Histological comparison of intervertebral disc degeneration in non-chondrodystrophic and chondrodystrophic dogs in different stages of degeneration

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Abstract

In dogs and humans intervertebral disc (IVD) degeneration is a common cause of painful diseases like type I and II IVD herniation. However, IVD degeneration does not always lead to clinical signs of disease. Hans J. Hansen (1952) distinguished different types of IVD herniations in the two different dog types: chondrodystrophic (CD) and non-chondrodystrophic (NCD). CD dogs have short legs due to genetically disturbed endochondral ossification processes in the growth plates of tubular bones. These dogs show accelerated degeneration of IVDs. In certain circumstances under compression, they can suddenly result explosive IVD herniations. Hansen termed these herniations type I herniations, causing severe trauma and hemorrhage to the spinal cord. NCD dogs have tubular bones of normal length and commonly show, if at all, slower developing herniations (Hansen type II herniations) at average 7-8 years of age. Hansen adjudged type I herniations to chondroid degeneration of the nucleus pulposus (NP) in the IVD, whereas type II herniations were the result of fibroid degeneration of the NP. However, Hansen's research was not based on objective microscopic and macroscopic grading schemes. Further histopathological investigation of the degenerative process of CD and NCD dogs is thus required. In this project the histopathological process of IVD degeneration in CD and NCD dogs were compared objectively by using macroscopic and microscopic grading schemes. IVDs were sampled from cadavers of both dog types. Degeneration of the IVDs were graded according to Thompson's macroscopic grading scheme. Histological survey was done on blinded slides using the Boos scoring system for histopathological evaluation that was modified and validated for canines by De Nies et al (2010). Macroscopic and histological scoring of the IVDs were combined and statistically analyzed to elucidate differences between the CD and NCD dogs. One quantitative difference was found between CD and NCD dogs in Thompson grade I: the NP of CD dogs contained significantly more chondrocytes than the NP of NCD dogs. In all Thompson grades in both dogs types only chondrocytes and no fibrocyte-like cells were found in the degenerating NPs. Therefore the term 'fibroid degeneration' seems to be redundant. In conclusion, CD and NCD dogs are similar regarding the fundamental histological steps of IVD degeneration. However, the process of IVD degeneration appears to be highly accelerated in CD dogs.

Key words: intervertebral disc degeneration, chondrodystrophic, non-chondrodystrophic, Thompson grading scheme, Boos system

Introduction

Anatomy of the vertebral column

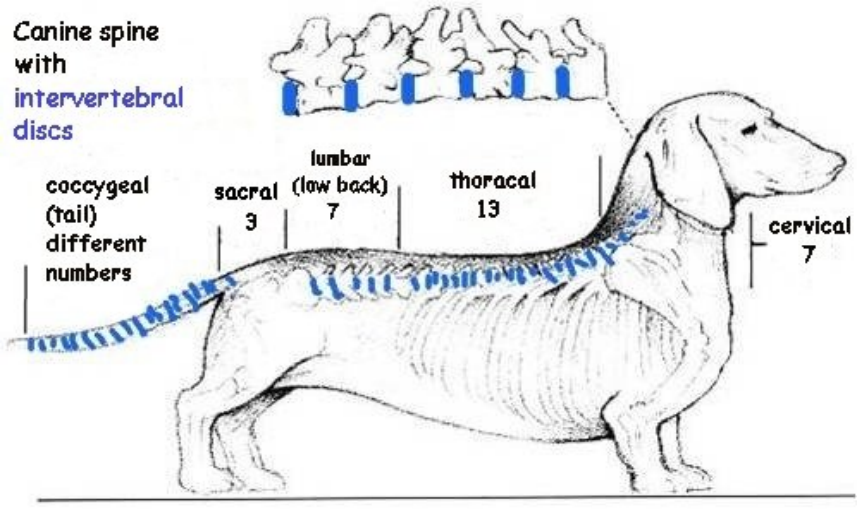

Fig.1 Gross anatomy of the canine spine[19]

The canine vertebral column (backbone or spine) consists of 7 cervical, 13 thoracic, 7 lumbar, 3 fused sacral and approximately 20 coccygeal vertebrae (Fig.1). From the second vertebra to the sacrum, the vertebrae are connected by intervertebral discs (IVDs) which provide restricted movement to the spine. The exact number of vertebrae differs, because dog breeds can have different numbers of the coccygeal vertebrae.[9,12] The spine extends from the cranium, thorax, sacrum, to the tail. Through the spine extends the vertebral canal that houses and protects the spinal cord.

