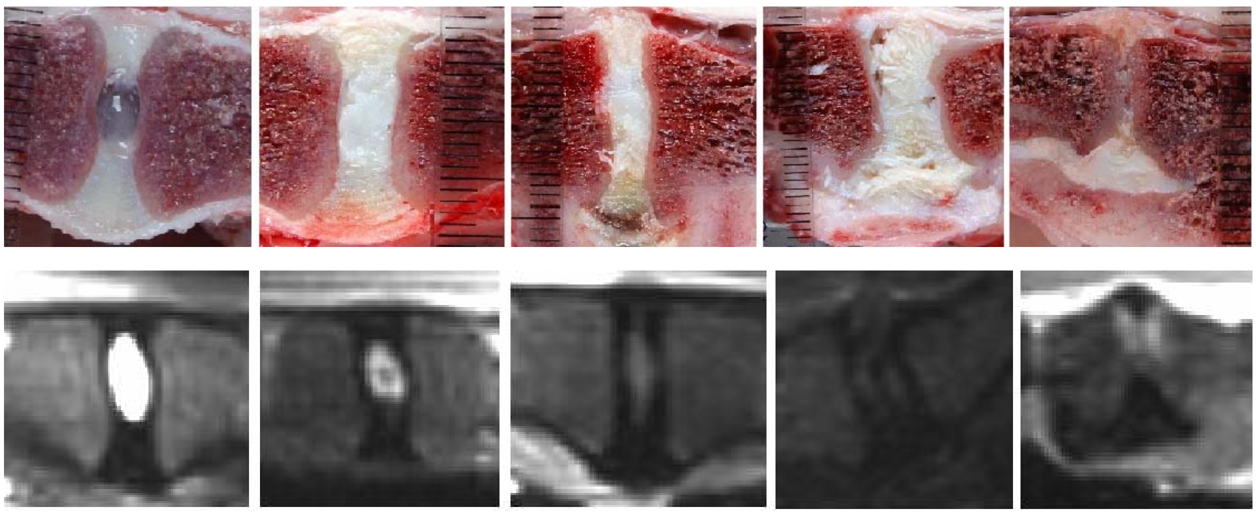
Validation of the macroscopic scoring scheme according to Thompson for pathological changes in intervertebral disc degeneration in canine cadaveric spines and correlation with imaging findings using low field magnetic resonance imaging



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Prefatory note

Every student studying Veterinary Medicine at the University of Utrecht has the opportunity to fulfil a research project after their doctoral exam, eventually succeeding this part in order to graduate for their veterinary exam. This research project: "Validation of the macroscopic scoring system according to Thompson for pathological changes in intervertebral disc degeneration in canine cadaveric spines and correlation with imaging findings using low field magnetic resonance imaging", is carried out by drs. E.B.Pickee as a cooperative effort of the departments of pathobiology and clinical sciences of companion animals at the faculty of Veterinary Medicine at the Utrecht University.
This project is a part of a larger project about the understanding of intervertebral disc degeneration in dogs which is performed by veterinarian and PhD student Niklas Bergknut. Supervision was performed by veterinary pathologist Dr. Guy Grinwis, veterinary neurosurgeon Dr. Björn Meij and veterinarian/PhD student drs. Niklas Bergknut.

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Summary

Intervertebral disc degeneration (IVDD) is a common cause of chronic back pain in humans and dogs. In human pathology the five-category grading scheme for gross pathological changes, according to Thompson (1), is most often used as the golden standard in IVDD research. The aim of this study was to validate the Thompson scheme in canines and to correlate the findings on gross pathology with imaging findings using low field (0.2 Tesla) magnetic resonance imaging (MRI).

A total of 183 intervertebral segments, obtained from 19 randomly selected dogs older than one year of age, euthanized for various unrelated reasons were used for this study. Sagittal T2-weighted MRI of the thoracic and lumbar spine was performed within 24 hours post mortem. Immediately after MRI the spines were cut in the mid-sagittal plane and high resolution photographs were taken of each intervertebral segment (endplate-disc-endplate). The MR images and the photographs were entered into a computer program enabling randomized and blinded scoring by four individual scorers. The 183 segments were macroscopically scored according to Thompson (1). The MR images were scored according to the grading system by Pfirrmann (2). Cohen's weighted kappa analysis was used for the inter- and intra-observer agreement of the Thompson score (resp. κ 0.90, and κ 0.833) and for the correlation between the results of MRI and macroscopic scoring (κ 0.70). It is concluded that the Thompson score can be used for grading canine IVDD with a high inter- and intraobserver agreement. Correlation between macroscopic grading of spine segments according to Thompson and grading of low field MR images according to Pfirrmann was substantial and therefore low field MRI can be used for clinical diagnosis of canine IVDD although there are some limitations.

1. Introduction

1.1 Relevance and prevalence

Intervertebral disc degeneration (IVDD) is a common problem in both humans and dogs and it is one of the most common causes of euthanasia in dogs under the age of 10 years. It is a problem that has very large socio-economical effects. In humans lower back pain alone has a lifetime prevalence of 60 to 80% (3). Diseases which are associated with intervertebral disc degeneration impose an economic load which is similar to coronary heart disease and exceeds that of other important health problems such as diabetes, Alzheimer's disease and kidney diseases (4). This makes it one of the largest human health issues in the western world and its costs, only in the UK, amounts to about £12 billion a year (5, 6).
In canines, disc problems are most commonly seen in chondrodystrophic breeds with a genetic defect resulting in abnormal chondrocyte proliferation. The dachshund and basset hound are classified as chondrodystrophic and account for 45% to 70% of all canine IVDD cases (7). Intervertebral disc degeneration predisposes the canine intervertebral disc to protrusion-extrusion injury, which can result in damage to the spinal cord. Such occurrences are relatively common and the resultant neurological consequences, which have a reported incidence rate of 23 cases per 1000 dogs, are some of the most frequently encountered neurological disorders in veterinary practice (8).

1.2 The canine as a model

Much research has been done concerning intervertebral disc degeneration in humans and animals. Typically animal models are often used to study intervertebral disc degeneration. These animal models are essential in making the transition from scientific concepts to clinical applications. The animal model must be ethical, controllable, reproducible and cost-effective and most important, the animal model must be similar in nature to the human pathological process (9). The structure, maturation and degeneration of canine intervertebral discs resemble those of humans (31). Very important variables in comparing discs are disc size and cell type. Size affects rates of biologic processes (32) where organisms tend to maximize surface area to increase metabolic capacity, and keep the distances small to optimize diffusive transport (30). In contrast with other animal models the canine disc resembles the human disc in shape and size and is therefore a suitable model to study disc degeneration and, in particular, acute degenerative changes of the disc. There are however some important differences between the canine and human intervertebral discs. In humans there is only one common form of IVDD; the fibrinoid disc degeneration. In dogs however, two types of IVDD can be distinguished. These are the chondroid and the fibrinoid intervertebral disc degeneration, respectively most often causing Hansen type I and Hansen type II disc herniation. Chondroid disc degeneration is seen only in chondrodystrophic dogs and humans with pituitary dwarfism. Fibrinoid disc degeneration is mostly seen in dogs, commonly over seven years of age, and humans with a normal bone structure (nonchondrodystrophic), it is then referred to as "normal senile remodelling" (11). Chondrodystrophic breeds are characterized by a disturbance of epiphyseal chondroblastic growth referred to as achondroplasia. The achondroplasia is believed to be responsible for an early onset of disc degeneration, as reflected by chondroid transformation of the nucleus pulposus before the age of one (12). In humans, aging and degeneration of the intervertebral disc has been studied frequently. It was only when the five-category grading scheme according to Thompson was developed, that a golden standard was set and interpretation of these studies was possible. In canines such a golden standard has not been established. In most of the previous studies of IVDD in dogs, histology, myelography, and radiography have been used, but in most studies degenerative changes are termed differently, making it hard to compare results from various investigators (13). The validation of the reproducibility of an animal model allows results from different scientific researchers to be compared (9).

