Intervertebral Disc Degeneration in Dogs

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Dedication

To Annette, Niels, Thom and Anna, for all your love and laughter.

Intervertebral Disc Degeneration in Dogs

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(met een samenvatting in het Nederlands)

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Cover: Midsagittal histological sections of a healthy (left) and a moderately degenerated (right) canine intervertebral disc stained with picosirius red and alcian blue (photo: J. Fama).

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Intervertebral Disc Degeneration in Dogs

Abstract

Back pain is common in both dogs and humans, and is often associated with intervertebral disc (IVD) degeneration. The IVDs are essential structures of the spine and degeneration can ultimately result in diseases such as IVD herniation or spinal instability. In order to design new treatments halting or even preventing IVD degeneration, more basic knowledge of the disease process is needed.

The aim of this thesis was to increase the knowledge of IVD degeneration in dogs and to evaluate the similarities and differences between IVD degeneration in dogs and humans, in order to establish whether spontaneous IVD degeneration occurring in both chondrodystrophic (CD) and non-chondrodystrophic (NCD) dog breeds can be used as translational animal models for human spine research.

The key findings of the thesis were:

- The division of the processes underlying canine IVD degeneration into chondroid or fibroid degeneration appears to be inaccurate. The biochemical, histopathological, and morphological alterations examined during the process of IVD degeneration were found to be similar in CD and NCD dog breeds.
- IVD degenerative diseases were most common in CD breeds, especially in Dachshunds, and were 1.5 times more common in male than female dogs. Case fatality rates were found to be higher than previously suggested, with rates of 34% in the overall population, around 20% in most CD breeds, and over 50% in the NCD breeds at highest risk such as the Doberman and the German Shepherd Dog.
- IVD degeneration in dogs could accurately be diagnosed, early in the degenerative process, by using low-field magnetic resonance imaging (MRI). The MRI based grading scheme used in humans could reliably be used in dogs, and was found to be highly correlated with pathological changes found *post mortem*. Early diagnosis facilitates the possibility of preemptive treatments.
- A new nucleus pulposus prosthesis, made of an intrinsically radiopaque hydrogel, was tested *ex-vivo* in dogs. Surgical implantation of the prosthesis in canine lumbosacral IVDs via a dorsal laminectomy was clinically applicable. After absorbing fluid from the surrounding tissue the swollen implant could restore disc height, which could be monitored by radiography, computed tomography and MRI.
- Many similarities were found between the processes of IVD degeneration in humans and CD and NCD dog breeds. Both dog-types may serve as translational animal models of spontaneous IVD degeneration for human research. Synergistic effects of studying IVD degeneration in veterinary patients could lead to new treatment modalities for both dogs and humans, a reduced need for animal testing, and lower cost of research. It is also likely that spontaneous IVD degeneration in dogs more resembles the true disease process, as it occurs in humans, than induced IVD degeneration in experimental animals.

Keywords: Intervertebral disc degeneration, dog, canine, herniation, spontaneous animal model.

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Content

This thesis is based on the following publications:

I. **Bergknut N**, Egenvall A, Hagman R, Gustas P, Meij BP, Hazewinkel HAW, Lagerstedt A-S. Incidence and mortality of diseases related to intervertebral disc degeneration in a population of over 600,000 dogs. *Journal of the American Veterinary Medical Association* provisionally accepted (2010).

II. **Bergknut N,** Auriemma E, Wijsman SJCM, Voorhout G, Hagman R, Lagerstedt A-S, Hazewinkel HAW, Meij BP. Pfirrmann grading of intervertebral disc degeneration in chondrodystrophic and non-chondrodystrophic dogs with low-field magnetic resonance imaging. *American Journal of Veterinary Research* accepted (2010).

III. **Bergknut N**, Grinwis GCM, Pickée EB, Auriemma E, Lagerstedt A-S, Hagman R, Hazewinkel HAW, Meij BP.Validation of macroscopic grading of canine intervertebral disc degeneration according to Thompson and correlation with low-field magnetic resonance imaging findings. *American Journal of Veterinary Research* accepted (2010).

IV. **Bergknut N**, Rutges JPHJ, Smolders LA, Kranenburg HC, Hagman R, Lagerstedt A-S, Grinwis GCM, Voorhout G, Creemers LB, Dhert WJA, Hazewinkel HAW, Meij BP. The dog as an animal model for human intervertebral disc degeneration? *Spine* under revision (2010).

V. **Bergknut N**, Smolders LA, Koole LH, Voorhout G, Hagman R, Lagerstedt A-S, Saralidze K, Hazewinkel HAW, van der Veen AJ, Meij BP. An *ex-vivo* investigation of the properties of a new nucleus pulposus prosthesis in canine spines. *Biomaterials* (2010) Sep;31(26):6782-8.

Abbreviations

AF	Annulus fibrosus
CD	Chondrodystrophic
CSM	Cervical spondylomyelopathy
CT	Computed tomography
DLSS	Degenerative lumbosacral stenosis
DYAR	Dog years at risk
EP	Endplate
IVD	Intervertebral disc
IVDD	Intervertebral disc degeneration
MRI	Magnetic resonance imaging
NCD	Non-chondrodystrophic
NP	Nucleus pulposus
SE	Standard error

Chapter 1

Aim and scope of the thesis

Background

The canine intervertebral disc (IVD) is a versatile structure and is responsible for the stability and flexibility of the vertebral column[1,2]. Degeneration of the IVD is a common phenomenon in dogs and is characterized by degradation of the extracellular matrix, mainly proteoglycans and collagen[3,4]. Once the degenerative process has started, a cascade of events is triggered that can ultimately lead to structural failure of the IVD and clinical signs of disease[5,6]. Common diseases related to IVD degeneration in dogs include degenerative lumbosacral stenosis (DLSS)[7], cervical spondylomyelopathy (CSM)[8], and Hansen type I and II IVD herniation[9,10]. These diseases are referred to as "IVD degenerative diseases" in this thesis. Herniation of the IVD is the most common cause of neurological deficits in dogs[5,9], with a lifetime prevalence estimated at 2%[2,11]. IVD degeneration is, however, not synonymous with IVD disease. While IVDs giving rise to clinical signs of disease inevitably will be degenerated, degenerated IVDs are common incidental findings in dogs [3,12-14].

The canine species can be divided into chondrodystrophic (CD) and non-chondrodystrophic (NCD) breeds based on their physical appearance. In CD breeds, endochondral ossification of the long bones is disrupted, resulting in disproportionally short extremities. This trait has in the past been favored in selective breeding programs[3,15], but unfortunately chondrodystrophy is also linked with IVD degeneration, which has resulted in breeds, such as the Dachshund, with disproportionally short legs and a high prevalence of IVD herniation. IVD degeneration in CD breeds is reported to develop early, often before 1 year of age[3]. However, some large NCD breeds, such as the German Shepherd Dog and the Doberman, can also develop IVD degeneration, but then usually later in life[15]. Although degeneration of the IVD is considered to be multifactorial,[6] the main factors are considered to be genetic in CD breeds and trauma or "wear and tear" in NCD breeds[3,5].

Although IVD degenerative diseases in dogs have been the focus of numerous studies over the past 60 years, most of these studies were limited to diagnostics and treatments, leaving the process of degeneration largely unexplored. Considerably more studies, focusing on the pathogenesis of IVD degeneration, have been conducted in humans and laboratory animals[6,16-18]. Although the clinical presentation, diagnostics, and treatments are largely similar in humans and dogs[4,19-21], few comparative studies have been performed[17,19,22,23]. Despite the lack of comparative data, the dog has frequently been used as a model of human disease when developing new surgical procedures and for biomechanical research of the spine[1,24-28]. Before results based on translational studies between dogs and humans can be accurately evaluated, basic comparative studies are needed to determine the similarities and differences between the process of canine and human IVD degeneration.

Hypothesis

1. The morphological process of IVD degeneration in CD and NCD breeds is more similar than previously reported, with the only difference being that degeneration takes place earlier in life and proceeds more rapidly in CD breeds.

2. Spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human IVD research.

Aims

The first aim of this thesis was to increase the knowledge of IVD degeneration in dogs with regards to the morphological processes of degeneration and the demographics of IVD degenerative diseases, and also to validate grading schemes enabling objective grading and monitoring of the process of IVD degenerations in dogs.

The second aim of this thesis was to evaluate the similarities and differences between IVD degeneration in dogs and humans, in order to establish whether spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human research. The reason for wanting to use dogs as models for human IVD degeneration is threefold. *Firstly*, relevant animal models are needed to successfully design new treatments for IVD degeneration in humans. Spontaneously occurring IVD degeneration in an animal, living in the same environment as humans, is likely to mimic the human situation better than induced IVD degeneration in laboratory animals, an approach that is commonly used today. *Secondly*, new treatments for IVD degenerative disease in humans, designed in dogs, will also benefit dogs as veterinary patients. *Thirdly*, by using canine veterinary patients for relevant clinical trials and also to study the process spontaneously occurring IVD degeneration *in vivo* as well as *post mortem*, the number of laboratory animals used for IVD research can hopefully be reduced.

To explain how the main objectives of this thesis were intended to be met, and the hypotheses tested, the specific aims of each separate chapter are described below.

The aim of **Chapter 2** was to review current literature on canine IVD degeneration, thereby explaining, and hopefully increasing the understanding of this process, as it is known in dogs.

The aim of the study described in **Chapter 3** was to increase insight into the age and breed distribution of IVD degenerative diseases in dogs. To this end, a large population-based study was performed, with the view of using the data obtained as a platform for future genetic studies of IVD degenerative diseases in dogs.

The aim of the study reported in **Chapter 4** was to evaluate whether the magnetic resonance imaging (MRI) based grading system by Pfirrmann[29] for grading of IVD degeneration in human lumbar discs is applicable for use in both CD and NCD breeds and for intervertebral discs at all locations of the vertebral column.

The aims of the study described in **Chapter 5** were to validate the Thompson[30] grading system for gross pathological changes of IVD degeneration in dogs, and to investigate the agreement between pathology findings and low-field MRI findings.

The aim of the study reported in **Chapter 6** was to investigate whether spontaneous IVD degeneration occurring in CD and NCD dog breeds can be used as valid translational models for human IVD degenerative research, by comparing the morphological appearance, histological structure, and biochemical characteristics in different stages of IVD degeneration in dogs and humans.

The aim of the study described in **Chapter 7** was to perform a translational study where a novel nucleus pulposus prosthesis (NPP), intended ultimately for clinical use in humans, was tested *ex-vivo* in canine lumbosacral segments (L7-S1). A clinically adapted mode of implantation of the NPP in the nuclear cavity of the L7-S1 intervertebral disc was investigated. Swelling, fit, and restoration of disc height of the NPP *in situ* were monitored by radiography, computed tomography and MRI.

The results of these studies are summarized and discussed in **Chapter 8,** and the general findings and conclusions are presented in English, Dutch, and Swedish in **Chapter 9**.

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Chapter 2

General Introduction

Intervertebral Disc Degeneration in the Dog

Abstract

Intervertebral disc degeneration is common in dogs and is closely associated with intervertebral disc (IVD) diseases, such as type I and II IVD herniation, cervical spondylomyelopathy, and degenerative lumbosacral stenosis. Although there have been many reports and reviews on the clinical aspects of canine IVD disease, little is known about the degenerative process leading to IVD disease in dogs. This review discusses the physiology of the healthy disc and the morphological, histopathological, biochemical, and biomechanical effects of IVD degeneration, with special attention being paid to the distinction between chondrodystrophic and non-chondrodystrophic dog breeds. Finally the possibility of disc regeneration in dogs with IVD diseases is discussed.

Introduction

The canine spine consists of 7 cervical, 13 thoracic, 7 lumbar, 3 (fused) sacral, and a variable number of coccygeal vertebrae[1,2]. The vertebral bodies of C2-S1 and all coccygeal vertebrae are interconnected by an intervertebral disc (IVD)[1,3]. The IVD is responsible for both the stability and flexibility of the vertebral column. It is a versatile structure composed of four essentially different parts: a central nucleus pulposus (NP), an outer annulus fibrosus (AF), the transition zone (TZ) between the AF and NP, and cartilaginous endplates (EPs) in between the IVD and the subchondral bone (Fig. 1).

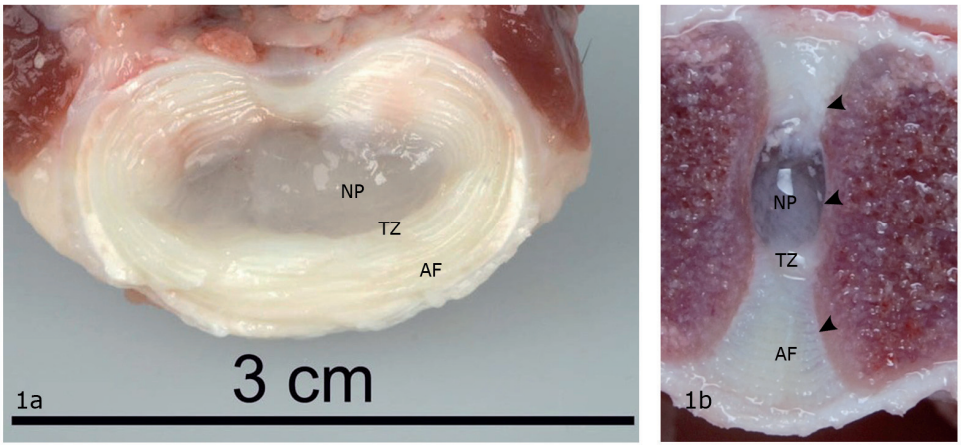

Figure 1. Transverse (a) and sagittal (b) section through a L5-L6 intervertebral disc of a mature non-chondrodystrophic dog, showing the nucleus pulposus (NP), transition zone (TZ), annulus fibrosus (AF), and endplates (EP).

Degeneration of the IVD is a common phenomenon in dogs and is associated with IVD degenerative disease[4,5]. The medical definition of degeneration is: '*The change of tissue to a lower or less functionally active form. True degeneration is defined by actual chemical change of the tissue itself'*. However, it is important to stress that IVD degeneration is not the same as IVD disease[2]. IVD degeneration is known to predispose dogs to Hansen type I and II IVD herniation[2] and is highly associated with degenerative lumbosacral stenosis (DLSS)[6,7] and cervical spondylomyelopathy (CSM)[8]. Dogs displaying clinical signs of IVD disease, such as IVD herniation, DLSS, or CSM, will inevitably have IVD degeneration; however, degenerated IVDs are also common incidental findings in dogs without clinical signs of disease [2,8-10].

The first case report on IVD degenerative disease in a dog was published in 1881 and involved a Dachshund with sudden onset of hind limb paralysis[11]. Although the mass that compressed the spinal cord in this Dachshund was described as a "chondroma located only to the epidural space", it is more likely that this was the first description of IVD herniation in a dog. Shortly thereafter, in 1896, a more comprehensive study of IVD herniation in dogs was published, although the disease was not yet recognized as herniation of the IVD, but as enchondrosis intervertebralis[12], a reactive inflammation in the epidural space. It would take another 40 years before the disease called enchondrosis intervertebralis was correctly described in the veterinary literature as being a herniation of NP material from the IVD into the spinal canal, causing compression of the spinal cord[13]. Pioneering studies of IVD degeneration in dogs, which are still commonly referred to, were performed during the 1950s by the Swedish veterinarians Hansen and Olsson[2,14-16]. Since their studies, numerous publications have described the clinical aspects of IVD degenerative diseases, but very few have revisited the fundamental aspects of IVD degeneration[17-27]. Substantial research has been performed on IVD degeneration in humans, and veterinary articles on IVD degeneration often translate the degenerative processes from humans to dogs[28,29]. Although there are similarities between IVD degenerative diseases in dogs and humans that would warrant such translation, extrapolation of human data to dogs may lead to erroneous conclusions regarding the degenerative process in dogs.
The aim of this introduction was to review and compile current literature on canine IVD degeneration, with a view to increasing our understanding of the degenerative processes active in dogs.

Embryology of the canine spine and IVD

In the early mammalian embryo, the body plan of the mature organism is established during the process of gastrulation, in which three individual somatic germ layers are formed: an outer ectodermal layer, a middle mesodermal layer, and an inner endodermal layer[30,31]. A longitudinal column of mesoderm, the notochord, establishes the cranial/caudal and posterior/anterior axes of the developing embryo (Fig. 2). The notochord can be used as a reference axis, dividing the embryo into left and right sides[30,32,33]. Ectoderm directly posterior to the notochord gives rise to the neural plate, which is composed of so-called neuroectoderm. The neural tube and neural crest cells (positioned dorsolateral to the neural tube) are formed from the neuroectoderm and give rise to the central nervous system and peripheral nervous system, respectively[30,33].
During the development of the neural tube, mesoderm adjacent to the developing neural tube forms a thickened column of cells, the paraxial mesoderm. The paraxial mesoderm ultimately develops into discrete blocks and from then on these are referred to as somites.

Most components of the axial skeleton, the associated musculature, and the overlying dermis derive from these somites: each somite is divided into: 1) dermatome, which gives rise to dermis, 2) myotome, which gives rise to epaxial musculature, and 3) sclerotome, which gives rise to vertebral structures[30,33]. The notochord induces its surrounding mesenchymal cells to secrete epimorphin, which attracts sclerotomal cells to the region around the notochord and neural tube[34,35]. Sclerotomal cells migrate medially and ventrally on either side of the neural tube and form a continuous tube of mesenchymal cells, the perichordal tube, which completely surrounds the notochord[34]. Subsequently, increased proliferation of cells at regular intervals along the length of the perichordal tube creates an alternating series of dense and less dense accumulations of cells, a process called resegmentation[34,35]. While the bodies of the vertebrae develop from the less dense accumulations, the dense accumulations form the AF and TZ of the IVD, intervertebral ligaments, vertebral arches, and vertebral processes, of which the latter two eventually fuse with their corresponding vertebral body[34,35]. The formation of the vertebral bodies results in segmentation of the notochord, which persists as separate portions in each intervertebral space. These separate portions of notochord expand, forming the NP of the individual IVDs[32,34-36].

Anatomy and physiology of the IVD

The canine spine can be subdivided into individual functional spinal units each of which is composed of an IVD, two adjacent vertebrae, two facet joints, and the surrounding ligamentous structures[37]. The dorsal and ventral longitudinal ligaments are situated dorsal and ventral to the IVD and vertebral bodies, respectively[1,3]. In addition, an intercapital ligament (*ligamentum conjugale costarum*) is situated between the heads of each pair of ribs in the region from T1-T2 to T9-T10, passing on the left and right sides beneath the dorsal longitudinal ligament[1-3]. The IVDs at different spinal levels have a different size and shape. Overall, the cervical discs are the thickest IVDs, followed by the lumbar, thoracic, and coccygeal discs, respectively[3]. An exception is the lumbosacral IVD, which is the largest IVD of the canine spine. The size and conformation of the IVD and its surrounding ligaments are decisive for the mobility of the spinal segment: the cervical spine and lumbosacral junction are relatively mobile, whereas the thoracic spine is relatively rigid and stiff[2,3,38-41].

The healthy IVD is composed of four distinct components, each of which exhibits specialized physical-mechanical properties designed for specific functions. The central NP is a mucoid, translucent, oval-shaped structure, mainly composed of chemically

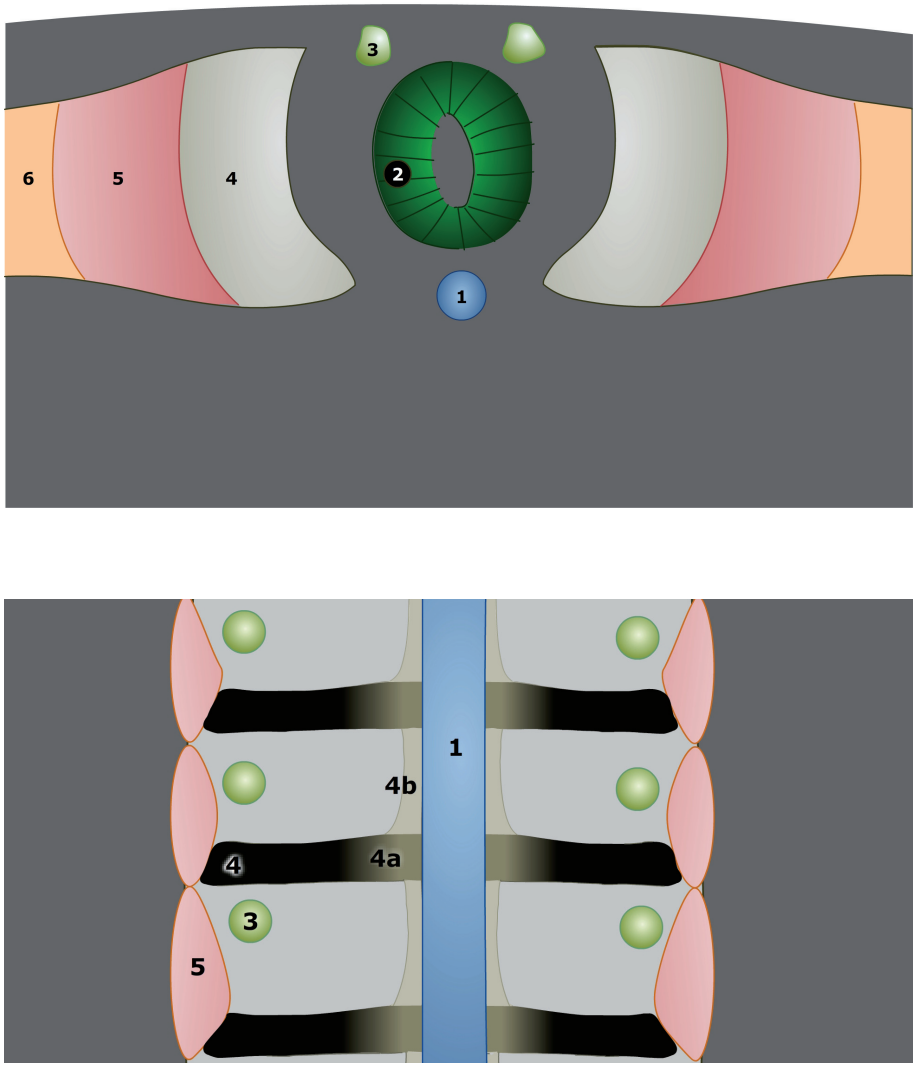

Figure 2A. Schematic image of a transverse (top) and dorsal (bottom) cross-section through the canine embryo, with the 1) notochord, 2) neural tube, 3) neural crest cells, 4) sclerotome, 5) myotome, and 6) dermatome.

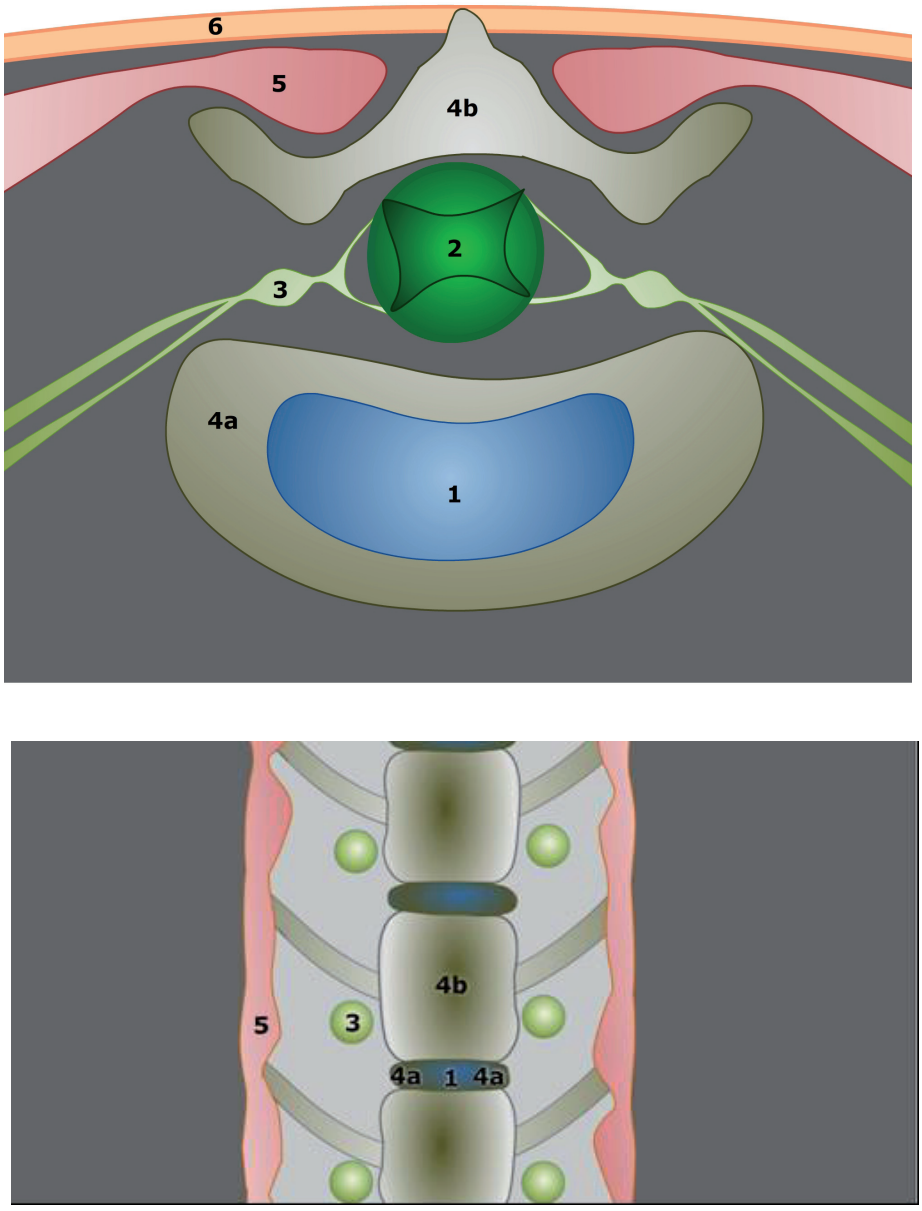

Figure 2B. Schematic image of a transverse (top) and dorsal (bottom) cross-section through the dorsal part of a mature dog, with the 1) nucleus pulposus, 2) spinal cord, 3) spinal nerves, 4a) annulus fibrosus and transition zone, 4b) vertebra, 5) epaxial musculature, and 6) skin. The colors of the structures of the mature animal correspond with the colors of their embryological origin.

trapped water molecules (>80%) and a negatively charged extracellular matrix, which attracts water toward the center of the IVD by osmosis[42-46]. The NP is surrounded ventrally, dorsally, and laterally by the AF, a dense network of multiple, organized, concentric fibrous lamellae. In each lamella, the fibers run parallel and are tilted with respect to the axis of the spine, with the direction of tilt alternating in successive lamellae[42,47]. The inner lamellae of the AF are generally thicker than the outer layers, and the ventral part of the AF is 2 to 3 times thicker than the dorsal part[2,47-49]. Near the center of the IVD, the AF loses its distinctive structure and form to become more cartilaginous and less fibrous[2,3,42]. This zone of transition from a fibrous to a more cartilaginous/mucoid structure, the TZ, forms the interconnection between the NP and AF. The TZ is often not considered a separate structure, but as the innermost AF[40]. The cranial and caudal borders of the IVD are formed by the cartilaginous endplates (EPs), situated in between the NP/AF and the epiphyses of the respective cranial and caudal vertebral bodies[42,48,49]. The tissue of the EPs is hyaline cartilage-like, lacking the fibrous appearance of the fibrocartilaginous AF[42]. The collagen and elastin fibers of the inner AF are strongly connected with the EPs, whereas the fibers of the outer AF form connections with the bony vertebral body epiphyses (Sharpey's fibers), thereby forming a compartment completely enclosing the NP[2,42,50].

The outer layers of the AF have a limited blood supply, but there is no direct blood supply to the inner layers of the AF or to the NP; however, nearby vascularization consists of terminal branches of the vertebral epiphysial arteries, which give rise to a densely woven network adjacent to the cartilaginous EPs[51]. The EP region directly adjacent to the NP is most densely vascularized[52]. The capillary buds empty into a subchondral postcapillary venous network or into medullary veins[52]. Innervation of the actual IVD tissue is sparse: nerve endings have only been found in the outer lamellae of the AF, and not in the NP, TZ, and inner AF[2,53,54]. However, surrounding soft tissue structures, such as the dorsal longitudinal ligament, are profusely innervated[2,53].

The EP plays an essential role with respect to the nutrition of the IVD. Most small molecules, such as oxygen and glucose, are supplied to the avascular IVD through diffusion and osmosis from the capillary buds into the adjacent vertebrae, through the semipermeable EPs, to the cells of the NP, TZ, and AF[55-59]. Additional nutrients and oxygen are supplied via the outer, vascularized parts of the AF[55,57,58,60]. For larger molecules with low diffusion rates, such as albumin and enzymes, bulk fluid flow ('pumping mechanism'), which is created by the physiological loading of the IVD and changes in posture, is an important mechanism[55-57,59,60].

Histology of the healthy IVD

The IVD is a supportive tissue mainly composed of matrix produced by a relatively small population of cells. Although cells are essential for the growth, repair, and metabolism of the IVD, its mechanical function can be explained largely in terms of the characteristics of the extracellular matrix. The cells in distinct regions of the IVD are highly specialized to produce, maintain, and organize a matrix well-suited to fulfill the physical-mechanical function of each specific IVD component[42,43,61,62].

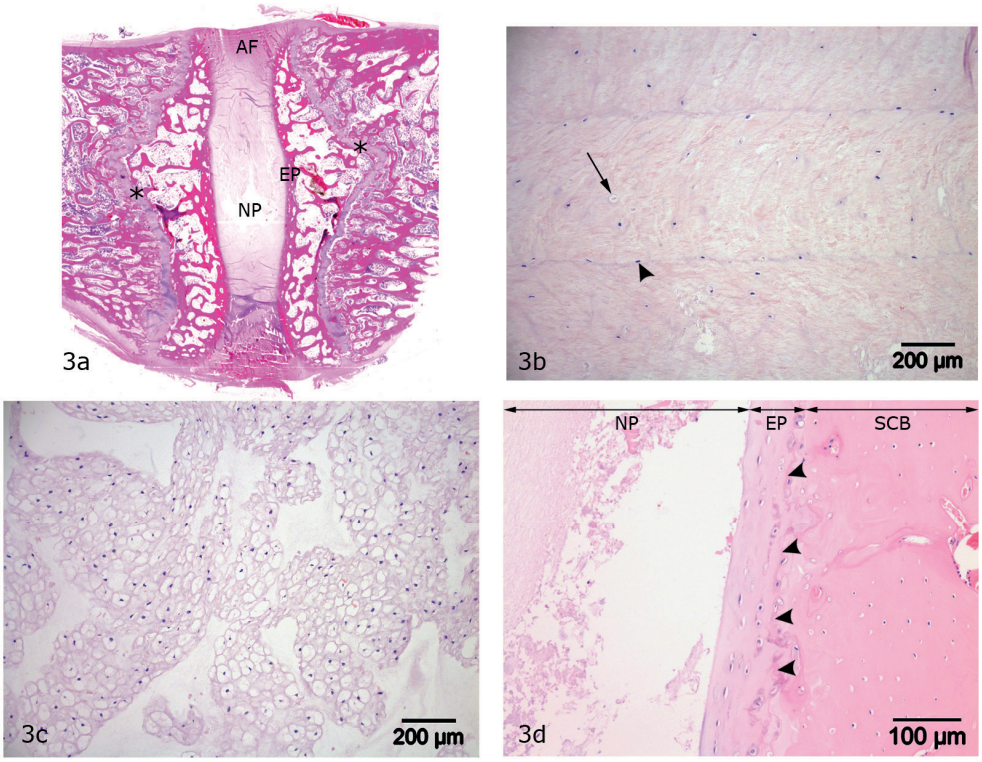

Figure 3. a) Midsagittal histological section (H&E) of a healthy, immature canine intervertebral disc, still with active growth plates in the vertebral bodies (*). **b)** Annulus fibrosus (AF), showing the lamellar layers with fibrocyte-like cells (arrowhead) and chondrocyte-like cells (arrow). **c)** Nucleus pulposus (NP), showing clustered notochordal cells. **d)** Cartilaginous endplate (EP), showing chondrocyte-like cells in a hyaline-type matrix. The border between endplate (left) and subchondral bone (SCB) (right) is indicated with arrowheads.

In the healthy IVD, the notochordal cell is the main cell type of the NP (Fig. 3c)[2,20,40,63]. These large cells are derived from the embryonic notochord and are characterized by cytoplasmatic vesicles, the contents and functions of which are still debated[2,64-67]. However, there are clear indications that these vesicles are unique organelles with osmoregulatory functions, and that they are potentially involved in the swelling and stretching of the embryonic notochord and in the regulation of osmotic stresses in the NP[68]. The notochordal cell has relatively few mitochondria and is therefore thought to rely mainly on anaerobic metabolism.[64] Notochordal cells are found in clusters[64,65,69] and are connected through gap junctions, tight junctions, desmosomes, and actin filaments[40,64-66,70,71]. Disruption of the physiological clusters results in cell death, and therefore they are thought to play an essential role in the physiology and function of the notochordal cell[66]. The notochordal cell islands produce an amorphous basophilic matrix rich in proteoglycans and collagen type II, which is distributed between the notochordal cell clusters and the TZ[2,40,63,64,70,72]. The notochordal cell is the original cell of the NP and is considered to be a potential progenitor cell or supporter cell of the healthy NP, as it has been shown to maintain a healthy IVD matrix by producing a high-quality matrix and by stimulating the production of proteoglycans by other cell types[63,72-76].

The TZ forms the border between the NP and AF and contains chondrocyte-like cells, embedded in a loose acidophilic fibrous matrix network[2,17,20,49]. In the notochordal cell-rich IVD, there is a clear distinction between the TZ and the matrix surrounding the notochordal cells[40].

Microscopically, the lamellae of the AF can be seen as separate fibrocartilaginous layers composed of parallel organized eosinophilic fibrous bundles[2,20,40]. The outer part of the AF contains fibroblast-like and fibrocyte-like cells, which are ellipsoidal in shape and organized with their long axis parallel to the fibrous bundles[17,20]. The cell population changes from fibrocyte-like cells in the outer layers of the AF to a mixed population of fibrocytes and chondrocyte-like cells in the inner layers[2,20,40,49].

The canine EP, which resembles hyaline cartilage, forms the boundary between the subchondral bone and the AF and NP. It consists of dorsoventrally organized layers of matrix and chondrocyte-like cells that run parallel to the subchondral bone[49,50]. The proportional thickness of the canine EP in relation to disc height or the average number of cell layers in the healthy canine IVD is not known, and only a few studies have described the basic histological appearance of the canine IVD[2,14].

Biochemical structure of the healthy IVD

The healthy NP is relatively densely populated by notochordal cells that synthesize and remodel the matrix, a complex network of negatively charged proteoglycans interwoven in a mesh of collagen fibers (mainly collagen type II) [77]. The proteoglycan molecules

consist of a protein backbone with negatively charged glycosaminoglycan (GAG) side chains. The most common side chains are chondroitin sulfate and keratin sulfate, which are covalently bound to the central core protein [22,23,77]. These negatively charged GAGs repel each other, giving the proteoglycans the appearance of a bottle-brush. The most common proteoglycan in the healthy IVD is aggrecan, which is a large aggregate of proteoglycan molecules[26,27]. The proteoglycans are in turn aggregated with hyaluronic acid, forming larger molecules. The accumulation of these negatively charged proteoglycan molecules creates a high osmotic gradient, attracting enough water into the NP to maintain intradiscal pressure[22,23,26]. Over 80% of the healthy NP is composed of water [78,79]. In addition to proteoglycans, other molecules, such as versican and several integrins, are produced by the notochordal cell clusters[72]. Other constituents of the extracellular matrix include other types of collagen and other proteoglycans such as decorin, biglycan, and fibromudlin; however, these have been reported in the human NP[43] and it is not known whether they occur in the canine NP.