The spine is supported by a number of spinal ligaments and muscles (Fig.2). On the ventral and dorsal side the spine is supported from vertebra to vertebra by the continuous ventral and dorsal longitudinal ligament. From the cervical to sacral region ligamenta flava between the vertebrae are visible from the inside of the vertebral canal. In the lumbar region the supraspinous and interspinous ligaments have an important biomechanical role to resist flexion-extension moments.[18] From the 2nd to 9th thoracic vertebrae the costal conjugal ligament extends as a broad ligament between the costal capitula, as a roof over the discs and positioned under the dorsal longitudinal ligament.[9]

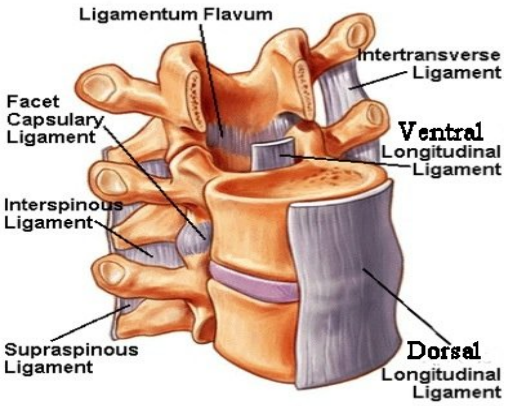

Fig.2 Anatomy of the vertebrae and supporting ligaments [20]

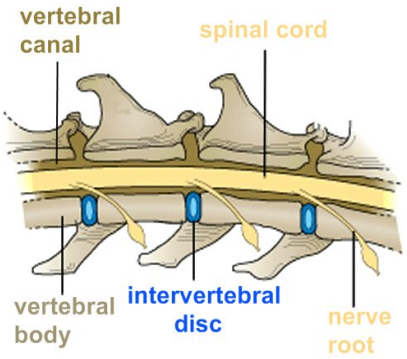

Fig.3 Position of the IVDs in the in the spine

Adjacent vertebrae articulate by small ligament-like synovial joints or facet joints (Fig.3). These joints are filled with synovial fluid. The transverse and dorsal processes of vertebrae differ in shape and length along the spine. The dorsal processes in the thoracal region are highest and can be used as a landmark for Th1 to distinguish from the cervical vertebrae that have much lower dorsal processes. Except from the lumbosacral disc, the cervical IVDs are longest of all, whereas the thoracic discs are shortest.[8,9,10]

Anatomy and function of the IVD

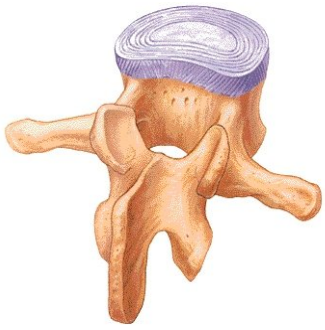

Fig.4 Vertebra and intervertebral disc[23]

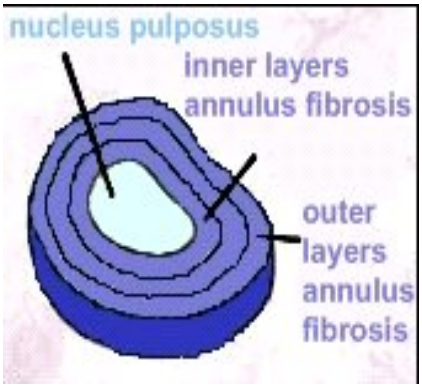

Fig.5 Anatomy of the intervertebral disc[23]

To maintain flexibility and stability to the spine during movement (bending and torsion) the vertebrae from C2 to the sacrum are contiguously linked by IVDs (Fig.4).[9,10,12,17] According to *in vitro* biomechanical tests, the disc is shown not to act as a shock absorber.[17] Shock absorption is maintained by musculature. Indeed IVDs have to withstand compression mostly arising from muscular compression during movement. In the dog, about 17% of the spine is occupied by the IVDs.[17] Each IVD consist of a ligament-like band, called the annulus fibrosis (AF) which form the outer

structure of the IVD (Fig.5). Within the AF the nucleus pulposus (NP) is found. In healthy state the NP is a non-compressible, transparent and hydrophilic semi-fluid gel.[1,9] In this conformation the NP is confined under pressure, with the AF and endplates (EP) containing the NP. Cartilaginous EPs bound the IVD cranial and caudal to the associated vertebral bodies.[1,8,9,10] In young animals soft, translucent hyaline cartilage lines the surface of the EP. In young animals the center of the EP is fenestrated by vessels through vesicular canals to communicate with the spongiosa of the vertebral body. During maturation the vessels disappear and can be distinguishable as little scar tissue spots in the EP. Where the EP contacts the vertebral body, the vertebral body has no compact bone.[8]

Physiology and histology of the IVD

Compression resistance properties

The pressure in the IVD depends on water content of the NP and resistance of the AF.[9,10] The NP spreads compressive forces over the whole surface of the vertebral bodies, while the AF functions as a constraining structure surrounding the NP. It results in tensioning of the AF.[11] Healthy NPs consist of a non-compressive hydrophilic semi-fluid gel[9], which is composed of a hydrophilic matrix that is supported by notochordal cells. Notochordal cells are the predominant cell population of the healthy canine NP.[17] The NP exists for 80% of water and the AF for 60%. Collagen accounts for only 5% in weight of the NP. Type I collagen dominates in the outer lamellae of the AF and type II collagen is the main content in the inner AF and nucleus. [9] Fibroblasts and fibrocyte-like cells in the AF form fibers to maintain the connective tissue matrix and renew the ground substance of the extracellular matrix in the NP.[15,16] Fibroblasts and fibrocyte-like cells both synthesize elastin, reticular fibers and collagen with fibroblasts being most active in synthesizing matrix.[14] Collagen fibers form a 3 dimensional extracellular matrix. These collagen fibrils are expanded by negatively charged proteoglycan molecules that attract H_2O molecules. Proteoglycan molecules are interconnected by hyaluronic acid. These proteoglycan molecules exists of central core proteins and covalently bound glycosaminoglycans (GAG's). These GAGs attract H_2O molecules because of exchanging properties of ions (and not because of chemical binding of H2O molecules). The GAG concentration is highest in the NP, but also high in the matrix between lamellae of the AF and in the transitional zone.[17]

