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Prostatic Collagen Architecture in Neutered and Intact Canines

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Abstract

Background—Prostate stiffness and increased collagen content both associate with the presence of urinary symptoms in men but mechanisms responsible, including impact of age and androgens, are unknown. Dogs develop prostate-related urinary dysfunction similar to humans, but mechanisms are also unknown. Mice have been used to examine how prostatic collagen accumulation affects voiding but whether mouse prostatic collagen organization resembles human or dog has not been evaluated. Here we have constructed the first comprehensive, comparative maps of collagen architecture in canine, human, and mouse prostate and test whether canine prostatic collagen content is increased by aging and reduced by castration.

Methods—Complete transverse prostate sections were stained with picrosirius red and imaged with confocal microscopy to reveal and compare collagen architecture across species. Canine prostatic collagen fiber length, diameter, and density in prostatic urethral, periurethral, peripheral, and capsular regions were quantified and compared among four experimental groups: young intact, young neutered, old intact, and old neutered dogs.

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Results—Surprisingly, the majority of collagen was localized to the prostatic urethra in canine, human and mouse. In canine and human, capsular regions also featured a dense collagen network but it appeared less dense than around prostatic urethra. Older, intact male canines exhibited overall denser prostate collagen fibers and had thicker capsular fibers than young, intact males. Prostatic glandular regions undergo dramatic atrophy and regression following castration, and our finding of neutered animals having increased collagen fiber density in both periurethral and peripheral regions is consistent with glandular contraction and increased proportion of stroma.

Conclusions—Collagen architecture in dog appears similar to that in humans when cross sections are compared side-by-side. Canine collagen organization is affected by both age and androgen status, suggesting these factors may contribute to collagen accumulation in some males.

Keywords

LUTS; capsule; fibrosis; age; one health

1 Introduction

Lower urinary tract symptoms (LUTS) encompass a range of bothersome urinary disorders including urgency, weak stream, straining, and increased urinary frequency, especially at night. Symptoms generally become more severe with advancing age and are sometimes associated with dangerous and expensive complications i.e. urinary retention, hydronephrosis, etc..^{1,2} It is therefore critical to identify etiologic factors and improve treatment efficiency. Prostate enlargement is a leading risk factor of male LUTS^{3–5} but mounting evidence implicates additional factors including prostatic fibrosis.^{6–13} Human periurethral prostatic fibrosis correlates with tissue stiffness and LUTS severity.¹³ Prostatic fibrosis also correlates with histological inflammation, a predictor of clinical progression in men with urinary dysfunction.^{9,14}

A significant barrier to pinpointing the etiology of prostate-related urinary dysfunction has been scarcity of high-quality prostate tissue from which to survey regional collagen content and correlate it with other known risk factors such as gland volume, inflammation, age, and circulating hormone levels. Previously published studies focus solely on periurethral tissue obtained from radical prostatectomy (RP) or transurethral resection of their prostate (TURP) surgeries.¹¹ Tissues obtained from TURP are often damaged by cautery instruments, thereby complicating collagen analysis, and peripheral zone and capsule are not typically collected during TURP, limiting regions that can be assessed. RP specimens are only removed under conditions of severe urinary symptoms or prostate cancer; there is no disease-free or treatment-free and age-matched control prostate for comparative analyses. For these reasons, many researchers are turning to non-human species to identify mechanisms for, and physiological consequences of, prostatic fibrosis.

Mice are commonly used to study prostate disease, and although urinary dysfunction can be driven experimentally, it does not develop spontaneously in mice.^{15,16} In addition, the anatomical organization of mouse prostatic ducts differs from that of humans, with mice having a multi-lobed prostate in place of a zonal gland. Canine prostate has anatomical and histological features that resemble human prostate, and intact canines are the only non-

human species known to spontaneously develop benign prostatic enlargement and associated sequelae including urinary, ejaculatory, excretory, systemic, and/or locomotive dysfunction.¹⁷

In this report, we stained whole transverse sections of human, canine, and mouse prostate using a fluorescent picrosirius red staining method developed in our laboratory¹⁸ and compared collagen organization across species, noting similarity between dog and human. We also used publicly accessible software developed at the University of Wisconsin-Madison¹⁹ to quantify collagen and test the hypothesis that prostatic collagen fibers in old intact dogs are longer, thicker, and denser than those in young intact or old castrated dogs.

