Sterilized screw storage boxes are commonly used in the hospital setting to house multiple screws in a range of sizes and lengths. This allows for any selection to be made intraoperatively based on the size or length needed for a particular patient and fracture configuration. The need for this duplicity in screw lengths leads to screws not being used at each procedure and undergoing the autoclave process multiple times. Less-often-used screw size and length combinations could be autoclaved hundreds or even thousands of times before insertion in a clinical case. Autoclaves use moist heat to coagulate and denature proteins in microorganisms, thereby sterilizing the item in the process. Steam sterilization is effective, relatively inexpensive, works on a wide range of materials, and is nontoxic to health-care workers. As such, it is one of the most common forms of sterilization used for instruments and implants that are heat and moisture tolerant.

Effect of repeated steam sterilizations on insertional torque, torque to failure, and axial pullout strength of 3.5-mm and 2.0-mm cortical bone screws

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OBJECTIVE
The objective of this study was to determine the effects of repeated steam sterilization cycles on the biomechanical properties of surgical screws.

METHODS
42 3.5-mm and 42 2.0-mm self-tapping, cortical screws were divided into 3 groups per size and underwent autoclave sterilization for 1 (G1), 50 (G50), or 100 (G100) cycles and testing from August 2018 through June 2021. Sixty screws were then inserted into canine cadaver femurs, and biomechanical properties were measured, including peak insertional torque, torque to failure, and pullout strength, each normalized to cortical thickness. Scanning electron micrographs were taken from 24 screws, and images were blindly analyzed by 5 trained examiners.

RESULTS
The mean normalized insertion torque for 3.5-mm screws was significantly different between G1 and both G50 and G100. The mean normalized torque to failure for 3.5-mm screws was significantly different between G1 and both G50 and G100. Axial pullout testing was found to be significantly different for 2.0-mm screws between G1 and G100. Scanning electron micrographs surface scoring identified a significant difference in 3.5-mm screws at the screw tip.

CONCLUSIONS
The results indicate that biomechanical changes occur with repeated steam sterilizations. Specifically, peak insertional torque and torque to failure are decreased with increased sterilizations for 3.5-mm screws, whereas 2.0-mm screws were altered in pullout testing after 100 sterilizations. It is suspected that numerous sterilizations negatively alter the physical-mechanical properties of certain screw sizes.

CLINICAL RELEVANCE
The biomechanical properties of the bone-implant interface could negatively be affected by multiple steam sterilizations during clinical setting.

Keywords: multiple autoclave sterilizations, cortical bone screws, biomechanical properties, scanning electron microscopy, screw boxes

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It has been reported, largely in human orthodontics and endodontics, that repeated sterilization cycles affect the properties of implants and tools. In one study, the insertion torque of titanium orthodontic miniscrews was significantly affected by the number of sterilization cycles, but the clinical relevancy was not known. There was a significant reduction in torsional strength with repeated autoclaving of endodontic files in another study. Deleterious effects of repeated sterilization have also been seen in dental scaling instruments, with those made of carbon steel being more susceptible to the harsh effects of autoclave sterilization than those of stainless steel. Most of these studies autoclaved their devices 10 times. To the authors’ knowledge, no studies have been performed in human or veterinary medicine evaluating the effects of repeated sterilization on 316L stainless steel surgical screws. It is not uncommon in a hospital setting for a single screw within a screw box to undergo 100 or more sterilization cycles within a year if that particular implant is not chosen for use and replaced with a new screw. Additionally, the authors are not aware of any standard recommendations for the rotation of surgical screws within a screw storage box nor recommendations to discard screws after undergoing a certain number of autoclave sterilizations.

When cortical screws are placed for fracture reduction and stabilization, there is an expectedly repeatable interaction between the bone and implant. Alteration of a screw's biomechanical properties may have significant effects on these interactions, thus negatively affecting the stability of the construct and clinical outcome. Significant increases in insertional torque may lead to plastic deformation of the screw and mechanical failure of the screw or the bone, whereas significant decreases in insertional torque and pullout strength occur when there is a decrease in the ratio between screw size and the pilot hole drilled. If biomechanical properties are affected by repeated sterilizations, implant management techniques may need to be altered to avoid the risk of catastrophic implant failure.