Nutrition

Healthy matured IVDs itself do not contain any blood vessels or nerves. The IVD is dependent on diffusion of nutrition and oxygen from nearby blood vessels, situated in the vertebral bodies, through the EPs which are permeable to nutritional and small molecules.[10]

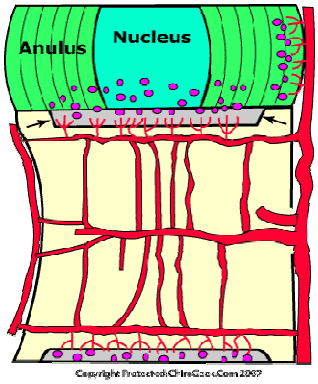

Fig.6 Transport of nutrition to the intervertebral disc[24]

Diffusion is possible through interfibrillar pores that pass parts not wider than 1,5nm (fig.6). For small uncharged solutes these two routes are equally important.[8]

Pathophysiology and pathogenesis of IVD degeneration

The IVD is the only non-vascular organ in the body and nutrition and oxygen is provided by slow delivery processes like diffusion. IVDs are adapted for these conditions by having a slow rate of metabolism: in humans GAG's has a turnover of 500 days and of the turnover of collagen or even longer.[31] IVD degeneration is associated with decreased nutrition and de-oxigenation like in the process of aging. Parameters like pressure resistance of the NP and resistance of the AF are subject to degenerative changes, leading to IVD insufficiency. If the NP fails in resisting compressive forces, the AF has to resist compressive forces. The AF is constructed to resist pulling forces and therefore ruptures if the NP becomes more degenerated (in higher Thompson grades). The IVD can bulge into the vertebral canal, exert pressure on the spinal cord and associated nerve roots. IVD herniations are commonly seen in older dogs with an average age of 7-8 years. (Fig.7).[9,12] IVD herniation can lead to clinical signs like pain and neurological deficit, when the spinal cord or spinal nerves are compressed. Damage is usually limited if the IVD herniates into the vertebral body, which occurs seldom. Ventral herniations and herniations into the vertebral bodies through the EP, cause no clinical signs, because no nerves are affected.

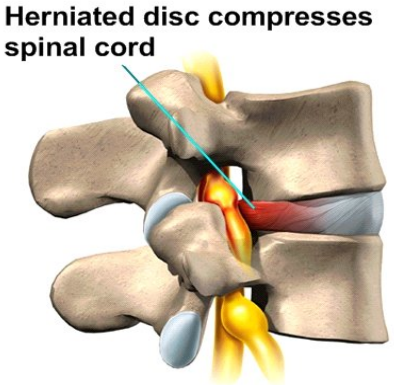

Fig.7 Herniated IVD pressures on nerve roots or spinal cord[26]

IVD degeneration can be graded macroscopic, by evaluating morphological macroscopic changes of mid-sagittal cut IVDs, according to Thompsons grading scheme. This scheme is originally designed to grade human IVD degeneration, but was recently validated for macroscopic grading of canine IVD degeneration.

Table 1 Thompson grading system; degenerative changes specified and categorized from grade I to V. [30]

Grade	Nucleus	Anulus	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 anular-nuclear demarcation	Focal defects in cartilage	Early chondrophytes or osteophytes at margins
IV	Horizontal 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 anulus		Diffuse sclerosis	Osteophytes greater than 2 mm

Thompson grade I describes the healthy IVD and grade V the final stage of degeneration (table 1). In each grade the degenerative changes in NP, AF, EP and vertebral body are specified. Herniations of disc material can occur in all Thompson grades, but is more likely in higher Thompson grades, because herniation is often a result of degeneration with secondary loss of NP. In the initial stages of degeneration, a transformation from the translucent NP into a more fibrous NP is visible. This transformation is due to a process of chondrification. Chondrification involves a decrease in GAG's, an increase in collagen (30-40% dry weight within 6-12 months). Chondroid degeneration is histologically recognizable as an invasion of chondrocyte-like cells into the NP and AF where they form aggregations ('nests'), producing cartilaginous matrix that surrounds them. The nucleus of CD dogs has 40-50% less proteoglycans, 40% less glycoprotein and noncollagenous protein, more collagen and 30-50% less chondroitinsulphate than NCD dogs.[12] Degeneration is associated with decreased nutrition and de-oxygenation of the IVD. This leads to further degeneration; if fibroblasts and other cells produce under anaerobe circumstances, they produce lactate that lowers pH grades.[10] Lowered pH grades activates matrix-degrading enzymes. Dogs that exercise often have an increased aerobic metabolism of NP and AF.[6,12] Through anaerobic metabolism, which is more common in older dogs and in patients suffering from systemic disease, the ratio of GAG subunits Keratansulphate (KS) : chondroitinsulphate (CS) increases, because the synthesis of KS is easier in hypoxic environment.[12] During the aging process the weight of the GAG molecules in the NP is increased, but this does not affect their hydrophilic properties.[10]

