ELSEVIER

EVALUATION OF TWO TECHNIQUES OF PARTIAL URETHRAL OBSTRUCTION IN THE MALE RAT MODEL OF BLADDER OUTLET OBSTRUCTION

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ABSTRACT

Objectives. To perform a comparison to determine which of two methods of partial urethral ligation produces the most consistent outcome and fewest side effects. Such a study has not been previously reported. Partial urethral ligation is a means of causing reproducible bladder outlet obstruction. In the male rat model, partial urethral obstruction can be performed either by perineal incision and bulbous urethral ligation or retropubic incision and midprostatic obstruction.

Methods. Fifteen male Sprague-Dawley rats were studied. Five were selected for bulbous urethral obstruction through a perineal incision, five for midprostatic obstruction using a retropubic approach, and five for a sham operation through a perineal incision.

Results. The operative time was shorter and morbidity lower with the perineal approach compared with the retropubic approach. Inflammation or infection, or both, were seen in the prostate, bladder, proximal urethra, ureters, and kidneys in the rats in which a midprostatic obstruction was performed. The proximal urethra and prostate were mildly inflamed in those rats that underwent bulbous obstruction. Sham-operated rats exhibited mild prostatitis only.

Conclusions. The perineal approach to the bulbous urethra is the method of choice for creating a partial urethral obstruction model of bladder outlet obstruction in the male rat. UROLOGY **66:** 1127–1133, 2005.

The use of animal models that faithfully recreate the urinary symptoms related to bladder outlet obstruction in men are critical to evaluate potential therapeutic methods.[1,2] Several methodologic approaches in experimental animals are used to create the model; one simply performed, convenient approach is partial urethral obstruction (PUO) in the rat. Although both sexes have been used for the model, the female rat has been used most commonly because of the simpler anatomy and the absence of possible confounding factors resulting from changes to the accessory sex organs or the absence of changes in the cystometric parameters.[3–6] Nevertheless, sex-specific alterations and therapies related to the symptoms of bladder outlet obstruction in the male may exist that need evaluation. The development of a relevant, reproducible, male animal model, with a comparison of the possible side effects of surgical obstructive techniques, would be useful. Therefore, the purpose of this study was to compare the effects of two techniques of PUO in the male rat to determine the best model.

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MATERIAL AND METHODS

A total of 15 Sprague-Dawley male rats were studied. The Albert Einstein College of Medicine Vivarium provided housing and maintenance. The rats were kept on a 12-hour light-dark cycle and had ad libitum access to food and water. The Albert Einstein College of Medicine Institute for Animal Studies preapproved all animal protocols used in this study. Five rats were selected for bulbous urethral ob-

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TABLE I. *Six-week results for male rats that underwent partial urethral obstruction*

	Approach		
	Sham	**Perineal**	**Suprapubic**
Bladder weight, mean ± SEM (mg)	232 ± 15.3	701.167 ± 177.1	770.800 ± 147.6
Rats (n)	5	5	5

struction, five for midprostatic obstruction, and five for sham operations through a perineal incision. Anesthesia was induced by intraperitoneal injection of pentobarbital solution (1 mg/mL) using the following formula: rat weight in grams/1.25 = milliliters of pentobarbital. After anesthetic induction, the abdominal wall and perineum were shaved with an electric razor and sterilized with povidone iodine. Sterilized microsurgical instruments were used for surgery.

Midprostatic Urethral Obstruction: Retropubic Approach

In 5 rats, a 2-cm midline vertical incision was made along the lower abdominal ventral wall, followed by dissection of the underlying muscle and subcutaneous tissue. The prostate was then elevated gently to reach the area of the bladder neck. The prostatic urethra was isolated carefully so that the seminal vesicles and ureters at the ureterovesical junction were not jeopardized. A sterile metal bar with a 0.91-mm diameter was placed on the prostatic urethral surface, and a 3-0 polypropylene suture was used to place a tie around both the prostatic urethra and the bar. As soon as the suture was secured, the bar was removed, leaving the prostatic urethra partially obstructed. A 4-0 silk suture was used to reapproximate the muscle layer, and a 4-0 nylon suture was used to close the skin. The incision was then cleaned with povidone iodine, and the rat was placed in its cage under a heating lamp after surgery.

Bulbous Urethral Obstruction: Perineal Approach

In 5 rats, a 1-cm midline vertical incision was made from the penoscrotal junction to the midscrotum to gain access to the bulbous urethra. The urethra was then isolated from the cavernous bodies, and the remaining part of the procedure, including the obstruction and closure, was performed as described above.

