INTRODUCTION: The current standard of care for intervertebral disc (IVD) herniation, surgical discectomy, does not repair annulus fibrosus (AF) defects which is partly due to the lack of effective methods to do so, and is why new repair strategies are widely investigated and tested preclinically. There is a need to develop a standardized IVD injury model in large animals to enable comparison and interpretation across preclinical study results. The purpose of this study was to compare the most frequently used \textit{in vivo} IVD injury models in sheep in order to determine the AF defect type combined with nucleus pulposus (NP) removal that would better mimic degenerative human spinal pathologies.

METHODS: The study was ethically approved by relevant authorities (Cantonal authorities in Graubünden, Switzerland: Permission # 19/2019) and performed in a facility accredited by the AAALAC. Six healthy skeletally mature sheep were enrolled and randomly assigned to one of two observation periods (1- and 3-months), and underwent creation of 3 different full thickness AF defect types (slit [longitudinal 16-mm], cruciate [2 cuts of 8-mm], box [rectangle of 5-mm x 3-mm]) in conjunction with 0.1 g NP removal in three lumbar levels between L1-L4 using a lateral retroperitoneal surgical approach. L4-L5 IVD was left intact and used as control (Fig. 1A). Postoperatively, the spine was monitored \textit{in vivo} by clinical CT scans immediately after surgery, at 2 weeks and at euthanasia (1- or 3-months), and \textit{ex vivo} after euthanasia by MRI and histology. The main outcomes (disc height loss, Pfirrmann grading, semiquantitative histopathology grading for IVD degeneration) were assessed with nonparametric Friedman two-way analysis of variance for paired samples for the AF defect types with fixed effect of the sheep and respective observation periods. For all analysis, statistical significance was set at \( p < 0.05 \).

RESULTS: All AF defect types led to detectable degenerative changes mimicking human IVD degenerative pathologies. The injured IVDs had a mean disc height variation postoperative of 90 ± 5\% (Fig. 1B). The AF defect types were not equal in term of disc height loss at 3 months postoperatively; the cruciate and box-cut AF defect showed significant decreased disc height compared to their preoperative height (\( p=0.00419 \) and 0.0295 for cruciate and box-cut, respectively) with the box-cut defect creating the greatest disc height loss postoperatively compared to cruciate defect (\( p=0.0304 \)). Contrariwise, the slit AF defect showed restoration of normal height at 3 months postoperatively when compared to preoperative height. MRI showed bulging of disc tissue in 83\% of injured IVD equally distributed between the AF defect types, but no herniation. On MRI, more pronounced degenerative changes were observed in the injured IVDs (Fig. 2A) indicated by their significantly higher Pfirrmann grade (Fig. 2B) compared to control IVD (\( p=0.0022, 0.004, 0.0256 \) for the slit, cruciate, box-cut AF defect respectively) but no difference was detected between the type of AF defect (\( p=0.2327, 0.7567, 0.4630 \)). All injured IVDs showed a combination of histological changes such as defects involving the AF and NP along with degenerative and inflammatory alterations and limited reparative changes. Injured IVDs at 1- and 3-months had statistically significant increases in total histopathological severity score compared to control IVD (\( p=0.0004, 0.006, 0.0009 \) for the slit, cruciate and box-cut AF defect respectively) but no significant difference between the types of AF defect created (\( p=0.1456, 0.2970, 0.9725 \)).

DISCUSSION: This study involved a thorough evaluation of the 3 most frequently used sheep IVD injury models for simulating severe AF injury and herniation, with all surgical AF defect techniques with partial NP removal reproducibly initiated degenerative changes detectable using medical imaging techniques and histopathological analysis. However, postoperative IVD height loss was greatest for cruciate and box-cut AF defects. Degenerating IVD are observed to lose disc height over time because of NP dehydration and tissue loss, resulting in a reduced capacity to resist the compressive loads applied to the spine.\textsuperscript{2} The postoperative loss of total disc height was a consistent finding across all AF defect types in the shorter term (up to 1 month postoperatively). The IVD height restoration observed with the slit AF defect could result from a less invasive disruption of the AF leading to earlier healing along with a better ability to withstand internal pressurization and to resist changes in corresponding multidirectional vertebral motion than a full thickness cruciate and box-cut AF excision.\textsuperscript{2} The ability of the different AF defects to precipitate IVD degeneration is a critical aspect of preclinical IVD injury models, and warrants consideration for potential implications in human surgery. All injured IVDs reliably showed T2 signal changes and MR-images compatible with IVD degeneration resulting in an increased Pfirrmann grade. However, the similarity of results between 1- and 3-months postoperatively limits the ability to clearly differentiate between induction and progression of IVD degeneration mimicking human pathology, and acute static MRI signal changes due to the structural damage inflicted to the IVD structure during the surgery. Nevertheless, similarities in total histopathological severity score between the AF defects showed that they act similarly in term of histological degeneration and repair processes over a 1-to-3-month observation period.

SIGNIFICANCE/CLINICAL RELEVANCE: This study showed that the most frequently used IVD injury models in sheep do not all generate comparable IVD degeneration signs. While all IVD injury models produce changes that mimic human degenerative pathologies, the slit AF defect should be avoided since disc height recovered suggesting some healing. Furthermore, cruciate defect is a common surgical technique in discectomy procedures and was shown to be a relevant and reproducible AF defect for new AF repair strategies in sheep preclinical investigations.

![Figure 1](https://example.com/figure1.png) CT imaging shows IVD height loss in all 3 defect types. Different superscript letters within the same observation period indicate a significant difference between the AF status (\( p < 0.05 \)).

![Figure 2](https://example.com/figure2.png) MR-imaging shows increased degeneration grade for all defect types. Asterisks indicates a significant difference between the AF status (\( p < 0.05 \)).