A Preliminary Evaluation of Ovine Bladder Mucosal Damage Associated With 2 Different Indwelling Urinary Catheters

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OBJECTIVE To determine whether a new catheter design with a low-profile, open-ended rounded rather than pointed Foley catheter tip can reduce mucosal damage to the bladder of ewes.

METHODS AND MATERIALS Six ewes were randomly assigned to 1 of 2 indwelling urinary catheters—a 16 Fr Foley catheter or a 16 Fr open-tip CystoSure catheter. After 96 hours, all the animals were sacrificed and their bladder and urethra harvested for analysis.

RESULTS Image analysis of the bladder surfaces demonstrated a significant decrease in the percentage of bladder area covered by ulceration and inflammation in sheep with CystoSure catheters compared with Foley catheters ($P < .002$) as well as a trend toward less edema ($P = .17$). Macromorphologic evaluations were confirmed with immunohistochemical markers of cell proliferation and inflammation.

CONCLUSION In this pilot study, we were able to demonstrate that a new catheter design with an open-ended rounded rather than pointed tip and a reduced balloon base-to-tip profile may reduce mucosal damage to the bladder of ewes. Based on the findings from this trial, we believe this new catheter design with its low-profile, rounded tip may reduce bladder mucosal injury, which is a risk factor for catheter-associated urinary tract infections. UROLOGY 110: 248–252, 2017. © 2017 Elsevier Inc.

Urinary catheterization is an essential component of modern health care at many different levels. Unfortunately, subsequent catheter-associated urinary tract infections (CAUTIs) are significant nosocomial hazards with ~250,000 infections per year in the United States, costing ~$250 million[1] and contributing significantly to the proliferation of multidrug-resistant bacteria that are associated with approximately 23,000 death per year in the United States.[2] Significant solutions for CAUTIs are needed, but successes to date have been limited.

The most commonly used urinary catheter today, the Foley catheter, has been a universal standard for bladder drainage in women and men since the introduction in 1933 by the C.R. Bard Company of a balloon-based catheter designed by Dr. Fredrick Foley.[3] Although the design of this catheter is excellent for emptying the bladder, when the bladder is draining, a vacuum is created that draws the bladder mucosa at the dome into the eyelets of the catheter and forces the pointed tip of the Foley into the bladder wall, eliciting damage to the mucosal integrity[4] and thereby potentially increasing the risk of bacterial invasion. In this pilot study, we investigate whether a new catheter design with a low-profile, open-ended rounded rather than pointed tip can reduce mucosal damage to the bladder of ewes.

METHODS AND MATERIALS

Animals and Experimental Design

Six nonpregnant, nonlactating Rambouillet ewes between 5 and 7 years of age and of similar genetic background were randomly assigned to 1 of 2 indwelling urinary catheters—a 16 Fr Foley

Financial Disclosure: *James A. Greenberg is the inventor of CystoSure and a shareholder in Emmy Medical LLC, the manufacturer of the CystoSure urinary drainage catheter. The research grant provided by Emmy Medical LLC to North Dakota State University paid for the sheep, supplies, laboratory fees, and so on but was not used as salary of the remaining authors.*

Funding Support: *This study was supported by an educational grant from Emmy Medical LLC, the manufacturer of the CystoSure urinary drainage catheter, to the Department of Animal Sciences at North Dakota State University. The collection and analysis of the data was performed independent of the sponsor. Dr. Greenberg, who is the inventor of CystoSure and a shareholder in Emmy Medical, LLC was involved in the data interpretation, writing the manuscript and the decision to submit the paper for publication.*

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Submitted: July 4, 2017, accepted (with revisions): August 15, 2017