Control Group: Sham Operation

In 5 rats, the penoscrotal incision was made, and the urethra was isolated as described above, but the urethral ties were not placed.

Cystometric Evaluation

After 6 weeks, the animals were anesthetized and underwent placement of a retropubic catheter, as described previously.[7] Cystometric evaluation in awake animals was performed 48 hours later. Data were collected and analyzed after the animals were seen to be resting quietly in the cage, usually 20 minutes after the infusion began. Pressures and volume measurement were recorded on a personal computer on-line using PowerLab software (ADI, Castle Hill, Australia). Bladder function was evaluated using the following criteria: bladder capacity (volume of infused saline at micturition), basal pressure (lowest average baseline bladder pressure recorded during cystometry), threshold pressure (bladder pressure immediately before micturition), micturition pressure (peak bladder pressure during micturition), micturition volume (volume of urine voided), postvoid residual volume (volume of saline infused minus voided volume), mean intermicturition pressure (mean pressure measured between micturitions [all volumes are in milliliters and pressure measurements in centimeters of water]), spontaneous activity, and an approximate index of the number and pressure of overactive detrusor contractions, measured as the mean intermicturition pressure minus the average basal pressure. Data were analyzed using Chart4 Windows (ADI).

Histologic Examination

After the experimental protocol was complete, the animals were killed, and the organs from each of the groups were fixed in formalin and processed for histologic evaluation. The tissue sections were stained with hematoxylin-eosin and evaluated independently by a veterinary pathologist.

Statistical Analysis

Statistical analysis was done using SigmaStat software (Systat Software, Point Richmond, Calif). One-way analysis of variance was performed for each of the cystometric parameters. When the analysis of variance results were significant, the Tukey test was used for post hoc pair-wise comparisons. Data with $P < 0.05$ were considered significant.

RESULTS

No operative or perioperative mortalities occurred in any group. The average operative time with the perineal method was approximately 15 minutes compared with 35 minutes for the retropubic approach. The bladder weights increased threefold in the obstructed groups (Table I). Complete cystometric data evaluation was completed in 4 animals from each group (Table II). Representative cystometric tracings from each group are shown in Figure 1.

The data showed that retropubic obstruction caused increased micturition pressure and postvoid residual volume that was significantly greater than that in the sham and perineal obstruction groups. Both methods of obstruction caused cystometric changes in the bladder consistent with detrusor overactivity, that is, increased spontaneous activity. However, the perineal approach created significantly greater bladder capacity, spontaneous activity, intermicturition, and threshold pressure at a lower micturition pressure and postvoid residual volume than in the retropubic group.

TABLE II. *Results of cystometric evaluation*

Group	MP (cm H_2O)	TP (cm H_2O)	IMP (cm H_2O)	BP (cm H_2O)	SA (cm H_2O)	BC (mL)	MV (mL)	IV (mL)	RV (mL)
Sham	40.84 ± 0.36	19.62 ± 0.26	14.39 ± 0.27	11.97 ± 0.40	2.43 ± 0.29	1.21 ± 0.02	1.20 ± 0.02	1.20 ± 0.02	0.01 ± 0.01
Perineal	40.67 ± 0.35	21.89 ± 0.46	21.79 ± 0.29	11.70 ± 0.17	10.17 ± 0.34	2.14 ± 0.01	2.13 ± 0.01	2.13 ± 0.01	0.01 ± 0.01
Retropubic	47.39 ± 0.64*†	19.65 ± 0.27*‡	18.03 ± 0.46*§	10.60 ± 0.31*‖	7.43 ± 0.23*§	1.88 ± 0.01*§	1.12 ± 0.02*§	1.12 ± 0.02*§	0.77 ± 0.01*†

Key: MP = micturition pressure; TP = threshold pressure; BP = basal pressure; BC = bladder capacity; MV = micturition volume; IMP = intermicturition pressure; SA = spontaneous activity; IV = infused volume; RV = residual volume.

Results expressed as mean ± standard error of the mean; n = 4 animals in each group.

* $P < 0.001$, groups significantly different.

† $P < 0.001$, retropubic vs. perineal and sham different.

‡ $P < 0.003$ perineal vs. sham and retropubic different.

§ $P < 0.001$, each group different from each other.

‖ $P < 0.05$ sham vs. retropubic different.

