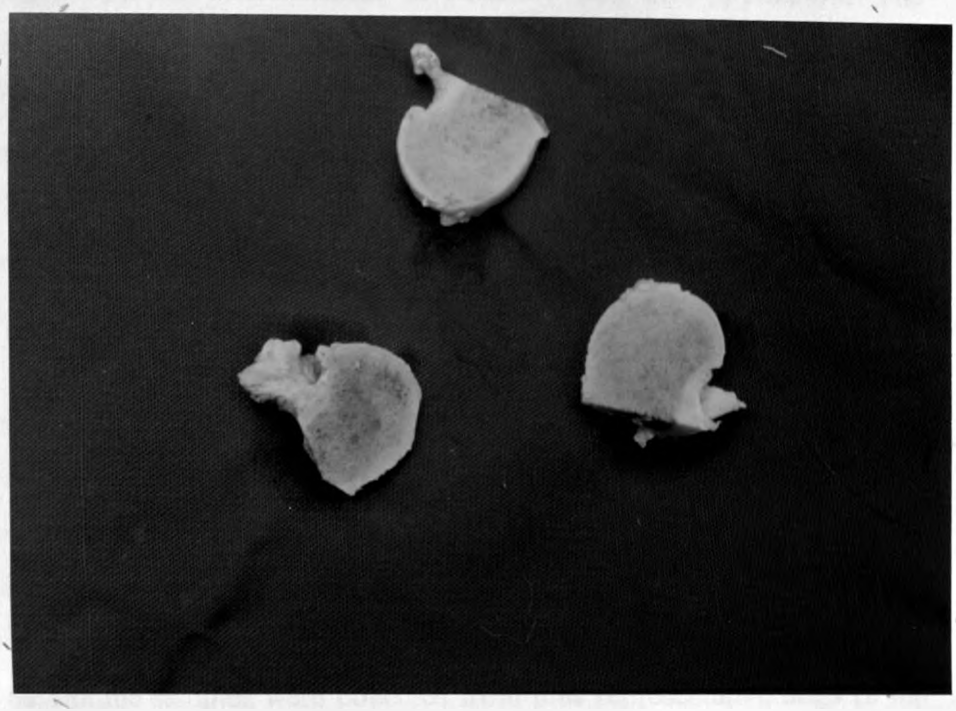


Figure 7. Slabs of articular cartilage from the femoral head, fixed in 10 % formalin solution for 14 days, decalcified in 10 % nitric acid for 3 weeks and ready for histological processing.

The slabs were embedded in paraffin, cut at 5 μm using a microtome and stained with Safranin-O, fast green and iron hematoxylin. Five histological sections of articular cartilage from each of the 22 representative hip joints were examined by a light microscope for abnormalities in structure and cell population. The distribution and intensity of Safranin-O stain and tidemark integrity were also evaluated. Numerical scores were assigned as the histologic–histochemical grade (also referred to as Mankin scores) according to previously described criteria (Dew and Martin, 1992), illustrated in Table 6. Articular cartilage degradation was further graded using previously described criteria (Dew and Martin, 1992), illustrated in Appendix XI.

3.4.5.0 Electron microscopic evaluation of synovial membrane and articular cartilage degradation

3.4.5.1 Changes in the synovial membrane

Synovial membrane samples were collected from four representative dogs (8 hip joints) for electron microscopic evaluation. The samples were immersed in 3 % glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) and fixed for 4 hour at 4 ° C. After the material was washed with buffer, it was postfixed in 1 % osmium tetroxide for 1 hour at 4 ° C and then soaked for 1 hour in 0.5 % uranyl acetate. The samples were dehydrated in a graded series of ethanol and embedded in plastic (Epon Araldite). 5 blocks were prepared from each joint.

Semi-thin sections were prepared using an ultramicrotome, stained in toluidine blue and examined with a light microscope for orientation of special areas of interest to be obtained for ultrastructural study. Gray to silver ultrathin sections were picked up on copper-coated 200 mesh grids and stained in 2 % uranyl acetate and lead citrate. The sections were examined in transmission electron microscope (JEOL 1010, GMBH, Germany). The morphology of synoviocytes was qualitatively evaluated to outline their response in chronic synovitis.

Table 6. Criteria for histological grading of articular cartilage based on structure, cells, Safranin-O staining and tidemark integrity.

(I)	**Structure**		**Grade**
	(a)	Normal.	0
	(b)	Surface irregularities.	1
	(c)	Pannus and surface irregularity.	2
	(d)	Clefts to transitional zone.	3
	(e)	Clefts to radial zone.	4
	(f)	Clefts to calcified zone.	5
	(g)	Complete disorganization.	6
(II)	**Cells**		
	(a)	Normal.	0
	(b)	Diffuse hypercellularity.	1
	(c)	Cloning.	2
	(d)	Hypocellularity.	3
(III)	**Safranin-O staining**		
	(a)	Normal.	0
	(b)	Slight reduction.	1
	(c)	Moderate reduction.	2
	(d)	Severe reduction.	3
	(e)	No dye noted.	4
(IV)	**Tidemark integrity**		
	(a)	Intact.	0
	(b)	Crossed by blood vessels.	1

Dew and Martin (1992).

3.4.5.2 Articular cartilage degradation

Tissue for electron microscopy was obtained adjacent to those removed for light microscopy. Samples from 4 representative (8 hip joints) dogs were fixed in buffered 3% glutaraldehyde (pH 7.2). Cartilage was cut into 2 mm wide and up to 8 mm long and decalcified in EDTA for 4 weeks. Slices were postfixed in 1% osmium tetroxide and 0.5% uranyl acetate.

All cartilage was embedded in Epon-Araldite. 5 blocks of articular cartilage from the femoral head of 4 representative dogs were sectioned perpendicularly to the surface. Histological sections were stained with toluidine blue and examined under a light microscope for orientation.

Ultrathin (gray to silver thin) sections were picked up on copper coated 200-mesh grids and stained in 2 % uranyl acetate and lead citrate as described (Griesen, et al., 1982, Lucchinetti, et al., 2002). Electron microscopy was conducted as described. Micrographs from interesting areas of the samples were examined. Electron micrographs were also taken and saved on computer software for ease of evaluation and inclusion in the thesis.

3.5 Statistical analysis

The minimum sample size (36) used was determined by assuming an estimated prevalence rate of 10 % for hip osteoarthritis in dogs in Kenya and a confidence level of 90 %, according to Thrusfied (1995). The distribution of the clinical, radiographic and pathological grades was expressed as percentages. Analysis of variance (ANOVA) and t-test were used to compare data on ligamentum capitis femoris ($p < 0.05$). Correlation coefficients (Pearson correlation coefficient (r), Kappa (K) statistics) were determined between the volume of ligamentum capitis femoris and radiographic grades of osteoarthritis, and radiographic and pathological grades of osteoarthritis (Freund and Wilson, 1997).

CHAPTER FOUR

4.0 RESULTS

4.1.0 General

No significant difference ($p<0.05$) was observed between the mean body weight of dogs that were normal (27.1 kgs), with mild (24.9 kgs) and those with severe lameness (28.3 kgs). Fourteen of the 21 males (39.9 %) had hindlimb lameness, while 7 of the 15 females (22.2 %) had hindlimb lameness (a ratio of 1.75 males: 1 females).

4.2.0 Clinical features of osteoarthritis of the hip joint

The distribution of the results of clinical examination of dogs (normal, mild or severe hindlimb lameness) is presented in Table 7 and Figure 8. Of the 36 animals that were admitted into the study, 14 (38.9 %) were clinically normal, 6 (16.7 %) were found to have mild lameness of the hindlimbs while 16 (44.4 %) had severe and debilitating lameness that required euthanasia. Thus 22 (61.1 %) animals examined in the study had either mild or severe signs of hindlimb lameness related to the hip joints.

The 14 dogs that were classified as normal had good pelvic limb muscle cover, no signs of pain or gait abnormality, and with no changes on the physical conformation of the animals (Figure 9).

The 6 dogs showing mild lameness, presented mild pain while walking, slight atrophy of the muscles of the hindlimbs, severe pain on flexion and extension of the hip joint, crepitus and resentment when the hip joint was abducted or manipulated. Intermittent signs of lameness were elicited by a short walk. Figure 10 A and B illustrate two dogs which presented mild hindlimb lameness.

The 16 dogs classified as having severe hindlimb lameness had a history of prolonged lameness, previous medication for lameness, complete disability leading to dragging of the feet on the floor, or inability to rise or walk. The more severely affected dogs had decubital wounds on the dorsal parts of the paws and reduced pelvic paw position reflex. These dogs also had severe atrophy of the hamstring muscles and associated compensatory enlargement of the pectoral muscles.

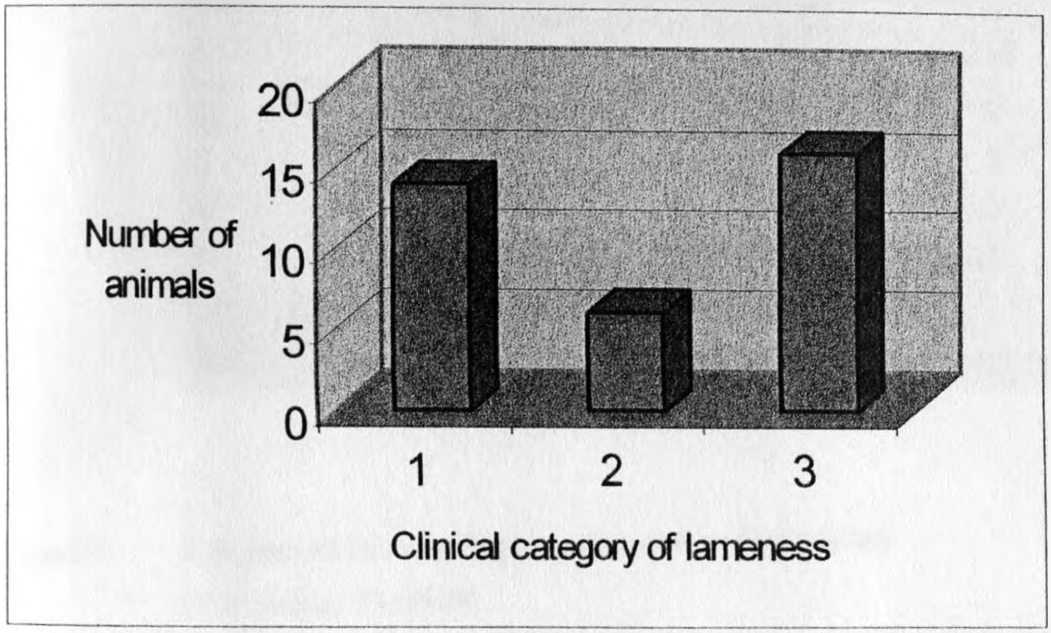
Seven animals in this category had a wobbling or swaying gait and inability to walk in a straight path. Conformational deformity of the back and pelvic area due to chronic pain occurred frequently in this category. Figures 11 A, B and C illustrate some of the clinical signs of the animals with severe hindlimb lameness caused by osteoarthritis of the hip joint.

A poor prognosis for medical and surgical management of osteoarthritis of the hip joint was recorded in the 16 adult German shepherd dogs in the study due to severe clinical manifestations and old age.

Table 7. Clinical categories of hindlimb lameness in 36 adult German shepherd dogs in Kenya.

Clinical Categories	Code number of study animal						Total
Normal	8	9	12	16	20	25	14
	28	30	31	36	38	39	
	40	41					
Mild Lameness	7	32249	17	27	33	13	6
Severe Lameness	1	11	14	15	18	19	16
	22	23	24	26	29	34	
	32	10	32510	35			

Figure 8. Distribution of the clinical categories of hindlimb lameness in 36 adult German shepherd dogs in Kenya.



Key:

Category	1	=	Normal.
Category	2	=	Mild Lameness.
Category	3	=	Severe Lameness.

Figure 9. A 7-year-old German shepherd dog with normal posture.
Case number 31359-28.
Note the abundant muscle cover on the hindquarters and the hip joint area.

Figure 10 A. A 9-year-old German shepherd dog (crossbreed) with signs of mild hindlimb lameness.
Case number 31359-27.
The dog adopted an adducted posture while standing.

Figure 10 B. An 11-year-old German shepherd dog with mild hindlimb lameness.
Case number 31359-19.
Notice the dropped hip joints and the muscle atrophy of the hindlimbs while the dog is standing.

Figure 11 A. An 8-year-old German shepherd (crossbreed) dog with severe lameness and change in conformation of the hindlimbs.
Case number 31359-14.
Notice the wobbly posture and the muscle atrophy of the hindlimbs.

Figure 11 B. A 15-year-old German shepherd dog with severe hindlimb lameness leading to recumbency.
Case number 31359-24.
The dog was lying on the floor, unable to bear weight on its hindlimbs.

4.3.0 Radiographic findings

Data on radiographic findings is presented in Table 8, Appendix IV and illustrated in Figure 12.

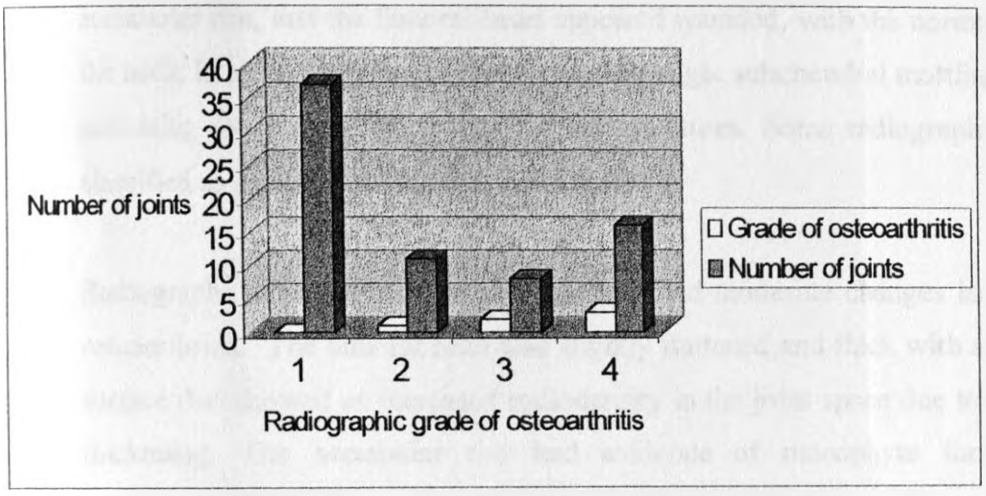
In the 72 hip joints that were radiographically examined, 37 (51.4 %) were normal and were assigned grade 0. These joints were from animals with no clinical signs of hindlimb lameness. The hip joints considered as normal represented approximately half of the total number of hip joints evaluated in the study.

48.6 % of the hip joints evaluated in this study were assigned radiographic grades 1, 2 or 3. Out of these, eleven (15.3 %) hip joints were scored as grade 1, 8 (11.1 %) as grade 2 and 16 (22.2 %) as grade 3. There was a distinct difference between hip joints placed in grade 1 and those in grade 2, and 3. However, careful radiographic examination was required to differentiate between radiographic features of joints in grade 0 and those in grade 1.

Table 8. Radiographic grades of osteoarthritis of hip joints from 36 adult German shepherd dogs in Kenya.

Radiographic grade	Number of joints	Percentage (%)
0	37	51.4
1	11	15.3
2	8	11.1
3	16	22.2
Total	72	100

Figure 12. Distribution of the radiographic grades of osteoarthritis for hip joints from 36 adult German shepherd dogs in Kenya.



Key:

Radiographic grade;

1 corresponds to grade 0

2 corresponds to grade 1

3 corresponds to grade 2

4 corresponds to grade 3

The 37 hip joints classified as normal, had well rounded femoral heads, fitting at least 50 % into the acetabulum, no remodeling or osteophytes, and no subchondral sclerosis. The radiographic appearance of some of the coxofemoral joints considered as normal is illustrated in Figure 13.

The radiographic appearance of hip joints scored as grade 1 had mild signs of osteoarthritis. These joints had a slight increase in radio-density around the acetabular rim, and the femoral head appeared rounded, with the normal shape of the neck. In some radiographs there appeared slight subchondral mottling, perhaps indicating evidence of affection of these structures. Some radiographs of joints classified as grade 1 are illustrated in Figure 14.

Radiographs of hip joints scored as grade 2 had moderate changes indicative of osteoarthritis. The femoral head was slightly flattened and thick with an irregular surface that showed an increased radiodensity in the joint space due to soft tissue thickening. The acetabular rim had evidence of osteophyte formation. A radiograph of hip joints assigned grade 2 is illustrated in Figure 15.

Hip joints scored as grade 3 had signs of severe osteoarthritis. These included flattening of the femoral head, extensive periarticular osteophytes, increased radiodensity in the joint cavity and periarticular tissue and shortening and thickening of the femoral neck. In more severe cases there was obvious subluxation or complete luxation of the femoral head. A radiograph of hip joints assigned grade 3 is illustrated in Figure 16.

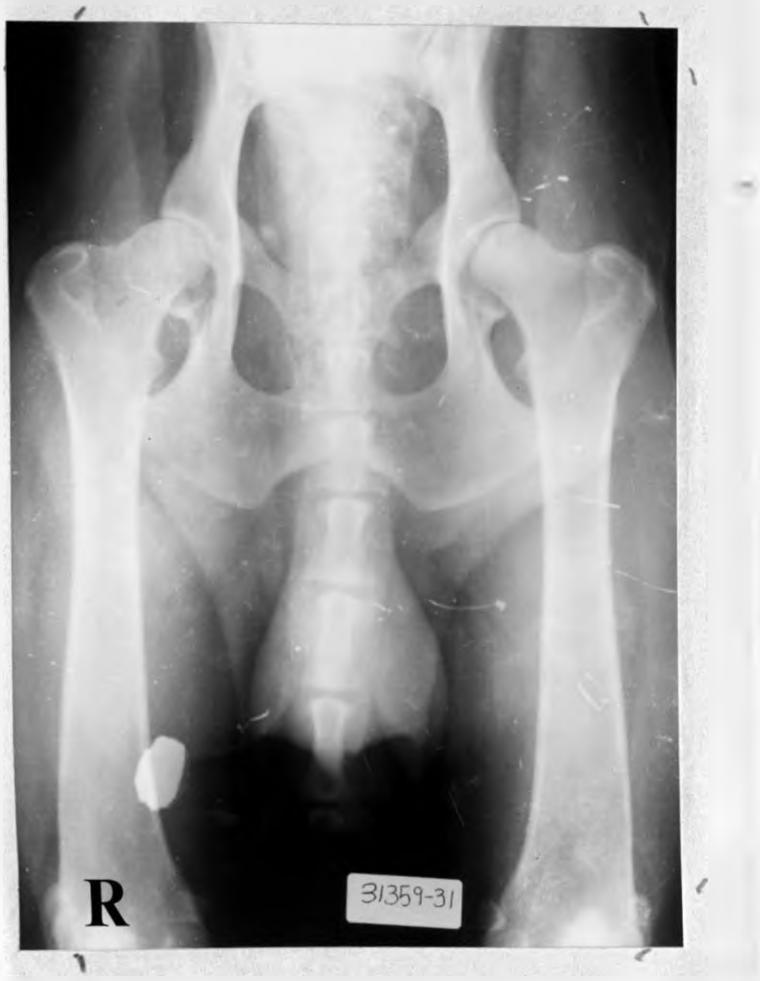


Figure 13. A ventrodorsal pelvic radiograph of a 10-year-old female German shepherd dog with normal hip joints graded zero.
Case number 31359-31.
Note the smooth and round femoral head fitting in the acetabulum.