The healthy AF is a less cellular structure than the NP, and sparsely distributed fibrocyte-like cells produce and maintain the structure of the lamellar layers. The AF fibers in the lamellae are made up of collagen fibrils aggregated together with elastic fibers and coated by proteoglycans[80]. The outer part of the AF contains mostly collagen type I, whereas the inner part (TZ) contains predominantly collagen type II. These structural differences are inherent to the functions of the respective tissue parts. The AF has a lower water content than the NP, and consists of about 60% water[78,79].

Little is known about the biochemical structure of the canine EP. Since considerable research has been performed in humans regarding the constituents of the EP, this information will be briefly presented. However, it should be noted that the canine EP differs considerably from the human EP[37], and therefore care should be taken when extrapolating findings from humans to dogs. The biochemical composition of the healthy EP appears to be highly similar to that of articular cartilage[81]. The EP has a highly hydrated matrix (50-80%) composed of proteoglycans (mainly aggrecan), interconnected with hyaluronic acid and link proteins, and collagen (mainly type II)[81-83]. The biochemistry of the EP is critical for maintaining the integrity of the IVD, since especially the proteoglycans within the matrix appear to regulate the transport of solutes into and from the IVD[84]. Also, collagen type X, a calcium binding molecule, has been found in the canine EP and has received considerable attention because it is thought to be involved in EP calcification[85,86].

The process of remodeling and breakdown of the extracellular matrix in the IVD is regulated by an array of enzymes, such as matrix metalloproteinases (MMPs), a disintegrin, and metalloproteinases (ADAMs), produced by the cells of the IVD. Much is known about the activity of these regulatory enzymes in humans,[43,87,88] but considerably less is known about the their involvement in IVD remodeling in dogs [74,89,90]. However, it

is likely that MMPs and ADAMs are involved in both remodeling and degeneration of the canine IVD in much the same way as in humans, although this remains to be proven.

Biomechanical function of the healthy IVD

The biomechanical function of the IVD in each spinal unit is to transmit compressive forces between vertebral bodies and to provide mobility as well as stability to the spinal segment[91,92]. The NP, AF, TZ, and EPs of the IVD work as one functional unit, with each component providing different specialized functions. The NP is a highly hydrated structure that exerts swelling pressure inside the disc. The mucoid NP is confined by the surrounding AF and EPs, which protect the NP against lateral shearing induced by the applied load and its own internal swelling pressure[42,43,46,93]. The fibers of the AF provide reinforcement when stretched as the IVD is twisted, bent, and/or compressed. The inner annular fibers and TZ mainly resist compressive forces, whereas the outer annular fibers cope with tensile forces[42,43,46]. In this particular conformation, the NP provides the main resistance to compressive loads, the AF copes with tensile forces, and the partly deformable EP contains the NP while providing nutrients to the IVD cells. Owing to the specialized conformation of these structurally and functionally divergent entities, the IVD concurrently provides mobility and stability to compressive, tensile, and shear stresses of the spine[42,43,46,91].

Pathophysiology of IVD degeneration

Degeneration of the IVD is a complex, multifactorial process that is characterized by deterioration of the quality of the IVD matrix, resulting in a reduced IVD function. IVD degeneration has been described as an aberrant, cell-mediated response to progressive structural failure of the IVD[94]. It is also characterized by a progressive decrease in the ability of the IVD to absorb and retain water within the NP and thereby its function as a hydraulic cushion is reduced. This decreased function results in structural, cellular, and biomolecular changes of the NP, AF, and EPs.[2,18,22-24,43,45,94] The process of IVD degeneration and deterioration of the matrix involves the interplay of several factors, such as genetic, repeated physical-mechanical overload, impaired metabolite transport and nutrition to the cells within the IVD matrix, cell senescence and death, altered levels of enzyme activity, (post-translational) changes in matrix macromolecules, and changes in the water content.[94-96] As a result of structural failure, the local mechanical environment of the cells becomes abnormal. These changed circumstances in combination with the avascular and low cellular nature of the IVD limit the potential of IVD cells to repair the matrix. The degenerated IVD can be further damaged by stress at levels that are considered physiological for the healthy IVD. Consequently, a vicious

cycle of continued damage and failed regeneration is triggered, resulting in degeneration rather than healing. It is difficult to characterize IVD degeneration, because the pathological process is difficult to distinguish from the processes associated with physiological aging of the disc. Although the definition proposed above may partially distinguish truly degenerative changes from age-related ones, it cannot be applied to the initial and early stages of IVD degeneration. Therefore, in this thesis, the term IVD degeneration is used to describe deterioration of the quality of the IVD matrix due to pathological reasons as well as age-related changes and the associated structural changes of the disc as described below.

Macroscopic aspects of IVD degeneration

To assess the degree of IVD degeneration in a reproducible and objective manner, universally applicable grading schemes are needed. In human medicine, the gold standard for IVD degeneration is the five-category grading scheme for gross pathological changes described by Thompson et al. (1990)[97]. The Thompson grading scheme has, however, not been validated for use in dogs. This grading scheme is based on the morphological appearance of the NP, AF, EP, and the vertebrae, viewed on midsagittal sections (Table 1 and Fig. 4) and is likely to be applicable for use in dogs, although this remains to be proven.

Degeneration commonly starts in the NP. Macroscopically, this is characterized by the mucoid NP changing from a translucent gray color to a non-translucent white-gray color, ultimately accompanied by cleft formation. As the NP degenerates, the AF lamellar structure buckles inward, becomes disorganized, and starts to degenerate. The TZ will widen and become irregular, thereby making it difficult to distinguish AF from NP tissue.[2,17,20,63] Once degeneration and weakening of the IVD have become so extensive as to cause loss of its central axial load-bearing properties, the cartilaginous EP thickens and becomes irregular and may fracture. Bony proliferations, such as osteophytes and ventral spondylosis, start to develop at the peripheral margins of the spinal column [97,98]. Continued degeneration will lead to highly irregular and sometimes breached EPs and subchondral bone. The IVD space will be greatly reduced and completely collapses in extreme cases, with bulging of the degenerated AF or even herniation of the IVD. Based on the Thompson grading scheme, this gradual process of IVD degeneration can be divided into five grades, ranging from a completely healthy IVD (grade I) to a severely degenerated IVD (grade V) (Table 1). However, herniation and prolapse of the IVD, which are considered consequences of the degenerative process, are not included in this grading scheme[2].

Table 1. Description of the five categories of the macroscopic grading scheme for gross pathological changes of intervertebral discs according to Thompson

Grade	Nucleus pulposus	Annulus fibrosus	End-plates	Vertebral bodies
I	Bulging gel	Discrete fibrous lamellae	Hyaline, uniform thickness	Rounded margins
II	White fibrous tissue peripherally	Mucinous material between lamellas	Irregular thickness	Pointed margins
III	Consolidated fibrous tissue	Extensive mucinous infiltration; loss of annular-nuclear demarcation	Focal defects in cartilage	Early chondrophytes or osteophytes at margins
IV	Horizontal (vertical) clefts parallel to end-plate	Focal disruptions	Fibrocartilage extending from subchondral bone; irregularity and focal sclerosis in subchondral bone	Osteophytes < 2 mm
V	Clefts extend through nucleus and annulus		Diffuse sclerosis	Osteophytes > 2 mm

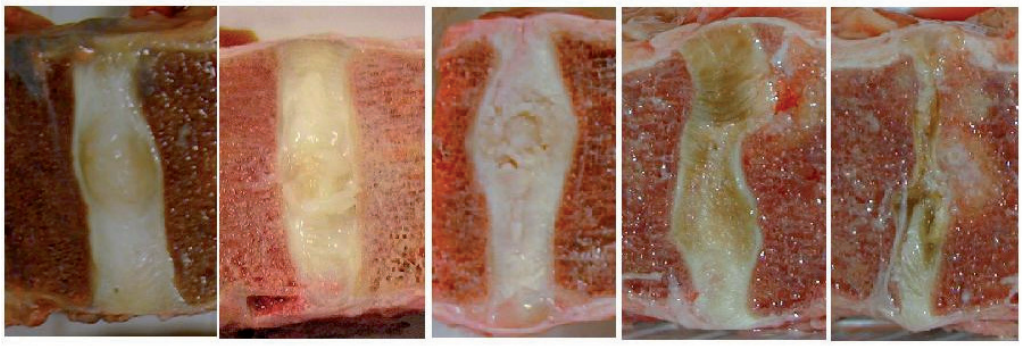

Figure 4. Midsagittal photographs of human intervertebral discs depicting the different Thompson grades. From left to right; Thompson grade I, II, III, IV and V.

Magnetic resonance imaging (MRI) of IVD degeneration

The only diagnostic modality currently available to evaluate the status of an IVD *in vivo* is MRI. T2-weighted MRI scans are best suited for evaluation of IVD degeneration as they best depict the glycosaminoglycan and water content of the disc, which is negatively correlated with the extent of disc degeneration[99,100]. The Pfirrmann system is the most widely used system to grade human IVD degeneration on the basis of MRI findings[101-103]. It is based on the system for grading gross pathological changes in intervertebral discs of Thompson et al.,[101-103] and, like that system, the Pfirrmann system divides the process of IVD degeneration into five grades, ranging from a completely healthy IVD (grade I) to a severely degenerated IVD (grade V) (Table 2 and Fig. 5). For canine IVD degeneration, two different MRI grading systems have previously been proposed[104,105]. In view of enabling translational studies and gaining synergistic effects between human and veterinary research the use of the Pfirrmann system also in dogs would be more practical, but so far the system has not been validated in dogs.

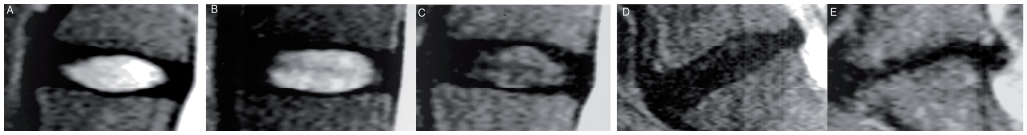

Figure 5. Midsagittal, T2-weighted MR images of human intervertebral discs depicting the five different Pfirrmann grades. From left to right; Pfirrmann grade I, II, III, IV and V. *Reprinted with permission from Pfirrmann et al. Spine 2001*[103].

The Pfirrmann grading system focuses only on changes in the structure of the disc itself (T2-weighted signal intensity, disc structure, NP and AF distinction, and disc height) and does not take changes related to IVD herniation into account, such as bulging, protrusion or extrusion of the disc. Yet it is imperative to include these aspects to obtain a complete picture of the status of the disc. To accurately appreciate the extent of potential bulging, protrusion, or extrusion of the disc, transverse MR images are needed in combination with the midsagittal images used for Pfirrmann grading. In order to completely evaluate the status of IVD in dogs, the Pfirrmann grading system should be used in combination with information about disc herniation (if present), such as protrusion or extrusion.

Table 2. Description of the five categories of the MRI-based grading scheme according to Pfirrmann[103]

Grade	Structure	Distinction between NP and AF	Signal intensity	Height of intervertebral disc
I	Homogenous, bright white	Clear	Hyperintense, isointense to CSF	Normal
II	Inhomogeneous with or without horizontal bands	Clear	Hyperintense, isointense to CSF	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointense	Collapsed disc space

NP= nucleus pulposus; AF = annulus fibrosus; CSF=cerebrospinal fluid

Histopathology of IVD degeneration

As the IVD is a tissue with a relatively low cellular density and its functionality is determined by its extracellular matrix, it is useful to apply specific staining methods for the extracellular matrix components in combination with standard hematoxylin–eosin staining. An improved staining method for IVD tissue, using alcian blue (staining proteoglycans) and picosirius red (staining collagens), has been proposed [106]. Histological changes in the IVD are frequently referred to as the gold standard for IVD degenerative research[104,107]. A new scheme for grading histopathological changes in the canine IVD has recently been proposed [108] and evaluates not only the cellular changes in the AF, NP, and EPs, but also matrix changes, using an alcian blue and picosirius red staining (Fig. 6). The grading system also considers changes surrounding the IVD, such as new bone formation and sclerotic changes of the subchondral bone.

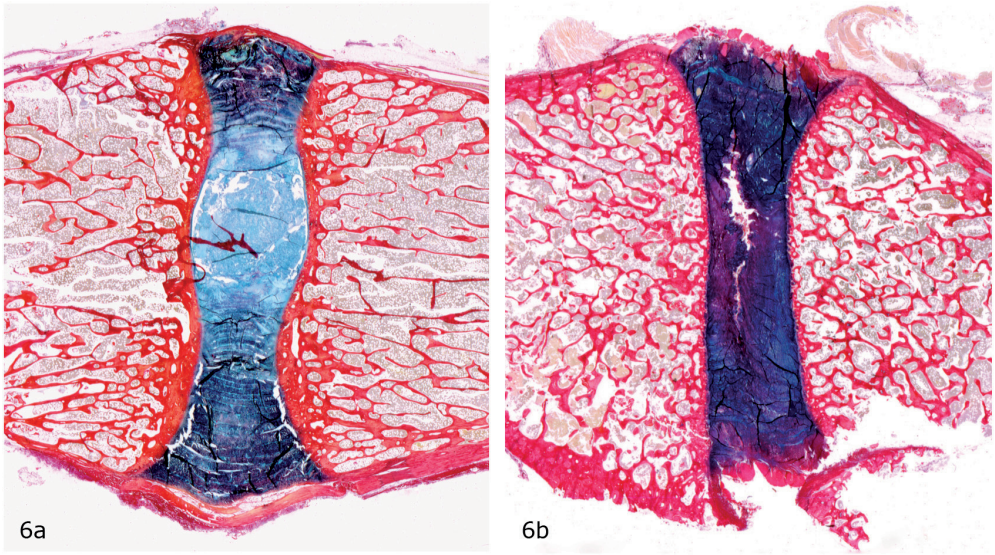

Figure 6. Midsagittal histological sections of **a)** a healthy and **b)** a moderately degenerated canine intervertebral disc (IVD) stained with picosirius red and alcian blue. Alcian blue stains proteoglycans light blue and picosirius red stains principally collagen type I red. In the healthy IVD **(a)** a clear distinction can be made between the nucleus pulposus (NP) containing chiefly proteoglycans, and the annulus fibrosus (AF) staining dark blue/purple, which indicates a mixture of proteoglycans and collagen type I. In the degenerated IVD **(b)** no clear distinction between the NP and AF can be made, with increasing collagen staining seen throughout the IVD. A cleft transecting the NP can also be seen.

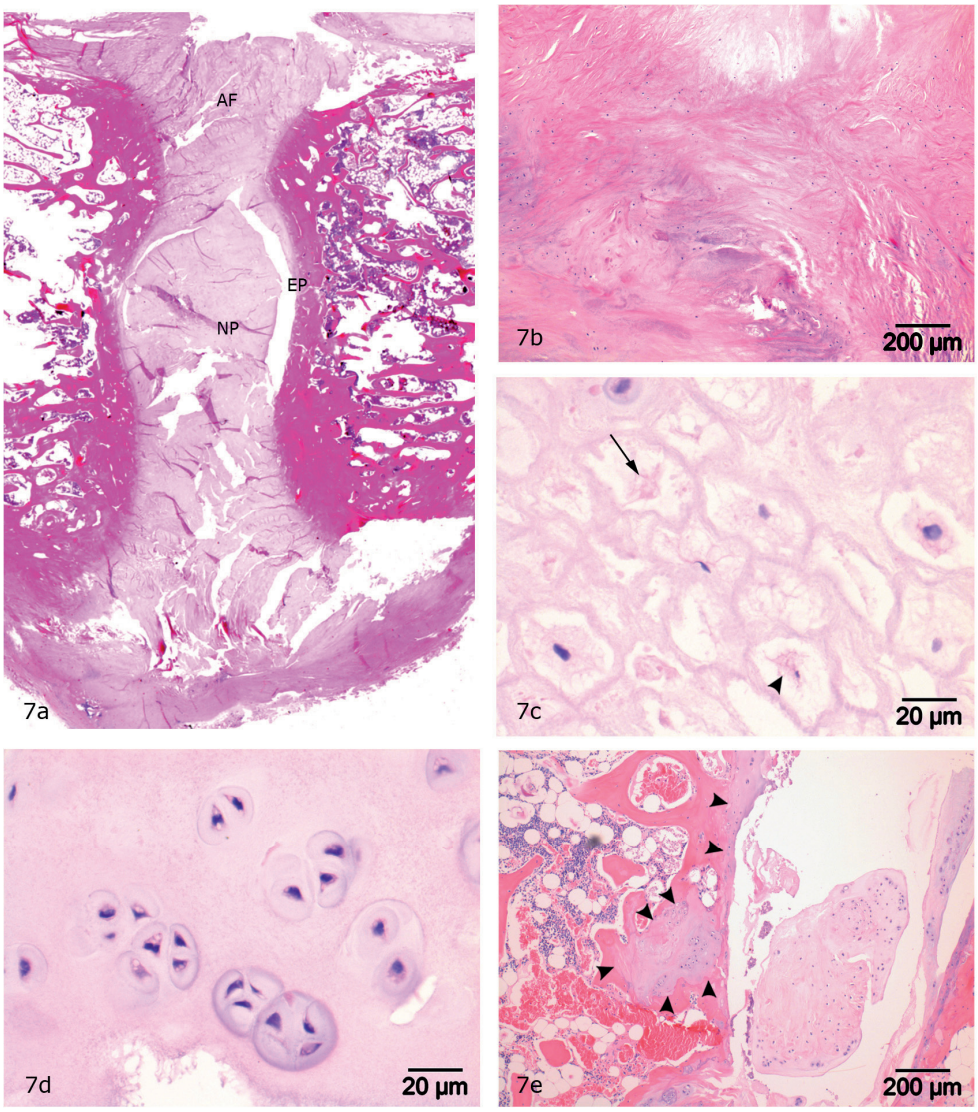

Figure 7. a) Midsagittal histological section (H&E) of a degenerating, canine intervertebral disc. b) Annulus fibrosus, showing disorganization of the lamellar structure and an increase in chondrocyte-like cells. c) Nucleus pulposus, showing dead (arrow) and dying (arrowhead) notochordal cells. d) Nucleus pulposus, showing small groups of chondrocyte-like cells (cell-nests). e) Cartilaginous endplate, showing endplate irregularities and damage. The irregular border between the endplate and the subchondral bone is marked with arrowheads.

The histopathological changes taking place in the course of IVD degeneration were described by Hansen in 1952[2]. Although the techniques of histological imaging have evolved considerably since then and advanced immunohistochemical methods are now available, there are surprisingly few recent studies that investigated the histopathology of the canine IVD[17,20,65,109].

The early stage of the degenerative process is characterized by cellular changes within the NP. The notochordal cell clusters are lost, resulting in smaller notochordal cell islands or single notochordal cells. In addition, dying notochordal cells can be observed (Fig 7). Concurrently, the TZ gradually expands into the NP, and fibrocartilaginous strands containing single or clusters of chondrocyte-like cells can be seen to transect the NP. In essence, chondrocyte-like cells replace the notochordal cell population and the extracellular matrix enlarges, consisting largely of disorganized collagen fibers. The chondrocyte-like cells either migrate from the TZ into the NP and/or are a differentiated cell lineage from the notochordal cells. As degeneration progresses, the notochordal cells disappear and chondrocyte-like cells invade the entire NP, forming continuously larger clones, a process referred to as *chondrification*[2,17,20,63,65]. The degenerated NP tissue with chondrocyte-like cells and cell clones dispensed in a collagen network resembles to some extent hyaline cartilage. This hyaline-like tissue is transected by strands of extracellular tissue, mainly collagen, so that the NP is divided into lobules, which gives the degenerated NP a mixed appearance of hyaline cartilage and fibrocartilaginous tissue. Degeneration of the extracellular matrix of the NP consists of a decrease and degradation of proteoglycans [19,21,26] and a shift from collagen II to collagen I fibers, which can be seen histologically by combined staining with alcian blue and picosirius red [106] (Fig. 6). The degeneration and subsequent dehydration of the NP[22,23] can result in secondary cleft and crack formations[104].

Histologically, the degeneration of the AF is characterized by the disorganization of the lamellar fibers and the ingrowth of chondrocyte-like cells from the TZ. The chondrocyte-like cells spread outward from the TZ as degeneration and fibrillar disorganization of the AF progresses. The reduction in size of the NP and cleft formation in the NP can initially cause the AF lamellae to buckle inward, contributing to the disorganization and weakening of the AF. Cross-links between the annular fibers, which prevent lamellar movement in the AF, are found in increasing numbers in degenerated IVDs[110-112]. The inability of normal AF movement combined with NP degeneration and loss of IVD height may eventually force the AF to bulge outward, causing a type II herniation[2,104]. If the annular damage is too severe with transecting cracks, NP tissue may extrude through the AF into the spinal canal, causing a type I herniation[2]. Type I herniation can also occur in AFs showing fewer signs of degeneration due to an altered biomechanical loading occurring secondary to a stiffer, degenerated, or even calcified NP. A sudden increase in stress can cause the AF to fail at its weakest point, which is often the thinner, dorsal AF, resulting in an explosive extrusion of NP material.

In the early stages of degeneration, thickening of the EPs may be seen, which might further impair nutrient transport into the NP and thereby speed up the rate of degeneration. With increasing degeneration, the EP become increasingly irregular and may breach at several places. The breaches usually occur in the central parts of the EPs and can give rise to a "Schmorls node", which is herniation of the NP into the vertebral body[113]. This is frequently described in humans but is found less often in dogs.

Biochemical changes of the degenerating IVD

Little is known about the biochemical changes accompanying canine IVD degeneration, and only the GAG content and composition of canine NP, TZ, and AF have been investigated in detail[19,22,23,26,77]. A decrease in GAG content and degradation of GAG molecules, by substitution of the long chondroitin sulfate side chains with shorter keratan sulfate side chains, are strongly associated with increasing severity of IVD degeneration. IVD degeneration is also associated with an increase in the collagen content, which first occurs in the NP, followed by similar changes in the AF as degeneration progresses[22-27]. Even less is known about the degenerative changes taking place in the canine EP. Degeneration of the human EP is accompanied by a decreased water, collagen II, and proteoglycan content with increasing degeneration[82]. In addition, the EPs may undergo substantial mineralization[114,115]. The degeneration and calcification of the EP combined with sclerosis of the subchondral bone can lead to obstruction of capillary buds and may disturb the physiological transport of solutes to and from the IVD[84,86,114-116].

It is clear that IVD degeneration involves degradation of the extracellular matrix, leading to reduced functionality of the disc, but little is known about the biochemical agents (enzymes) involved in the process of canine IVD degeneration. However, considerably more is known from IVD research in humans and other animal species. Since it is possible that similar biochemical changes occur in degenerating canine IVDs as in IVDs from other species, the biochemical changes described below also include changes reported for species other than dogs.

Some of the more important enzymes involved in IVD remodeling and degeneration are the MMPs, with MMP-1 and -2 being especially important as they are responsible for the breakdown of collagen types I and II, respectively. A correlation between an increase in MMP-2 and more severe IVD degeneration has been found in humans[87]. Another important enzyme group is the ADAMs, with A Disintegrin And Metalloprotinease with Thrombospondin Motifs 4 (ADAMTS- 4) being the most prominent, causing the breakdown of aggrecan[117]. The most important inhibitors present in the IVD are protease inhibitors called tissue inhibitors of metalloproteinase (TIMPs), which inhibit the MMPs.

As the blood flow of the IVD is limited to the outer layers of the AF, the cellular response to degeneration is limited to macrophages and some lymphocytes [118,119]. For the same reason, there are few plasma-derived inflammatory mediators in the IVD, thus most inflammatory agents and regulatory enzymes are likely to be derived from the resident cell population or invading cells, such as macrophages. Inflammatory mediators, such as tumor necrosis factor alfa (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), have been identified in degenerated IVDs. Inflammatory mediators have been shown to alter the expression pattern of the IVD cells by up-regulating the production of MMPs and down-regulating the production of matrix molecules, such as collagens and aggrecan, thereby accelerating the process of degeneration[120-123]. However, more research into the pathogenesis of IVD degeneration, specifically in the dog, is needed to obtain a better understanding of the degenerative process.

Biomechanical effects of IVD degeneration

The inability of the disc to fulfill its physiological function interferes with the normal function of the vertebral column, thereby influencing other components of the spinal unit, such as ligaments, facet joints, and vertebral bodies[42,91,124]. Therefore, the deficits in the biomechanical quality and integrity of the IVD caused by degeneration can be detrimental to the function and integrity of the functional spinal unit as a whole. The significance of the IVD as a stabilizing and mobilizing component has been highlighted by biomechanical studies investigating the effects of IVD degeneration and removal of IVD components on spinal biomechanics. Although most of these studies were performed with human material, these will also be discussed[125]. Biomechanical loading of the spine is largely comparable in dogs and humans[126,127], and thus the biomechanical effects described for the human spine are likely to be similar to those for the canine spine.

IVD degeneration has significant effects on spinal biomechanics. Disruption of the natural NP and AF structures results in a less functional IVD. With increasing Thompson grades from I to IV, the stabilizing function of the IVD in relation to the rotational biomechanics (i.e., flexion/extension, lateral bending, axial rotation) is lost, with a concurrent increase in the mobility of the affected spinal segment. This increase in mobility is most apparent in axial rotation. However, Thompson grade V IVD degeneration leads to a decreased laxity and 'restabilization' of the spine due to the formation of osteophytes/spondylosis and collapse of the IVD space[41,128-132]. In addition, the degenerative process leads to a stiffer IVD and thereby to a decrease in the ability to function as a hydraulic cushion[133].

Since the function of the spinal unit can be considered to reflect the combined functions of the individual components, a decreased functionality of one part of the IVD can affect the function of the entire spinal unit. Degeneration usually starts in the NP, where dehydration leads to a decrease in NP size and a decrease in intradiscal pressure, resulting in increased stress on the AF with a compensatory increase in functional size.[134,135] Also, IVD degeneration causes compressive loads to be distributed more peripherally, onto the AF[135,136]. As a result, the load on the AF is increased and altered, resulting in bulging of the IVD and annular tears[137]. Degeneration of the IVD results in an uneven distribution of load onto the EP when the spine rotates[138], and makes the EP more susceptible to damage[139]. Although the EPs are deformable when axially loaded[93], they appear to be a weak link in the functional spinal unit, so that the EP cracks relatively early in the degenerative cascade, resulting in a disturbed nutritional supply[130,140,141]. Decreased IVD function results in altered and increased facet joint loading[142,143], which can lead to secondary osteoarthritic changes. The altered loading pattern can also affect the adjacent vertebrae, leading to remodeling and sclerosis of the vertebral bodies[140,144].

IVD degeneration in chondrodystrophic vs. non-chondrodystrophic dog breeds

Within the canine population, two different types of breeds can be distinguished on the basis of their physical appearance: the chondrodystrophic (CD) dog breeds and the non-chondrodystrophic (NCD) dog breeds. CD breeds are characterized by a disturbed endochondral ossification, resulting in disproportionally short limbs[20,145]. Chondrodystrophic breeds include the Dachshund, French Bull Dog, miniature Poodle, Pekingese, Beagle, Lhasa Apso, Welsh Corgi, and the American Cocker Spaniel[2,9,20]. Other distinguishing factors between these two types of dog breeds are the age of onset, frequency, and characteristics of IVD degeneration. CD dog breeds often suffer from IVD disease at a relatively early age (3-4 years), and IVD-related problems mainly occur in the cervical and thoracolumbar spine[2,9]. In contrast, in NCD dog breeds IVD-related problems are generally seen in the lower cervical or lumbar spine and at a significantly later age (>6 years)[2,4,5]. NCD breeds frequently affected by IVD disease include the German Shepherd, Doberman, Rottweiler, and the Labrador Retriever[4,5]. These differences in age, prevalence, and occurrence of IVD degenerative diseases are indicative of differences in the degenerative process between these two types of breed. IVD degeneration has traditionally been divided into chondroid and fibroid degeneration, which occur in CD breeds and NCD breeds, respectively [2].

Gross morphology

When examining the morphology of the IVD in both types of dog breeds, it is apparent that the degenerative process starts relatively early in life in CD breeds, with the metamorphosis from a mucoid, semi-fluid NP toward a fibrous, sturdy NP starting at 3-4 months of age (Fig. 8).

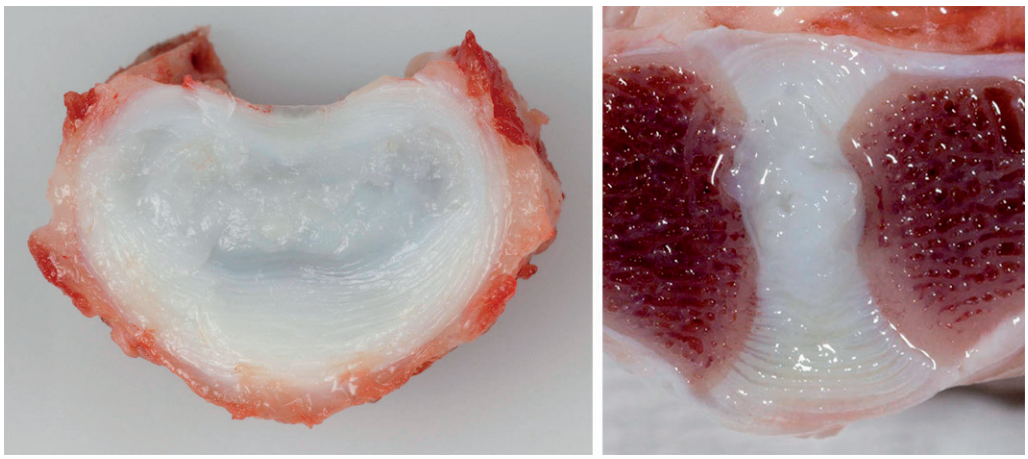

Figure 8. Transverse **(left)** and sagittal **(right)** section through a L5-L6 intervertebral disc of a 2-year-old chondrodystrophic dog, showing a fibrocartilaginous nucleus pulposus, a widened transition zone, and a normally structured annulus fibrosus.

This transformation is complete by 1 year of age in most IVDs throughout the spinal column[2,9,20], with the exception of the cervical spine, where a small proportion of the IVD may still exhibit a mucoid NP[2]. This metamorphosis of the NP is associated with a decrease in size of the original NP and with a concurrent increase in the width of the TZ[2,17,20]. Degenerative changes in the AF, although not prominent, also become apparent relatively early in life, which, from a biomechanical point of view, is likely a consequence of NP degeneration[2,135,136]. A process frequently observed in IVD degeneration in CD breeds is calcification of the NP and occasionally of the AF (Fig. 9)[2,146-148]. In CD breeds, degeneration can progress rapidly, resulting in herniation before 3 years of age[2]. Hansen type I IVD herniation is generally encountered in the cervical and thoracolumbar spine[2,14-16]. Prolapse in the midthoracic area is rarely observed due to the presence of the intercapital ligament, which prevents dorsal and dorsolateral IVD herniation[2,9].

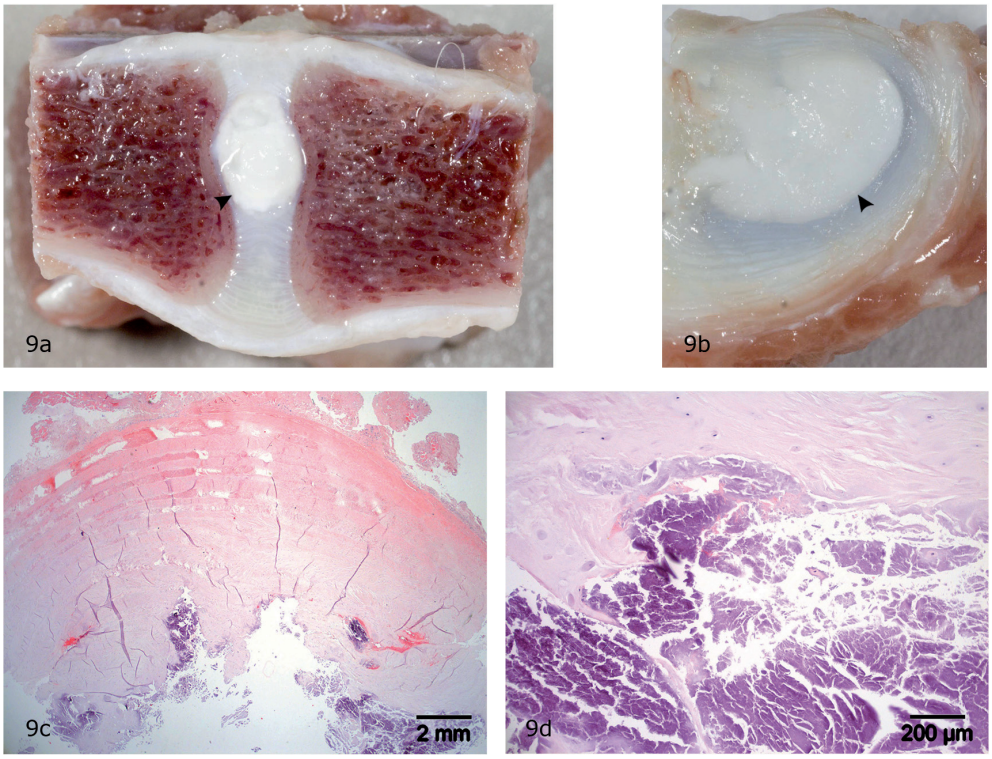

Figure 9. Midsagittal **(a)** and a transverse **(b)** section of an intervertebral disc from a 2-year-old chondrodystrophic dog with extensive mineralization of the NP (arrowhead). **c)** Transverse histological section (H&E) of the same intervertebral disc depicting the relatively normal structure of the annulus fibrosus combined with a severely mineralized nucleus pulposus. **d)** Magnification of (c) showing the mineral deposits.

In NCD breeds, the morphological changes described for CD breeds also occur, but later in life (> 6 years)[2,20]. The transition from a mucoid NP to a fibrous NP occurs, but mostly in single IVDs and generally in spinal levels exposed to a higher workload and mobility (L7-S1 and lower cervical levels)[5,8]. Calcification of the IVD is rarely observed in NCD breeds[2]. The IVD degeneration observed in these dogs is more gradual and results in fibrocartilaginous metamorphosis of the NP with partial rupturing of the AF, resulting in bulging of the IVD. This type of herniation is referred to as Hansen type II IVD herniation[2].

Histopathology

Histological examination of the IVD from CD breeds shows that degenerative changes in the NP can be observed as early as 2 months of age. The main cellular change in the NP involves the disappearance of notochordal cells, which are replaced by a less cell dense population of chondrocyte-like cells[2,17,20,63]. This cellular process occurs concurrently with transformation of the mucoid NP into a fibrous NP and enlargement of the TZ width, and is completed at the age of 1 year in most cases [2,17,20,63]. In NCD breeds, the notochordal cell is the main cell type of the NP during the majority of life [2,20,63]. A similar shift in cell population as described in CD breeds is also observed in NCD breeds; however, this process occurs relatively infrequently, does not occur in all IVDs, and generally starts later in life (> 6 years) [2,20,63-65]. Degeneration of the AF can be observed in both types of dog breed and is characterized by disorganization and rupture of the lamellar layers. However, in CD breeds severe damage to the AF does not occur until the NP is severely degenerated[2]. In NCD breeds, the degenerative process is less acute, with moderate fissures and disorganization of the AF. In addition, degenerative changes of the IVD occur simultaneously in the AF and NP in NCD breeds[2].