In this project it was attempted to randomly score ten intervertebral discs (T11-S1), of each of 19 nonchondrodystrophic and chondrodystrophic dogs, using the macroscopic grading scheme according to Thompson and the MRI based grading system according to Pfirrmann (1,2). By analyzing the scoring data obtained from the gross morphology scoring using the human grading scheme according to Thompson (1), of the intervertebral discs of the 19 dogs performed by the four observers, it is possible to determine if this scoring scheme can also be applied to canines, which can then be used as a golden standard for future studies. The applicability of the MRI based grading system according to Pfirrmann on canines has recently been investigated, and the outcome was positive with a high inter- en intra-observer agreement (29). The outcome of both scoring schemes will also be statistically compared to see if they correlate and to which extent. If the systems can be applied to canines, it will also make it easier to use canines as an animal model for human intervertebral disc research.

1.3 The intervertebral disc

1.3.1 Development

In the embryonic stadium, the intervertebral discs arise from both the mesenchyme and the notochord. During embryogenesis, the mesemchymal cells surround the notochord, these mesemchymal cells will differentiate into the annulus fibrosus, which is made of fibrocartilage, and the vertebral bodies, which are made of bone. The notochord will virtually disappear in the osseous sites, but inside the primitive annulus fibrosus it will persist, where it is thought that the entrapped notochordal cells synthesize the nucleus pulposus (14). In humans all the notochordal cells become chondrocytes (at the age of 6-10 years) and the disappearance of notochordal cells precedes the onset of disc degeneration, but it remains unclear if the disappearance of these cells might have a role in initiating disc disease (15). However, notochordal cells appear to be specially suited to sustain nuclear volume and inhibit degeneration. An observation commonly seen in animal models is that notochordal cell loss leads to degeneration of the intervertebral disc (7). The content of notochordal cells and chondrocytes and the transition times from notochordal to chondrocytic cells in the nucleus pulposus vary among species (16). Nonchondrodystrophic breeds lose their notochordal cells at the age of 60 months, chondrodystrophic breeds lose their notochordal cells at the age of 12 months (15). The functional significance of notochordal cells in an experimental model of degenerative disease has never been studied directly. As notochordal cells appear to influence the metabolic behaviour of other cell types, it is not known how the presence of notochordal cells may influence the lifespan or biology of chondrocytic, or fibrocytic cells in animal models of disc degeneration (15).

1.3.2 Intervertebral structures

The intervertebral discs lie between the vertebral bodies, linking them together, along almost the entire length of the spinal column (Fig. 1). They are the main joints of the spinal column and represent thirty percent of its height. Only the atlanto-axial joint does not have an interposing intervertebral disc. The dorsal and ventral longitudinal ligaments run over the dorsal and ventral side of the spinal column. When the musculature is removed from the vertebral column, laterally the surface of the intervertebral discs become visible (17) The most important role of the discs is

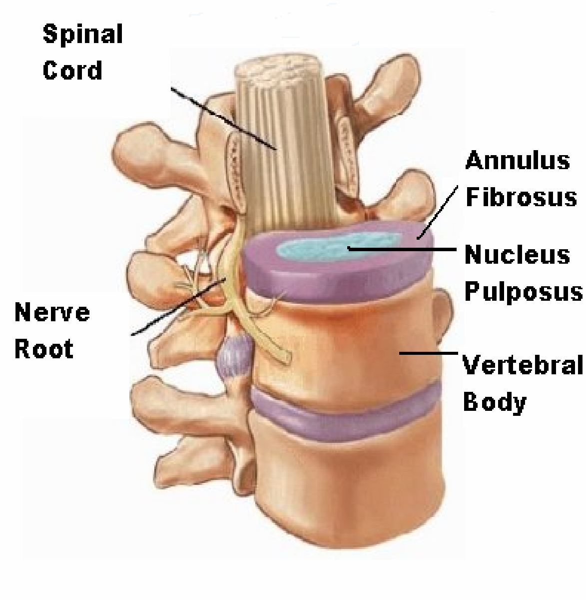

Fig.1 *vertebrae with intervertebral structures*

Bron:http://www.wolfhouse.dk/articles/images/img_fce_figure1.jpg mechanical, for they constantly have to transmit loads arising from the muscle activity and body weight through the spinal column. On one hand the intervertebral structures have to be able to resist and impart stability against deforming loads, but they will still have to allow the spine to be flexible. When the spine is cut in the sagittal plane, three important structures which form the intervertebral disc can be distinguished. These are a gelatinous core, known as the nucleus pulposus, which is surrounded by a thick outer ring of concentric fibrous cartilaginous lamellae named the annulus fibrosus. These two structures are on the cranial and caudal side sandwiched by the cartilage end-plates, which are connected to the vertebral bodies (18, 19).

1.3.3 Nucleus pulposus

The nucleus pulposus is synthesized by the remaining notochordal cells inside the annulus fibrosus. In the young animal, the content of the nucleus is 80% to 88% water giving the nucleus its gelatinous and translucent appearance. The water in the nucleus is attracted by, and linked to an important component of the ground substance, these are large molecules known as proteoglycans. Each proteoglycan is a complex molecule formed by a protein backbone and many subunits know as glycosaminoglycans. These glycosaminoglycans side chains are highly negatively charged, because of this charge they strongly reject each other and form a ninety degree angle with the backbone, this is why the proteoglycan assumes the characteristics of a bottle brush (figure 2) (17). The same high negative charge creates a significant osmotic gradient which attracts and binds water molecules into the wide spaces around the glycosaminoglycan molecules. The importance of this osmotic gradient is very high, for there are no blood vessels in the intervertebral disc making the intervertebral disc the largest avascular structure of the body. Thus the discs are solely dependent of nutrients carried by the water which is transported due to the osmotic gradient and the hydro-static pressure of the disc. There are two routes for nutrient supply into the disc, via the endplate and the annulus periphery, the endplate route being the far most important one (20). Although the nucleus exist almost entirely out of ground substance, different cell types can also be found (chondrocytes, fibrocytes, and notochordal cells), which prevalence is depending on the age of the disc. In the young disc, the chondrocyte is the most present cell type, and in the older disc the fibrocyte becomes more common (17).

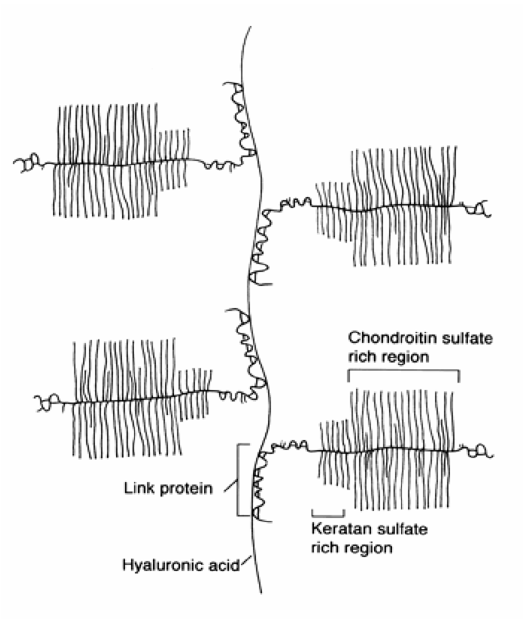

Fig.2 *proteoglycan, bottle-brush*

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1.3.4 Annulus fibrosus

The annulus fibrosus arises from the embryonic mesenchyme. It is a fibrocartilage structure that envelops the nucleus pulposus. When the annulus is viewed in transverse section, concentric rings of fibrous tissue can be seen, which entirely encircle the nucleus pulposus (Fig. 3). When transected in the sagittal plane, the fibrous rings will give the annulus a banded appearance. When viewed under a microscope, these bands are seen as layers of complex fibrocartilaginous lamellae, composed of many parallel situated fibrous bundles. The fibrous lamellas are thoroughly connected to the cartilaginous endplates and its accompanying vertebrae, surrounding the nucleus from one vertebra to the next. Each lamellae has a different directional arrangement; this characteristic determines the mechanical features of the disc (21). In human lumbar discs, 15 to 38 concentric layers have been described, in canines this is unknown. Fifty percent of these fibrous rings do not completely surround the nucleus pulposes, this percentage is even rising with age. The openings of these incomplete formations are mostly situated on the dorsolateral side of the annulus fibrosus, causing a natural weakness at this area. Almost the entire annulus is composed of fibrous tissue, in which long typical fibrocytes are located, who maintain the tissue producing mainly collagen type I. Innervation of the intervertebral disc in humans is provided by branches from the sinuvertebral nerve, in canines these nerves have not been found. In both humans and canines the inner part of the annulus and nucleus are not innervated. In the outer part of healthy, human annulus tissue some nerve-endings can be found in contrast to the canine annulus which is not innervated. The canine dorsal longitudinal ligament however, is innervated in contrast to its human equivalent (17).