In certain conditions these degenerative changes can be accelerated in predisposed dog breeds. Hansen has characterized specific dog breeds as 'chondrodystrophic', differing from 'non-chondrodystrophic' dog breeds in having short legs in comparison to the rest of their body. These dogs have a genetic defect in the growth plates of

tubular bones which results in a disturbed process of endochondral ossification, resulting in short tubular bones. They also show IVD degeneration earlier in life than non-chondrodystrophic dogs, leading to IVD diseases in relative younger dogs (from 3 years of age).[9]

Classification and pathophysiology of IVD degeneration and herniation according to H.J. Hansen

In 1952 Hans-Jürgen Hansen described IVD degeneration as a vital process of exchange of cell types that leads to functional loss rather than degeneration by dying or functional failing of cells. Linked to the differences between CD and NCD dogs, he described two different types of IVD herniation.[9]

Hansen type I IVD herniation
IVD herniation Hansen type 1 is mainly seen in CD dogs. This type of herniation occurs in an explosive fashion, causing trauma to the spinal cord and hemorrhage from the vertebral sinuses. The pathogenesis of type I herniation is assumed to be the result of invasion of chondrocyte-like cells in the NP and AF and a loss of notochordal cells from the NP. The gelatinous NP transforms towards a more hyaline cartilage like appearance.[3] This process, also referred as chondrofication or chondroid degeneration, occurs along the entire vertebral column from 2 months of age in most CD breeds. Within 1 year 75% - 90% of the IVDs are transformed into a grey-white to yellow hyalo-cartilaginous tissue. Disc calcifications also occurs secondary to degenerative changes of the chondroid metamorphosis.[3,9]

Hansen type II IVD herniation
This senile degeneration is seen commonly in NCD breeds. Herniations are smaller and more regular than herniations of Hansen type I. The NP bulges into the weakened AF and usually one or only a few discs are involved. The outer AF and posterior longitudinal ligament usually stays intact with no hemorrhage. They are believed to be a result of fibrous metaplasia, involving a change in the NP cell population of predominant notochordal cells to mainly fibrocyte-like cells. This exchange has been characterized as a fibrous collagenization of the NP with concurrent degeneration of the AF. Degeneration that causes Hansen Type II is thought to be the result of senile disc degeneration by successive collagenization of the more fibroid NP.[1,2,7,9]

Hansen clearly stated that in CD dogs the NPs of degenerating IVDs display chondroid metaplasia, whereas in NCD breeds NP degeneration is characterized by fibroid metaplasia. However, Hansen also described many similarities in the histopathological appearances of IVDs in the two different dog types. Moreover, the histopathological examination of the IVDs was not performed objectively by the use of objective macroscopic of histological grading schemes.[9]

Aim and Hypothesis

Aim

The aim of this study was to compare the histological appearance of IVDs from CD and NCD dogs, within the same Thompson grades[30] to establish if there are differences between the degenerative process in CD and NCD dog breeds. The aim was also to see if fibrocyte like cells could be identified within the NP in degenerating IVDs from NCD dog breeds, which was described by Hansen.

Hypothesis

The degenerative process in CD and NCD dog breeds is similar based on qualitative and quantitative histopathological appearances.

Material and Methods

Material collection and processing

For this histological study slides were used from previous studies, collected from randomly selected fresh canine cadaveric spines. Most dogs were patients that died or were euthanized at the Faculty of Veterinary Medicine, Utrecht University and subsequently donated for research. IVDs from thirty-four different dogs were used for this project. The spinal segments were cut mid-sagitally using a belt saw, cooled with water to prevent burning of the cut surface. Information of each IVD (breed, age) was registered and the spinal cut surfaces were photographed for macroscopic grading according to Thompson grading scheme. Subsequently, each IVD was fixed using 4% neutral buffered formalin, decalcified in EDTA, embedded in paraffin, routinely stained with Hematoxilin and Eosin (H&E) stain and with Alcian Blue/ Picrosirius Red stain. Histological samples (both H&E and Alcian Blue/Picrosirius Red) from 7 CD and & 7 NCD dogs, for Thompson grade I, II and III, were selected for this study (42 IVD samples in total).

Modified Boos system and additional scoring of fibrocyte-like cells

In total 84 slides (42 H&E and 42 Alcian Blue/Picrosirius Red) were blinded and graded histologically according to the 9 parameters in the modified Boos system. For histology magnifications 4 x, 10 and 40 x were used. All slides were scored twice to ensure good intra-observer reliability. Each slide was scored for following parameters of the modified Boos system (Table 2).

Table 2 Histological parameters of the modified Boos grading system for the canine intervertebral disc.

