1   **Factors Driving Unique Urination Phenotypes of Male and Female 9-week-old C57BL/6J**

2   **Mice**

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23   **ABBREVIATED TITLE: Sex Differences in Urinary Physiology of C57BL/6J Mice**

24   **ABSTRACT (250 word limit)**

25   Laboratory mice are used to identify causes of urinary dysfunction including prostate-

26   related mechanisms of Lower Urinary Tract Symptoms (LUTS). Effective use of mice for this

27   purpose requires a clear understanding of molecular, cellular, anatomical, and endocrine

28   contributions to voiding function. Whether the prostate influences baseline voiding function has

29   not been specifically evaluated, in part because most methods that alter prostate mass also

30   change circulating testosterone concentrations. We performed void spot assay and cystometry

31   to establish a multi-parameter "baseline" of voiding function in intact male and female 9-week-

32   old (adult) C57BL/6J mice. We then compared voiding function in intact male mice to that of

33   castrate males, males (and females) treated with the steroid five alpha reductase inhibitor

34   finasteride, or males harboring alleles (*Pbsn4$^{cre/+}$;R26R$^{Dta/+}$*) that significantly reduce prostate

35   lobe mass by depleting prostatic luminal epithelial cells. We evaluated aging-related changes in

36   male urinary voiding. We also treated intact male, castrate male, and female mice with

37   exogenous testosterone to determine the influence of androgen on voiding function. The three

38   methods used to reduce prostate mass (castration, finasteride, *Pbsn4$^{cre/+}$; R26R$^{Dta/+}$*) changed

39   voiding function from baseline but in a nonuniform manner. Castration feminized some aspects

40   of male urinary physiology (making them more like intact female) while exogenous testosterone

41   masculinized some aspects of female urinary physiology (making them more like intact male).

42   Our results provide evidence that circulating testosterone is responsible in part for baseline sex

43   differences in C57BL/6J mouse voiding function while prostate lobe mass in young, healthy

44   adult mice has a lesser influence.

45   **KEY WORDS (3 to 5):** LUTS, Void Spot Assay, Cystometry, BPH, Animal Model

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47

48 ## 1. INTRODUCTION

49   Lower urinary tract symptoms (LUTS) are prevalent in adult men and women, negatively

50 affect quality of life, and associate with depression.[1, 2] It was once believed that LUTS in aging

51 men derive almost exclusively from prostatic enlargement and urethral occlusion.[3] The rationale

52 was that prostatic volume[4] and LUTS prevalence and severity[5, 6] increase with age, and that

53 prostate resection generally alleviates LUTS in aging males.[7] Recent studies suggest a disease

54 process more complex than previously appreciated. Prostatic volume does not strongly

55 correlate with urodynamic patterns or LUTS severity when measured in the same cohort of

56 men.[5, 8] Some men with above average prostate volume do not experience clinically significant

57 LUTS while others with below average prostate volumes experience severe LUTS.[9, 10] It is now

58 becoming clear that male LUTS arise from multiple mechanisms in addition to prostatic

59 enlargement.[11] There is growing support for the roles of prostatic urethral collagen

60 accumulation,[12] inflammation,[13] and smooth muscle hypercontracticility[14, 15] in progressive

61 LUTS. There is also a growing need for validated model systems to rigorously test these new

62 mechanisms and new pharmacological interventions.

63   The use of mice to study urinary voiding dysfunction is extensive and widespread. A search

64 of the term "Urology" in PubMed revealed 17,815 publications in 2018: 27 were studies in dog,

65 575 in rat, and 1003 in mouse. The practice of using male mice for human urinary voiding

66 translational studies has been controversial because not all aspects of mouse and human

67 prostate anatomy are the same. Similarities include anatomical location (base of the bladder)

68 and a narrowing of the urethra in the region where prostatic ducts drain (prostatic urethra).

69 Major differences include prostatic encapsulation and compaction. A portion of the mouse

70 prostate gland lies within a muscular sphincter (rhabdosphinter) but the majority of prostate

71 tissue branches into four bilaterally symmetrical prostate lobes (anterior, dorsal, lateral, and

72 ventral) that are not encapsulated.[16, 17] The human prostate gland, in contrast, is spherical and

73 has a fibromuscular capsule.[18] Several studies demonstrate a clear influence of prostate

74   pathologies on mouse urinary voiding behaviors.[19-22] Influence of the mouse prostate on

75   baseline voiding function has not been specifically evaluated, in part because many methods to

76   alter prostate mass also alter circulating testosterone concentrations.

