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# Biomechanical and Radiographic Evaluation of An Ovine Model for The Human Lumbar Spine

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## Abstract

While various species of animal models have been used in preclinical investigations of spinal implant devices to assess their biological adaptation and biomechanical performance, few studies have made comprehensive comparisons to validate their suitability of modelling the human spine. The purpose of this study was to assess essential biomechanical behaviours and disc morphology of the ovine lumbar model. Flexibility testing was conducted on the spines (L3-L4 and L4-L5) of nine skeletally matured sheep. Segmental rotation and intradiscal pressure were measured and load sharing between the intervertebral disc and posterior elements were calculated on the basis of a simplified parallel spring model. Following the tests, the spinal segments were sectioned into a series of sagittal slabs, and transverse radiographs of these slabs were taken to evaluate the variation in the disc height and end-plate curvature. Comparing the biomechanical and radiographic results with published data on the human lumbar spine, good comparability between the ovine and cadaveric lumbar spines was found in terms of the general disc shape and in most of the biomechanical parameters including the range of motion, neutral zone, and load sharing between the intervertebral disc and posterior elements. A few distinctive differences were also found between the two, including flatter sagittal alignment, smaller disc dimensions, and greater lateral bending motion in the ovine model. [PUBLICATION ABSTRACT]

## Keywords

lumbar spine, animal model, disc morphology, load sharing, biomechanics

## 1 INTRODUCTION

Animal models have often been used in preclinical investigations of spinal implant devices to assess their biological adaptation and biomechanical performance [1-8]. However, few comprehensive comparisons have been made to validate the suitability of these models. With the emergence of the new motion-preserving implants for spinal surgery, a good animal spine model should also demonstrate similar load-sharing characteristics between its major spinal structures as in human spine. Wilke et al. [9] compared the range of motion, neutral zone, and segmental stiffness of an ovine spine model with historical data from the human spine in six loading directions. Their results showed that the two species have comparable biomechanical ranges of motion in flexion-extension, right-left lateral bending, and right-left axial rotation at the L4-L5 spinal segment. Distribution of the spinal load between the anterior and posterior structures in their ovine model and how it compares with the human cadaveric model was not investigated. Kandziora et al. [10] examined the suitability of an ovine cervical spine model in terms of its biomechanical and anatomical characteristics. The range of motion and stiffness of each cervical segment as well as linear anatomic parameters of each vertebra were compared for two spines.

The objectives of this study were, first, to assess the essential biomechanical behaviours of the ovine spine including the range of motion and neutral zone, to compare the results with those from previously published human cadaver studies [11-15] and to characterize ovine lumbar load-sharing characteristics between the intervertebral disc and posterior elements and, second, to establish the disc height, end-plate curvature, and sagittal alignment of the ovine lumbar spine. All tasks were performed for the purpose of further contributing to the quantifiable information available for the sheep as an animal model for the human lumbar spine.

# 2 METHODS

Lumbar spines from nine skeletally matured female Rambouillet X Columbia sheep were procured for this study and stored at -29 °C until testing. Two frequently used disc levels L3-L4 and L4-L5 were selected for the analysis. All paraspinal musculatures were removed prior to testing and the multilevel lumbar motion segments (L3-L5) were tested using non-constrained pure moment flexibility protocol on a customized spine-loading frame.

## 2.1 Biomechanical evaluation

Pure moments in the six loading directions of flexion, extension, right lateral bending, left lateral bending, right axial rotation, and left axial rotation were applied to the completely unconstrained spine segmental levels via a pulley-weight system [16-18]. Moments were applied in six incremental loading steps (0, 0.5, 2.5, 4.5, 6.5, and 8.5Nm). A six-axis load cell (AMTI, Watertown, Massachusetts, USA) was mounted in series with the caudal base of the vertebra to verify the moments and forces applied to the specimen. The three-dimensional motion of the intervertebral joints were recorded with the Vicon motion analysis system (ViconPeak, Oxford, UK) which consists of three cameras tracking infrared markers that were attached to each vertebra of the specimen. The range of motion and neutral zone (at 0.5Nm) were determined on the basis of Euler angle rotations, and the segmental stiffness was derived from the linear portion of the load-displacement plot. In addition, a miniature pressure transducer (Precision Measurement Company, Ann Arbor, Michigan, USA) was implanted in the nucleus of the L3-L4 and L4-L5 discs to measure the intradiscal pressure during the flexibility tests. A small compressive preload (10 N) was applied in a 'follower-load' fashion [19] throughout the testing sequence to help to keep the transducer in place. The extra moment generated by the preload on the spine was negligible.

