Anatomical and histological equivalence of the human, canine, and bull vas deferens

Dean E. Leocadio, MD,[1] Allen R. Kunselman, MA,[2] Timothy Cooper, DVM,[3] Jairo H. Barrantes, MD,[4] J.C. Trussell, MD[1]

[1]Department of Urology, State University of New York at Upstate, Syracuse, New York, USA
[2]Health Evaluation Sciences, Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA
[3]Department of Comparative Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA
[4]Department of Pulmonary and Critical Medicine, Wayne State University, Detroit, Michigan, USA

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Introduction: *Several animal models have been utilized for in-vitro experimentation and surgical training exercises of the vas deferens. The canine model is currently the standard for both in-vivo and ex-vivo study. Due to increasing costs associated with experimentation on canines, and in keeping with the principles of refine, reduce, and replace, a novel model that is cost-effective and easily obtained is desired. We compared morphology of the bull vas deferens to that of the human and the canine.*

Materials and methods: *Bilateral vas deferens tissue from the human (n = 6), canine (n = 6), and bull (n = 5) were compared. Outer diameter (OD), inner diameter (ID), and microscopic measurements of the luminal mucosa and muscularis were then determined from each of these tissues. Histological comparisons were performed by a single pathologist. Data was analyzed using Two One-sided Tests (TOST) Analysis of Equivalence.*

Results: *According to the TOST statistical analysis, the vassal ID was equivalent for all three species. Similarly, equivalent microscopic measurements were noted for both vassal mucosal (human-canine and human-bull) and muscularis thicknesses (canine-bull). Lastly, all three species had similar histological characteristics.*

Conclusions: *The vas deferens' of the human, canine, and bull are equivalent in many ways, including histological similarities. It is reasonable to conclude that the bull vas could be substituted for the human vas for both in-vitro testing and microscopic vasovasostomy simulation exercises. Specimens are cost-effective, provide ample tissue length, and are easy to obtain.*

Key Words: animal model, vas deferens, surgical training

Introduction

Several animal models, including the canine,[1] rabbit,[2] rat,[3] ram,[4] and primate[5] have substituted for the human vas deferens for in-vitro experimentation and surgical training exercises. Due to ease of animal handling and comparative anatomy, the canine model is currently the standard,[6] especially when subsequent semen analysis is desired. Housing live animals in a vivarium carries inherent costs and obligations. Due to increasing costs associated with experimentation on canines, a novel model that is cost-effective and easily obtained is desired.

It has been demonstrated that laboratory-based training in microsurgical techniques significantly adds to retention of learned technical skills by novice surgeons.[7] However, physical and/or financial limitations can be prohibitive in some training centers. Because of its availability and low cost, and in keeping with the principles of refine, reduce, and replace, we considered the use of bull vas deferens as an animal model for in-vitro research and surgical training, particularly microsurgical techniques such as the vasovasostomy. The objective of the present study is to compare both anatomical and histological parameters of the human, canine, and bull vas deferens.

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Address correspondence to Dr. JC Trussell, Department of Urology, State University of New York at Upstate, 750 East Adams Street, Syracuse, NY 13210 USA

Methods

After institutional review board (IRB) and institutional animal care and use committee (IACUC) approvals, vas deferens tissue was obtained from the human (n = 6), canine (n = 6), and bull (n = 5), Table 1. Outer diameter (OD), inner diameter (ID), and microscopic measurements of the luminal mucosa and surrounding muscularis were then determined from each of these tissues, Table 2. Histological measurements were performed by a single pathologist. Data were analyzed using TOST Analysis of Equivalence.[8]

Human vas deferens

Six patients underwent an elective vasectomy using a modified no-scalpel technique. The excised vas tissue was initially placed into saline.

After completing the vasectomy, the OD of the two segments of vas were measured in millimeters (to the nearest ½ mm) using a sterile ruler. Subsequently, a single set of sterile lachrymal duct probes were used to determine luminal ID. Each probe was measured by a micro-machinist to the nearest micron, Table 2. The lumen was serially probed until the next-larger lacrymal duct probe would no longer pass into the vas lumen. The size of the largest probe inserted into the vas lumen was recorded. Additional, non-manipulated vas tissue was placed in formalin and submitted to the pathology department for histological analysis.

Slides, prepared by the anatomical pathology department, were obtained and submitted in a blinded fashion for microscopic evaluation by a single pathologist. Representative photomicrographs, Figure 1, along with measurements in microns were obtained. Measurements included three separate regions, at the 12:00, 5:00 and 7:00 positions, Figure 1c, of both mucosal and muscularis thicknesses.