The purpose of this study was to determine the effects of a standard, repeated steam sterilization cycle on the insertional torque, torque to failure, and axial pullout strength of 2.0-mm and 3.5-mm stainless steel cortical screws inserted into cadaveric canine femurs.

We hypothesized that repeated sterilizations with steam would affect the biomechanical properties of the screws and that 2.0-mm screws would be more susceptible to these changes than 3.5-mm screws.

Methods

Mechanical testing was performed by the standards established by the American Society for Testing and Materials testing of medical bone screws. A total of 84 screws, 42 2.0-mm and 42 3.5-mm 316L stainless steel self-tapping, cortical bone screws, underwent autoclave sterilization. Both 2.0-mm and 3.5-mm screws were divided into 3 groups of 14 screws each. Group G1 underwent 1 autoclave sterilization cycle, group G50 underwent 50 autoclave sterilization cycles, and group G100 underwent 100 autoclave sterilization cycles. Screws were sterilized in a standard-wrapped, commercial screw box (Synthes) to mimic the clinical setting. Screws were sterilized using a prevacuum autoclave setting at 270 °C for 4 minutes with a 30-minute drying time in accordance with manufacturer instructions and industry standards (AMSCO 400 series; Steris).

Femurs were collected bilaterally from 15 dogs euthanized for reasons unrelated to the musculoskeletal system in accordance with guidelines approved by the Louisiana State University IACUC. The dogs were adult, intact, mixed-sex, and of various breeds. There were 8 males and 7 females between 9 and 14 kg. Soft tissues were removed, and specimens were wrapped in saline-moistened towels and stored at −20 °C until further analysis. Prior to testing, femurs were allowed to thaw for 24 hours at room temperature (22 °C). Femurs were transected approximately at the physis level, leaving the diaphysis intact using a band saw. Then, a 1.5-cm segment of the proximal and 1.5-cm segment of the distal femoral diaphysis were transected for individual testing methods. The thickness of the near cortex, medullary cavity, far cortex, and total diameter were measured in millimeters with calipers in a lateral-to-medial direction. After preparation, the proximal and distal diaphyseal segments were used for separate testing methods. Proximal segments underwent screw placement while measuring peak insertional torque followed by torque to failure, and distal segments underwent screw placement while measuring peak insertional torque followed by axial pullout.

Implant constructs

Ten screws per group and size were inserted by hand by a single investigator following the standard Association of the Study of Internal Fixation technique for a position screw. Using an orthopedic drill (electric drill; ConMed Linvatec) with a drilling speed of 760 rpm, the bicortical thread hole was drilled, guided by a custom C-clamp drill guide to ensure placement perpendicular to the long axis of the bone. For 2.0-mm cortical screws, a 1.5-mm drill bit was used for each threaded hole, and for a 3.5-mm cortical screw, a 2.5-mm drill bit was used. A new drill bit was used for each group.

Torque testing

Screws were placed in the proximal and distal diaphysis using a custom screwdriver with a digital torque wrench (Mark-10 MTT03-50Z Digital Torque Gauge Series TT03; Mark-10) fitted to the shaft of an Association of the Study of Internal Fixation hex drive screwdriver. The torque wrench measured peak force in N-cm with an accuracy of 0.01. Peak insertional torque was achieved when the screw fully engaged the far cortex and at least 2 mm of the self-tapping screw tip exited the far cortex. Once peak insertional torque was measured, maximum torque
at failure was measured in the proximal diaphyseal segments, and values were recorded.

**Axial pullout**

All screws undergoing axial pullout testing were placed in distal diaphyseal segments using the technique previously described, and peak insertional torque values were recorded. Perpendicular pullout forces were tested using a 313 Universal Test Machine (TestResources) *(Figure 1).* All constructs were tested for failure, defined as failure of the bone, failure of the screw-bone interface (screw pullout from the bone), or failure of the implant. A preload of approximately 1 N was applied to each screw prior to the start of the test and extracted at a controlled displacement rate of 5 mm/min until bone or screw failure occurred. Load, displacement, and time data were acquired at 0.02-second intervals throughout the testing period by analog/digital conversion and stored in a computer data file. A load-versus-displacement curve was created from load data recorded by the data acquisition device of the materials testing machine.