https://doi.org/10.1016/j.urology.2017.08.020
0090-4295

catheter or a 16 Fr CystoSure catheter (see supplementary Fig. S1). The Foley catheters were uncoated, 100% latex-free silicone 16 Fr. Foley catheters with a 5-cc balloon (part number 1656816, Bard Medical, Covington, GA). The Foley catheters were closed-tip, side-open catheters. With the balloon inflated to the recommended 10 cc of saline, the balloon measured 26 mm in diameter and 25 mm in height with a base of balloon to catheter tip height of 47 mm. The CystoSure catheters were uncoated 100% latex-free silicone 16 Fr. CystoSure catheters with a 5- cc balloon (part number 10-400, Emmy Medical LLC, Holliston, MA). The CystoSure catheters were open-tip catheters. With the balloon inflated to 10 cc of saline, the balloon measured 30 mm in diameter and 19 mm in height with a base of balloon to catheter tip height of 23 mm. Randomization was done using numbers generated from www.random.org. All ewes were penned in metabolic crates at the Animal Nutrition and Physiology Center on the North Dakota State University campus. On day 1, all catheters were sterilely inserted following the manufacturer guidelines for insertion in humans. All the catheters were connected to Bard 100% silicone 2000-mL drainage bags with antireflux chambers (part number 947416, Bard Medical). The drainage bags were uniformly secured to the crates in a manner that allowed the animals to be upright or sitting without tension on the catheter. The height from the ewe's urethra to the top of the drainage bag was recorded for each animal in both the upright and sitting positions to control for the vacuum pressure of the drainage. After 96 hours, all the animals were sacrificed and their bladder and urethra harvested for analysis. All animal procedures performed were approved by the North Dakota State University Institutional Animal Care and Use Committee (#A17030).

Tissue Collection and Processing

Immediately after dissection, each bladder was cut to visualize inside area, and images were acquired from a higher resolution imaging system (Canon EOS 6D with 50 mm Lens, Canon Inc., Tokyo, Japan) under controlled illumination for analysis. The researchers performing the image and histologic analyses were blinded to which animals received which catheters. For each bladder, at least 3 random cross-sectional samples (~1 cm^3) were collected from inflamed areas with visible ulceration and hemorrhage surrounded by edema and healthy-appearing areas from the upper and middle parts of bladder away from the urethra without any signs of inflammation. All the samples were immersion fixed in neutral buffered formalin for immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed as previously described to detect Ki67 (a marker of proliferating cells),[5] CD3 (a marker of T-lymphocytes),[6] and CD163 (a marker of macrophages).[7] Briefly, paraffin-embedded bladder tissues were sectioned at 3 μm and mounted onto slides. Sections were rinsed several times in Tris Buffered Saline containing Triton-X100 (0.3%, v/v) and then treated for 20 minutes with blocking buffer (Tris Buffered Saline containing normal horse serum [2.5%, vol/vol]) followed by incubation with specific primary antibody for Ki67 (1:250; mouse monoclonal, VP-K452; Vector Laboratories, Burlingame, CA), CD3 (1:600; rabbit polyclonal, A045229-2; Agilent, Santa Clara, CA) or CD163 (1:400; mouse monoclonal, MCA1853; Bio-Rad, Hercules, CA) overnight at 4°C. Primary antibodies were detected using a secondary antimouse or antirabbit antibody coupled to peroxidase (ImmPRESS Kit; Vector Laboratories). For color development, silver gray substrate was used (Vector Laboratories). The sections were then counterstained with nuclear fast red (Electron Microscopy Sciences, Hatfield, PA) to visualize cell nuclei. Control sections were incubated with normal mouse IgG (4 μg/mL) in place of primary antibody.

Image Analysis

Image of each bladder was analyzed using FLIR Research IR Software (FLIR Systems Inc., Wilsonville, OR) to determine the proportion of inflamed areas characterized by red surface including hemorrhage and ulceration, and edema (eg, swollen smooth surface) out of total bladder area. For each stained tissue section, images (n = 5) were generated at 200× using a Zeiss Imager M2 microscope equipped with an AxioCam HRm camera (Zeiss Inc., Thornwood, NY). Images of epithelium and lamina propria, separately, stained for Ki67, and lamina propria, stained only for either CD3 or CD163, were analyzed using for (Image Premiere, Media Cybermetrics, Silver Springs, MD) for image analysis.

Statistical Analysis

To compare the effects of 2 catheter types, data were analyzed using the general linear models tools from SAS/STAT software