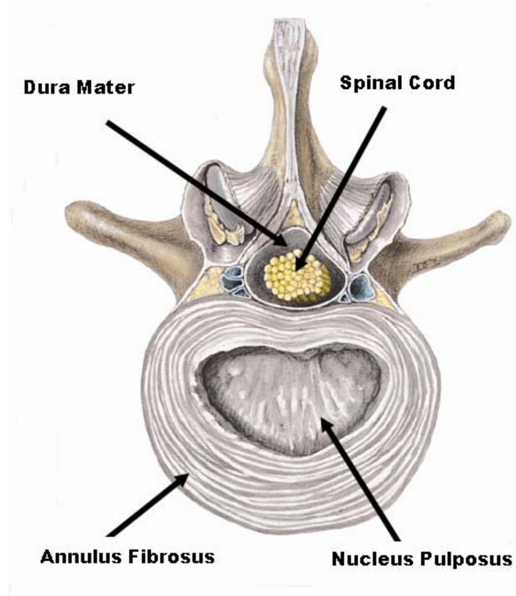

Fig. 3 Concentric layered annulus
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1.3.5 Hyaline cartilage end-plates

The cartilaginous end-plate is a very thin layer of hyaline cartilage, with fibres running parallel and horizontally (19). It represents the caudal and cranial component of the intervertebral disc. The end-plates connect the intervertebral discs to the associated vertebral bodies. The cartilaginous plate is about 1-to-2 mm thick at the periphery, but towards the centre of the disc it becomes very thin and barely visible. In the centre of each end-plate, where the nucleus normally is situated the plate is slightly concave to ensure an optimal surrounding of the nucleus (17). It is well known that the cartilaginous end plate is an important structure concerning the nutrition of the intervertebral disc. This important function is obvious when realizing that a fall in nutrient supply to the intervertebral disc is associated with disc degeneration (20). Because there are no blood vessels supplying oxygen and nutrients directly to the intervertebral discs, it gets its nutrients and oxygen solely by diffusion across the surface of the cartilaginous end plates (13). Like other cartilages, the endplate acts as a selectively permeable barrier to solutes letting important nutrients in and waste products out of the disc. These essential nutrients are glucose and substrates for matrix production such as amino acids and sulphate (20).

1.4 Intervertebral disc degeneration

Common features associated with disc degeneration include nuclear dehydration, proteoglycan depletion, diminished cellularity, annular disorganization and focal disruption. The initiating and perpetuating factors are elusive and thought to include cumulative trauma, impaired nutrition, and genetic predisposition, among others (7). The changes that take place in the disc with increasing age are that the nucleus becomes less hydrated and more collagenous, the boundary between nucleus and annulus becomes increasingly vague and the annular rings thicken and appear more disorganized. Eventually cracks, fissures and disruptions appear in the endplate, nucleus and annulus which causes the disc to thin and distort. It is difficult to distinguish aging from pathological changes and at present there are no clear markers, either morphological or biochemical, distinguishing these two processes (28).
Instead of degeneration of the discs it is better to speak of degenerative changes in the discs. The latter describes both the normal senile remodelling and the pathological changes that can occur in the intervertebral disc, as degeneration refers only to the pathological changes. In nonchondrodystrophic dogs those two changes both take place (22). According to Hansen et al. intervertebral disc degeneration in dogs can be divided into two categories. These are the fibrinoid degeneration; this type of degeneration is taking place in the intervertebral disc of nonchondrodystrophic dogs, and the chondroid degeneration, which takes place in the chondrodystrophic dogs (11). Chondrodystrophic breeds are characterized by a disturbance of epiphyseal chondroblastic growth referred to as achondroplasia. The achondroplasia is believed to be responsible for premature disc degeneration, as reflected by chondroid transformation of the nucleus pulposus prior to 1 year after birth (12). Although the process of fibrinoid intervertebral disc degeneration in nonchondrodystrophic dogs closely resembles the degenerative changes in humans (22), the alterations in the discs of chondrodystrophic breeds also show many similarities to the aging pattern reported for the human disc (12). The fibrinoid type of degeneration is characterized by organizational and biochemical changes of the annulus and nucleus and is typically confined to a single disc along the vertebral column**.** The dachshund and basset hound are classified as chondrodystrophic breeds that have, in addition to short limbs, high incidence of disc degeneration and herniation of early onset, accounting for 45% to 70% of all canine cases. In contrast to the nonchondrodystrophic dog, degeneration in chondrodystrophic species occurs simultaneously at all levels with chondroid metamorphosis of the nucleus, matrix disintegration, calcification and

localized areas of cell death (7). Generally, degeneration of the intervertebral disc is associated with a loss of water from the nucleus pulposus, which is the result of a decrease in proteoglycan concentration. The gel like nucleus will function less as a hydraulic cushion because of the loss of water, which will influence the normal function of the vertebral column (22). In particular, nuclear volume is the essential feature of disc health that, when diminished, triggers a number of remodelling events that characterize disc disease (7). With the onset of degeneration more and more collagen is formed in the intervertebral disc, hence it is becoming a consolidated mass. Reorganization takes place and collagen type II is being replaced by collagen type I, the first (type II) especially suitable for dealing with compressive forces and the latter (type II) being better in dealing with tensile forces (22). The degenerative changes eventually result in the alteration in mechanical function of the disc and eventually lead to tissue failure. In the end degenerated nuclear material can burst from the marginal area of the annular rings and press on the spinal cord, or the degenerated intervertebral disc collapses leaving almost no intervertebral disc space (Fig. 4).

There are many causes which can initiate disc degeneration, such as trauma, altered mechanical loads, altered biochemical and nutritional factors, changes in metabolism and cell survival, and predisposing genetic factors. With increasing age comes an increased incidence of degenerative changes, including cell death, cell proliferation, mucous degeneration, granular change and concentric tears. It is difficult to differentiate changes that occur solely due to aging from those that might be considered 'pathological' (23).

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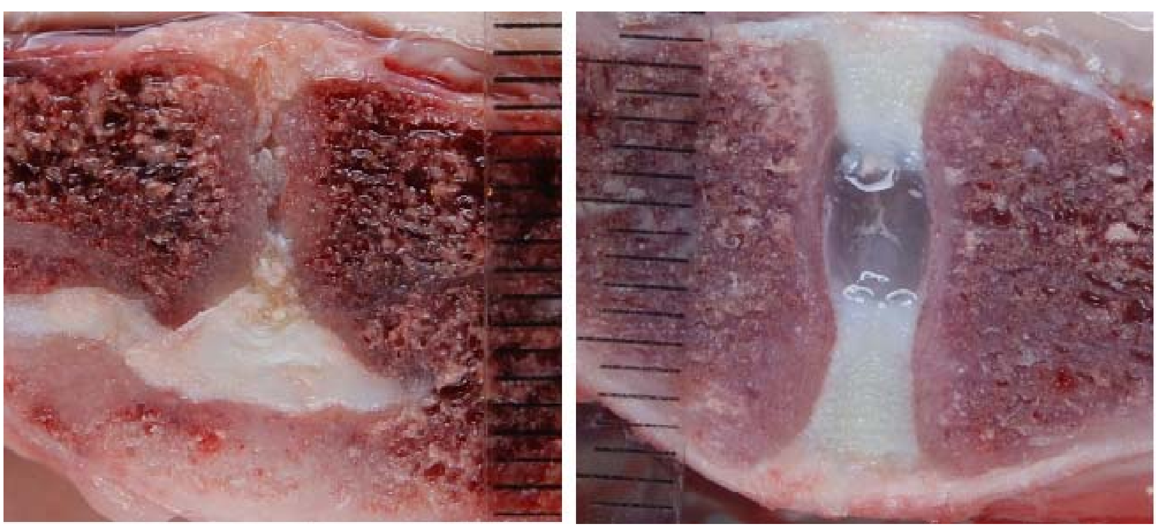

Fig. 4. On the left a Thompson grade V degenerated canine disc, diffuse sclerosis of the endplates and spondylosis can be seen and the disc space is almost entirely collapsed. On the right a healthy Thompson grade 1 intervertebral disc, with a bulging clear nucleus, a concentric layered annulus fibrosus and a uniformly thick endplate without disruptions. Osteophyte formation and subchondral sclerosis are also absent.

