

CT-Guided Oxygen-Ozone Intradiscal Injection Therapy for Thoracolumbar Intervertebral Disc Herniations (Hansen Type II) in Dogs

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1 **CT-guided oxygen-ozone intradiscal injection therapy for thoracolumbar intervertebral**
2 **disc herniations (Hansen Type II) in dogs**

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5

6 **Abstract**

7 **Background:** The study aims to investigate the treatment of thoracolumbar herniated intervertebral discs (IVD)
8 in dogs using Oxygen-Ozone (O₂-O₃) intradiscal injection and assess the results using MRI which achieve more
9 accurate details and procedures.

10 **Results:** All indices were improved after the treatment, while, three were statistically significant at P<0.5. All 3
11 dogs returned to their normal life after this study.

12 **Conclusions:** Intradiscal O₂-O₃ injection reduces the size of the herniated disc via disc shrinkage and decreasing
13 the pressure of herniated part on the spinal cord.

14 **Keywords:** Oxygen-ozone injection, disc shrinkage, intervertebral disc herniation, MRI, dog

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16 **Background**

17 Intervertebral discs are located between all
18 vertebral bodies of the vertebral column,
19 except for the first and second cervical
20 vertebrae (C1 and C2), and sacrum.
21 Intervertebral discs possess a complex
22 structure: a thicker fibrous cartilage outer ring
23 (the annulus fibrosus), and a more gelatinous
24 core (the nucleus pulposus). They function as
25 shock absorbers between each vertebra in the
26 vertebral column, allow slight movement of
27 vertebrae, function as ligaments to maintain
28 the vertebrae, and absorb shock to the spine.
29 Dehydration of the nucleus pulposus occurs
30 when it loses water [1]. However, the
31 dehydrated IVDs will no longer be able to
32 function as proper shock absorbers.
33 Intervertebral disc disease (IVDD) usually
34 pushes the nucleus pulposus against the spinal
35 canal and compresses the spinal cord. Effects
36 of IVDD range from bulging to protrusion or
37 extrusion of IVD [2]. A slow aging process in
38 non-chondrodystrophic dogs mainly results in
39 IVD degeneration [3]. If the nucleus pulposus
40 extrudes into the spinal canal and hits the
41 spinal cord (concussion), it will require
42 surgical intervention. More often, it slowly

43 pushes its way into the spinal canal, resulting
44 in pressure over the spinal cord (compression)
45 [2].

46 IVDD is diagnosed using a combination of
47 physical and neurological examinations, along
48 with radiographs such as X-rays. In more
49 advanced methods, imaging techniques such as
50 MRI are obtained from the spine [3].

51 MRI scanners employ strong magnetic
52 fields, magnetic field gradients, and radio
53 waves to generate diagnostic images. It is the
54 best diagnostic tool for the diagnosis of IVD
55 degeneration prior to herniation or extrusion.
56 With the invention of MRI, since it provided
57 more detailed images from the spinal cord,
58 myelography became obsolete [4].

59 O₂-O₃ injection for the treatment of disc
60 herniation has been primarily proposed in
61 human medicine. It is now frequently used as a
62 minimally-invasive treatment for IVD
63 herniation. Aside from being a cost-effective
64 procedure, intradiscal injection of this gas
65 demonstrated acceptable clinical results.
66 Along with shrinking the herniated disc, O₂-O₃
67 gas possesses anti-inflammatory and analgesic
68 effects on the compressed spinal cord [5].
69 Many studies have confirmed the positive
70 effects of ozone therapy in human medicine [6,

71 7, 8]. In veterinary medicine, HYUN-JUNG
 72 HAN *et al.* studied the impact of intradiscal
 73 O₂-O₃ injection on improving IVD herniation.
 74 In their study, CT was employed to measure
 75 the A-index, which measures the disc
 76 herniation area, according to the following
 77 formula [5]:

$$78 \quad A - \text{index (\%)} = \frac{\text{Area of disc herniation} \times 100}{\text{Area of the spinal canal}}$$

79 However, IVD is visible in a CT only if the
 80 disk is calcified. Therefore, using CT, IVD
 81 herniation, and spinal cord compression can be
 82 measured with very low accuracy. Therefore,
 83 using CT results increases the risk of false
 84 smaller measurements on disks that are not

85 shrunk. Moreover, in CT, the difference
 86 between a disk and other tissues are not
 87 distinguishable [4].

88 To increase accuracy and introduce a
 89 diagnostic strategy that poses less radiation on
 90 the animal [2], this study was conducted this
 91 study using MRI. Meanwhile, multiple indices
 92 were measured to evaluate the effect of O₂-O₃
 93 therapy on IVDD more accurately.

94 Results

95 The quantitative indices for the 13 affected
 96 intervertebral discs were measured using Marco
 97 PACS software and the mean data values were
 98 presented in Table 1.

