PRIZE AWARD: Best Basic Science Abstract

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THE EFFECT OF OVARIECTOMY ON BLADDER FUNCTION AND MYOSIN ISOFORM EXPRESSION IN FEMALE RABBITS WITH PARTIAL BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study:
The effect of estrogen on the regulation of bladder smooth muscle contraction is not well known. Prior studies with male rabbits have demonstrated that partial bladder outlet obstruction (PBOO) affects bladder function and the expression of carboxy (SM2/SM1)[1] and amino terminal(SM-B/SM-A)[2] smooth muscle myosin heavy chain (SMMHC) isoforms. Separately, estrogen has been shown to preferentially affect the carboxy terminal smooth muscle myosin isoforms.[3] Previous studies have not examined gender differences or the effects of ovariectomy on smooth muscle contractile function during bladder wall remodeling. Using the female rabbit model of PBOO, the objective of this study was to evaluate the effect of ovariectomy on urinary function, detrusor smooth muscle contraction, and smooth muscle myosin isoform expression.

Study design, materials and methods
Twenty female New Zealand white rabbits were divided evenly into 4 groups: Intact (non-ovariectomized) sham-operated (InSham), intact PBOO (InPBOO), ovariectomized sham-operated (OvxSham), and ovariectomized PBOO (OvxPBOO). Metabolic cage data (24 hour) assessing urinary frequency and volume per void (ml/void) were recorded preoperatively and 14 days post-obstruction. At the time of sacrifice, bladders were removed and weighed. Detrusor muscle strips were dissected from the mid bladder region and used for in vitro studies. Maximal contractile responses to KCl (125 mM), electrical field stimulation (EFS, 32 Hz), and carbachol (100 □M) were recorded. Maximum contractile force was expressed as mg/100 mg of tissue weight. Western blot experiments were used to determine protein expression for total smooth muscle myosin heavy chain and GAPDH. RT-PCR was performed to determine the relative expression of the SMMHC carboxy terminal isoforms SM2/SM1 and the amino terminal isoforms SMB/SMA. Results were expressed as the relative expression of SM2 [SM2/(SM1+SM2)] and SMB [SMB/(SMB+SMA)]. Data are presented as mean ± SEM and compared using Student's t test or two-way analysis of variance (ANOVA) for multiple groups.

Results
A significant increase in urinary frequency and a decrease in volume/void were demonstrated in both the intact and ovariectomized PBOO groups compared to their respective sham-operated groups (Table). Ovariectomy did not influence urinary frequency and volume per void in the PBOO animals ($P = 0.94$ and 0.77, respectively). Detrusor smooth muscle from animals that underwent PBOO (intact and ovariectomized) had significantly lower maximum tension generated in response to all stimulants compared to the intact sham animals ($P < 0.001$). Although there was a trend towards an increase in the maximum contractile response to all stimulants by the detrusor smooth muscle from ovariectomized animals with PBOO compared to intact animals with PBOO, these differences did not reach significance. Among the sham-operated animals, ovariectomy resulted in a significant decrease in the maximum force generated by KCl (OvxSham = 2.87 ± 1.08 vs. InSham = 6.56 ± 0.99 mg/100 mg tissue weight, $P = 0.04$).

RT PCR data showed that PBOO resulted in a statistically significant decrease in the relative expression of SM2 in both intact and ovariectomized animals compared to the intact sham-operated animals(all $p < 0.001$) while the relative expression of SM-B was significantly reduced after PBOO among the intact animals ($p < 0.001$) only. However, the western blot data did not demonstrate a significant difference in total smooth muscle myosin heavy chain protein expression between all groups. Ovariectomy resulted in a higher relative expression of SM2 in the ovariectomized sham rabbits compared to the intact sham animals (see Figure, $P = 0.003$) with no difference in the relative expression of SM-B. In contrast, among the PBOO animals, ovariectomy did not alter the relative expression of SM2 but did result in a significantly higher expression of SM-B in the ovariectomized animals with PBOO compared to the intact PBOO rabbits (see Figure, $P = 0.02$). Among the intact rabbits, the percent decrease in the relative expression of SM-B after PBOO was twice that seen among the ovariectomized animals (~48% vs. ~24%). Conversely, the percent decrease in the relative expression of SM2 after PBOO in the intact animals was half that seen among the ovariectomized animals (~17% vs. ~32%)