77   To address these issues, we used multiple experimental groups and contemporary

78   methods to determine the influence of androgens and prostatic mass on baseline voiding

79   function, lower urinary tract anatomy, and histology. Our companion publication focuses on

80   histology. This report exclusively discusses anatomy and physiology and provides an expansive

81   urinary physiology data set in wild-type C57BL/6J mice that can be mined for hypothesis

82   generation and validation (Tables 2-10). We first evaluated baseline voiding function in nine-

83   week-old male and female C57BL/6J control mice. We then used three different approaches to

84   reduce prostatic mass in male mice: surgical castration, treatment with a steroid 5 alpha

85   reductase inhibitor (finasteride), and a genetic approach to ablate prostate luminal epithelial

86   cells. We also treated intact male, castrate male, and female mice with testosterone to control

87   for the influence of androgen. We identified clear sex differences in baseline urinary function,

88   even though relative bladder mass and volume do not significantly differ between male and

89   female mice. Exogenous testosterone changes several parameters of female mouse urinary

90   function in the direction of intact males (masculinization). Castration feminizes male mouse

91   urinary function, but neither finasteride treatment nor a genetically-induced reduction in prostate

92   mass feminizes male mouse urinary function. We conclude that circulating testosterone is

93   responsible, in part, for sex differences in baseline mouse voiding function while prostatic lobe

94   mass plays a lesser role.

95   **2. MATERIALS AND METHOD**

96   **2.1   Mice**

97   All experiments were conducted under a protocol approved by the University of Wisconsin

98   Animal Care and Use Committee and in accordance with the National Institutes of Health Guide

99   for the Care and Use of Laboratory Animals. Mice were housed in Udel® Polysulfone

100 microisolator cages on racks or in Innocage® disposable mouse cages on an Innorack®; room

101 lighting was maintained on 12 hour light and dark cycles; room temperature was maintained at

102 20.5 ± 5 C; humidity was 30–70%. Mice were fed 8604 Teklad Rodent Diet (Harlan

103 Laboratories, Madison WI) and feed and water were available *ad libitum*. Cages contained corn

104 cob bedding. All endpoint measurements were collected in nine-week-old mice unless specified

105 otherwise.

106   All mice used in this study were purchased from Jackson Laboratories (Bar Habor, ME)

107 and included C57BL/6J (Stock #000664), Tg(Pbsn-*cre*)4Prb/J (Pbsn4*cre*, stock number

108 026662) bred onto the C57BL/6J background for four to five generations,[23] B6.Cg-

109 Gt(ROSA)26Sor^tm14(CAG-tdTomato)Hze/J   (R26R-Tdtomato,   Jax   stock   #007914),[24]   and

110 Gt(ROSA)26Sortm1(DTA)Jpmb/J (R26R-Dta, Jax stock #006331)[25]. The genotype of mice with

111 depleted prostatic epithelial cells was Pbsn4^cre/+; R26R^Dta/+ and their respective littermate

112 controls were Pbsn4^cre/+; R26R^TdTomato/+.

2.2   Castration/Sham Castration

114 Castration was performed at six weeks of age. Mice were anesthetized with isoflurane and

115 given ketoprofen (0.5 mg/kg sc) as an analgesic. A midline incision was made in the scrotum,

116 and the testes were either removed (castrate) or examined (sham controls). The scrotum was

117 closed using a simple interrupted pattern.

2.3   Testosterone Capsule Preparation and Implantation

119 Capsules were prepared as previously described.[26] Silastic tubing (Dow Corning, Cat. #

120 508-008, Silastic Laboratory Tubing, 1.57 mm inside diameter X 3.18 mm outside diameter) was

121 cut to 16 mm. The wooden stick of cotton tipped applicators (Fisher Scientific Cat. #23-400-100)

122 was cut into 5 mm pieces and inserted 3 mm into the tubing to plug the ends. 10 mm of the

123 sham capsule was left empty. 10 mm of the testosterone capsule was filled with 4-androsten-

124 17beta-ol-3-one (Testosterone, T, ≥99% pure, Steraloids Inc. Cat. # A6950-000). Silastic

125 capsules were sealed with silastic medical adhesive, type A (Dow Corning, purchased from

126   Factor II Inc, [Product No. A-100]). Testosterone filled silastic capsules have been shown to

127   effectively increase testosterone in C57BL/6J mice when implanted as described.[27]

128   Mice were anesthetized with isoflurane for silastic capsule implantation and given

129   ketoprofen (0.5 mg/kg sc). An incision was made on the caudal aspect of the back just to the

130   right of midline. Capsules were inserted parallel to the spine and the incision was closed with

131   wound clips.