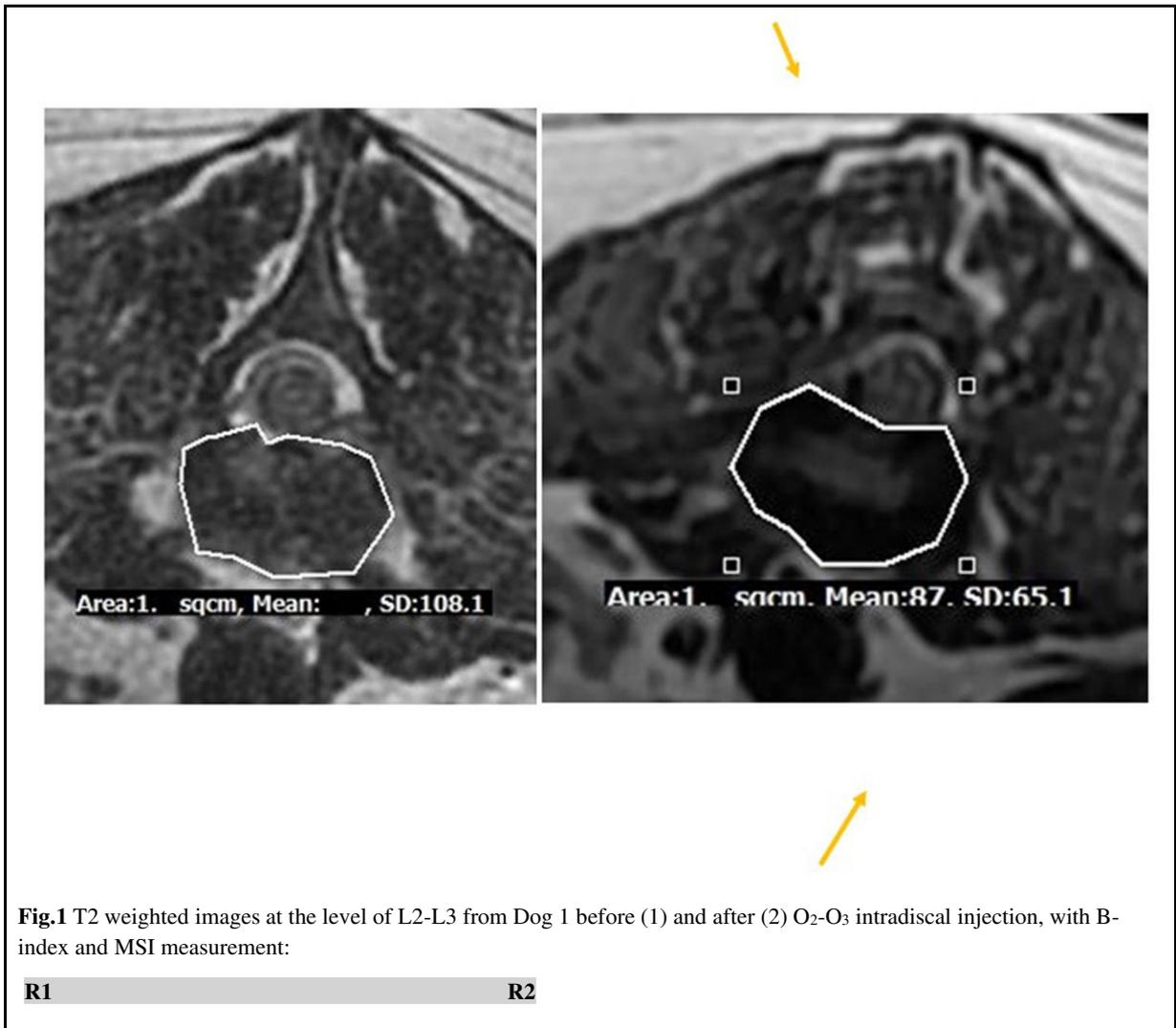
99 **Table 1** Mean data values before and after intradiscal O₂-O₃ injection in 13 IVDs: Astrisks indicates the significant
 100 decreasing in MSI and A-index mean data values after treatment.
 101

Stage	PG	SS	MSU	MSI	A-Index	B-Index
Before:						
Mean	2.2308	1.1538	1.1538	398.6154	12.5708	271.5385
Std. Error of Mean	0.36080	0.22206	0.10415	39.83726	1.80314	25.03449
Std. Deviation	1.30089	0.80064	0.37553	143.63527	6.50132	90.26315
After:						
Mean	2.6923	1.3077	1.0000	206.6154*	6.3554*	253.4615
Std. Error of Mean	0.28610	0.13323	0.16013	18.95154	1.44976	23.57057
Std. Deviation	1.03155	0.48038	0.57735	68.33074	5.22717	84.98492
Total (before and after):						
Mean	2.4615	1.2308	1.0769	406.5621	9.4631	262.5000
Std. Error of Mean	0.23026	0.12779	0.09484	2.261107	1.29269	16.94164
Std. Deviation	1.17408	0.65163	0.48358	8.152538	6.59147	86.38576

102 The patients went through comprehensive 112 minutes, and was determined according to the
103 neurological examination, radiographic studies, and 113 patient's time of entrance, the process for
104 1.5 Tesla MRI. A total of 13 IVDs were injected in 114 anesthesia, involved IVD detection, and final
105 3 dogs (from bulging to protrusion). In specific, 8 115 injection and removing the spinal needle (for each
106 IVDs were injected in dogs 1 and 3, while 5 IVDs 116 involved IVD). All patients recovered from the
107 were injected in dog 2. 117 procedure normally. Following the injection, some

108 After determining the definite location of the 118 gas contents were seen through surrounding
109 involved IVDs, the dogs were sedated for 119 musculature, and even in the spinal canal. In any
110 intradiscal O₂-O₃ injection under CT scan guidance. 120 case, such incidents generally had no side-effects
111 This intervention lasted approximately 20 to 25 121 on recovery or the study in Fig. 1.

