Porcine Functional Spine Unit in orthopedic research, a systematic scoping review of the methodology

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Abstract
Purpose: The aim of this study was to conduct a systematic scoping review of previous in vitro spine studies that used pig functional spinal units (FSU) as a model to gain an understanding of how different experimental methods are presented in the literature. Research guidelines are often used to achieve high quality in methods, results, and reports, but no research guidelines are available regarding in vitro biomechanical spinal studies.

Methods: A systematic scoping review approach and protocol was used for the study with a systematic search in several data bases combined with an extra author search. The articles were examined in multiple stages by two different authors in a blinded manner. Data was extracted from the included articles and inserted into a previously crafted matrix with multiple variables. The data was analyzed to evaluate study methods and quality and included 70 studies.

Results: The results display that there is a lack of consensus regarding how the material, methods and results are presented. Load type, duration and magnitude were heterogeneous among the studies, but sixty-seven studies (96%) did include compressive load or tension in the testing protocol.

Conclusions: This study concludes that an improvement of reported data in the present field of research is needed. A protocol, modified from the ARRIVE guidelines, regarding enhanced report-structure, that would enable comparison between studies and improve the method quality is presented in the current study. There is also a clear need for a validated quality-assessment template for experimental animal studies.

Introduction
Many different spinal pathologies can cause back pain but in most cases the cause is still unknown. Further basic research is therefore crucial to gain additional information regarding causal relationship between spinal loads, back pain, and spinal pathologies. Research regarding spinal loading is often done using biomechanical test models [1]. To achieve high research quality, it is vital to validate and in a detailed manner describe the study method. Research guidelines are recommendations on how to ensure high study quality depending on study type. The research guidelines help to minimize unnecessary studies, maximize information published and allow reproducibility and comparability across studies. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [2] is a worldwide accepted checklist that support authors of in vivo experimental studies to achieve high quality aspects regarding the study design, method, material, analyza-
tion and report of studies and there are several check-
lists regarding different in vitro experimental studies, but not any specific for functional spinal units and biomechanical experiments.

Spines from human cadavers and animals are commonly used in varying experimental models for spinal research. Frequently used animals are calves, deer, dogs,
goats, pigs, and sheep [3–7]. The porcine lumbar spine resembles the human lumbar spine in both biomechanical properties, load response and tissue structure, and is a well-used experimental model [8–16].

The material and specimen complexes used in biomechanical studies can be of many compositions ranging from a complete spine to small tissue samples from any part of the spine. A Functional Spinal Unit (FSU) consists of an upper and a lower vertebra with an intact intervertebral disc and is an international well-established research model for spine studies. In many biomechanical experimentation settings, the FSU is attached in some way superiorly and inferiorly to a device, which may induce a load on the specimen. The load can be of different vectors/angles, magnitudes/sizes, or a combination of these, and of variable rate and durations depending on study question, method, and protocols [16–18].

There is currently no common consensus regarding the methodology of in vitro spinal experimental biomechanical studies nor an established research presentation guideline, which is why there is a need to conduct a systematic scoping review and present a basic research guideline to achieve comparability, reduce unnecessary experiments and increase study quality.

**Aim**

The aim of this study was to conduct a systematic scoping review of previous in vitro biomechanical studies that used porcine functional spinal units (FSU) to gain an understanding of how different experimental methods are presented, summarize the study outcomes, and suggest future reporting guidelines.

**Material and methods**

The study methodology was a systematic scoping review [19, 20]. The search inclusion criteria were 1. Pig spine, 2. FSU specimen, 3. Not operated nor instrumented (preparation and testing fixation were accepted), 4. Article published in English language in a peer reviewed journal, 5. No publication date limit.

**Study search protocol and search strategy**

A modified version of the Systematic Review Protocol for Animal Intervention Studies (SYRCLE) [21] and the PRISMA-ScR Checklist [22] was used as a general study protocol to ensure systematic approach. The search strategy was a two-phase process: 1. Database search, and 2. Complementary search of first and last author of included studies from phase 1.
Several pilot searches were done according to the inclusion criteria and the final search was done in collaboration with a medical research librarian in the databases of PubMed, Embase, Cochrane and Web of Science in 2021–04-14. The search protocol: Search ((((((Spine[mh] OR Vertebral Column[tiab] OR Vertebral Columns[tiab] OR Spinal Column[tiab] OR Spinal Columns[tiab] OR Vertebra[tiab] OR Vertebrae[tiab] OR Spine[tiab] OR spinal[tiab]))) AND (Mechanical Phenomena[Mesh] OR Biomechanic[tiab] OR Biomechanical[tiab] OR Mechanobiological[tiab] OR Kinematics[tiab])) AND (pig[tiab] OR pigs[tiab] OR piglet[tiab] OR piglets[tiab] OR porcine[tiab] OR porcines[tiab] OR Swine[mh] OR swine[tiab]))) AND strength)) NOT (Editorial[ptyp] OR Letter[ptyp] OR Comment[ptyp] OR Case reports[ptyp]) Filters: English, Title, Abstract, Keywords. No letter, comment, editorial.

The complementary author search (phase 2) was done in PubMed and included all primary and last authors from the accepted studies from the database search (phase 1).

The flowchart of the selection method is presented in Fig. 1 [23]. Each abstract was examined by all three authors individually. All abstracts which were considered relevant by two authors were cleared for the next step. The abstracts which were approved by only one author were discussed by all three authors to determine whether they were cleared for inclusion.

The approved articles (n=92) were then read and assessed by the authors. The articles were divided so that each article was read by two of the authors individually. The articles were judged in accordance with the study protocol. Out of the 92 articles that were read, 33 were accepted for data extraction.

All first and last authors of the 33 accepted studies were then included in the complementary author search that involved 38 unique authors. The author search presented an additional 77 new abstracts that were screened according to the previous selection method, and which 37 were accepted for data extraction. In total 70 studies were included in the present study.