132   **2.4   Finasteride Treatment**

133   Finasteride (Alfa Aesar, J63454, Ward Hill, MA) was dissolved in 100% EtOH and diluted

134   in corn oil to make a 10% EtOH/ 90% corn oil dosing solution. The solution was stored at 4°C

135   for the duration of the experiment. Mice were given finasteride (50 µL of a 40 µg/µL solution)

136   daily via oral gavage.

137   **2.5   Void Spot Assay**

138   We followed the recommended guidelines of reporting VSA data.[28-30] VSA was

139   performed in the vivarium where mice were housed one day prior to cystometry and euthanasia.

140   Whatman grade 540 (Fisher Scientific no. 057163-W) filter papers (27 × 16 cm) were placed in

141   the bottom of Udel® Polysulfone microisolator cages. Mice were placed in the cage (singly

142   housed) with food *ad libitum* but no water for four hours starting from 8-11 AM GMT. VSA was

143   performed once a week starting at six weeks of age, allowing for three acclimation sessions

144   prior to the session at nine weeks of age which was used for analysis. Filter papers were dried

145   and imaged with an Autochemi AC1 Darkroom ultraviolet imaging cabinet (UVP, Upland, CA)

146   equipped with an Auto Chemi Zoom lens 2UV and an epi-illuminator. Image capture settings

147   were adjusted using UVP VisonWorksLS image acquisition software. Images were captured

148   using an Ethidium Bromide filter set (570-640 nm) and 365 nm epi-illumination. Void Whizzard

149   was downloaded from http://imagej.net/Void_Whizzard and run according to the user guide.[30]

150   Analyzed parameters included: Total Spot Count, Total Void Area (cm^2), % area in center of

151   paper, % area in corners of paper, and mass distribution of spots (0-0.1, 0.1-0.25, 0.25-0.5, 0.5-

152   1, 1-2, 2-3, 3-4, 4+ cm).

153   **2.6   Anesthetized Cystometry**

154   Cystometry was performed with minimal alterations to previously published protocols.[31,]

155   [32] Mice were anesthetized with urethane (1.43 g/kg sc). Thirty minutes after urethane dosing, an

156   incision was made in the ventral abdomen to expose the bladder. Bladder length and diameter

157   were measured for volume calculation. A purse-string suture was placed in the bladder dome.

158   Polyethylene cystostomy tubing (PE50, outer diameter 0.58mm, inner diameter 0.28mm) was

159   inserted into the bladder through the center of the suture and purse-string secured to hold the

160   tubing in place with 2-3 mm of tubing within the bladder. The abdominal wall and skin were

161   closed separately in a simple interrupted pattern. The exterior tubing was secured to the ventral

162   abdominal skin with two simple interrupted sutures. Mice were placed on a heat pad for one

163   hour after the procedure.

164   The exposed tube was connected to a three-way stopcock, and the other two arms of

165   the stopcock were connected to an infusion pump (Harvard Apparatus, Holliston, MA) and

166   pressure transducer (Memscap AS, Norway). Intravesical pressure was recorded continuously

167   using a PowerLab data collection system (ADI Instruments, Colorado Springs, CO). Room-

168   temperature sterile saline (0.9%) was infused into the bladder at a rate of 0.8 mL per hour.

169   Mice were placed in lateral recumbency above a force transducer (Model FT03, grass

170   Instruments) with a 3D printed urine collection funnel. The force transducer was calibrated with

171   known volumes of saline to create a pressure-volume conversion. The mass of voided urine

172   was recorded continuously using PowerLab.

173   At least one hour of voiding activity was recorded. Three to five consecutive voids,

174   occurring after stabilization of micturition cycles, were used for analyses. Multiple parameters

175   were measured including: Void Duration, Intervoid Interval, Baseline Pressure, Normalized

176   Threshold Pressure, Normalized Peak Void Pressure, Number of Non-Voiding Contractions,

177 Voided Volume, Compliance, Volume Flow Rate, Mass Based Flow Rate, and Efficiency

178 (Calculations used for analysis of cystometric tracings are described in Figure 1, Table 1).