2 Materials and Methods

2.1 Collection of the spines

The caudal spines (T11-S1) from 19 randomly selected dogs, of various nonchondrodystrophic and chondrodystrophic breeds, which were older than one year were used. The intervertebral discs between T11 and S1 are most likely to show the different types of degeneration. Therefore, intervertebral discs arising from these segments may provide the widest range of samples (13, 12). The animals used in this study came from different places and were kept for different reasons, 4 of the dogs were companion animals admitted for autopsy at the Department of Veterinary Pathology at Utrecht University, 6 of them were previously used for educational purposes, 3 had been used for research purposes, and the remaining 6 dogs had been used for hunting. None of the animals were euthanized for spine related problems. The spines were sampled no longer than 24 hours post mortem.

2.2 Processing the spines

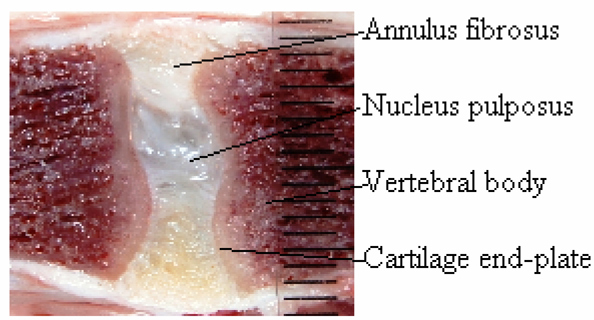

Fig 5. Midsagittal segment consisting of four tissues

The spinal columns were dissected at the pathology division of the department of pathobiology at the Faculty of Veterinary Medicine, Utrecht University. After dissection, the spines were wrapped in moist towels and put into plastic bags to prevent dehydration. The bags were immediately brought to the imaging department, where T2-weighted sagittal MR images were taken. Immediately after MR imaging, the spines were brought back to the pathology department where the vertebral bodies were cut in the midline (sagittal plane) by the use of a belt saw, and high resolution macroscopic photos were taken with the use of a digital reflex camera (Canon, 10 mega pixel) These photos were later used for the gross pathology grading according to Thompson (1). After transection of the spine and photographing, 3-4 mm slices were sawed from the sagittal plane of the spine through the middle of the vertebral body. This resulted in ten 3 to 4-mm-thick segments consisting of the nucleus pulposus, the annulus fibrosus, the cartilage endplates and the 2 halves of the vertebral bodies (Fig 5). These midsagittal segments were then fixated in 4% neutral buffered formalin, decalcified in EDTA and will later be used for histopathological examination and grading according to Boos (24). The histological grading is part of the PhD project performed by drs. Niklas Bergknut. After collecting the spinal segments on one side of the transected spine, the other side was used to gather samples of the nucleus pulposus and annulus fibrosus. Immediately after removing the nucleus it was snap frozen in liquid nitrogen and stored in a minus 80°C freezer for further biochemical analysis.

The snap frozen nuclei are also being used in the previously mentioned PhD study, with the goal to compare IVDD in dogs to the equivalent in humans to find out whether dogs with IVDD could function as a naturally occurring model for human research. This will be done in collaboration with Joost Rutges, MD and PhD-student at the University Medical Center in Utrecht (UMC-U).

2.3 Magnetic resonance imaging of the dissected spines

Immediately after dissection the spines were brought to the radiology department for MR imaging. The MRIs of the spines were performed using a low field 0.2 T open magnet (Magnetom Open Viva, Siemens AG, Utrecht University). The imaging protocol included sagittal T2-weighted spin echo (repetition time [TR] 3835-4450 msec/echo time [TE] 117 msec) images. Sagittal T2-weighted images were used because in this way the changes of the nucleus pulposus were best seen (25). The most important reason for using the T2-weighted images was that in the study by Pfirrmann et al. they also used sagittal T2-weighted images. The obtained MR slices were 3-mm thick, the slice which best depicted the intervertebral discs was used for the Pfirrmann scoring. The MR images were put into a computer program which enabled easy access and scoring according to Pfirrmann (2).

2.4 Photography of the discs

The midsagittal plane was chosen to permit visualization of all (possible) tissues affected by IVDD. Because of the high speed of the band saw, water was poured on the saw, in order to keep the temperature down and prevent burn marks. The water also lubricated the sawing process and drained off the particles which originated from it. After the sectioning, the lateral cut surface was briefly wiped using a wet paper towel in order to remove occasional debris.

After cleansing, the transected spines were placed in the photography room, on a special table with multiple lights illuminating the spine from different directions. High resolution photographs were taken with the use of a digital reflex camera (Canon, 10 megapixel) and two accompanying flashbulbs. The camera was set on auto focus, in order to get the same quality of photographs with each intervertebral segment. Along and parallel to each disc a translucent ruler was placed, which enabled comparing different sized discs of different dogs. The digital photographs were further processed in a computer program (Microsoft Office Picture Manager 2006); the photographs of the segments were modified in such a way that one segment was filling up a whole picture, the pictures have not been processed in any other way, i.e. concerning sharpness or the brightness. In this way the visibility of the discs was excellent, and it was not possible for the observers to see the origin of the discs enabling objective grading.

2.5 Grading of the photographs (gross morphology)

The grading of the photographs was done by a five category grading scheme according to Thompson that was developed for assessing the gross morphology of midsagittal sections of the human lumbar intervertebral disc. Thompson calculated that five was a sufficient number of categories to distinguish the pathologically distinctive features of the specimen. These distinctive pathological features concern changes of the nucleus pulposus, annulus fibrosus, the cartilaginous and bony end-plates, and the periphery of the vertebral body. With this grading scheme, a golden standard for the degree of intervertebral disc degeneration was set.
The five categories according to Thompson are described in Table 1; images of the discs typical of each category are illustrated in Figure 6 (1).

Table 1. Description of morphological grades according to Thompson (1).

Grade	Nucleus	Annulus	End-plate	Vertebral body
I	Bulging gel	Discrete fibrous lamellas	Hyaline, uniformly thick	Margins rounded
II	White fibrous tissue peripherally	Mucinous material between lamellas	Thickness irregular	Margins pointed
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 less than 2 mm
V	Clefts extend through nucleus and annulus		Diffuse sclerosis	Osteophytes greater than 2 mm

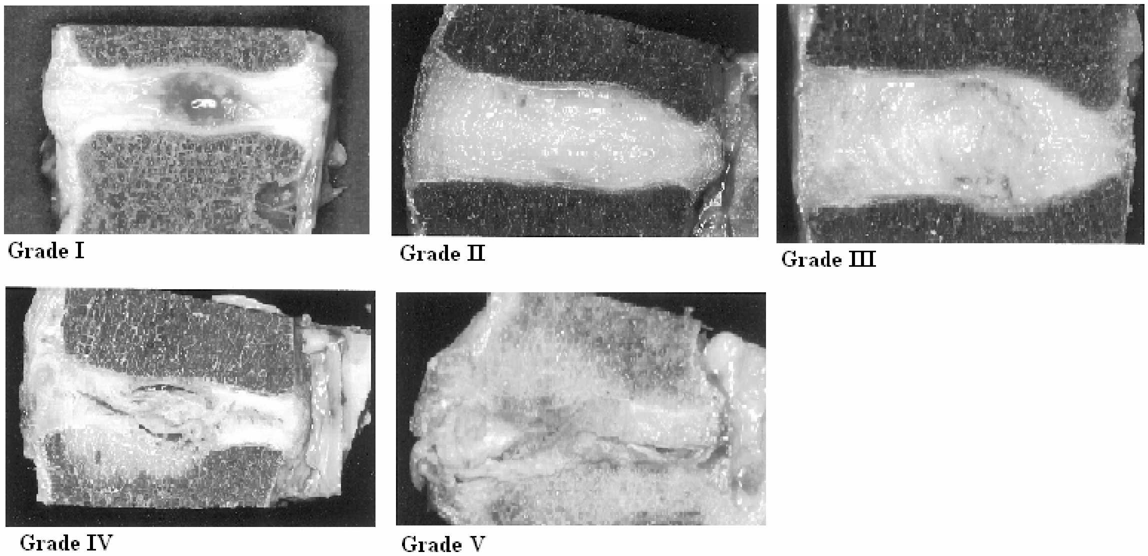

Fig 6. The appearance of typical discs in humans of each grade according to Thompson (1).