122



123

124

125 After 5 weeks from O₂-O₃ injection, the clinical
126 signs of all three patients were improved. Then,
127 they were reevaluated by 1.5 Tesla MRI, which
128 showed progressive shrinkage of involved IVD in
129 all patients.

130 Reduction of the involved IVD protrusions,
131 after O₂-O₃ injection, was detected on MRI scans
132 taken 5 weeks after the procedure. The results of
133 measured indices were as following:

- 134 1. Pfirrmann Grading: this index showed
135 degeneration of IVD contents, except for 3
136 IVD.
- 137 2. Schneiderman Score: this index was not
138 significantly changed.
- 139 3. MSU Classification: this index did not
140 experience considerable changes, which
141 may be due to the large grading of this
142 classification. Therefore, there were no
143 significant changes in IVD protrusion
144 status.
- 145 4. MSI: this index was changed considerably
146 in all IVDs, up to one-third in some.
- 147 5. A-index: this index successfully changed,
148 except in the 2 IVDs, which experienced
149 minor increases.
- 150 6. B-index: the variations of this index was
151 interesting, except in the 2 IVDs. In
152 specific, there was a mild increase in the
153 one IVD and no change in only one IVD.

154 The results are shown in Tables 1. They were
155 evaluated using SPSS software and Paired T-test.

156 Discussion

157 The present study demonstrates the preliminary
158 quantitative evaluation of IVD size, 5 weeks after
159 intradiscal O₂-O₃ injection in dogs. Data confirmed
160 that the degeneration of IVDs occurs following
161 intradiscal O₂-O₃ injection (up to 100% according
162 to MSI index). However, A and B indices were
163 enhanced in 85% of IVDs, which may need more
164 time due to the chronicity of IVD shrinkage.
165 Previous studies on human subjects demonstrated
166 that IVD shrinkage following intradiscal O₂-O₃
167 injection is influenced by several factors, including
168 the extension of the herniated IVD contents, degree
169 of protrusion, and IVD classification [8].

170 According to HYUN-JUNG HAN *et al.* [5],
171 comparing our data indicates that the volume of
172 disc herniation is not related to the disc shrinkage.

173 As the results confirm, degenerative changes
174 were delineated in 77% of IVDs in the first variant.
175 On the other hand, the 2nd and the 3rd indices were
176 not significantly changed, which can be due to the
177 progressive time-consuming process of IVD
178 degeneration.

179 Pfirrmann and Schneiderman scheme
180 classifications did not experience significant
181 changes. In current study, only one radiologist was
182 responsible for the interpretation of the MR images,

183 which justifies the possibility of error in the results
184 [11]. To overcome this shortcoming, factors should
185 be evaluated by different radiologists to ensure
186 inter-observer reliability [11]. Another significant
187 issue regarding these two factors is their detectable
188 progressive variation. Hence, more time is needed
189 to alter the MRI intensity of IVD.

190 MSU classification should be evaluated using
191 MRI [12]. As mentioned there, it is measured
192 during a 5-year clinical follow-up on the cases.
193 Therefore, more time is required to evaluate and
194 determine the statistical meaningfulness of this
195 index.

196 To determine and quantify the IVD
197 degeneration, the MSI (4th index) was evaluated. A
198 significant change was detected before and after the
199 treatment, which was statistically meaningful
200 (0.0001, $P < 0.05$). MRI enables assessment of the
201 intensity of the IVD material before and after O₂-O₃
202 intradiscal injection.

203 Another valid index to measure the disc
204 shrinkage and decompression of the spinal cord is
205 the 3rd variant (A-index) [5]. In our study, this
206 index was statistically meaningful (0.006, $P < 0.05$).
207 This variant can be precisely estimated using 1.5
208 Tesla MRI because MRI delineates the accurate
209 margin of the herniated material and compression
210 of spinal cord parenchyma.

211 The last variant (B-index) is measured to
212 evaluate the IVD shrinkage during the 5-week

213 experiment period. The measured B-index value
214 was also statistically valid, which was measured up
215 to 0.003 ($P < 0.05$). In addition, this variant was
216 measured by MRI, which provides a gold standard
217 of IVD diagnosis (due to the cartilaginous structure
218 of the IVD).