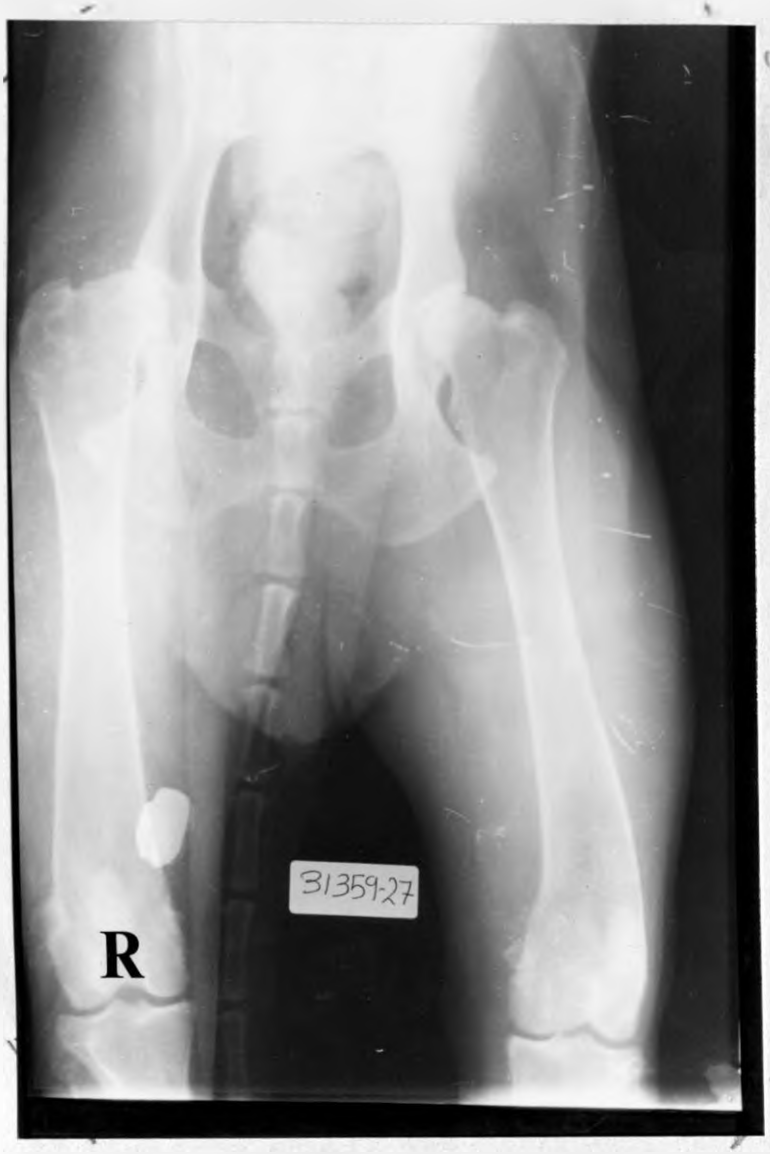


Figure 14. A ventrodorsal pelvic radiograph of a 10-year-old female German shepherd dog with osteoarthritis of the hip joints (grade 1 on the left and grade 2 on the right joint).
Case number 31359-27.
The femoral neck of the right joint appears thick and the femoral head is subluxated.

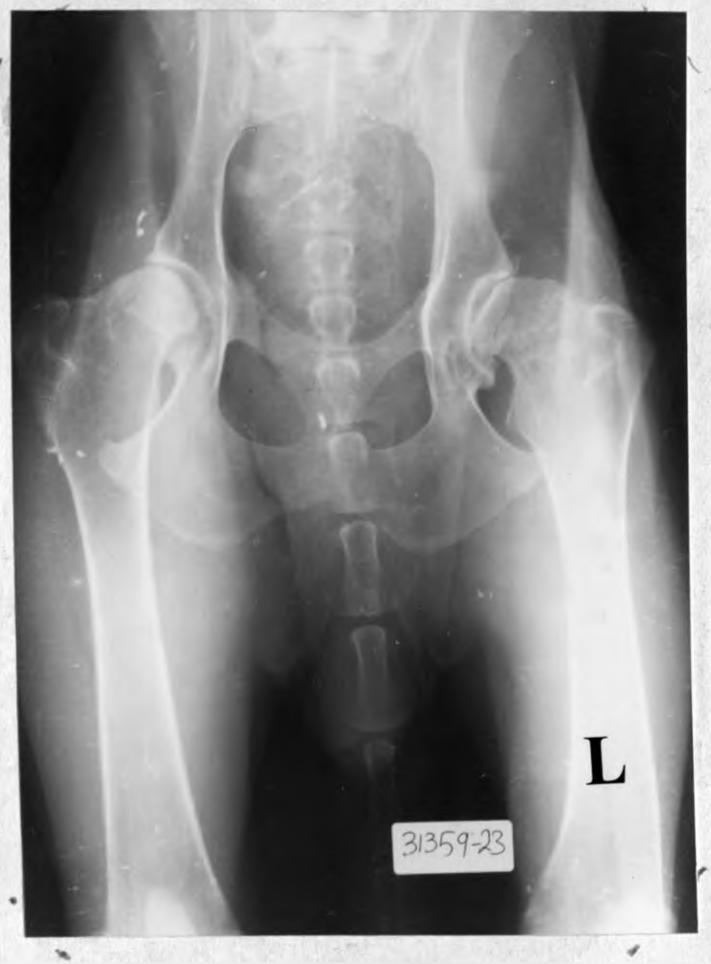


Figure 15. A ventrodorsal pelvic radiograph of an 8-year-old female German shepherd dog with osteoarthritis of the hip joints graded 2.
Case number 31359-23.
Note the thick femoral neck, flat femoral head and remodeling of the acetabulum in both joints.

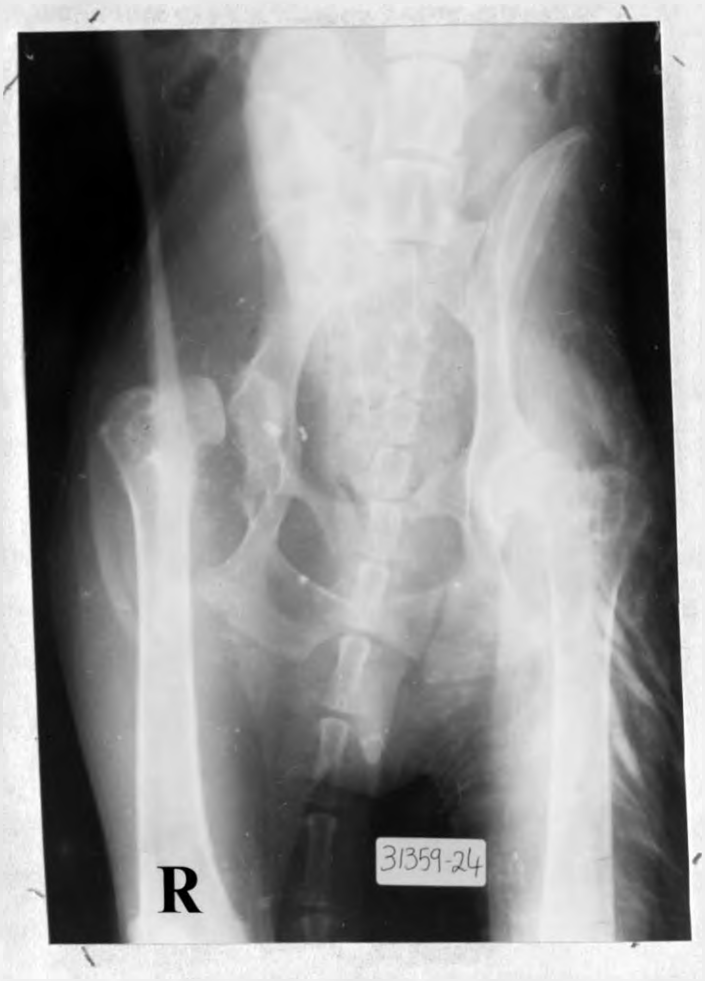


Figure 16. A ventrodorsal pelvic radiograph of a 15-year-old male German shepherd dog with severe bilateral osteoarthritis of the hip joints graded 3.
Case number 31359-24.
Note the extensive remodeling of the acetabulum and flattening of the femoral head in both joints. There is subluxation of the femoral heads, particularly on the right hip joint the subluxation is very obvious.

4.4.0 Pathology

4.4.1.1 Volume of ligamentum capitis femoris

Ligamentum capitis femoris was present in 46 (71.9 %) and missing in 18 (28.1%) hip joints [bilateral (21.9 %) in 7 dogs, in the right joint (4.7 %) of 3 dogs and in the left joint (1.6 %) of one dog]. Data on the volume of ligamentum capitis femoris in 64 hip joints is presented in Appendix IV

4.4.1.2 Relationship between the volume of ligamentum capitis femoris and radiographic grades of hip joints

Table 9 presents the volume of ligamentum capitis femoris for each radiographic grade accorded to the hip joints. The mean volume of ligamentum capitis femoris in hip joints with radiographic grade 0 was 0.8212 ± 0.3462 mls, while the mean for hip joints with radiographic grade 1, was 0.6545 ± 0.2544 mls.

Although 4 out of the 7 hip joints radiographically scored as grade 2 had no ligaments (volume = 0 mls), the mean volume of ligamentum capitis femoris for the group was 0.3143 ± 0.5551 mls. Thirteen hip joints scored as grade 3 had no ligaments present, and the mean volume of ligamentum capitis femoris was therefore 0 mls. The volume of ligamentum capitis femoris for the various radiographic grades is graphically represented in Figure 17.

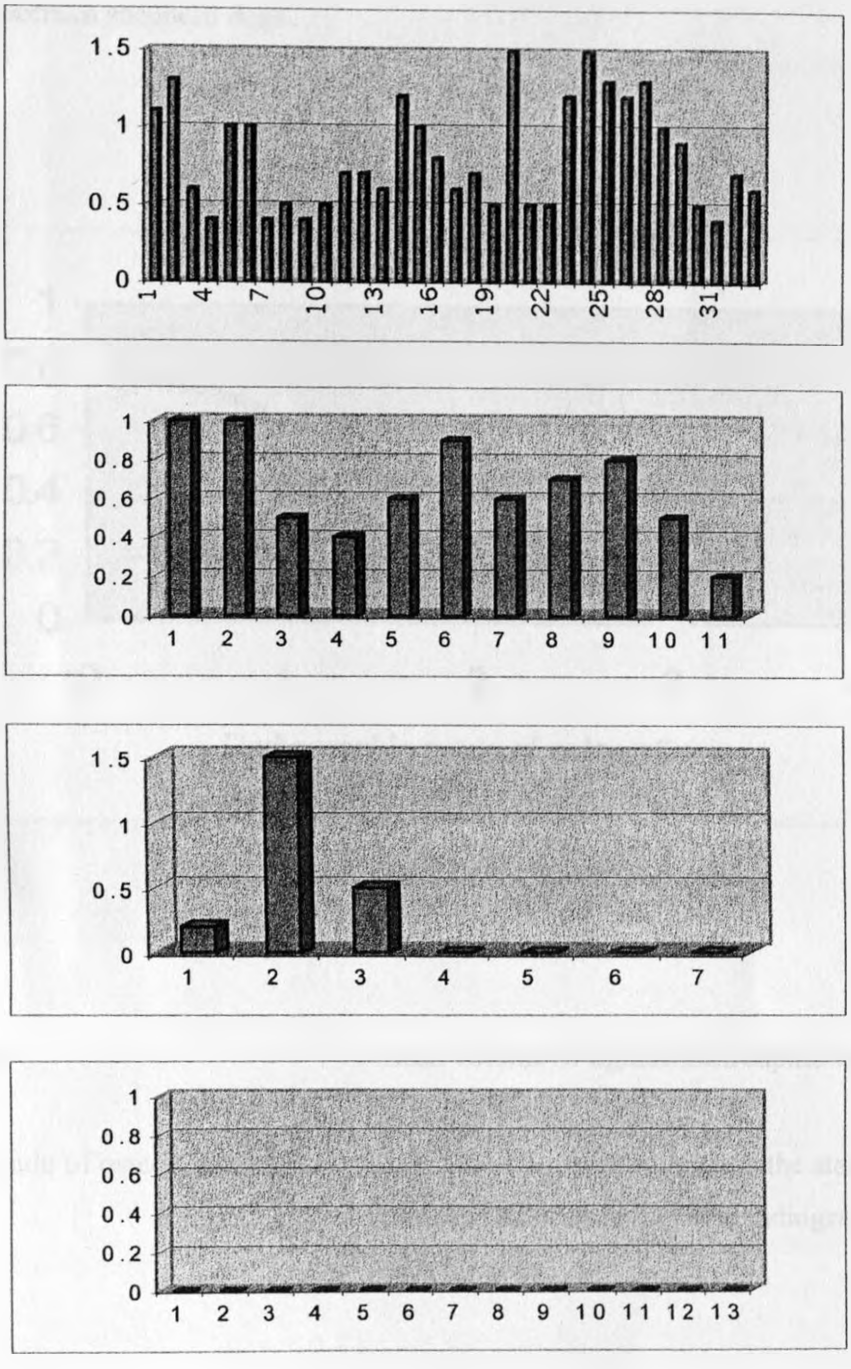
The relationship between the mean volume of ligamentum capitis femoris for the four radiographic grades of the joints is graphically illustrated in Figure 18. The hip joints with a radiographic score of grade 0 and grade 1 had comparatively higher mean volume of ligamentum capitis femoris of 0.82 mls and 0.65 mls respectively. Conversely, in hip joints that were radiographically scored as moderately osteoarthritic (grade 2), the mean volume of ligamentum capitis femoris was 0.31 mls.

Table 9. The volume of ligamentum capitis femoris in milliliters in relation to radiographic grades of osteoarthritis of the hip joints from 32 adult German shepherd dogs in Kenya.

Radiographic grade of hip joints	Volume of ligamentum capitis femoris in milliliters.						
0 N=33. Mean = 0.8212 ± 0.3462	1.1	1.3	0.6	0.4	1.0	1.0	0.4
	0.5	0.4	0.5	0.7	0.7	0.6	1.2
	1.0	0.8	0.6	0.7	0.5	1.5	0.5
	0.5	1.2	1.5	1.3	1.2	1.3	1.0
	0.9	0.5	0.4	0.7	0.6		
1 N=11. Mean = 0.6545 ± 0.2544	1.0	1.0	0.5	0.4	0.6	0.9	0.6
	0.7	0.8	0.5	0.2			
2 N=7. Mean = 0.3143 ± 0.5551	0.2	1.5	0.5	0	0	0	0
3 N=13. Mean = 0	0	0	0	0	0	0	0
	0	0	0	0	0	0	

N= number of samples.

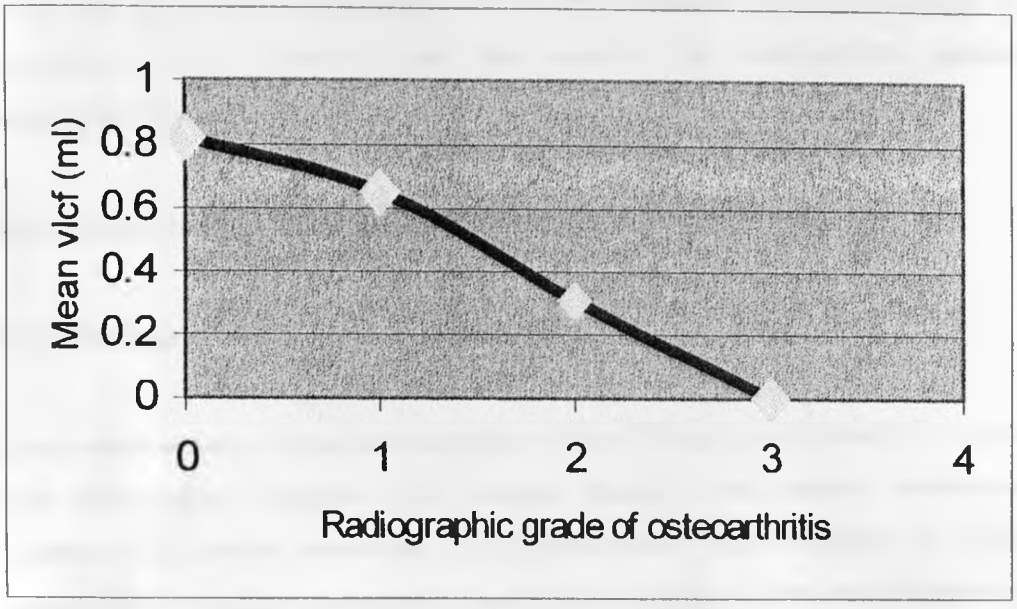
Figure 17. The volume of ligamentum capitis femoris in milliliters in relation to various radiographic grades of osteoarthritis in 32 adult dogs.



Key:

1. The volume of ligamentum capitis femoris of the hip joints radiographically graded as 0, 1, 2, and 3, from top to bottom, respectively.
2. X-axis (horizontal) represents the volume of ligamentum capitis femoris in milliliters; Y-axis (vertical) represents number of joints sampled.

Figure 18. The relationship between the mean volume of ligamentum capitis femoris and the radiographic grades of osteoarthritis of the hip joints in 32 adult German shepherd dogs.



Key:

Mean vlcf (mls)	=	mean volume of ligamentum capitis femoris in milliliters.
Radiographic grade of osteoarthritis	=	determined by the author from the standard extended ventrodorsal pelvic radiographs for each hip joint.

The mean volume of ligamentum capitis femoris was significantly different ($p < 0.05$) between the groups grades 1 and 3, 1 and 4, 2 and 3, 2 and 4, 3 and 4 (Appendix VI). However, there was no significant difference between the mean volume of ligamentum capitis femoris in hip joints that were normal and in those with mild osteoarthritis grade 1 and 2.

There was an inverse correlation ($r = - 0.75$) between the mean volume of ligamentum capitis femoris and the severity of osteoarthritis graded radiographically in the study.

4.4.2.0 Pathological findings in synovial membrane and joint cavities

4.4.2.1 Gross findings

Gross examination of synovial membrane from 56 hip joints revealed various gross pathological changes. The changes ranged from normal anatomical appearance to severe synovitis, chondrometaplasia and formation of "joint masses". There was no appreciable change affecting the colour and thickness of synovial membrane samples from 27 (48.2 %) hip joints. These samples had either mild or no villous hypertrophy, and the inner surface of the joint capsules appeared white, smooth and without gross alterations.

Conversely, synovial membrane samples from 29 (51.8 %) hip joints had mild to severe gross thickening (Figure 19). The synovial membrane had irregular surface projections of hypertrophic villi and was grossly enlarged. These changes are consistent with chronic synovitis. The samples were collected from hip joints that were also affected by osteoarthritis.

There was grossly palpable firm cartilaginous material in synovial membrane from 21 (37.5 %) hip joints. These samples had firm consistency and white appearance due to the presence of cartilaginous material.

Figure 19. Gross thickening of synovial membrane of dogs with severe osteoarthritis of the hip joints (22 R and 24 R) and synovial membrane from a normal hip joint (28 L).

Case numbers 31359-22, 31359-24 and 31359-28.

The synovial membrane sample in the middle (28 L) appears thinner compared to the other two samples, which are thicker. The samples were preserved in 10 % formalin solution prior to photography.

In addition, samples had abundant villous hypertrophy, which together with the gross thickening, significantly altered the normal appearance of the synovial membrane.