The grading was performed by four individual observers; observer A: EP, observer B: NB, observer C: BM and observer D: GG. All the observers read the Thompson article, and used Table 1 and Figure 6 for the grading of the photographs. All observers have a special interest in spinal pathology; a veterinary neurosurgeon (BM), a veterinary pathologist (GG), a PhD student working on IVDD (NB) and a veterinary student (EP). The pictures were presented randomly and in duplicate in the Microsoft program Excel (part of Microsoft Office Professional edition 2003). The photographs in the computer program could be accessed by clicking on a hyperlink, in this way easy access to the photos was possible and blinded and objective scoring was obtained. The individual scores were entered in the program. It was unknown to each observer from where the pictures (segments) originated, the observers were asked to blindly grade the 370 pictures without knowing that they were presented in duplicate. They were presented in duplicate to investigate the intra-observer agreement. The inter-observer agreement was also investigated; this was done by statistically comparing the individual scores of the observers.

2.6 Grading of the magnetic resonance images

The grading of the MR images was done by using a human magnetic resonance classification system for the assessment of lumbar intervertebral disc degeneration according to Pfirrmann. This system is done on T2-weighted magnetic resonance images, and includes 5 categories of the different stages of degeneration seen in human lumbar intervertebral discs. Pfirrmann also developed an algorithm (Fig. 8) which facilitates the grading and classification of disc degeneration.
The grading system and algorithm are based on MRI signal intensity, disc structure, distinction between nucleus and annulus and disc height. The signal loss of the disc on T2-weigthed MRIs correlates with the progressive degenerative changes of the intervertebral disc. The brightness of the nucleus has been shown to correlate directly with the proteoglycan concentration, but not with the water or collagen content (2). On the human side research already has already been done on the correlation between the MRI grading system and the macroscopic grading scheme according to Thompson. It is reported that the MRI grading system is reliable for assessing the macroscopic disc morphology (2).
Recent, yet unpublished research at the university of Utrecht department of veterinary sciences has proven that the human magnetic resonance classification system according to Pfirrmann can be applied when scoring canine IVDD (29).

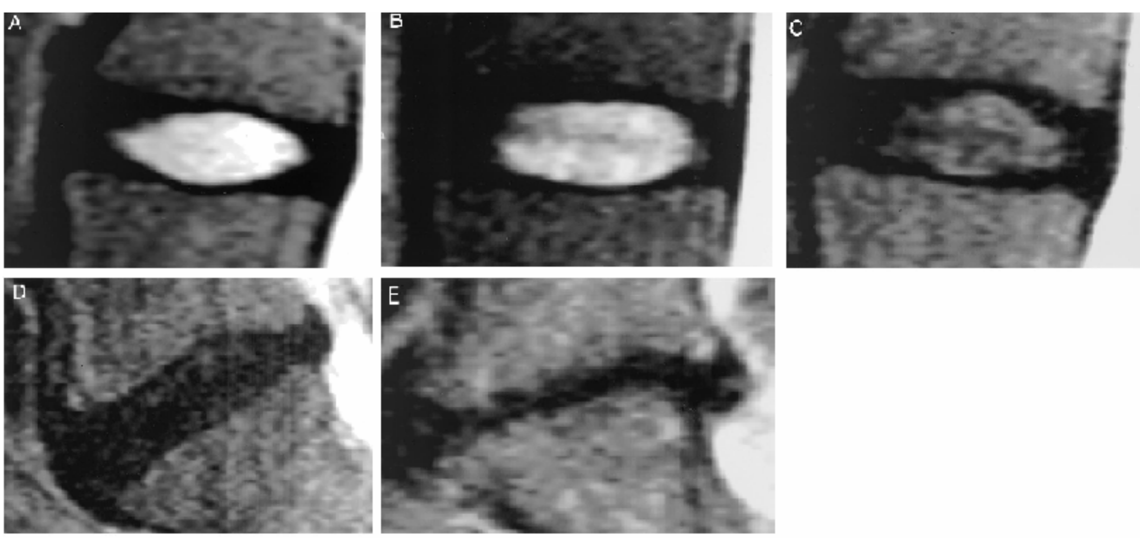

Fig 7. A–E, MRI Grading system for the assessment of lumbar disc degeneration in humans according to Pfirrmann (2).
A=Grade I B=Grade II C=Grade III D=Grade IV E=Grade V

The scoring of the canine MRIs was performed by 4 individual observers, observer A: EP, observer B: NB, observer C: BM and observer D: EA. The observers have a special interest in spinal pathology; a veterinary radiologist (EA), a veterinary neurosurgeon (BM), a PhD student on canine IVDD (NB), and a student in veterinary sciences (EP). Three of the four observers (NB, BM, EP) also graded the photographs according to Thompson. All the observers have read the Pfirrmann article and used Table 2 and Figure 7 for grading the MR images. The MR images were also put in the computer program Microsoft Excel (part of Microsoft Office Professional edition 2003) by the use of a hyperlink, the grades were also entered in the computer program. In order to assure that the right discs were scored, a list with information concerning which discs had to be scored was provided.

Table 2 Description of MRI grades according to Pfirrmann (2).

Grade	Structure	Distinction of Nucleus and Annulus	Signal Intensity	Height of Intervertebral Disc
I	Homogenous, bright white	Clear	Hyperintensive, isointensive to cerebrospinal fluid	Normal
II	Inhomogeneous with of without horizontal bands	Clear	Hyperintensive, isointensive to cerebrospinal fluid	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

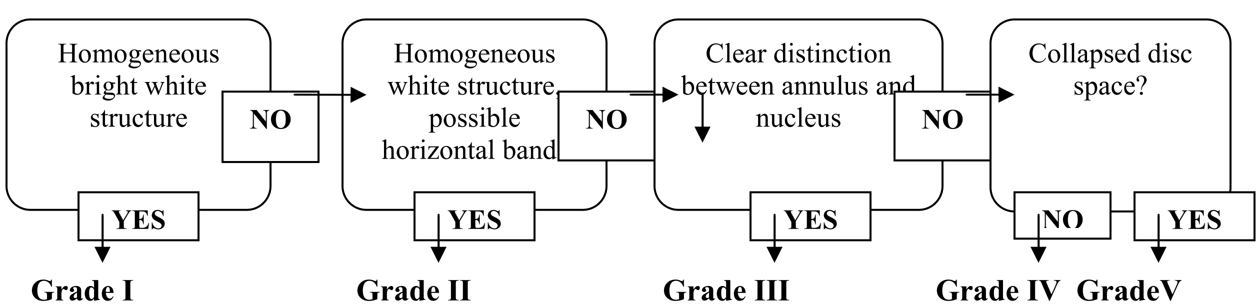

Fig 8. Algorithm for the grading scheme and for the assessment of the lumbar disc degeneration grade according to Pfirrmann (2).

2.7 Analysis of Data

2.7.1 Thompson score

The grades given by the four individual observers to the photographs according to Thompson resulted in eight series of grades given to ten segments per dog over a total of 19 dogs. By calculating the intra- and interobserver reliability of the scoring, something can be said about the applicability of the Thompson grading scheme on canine IVDD. The Thompson scores were statistically analyzed using Cohen`s weighted Kappa. This statistical method calculates the percentage of agreements of the grades given to a picture, corrected by the chance that the same grade is given by coincidence. Weighted Kappa is used, because the grades are classified categories. By using the weighted Kappa, more weight is given to grades that deviate only one grade from the other and less weight is given to grades that differ more than one grade from each other. The weights which were used are; 1.0 for total agreement, 0.94 for 1 grade disagreement, 0.75 for 2 grades disagreement and 0.44 for 3 grades disagreement and 0 for 4 grades disagreement.

