

Measurement and Quantification of Cystometric Bladder Pressure Spectra in an in-vivo Sheep Model: A Feasibility Study

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Abstract—Cystometry is a standard procedure for the clinical evaluation of lower urinary tract disorders such as detrusor overactivity (DO). The utility of this procedure for DO diagnosis, however, is limited by the use of physician observations of bladder contractions and patient reported filling sensations. Although a number of preclinical and clinical studies have observed and developed methods to characterize bladder pressure dynamics, these techniques have not been scaled for routine clinical application. The goal of this study was to evaluate the feasibility of using an awake large animal model to characterize bladder pressure signals from cystometry as bladder pressure spectra and quantify changes in spectra during bladder filling. Two adult female sheep were trained for quiet catheterization in a minimally supportive sling and underwent multiple awake and limited anesthetized cystometry tests. In each test, bladder pressure was measured during continuous filling or with filling that included periods of no filling (constant volume). A Fast-Fourier Transform (FFT) - based algorithm was then used to quantify changes in pre-voiding bladder pressure spectra. Changes in Spectral Power (SP) and Weighted Average Frequency (WAF) were calculated during filling. To visualize temporal changes in bladder pressure frequencies during filling, Continuous Wavelet Transform (CWT) was also applied to cystometry data. Results showed that a significant increase in SP and decrease in WAF were both associated with bladder filling. However, during awake constant volume tests, SP significantly increased while changes in WAF were nonsignificant. Anesthetized tests demonstrated comparable values to awake tests for WAF while SP was considerably reduced. CWT facilitated visualization of spectral changes associated with SP and WAF as well as apparent non-voiding contractions during awake and anesthetized volume tests.

Clinical Relevance—Bladder pressure spectra during cystometry are detectable in sheep and the changes during filling are similar to those observed in human retrospective clinical data. Sheep cystometry may be a valuable testbed for establishing and testing quantitative pressure spectra for use as a clinical diagnostic tool.

I. INTRODUCTION

Cystometry is a standard medical procedure for the clinical evaluation of lower urinary tract symptoms [1], [2]. While cystometry is routinely used in preclinical animal models to quantify bladder performance and pressure dynamics, clinical

cystometry tests can be inconsistently performed and lack incorporation of standard objective measures or methods [3]. However, quantification of preclinical animal bladder pressure spectra has driven early investigation of bladder pathologies such as overactive bladder (OAB) and detrusor overactivity (DO) [4]–[6]. Furthermore, studies have extended these methods to retrospective human data using spectral transforms such as FFT and Wavelets [7]–[10]. Analysis of filling related bladder pressure signals in in-vitro preparations [11], [12], ex-vivo preparations [13], [14], in-vivo animal models [4], [15], and clinical studies [7], [10], [16], have revealed the presence of autonomous contractions of the bladder, also known as Non-Voiding Contractions (NVCs) which have been implicated in the pathogenesis of DO [17]. However, the clinical utility of these signals and objective measures of analysis remains in their infancy. To facilitate translation to prospective human testing with available clinical equipment, our team sought to quantify bladder spectral characteristics in a large animal model that has been developed as a therapy-diagnostic testing platform [18], [19].

In this pilot study, we evaluated the feasibility of measuring and quantifying changes in bladder pressure spectra associated with filling in sheep. We used clinical grade cystometry equipment with an FFT-based algorithm previously developed in human retrospective analysis [10], [12]. Our primary goal was to quantify changes in bladder pressure spectra during repeated continuous filling tests in awake, conscious sheep and in a limited number of constant volume and anesthetized cystometries. We used CWT to visualize spectral changes during filling.

II. METHODS

A. Animal Training

Experimental procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee. Two female sheep (breed: Friesen; age: 17 mo., 19 mo.; 70.1 kg, 64.4 kg) were studied. Each animal was housed with another sheep in a conjoined 325 ft² pen with controlled temperature (20 – 23 °C), under a 12h:12h light:dark cycle and fed once per day with ad lib water. Enrichment was provided by daily interactions with staff and with other sheep. Animal training occurred over a period of one month prior to cystometric testing and included sequential phases of halter training, sling training and catheter training with an 8 Fr Foley (Bard Medical, USA). Trial cystometry tests were also performed during the catheter training with a maximum of five fills per day. At all stages of training, treats were provided to the sheep for positive reinforcement.