179 **2.7   Statistical Analysis**

180 Statistical analyses were performed with Graph Pad Prism 8.0.2 (Graphpad Software, La

181 Jolla, California). Shapiro-Wilk test was used to test for normality and transformation was

182 applied to normalize data when possible. The F-test was used to test for homogeneity of

183 variance for pairwise comparisons. Welsh's correction was applied when variances was

184 unequal. When variance was equal, comparisons between two groups were made using

185 Student's t-test. The Mann Whitney test was applied when data could not be normalized through

186 transformation. Bartlett's test was used to test for homogeneity of variance for multiple

187 comparisons. Welsh's ANOVA was applied when variance was unequal followed by Tamhane's

188 T2 multiple comparisons test. When variance was equal, comparisons between groups were

189 made using ordinary one-way ANOVA followed by Sidak's multiple comparisons test. If data

190 could not be normalized through transformation, the Kruskal-Wallis test was applied with Dunn's

191 multiple comparisons test. A $p < 0.05$ was considered statistically significant. We have also

192 noted changes that approach significance $p < 0.10$ when they are consistent with our

193 hypothesis. These changes are marked with "$\triangle$" in tables and referred to in text as "trends"

194 accompanied by the appropriate p-value. All numerical data are presented as mean +/-

195 standard error of the mean (SEM).

196
197 **3. RESULTS**

198 **3.1   Male and Female Mouse Baseline Urinary Voiding Physiology Characteristics**

199 We evaluated several voiding parameters in male and female mice to determine the impact

200 of sex on urinary voiding physiology (Figure 2-A, Figure 4-A, and Table 2). Body mass is greater

201 in males than females, consistent with previous findings in C57BL/6J mice.[31] Relative bladder

202 mass and volumes were determined by normalizing to body mass. Male and female relative

203 bladder weight and volume do not significantly differ, consistent with previous studies.[33]

204 We used void spot assay (VSA) as a first approach to evaluate voiding behaviors. The VSA

205 procedure has been refined considerably in recent years. Methodology improvements minimize

206 experimental bias, new software enables rigorous and unbiased assessment, [28, 30] and 12

207 endpoint measurements collected in this study confer a more robust and multidimensional

208 perspective than the five or less measurements collected in prior comparisons of male and

209 female C57BL/6J mice.[31, 33] We identified several differences between control male and female

210 mice. Female mice deposit more total urine (measured by area, cm^2) in a four-hour period than

211 males. Our findings differ from that of a previous study involving older mice and using a method

212 of VSA analysis that excluded urine spots <0.66 cm^2.[31] It is worth noting that we recently

213 showed that small void spots are not caused by mice tracking urine from deposited voids, a

214 rationale for their previous exclusion.[30]

215 Previous studies excluding small and large void spots, or spots in cage corners, did not

216 observe male-female differences in voiding patterns.[31, 33] When we evaluated the spatial

217 distribution of voids (center, corners, and in-between), we found that female mice trend toward

218 depositing less urine in the center of the cage (p = 0.0524). We also evaluated the categorical

219 distribution of VSA spots (0-0.1, 0.1-0.25, 0.25-0.5, 0.5-1, 1-2, 2-3, 3-4, and 4+ cm^2) and found

220 that female mice deposit a greater proportion of large voids (4+ cm^2) than males.

221 We next used anesthetized cystometry (CMG) to evaluate voiding function. This method

222 has also evolved in recent years. New practices include: 1) A novel method of urethane delivery

223 (s.c.) that minimizes spontaneous body movements and their associated influence on

224 intravesicular pressures, 2) computer generated traces that improve accuracy of measured

225 trace characteristics, and 3) simultaneous collection of intravesical pressure and voided urine

226 mass allowing for calculation of additional void characteristics. We now routinely collect 11 CMG

227 endpoint measurements compared to the four to five collected in previous studies. We found

228   that peak void pressures are higher in females than males, consistent with a previous study.[31]

229   Also consistent with previous studies, we did not observe sex differences in intervoid interval,

230   number of non-voiding contractions, and voided volume.[31, 33] Novel findings from this study are

231   that female mice have higher threshold pressures, shorter void durations, and less bladder

232   compliance than males.

233   The mouse urinary voiding behaviors we report in this study are specific to C57BL/6J

234   mice and may be different across the multitude of mouse strains, with mice of difference age, as

235   well as health or disease status. Sex differences and mouse urinary phenotype were previously

236   reported to be strain specific.[31] Age and disease state in a genetically, surgically, or otherwise

237   altered mice, could also impact sex differences in mice. We recently documented an aging-

238   related voiding dysfunction between 2-month and 24-month-old male C57BL/6J mice.[19] Here,

239   we honed in on urinary function in young adult (1.5 – 3.5-month-old) mice. We performed VSA

240   on 6, 7, 8, 9, 10, 12, and 14-week old male C57BL/6J mice and all measured endpoints

241   changed with age (Figure 3, Table 3).