Canine vas deferens

Six adult mongrel canines weighing at least 35 pounds and at least 6 months of age were utilized for a survivable vas deferens surgery. The canines were purchased from an authorized animal supplier of canines (Marshall Farms). After the appropriate quarantine, the canines underwent a surgical procedure involving a minor extraperitoneal, lower abdominal incision just caudal to the external inguinal ring. While under general anesthesia, the vas deferens was dissected free of surrounding adventitia, sharply divided, and a proprietary device inserted between the divided vassal ends. All six canines recovered without incident. Approximately, 3 months later, the canines were euthanized using a standard Institutional Animal Care and Use Committee (IACUC) protocol of IV Phenobarbitol sodium "sleep away." At necroscopy, the proprietary device was examined then explanted, along with vas deferens tissue attached to the device for one centimeter in both the abdominal and scrotal directions. A separate portion of vas was excised from the remaining abdominal vassal segment. As with the human vas tissue, outer diameter was measured to the nearest ½ mm using a ruler. The lumen was probed with the same set of lachrymal duct probes used to measure the lumen of the human and bull vas. Additional vas tissue was placed into buffered formalin for storage and subsequent histological evaluation.

TABLE 1. **Tissue handling**

	Portion of vas used
Human	Scrotal
Bull	Scrotal
Canine	Caudal to external ring
	Measure OD and ID
Human	Fresh
Bull	Fresh
Canine	Fresh
	Histology
Human	Formalin: < 5 days
Bull	Formalin: < 13 mo.
Canine	Formalin: < 18 mo.

OD and ID = outer diameter and inner diameter measurements of vas deferens

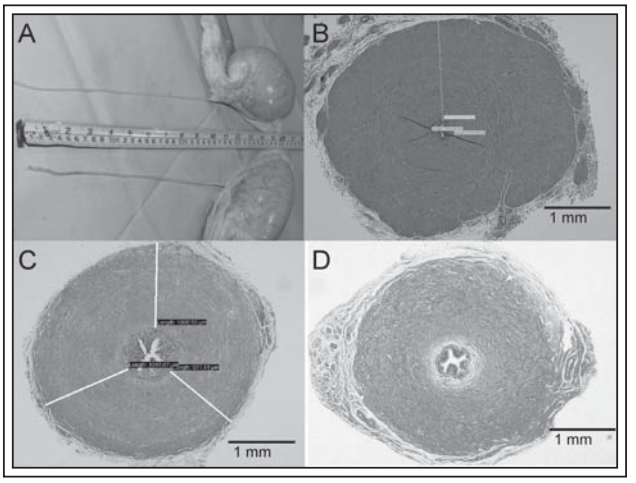

Figure 1. A. Gross specimen of bull vas deferens with associated testicles. **B, C, D.** Cross-sectional microscopic images of human, bull, and canine vas deferens specimens, respectively.

TABLE 2. **Diameter and luminal measurements**

	OD (mm)	ID (probe)°	ID tight (probe)°
Human			
1-L	3	2	3
1-R	3	2	3
2-L	3	2	3
2-R	2.5	1	2
3-L	3	2	3
3-R	3	3	4
4-L	3.5	4	5
4-R	2.5	3	4
5-L	3	2	3
5-R	3	2	3
6-L	2.5	3	4
6-R	2.5	3	4
Canine			
1-L	2	0	1
1-R	2	1	2
2-L	3	1	2
2-R	3	1	2
3-L	2.5	1	2
3-R	2.5	0	1
4-L	2	0	1
4-R	2	0	1
5-L	2.5	0	1
5-R	3	0	1
6-L	3	1	2
6-R	3	1	2
Bull			
1-L (10 cm)*	2.5	1	2
1-R (10 cm)	3	3	4
1-L (20 cm)	2.5	2	3
1-R (20 cm)	2	1	2
1-L (30 cm)	2.5	2	3
1-R (30 cm)	2	1	2
Bull (cont'd)			
2-L (10 cm)	3	3	4
2-R (10 cm)	3	4	5
2-L (20 cm)	2.25	3	4
2-R (20 cm)	2,25	3	4
2-L (30 cm)	2	3	4
2-R (30 cm)	2	3	4
3-L (10 cm)	2	3	4
3-R (10 cm)	2	3	4
3-L (20 cm)	2.5	2	3
3-R (20 cm)	2.5	2	3
3-L (30 cm)	2	1	2
3-R (30 cm)	2	1	2
4-L (10 cm)	2	3	4
4-R (10 cm)	2.5	4	5
4-L (20 cm)	2	2	3
4-R (20 cm)	2.5	3	4
4-L (30 cm)	2.5	1	2
4-R (30 cm)	2.5	1	2
5-L (10 cm)	3	4	5
5-R (10 cm)	2.5	3	4
5-L (20 cm)	3	3	4
5-R (20 cm)	2.5	3	4
5-L (30 cm)	2	2	3
5-R (30 cm)	2	2	3