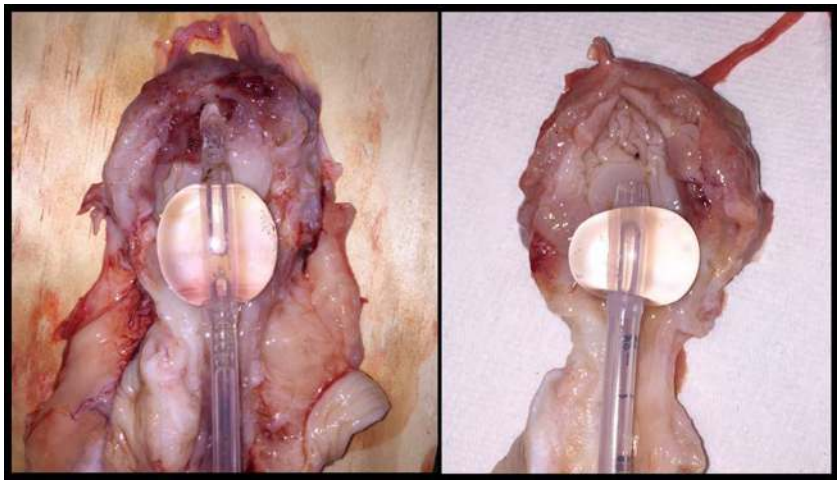

Figure 1. Bladders with catheters in situ: Foley catheter (left); CystoSure catheter (right).

and presented as means ± standard error of the mean (SAS Institute Inc., Cary, NC). The model included 2 catheter types, inflamed vs noninflamed areas, and their interactions. When the F-test was significant ($P < .05$), differences between specific means were evaluated by using the least significant differences test.

RESULTS

There was no difference in the sheep groups with regard to age ($P = .64$; 95% confidence interval (CI) −2.18 to 1.52) or weight ($P = .17$; 95% CI −43.57 to 10.90).

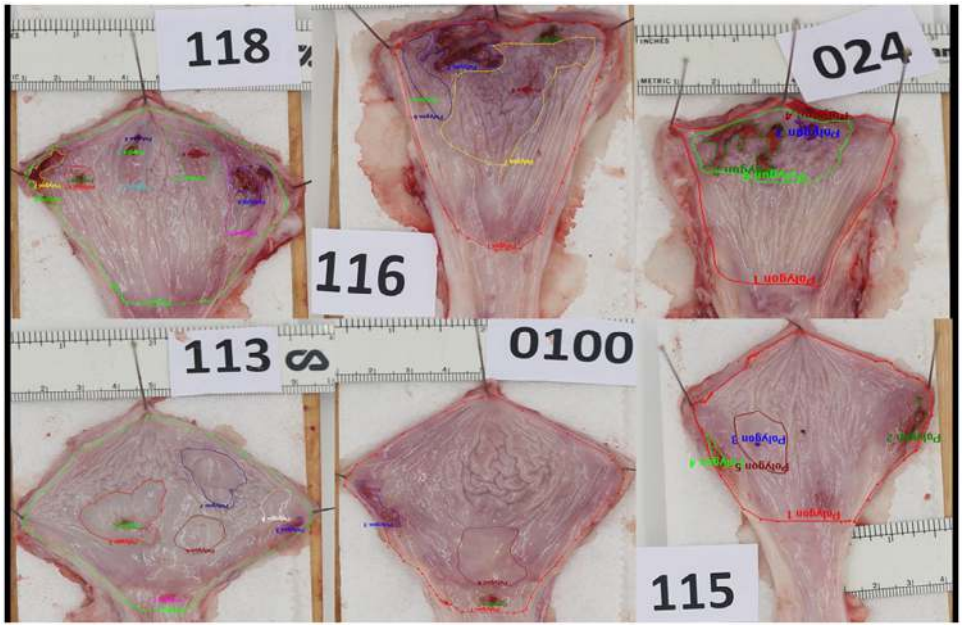

Figure 2. Bladders from ewes with Foley catheters (top) and CystoSure catheters (bottom). Image analysis demonstrating noninflamed and inflamed areas characterized by red spots of ulceration and edema outlined by polygons. Area of each polygon was determined using image analysis software, and a percentage of area covered by ulceration and edema for each bladder was calculated.