219 According to A and B indices, we concluded
220 that the IVD shrinkage occurred gradually in 5
221 weeks. On the other hand, according to HYUN-
222 JUNG HAN *et al.* [5], there is no relationship
223 between disc shrinkage and the volume of disc
224 herniation. However, more time is needed to
225 evaluate other factors (due to Pfirrmann,
226 Schneiderman, and MSU classification data in our
227 study). Therefore, intradiscal ozone-oxygen
228 injection yields progressive decompressive and
229 anti-inflammatory effects on the spinal cord.
230 Moreover, it has preventive influences on
231 deterioration or recurrence of clinical signs.
232 Nevertheless, more time is required to compare the
233 results with laminectomy or fenestration [2].

234 We also introduced intradiscal O₂-O₃ injection
235 as an alternative therapy for herniated IVDD with
236 low invasion, long-lasting effects. Moreover,
237 compared with glucocorticoid therapeutics and
238 surgery, this method is a cost-effective solution.
239 However, despite being useful in chronic patients,
240 intradiscal injection of O₂-O₃ is not a desirable
241 therapy in patients that require rapid
242 decompression. In specific, it is beneficial in

243 patients who demonstrate clinical improvements by 272 considerably decreases the risk of abscess
244 anti-inflammatory therapies and have frequent 273 formation from skin contamination [16, 17].

245 relapses. All the dogs included in this study had 274 The other noticeable advantage of this method
246 chronic problems and did not require emergency 275 is its simplicity and short anesthesia duration,
247 surgery. Samples with no deep pain perception in 276 which plays an essential role in these patients. In
248 the clinical examination were excluded since they 277 addition, it requires minimal hospitalization
249 need emergency operation to decompress the spine. 278 (approximately 24 hours) for post-surgical checks

250 The density of the involved disc is decreased 279 of the side-effects. In laminectomy and other
251 after O₂-O₃ intradiscal injection. This intradiscal 280 surgical methods, hospitalization time is
252 shrinkage creates free space for the compressed 281 considerably more. Furthermore, the cost of this
253 spine to move regardless of herniated disc materials 282 procedure is significantly lower than surgery or
254 in the spinal cord. This confirmed by MRI data and 283 other alternatives, specifically in the long-term, as
255 clinical signs. Disc shrinkage also guaranteed that 284 this method prevents further problems with the
256 the treated IVD would not remain herniated or 285 injected IVD.

257 extruded, and thus, the possibility of recurrence of 286 **Conclusions**
258 pain and clinical signs were reduced considerably 287 In this study, a recognizable disc shrinkage was
259 [5]. Therefore, the O₂-O₃ therapy offers a 288 confirmed in 1.5 Tesla MRI. However, the degree
260 replacement for previous conservative methods, 289 of this shrinkage requires more time and is related
261 which may prevent further extrusion or recurrence 290 to the extent of herniated disc material
262 of herniation of IVD [14]. 291 degeneration. Nevertheless, no specific

263 There are other advantages in using the O₂-O₃ 292 complications were detected. In conclusion, it is
264 mixture as a non-harmful gas with analgesic and 293 observed that intradiscal O₂-O₃ mixture injection
265 anti-inflammatory effects, even when this gas is in 294 under CT scan guidance can be helpful, and
266 direct contact with the spinal cord. The anti- 295 minimally invasive, in the decompression of disc
267 inflammatory feature of the ozone-oxygen mixture 296 herniations.

268 functions via inhibiting inflammatory inducers and 297 **Methods**
269 pain-producing mediators such as prostaglandins 298 *Animals:* Among the dogs admitted to the Small
270 [15]. Moreover, due to its strong bactericidal 299 Animal Teaching Hospital (Faculty of Veterinary
271 activity, it has fewer complications, which 300 Medicine, University of Tehran), 3 dogs with