Morphology AF
0 well organized, half ring-shaped, collagen lamellae
1 Mild disorganized; some loss of half ring-shaped structure (>25%), most lamellar layers still distinguishable
2 Moderate disorganized; partly ruptured AF, loss of half ring-shaped structure (25-75%)
3 completely ruptured AF; no or few(>25%) distinguishable half ring-shaped collagen lamellae
Chondrocyte-like cell proliferation in the NP
0 no proliferation
1 increased chondrocyte-like cell density
2 connection of two chondrocyte-like cells
3 small size clones (i.e. several chondrocyte-like cells group together, i.e. 2-7 cells)
4 moderate size clones (i.e. > 8 cells)
5 huge clones (i.e. 15 cells)
6 scar/tissue defects
Presence of notochordal cells in the NP
0 abundantly present (>50%)
1 rarely present (1-50%)
2 absent
Chondroid metaplasia in the AF
0 no chondrocyte-like cell morphology, just spindle shaped fibroblasts
1 mild chondroid metaplasia (i.e. limited to inner most layers of AF)
2 moderate chondroid metaplasia (i.e. chondroid cell up to half of the AF)
3 marked (i.e. chondroid cells up to outer layers of the AF)
Cleft formation
0 absent
1 rarely present
2 present in intermediate amounts
3 abundantly present
4 scar/ tissue defects
Matrix staining of NP: Alcian Blue/Picrosirius Red
0 blue stain dominates
1 mixture of blue and red staining
2 red stain dominates
Regularity endplate
0 regular thickness; homogeneous structure
1 slight irregular thickness
2 moderate irregular thickness
3 severe irregular thickness with interruption of the EP
New bone formation (osteophytes)
0 absent
1 rarely present
2 present in intermediate amounts
3 abundantly present; tendency towards bridging/ complete bridging
Bone sclerosis
0 no sclerosis (< 2 x thickness of dorsal the cortex of the vertebral body)
1 mild sclerosis (2-4 x thickness of the dorsal cortex of the vertebral body)
2 moderate sclerosis (>4 x thickness of the dorsal cortex of the vertebral body)
3 severe subchondral bone irregularities

All slides were in addition also evaluated on the presence of fibrocyte-like cells in the NP. This can be seen as an additional parameter to the modified Boos system.

Statistical analysis

For statistical data analysis the scores according to the modified Boos system of the second round were used, as the scores obtained in the second round were deemed more reliable as there is a learning curve in using any grading system.

Parameter scores and total scores
Each slide was scored on individual parameters (10 in total: 9 parameters of the modified Boos system plus 1 additional score for fibrocyte-like cells in the NP) and a total score was created by adding up the scores for individual parameters. These total scores and scores for individual parameters were used for further statistical data analysis.

Computer analysis
The Boos scores were determined blinded and thereafter data such as Thompson score and dog type (CD or NCD) were added for the statistical analysis. SPSS 16.0 (IBM Company Chicago, USA) was used to investigate if CD and NCD dogs had significant different scores per individual parameter or for the total scores. Normal distribution of the data was confirmed using PP and QQ plots. The student's T-test was used to calculate statistical differences between the CD and NCD dogs regarding the 10 evaluated parameters per Thompson grade. Furthermore intra-observer reliability was evaluated by (weighted) Kappa analysis. Kappa analysis calculates the reliability of qualitative scores between observers or different scoring rounds of one observer.[32] It gives an indication if the scores are chosen at random or deliberately chosen. Scores with a difference between the first and second round of two or even more have more weight in this calculation than a difference of only 1 point. The results of this weighted Kappa analysis can be interpreted according to table 3.

Table 3 Interpretation of (weighted) Kappa analysis results [32]

Strength of agreement	**Value of *K***
Poor	< 0.20
Fair	0.21 - 0.40
Moderate	0.41 - 0.60
Good	0.61 - 0.80
Very good	0.81 - 1.00

Results

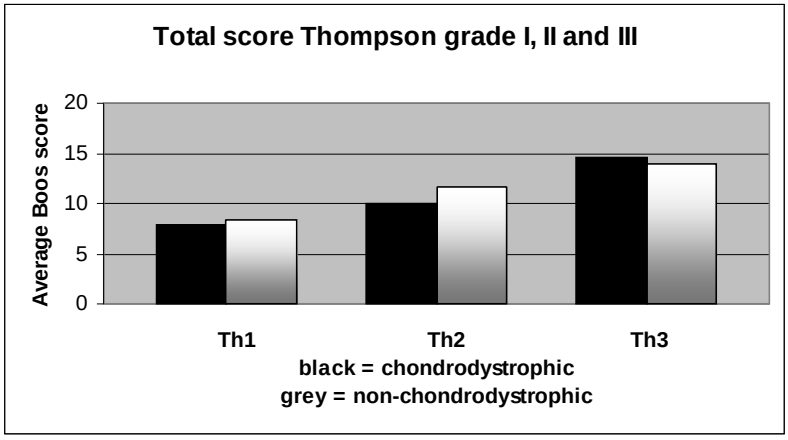

Fig.13 Mean total Boos scores of chondrodystrophic and non-chondrodystrophic dogs in Thompson grade I, II and III

Total scores of all histological parameters that represent IVD degeneration were not significantly different between the CD and NCD dogs in all three Thompson grades (Fig.13 and Table 4).