There were "joint masses" in 12 (21.4 %) out of the 56 hip joints. These masses were found either as free within the joint cavity or embedded within the synovial membrane tissue. The largest mass was 1.0 x 1.5 cm in diameter, while the smaller masses were approximately 0.2-0.1 cm. Masses embedded in synovial membrane were irregular in shape and consistency. On the other hand, the free masses were round, white, smooth and firm in consistency. Figure 20 illustrates the gross appearance of a joint mass from one of the hip joints.

4.4.2.2 Histological findings

The results of histological examination of 24 selected samples of synovial membrane are presented in Appendix VII and summarized in Table 10. The degree of synovitis was graded as grade 4 (very severe) in 45.8% of the samples, grade 3 (severe) in 16.7 %, grade 2 (moderate) in 25 %, grade 1 (mild) in 8.3 % and grade 0 (normal) in 4.2 % of the samples.

The major histological findings varied from normal, mild, moderate, severe to very severe synovitis. Figure 21 shows histological appearance of synovial membrane harvested from a normal hip joint.

The main alterations in mild synovitis included infiltration with spindle-shaped inflammatory cells, without villous hypertrophy (Figure 22). Samples with moderate synovitis showed increased inflammatory cell infiltration with several layers of predominantly round cells (rather than the normal two layers of spindle-shaped cells lining the inner synovial layer) and villous hypertrophy.

Table 10. Distribution of the histological scores of synovial membrane from osteoarthritic hip joints of 24 adult German shepherd dogs in Kenya

Synovial score	Description of histological features	Sample	Percentage (%)
0	Synovial membrane lined with one to two cells, no villous formation, no hyperplasia.	1	4.2
1	Inflammatory cell infiltration, mild fibrosis and synovial hyperplasia, villi present.	2	8.3
2	Extensive hyperplasia, and cell infiltration, moderate fibrosis, roundish chondrocyte-like cells.	6	25.0
3	Extensive infiltration and fibrosis, gross thickening and disorganization of the synovium, villous hypertrophy.	4	16.7
4	Fragments of cartilage-like tissue, predominantly roundish chondrocyte-like cell, extensive fibrosis and villous hypertrophy.	11	45.8
Total		24	100.0

Figure 20. A 'joint mass' from the joint cavity of an 11-year-old German shepherd dog with hindlimb lameness.
Case number 31359-33.

Figure 21. Photomicrograph of synovial membrane from a normal hip joint of a 7-year-old German shepherd dog (Hematoxylin eosin; X 4).

Case number 31359-28.

Notice the thin layer of cells (↓) and the synovial stroma composed of collagen fibers (→).

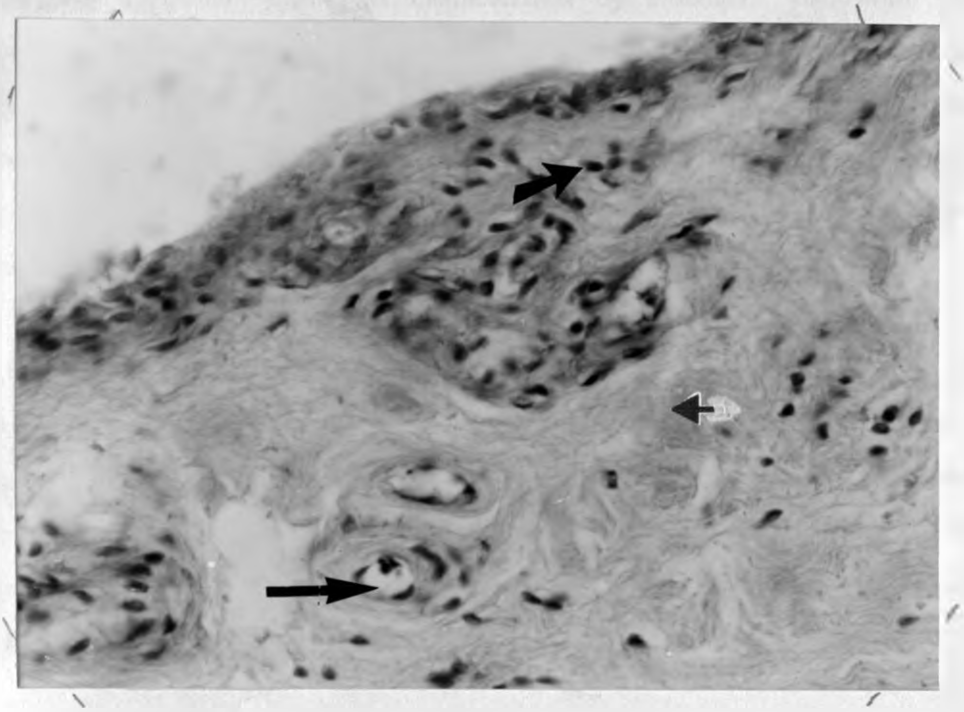


Figure 22. Photomicrograph of synovial membrane from the hip joint of a 10-year-old German shepherd dog with mild synovitis (Hematoxylin eosin; X 10). Case number 3135-17.

There is infiltration with inflammatory cells (fibroblasts and macrophages) ( ), capillary vascularization ( ) and abundant collagen fibres ( ).

Abundant fibrosis occurred in the synovial intima, which was also interspersed with mononuclear inflammatory cells. There was a proliferation of endothelial cells forming new capillaries in the inflamed synovium (Figure 23).

Histological examination of samples with synovial thickening revealed massive proliferative synovitis. This was characterized by extensive infiltration by inflammatory cell, capillary neovascularization, extensive villous hypertrophy and chondrometaplasia. Chondrometaplasia was noted on the periphery of the synovial membrane, in which case the cartilaginous material was freely detached from the rest of the synovial tissue. Chondrometaplasia was also noted on synovial villi and within the intimal layer of the synovium (Figure 24 A and B).

The free masses occurred as joint masses. The histological appearance of one of the joint masses is illustrated in Figure 25. The superficial layers of the masses comprised of chondrocytes that were enmeshed within a disorganized fibrocellular matrix. The cartilage closely simulated articular cartilage. The deeper portions of the masses revealed presence of bone marrow, which is consistent with osseous transformation of the affected synovium.

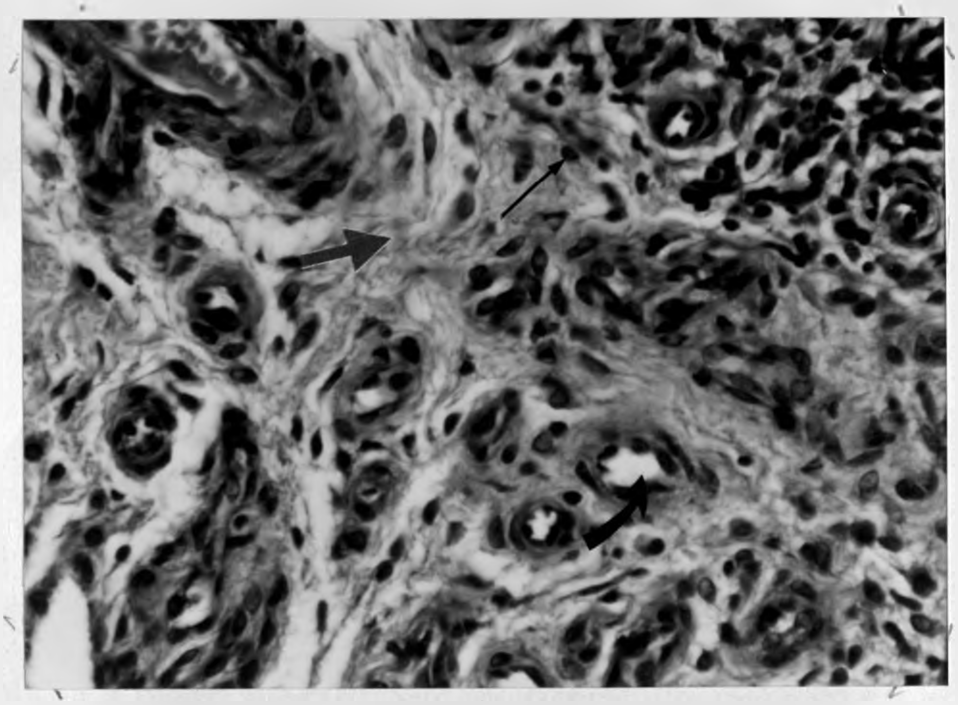


Figure 23. Photomicrograph of synovial membrane from the hip joint of a 10-year-old German shepherd dog with moderate synovitis (Hematoxylin eosin; X 10).
Case number 31359-27.
Note the infiltration (⟶) of the synovial membrane with inflammatory cells, capillary vascularization (➘) and fibrosis (➡).

Figure 24 A. Photomicrograph of synovial membrane from the hip joint of an 11-year-old German shepherd dog with synovial chondrometaplasia (Hematoxylin eosin; X 10).

Case number 31359-18.

Notice the villous hypertrophy (↓), roundish 'chondrocyte-like' cells (⟶) and collagen fibres. The empty spaces (⬇) in the section are artifacts due to tissue damage during preparation perhaps indicating edema.

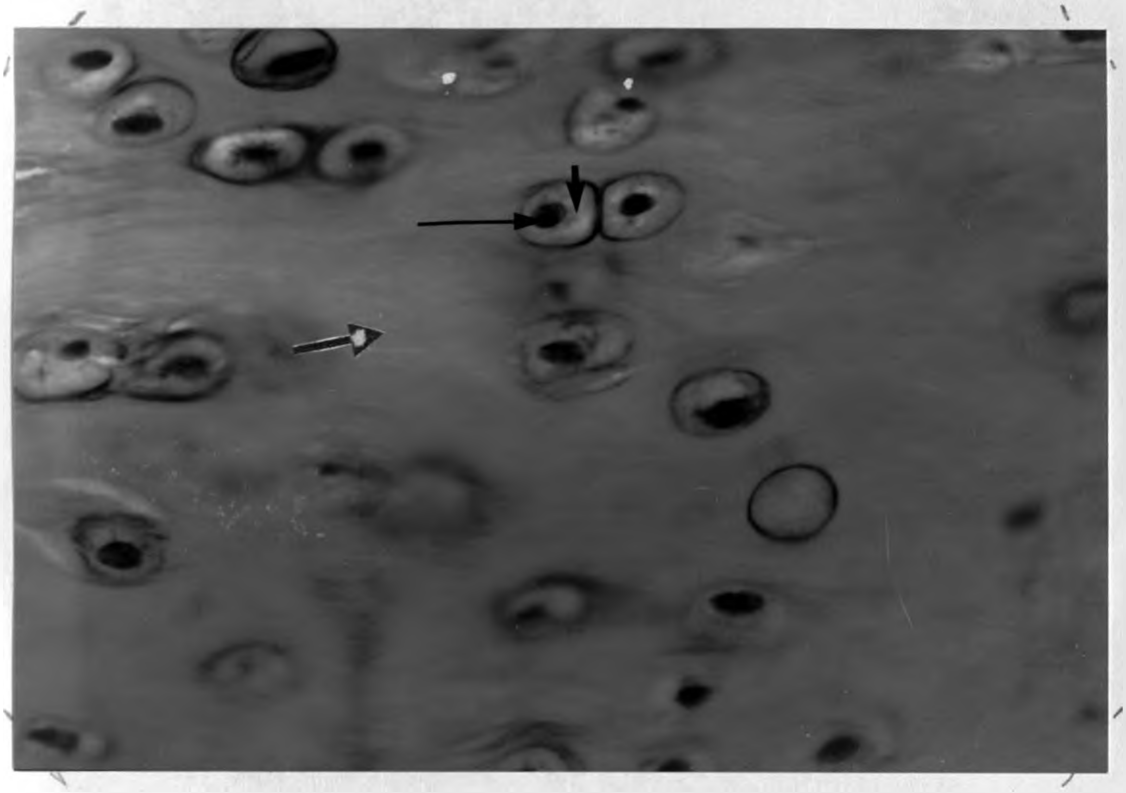


Figure 24 B Photomicrograph of synovial membrane from the hip joint of an 11-year-old German shepherd dog with synovial chondrometaplasia. (Hematoxylin eosin; X 40).
Case number 31359-18.
Notice the roundish cells (⟶) surrounded by clear zone (↓) appearing like lacunae. The collagen fibres (⟶) contributed to the gross thickening of the synovial membrane.

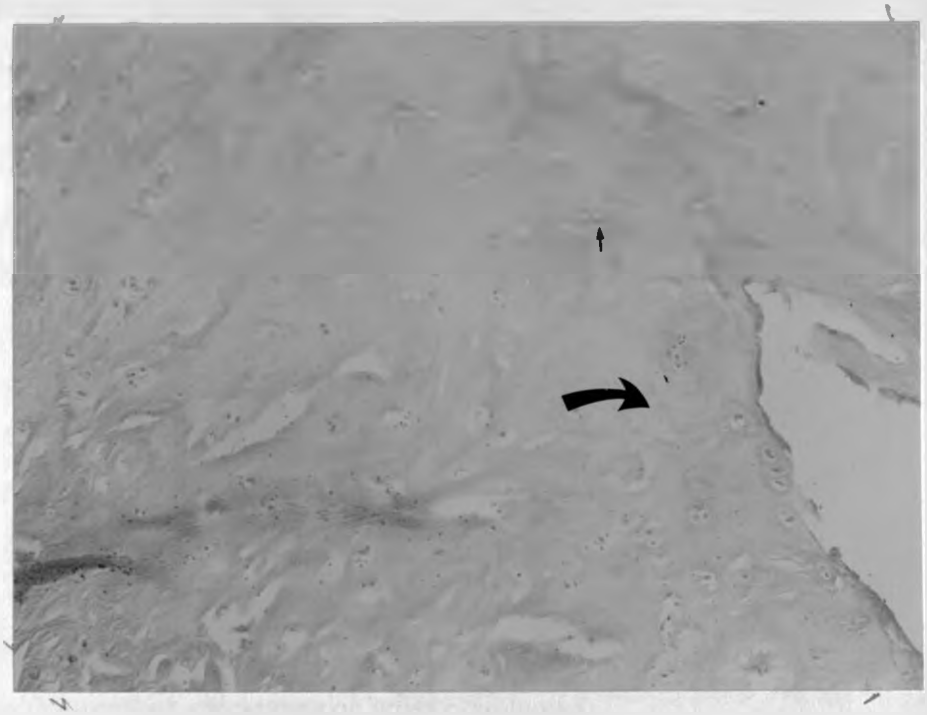


Figure 25. Photomicrograph of a section of a 'joint mass' from the hip joint cavity of a 5-year-old German shepherd dog with severe osteoarthritis (Hematoxylin eosin; X 10).
Case number 31359-10.
Note the osteoid deposition (↑) in the chondroid matrix (➘).

4.4.3.0 Pathological findings on articular cartilage in osteoarthritis of the hip joints

4.4.3.1 Gross findings

Gross examination of articular cartilage from 64 hip joints revealed varied pathological features. These ranged from normal joint surfaces, to mild, moderate and severe osteoarthritis. The distribution of graded hip joint lesions observed at gross pathological examination is presented in Table 11 and Figure 26.

Twenty-two (34.4 %) hip joints were classified as showing normal features, while 11 (17.2 %) showed mild osteoarthritis. Seven (10.9 %) hip joints had moderate osteoarthritis, whereas 24 (37.5 %) hip joints had severe osteoarthritis.

Based on postmortem findings, majority (65.6 %) of the hip joints in the study were classified as having mild, moderate or severe osteoarthritis, while 34.4 % had normal anatomical appearance on gross examination.

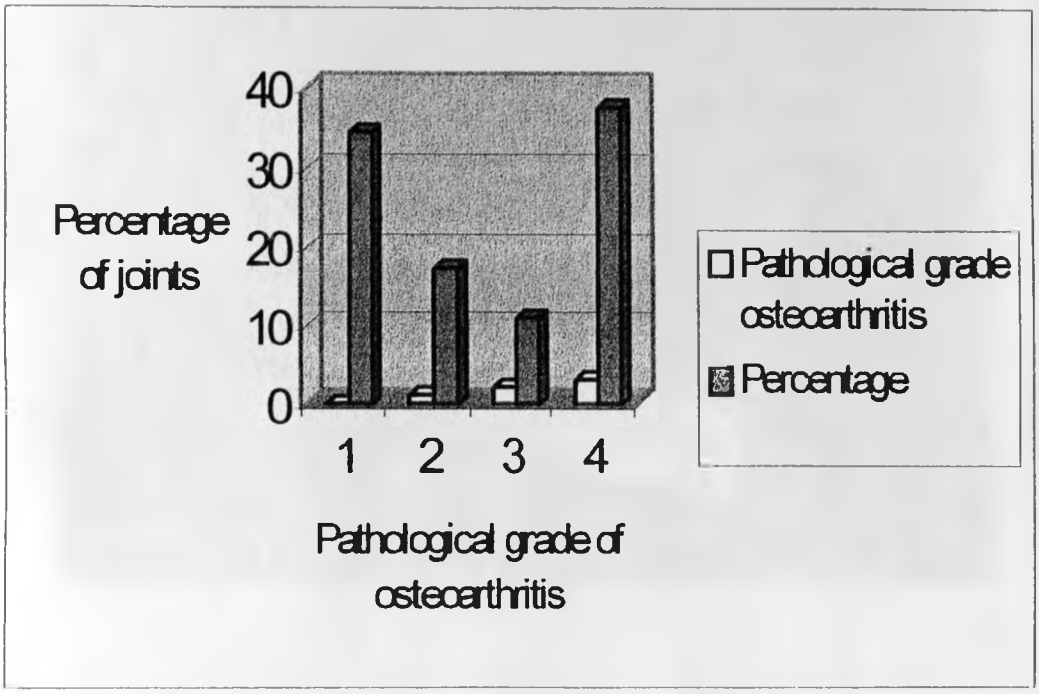
Joints that were defined as normal had their articular surfaces appearing white in colour, smooth and glistening, and with no visible surface alterations. Examination of the periarticular surfaces also revealed no excessive bone growth (osteophytes). The gross anatomical appearance of normal articular cartilage of an adult canine hip joint from one of the animals in the study is illustrated in Figure 27.

The hip joints affected by mild osteoarthritis revealed articular cartilage, which was smooth, white in colour and glistening appearance. However, osteophyte formation and evidence of small clefts in the articular cartilage was found in some joints.

Table 11. Classification of articular cartilage from hip joints of 32 adult German shepherd dogs on the basis of gross pathological changes.

Group	Hip joint						Total	Percent
Normal (0)	8R	8L	9R	9L	12R	12L	**22**	**34.4**
	16R	16L	20L	20R	25R	25L		
	28R	28L	30R	30L	31R	31L		
	32R	32L	38R	38L				
Mild (1)	11R	11L	19L	26R	27R	27L	**11**	**17.2**
	36R	36L	39L	41R	41L			
Moderate (2)	13R	13L	34R	34L	17L	22L	**7**	**10.9**
	39R							
Severe (3)	1R	1L	7R	7L	10R	10L	**24**	**37.5**
	17R	18R	18L	19R	22R	23R		
	23L	24R	24L	26L	29R	29L		
	33R	33L	35R	35L	32510R	32510L		
					Total		**64**	**100**

Figure 26. Graphical presentation of the gross pathological grades of osteoarthritis of the hip joints from 32 adult German shepherd dogs.



Key:

Pathological grade of osteoarthritis:			type of lesion.
1	=	grade 0	normal cartilage.
2	=	grade 1	mild osteoarthritis.
3	=	grade 2	moderate osteoarthritis.
4	=	grade 3	severe osteoarthritis.