We found regional differences in canine prostatic collagen density. Surprisingly, collagens in the urethral region were denser than those in the capsule for all groups. Aging and neuter status impacts canine prostatic collagen characteristics in different ways. For example, old male prostates had thicker capsular collagen fibers and denser peripheral region fibers than young intact male prostates. Intact male prostates have longer capsular collagen fibers than neutered male prostates. Intact male prostates also had less dense periurethral and peripheral region fibers than neutered consistent with glandular contraction of the prostate and increased proportion of stroma. These results support the hypothesis that aging and androgens influence canine prostate collagen organization. Since changes occur with age and androgens, these changes could contribute to LUTS and further studies are needed to correlate these parameters with symptom severity. These results also shed new light on the natural history of collagen organization in canines, which spontaneously develop benign prostatic disease and related sequelae like humans

2 Materials and Methods

2.1 Prostate tissue collection, preparation and processing

2.1.1 Dog—Thirty-one canine prostates were collected post-mortem from dogs euthanized for reasons other than prostatic disease (Figure 1-A). Prostates were from Dane County Humane Society (Madison, WI), the University of Wisconsin-Madison Veterinary Teaching Hospital Necropsy Service (Madison, WI), Covance (Madison, WI), and the Mayo Clinic (Rochester, MN). Dogs ranged in age from 0.83 years to 15 years with a mean of 5.95 years and median of five years. They ranged in weight from 4.82 kg to 47.10 kg with a mean of 27.02 kg and median of 28.5 kg. Weight information was not recorded for six dogs at time of euthanasia. To ensure representation across the canine population, prostates were obtained from multiple breeds: Golden Retriever (4), Beagle (2), Border Collie (2), Pit Bull (2), Boxer (1), Cocker Spaniel (1), Doberman Pinscher (1), French Bulldog (1), Labrador Retriever (1), Miniature Poodle (1), Rat Terrier (1), Rottweiler (1), Shih Tzu (1), Standard Poodle (1), Weimeriner (1), mixed breed (8), and unknown breed (2). Prostates were classified based on age: young (< 3 years), middle aged (3–5 years), and old (> 5 years) and by androgen status (intact and neutered). Neutered dogs were defined as those that had undergone bilateral orchiectomy at least one month prior to euthanasia, allowing adequate time for castration related prostatic atrophy. Intact dogs were defined as those with two palpable testicles present in scrotum at euthanasia. Dogs with unilateral and bilateral cryptorchidism were excluded. Benign prostatic enlargement is most common in dogs >6

years of age, but may begin as early as 2.5 years of age. To maximize group size and comparisons between young and old, three to five-year-old (middle aged) dogs were excluded from this study, leaving four groups: old intact (n = 4), young intact (n = 9), old neutered (n = 14), and young neutered (n = 4). Demographic information is shown in Table 1.

Prostates were fixed and stored in neutral-buffered formalin. Prostates were bisected by a single dorsal-ventral cut at cranial-caudal midline and then cut into wedges suitable for embedding (Figure 1-B). Wedges were oriented as shown in Figure 1-C, embedded in paraffin, and sectioned by the University of Wisconsin-Madison Veterinary Teaching Hospital Histology Service. Twenty serial sections were taken from each embedded wedge. The first and eleventh section were stained with hematoxylin and eosin and the second and twelfth were stained with picosirius red.