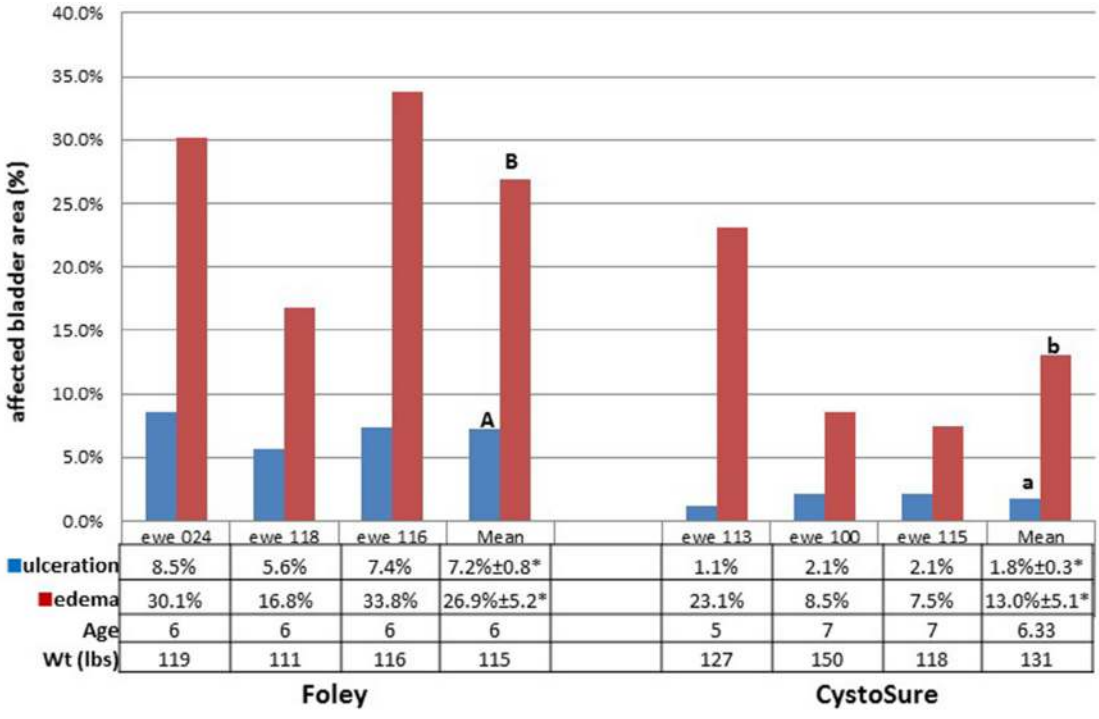

Figure 3. Percentage of total bladder area with ulcerations (**A,a** $P < .002$) and edema (**B,b** $P = .17$) in sheep with Foley and CystoSure catheters. *standard error of mean (SEM). (Color version available online.)

Macro-morphologic evaluation of bladder surface demonstrated the presence of noninflamed and inflamed areas characterized by ulceration and hemorrhage (red areas) and edema (Figs. 1, 2). There was a significantly lower ($P < .002$) percentage of bladder area covered by ulceration and inflammation and a trend toward less ($P = .17$) edema in sheep with CystoSure catheters compared with Foley catheters (Fig. 3). Ki67, CD3, and CD163 immunopositive cells were detected in the epithelium and the lamina propria in both inflamed and noninflamed areas of the bladder. Ki67 was expressed in cell nuclei, but CD3 and CD163 were expressed on the cell cytoplasm (Fig. 4). In noninflamed areas, minimal staining of Ki67, CD3, and CD163 was observed within the epithelium, but in the lamina propria a few cells expressed Ki67 and some cells expressed CD3 and CD163(Fig. 4A,C,E). In inflamed areas, Ki67 was highly expressed in the epithelium and in the lamina propria; small numbers of CD3 and CD163 immunopositive cells were present in the epithelium but large numbers in the lamina propria (Fig. 4B,D,F).