Data extraction
The data relating to the predefined variables were then inserted into previously crafted matrices (Table 1, 2, 3). Two authors screened the articles individually and compared the data extraction results. If in disagreement or if an uncertainty arose, a second was conducted in collaboration. The variables in the matrices included material type, sample size, mechanical load, test apparatus, study question and outcome of the study.

| Study nr | Year | Journal | Reference |
|----------|------|---------|-----------|
| 1        | 2015 | Acta of bioengineering and biomechanics | [24] |
| 2        | 2004 | Spine   | [25] |
| 3        | 2005 | Clinical biomechanics | [26] |
| 4        | 2016 | Spine Journal | [27] |
| 5        | 2005 | Spine   | [17] |
| 6        | 2005 | Clinical biomechanics | [28] |
| 7        | 2001 | Clinical biomechanics | [29] |
| 8        | 2004 | Clinical biomechanics | [30] |
| 9        | 2008 | Spine   | [31] |
| 10       | 2009 | Clinical biomechanics | [32] |
| 11       | 2003 | J Orthop Res | [33] |
| 12       | 2012 | Spine   | [34] |
| 13       | 2012 | Medical engineering & physics | [35] |
| 14       | 2001 | Clinical biomechanics | [36] |
| 15       | 2015 | The spine journal | [37] |
| 16       | 2012 | Journal of biomechanics | [38] |
| 17       | 2013 | Clinical biomechanics | [39] |
| 18       | 2013 | Medical engineering & physics | [40] |
| 19       | 2013 | Medical engineering & physics | [41] |
| 20       | 2007 | Spine   | [42] |
| 21       | 1998 | Spine   | [43] |
| 22       | 2007 | Journal of biomechanics | [44] |
| 23       | 2009 | Clinical biomechanics | [45] |
| 24       | 2005 | Spine   | [46] |
| 25       | 2008 | Clinical biomechanics | [47] |
| 26       | 2007 | Spine   | [48] |
| 27       | 2010 | Knee surgery, sports traumatology, arthroscopy | [18] |
| 28       | 2006 | Spine   | [49] |
| 29       | 2010 | European Spine J | [50] |
| 30       | 2010 | Spine   | [51] |
| 31       | 2016 | Journal of biomechanics | [52] |
| 32       | 2020 | Journal of biomechanics | [53] |
| 33       | 2008 | Journal of biomechanics | [54] |
| 34       | 2011 | Spine   | [55] |
| 35       | 2002 | Journal of biomechanics | [56] |
| 36       | 2002 | Stud Health Technol Inform | [57] |
| 37       | 2001 | Spine   | [15] |
| 38       | 2008 | Journal of biomechanics | [58] |
| 39       | 2005 | Spine   | [59] |
| 40       | 2011 | BMC Musculoskelet Disord | [60] |
| 41       | 2001 | Journal of biomechanics | [61] |
| 42       | 1998 | Magn Reson Imaging | [62] |
| 43       | 2019 | Journal of biomechanics | [63] |
| 44       | 2020 | Journal of biomechanics | [64] |
| 45       | 2020 | Journal of biomechanics | [65] |
| 46       | 2019 | Ultrasound Med Biol | [66] |
| 47       | 2020 | The Spine Journal | [67] |
| 48       | 2016 | J Biomech Eng | [68] |
| 49       | 2010 | J Biomech Eng | [69] |
Results
The systematic scoping review included 70 studies that had been published between 1997–2021. The included studies are presented in Table 1.

Specimens
Material information is presented in Table 2. Basic information regarding breed was in general not specified and only mentioned as "domestic" or "landrace" when mentioned. Forty-one (58%) studies mentioned the weight of the pigs, of which 25 (60%) were between 60–80 kg. Thirty-four (65%) studies stated the age of the pigs (some used young/immature), out of which 13 (28%) used pigs that were 4–6 months old. The level of the used FSUs in the included studies were 42 (60%) on cervical, 25 (36%) on lumbar and 1 (1.5%) on thoracic FSU’s.

Preparation
There were clear similarities in the preparation of the specimens: Fifty (72%) studies had frozen the specimens and then thawed them prior to testing, 51 (72%) kept the specimens moistened during the procedure and 51 (73%) used a preload to reduce post-mortem swelling.

Load protocols
Loading was done in many ways with varying degrees of reported information (Table 3): Sixty-seven (96%) studies used compressive load or tension, three did not. Forty-four (63%) had an angular load (flexion/extension), out of which only 23 (53%) specified the angle. Load duration and magnitude were heterogeneous among the studies. Load protocols ranged from simple one directional compression-tension to multi direction six degrees of freedom (6DF) loadings that required complex lay-out of both test equipment and procedure. A majority of these were performed in custom made testing apparatus or modified material testing machines. Repeated testing in different directions required submaximal loading and the level used varied between the studies but were calculated to be within the apparent linear region of the stress- strain curve or within the physiological range of motion (ROM). Pre-loading (300–500 N) the specimens for 15 to 180 min were the most common way to counter swelling, but 19 (27%) lacked any information regarding this.

Study apparatus and validated tests
Sixty-eight (97%) studies mentioned the model of the test-device used, out of which 49 (72%) used an Instron mechanical testing system of model 8511/8872/8874. There was no mention of whether the machine was validated, or when it was last calibrated in any study.

Biomechanical properties
Table 4 summarizes the mechanical properties in six degrees of freedom, three translations presented as axial shear (often referred to as compression/tension), Lateral shear and A-P shear. Three rotations; sagittal rotation (flexion/extension bending), coronal rotation (lateral bending) and horizontal rotation of the porcine FSU were derived from the articles included in this study. The nomenclature varied in the articles probably due to different scientific traditions. Both alternatives are added in the table to facilitate understanding of it.