2.1.2 Mouse and Human for comparative transverse sections—Mouse specimens (n = 8) were obtained from young adult (p50 – 1 year-old) wild-type C57BL/6J (stock number 000664) mice acquired from Jackson Laboratories (Bar Harbor, ME). Mice were housed in an Udel® Polysulfone microisolator cage; the room was on 12-h light and dark cycles; room temperature was typically $20.5 \pm 5^\circ\text{C}$; humidity was 30–70%. Mice were fed 8604 Teklad Rodent Diet (Harlan Laboratories, Madison WI) and water ad libitum; cages contained corncob bedding. Mice were euthanized by CO₂ asphyxiation at post-natal-day 50, whole lower urinary tract collected, fixed 24h in 4% PFA, embedded in paraffin, and sectioned in the transverse plane. All mouse procedures were approved by the University of Wisconsin Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Two slides from one human prostate gland were provided for this experiment by Dr. Douglas Strand, Department of Urology, University of Texas Southwestern Medical Center, Dallas, Texas. Tissues were formalin-fixed and paraffin-embedded in the transverse plane. The human prostate specimen was obtained from an 18–29-year-old organ donor at the Southwest Transplant Alliance in Dallas, TX from March 2017 to April 2018. A cystoprostatectomy is performed after transplantable organs are harvested and immediately transported to UT Southwestern Medical Center for processing under IRB STU 112014–033.

2.2 Picosirius Red Staining and Imaging

Picosirius red staining was achieved by incubating slides for 1 hr. at 25°C in 0.5 g of Direct Red 80 (2610–10-8; Sigma-Aldrich Corp., St. Louis, MO) in 500 mL of saturated picric acid (P6744–1GA; Sigma-Aldrich Corp.). Slides were washed twice with acidified water (0.5% acetic acid), dehydrated in a graded ethanol series, cleared in xylene, mounted with Richard-Allan toluene-based mounting medium (4112APG; Thermo Fisher Scientific).

2.2.1 Dog—Two slides were imaged per dog using a fluorescent detection method¹⁷ optimized by our laboratory and three representative images were taken from each slide in each of four prostatic regions of interest (Figure 2-A): urethral, periurethral, peripheral, and capsular. Images were taken randomly throughout each region in order to obtain a random

sample of all areas contained in each region regardless of glandular/stromal content. Because of this there was no correction for reduced glandularity in neutered compared to intact and thus we expected an increased density of collagen in neutered animals. We do not anticipate the reduced glandularity in neutered to have any impact on length or diameter measures. The urethra region was defined as the region within 459 μm of the lumen. The periurethral region extended outward 918 μm from where the collagen switched from circumferentially wrapping the urethra to branching outward in a web-like fashion. The peripheral region extended 918 μm from the capsular region. Images were analyzed using publicly accessible CT FIRE software developed at the University of Wisconsin-Madison.¹⁹ This software detects and quantifies fiber-like structures in images (Figure 2-B). For this study, fiber length, diameter, and density were measured. Length and diameter were measured in μm and density was measured in number of fibers per mm^2 .

2.2.2 Mouse and Human for comparative transverse sections—One slide from human and one slide from mouse were imaged in whole at 10X and 20X respectively using Keyence BZ-X700 microscope and images were stitched and compressed into whole transverse images.

2.4 Statistical Analysis

Statistical Analysis was performed using RStudio (Version 1.0.153 – © 2009–2017 RStudio, Inc.). Homogeneity of variance was tested using Bartlett's Test and all groups determined homogeneous ($p > 0.05$). Groups were compared using analysis of variance (ANOVA) followed by Tukey's post-hoc analysis. The significance threshold was set at $p < 0.05$ and trend threshold at $p < 0.10$.

3 Results

Canines and humans spontaneously develop prostate-related urinary complications and mice are increasingly used to model prostate-related urinary dysfunction in higher order animals. Collagen architecture was previously described in portions of human prostate¹³ but never across the entire human or canine gland. To provide a basis for species comparisons, we stained complete sections from dog (Figure 3-A), human (Figure 3-B), and mouse (Figure 3-C) prostate with picrosirius red and visualized staining with fluorescence microscopy. The prostatic urethra harbored the most visually prominent picrosirius red staining of collagen in all three species, even more prominent than prostatic capsule in dog and human. Unlike the multi-lobed mouse prostate, and human prostate, a prominent collagenous medial septum separates the canine prostate into right and left lobes. Collagenous capsular trabeculae (Figure 2-A) further divide canine prostate lobes into lobules consisting of tubuloalveolar glands lined by columnar epithelium. Fibers also bisect lobules dividing the gland into smaller units. Smaller collagen fibers follow ductal architecture, wrapping circumferentially around the prostatic urethra and branch into the proximal and peripheral regions, to form a basket-like network around ducts. Fibers then revert to a circumferential pattern in the capsular region. Quantitative characteristics of canine prostatic collagen fibers are described below and listed in Table 2, and representative images from each group/region are given in Figure 4.