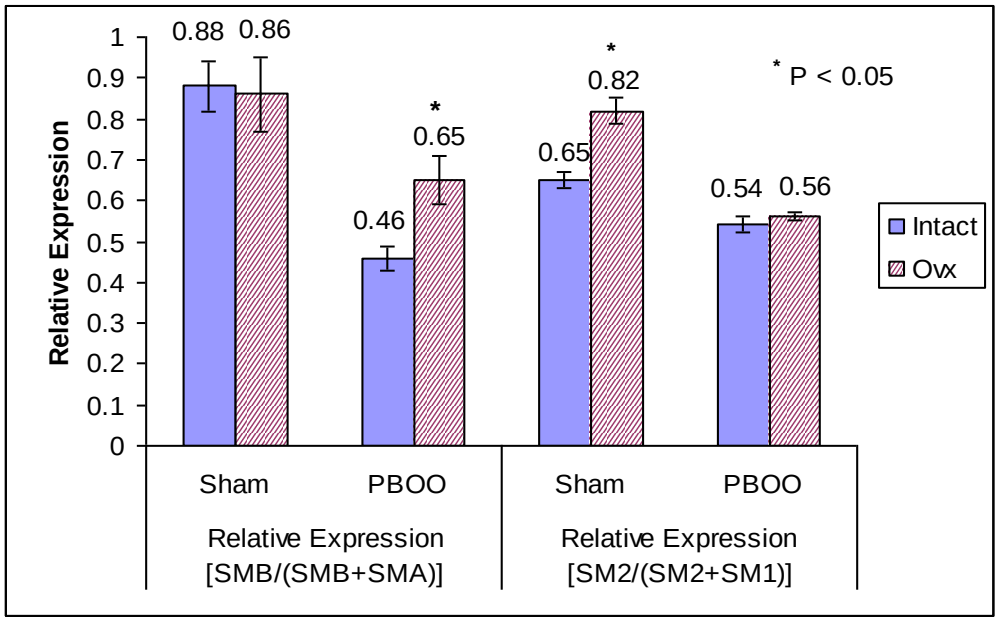
Interpretation of results
Ovariectomy did modulate the degree of changes in carboxy and amino terminal smooth muscle myosin isoforms expression after PBOO. The increased relative expression of SM2 by the ovariectomized sham-operated animals compared to the intact sham-operated animals may, in part, explain the decreased *in vitro* contractile response to KCl of detrusor smooth muscle from the OvxSham group compared to the InSham. Consistent with prior data, the relative expression of SM-B decreases after PBOO but this effect appears to be blunted in the ovariectomized animals. Alternatively, the increase in the relative expression of SM2 associated with ovariectomy alone in sham-operated animals appears to be modulated by partial bladder outlet obstruction.

Concluding message
These findings highlight the importance of identifying gender differences in bladder wall remodeling. Our results may help clarify possible mechanisms in lower urinary tract dysfunction in postmenopausal women.

Table

	Intact			OVX		
	Intact Sham Mean ± SEM (N = 5)	**Intact PBOO Mean ± SEM (N = 5)**	**P value**	**Ovx Sham Mean ± SEM (N = 5)**	**Ovx PBOO Mean ± SEM (N = 5)**	**P value**
Voids/24 h	11 ± 4	44 ± 9	0.01	15 ± 6	45 + 10	0.03
Vol/void (ml)	21.6 ± 7	3.2 ± 1.2	0.03	17.8 ± 6	2.8 + 0.5	0.04
Bladder weight (g)	3 ± 0.6	17.9 ± 4	0.01	4.8 ± 0.7	9.9 + 2.5	0.08
Rabbit weight (kg)	3.4 ± 0.2	3.4 ± 0.1	1.00	3.4 ± 0.1	3.3 ± 0.1	0.50

Figure



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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Pennsylvania Institutional Animal Care and Use Committee (IACUC) Protocol # 802084**