In CD breeds, calcification of the NP can frequently be observed in the perinuclear region and less frequently in the central part of the NP; calcification is rarely observed in NCD breeds [2,146-148]. Hansen (1952) stated that the calcification found in degenerating IVDs is dystrophic calcification, secondary to tissue necrosis, rather than endochondral ossification[2]. This has been supported by more recent studies of humans and merino sheep [147,149-151]. Different calcium deposits have been described in the human IVD[149,150] but it has been suggested that the mineral deposits found in CD breeds consist of hydroxyapatite [147]. There is a familial form of dystrophic calcification seen in merino sheep and rarely also in humans, the calcifications of which have a similar appearance to those seen in CD breeds[147,151]. Moreover, this deposition of mineral is highly sensitive to pH changes in the surrounding matrix. Mineral deposits of hydroxyapatite are formed in an alkaline environment and can be dissolved under acidic conditions. Dissolution of the calcifications could explain the 'disappearing' calcifications seen in some dogs in a longitudinal radiological study[152]. However, it still remains to be proven that the calcifications seen in CD breeds are indeed composed of hydroxyapatite.

On the basis of these differences in degeneration between the two types of dog breed, Hansen (1952) termed the degeneration occurring in CD and NCD breeds 'chondroid' and 'fibroid' degeneration, respectively. It should be noted that, even though differences exist in the age, onset, and progression of degeneration, Hansen emphasized the fact that both groups showed many similarities regarding the fundamental processes involved in the degenerative cascade. A more recent study also supports the theory that the degenerative processes occurring in CD and NCD breeds are similar[108]. Moreover, Hansen may have

erroneously drawn the conclusion that the notochordal cells in the degenerating NP of NCD breeds transform into fibrocyte-like cells, something he did not observe in CD breeds. However, the 'fibrocyte-like cells' shown in photographs in his thesis bear a strong resemblance to apoptotic notochordal cells [108,153] seen in the IVDs of both CDs and NCD breeds.

Biochemical changes

Biochemical analysis of the IVD of both types of dog breed shows differences in proteoglycan and collagen content. The NP of a young NCD-breed dog is significantly richer in proteoglycans than that of a CD-breed dog[18]. In addition, after approximately 30 months of age the NP of CD-breed dogs shows a sharp decline in proteoglycan content, whereas the proteoglycan content of the NP of NCD-breed dogs stays more-or-less constant throughout life[22,23]. Also, the composition of proteoglycans differs between the two types of breed. In the NP of CD-breed dogs, the concentration of chondroitin sulfate side-chains starts to decline before the age of 1 year (1-5 months), with a concurrent increase in, and ultimately a complete replacement by, keratan sulfate[22]. In the NP of NCD-breed dogs, the chondroitin sulfate content remains steady up to 21-30 months of age, after which it decreases slightly over time, with a concurrent increase in keratan sulfate. This change in GAG content is less dramatic than that seen in the NP of CD-breed dogs[22,23]. These differences are most pronounced in the NP, and less apparent in the TZ and AF. In addition to differences in proteoglycan concentration and composition, significant differences exist in the collagen content of the IVD. Before the age of 1 year, the mean collagen content of the NP at all spinal levels is 25% in CD-breed dogs, much higher than that in NCD-breed dogs[18,24]. However, the NP of the L7-S1 IVD becomes more collagen rich later in life (at 60 months of age) in NCD-breed dogs and becomes more similar to that of CD-breed dogs aged 21-30 months[24]. In addition, the ratio collagen/non-collagenous protein of the NP and AF from CD-breed dogs increases considerably with age, starting before the age of 1 year for the NP and at 30 months for the AF[24]. In contrast, the IVD of NCD-breed dogs displays a marked uniformity in collagen/non-collagenous protein, except after 124 months. Similar to the NP from CD-breed dogs, the NP collagen/non-collagenous protein ratio increases from 24-30 months of age in NCD-breed dogs; however, this change is less pronounced than that in CD-breed dogs[24]. The differences in extracellular matrix between the CD and NCD breeds could reflect the rapid deterioration of hydroelastic properties and, consequently, hydraulic function of the IVD in CD breeds[18,22-24].

In conclusion, it appears that the degenerative process in CD and NCD breeds follows a similar fundamental pattern. However, morphological, histopathological, and biochemical differences indicate that different etiological factors are at play in the two types of dog breed. A genetic component, which is linked to the genetic origin that

characterizes CD breeds, appears to be most important in IVD degeneration in CD breeds[2,9,20]. The cellular changes observed in the NP of young CD-breed dogs are likely to be of genetic origin and appear to be a progressive manifestation of the chondrodystrophy which characterizes these animals. In NCD breeds, a multifactorial etiology is more likely, involving an interplay of genetic factors, trauma, and "wear and tear" of the IVD.

Given the association between the high incidence of IVD degeneration and the disappearance of notochordal cells from the NP, the latter process could be a key factor in the initiation of the degenerative process in both types of dog breed[2,20,63-66,73-75,154,155]. However, just as with "the chicken or the egg" dilemma, it is not clear whether degeneration leads to the disappearance of notochordal cells or whether the disappearance of notochordal cells initiates degeneration.

Regeneration of the IVD

In the last decade, there has been increasing interest in ways to reverse the degenerative process, i.e. regeneration of the IVD. Regeneration of the IVD involves the prevention, inhibition, and/or reversal of degenerative processes by concomitantly stimulating the synthesis of extracellular matrix while at the same time decreasing, and ideally reversing, itsdegradation[156,157]. Different strategies for biological repair of the degenerated IVD are available. The integrity of the IVD structure, the physiological status, the quality of the matrix, and the viability and activity of the cells in the NP, AF, and EP are factors to be considered. Therefore, at each stage of IVD degeneration, reversing the process requires different regenerative approaches. Potential strategies include the application of growth factors (GFs), anti-catabolic agents, or cell-based strategies.

Application of growth factors and anti-catabolic agents

GFs can have beneficial anabolic effects on the extracellular matrix by stimulating cell proliferation, differentiation, and/or migration, or by stimulating the cells to enhance matrix repair and production[156-158]. In the early stages of degeneration, when the IVD cells are metabolically impaired but still functional, regeneration might be achieved by a single administration/injection of GFs or anti-catabolic agents, which provides a short-term beneficial effect[156-159]. In more advanced stages of IVD degeneration, in which prolonged stimulation of the cells is desired, gene therapy could be used. Gene therapy involves incorporating a GF gene into the cells of the IVD via a viral transport vector (transfection), thereby providing a constant production of the desired GF to positively influence matrix health. Gene delivery can be achieved by either direct *in vivo*

transfection or an *ex vivo* process in which target cells are harvested, transfected *in vitro*, and then inserted into the affected disc[156-159].
The regenerative potential of numerous GFs and anti-catabolic agents, administered by a single injection, gene therapy, or both, has been investigated in canine IVD cells and in other species, such as rabbits, cattle, and humans. GFs investigated specifically in the dog are insulin-like-growth-factor-1 (IGF-1), transforming growth factor beta (TGF-β), platelet derived growth factor (PDGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF)[160]. The administration of these GFs in canine NP, TZ, and AF organ culture had a beneficial effect on extracellular matrix production and cell proliferation, significantly increasing the synthesis of proteoglycans by IVD cells, with TGF-β being the most potent[160]. Other potent GFs assessed in other species include bone morphogenetic proteins (BMPs)-2, -7 (OP-1), -12, -13, growth and differentiation factor-5 (GDF-5), tissue inhibitor of metalloproteinase (TIMP-1), LIM mineralization protein (LMP)-1, Link N, and Sox-9[156-159].

Cell-based regenerative strategies
In more advanced stages of degeneration, the cells may have an impaired metabolic function and fail to respond to biological stimulation by GF injection or gene therapy. In this case, the implantation of healthy cells, capable of producing a healthy extracellular matrix and of remodeling a degenerated extracellular matrix, or gene-manipulated cells into the IVD after discectomy can be considered[159]. The cell-based therapies aimed at regenerating the IVD currently described are autologous IVD chondrocyte-like cell transplantation and mesenchymal stem cell (MSC) transplantation of either adipose tissue derived or bone marrow derived stem cells[161]. Scaffolds, designed as a functional microenvironment for the transplanted cell to improve cellular proliferation, migration, and matrix production, can also be applied to improve the regenerative potential of the transplant. Examples of scaffolds include atelocollagen[162,163], injectable hyaluronic acid [107], chitosan based, and poly (L-lactic-co-glycolic acid) (PLGA)[164].

The concept of IVD regeneration in dogs using cell transplants has been evaluated in several studies. Transplantation of autologous chondrocyte-like cells in NCD-breed dogs[165] and CD-breed dogs[164] after partial discectomy of the lumbar IVD resulted in a deceleration of degeneration and potential regeneration, assessed by means of radiography, MRI, gross pathology, histology, and matrix staining. Transplantation of allogenic MSCs in degenerated Beagle IVDs[166] and autologous IVDs from NCD-breed dogs[107] resulted in a deceleration of degeneration, assessed by means of morphological examination, MRI, radiography, gene expression analysis, and matrix protein analysis. Transplantation of chondrocyte-like cells and MSCs has also been evaluated in other animal species, such as rabbits, rats, and goats[161]. However, given the importance of

notochordal cells to the health of the IVD matrix, further research into this cell type for use in IVD regeneration strategies seems appropriate[2,20,63-66,73-75,154,155].

Conclusion

The IVD is an essential structure of the spine, and the capacity of the IVD to fulfill its physiological function is largely dependent on the quality of its extracellular matrix and, therefore, on the ability of cells of the IVD to synthesize, remodel, and maintain a biochemically healthy matrix. Degeneration of the IVD involves deterioration of the matrix quality, ultimately leading to structural failure of the tissue. IVD degeneration is commonly seen in both CD and NCD dog breeds and can lead to debilitating disease such as IVD herniation or spinal instability. IVD degeneration is a gradual process involving the NP, AF, TZ, EP, and all surrounding spinal structures. Histopathologically, a shift from the native notochordal cell population to a 'suboptimal' chondrocyte-like cell population appears to be the initiating stage of the degenerative process. At the same time, compensatory changes in the surrounding AF, EPs, and vertebral bodies can often be seen. These cellular changes in the NP result in an aberrant homeostasis of the matrix and the enzymes involved in matrix regulation. Since the individual components of the IVD are meant to function synergistically and are dependent on one another, deterioration of one component leads to degeneration of the other, resulting in a vicious degenerative cycle that can ultimately result in bulging or herniation of the IVD.

The distinction between CD and NCD breeds regarding IVD degeneration is widely accepted by the veterinary community. Although these types of dog breed differ significantly in the age of onset and the progression of the morphological, histopathological, and biochemical changes associated with the degenerative process, it seems that the fundamental steps in the degenerative cascade are similar in both types of breed. In CD breeds, a genetic cause is likely to initiate IVD degeneration, which may also explain the calcifications of the IVD observed in this group. In NCD breeds, a multifactorial etiology is more plausible, caused by trauma or 'wear and tear' of the IVD.

Interest in the regenerative repair of the IVD has increased over the past years. The application of factors to positively influence matrix homeostasis, leading to a healthier IVD, seems to have beneficial effects. In more advanced stages of degeneration, cell-based therapies may facilitate the regenerative process. Although some regenerative treatment strategies show promising results, the underlying disease process is not yet fully understood. Thus more fundamental research is needed into the pathogenesis of IVD degeneration before we are likely to find out how to permanently halt or reverse IVD degeneration.

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Chapter 3

Incidence and mortality of diseases related to intervertebral disc degeneration in a population of over 600,000 dogs

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Abstract

Common canine diseases related to intervertebral disc (IVD) degeneration include degenerative lumbosacral stenosis (DLSS), cervical spondylomyelopathy (CSM), and Hansen type I and II IVD herniation.

Objective: The aim of this study was to investigate the incidence and mortality of IVD degenerative diseases by breed, age, and gender, as reflected by insurance claims for veterinary care and mortality in a large dog population.

Material and methods: Data on more than 600,000 dogs spanning a 12-year period (1995-2006), resulting in 2,772,423 dog-years at risk (DYAR), were used to calculate incidence and mortality rates. Incidence rates (based on health insurance claims) were calculated for dogs $\leq$ 12 years of age and mortality rates (based on life insurance claims) were calculated for dogs $\leq$ 10 years of age.

Results and conclusion: The incidence rate (±SE) of IVD-related diseases in the population, based on dogs with settled veterinary care, was 27.8 ± 0.3 dogs per 10,000 DYAR. By breed, it was highest in the Miniature Dachshund, followed by the Standard Dachshund and Doberman (237.1 ± 12.0, 141.2 ± 3.0, and 88.6 ± 8.5 dogs per 10,000 DYAR, respectively). The incidence of IVD-related disease was higher in male dogs than in female dogs (33.6 ± 0.5 and 22.2 ± 0.4 dogs per 10,000 DYAR, respectively) and it increased with age.

The overall mortality rate due to IVD degenerative diseases was 9.4 ± 0.2 deaths per 10,000 DYAR, with the mortality rate being 1.6 times higher in male dogs than in female dogs. The case fatality rate (ratio of mortality rate to incidence rate of IVD-related diseases) was about 1:2 in the large-breed dogs and 1:5 in the small-breed dogs at highest risk. The incidence and mortality rates of IVD degenerative diseases were significantly higher in chondrodystrophic breeds than in non-chondrodystrophic breeds, with the exception of lumbosacral disease, which was more common in non-chondrodystrophic breeds. IVD degenerative disease was over represented in some breeds and absent in others, suggesting that there is a significant genetic factor involved in the occurrence of IVD degenerative diseases.

Introduction

Common canine diseases related to intervertebral disc (IVD) degeneration include degenerative lumbosacral stenosis (DLSS), cervical spondylomyelopathy (CSM), and Hansen type I and II IVD herniation[1,2]. The lifetime prevalence (the proportion of dogs developing IVD herniation at some point during their lifetime) of IVD herniation has been conservatively estimated at 2%[3,4]. IVD degenerative diseases are generally more common in chondrodystrophic breeds than in non-chondrodystrophic breeds and in older dogs than in younger dogs[5-7]. Degeneration of the IVD is generally considered to be multifactorial,[7,8] but a genetic influence is apparent in some breeds[9-11]. IVD degeneration is not synonymous with IVD disease, while IVDs that give rise to clinical signs inevitably show degeneration; degenerated IVDs are common incidental findings[2,5,12-15].

Some countries have introduced radiographic screening programs to reduce the occurrence of IVD herniation, by excluding dogs with a high number of visible calcified IVDs from breeding purposes[16-18]. However, this may not be an efficient screening method as calcified IVDs can occur in dogs without herniation, and IVD herniation can occur without the presence of IVD calcification[19]. A more appropriate approach would be to screen dogs at high risk of acquiring IVD degenerative disease by using DNA markers, but such markers are not currently available. In order to identify high risk dogs, in the absence of such markers, we investigated the incidence and mortality rates of IVD degenerative diseases by breed, age, and gender. To this end, we used insurance claims for veterinary care or deaths due to IVD degenerative disease in the Swedish dog population. In Sweden, the insurance company Agria* annually insures approximately 40% of the Swedish dog population (about 200,000 – 250,000 dogs), and these dogs are considered fairly representative of the entire Swedish dog population[20-22]. The insurance company offers an insurance policy covering costs for veterinary care and a life insurance policy. In general the veterinary care policy is valid for dogs up until the age of 12 years, whereas the life insurance policy only pays out if the dog dies because of disease or accident before the age of 10 years. Most dogs with veterinary care insurance are also covered by life insurance. The insurance process has previously been described in detail[21].

The aim of this study was to increase the knowledge of the breed and age distribution of IVD degenerative diseases in dogs, thereby facilitating early diagnosis and possibly preemptive treatments in high risk dogs. Secondly we aimed to lay a foundation for future genetic studies of IVD degenerative diseases, hoping that there one day will be DNA markers identifying dogs at high risk of developing disease.

* Agria Pet Insurance, Stockholm, Sweden

Materials and Methods

Study population

The study population was all dogs with veterinary care and/or life insurance at Agria* in the period 1995-2006. Insurance claims and deaths, within this period, related to IVD degenerative diseases were entered as cases in the calculation of incidence rates and mortality rates. Incidence rates for veterinary care were calculated using only the first reimbursed claim with this diagnosis for each individual dog. The insurance process has previously been described in detail[21].

Data management

The following data were collected: the date when the dog entered or left the insurance program, type of insurance (veterinary care / life insurance), the dog's breed (according to the Swedish Kennel Club's standard), gender, and dates of birth and death, and postal code of the owner (urban versus rural) and diagnostic codes for insurance claims (veterinary care / life insurance). All life insurance claims were included, regardless of whether they were reimbursed or not, whereas only veterinary care claims that had been reimbursed were included.

Insurance claims, consisting of one or several receipts of payment for veterinary care, had to be accompanied by a diagnostic code for the primary clinical complaint assigned by the attending veterinarian, using a standardized diagnostic registry[23]. Dogs with a diagnosis of IVD degenerative diseases such as DLSS, CSM, unspecific, or anatomically specified IVD herniation were identified. Nineteen diagnostic codes, grouped in five different ways, were used to retrieve the cases. An overall group "Diseases related to IVD degeneration" included all 19 diagnostic codes. The subgroup "Unspecific IVD herniation" included all diagnostic codes for IVD herniation, without reference to anatomic site. Three groups were made up of diagnostic codes related to anatomic site: cervical, thoracolumbar, or lumbosacral.

Incidence, mortality, and case fatality calculations

Incidence rates (± standard error) were calculated on the basis of the first veterinary care insurance claim with a relevant diagnosis for dogs aged 12 years or younger. Mortality rates (± standard error) were calculated based on life insurance claims of dogs that had died aged 10 years or younger of a relevant disease. The denominator was the number of dog–years at risk (DYAR). The numerators were the dogs with at least one reimbursed veterinary care claim (incidence) or the number of dogs with life insurance that had died (mortality). Incidence and mortality rates for IVD degenerative diseases were calculated by breed, gender, and location of residence (urban versus rural) overall and for each diagnostic subgroup. Incidence and mortality rates are expressed per 10,000 DYAR[24].

Breed-specific incidence rates were calculated for breeds with more than 12,000 DYAR. Mortality rates were calculated for all breeds, where incidence rates for veterinary care insurance had been calculated, and which had more than 9000 DYAR for the life-insurance.

An approximate case fatality rate, i.e., the proportion of dogs that died of the disease they were diagnosed with, was calculated by dividing the mortality rate by the incidence rate of IVD degenerative disease.

Age-related measures

The overall risk of developing IVD degenerative diseases, relative to age was calculated for the entire study population, as well as for the three breeds at highest risk. For this analysis, the veterinary care and life-insurance datasets were combined. The proportional hazards regression (PHREG) procedure was used for Cox regression[†] in order to provide baseline survival curves that reflect the probability, related to age, that dogs in the population would develop IVD degenerative disease. The date of the case was the first diagnosed event of IVD degenerative disease, be it a claim for veterinary care or for life insurance. The dog's age when insurance was started or stopped (censoring, becoming a case or death) was used as time variable. There were no independent variables; each dog was entered on the first day it appeared in the dataset and if neither a case nor censored during the period, it was censored on the last day in the period (31st December 2006). Age-specific hazards (combined veterinary care and life insurance) were constructed using the SMOOTH macro, which computes age-specific hazards from the survival function computed by PHREG[25], producing a smoothed estimate of the hazard curve using a kernel smoothing method. Confidence intervals of 95% were also calculated and included in the graphs. The WIDTH parameter was set to one-tenth of the range of event times.

Correlation between IVD degenerative diseases and chondrodysplasia

Spearman rank correlation was used to evaluate the correlation between the incidence of IVD degenerative diseases (from the veterinary care insurance data) and chondrodystrophic versus non-chondrodystrophic dog breeds. In the absence of a comprehensive list of breeds regarded as chondrodystrophic, the following breeds were classified as chondrodystrophic[1,4,7,12,26-34]: Basset Hound, Beagle, Standard Dachshund, Miniature Dachshund, English Bulldog, French Bulldog, American Cocker Spaniel, Bichon Frisé, Jack Russell Terrier, Drever, Pug, Miniature Poodle, Cavalier King Charles

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Spaniel, Shih Tzu, Papillion and Tibetan Spaniel. Correlations were calculated using the software SPSS[‡].

Results

Population

The veterinary care insurance covered 665,249 dogs with 2,772,423 DYAR and the life insurance covered 552,120 dogs with 2,055,261 DYAR during the 12-year period. IVD degenerative diseases were reported in 186 of the 308 breeds included in the registry. In the breed-specific analysis 50 of these breeds had sufficient DYAR to meet the inclusion criteria for calculation of incidence rates of veterinary care (12,000 DYAR), and 52 breeds met the inclusion criteria for calculation of mortality rates (9,000 DYAR).

Incidence rate of IVD degenerative diseases

The mean incidence rate of IVD degenerative diseases in the entire population was 27.8 ± 0.3 dogs per 10,000 DYAR. This was based on a total of 7708 dogs with reimbursed insurance claims for veterinary care for IVD degenerative diseases. Male dogs were more commonly affected than female dogs (33.6 dogs per 10,000 DYAR, n = 4578 versus 22.2 dogs per 10,000 DYAR, n = 3130, respectively). The male/female ratio was 1.5/1. The incidence rate of IVD degenerative diseases was higher in dogs from urban areas than in dogs from rural areas (34.7 dogs per 10,000 DYAR, n = 2401, versus 25.5 dogs per 10,000 DYAR, n = 5307, respectively). Dachshund breeds were at highest risk, and nine of the ten breeds at high risk were chondrodystrophic or miniature breeds. The Doberman was the only non-chondrodystrophic breed at high risk (Table 1).

In total 4629 dogs were classified with unspecific IVD herniation, giving an overall incidence rate of 17.8 ± 0.9 dogs per 10,000 DYAR. The male/female ratio was 1.6/1. The top ten breeds at highest risk were all chondrodystrophic, with Dachshunds being overrepresented. The incidence rate of unspecific IVD herniation was 188.0 ± 11.0 and 108.0 ± 2.6 dogs per 10,000 DYAR for the Standard and Miniature Dachshund, respectively. Cervical IVD herniation or CSM was diagnosed in 844 dogs, yielding an overall incidence rate of 3.0 ± 0.1 dogs per 10,000 DYAR. The male/female ratio was 1.6/1. Cervical IVD herniation was found in both chondrodystrophic and non-chondrodystrophic dogs, with Dobermans being clearly over represented in the latter (Table 2). Thoracolumbar IVD herniation was diagnosed in 1045 dogs, with an overall incidence rate of 3.7 ± 0.1 dogs per 10,000 DYAR. The male/female ratio was 1.6/1. Of the 15 breeds at highest risk, 11 were chondrodystrophic, with Dachshunds again being

[‡] SPSS 17.0, Chicago, IL

over represented (Table 3). Lumbosacral IVD herniation and DLSS were diagnosed in 1574 dogs, giving an overall incidence rate of 5.6 ± 0.1 dogs per 10,000 DYAR. The male/female ratio was 1.4/1. The 15 breeds at highest risk were all non-chondrodystrophic, with German Shepherd Dogs being clearly over represented (Table 4).

Mortality of IVD degenerative diseases

In total, 1924 dogs died of IVD degenerative diseases, giving an overall mortality rate of 9.4 ± 0.2 deaths per 10,000 DYAR. More male dogs than female dogs died of IVD degenerative diseases (11.5 ± 0.3 deaths per 10,000 DYAR, n = 1174 versus 7.2 ± 0.3 deaths per 10,000 DYAR, n = 750, respectively). Mortality rates were similar in dogs from urban areas (9.2 ± 0.2 deaths per 10,000 DYAR, n = 440) and rural areas (9.4 ± 0.4 deaths per 10,000 DYAR, n = 1484). The mortality rates due to IVD degenerative diseases were higher in chondrodystrophic breeds than in non-chondrodystrophic breeds, with 7 of the top 10 breeds with the highest risk of mortality being chondrodystrophic (Table 1).

Case fatality of IVD degenerative diseases

The overall case fatality rate of IVD degenerative diseases was 34%. Case fatality rates were considerably higher in non-chondrodystrophic breeds than in chondrodystrophic breeds, with rates of 63% in Dobermans and 65% in German Shepherd Dogs compared with 24% in Miniature Dachshunds and 25% in Miniature and Standard Dachshunds.

Table 1. Rates per 10,000 dog-years at risk (DYAR) ± SE of diseases related to intervertebral disc degeneration in the 15 breeds at highest risk and the 5 breeds at lowest risk out of 50 breeds insured for veterinary care with more than 12,000 DYAR.

		Incidence rate		Mortality rate	
	Total no. DYAR for veterinary care	**Rates ± SE**	**No. of affected dogs**	**Rates ± SE**	**No. of affected dogs**
Population	2,772,423	27.8 ± 0.3	7,708	9.4 ± 0.2	1,924
Male dogs	1,363,175	33.6 ± 0.5	4,578	11.5 ± 0.3	1,174
Female dogs	1,409,248	22.2 ± 0.4	3,130	7.2 ± 0.3	750
Rural	2,079,943	25.5 ± 0.4	5,307	9.4 ± 0.2	1,484
Urban	692,480	34.7 ± 0.7	2,401	9.2 ± 0.4	440
High-risk breeds					
Miniature Dachshund	15,433	237.1 ± 12.0	366	56.7 ± 6.9	67
Standard Dachshund	155,240	141.6 ± 3.0	2,196	35.7 ± 1.7	446
Doberman	12,411	88.6 ± 8.5	110	56.0 ± 7.4	57
Beagle	20,509	68.3 ± 5.8	140	15.0 ± 2.9	26
American Cocker Spaniel	13,495	60.8 ± 6.7	82	14.1 ± 3.6	15
Cocker Spaniel	42,059	52.1 ± 3.5	219	15.6 ± 2.3	48
Cavalier King Charles Sp.	55,124	49.9 ± 3.0	275	10.3 ± 1.5	46
Tibetan Spaniel	14,994	48.7 ± 5.7	73	8.5 ± 2.8	9
Shih Tzu	18,938	39.6 ± 4.6	75	9.2 ± 2.7	12
Papillon	28,085	39.5 ± 3.8	111	6.8 ± 1.9	13
Rottweiler	40,191	38.3 ± 3.1	154	18.3 ± 2.3	61
Dalmatian Dog	17,347	36.9 ± 4.6	64	8.6 ± 2.6	11
German Shepherd Dog	188,356	36.3 ± 1.4	683	23.5 ± 1.3	350
Miniature Schnauzer	27,957	35.8 ± 3.6	100	5.9 ± 1.7	12
Bernese Mountain Dog	18,606	31.7 ± 4.1	59	18.9 ± 3.4	31
Low-risk breeds					
Finnish Spitz	15,329	2.0 ± 1.1	3	0.0 ± 0.0	0
Finnish Hound	19,229	3.1 ± 1.3	6	5.5 ± 1.7	10
Swedish Elkhound	48,176	3.3 ± 0.8	16	1.6 ± 0.6	7
Collie	41,172	3.4 ± 0.9	14	1.3 ± 0.7	4
Samoyed	15,514	3.9 ± 1.6	6	1.7 ± 1.2	2

Table 2. Rates per 10,000 dog-years at risk (DYAR) ± SE of cervical intervertebral disc herniation and cervical spondylomyelopathy in the 15 breeds at highest risk and the 5 breeds at lowest risk out of all breeds insured for veterinary care with more than 12,000 DYAR.

	Incidence rate		Mortality rate	
	Rates ± SE	**No. of affected dogs**	**Rates ± SE**	**No. of affected dogs**
Population	3.0 ± 0.1	844	0.9 ± 0.1	187
Male dogs	3.7 ± 0.2	518	1.2 ± 0.1	127
Female dogs	2.3 ± 0.1	326	0.6 ± 0.1	60
Rural	2.7 ± 0.1	570	1.0 ± 0.1	137
Urban	3.9 ± 0.2	274	0.9 ± 0.1	50
High-risk breeds				
Doberman	58.6 ± 6.9	73	40.3 ± 6.3	41
Miniature Dachshund	24.4 ± 3.9	40	5.1 ± 2.1	6
Beagle	13.5 ± 2.5	28	2.9 ± 1.3	5
Standard Dachshund	13.0 ± 0.9	210	1.7 ± 0.4	21
Rottweiler	8.9 ± 1.5	36	4.2 ± 1.1	14
Cavalier King Charles Spaniel	8.8 ± 1.3	49	1.6 ± 0.6	7
Whippet	7.0 ± 2.32	9	0.0 ± 0.0	0
Dalmatian Dog	6.87 ± 1.98	12	1.6 ± 1.1	2
American Cocker Spaniel	6.56 ± 2.19	9	1.9 ± 1.3	2
Cocker Spaniel	5.64 ± 1.15	24	1.3 ± 0.7	4
Miniature Schnauzer	4.61 ± 1.28	13	1.5 ± 0.9	3
Soft Coated Wheaten Terrier	4.1 ± 1.3	10	0.5 ± 0.5	1
German Spaniel	4.07 ± 1.54	7	0.7 ± 0.7	1
Tibetan Spaniel	3.96 ± 1.62	6	1.0 ± 1.0	1
Drever	3.59 ± 0.8	20	0.8 ± 0.4	4
Low-risk breeds				
Swedish Elkhound	0.0 ± 0.0	0	0.0 ± 0.0	0
Collie	0.0 ± 0.0	0	0.0 ± 0.0	0
Münsterländer	0.0 ± 0.0	0	0.0 ± 0.0	0
Papillon	0.4 ± 0.4	1	0.0 ± 0.0	0
Golden Retriever	0.2 ± 0.1	3	0.0 ± 0.0	0

Table 3. Rates per 10,000 dog-years at risk (DYAR) ± SE of thoracolumbar intervertebral disc herniation in the 15 breeds at highest risk and the 5 breeds at lowest risk out of all breeds insured for veterinary care with more than 12,000 DYAR.

	Incidence rate		Mortality rate	
	Rates ± SE	**No. of affected dogs**	**Rates ± SE**	**No. of affected dogs**
Population	3.7 ± 0.1	1,045	0.6 ± 0.1	131
Male dogs	4.5 ± 0.2	622	0.8 ± 0.1	81
Female dogs	3.0 ± 0.1	423	0.5 ± 0.1	50
Rural	3.5 ± 0.1	798	0.7 ± 0.1	104
Urban	3.8 ± 0.2	247	0.6 ± 0.1	27
High-risk breeds				
Miniature Dachshund	41.0 ± 5.0	67	4.2 ± 1.9	5
Standard Dachshund	27.2 ± 1.3	437	3.8 ± 0.6	48
American Cocker Spaniel	11.7 ± 2.9	16	0.9 ± 0.9	1
Beagle	8.6 ± 2.0	18	0.6 ± 0.6	1
Papillon	8.1 ± 1.7	23	0 ± 0	0
Tibetan Spaniel	7.9 ± 2.3	12	1.0 ± 1.0	1
Miniature Schnauzer	7.1 ± 1.6	20	0.5 ± 0.5	1
Cocker Spaniel	7.0 ± 1.3	30	3.3 ± 1	10
Shih Tzu	6.3 ± 1.8	12	0.8 ± 0.8	1
Border Terrier	5.9 ± 1.4	17	0.4 ± 0.4	1
Cavalier King Charles Spaniel	5.7 ± 1.0	32	0.7 ± 0.4	3
Bichon Frise	3.7 ± 1.2	10	0.5 ± 0.5	1
Drever	3.6 ± 0.8	20	1.7 ± 0.6	9
Miniature Poodle	3.4 ± 0.8	20	1 ± 0.5	4
Nova Scotia Duck Tolling Retriever	3.0 ± 1.3	5	1.3 ± 0.9	2
Low-risk breeds				
Finnish Hound	0.0 ± 0.0	0	0.0 ± 0.0	0
Petit Basset Griffon	0.0 ± 0.0	0	0.0 ± 0.0	0
Finnish Spitz	0.0 ± 0.0	0	0.0 ± 0.0	0
Tervuren	0.0 ± 0.0	0	0.0 ± 0.0	0
English Springer Spaniel	0.2 ± 0.2	1	0.0 ± 0.0	0

Table 4. Rates per 10,000 dog-years at risk (DYAR) ± SE of lumbosacral degenerative diseases in the 15 breeds at highest risk and the 5 breeds at lowest risk, out of all breeds insured for veterinary care with more than 12,000 DYAR.

	Incidence rate		Mortality rate	
	Rate ± SE	**No. of affected dogs**	**Rate ± SE**	**No. of affected dogs**
Population	5.6 ± 0.1	1,574	2.6 ± 0.1	586
Male dogs	6.7 ± 0.2	916	3.3 ± 0.2	333
Female dogs	4.6 ± 0.2	658	1.9 ± 0.1	193
Rural	5.1 ± 0.2	1,072	2.8 ± 0.2	393
Urban	7.2 ± 0.3	502	2.5 ± 0.1	193
High-risk breeds				
German Shepherd Dog	27.9 ± 1.2	526	18.1 ± 1.1	270
Doberman	17.5 ± 3.7	22	7.9 ± 2.8	8
Rottweiler	15.9 ± 2.0	64	8.1 ± 1.6	27
Bernese Mountain Dog	15.5 ± 2.9	29	10.4 ± 2.5	17
Boxer	14.0 ± 2.7	27	6 ± 2	9
Dalmatian Dog	13.8 ± 2.8	24	1.6 ± 1.1	2
Irish Setter	11.3 ± 2.5	20	1.5 ± 1.1	2
Labrador Retriever	9.3 ± 0.8	128	2.1 ± 0.5	22
Nova Scotia Duck Tolling Retriever	7.2 ± 2.1	12	0.7 ± 0.7	1
Flat Coated Retriever	6.5 ± 1.4	22	2.2 ± 0.9	6
German Pointer	6.0 ± 1.5	17	3 ± 1.1	7
Standard Poodle	6.0 ± 1.5	15	1.1 ± 0.7	2
Tervuren	5.3 ± 1.9	8	1.7 ± 1.2	2
Golden Retriever	5.2 ± 0.6	81	1.2 ± 0.3	14
English Springer Spaniel	5.2 ± 0.9	31	0.6 ± 0.4	3
Low-risk breeds				
Yorkshire Terrier	0.0 ± 0.0	0	0.0 ± 0.0	0
Petit Basset Griffon	0.0 ± 0.0	0	0.0 ± 0.0	0
Finnish Spitz	0.0 ± 0.0	0	0.0 ± 0.0	0
Tibetan Spaniel	0.0 ± 0.0	0	0.0 ± 0.0	0
Drever	0.2 ± 0.2	1	0.2 ± 0.2	1

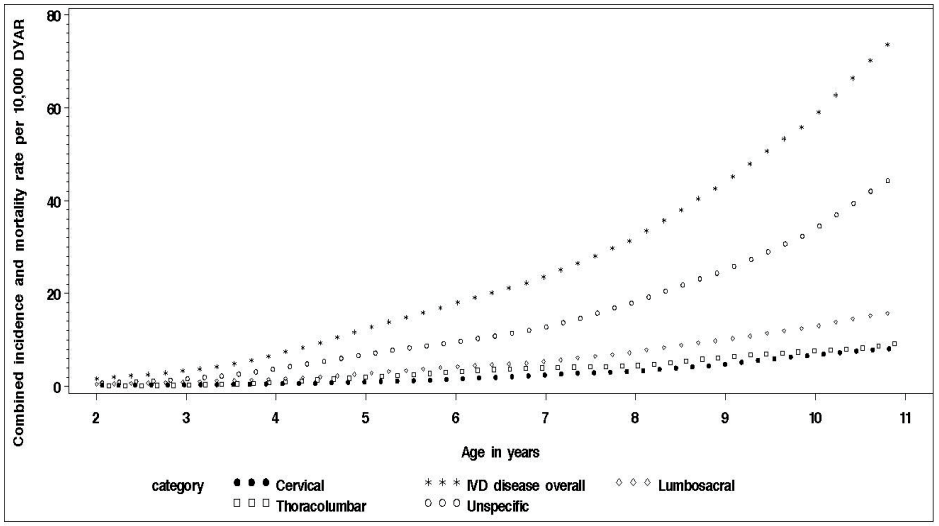
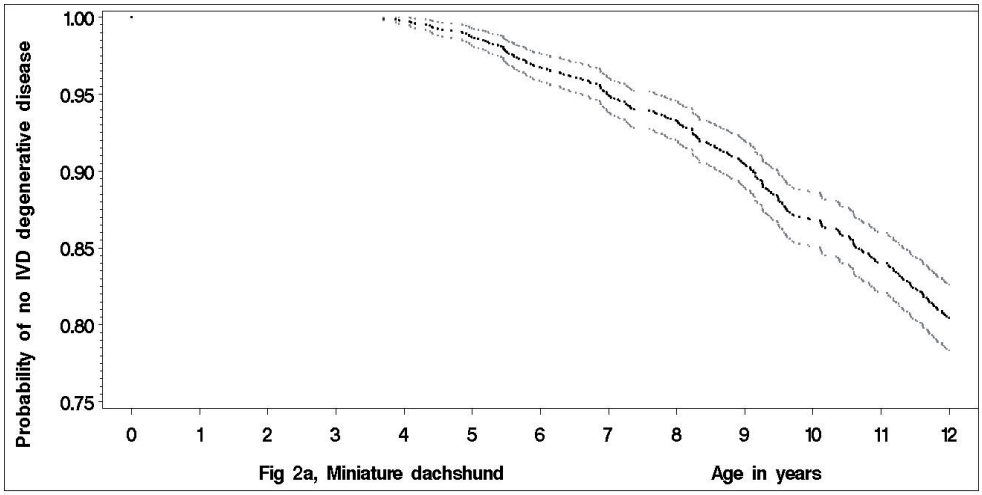


Figure 1. Age in relation to the hazard of the overall category "diseases related to intervertebral disc (IVD) degeneration" and the individual subgroups groups "unspecific IVD herniation", "cervical IVD herniation and cervical spondylomyelopathy", "thoracolumbar IVD herniation", and "lumbosacral disease". The hazard was calculated using combined veterinary care and life insurance data, censoring dogs at first diagnosis so each dog is only counted once.