Table 4 Means and P-values obtained with students T-test of CD and NCD dogs for each histological parameter in Thompson grade I, II and III

	Thompson grade I			**Thompson grade II**			**Thompson grade III**		
Parameter	**CD**	**NCD**	**p-value**	**CD**	**NCD**	**p-value**	**CD**	**NCD**	**p-value**
Morphology AF	0,26	0,43	0,61	0,71	0,57	0,73	1,14	1	0,78
Chondrocytes NP	2,57	1,14	0,04*	2,14	2,86	0,2	3	2,86	0,81
Notochord cells NP	1,29	0,71	0,18	1,71	1,29	0,31	1,86	1,43	0,22
Chondrocytes AF	2,43	2,71	0,43	2,86	2,71	0,55	3	2,86	0,34
Tear/cleft formation	0,14	0,43	0,27	0,43	0,86	0,36	1,86	1,43	0,45
Staining NP AB/PR	0,43	0,57	0,7	0,29	0,86	0,1	0,29	0,14	0,66
Regularity endplate	1	0,71	0,34	1,29	1,29	1	1,29	1,71	0,37
New bone formation	0	0	-	0	0,29	0,34	0,71	0,71	1
Bone sclerosis	0,57	0,86	0,4	0,57	1,29	0,11	1,43	1,86	0,48

The histological parameters show no significant differences in degenerating IVDs of CD and NCD dogs up to Thompson grade III with the exception of one parameter. In Thompson grade I CD dogs showed more chondrocyte-like cells in the NP than NCD dogs (Fig.14).

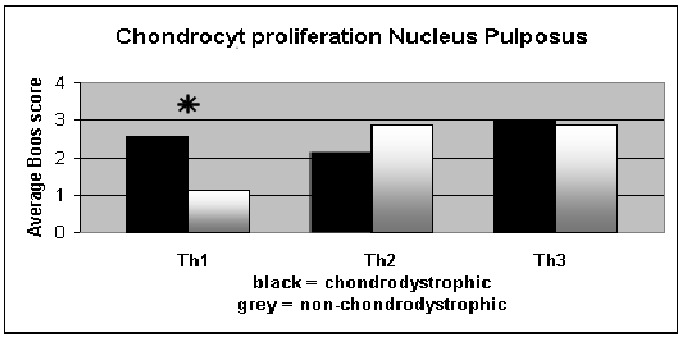

Fig. 14 Boos scores for chondrocyte-like cell proliferation in the NP in CD and NCD dogs in Thompson grade I, II and III

Table 5 Mean fibrocyte-like cell counts in the NP of CD and NCD dogs in Thompson grade I, II and III.

	Thompson grade I		Thompson grade II		Thompson grade III	
Parameter	**CD**	**NCD**	**CD**	**NCD**	**CD**	**NCD**
fibrocyte-like cells NP	0	0	0	0	0	0

Scores for fibrocyte-like cell counts in the NP were registered separately (Table 5), as this parameter is not part of the validated histological scoring system, but it is still of interest to know if fibroid metaplasia is seen in the NPs of NCD dogs like Hansen stated. No fibrocyte-like cells were found in the NP of CD or NCD dogs in any of the evaluated Thompson grades.

Tables of the remaining, individual parameters have been included in the appendix (Figs.15 to 21). These tables show no significant difference between CD and NCD dogs.

Table 6 Results and interpretation of weighted Kappa analysis to verify intra-observer reliability

Variables	Observer agreement	Weighted Kappa score	Interpretation
Morphology AF	0,81	0,84	*very good*
Chondrocytes AF	0,81	0,46	*Moderate*
Regularity endplate	0,47	0,49	*Moderate*
Tear/cleft formation	0,61	0,66	*Good*
Bone sclerosis	0,67	0,58	*Moderate*
New bone formation	0,83	0,82	*very good*

According to Landis and Koch's interpretation the intra-observer reliability is from moderate to very good and of acceptable value.

Discussion

Comparison of total Boos scores of CD and NCD dogs in each Thompson grade is not accurate

In this research a histological comparison was made between CD and NCD dogs based on 10 histological parameters in Thompson grades I, II and III. The mean total Boos scores can be seen as a gross numeric presentation of the total degree of degeneration; the mean total Boos scores increase in ascending Thompson grades. Statistical analysis to compare dog types per Thompson grade based on total Boos scores can only be informative, unless the individual histological parameters are also assessed individually. Some histological parameters are graded with similar weight in the Thompsons macroscopical grading scheme (Table 7).

Table 7 Morphological degenerative changes influence Boos scorings and Thompson grading with comparable weight.

Modified Boos system	**Thompson grading scheme**
'morphology Anulus Fibrosis':	*Anulus Fibrosis*:
0 '*well organized'*	Grade 1: '*discrete fibrous lamellas'*
1 '*mild organized'*	Grade 2: '*Mucinous material between lamellas'*
2 '*moderate organized'*	Grade 3: '*loss of annular-nuclear demarcation'*
3 '*completely ruptured AF'*	Grade 4: '*Focal disruptions'*
	Grade 5: '*Clefts extend through nucleus and anulus'*
'cleft formation':	
0 '*absent'*	
1 '*rarely present'*	
2 '*present in intermediate amounts'*	Grade 4: *Nucleus*: '*horizontal clefts parallel to EP'* and: *Anulus*: '*Focal disruptions'*
3 '*abundantly present'*	
4 '*scar/tissue defects'*	Grade 5: '*Clefts extend through nucleus and anulus'*
'regularity endplate':	*Endplate*:
0 '*regular thickness*'	Grade 1: '*hyaline, uniformly thick'*
1 '*slight irregular thickness*'	Grade 2: '*Thickness irregular*'
2 '*moderate irregular thickness*'	Grade 3: '*focal defects in cartilage*'
3 '*severe irregular thickness with interruption of the EP'*	
'Bone sclerosis'	
0 '*no sclerosis*'	
1 '*mild sclerosis'*	Grade 4: '*fibrocartilage extending from subchondral bone: irregularity and focal sclerosis in subchondral bone*'
2 '*moderate sclerosis*'	
3 '*severe sclerosis*'	Grade 5: '*Diffuse sclerosis*'

Comparable agreement can be seen in Boos parameter '*new bone formation*' and Thompsons description for '*vertebral body*'. *If* significant differences would be found in total Boos scores each Thompson grade between CD and NCD dogs, it could be argued that these differences would be mainly attributed to following Boos parameters:
Chondroid metaplasia in the AF
Presence of notochordal cells in the NP
Chondrocyte-like cell proliferation in the NP
A comparison of total Thompson scores and the total of all morphological parameters of the Boos system would result in no significant difference, because the morphological histological changes result in the same macroscopic changes, scored by Boos system.