3.1 Fiber Length

The longest prostatic collagen fibers are in the capsular region of old intact male dogs ($31.53 \pm 0.52 \mu\text{m}$, mean $\pm$ standard error of the mean fiber length) while the shortest are in the peripheral region of young intact males ($26.21 \pm 0.17 \mu\text{m}$). The rank order of collagen fiber length by prostatic region is the same for all four experimental groups: capsular > urethral > periurethral > peripheral.

3.2 Fiber Diameter

The thickest fibers are in the old intact male periurethral region ($2.624 \pm 0.02 \mu\text{m}$) while the thinnest fibers are in the young neutered male capsular region ($2.209 \pm 0.02 \mu\text{m}$). For all groups except old intact, the prostatic urethral region followed by capsular region harbor the thinnest collagen fibers. In the case of old intact males, capsular fibers are thicker than urethral fibers. The fact that this shift toward thicker fibers in the capsule is only found in intact males, makes the likelihood of it contributing to LUTS greater than other changes in collagen characteristics. Periurethral collagen fibers in old intact and young males are thicker than peripheral fibers. Peripheral fibers are thicker than periurethral fibers in old neutered males.

3.3 Fiber Density

Fiber density had the greatest variability between groups. Collagens are most dense in the prostatic urethral region of young neutered males (481 ± 6.69 fibers per mm^2) and least dense in the capsular region of young intact males (322 ± 3.50 fibers per mm^2). The capsular region consistently harbors the least dense collagen fibers of all prostate regions regardless of age or neuter status. However, collagen fiber density in other regions differs with age and neuter status. The rank order density across these other prostatic regions of old intact males is prostatic urethral > peripheral > periurethral region, for old neutered males is periurethral > urethral > peripheral, and for young intact and neutered males is urethral > periurethral > peripheral.

3.4 Impact of age

Age only influences prostatic collagen architecture of intact male dogs. Prostatic capsular fibers are thicker in old intact than in young intact males, $p = 0.0470$ (Figure 5-A). Aging increases prostatic collagen density, as peripheral region prostatic collagen fibers are significantly denser in old versus young intact males, $p = 0.0251$ (Figure 5-B). Aging has little impact on prostatic collagen fiber length (it did not differ between old and young dogs).

3.5 Impact of castration

Castration reduced collagen fiber length. Capsular collagen fibers in old intact males are trending towards longer than those in old neutered males, $p = 0.0793$, and are significantly longer in young intact versus neutered males, $p = 0.0041$ (Figure 5-a). Capsule fibers are thicker in old intact than in old neutered males, $p = 0.0118$ (Figure 5-b). As anticipated by our method of determining density, neutering also increases prostatic collagen density. Periurethral region collagens of old and young intact were significantly less dense than that of old ($p < 0.0001$) and young ($p < 0.0001$) neutered (Figure 5-c and d). Peripheral region

collagens of old intact males trended towards being less dense than that of old neutered males ($p = 0.0853$) and are significantly less dense in young intact versus neutered males, $p < 0.0001$ (Figure 5-e). Capsular collagen fibers are also less dense in young intact versus neutered males, $p < 0.0001$ (Figure 5-f).