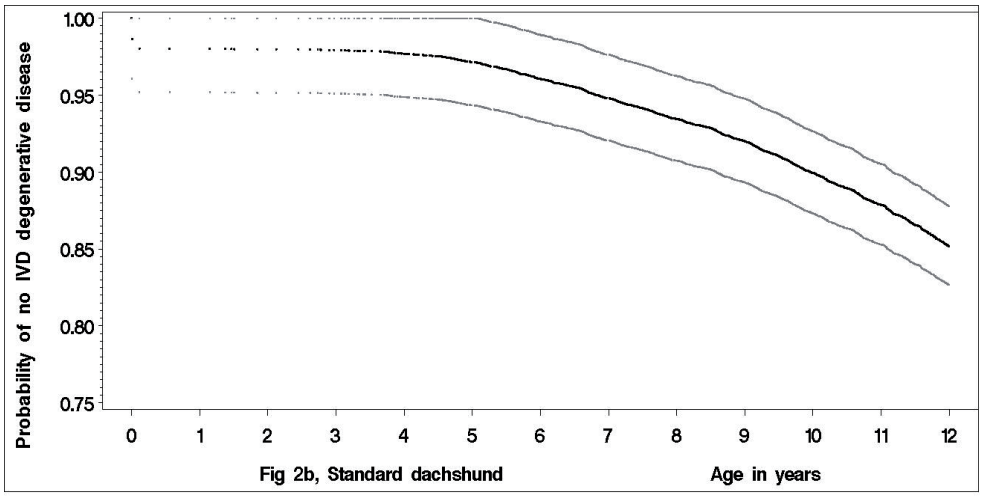
Effect of age and breed

The hazard (combined incidence rate) of IVD degenerative diseases increased with age (Fig. 1 and 2). When combining life and veterinary care insurance claims, the three breeds at highest risk of developing IVD degenerative diseases at some point in their lives (before the age of 12 years) were in increasing order: Miniature Dachshund, Doberman, and Standard Dachshund. If the dogs would live to the age of 12 years; 20% of the Miniature Dachshunds, 17.5% of the Dobermans and 15% of the Standard Dachshunds would have had at least one event of IVD degenerative disease (Fig. 2a-c). The proportion of dogs with IVD degenerative disease in the entire population, before the age of 12 years, was 3.5% (Fig. 2d). The risk of IVD degenerative disease increased with age (Fig.1 and 2). The German Shepherd Dog, which is the breed at highest risk of lumbosacral IVD degenerative disease in this study, had a "life-time prevalence" of IVD degenerative diseases at 7% before the age of 12 years.

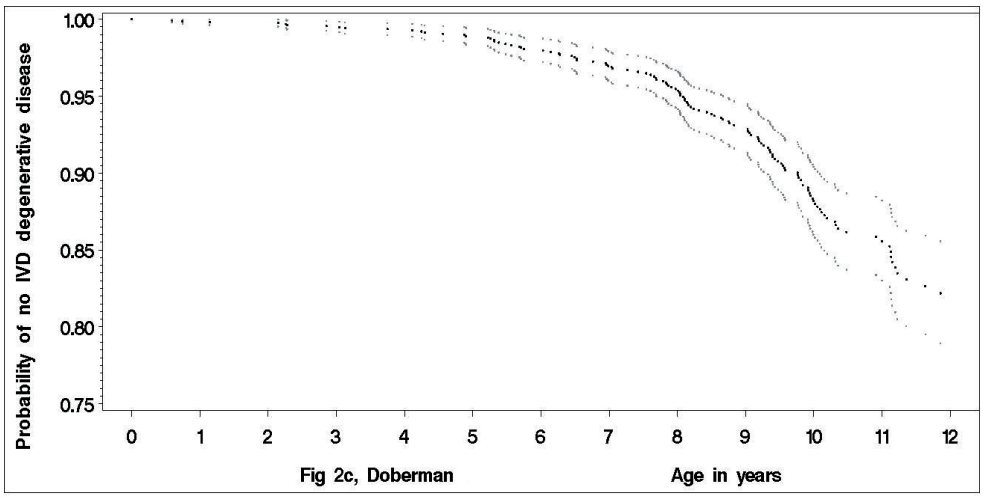


2a

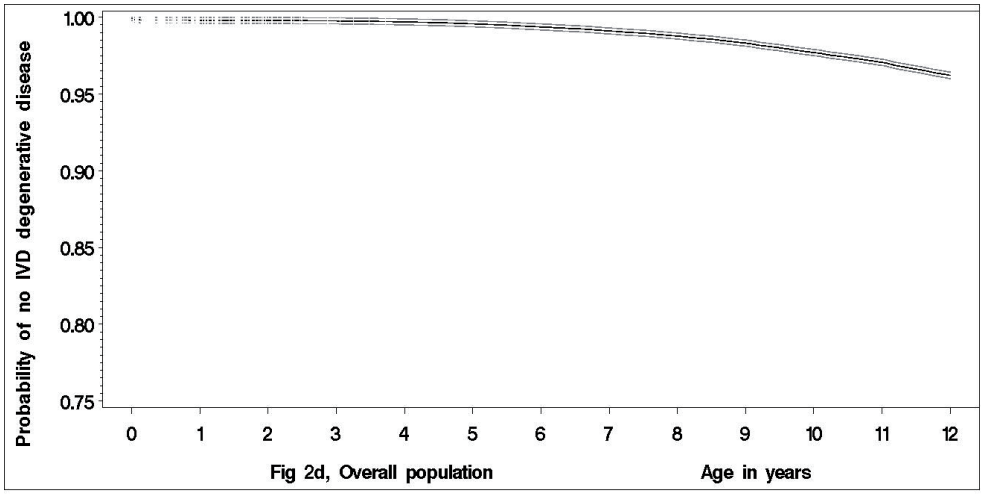
Figure 2. Proportion of dogs that have not developed intervertebral disc (IVD) degenerative disease, plotted by age using life insurance and veterinary care data combined, including 95% confidence intervals for **a)** Miniature Dachshund, **b)** Standard Dachshund, **c)** Doberman, **d)** The overall dog population.
The dogs were censored at first diagnosis, regardless if it was for veterinary care or life insurance, so that each dog was only counted once.



2b


Probability of no IVD degenerative disease
1.00
0.95
0.90
0.85
0.80
0.75
0 1 2 3 4 5 6 7 8 9 10 11 12
Fig 2c, Doberman
Age in years

2c


Probability of no IVD degenerative disease
1.00
0.95
0.90
0.85
0.80
0.75
0 1 2 3 4 5 6 7 8 9 10 11 12
Fig 2d, Overall population
Age in years

2d

Correlation between IVD degenerative diseases and chondrodysplasia

IVD degenerative diseases in general, thoracolumbar IVD herniation, and unspecified IVD herniation were significantly correlated to being of a chondrodystrophic breed, with R= 0.47, 0.77 and 0.73, respectively, $P < 0.01$ for all correlations. Cervical IVD degenerative disease was not significantly correlated with chondrodystrophic breed, and lumbosacral IVD degenerative disease was significantly and negatively correlated with chondrodystrophic breed ($R = -0.73$, $P < 0.01$).

Discussion

In the present study a conservative "lifetime prevalence", before the age of 12 years, was found to be 3.5% (Fig. 2 d) in the overall population. Combined with the relatively high case fatality rate <10 years of age (34%) it can be concluded that IVD degenerative diseases are responsible for the death of on average 1 in 100 dogs. However, the incidence rate of IVD degenerative diseases was much higher in some breeds, and particularly in chondrodystrophic dogs with a "lifetime prevalence" of 20% (Fig. 2a) in the most affected breed. The risk of IVD degenerative diseases was found to increase with age.

Although IVD degenerative diseases occurred in different breeds, the incidence of these diseases was especially high in chondrodystrophic breeds, mainly Dachshunds. Moreover, IVD degenerative diseases were not diagnosed at all in some common breeds. This large variation in incidence between different breeds is suggestive of a significant genetic component in the development of IVD degenerative diseases. For example, thoracolumbar IVD herniation was about 30 times more common in Miniature Dachshunds than in German Shepherd Dogs, whereas DLSS was most common in German Shepherd Dogs but was not diagnosed at all in Miniature Dachshunds. This strong genetic influence on IVD degenerative diseases suggests that screening programs, such as currently conducted in Denmark to reduce the occurrence of IVD herniation in Dachshunds[16], could be successful in reducing the occurrence of these diseases provided that the elimination criteria used is linked with increased heritability of disease. However, Rohdin *et al.* have suggested that eliminating dogs from breeding programs based on the number of radiographically visible IVDs might not be the best approach[19]. The results from the present study support the need for future genetic studies attempting to identify DNA markers of IVD degeneration, which may aid potential screening programs in reducing the incidence of disease.

In addition to the strong genetic component, there is also evidence that external factors, such as physical activity or environmental factors, influence the development of IVD

degenerative diseases. For example, there are currently about 4500 active service dogs in Sweden, employed mainly by police or military services and serving as guide dogs for blind people. Most of the police dogs (75%) and military dogs (65%) are German Shepherd Dogs, the breed at highest risk of DLSS.[35,36]. Of the police dogs, 16.5% are retired early (< 8 years of age) because of back problems [37]. The "life-time prevalence" of IVD degenerative disease found in the German Shepherd Dog in this study was 7% in dogs under 12 years of age. The higher percentage of affected police German Shepherd Dogs, already at the age of 8 years, indicates that factors other than genetic, such as high physical workload, can significantly accelerate the rate and occurrence of IVD degenerative diseases in these dogs.

The overall case fatality rate of IVD degenerative diseases was 34%, with considerably higher rates in large breed non-chondrodystrophic dogs than in small breed chondrodystrophic dogs, of the breeds at highest risk. However, case fatality rates were probably underestimated because dogs older than 10 years were included in the life insurance data whereas the veterinary care data included dogs up to 12 years of age. The higher case fatality rates found in large breed dogs could be dependent on a number of factors: 1) most of the IVD degenerative diseases seen in small breed dogs were IVD herniation, which responds better to conservative treatment, than DLSS and CSM, which were more common in large breed dogs; 2) Large breed dogs generally have a shorter life expectancy; and 3) the management of convalescence in paraparetic/paraplegic dogs is more difficult and physically demanding for owners of larger dogs.

The mortality rate for IVD degenerative disease was slightly higher in dogs from rural areas (9.4 ± 0.2 deaths per 10,000 DYAR) than in dogs from urban areas (9.2 ± 0.2 deaths per 10,000 DYAR), yet the incidence rate for veterinary care of IVD degenerative disease was on average 1.3 times higher in urban dogs than in rural dogs among the 10 breeds at highest risk. The greatest difference was seen in the Standard Dachshund, which had a 1.8 times higher risk of IVD degenerative disease if it lived in an urban area. Possible explanations for this difference include environmental differences between dogs living in urban and rural areas, genetic differences within the breeds, or that different breeds are kept in urban and rural areas. Other explanations may be that dogs in rural areas might be less likely to receive a thorough diagnostic work-up and care because of a relative lack of advanced, specialist facilities, or that the costs of diagnosis and treatment might have been too low in rural areas to claim insurance and the dog would thus not have been included as a case.

Veterinary care data for DLSS, calculated using information from the same database, have recently been published[11]. There are some differences in the data extraction and presentation, which explains the discrepancies between the two studies. An important,

difference is that we considered dogs to have an IVD degenerative disease only once in a life-time, whereas in the previous study[11] a dog could have an IVD degenerative disease more than once (a maximum of once yearly), which led to the incidence being slightly higher in the previous study[11]. Moreover, we did not include dogs older than 12 years, whereas the previous study[11] had no upper age limit. Lastly, we used a cut-off value for inclusion of breeds of 12,000 DYAR whereas the previous study[11] used 3,600 DYAR, so that we excluded less common breeds in the present study.

A limitation of the present study is the assumption that veterinarians make the correct diagnosis and use the correct diagnostic codes. To limit this, we used a strict selection of the diagnostic codes to identify dogs as cases. This selection will have led to a number of false negatives as dogs assigned unspecific diagnostic codes by the attending veterinarian, such as "back problem without further specification" or "pain on palpation of the back", were not included as cases in this study. These two diagnostic codes alone were assigned to more than 3,000 patients during the study period. In addition, the diagnosis of IVD degenerative diseases on clinical examination only and treated conservatively will end up with a veterinary fee lower than the deductible for Agria's veterinary care insurance and these cases have not be included in this study. The strict selection of diagnostic codes, in combination with cases for which no insurance claims are filed is likely to have led to some degree of underestimation of the true incidence of IVD degenerative diseases in the Swedish dog population.

The high number of DYAR set as inclusion criteria for the breed-specific presentation of results excluded many less common breeds. In some of these excluded breeds, such as the Basset Hound, Long-haired Dachshund, and Clumber Spaniel, the incidence of IVD degenerative disease was high. Although the high cut-off increases the precision of the rates, it also leads to potential high-risk breeds not being identified.

Another potential confounder is owner-dependent breed bias. In this study, hunting dogs such as Finnish Hound, Finnish Spitz, and Swedish Elkhound were found to be among the breeds with the lowest risk of IVD degenerative diseases. It could be argued that these dogs are bred for hunting and thereby generally healthier than other breeds. It could, however, also be argued that the owners of hunting dogs might be less likely to bring their dogs to the veterinarian for treatment and thus the insurance data might not reflect the true situation. However, it is logical to assume that also the owners of hunting dogs chose to have insurance because they want to use it when relevant, hence owners of insured hunting dogs are likely to make use of this insurance in much the same way as any other dog owner.

Although previous studies of the epidemiology of IVD degenerative disease involved smaller cohorts of patients treated at academic or referral clinics, the outcomes of these studies were similar to ours[7,35,38]. Another large study was performed 40 years ago and reported similar findings in a North American dog population (8117 cases with 356,954 DYAR).[4] In contrast to our and previous studies[4,39], Dallman et al.[40] investigated 105 dogs and reported that cervical IVD disease was more common in female dogs than in male dogs. From this it can be concluded that population-based epidemiologic studies will greatly reduce the risk of finding biased results.

Conclusions

Although IVD degenerative diseases are found in a great variety of breeds, CD breeds and a small number of NCD breeds such as German Shepherd Dogs and Dobermans are strongly over-represented, indicating a strong genetic influence in the pathogenesis of these diseases. IVD degenerative diseases have a relatively high mortality rate, 34% in the overall population and over 50 % in the large breed dogs at highest risk.

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Chapter 4

Pfirrmann grading of intervertebral disc degeneration in chondrodystrophic and non-chondrodystrophic dogs with low-field magnetic resonance imaging

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Abstract

Objective: The purpose of this study was to assess whether the Pfirrmann system for grading lumbar intervertebral disc (IVD) degeneration in humans can also be used in dogs.
Material and methods: MR images of spinal segments from 202 dogs were reviewed by three independent observers who graded the extent of IVD degeneration in each visible intervertebral disc, using the classification system developed by Pfirrmann et al. for human lumbar intervertebral discs. Grading was validated against two factors associated with the extent of disc degeneration, namely, type of dog (chondrodystrophic or non-chondrodystrophic) and age.
Results: The inter- and intraobserver agreement on Pfirrmann grading of IVD degeneration was good **(**kappa scores ranging between 0.81 and 0.93). Increasing extent of disc degeneration was positively correlated with increasing age and with chondrodystrophic dog breeds.
Conclusion: The Pfirrmann system can be reliably used to grade IVD degeneration in dogs of different breeds and ages. Findings confirmed that increasing extent of IVD degeneration is positively correlated with increasing age and with predisposition to chondrodystrophy.

Introduction

Intervertebral disc (IVD) degeneration is a common disorder in dogs (Agria, 2000, Hoerlein, 1953, Gage, 1975), and in the Swedish dog population IVD degeneration-related diseases are one of the top five reasons for euthanasia of dogs younger than 10 years (Agria, 2000, Egenvall et al., 2000, Egenvall et al., 1998). IVD degeneration-related diseases include Hansen type I and II intervertebral disc herniation, degenerative lumbosacral stenosis, and cervical spondylomyelopathy (Sharp and Wheeler, 2005). IVD degeneration is more common in chondrodystrophic breeds than in non-chondrodystrophic breeds and in older dogs than in younger dogs (Hoerlein, 1953, Hansen, 1951, Bray and Burbidge, 1998a). It is important to note that IVD degeneration is not synonymous with IVD disease. An IVD causing clinical signs of disease will inevitably be degenerated, but degenerated IVDs are also common findings in dogs without clinical signs of disease (Hansen, 1952, Hoerlein, 1953, Jones and Inzana, 2000, da Costa et al., 2006)

There are many similarities between canine and human IVD degeneration (Hansen, 1951, Lotz, 2004, Bray and Burbidge, 1998b, Zimmerman et al., 1992) hence dogs are sometimes used as spontaneous models of human IVD degeneration research (Alini et al., 2008). In both humans and dogs, it can be difficult to diagnose IVD degeneration-related diseases and the use of diagnostic imaging is essential for correct diagnosis. Even then, it is sometimes difficult to distinguish discs with pathological degeneration from discs showing normal age-related changes known as “normal senile remodeling” (Adams and Roughley, 2006). Moreover, not all degenerated discs give rise to clinical symptoms, irrespective of the cause of degeneration (Bendix et al., 2008, Carragee and Hannibal, 2004).

Intervertebral disc herniation in dogs is believed to result from IVD degeneration (Hansen, 1951, Bray and Burbidge, 1998a). The current surgical treatment is laminectomy in combination with nucleotomy of the herniated disc. However, in humans with IVD degeneration-related diseases, nucleotomy has been shown to further destabilize the spine (Frei et al., 2001, Zollner et al., 2000) and it is likely that this is also true in dogs (Bray and Burbidge, 1998b, Zimmerman et al., 1992). Although spinal instability in humans is currently treated by spinal fusion, there is a desire to move away from this type of salvage technique and to focus on regenerative treatment strategies to achieve functional repair or even regeneration of the degenerated intervertebral disc. Such strategies require early identification of the degenerative process with reliable and accurate diagnostic methods and grading systems. These types of regenerative treatments will shortly be realized in humans but, due to difficulties in identifying canine patients with early signs of disease, is currently not applicable in dogs. However with the rapid

development of veterinary diagnostic methods, early therapy preventing severe clinical signs will hopefully be available also for dogs within a not too distant future. Also when performing clinical research studies, attempting to stop IVD degeneration or even stimulating regeneration, the Pfirrmann grading scheme is a most valuable tool to follow progression of degeneration or regeneration of the IVDs in vivo.

The Pfirrmann system is the most widely used system to grade human IVD degeneration on the basis of magnetic resonance imaging (MRI) findings (Wilke et al., 2006, Kettler and Wilke, 2006, Pfirrmann et al., 2001). It is based on the system for grading gross pathological changes in intervertebral discs proposed by Thompson et al., which is the most commonly used gold standard in humans (Pfirrmann et al., 2001, Thompson et al., 1990). For canine IVD degeneration, two MRI grading systems have been proposed: one using histopathology (Seiler et al., 2003) and the other using morphological appearance (Sether et al., 1990) as gold standard. A third grading system has been proposed for canine thoracolumbar discs but has not yet been validated (Besalti et al., 2006). None of these three systems have been validated for inter- and intraobserver agreement. There is also no validated grading system available for intervertebral discs in all locations of the spine.

The aim of this study was to evaluate whether the MRI-based grading system by Pfirrmann for grading of IVD degeneration in the human lumbar discs is applicable for use in all types of dogs and for intervertebral discs of all locations of the spine. The aim was also to validate the Pfirrmann system by determining the inter- and intraobserver agreement between Pfirrmann scores, and to evaluate whether biological factors known to increase the extent of IVD degeneration are correlated with higher Pfirrmann scores (Hansen, 1951, Hansen, 1952).

Materials and Methods

Inclusion criteria

All mid sagittal T2-weighted MR images obtained of any part of the spine of patients attending the Clinic of Companion Animals, Utrecht University, The Netherlands, between 2002 and 2008 were included (N=217). Although the majority of the patients included in this study were investigated for suspected IVD degenerative diseases, there were still many patients examined for other reasons, such as suspected syringomyelia, neoplasia, fibrocartilaginous emboli, and degenerative myelopathy. Poor image resolution led to the exclusion of data for 15 small-breed dogs. These images were excluded in consensus between the three observers. In total 994 discs from 202 dogs of different breeds, age, and sex were assessed and included in the study. Of these 202 dogs,

66 were considered on the basis of their breed to be chondrodystrophic and the remaining 136 to be non-chondrodystrophic. In the absence of a comprehensive list of breeds regarded as chondrodystrophic, the following breeds were classified as chondrodystrophic as they had previously been identified as such in the literature (Hansen, 1952, Bray and Burbidge, 1998a, Braund et al., 1975, Sharp and Wheeler, 2005, Goggin et al., 1970, Priester, Gage, 1975, Brisson et al., 2004, Forterre et al., Besalti et al., 2006, Eigenmann et al., 1988, Windsor et al., 2008, Parker et al., 2009): basset hound, beagle, dachshund, miniature dachshund, English Bulldog, French Bulldog, Jack Russell terrier, pug, miniature poodle, Shih Tzu and Welsh corgi. Moreover, the term IVD degeneration was applied to all discs showing signs of degeneration on MRI, regardless of the cause of the degeneration and whether the degeneration was symptomatic or not.

Imaging technique

The MRI was performed using a 0.2 T open magnet[1]. Only T2-weighted MR images (TR 3835-4450 msec/ TE 117 msec) were used. The obtained slices were 3-mm thick. For smaller dogs a Multipurpose Flex Coil, 21 cm in diameter, and for larger dogs a circular polarization, flexible Body/ Spine coil, was used.

Image assessment

The 5-category system developed by Pfirrmann et al to grade IVD degeneration in human lumbar discs was used (Pfirrmann et al., 2001) (Table 1). T2-weighted sagittal MR images were graded by three independent observers, namely, a veterinary student (SW), a PhD student (NB), and a board certified veterinary radiologist (EA). The MR images were examined on standard computer screens using the software Web1000 5.1[2]. Each MR image depicted a part of the spine containing several intervertebral discs.

Figure 1: MR images of human intervertebral discs depicting T2-weighted, midsagittal images of Pfirrmann grades I, II, III, IV, and V from left to right. *Reprinted with permission from Pfirrmann et al. Spine, 2001.*

[1] Magnetom Open Viva, Siemens AG, Munich, Germany

[2] Clinical Review Station, Agfa Gevaert N.V.

The same discs and sagittal slices were viewed at the automatic settings given by the software. All observers were well acquainted with the Pfirrmann grading procedure and had image examples of the various grades of degeneration at hand during the grading procedure (Figure 1).
If criteria from more than one category were found within the same IVD, the higher grade was selected since the IVD was showing signs of progressing degeneration. The MR images were graded twice with a 2-week interval for observer SW (since SW only worked on the project during a short time period) and a 6-month interval for observers NB and EA. Despite the short interval for observer SW, bias due to recollection of what grade was given to which of the 994 images was deemed unlikely. To ensure that the short interval did not affect SW's results a statistical comparison was made between the three observers intra observer agreements.

Table 1. Description of the five categories of the MRI-based grading scheme according to Pfirrmann.

Grade	Structure	Distinction between NP and AF	Signal intensity	Height of intervertebral disc
I	Homogenous, bright white	Clear	Hyperintense, isointense to CSF	Normal
II	Inhomogeneous with or without horizontal bands	Clear	Hyperintense, isointense to CSF	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointense	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointense	Collapsed disc space

NP= nucleus pulposus; AF = annulus fibrosus; CSF=cerebrospinal fluid

Biological validation
To ensure that the results produced using the Pfirrmann system correlated with variables known to be associated with increasing disc degeneration, we validated the Pfirrmann scores against the dog's age and predisposition to chondrodystrophy (i.e., chondrodystrophic or non-chondrodystrophic). Previous studies have described the stronger association between IVD degeneration and older dogs as well as with chondrodystrophic breeds (Bray and Burbidge, 1998a, Seiler et al., 2003). This is why these parameters were used as a type of gold standard for the biologic validation.

Statistical analysis

Cohen's weighted Kappa analysis was used to evaluate inter- and intraobserver agreement. The interpretation of the Kappa (**κ**) values was slight (**κ** 0-0.20), fair (**κ** 0.21-0.40), moderate (**κ** 0.41-0.60), substantial (**κ** 0.61-0.80), and almost perfect agreement (**κ** 0.81-1.00) (Landis and Koch, 1977a, Landis and Koch, 1977b, Koch et al., 1977).

The Pfirrmann grades awarded by the three observers for each MR image were averaged and the averaged grade was used to correlate the degree of degeneration seen on MRI with the age and type of dog (chondrodystrophic or non-chondrodystrophic). Spearman's rank test was used to test for correlation. $P<0.05$ was considered significant.

Results

Animals

Of the total of 202 dogs from which IVDs were assessed, 109 were male and 93 were female. The ages of the dogs varied from 1-13 years (mean 5.4 years). Dogs from 58 different breeds, and IVDs in all different locations of the spine from C2-C3 to L7-S1 were included in the study.

Descriptive results

In total, 994 discs (368 discs from chondrodystrophic dogs and 626 discs from non-chondrodystrophic dogs) were graded on T2-weighted MR images depicting a part of the spine containing several intervertebral discs (Figure 2).

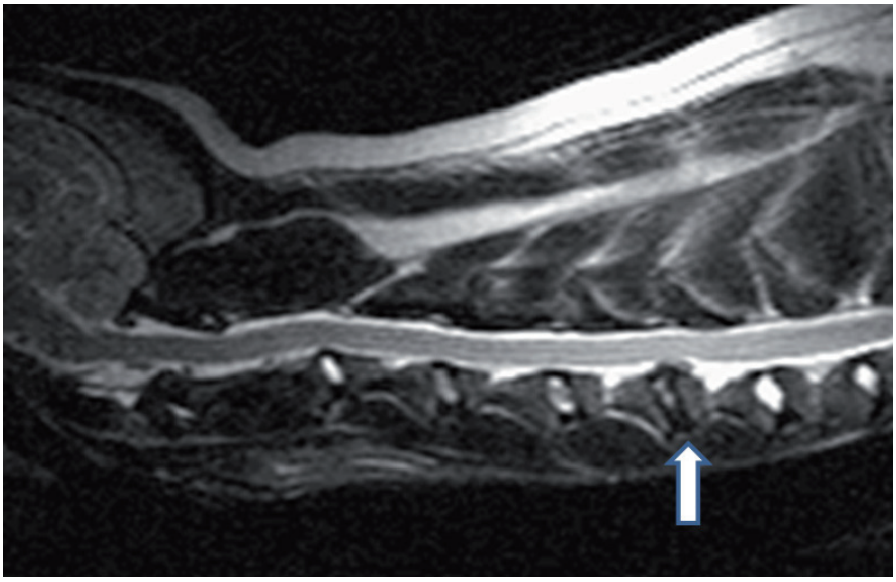


Figure 2: A midsagittal, T2-weighted image of the canine cervical spine. The C5-6 disc (arrow) shows signs of degeneration (Pfirrmann grade III).

Of the discs evaluated, 26.6% had grade I, 29.5% grade II, and 29.5% grade III degeneration; only 13.7% of the discs had grade IV and 0.7% had grade V degeneration (Figure 3, Table 2). In total, 69.9% (250/368) of the discs from chondrodystrophic dogs showed grade III–V degeneration and 70.3% (440/626) of the discs from non-chondrodystrophic dogs showed grade I–II degeneration (Table 2). In the chondrodystrophic dogs, Pfirrmann grades III and IV were predominant from 2 years of age (Figure 4a) whereas in the non-chondrodystrophic dogs Pfirrmann grades I-II were predominant in all age groups (Figure 4b). In contrast to other chondrodystrophic dogs, most IVDs of the Jack Russell terriers fell in the lower half of the Pfirrmann grades irrespective of the dog's age.

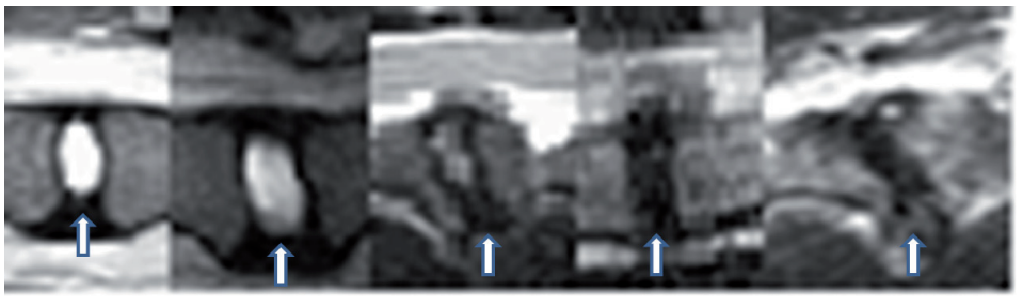


Figure 3: MR images of canine intervertebral discs (arrows) depicting T2-weighted, midsagittal images of Pfirrmann grades I, II, III, IV, and V from left to right.

Table 2. Distribution of Pfirrmann grades in 994 discs, in numbers and (%), of chondrodystrophic (C) and non-chondrodystrophic (NC) dogs.

Disc grade	NC n (%)	C n (%)	NC + C n (%)
I	211 (33.7 %)	54 (14.7 %)	265 (26.6 %)
II	229 (36.6 %)	64 (17.4 %)	293 (29.5 %)
III	130 (20.8 %)	163 (44.3 %)	293 (29.5 %)
IV	50 (8.0 %)	86 (23.3 %)	136 (13.7 %)
V	6 (0.9 %)	1 (0.3 %)	7 (0.7 %)
Total	626 (100 %)	368 (100 %)	994 (100 %)

Intraobserver agreement

The intraobserver agreement was almost perfect and ranged from **κ** 0.93 ± 0.032 to 0.89± 0.031 (Table 3). In 13.2–22.2% of the cases there was a difference of 1 grade between the scores given by the same observer; a difference of 2–3 grades was seen in less than 1% of all intraobserver comparisons (Table 3). No significant difference was found between the three observers' intraobserver agreement.

Table 3. Overview of intra- and interobserver agreement of the three observers (SW, NB and EA) in two grading rounds. Weighted kappa ± standard error (SE) given in the first column followed by absolute numbers of intervertebral discs and (%) of total number of scored discs.

	Kappa ± SE	Perfect agreement	Disagreement of 1 grades	Disagreement of 2 grades	Disagreement of 3 grades
Intraobserver					
SW	0.93±0.032	855 (86.0 %)	131 (13.2 %)	7 (0.7 %)	1 (0.1%)
NB	0.89±0.031	763 (76.8%)	221 (22.2%)	10 (1.0%)	0 (0%)
EA	0.92 ±0.032	836 (84.1%)	156(15.7%)	2 (0.2%)	0 (0%)
Interobserver					
SW-NB	0.81±0.030	618 (62.2%)	340 (34.2 %)	35 (3.5 %)	1 (0.1%)
SW-EA	0.87±0.031	720 (72.4%)	263 (26.4 %)	11 (1.1 %)	0 (0 %)
NB-EA	0.85±0.031	669 (67.3%)	322 (32.4 %)	3 (0.3 %)	0 (0 %)

Interobserver agreement

Interobserver agreement ranged between **κ** 0.81 ± 0.030 and 0.87 ± 0.031, which represents almost perfect agreement. In 26.4–34.2% of the cases, there was a grading difference of 1 grade between two observers; a difference of 2 grades was seen in 0.3–3.5% of the cases, and in one case a difference of 3 grades was seen (Table 3).

Biological validation

A significant, positive correlation between higher Pfirrmann grade and type of dog (chondrodystrophic or non-chondrodystrophic) was found ($r = 0.343$; $p<0.01$): higher grade degeneration was seen more often in chondrodystrophic dogs than in non-chondrodystrophic dogs. In addition, higher Pfirrmann grade was positively correlated with the age of the dog ($r = 0.169$; $p<0.01$).