2.7.2 Correlation Thompson and Pfirrmann

The outcomes of both grading systems were statistically evaluated using correlation analysis. In order to get a good and reliable comparison between the five grade grading scheme according to Thomson and the five grade grading scheme according to Pfirrmann, the grades given by the observers were averaged.
In the case of the Pfirrmann scores, when at least three of the four observers have given the same grade to an MR image, it was this grade that was used as the mean grade. In the other cases the MR images were reviewed in unison, by at least three observers and a consensus decision was made, in these cases this grade was used as the mean grade. The Thompson scores were also averaged, each disc was graded in a total of 8 times (discs were scored in duplicate), when a clear majority (at least 5) of the grades were the same, this grade was chosen as the mean grade. In all other cases and in the cases where there was a deviation of more than 1 grade between the 4 individual scores, the photographs were reviewed in unison by at least three observers and a consensus decision was made.
The averaged grades given to the photographs and the averaged grades given to the MR images were statistically analyzed, to find the degree of correlation. Cohen`s weighted Kappa (**κ)** was used to calculate this correlation in the same way as is mentioned above.

κ (kappa)			
<.00 <			'poor'
.00	-	.20	'slight'
.21	-	.40	'fair'
.41	-	.60	'moderate'
.61	-	.80	'substantial'
.81	-	1.00	'almost perfect'

Table 3. Interpretation of the Kappa score

3 Results

3.1 Donor animals

As can be seen in Table 4, 12 of the 19 spines originated from female dogs and 7 from male dogs. All dogs were over one year of age, and represent a relatively even distribution up to the age of 16. The dogs that were sampled had different backgrounds. Three dogs were put down for other research related reasons which did not concern the spine or other parts of the locomotory apparatus. Four dogs were patients admitted for autopsy by the university clinic for companion animals and were included after their owners consent, and 6 dogs had been used for educational purposes. The remaining 6 dogs had been used for hunting and were euthanized because of behavioural problems. All the dogs used in this project did not die of, or were euthanized because of diseases relating to the spine.
The vertebral segments that were chosen for this research project were T11 to S1 due to the fact that they are most likely to display a wide variety of degrees of disc degeneration (13, 12).

Dog	Breed	Purpose	Age	Weight	Sex
1	Mongrel	Education	15y4m	13,5 kg	Female
2	Mongrel	Research	1y5m	22,2 kg	Female
3	Beagle	Education	2y1m	11,2 kg	Female
4	Beagle	Education	2y1m	11,0 kg	Female
5	Beagle	Education	2y4m	11,7 kg	Female
6	Mongrel	Research	1y4m	20,0 kg	Female
7	Foxhound	Hunting	7y0m	39,0 kg	Female
8	Foxhound	Hunting	10y0m	44,0 kg	Male
9	Foxhound	Hunting	9y0m	37,0 kg	Female
10	Bouvier	Companion	7y5m	42,0 kg	Male
11	Flatcoated Ret.	Companion	1y0m	18,7 kg	Female
12	Welsh Terrier	Companion	16y0m	8,5 kg	Male
13	Mongrel	Research	1y4m	21,0 kg	Female
14	Kerry beagle	Hunting	3y0m	29,0 kg	Male
15	Kerry Beagle	Hunting	3y0m	29,0 kg	male
16	Kerry Beagle	Hunting	8y0m	31,0 kg	Male
17	Bouvier	Companion	11y10m	43,7 kg	Male
18	Beagle	Education	9y9m	12,1 kg	Female
19	Beagle	Education	10y0m	9,5 kg	Female

Table 4. Breed, age, weight and sex of the dogs.

3.2 Thompson grading

3.2.1 Description of gross morphology changes

Most of the discs photographed were graded Thompson grade I (61/182, 33.5%), II (74/182, 40.7%), and III (27/182, 14.8%), and less Thompson grade IV (16/182, 8.8%) and V (4/182, 2.2%) were given (Table 11). The gross morphology of ten intervertebral discs typical for their grade, used in this study, can be observed in figure 9, accompanied by the averaged grades given. The following intervertebral changes were frequently observed. Nucleus pulposus degeneration was especially visible in the form of consolidation, whitening, the presence of clefts, a decrease in disc height and less apparent distinction to the annulus fibrosus. The degenerated annulus, showed less discrete fibrous lamellas, formation of mucinous infiltration between the lamellas, loss of anular-nuclear demarcation and presence of tears and gaps. End-plate degeneration could be observed as irregular thickness of the hyaline cartilage, sclerosis in the subchondral bone and focal disruptions. Most of the degeneration concerning the vertebral body could be identified as osteophyte formation of different sizes, pointed margins and even bridging and a relatively large amount of spondylosis could be observed. In some cases the presence of spondylosis did not say anything about the degree of degeneration of the intervertebral disc, i.e. there were vertebras with healthy discs showing severe spondylosis.

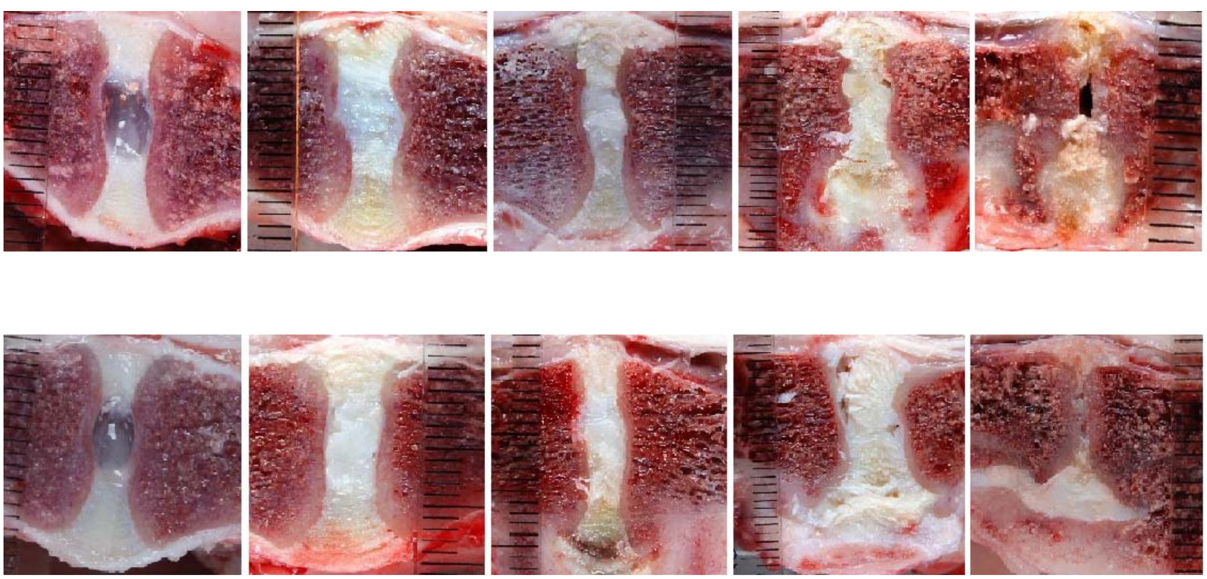

Fig 9. In the top and bottom row intervertebral discs of different canines used in this research are depicted, from left to right; Thompson grade I, II, III, IV and V.

3.2.2 Intra-observer reliability

Each photograph has been scored twice since they were provided in duplicate. The intra-observer reliability has been calculated according to Cohen`s weighted Kappa analysis by comparing these duplicate grades for each individual observer and is expressed in a kappa value. The mean intra-observer reliability ± standard error (SE) for observer A is 0.944 ± 0.065, for observer B 0.909 ± 0.064, for observer C 0.875 ± 0.068, and for observer D 0.882 ± 0.064.

All the observers scored a weighted kappa value of at least 0.875, indicating that the intra-observer reliability is almost perfect in all cases (Table 3). In the pivot Tables 6, 7, 8 and 9 the relation between Thompson grading of photograph 1 and 2 by each observer is visible.

Observer	Occupation
A	Veterinary student (EP)
B	PhD student on canine IVDD (NB)
C	Veterinary neurosurgeon (BM)
D	Veterinary pathologist (GG)

Table 5. Observers Thompson grading scheme.