4 Discussion

Prostatic collagen content and stiffness are associated with LUTS severity in men but how prostatic collagens are organized and mechanisms driving their accumulation are only beginning to be explored. Here, we provided a number of critical resources to advance this research area. First, we provide the first direct comparisons of canine, human, and mouse prostatic collagen organization to improve understanding of model systems and their relationship to humans. We concluded that canine prostatic collagen architecture is very similar to that of humans, particularly with respect to the dense collagen matrix surrounding urethral epithelium. We showed in side-by-side comparison that the canine prostate contains a capsule similar to human prostatic capsule; thereby addressing a long-standing controversy over existence of a capsule in canine prostate.^{21,22} A surprising discovery was that the circumferential band of collagens directly surrounding the urethra in human and canine prostate contained the most visually prominent picrosirius red staining of collagen even more prominent than the capsule. This observation was possible due to the novel way in which we visualized collagens in transverse sections from the whole gland to facilitate comparisons between regions. Though it has been widely speculated that the prostatic capsule contributes to LUTS by restricting prostate gland enlargement, the new finding that the urethral region harbors a substantial collagen network raises new questions about its potential role as a structural and signaling molecule in gland homeostasis and urinary physiology.

Previous studies correlated prostatic rigidity, collagen content, and elastin deficiencies with LUTS.^{6,9–11,13,23,24} This study is the first to evaluate collagen content across the entire gland, including urethral and capsular tissue, offering new results to address two mechanisms by which prostatic fibrosis could drive LUTS. The first potential mechanism is that collagen accumulation in the prostatic capsule increases its rigidity, and in conjunction with an expanded transition zone due to BPH, increases urethral pressure to drive an obstructive urinary phenotype. One piece of evidence supporting this mechanism is the established success of the transurethral incision of the prostate (TUIP) procedure, in which the prostatic capsule is incised to relieve pressure on the underlying urethra.²⁵ A second mechanism by which prostatic collagen accumulation could impair urinary flow is through a direct stiffening of the prostatic urethra to reduce its compliance. In the resting state, the lumen of the prostatic urethra is collapsed. Micturition causes the urethra to expand and accommodate urine passage.²⁶ A lack of urethral compliance due to urethral fibrosis could underlie the obstructive phenotype seen in some men with LUTS. Our study did not find any changes in urethral collagen with age or castration, however changes in urethral collagen could be specific to human, or only occur in the most severe LUTS cases.

This study leverages novel software to quantitatively assess collagen characteristics in different regions of the prostate, and determines changes in collagen fibers with age or

castration. Without this software we would not have been able to do individual fiber analysis with diameter and length measurements. This detailed analysis can be applied to future studies in order to continue to improve collagen analysis and correlate with LUTS severity. We found age and neuter status have different impacts on collagen characteristics. Aging affected collagens in intact but not neutered male prostate. Suggesting a protective effect in castrated animals which correlates with the protective effect castration has on development of LUTS and BPH. Old intact male prostates have thicker capsular collagen fibers and denser peripheral region fibers than young intact and neutered male prostates.

Castration increases periurethral and peripheral region collagen fiber density. Due to our methods we were not surprised that castration increases collagen fiber density, however, we considered the several possible explanations. First, it is possible that collagen density increases directly in response to castration. A study in rats found that castration increased levels of fibroblastic growth factors (FGF) 2, 7, and 8.²⁷ Further, in cornea, FGF2 induces the secretion of type I collagen.²⁸ If similar increases of FGF are present in canine following castration, and if FGF2 also induces the secretion of type I collagen in canine prostate, it could explain the increase in collagen density found with castration in the periurethral and peripheral regions. Transforming Growth Factor- β (TGF- β) mRNA expression is also increased following castration²⁹ and known to drive fibroblast to myofibroblast transition, epithelial-mesenchymal transition, and extracellular matrix deposition.³⁰ Another potential mechanism by which prostatic collagens could become denser upon castration is that the glandular atrophy causes the prostate to collapse into a smaller space, bringing collagen fibers into closer proximity. Comparisons of percent glandular tissue and stroma tissue in these various age and androgen status groups do not to our knowledge exist and warrant future study. Impact of collagen on prostate cancer risk was not directly addressed in this study. However, neutered canines are more likely than intact to develop prostate cancer.²⁰ Further study is needed, but increased density in the periurethral and peripheral region in neutered canines could be related to the increased incidence of neoplasia in male neutered dogs.