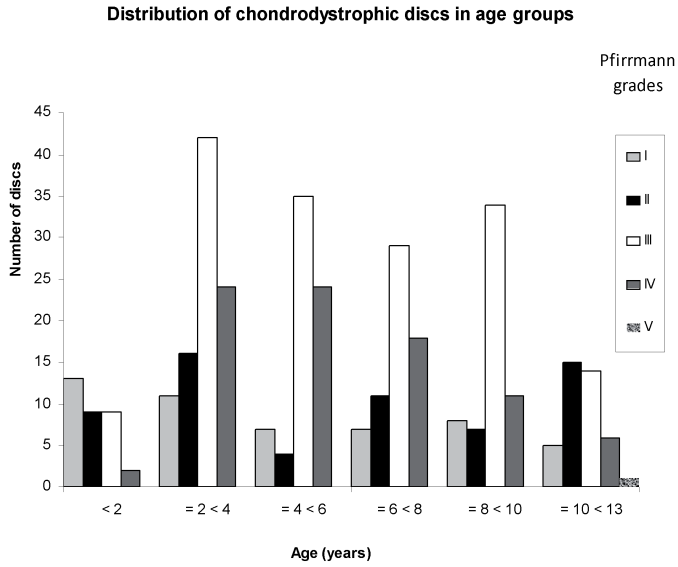
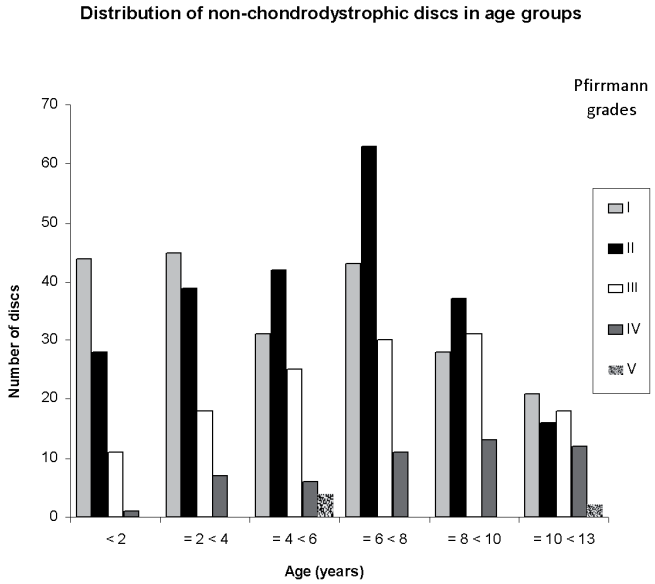


Figure 4a (above) and 4b (below): Distribution of Pfirrmann grades in relation to age in chondrodystrophic dogs (4a) and in non-chondrodystrophic dogs (4b). For each age group, the number of intervertebral discs per Pfirrmann grade is given; each bar represents one Pfirrmann grade. In the chondrodystrophic dogs it can be seen that higher Pfirrmann grades predominate from 2 years of age.



Discussion

This study showed that the Pfirrmann system was highly reproducible for grading IVD degeneration at all spine locations in dogs of various breeds and ages. Moreover, the Pfirrmann score was associated with predisposition to chondrodystrophy and with age, factors associated with IVD degeneration. Thus the Pfirrmann system would appear to be suitable for grading IVD degeneration in dogs.

Perfect agreement was found between the observers in 62.2–72.4% of the discs viewed; in the remaining cases there was a discrepancy of 1 or more grades between the observers. These discrepancies could be because the Pfirrmann system is not a continuous scale and the cut-off point between two categories is not always clear. For example, the discriminating features between grade I and II (homogeneous versus inhomogeneous bright signal of the nucleus) and grade III and IV (ability to discriminate between annulus and nucleus) are to some extent subjective, which could explain why in 0.3–3.5% of cases there was a discrepancy of 2 grades in the disc score and a difference of 3 grades in one case. In addition, human error, such as viewing the wrong sagittal image while grading, or typing errors, is also a potential cause of discrepancies in the cases where discs were graded with a 2 or 3 grade difference.

While most patients included in this study were investigated for suspected intervertebral disc-related problems, others were examined for other reasons, which could explain the broad variation in both healthy and degenerated discs seen. Only 7 discs were scored Pfirrmann grade V (Table 2). Whether this is representative of end-stage disc degeneration in the canine population cannot be determined from this single study. We found no studies in the literature of the association between extent of disc degeneration and severity of clinical signs in dogs. Previous studies have shown that chondrodystrophic dogs are more prone to disc degeneration than non-chondrodystrophic dogs, and that older dogs are more likely to have degenerated discs than younger dogs (Gage, 1975, Hansen, 1951, Bray and Burbidge, 1998a, Ghosh et al., 1977a, Ghosh et al., 1977b).
Since the breed standard for the Jack Russell terrier state that they should have abnormally short limbs in relation to their body length, they were classified as being chondrodystrophic. Surprisingly most IVDs of the Jack Russell terriers fell in the lower half of the Pfirrmann grades irrespective of the dog's age, which marked a clear difference compared with the other breeds assigned to the chondrodystrophic category. This indicates that chondrodysplasia causing abnormally short limbs is not necessarily linked with IVD degeneration.

In veterinary medicine, low-field (0.2 Tesla) MRI is generally more available than high-field MRI. The relatively low resolution of T2-weighted, sagittal images from low-field MRI was a problem when viewing the intervertebral discs of miniature breeds, and we had to discard the MR images of 15 smaller breed dogs that had a poor image quality. The image resolution provided by the low-field MRI was sufficient to give clear images of IVDs in most dogs but as the clarity of the images decreased with decreasing IVD size, it may have been relatively inaccurate for small-breed dogs (the breeds ranged in size from Bullmastiff to miniature Dachshund). An additional potential problem is that the coil of the MRI covers a limited field of view, giving a brighter signal in the focus area of the magnetic field than in areas of the spine located further away from the coil. This is known as the "coil-effect" and can lead to falsely higher Pfirrmann grades for discs located away from the centre of the coil on T2-weighted MR images (Reicher et al., 1986, Seeger, 1989). T2-weighted MRI scans are used for Pfirrmann grading because they best depict the glycosaminoglycan and water content of the disc, which is negatively correlated with the extent of disc degeneration (Pearce et al., 1991, Benneker et al., 2005).

The Pfirrmann grading system focuses on characteristic changes in the structure of the disc itself (T2-weighted signal intensity, disc structure, nucleus and annulus distinction and disc height) and not on changes in the tissues surrounding the disc (endplate sclerosis, vertebral osteophytes and disc herniation). However, it is imperative to include these aspects to get a complete picture of the status of the disc. To accurately appreciate the extent of potential bulging, protrusion, or extrusion of the disc transverse MR images are needed in combination with the midsagittal images used in Pfirrmann grading. Besalti et al.(Besalti et al., 2006) have proposed grading thoracolumbar canine IVD degeneration on MR images based on four criteria: IVD degeneration, bulging of the disc, disc protrusion, and disc extrusion. These basic four criteria are also in line with the recommendations from a joint taskforce of the North American Spine-, Spine Radiology-, and Neuroradiology Societies (Fardon and Milette, 2001). Although Besalti developed this system further, the grading scheme has never been validated or compared against a gold standard. In humans, grading disc degeneration on MR images using the criteria normal, bulging, extrusion, or protrusion has only moderate interobserver agreement (Milette et al., 1999, Brant-Zawadzki et al., 1995). In order to evaluate the status of intervertebral discs in dogs correctly, the Pfirrmann grading system should be used in combination with information about disc herniation (if present), such as protrusion or extrusion, both of which cause stenosis of the spinal canal.

Conclusions

The inter- and intraobserver reliability of the Pfirrmann system used in dogs was almost perfect, with **κ** scores ranging between 0.81 and 0.93. Moreover, the extent of disc degeneration was significantly associated with breed predisposition to chondrodystrophy and with the animal's age. On the basis of these results, we conclude that the Pfirrmann grading system can be used to evaluate IVD degeneration in dogs.

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Chapter 5

Reliability of macroscopic grading of canine intervertebral disc degeneration according to Thompson and comparison with low-field magnetic resonance imaging findings

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Abstract

Objective: The aims of this study were 1) to evaluate the reliability of the Thompson grading system for gross pathological changes of intervertebral disc (IVD) degeneration in dogs, and 2) to investigate the agreement between the findings on gross pathology with imaging findings using low field (0.2 Tesla) magnetic resonance imaging (MRI).

Animals: Spinal columns from 19 dogs of different age, breed, and origin.

Procedure: In total 182 intervertebral segments were collected from 19 canine cadavers. Sagittal T2-weighted MRI of the lower spine was performed within 24 hours post mortem. The spines were subsequently divided in the mid-sagittal plane and high-resolution photographs were taken of each intervertebral segment (endplate-disc-endplate). The MR images and photographs were graded in a randomized and blinded fashion by four independent observers, using Pfirrmann and Thompson grading criteria, respectively.

Results: Cohen's weighted Kappa analysis showed that the interobserver agreement for Thompson scores ranged from 0.76 to 0.88 and that for intraobserver agreement from 0.88 to 0.94. The agreement (kappa) between Pfirrmann and Thompson grading was 0.70.

Conclusions and clinical relevance: The Thompson scheme can be used to grade canine IVD degeneration with a high inter- and intraobserver agreement and shows substantial agreement with low-field MRI findings graded with the Pfirrmann system. This suggests that low-field MRI can be used to diagnose IVD degeneration in dogs.

Introduction

Intervertebral disc degeneration (IVD degeneration) is common in dogs and humans and is often associated with severe back problems (Frymoyer and Cats-Baril, 1991, Maniadakis and Gray, 2000, Hoerlein, 1953, Gage, 1975). Diseases related to IVD degeneration, such as disc herniation, degenerative lumbosacral stenosis, and cervical spondylomyelopathy, are common reasons for euthanasia in dogs. In humans, back problems have a lifetime prevalence of 60-80%, and 5% of affected individuals are subsequently unable to work (Frymoyer and Cats-Baril, 1991, Freburger et al., 2009). The diagnosis of intervertebral disc disease must be based on physical and neurological examination findings, as well as history and imaging findings (Sharp and Wheeler, 2005). Advanced imaging modalities, such as magnetic resonance imaging (MRI) and computed tomography (CT), have long been used in human medicine and are increasingly available in veterinary practice, where they have greatly facilitated the diagnosis of IVD disease in dogs (Suwankong et al., 2006). In order to detect and treat signs of IVD disease earlier in the pathogenesis current diagnostic imaging techniques need to be refined further. To achieve this, an accurate and reliable macroscopic gold standard is needed.

It is important to distinguish between IVD degeneration and IVD disease. An IVD giving rise to clinical signs through disc herniation or spinal instability will inevitably be degenerated; however degenerated IVDs are common incidental findings in dogs without clinical signs of disease (Hoerlein, 1953, Hansen, 1952, Jones and Inzana, 2000, da Costa et al., 2006). In this article the expression “IVD degeneration” is applied to discs that are degenerated due to genetic or pathologic reasons as well as discs that have simply gone through age-related changes. The reason for this is the difficulty of separating these conditions from each other as well as the fact that age-related changes, so called senile remodelling, can in time led to a pathologic condition in the disc.

There are many similarities between canine and human IVD degeneration; they have similar clinical presentations, diagnostic methods, treatments, as well as similar loading patterns and macro- and microscopic appearances (Zimmerman et al., 1992, Bray and Burbidge, 1998b, Lotz, 2004, Hansen, 1951). In general, in chondrodystrophic humans and dogs as well as achondroplastic humans, IVD degeneration is of the chondroid type. However, in humans and dogs without these disorders, IVD degeneration is generally of the fibrous type (Hansen, 1952, Bray and Burbidge, 1998b, Hansen, 1951, An and Masuda, 2006, Singh et al., 2005, Bray and Burbidge, 1998a, Alini et al., 2008).

In human medicine, there are well-established systems for scoring the severity of disc

degeneration based on histology (Boos et al., 2002), radiography (Lane et al., 1993), MRI, and gross pathology. The most commonly used gold standard for IVD degeneration in humans is the 5-category grading scheme for gross pathological changes developed by Thompson (Thompson et al., 1990), although grading of disc degeneration on MR images according to Pfirrmann (Pfirrmann et al., 2001), developed for human lumbar intervertebral discs, is also used extensively (Griffith et al., 2007, Rajasekaran et al., 2008, Scuderi et al., 2008). Such IVD degeneration scoring systems are not generally used in veterinary medicine. There is one grading scheme proposed for MRI of canine lumbar discs, using histopathology as the gold standard (Seiler et al., 2003). The appearance of degenerated canine intervertebral discs on MRI and their gross morphology were described by Sether et al.(Sether et al., 1990), and disc protrusion on MRI and CT was correlated with surgical findings by Suwankong et al.(Sharp and Wheeler, 2005, Suwankong et al., 2006, Coates, 2000). The Thompson scheme has, to the authors' knowledge, not been used to grade canine IVD degeneration and there is no other scoring system available for evaluating the gross pathological changes of canine IVD degeneration.

The aims of this study were 1) to evaluate the reliability of the Thompson grading system for gross pathological changes of IVD degeneration in dogs, and 2) to investigate the agreement between pathology findings and imaging (low-field MRI) findings.

Materials and methods

Dogs

Nineteen cadavers of dogs of different breeds and ages were included in this study (Table 1). Dogs younger than 1 year of age were not included as they rarely show disc degeneration (Bray and Burbidge, 1998a). The dogs were either research dogs or patients from the Utrecht University Clinic for Companion Animals and had been euthanized for reasons unrelated to this study.

Collection and processing of the spines

The T11-S1 spinal segments from the 19 dogs were dissected within 24 hours post mortem at the Department of Pathobiology[1]. Five of the spinal segments were cut at T12-L1 instead of at T11, which resulted in a total of 182 intervertebral segments used for this study. The spinal segments were wrapped in moist towels and T2-weighted, sagittal MRI was performed within 1 hour of segment dissection, using a low-field (0.2 Tesla) open

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magnet[2] with a Multipurpose Flex Coil with 16 cm in diameter. The spines were surgically removed before imaging since the entire dogs could not be transported to the MRI facility; this could potentially lead to somewhat decreased MRI resolution as the coil diameter was
slightly larger than the diameter of the spines leading to decreased signal to noise ratio. The imaging protocol included sagittal T2-weighted spin echo (repetition time [TR] 3835-4450 msec/echo time [TE] 117 msec) images. The obtained MR slices were 3-mm thick and the slice which best depicted the intervertebral discs was selected for grading of IVD degeneration according to Pfirrmann et al.(Pfirrmann et al., 2001).

Table 1. Breed, age, body weight, and gender of 19 dogs from which the caudal spines were used to compare MRI and gross morphology of the intervertebral discs.

Dog	Breed	Age (y)	Weight (kg)	Gender
1	Mixed breed	15	14	Female
2	Mixed breed	1	22	Female
3	Beagle	2	11	Female
4	Beagle	2	11	Female
5	Beagle	2	12	Female
6	Mixed breed	1	20	Female
7	Foxhound	7	39	Female
8	Foxhound	10	44	Male
9	Foxhound	9	37	Female
10	Bouvier	7	42	Male
11	Retriever, Flatcoated	1	19	Female
12	Welsh Terrier	16	9	Male
13	Mixed breed	1	21	Female
14	Kerry Beagle	3	29	Male
15	Kerry Beagle	3	29	Male
16	Kerry Beagle	8	31	Male
17	Bouvier	11	44	Male
18	Beagle	9	12	Female
19	Beagle	10	10	Female

[2] Magnetom Open Viva, Siemens AG, Munich, Germany

After MRI, the spines were cut through the midline (sagittal plane) with a water-cooled belt saw and the midsagittal cut surfaces of the intervertebral segments were cleaned to remove debris. The spines were placed on a table with multiple lights illuminating the spine from different directions and high-resolution photographs were taken using a digital single lens reflex camera[3] equipped with a macro flash. A transparent ruler was placed on the cut surface as a size marker. The digital photographs were cropped using Microsoft Office picture manager[4] so that a single intervertebral segment was visible in each picture. This was done to prevent the observers who graded IVD degeneration from knowing which intervertebral segment it was and also to prevent comparison with adjacent intervertebral segments. The photographs were used for the gross pathology grading of IVD degeneration according to Thompson et al. (Thompson et al., 1990).

Pfirrmann grading of the MR images

The system for grading IVD degeneration in human lumbar discs on midsagittal MR images according to Pfirrmann (Pfirrmann et al., 2001) has been shown to be reliable for grading canine intervertebral discs (Bergknut et al., 2010). The grading system is based on MRI signal intensity, disc structure, disc height, and distinction between nucleus and annulus (Table 2).

Table 2. Description of the five categories of the MRI-based grading scheme according to Pfirrmann.

Grade	Structure	Distinction of NP and AF	Signal intensity	Width of intervertebral disc
I	Homogenous, bright white	Clear	Hyperintense, isointensive to CSF	Normal
II	Inhomogeneous with or without horizontal bands	Clear	Hyperintense, isointensive to CSF	Normal
III	Inhomogeneous, gray	Unclear	Intermediate	Normal to slightly decreased
IV	Inhomogeneous, gray to black	Lost	Intermediate to hypointensive	Normal to moderately decreased
V	Inhomogeneous, black	Lost	Hypointensive	Collapsed disc space

NP= nucleus pulposus; AF = annulus fibrosus; CSF=cerebrospinal fluid

[3] 10 megapixels, Nikon, Tokyo, Japan
[4] Microsoft Office picture manager, Redmond, USA

The MR images were converted to JPEG files and accessed via hyperlinks placed in Excel[5] to prevent identification of the images by the observers. Grading was performed on routine T2-weighted MR images, with Pfirrmann categories ranging from a healthy intervertebral disc (grade I) to end-stage intervertebral disc degeneration (grade V). Four blinded observers independently graded the MR images, namely, a veterinary student (EP), a PhD student (NB), a board certified veterinary surgeon (BM), and a board-certified veterinary radiologist (EA).

Thompson grading of the photographs

The grading scheme of Thompson et al. (Thompson et al., 1990) is a 5-category scheme for assessing the gross morphology of midsagittal sections of human lumbar intervertebral discs. Pathological changes of the nucleus pulposus, the annulus fibrosus, the end-plates, and periphery of the vertebral body (Table 3) were graded by the same veterinary student (EP), PhD student (NB), and board certified veterinary surgeon (BM) and additionally by a board-certified veterinary pathologist (GG). The photographs were presented to the observers randomly and in duplicate, via hyperlinks, in Excel.

Statistical analysis

The inter- and intraobserver reliability of the Thompson grading was statistically analyzed using Cohen's weighted Kappa analysis, which calculates the percentage of agreement among the grades given to a picture corrected by the chance that the same grade is given by chance. The weighted Kappa gives more weight to grades that deviate only one grade from one another and less weight to grades that differ by more. The interpretation of the Kappa values is slight (0-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) and almost perfect agreement (0.81-1.00) (Landis and Koch, 1977b, Landis and Koch, 1977a, Koch et al., 1977).

To calculate the interobserver reliability (the frequency of agreement between the four observers), the grades given by the observer with the highest intraobserver agreement (Kappa value 0.94, observer EP) was selected and compared with the grades given by the other three observers.

After evaluation of the inter and intraobserver reliability, the grades given by the four observers were averaged for every single IVD included in the study (both for the MR images and for the photographs) in order to enable analysis of the agreement between the Thompson scores and the degree of degeneration seen on MRI. In the cases where there was a deviation of more than one grade between the four observers, the image was re-reviewed in unison and a consensus decision was made. The degree of agreement between the averaged grades given to the photographs and the MR images of each

[5] Microsoft Office Professional edition, 2003, Redmond, USA

individual intervertebral segment were then investigated using Cohen's weighted Kappa analysis.

Table 3. Description the five categories of the macroscopic grading scheme for gross pathological changes of intervertebral discs according to Thompson.

Grade	Nucleus pulposus	Annulus fibrosus	End-plates	Vertebral bodies
I	Bulging gel	Discrete fibrous lamellas	Hyaline, uniform thickness	Rounded margins
II	White fibrous tissue peripherally	Mucinous material between lamellas	Irregular thickness	Pointed margins
III	Consolidated fibrous tissue	Extensive mucinous infiltration; loss of annular-nuclear demarcation	Focal defects in cartilage	Early chondrophytes or osteophytes at margins
IV	Horizontal (vertical) clefts parallel to end-plate	Focal disruptions	Fibrocartilage extending from subchondral bone; irregularity and focal sclerosis in subchondral bone	Osteophytes < 2 mm
V	Clefts extend through nucleus and annulus		Diffuse sclerosis	Osteophytes > 2 mm

Results

Descriptive section

The frequency of IVD degeneration on the MR images (Figure 1) and photographs (Figure 2) decreased in a similar pattern from grade I to V. In total, IVD degeneration was graded Pfirrmann grade I in 39.0% of intervertebral segments (71/182), grade II in 33.5% (61/182), grade III in 22.5% (41/182), grade IV in 3.3% (6/182), and grade V in 1.6% (3/182) on MR images. IVD degeneration was graded Thompson grade I in 33.5% of intervertebral segments (61/182), grade II in 40.7% (74/182), grade III in 14.8% (27/182), grade IV in 8.8% (16/182), and grade V in 2.2% (4/182) on photographs (Table 4).

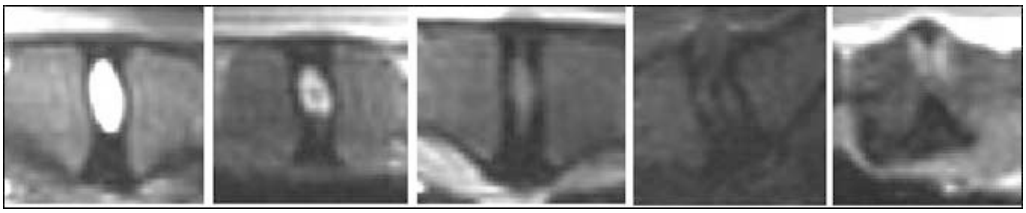

Fig 1. Midsagittal, T2-weighted MR images of canine intervertebral discs depicting the five different Pfirrmann grades. From left to right; Pfirrmann grade I, II, III, IV and V.

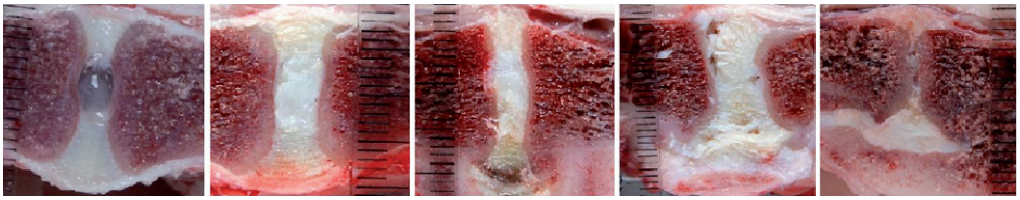

Fig 2. Midsagittal photographs of canine intervertebral discs depicting the different Thompson grades. From left to right; Thompson grade I, II, III, IV and V.

Table 4. Agreement between the mean Thompson and Pfirrmann grades for intervertebral discs from 19 dogs. The percentage shows the proportion of the intervertebral discs that received the same grade on MRI as on the gross morphology.

		Mean Pfirrmann grades					
	Grade	**I**	**II**	**III**	**IV**	**V**	**Total**
Mean Thompson grades	**I**	**50 (82%)**	11				61
	II	20	**39 (53%)**	15			74
	III	1	7	**18 (67%)**	1		27
	IV		4	7	**3 (19%)**	2	16
	V			1	2	**1 (25%)**	4
	Total	71	61	41	6	3	182

***Intraobserver reliability of the Thompson sche*me**

Intraobserver reliability was almost perfect for all observers (Table 5), with Kappa scores ranging from 0.94 ± 0.065 to 0.88 ± 0.068. In most cases (76-89%), both photographs of the same intervertebral segment received the same score. Discrepancies between the scores of duplicate photographs were generally a single grade only, although in two cases there was a discrepancy of two grades and in one case there was a difference of three grades (Table 5).

Interobserver reliability of the Thompson scheme

Interobserver reliability was almost perfect for 5 of 6 comparisons (Kappa values ranged from 0.83 ± 0.068 to 0.88 ± 0.064) and was substantial for 1 of 6 comparisons (Kappa 0.76 ± 0.068) (Table 6).

Table 5. The intraobserver reliability (κ-value and standard error (SE)) of four observers (Obs) using the Thompson scheme to grade intervertebral disc degeneration on 182 photographs (Ph) in duplicate. Agreement between observations is displayed in absolute numbers and (%).

Observers	Kappa grade and SE	Perfect agreement	1 Grade disagreement	2 Grades disagreement	3 Grades disagreement
Obs 1 Ph 1-2	0.94 ± 0.065	163 (89%)	20 (11%)	0 (0%)	0 (0%)
Obs 2 Ph 1-2	0.91 ± 0.064	155 (85%)	28 (15%)	0 (0%)	0 (0%)
Obs 3 Ph 1-2	0.88 ± 0.068	139 (76%)	41 (22.5%)	2 (1%)	1 (0.5%)
Obs 4 Ph 1-2	0.88 ± 0.064	152 (83%)	30 (16.5%)	1 (0.5%)	0 (0%)

Agreement between MRI and Thompson scheme

The agreement between mean Thompson scores and mean Pfirrmann scores was substantial (Kappa 0.70). The agreement between the Thompson and Pfirrmann scores was lower for the segments with IVD degeneration grades IV and V than for the segments with IVD degeneration grades I to III (Table 4); however, there were fewer segments with severe (grades IV and V) IVD degeneration than with less severe IVD degeneration (grades I–III).

Table 6. The interobserver reliability of observer (Obs) 2, 3, and 4 compared with observer 1 when applying the Thompson scheme to grade canine intervertebral disc degeneration on photographs (Ph) expressed in kappa value (κ) and standard error (SE). Photograph 1 is equal to the first grading round for each observer and photograph 2 is the second grading round, i.e. in the top left corner of the table, the agreement of the first grading round of observers 1 and 2 is displayed.

	Obs 2, Ph 1	**Obs 2, Ph 2**	**Obs 3, Ph 1**	**Obs 3, Ph 2**	**Obs 4, Ph 1**	**Obs 4, Ph 2**
Obs 1, Ph 1	0.88 ± 0.064		0.76 ± 0.068		0.83 ± 0.063	
Obs 1, Ph 2		0.86 ± 0.064		0.83 ± 0.068		0.84 ± 0.063

Discussion

Most diagnostic methods and treatments for companion animals applied in veterinary medicine are adapted from their human counterparts. However, this is not always possible because of anatomical or physiological differences between the species, and for this reason new diagnostic methods, treatments, or surgical techniques need to be validated in the intended species before being implemented in veterinary practice. In this study, we found the intraobserver and interobserver agreement for grading IVD degeneration with the Thompson scheme was almost perfect, showing that the system can be used to reliably grade, post mortem, IVD degeneration in dogs.

In order to develop treatments for dogs with IVD degeneration aimed at an earlier stage in the pathogenesis, new diagnostic imaging methods with a high sensitivity and specificity for detecting early signs of disease are needed. To develop and validate diagnostic methods for early IVD degeneration detection a reliable pathological-anatomical gold standard, such as the Thompson scheme, is needed. An adequate grading

scheme for IVD degeneration should be credible, reproducible, and able to distinguish between different stages of disease. Since the Thompson scheme fulfils these criteria and is the current gold standard in human IVD degeneration research, (Singh et al., 2009, Patel et al., 2007, Rutges et al., 2008) we chose this method to grade IVD degeneration in dogs. Other methods used to determine the degree of disc degeneration include histopathology and measurement of the glycosaminoglycan content of the nucleus (Bray and Burbidge, 1998a, Ghosh et al., 1977b, Ghosh et al., 1977a, Nguyen et al., 2008, Gruber et al., 2002). Although these methods have their advantages, they lack the simplicity and overview gained with the Thompson scheme.
Although the Thompson grading system worked well when used on canine intervertebral discs, we noted inter-species differences in spinal lesions and especially the relatively common appearance of ventral/anterior spondylosis in the dogs. In humans, spondylosis is generally associated with advanced stages of IVD degeneration (Adams and Roughley, 2006), whereas in the dogs spondylosis was also encountered in less affected and even in healthy intervertebral discs. This has previously been recognized in the veterinary literature (Seiler et al., 2003, Wright, 1980). Although we tried to prevent the presence of spondylosis in spinal segments with healthy or mildly degenerated IVDs from affecting grading, the presence of spondylosis could still subconsciously have biased the observers. According to the Thompson scheme, osteophytes should only be present in the higher degeneration grades and osteophytes larger than 2 mm should only be found in grade V discs. Hence the presence of spondylosis was only considered clinically relevant to IVD degeneration when present in severely degenerated IVDs. Spondylosis in dogs is associated with type II disc herniation rather than with type I herniation (Levine et al., 2006), supporting the hypothesis that fibrous metaplasia of the intervertebral discs in dogs resembles IVD degeneration in humans.
There were two cases with an intra observer discrepancy of 2 grades and a third case with an intra observer discrepancy of 3 grades, during Thompson grading. No explanation could be found for these individual cases and there was also no common feature distinguishing these three intervertebral segments from the rest. It is therefore not clear if these cases were clerical errors or if the observer indeed perceived them of being markedly different between the two grading rounds.

There are several factors that influence the grading of canine disc degeneration on MRI according to Pfirrmann. Firstly, the dogs were of different ages, shapes and sizes. Secondly, the resolution of the discs is markedly lower in small breeds (due to the smaller size of the IVDs, the image resolution is the same for small and large dogs) than in large breeds when using low field MRI (Cihangiroglu et al., 2004, Nijeholt et al., 2001). This could lead to inaccurate grades being given to intervertebral discs of small dogs. Thirdly, T2-weighted MRI scans are used since they best depict the glycosaminoglycan and water content of the disc, which in turn is correlated to the

degree of disc degeneration. This means that the brighter the nucleus appears on a T2-weighted MRI, the higher the glycosaminoglycan content is and the healthier the disc is (Pfirrmann et al., 2001, Ghosh et al., 1977b, Ghosh et al., 1977a, Adams and Roughley, 2006, Pearce et al., 1991). The so called "coil-effect" of the MRI causes a brighter signal in the focus area of the magnetic field than in the adjacent areas of the spine (Reicher et al., 1986, Seeger, 1989) which can lead to falsely low nucleus pulposus signals, and thus falsely high Pfirrmann scores. Moreover the spines were surgically removed before imaging due to logistical reasons. This will have resulted in somewhat higher resolution of the MR images compared to if the spines were imaged *in situ.*
The overall agreement between the mean Thompson scores and the mean Pfirrmann scores was substantial (κ 0.70). Most Thompson grade I and II discs were scored Pfirrmann grade I and II, and the deviation was not greater than one grade. However, for Thompson grade III, IV, and V discs, the Pfirrmann score deviated by up to two grades, and for Thompson grade IV and V less than 50% were given the same Pfirrmann grades. Since relatively few discs were graded Thompson IV (8.8%) and V (2.2%) and Pfirrmann grade IV (3.3%) and V (1.6%), the low frequency of occurrence may have contributed to the poor agreement between Thompson and Pfirrmann grades IV and V. Another factor that may have contributed to the low agreement is the intrinsic difference between the two grading systems, namely, that the Thompson system is based on changes seen in the nucleus, annulus, end-plate and vertebral body whereas the Pfirrmann system considers only changes in the nucleus and annulus. Future studies should focus on the agreement between MRI and macroscopic appearance on high grade degenerated IVDs to further bring clarity to this.

The high agreement between macroscopic changes in intervertebral discs (graded with the Thompson system) and low-field MRI findings (graded with the Pfirrmann system) suggests that, given the increasing availability of MRI in veterinary practice, MRI will become an increasingly useful tool to diagnose IVD degeneration in dogs in a clinical setting. However, as previously stated dogs commonly have degenerated IVDs without any clinical signs so the finding of degenerated IVDs on MRI will always have to be combined with the results of clinical and neurological examinations in order to reach a correct diagnosis.

Conclusions

This study demonstrates that the Thompson scheme is a reliable method for grading canine IVD degeneration with a high inter- and intraobserver agreement. Further, the agreement between macroscopic grading of intervertebral segments according to Thompson and grading of low-field MR images according to Pfirrmann was substantial,

which suggests that low-field MRI can be used to diagnose IVD disease in dogs. This is however more true for lower than high grade degenerated IVDs. It is also important to recognize that degenerated discs are frequently found in asymptomatic dogs and that finding of degenerated IVDs on MRI will always have to be combined with results of clinical and neurological examinations in order to reach a correct diagnosis.

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Chapter 6

The dog as an animal model for intervertebral disc degeneration?

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Abstract

Study design: Prospective observational and analytic study.
Objective: To investigate whether spontaneous intervertebral disc degeneration (IVDD) occurring in both chondrodystrophic (CD) and non-chondrodystrophic dogs (NCD) can be used as a valid translational model for human IVDD research.
Summary of background data: Different animal models are used in IVDD research, but in most of these models IVDD is induced manually or chemically, rather than occurring spontaneously.
Methods: 184 IVDs from 19 dogs of different breeds were used. The extent of IVDD was evaluated by macroscopic grading, histopathology, glycosaminoglycan (GAG) content, and matrix metalloproteinase 2 (MMP-2) activity. Canine data were compared to human IVD data acquired in this study or from the literature.
Results: Gross pathology of IVDD in both dog types (CD and NCD) and humans showed many similarities, but the cartilaginous endplates were significantly thicker and the subchondral cortices significantly thinner in humans than in dogs. Notochordal cells were still present in the IVDs of adult NCD, but were not seen in the CD breeds or in humans. Signs of degeneration were seen in young dogs of CD breeds (<1 year of age), whereas this was only seen in older dogs of NCD breeds (5-7 years of age). The relative GAG content and MMP-2 activity in canine IVDD were similar to those in humans: MMP-2 activity increased and GAG content decreased with increasing severity of IVDD.
Conclusions: IVDD is similar in humans and dogs. Both CD and NCD breeds may therefore serve as models of spontaneous IVDD for human research. However, as with all animal models, it is important to recognize inter-species differences, and indeed the intra-species differences between CD and NCD breeds (early versus late onset of IVDD, respectively) in order to develop an optimal canine model of human IVDD.

Key points:

- Spontaneous IVDD is common in dogs, and especially in chondrodystrophic breeds.
- The gross pathology and histology of IVDD showed many similarities in humans and dogs.
- MMP-2 activity is increased and GAG content is decreased with increasing severity of IVDD in both dogs and humans.
- The early/late onset of IVDD and early/late disappearance of notochordal cells in chondrodystrophic versus non-chondrodystrophic dogs provides two different canine models of spontaneous IVDD.

Introduction

Low back pain is a common disorder with a lifetime prevalence over 70% in the global population[1]. It is the main cause of lost workdays in the USA, with estimated direct medical costs of 12-25 billion USD annually[2,3]. Intervertebral disc degeneration (IVDD) and herniation are considered the main causes of acute and chronic low back pain[4-8]. Before new treatments for IVDD are tested in clinical trials, their safety and functionality should be extensively tested in *ex vivo* and *in vivo* animal studies. Different animal models have been used in IVDD research, [9-12] but in most of these models IVDD is induced manually or chemically, rather than occurring spontaneously. Animal models of spontaneous IVDD are the Sand rat[13-15], Pintail mouse[16], baboon,[17] and the dog[18-20]. Unlike mice and baboons, dogs commonly suffer from back pain due to IVDD, and are diagnosed and treated for this. Moreover, the clinical presentation, macroscopic and microscopic appearance, diagnostics, and treatment of IVDD are similar in humans and dogs[8,21-23]. Decompressive surgery and spinal fusion are common treatments for IVDD in both humans and dogs.

In the dog, herniation of the intervertebral disc (IVD) is the most common cause of neurological deficits,[24] and IVDD-related diseases are common reasons for euthanasia in dogs younger than 10 years[25]. The dog has frequently been used as a translational model for surgical procedures and biomechanical studies of the spine[26-31]. In most of these studies purpose-bred, research dogs have been used. However, the availability of veterinary IVDD patients as a study population for preclinical trials has not yet been utilized, although it is likely to be beneficial not only for humans but also for dogs as veterinary patients.