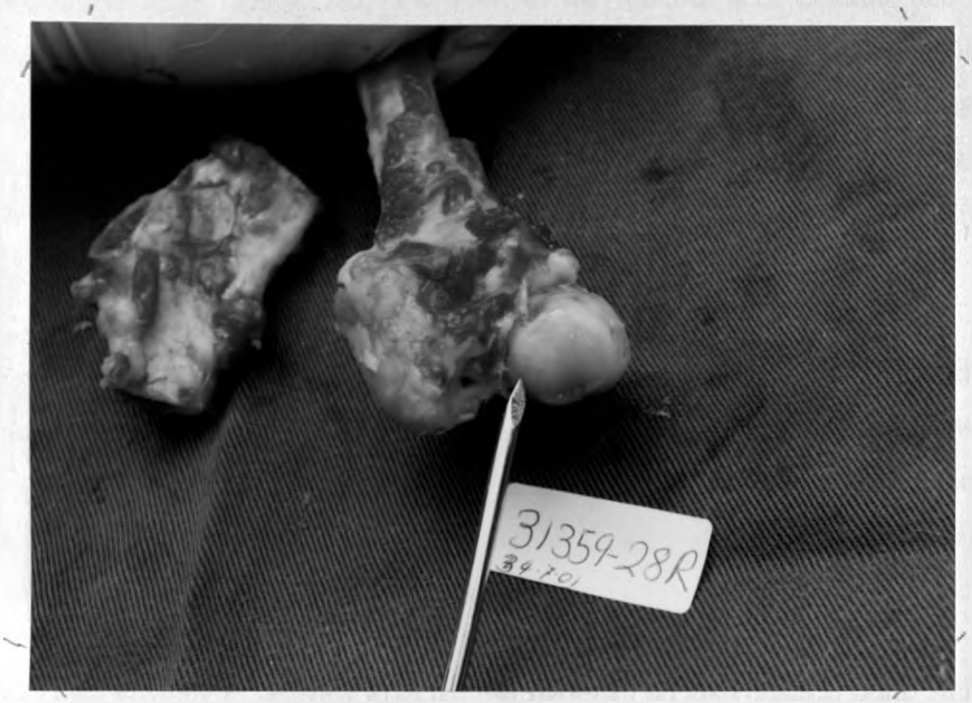


Figure 27. Articular cartilage of the femoral head from a 7-year-old German shepherd dog with no signs of hindlimb lameness.
Case number 31359-28.
Notice the smooth glistening surface of the articular cartilage (pointer).

The hip joints with mild osteoarthritis showed flaking due to erosion of cartilage from portions of the femoral head. This appeared as small patches of discoloration with loss of articular surface contour as illustrated in Figure 28.

The articular cartilage classified as having moderate osteoarthritis was marked by a significant degree of flaking and fibrillation affecting approximately 30 % of the articular cartilage. There was alteration of the femoral neck contour due to formation of periarticular osteophytes. There were lesions on the acetabular articular cartilage as well. The main lesions were periarticular osteophyte formation around the acetabular rim, ossification of the acetabular fossa and erosion of articular cartilage (fibrillation). Figure 29 illustrates the appearance of a hip joint classified as having moderate osteoarthritis.

The articular cartilage with severe osteoarthritis had gross remodeling, visible osteophytes, fibrillation and eburnation (where the articular cartilage was completely eroded, exposing the subchondral bone). These changes were also visible on articular cartilage of the acetabulum of the affected joints (Figure 30 A, B and C).

Joints that were more severely affected had flattened femoral heads (Figure 30 D). The acetabulum appeared shallow or completely flattened due to excessive fibrillation and wearing of the subchondral bone. The most severe case in the study was that of a 15-year-old German shepherd dog. In this case, there was complete disorganization of the architecture of the acetabulum, flattening of the femoral head and thickening of the femoral neck. The prolonged subluxation and excessive abrasive wearing of the subchondral bone of the articular surfaces contributed to this physical alteration. The important observation in this particular case was the long course of the condition without coexisting overt clinical and radiographic diagnosis of canine hip dysplasia and osteoarthritis. This would not have been detected without the clinical and radiographic examination in the study.

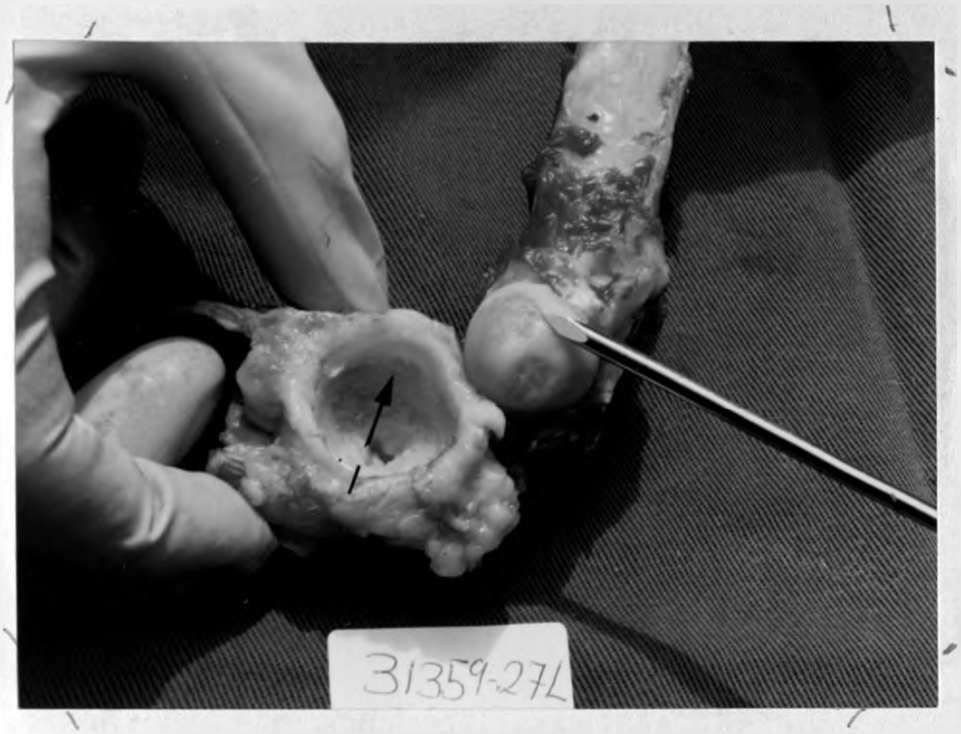


Figure 28. Articular cartilage from a 10-year-old German shepherd dog with mild osteoarthritis of the hip joint.
Case number 31359-27.
Notice the flaking of articular cartilage of the femoral head (metal pointer) and the acetabulum (arrow).

Figure 29. Articular cartilage of the femoral head of a 10-year-old German shepherd dog with moderate osteoarthritis.
Case number 31359-13.
There is fibrillation of articular cartilage (arrow).

Figure 30 A. Articular cartilage from an 8-year-old German shepherd dog with severe osteoarthritis of the hip joints.
Case number 31359-22.
Notice the complete erosion of articular cartilage of the femoral head exposing the subchondral bone (metal pointer).

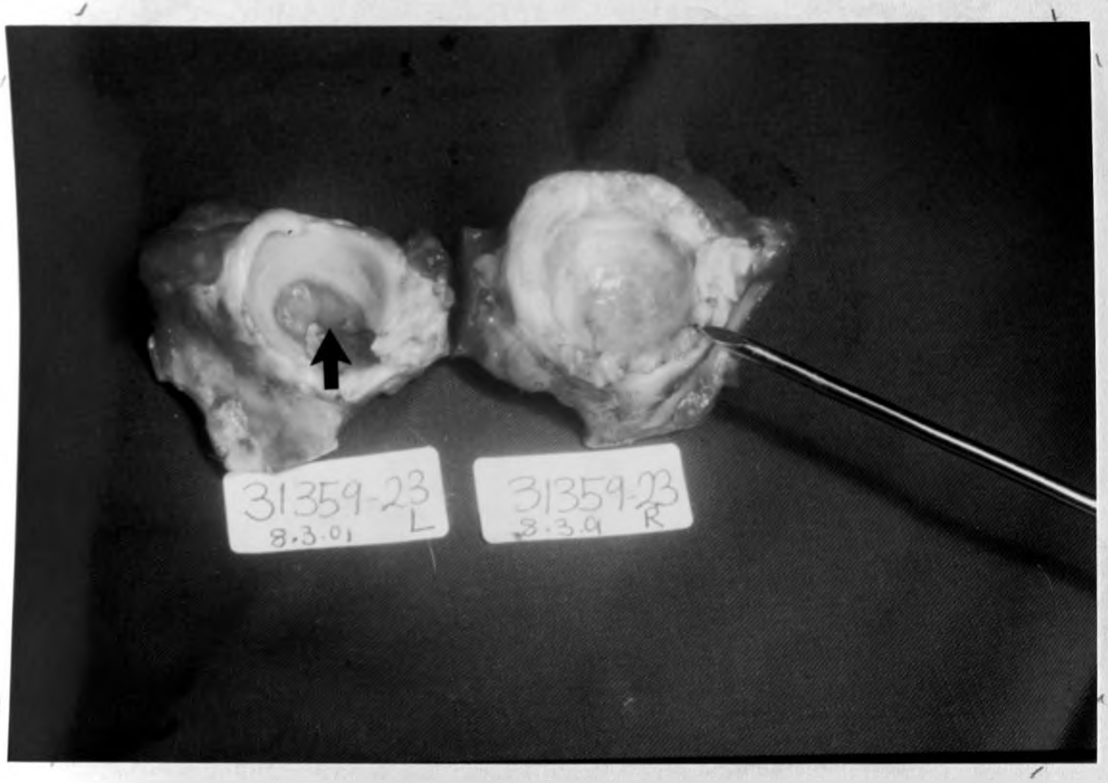


Figure 30 B. Articular cartilage of the acetabulum from an 8-year-old German shepherd dog with severe osteoarthritis of the hip joints.
Case number 31359-23.
Notice the osteophyte formation on the rim of the right acetabulum (metal pointer). The acetabular fossa of the right hip joint appears completely ossified (arrow) while the acetabulum on the left side is partially ossified.

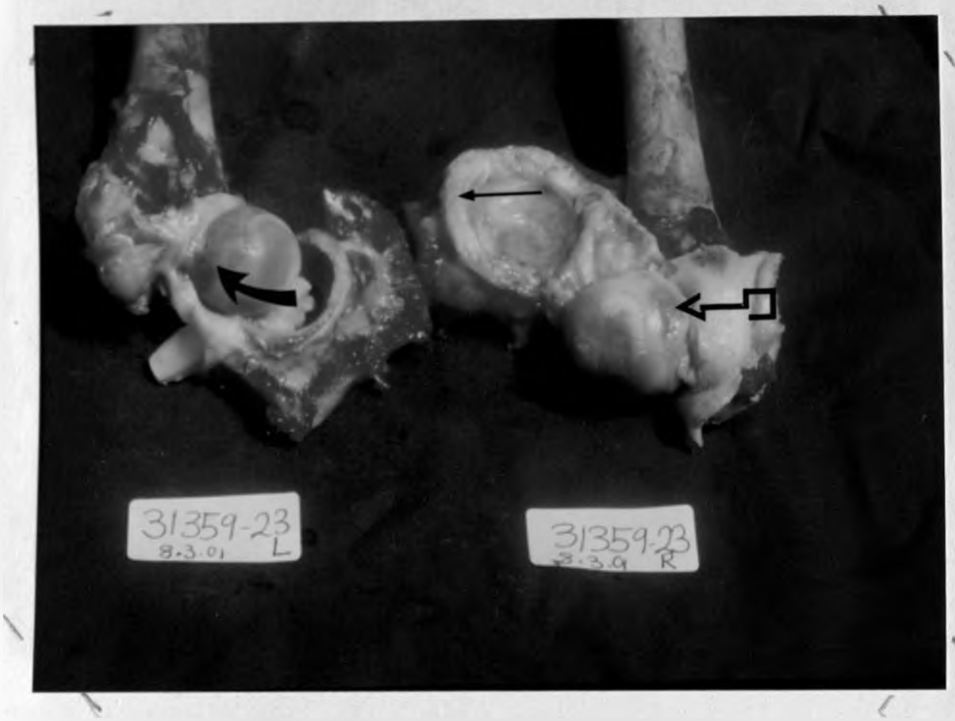


Figure 30 C. Articular cartilage of the acetabulum and femoral head from an 8-year-old German shepherd dog with severe osteoarthritis of the hip joints.
Case number 31359-23.
Notice the extensive fibrillation ( ), osteophyte formation ( ) and thickened synovium ( ).

Osteophyte formation was observed in 45 % of the hip joints. This phenomenon is shown in Figure 30 D. Cartilage fibrillation, eburnation of subchondral bone and flattening of the femoral head were also observed as steps towards progressive degeneration of the articular surfaces and failure of the repair process.

4.4.3.2 Correlation between the gross pathological grades and radiographic features

The comparison between the radiographic grades of osteoarthritis and the gross pathological grade of the hip joints from 32 adult German shepherd dogs is presented in Appendix VIII and summarized in Table 12.

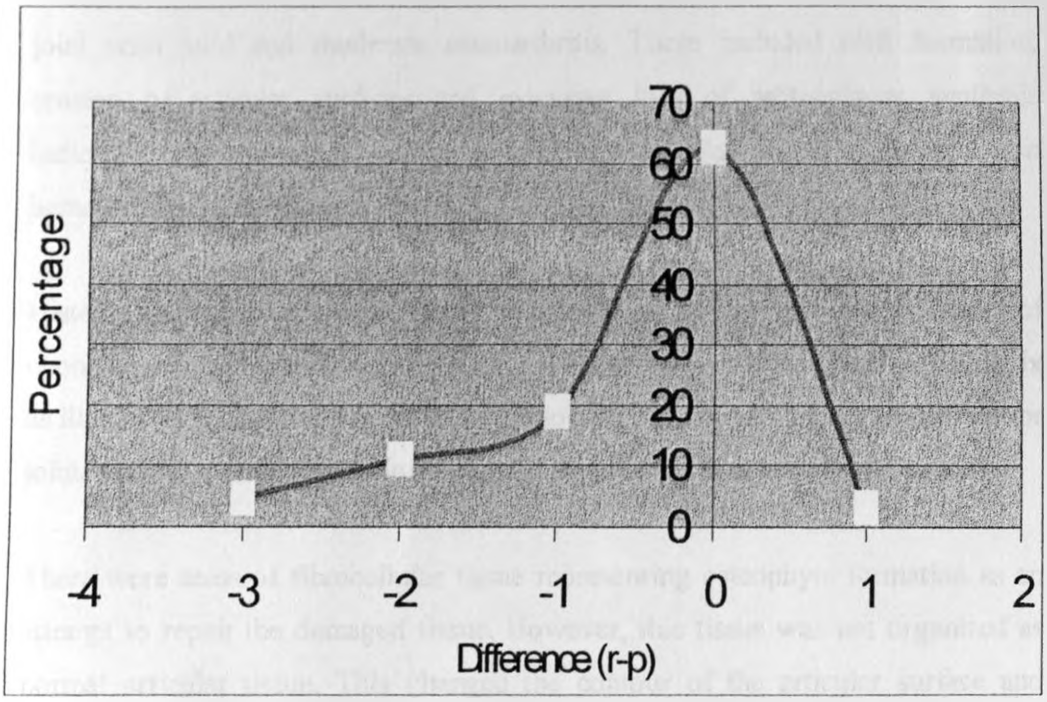
There was a normal distribution curve of the data with the mean deviation between the scores at zero. 62.5 % of the data correlated between the radiographic grade and the gross pathological data. This meant that 62.5 % of the radiographic findings were confirmed as accurate on gross pathological examination. 3.1 % of the readings were with an error +1, while 18.8 % had an error of -1, 10.9 % had an error of –2, while 4.7 % had an error of –3 (Figure 31).

The majority (84.4 %) of the data was clustered within the deviation of –1 and +1 between the radiographic grade and the pathological grade of the same joint examined by the author. There was a probability of 84.4 % that radiographic features would be confirmed by gross pathology of the same hip joint with an error –1 and +1 grade. There was a positive correlation between the radiographic grade of osteoarthritis and the pathological grade when data on the same hip joint was compared. The statistical analysis for correlation is shown in Appendix IX. There was a positive correlation between the two diagnostic tests ($r=0.64$). The test of agreement between radiography and pathology was also statistically significant (Kappa Coefficient (K)=0.5977) (Appendix IX).

Table 12. Distribution of the difference between radiographic and pathological grades of osteoarthritis of hip joints from 32 adult German shepherd dogs in Kenya.

Difference	Number of joints	Percentage
1	2	3.1
0	40	62.5
- 1	12	18.8
- 2	7	10.9
- 3	3	4.7
Total	64	100.0

Figure 31. The distribution of the curve between the radiographic grade and the pathological grade of hip joints from 32 adult German shepherd dogs.



Key: r = radiographic grade.

p = pathological grade.

r-p = difference between radiographic grade and pathological grade.

4.4.4 Histological and histochemical features of articular cartilage degradation

A wide range of histological changes were observed. These varied from mild to severe.

Samples collected from joints previously determined to be normal on radiographic examination revealed histological changes in parts of the articular cartilage (Figure 32 A). There were histological changes observed in samples from hip joint with mild and moderate osteoarthritis. These included cleft formation, erosion of articular surfaces and extensive loss of proteoglycan synthesis indicated by pale green colour of matrix with Safranin-O stain and iron hematoxylin.

Histological samples from severe osteoarthritic joints showed evidence of chondrocyte necrosis, cloning, hypocellularity and deep clefts affecting the matrix as illustrated in Figure 32 B and C. Histological evaluation was not conducted for joints with extensive fibrillation because only subchondral bone was available.

There were areas of fibrocellular tissue representing osteophyte formation as an attempt to repair the damaged tissue. However, this tissue was not organized as normal articular tissue. This changed the contour of the articular surface and indicated efforts by the femoral articular cartilage to repair its architecture.

Although articular cartilage from severely osteoarthritic hip joints had fibrillation, the periphery of femoral heads had intact cartilage. However, histological sections from such areas had articular cartilage erosion, loss of proteoglycans and chondrocyte necrosis, indicative of degenerative joint disease.

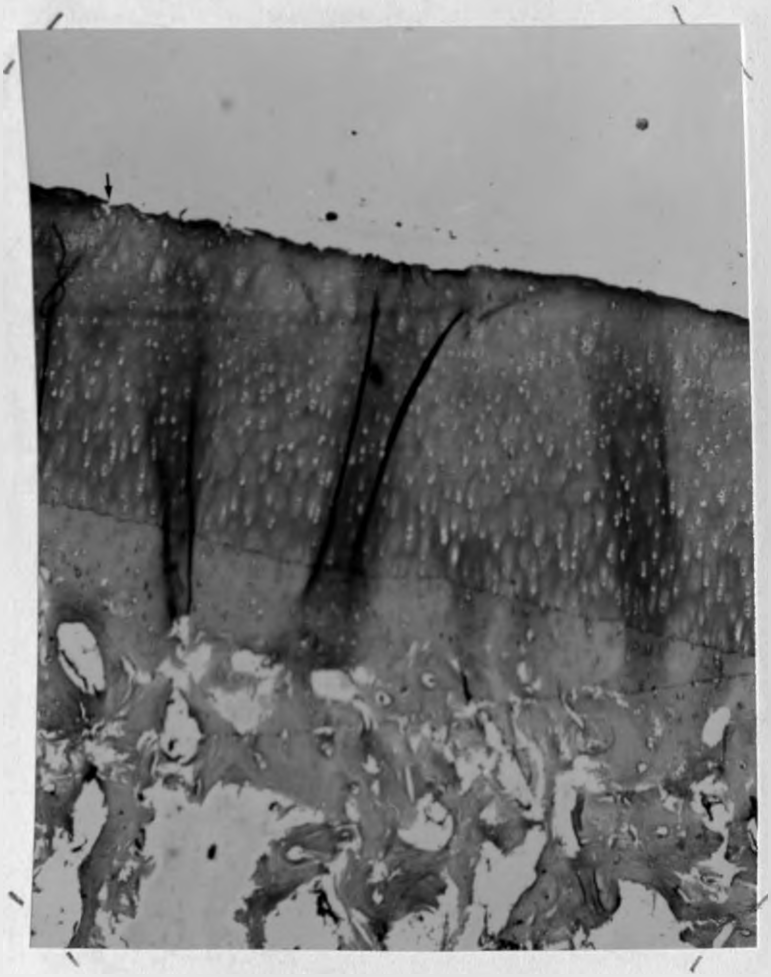


Figure 32 A. Photomicrograph of articular cartilage from a 10-year-old German shepherd dog with normal hip joints (Safranin-O; X 10).
Case number 31359-8.
Notice the red colour of cartilage matrix indicating presence of some proteoglycans. The articular surface appears rough (↓).