Discussion
The primary result of this study was the conclusion that there is a lack of consensus regarding how the material, methods and results should be documented and presented to achieve comparability and high-quality studies. We found that while many of the included studies used similar test materials when looking at age, weight, and spinal level, very few mentioned the breed of the pig and only as “domestic/landrace”. The spine level used in the included studies varied. Several studies used lumbar vertebrae, but many used cervical vertebrae as displayed in Table 2. There is some evidence that porcine cervical vertebrae is more similar to the human lumbar vertebrae in terms of ROM and morphology as well as failure
| Study | Level | Breed       | Weight | Age          | Sample size | Previously frozen | Environmental considerations                                                                 | Test Equipment |
|-------|-------|-------------|--------|--------------|-------------|-------------------|-----------------------------------------------------------------------------------------------|----------------|
| 1     | Lumbar| na          | na     | 18 months    | 6           | Yes               | 12 h hydration with phosphate buffer saline solution                                            | Instron 8874   |
| 2     | Cervical | na       | 80 kg  | 6 months     | 52          | Yes               | Wrapped in paper wet with saline                                                              | Instron 8511   |
| 3     | Cervical | na       | 80 kg  | 6 months     | 16          | Yes               | Wrapped in paper wet with saline                                                              | Instron 8511   |
| 4     | Cervical | na       | 80 kg  | 6 months     | 14          | na                | Heated to body temperature                                                                    | Instron 8511   |
| 5     | Lumbar | Domestic   | 65–73 kg | 4 months  | 16          | No, refrigerated  | In a plastic bag                                                                              | MTS Teststar   |
| 6     | Lumbar | Domestic   | ~80 kg  | 5 months     | 24          | No, refrigerated  | In a plastic bag                                                                              | MTS Teststar   |
| 7     | Cervical | na       | 80 kg  | 6 months     | 26          | Yes               | na                                                                                           | Instron 8511   |
| 8     | Lumbar | na          | na     | na           | 32          | na                | Contained in plastic sleeve filled with saline                                               | Custom made    |
| 9     | Lumbar | na          | na     | na           | 6           | na                | na                                                                                           | Instron 8874   |
| 10    | Cervical | na       | na     | na           | 16          | Yes               | Wrapped in saline-soaked cloth                                                                 | Instron 8872 cus-tom build |
| 11    | Lumbar | na          | na     | > 16 weeks   | 6           | na                | Immersed in an isotonic saline bath cooled to approximately 4 °C                               | Custom made, 6 DOF |
| 12    | Cervical | na       | na     | na           | 48          | Yes               | Moistened with saline every 20 min                                                            | Instron 8872   |
| 13    | Cervical | na       | na     | na           | 14          | na                | Wrapped in saline-soaked gauze                                                                 | Instron 8872   |
| 14    | Cervical | na       | 80 kg  | 6 months     | 48          | Yes               | Wrapped in saline-soaked towel                                                                | Instron 8511   |
| 15    | Lumbar | na          | 60 kg  | na           | 12          | Yes               | Sprayed with saline and wrapped in plastic                                                    | Custom spine simulator AMTI MC3-A-1000 |
| 16    | Cervical | na       | na     | na           | 96          | Yes               | Instron 8872 cus-tom build                                                                    | Instron 8872 cus-tom build |
| 17    | Cervical | na       | na     | na           | 32          | Yes               | Wrapped in saline-soaked gauze                                                                 | Instron 8872 cus-tom build |
| 18    | Cervical | na       | na     | na           | 30          | Yes               | na                                                                                           | Instron 8872 Koll-morgen/Danaher Motion AKM23D |
| 19    | Cervical | na       | na     | na           | 31          | Yes               | Wrapped in saline-soaked gauze                                                                 | Instron 8872 Koll-morgen/Danaher Motion AKM23D |
| 20    | Thoracic | Domestic | 56–61 kg | Immature | 14          | Yes               | Wrapped in moist gauge                                                                        | Instron 8872   |
| 21    | Lumbar | Domestic   | 66±3 kg | Young       | 12          | Yes               | na                                                                                           | MTS Teststar   |
| 22    | Cervical | na       | na     | na           | 218         | Yes               | Wrapped in saline-soaked gauze                                                                 | Instron 8872   |
| 23    | Cervical | na       | na     | na           | 50          | Yes               | na                                                                                           | Instron 8872 Koll-morgen/Danaher Motion AKM23D |
| 24    | Cervical | na       | 50–80  | na           | 10          | Yes               | Wrapped in saline-soaked gauze                                                                 | Instron 8872   |
| 25    | Lumbar | Domestic   | 90–100 kg | 8 months | 10          | Yes               | Wrapped in saline-soaked cloth                                                                 | Instron 8874   |
| 26    | Cervical | na       | Mean 80 kg | Mean 6 months | 16      | Yes               | na                                                                                           | Instron 8511   |
| Study | Level | Breed | Weight | Age     | Sample size | Previously frozen | Environmental considerations | Test Equipment             |