Thompson score		Observer A Photo 2					
	category	I	II	III	IV	V	total
	I	55	2				57
Observer A	II	2	59	7			68
Photo1	III		4	28	3		35
	IV			2	17		19
	V					4	4
	total	57	65	37	20	4	183

Table 6. Thompson grades given to the in duplicate (photo 1 and photo 2) presented photographs by observer A

Thompson score		Observer B Photo2					
	category	I	II	III	IV	V	total
Observer B	I	55	3				58
Photo1	II	2	65	11			78
	III		3	25	3		31
	IV			4	8	1	13
	V				1	2	3
	total	57	71	40	12	3	183

Table 7. Thompson grades given to the in duplicate (photo 1 and photo 2) presented photographs by observer B

Thompson score		Observer C Photo2					
	category	I	II	III	IV	V	total
Observer C	I	73	10		1		84
Photo1	II	6	25	6			37
	III	1	6	17	4		28
	IV		1	4	10	3	18
	V				2	14	16
	total	80	42	27	17	17	183

Table 8. Thompson grades given to the in duplicate (photo 1 and photo 2) presented photographs by observer C

Thompson score		Observer D Photo2					
	category	I	II	III	IV	V	total
Observer D	I	48	4				52
Photo1	II	7	72	5			84
	III		5	20	5		30
	IV			2	12	1	15
	V			1	1		2
	total	55	81	28	18	1	183

Table 9. Thompson grades given to the in duplicate (photo 1 and photo 2) presented photographs by observer D

3.2.3 Inter-observer reliability

Observer A had the highest intra-observer reliability (kappa value of 0.94) of the four observers. The individual scores of the other observers (B, C, and D) were compared with observer A to calculate the inter-observer reliability. The inter-observer kappa values and standard errors can be observed in Table 10.

Observer	B Photo 1	B Photo2	C Photo 1	C Photo 2	D Photo 1	D Photo 2
A Photo1	**κ** 0.878 SE 0.064		**κ** 0.760 SE 0.068		**κ** 0.831 SE 0.063	
A Photo 2		**κ** 0.864 SE 0.64		**κ** 0.829 SE 0.068		**κ** 0.835 SE 0.063

Table 10. Inter-observer reliability expressed in a kappa value (**κ**) and standard error (SE) of observers B, C and D compared with observer A.

The inter-observer reliability of the observers ranged from 0.760 to 0.878, with an average of 0.833. This indicated that the inter-observer reliability can be defined as almost perfect (Table 3). In the attachment the pivot tables of the inter-observer comparison of the Thompson scores can be seen.

3.3 Pfirrmann grading

<u>3.3.1 Description of MRI changes</u>

Most MR images were graded Pfirrmann grade I (71/182, 39.0%), II (61/182, 33.5%) and III (41/182, 22.5%) and less were graded Pfirrmann grade IV (6/182, 3.3%) and V (3/182, 1.6%) (Table 11). Due to the low field (0.2 Tesla) MR imaging technique the sharpness of the images is not quite optimal, which can be observed in figure 10. Though, most degenerative changes needed for the Pfirrmann grading could be seen. The following degenerative changes in the intervertebral segments were most frequently seen in the MR images. A decrease in nucleus signal intensity, ranging from bright white to black, the hypointensive nuclear signal sometimes gave the nucleus a more or less banded appearance. A decrease in distinction between the nucleus pulposus and the annulus fibrosus were also common observations and a decrease in the height of the intervertebral disc was occasionally seen.

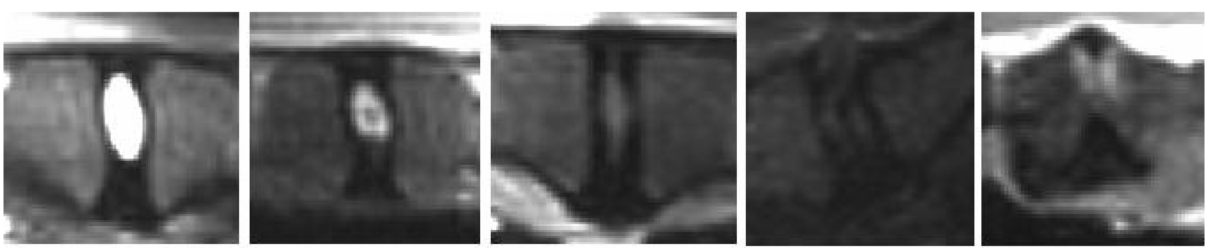

Fig 10. MR images of intervertebral discs of different dogs used in this research, from left to right; Pfirrmann grades I, II, III, IV and V

3.4 Correlation Thompson and Pfirrmann score

The means of the individual scores of the Thompson score and the Pfirrmann score were correlated by the use of Cohen`s weighted kappa. The outcome was **κ** 0.70, indicating a substantial correlation (Table 3). The details can be observed below in Table 11 and Figure 11, which depict the mean grades of the intervertebral discs given by the four observers according to Thompson and Pfirrmann scoring scales. The best correlation is found between Thompson grade I and Pfirrmann grade I, where 50 Pfirrmann grades I were given to 61 Thompson grade I discs. Thompson grade IV showed the lowest correlation with the Pfirmann grades (3/16, 19%) since Thompson grade IV was graded as Pfirrmann grade II (4/16, 25%), III (7/16, 43.8%), and V (2/16, 12.5%) Thus 13/16 (81%) of Thompson grade IV deviated for 1 or 2 grades from Pfirrmann grade IV.

		Averaged Pfirrmann score					
	Category	I	II	III	IV	V	total
Averaged Thompson	I	50(82%)	11				61(33.5%)
	II	20	39(53%)	15			74(40.7%)
	III	1	7	18(67%)	1		27(14.8)
	IV		4	7	3(19%)	2	16(8.8%)
	V			1	2	1(25%)	4(2.2%)
	total	71(39.0%)	61(33.5%)	41(22.5%)	6(3.3%)	3(1.6%)	182

Table 11. Pivot table showing the correlation of the mean Thompson and mean Pfirrmann grades.

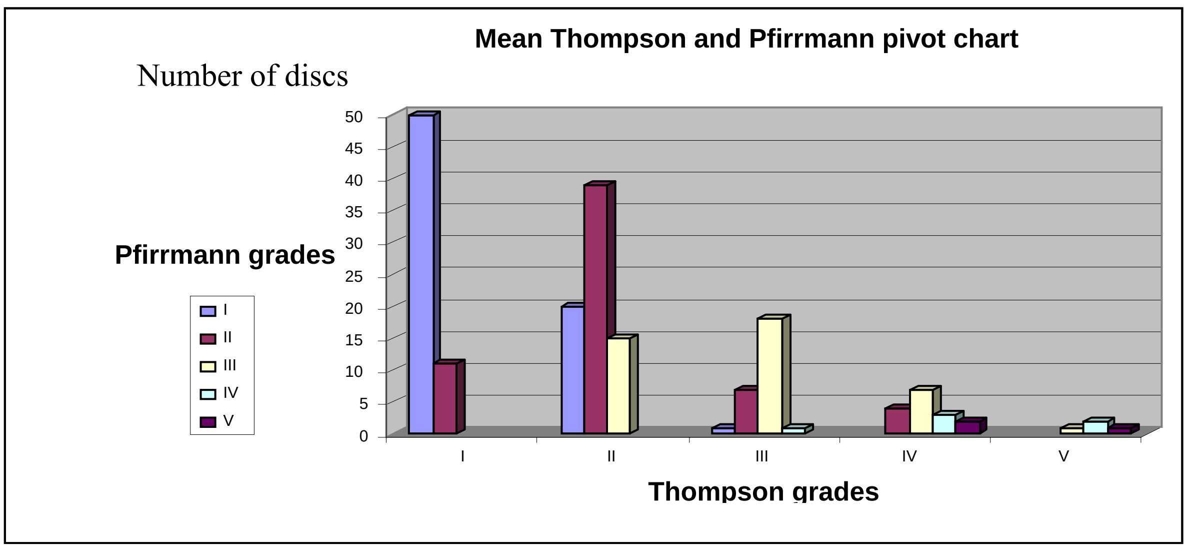

Figure 11. Mean Pfirrman grades given to the intervertebral discs compared with given Thompson grades.