242   **3.2   Prostate Mass Reduction Minimally Impacts Baseline Male Mouse Urinary**

243   **Physiology**

244   We hypothesized that the prostate may contribute to sex differences in C57BL/6J mouse

245   voiding behaviors. We used three strategies to reduce prostate mass and evaluate the resulting

246   impact on voiding function: Castration, 5 alpha reductase inhibitor (finasteride) treatment, and

247   genetic prostatic luminal cell ablation. We specifically tested whether our three methods reduce

248   prostate mass as expected, cause a consistent directional change in VSA and/or CMG voiding

249   characteristics, and whether the directional change is consistent with "feminization" of urinary

250   physiology (i.e. same directional change as female compared to male).

251   The influence of castration on male voiding function is summarized in Figure 2-B and

252   Table 4. Castration, more than any other method to reduce prostate lobe mass, causes the

253   greatest magnitude of prostate lobe mass reduction, but also reduces body, bladder, seminal

254 vesicle mass, and bladder volume as reported previously in Swiss mice.[34, 35] Castrate and intact

255 male mice did not overwhelmingly differ in VSA-measured voiding function, but there were some

256 differences consistent with "feminization" of male urinary physiology. Castrate male mice, like

257 female mice, deposit more urine in a four-hour monitoring period compared to intact males. This

258 is consistent with a previous finding of increased urine mass/time in castrate Swiss mice.[35]

259 Castrate mice are similar to intact males in the percent of voids deposited in the center of the

260 cage and the number of 4+ cm^2 spots. Castrate males and intact females have a shorter void

261 duration and a lower bladder compliance than intact males. However, castrate males have a

262 lower baseline bladder pressure than intact control males, an endpoint that distinguishes them

263 from females. Additionally, castrate males trend toward a shorter intervoid interval and lower

264 voided volume than intact males ($p = 0.0968$ and $p = 0.0977$ respectively).

265   We next reduced prostate mass by treating mice for two weeks with finasteride (100

266 mg/kg BID via oral gavage) to block conversion of testosterone to the more potent ligand,

267 dihydrotestosterone, and reduce androgen concentration in a non-surgical manner. This dosing

268 paradigm reflects that of a previous study in rats, which reported 24h of sustained serum

269 finasteride concentrations following a single 100 mg/kg oral dose and a prostate mass reduction

270 two weeks after treatment.[36] Results are summarized in Figure 2-C and Table 5. Males treated

271 with finasteride (Males w/ Fin) have significantly smaller seminal vesicles and smaller anterior,

272 ventral, and lateral prostates than oil-treated control males. Dorsal prostate mass does not differ

273 between groups. VSA-measured voiding function in males w/ Fin does not differ from control

274 males. CMG-measured endpoints differed between groups (males w/ Fin had a significantly

275 larger intervoid interval and voided volume, and trended ($p=0.0755$) toward more non-voiding

276 contractions than control males). The finasteride-mediated directional changes in voiding

277 function were different than those caused by castration. We treated female mice (which have a

278 very low baseline level of testosterone) with finasteride and performed VSA and CMG to

279 determine the impact of finasteride on female urinary function. Finasteride causes some

280   changes in VSA-measured voiding function in females but no changes in CMG-measured

281   function (Table 6).

282   To address the impact of reducing prostate mass without surgery or hormone treatment,

283   we used a genetic strategy to deplete prostatic luminal epithelial cells (Pbsn4$^{cre/+}$; R26R$^{Dta/+}$).

284   Results are summarized in Figure 2-D and Table 7. C*re*-driven epithelial cell death reduces

285   dorsal and lateral prostate mass without significantly changing mass of other prostate lobes or

286   seminal vesicle. There were no statistical differences in VSA- or CMG-measured voiding

287   function between Pbsn4$^{cre/+}$; R26R$^{Dta/+}$ mice and their genetic controls (Pbsn4$^{cre/+}$; R26R$^{Td/+}$).