*Centimeters in vas length from cranial aspect of bull testis
L = left; R = right; OD = outer diameter; ID = inner diameter
°Lachrymal duct probe measurements are as follows:
00 = 0.622 mm, 0 = 0.710 mm, 1 = 0.800 mm,
2 = 0.883 mm, 3 = 1.000 mm, 4 = 1.090 mm

Bull vas deferens

Five bull scrotums were obtained from a USDA licensed slaughterhouse (# 9442). Within 4 hours of slaughter, the refrigerated scrotal contents were explored and the testes and related cord structures were harvested. During dissection, care was taken not to injure or stretch the vas deferens. The vas length was measured using a ruler. At 10 cm, 20 cm, and 30 cm (from the craniad region of the testis) a 1 cm segment of vas was sharply excised. The vas width was recorded to the nearest ½ mm using a ruler. The same lachrymal duct probes used for the human and canine were used to determine luminal size. Additional adjacent tissue was excised and placed in formalin for subsequent histological analysis.

Histological evaluation

Tissues were fixed in 10% neutral buffered formalin (NBF). Tissues were processed in an automated Tissue-Tek VIP processor and paraffin-embedded with a Tissue-Tek TEC embedding station. Sections were cut at 6 µm intervals for routine hematoxylin and eosin (H&E) staining and were mounted on charged plus slides for immunohistochemistry (IHC). All tissues

were examined by an ACVP diplomat pathologist blinded to treatment/genotype/intervention. Images were obtained with an Olympus BX51 microscope and DP71 digital camera using MicroSuite Basic 2.6 imaging software. Both the mucosa and muscularis widths were microscopically measured in microns. The measurements were taken in three distinct locations (12:00, 5:00, and 7:00 o'clock). Lastly, histological characteristics of the muscularis and mucosa were recorded for each specimen.

Statistical analysis

The goal of this study was to assess equivalence between human and dog, human and bull, and bull and dog with respect to gross and microscopic vas deferens measurements. Prior to any analysis, the bounds to test for equivalence were determined to be -0.3 mm to 0.3 mm for inside diameter as determined by lachrymal duct probe insertion, -0.5 mm to 0.5 mm for outside diameter, -250 micrometers to 250 micrometers for muscle thickness, and -25 micrometers to 25 micrometers mucosa thickness. Two one-sided tests (TOST) were performed to test for equivalence using a significance level of 0.05.[9] All analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA).

TABLE 3. **TOST analysis of equivalence**

Comparison	95% confidence interval*	Outcome
ID H-C	(0.00, 0.20)	E
ID H-B	(-0.10, 0.00)	E
ID C-B	(-0.25, 0.00)	E
OD H-C	(-0.18, 0.65)	NE
OD H-B	(-0.00, 0.66)	NE
OD C-B	(-0.22, 0.54)	NE
Muscle H-C	(0.00, 958.96)	NE
Muscle H-B	(0.00, 852.52)	NE
Muscle C-B	(-210.00, 0.00)	E
Mucosa H-C	(-1.11, 22.08)	E
Mucosa H-B	(-14.04, 6.32)	E
Mucosa C-B	(-26.17, 0.00)	NE

*alpha = 0.05 TOST for equivalence
ID = inner diameter
OD = outer diameter;
H = human; C = canine; B = bull
E = equivalent
NE = not equivalent

Results

According to TOST statistical analysis, equivalence was noted for both the ID of all three species in addition to the microscopic mucosal measurements of the human to canine and human to bull (the canine to bull measurements were not equivalent), Table 3. Although variation in the human smooth muscle thicknesses (which ranged from 2.5 mm to 3.5 mm) resulted in no equivalence in the OD measurements, equivalence was noted with the canine to bull microscopic smooth muscle thickness.

In all three species the mucosa was lined by a tall columnar pseudostratified epithelium with a prominent basal cell layer. Surrounding this, the muscularis of all three species consists of medium sized fascicles of smooth muscle separated by collagenous trabeculae. These smooth muscle fascicles were oriented in multiple semi-distinct but interwoven layers. Regarding the bull specimens, individual fascicles are generally larger with less intervening collagen. A Masson's trichrome (+/- VVG) preparation was not performed, but would have improved the illustration of the muscular layers. Smooth muscle fascicles in the human appear to more closely resemble those of the dog rather than the bull.

