Hypothesis / aims of study
Hypothesis: Menopause negatively impacts the vagina and its supportive connective tissues and increases susceptibility to pelvic organ prolapse.
Aim: To test the effects of surgical menopause on the structural and ultrastructural properties of the rat vagina and its supportive tissues.

Study design, materials and methods
Six virgin Sprague-Dawley rats 6 weeks status-post a bilateral oophorectomy, 8 sham-operated rats, and 8 virgin rats of similar age, were sacrificed according to IACUC guidelines. The in situ relationship between the vagina and its supportive tissues were studied grossly in all of the rodents. The specimens were reduced to the bony pelvis and associated musculature, the levator ani muscles, the vagina and supportive connective tissue, cervix, uterine body, sacrum and L1-7. The lumbar spine was potted in polymethylmethacrylate and mounted in a custom made fixed cylindrical clamp. Each specimen was positioned such that the longitudinal axis of the vagina was aligned with the loading axis. A customized soft-tissue clamp was used to secure the distal 5 mm of the vagina to a load cell that was rigidly fixed to the crosshead of the machine. Prior to each test, the specimens were preloaded to 0.2 N and preconditioned at 25 mm/min between 0 and 2.5 mm of elongation for ten cycles. A load to failure test was performed at the same elongation rate. The parameters describing the structural properties of the vagina and its support tissues were determined from the resulting load-elongation curve. These included the stiffness, ultimate load, ultimate elongation at failure, and energy absorbed to failure. Scanning electron microscopy was used to inspect the vaginal epithelium for evidence of tissue failure. Collagen fibril diameter, fibril density, and fibril area fraction before and after failure were determined by quantitative analysis of transmission electron micrographs. Biomechanical and collagen fibril parameters were compared using an F-test for equality of variances and unpaired t-test for comparison of means. Simultaneous video recording of the tests was performed and is available for presentation.

Results
The rat vagina and cervix are supported by anatomical structures analogous to those seen in the humans. Biomechanical testing showed that the mean ultimate failure load of the oophorectomized rats was significantly decreased to 11.36 N from the sham operated failure loads of 14.0 N (p = 0.002). The vagina and its supportive tissues were substantially less stiff in the oophorectomized rats (1.86 vs. 2.83 N/mm, p = 0.002) while the ultimate elongation and energy absorbed to failure were similar (p = 0.135 and 0.43). Although the energy absorbed to failure was higher in sham operated rats relative to virgin controls (71.3 N-mm vs. 51.1 N-mm, p = 0.02), there was no difference in the ultimate failure load, stiffness and ultimate elongation in these two groups (p > 0.05). As demonstrated by video analysis, the primary mechanism of failure resulted from the disruption of the lateral and posterior connective tissue attachments to the pelvic sidewall, the pelvic diaphragm and the pubic symphysis. Meticulous inspection of the vaginal epithelium by SEM confirmed that it remained intact following each load to failure test. Analysis of ultrathin sections of the vaginal wall by TEM, however, demonstrated that failure resulted in a complete disruption of collagen architecture with a decrease in fibril diameter, fibril density and fibril area fraction (p < 0.0001, p = 0.009 and p < 0.002).
Interpretation of results
Surgical menopause results in a significant and rapid decline in the structural properties of the vagina and its supportive tissues. Failure of vaginal support corresponds to a complete disruption of collagen architecture. These findings provide a biological mechanism for the increased incidence of prolapse seen in menopausal women. Further studies will be needed to determine whether supplementation with sex steroid hormones can diminish this deterioration in the vagina and its supportive tissues that occurs following oophorectomy.

References
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