Dogs can be divided into chondrodystrophic (CD) and non-chondrodystrophic (NCD) breeds based on their physical appearance. In CD breeds, endochondral ossification of the long bones is disrupted, resulting in short, bow-shaped extremities. This trait is strongly linked with IVDD and has in the past been favored in selective breeding for some breeds[24,32], such as dachshunds, with short legs and a high prevalence of IVDD. The disease is reported to develop by 1 year of age in CD breeds[32]. However, NCD breeds, and especially large-breed dogs, also often develop IVDD-related diseases but then mostly later in life[24]. The main factors responsible for IVDD are considered to be trauma or “wear and tear” in NCD breeds, but genetic in CD breeds[24,32].

Aim of the study

To investigate whether spontaneous IVDD occurring in CD and NCD breeds can be used as valid translational models for human lumbar IVDD research, by comparing the morphological appearance, histological structure, and biochemical characteristics in different stages of IVDD in dogs and humans.

Materials and methods

Study population and processing of the spines

Lower spine segments from 19 dogs (of different breeds, ages, and sex) older than 1 year, without a history of IVDD-related diseases, that died or were euthanized for reasons unrelated to this study were dissected within 24 hours *post mortem*. The spinal units (endplate-disc-endplate) of the lower spines were isolated, resulting in 184 intervertebral segments (137 from NCD and 47 from CD), and cut through the sagittal midline. The midsagittal plane of each spinal unit was subsequently photographed with a high-resolution digital camera (10 megapixels, Nikon, Tokyo, Japan) for gross morphological grading according to Thompson et al.[33]. Thereafter 3-to 4-mm thick midsagittal slices were cut and stored in 4% neutral-buffered formalin for histopathological examination. The remaining nucleus pulposus (NP) material was snap frozen (in 123 of the 184 IVDs) for glycosaminoglycan (GAG) analysis and matrix metalloproteinase 2 (MMP-2) zymography.

Twenty-five histological sections (5 per Thompson grade) and 20 photographs (randomly selected) of adult human lumbar IVDs, collected with permission of the Medical Ethics Committee, were obtained from the Biobank, Department of Pathology, University Medical Centre Utrecht (UMC-U).

Normal anatomy and gross pathology

All photographs were assessed by three independent observers (NB, JR, and BM) and graded I-V using the criteria of Thompson et al.[34], which have been validated for use in dogs[33]. The results were compared between human and canine IVDs, as well as between CD and NCD.

The midsagittal photographs of all Thompson grade I canine and human lumbar IVDs were used to measure the width (anterior-posterior), height (superior-inferior), and area of the midsagittal surface of the IVD and NP. All measurements were obtained using the software Image J (National Institutes of Health, Bethesda, MD). In the photographs of the canine IVDs, but not in those of the human IVDs, a transparent ruler was included to enable calculation of the dimensions. The dimensions (mm) of human lumbar IVDs were obtained from the literature[35-37]. Ratios were calculated for IVD height/width, midsagittal NP area/IVD area, and NP width/IVD width for all Thompson grade I IVDs.

Histopathology

The midsagittal, intervertebral segments were fixed in 4% neutral-buffered formalin and decalcified in EDTA. After decalcification, all discs were embedded in paraffin. With a microtome 5-µm sections were cut, deparaffinized, and stained with Hematoxylin/Eosin or Alcian blue/Picrosirius red[38].

Thirty-five canine samples were used for histological evaluation: seven samples of each Thompson grade were randomly selected (from CD and NCD pooled) and evaluated by three independent observers (NB, JR, GG), using the modified Boos grading scheme recently described for use in dogs[39]. The three observers also examined 25 human IVD sections (5 per Thompson grade) for comparison.
The height of the entire IVD (superior-inferior) including endplates and the thickness and number of cell layers of each endplate were measured on histological sections of canine and human IVDs graded Thompson I.

Glycosaminoglycan assay
The sulfated GAG content of 123 canine NP samples was measured using the Farndale (Dimethylmethylene Blue) assay[40]. The relationship between GAG content and severity of degeneration in dogs was compared with that previously reported for humans[41,42].

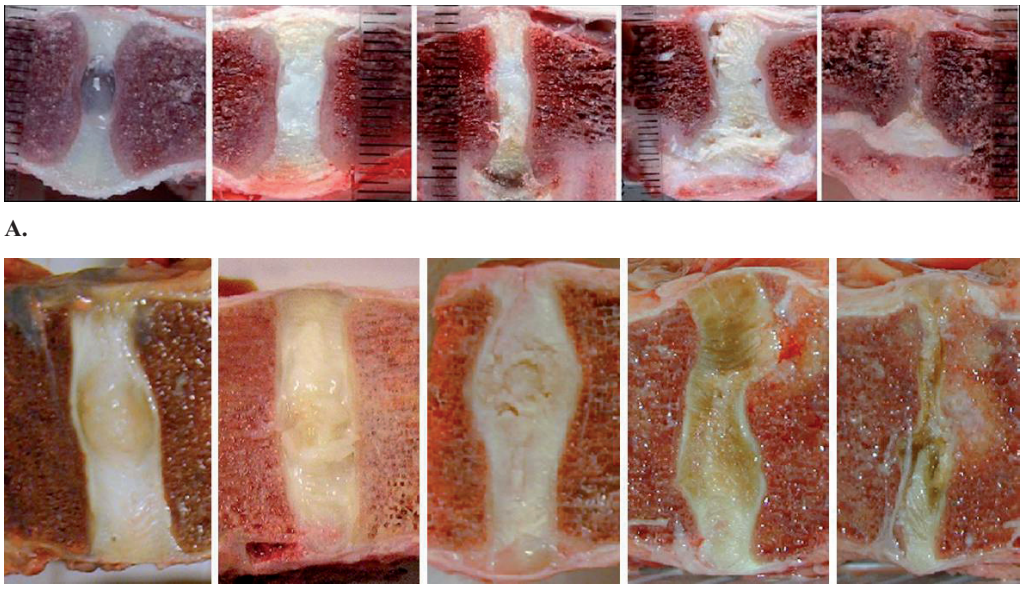
MMP-2 zymography
MMP-2 activity was assayed by gelatin zymography, as described previously[43]. The protein content of the NP samples was measured[44] to standardize the amount of tissue extract loaded onto precast gels (Bio-rad Laboratories Hercules, CA) for the measurement of MMP-2 activity. High-resolution pictures were taken of the gels, in which the activity of the MMP-2 enzyme was shown as a clear band against a darker background. Enzyme activity was assessed by evaluating the amount of gel degraded by each individual NP sample[45]. The background staining of each gel was used as baseline, and the relative destaining of the individual bands were calculated using the Quantity One software (Bio-rad Laboratories Hercules, CA). MMP-2 activity was calculated and grouped per Thompson grade in the same way as for human IVD samples in a previous study[43].

Statistical analysis
Differences between dogs and humans in the ratios IVD height/width, NP area/IVD area and NP width/IVD width of Thompson grade I IVDs were analyzed by means of an independent sample T-test. Normal distribution was verified through Q-Q plots. Pearson correlation test was used to evaluate the correlation between IVD dimensions and the weight/size of the dogs and between GAG content and Thompson grades. A Mann-Whitney U test was used to test for the association between the type of dog and Thompson grades. Differences in GAG concentrations and MMP-2 activity between the five Thompson grades were analyzed by Kruskal–Wallis non-parametric 1-way analysis of variance (ANOVA) with Bonferroni correction. Statistical significance was set at $P < 0.05$.

Results

Normal anatomy and gross pathology

The overall appearance of the IVDs from humans and dogs was similar, with healthy IVDs from both species consisting of an annulus fibrosus (AF) with a clear lamellar structure and a gelatinous central NP. All five grades of IVDD described in humans by Thompson et al.[34] were also seen in dogs (Fig. 1).



A.

B.

Fig 1. Midsagittal images of A) canine intervertebral discs and B) human intervertebral discs depicting, from left to right, Thompson grade I, II, III, IV, and V.

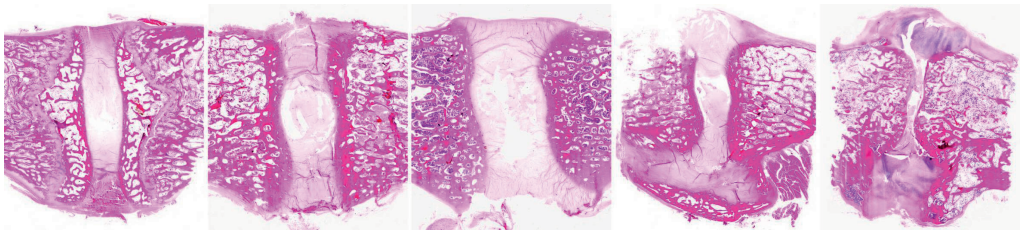
However, growth plates were found in the intervertebral segments of the growing dogs (1 year old), which is not the case in growing humans[46]. Dogs normally have 7 lumbar vertebrae in comparison with 5 in humans. The cartilaginous endplates were thicker in human IVDs, and subsequently more pronounced endplate irregularities were found in humans with increasing severity of IVDD. In both dogs and humans, radial clefts and fissures in the NP parallel to the endplate, were first detected in Thompson grade III IVDD, whereas more extensive clefts and fissures, transecting the AF, were seen first in Thompson grade IV IVDs in both humans and dogs.

Although the canine IVDs were smaller than the human IVDs, the ratio of NP area/IVD area was similar in the two species (P=0.18) (Fig. 1, Table 1); however, the ratios IVD

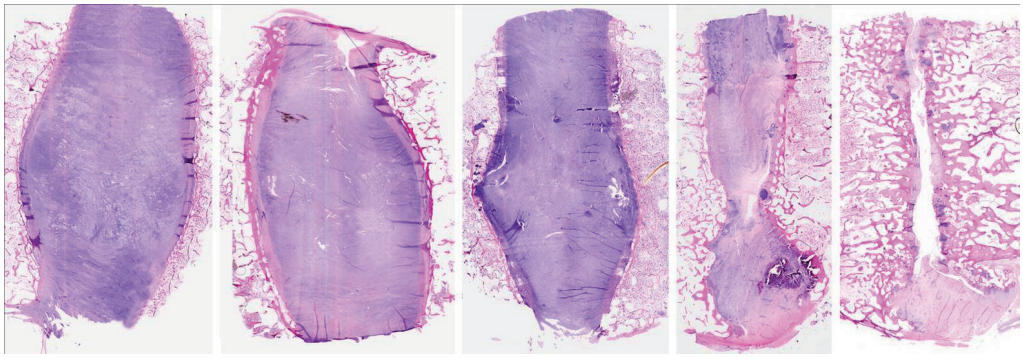
height/width, NP/IVD width, and endplate thickness/IVD height were significantly different in healthy humans and dogs. As in humans, the height of the canine lumbosacral IVD (5.4 ± 1.1 mm) was greater than that of the lumbar IVDs (3.5 ± 0.6 mm).
IVD height at all spinal levels was significantly correlated with weight in dogs (r = 0.8, P = 0.01), as was IVD width and weight (r = 0.6, P = 0.01). The CD and NCD breed dogs were of comparable age (5.2 years for CD dogs and 5.5 years for NCD dogs). Higher Thompson grades (more degenerated IVDs) were seen more often in CD dogs than in NCD dogs (Z = -3.6, P = 0.0001), (Table 3). There were too few human samples to allow analysis; however, the distribution of Thompson grades in the human IVDs were more similar to that of NCD dogs than to that of CD dogs (Table 3).

Histopathology

The overall histological appearance of the IVDs in different stages of degeneration was similar in humans and dogs (Fig 2): the AF and NP had a similar appearance, and the cell populations and density were comparable, with fibrocyte-like cells in the AF and chondrocyte-like cells in the endplate and NP.



A.



B.

Fig 2. Midsagittal, hematoxylin/eosin-stained histological sections of A) canine intervertebral discs and B) human intervertebral discs, depicting the histological appearance of, from left to right, Thompson grade I, II, III, IV, and V.

Notochordal cells were present in the NP of 7/7 canine grade I IVDs and in 2/7 grade II IVDs. All IVDs containing notochordal cells were from NCD. Notochordal cells were not found in any IVDs from CD dogs or humans.
In both humans and dogs, increasing Thompson grade was accompanied by degeneration of the NP with increasing cell cluster size, increasing disorganization of the AF lamellae, and increasing appearance of clefts and cracks in the IVD. However, the endplates had more chondrocyte layers in human IVDs than in canine IVDs, whereas subchondral bony cortices were found to be thicker relative to the total IVD height and endplate thickness in dogs than in humans. The absolute thickness of the subchondral bony cortices was comparable in the two species (Table 1).

Table 1. Measurements and dimensions of the mid-sagittal surface of canine and human lumbar intervertebral discs (IVDs) as determined by gross- and histopathological examinations. The P-values reflect significant differences between the values obtained in dogs and humans. NP = Nucleus pulposus, EP = Endplate, SD = Standard Deviation. N/A = Not available

Measurement	**Dog**		**Human**		
	Mean ± SD	Range	Mean ± SD	Range	P-value
Gross pathology					
Height IVD (mm)	3.5 ± 0.6	2.4-4.7	10 §	6-14 §	**N/A**
Width IVD (mm)	15.9 ± 2.0	11.0-18.9	35 §	27-45 §	**N/A**
Ratio IVD height/width	0.22 ± 0.03	0.14-0.28	0.29 ± 0.05	0.23-0.34	*<0.01***
Ratio NP/ IVD width	0.30 ± 0.05	0.21-0.41	0.38 ± 0.05	0.31-0.44	*<0.01***
Ratio NP/ IVD area	0.25 ± 0.05	0.17-0.34	0.28 ± 0.02	0.24-0.31	*0.18*
Histology					
Thickness EP (mm)	0.22 ± 0.06	0.1-0.42	1.58 ± 0.35	1.25-2.51	*<0.01***
EP thickness/ IVD height (%)	6%	3-11%	13%	9-19%	*<0.01***
Number of cell layers in EP	5	3-8	21	18-23	*<0.01***
Thickness cortex (mm)	0.90 ± 0.36	0.27-1.78	0.66 ± 0.33	0.25-1.59	*0.06*

§ Measurements obtained from the literature[35-37].
** Statistically significant at $P < 0.01$.

The pattern of Alcian blue/Picosirius red staining (staining GAG and collagen I, respectively) was similar in human and canine IVDs: Thompson grades I and II IVDs showed predominantly blue staining of the NP, whereas the NP of IVDs of grade III or higher IVDs was stained red and blue or predominantly red.

Glycosaminoglycan assay

As only 1 of the 123 canine IVD samples used for the GAG and MMP-2 analyses was Thompson grade V, it was grouped with the Thompson grade IV IVDs for statistical analysis (Thompson IV+V). In dogs, the mean GAG concentrations in the NP (wet weight) were negatively correlated with increasing Thompson grades ($r = -0.84$, $p = 0.0001$), (Fig. 3), as has been reported in humans [41,42].

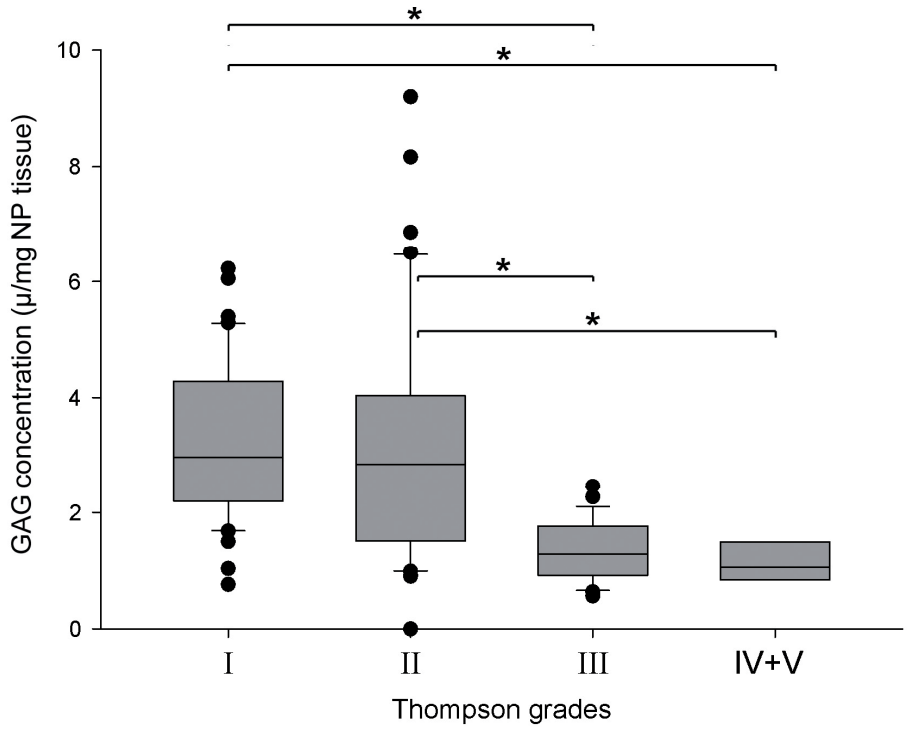


Fig 3. Box-plot displaying the glycosaminoglycan (GAG) concentration in the nucleus pulposus of canine intervertebral discs in relation to the Thompson grade. * $P < 0.05$, ● = outliers.

MMP-2 zymography

The mean relative activity of MMP-2 in canine IVDs increased significantly with increasing Thompson grade over grades I to III, but decreased in the group Thompson IV+V (Fig. 4). A similar pattern has been reported in humans[43].

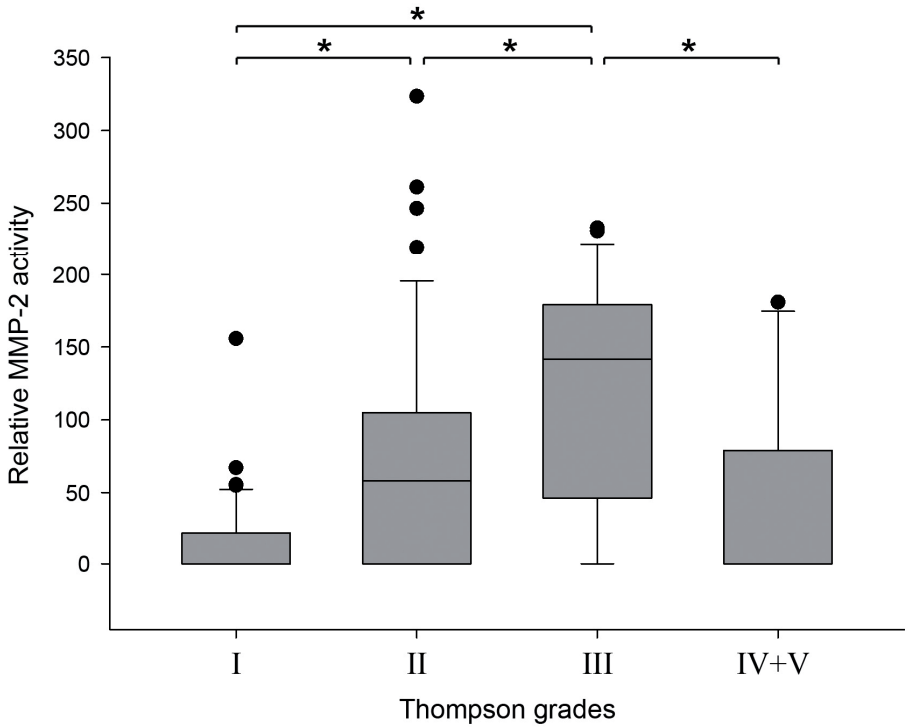


Fig 4. Box-plot displaying the MMP-2 activity in the nucleus pulposus of canine intervertebral discs in relation to the Thompson grade. * P <0.05, ● = outliers.

Discussion

We found that the gross pathology, histopathology, GAG content, and MMP-2 activity of human and canine IVDs were similar in all different stages of IVDD. In addition, dogs are the only animals that develop IVDD-related diseases that are diagnosed and treated, both medically and surgically, in the same way as in humans. Combined, these facts indicate that canine IVDD could prove a suitable model of spontaneously occurring IVDD for human research.

This study also shows the common occurrence of asymptomatic degenerated IVDs in dogs, just as in humans [47] and in general similar pathological changes were seen in degenerated IVDs from humans and dogs. Even the ratios of the different dimensions of the IVDs were similar in dogs and humans, although the canine IVDs were generally smaller relative to weight. Some differences were however found, such as the absence of growth plates in growing human vertebrae and the thicker cartilaginous endplates in humans. Whereas in dogs most vertebral growth takes place in the growth plates, in humans vertebral growth takes place in the junction between the vertebrae and the endplates[46], which may explain the thicker endplates found in humans.

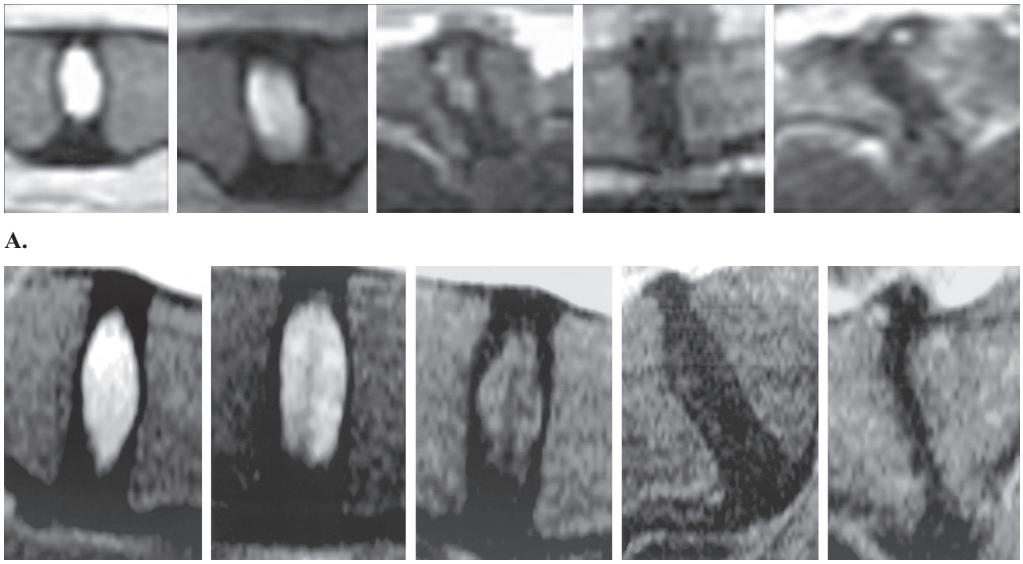
Also at the histological level, comparisons between dogs and humans revealed similar pathological changes due to degeneration. The hallmarks of IVDD, including chondroid cell clusters, disorganization of the AF, and increasing appearance of clefts and cracks, were found in dogs and humans with increasing severity of IVDD. Notochordal cells were found in the healthy IVDs of adult NCD dogs but not in the IVDs of adult humans or CD dogs; notochordal cells are present in the NP in children and CD dogs younger than 1 year[32,48]. Apart from this discrepancy, the histological changes within each Thompson grade were similar in NCD and CD dog breeds and in humans, suggesting similar pathological processes.

Also at the biochemical level the changes occurring during IVDD were similar. In humans, MMP-2 activity is positively correlated with increasing Thompson grade up to grade IV and decreases slightly in grade V[43]. We found a similar trend in dogs, with the exception that MMP-2 activity was already reduced in grade IV+V degeneration. The GAG content in canine IVDs was negatively correlated with increasing Thompson grades, as previously described for human IVDD [41,42]. Previous studies have shown that the GAG content of IVDs from CD dogs is lower than that of IVDs from NCD dogs of similar age [49,50]. This is consistent with IVDD occurring at a lower age in CD than in NCD dogs.

A previous study has also demonstrated the striking similarities between the MRI appearance of IVDD in the different stages of degeneration in humans and dogs, even enabling the use of the human MRI grading system for lumbar IVDD [51] in veterinary practice [52] (Fig. 5). Pfirrmann grading of canine lumbar IVDD is strongly correlated with Thompson grades[33]. In humans, degeneration of the IVD on T2-weighted MRI is negatively correlated with the GAG content [53]. Our results and those of previous canine studies[33,52,54] indicate a similar trend in dogs, supporting the use of the dog as a translational model for studies of IVDD in humans.

The fact that dogs walk on four legs and humans on two is often raised as a reason to not use dogs as models for human IVDD since it is believed that humans have a higher axial loading on the spinal segments due to gravity. However, the axial loading patterns of human and canine IVDs have been shown to be comparable or even higher in dogs [30,55,56].

The gross morphological and histological appearances of IVDs in different stages of degeneration was similar in CD and NCD dogs, but IVDD manifested much earlier in CD dogs, as reported previously[24,32,57]. All IVDs from adult CD dogs showed histological signs of degeneration, regardless of the dog's age, whereas similar signs were found in only 54.7% of the NCD dogs and most often in older dogs. These findings support the theory that the etiology of IVDD is different in the two groups of dogs, which could thus make two different IVDD models, early (CD dogs) and late (NCD dogs) onset of IVDD.



A.

B.

Fig 5. T2-weighted, midsagittal magnetic resonance images of A) canine intervertebral discs and B) human intervertebral discs depicting, from left to right, Pfirrmann grade I, II, III, IV, and V. Figure 5B is reproduced with permission; *Pfirrmann et al. Magnetic resonance classification of lumbar intervertebral disc degeneration, Spine 2001.*

The IVDD commonly seen in CD dogs is believed to be of genetic origin, as all IVDs show signs of degeneration from an early age. In contrast, the IVDD seen in NCD dogs is more likely to be caused by "wear and tear", as in most humans, [22,24] although a genetic influence has also been suggested in some NCD breeds. This is supported by the higher correlation of age with IVDD in older NCD dogs and by the higher prevalence of IVDD in working dogs, and in the lumbosacral IVD, which is subjected to higher mechanical loads [58,59]. Although IVDD in CD and NCD breeds appears to have different

etiologies, the pathology is similar. Early versus late onset of clinical IVDD in CD and NCD breeds, respectively, enables longitudinal case-control studies of spontaneously occurring IVDD in canine veterinary patients.

Relevant animal models are needed to improve the treatment of IVDD. As no animal model can perfectly mimic the complex processes of IVDD in humans,[60] the similarities and differences between humans and animals should be considered when using animal models. Animal models of induced IVDD can result in substantial and reproducible IVDD in a short time, but it is likely that the pathological pathways differ from those involved in spontaneous IVDD, and thus the extrapolation of data from induced animal models to humans could lead to erroneous conclusions.
This study has shown that the many similarities between canine and human IVDD could make the dog a suitable animal model of human IVDD. In addition, canine IVD material for research (*ex vivo*) is substantially easier to obtain than human. Another advantage with using dogs as an animal model is the potential of using veterinary IVDD patients as a study population for the investigation of mechanisms of degeneration and potential new treatments. This would reduce the use of laboratory animals as models of human disease and may also lead to better treatments for canine patients. These facts together with our findings suggest that the dog is one of the most appropriate animal models for spontaneous IVDD.

Conclusions

There are many similarities between IVDD in humans and in CD and NCD breeds, and both types of dog breeds could serve as animal models of spontaneous IVDD for human research. However, when employing the dog as a model for human IVDD research it is important to recognize the specific inter-species differences as well as the difference of IVDD between CD and NCD dogs (early versus late onset, respectively).

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Chapter 7

The performance of a hydrogel nucleus pulposus prosthesis in an ex vivo canine model

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Abstract

A nucleus pulposus prosthesis (NPP) made of the hydrogel N-vinyl-2-pyrrolidinone copolymerized with 2-(4′-iodobenzoyl)-oxo-ethyl methacrylate has recently been developed. The special features of this NPP, i.e. intrinsic radiopacity and its ability to swell *in situ* to fill the nucleus cavity and restore disc height, were investigated *ex-vivo* in canine spinal specimens. L7-S1 intervertebral discs were isolated from three canine spinal specimens, and the dimensions of the nuclei pulposi were measured. Based on these averaged measurements, the NPP prototype was made and inserted in its dry form (xerogel) into a canine cadaveric spinal segment and allowed to swell overnight at 38 ºC. The integrity of the NPP and the filling of the nucleus cavity were assessed before and after swelling, using radiography, computed tomography, and magnetic resonance imaging. The ability of the NPP to restore disc height was assessed on radiographs of 10 spinal specimens. Thereafter the NPP was macroscopically assessed *in situ* by dissection of the spinal specimen.
Both on imaging and macroscopically, 9/10 NPPs appeared to have a near perfect fit and disc height was restored in 8/10 spinal segments. The NPP may thus be an acceptable treatment option for low back patients meeting the requirements for NPP treatment.

Introduction

Low back pain (LBP) is a major health problem in the Western world, (Frymoyer and Cats-Baril, 1991, Maniadakis and Gray, 2000) and its incidence has increased dramatically over the last two decades (Freburger et al., 2009). The most common cause of back pain is believed to be intervertebral disc (IVD) degeneration (Cheung et al., 2009, Adams, 2004). IVD degeneration can apart from discogenic pain also lead to spinal instability and stenosis of the spinal canal causing severe clinical symptoms. Most patients suffering from IVD degeneration responds well to conservative and medical treatment. Surgical treatment is preserved for patients refractory to medical treatment. The most common surgical treatment of these patients is decompressive surgery combined with spinal fusion. The primary aim of this procedure is to reduce pain. It does however leave the patients with altered biomechanical properties of the spine which can lead to adjacent segment degeneration (Min et al., 2008, Yang et al., 2008). For patients with IVD degeneration at a single level, without spinal canal stenosis, total disc replacement can be a treatment option. Total disc replacement, contrary to spinal fusion, preserves near normal biomechanical functionality of the spinal segment. However, severe complications such as implant migration and failure may occur (van Ooij et al., 2007, Resnick and Watters, 2007).

New treatments aim at being minimally invasive and at intervening earlier in the degenerative process by restoring the nucleus pulposus (NP) function and/or supporting regeneration of the IVD. Regenerative treatments have been investigated but in experimental settings only (Hiyama et al., 2008, Ganey et al., 2003, Masuda et al., 2006), and although minimally invasive surgical procedures coagulating the NP or the posterior annulus are currently used, they are considered controversial (Raj, 2008, Felder-Puig et al., 2009). One drawback is that they reduce IVD height and do not restore the normal biomechanical function of the NP, leading to further degeneration of the spinal segment (Putzier et al., 2005). Another treatment option is implantation of a NP prosthesis (NPP) after nucleotomy of the degenerated disc, but this procedure requires that the annulus fibrosus is intact. The Prosthetic Disc Nucleus (PDN)[1], which is commercially available, has been implanted in over 5500 human patients worldwide (Coric and Mummaneni, 2008, Shim et al., 2003). Criteria for the use of any NPP are: 1) disc degeneration manifest by morphologic changes in the NP, 2) a competent annulus fibrosus, and 3) a minimal IVD height of 5 mm (Boelen et al., 2006, Guyer and Ohnmeiss, 2003). These requirements mean that the implant is suitable for only a small group of patients with low back pain, but in these patients implantation will restore disc height and mobility of the spinal segment, thereby relieving pain and may also prevent

[1] Raymedica, Inc., Bloomington, MN

further degeneration of the spinal segment (Meakin, 2001, Meakin et al., 2001, Di Martino et al., 2005, Berlemann and Schwarzenbach, 2009)as well as adjacent segment degeneration, which is a common problem after spinal fusion (Min et al., 2008, Yang et al., 2008). Although preclinical and preliminary clinical data show promising results (Jin et al., 2003, Coric and Mummaneni, 2008, Shim et al., 2003), long term clinical studies are still lacking.

However, most commercial NPPs are not designed to completely fill the nuclear cavity. Complete filling of the nuclear cavity and even distribution of the load over the endplates can halt further degeneration of the annulus fibrosus, whereas incomplete filling is likely to lead to incorrect biomechanical loading and to progression of annular degeneration or even implant migration (Meakin, 2001, Meakin et al., 2001, Di Martino et al., 2005). Boelen *et al.* proposed applying custom-made synthetic hydrogel NPP implants designed to precisely fill the nuclear cavity (Boelen et al., 2007). The NPP is made of a radiopaque synthetic hydrogel biomaterial in its water-free state, taking into account the increase in size due to three-dimensional swelling of the material *in situ*. The NPP is implanted in its dry state, so that only a small annular opening is needed, and then absorbs fluid from the surrounding tissue and swells to fill the nucleotomy cavity and thus restore disc height. The radiopacity of the implant enables imaging by radiography or computed tomography (CT), and the absorbed fluid makes magnetic resonance imaging (MRI) possible.

The aim of this study was to test this NPP *ex-vivo* in canine lumbosacral segments (L7-S1). A clinically adapted mode of implantation of the NPP in the nuclear cavity of the L7-S1 intervertebral disc is reported. Swelling, fit, and restoration of disc height of the NPP *in situ* were monitored by radiography, CT, and MR imaging.

Materials and Methods

Manufacturing of the nucleus pulpous prosthesis

The NPPs were made in a bean-shaped form, to copy the shape of the normal NP. The biomaterial is a chemically cross-linked hydrogel that is synthesized through a free-radical polymerization reaction from four reactive vinyl-type monomers: N-vinyl-2-pyrrolidinone (NVP), 2-hydroxyethyl methacrylate (HEMA), 2-(4′-iodobenzoyl)-oxo-ethyl methacrylate (4-IEMA), and allylmethacrylate (AMA). NVP and HEMA are responsible for the hydrophilic nature of the material, 4-IEMA provides intrinsic radiopacity to the NPPs as the material contains covalently linked iodine, and AMA results in chemical cross-linking (Boelen et al., 2006). The NPPs were manufactured in

two steps: (i) polymerization and (ii) computer-controlled machining of the hydrogel in its dry state.

All chemicals were purchased from Acros[2]. NVP and HEMA were distilled under reduced pressure to remove inhibitory additives. The monomer 4-IEMA was synthesized from HEMA and 4-iodobenzoylchloride as described previously. AMA and 2,2´-Azobis-(isobutyronitrile) (AIBN) were used as received. NVP, HEMA, 4IEMA, and AMA were weighed into a 500-mL round-bottom flask in the molar ratio 71.8 : 20.4 : 5.8 : 2. The total mass was about 200 g. AIBN (0.03 mole %) was added and completely dissolved in the monomer mixture. The reaction mixture was divided over a number of Teflon tubes (inner diameter 15 mm, wall thickness 1 mm, length 300 mm), which were closed with a stopper at one end. The tubes were filled to maximally 60%. The monomer-filled tubes were partially immersed in a thermostated oil bath and a computer-controlled time/temperature profile was run as follows: (i), constant temperature at 40 ºC for 1 h; (ii) slow heating to 50 ºC over 1 h; (iii), constant temperature at 50 ºC for 6 h; (iv), slow heating to 60ºC over 1h; (v) constant temperature at 60 ºC for 6 h; (vi) slow heating to 80 ºC over 1 h; (vii), constant temperature at 80 ºC for 4 h; (viii), slow heating to 100 ºC over 1 h; (ix), constant temperature at 100 ºC for 4 h; (x) slow heating to 130 ºC over 1 h; (xi), constant temperature at 130 ºC for 2 h; (xii) slow cooling to ambient temperature (over 8 h). The procedure yielded transparent, glassy rods, which were removed from the Teflon tubes.

NPPs were machined from the polymer rods, using a five-axes computer-controlled lathe/milling system. As the material is a hydrogel, cooling with water had to be avoided, which meant that the machining process was performed slowly.