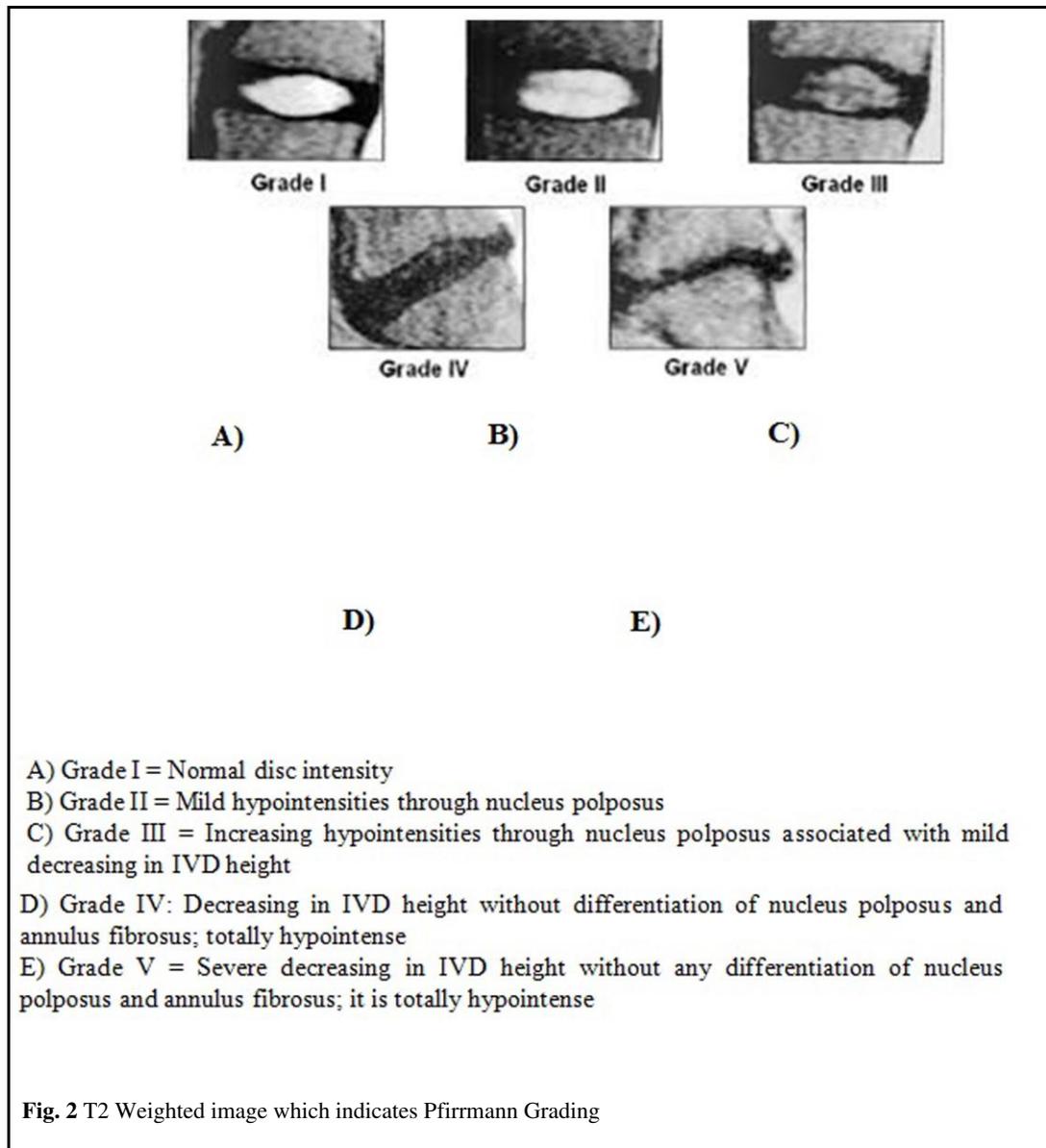
301 various types of neurologic abnormalities were 331 and hopping test before injection. None of the
302 included in this study based on their owner's 332 samples had a history of glucocorticoid therapy or
303 request. The animals had clinical signs related to 333 surgery beforehand.
304 thoracolumbar IVDD and positive deep pain 334 **Premedication and Anesthesia**
305 perception (DPP). In addition, dogs under 8 year- 335 The dogs were given intravenous Pantoprazole (1
306 old with no clinical signs relevant to thoracolumbar 336 mg/kg) and subcutaneous Metoclopramide (0.5
307 IVDD, along with samples with other major 337 mg/kg), four to six hours before anesthesia. They
308 diseases such as cardiac or endocrinopathy, were 338 were sedated using intravenous injection of
309 excluded. 339 Ketamine (5 mg/kg) and Diazepam (0.2 mg/kg) and

310 Samples 1 and 3 were female, while sample 340 were maintained in sedation with intravenous
311 number 2 was male, and were 12, 10, and 9 years 341 Ketamine (4 mg/kg).
312 old, respectively. The patients demonstrated long- 342 **Diagnostic Imaging:** The CT-scan device
313 lasting periodic problems in gaining weight. Their 343 (Siemens, SOMATOM Spirit 2, 70KV, 100 mAs,
314 body weight ranged from 7 to 28 Kgs. The patients 344 Germany) was employed for 1 mm thick slices at
315 were gone through complete neurological 345 1.5 mm intervals under general anesthesia for
316 examination by an experienced surgeon and were 346 intradiscal injections. MRI scan (General Electric
317 diagnosed with thoracolumbar intervertebral disc 347 Medical System, 1.5 Tesla) was performed before
318 herniation (IVDH) on physical, neurological, 348 and five weeks after O₂-O₃ therapy. Index
319 diagnostic imaging views (radiography and MRI), 349 measurements were performed using Marco PACS
320 complete blood count profile, and serum 350 software.
321 biochemical analysis. Different ranges of disc 351 **Intradiscal O₂-O₃ Injection**
322 herniation were detected in thirteen intervertebral 352 A 0.5 ml O₂-O₃ mixture (O₃ concentration of 32
323 joint spaces. Deep pain perception (DPP) was 353 µg/µl) was injected into the intradiscal area of
324 positive in all patients, and they had no other 354 thirteen IVDs obtained from three dogs under CT
325 systemic diseases. However, they had difficulty in 355 guidance and general anesthesia. Medical O₂-O₃ gas
326 jumping and running. 356 was produced by the 5th generation technology

327 On the contrary, none had urinary or fecal 357 medical ozone generator (Gardina, MC80, Spain).
328 incontinence. Sample 1 had delayed right hind limb 358 **Measurements**
329 paw replacement and hopping test, while samples 2 359 1) Pfirrmann Grading: Pfirrmann grading
330 and 3 both had bilateral delayed paw replacement 360 was used to evaluate the degenerative

361 changes of Nucleus Polposus in T2W 364
 362 images, before and 5 weeks after 365
 363 intradiscal injection. This grading is 366

normally used in spinal cord researches
 and has different classifications (grade I to
 V in Fig. 2 [9, 10].