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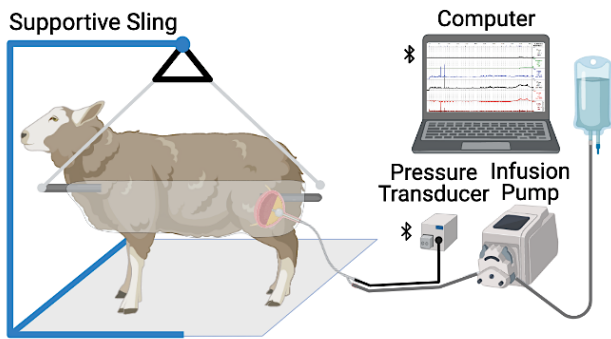


Fig. 1. Experimental setup for awake cystometry tests with sling supported sheep and equipment.

B. Awake Cystometry

Test sessions were conducted twice per week in a temperature-controlled room. After acclimating to the testing room, the sheep were gently encouraged into a supportive sling (Lumex LF1030, GF Health Products, Inc., Atlanta GA) and the front legs were lifted 3-4 inches while the hind legs remained on the ground. The urethral opening was cleaned with Betadine. A T-DOC air-charged pressure catheter (7 Fr, Laborie, ON, Canada) was lubricated and carefully inserted into the urethral opening using the index finger as a guide. Catheter position in the bladder was confirmed by draining urine from the catheter.

After catheterization, the saline infusion line was secured to the catheter lumen and the pressure transducer connection was secured to the UDS Roam (Laborie, ON Canada). Sterile saline (41 – 43°C) was infused at a rate of 20 ml/min into the bladder using the Laborie GOBY HUB infusion pump (Laborie, ON Canada) as depicted in Fig. 1. Bladder pressure

was monitored throughout the filling and voiding cycle. Infusion of saline was stopped as soon as any behavioral signs of voiding (ex. twitching of the perineum and tail, perking up of the ears, attempts to squat) or a significant increase in bladder pressure was observed (≥ 40 cmH₂O) and the sheep was allowed to void around the catheter. Voided saline was collected in a graduated container for bladder capacity measurement.

In addition to continuous filling, we also conducted several pressure measurements with the pump off to quantify pressure dynamics at constant bladder volumes. Two constant volume levels were used: A low constant volume level, at 15% capacity, and a high constant volume level, at 85% capacity, were held for 4 minutes each. Capacity was estimated as the mean maximum infused volume for the two most recent continuous cystometries. Between consecutive cystometries, the animal was rewarded with a treat and allowed to rest quietly for 3-4 minutes.

C. Anesthetized Cystometry

A limited set of continuous and constant volume tests were conducted under anesthesia. Prior to anesthetized cystometry, sheep were fasted overnight with ad lib water. Animals were initially sedated with Ketamine (2.5-4.0 mg/kg, IM) and Midazolam (0.4-0.5 mg/kg, IM), positioned supine, and intubated. Induction was achieved with a mixture of Isoflurane (0.5-4%, IN) and Propofol (2-6 mg/kg, IV). A ventilator maintained respiratory rate between 8-20 breaths minute⁻¹, oxygen saturation above 95%, and end tidal carbon dioxide between 35-50 mmHg (Narkomed 2A, Drager). ECG and heart rate were continuously monitored (Datex-Ohmeda, Madison, WI). Body temperature was maintained between 36-41°C with an external heating/cooling blanket. Animals