Determining the size of the implants

Four lumbosacral spines were isolated from the cadavers of healthy mixed-breed dogs (weight range 22.9-24.0 kg; age range 15-22 months) euthanized in an unrelated experiment. Three spines were used to determine the dimensions of the implants and the fourth spine was used for the implantation of the NPP (see below). MRI was performed using a 0.2 T open magnet[3]. For optimal resolution of the IVDs, a mixed signal sequence was used (DESS - Dual Echo Steady State) with a repetition time of 41 ms, an echo time of 12 ms, and a slice thickness of 1.194 mm. The MR images were obtained and assessed on standard computer screens using the software Web1000 5.1[4]. MRI showed the L7-S1 IVDs to be normal. The IVDs were then isolated by careful dissection for measurement of the width, height, and thickness of the NPs with a Vernier caliper

[2] Acros, Landsmeer, The Netherlands

[3] Magnetom Open Viva, Siemens AG, Utrecht, the Netherlands

[4] Clinical Review Station, Agfa Gevaert N.V.

(accuracy 0.05 mm). The NPPs were made on the basis of the mean dimensions of these three lumbosacral IVDs. Based upon our experience during pilot studies, the implants were made 20% smaller to compensate for residual NP material after nucleotomy. A swelling factor of 1.3 in each dimension was taken into account when making the implants, representing a volume swelling factor of 2.2 (1.3^3) (Boelen et al., 2006, Boelen et al., 2007). The custom-made NPPs measured (width x height x thickness) 10.4 x 4.5 x 2.4 mm in the unswollen state, and 13.5 x 5.9 x 3.1 mm in the swollen state (Fig. 1).

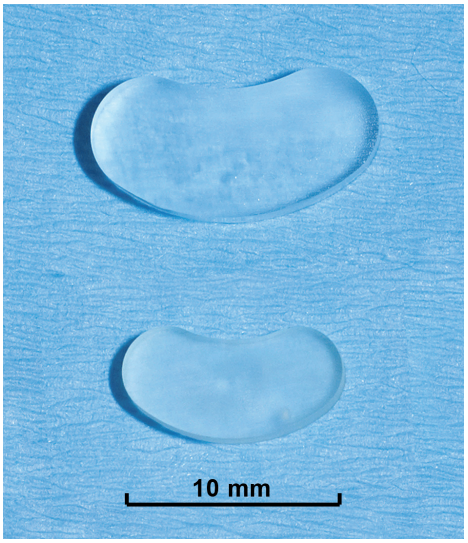


Fig 1. The nucleus pulposus prosthesis: dry state/xerogel (bottom) and hydrated/swollen (top).

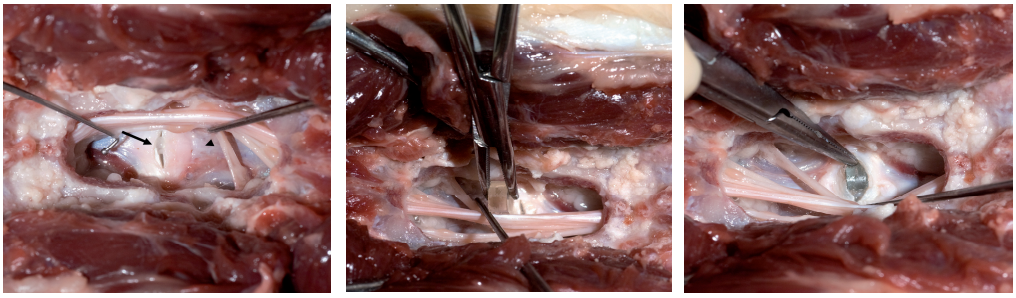
Surgical implantation and imaging of the NPP in situ

Implantation of the custom-made NPP was studied using the spine from the fourth healthy mixed-breed dog. A dorsal (posterior approach) laminectomy was performed over the lumbosacral region of the cadaveric spinal segment, followed by nucleotomy of the L7-S1 disc. The NPP was inserted into the nuclear cavity through a 5-mm transverse incision in the dorsal (posterior) annulus fibrosus (Fig. 2), and 5 mL phosphate-buffered saline (PBS) was subsequently injected into the cavity and surrounding tissue to initiate implant hydration (Boelen et al., 2006, Boelen et al., 2007) .

The annular incision was closed with a mattress suture (Polydioxanone 2-0[5]) and reinforced with tissue glue (Dermabond[5]). Lastly, a piece of polypropylene mesh[5] (1.0 x 1.5 cm) was glued over the annular defect. The spinal segment was wrapped in PBS-soaked gauges and placed in a plastic bag to prevent implant and specimen dehydration. To achieve complete swelling of the implant, the spinal segment was incubated at 38°C

[5] Johnson & Johnson, New Jersey, USA

(i.e., the normal canine body temperature) for 18 h. Imaging was performed using digital radiography[6], CT[7], and MRI[3] to monitor the positioning, swelling, and fit of the NPP *in situ*.



A. **B.** **C.**

Fig. 2 A) Dorsal view of the canine L7-S1 intervertebral disc (IVD) prepared for implantation of a nucleus pulposus prosthesis (NPP). Access to the IVD is gained via dorsal laminectomy followed by transverse incision of the dorsal annulus fibrosus (arrow). The gelatinous nucleus pulposus is removed. The cauda equina is gently retracted (arrow head). B) The NPP is carefully inserted through the incision in the dorsal annulus fibrosus. C) The NPP just before being pushed beyond the annulus fibrosus into the nuclear cavity.

A.

B.

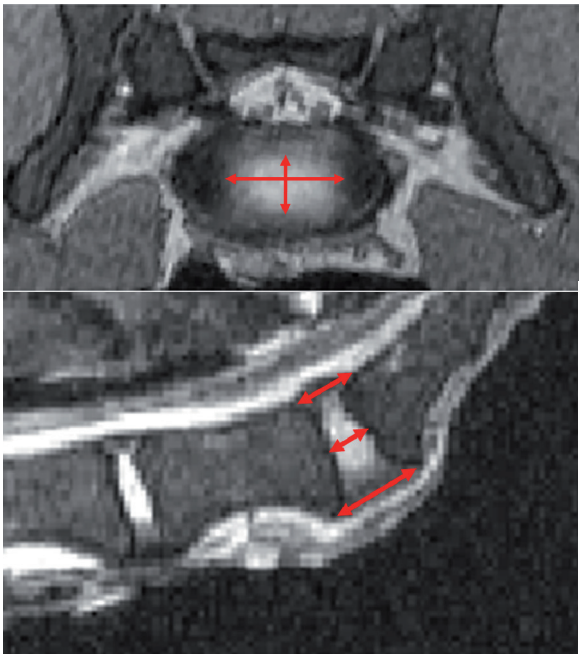


Fig. 3 **A)** Transverse, mixed signal (DESS) MR-image, used to measure the width and height of the nucleus pulposus (red arrows) and annulus fibrosus. **B)** Mid-sagittal, mixed signal (DESS) MR-image, used to measure the thickness of the disc in the middle, on the dorsal (posterior) and ventral (anterior) side (red arrows).

[6] Philips Bucky Diagnost, Philips, NV, Eindhoven, the Netherlands

[7] Philips Secura, Single slice spiral scanner, Philips, NV, Eindhoven, the Netherlands

Assessment of the accuracy of IVD dimensions measured by MRI versus in situ with calipers

The following dimensions of the lumbosacral spine were measured on MR images and *in situ* with a Vernier caliper: annulus fibrosus width (left to right, at widest point), annulus fibrosus height (ventral [anterior] to dorsal [posterior], at widest point), NP width (left to right, at widest point), NP height (ventral to dorsal, at widest point), thickness of the disc (cranial-caudal [superior-inferior]) on the dorsal side, and thickness of the disc (cranial-caudal) on the ventral side. The dimensions were measured on mid-sagittal and transverse, mixed signal (DESS) images, using the automated settings and measurement tool provided by the software Web1000 5.1[4](Fig. 3).

Assessment of IVD height

Canine cadaveric spine segments (L5-Cd1) from 10 healthy female mixed-breed dogs (weight range 16.1-20.4 kg; age range 26-44 months), euthanized in an unrelated experiment, were used. Dorsoventral and lateral radiographs were taken using digital radiography[8] to assure that no congenital or acquired anomalies were present. The spinal specimens were subjected to the same procedures described above and radiographs were made in: 1) the native spine, 2) after dorsal laminectomy and nucleotomy, and 3) after implantation and swelling of the NPP. The radiographs were subsequently blinded and the L7-S1 IVD height was measured by three independent observers on standard computer screens, using the software Sante Dicom viewer[9] and the same settings and magnification for all three observers. A single line, perpendicular to the endplates, positioned in the center of the IVD was used to measure IVD height.

Data and statistical analysis

MRI and caliper measurements were compared using a paired Student's *t*-test. The linear correlation between these two sets of measurements was also evaluated, using Pearson's correlation. The mean disc height ± standard deviation (SD) on radiographs was calculated for the three conditions and a linear mixed model was used to compare them. "Observer" (three levels: observer 1-3) was set as a fixed effect, whereas "spine" (10 levels: specimen 1-10) was assigned to random effects. "Condition" (three levels: condition 1-3) was used both as a fixed and a random effect. A Bonferroni correction was applied to compensate for the multiple comparisons. The level for statistical significance was set to $P < 0.05$.

[8] Digital vet Premium, Sedecal, Madrid, Spain

[9] Santesoft, Athens, Greece

Results

Surgical implantation and imaging of the NPP in situ

The dorsal (posterior) approach, which is routinely used in canine lumbosacral IVD surgery, enabled accurate insertion of the NPP. The implant was visualized before and after swelling by radiography (Fig. 4), CT (Fig. 5), and MRI (Fig. 6). No artifacts were observed on CT or MR imaging. The NPP conformed to fill the nuclear cavity, as was also seen after isolation of the implant from the IVD at the end of the experiment (Fig. 7).

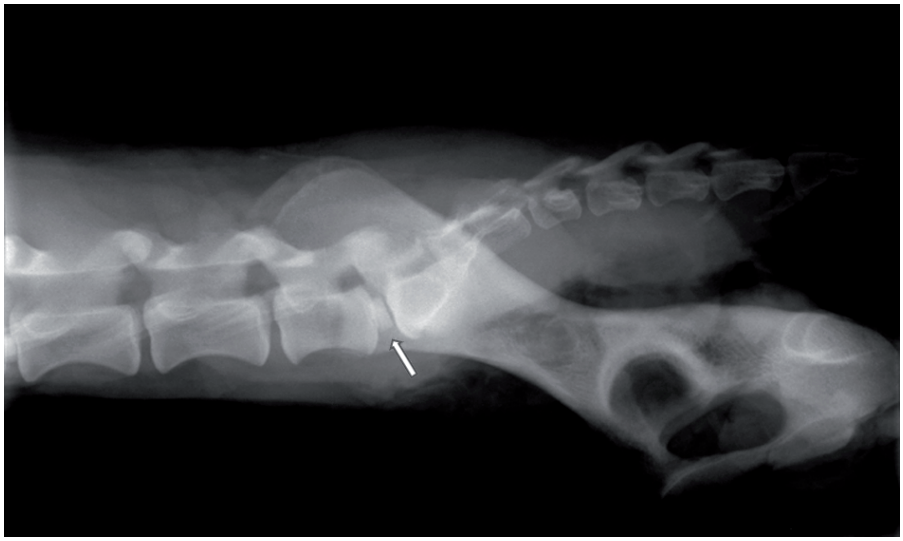


Fig. 4 Lateral radiograph of the canine lumbosacral region with a swollen nucleus pulposus prosthesis indicated by an arrow.

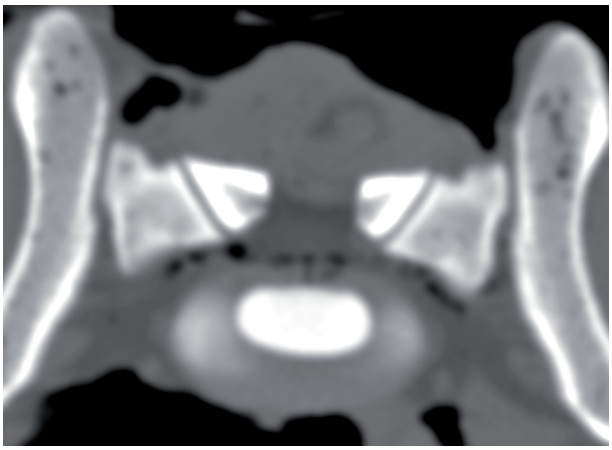
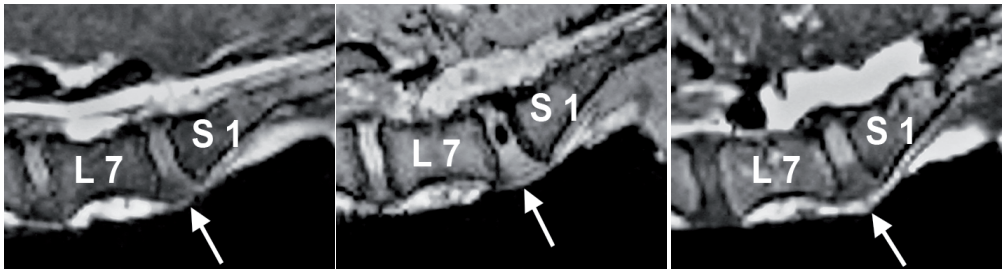


Fig. 5 Transverse CT images of the L7-S1 intervertebral disc with a swollen nucleus pulposus prosthesis in the center of the figure.



A. **B.** **C.**

Fig. 6 T2-weighted, sagittal magnetic resonance image of the canine lumbosacral segment. A) The native spinal segment with a normal L7-S1 intervertebral disc (IVD). B) After dorsal laminectomy, nucleotomy, and insertion of the nucleus pulposus prosthesis (NPP). The NPP before swelling (arrow). C) The same spinal segment after incubation at 38°C for 18 h, showing the swollen NPP (arrow).

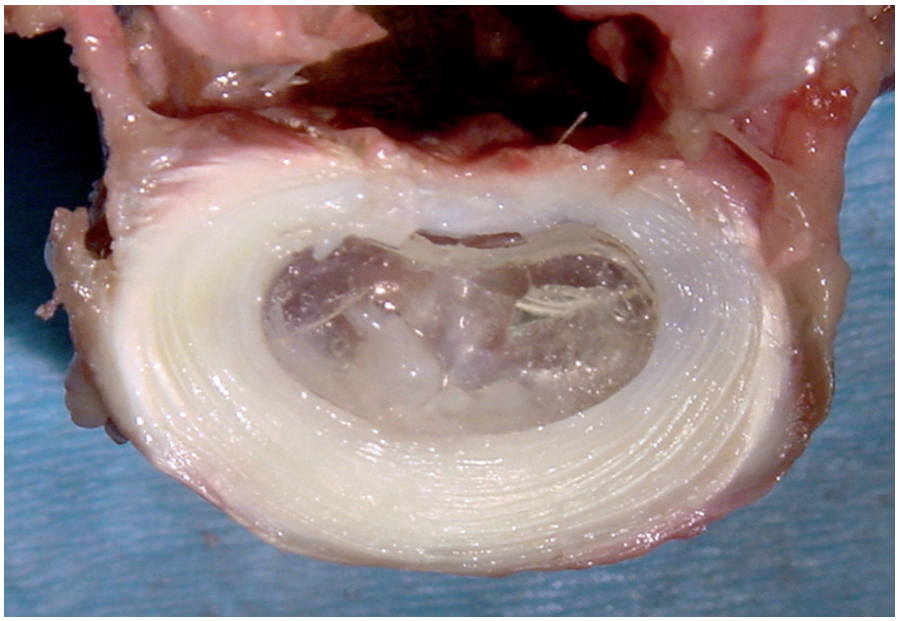


Fig. 7 Transverse section of a canine L7-S1 intervertebral disc with the hydrated nucleus pulposus prosthesis (NPP) in place. The insertion tunnel through the dorsal annulus can still be visualized. Minor damage was caused to the top right corner of the NPP during surgical extraction.

Assessment of the accuracy of IVD measured by MRI versus in situ by caliper

The MRI and caliper measurements of the IVD dimensions were not significantly different (Student's *t*-test) and were linearly correlated ($r = 0.965$; $P = 0.001$) (Fig. 8).

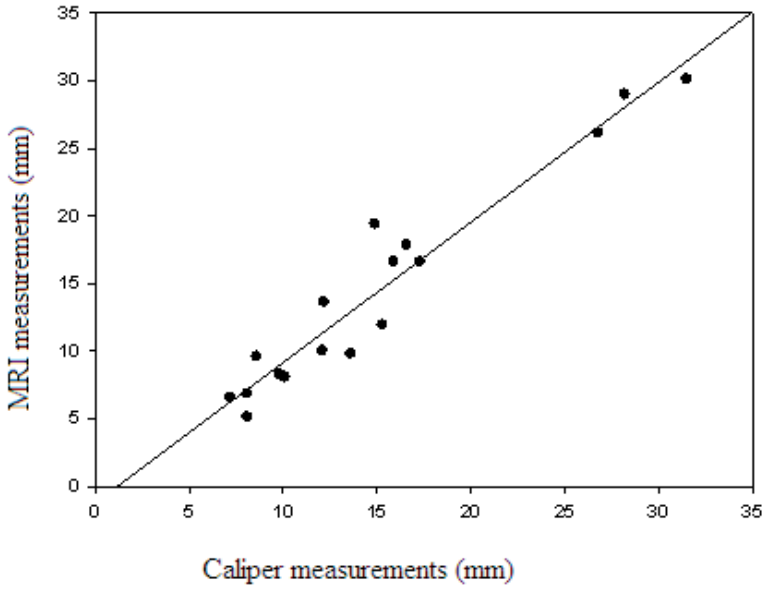


Fig. 8 Scatter diagram showing the linear relationship between MRI and caliper measurements of the dimensions of canine L7-S1 intervertebral discs.

Assessment of IVD height

In 8 of 10 spinal segments IVD height was restored after swelling of the NPP. In 1 of the other 2 spinal segments, the NPP was fractured and herniated through the annular incision; no explanation could be found for the failure to restore IVD height in the second segment. The mean (±SD) disc height for the spines in the native state, after nucleotomy, and after implantation and swelling of the NPP was 6.08 ± 0.59 mm, 5.44 ± 0.67 mm, and 6.29 ± 0.58 mm, respectively. There was a significant difference between the mean disc height in the native spine and that after nucleotomy, and also between the mean disc height after nucleotomy and that after implantation and swelling of the NPP. No significant difference was found between the mean disc height in the native spine and that after implantation and swelling of the NPP (Fig. 9).

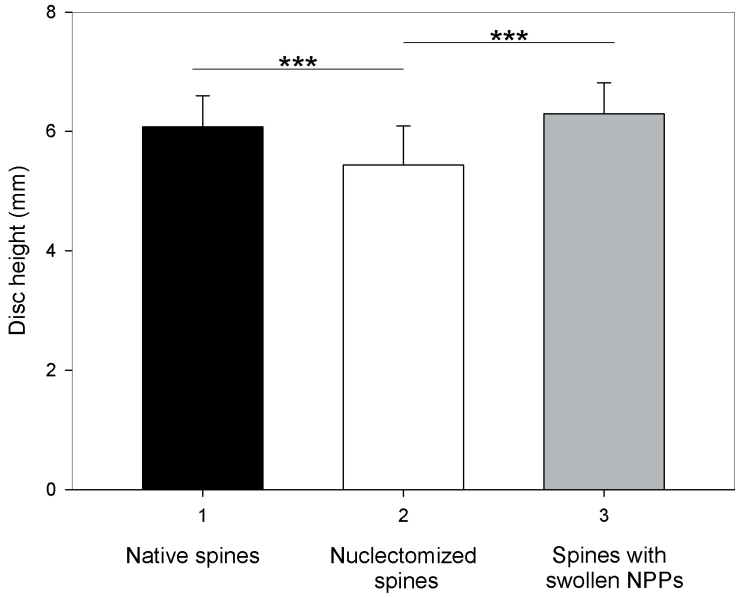


Fig. 9 The mean canine lumbosacral intervertebral disc height in 1) the native spine, 2) after laminectomy and nucleotomy, and 3) after implantation and swelling of the nucleus pulposus prosthesis (NPP). *** = $P \leq 0.0001$.

Discussion

We found that the NPP conforms well to fill the nuclear cavity, as reported previously (Boelen et al., 2006). An adequate fit with complete filling of the nuclear cavity is required to provide an even pressure over the endplates, which may prevent further degeneration of the IVD (Meakin, 2001, Meakin et al., 2001, Di Martino et al., 2005, Berlemann and Schwarzenbach, 2009). Because it is unlikely that surgeons can completely remove the remaining NP tissue from the IVD, to leave a nuclear cavity with smooth edges, it is essential that the nuclear implant should be able to conform to fill the space provided. In practice, the implant should be somewhat smaller than the NP, to prevent stress points along its margins.

The NPP is, due to its intrinsic radiopacity and fluid absorbing capacity, easy to visualize using radiography, CT, and MRI, which facilitates optimal positioning of the implant intraoperatively (radiography) and enables postoperative monitoring of in the implant to detect whether structural changes occur with time (CT and MRI). Low-field 0.2 Tesla MRI provides low-resolution images, but these were sufficient to allow

accurate measurement of the IVD dimensions, as evidenced by the strong linear correlation between MRI and caliper measurements. High-field MRI using magnets with 1.5 Tesla or higher, which are commonly available in human hospitals, provide high-resolution MR images, which should make it possible to measure the actual size of the NP of patients *in vivo* in order to make customized NPPs. No NPP-induced artifacts were observed on the MR images. Such artifacts sometimes occur with NP implants containing metallic markers for radiographic localization (Yang et al., 2009, Rupp et al., 1993).
The fact that the NPP is inserted in its dehydrated (xerogel) state facilitates its insertion in the annular incision of the lumbosacral IVD. When the implant absorbs water it swells and locks itself in place between the vertebrae, decreasing the risk of implant migration. However, fragmentation and implant migration occurred of one NPP in this study, this has also been reported when using the same NP implant in another study (Smolders et al., 2009). To prevent herniation the authors recommend improving the annular-closing technique further and also to improve the physical-mechanical characteristics of the biomaterial to prevent fragmentation.
Despite these changes, the NPP was found to restore the biomechanical properties of 10 canine lumbosacral junctions evaluated *ex vivo* by way of controlled biomechanical testing in the motion directions flexion/extension, bilateral bending, and axial rotation. Each specimen was tested in the native state, after a removal of the NP (nucleotomy), and after insertion of the NPP. Nucleotomy induced significant instability in each motion direction, which was significantly restored towards the native situation after insertion and swelling of the NPP (Smolders et al., 2009).

Although the NPPs in this study were all inserted via a dorsal (posterior approach) laminectomy, the authors have also successfully implanted a number of NPPs via an anterior-lateral approach in canine cadaveric lumbar IVDs other then the L7-S1. This is likely to be the approach used for implantation of NPPs in humans.

In general, IVD height was significantly restored by the swollen NPP and even a trend towards overcompensation was noted. Although this difference was not significant, this could imply that the implants may have been too large. This could be because of the difference in the body weight between the 3 dogs, used to obtain the dimensions for the NPP, and the body weight of the 10 dogs used to assess IVD height. The reason that IVD height was not restored in two of the specimens could be explained by fragmentation and migration of part of the implant in one case, but in the other case the implant was retrieved intact and no satisfactory explanation could be found.

The results indicate that an NPP made from cross-linked NVP/HEMA/4-IEMA/AMA hydrogel has promising qualities. The implants can restore IVD height, and the intrinsic

characteristics of the NPP facilitate imaging by radiography, CT, and MRI. Previous studies have demonstrated the biocompatible nature of the material (Boelen et al., 2006), and the capacity to restore biomechanical properties to nucleotomized spinal segments (Smolders et al., 2009). Interestingly, the NPP concept may not only be applicable to treat human patients but may also be used for companion animals (dogs) suffering from low back pain. This opens the possibility to evaluate the experimental implant not only in an animal model, but also in animal patients.

Conclusions

A NPP made from cross-linked NVP/HEMA/4-IEMA/AMA has clinically valuable characteristics, i.e., intrinsic radiopacity and the ability to swell *in situ* to fit the space provided. The dry-state xerogel implant can be inserted through a small annular opening and when it swells (2.2-times volume) it locks into place between the adjacent vertebral bodies and is contained by the annular ring, thereby restoring the height of the nucleotomized lumbosacral IVD. These properties make the NPP potentially suitable for clinical use in early stages of IVD degeneration. However, before *in vivo* testing occurs, physical-mechanical improvements to the hydrogel are needed to prevent fragmentation, and an annular closure technique has to be developed to prevent implant migration.

Acknowledgments

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Chapter 8

General Discussion

General Discussion

The first aim of this thesis was to increase the knowledge of intervertebral disc (IVD) degeneration in dogs with regards to the morphological process of degeneration and the demographics of IVD-related diseases in dogs, and also to validate grading schemes enabling objective grading and monitoring of the degenerative processes in canine IVDs.

The studies presented in **Chapters 2, 3, 4, 5,** and **6** increased our knowledge of the morphological process of canine IVD degeneration and made use of a new magnetic resonance imaging (MRI) grading scheme for evaluating and monitoring IVD degeneration in dogs, thereby facilitating early diagnosis and potentially making preemptive treatment for high-risk canine patients possible. The aforementioned studies also tested the first hypothesis of this thesis: *The morphological process of IVD degeneration in chondrodystrophic (CD) and non-chondrodystrophic (NCD) breeds is more similar than previously reported, with the only difference being that degeneration takes place earlier in life and proceeds more rapidly in CD breeds.* The basis for this hypothesis and our findings regarding canine IVD degeneration are described below.

In **Chapter 2,** the literature on canine IVD degeneration was reviewed, to gain insight into current knowledge of the processes underlying IVD degeneration in dogs. This information combined with our own findings from the studies presented in **Chapters 4, 5,** and **6** led to the conclusion that IVD degeneration cannot correctly be divided into chondroid and fibroid IVD degeneration, as previously suggested[1]. The distinction between chondroid degeneration in CD breeds and fibroid degeneration in NCD breeds has been widely accepted by the veterinary community, and is largely based on the studies of Hansen in the 1950s[1-3]. In his fundamentally important studies of IVD degeneration in dogs, Hansen accurately identified that CD and NCD breeds differ significantly in the age of onset and speed with which the degenerative changes progress, but considered that two essentially different degenerative processes were active. However, from the recent literature and our findings it would appear that the fundamental steps in the degenerative cascade are similar in the two types of dog breed. Degeneration in both types is characterized initially by the degradation and loss of proteoglycans, a change from collagen type II to collagen type I, and a gradual loss of notochordal cells, which are replaced by a less dense cell population of chondrocyte-like cells. These matrix and cellular changes are similar in the two types of breeds, but occur earlier and faster in CD breeds[4-9] **(Chapters 4, 5,** and **6)**. As Hansen also pointed out, we found that most of the IVDs of CD breeds started to degenerate simultaneously and earlier in life (<1 year of age) than in NCD breeds. In NCD breeds often only one IVD was degenerated, with the other IVDs remaining healthy. Healthy IVDs, full with notochordal cells, were found in young dogs of CD and NCD breeds, but only in older NCD breeds

(>6-years of age) and not in older CD breeds **(Chapter 6)**. Morphologically, all stages of the degenerative process, from Thompson grade I to Thompson grade V, can be identified in the IVDs of dogs of both CD and NCD breeds. But again, degeneration occurs earlier in life and more rapidly in CD breeds[10,11]**(Chapter 5)**. The same is true for the degenerative changes seen on MRI graded according to Pfirrmann[12,13] **(Chapter 4)**.

This erroneous assumption that two different pathological processes are active in canine IVD degeneration is because Hansen referred to the cells he found in the nucleus pulposus (NP) of older NCD-breed dogs as fibrocyte-like cells and hence called the process of IVD degeneration in NCD breeds fibroid degeneration. To illustrate these fibrocyte-like cells, Hansen presented a histological image of the NP from a 10-year-old Airedale Terrier (figure 36 of Hansen's thesis from 1952[1]). However, on closer examination the cells have an uncanny resemblance to what now would be referred to as dying notochordal cells[14,15]. Although his conclusion was not quite correct, the distinction Hansen made between degenerating IVDs in CD and NCD breeds was logical because these breeds show significant differences, not only in the onset of degeneration, but also in the pathological end-stage. In CD breeds, the end-stage of IVD degeneration can involve extrusion of degenerated and calcified NP tissue into the spinal canal, where it compresses the neural structures, giving rise to clinical signs of disease (Hansen type I herniation). In NCD breeds, the end-stage of IVD degeneration usually results in bulging of the disc or even protrusion of the degenerated and dorsally bulging annulus fibrosus (AF) (Hansen type II herniation), which also causes clinical signs of disease due to compression of neural structures. Hansen already noted in his thesis that the distinction between type I herniation in CD breeds and type II herniation in NCD breeds was not consistent, as type II herniation is occasionally seen in CD breeds and type I herniation in NCD breeds. Thus a clear distinction between IVD degeneration as seen in CD and NCD breeds cannot be made based on the type of herniation displayed in the end-stage of disc degeneration. However, as more recent evidence indicates that the pathological processes underlying IVD degeneration in CD and NCD breeds are similar (**chapter 4, 5,** and **6**), but generally have a different etiology, with principally a genetic cause in CD breeds and a multifactorial origin of trauma and "wear and tear" being most common in NCD breeds (**chapter 3**), the distinction between IVD degeneration in CD and NCD breeds should be based on the etiology of degeneration rather than on the pathological process itself.

There is, however, one other important difference between the processes of IVD degeneration in CD and NCD breeds, namely, the common occurrence of mineral deposits in the degenerating IVDs of CD breeds, whereas such deposits are rare in the degenerating IVDs of NCD breeds [1,16-18]. It is likely that these mineral deposits affect the process of degeneration. Instead of a gradually collapsing IVD, due to dehydration and

concurrent loss of NP volume, the NP cavity in CD breeds is often filled with mineral deposits, thereby maintaining IVD height and preventing inward collapse of the AF with subsequent disorganization and degeneration, which is often seen in NCD breeds. The AF of the IVDs with a mineralized NP will ultimately also degenerate, but often not until the NP is severely degenerated[1]. It is also possible that this mineralization is the cause of the different types of herniation seen in the pathological end-stage of IVD degeneration in the two types of dog breed. In the degenerated IVD of NCD-breed dogs, there is less NP material left to extrude into the spinal canal, whereas the degenerated NP of CD-breed dogs is often full of mineralized matrix that can readily be extruded into the spinal canal. However, it remains unclear whether the herniation of mineralized NP tissue occurs secondary to the progressive degeneration of the AF, which finally ruptures, or whether herniation is principally due to altered biomechanical loading of the spinal segment because of the calcified NP. Most likely, Hansen type I herniation is a combination of both degeneration of the AF and altered loading of the spinal segment, but this remains to be proven. Hansen stated that the calcifications are of a dystrophic origin rather than part of endochondral ossification[1]. This is supported by more recent studies in humans and sheep[17,19-21]. Deposition of different calcium salts has been described in the human IVD[19,20]. It has been suggested that the mineral deposits found in CD breeds consist of hydroxyapatite [17], as is also described in a hereditary form of dystrophic calcification seen in merino sheep and humans[17,21]. However, it still remains to be proven that the calcifications seen in young CD-breed dogs are indeed composed of hydroxyapatite.

Regarding the demographic characteristics of canine IVD degenerative diseases, the study presented in **Chapter 3** reported new findings and confirmed the results of previous studies in smaller and geographically more limited study populations[1,22-26]. In accordance with most previous studies, we found that IVD degenerative disease in general (i) has a conservative life-time prevalence of about 3.5% in dogs younger than 12 years; (ii) is most common in CD breeds, especially in Dachshunds, and (iii) is 1.5 times more common in male than female dogs. With regard to the breed predilection for the site of the disease, the CD breeds and especially Dachshunds were most commonly affected by thoracolumbar IVD herniation. Large-breed NCD dogs, especially German Shepherd Dogs, were most commonly affected by degenerative lumbosacral stenosis (DLSS). Cervical IVD degenerative diseases were equally divided over CD and NCD breeds, with Dobermans and Dachshunds being at highest risk of cervical disease. The novel findings of the study presented in **Chapter 3** were the high case fatality rates (ratio of deaths to incidence rate of IVD-related diseases), which were 1:3 in the overall population, 1:5 in the CD breeds, and more variable in the NCD breeds, with an overall rate of 1:2 in the high-risk NCD breeds. Moreover, this was the first large-scale epidemiological study of IVD degenerative diseases to include low-risk breeds. A

number of dog breeds, mainly hunting dogs, have a low reported incidence of IVD degenerative disease. The fact that these diseases were found to be overrepresented in some breeds and rare in others suggests that there is a genetic component involved in the occurrence of IVD degenerative diseases, not only in CD breeds but also in some NCD breeds, such as DLSS in the German Shepherd Dog and cervical spondylomyelopathy (CSM) in the Doberman. As IVD degeneration in these breeds mostly affects only a single IVD, it is likely that the genetic component involved in initiating IVD degeneration in these breeds does not affect the IVDs primarily but secondarily, through malformation of the vertebrae or misalignment of the facet joints, as proposed earlier[27,28]. However, in most other NCD breeds it is more plausible that IVD degeneration has a multifactorial origin that is less influenced by genetic factors and more by physical factors causing "wear and tear" of the disc. A breed can be considered as a subgroup of the species[29]. All breed-associated diseases with proven high incidence rates in comparison with other breeds are suspected to have a genetic basis[30]. Genetic similarity within a breed is mainly based on multiple common ancestries, which increases the chances of distributing the risk factors within the subgroup[31]. Using this rationale, there is compelling evidence that early IVD degeneration in CD breeds, DLSS in the German Shepherd Dog and CSM in the Doberman, are indeed hereditary disorders.

An interesting incidental finding in the study described in **Chapter 4** was that most IVDs of Jack Russell terriers, despite their CD phenotype, had a low Pfirrmann grade irrespective of the dog's age, in marked contrast with the other CD breeds. This indicates that genetic factors causing chondrodysplasia in dogs might not necessarily be responsible for IVD degeneration. If chondrodysplasia and IVD degeneration are indeed caused by different genetic factors, they are, however, likely to be closely linked in most dog breeds. Another finding highlighting the complexity of the association between chondrodysplasia and IVD degeneration is based on the recent publication by Parker et al., who showed that an expressed fgf4 retrogene is a likely cause of chondrodysplasia in dogs[32]. This retrogene is not expressed in the Beagle, one of the CD breeds at highest risk of developing IVD degenerative disease, which indicates that different genetic factors could be involved in the process of chondrodysplasia and associated IVD degeneration in dogs. More genetic research is needed to elucidate the etiology of IVD degenerative diseases in dogs. The gene(s) involved in the etiology of these abnormalities can possibly be identified using association analysis, by comparing the frequency of marker alleles in an appropriate cohort (with respect to breed, gender, and age) of cases and controls. Detailed knowledge of the genes involved will increase our understanding of the pathogenesis of the diseases and may have a crucial role in developing novel therapies. The latter can include specific interventions in cellular processes to prevent, slow, or stop the sequence of events leading to IVD degeneration.

It is important to point out that IVD degeneration is not synonymous with IVD disease. While IVDs that give rise to clinical signs inevitably show degeneration, degenerated IVDs are commonly reported incidental findings[1,10,12,28,33,34]. This was evident in the study reported in **Chapter 4,** where MRI often revealed only one or two herniated IVDs that gave rise to clinical signs and asymptomatic degenerated IVDs elsewhere in the spinal segment in CD breeds, whereas in NCD breeds often only the IVD giving rise to clinical signs was found to be degenerated. In the study reported in **Chapter 5,** it was apparent that IVDs can be degenerated without giving rise to clinical signs of disease, as many of the 19 dogs (both CD and NCD breeds) included in the study had severely degenerated IVDs but none had a history of clinical signs of IVD disease.