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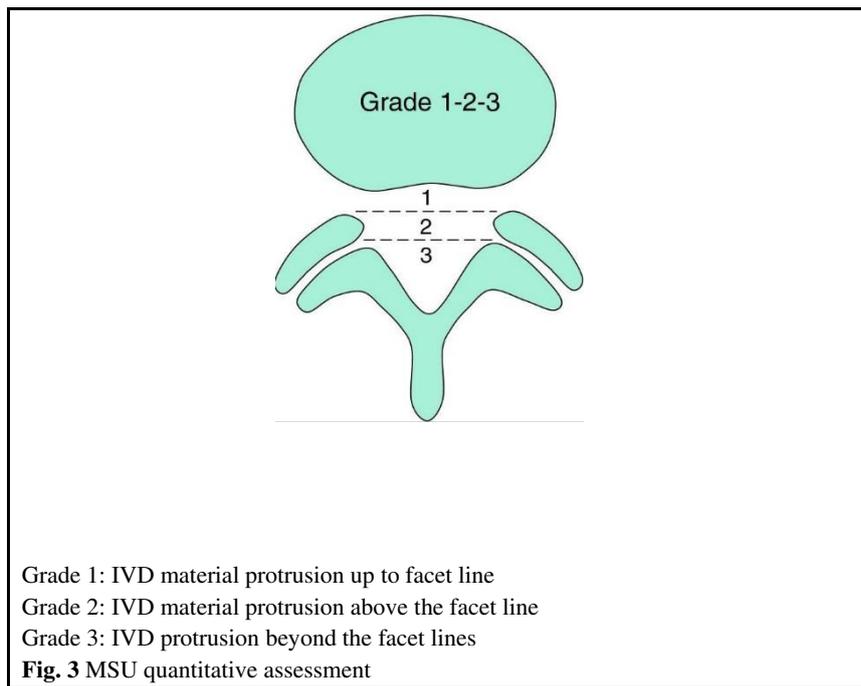
368 2) Schneiderman classification scheme: This 374
 369 scheme evaluates the degenerative 375
 370 changes in height and signal intensity of 376
 371 the intervertebral IVDH discs in T2W 377
 372 images, before and 5 weeks after 378
 373 intradiscal injection in Table 1 [11]. 379

3) MSU index: MSU (Michigan State
 University) index indicates the size of disc
 herniation and its location in the spinal
 canal. Moreover, it assesses the degree of
 disc herniation before and 5 weeks after
 intradiscal injection. It was introduced by
 the MSU classification system and is

381 defined as a simple and reliable method to 383 (See in Fig. 3).
 382 measure herniated IVD objectively [12] 384

385 **Table 2** Schneiderman classification scheme [11]
 386

Grade	0	1	2	3
387 Intervertebral disc intensity	Normal height of IVD	Speckled pattern or heterogeneous with decreased intensity in nucleus pulposus	Diffuse loss of signal in nucleus pulposus and annulus fibrosus	Signal void in IVD intensity
388	Normal signal intensity in annulus fibrosis and nucleus pulposus			Severe decreasing in IVD height
389				
390				



391

392 4) MSI: Evaluation and comparison of the 402
 393 Mean Signal Intensity (MSI) of the IVD 403
 394 were performed before and 5 weeks after 404
 395 intradiscal injection [13]. 405

396 5) A-index: Measurement of the disc 407
 397 herniation area, in comparison with the 408
 398 spinal canal area, was performed before 409
 399 and 5 weeks after intradiscal injection, as 410
 400 proposed in Fig. 1 [1]. 411

401

6) B-index: Measurement and comparison of 412
 herniated intervertebral disc area, before 413
 and 5 weeks after intradiscal injection, was 414
 performed using the following equation:

$$B - index (\%) =$$

$$\frac{\text{Intervertebral disc surface area before ozone-oxygen injection} \times 100}{\text{Intervertebral disc surface area after 5 weeks after ozone-oxygen injection}}$$

The injection sites in the dorsal thoracolumbar 415
 area were first marked by the CT guide and were 416
 then surgically scrubbed (See in Fig. 4).



Fig. 4 Preparation of the patient and injection of the spinal needle in the L4-L5 IVD under CT scan guidance

413

414 The skin was perforated using a 2.5 inch 22 G
 415 spinal needle, and the needle was guided through
 416 epaxial muscles to the center of the herniated disc
 417 from the lateroventral side of the articular facet
 418 (See in Fig. 5).