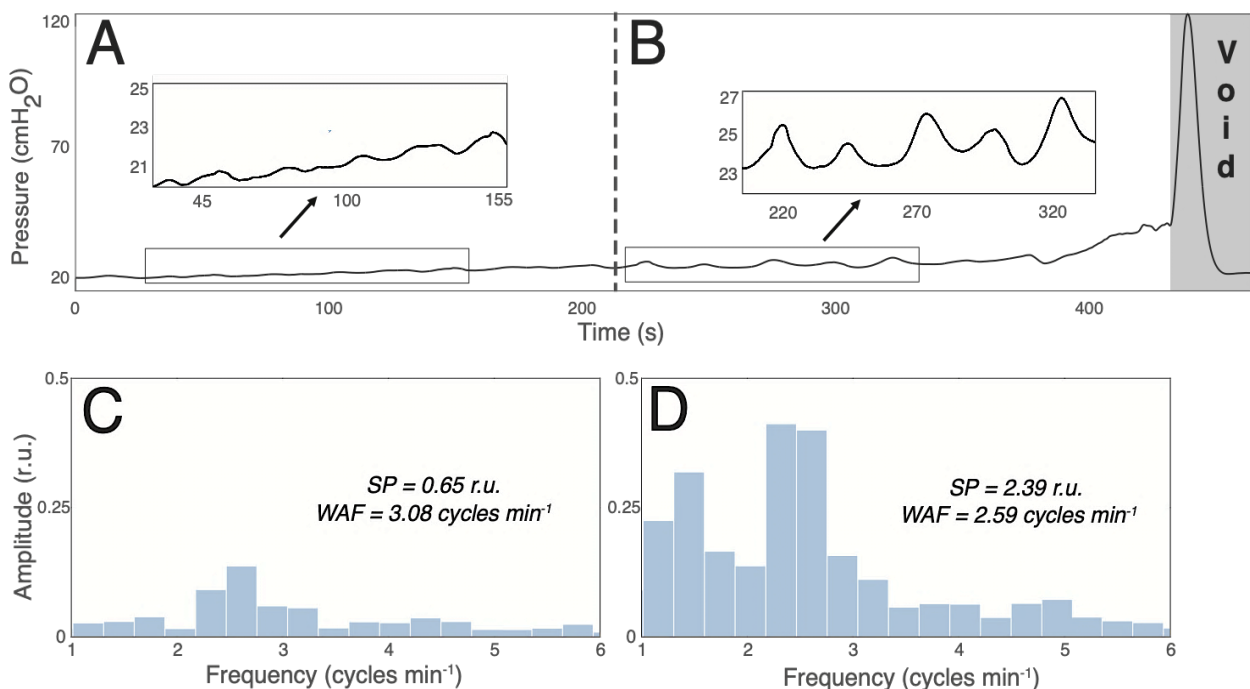


Fig. 2. Bladder pressure signals during Early Fill (A) and Late Fill (B) segments are shown highlighting obvious durations of NVCs (insets). Corresponding FFT spectra of A and B demonstrating smaller SP and larger WAF in Early Fill (C) relative to Late Fill (D) spectra.

were continuously infused with intravenous 0.9% normal saline (5-10 ml/kg/h) via the right femoral vein.

Both sheep underwent one session each of anesthetized tests including multiple continuous and constant volume cystometry tests. A significant increase in bladder pressure (≥ 40 cmH₂O) accompanied by leakage around the catheter was used as an indicator of maximum bladder capacity and cystometry termination. A 60 cm³ syringe was used to manually evacuate the bladder. Continuous and constant tests were repeated up to 4 times per session with 3-4 minutes between tests.

D. Data Analysis

Analysis of pressure data was performed using an FFT-based algorithm, similar to those developed in [10], [12], based upon targeting the frequency range of 1 - 6 cycles per min⁻¹ for NVCs as a signal of interest [7], [16], [13], [20]. Pressure data were acquired at a sampling rate of 10 samples per second using the Laborie GOBY HUB (Laborie, ON Canada) and uploaded into MATLAB (MathWorks, Natwick, MA) where vesical pressure signals were smoothed using a 10-point moving average. Only pre-void data was used for analyses. A high amplitude artifact filter was applied to eliminate sudden rapid changes in signals caused by minor movements such as head turns, breath-sighs and foot shuffling. A 50-point moving average filter was then applied to improve processing and visualization of signals within the frequency range of 1-6 cycles min⁻¹. Prevoid continuous cystometry data were divided equally into two segments: Early Fill and Late Fill, for analysis while signals in 15% capacity and 85% capacity segments were considered for analysis in constant volume tests. A Hanning window was applied to all segments followed by FFT to compute their respective amplitude spectra. Two metrics: Spectral Power (SP) and Weighted Average Frequency (WAF), were calculated from the amplitude spectra $[X(f)]$, using (1). and (2). A two-sample, 1-tailed T-test was used to compare spectral parameters per fill level segment within each sheep,

during cystometry tests. Statistical significance was determined at $P < 0.05$.