Most studies of prostatic collages and their influence on LUTS have focused on collagens as structural molecules. However, there is growing recognition in the breast cancer field that collagens also function as signaling molecules which influence tumor initiation and progression.³¹ One mechanism supported by recent data is that collagens drive myodifferentiation of fibroblasts and epithelial-mesenchymal transition through soluble growth factors and chemokines.^{32–34} These influences of collagen architecture on fibroblasts and microenvironment could occur concurrently with the changes in prostate scaffolding, leading to further changes in urinary function.

5 Conclusions

Our results support the hypothesis that in canines, collagen fibers are longest in the capsule, thickest in the periurethral region, and densest in the prostatic urethra. The overall prostatic collagen network in old canines is denser and consists of longer and thicker fibers than in young canines, and neutered canines are exempt from these age-related changes. These

results shed new light on the natural history of collagen dynamics in canines, which, like humans, spontaneously develops benign prostatic enlargement and related sequelae.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Prostate sections. (A) Urinary tracts were isolated in euthanized canines and the urethra cut cranial and caudal to the prostate. (B) Whole prostate was then sectioned via three dorsal-ventral cuts into two wedges. Wedge C' and C'' were oriented as shown in (C), embedded in paraffin and sectioned. Exceptionally large prostates were transected along the urethra prior to fixation to allow further penetration of fixative, and resulting in cut from urethra to dorsal capsule in sections (cut-line in C).

Figure 2.

Collagen analysis of prostate regions. (A) Dog prostates are divided into a right and left lobe by a median collagenous septum. Lobes are further divided by collagenous capsular trabeculae (arrowhead). We divided the prostate into four regions to assess collagen architecture across the gland: prostatic urethra (U), periurethral region (Pu, extends 918 μm outward from the urethra), peripheral region (Pp, extends 918 μm inward from the capsule), and capsule. Three representative images were taken from each region (ex. [b']) per section, and two sections were taken per prostate. (B) CT FIRE recognized and traced fibers (b'') on the representative image (b) and gave length and diameter of each fiber.

Figure 3.

Comparison of (C) mouse, (B) human, and (A) dog prostate. Top row shows a schematic comparison of dog, human, and mouse lower urinary tract. Note the lobar organization of mouse prostatic ducts (anterior prostate [AP], ventral prostate [VP], dorsolateral prostate [DLP]) compared to the spherical organization of human and canine ducts. Prostates from all three species were oriented on the transverse plane and sectioned to approximate cranial-caudal midline (dashed line). Bottom row shows fluorescent picosirius red stained transverse sections from dog, human, and mouse prostate. Urethral lumen is labelled with “U”.

Figure 4.

Representative images by region and group. Whole prostate sections were stained with picrosirius red. Representative images were captured using fluorescent microscopy and used for CT FIRE analysis.

Figure 5.

Age and androgen related changes in collagen length, diameter, and density. Regions of interest from picrosirius red stained canine prostatic tissue sections were quantitatively evaluated for collagen fiber thickness, density, and length using CT fire software. Capital letters denote changes with age, lower-case letters denote changes with castration. (A) Capsule fibers are thicker ($p = 0.0470$) and (B) peripheral fibers are denser ($p = 0.0251$) in old versus young intact males. (a) Capsular collagen fibers are longer in young intact versus neutered males ($p = 0.0041$). (b) Capsule fibers are thicker in old intact than in old neutered males ($p = 0.0118$). (c & d) Periurethral region collagen fibers of intact males are less dense than that of neutered males (for old and young, $p < 0.0001$). (e) Peripheral region and (f) capsular region fibers are less dense in young intact versus neutered males ($p < 0.0001$ for both comparisons).

Table 1

Demographic information.