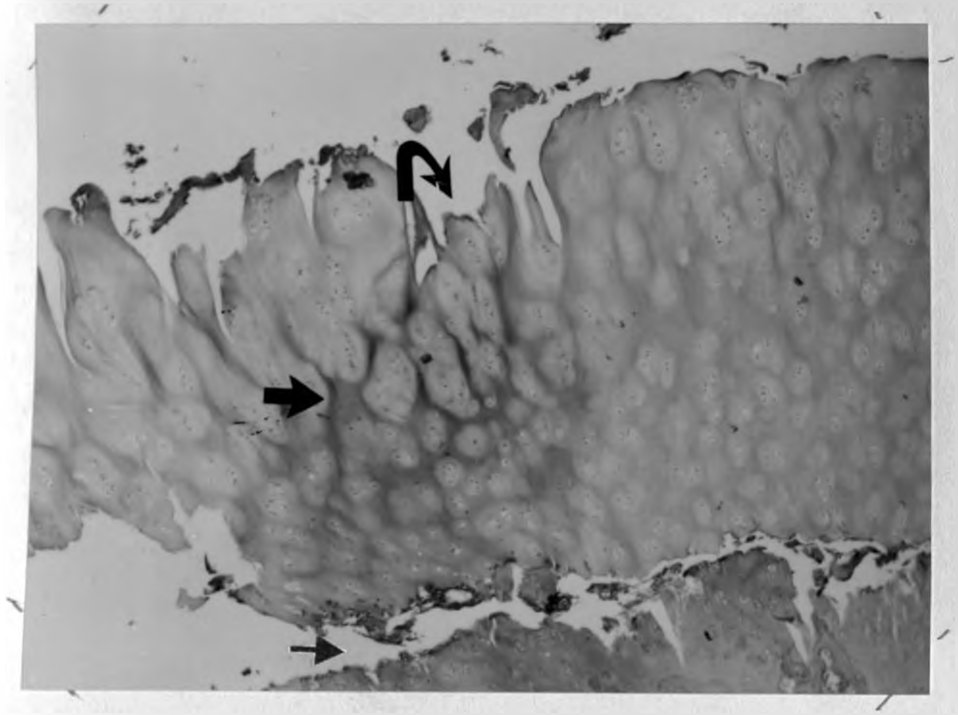


Figure 32 B. Photomicrograph of articular cartilage from a 10-year-old German shepherd dog with severe osteoarthritis of the hip joints.
(Safranin-O stain; X 40).
Case number 31359-10.
Notice the pale greenish colour (➡) of the matrix indicating loss of proteoglycan, cleft formation (↷) and disruption of the articular cartilage exposing the subchondral bone (➡).

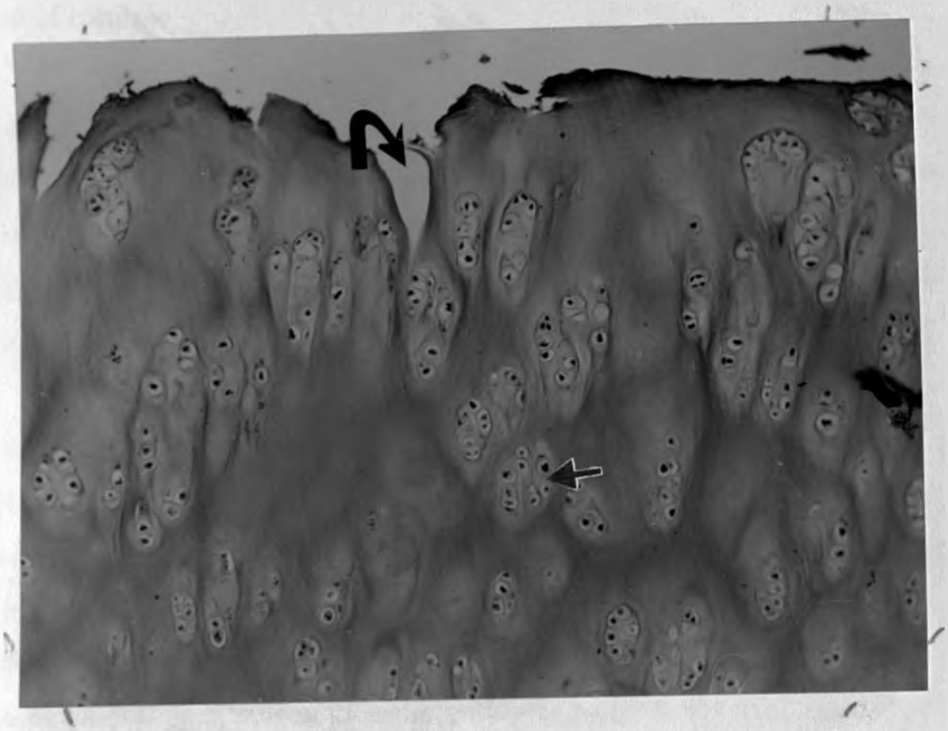


Figure 32 C. Photomicrograph showing cleft formation and chondrocyte cloning of articular cartilage of the femoral head of an 8-year-old German shepherd dog with severe osteoarthritis (Safranin-O; X 60).
Case number 31359-11.
Notice the cleft formation ( ) and chondrocyte cloning ( ) of articular cartilage appearing as clusters of chondrocytes.

Samples from hip joints with the most severe osteoarthritis showed total loss of articular cartilage, exposing the subchondral bone. Small portions of articular cartilage occasionally remained on the edge of the femoral head, with almost total erosion of cartilage.

The results of the histochemical grading of articular cartilage lesions are presented in Appendix X and summarized in Table 13. Fifteen (68.2 %) out of the 22 representative samples of articular cartilage histologically examined had Mankin scores between 10-15. Six (27.3 %) out of the 22 samples had Mankin scores between 5-9 while one sample (4.5 %) had a score below 5.

The histological scores above 7 were recorded in 15 out of the 22 (68.2 %) samples evaluated. These represented severe articular cartilage changes, which included extensive erosion and complete disorganization of the cartilage structure. The scores for Safranin-O staining were high in almost all the samples indicating the extensive loss of proteoglycans within the articular cartilage matrix. The histological sections appeared green or pale green due to the stain on collagen fibres. There was a wide variation in the histological appearance of samples from the same hip joint. Severe histological changes were noted in some samples from hip joints that were clinically and radiographically graded as normal.

Comparison of the histological scores with the radiographic grades, pathological grades and the clinical categories of the same hip joint is illustrated in Table 14. Although 9 out of the 22 samples (40.9 %) had very severe histological changes, the clinical, radiographic and gross pathological findings of these samples were normal. On the other hand, histological scores for 13 out of 22 samples (59.1 %) corresponded to the expected clinical, radiographic and pathological grades.

Table 13. Distribution of the mean histological grades of articular cartilage degradation of femoral heads from 22 adult German shepherd dogs.

Range of histological score	Number of joints	Percentage (%)
0 - 5	1	4.5
5 - 9	6	27.3
10 -15	15	68.2
Total	**22**	**100.0**

Table 14. Comparison between histological, radiographic and gross pathological grades and clinical categories of hindlimb lameness.

Sample (joint)	Histological grade range: 0-15	Radiographic grade range: 0-3	Pathological grade range: 0-3	Clinical category range: 1-3	Comment
7 L	5.4	0	3	2	A
7 R	4.2	0	3	2	N
8 L	10.2	0	0	1	N
8 R	12	0	0	1	N
10 R	11.4	3	3	3	A
10 L	12.2	3	0	3	A
11 R	13.4	1	1	3	A
11 L	5.2	1	1	3	N
12 R	10.8	0	0	1	N
13 L	6	0	2	2	A
16 L	11	0	0	1	N
16 R	5.6	0	0	1	A
17 R	12.4	0	3	2	A
17 L	6.8	0	2	2	A
18 L	13.2	2	3	3	A
19 R	6.8	0	3	3	A
20 R	11.4	0	0	1	N
20 L	11.2	0	0	1	N
22 L	13.2	2	2	3	A
22 R	10	2	3	3	A
23 R	12.2	2	3	3	A
28 L	11.4	1	0	1	N

A = Histological grade corresponded to the radiographic, pathological and clinical grades

N = Severe histological grades did not correspond to radiographic, pathological and clinical grades.

4.4.5.0 Electron microscopic features of synovial membrane and articular cartilage of hip joints with osteoarthritis

4.4.5.1 Synovial membrane findings

Figure 33 and 34 illustrate the electron microscopic features of synovial membrane harvested from severely osteoarthritic joints. There was extensive fibrosis of the synovial intimal layer, which was characterized by presence of electron dense bands of collagen fibres in various orientations. There was extensive deposition of electron dense particles within the intimal layer, adjacent to the synovial cells and within the collagen fibres. This observation was considered to indicate calcification.

Wide variation was noted in the structure of the synovial cells. Synovial hyperplasia and metaplasia were frequently observed. The synovial metaplasia appeared as cells containing more than one nucleus within the same cell membrane (Figure 34).

Vascularization of synovial membrane was also confirmed by electron microscopy. Numerous capillaries were seen in the synovium. This was a consistent finding in normal synovia and in those with acute and chronic synovitis. Necrotic synoviocytes and secretory vesicles from synoviocytes were also encountered (Figure 35 and 36).

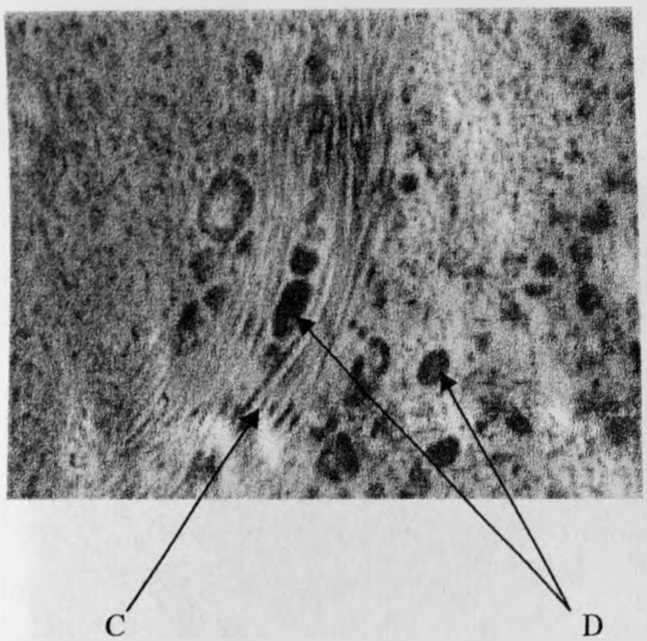


Figure 33. An electron micrograph of synovial membrane from the right hip joint of a 5-year-old German shepherd dog with severe osteoarthritis.
(Case number 31359- 10).
Notice the collagen fibres (C) in different orientations and the extracellular electron dense deposits (D) indicative of calcification.
Uranyl acetate and lead citrate; X 5,000.

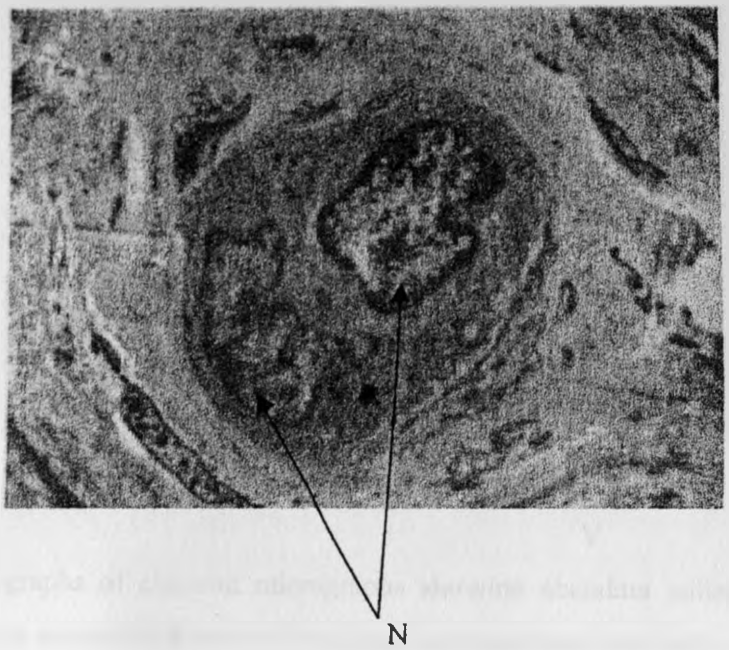


Figure 34. An electron micrograph of a synoviocyte with two areas of lobulated nuclear material (N), in synovial membrane from an 11-year-old German shepherd dog with severe osteoarthritis of the hip joint.
Case number 31359-18.
Uranyl acetate and lead citrate; X 2,500.

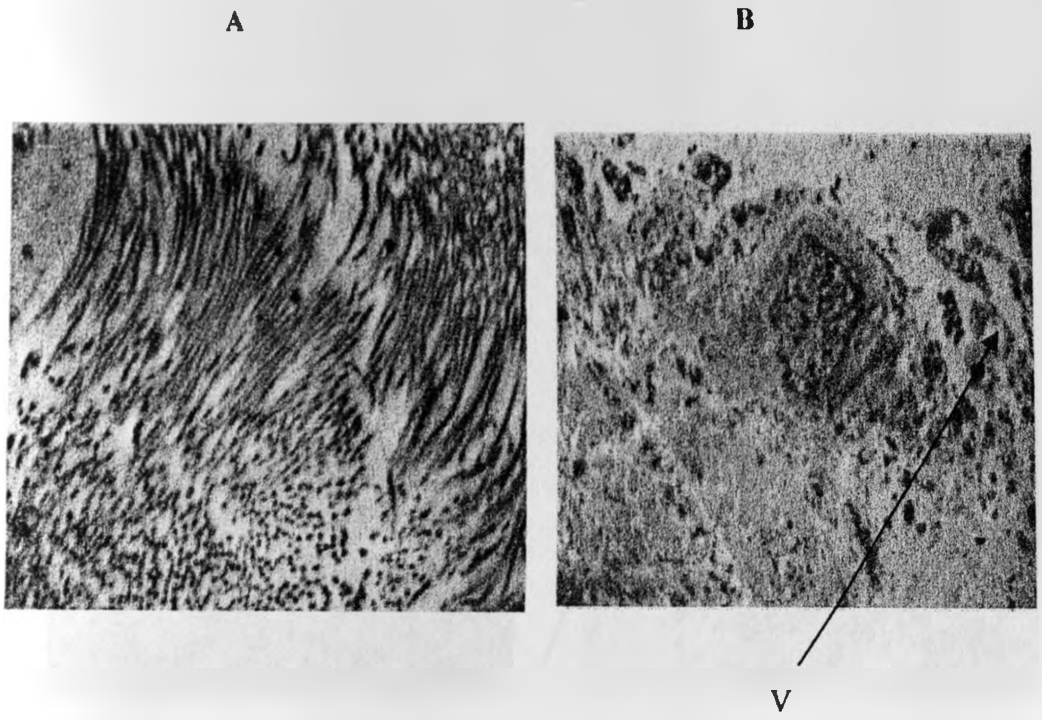


Figure 35. Photographs of electron micrographs showing abundant collagen fibres (A) and necrotic cell-types (B) of synovial membrane from an 11-year-old German shepherd dog with severe osteoarthritis of the hip joints.
Case number 31359-19.
Notice the abundant collagen fibres (A), fragmentation of nuclear and cytoplasmic material (B) and the secretory vesicles (arrow V).
Uranyl acetate and lead citrate; X 4,000 (A) and x 2,500 (B).

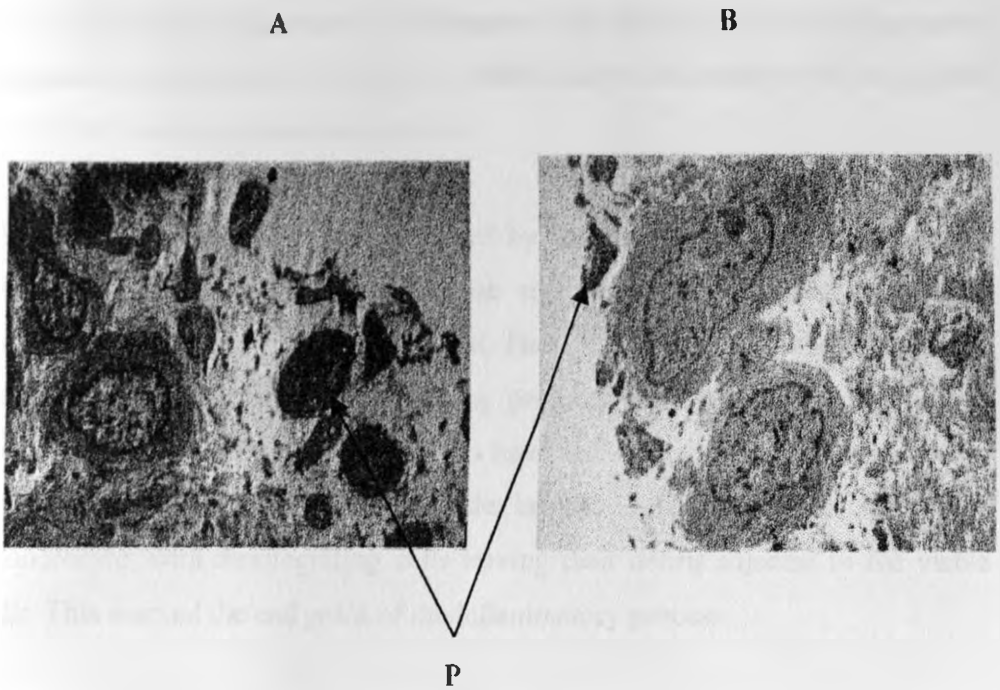


Figure 36. Photographs of electron micrographs of synoviocytes showing secretion of electron dense material and necrotic synoviocytes from an 11-year-old German shepherd dog with severe osteoarthritis of the hip joint.
Case number 31359-18.
Note the electron dense particles (P). The necrotic synoviocytes appear less intensely stained in A and B.
Uranyl acetate and lead citrate; X 2,000.

4.4.5.2 Articular cartilage findings

Electron microscopic evaluation of articular cartilage revealed proliferation (Figure 37 A and B), degeneration and death of chondrocytes. The proliferative tendency was demonstrated by presence of two, three or four chondrocytes within the same lacuna (Figure 37 C and D).