In the study described in **Chapter 4,** it was concluded that the MRI grading system originally designed for use in humans can be reliably used to evaluate IVD degeneration in dogs. This conclusion was based on the high inter- and intraobserver reliability and biological validation showing that IVD degeneration was significantly associated with the CD phenotype and with increasing age. For the Pfirrmann grading system to be clinically useful in veterinary practice, it does however need to be used in combination with information about disc herniation (if present), such as protrusion or extrusion.

In the study reported in **Chapter 5**, the Thompson scheme was found to be a reliable method for grading canine IVD degeneration with a high inter- and intraobserver agreement. Further, there was substantial agreement between macroscopic grading of intervertebral segments according to Thompson and grading of low-field MR images according to Pfirrmann, which suggests that low-field MRI can be used to accurately identify IVDs in different stages of degeneration in dogs. MRI can thus be useful for monitoring progression of IVD degeneration in high-risk breeds and can ultimately lead to identification of IVDs suitable for preemptive treatments. Currently, the only preventive treatment used in dogs is IVD fenestration,[35] which is aimed at preventing herniation by removing most NP tissue. However, fenestration of the IVD changes the biomechanical properties of the spinal segment, which becomes more unstable[36-40]. Also the morbidity and mortality of spinal surgery are considerable, so instead of further damaging the IVD by fenestration, a better prophylactic treatment would be to halt the process of degeneration or even to regenerate the degenerated IVD. This may be achieved through the application of growth factors, anti-catabolic agents, or cell-based strategies, which have been investigated in numerous studies over the past decade[41-47]. Different regenerative treatment strategies are generally considered to be required in different stages of degeneration, which is why an objective and accurate MRI grading scheme for IVD degeneration is needed.

The second aim of this thesis was to evaluate the similarities and differences between the process of IVD degeneration in dogs and humans, in order to establish whether spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human research. The studies reported in **Chapter 6,** but also in **Chapters 2, 4,** and **5**, revealed that there are clear similarities between the degenerative process in dogs and humans. The basis for the second hypothesis (*Spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human IVD research)* and implications of this are discussed below.

The common appearance of spontaneously occurring IVD degeneration in dogs, as in humans, was made evident in **Chapters 3, 4** and **5**, and in **Chapter 3** the common occurrence of IVD degenerative diseases in dogs was also displayed. In **Chapter 6** many similarities between the process of IVD degeneration in humans and CD and NCD dog breeds (Chapter 6) suggests that both types of dog breeds would be suitable as translational animal models of spontaneous IVD degeneration for human research. Although the dog has frequently been used as a translational model for surgical procedures and biomechanical studies of the spine by the human medical community[48-53] and some previous studies have discussed the translational aspects between canine and human IVD degeneration[54-56], few extensive comparative studies have been performed.

When using the dog as a model for human research, it is important to recognize the specific interspecies differences as well as the differences in IVD degeneration between CD and NCD breeds (early versus late onset, respectively). The fact that dogs spontaneously develop IVD degeneration at different ages makes them suitable as models for different types of studies. CD breeds, which develop degeneration in most of their IVDs early in life, are best suited for longitudinal studies investigating the process of IVD degeneration, or for preclinical studies of interventional treatments aiming to prevent, stop, or slow the course of degeneration. NCD breeds especially the German Shepherd Dog, are thought to have a similar disease process as humans with lumbosacral IVD degeneration, i.e. the degeneration of the lumbosacral disc in the German Shepherd Dog develops over a longer period (years) of chronic IVD stress, "wear and tear"[24]. These dogs would thus make suitable models for investigating the development of IVD degeneration of the human lumbosacral disc, and here veterinary patients could also be used for preclinical studies of new treatments for IVD degenerative diseases. By performing studies with veterinary patients, the number of dogs used as laboratory animals as models of human disease would be reduced, and it would also be substantially cheaper than using research animals. It is also likely that spontaneous IVD degeneration in dogs resembles the true disease process, as it occurs in humans, better than induced IVD degeneration in laboratory animals does.

Given the similarities between the processes of IVD degeneration in dogs and humans, if the etiology and pathogenesis of IVD degeneration are elucidated in dogs, it is likely that similar mechanisms are responsible for the disease in humans. It will probably be easier to elucidate the processes of IVD degeneration in dogs than in humans, because of the genetic and phenotypic differences between different breeds, as discussed above, combined with the limited genetic variations within breeds. Canine IVD material is also easier to obtain than human IVD tissue, and it can be obtained from different sources without using purpose-bred research dogs, namely, veterinary patients operated for IVD hernias, veterinary patients post-mortem with the owner's consent, or from research dogs used in unrelated experiments. The fact that dogs walk on four legs and humans on two is often raised as a factor limiting the use of dogs as models of human IVD degeneration, since it is incorrectly believed that humans have a higher axial loading of the spinal segments due to gravity. However, the axial loading patterns of human and canine IVDs have been shown to be comparable or even higher in dogs and other quadrupeds [52,57,58].

Some differences between IVD degeneration in humans and dogs were, however, found, such as the absence of growth plates in growing human vertebrae and the thicker cartilaginous endplates in humans. Whereas in dogs the majority of vertebral growth takes place in the growth plates, the growth of human vertebrae takes place in the junction between the vertebrae and the endplates. This may be the explanation for the relatively thicker endplates found in humans (endplate thickness/total IVD height). The importance of these differences in endplate thickness between dogs and humans, with regard to the rate of osmosis, needs to be evaluated further in order to improve the accuracy of extrapolating findings from dogs to humans. Currently not much is known about the rate of nutrient osmosis into the canine IVD. This needs to be explored further, as this knowledge is not only of importance for translational studies, but also for developing cell-based therapies to treat degenerating canine IVDs. As there is no blood supply providing nutrients to the NP, implanted cells will be dependent chiefly on the osmosis of nutrients through the endplates for their nutritional supply.

In the study described in **Chapter 7,** canine spines were used, *ex-vivo,* in a translational study testing a nucleus pulposus prosthesis (NPP) that is intended for use in humans. A clinically adapted mode of implantation of the NPP into the nuclear cavity of the canine L7-S1 IVD was used. Swelling, fit, and restoration of disc height of the NPP *in situ* were monitored by radiography, CT, and MR imaging. The canine spines were found suitable for this type of translational studies, and a NPP might also be a viable treatment option, not only for humans but also for selected veterinary patients suffering from IVD degenerative disease.

Key findings

- The classic division of the processes underlying canine IVD degeneration into chondroid or fibroid degeneration appears to be inaccurate. The fundamental pathological changes with regards to biochemical, histopathological, and morphological alterations during the process of IVD degeneration were found to be similar in CD and NCD breeds. A more appropriate distinction is based on the etiology of disease, as evidence points to there being a principally genetic cause in CD breeds, whereas a multifactorial etiology, including "wear and tear", is more plausible in most NCD breeds.
- IVD degenerative diseases in dogs had a conservative life-time prevalence of 3.5% in dogs younger than 12 years. These diseases were most common in CD breeds, especially in Dachshunds, and were 1.5 times more common in male than female dogs. Case fatality rates of IVD degenerative diseases were found to be higher than previously suggested, with rates of 34% in the overall population, around 20% in most CD breeds, and more variable in the NCD breeds with over 50% in the breeds at highest risk.
- The Thompson scheme proved to be a reliable method for macroscopic grading of canine IVD degeneration. Further, there was substantial agreement between macroscopic grading of IVD degeneration according to Thompson and grading of low-field MR images according to Pfirrmann, which suggests that low-field MRI can be used to accurately identify IVD degeneration in dogs.
- Many similarities were found between IVD degenerative processes in humans and both CD and NCD dog breeds. Both types of dog breed could thus serve as translational animal models of spontaneous IVD degeneration for human research, offering diverse possibilities for the use of these two animal models with early onset (CD) versus late onset (NCD) IVD degeneration.
- Veterinary patients suffering from IVD degenerative diseases can be used in preclinical trials, and also to study the process of IVD degeneration. Synergistic effects from this approach could lead to new treatment modalities for both dogs and humans, a reduced need for laboratory animals, and lower research costs. It is also likely that spontaneous IVD degeneration in dogs resembles the true disease process, as it occurs in humans, better than induced IVD degeneration in laboratory animals does.

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Chapter 9

Summary

Summary of the thesis

The intervertebral disc (IVD) is an essential structure of the spine, and is largely responsible for both the stability and flexibility of the vertebral column. Degeneration of the IVD involves deterioration of the matrix, which can ultimately result in structural failure and serious debilitating diseases such as IVD herniation or spinal instability. The cause of IVD degeneration in dogs is generally believed to be multifactorial, with the more common causes being genetic predisposition, trauma, or 'wear and tear'. IVD degenerative diseases can be seen in most dog breeds, but are especially prevalent in chondrodystrophic (CD) breeds and in a few non-chondrodystrophic (NCD) breeds such as the Doberman and the German Shepherd Dog.

Although IVD degenerative diseases in dogs have been the focus of numerous studies over the past 60 years, most of these studies were limited to diagnostics and treatments, leaving the process of degeneration largely unexplored. Considerably more studies focusing on the pathogenesis of IVD degeneration have been conducted in humans and laboratory animals. Although the clinical presentation, diagnostics, and treatments are largely similar in humans and dogs, few comparative studies have been performed. Before results based on translational studies between dogs and humans can be accurately evaluated, basic comparative studies are needed to ascertain the similarities and differences between IVD degenerative processes in dogs and humans.

The **first aim** of this thesis was to increase the knowledge of IVD degeneration in dogs with regards to the morphological processes of degeneration and the demographics of IVD degenerative diseases, and also to validate grading schemes enabling objective grading and monitoring of the process of IVD degenerations in dogs. Such knowledge will facilitate early diagnosis and possibly preemptive treatments for high-risk patients.

The **second aim** of this thesis was to evaluate the similarities and differences between IVD degeneration in dogs and humans, in order to establish whether spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human research. The reason for wanting to use dogs as models for human IVD degeneration is threefold. *Firstly*, relevant animal models are needed to successfully design new treatments for IVD degeneration in humans. Spontaneously occurring IVD degeneration in an animal, living in the same environment as humans, is likely to mimic the human situation better than induced IVD degeneration in laboratory animals, an approach that is commonly used today. *Secondly*, new treatments for IVD degenerative disease in humans, designed in dogs, will also benefit dogs as veterinary patients. *Thirdly*, by using canine veterinary patients for relevant clinical trials and also to study the process spontaneously occurring IVD degeneration *in vivo* as well as *post*

mortem, the number of laboratory animals used for IVD research can hopefully be reduced.

The studies of this thesis investigated two hypotheses: 1) The morphological processes of IVD degeneration in CD and NCD breeds are more similar than previously reported, with the only difference being that degeneration takes place earlier in life and proceeds more rapidly in CD breeds. 2) Spontaneous IVD degeneration occurring in both CD and NCD dog breeds can be used as translational animal models for human IVD research.

The specific aims and conclusions of the studies presented in the chapters of this thesis are described below.

The study described in **Chapter 2** was a review of the literature on canine IVD degeneration, thereby investigating and explaining the process of IVD degeneration as it is known in dogs. The chapter also highlighted the differences in the degenerative process in CD and NCD breeds, which were found to be fewer than previously reported.

The aim of the large population-based study presented in **Chapter 3** was to increase insight into the age and breed distribution of IVD degenerative diseases in dogs. The lifetime prevalence of IVD degenerative diseases in dogs younger than 12 years was found to be 3.5%. The case fatality rate of these diseases was found to be 34% overall and considerably higher (>50%) in some large-breed dogs such as the Doberman and the German Shepherd Dog. Although IVD degenerative diseases were found in a great variety of dog breeds, CD breeds and a small number of NCD breeds were clearly over-represented, indicating substantial genetic influence in the pathogenesis of these diseases in these breeds.

The aim of the study described in **Chapter 4** was to evaluate whether the magnetic resonance imaging (MRI)-based grading system of Pfirrmann et al. for grading IVD degeneration in human lumbar discs is applicable for use in both CD and NCD dog breeds and for intervertebral discs at any location in the vertebral column. The aim was also to validate the Pfirrmann system by determining the interobserver and intraobserver agreement between Pfirrmann scores, and to evaluate whether biological factors known to increase the extent of IVD degeneration are correlated with higher Pfirrmann scores. The interobserver and intraobserver reliability of the Pfirrmann system used in dogs was very high, with κ scores ranging between 0.81 and 0.93. Moreover, increasing severity or grade of disc degeneration was significantly associated with CD phenotype and older age. On the basis of these results, it was concluded that the Pfirrmann grading system can be used to evaluate IVD degeneration in both CD and NCD breeds.

The aims of the study presented in **Chapter 5** were to validate the Thompson grading system for gross pathological changes of IVD degeneration in dogs, and to investigate the agreement between pathology findings and low-field MRI findings. This study demonstrated that the Thompson scheme is a reliable method for grading canine IVD degeneration with a high interobserver and intraobserver agreement. Further, the agreement between macroscopic grading of intervertebral segments according to Thompson and grading of low-field MR images according to Pfirrmann was substantial, which suggests that low-field MRI can be used to diagnose IVD degeneration in dogs. This is however more true for lower than high grade degenerated IVDs. It is also important to recognize that degenerated discs are frequently seen in asymptomatic dogs and that the findings of degenerated IVDs on MRI will always have to be combined with the results of clinical and neurological examinations in order to reach a correct clinical diagnosis. In the studies reported in **Chapters 4** and **5**, IVDs from both CD and NCD dogs were found in all grades of degeneration, indicating that the morphological process of degeneration is similar in the two types of dog breed.

The aim of the study presented in **Chapter 6** was to investigate whether spontaneous IVD degeneration occurring in CD and NCD breeds can be used as valid translational models for human lumbar IVD degenerative research, by comparing the morphological appearance, histological structure, and biochemical characteristics of different stages of IVD degeneration in dogs and humans. There were many similarities between IVD degeneration in humans and CD and NCD dog breeds, and both CD and NCD breeds could serve as models of spontaneous IVD degeneration for human research. However, when employing the dog as a model for human IVD research, it is important to recognize the specific interspecies differences as well as the difference in IVD degeneration between CD and NCD breeds (early versus late onset, respectively).

The aim of the study reported in **Chapter 7** was to perform a translational study in which a nucleus pulposus prosthesis (NPP), intended ultimately for clinical use in humans, was tested *ex-vivo* in canine lumbosacral segments (L7-S1). A clinically adapted mode of implantation of the NPP in the nuclear cavity of the L7-S1 intervertebral disc was investigated. Swelling, fit, and restoration of disc height of the NPP *in situ* were monitored by radiography, computed tomography, and MRI. From this study, it was concluded that the canine spine can successfully be used for this type of translational study. It was also concluded that the new NPP has clinically valuable characteristics, i.e., intrinsic radiopacity and the ability to swell *in situ* to fit the space provided. These properties make the NPP potentially suitable for clinical use in early stages of IVD degeneration not only in humans but possibly also in dogs. However, before *in vivo* testing occurs, physical-mechanical improvements to the hydrogel are needed to prevent

fragmentation and an annular closure technique has to be developed to prevent implant migration.

The results of all the studies were summarized and discussed in **Chapter 8,** and the thesis was concluded with summaries in English, Dutch, and Swedish **(Chapter 9)**.

Key findings

- The classic division of the processes underlying canine IVD degeneration into chondroid or fibroid degeneration appears to be inaccurate. The fundamental pathological changes with regards to biochemical, histopathological, and morphological alterations during the process of IVD degeneration were found to be similar in CD and NCD breeds. A more appropriate distinction is based on the etiology of disease, as evidence points to there being a principally genetic cause in CD breeds, whereas a multifactorial etiology, including "wear and tear", is more plausible in most NCD breeds.
- IVD degenerative diseases in dogs had a conservative life-time prevalence of 3.5% in dogs younger than 12 years. These diseases were most common in CD breeds, especially in Dachshunds, and were 1.5 times more common in male than female dogs. Case fatality rates of IVD degenerative diseases were found to be higher than previously suggested, with rates of 34% in the overall population, around 20% in most CD breeds, and more variable in the NCD breeds with over 50% in the breeds at highest risk.
- The Thompson scheme proved to be a reliable method for macroscopic grading of canine IVD degeneration. Further, there was substantial agreement between macroscopic grading of IVD degeneration according to Thompson and grading of low-field MR images according to Pfirrmann, which suggests that low-field MRI can be used to accurately identify IVD degeneration in dogs.
- Many similarities were found between IVD degenerative processes in humans and both CD and NCD dog breeds. Both types of dog breed could thus serve as translational animal models of spontaneous IVD degeneration for human research, offering diverse possibilities for the use of these two animal models with early onset (CD) versus late onset (NCD) IVD degeneration.
- Veterinary patients suffering from IVD degenerative diseases can be used in preclinical trials, and also to study the process of IVD degeneration. Synergistic effects from this approach could lead to new treatment modalities for both dogs and humans, a reduced need for laboratory animals, and lower research costs. It is also likely that spontaneous IVD degeneration in dogs resembles the true disease process, as it occurs in humans, better than induced IVD degeneration in laboratory animals.

Samenvatting

De tussenwervelschijf (TWS) is verantwoordelijk voor zowel de stabiliteit als de flexibiliteit van de wervelkolom. Degeneratie van de TWS kan resulteren in een structureel defect en ziekten zoals hernia nuclei pulposi of instabiliteit van de wervelkolom. In het algemeen wordt aangenomen dat de oorzaak van TWS degeneratie multifactorieel is, waarbij genetische predispositie, herhaalde overbelasting of 'slijtage' als de belangrijkste oorzaken worden gezien. Aandoeningen ten gevolge van TWS degeneratie komen voor bij de meeste hondenrassen, maar vooral bij chondrodystrofische (CD) rassen en een paar niet-chondrodystrofische (NCD) rassen, zoals de Doberman en de Duitse Herdershond. Hoewel aandoeningen ten gevolge van TWS degeneratie bij honden het onderwerp is van talrijke studies gedurende de afgelopen 60 jaar, beperkten de meeste van deze studies zich tot de diagnostiek en behandeling, waardoor het proces van degeneratie grotendeels onduidelijk is gebleven. Aanzienlijk meer onderzoek, gericht op de pathogenese van TWS degeneratie, is verricht bij mensen en proefdieren. Hoewel de klinische presentatie, diagnostiek en behandelingen grotendeels vergelijkbaar zijn bij mensen en honden die lijden aan TWS degeneratie, zijn weinig vergelijkende studies uitgevoerd. Voordat de resultaten van translationeel onderzoek kunnen worden toegepast, zijn fundamentele vergelijkende studies nodig die de overeenkomsten en verschillen tussen het proces van TWS degeneratie bij honden en mensen beschrijven.

Het eerste doel van dit proefschrift was erop gericht om de kennis te vergroten met betrekking tot het morfologische proces van TWS degeneratie, de demografie van TWS gerelateerde ziekten bij honden te bestuderen, en om graderingsystemen voor TWS degeneratie voor gebruik in honden te valideren waardoor een objectieve indeling en monitoring van het degeneratieve proces van de TWS bij honden mogelijk wordt.

Het tweede doel van dit proefschrift was om de overeenkomsten en verschillen tussen het morfologische proces van TWS degeneratie bij honden en mensen te onderzoeken. Zodoende kan worden vastgesteld of spontane TWS degeneratie bij zowel de CD als NCD hond kan worden gebruikt als translationeel diermodel voor onderzoek van TWS degeneratie bij de mens. De reden voor het gebruik van honden als diermodel voor onderzoek van TWS degeneratie bij de mens is drieledig. Ten eerste, om met succes

nieuwe preventieve en therapeutische behandelingen voor TWS degeneratie bij de mens te ontwerpen en ontwikkelen zijn relevante diermodellen nodig. Een model van een dier waarbij TWS degeneratie spontaan optreedt, bootst waarschijnlijk beter TWS degeneratie bij de mens na dan geïnduceerde TWS degeneratie in proefdieren. Ten tweede zullen nieuwe behandelingen voor aandoeningen van de TWS bij de mens, die dan ontwikkeld worden bij honden, tevens ten goede komen aan de veterinaire honden-patiënten. Ten derde, door het uitvoeren van klinische studies bij honden patiënten met TWS degeneratie kan het aantal proefdieren hopelijk worden verminderd.

De hypothesen van dit proefschrift waren: 1) Het morfologische proces van TWS degeneratie bij CD- en NCD rassen is meer vergelijkbaar dan wat tot nu toe in de literatuur werd beschreven en 2) Spontane TWS degeneratie bij CD en NCD rassen kunnen gebruikt worden als translationeel diermodel voor TWS degeneratie onderzoek bij mensen.

De verschillende hoofdstukken worden hieronder nader beschreven.

Het doel van **hoofdstuk 2** was om de huidige beschikbare literatuur over TWS degeneratie bij honden te beschrijven. De bedoeling van dit onderzoek was om de kennis van het proces dat betrokken is bij TWS degeneratie bij honden te vergroten. In dit hoofdstuk worden ook de verschillen van TWS degeneratie tussen CD en NCD rassen uitgelicht. Het degeneratieve prcoes in CD en NCD rassen bleek uiteindelijk meer vergelijkbaar te zijn dan voorheen werd aangenomen.

Het doel van **hoofdstuk 3** was om middels een groot populatieonderzoek, het inzicht over de leeftijd en ras van de TWS degeneratieve ziekten in de verschillende honden soorten te vergroten. De levensduur prevalentie van TWS degeneratieve ziekten bij honden onder de leeftijd van 12 jaar bleek 3,5% van de Zweedse honden populatie te zijn. De mortaliteit van deze ziekten bleek 34% te zijn en is aanzienlijk hoger (> 50%) in sommige grote honden, zoals bij de Doberman en de Duitse Herdershond. Hoewel aandoeningen van de TWS bij veel hondenrassen voorkwamen, waren de CD rassen en een klein aantal van de NCD rassen sterk oververtegenwoordigd. Dit kan duiden op een sterke genetische component in de pathogenese van deze ziekten bij de hond.

Het doel van **hoofdstuk 4** was om te boordelen aan de hand van *magnetic resonance imaging* (MRI) beelden of het humane classificatie schema van TWS degeneratie volgens Pfirrmann ook van toegepast kan wordenbij zowel CD als NCD honden. Daarnaast was

het doel om het Pfirrmann systeem te valideren voor het gebruik bij honden door het bepalen van de inter-en intraobserver overeenkomst, en de overeenkomst tussen Pfirrmann scores en biologische factoren waarvan bekend is dat deze de mate van TWS degeneratie verhogen. De inter-en intraobserver betrouwbaarheid van het gebruik van het Pfirrmann systeem bij honden was erg hoog met κ scores variërend tussen 0.81 en 0.93. Bovendien was een hogere graad van degeneratie significant gecorreleerd met het CD fenotype en met oudere leeftijd. Op basis van deze resultaten concluderen we dat de indeling volgens Pfirrmann kan worden gebruikt om TWS degeneratie te evalueren bij CD en NCD hondenrassen.

De doelstellingen van **hoofdstuk 5** waren om het Thompson systeem te valideren voor macroscopische pathologische veranderingen van TWS degeneratie bij honden en om de overeenkomst tussen pathologische bevindingen en MRI bevindingen te onderzoeken. Deze studie toonde aan dat het Thompson systeem een betrouwbare methode is om TWS degeneratie bij de hond te graderen met een hoge inter-en intraobserver overeenkomst.Verder was de overeenkomst tussen macroscopische indeling van de tussenwervelschijfsegmenten volgens Thompson en de indeling van lage-veldsterkte MR beelden volgens Pfirrmann substantieel, hetgeen erop wijst dat de lage-veldsterkte MRI kan worden gebruikt om TWS degeneratie bij honden te diagnosticeren. Gedegenereerde TWSs worden ook vaak aangetroffen bij asymptomatische honden en het beoordelen van de gedegenereerde TWSs op MRI dient altijd gecombineerd te worden met de resultaten van het klinische en neurologisch onderzoek.

In **hoofdstuk 4 en 5** werden TWSs van zowel CD en NCD honden gevonden in alle graderingen van degeneratie, waaruit blijkt dat het morfologische proces van degeneratie vergelijkbaar is bij de twee types hondenrassen.

Het doel van **hoofdstuk 6** was om te onderzoeken of spontane TWS degeneratie in CD- en NCD rassen kan worden gebruikt als translationeel model voor onderzoek naar humane lumbale TWS degeneratie. Dit is getracht door een vergelijking te maken tussen de morfologische verschijning, histologische structuur en biochemische eigenschappen in verschillende stadia van TWS degeneratie bij honden en mensen. Er zijn veel overeenkomsten tussen de TWS degeneratie bij mensen en CD- en NCD hondenrassen. Beide honden types kunnen dienen als spontane TWS degeneratie model voor humaan TWS onderzoek. Echter, wanneer de hond gebruikt wordt als model voor TWS onderzoek ten behoeve van de mens is het van belang zowel de specifieke inter-species

verschillen te erkennen als het verschil van TWS degeneratie tussen de CD en NCD honden (vroeg versus later begin van degeneratie).

Het doel van **hoofdstuk 7** was om een translationeel onderzoek uit te voeren waarbij een nucleus pulposus prothese (NPP) van een nieuw type hydrogel, die uiteindelijk bestemd is voor klinisch gebruik bij de mens, ex vivo werd getest in lumbosacrale segmenten (L7-S1) van hondenruggen. Een klinisch aangepaste wijze van implanteren van de NPP in de nucleaire holte van het L7-S1 segment werd onderzocht. Zwelling, opvulling en het herstel van de hoogte van de TWS door de NPP *in situ* werd beoordeeld met radiografie, Computed Tomography en MRI. Uit deze studie werd geconcludeerd dat de hond voor een dergelijk onderzoek uitstekend kan worden gebruikt als translationeel model. Tevens werd geconcludeerd dat de nieuwe NPP klinisch waardevolle eigenschappen heeft, dat wil zeggen, intrinsieke radiopaciteit en de mogelijkheid *in situ* te zwellen en om de nucleaire ruimte op te vullen. Deze eigenschappen maken de NPP potentieel geschikt voor klinisch gebruik, niet alleen bij de mens, maar mogelijk ook bij de hond. Echter, voordat *in vivo* testen kunnen plaatsvinden, zijn fysisch-mechanische verbeteringen aan de hydrogel NP prothese nodig om fragmentatie te voorkomen en is er een verbeterde techniek nodig om de annulus fibrosus af te sluiten om zodoende implantaatmigratie te voorkomen.

De resultaten van alle studies werden samengevat en besproken in **hoofdstuk 8** en het proefschrift werd afgesloten met samenvattingen in het Engels, Nederlands en Zweeds **hoofdstuk 9**.

Belangrijkste bevindingen

- De klassieke tweedeling van TWS degeneratie hij de hond in kraakbenige of fibroide degeneratie lijkt onjuist te zijn. De pathologische veranderingen met betrekking tot biochemische, histopathologische en morfologische veranderingen tijdens het proces van TWS degeneratie bleken vergelijkbaar in CD en NCD honden. Aangezien er bewijs lijkt te zijn dat een genetische oorzaak in de CD honden en een meer multifactoriële etiologie, waaronder 'slijtage', de onderliggende oorzaak is bij de meeste NCD honden, kan er beter onderscheid worden gemaakt op basis van een verschil in etiologie van de ziekte.

- Aandoeningen van de TWS bij honden hebben een prevalentie van 3,5% bij honden vóór de leeftijd van 12 jaar en komen het meest voor bij CD honden (vooral Teckels) en komt 1.5 x vaker voor bij mannelijke dan bij vrouwelijke honden. De mortaliteit van de TWS degeneratieve ziekten bleek hoger te zijn dan eerder werd gesuggereerd met 34% in de totale honden bevolking, ongeveer 20% in de meeste CD rassen en meer variabel in de NCD rassen met meer dan 50% in de rassen welke het hoogste risico lopen op TWS ziekten.

- Het Thompson graderingsysteem is een betrouwbare methode is om macroscopische TWS degeneratie bij de hond in te delen. De overeenkomst tussen de macroscopische indeling van TWS degeneratie volgens Thompson en de indeling van lage-veldsterkte MR beelden volgens Pfirrmann was substantieel. Dit wijst erop dat de lage-veldsterkte MRI kan worden gebruikt om nauwkeurig TWS degeneratie bij honden vast te stellen.

- De processen van TWS degeneratie bij de mens en de CD en NCD honden zijn in grote mate identiek. Beide types hondenrassen kunnen dus dienen als translationeel spontaan TWS diermodel voor humaan TWS degeneratie onderzoek. Dit biedt diverse mogelijkheden voor het gebruik van deze twee diermodellen die gekenmerkt zijn door vroege (CD) versus late (NCD) TWS degeneratie.

- Veterinaire patiënten die lijden aan aandoeningen van de TWS kunnen gebruikt worden voor preklinische proeven en ook om het proces van de TWS degeneratie te bestuderen. Dit zou kunnen leiden tot nieuwe behandelingen voor zowel honden en mensen, een reductie van het aantal proefdieren en lagere kosten van het onderzoek. Het is ook waarschijnlijk dat spontane TWS degeneratie bij honden het ware ziekte proces bij mensen meer nabootst dan de geïnduceerde TWS degeneratie bij proefdieren.

Sammanfattning av avhandlingen

Ryggproblem är vanligt förekommande hos både människor och hundar och är ofta associerade med degeneration av intervertebral diskerna (IVD). IVD är lokaliserade mellan ryggkotorna och de utgör en essentiell del av ryggraden där de bidrar till både stabilitet och flexibilitet. Degeneration av IVD kan leda till både diskbråck och instabilitet av ryggraden. Det finns olika orsaker till att diskerna i ryggen börjar degenerera, men förslitning, trauma och ärftliga faktorer tros vara de vanligaste. Sjukdomar associerade med degeneration av IVD ses hos de flesta hundraser men de är särskilt vanligt förekommande hos chondrodystrofa (CD) hundraser och några få icke-chondrodystrofa (NCD) hundraser som Dobermann och Schäfer.

Trots att många studier har gjorts under de senaste 60 åren av sjukdomar associerade med IVD-degeneration hos hundar så är förhållandevis lite känt om sjukdomsprocessen då de flesta studierna har inriktat sig på diagnostik och behandling istället för de underliggande orsakerna. Avsevärt mer är känt om degeneration av IVD hos människor, men trots att sjukdomspresentation, diagnos och behandling är väldigt likartade mellan diskrelaterade sjukdomar hos hundar och människor har få komparativa studier gjorts. Innan resultat från humana studier pålitligt kan tillämpas på hundar – eller tvärtom – måste grundläggande komparativa studier utreda likheterna och skillnaderna mellan den degenerativa processen av IVD hos hundar och människor.

Målsättningen för den här avhandlingen var att öka kunskaperna om den degenerativa processen av IVD hos hundar med tyngdpunkt på den morfologiska processen samt demografin av dess associerade sjukdomar. Målet var också att utvärdera om graderingssystem för IVD:s degeneration, som används för människor, kan användas även för hundar.

Målsättningen var även att utvärdera skillnader och likheter mellan den degenerativa processen av IVD hos hundar och människor, för att utvärdera om naturligt förekommande degeneration av IVD hos både CD och NCD hundraser kan användas som modeller för human forskning. Det finns tre anledningar att använda hundar som modell för human forskning av IVD:

- För att utveckla nya behandlingsmetoder för ryggproblem hos människor, orsakade av degeneration av IVD, behövs relevanta djurmodeller.

- Nya behandlingsmetoder för ryggproblem hos människor, testade på hundar, kan även komma hundar som veterinära patienter till nytta.
- Studier och kliniska prövningar kan till del utföras på hundar som är veterinära patienter istället för försöksdjur, vilket därigenom kan minska antalet försöksdjur som används till forskning av ryggproblem.

De två hypoteser som testats i avhandlingen är: 1) Den degenerativa processen av IVD som ses i CD och NCD hundraser är väsentligt mer likartad än vad som tidigare beskrivits, med den tydliga skillnaden att degenerationen börjar vid en lägre ålder och i samtliga IVD samtidigt hos CD hundraser. 2) Naturligt förekommande degeneration av IVD hos både CD och NCD hundraser kan användas som modeller för human forskning.

De viktigaste resultaten från den här avhandlingen var:

- Uppdelningen mellan chondroid och fibroid degeneration av IVD som tidigare beskrivits i den veterinärmedicinska litteraturen förefaller vara inkorrekt. De biokemiska, histologiska och morfologiska förändringarna i den degenerativa processen av IVD som studerats i den här avhandlingen var likartade i diskerna från CD och NCD hundraser. Det förefaller dock att degenerationen av IVD har olika etiologier hos de olika hundtyperna, med genetiskt betingad degeneration hos de CD hundraserna medan förslitning är en mer vanligt förekommande orsak hos de NCD hundraserna.
- Sjukdomar associerade med degeneration av IVD diagnosticeras hos 3,5% av den svenska hundpopulationen yngre än tolv år. Dessa sjukdomar sågs oftast hos CD hundraser och var särskilt vanliga hos taxar. Hanhundar drabbades 1,5 gånger oftare än tikar. Dödligheten av hundar som diagnosticerats med sjukdomar associerade med degeneration av IVD var 34% i hela hund populationen, ca 20% hos de CD hundraserna och mer varierande hos de NCD hundraserna med över 50% hos de mest drabbade raserna såsom Dobermann och Schäfer.
- Tidig degeneration av IVD hos hundar kunde med hög säkerhet diagnosticeras med hjälp av lågfältsmagnetröntgen. Graderingssystemet för förändringar av degenererande IVD, sett på magnetröntgen, som används inom humanvården kunde tillämpas på hundar och hade hög korrelation med patologiska förändringar funna *post mortem*. Även det human-medicinska graderingssystemet för patologiska förändringar av degenererande IVD, enligt Thompson *et al.* kunde tillämpas med hög säkerhet på IVD från hundar.
- Många likheter fanns mellan den degenerative processen av IVD som ses hos både CD och NCD hundraser, och den hos människor. Både typerna av

hundraser kan således användas som modeller för naturligt förekommande degeneration av IVD för human forskning.

- Kirurgisk implantering av en ny nucleus pulposusprotes tillverkad av en röntgentät hydrogel, framför allt avsedd för humant bruk, testades i en *ex vivo* studie i lumbosacrala disker från hundar. Kirurgisk implantering via en dorsal laminektomi av lumbosacraldisken var kliniskt applicerbar hos hund och kan potentiellt användas även för att behandla veterinära patienter som lider av lumbosacral instabilitet. Protesen var väl synlig både i röntgen, datortomografi och magnetröntgen och orsakade inga artefakter av bilderna. Efter att nucleus pulposusprotesen absorberat vätska från omliggande vävnad, fyllde den upp tomrummet i diskerna orsakat av nuclectomierna och återställde ett normalt anatomiskt avstånd mellan ryggkotorna.
- Veterinära patienter (hundar) som drabbats av ryggproblem associerade till degeneration av IVD kan användas för att studera den degenerativa processen som leder till klinisk sjukdom. De kan även inkluderas i prekliniska studier för att, longitudinellt, utvärdera nya behandlingar för humant bruk. Synergieffekter av att utföra studier på veterinära patienter kan förhoppningsvis leda till nya och bättre behandlingar för ryggproblem hos både hundar och människor, ett minskat behov av försöksdjur samt minskade forskningskostnader. Det är även sannolikt att sjukdomsprocessen hos veterinära patienter med naturligt förekommande degeneration av IVD är mer lik sjukdomsprocessen som uppstår hos människor än inducerad degeneration av IVD hos de försöksdjur som oftast nyttjas idag.

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