After the animals were killed, the gross and microscopic anatomic changes of the experimental organs were examined. Inspection of the animals in which midprostatic urethral obstruction was performed showed diffusely enlarged prostatic lobes containing abscesses (Fig. 2). The seminal vesicles were enlarged and congested with secretions (Fig. 3). The histologic appearance of the kidneys was that of pyelonephritis and secondary hydronephrosis along with bilateral hydroureters. The urinary bladder was thickened and exhibited inflammation consistent with cystitis (Fig. 4). The prostatic tissue revealed inflammatory changes and abscesses at multiple foci in each of the lobes, excluding the anterior prostate, which was normal. Sections from the kidneys demonstrated polymorphonuclear cells and microabscesses, suggesting bacterial infection. Sections from the ureters also showed signs of inflammation. The prostatic urethral tissue examined from sections proximal to the point of obstruction also showed inflammatory changes. No inflammation was seen in tissue distal to that point. On examination of the dilated seminal vesicles, no inflammatory changes were seen. Examination of the bladder wall revealed hypertrophied muscle intermixed with fibrotic and inflammatory changes. Histologic signs of infection were seen at the bladder neck, including polymorphonuclear cells and monocytes.

Gross anatomic examination of the rats in which the bulbous urethral obstruction was performed revealed an enlarged bladder in each case. However, unlike the more proximally obstructed rats, these rats had normal-appearing kidneys, ureters, and prostates. Most of the prostatic tissue appeared normal, with a few areas of mild inflammation in the acini and adjacent interstitium. The seminal vesicles, which were slightly enlarged, kidneys, and ureters did not show any signs of inflammation. Proximal sections of the urethra showed mild inflammation, with several neutrophils in the lamina propria. Distal sections of the urethra past the point of obstruction showed no signs of inflammation. The bladder wall revealed hypertrophied muscle without any fibrotic or inflammatory changes. Gross anatomic study of the rats in which sham operations were performed did not reveal any abnormalities. Only the prostate gland showed mild inflammation in a few areas of the acini and adjacent interstitium.

COMMENT

Partial urethral obstruction in male rats resulted in more detrusor overactivity, which may