288   The results of the three different methods of prostate mass reduction show the complex

289   interplay of hormones and anatomical urinary tract variation that result in altered urinary function

290   in male mice. We were surprised that the three methods of prostate reduction had inconsistent

291   and minimal effects on urinary function. This is possibly due to variability in the localization and

292   extent of prostate mass reduction among our three methods. In our companion paper, we

293   explore this further through histologic evaluation of the prostate and urethra structure following

294   each of our prostate mass manipulations. It is also noteworthy that we evaluated prostate mass

295   reductions in our study; increases in prostate mass could have discrete impacts on urinary

296   function.[37]

297   **3.3   Exogenous   Testosterone   Supplementation   Masculinizes   Female   Urinary**

298   **Physiology**

299   Because male castration feminized some of male urinary physiology, and because other

300   methods of prostate mass reduction failed to recapitulate the resulting changes in male voiding

301   function, we next tested if circulating testosterone underlies sex differences in mouse urinary

302   function. We supplemented female mice with exogenous testosterone. We assessed the

303   supplemented mice for directional changes in VSA/CMG parameters consistent with

304   "masculinization" of urinary physiology (i.e. the parameters changed in the same direction as

305   males). Female mice were divided into two groups: control or with silastic testosterone capsule

306   implants (Female w/ T). Capsules were implanted at 6 weeks of age and assessment took place

307   at 9 weeks of age. Results are summarized in Figure 4-B and Table 8.

308   Females w/ T have a significantly greater body mass but similar bladder mass and

309   volume compared to female controls. A previous study reported that testosterone does not

310   change female mouse body weight but increases bladder weight.[35] However, this study used

311   mice of a different age, strain, environment, and method of testosterone delivery.

312   Testosterone supplementation caused several changes in female voiding function

313   measured by VSA. Similar to intact males, Female w/ T mice deposit significantly less urine

314   during a four hour monitoring period, void fewer spots greater than 4 cm^2, and void a larger

315   percentage in the center of the paper than female controls. Also similar to males, Female w/ T

316   mice have a significantly lower threshold pressure, a lower peak void pressure, and higher

317   bladder compliance than control females. Females w/ T, unlike males, have more void spots --

318   specifically more 0 to 0.1cm^2 spots than female controls.

319   Female voiding parameters affected by exogenous testosterone include many of the

320   same parameters that distinguish male from female voiding function. Thus, we conclude that

321   even though female lower urinary tract anatomy differs from that of males, exogenous

322   testosterone "masculinizes" female voiding patterns. The clear relationship between

323   testosterone and sex differences in urinary function was a surprising finding of this study.

324   Androgen receptor expression and activity is well described in the reproductive tract, but its

325   expression and activity in the urinary tract isn't well characterized. A previous report

326   documented androgen receptor expression in occasional stromal cells of the bladder and weak

327   staining in the kidney.[38] Another found that pelvic ganglia contain androgen sensitive autonomic

328   nerves.[39] This finding raises the possibility that androgens can masculinize autonomic signaling

329   in the female lower urinary tract to drive urinary voiding patterns similar to intact male mice.

330   Further studies to compare androgen receptor expression of the entire male and female lower

331 urinary tract are needed to better elucidate the influence of androgens on autonomic signaling in

332 the bladder, prostate and urethra.

333 A VSA feature not accounted for by "masculinization" of urinary function was the

334 increase in spot count, specifically small spots (0-0.1 cm^2). We implanted testosterone capsules

335 into 8-week-old male mice (Male w/ T) and assessed voiding one week later (Table 9). Males w/

336 T trend toward an increase in total void spot count, p=0.0760, and small (0-0.1 cm^2) spot count,

337 p=0.0696, compared to male controls, making increased voiding frequency a common feature of

338 exogenous testosterone supplementation in females and intact males (overall p<0.0001 and

339 p=0.0760, small p<0.0001 and p=0.0696).

340 To determine whether restoration of physiologic testosterone induces voiding frequency

341 in castrate mice, we implanted castrate males with testosterone capsules (Castrate w/ T) at

342 eight weeks of age and assessed at nine weeks of age (Table 10). Spot count did not increase

343 when castrate mice were supplemented with testosterone. Therefore, testosterone increases

344 voiding frequency when increased to supra-physiologic concentrations, but not when depleted

345 and then returned to physiologic concentrations.

346 A "frequent voider" pattern, as detected by VSA (>100 urine spots deposited on a filter

347 paper in a four hour monitoring period), was previously noted in approximately 10% of 9-week-

348 old C57BL/6J mice.[29] In our study, 14.29% of testosterone treated females and 25% of

349 testosterone treated males were frequent voiders, but no control females and just 11% of

350 control males were frequent voiders. These results further support the notion that supra-

351 physiologic testosterone increases voiding frequency and are consistent with increased voiding

352 frequency in rats and dogs supplemented with testosterone.[40]

353 **4. CONCLUSIONS**

354 We used surgical, pharmacological, and genetic approaches to reduce mouse prostate

355 mass, and also exposed C57BL/6J mice to exogenous testosterone to determine the influence

356 of the prostate and testosterone on voiding function. We characterized male and intact female

357   urinary phenotypes using contemporary methodologies and highlighted unique sex differences

358   in urinary voiding phenotype.