|-------|-------|-------|--------|---------|-------------|-------------------|-----------------------------|---------------------------|
| 27    | Lumbar| Domestic | 65–70 kg | 6 months | 8           | No, refrigerated  | Wrapped in saline-soaked gauze | na                        |
| 28    | Lumbar| Domestic | 80 kg   | Immature | 69          | Yes              | Sprayed with saline         | Instron 8872              |
| 29    | Lumbar| Domestic | mean 78 kg | Mean 7 months | 8 | Yes | Kept wet by saline-soaked gauze | Instron 8872 |
| 30    | Cervical| na | ~80 kg | Mean 6 months | 22 | Yes | Wrapped in saline-soaked cloth | Instron 8872 cus- |
| 31    | Lumbar| na | na | na | 9 | Yes | Wrapped in saline-soaked gauze | Custom built pendulum design |
| 32    | Cervical| na | na | na | 22 | Yes | na | Pressure transducer (model DPG1000DR) |
| 33    | Cervical| na | 80 kg | 6 months | 16 | Yes | na | Pressure transducer needle (OrthoAR) |
| 34    | Cervical| na | na | 6–8 months | 20 | Yes | Wrapped in saline-soaked gauze | Instron 8872 |
| 35    | Lumbar| na | na | na | 1 | na | Circulating isotonic saline at 4C | Custom built load device |
| 36    | Lumbar| na | na | > 16 weeks | 6 | na | Physiological fluid environment | Custom built load device |
| 37    | Lumbar| na | na | na | 7 | na | Room temperature in ambient air | Custom built load device |
| 38    | Lumbar| na | na | 10 months | 8 | Yes | Tested in a saline bath at 37 C | Instron 8872 |
| 39    | Lumbar| na | na | 10 months | 8 | Yes | Tested in a physiologic saline bath (39 °C) | Instron 8872 |
| 40    | Lumbar| na | na | na | 8 | na | Wrapped in a saline soaked cloth | Instron 8872 |
| 41    | Lumbar| na | na | na | 6 | na | na | Custom built load device |
| 42    | Lumbar| na | na | na | 1 | Yes | na | Encapsulated with plastic-backed saline soaked gauze |
| 43    | Cervical| na | na | 5–18 months | 48 | Yes | Encapsulated with plastic-backed saline soaked gauze | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
| 44    | Cervical| na | na | na | 32 | Yes | Temperature-controlled laboratory at 21 °C | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
| 45    | Cervical| na | na | na | 12 | Yes | na | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
| 46    | Cervical| na | na | na | 24 | Yes | Room temperature and surrounded by a water | Instron 8511 |
| 47    | Cervical| na | na | na | 20 | Yes | na | Instron 8872 |
| 48    | Cervical| na | na | na | 21 | na | Superficial moistening every 20 min | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
| 49    | Cervical| na | na | na | 30 | Yes | Saline soaked cloth wrapped in plastic | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
| 50    | Cervical| na | na | na | 4 | Yes | na | Instron 8511 |
| Study | Level  | Breed          | Weight | Age         | Sample size | Previously frozen | Environmental considerations | Test Equipment                        |
|-------|--------|----------------|--------|-------------|-------------|-------------------|------------------------------|---------------------------------------|
| 51    | Cervical | na             | 85 kg  | 6 months    | 126         | Yes               | Hydrated with a saline mist every 15 min | Instron 8872                           |
| 52    | Cervical | na             | na     | na          | 14          | Yes               | Misted with a 0.9% saline solution | Instron 8872                           |
| 53    | Cervical | na             | 85 kg  | 6 months    | 126         | Yes               | Misted with a saline solution every 15 min | Instron 8872                           |
| 54    | Cervical | na             | na     | na          | 18          | Yes               | Wrapped with saline soaked plastic-backed cloth | Instron 8511 + custom device          |
| 55    | Cervical | na             | na     | na          | 64          | Yes               | Wrapped in a saline-soaked plastic backed cloth | Instron 8511 + custom device          |
| 56    | Lumbar  | Domestic       | 55 kg / 195 kg | 4 months / 2–3 years | 12          | Yes               | na                           | MTS Teststar                       |
| 57    | Lumbar  | Domestic       | 75–80 kg | 6 months    | 19          | No, refrigerated  | Wrapped in saline-soaked gauze | MTS Teststar                       |
| 58    | Cervical | na             | 80 kg  | 6 months    | 30          | na                | Saline-soaked cloth and plastic wrap | Instron 8511                           |
| 59    | Cervical | na             | 80 kg  | 6 months    | 30          | na                | Wrapped in cloth soaked in saline along with plastic wrap | Instron 8511                           |
| 60    | Cervical | na             | 80 kg  | 6 months    | 10          | na                | Saline-soaked cloth and plastic wrap | Instron 8511                           |
| 61    | Lumbar  | na             | na     | 6–8 months  | 5           | Yes               | Wrapped in a saline soaked towel rehydrated every 20 min | Instron 591 + Instron 8874           |
| 62    | Cervical | na             | 80 kg  | na          | 18          | Yes               | Saline (0.9% NaCl) soaked plastic-backed material and a layer of polythene film | Instron 8511                           |
| 63    | Cervical | na             | 80 kg  | 6 months    | 50          | na                | Wrapped in a saline soaked cloth and plastic wrap | Instron 8511                           |
| 64    | Cervical | Domestic       | 80 kg  | na          | 26          | Yes               | na                           | Instron 8511                           |
| 65    | Cervical | Domestic       | 80 kg  | 6 months    | 56          | Yes               | na                           | Instron 8511                           |
| 66    | Cervical | na             | 80 kg  | na          | na          | Yes               | na                           | Instron 8511                           |
| 67    | Lumbar  | na             | na     | na          | 1           | Yes               | Sprayed and wrapped in paper towel soaked with 0.9% saline solution, triple sealed in plastic bag | Instron 8511 + Dynamic six-axis spine simulator, dSPACE Ltd |
| 68    | Lumbar  | Organically farmed pig | 60 kg  | 8–12 months | 1           | No                | wrapped in plastic film at room temperature (20°C) | Zwick 25–200                      |
| 69    | Cervical | na             | na     | na          | 48          | Yes               | 3% weight/volume saline soaked tissue | Instron 8872 Kollmorgen/Danaher Motion AKM23D |
mechanisms than porcine lumbar vertebrae [16] and is therefore proposed as a good model for lumbar spine studies.