4 Discussion

As has been mentioned previously, there have been many studies regarding intervertebral disc disease. Interpretation of these studies was sometimes difficult as they did not have a common golden standard. With the introduction of the gross morphology grading scheme according to Thompson this golden standard was set. However, there are some important features a golden standard (for IVDD) should adhere to, for instance; it should be credible, reproducible, able to distinguish categories, and conform to accepted distinctive marks of aging and degeneration (1). The Thompson gross morphology grading scheme shows compliance with these characteristics, making it a very suitable and applicable golden standard.
The intra- and interobserver reliability for the Thompson grading applied to canine intervertebral segments is almost perfect (resp. **κ** 0.90, and **κ** 0.833). All the degenerative criteria used in the Thompson grading were found in the canine intervertebral discs of this study, though some were found more frequently than others. A major morphological finding in the canine spine and difference with the human spine was that relatively many canine vertebras were affected with spondylosis. It was noticeable that the occurrence of this finding could not be related to the degree of degeneration of that specific segment or spine. The four observers agreed not to let the presence of spondylosis influence their grade determination. This agreement assured the interobserver grading to be compared and the grading to be the same as been performed on humans, although it cannot be determined if and to what degree the grades have been influenced by its presence. Another point that occasionally could have influenced the gross morphology grading according to Thompson, were the artefacts caused by the procedure to obtain the macroscopic photographs. After increasing use the blade of the belt saw, used to cut the spines, became blunt causing the saw to cut less smoothly through the spines which could lead to artefacts (extract pieces of disc material) producing discs that did not resemble their original state, i.e. creating a false positive.

The overall correlation between the mean Thompson scores and the mean Pfirrmann scores is substantial (**κ** 0.70). For Thompson grade I and II most of the time the same Pfirrmann grade is given and the deviation is not greater than 1 grade. However, for Thompson grade III, IV and V the Pfirrmann score deviation ranges up to 2 grades and for Thompson grade IV and V less than 50% of the same Pfirrmann grades were given. Relatively few discs were graded Thompson IV (8.8%) and V (2.2%) and Pfirrmann grade IV (3.3%) and V (1.6%). This low frequency of occurrence of these grades may cause the poor correlation between Thompson and Pfirrmann grades IV and V. In total almost 190 discs have been scored (in duplex), and the intervertebral discs originated from 19 dogs. This means that relatively a large number of discs (10) per dog were used. Possibly because of this high disc per dog ratio, the variation of the different discs grades is more limited than if more dogs were used yielding the same number of discs.
Three of the four observers who performed the Thompson grading, also performed the Pfirrmann grading, which could affect the scoring behaviour of these observers. For instance, when an observer has performed the Pfirrmann grading, the observer knows

how many grade IV or grade V he has scored in total. In this way the observer could have been biased and was able to use this information when performing the Thompson scoring.

The MR images used for the grading according to Pfirrmann were generated by the use of a 0.2 Tesla open magnet. The low field MR images are limited in depicting the different intervertebral structures which needs to be evaluated. This limited the possibility to discriminate and view the different vertebral structures. The Pfirrmann grade is based upon the intensity of the T2-weighted signal (2). In the majority of the MR images evaluated in this research, the signal intensity of the discs tends to weaken when the discs were situated further away from the coil. Since a weak nucleus pulposus signal will receive a higher Pfirrmann grade (II instead of I, or III instead of II) compared with a strong nucleus signal, this could easily influence the scoring creating a false increase of discs with a higher Pfirrmann score. Other criteria where the Pfirrman grades are based upon are; the homogeneity of the nucleus pulposus, distinction between nucleus and annulus, and the height of the intervertebral disc. However, there are more important structural changes which can take place during intervertebral disc degeneration, such as nuclear calcification, alteration of the shape of nucleus pulposus and changes concerning the vertebral bodies such as sclerosis and osteophyte formation (26). These are important considerations and should be taken into account when the grades of the Thompson scores and the Pfirrmann scores are being compared. An important difference is that the criteria for the Thompson grades are far more complete, covering all the intervertebral structures, whereas the Pfirrmann criteria does not. Moreover, Thompson grades are based upon changes seen in the nucleus, annulus, end-plate and vertebral body. The Pfirrmann grade criteria do not consider the end-plate and vertebral body at all; it is possible that these differences can explain the different Pfirrmann and Thompson grades given to the same discs. Also, it cannot be expected to grade low field MR images according to criteria such as changes in the end-plate and vertebral body. MR images are also limited in depicting intervertebral disc degeneration when only small tears in the outer layers of the annulus fibrosus are present since that will not result in changes in signal intensity. It is only at a later stage that this would be highlighted by a reduced or absent MRI signal due to the relative change in proteoglycan and water content (27). It is likely that MR images produced with MRI machines containing more powerful magnets (1.5 to 3.0 Tesla) can depict more subtle changes which can also be seen with the naked eye, making the different grading schemes more comparable. However, MRI will always have its limitations, for instance mineralization of the disc could be mistaken for changes associated with other lesions (13).

Although there are some limitations to the use of low field MRI in imaging canine IVDD we found a clear and “substantial” correlation to our golden standard. Considering that the image quality of MRI is likely to only improve in the future, especially with the use of stronger magnetic fields, MRI will be a very useful tool to correctly diagnose IVDD in dogs in a clinical setting.

5 Conclusions

The Thompson grades given to the photographs have a mean intra-observer reliability of **κ** 0.90 and a mean inter-observer reliability of κ 0.83, which means an almost perfect correlation for both the inter- and intra-observer reliability according to the Cohen`s weighted kappa analysis. Thus, it can be concluded that the Thompson macroscopic scoring scheme can be applied on the canine intervertebral disc.
The correlation between the two scoring methods, the MRI based five category grading scheme according to Pfirrmann and the five category macroscopic grading scheme according to Thompson, is substantial according the Cohen`s weighted kappa statistical analysis. Although the correlation is substantial there are some limitations. A conservative estimation of the expected Thompson score on gross morphology can be provided based on the Pfirrmann score on MRI. However there is a reasonable chance that the predicted Thompson grade deviates with one grade and sometimes even with two grades from the actual Thompson score, especially in case of grade III, IV and V.

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One of the joys of performing this research was working with many people from different departments at the faculty of veterinary medicine at the University of Utrecht. Besides being very cooperative, friendly and motivating, without these people this research simply wouldn't be possible and therefore I would like to kindly thank all the people who helped me completing this research project.

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ATTACHEMENT

Inter-observer (A-B, A-C, A-D) comparison Thompson grading

Observer B Photo1

Thompson score

Observer A Photo1

category	I	II	III	IV	V	Total
I	53	4				57
II	5	58	4	1		68
III		16	19			35
IV			8	11		19
V				1	3	4
total	58	78	31	13	3	183

Observer B Photo2

Thompson score

Observer A Photo2

category	I	II	III	IV	V	total
I	53	4				57
II	4	53	8			65
III		14	20	3		37
IV			12	7	1	20
V				2	2	4
total	57	71	40	12	3	183

Observer C Photo1

Thompson score

Observer A Photo 1

category	I	II	III	IV	V	total
I	50	6	1			57
II	31	25	10	2		68
III	3	5	15	7	5	35
IV		1	2	9	7	19
V					4	4
total	84	37	28	18	16	183

Observer C Photo 2

Thompson score

	category	I	II	III	IV	V	total
Observer A	I	55	1	1			57
	II	23	31	10	1		65
Photo 2	III	2	10	13	9	3	37
	IV			3	7	10	20
	V					4	4
	total	80	42	27	17	17	183

Observer D Photo 1

Thompson score

	category	I	II	III	IV	V	total
Observer A	I	49	8				57
	II	3	57	8			68
Photo 1	III		18	17			35
	IV		1	5	12	1	19
	V				3	1	4
	total	52	84	30	15	2	183

Observer D Photo 2

Thompson score

	category	I	II	III	IV	V	total
Observer A	I	50	7				57
	II	2	57	6			65
Photo 2	III		19	16	2		37
	IV		1	8	10	1	20
	V				3	1	4
	total	52	84	30	15	2	183