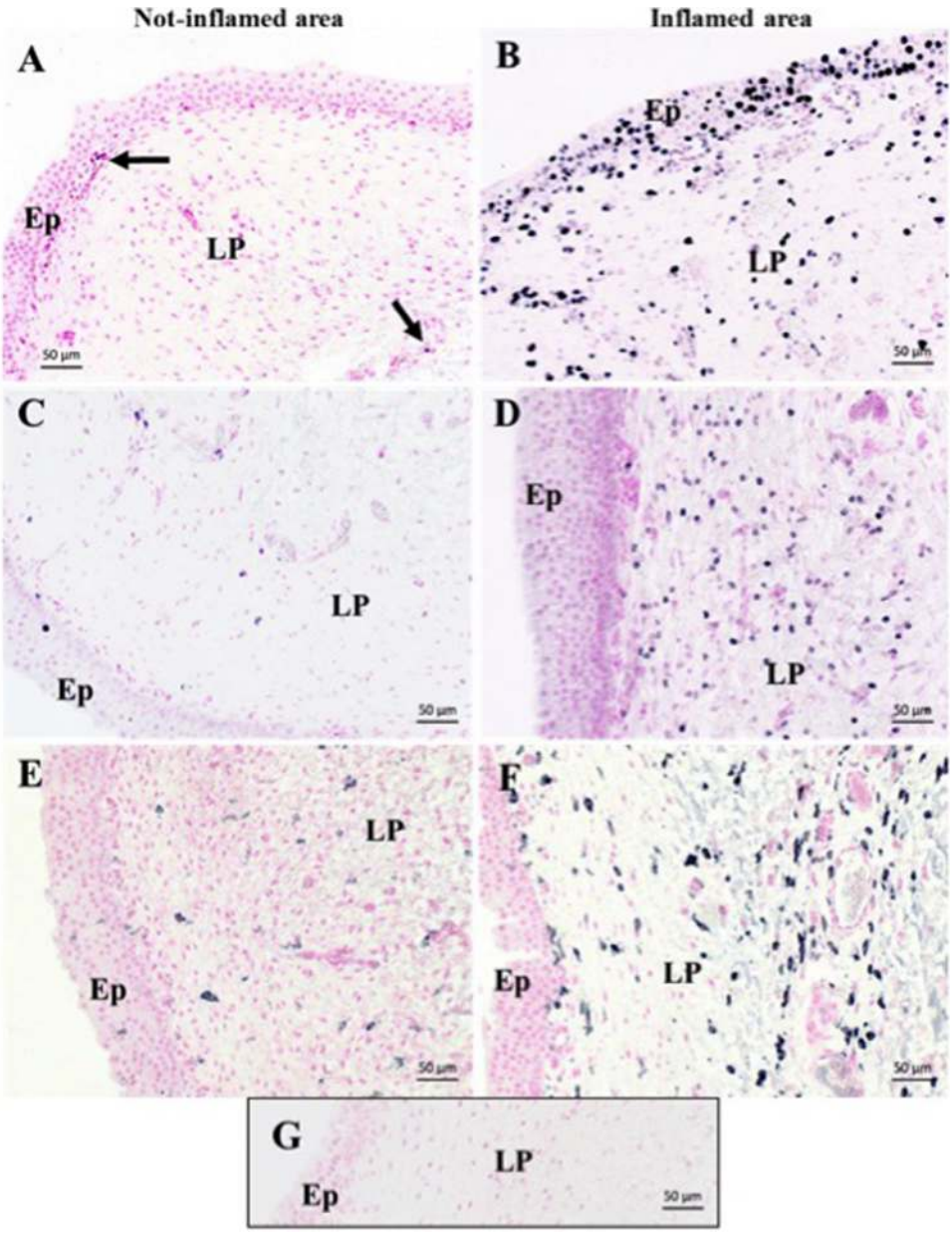

Figure 4. Representative images of Ki67 (a marker of proliferating cells; **A** and **B**), CD3 (a marker of T-lymphocytes, **C** and **D**), and CD163 (a marker of macrophages, **E** and **F**) staining in noninflamed (left columns, **A, C,** and **E**) and inflamed areas (right columns, **B, D,** and **F**) of tissues collected from bladders with CystoSure catheter. Blackish color indicates positive staining, and pinkish color cell nuclei (stained with fast red). **(G)** Control staining when primary antibody was omitted. Note more staining in inflamed than noninflamed areas (left column and right column, respectively). Note minimal staining of Ki67 (arrows) in noninflamed areas **(A)**. EP, epithelium; LP, lamina propria.

DISCUSSION

Despite vigorous efforts aimed at reducing CAUTIs, the problem perseveres as a significant challenge. To date, most risk-reducing strategies have focused either on behavioral changes such as minimizing the time in which transurethral catheters are indwelling,[8] replacing transurethral catheters with suprapubic catheters,[9] or technological solutions that have focused on bio-coatings to reduce bacterial adherence.[10] Despite the long-recognized phenomenon of Foley catheter tip-induced bladder mucosal damage, few technologies have sought to remedy this particular risk.