$$SP = \sum_{f=1}^6 X(f) \quad (1)$$

$$WAF = \sum_{f=1}^6 [X(f) * f] / SP \quad (2)$$

To visualize pressure dynamics during bladder filling, Continuous Wavelet Transform (CWT) was applied to the cystometry pressure time series using the ‘Morlet’ mother wavelet. Edge effects were reduced by applying a cone of influence [8]. CWT spectra were limited to the frequency range of interest and colormap gain was adjusted to optimize signal visualization.

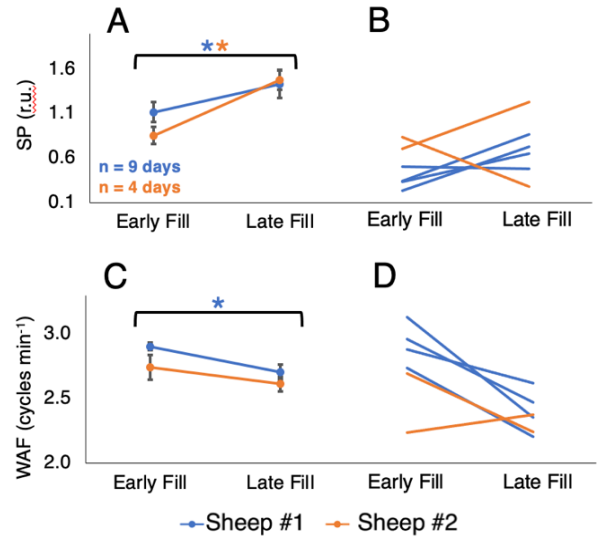


Fig. 3. Spectral Power (SP) and Weighted Average Frequency (WAF) for awake (A, C) and anesthetized (B, D) continuous filling cystometry tests (Mean \pm SEM for awake tests, * $P < 0.05$).

III. RESULTS

A. Continuous Filling Cystometry

Spectral pressure measurements during awake continuous filling showed clear relationships to fill level (Fig. 2) and were comparable across the two sheep and the 13 days of testing.