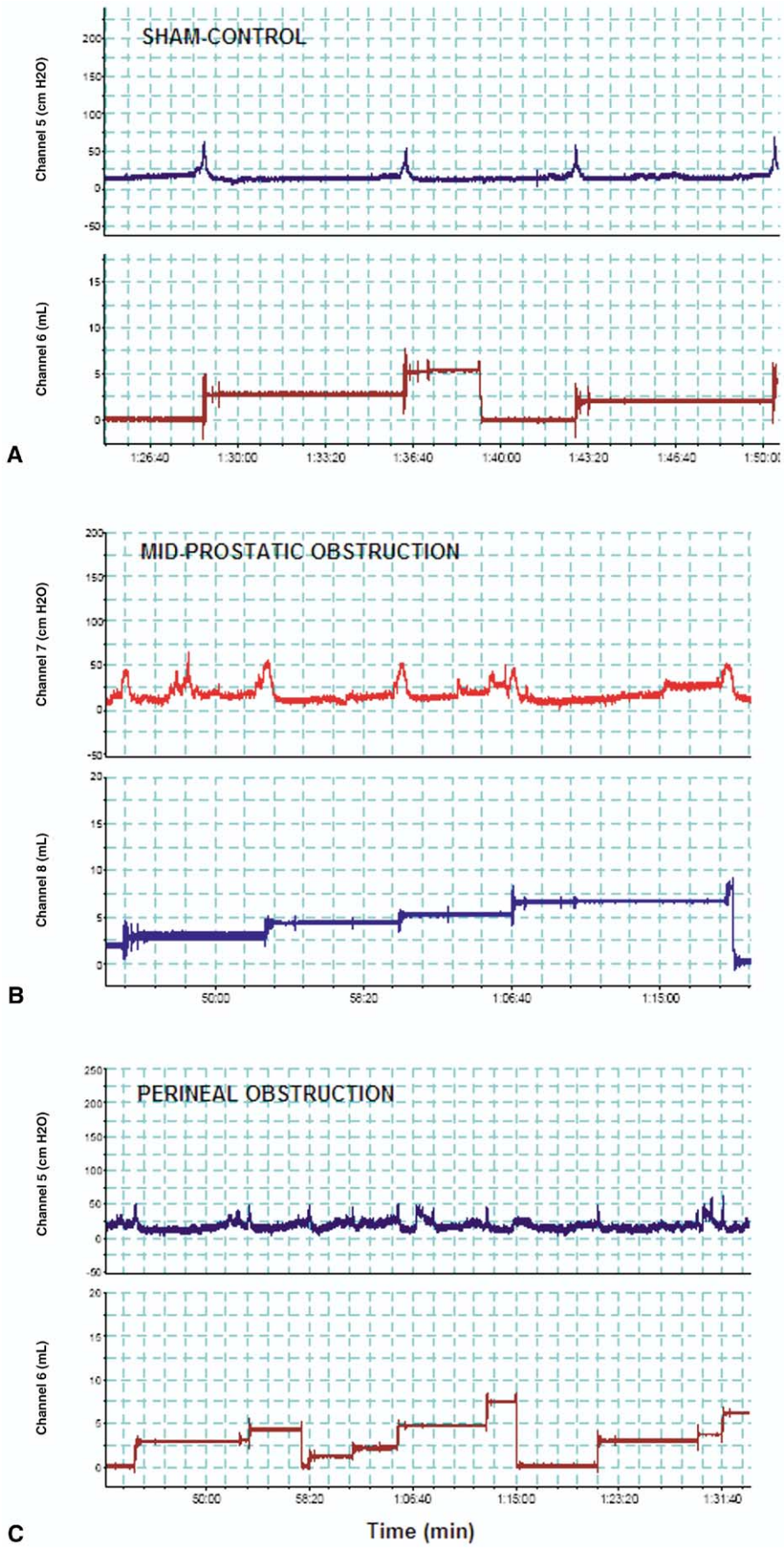

FIGURE 1. *Cystometric tracings from (A) control rats, (B) midprostatic obstruction rats, and (C) perineal obstruction rats.*

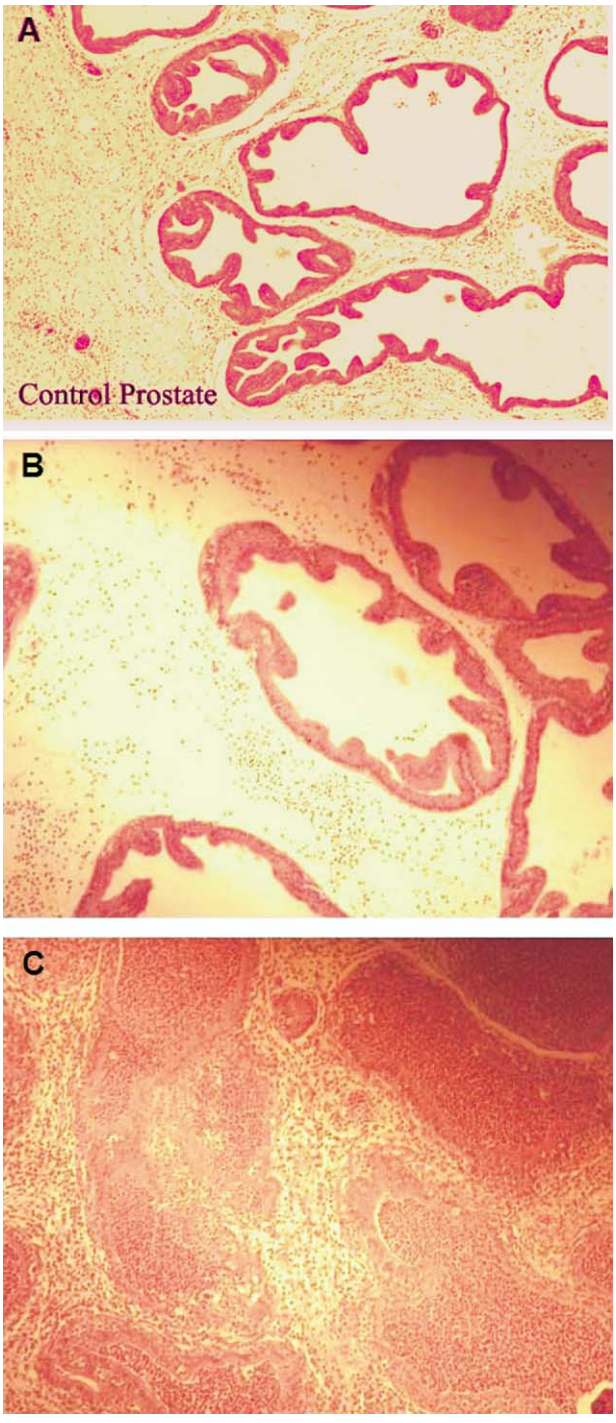

FIGURE 2. *Photomicrographs of prostate glands from three groups of animals. Note increased inflammatory response in retropubically obstructed animals compared with sham and perineal PUO animals. (A) Section of prostate from sham-operated animal. (B) Prostate from perineal PUO rats. (C) Prostate from retropubic rats. Hematoxylin-eosin stain, original magnification ×20.*