Urinary tract infections result from the interaction between infecting uropathogens and the urinary tract epithelium. Simplified, the pathway from normalcy to infection can be reduced to 3 basic stages—introduction, colonization, and invasion. Once uropathogens have been introduced into the sterile bladder environment and colonization has gained a foothold, the final phase of the infection process involves invasion through the surface epithelium into the underlying bladder tissues. Significant defense mechanisms against invasive bacteria include an intact immune system, functional bladder surface mucus, and an intact epithelial surface. Bladder surface mucus imparts a barrier function through extremely hydrophilic, highly anionic polysaccharide components (eg, glycosaminoglycans) that trap water at the outer layer of the epithelial umbrella cell, creating a critical interface between urine and the bladder. The result is a highly impermeable urothelium that bolsters the defense against invasive bacteria into the bladder interstitium.[11] The final barrier to bacterial invasion is the integrity of the urothelium itself. Disruptions to this barrier through either trauma or tumor invasion allows direct entry of bacteria into the nutrient-rich interstitial tissues, where they can further replicate, disseminate, and elicit the hallmarks of a urinary tract infection.

In this pilot study, we have demonstrated on a proof-of-concept basis that a new catheter design with an open-ended rounded rather than pointed tip and a reduced balloon base-to-tip profile may reduce mucosal damage to the bladder of ewes. Bladders with Foley indwelling catheters displayed significantly more evidence of both mucosal ulceration and edema than bladders with the new round-tip indwelling catheters. Bladder surface areas with macromorphologic appearances characterized by ulceration, hemorrhage, and edema were confirmed histologically to have extensive immunohistochemical evidence of inflammation. The strength of this study was our strict adherence to controlling as many of the variables as possible and the blinding of our pathologist to the catheters used. Both catheters used were 16 Fr and 100% silicone. All the catheters were indwelling for the same amount of time and the bladder-to-collection bag heights were uniform. The weakness of this pilot study was our small sample size that precluded a more robust statistical analysis.

Because the progression from bacteriuria to cystitis requires the invasion of uropathogens across the bladder epithelial surface into the underlying bladder wall, an intact mucosal surface almost certainly serves as a barrier to infection, whereas a damaged surface likely predisposes to one. The findings from this study indicate that this new catheter design with its low-profile, rounded tip may reduce bladder mucosal injury, which is a risk factor for CAUTIs. Further, despite interspecies anatomic considerations, these findings should translate into humans and merit additional larger clinical trials.

References

1. AHRQ Newsroom Press Release: AHRQ Patient Safety Toolkit Helps Hospitals Reduce Catheter-Associated Urinary Tract Infections (CAUTI). Published November 19, 2015. Available at: https://www.ahrq.gov/news/newsroom/press-releases/2015/cautitoolkit.htm. Accessed January 29, 2017.
2. Centers for Disease Control and Prevention. *Antibiotic Resistance Threats in the United States, 2013*. Atlanta, GA: CDC; 2013.
3. Foley FEB. A hemostatic bag catheter. *J Urol*. 1937;38:137-139.
4. Milles G. Catheter-induced hemorrhagic pseudopolyps of the urinary bladder. *JAMA*. 1965;193:968-969.
5. Grazul-Bilska AT, Johnson ML, Borowicz PP, et al. Placental development during early pregnancy in sheep: effects of embryo origin on vascularization of the placenta. *Reproduction*. 2014;147:639-649.
6. Lucy MC, Evans TJ, Poock SE. Lymphocytic foci in the endometrium of pregnant dairy cows: characterization and association with reduced placental weight and embryonic loss. *Theriogenology*. 2016;86:1711-1719.
7. Herrmann-Hoesing LM, Noh SM, Snekvik KR, et al. Ovine progressive pneumonia virus capsid antigen as found in CD163- and CD172a-positive alveolar macrophages of persistently infected sheep. *Vet Pathol*. 2010;47:518-528.
8. Centers for Disease Control and Prevention. *Guidelines for Prevention of Catheter-Associated Urinary Tract Infections, 2009*. Atlanta, GA: CDC; 2009.
9. Kidd EA, Stewart F, Kassis NC, Hom E, Omar MI. Urethral (indwelling or intermittent) or suprapubic routes for short-term catheterisation in hospitalised adults. *Cochrane Database Syst Rev*. 2015;(12):CD004203. doi:10.1002/14651858.CD004203.pub3.
10. Lam TBL, Omar MI, Fisher E, Gillies K, MacLennan S. Types of indwelling urethral catheters for short-term catheterisation in hospitalised adults. *Cochrane Database Syst Rev*. 2014;(9):CD004013. doi:10.1002/14651858.CD004013.pub4.
11. Parsons CL. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. *Urology*. 2007;69(4A):9-16.

APPENDIX

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.urology.2017.08.020.