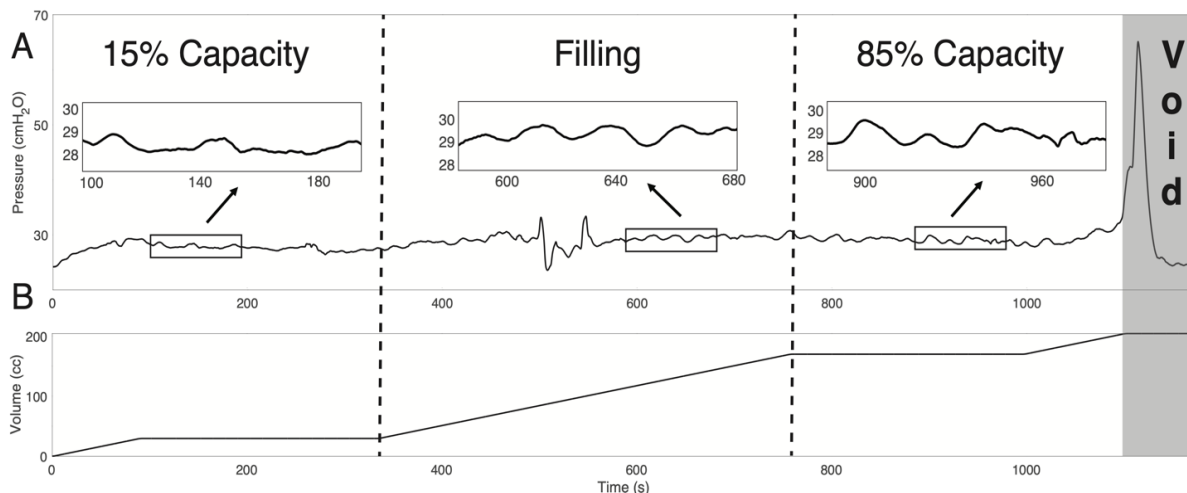


Fig. 4. Representative plot of bladder pressure signal during awake constant volume test showing at 15% capacity and 85% Capacity as well as filling and voiding states (A) and (B) corresponding infused volume. NVCs are visible during all phases of filling (inset).

SP increased during filling (Fig. 3A) for both Sh1 (Early Fill = 1.12 ± 0.11 , Late Fill = 1.43 ± 0.15 , $P < 0.05$, $N = 9$ days) and Sh2 (Early Fill = 0.85 ± 0.1 ; Late Fill = 1.48 ± 0.11 , $P < 0.05$, $N = 4$ days). WAF decreased during filling (Fig. 3C) for Sh1 (Early Fill = 2.91 ± 0.03 , Late Fill = 2.71 ± 0.06 , $P < 0.05$, $N = 9$ days) but not Sh2 (Early Fill = 2.74 ± 0.1 , Late Fill = 2.61 ± 0.05 , $P = 0.19$, $N = 4$ days). In terms of spectral pressure measurement repeatability across both sheep and all fill segments that were repeatedly tested ($N = 114$: Early and Late Fills combined), the average deviation for SP was 0.30 ± 0.26 (mean \pm SD) and ranged from 0.01 to 1.37 relative units. The average deviation for WAF was 0.16 ± 0.12 cycles min^{-1} and ranged from 0.001 to 0.51 cycles min^{-1} .

For limited anesthesia tests, SP increased with filling (Fig. 3B) for Sh1 but not Sh2 while, mean WAF decreased (Fig. 3D) for Sh1 but not Sh2. No statistical comparisons were performed due to the small number of tests.

B. Constant Volume Cystometry

For constant volume tests, spectral pressure measurements retained similar relationships to bladder filling (Fig. 4). SP increased significantly between fill levels (Fig. 5A) for Sh1 (15% = 0.77 ± 0.07 , 85% = 1.69 ± 0.16 , $P < 0.05$, $N = 11$) but not Sh2 (15% = 1.42 ± 0.80 , 85% = 1.56 ± 0.39 , $P = 0.38$, $N = 3$). WAF did not significantly change with fill level (Fig. 5C) for either Sh1 (15% = 2.97 ± 0.09 , 85% = 2.95 ± 0.08 , $P = 0.40$, $N = 11$) or Sh2 (15% = 3.01 ± 0.16 , 85% = 2.95 ± 0.12 , $P = 0.32$, $N = 3$). During anesthetized constant volume tests, SP moderately increased across volume levels (Fig. 5B) for Sh1 but not Sh2. However, WAF appeared to decrease (Fig. 5D) for both Sh1 and Sh2. The SP of anesthetized constant volume tests was generally smaller than for the awake tests. Statistics were not calculated for constant volume tests under anesthesia due to small sample size.