Fig. 5 . O₂-O₃ intradiscal injection under CT guidance
 Red arrow: Spinal needle in L4-L5 IVD
 Yellow arrows: O₂-O₃ gas bubbles injection

419

420 The operation was under constant monitoring
 421 with frequent CT images. Next, the stylet needle
 422 was extracted, and a 0.5 ml fresh ozone-oxygen gas
 423 mixture was injected. Finally, the stylet was
 424 extracted, and the injection site was compressed for
 425 about 1 minute. All the dogs were monitored for 24
 426 hours afterward and were discharged the following
 427 day. Owners were instructed to limit the dog
 428 activity during the first 2 weeks and increase their
 429 activity gradually in the next 3 weeks. Clinical and
 430 MRI scans were performed for disc shrinkage, its
 431 size, and spinal cord compression.

432 *Statistical Analysis:* The data were assessed using
 433 SPSS. A paired T-test was used to identify the
 434 correlations between measurements before and
 435 after injection. P<0.05 was considered as the level
 436 of significance.

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443 **Authors' contributions**

444 All authors have read and approved the final manuscript.

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Figures

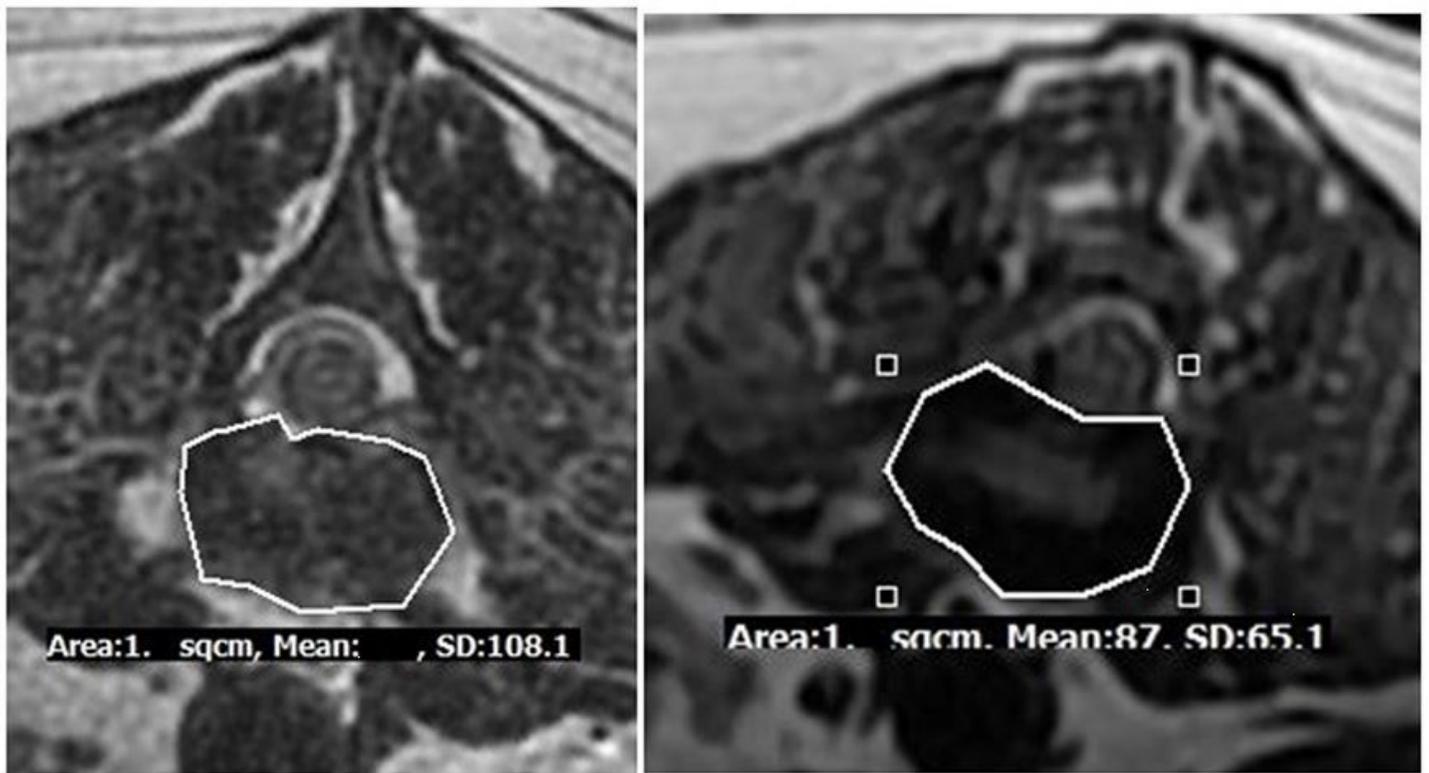
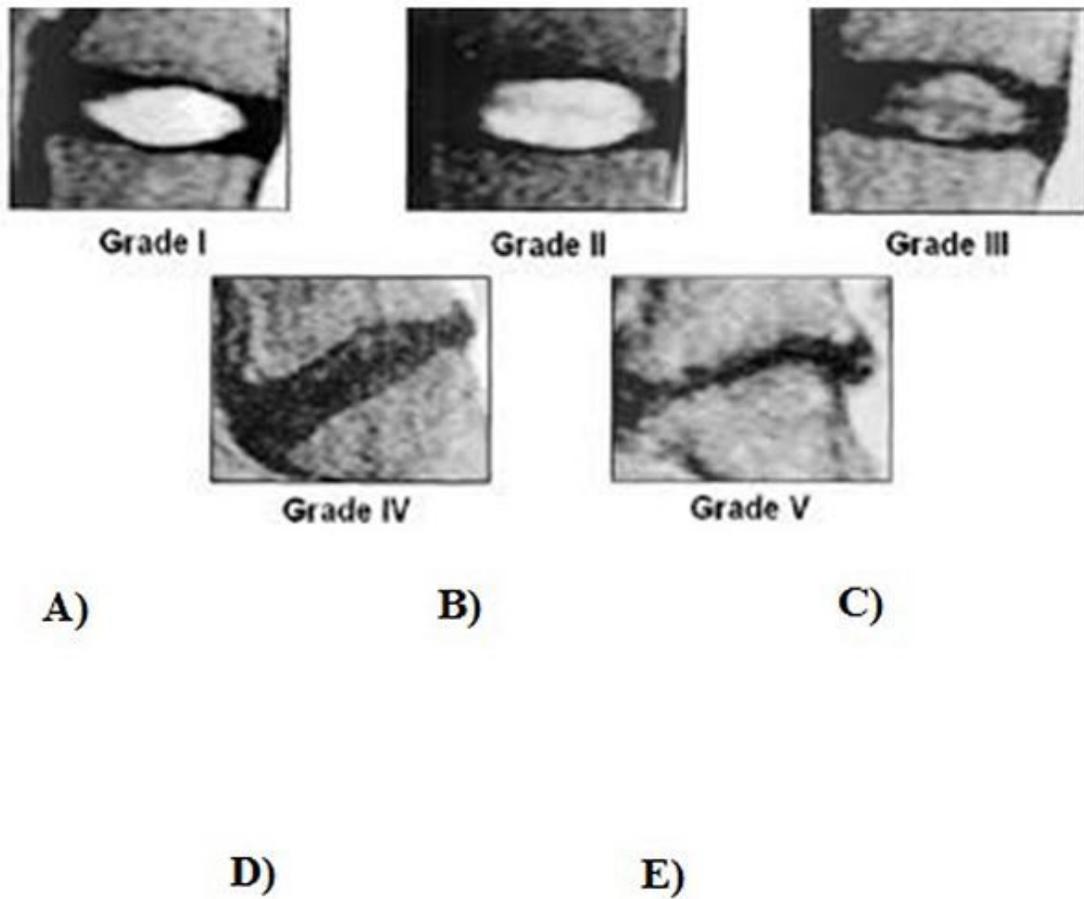