**Statistical analysis**

Statistical analysis was performed with commercial software (JMP Statistical Discovery; SAS Institute Inc). The normality of data was assessed by use of a D’Agostino and Pearson test. All values (insertional torque, torque to failure, and pullout force) were normalized to the measured total cortical thickness of the bone in millimeters prior to analysis. Normally distributed data are reported as mean ± SD, and groupwise comparisons were performed with a 1-way ANOVA. Mean separation was accomplished with a Fisher least significant difference test. Non-normally distributed data (scanning electron micrographs topography scores) were evaluated by the median of those indices through nonparametric analysis. For all analyses, significance was set at values of $P \leq 0.05$.

**Results**

The mean peak insertional torque for 3.5-mm screws was statistically lower for G50 ($P = 0.003$) and G100 ($P = 0.037$) when compared to G1. There was no significant difference between groups for the 2.0-mm screws. The mean peak insertional torque ± SD measured in N·cm and normalized to cortical thickness was 14.84 ± 1.43 (G1), 11.35 ± 2.11 (G50), and 12.45 ± 2.38 (G100) for 3.5-mm screws and 3.52 ± 0.72 (G1), 3.81 ± 1.16 (G50), and 3.75 ± 1.10 (G100) for 2.0-mm screws.

The mean torque to failure for 3.5-mm screws was statistically lower for G50 ($P = 0.002$) and G100 ($P = 0.001$) when compared to G1. There was no significant difference between groups for the 2.0-mm screws. The mean torque to failure ± SD measured in N·cm and normalized to cortical thickness was 20.22 ± 1.50 (G1), 16.55 ± 2.43 (G50), and 17.08 ± 1.84 (G100) for 3.5-mm screws and 6.92 ± 1.89 (G1), 8.57 ± 1.69 (G50), and 7.08 ± 1.62 (G100) for 2.0-mm screws.

**Scanning electron microscopy**

Four unused screws from each group and size were analyzed using a scanning electron microscope (SEM) (Field Electron and Ion Company Quanta 3D DualBeam; Focused Ion Beam [FIB] with a high-resolution Field Emission Gun SEM). Using a method of analysis previously described,5,10 trained examiners analyzed the micrograph from 2 different areas of each screw (the tip of the screw at 250X magnification and the first thread of the cutting surface of the fluted tip at 600X magnification) *(Figure 2).* Examiners were provided grades according to the following scale: 1, no alteration; 2, minor and well-marked alteration; and 3, moderate to major alteration. The micrographs were observed by 5 examiners and classified according to the scale.

Figure 1—Representative diagram of pullout test.

Figure 2—Locations of the analyzed scanning electron micrographs. The tip of the screw at 250X magnification and the first thread of the cutting surface of the fluted tip at 600X magnification.
The difference in load at failure was not statistically significant between autoclave groups in 3.5-mm screw sizes. For the 2.0-mm screws, there was not a significant difference between G1 and G50, but a significant difference was found between G1 and G100 ($P = .027$). The mean load at failure ± SD measured in Newtons during pullout testing and normalized to cortical thickness was 196.7 ± 60.04 (G1), 223.1 ± 63.37 (G50), and 227.2 ± 57.4 (G100) for 3.5-mm screws and 228.5 ± 57.23 (G1), 201.9 ± 33.54 (G50), and 168.1 ± 52.32 (G100) for 2.0-mm screws.

Scanning electron micrographs by scoring scale and the 2 locations analyzed by the examiners are illustrated in Figure 2 and Figure 3. Only micrographs at the tip of 3.5-mm screws had a significant group effect (Table 1).