	All n=31	Young Neutered n=4	Old Neutered n=14	Young Intact n=9	Old Intact n=4
Age					
Mean	5.95	1.91 ^b	9.43	1.33 ^a	8.23
Median	5.00	1.90	9.5	1.00	8.80
Range	0.83– 15.00	0.83– 3.00	5.00– 15.00	0.83– 3.00	5.00– 10.30
Weight (kg)					
Mean	27.02	25.20	28.54	22.75	31.40
Median	28.5	26.60	31.00	26.00	35.25
Range	4.82– 47.10	14.00– 35.00	4.82– 40.10	5.30– 35.70	8.00– 47.10
# Dogs Weight unknown	6	1	2	3	-
Breed					
Beagle	2	-	-	2	-
Border Collie	2	-	2	-	-
Boxer	1	1	-	-	-
Cocker Spaniel	1	1	-	-	-
Doberman Pinscher	1	-	-	1	-
French Bulldog	1	-	-	-	1
Golden Retriever	4	-	4	-	-
Labrador Retriever	1	-	1	-	-
Miniature Poodle	1	1	-	-	-
Pit Bull	2	-	-	1	1
Rat Terrier	1	-	1	-	-
Rottweiler	1	-	-	-	1
Shih Tzu	1	-	1	-	-
Standard Poodle	1	-	1	-	-
Weimeriner	1	-	-	-	1
Mixed	8	1	2	5	-
Unknown	2	-	2	-	-
Acquired From					
Covance Laboratories	2	-	-	2	-
DCHS	3	-	-	3	-
Mayo Rochester	2	-	-	2	-
UW-VMTH	23	4	14	2	4

^a old intact were significantly older than young intact ($p = 4 \times 10^{-7}$)^b old neutered were significantly older than young neutered ($p = 1.5 \times 10^{-6}$)

Table 2

Collagen fiber characteristics by region and group.

	Young Neutered n=4	Old Neutered n=14	Young Intact n=9	Old Intact n=4
Prostatic Urethra Fibers	(mean ± SE)	(mean ± SE)	(mean ± SE)	(mean ± SE)
Length (μm)	27.30 ± 0.14	28.17 ± 0.10 ^b	28.37 ± 0.10 ^b	28.57 ± 0.29 ^b
Diameter (μm)	2.246 ± 0.03	2.266 ± 0.01	2.273 ± 0.01	2.398 ± 0.03
Density (fibers/mm ²)	481 ± 6.69	464 ± 1.31 ^b	454 ± 3.38 ^b	454 ± 6.69 ^b
Periurethral Region Fibers				
Length (μm)	26.50 ± 0.08 ^b	27.43 ± 0.05 ^b	26.94 ± 0.11 ^b	26.78 ± 0.07 ^b
Diameter (μm)	2.378 ± 0.02	2.437 ± 0.01 ^{a, b}	2.475 ± 0.02 ^{a, b}	2.624 ± 0.02
Density (fibers/mm ²)	477 ± 8.95 ^B	474 ± 1.32 ^{b, B}	360 ± 2.78 ^a	394 ± 8.95
Peripheral Region Fibers				
Length (μm)	26.39 ± 0.13 ^b	26.83 ± 0.04 ^{a, b}	26.21 ± 0.17 ^{a, b}	25.56 ± 0.13 ^{a, b}
Diameter (μm)	2.356 ± 0.04	2.505 ± 0.01 ^{a, b}	2.315 ± 0.02	2.530 ± 0.04
Density (fibers/mm ²)	455 ± 11.07 ^B	458 ± 1.37 ^b	394 ± 5.06 ^a	403 ± 11.07 ^A
Capsule Fibers				
Length (μm)	27.82 ± 0.25 ^B	29.30 ± 0.10 ^a	31.38 ± 0.19 ^a	31.53 ± 0.52 ^a
Diameter (μm)	2.209 ± 0.02	2.234 ± 0.01 ^B	2.264 ± 0.01	2.470 ± 0.02 ^A
Density (fibers/mm ²)	424 ± 3.11 ^B	416 ± 2.30 ^a	322 ± 3.50 ^a	372 ± 3.11 ^a

^a significant difference from prostatic urethra^b significant difference from capsule^A significant difference with increased age^B significant difference with castration