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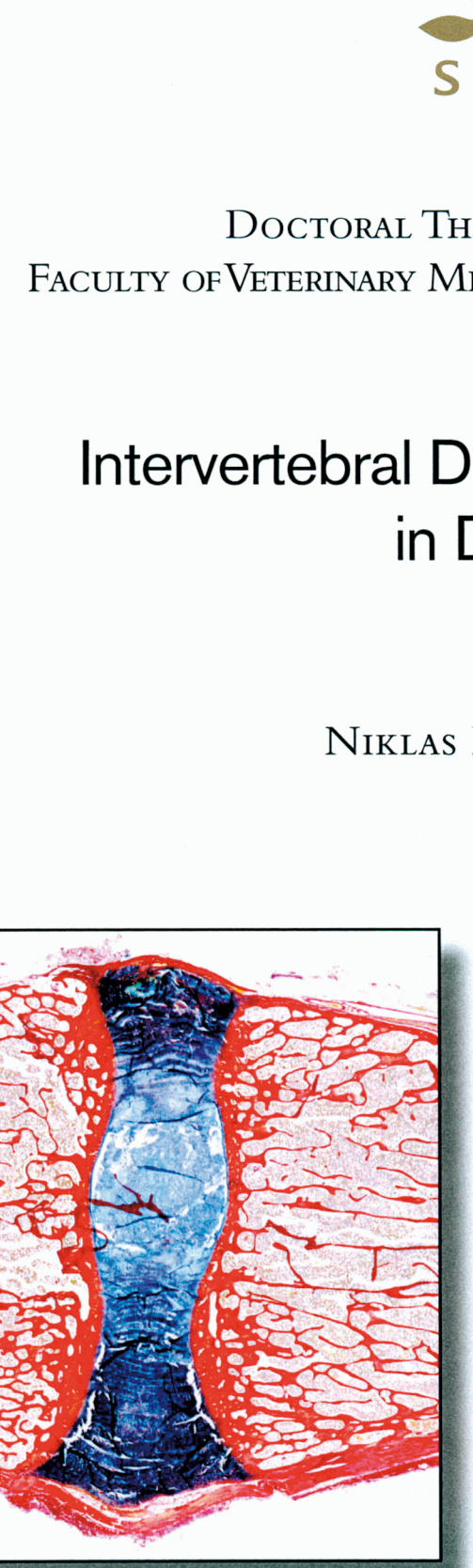

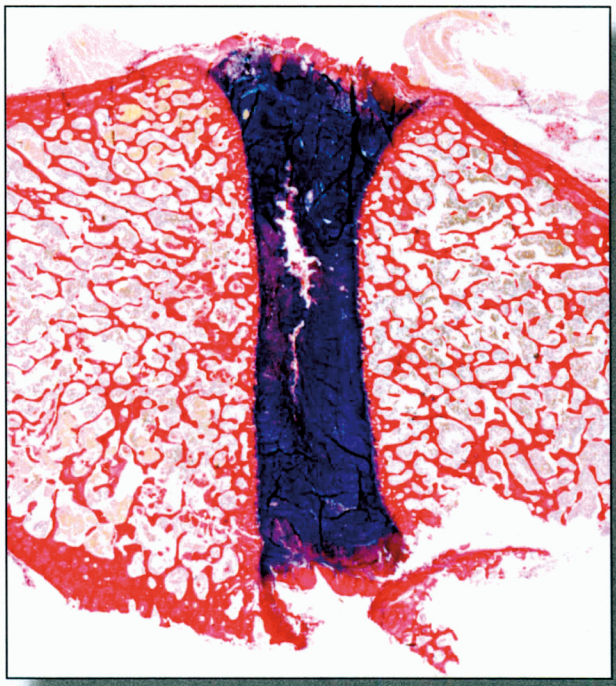

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Intervertebral Disc Degeneration in Dogs

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Dedication

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

Intervertebral Disc Degeneration in Dogs

Tussenwervelschijf Degeneratie bij de Hond
(met een samenvatting in het Nederlands)

Intervertebral Diskdegeneration hos Hund
(med en sammanfattning på Svenska)

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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 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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Back pain related to intervertebral disc (IVD) degeneration is common in both dogs and humans. In this thesis IVD degeneration was studied in both species. The results show that translational studies between dogs and humans are possible which may lead to synergistic effects, resulting in similar and novel treatment modalities for dogs and humans, a reduced need for animal testing, and lower cost of research. Spontaneous IVD degeneration in dogs may also be a better animal model than induced IVD degeneration in experimental animals.

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