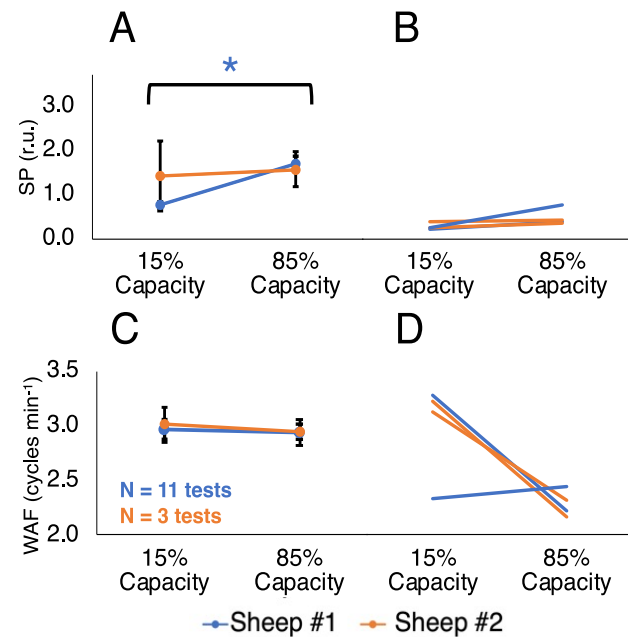


Fig. 5. Spectral Power (SP) and Weighted Average Frequency (WAF) for awake (A, C) and anesthetized (B, D) constant volume cystometry tests (Mean \pm SEM for Sh1 awake tests, * $P < 0.05$).

C. Apparent NVCs and CWT Spectra

On numerous cystometry tests we observed bladder contraction signals matching published descriptions of non-voiding contractions (NVCs). Examples of these apparent NVCs are shown in Fig. 2, Fig. 4, and Fig. 6 (A & B). These signals had amplitudes of 1-4 cmH_2O and a frequency of 1-4 cycles min^{-1} . Corresponding CWT scalograms (Fig. 6 (B & D)) show spectral signals that match these NVC parameters. The amplitude and frequency of these apparent NVCs and associated CWT spectra were reduced during anesthetized cystometry.

D. Cystometry Testing

In total, we conducted 75 continuous filling tests over 13 awake cystometry testing days from the two sheep (59 tests over 9 days for Sh1; 16 tests over 4 days, Sh2). 16 of the 75 cystometry tests (21%) were discarded due to sensor malfunction, incomplete filling or excessive animal movements, resulting in 46 (Sh1) and 13 (Sh2) tests available for analyses. One anesthetized cystometry session was conducted per animal composed of 4 (2) tests for Sh1 (Sh2). Mean bladder capacities across the awake continuous cystometry sessions were 148.4 ± 15.5 ml for Sh1 and 207.2 ± 13.6 ml for Sh2 and capacities for anesthetized sessions were 221.8 ± 8.4 ml and 214.0 ± 22.0 ml for Sh1 and Sh2, respectively. Bladder capacities were similar for both continuous and constant volume cystometries.

IV. DISCUSSION

In this feasibility study, we utilized a novel FFT-based algorithm to quantify filling related bladder pressure spectra in a large animal model of awake and anesthetized cystometry. In cystometries with a sufficient number of tests for statistical analysis, SP increased during filling while WAF decreased. Additionally, apparent NVCs were observed during cystometry and had notably lower amplitude during anesthesia cystometries. These findings build upon our prior preclinical and clinical studies [10], [12]. Furthermore, we demonstrate that sheep allow direct use of human scale clinical cystometry equipment to capture these signals and are a viable model to establish objective measures of bladder function.

We previously used the FFT algorithm to demonstrate that SP significantly increases while WAF non-significant decreases with porcine bladder strip stretching [12] and bladder filling in clinical retrospective data [10]. These changes in spectral measures match with present findings suggesting that large animal models, can link preclinical and clinical models. Changes in spectral measures, SP and WAF, correlated with the amplitude and frequency of apparent NVCs. Several studies have utilized rodent, feline, pig and human models to indicate that NVCs amplitude and frequency also change with filling [4], [13], [7]. In this study, oscillations were also visible with the pump off further indicating these signals may be a product of natural bladder function and volume, and not simply induced during rapid cystometric filling. Both NVC amplitude and SP were comparatively lower during anesthetized tests, similar to