Each specimen underwent non-destructive flexibility testing at two stages: first, intact and, second, with the posterior elements removed. Load sharing between intervertebral disc and the posterior structures was calculated on the basis of a compound parallel spring model [20]. The percentage of the applied moment M transmitted through the posterior structures, namely $M_{post}$, was determined from $M_{post}/M = 1 - k_{disc}/k_{int}$, where $k_{int}$ and $k_{disc}$ are the segmental stiffness of the intact spine (stage 1) and of the spine without the posterior elements (stage 2) respectively.

Data from intradiscal pressure measurement were used to verify the amount of load shared by the posterior elements. The load-sharing index LSpost for posterior elements was defined for this purpose: $LS_{post}(\%) = 1 - P_{int}/P_{disc}$, where $P_{int}$ and $P_{disc}$ are the maximum intradiscal pressure reading of the intact spine and the spine with the posterior elements removed respectively.

## 2.2 Radiographic evaluation

Immediately following the flexibility tests, the specimens were wrapped in a wet paper towel, sealed in a plastic zipper bag, and stored at -29 °C. Seven of the ten frozen specimens were sectioned into a series of four consecutive sagittal sections with an approximate thickness of 6.0mm using a band saw at both L3-L4 and L4-L5 levels. Transverse radiographs of the slabs were taken using a high-resolution radiographic unit (Faxitron, Hewlett-Packard, McMinnville, Oregon, USA) and high-resolution film

(Ektascan B/RA 4153, Eastman Kodak, Rochester, New York, USA). The digital images of these radiographs were obtained using IM50 imaging software (Leica Microsystems) and a 24mm lens (Nikkor fm24, Nikon, Tokyo, Japan).

The following measurements were made from the digitized images:

(a) the height of the disc space in the anterior, central, and posterior margins;
(b) the sagittal alignment angle defined by the posterior edge of the vertebral bodies.

The disc height measurements used were collected by first identifying the four 'corners' of the disc located on both the anterior and the posterior edges of the two adjoining end plates, as seen from the markers in Fig. 1. A bisectrix was then created by connecting the markers from the anterior and posterior midpoints (dashed line in Fig. 1). The anterior disc height was defined as the sum of the perpendicular distance from the bisectrix to both anterior markers, and the posterior disc height was defined as the sum of the perpendicular distances from the bisectrix to both posterior markers. The same process was used to determine the disc height at the middle of the vertebral body.

The angle of sagittal alignment was determined by the angle formed between the lines connecting the anterior and posterior 'corner' points on the inferior aspect of the superior vertebra and the superior aspect of the inferior vertebra. All measurements were performed by two observers in a blinded fashion, and the mean and standard deviation for each measurement were reported.

### 2.3 Statistical analysis

For biomechanical evaluation, a statistical test consisting of multivariate analysis of variance (MANOVA) ($p < 0.05$) was performed to compare the difference between the two disc levels in the following parameters: the range of motion, neutral zone motion, percentage moment Mpost transmitted through the posterior structure as calculated from the spring model, and the posterior element load share index LSpost. Data from all loading directions were included as the dependent variables. For radiographic evaluation, the MANOVA test was used to compare the level differences in disc height measurement at the three regions and sagittal alignment angle. If no significant difference was found between disc levels ($p > 0.05$), the data would be pooled together to compare the mean with the published human data using one sample analysis (t test of means). For disc height measurement, the regional differences were evaluated with an analysis of variance test ($p < 0.05$) and post-hoc Bonferroni comparisons ($p < 0.0167$).