Although mitotic bodies were not encountered, the nucleus of such cells appeared lobulated or disintegrated (Figure 38). Multiplication of chondrocytes, evidenced by mitotic bodies, was not encountered.

Chondrocyte degeneration was indicated by the loss of cell membrane outline. The cells had no rough endoplasmic reticulum and exhibited shrinkage, fragmentation or loss of nuclear material. There was shrinking of the cytoplasmic material in the chondrocytes, indicating progressive degeneration (Figure 39). Degenerating chondrocytes appeared to have left behind dark staining electron dense particles and cell debris within the lacuna. Some lacunae had one viable chondrocyte, with disintegrating cells leaving their debris adjacent to the viable cells. This marked the end point of the inflammatory process.

Although some chondrocytes were devoid of nuclear material, they contained lipid bodies and rough endoplasmic reticulum within the cytoplasm (Figure 38).

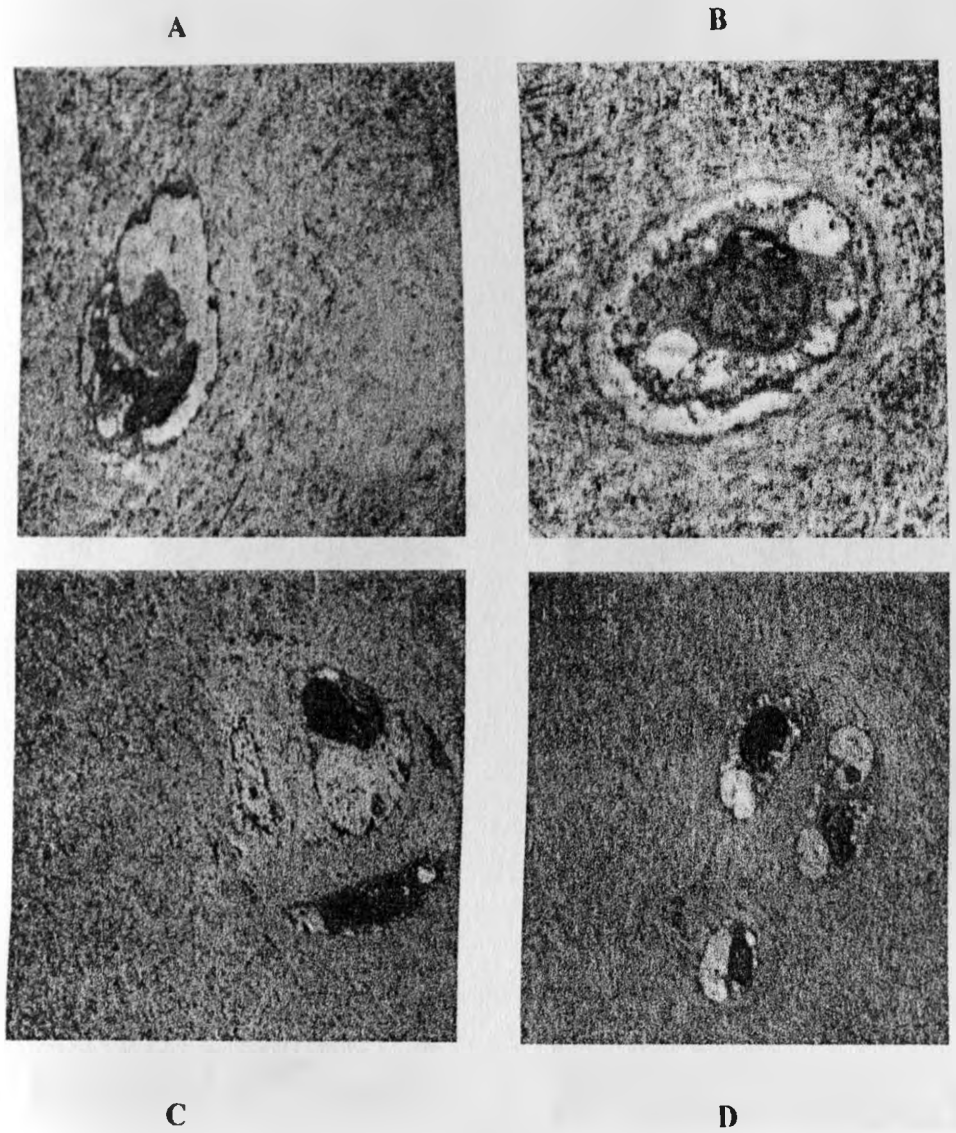
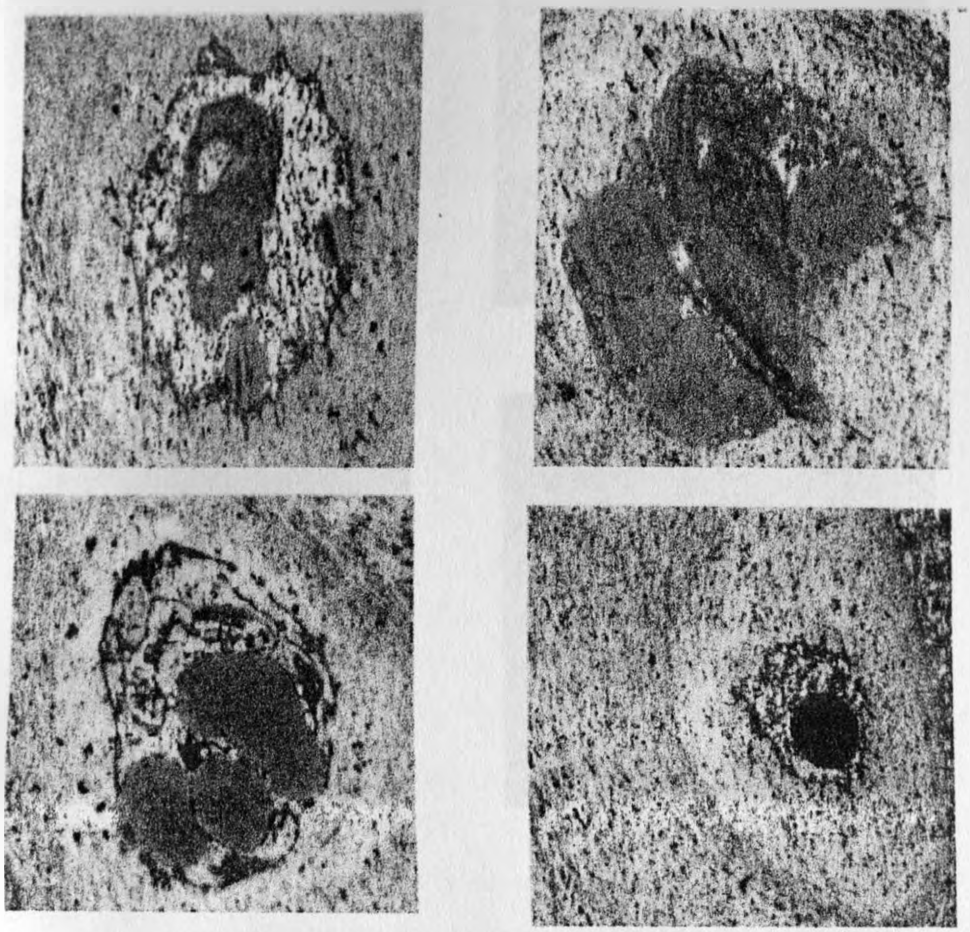


Figure 37. Electron micrographs showing chondrocyte degeneration (A and B), and chondrocyte clustering (C and D) of articular cartilage from an 11-year-old German shepherd dog with severe osteoarthritis of the hip joints.
Case number 31359-19.
Notice the fragmentation of the nuclear and cytoplasmic material (A and B). There are 3 cells within the lacuna in C and D respectively.
Uranyl acetate and lead citrate; X 1200.

A

B



C

D

Figure 38 Electron micrographs of chondrocytes from a 5-year-old German shepherd dog with severe osteoarthritis of the hip joints.

Case number 31359-10.

Notice the lipid bodies in A, B, C, and lobulation of the nuclear material (A and B). The nucleus is not evident in C while the lacuna in D appears prominent due to the reduced cytoplasm of the chondrocyte.

Uranyl acetate and lead citrate; X 2,000.

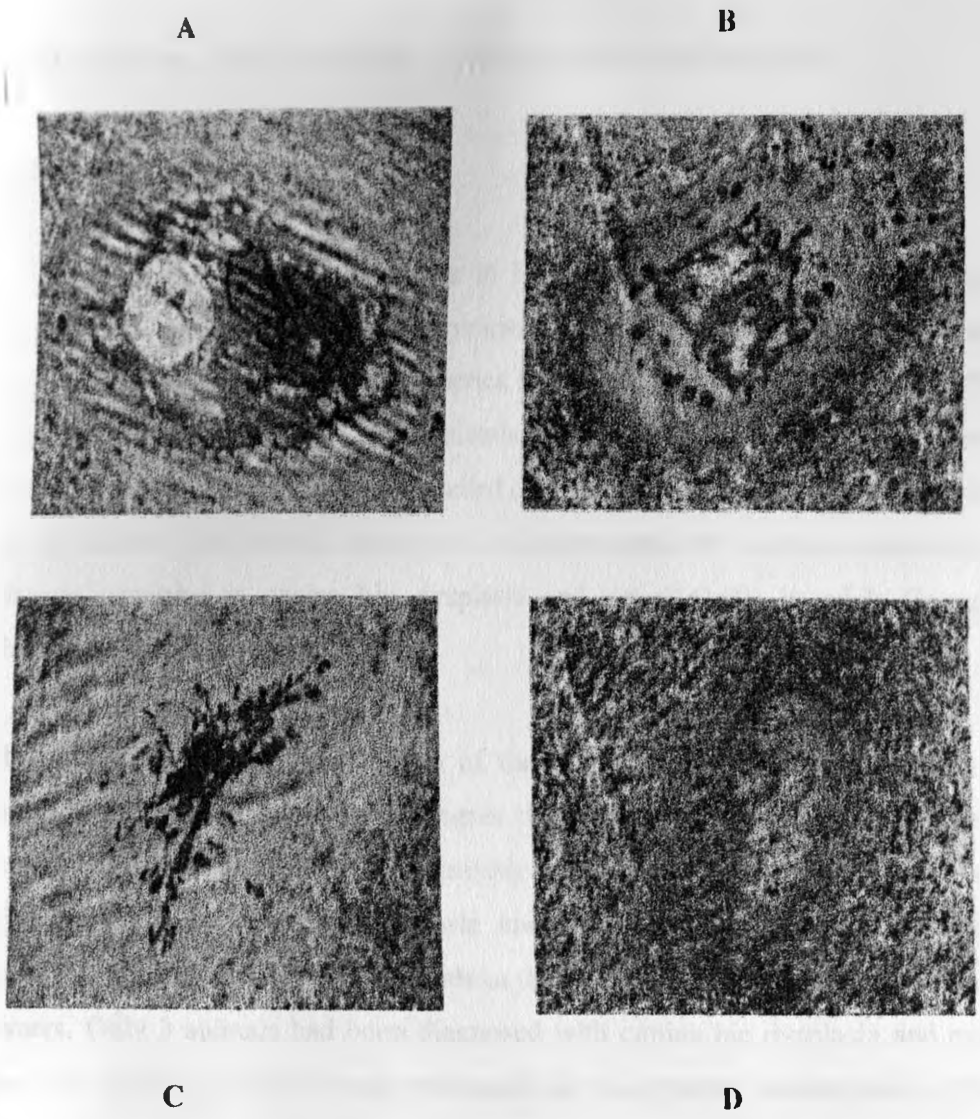


Figure 39 Electron micrographs showing progressive degeneration of chondrocytes from articular cartilage of a 10-year-old German shepherd dog with moderate osteoarthritis of the hip joint.

Case number 31359-17.

Notice the lobulation of nuclear material (A), progressive fragmentation of nuclear and cytoplasmic material in chondrocytes (B and C), eventually leaving the lacuna with cell debris (D).

Uranyl acetate and lead citrate; X 2000.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The existence of canine hip dysplasia in Kenya and the East Africa region has largely been derived from clinical empiricism rather than scientific data. However, scientific reports from Europe and America indicate that canine hip dysplasia is the most important joint disease in small animals (Smith, 1997, Lust, 1997). The present study, therefore, represents the first detailed original work on the nature and severity of the clinical, radiographic, gross and histopathologic and electron microscopic changes occurring in canine hip dysplasia and osteoarthritis in adult German shepherd dogs in Kenya.

The current study found that 44 % of the dogs were affected by canine hip dysplasia and osteoarthritis to the degree that they were physically debilitated, leading the owners to request for euthanasia of such dogs. Although the observed clinical signs of canine hip dysplasia and osteoarthritis were distinct, most animals carried on with their lives, without these signs being noticed by many dog owners. Only 3 animals had been diagnosed with canine hip dysplasia and even then, the referring veterinarians conducted no radiographic examination of the affected hip joints. In order to increase awareness, clinicians and dog owners need continuing education on the causes and clinical signs of canine hip dysplasia.

In order to detect the existence of this condition, adult dogs with hindlimb lameness should undergo thorough physical examination of the hip joints. This should include an evaluation of extended ventrodorsal pelvic radiographs. Arthroscopy, ultrasonography, computed tomography scanning and stress radiographic diagnostic methods should be further researched in Kenya, to facilitate accurate, timely diagnosis and prognosis.

Clinical data indicated that the adult form of canine hip dysplasia was commonly encountered. This is because relatively old dogs were selected for the study. The distinct clinical signs including severe debility, muscle atrophy, wobbling gait, changes in body conformation, vocalization and pain on flexion and extension and crepitus of the hip joint, were consistent with previous descriptions of the severe form of the disease (Brinker, et al., 1990, Lust, 1997). Other clinical signs indicative of mild osteoarthritis were moderate hindlimb lameness and pain on flexion and extension of the hip joint. Other workers (Brinker, et al., 1990) have reported these signs before.

Decubital wounds are a well-known sequel to chronic disease in recumbent animals. However, the development of decubital wounds on the dorsal aspects of paws and the excessive wearing of the toenails of affected hindlimbs have not been reported in scientific literature on canine hip dysplasia (Morgan, 1997, Smith, 1997). Clinical evaluation indicated that the paws were being dragged on the floor, further suggesting that the wounds were a result of reduced nerve function (delayed pelvic paw position reflex). Although Sorjonen, et al. (1990) described a case of ischiatic nerve entrapment in association with severe canine hip dysplasia, this study found similar clinical manifestations in 5 dogs affected with canine hip dysplasia without a coexisting lesion on ischiatic nerve. Ossification of the spinal meninges has been observed during other postmortem examinations of adult German shepherd dogs with hindlimb lameness. Evaluation of this etiological factor in contributing to hindlimb lameness in adult German shepherd dogs requires additional research, to determine its significance.

It is proposed that the decubital wounds on the dorsal aspect of the paws of the affected hindlimbs in this study was the result of ischiatic nerve entrapment. This was based on the associated delayed pelvic paw position reflex, and crepitus on flexion and extension of the hip joint. Further transformation to synovial chondrometaplasia and osteochondromatosis observed in this study could also have contributed to tissue rigidity and impaired nerve function. This was

clinically manifested as limited range of joint motion of affected hips. Liu and Thacher (1990) reported neurological deficit consequent to multiple cartilaginous exostoses involving the spinous processes of several thoracic and lumbar vertebrae in a 3-month old female Scottish terrier dog. Due to the attention placed on the hip joint, the extent to which osteochondromatosis affected other parts of dogs in this study was not determined and would form a component of a future study.

Whereas Jubb, et al. (1985) reported that articular cartilage has no nerve supply, Kinzel, et al. (1998) described the sensory innervation of the hip joint capsule in dogs. Accordingly, the craniolateral area of the hip joint capsule is innervated by rami articulares of N. gluteus cranialis, the caudolateral area by rami of N. ischiadicus and the medial area by rami articulares of N. femoralis. Synovial chondrometaplasia and osteochondromatosis observed in this study could impair the normal functions of the nerve structures, causing pain and physical disability of the affected hip joints.

Further investigations are needed to determine the relationship between nerve function and synovial chondrometaplasia and osteochondromatosis in canine hip dysplasia. Free nerve endings have been qualitatively and quantitatively measured in the acetabular labrum in 23 fresh human cadavers (Kim and Azuma, 1995) and in human patients undergoing hip surgery (Birnbaum, et al., 1997, Leunig, et al., 2000). Kinzel, et al. (1998) demonstrated the important role played by the synovial membrane and its nerve supply in nociceptive and proprioceptive mechanisms of the hip joint. More recently, Kinzel, et al. (2002) reported 10 years experience with denervation of the hip joint capsule for treatment of 269 cases of canine hip dysplasia and arthritis in Germany. Alteration in the anatomical properties of these structures may thus affect their physiological functions, as was manifested in the 5 dogs in this study.

Nutritional processes take place within the joint cavity by diffusion of metabolites across the synovial membrane, combined with the vascular supply within these tissues. Cartilaginous and osseous transformation of the joint capsule could impair diffusion of metabolites. The healing potential of surgical incision of the joint capsule has not been determined in hip joints with synovial osteochondromatosis. Further studies are needed to determine the effect of synovial chondrometaplasia and osteochondromatosis in causing pain to inflamed joint capsules in canine hip dysplasia.

The occurrence of 'joint mice' in the canine hip joint has previously not been reported in the literature and was an important finding in the study. Previous reports (Flo, et al., 1987, Edinger and Manley, 1998) have associated joint mice with osteochondritis of the shoulder joint, tarsocrural, talus and the elbow joints in dogs. Pathological examination in this study further demonstrated the presence of joint masses in 12 joints from dogs with severe canine hip osteoarthritis. It is a fact that clinicians would face a diagnostic challenge in handling adult dogs affected with joint masses within the hip joint. This study has shown that osteochondromatosis should be included in the differential diagnosis of hindlimb lameness in adult dogs.

Surgical management of canine synovial osteochondromatosis in the shoulder and elbow joints involves synovectomy (Flo, et al., 1987), arthrodesis (Edinger and Manley, 1998) or arthroscopic removal and curettage (Cook, et al., 2001). Such procedures are either not practical (for example arthrodesis of the hip joint) or would require highly specialized equipment and expertise. The observations made in this study indicate that the prognosis for hip joints with joint masses is likely to be very poor. This further emphasizes the need for early and accurate diagnosis that would lead to timely and rational management advice to the dog owners.

This study demonstrated the value of gross pathology in confirming the clinical and radiographic signs of osteoarthritis of the hip joint, and determining the

nature and severity of the condition in adult dogs. It also confirmed the existence of severe inflammatory changes affecting articular cartilage and synovial membrane from adult dogs with mild or severe osteoarthritis of the hip joint. Similar studies should be conducted in dogs that are euthanised for various reasons to further document the changes in hip joints.

Ligamentum capitis femoris participates in the stabilization of the hip joint in most animals. The integrity of the synovium and ligamentum capitis femoris in joints affected by trauma and laxity has been reported in acute asymptomatic canine hip dysplasia and osteoarthritis (Lust and Summers, 1981, Madsen, 1997). The size of the ligament increased due to hyperplasia, edema and chronic fibrosis. This was determined by a previously described water displacement technique (Lust and Summer 1981, Burton-Wurster, et al., 1999), in young dogs specifically bred for their genetic predisposition to canine hip dysplasia. This is the first report on the volume of ligamentum capitis femoris in adult dogs with normal and osteoarthritic hip joints, previously not published in scientific literature, until recently (Mande, et al., 2003).