In dogs, the submucosa was loose, possibly edematous, versus the more compact and fibrous submucosa of the bull. However, this may be an artifact of experimental technique/harvest protocol. The human submucosa is more similar to the compact submucosa of the bull.

The nuclei of the human samples frequently had cytoplasmic intra-nuclear invaginations (pseudoinclusions), a feature not noted in the other two species. The epithelia were otherwise comparable morphologically.

Discussion

In-vivo animal models of the human vas deferens have been used for pharmacological experimentation[10] in addition to the development of novel surgical techniques. For instance, Cilip et al used isolated canine vas tissue in their study of laser treatment as a potential noninvasive vasectomy technique.[1] Furthermore, an in-vivo canine model was used by Roberts et al to demonstrate the ability of high-intensity focused ultrasound to ablate the epididymis.[11] Lastly, groups led by Lohiya[5] and Vrijhof[2] conducted pre-clinical trials of novel vasectomy reversal techniques utilizing primate and rabbit animal models, respectively.

In-vitro work by Saunders et al utilized the bull vas to determine that a 3-suture vasovasostomy reinforced with BioGlue had similar distraction strength when compared to a suture-only 2-layer anastomosis.[12] During this study, it was noted (not published) that the tissue handling qualities of the bull vas were similar to those encountered with an actual human vasovasostomy. Specifically, it was necessary to gently dilate the vassal lumen with jeweler forceps to visualize passage of the 9-0 nylon passing through the muscular layer in a similar, tactile fashion to that of a human vas. This operative experience is corroborated by the histological similarities between all three species previously noted in this study.

Previous studies have determined the human and canine vas deferentia are similar both grossly[6] and histologically.[13] For instance, Bruschke et al reported the "no resistance" luminal measurement was 0.85 mm +/- 0.071 mm for the human and 0.77 mm +/- 0.075 mm for the canine. For "significant resistance," the measurement was 1.06 mm +/- 0.124 mm for the human and 0.98 mm +/- 0.085 for the canine. The mean outside diameter for the human vas was 2.85 mm +/- 0.43 mm with variations often noted when comparing the left to the right vas from the same subject. Although a relationship was found between the OD and ID, it was not sufficient to predict luminal diameter based on the vas OD.[6]

As operative time and cost constraints increase, the use of surgical simulation models become more and more advantageous,[14] especially when microsurgical skills are considered. Grober et al demonstrated that microsurgical skill retention was greatly aided by the use of bench models/simulators using isolated animal vas deferens.[7] Moreover, these technical skills were noted to be maximized by repetitive wet-lab exposure. For some training programs, the use of rats has been the standard model for microsurgical training. However, stringent laws and related costs for use of the rat model have required some programs to instigate finding alternate methods of training.[15] As an alternative to wet labs, bench models using fresh, freeze-dried,[16] cadaver, or even artificial materials (such as silicone tubing) are popular for teaching surgical skills.[17] In fact, Anastakis demonstrated that bench models were equivalent to cadaver models for technical skill training and should therefore, be used early in residency training to accelerate technical skill acquisition.[17] We believe one option, for vas deferens-related microsurgery, would include fresh bull vas tissue which allows for a lengthy portion of tissue that is remarkably similar in size, and texture, to that of the human vas.

The current study demonstrates similarities between the human, canine, and bull vas deferens in both luminal diameters and histological characteristics. Therefore it is reasonable to conclude that the bull vas could be a substitute for the human vas in both in-vitro testing in addition to microscopic vasovasostomy simulation laboratory training exercises. Bull testicles and attached cord structures are easily obtainable from local abattoirs, and each specimen provides an ample section of vas tissue.

This study does have limitations which are not limited to: (1) a small number of specimens, (2) the canine tissue was manipulated prior to microscopic analysis (specifically, the tissue analyzed was near an implantable foreign body which may have caused changes relating to obstruction, scarring and/or inflammation), (3) prolonged storage of some canine and bull vas segments in formalin, likely caused some tissue dehydration, and (4) some histological changes may have occurred due to inadvertent tissue stretch at the time of dissection.

In conclusion, the human, bull, and canine vas deferens are equivalent with regard to gross and histological comparisons. There are some minor variations between intra and inter-species muscle thickness, yet each specimen should be considered suitable for in-vitro testing or lab simulation of microsurgical techniques. □

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