## 3 RESULTS

### 3.1 Flexibility test and load-sharing characteristics

The results of the range of motion and neutral zone motion from the flexibility tests are presented in Figs 2 and 3. MANOVA tests showed no statistically significant differences between the two disc levels in the range of motion ($p = 50.57$) and neutral zone motion ($p = 0.26$), so the data from L3-L4 and L4-L5 were pooled together. No statistically significant difference was found in any loading direction when comparing the range-of-motion data from the ovine model with in-vitro measurement of the human lumbar spine reported in the literature [11, 13, 21]. However, compared with the in-vivo measurement of the human lumbar spine [14], the range of motion of the ovine lumbar segments in flexion-extension was 30-50 per cent smaller than in humans ($p = 0.0005$), while the range of motion in lateral bending was 20-50 per cent higher than in humans ($p < 0.0001$). The neutral zone motion from the ovine model showed no statistically significant differences from human cadaveric data in flexion-extension and axial rotation but was significantly lower than the human cadaveric data in lateral bending ($p < 0.0001$).

The results of load-sharing characteristics at disc levels L3-L4 and L4-L5 are presented in Fig. 4. The calculated percentage moment shared by the posterior elements, and the load-sharing index calculated from the intradiscal pressure measurement showed no statistically significant difference between the two disc levels (MANOVA test; $p = 0.16$ and $p = 0.18$ respectively). The largest amount of posterior structures load share occurred in axial rotation: 56.8 per cent for L3-L4 and 42.4 per cent at L4-L5. The smallest amount of posterior structures load share was found in lateral bending: 3.7 per cent and 6.6 per cent. The load-sharing index for the posterior elements calculated from intradiscal pressure measurement is presented in Table 1. The index indicated that over 70 per cent extension load and around 60 per cent axial rotation load was transmitted through the posterior elements. In lateral bending, the posterior elements of L3-L4 carried the least amount of load (8 per cent).

## 3.2 Intervertebral disc morphology and sagittal alignment

A MANOVA test showed no significant differences between the two disc levels in the disc morphologic measurements (p50.94).

### 3.2.1 Disc heights

At both L3-L4 and L4-L5 levels, the disc height in the anterior region (DHa) was the greatest: 6.31 ± 0.48mm and 6.28 ± 0.44mm respectively (Table 2). Variations in the disc height measurements between the four consecutive sagittal sections of each specimen were between 0.25 and 1.63mm and between 0.25 and 1.50mm respectively in the anterior and central regions and was between 0.88 and 2.50mm in the posterior disc region. The ovine lumbar disc height in all three regions was significantly smaller ($p < 0.0001$) than the 8-16mm anterior disc height in humans [22-24].

### 3.2.2 Sagittal alignment angle

The average sagittal alignment was similar between the two disc levels, 6.0° ± 1.8° lordosis for the L3-L4 level and 6.0° ± 1.4° lordosis for the L4-L5 level; both were significantly smaller ($p < 0.0001$) than the 10°-12° segmental lordosis at the L3-L4 level and 15°-21° at the L4-L5 level in humans [25, 26]. The ovine lordotic angle at these two discs was comparable ($p > 0.99$) with the 6.3° L2-L3 lordosis in humans reported by Campbell-Kyureghyan et al. [24].

# 4 DISCUSSION

The ovine lumbar spine model is one of the most frequently adopted models for preclinical investigations of biological and biomechanical behaviours of novel implants [5, 27-30]. However, there is a lack of comparative studies to address its suitability in modelling different aspects of human cadaveric spines. Wilke et al. [9] studied the in-vitro range of motion, neutral zone motion, and segmental stiffness of an ovine spine model in comparison with historical human cadaveric data. They concluded that these biomechanical parameters of sheep spine are 'quantitatively similar' to human spines, and therefore sheep can serve as an alternative for the evaluation of spinal implants. Their study did not characterize load-sharing properties of structures within each spinal segment, nor did the study compare the anatomical features between the two species. In 2001, Kandziora et al. [10] studied both the biomechanical and the anatomical properties of an ovine cervical model and conducted a direct comparison with human cadaveric cervical spines. They found less interspecies difference in the lower cervical spine, and, on the basis of similarities in the biomechanical and anatomical properties, they concluded that the ovine C3-C4 segment is the most reliable model for the corresponding human spine. In this study, the biomechanical and anatomical properties of two commonly used ovine lumbar segments were evaluated for their suitability as a human cadaveric spine alternative.