Analysis of the data revealed no distinct difference between the volume of ligamentum capitis femoris in hip joints that were normal and those that had mild osteoarthritis. However, the mean volume of ligamentum capitis femoris in hip joints with moderate osteoarthritis was lower than in the normal and mild osteoarthritic joints. The ligament was torn in all hip joints affected by severe osteoarthritis, consistent with previous reports (Morgan, 1997) describing rupture of ligamentum capitis femoris in end stage canine hip dysplasia and osteoarthritis. The study confirmed the volume of ligamentum capitis femoris as a useful research tool in studies of canine hip osteoarthritis. However, further studies on hip joints of other dog breeds are needed, to establish normal values and in varying severity of osteoarthritis in Kenya and East Africa region.

The mean volume of ligamentum capitis femoris decreased as the severity of osteoarthritis increased until the ligament was severed in hip joints with the most severe osteoarthritis. This contrasts with two previous studies (Lust, et al., 1980, and Lust and Summers, 1981) involving young dogs, in which the mean volume of ligamentum capitis femoris increased as the severity of hip joint subluxation increased. The decrease in the volume of ligamentum capitis femoris in this study could be accounted for by progressive damage due to physical stretching in aging dogs.

Progressive degeneration of the ligament could also have occurred subsequent to limited blood supply or inflammation of the ligament in chronic and severe osteoarthritis of the hip joints in adult dogs. It is further suggested that edema, inflammation and fibrosis could have contributed to the comparatively large volume of ligamentum capitis femoris in hip joints that were normal and those that had mild osteoarthritis in this study.

Synovial villous hypertrophy, hyperplasia, cellular infiltration and fibrosis are consistent findings in chronic synovitis and osteoarthritis in general (Jubb, et al., 1985). No detailed description was available in the literature on morphological and histological changes in synovial membrane in chronic hip osteoarthritis. This study demonstrated the preponderance for synovial villous hypertrophy, cellular infiltration and fibrosis. This contrasted with previous reports in which extensive villous hypertrophy, synovial chondrometaplasia and osteochondromatosis were not described in the pathology of canine hip dysplasia and osteoarthritis (Madsen, 1997, Morgan 1997).

Although most cases of synovial chondrometaplasia have been reported in adult dogs (Flo, et al., 1987, Gathumbi, 1991), one report (Liu and Thacher, 1990) described multiple cartilaginous exostoses affecting metatarsals, phalanges, scapula, ends of ribs and spinous processes of vertebrae in a 3 month-old female

Scottish terrier dog. Jubb, et al. (1985) observed that aging and chronic synovitis lead to osteochondromatosis and calcification.

Evaluation of adult dogs in this study demonstrated synovial chondrometaplasia and osteochondromatosis only in samples from dogs with clinical, radiographic, gross and histological features of severe hip dysplasia and osteoarthritis. On the other hand, no such findings were associated with samples from dogs of similar age group but without severe osteoarthritic hip joints. Synovial chondrometaplasia and osteochondromatosis observed in this study was thus, a consequence of chronic osteoarthritis of the hip joint, rather than aging alone.

The gross pathological alterations affecting articular cartilage observed in this study were consistent with those reported by other researchers in Europe and America (Morgan, 1997). The pathological and histological observations of joints also positively correlated with the radiographic manifestations of periarticular and intraarticular radiodensity. The increased radiographic density was due to fibrosis, chondrometaplasia and osteochondromatosis and osteophyte formation on articular cartilage around the affected hip joints.

The general radiographic and gross pathological alterations observed in this study were similar to those reported previously (Todhunter, et al., 1997). However, there was no description of joint mice and synovial chondrometaplasia in the hip joints despite the large number of dogs examined (Todhunter, et al., 1997). The only report (Gathumbi, 1991) on synovial osteochondromatosis of the hip joint of a dog did not indicate whether the joint had osteoarthritis. This study provides the first scientific data on synovial osteochondromatosis in several dogs with severe hip dysplasia and osteoarthritis.

The study further revealed that 64 % of the radiographic grades were consistent with gross pathological grades for the same hip joint. This implied that there was a probability of 64 % that radiographic grades could be accurately diagnosed and

confirmed by gross pathology. Further, 84.4 % of the radiographic grades were determined accurately within the error –1 and +1 difference.

The severe articular alterations leading to flaking, fibrillation and eburnation, accompanied with osteophyte formation and synovial chondrometaplasia and osteochondromatosis, all indicated end stage osteoarthritis. Acetabular alterations also included osteophyte formation, ossification of the acetabular fossa, complete fibrillation and remodeling of the acetabular contour. The most severe changes were flattening and eburnation of the acetabulum. This is proposed to be the result of constant mechanical abrasion, flaking, fibrillation and eburnation of articular surfaces. The ongoing biochemical processes resulting from the release of lysosomal enzymes from the traumatized chondrocytes and synoviocytes, also contribute to the pathological process. While osteophyte formation represent an attempt at repair and remodeling of the affected joint, histological evaluation of this tissue revealed abundant fibrous tissue with few chondrocyte-like cells with no particular arrangement such as seen in normal articular cartilage.

Histological examination of synovial membrane revealed extensive fibrosis, hyperplasia and degeneration of synoviocytes, as well as synovial chondrometaplasia. Additionally, the histological demonstration of bone and cartilage tissue in sections of joint masses was indicative of transformation of synovial tissue during chronic synovitis associated with osteoarthritis of the hip joint in adult dogs. It is necessary to consider treatment of extensive synovitis during surgical interventions to remove or replace osteoarthritic hip joints in adult dogs. Medical therapy for the inflamed synovium and articular cartilage may also be indicated.

The cellular transformation of normal synovial cells to chondrocyte-like cells, extensive fibrosis and preponderance for electron dense deposits indicating calcification, as demonstrated by light and electron microscopy, have rarely been reported in previous literature. The wide variation in the morphology and

distribution of chondrocytes and synoviocytes in the samples made their quantitation impracticable in this study. However, the electron microscopic findings validate the hypothesis that cell transformation occurs in adult dogs with chronic synovitis and osteoarthritis of the hip joint.

Electron microscopic studies revealed abundant collagen fibres within the synovial membrane, which contributed to thickening of the tissue. Observation of fibrosis confirmed the histological and gross pathological findings and is consistent with previous reports describing fibrosis and calcification related to chronic osteoarthritis in general (Jubb, et al., 1985). There was fibroplasia of the joint capsule in the most severe lesions in previous studies (Lust and Summers, 1981). However, calcification of synovial membrane reported in this study was not documented in previous studies of young dogs (Lust and Summers, 1981). This is probably due to the chronic nature of the condition in this study.

The presence of necrotic cells in synovial tissue could be due to either degeneration of cytoplasmic structure following enzymatic processes associated with the inflammation. This could also be a response to mechanical stress due to fibrosis and calcification, consequently reducing blood supply to the cells in the chronic stage of the disease. The observation of necrotic and uncertain cells in chronic synovitis was consistent with a previous report in young dogs (Lust and Summers, 1981).

Synovial metaplasia was demonstrated as cells containing more than one nucleus. This is perhaps the initial stage of synovial chondrometaplasia observed in gross and histological examination of tissue from dogs with severe hip osteoarthritis. Although synovial metaplasia and osteochondromatosis may indicate end stage osteoarthritis, information on such features in chronic synovitis and hip osteoarthritis in dogs is scanty. The results of this study indicate the need for further research on the pathobiology of synoviocytes in the overall progression of osteoarthritis with particular emphasis on the hip joint in adult dogs.

Electron microscopy of articular cartilage revealed the presence of degenerating chondrocytes, chondrocyte proliferation, chondrocyte death and empty lacuna. Degeneration of articular cartilage was marked in all the sampled tissue and interestingly, was demonstrated in tissue collected from animals that were clinically normal or had mild osteoarthritis. These observations contrasted with previous reports (Lust and Summers, 1981) in which chondrocyte degeneration and necrosis were not encountered, perhaps due to the relatively young animals studied and the early stage of the disease. The severe osteoarthritic changes observed in this study were probably due to the extensive inflammatory responses and attempts to repair the articular tissue.

Chondrocyte proliferation, death (apoptosis) and formation of osteophytes demonstrated in this study were also noted by Sandell and Aigner (2001) who summarized the reaction patterns of chondrocytes in human osteoarthritis into five categories; proliferation and cell death (apoptosis), changes in synthetic activity and degradation, phenotypic modulation of the articular chondrocyte and formation of osteophytes. Chondrocyte death has been the subject of recent controversy in the literature. Some researchers have encountered chondronecrosis (apoptosis) to a certain extent while others dismiss the concepts of chondrocyte death. The observation of chondronecrosis in this study adds to the existing scientific evidence of overwhelming catabolism in relation to the anabolic activity of the chondrocyte in osteoarthritis in dogs.

Lucchinetti, et al. (2002) reported chondrocyte necrosis following repetitive loading of bovine articular cartilage explants. It is proposed that the severity of osteoarthritis and the continued use of the inflamed hip joint could have progressively led to the chondrocyte necrosis reported in dogs in this study. The abrasive strain generated from the use of poorly lubricated articular surfaces in canine hip dysplasia and osteoarthritis could simulate the repetitive loading experiments. The excessive, repetitive loading imposed by Lucchinetti, et al. (2002) were not physiological and demonstrated the deleterious effects of

mechanical overload resulting in morphological and cellular damage similar to that seen in degenerative joint disease.

No recent scientific reports were found describing the electron microscopic changes in adult dogs with hip dysplasia and osteoarthritis. On the other hand, developments in the cell biology of osteoarthritis in reference to chondrocytes response have been reviewed in man (Fukui, et al., 2001, Sandell and Aigner, 2001). Diagnostic tools employing immunostaining for cellular proteins and staining for nuclear DNA and mRNA (McKenna, et al., 2000, Aigner, et al., 2001, Aigner and McKenna, 2002) are not available to researchers investigating canine hip dysplasia and osteoarthritis in developing countries.

Further studies are recommended to determine the nutritional management practices in dogs in Kenya. The studies would determine the extent to which diets influence the occurrence of osteoarthritis and provide guidelines on supplementary diets for dogs in Kenya. The current study provides baseline data on the clinical, radiographic and pathological features needed to actualize such studies.

Experimental models (Mande, et al., 1998a, Smith, et al., 2002, Marijnissen, et al., 2002), cartilage modifying agents (Mande, et al., 1998b, Innes, et al., 2000b) and pathophysiology of osteoarthritis in dogs (Mande, et al., 2000) have been reviewed. Although experimental models facilitate the study of early osteoarthritis, they take long to develop significant lesions and rarely reproduce the natural disease. This study provides the clinical, radiographic and pathological data on naturally occurring osteoarthritis and synovitis of the hip joint in adult dogs in Kenya, previously not reported in scientific literature.

5.2 CONCLUSIONS AND RECOMMENDATIONS

5.2.1 CONCLUSIONS

1. Clinical evaluation revealed that 44 % of adult dogs had severe osteoarthritis of the hip joints presenting with signs of lameness, pain, and physical disability. The disease is an important orthopedic problem affecting German shepherd dogs, despite current measures control (promoting breeding from dogs with lower hip scores) to its occurrence in Kenya. An important clinical presentation was the presence of decubital wounds on the dorsum of the paws of affected hindlimbs, attributed to neurological deficit associated with severe osteoarthritis.

2. The extended pelvic radiographic examination helped to assess the status of hip joints as normal or osteoarthritic. The procedure is recommended for routine examination of adult dogs with hindlimb lameness.

3. The inverse correlation ($r = -0.75$) between the mean volume of ligamentum capitis femoris and the radiographic grades of progressive severity of osteoarthritis of the hip joint, indicated the importance of the volume of ligamentum capitis femoris as a research tool for studies on hip dysplasia and osteoarthritis in adult dogs.

4. This is the first scientific report demonstrating a positive correlation ($r = 0.6$) between radiographic grades and gross pathological grades of osteoarthritis of the hip joints of dogs. Radiography and postmortem examination of hip joints should be routinely performed in dogs to improve the existing knowledge on canine osteoarthritis in Kenya.

5. Since the synovial membrane from 52 % of hip joints had villous hypertrophy, thickening, discoloration or transformation to cartilage or bone tissue, pathological changes are important features in chronic synovitis and osteoarthritis in adult dogs. These changes may affect the physiological functions of the hip joint capsule.

6. Based on the observation of synovial osteochondromatosis in 21 % of hip joints, it appears that synovial osteochondromatosis (joint mice) occurs more commonly than previously reported in scientific literature on adult dogs with hip osteoarthritis. The condition should be included in the differential diagnosis of hindlimb lameness in adult dogs.

7. Histology of articular cartilage and synovial membrane confirmed evidence of pathological changes in chondrocytes and synoviocytes, even in samples from animals found to be clinically normal, while the changes in severely affected animals were predictably extreme. Data derived from histological evaluation should be interpreted with reference to clinical and radiographic information.

8. Extensive fibrosis and calcification of synovial tissue and degeneration and metaplasia of synoviocytes, observed by electron microscopy, were associated with chronic synovitis and osteoarthritis in the study. Chondrocyte degeneration, proliferation and necrosis were also observed in advanced osteoarthritis.

5.2.2 RECOMMENDATIONS

Based on this study, the following recommendations are proposed;

1. Radiographic examination confirmed the severity of osteoarthritis of the hip joints of adult dogs. Further studies are needed to evaluate the occurrence and nature of hip dysplasia and osteoarthritis in young dogs. Such studies would determine the likelihood of the condition developing in aging dogs.

2. Although this study revealed that 61 % of adult dogs had mild and severe hip osteoarthritis, further epidemiological studies are needed to determine the prevalence of this condition in Kenya. An important output of the study is a catalogue of radiographs showing the various radiographic changes in hip joints, with a guide to the grade of osteoarthritis in dogs. This catalogue could be further developed and utilized for teaching, research and diagnostic purposes in Kenya and East Africa. Such literature is currently not available for the region.

3. With limited diagnostic facilities and expertise, radiographic examination and reference to a catalogue of the various grades, would assist veterinary clinician to determine the severity and prognosis for managing adult dogs afflicted by the condition. The information would be useful in future research as a reference guide and facilitate interpretation of radiographs of adult dogs with hip osteoarthritis in Kenya and East Africa.

4. The diagnostic accuracy could be further improved through training on current diagnostic techniques and radiographic interpretation. Additional interpretation by qualified veterinarians and examination of many pelvic radiographs could significantly improve the existing diagnostic capacity. This would ensure better understanding of the occurrence and severity of canine hip dysplasia and osteoarthritis in Kenya and East Africa.

5. It is necessary to improve the existing research capabilities by utilizing techniques in molecular biology to study the chondrocytes and synoviocytes in canine hip dysplasia and osteoarthritis in Kenya and East Africa. Collaboration with research institutions would facilitate exchange of scientific and clinical expertise on this condition.

6. The present study generated valuable material and institutional knowledge for teaching undergraduates and postgraduates, previously not available at the Faculty of Veterinary Medicine, University of Nairobi. The material will be used to increase awareness amongst the public, dog owners, donors and research partnerships, in order to consolidate efforts on improving the quality of life of dogs in Kenya. The routine postmortem examination of hip joints of dogs with chronic hindlimb lameness is recommended. This would improve the knowledge on the pathobiology of osteoarthritis, particularly changes in articular cartilage and synovial membrane in chronic osteoarthritis of hip joints of adult dogs in Kenya. Young and adult dogs from other breeds, and under different environmental conditions (nutritional management and occupation) should be included in such studies.

7. Long-term multidisciplinary research is recommended to determine the prevalence and effects of nutrition and genetics on radiographic and pathological features of hip osteoarthritis in large breed dogs in Kenya and East Africa. The effectiveness of the hip dysplasia scheme also needs to be evaluated. The East African Kennel Club, faculty of veterinary schools and dog owners should collaborate in order to control this debilitating condition.

CHAPTER SIX

6.0 REFERENCES

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CHAPTER SEVEN

7.0 APPENDICES

Appendix 1 A. The British Veterinary Association / Kennel Club: criteria for hip scoring scheme (1994).

Scores of 0-6 for each hip (except item 7)

1) Norberg Angle	(Determined as shown in Figure 5 A).
2) Subluxation:	Assess by degree to which femoral head is covered by dorsal acetabular edge and divergence of cranial joint space.
3) Cranial Acetabular Edge (C.A.E.)	Degree of flattening: 'Bilabiation' in advanced cases.
4) Dorsal Acetabular Edge (D.A.E.):	Exostosis (advanced cases only). Usually progressive from cranial end.
5) Cranial Effective Acetabular Rim (C.E.A.R.)	Exostoses and facets.
6) Acetabular Fossa (A.F.):	New bone (advanced cases).
7) Caudal Acetabular Edge (C.A.E.)	Exostoses (advanced cases) (Maximum score points 5).
8) Femoral head neck:	Exostoses.
9) Re-contouring:	Bone loss due to subchondral erosion (advanced cases).

Total score up to 53 for each hip = 106.

Appendix I B. Clinical classification of osteoarthritis by Pfizer Animal Health.

Osteoarthritis can be categorized into three levels of severity based on clinical signs. A patient with osteoarthritis may exhibit one or more of these signs:

MILD

- Slight stiffness and lameness when walking
- Mild pain during palpation of affected joint.
- Minimal licking of affected joint.
- 10-20% loss in range of motion.
- Some degeneration seen on radiographs.
- No palpable joint crepitus.

MODERATE

- Increased stiffness and lameness when walking, shortened gait.
- Moderate pain during palpation of affected joint.
- Occasional vocalization.
- Some licking of affected joint.
- 20-50% loss in range of motion.
- More pronounced degeneration seen on radiographs.
- Some palpable joint crepitus.
- Sitting preferred over standing.
- Reluctance to climb steps or jump up.
- Slow to rise from resting position.

SEVERE

- Reluctance to rise or walk more than five strides.
- Dog will not allow examiner to palpate joint
- Frequent vocalization
- Frequent licking of affected joint
- Over 50% decrease in range of motion
- Severe degeneration seen on radiographs
- Increased palpable joint crepitus
- Behavior changes
- Increased difficulty rising from resting position.

(1997 Pfizer Inc 1191 RIM9682 1/97).

Appendix 1 C. Criteria for the clinical categories of hindlimb lameness of study animals.

Category 1: Normal.

- Good muscle cover of the hindquarters.
- No clinical signs of lameness.
- Normal gait and posture.

Category 2: Mild Lameness.

- Mild hindlimb lameness attributable to the hip joints.
- Slight muscle atrophy.
- Pain on flexion and extension of the hip joints.
- Slight limit in range of motion.
- Slight inability to bear weight on hindlimbs.

Category 3: Severe Lameness.

- History of prolonged lameness of the hindlimbs.
- Extensive muscle atrophy of the hindlimbs.
- Wobbly gait and posture.
- Crepitus on manipulation of the hip joints.
- Non weightbearing or recumbency.
- Decubital wounds on dorsal aspects of paws.
- Pain and vocalization on flexion and extension of the hip joints.

Appendix II-III. Summary of data on study animals.