Most studies used similar procedures for preparation, i.e. specimens were kept frozen before use, a pre-load compression to balance swelling was applied and the specimen were kept moisturized during the experiment (Table 3). The preparation of the functional spinal units was in general done in similar style but were also usually reported in general terms. Most of the specimens used were frozen between harvesting and preparation. The literature report divergent findings regarding effects of freezing process. However no or minor impact on the outcome of the study protocol depending on intervention seems to be the general finding [90], however a load rate dependence has been noted [91]. The freeze temperature and storage time were seldom noted, which dependent on study intervention could be important. The thawing time of the specimens was often reported, but in some cases probably underestimated. The importance of a fully thawed specimen that has reached correct study temperature is vital, especially when time-dependent properties are investigated.

The method used to fixate the specimens to the stabilization cups varied among the studies, but the most common practices were by screws, cement such as PMMA or auto body plaster. The fixation methods are generally not validated and are more of a proven experience and how it affects the results are not known. Using a preload to supposedly balance post-mortem swelling of the specimen is conducted in several of the included studies (Table 3), and a study has displayed more in vivo related results compared to no physiological preload [57]. Most of the included studies reported that the specimens were moistened by using a hydrated gauze or similar during the test to counteract de-hydration and thus resemble the normal in situ conditions. This procedure is important [92] but the effect on FSU test results is not clear.

The method and load protocols that were used in the studies were heterogeneous regarding loading time, magnitude, and angle. Nearly every study used a compressive load, with or without an angular load superimposed. Out of the 44 studies that reported using an angular load, only 23 (Table 3) mentioned the specific angle(s) used. Using an angular load but omitting to report angle used makes it difficult to replicate the study, as well as making it impossible to compare it to similar studies. With few exceptions, the load duration and magnitude varied between the studies. Having varied durations and magnitudes between studies with completely different aims is no surprise, but even in those studies with similar aims did it vary.

No included study mentioned whether the technical equipment used in the experiment was validated, and none mentioned when the loading system was last calibrated or if a direct calibration using calibration weights and lengths is performed. Using a validated system would improve the evidence and quality provided by the study.

Load rate nomenclature was dependent on load mode, and expressed as force or stress rate, deformation or strain rate and torque rate. This varied between the studies, mainly because of different research questions. If appropriate parameters are reported, a transformation of load rate is feasible, making a comparison between studies possible. A conformity to a use of SI units would facilitate interpretation of data as well as simplify comparison between studies and is highly recommended.

To achieve an overall estimate of the mechanical properties presented, we chose to present range rather than mean and standard deviation since the values are derived from studies with inter varying loading pre-requisitions, sometimes the only common factor being the load mode or direction. Axial compression testing mode seems to be the most common loading mode in the articles as opposed to axial tension where there was insufficient information. These overall findings can aid in the layout of future studies necessary for adding knowledge about the loading mechanism of porcine FSU.

Table 2 (continued)

| Study | Level | Breed  | Weight | Age | Sample size | Previously frozen | Environmental considerations | Test Equipment                  |
|-------|-------|--------|--------|-----|-------------|-------------------|-----------------------------|---------------------------------|
| 70    | Cervical | na     | 60 kg  | na  | 28          | Yes               | na                          | pressure transducer, model DPG1000DR, 2000 PSI transducer |

Strengths and limitations

Selection and systematic bias

The search and selection process of search criteria was done through a stepwise process and addressed the MESH terms and included all useful synonyms available. The database search was completed with an author search to achieve less systematic drop out in the selection. The manual selection process of the studies was not validated but was done in a controlled manner where all studies were analyzed by several of the authors according to the preset protocol.
| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined | Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|-------------|---------|-----------|----------------|----------|-------|----------|-------|------|----------|----------------|---------------------------------|
| 1     | 1 mm     | Yes         | No      | No        | No             | No       | No    | No       | Na    | na   | 10 s     | na             | Disc pressure 0.62 MPa |
| 2     | 300 N/15 min | Yes      | No      | No        | No             | Yes      | No    | Yes      | Na    | 3000 N/s | To failure | na             | Failure load 3.8–6.5 kN |
| 3     | 300 N/15 min | Yes      | No      | Yes       | No             | No       | Yes  | No       | Na    | 0.5°/s | 6000 cycles | Axial 1472 N | na |
| 4     | 300 N/15 min | Yes      | Yes     | No        | No             | No       | Yes  | No       | Na    | 1000 N/s, 0.5 Hz | Ramp, 1000 cycles | Axial 1000 N |
| 5     | na       | Yes        | Yes     | Yes       | No             | No       | No   | Yes      | 17° flex 17° ext | 1 mm/s Ramp | na |
| 6     | na       | Yes        | Yes     | Yes       | No             | No       | No   | No       | 11° flex 12° ext | 1 mm/s Ramp | na |
| 7     | 260 N/15 min | Yes      | Yes     | Yes       | No             | Yes      | No   | Yes      | Na    | 45°/s 1 Hz | Max 86,400 cycles | Axial 260/867/1472 N | na |
| 8     | na       | Yes        | Yes     | Yes       | No             | No       | Yes  | Yes      | 5° flex 5° ext | 0.07 Hz | 1500 cycles | na |
| 9     | na       | Yes        | No      | No        | No             | No       | No   | No       | Na    | 40 N/s | Ramp     | Axial 500 N | Disc pressure Max 1.6 MPa |
| 10    | 300 N/15 min | Yes      | Yes     | Yes       | No             | No       | Yes  | No       | Na    | 0.5°/s | Max 10,000 cycles | 1500 N | Foramina pressure 6 kPa |
| 11    | 0, 200, 400 N | Yes      | Yes     | Yes       | Yes            | Yes      | Yes  | Yes      | Flex/ext/rot 0.8° lateral 1° | Axial 0.2 mm AP/Lat 0.3 mm | na |
| 12    | 300 N/15 min | Yes      | Yes     | Yes       | No             | No       | No   | Yes      | 4.2° flex 6.1° ext | 5 Hz | 120 min Static 1500 N ± 1250 N Static 1500 N |
| 13    | 300 N/15 min | Yes      | No      | No        | No             | No       | No   | No       | na    | 5 Hz | 120 min Static 1400 N ± 140 N Static 1400 N | Modulus 0.3–3.4 MPa Strain 1.3–2.2 |
| 14    | 300 N/30 min 1 KN/180 min | Yes      | Yes     | No        | No             | No       | Yes  | 21°      | 0.5°/s 3000 N/s | To failure | na |
| 15    | 500 N/30 min | Yes      | Yes     | Yes       | Yes            | Yes      | Yes  | Yes      | 4° flex, ext, lat | 0.1 Hz | 60 min | Axial 500 N | Failure load 5.6–1.1 kN |
| 16    | 300 N/15 min | Yes      | Yes     | No        | No             | Yes      | Yes  | Yes      | 7.9° flex 4.4° ext | 0.5°/s, 0.05 mm/s | na |