Discussion

These results suggest that numerous sterilizations alter the physical-mechanical properties of certain screw sizes. As the 3.5-mm screws were significantly affected in insertional torque and torque at failure, the changes may be more apparent in larger screws, contrary to the study hypothesis. The lack of statistical significance in the results of 2.0-mm screws, apart from pullout in screws undergoing 100 sterilizations, may be because there was no difference or because any difference that may exist was masked by the smaller surface area of the screw combined with the intrinsic variability of bone. The result that peak insertional torque was statistically lower with increased sterilizations for the 3.5-mm

![A](image1.jpg) ![B](image2.jpg) ![C](image3.jpg) ![D](image4.jpg) ![E](image5.jpg) ![F](image6.jpg)

**Figure 3**—Representative scanning electron micrographs of unused surgical screws at different locations (at the tip [A, C, and F] and at the first thread [B, D, and E]), graded by examiners in the study as grade 1 (no alterations [A]), grade 2 (minor and well-marked alteration [B and C]), or grade 3 (moderate to major alteration [D, E, and F]).

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Position</td>
<td>Screw size (mm)</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Grade</td>
<td>Thread</td>
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<td>Grade</td>
<td>Tip</td>
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<td>2.20</td>
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<tr>
<td>Grade</td>
<td>3.5</td>
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screws may be an indication that screw loosening in clinical cases could occur. Additionally, the torque to failure was lower for 3.5-mm screws with more sterilizations, which could also increase the risk of screws stripping or breaking with lower insertional torque.

The primary factors affecting pullout strength are the quality and shear strength of the bone, the major diameter of the screw, and the length of engagement of the thread. These primary factors affect load transfer at the thread-bone interface and determine the mean shear stress in the surrounding bone. However, because the shear force is not uniform, the geometry of the interface is also affected by local reaction forces and structural stresses due to secondary factors, including thread profile, pilot hole dimensions, and tapping.

Factors such as the geometry of the bone and differences in bone density render bone specimens nonuniform and have the potential to influence pull-out and insertional torque testing. These factors were minimized in sample selection by the normalization of raw data to the combined thickness of the cis- and transcortices. Still, the possibility of an effect does exist. Small sample sizes could have limited the statistically significant differences.

316L stainless steel is the most common metal used in veterinary surgery for the development of cortical orthopedic screws due to the optimum load-bearing characteristics, high ductility, wear and corrosion resistance, biocompatibility, and, not least importantly, low cost when compared to titanium, cobalt, and vanadium alloys. However, these metallic implants have a limitation due to their Young modulus. Cortical bone presents a Young modulus value between 5 and 30 gigapascals, whereas it is approximately 190 gigapascals for 316L stainless steel. This difference can lead to implant instability and failure due to the stress-shielding effect, which causes non-union between implant and bone, bone necrosis, and delay in the healing process. To improve 316L stainless steel’s performance, various techniques have been utilized, including surface coating to improve biocompatibility and minimize the corrosion rate.

Therefore, screws that undergo repeated sterilization could deteriorate the coating surface of the screws needed to improve its performance. Assessment of the surface roughness of the screw is valuable to assess damage of the coating surface. As mentioned earlier, the coating surface improves the performance of 316L stainless steel cortical screws. Therefore, a subjective assessment of the roughness of the surface of the screw revealed that only screw size 3.5 mm at the tip had a significant difference between groups, having more damage at the coating surface with more sterilizations. These results could be due to differences in the coating between sizes or small sample size.

The Young’s modulus, also known as stiffness or elastic modulus, measures a material’s resistance to deformation under stress. Since the bone and the screw have different Young’s moduli and they don’t vary, the proper force to analyze in this study is stress to failure which measures the force needed to approach the failure threshold of the screw-bone interface. The relationship between Young’s modulus and stress failure is directly proportional. Therefore, if the Young’s modulus of the screw is altered by decreasing it due to repeated sterilization, it should decrease the stress of failure of the construct and make it weaker. In this study, there was no difference in the stress to failure between groups.

Further testing is warranted using different sizes of screws (4.5 and 5.5 mm) to further understand how screw size affects results. There is variability in screw insertion when done by hand, which was minimized by having screws placed by just 1 surgeon.

Numerous studies have confirmed that cadaveric bone approximates the biomechanical properties of freshly harvested bone in both cats and humans even with prolonged storage.

In conclusion, multiple autoclave sterilizations could affect the biomechanical properties of the screw-bone construct, decreasing the performance of this type of screw and, in consequence, altering the performance or even failure of the construct. Further investigation is necessary to develop protocols that would track and limit the number of autoclave procedures that any screw would undergo.

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Disclosures

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