359   Urologic researchers and practitioners are beginning to appreciate that not all male LUTS

360   arise from urethral occlusion by an enlarged prostate and that additional factors (prostatic

361   urethra collagen accumulation,[12] prostatic inflammation,[13] and prostate smooth muscle

362   contraction[14, 15]) also drive LUTS. Mice are instrumental in studying these alternative

363   mechanisms. It is possible, for example, to induce prostatic inflammation and drive urinary

364   frequency and pelvic pain in mice modeling LUTS symptomology.[22, 41, 42] It is also possible to

365   knock-out or overexpress genes and cell types to validate mechanisms arising from clinical

366   studies and to use mice for pre-clinical safety and efficacy trials of novel therapeutics. Mice fuel

367   a creative cycle of translational research resulting in specific targeted therapies for men. In in

368   order to take full advantage of our models, further baseline studies are needed to determine

369   baseline molecular and cellular contributions to voiding function in our mouse models.

370

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377

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528

529 **FIGURE AND TABLE LEGENDS**

530 **Figure 1. A cystometry trace with annotated features that were used for calculating the**

531 **cystometry endpoints in Table 1.** BP 1 & 2 are baseline pressure, the lowest bladder pressure

532 before bladder filling (BP(1))/ immediately after voiding is complete (BP(2)). NVC is a non-

533 voiding contraction, a bladder pressure spike occurring without urination. TP is the threshold

534 pressure, the pressure when voiding initiates. PVP is the peak void pressure, the maximal

535 bladder pressure achieved during voiding.

536

537 **Figure 2. Summary statistics for anatomical and physiological differences across**

538 **experimental groups evaluated in this study including intact male and female mice,**

539 **castrate male mice (Castrate Male) mice treated with the steroid 5 alpha reductase**

540 **inhibitor finasteride (Males w/ Fin), or males genetically engineered to produce diphtheria**

541 **toxin in luminal epithelial cells, resulting in their depletion (Pbsn4cre/+;R26RDta/+).** All

542 results are mean fold differences relative to intact male control. Differences that are significantly

543 higher than intact males are shown in green and those that are lower than intact males are in

544 red. Endpoints that do not significantly differ from intact males are in gray. The numerical fold

545 difference relative to intact males is indicated by the bar size according to the legend at the top

546 of the figure. Female urinary physiology is different from male at baseline. Castration causes

547 some changes consistent with feminization of urinary function. Three different methods of

548 prostate mass reduction held no consistent effects on urinary physiology.

549

550 **Figure 3. Aging (from 6-to-14-weeks-old) alters void spot assay parameters in male**

551 **C57BL/6J mice.** Thirty mice underwent void spot assay (VSA) at 6 and 7 weeks, twenty at 8

552 and 10 weeks, and ten at 9, 12, and 14 weeks of age. The relationship between age and each

553 VSA parameter is shown as box-and-whisker plots. Age groups were compared and A-

554 designates a significant difference from 6-week-old mice, B- from 7-week-old mice, C- from 8-

555 week-old mice, and D- from 9 week-old-mice. Six to nine-week-old mice, and ten to fourteen-

556 week-old mice are statistically similar in void spot assay characteristics. (A) Spot count, (B) total