# cycles to disc failure
Hysteresis Moment 3–3.9 Nm
6 DF: Stiffness Linear 0.5–3.5 kN/mm Rotational 2–10 Nm/mm
Shear Force 2.2–2.7 kN Shear Stiffness 0.7–1.1 kN/mm
Table 3 (continued)

| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined | Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|-------------|---------|-----------|----------------|----------|-------|----------|-------|------|----------|----------------|--------------------------------|
| 17    | 300 N/15 min | Yes | Yes | Yes | No | No | Yes | Yes | na | 0.5°/s 1 Hz | Max 21,600 cycles | Axial 300 N | Shear failure Morphology/Site |
| 18    | 300 N/15 min | Yes | Yes | Yes | No | No | Yes | Yes | na | 0.5°/s 0.2 mm/s | 5 cycles | Axial 300 N | Shear Stiffness NZ 58–85 N/mm |
| 19    | 300 N/15 min | Yes | Yes | Yes | No | No | Yes | Yes | na | 0.15 mm/s | Ramp | Axial 1546 N ± 22 N | Shear Force 1.9–2.5 kN |
| 20    | 500 N | Yes | No | No | No | Yes | No | Yes | na | na | 30 s | Axial 0.5, 1.0, 1.5 Nm | Vertebral rotation 0.05–1.8° |
| 21    | na | Yes | No | No | No | No | No | No | na | 5 mm/min | Ramp | na | Failure load 7.9 kN |
| 22    | 300 N/15 min | Yes | No | No | No | No | No | No | na | 0.5 Hz | Max 1.2 h | 50, 70, 90% of calc strength | Fatigue # cycles to failure |
| 23    | 300 N/15 min | Yes | No | No | No | No | Yes | na | 0.5 Hz | Max 1.2 h | 10, 30, 50, 70, 90% of calc strength | Fatigue # cycles to failure Injury site |
| 24    | 300 N/15 min | Yes | No | No | No | No | No | No | na | 3000 N/s | Ramp | na | Failure strength 10.5 kN |
| 25    | na | Yes | Yes | Yes | No | No | No | Yes | 4° flex 4° lat | 1°/s | Step | 200–800 N | MD stress distribution 288–1611 kPa |
| 26    | 260 N/15 min | Yes | Yes | Yes | No | No | No | Yes | 15° flex 2° ext | 1 Hz | Max 14,400 cycles | Axial 1472 N | Fatigue Failure Injury site |
| 27    | na | Yes | No | No | No | No | No | No | na | 3 Hz, 5 mm/min | Ramp | 0–1000 N | Failure load 8.3 kN |
| 28    | na | Yes | No | No | No | No | Yes | Yes | na | 0.5 Hz | 1500 cycles | Axial 1600 N | Shear strength 1.0–2.4 kN |
| 29    | na | Yes | No | No | No | No | Yes | Yes | na | 0.1 mm/s | Ramp | Axial 1600 N | Shear strength 1.6–2.1 kN |
| 30    | 300 N/15 min | Yes | Yes | Yes | No | No | No | Yes | 17° flex 6° ext | 0.5 Hz | 7000 cycles | Axial 1472 N | Disc herniation Pathway |
| 31    | na | Yes | Yes | No | No | No | Yes | 5° | na | na | na | Axial 440–1123 N | Flexion stiffness 70–300 N/m rad |
| 32    | na | No | No | No | No | No | No | No | na | na | na | na | Differences in annular mechanical properties in pressurized and un-pressurized discs |
Table 3 (continued)

| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined | Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|-------------|---------|-----------|-----------------|----------|-------|----------|-------|------|----------|----------------|--------------------------------|
| 33    | na       | No          | No      | No        | No              | No       | No    | No       | na    | na   | na       | na             | Fracturing of end-plate as a result of injecting hydraulic solution into IVD |
| 34    | 300 N/ 15 min | Yes        | Yes     | Yes       | No              | No       | No    | Yes      | na    | 1 Hz | 6000 cycles | 1260–1540 N | Loss in disc height as a result of compression |
| 35    | 500 N/ 3 h  | Yes        | Yes     | Yes       | Yes             | Yes      | No    | 4°       | na    | na   | na       | na             | Obtaining the load–displacement properties of a motion-segment under “physiological conditions” |
| 36    | 0, 200, 400 N | Yes        | Yes     | Yes       | Yes             | Yes      | No    | No       | 2°    | na   | 87 s     | na             | Increased preload causes increased stiffness |
| 37    | na       | Yes        | No      | No        | No              | Yes      | No    | No       | na    | na   | 1 h      | 340 N          | Effects of torsion on IVD stress |
| 38    | 0.001 MPa (IVDP)/ 15 min | Yes | No      | No        | No              | No       | No    | No       | na    | na   | 3 cycles | 2.0 MPa (IVDP) | Deformation time–dependency of different FSU-parts under compression |
| 39    | 20 N/ 15 min | Yes        | No      | No        | No              | No       | No    | No       | na    | na   | 3 cycles | Avg 1694 N | IVD height loss after compression |
| 40    | na       | Yes        | Yes     | Yes       | No              | No       | No    | Yes      | na    | 1.5°/s | 7 h      | 250 N          | Neutral zone stiffness after compression |
| 41    | na       | Yes        | No      | No        | No              | Yes      | No    | Yes      | na    | na   | 340 N    | na             | IVD height loss after compression |
| 42    | 300 N/ 30 min | Yes        | No      | No        | No              | No       | No    | No       | na    | 1 h   | 1391 N   | na             | IVD fluid dynamic during compression |
| 43    | 300 N/ 15 min | Yes        | Yes     | Yes       | No              | No       | No    | Yes      | na    | 0.5 Hz | 12 h max | 10,800 cycles max | Fatigue test |
| 44    | 300 N/ 15 min | Yes        | No      | No        | No              | No       | No    | No       | na    | 0.5 Hz | 8,3 N/sec max | na             | Fatigue test |
| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|-------------|---------|-----------|----------------|----------|-------|----------------|------|-----------|----------------|----------------------------------|
| 45    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 0.5°/s | 3 cycles/na | 10 N, 300 N, 600 N and 1200 N | MD AF bulge change after compression validation of ultrasound to measure mechanical properties during experimentation |
| 46    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 0.5°/s | na | 15% of predicted UCT | Validation of ultrasound to measure mechanical properties during experimentation |
| 47    | 300 N/ 15 min | Yes | Yes | Yes | No | No | Yes | Yes | na | 0.5°/s | na | 300 N, 400 N | Facet joint capsule strain during compression and flexion/extension |
| 48    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | 4.3° flex and 5.1° ext | 0.5°/s | 120 min | 1500 N ± 1200 N | MD height loss, dynamic compressive stiffness |
| 49    | 300 N/ 15 min | Yes | Yes | Yes | No | No | Yes | Yes | na | 1 mm/s, 4 mm/s, 6 mm/s | To failure | 300 N, 1600 N | Ultimate anterior shear force, ultimate displacement, average stiffness and energy to failure |
| 50    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 0.5°/s | 5000 cycles | 1500 N | Interfacet spacing |
| 51    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 5, 10, 30 cycles/min | 5000 cycles | 10%, 20% and 40% of UCT | MD height loss and bulging |
| 52    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | 18.3° | 45°/s 1 Hz | 3600 cycles | 1500 N | Axial deformation, IVD pressure change, IVD height change |
| 53    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 5, 10, 30 cycles/min | 5000 cycles | 10%, 20% and 40% of UCT | Damage patterns |
| 54    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | na | 0.5°/s | 5 cycles | 1472 N | Facet joint fracturing, stiffness |
| 55    | 300 N/ 15 min | Yes | Yes | Yes | No | No | No | Yes | 132.3° flex and 62.3° ext | 0.5°/s | 7000 cycles | 1500 N | IVD herniation |
| 56    | na | Yes | No | No | No | No | No | No | na | 1700–2500 N/s | To failure | 1700–2500 N | Mean ultimate force at failure |
### Table 3 (continued)

| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined | Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|--------------|---------|-----------|----------------|----------|-------|----------|-------|------|----------|-----------------|---------------------------------|
| 57    | na       | Yes          | Yes     | Yes       | No             | No       | No    | Yes      | 9–15° | 1 Hz | 20,000 cycles/5.5 h | 700 N | Damage patterns on MRI and in histological slices |
| 58    | 300 N/15 min | Yes         | No      | No        | No             | No       | No    | Yes      | na    | na   | Max 85,000 cycles | 1500 N | Mean failure load |
| 59    | 300 N/15 min | Yes         | Yes     | Yes       | No             | No       | No    | Yes      | 14.8° flex and 4.3° ext | na   | 10,000 cycles | 1500 N | Nucleus pulposus migration with flex/ext + compression vs only compression |
| 60    | 300 N/15 min | Yes         | Yes     | Yes       | No             | No       | No    | Yes      | na    | na   | na       | 1500 N | Angular stiffness |
| 61    | 300 N     | Yes          | No      | No        | No             | Yes      | No    | na       | 14.8° flex and 4.3° ext | 2 Hz | 120 min | 300 N, 500 N, 600 N, 800 N, 1500 N | Degree of spondylolisthesis and spondylolysis |
| 62    | 260 N/879 s | Yes          | Yes     | Yes       | No             | Yes      | No    | Yes      | na    | 0.5°/s, 45°/s and 1 Hz | na   | 1472 N | Disc height loss, endplate fracture |
| 63    | 300 N/15 min | Yes         | Yes     | Yes       | No             | Yes      | No    | na       | 12° flex and 6° ext | 1 Hz | Max 10,000 cycles | 1500 N | Disc herniation |
| 64    | 300 N/15 min | Yes         | No      | No        | No             | Yes      | No    | 100 N/s  | na    | 100 N/s | To failure | 1500 N | Ultimate shear load at failure, deformation at failure, stiffness, energy absorbed |
| 65    | 300 N/15 min | Yes         | Yes     | No        | No             | No       | No    | Yes      | 10°   | 0.5°/s, 45°/s and 1 Hz | To failure | Maximum reported 2345 N | Ultimate load, deformation, energy, stiffness |
| 66    | na        | Yes          | No      | No        | No             | Yes      | No    | na       | na    | na   | na       | na | Damage patterns on MRI and in histological slices |
| 67    | 500 N/15 min | Yes         | Yes     | Yes       | Yes            | Yes      | No    | Yes      | na    | 0.5–5°/s | na | Maximum 500 N | Stiffness Matrix 6 DF |
| 68    | 500 N/3 h  | Yes          | Yes     | Yes       | Yes            | Yes      | No    | Yes      | na    | 0.1 Hz, 0.5 Hz | na | 500 N | Stiffness Matrix 6 DF |
| Study | Pre-load | Compression | Flexion | Extension | Lateral bending | Rotation | Shear | Combined | Angle | Rate | Duration | Load Magnitude | Mechanical properties reported |
|-------|----------|--------------|---------|-----------|----------------|----------|-------|----------|-------|------|----------|----------------|-----------------------------|
| 69    | 300 N/15 min | Yes | Yes | Yes | No | No | No | Yes | na | 0.5/s | Min 21,600 cycles to failure | 30, 50 and 70% ult compression tolerance | Endplate fatigue failure during cyclic compression loading with variable and consistent peak magnitudes |
| 70    | na | No | No | No | No | No | No | No | na | na | na | na | Effect of pressure-induced fracture on mechanical properties of AF |
A review based on additional animal species (such as calf, sheep, and dogs) would enhance the overall knowledge regarding how animal models are used in spinal research, how these studies report basic parameters regarding material and methods and thereby increase the external validity of the current study. This scoping review aimed to primarily address the field of porcine FSU to achieve higher quality in the methodology to achieve higher internal validity but with the potential limitation of external validity. Different animal models have different material properties and the use of porcine specimens in spine research has been widely accepted for many years but is highly dependent on research questions. Anatomical and ROM similarities between cervical porcine FSU and human lumbar FSU indicate that the porcine cervical FSU is a reasonably good model for research questions regarding ROM in the human lumbar spine [4–6, 16]. The present study did only include non-operated and non-instrumented FSUs that further reduced the available material but did enhance the possibility to compare the research results of basic loading parameters. Operated and instrumented specimens are intervened which may affect the basic loading parameters and the biomechanical properties of the FSU. Multisegmented spines were also excluded due to the difference in ROM and other loading parameters compared to FSU.