Results from the flexibility testing showed that the ovine lumbar spine matched well in the range of motion and neutral zone motion with those obtained from a previous study by Wilke et al. [9], and, most importantly, they are in general agreement with those obtained from the human cadaveric lumbar spine [13, 21]. Significant differences were found in the directions of flexion-extension and lateral bending when compared with in-vivo human motion. This might be partially attributed to the strong back muscles (massive flexor-extensor muscles) in an active in-vivo model which inevitably generates a larger range of motion. Another factor is due to the inherent deficiency found in most quadruped models where the range of motion in lateral bending is more dominant than or at least just as dominant as in flexion-extension. Nevertheless, the results indicated that the ovine lumbar model is a good replica for passive human motion segment.

Despite the horizontal position, the spine of a quadruped is thought to be loaded under high axial compression, like its human counterpart, as the strong back muscles work to maintain the spinal alignment against gravitational forces [31]. During normal walking, the asymmetrical thorax and pelvis loading patterns also subject the spine to high torsional loads. Unlike the human, the trunk extension during gallop results in a high extension moment on the posterior column in a quadruped [31].

In the present study, the load-sharing calculation between the intervertebral disc and posterior elements revealed that the posterior structures in the ovine lumbar spine act as major load barriers in axial rotation, extension, and flexion. This finding is in accordance with analytical and experimental results on the human spine from several early studies [32, 33]. The posterior column of the human spine as a major load barrier was also found in an ageing population by recent cadaveric studies [34, 35]. In these studies, Pollintine et al. [34, 35] demonstrated a significant load-bearing shift from the anterior column to the posterior column with ageing and spine degeneration. The amount of compressive load borne by the neural arch increased from below 20 per cent in non-degenerated spines to 40-90 per cent in spines from elderly donors.

Radiographic evaluation showed a substantial difference in the anterior disc height between the ovine model and a reported magnetic resonance imaging (MRI) study in asymptomatic humans, where the ovine disc ranges from 25 per cent to as much as 60 per cent smaller than the human disc but is in scale with its smaller anterior-posterior and medial-lateral dimensions of the vertebral body. Unlike humans, the lordotic angle of the ovine lumbar spine varies little from segment to segment and the value is similar to that of L2-L3 in humans.

Despite the size difference between ovine and human lumbar segments, sagittal section slabs showed a similar shape profile of the intervertebral disc across the medial-lateral margins. A slightly convex disc shape was found throughout the section series, especially in sections from the central disc region.

Similar mild convexity is reposted in a human lumbar spine with a normal disc in an MRI imaging study [23]. Although this mild convexity becomes severe with the progression of disc degeneration where gradually hardened disc tissues again push the gradually softened end plate through osteoporosis, resulting in severe concavity of the vertebral body [36]. Therefore, the ovine lumbar spine should be used to represent healthy discs only.

One of the main limitations of the study was that it did not include a study of human cadaveric lumbar spines under the same protocols to facilitate more direct comparisons between the two models. Secondly, the simplified spring model was built on the assumption that the spine segment is homogeneous from anterior to posterior, and removal of the posterior segment would not alter the load-bearing properties of the remaining structures. Finally, the study selected only the most frequently used L3-L4 and L4-L5 segments and was not a complete representation of the ovine lumbar spine. Nevertheless, all the test results showed little variation between the two lumbar segments.

In conclusion, good comparability between the ovine and cadaveric lumbar spine was found in terms of the general disc shape and in most of the biomechanical parameters, including the range of motion, neutral zone motion, and load sharing between the intervertebral disc and posterior elements. A few distinctive differences were also found between the two, including flatter sagittal alignment, smaller disc dimensions, and greater lateral bending motion in the ovine model. The present authors believe that the ovine lumbar model can be used as an alternative to human cadaveric spines. However, interpretation of the study results should always be made in light of the distinctive interspecies differences.

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