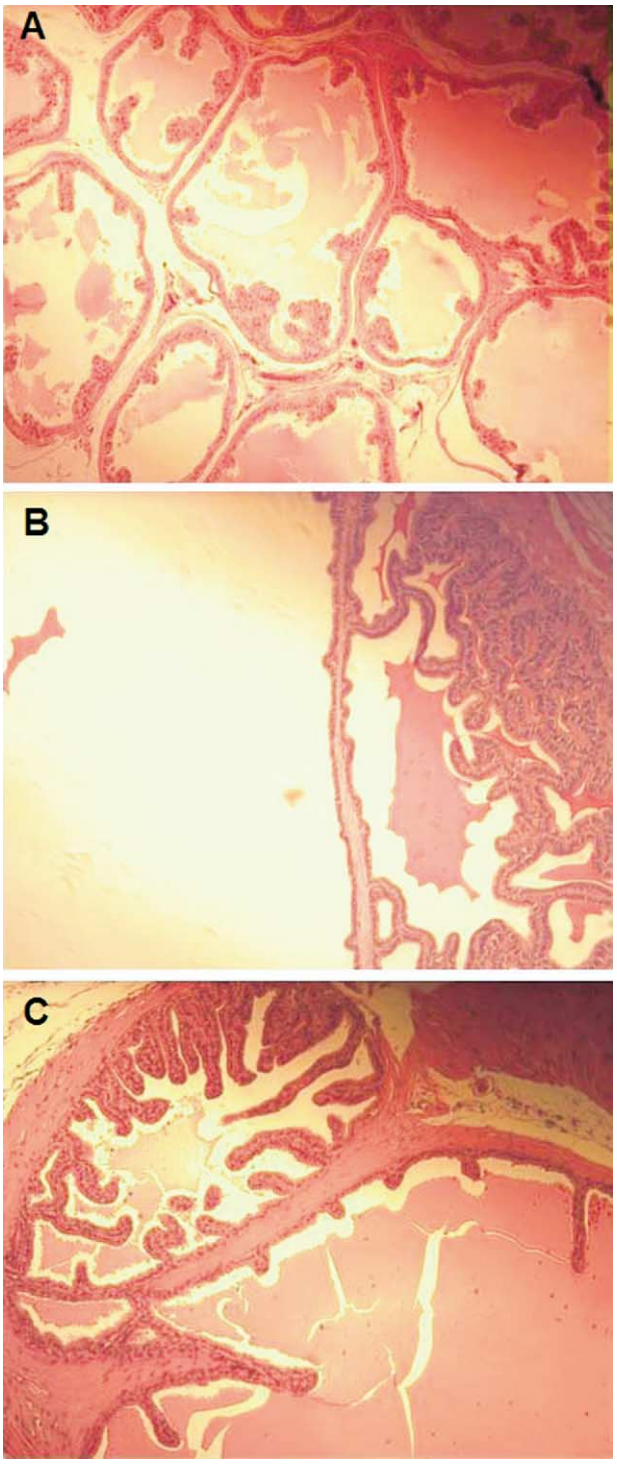

FIGURE 3. *Photomicrographs of sections of seminal vesicles of studied rats. (A) Seminal vesicle of control rat. (B) Seminal vesicles from animal after perineal obstruction. (C) Seminal vesicles from animal after retropubic obstruction. Hematoxylin-eosin stain, original magnification ×20.*

be analogous to that seen in some men with benign prostatic hyperplasia. No operative or perioperative mortalities occurred in any group. However, the operative time was shorter and the blood loss and morbidity were lower with the perineal technique. Cystometric findings of detrusor overactivity were observed in both groups, with those of the perineal group having a higher volume and higher pressure system in the