Cell amount related Boos parameters are crucial to verify if Hansens adjudged statement about the histological difference of IVD degeneration in CD and NCD dogs, is correct .
This histological research was mainly set up to verify if the histopathological distinction that Hansen considered, can be maintained. Hansen adjudged that degenerating NPs of CD dogs are the result of chondroid metaplasia and degeneration of the NPs of NCD dogs. he deemed, to be result of fibroid metaplasia. The results of this research indicates that chondroid metaplasia of the NP is the histopathological phenomenon that takes place in the process of IVD degeneration in CD and NCD dogs. To answer the research question, these parameters that evaluate amounts of chondrocyte-like cells, notochordal cells and fibrocyte-like cells were crucial.

Thompson grades expresses chronology and not the speed of degeneration.
Thompsons grading scheme describes which degenerative changes occur in chronological sequence, but it does not state anything about how fast these changes has occurred in time. It is thus possible to make an objective comparison between the IVD degeneration in young CD dogs and old NCD dogs. Since all the slides were graded first macroscopically, the sequence of degenerative changes can be interpreted.

Cellular and morphological Boos parameters are valuable to understand the sequence of degenerative changes in canine IVD degeneration in relation to cellular changes.
Morphological changes are assumed to be the result of presence or absence of cells, as cells can maintain or disrupt the tissue matrix.
In this research only one significant quantitative difference was found between CD and NCD dogs: in Thompson grade I CD dogs have more chondrocyte-like cells in their NP than NCD dogs. Thompson grade I is the initial state of degeneration. In CD dogs in Thompson grade I, the parameter ' morphology AF' had a low scoring result, so the AF was still in good condition whereas many chondrocyte-like cells were still invaded in the NP. The combination of the chronology provided by the Thompson grades, in combination with the morphological and cell parameters, we gain more detailed insight in the sequence of degenerative events and in which Thompson grade degeneration starts in the different dog types. In Thompson grade I CD dogs are in an early state of degeneration, low scores for morphological Boos parameters support this, but a high amount of chondrocyte-like cells in the NP of CD dogs is seen. This

can be interpreted as following: the invasion of chondrocyte-like cells in the NP occurs earlier and might be accelerated in CD dogs compared to NCD dogs.

Thompson grading system is accurate to grade the continuous biological process of IVD degeneration.
Despite IVD degeneration being a continuous biological process, grading of slides according to the 5 Thompson grades, is still objective and can result in accurate research results. Some IVD samples fell in-between two Thompson grades and were subsequently excluded from this research, only IVDs that clearly fitted in Thompson grade I, II or III were included. This is why the results of statistical analysis can be objective if based on Thompson's macroscopic grading scheme.

Reliability of the observer is confirmed by Kappa results.
Lower intra observer Kappa results are due to a process of learning the interpretation of histological morphology. In the first round the student had to become familiar with the histological appearance of IVDs. Due to different conditions during tissue processing and staining methods, cells can appear with some variations in shape and color. In the second round the observer became more experienced and recognized easier the cells in their surrounding area. For example this resulted in higher Boos scores in the parameter 'chondrocytes in the AF' in the second round.

Hansen potentially misinterpreted dying notochordal cells as fibrocytes. In a photograph in Hansen's thesis that shows dying notochordal cells in the NP of a NCD dog, the designation refers to these cells as being fibrocytes.

Further research:
The presence or absence of cells are pretty essential differences between degenerating IVDs in NCD and CD dogs, but as long as we do not know how these cells function, how they communicate with other cells and surrounding tissues the importance of these differences is difficult to interpret. Follow up studies of the function and metabolism of the cells in the IVDs, the extracellular matrix and shifts in collagen types, can be of interest to clarify essential biomechanical differences of degenerating IVDs between CD and NCD dogs. This is might be accomplished through specific histological staining or biomolecular research.
In this research 34 different dogs were used whereof some dogs had IVDs that were represented in two different Thompson grades. To enlarge statistical reliability, a larger study population would be appreciable.

Conclusion

Degenerating IVDs of CD and NCD dogs have similar qualitative histopathological appearances. In both dog types chondroid metaplasia of the NP is the histopathological phenomenon taking place in the process of IVD degeneration. No fibrocyte-like cells were found in the NP of any dogs. In Thompson grade I a quantitative difference between CD and NCD dogs was found: CD dogs have significant more chondrocyte-like cells in the NP than NCD dogs, which highlights an earlier start of the degenerative process in CD breeds.