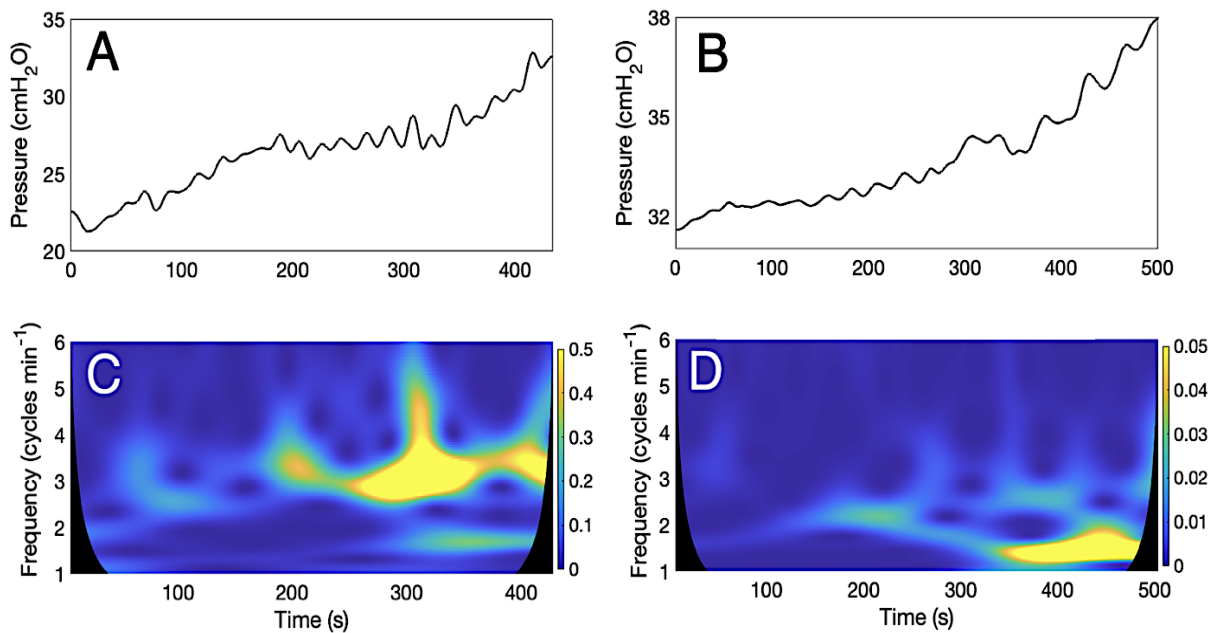


Fig. 6. Representative plots of bladder pressure signals (A, B) and corresponding CWT Scalograms (C, D) from awake (A, C) and anesthetized (B, D) pre-void continuous cystometry tests. Note CWT scale in C is 10 times greater than that of D, highlighting the reduced amplitudes of CWT signals measured under anesthesia vs awake cystometry.

previous descriptions of NVCs in anesthetized feline models [15].

Despite the correlation between apparent NVCs and bladder pressure spectral measures, we cannot attribute changes in captured spectra to NVCs alone. Non-terminal detrusor contractions (observed in one awake constant volume test, max amplitude ~ 39 cmH₂O) as well as abdominal pressures, which were not recorded in this study, may have impacted bladder spectra within the frequency range of 1-6 cycles min⁻¹. Simultaneous testing of abdominal pressure sensing in future studies will allow us to test impacts of non-bladder signals on bladder pressure spectra.

As a feasibility study, these results need to be replicated with a larger number of animals and tests. A benefit of our model is the compatibility of human-scale equipment with sheep bladder anatomy which should facilitate rapid prototyping, testing, and translation of clinically applicable technologies. The objective measures derived from our method could be used to assess repeatability of cystometry tests as well as to better understand the impacts of anesthesia. It will also facilitate comparison of air charged catheters, used in this study, with MEMS based pressure catheters to demonstrate capabilities to detect the pressure spectra, understand the potential impacts of sensors on the spectra or potential impacts of pressure drift or other characteristics of charged catheters [21]. As NVCs have been implicated in the pathogenesis of OAB [17], which is a common indication for cystometry [1], future studies can also include OAB models to further refine our quantitative analysis methods, investigate the potential of these signals in the development of bladder dysfunction, and develop measures of disease severity and therapeutic efficacy. While the use of bladder pressure spectra in urological research are at an early stage, large animal

models will be a useful testbed for establishing objective measures that can be rapidly translated in clinical studies.

V. CONCLUSION

This feasibility study demonstrates an initial successful measurement and quantification of filling-related changes in cystometric spectra using sheep. Use of this model will improve technologies for measuring these signals and can address the roles of these contractions in bladder functions. Improved links between clinical diagnostics and bladder physiology, in turn, will improve clinical urology practice.

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