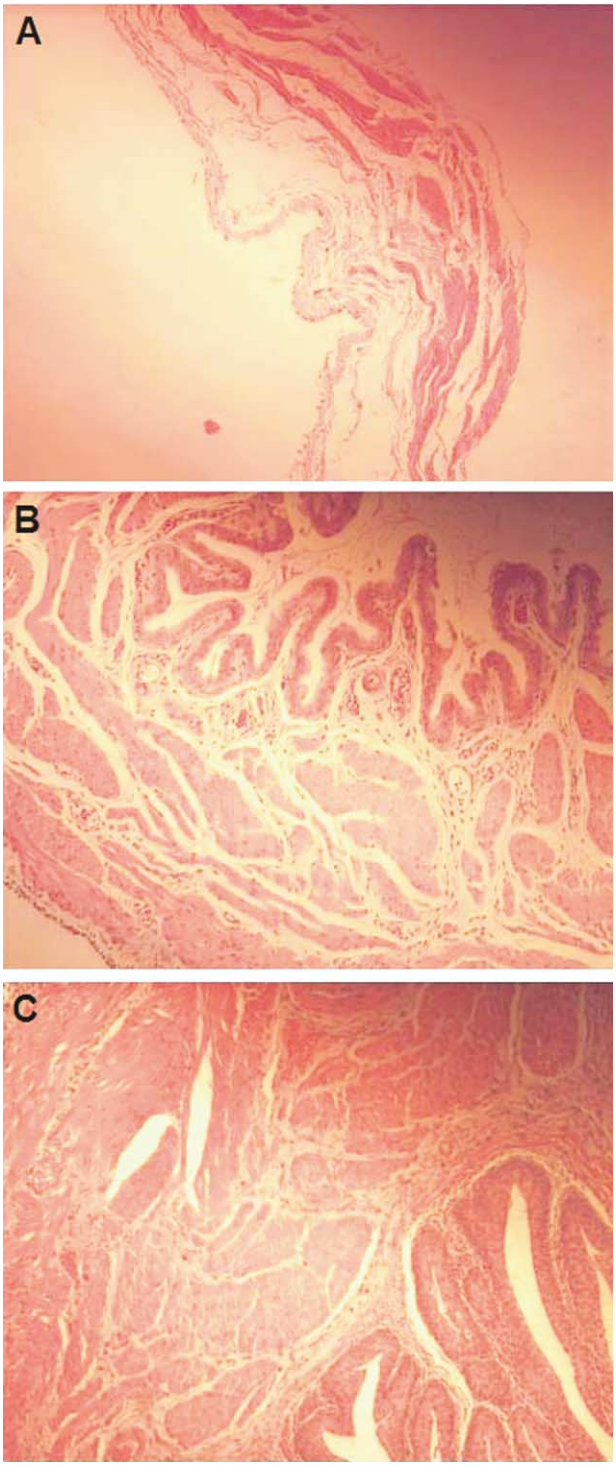

FIGURE 4. *Photomicrograph of histologic sections of bladders. (A) Control rat. (B) Perineal obstruction. (C) Retropubic obstruction. Bladders of obstructed animals showed smooth muscle hypertrophy greater in retropubic group than in perineal obstruction group. In these sections, mild inflammatory infiltrate is seen in both obstructed animals. Hematoxylin-eosin stain, original magnification ×20.*

face of normal bladder emptying. Thus, creation of the detrusor overactivity in the perineal group occurred without incomplete voiding and is a truer representation of the overactive bladder syndrome in men.

Therefore, the primary differences between the two models are a high complication rate observed after midprostatic obstruction and a more accurate model of the overactive bladder syndrome after PUO using the perineal approach. The prostatic tissue from the rats with midprostatic obstruction revealed diffuse inflammatory changes and histologic signs of infection. This was significantly different than in either the distally obstructed or unobstructed rat groups, in which only mild inflammation of the prostate was seen.

No significant differences were found in the gross or histologic appearance of the seminal vesicles between the groups. However, significant differences in the bladder, ureters, and kidneys were seen between the proximally and distally obstructed rats. In the rats with a midprostatic obstruction, the bladder showed signs of infection, fibrosis, and inflammation, as well as muscular hypertrophy, compared with the rats with more distal bulbous urethral partial obstruction in which only minimal inflammation and less muscular hypertrophy occurred. In the rats with midprostatic partial obstruction, histologic signs of infection were also seen in the ureters and kidneys in every case. Thus, the more proximal, partial midprostatic obstruction caused an ascending inflammation or infection, or both, which were not observed with the method of bulbous urethral partial obstruction. The latter seems to cause only a physiologic change in muscular thickening of the bladder wall, increased weight of the bladder, and dilation of the seminal vesicals, without secondary infection.

CONCLUSIONS

We report the complications of two methods of creating PUO to model the overactive bladder in the male rat. We conclude that the perineal approach, with partial obstruction of the bulbous urethra, is superior to midprostatic obstruction because of the ease of surgery and because it causes significantly less morbidity while more accurately reproducing the urodynamic changes observed in patients with overactive bladders.

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