Sample	Case No.	Code	Source	Age	Weight	Sex	Clinical	Radiographic grade	
Number		(owner)		years	(kilograms)	M or F	Grade	Left joint	Right joint
1	31359-1	CC	Clinic	12	34.2	M	3	1	1
2	32249	AT	Clinic	8	29.7	F	2	0	0
3	31359-7	BK	Clinic	7	25.6	M	2	0	0
4	31359-8	VS	Private	10	19.6	M	1	0	0
5	31359-9	VS	Private	6	26.9	M	1	0	0
6	31359-10	HA	Clinic	5	25.1	F	3	3	3
7	31359-11	VS	Private	8	18.3	M	3	1	1
8	31359-12	VS	Clinic	7	24.3	F	1	0	0
9	31359-13	VS	Clinic	17	21	F	2	0	0
10	31359-14	TL	Clinic	8	24.6	M	3	3	2
11	31359-15	AFC	Military	12	23.3	F	3	3	3
12	31359-16	OV	Clinic	6	28.3	M	1	0	0
13	31359-17	SH	Clinic	10	25.1	M	2	1	0
14	31359-18	IJ	Private	11	44.3	M	3	3	3
15	31359-19	IJ	Private	11	34.7	M	3	0	1
16	31359-20	KH	Clinic	7	23.3	M	1	0	0
17	31359-22	SF	Private	8	26.5	M	3	2	2
18	31359-23	KPDU	Military	8	27.8	F	3	2	2

Appendix II-III. Summary of data on study animals.

19	31359-24	AFC	Military	15	21.9	M	3	3	3
20	31359-25	DP	NCC	8	35.6	M	1	0	0
21	31359-26	VS	Private	7	25.6	M	3	3	0
22	31359-27	VS	Private	10	25.6	F	2	2	1
23	31359-28	LM	Private	7	24.2	M	1	1	0
24	31359-29	EN	Private	11	27.4	M	3	3	2
25	31359-30	AFC	Military	12	26.5	F	1	0	0
26	31359-31	CC	Clinic	10	41.5	F	1	0	0
27	31359-32	CC	Clinic	8	25.6	M	3	0	0
28	31359-33	AFC	Military	11	22.4	F	2	3	2
29	31359-34	KS	Clinic	10	36	M	3	0	0
30	32510	GM	Clinic	9	27.8	F	3	3	3
31	31359-35	AFC	Military	9	29.7	M	3	3	3
32	31359-36	AFC	Military	4	23.3	M	1	1	1
33	31359-38	CC	Clinic	11	29.7	F	1	0	0
34	31359-39	KPDU	Police	10	27.4	F	1	0	1
35	31359-40	KPDU	Police	10	19.6	F	1	0	0
36	31359-41	KPDU	Police	10	29.9	F	1	0	0
				9.25	**27.29**	15F:21M			

Appendix IV. Summary of radiographic grades of osteoarthritis and volume of ligamentum capitis femoris.

Sample	Case number	Radigraphic grade		Volume of ligament		Radigraphic grade	
		Joint	Grade	Left	Right	Joint	Grade
1	31359-1	1L	1	1	1	1R	1
2	32249	32249L	0	x	x	32249R	0
3	31359-7	7L	0	1.1	1.3	7R	0
4	31359-8	8L	0	0.6	0.4	8R	0
5	31359-9	9L	0	1	1	9R	0
6	31359-10	10L	3	0	0	10R	3
7	31359-11	11L	1	0.5	0.4	11R	1
8	31359-12	12L	0	0.4	0.5	12R	0
9	31359-13	13L	0	0.5	0.4	13R	0
10	31359-14	14L	3	x	x	14R	2
11	31359-15	15L	3	x	x	15R	3
12	31359-16	16L	0	0.7	0.7	16R	0
13	31359-17	17L	1	0.6	0.6	17R	0
14	31359-18	18L	3	0	0	18R	3
15	31359-19	19L	0	1.2	0.9	19R	1
16	31359-20	20L	0	1	0.8	20R	0
17	31359-22	22L	2	0.2	0	22R	2
18	31359-23	23L	2	1.5	0	23R	2
19	31359-24	24L	3	0	0	24R	3
20	31359-25	25L	0	0.6	0.7	25R	0
21	31359-26	26L	3	0	0.5	26R	0
22	31359-27	27L	2	0.5	0.6	27R	1
23	31359-28	28L	1	0.7	1.5	28R	0
24	31359-29	29L	3	0	0	29R	2
25	31359-30	30L	0	0.5	0.5	30R	0
26	31359-31	31L	0	1.2	1.5	31R	0
27	31359-32	32L	0	1.3	1.2	32R	0
28	31359-33	33L	3	0	0	33R	2
29	31359-34	34L	0	1.3	1	34R	0
30	32510	32510L	3	0	0	32510R	3
31	31359-35	35L	3	0	0	35R	3
32	31359-36	36L	1	0.8	0.5	36R	1
33	31359-38	38L	0	0.9	0.5	38R	0
34	31359-39	39L	0	0.4	0	39R	1
35	31359-40	40L	0	X	X	40R	0
36	31359-41	41L	0	0.7	0.6	41R	0

X - Animal was not available for postmortem

Appendix V. Number of the hip joints showing various radiographic grades of osteoarthritis.

Radiographic grade	Hip joint number												Total	Percentage
0	13R	13L	7R	7L	8R	8L	9R	9L	12R	12L	16R	16L	37	51.4
	17R	19L	20R	20L	25R	25L	26R	28R	30R	30L	31L	31R		
	32L	32R	34L	34R	38L	38R	39L	40R	40L	41R	41L	32249R		
	32249L													
1	1R	1L	11R	11L	17L	19R	27R	28L	36R	36L	39R		11	15.3
2	14R	22R	22L	23R	27L	29R	33R	18L					8	11.1
3	10R	10L	14L	15R	15L	18R	23L	24L	24R	26L	29L	33L	16	22.2
	32510R	32510L	35L	35R										
											Total		72	100

R represents right joint; L represents left joint.

Appendix VI. Analysis of variance (ANOVA) test on the mean volume of ligamentum capitis femoris based on various radiographic grades of osteoarthritis.

The data for the treatments was derived from appendix showing the ligamentum capitis femoris volume and the radiographic grade of osteoarthritis of each hip joint evaluated in the study.

The GLM Procedure

Class Level Information

Class	Levels	Values
TREAT	4	T1 T2 T3 T4

Number of observations 64

Dependent Variable: LCFV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	6.84259808	2.28086603	21.62	<.0001
Error	60	6.33099567	0.10551659		
Corrected Total	63	13.17359375			

R-Square	Coeff Var	Root MSE	LCFV Mean
0.519418	56.95705	0.324833	0.570312

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TREAT	3	6.84259808	2.28086603	21.62	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TREAT	3	6.84259808	2.28086603	21.62	<.0001

Parameter		Estimate		Standard Error	t Value	Pr > |t|
Intercept		-.0000000000	B	0.09009251	-0.00	1.0000
TREAT	T1	0.8212121212	B	0.10636792	7.72	<.0001
TREAT	T2	0.6545454545	B	0.13307546	4.92	<.0001
TREAT	T3	0.3142857143	B	0.15228414	2.06	0.0434
TREAT	T4	0.0000000000	B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Least Squares Means

TREAT	LCFV LSMEAN	LSMEAN Number
T1	0.82121212	1
T2	0.65454545	2
T3	0.31428571	3
T4	-0.00000000	4

Least Squares Means for effect TREAT
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: LCFV

i/j	1	2	3	4
1		0.1458	0.0004	<.0001
2	0.1458		0.0343	<.0001
3	0.0004	0.0343		0.0434
4	<.0001	<.0001	0.0434	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

The data was analyzed using a computer statistical software and significance determined at 95 percent confidence ($p < 0.05$).

Appendix VII. Histological scores of synovial membrane harvested from the hip joints of adult German shepherd dogs in Kenya.

Observation	Sample (joint)	Histological findings	Synovial score
1	19 L	Fragmentation of the villous tissue, extensive fibrosis, moderate infiltration with inflammatory cells.	2
2	18 L	Fragments of cartilage-like tissue, predominantly roundish cells at the periphery, and fibroblast-like cells, extensive fibrosis.	4
3	10 L	Extensive infiltration with inflammatory cells, extensive fibrosis leading to thickening, and disorganization, villous hypertrophy.	3
4	29 L	Capillary neovascularization, fragments of cartilage islands, villous hypertrophy, infiltration with inflammatory cells, extensive fibrosis.	4
5	29 R	Extensive fibrosis and villous hypertrophy, hyperplasia and moderate capillary neovascularization, predominant chondrocyte-like cells.	4
6	23 R	Extensive fragmentation of villi, appearing as cartilage islands, many roundish chondrocyte-like cells, extensive fibrosis, synovial metaplasia.	4
7	23 L	Extensive villous hypertrophy, synovial hyperplasia and evidence synovial metaplasia.	2.5
8	22 R	Extensive fibrosis but no villous hypertrophy, fibroblast-like cells and macrophage like cells evident.	1.5
9	22 L	Extensive hyperplasia and fibrosis, foci of synovial metaplasia, fibroblast and roundish chondrocyte-like cells.	2.5
10	28 L	Extensive hyperplasia, and cell infiltration, mild fibrosis and synovial metaplasia.	2
11	28 R	Extensive hyperplasia and mild fibrosis.	2
12	12 L	Hyperplasia and capillary neovascularization, villous formation, synovial metaplasia.	1.5
13	12 R	Synovial membrane lined with about one-two cells, no villous formation, no hyperplasia.	0
14	27 L	Fibroblast-like cells evident, as well as macrophage-like cells, extensive extensive fibrosis and synovial hyperplasia, cartilage islands present.	3.5
15	17 L	Extensive fibrosis and synovial hyperplasia, capillary vascularization, islands of cartilage and fragmentation of villi.	4
16	13 R	Fibroblastic hyperplasia no villous formation and mild capillary vascularization.	1
17	10 R	Fibrosis, and capillary vascularization, synovial metaplasia, evidence of cartilage islands and fragmentation of the villi.	4
18	18 R	Fibrosis, synovial metaplasia, cartilage like tissue on periphery of villi and as fragments.	4
19	19 R	Fibrosis and synovial hyperplasia, cartilage-like tissue present, synovial metaplasia and villous proliferation.	3.5
20	1 R	Extensive synovial hyperplasia, fibrosis and synovial metaplasia Villous hypertrophy.	3
21	1 L	Fibrosis and synovial hyperplasia, extensive capillary neovascularization roundish chondrocyte-like cells, and cartilage islands.	4
22	16 L	Inflammatory cell infiltration, fibrosis and synovial hyperplasia, evidence of fibroblast-like cells and roundish macrophage-like cells.	1
23	16 R	Moderate fibrosis and synovial hyperplasia, capillary vascularization, villi present.	1.5
24	26 L	Fibrosis and capillary neovascularization, synovial metaplasia, extensive villous proliferation and some cartilage like tissue.	3.5

Appendix VIII. Comparison between the radiographic grades and those determined by gross pathological examination of hip joints from 32 adult German shepherd dogs in Kenya.

Joint	Radiographic grade	Pathological grade	Difference
1R	1	3	-2
1L	1	3	-2
13R	0	2	-2
13L	0	2	-2
7R	0	3	-3
7L	0	3	-3
8R	0	0	0
8L	0	0	0
9R	0	0	0
9L	0	0	0
10R	3	3	0
10L	3	3	0
11R	1	1	0
11L	1	1	0
12R	0	0	0
12L	0	0	0
16R	0	0	0
16L	0	0	0
17R	0	3	-3
17L	1	2	-1
18R	3	3	0
18L	3	3	0
19R	1	3	-2
19L	0	1	-1
20R	0	0	0
20L	0	0	0
22R	2	3	-1
22L	2	2	0
23R	2	3	-1
23L	2	3	-1
24R	3	3	0
24L	3	3	0

Joint	Radiographic grade	Pathological grade	Difference
25R	0	0	0
25L	0	0	0
26R	0	1	-1
26L	3	3	0
27R	1	1	0
27L	2	1	1
28R	0	0	0
28L	1	0	1
29R	2	3	-1
29L	3	3	0
30L	0	0	0
30R	0	0	0
31L	0	0	0
31R	0	0	0
32L	0	0	0
32R	0	0	0
33L	3	3	0
33R	2	3	-1
34L	0	2	-2
34R	0	2	-2
32510L	3	3	0
32510R	3	3	0
35L	3	3	0
35R	3	3	0
36L	1	1	0
36R	1	1	0
38L	0	0	0
38R	0	0	0
39L	0	1	-1
39R	1	2	-1
41L	0	1	-1
41R	0	1	-1

Diff. Difference = (Radiographic- Pathological) grades

Appendix IX. Correlation coefficients between the radiographic and pathological grades of osteoarthritis of the hip joints from adult German shepherd dogs.

Frequency table of radiographic grades and pathological grades of hip osteoarthritis

Radiographic grade	Pathological grade				All
	0	1	2	3	
0	21.00	5.00	4.00	3.00	33.00
1	1.00	5.00	2.00	3.00	11.00
2	0.0	1.00	1.00	5.00	7.00
3	0.0	0.0	0.0	13.00	13.00
All	22.00	11.00	7.00	24.00	64.00

FREQUENCY	PATHOLOGY		
RADIOGRAPHY	NO	YES	TOTAL
NO	21	12	33
YES	1	30	31
TOTAL	22	42	64

Pearson Correlation Coefficient

Correlation (r)		0.6356
ASE		0.0822
95%	Lower Confidence limit	0.4746
95%	Upper Confidence limit	0.7967
Test of HO: Correlation = 0		
ASE under HO		0.0944
Z		6.7366
One-sided	Pr > Z	<0.0001
Two-sided	Pr > |Z|	<0.0001
Exact test		
One-sided	Pr >= r	1.393E-07
Two-sided	Pr >=	1.578E-07

Simple Kappa Coefficient

Kappa	(K)	0.5977
ASE		0.0937
95%	Lower Confidence limit	0.4141
95%	Upper Confidence limit	0.7813
Test of HO: Kappa = 0		
ASE under HO		0.1175
Z		5.0852
One-sided	Pr > Z	<0.0001
Two-sided	Pr > |Z|	<0.0001
Exact Test		
One-sided	Pr > = K	1.393E-07
Two-sided	Pr > = |K|	1.578E-07

Sample Size = 64

Appendix X. Histological grades of articular cartilage degradation of femoral heads from 22 adult German shepherd dogs.

Joint	Sample	I	II	III	IV	Mankin score	Joint	Sample	I	II	III	IV	Mankin score
7 L	1	0	1	3	0	**4**	**11 L**	1	0	1	3	1	**5**
	2	1	1	3	0	**5**		2	0	1	1	0	**2**
	3	2	2	2	0	**6**		3	5	2	3	1	**11**
	4	2	1	3	0	**6**		4	0	1	3	0	**4**
	5	2	2	2	0	**6**		5	0	1	3	0	**4**
					Mean	*5.4*						Mean	*5.2*
7 R	1	0	1	2	0	**3**	**12 R**	1	6	2	4	1	**13**
	2	1	2	3	0	**6**		2	0	1	4	0	**5**
	3	1	1	2	0	**4**		3	3	1	4	0	**8**
	4	0	1	3	0	**4**		4	6	3	4	1	**14**
	5	0	1	3	0	**4**		5	6	3	4	1	**14**
					Mean	*4.2*						Mean	*10.8*
8 L	1	0	1	4	0	**5**	**10 L**	1	6	2	4	0	**12**
	2	6	3	4	1	**14**		2	6	3	4	1	**14**
	3	5	1	4	1	**11**		3	3	3	4	1	**11**
	4	3	2	4	1	**10**		4	2	3	4	1	**10**
	5	4	2	4	1	**11**		5	6	3	4	1	**14**
					Mean	*10.2*						Mean	*12.2*
8 R	1	5	3	4	1	**13**	**13 L**	1	0	1	4	0	**5**
	2	6	3	4	1	**14**		2	2	1	4	0	**7**
	3	2	3	4	1	**10**		3	1	1	4	0	**6**
	4	2	3	4	0	**9**		4	1	1	4	0	**6**
	5	6	3	4	1	**14**		5	1	1	4	0	**6**
					Mean	*12*						Mean	*6*
10 R	1	5	2	4	1	**12**	**16 R**	1	0	1	4	0	**5**
	2	2	2	4	0	**8**		2	0	1	3	0	**4**
	3	3	2	4	1	**10**		3	1	2	3	0	**6**
	4	6	3	4	1	**14**		4	2	2	3	0	**7**
	5	6	2	4	1	**13**		5	1	2	3	0	**6**
					Mean	*11.4*						Mean	5.6
11 R	1	6	2	4	1	**13**	**16 L**	1	6	2	3	1	**12**
	2	6	2	4	1	**13**		2	4	2	3	1	**10**
	3	6	2	4	1	**13**		3	2	2	3	1	**8**
	4	6	3	4	1	**14**		4	6	1	4	1	**12**
	5	6	3	4	1	**14**		5	6	2	4	1	**13**
					Mean	*13.4*						Mean	*11*

Appendix X. Histological grades of articular cartilage degradation of femoral heads from 22 adult German shepherd dogs.

Joint	Sample	I	II	III	IV	Mankin score		Joint	sample	I	II	III	IV	Mankin score	
17 R	1	6	3	4	1	**14**		**22 L**	1	6	1	4	1	**12**	
	2	3	3	4	1	**11**			2	6	2	4	1	**13**	
	3	6	3	4	1	**14**			3	6	3	4	1	**14**	
	4	5	2	4	1	**12**			4	6	3	4	1	**14**	
	5	5	2	4	0	**11**			5	6	2	4	1	**13**	
					Mean	*12.4*							Mean	*13.2*	
17 L	1	3	2	3	0	**8**		**23 R**	1	6	2	4	1	**13**	
	2	2	1	3	0	**6**			2	4	1	4	1	**10**	
	3	0	1	3	0	**4**			3	4	2	4	1	**11**	
	4	0	1	3	0	**4**			4	6	2	4	1	**13**	
	5	6	2	3	1	**12**			5	6	3	4	1	**14**	
					Mean	*6.8*							Mean	*12.2*	
19 R	1	4	1	4	1	**10**		**28 L**	1	6	2	4	0	**12**	
	2	6	3	3	1	**13**			2	6	2	4	0	**12**	
	3	6	2	3	1	**12**			3	6	3	4	1	**14**	
	4	6	2	4	1	**13**			4	6	3	4	1	**14**	
	5	6	2	4	1	**13**			5	6	3	4	1	**14**	
					Mean	*6.8*							Mean	*11.4*	
20 R	1	6	3	4	1	**14**		**18 L**	1	6	2	4	1	**13**	
	2	6	3	4	1	**14**			2	6	3	4	1	**14**	
	3	3	2	4	1	**10**			3	6	3	4	1	**14**	
	4	5	3	4	1	**13**			4	4	2	4	1	**11**	
	5	0	1	4	1	**6**			5	6	3	4	1	**14**	
					Mean	*11.4*							Mean	*13.2*	
20 L	1	6	3	4	1	**14**		**22 R**	1	2	2	4	0	**8**	
	2	6	3	4	1	**14**			2	1	2	4	1	**8**	
	3	2	3	4	1	**10**			3	6	3	4	1	**14**	
	4	2	3	4	0	**9**			4	2	3	4	1	**10**	
	5	2	3	4	0	**9**			5	2	3	4	1	**10**	
					Mean	*11.2*							Mean	*10*	

Appendix XI. Criteria for grading of articular cartilage degradation.

Grade 0

Sections represented normal articular cartilage, which had a smooth, intact surface, uniform distribution of chondrocytes with 1 or 2 cells/lacuna, and uniformly red-stained extracellular matrix.

Grade 1

Articular cartilage sections had surface flaking and irregular loss of red-staining surface proteoglycans.

Grade 2

Articular cartilage had mild fibrillation of surface with extensive loss of proteoglycans and chondrocytes clustering.

Grade 3

Sections had severe eburnation of articular cartilage leaving no chondrocytes or matrix above the tidemark and subchondral bone.

(adapted from Dew and Martin, 1992).