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Appendix

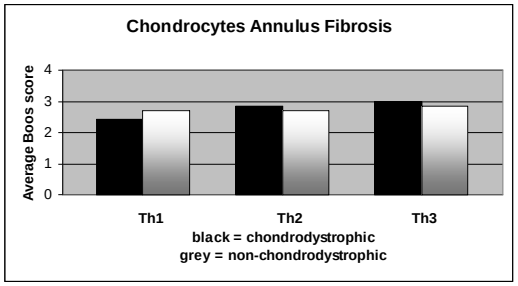

Fig.14 No significant differences in chondrocyte-like cell proliferation in the AF between CD and and NCD dogs.

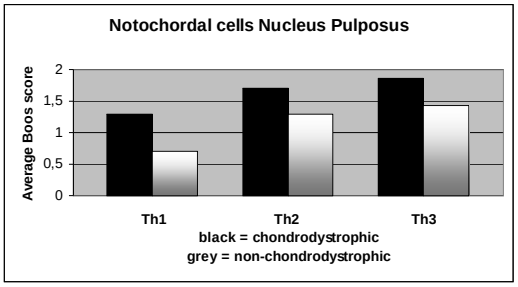

Fig.15 No significant differences in notochordal cells between chondrodystrophic and non-chondrodystrophic dogs.

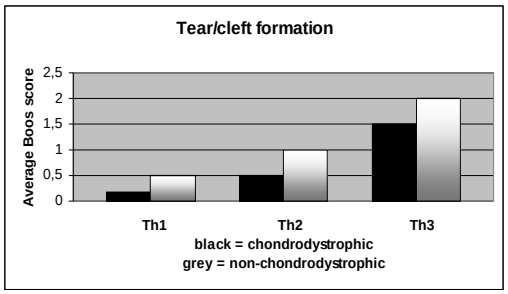

Fig.16 Tear and cleft formation is significant equal in chondrodystrophic and non-chondro-dogs.

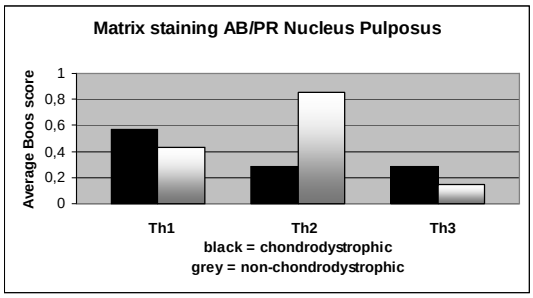

Fig.17 AB/PR staining results of the NP in chondrodystrophic and non-dystrophic trophyc dogs are significant equal.

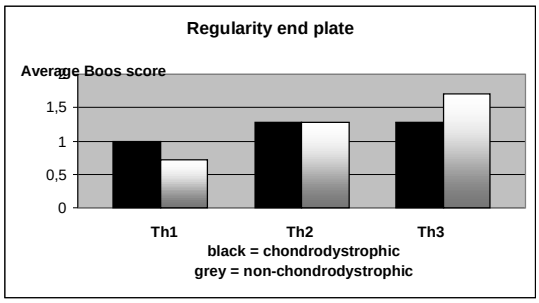

Fig.18 Chondrodystrophic and non-chondrodystrophic dogs display significant equal degenerative changes in end plates.

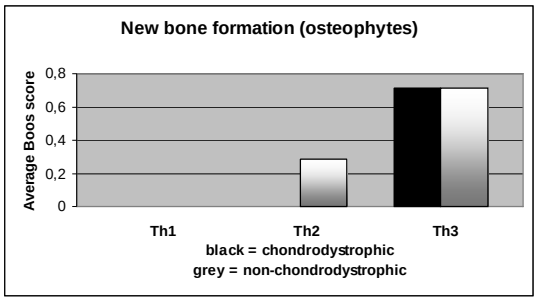

Fig.19 Chondrodystrophic and non-chondrodystrophic dogs show significant equal patterns in osteophyte formation

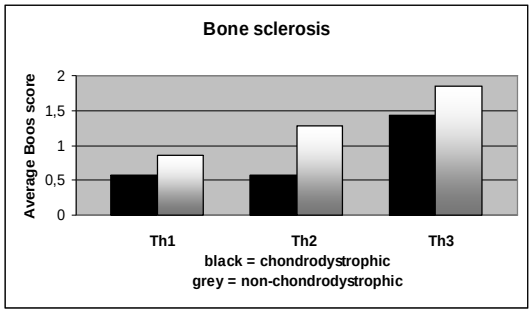

Fig.20 Chondrodystrophic and non-chondrodystrophic dogs show significant equal bone sclerosis.

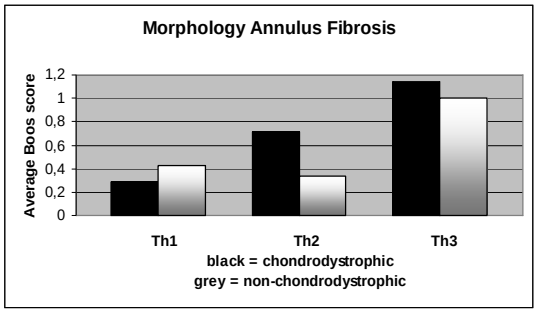

Fig.21 Chondrodystrophic and non-chondrodystrophic dogs show significant no different degenerative changes in the morphology of the AF.