Figure 1

1 T2 weighted images at the level of L2-L3 from Dog 1 before (1) and after (2) O2-O3 intradiscal injection, with B-index and MSI measurement: R1 R2



A) Grade I = Normal disc intensity

B) Grade II = Mild hypointensities through nucleus pulposus

C) Grade III = Increasing hypointensities through nucleus pulposus associated with mild decreasing in IVD height

D) Grade IV: Decreasing in IVD height without differentiation of nucleus pulposus and annulus fibrosus; totally hypointense

E) Grade V = Severe decreasing in IVD height without any differentiation of nucleus pulposus and annulus fibrosus; it is totally hypointense

Figure 2

T2 Weighted image which indicates Pfirrmann Grading

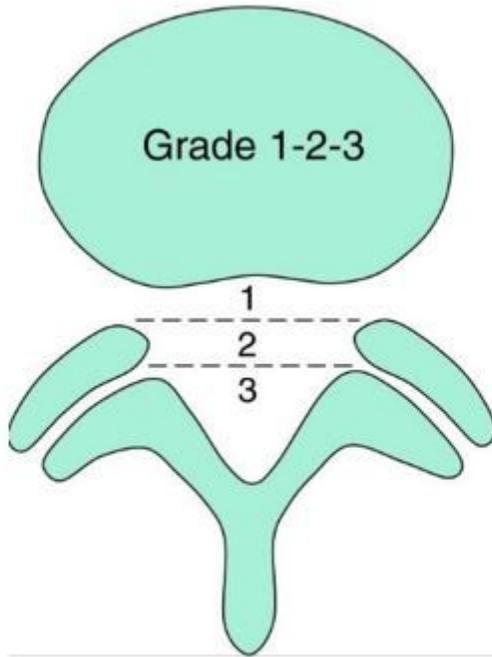


Figure 3

MSU quantitative assessment Grade 1: IVD material protrusion up to facet line Grade 2: IVD material protrusion above the facet line Grade 3: IVD protrusion beyond the facet lines



Figure 4

Preparation of the patient and injection of the spinal needle in the L4-L5 IVD under CT scan guidance



Figure 5

O2-O3 intradiscal injection under CT guidance Red arrow: Spinal needle in L4-L5 IVD Yellow arrows: O2-O3 gas bubbles injection