**Publication bias**

All included studies have been published in peer reviewed journals according to Table 1 and indexed in the Scopus or PubMed databases.

**Clinical use and significance**

This systematic scoping review highlight the importance to increase the scientific evidence level and quality in porcine FSU spinal research. We suggest that the results from this systematic scoping review may grant a better understanding of how future studies should be best conducted to present valid, reliable, and comparable data, which in turn may bring us closer to understanding the physical boundaries of the spine and to reduce unnecessary animal experimentation.

**Ethical considerations**

The usage of pigs for animal experimentation constitutes an ethical problem and means to minimize the number of animals used is a priority. One way could be to define a common accepted research protocol for in vitro spinal biomechanical testing. The similarities between the spinal properties of the pig compared to that of humans, is believed to be great enough to make it possible to draw parallels between the results from such studies with human biomechanical properties and thus justify them.

**Future considerations and study protocol suggestion**

Our study shows the importance of comprehensive reporting of relevant data concerning material, method, and methods of validation in experimental animal studies.

We suggest that future studies increase the information in the reports regarding study material and to validate the study method to enhance the internal and external validity of the study. We suggest that future study reports are based on the ARRIVE Guidelines [2] and the following basic template:

**Material:**

- Detailed material information (breed, weight, age etc.).
- Physical size of test material such as vertebral diameter and disc height
- Standardization and validation of material loading parameters, through compression to failure of one single included specimen
- Pre-test handling and preparation such as report of harvest, storage (temperature, time) and fixation to the testing equipment.

**Table 4** Mechanical properties

| Parameter /load mode          | Force   | Deformation /degrees | Stiffness | Stress range | Strain |
|-------------------------------|---------|----------------------|-----------|--------------|--------|
| Axial compression             | 0.58—17.0 kN | 1.8—6.6 mm         | 0.5—4.5 kN/mm | 0.5—7.7 MPa | na     |
| Axial tension                 | 45—112 N     | na                  | na        | na           | na     |
| Horizontal rotation           | na      | 0—6°                | 2.16—10.1 Nm/° | NA       | NA     |
| Flexion/extension bending-Sagittal rotation | 1.3—92 Nm   | 3.2—20.5°           | 0.54—8.7 Nm/° | NA       | NA     |
| Lateral bending-Coronal rotation | na     | na                  | 0.63—7 Nm/° | NA       | NA     |
| Shear A-P-Lateral             | 0.3—3.5 kN   | 0.66—18.8 mm        | 37—800 N/mm | na        | na     |

*na not available, NA not applicable*
Test conditions:

- Environmental conditions, temperature etc.
- Material conditioning, for example, means to minimize de-hydration.

Test apparatus validation

- Report of test apparatus
- Report of validation of test apparatus

Test protocol

- Preload
- Defined and reported load, time, frequency, angle and test protocol variations.
- Validated test protocol

Conclusion

Biomechanical testing on FSU units is a commonly used experimental spine research procedure. A notable variability in the amount of information that is reported in the materials and method section in the articles was identified in this review. A basic research guideline regarding improved report-structure, that would enable comparison between biomechanical experimental studies and increase the method quality, is presented in the present study. It is also evident that there is a clear need for a validated quality-assessment template for experimental animal studies.

Authors’ contributions

All three authors have been involved in all steps of this systematic scoping review. Credit statements: JH, LE and OT: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing, visualization. All authors read and approved the final manuscript.

Funding

Open access funding provided by University of Gothenburg. The study was made possible by salary funding by Gothenburg University (author 1), the Orthopedic department of Gothenburg University hospital (author 2) and by the R&D Centre Gothenburg and Södra Bohuslan (author 3). The study sponsors had no role in any part of the study.

Declarations

Competing interests

The authors state no inappropriate influence (bias) to this study.

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