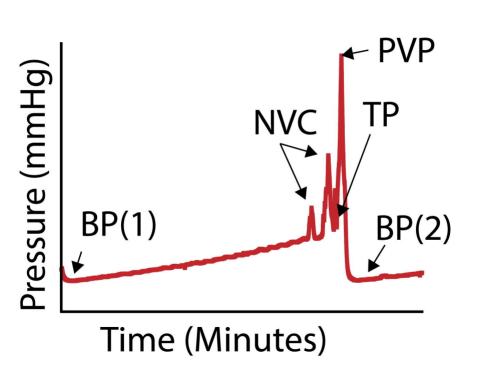557 urine area, (C) percent area in center, and (E-L) all spot sizes are altered with age.
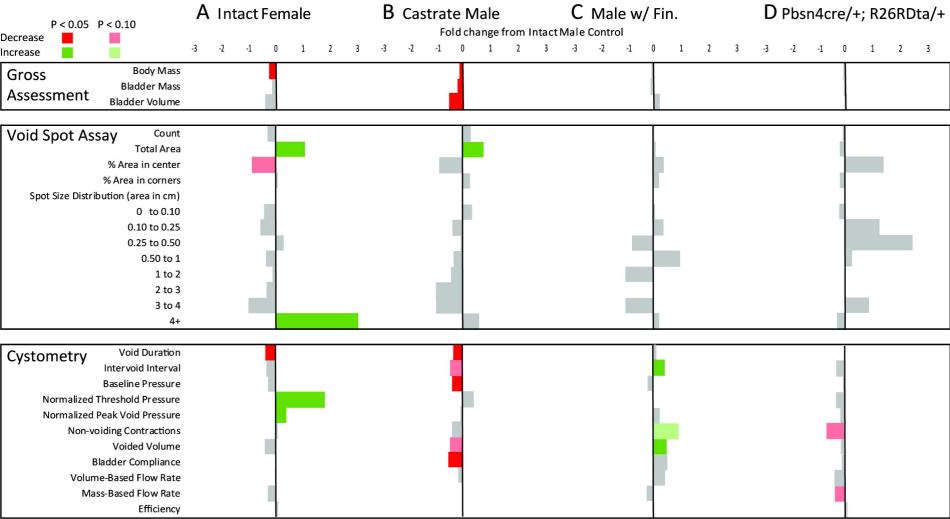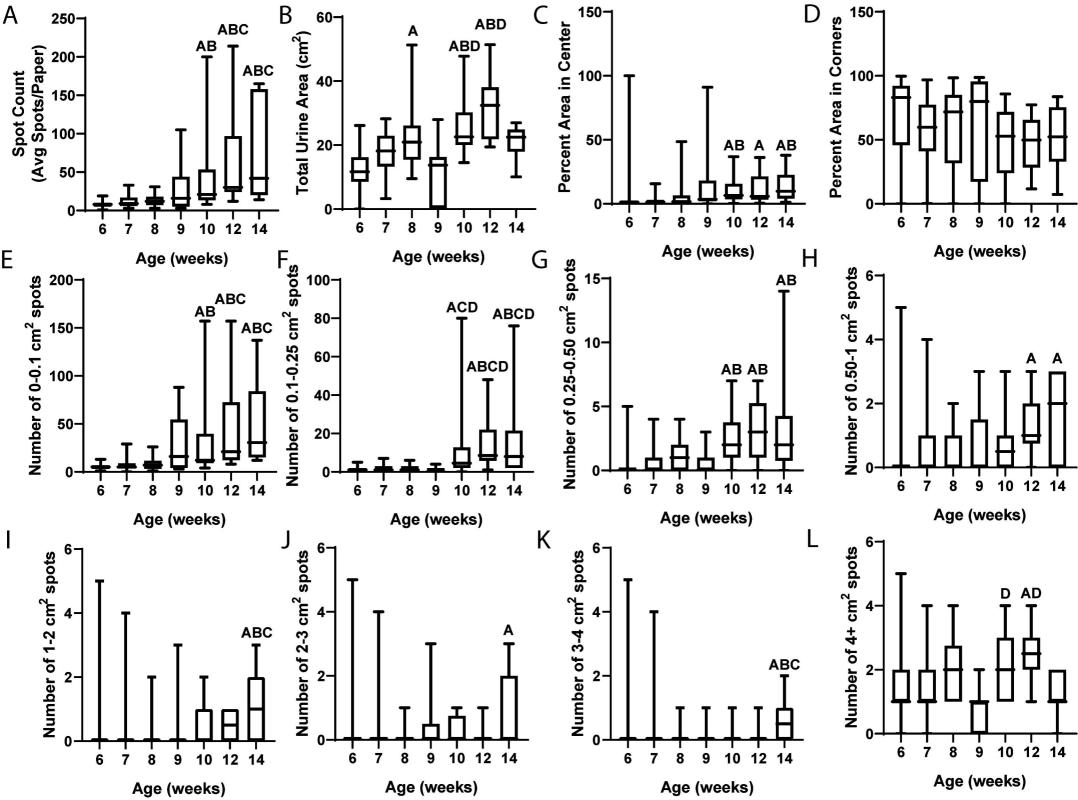
558

559 **Figure 4. Summary statistics for anatomical and physiological differences across**

560 **experimental groups evaluated in this study including intact male and female mice, and**

561 **female mice implanted with 1 cm silastic capsules containing crystalline testosterone**

562 **(Female w/ T).** All results are mean fold differences relative to intact female control. Differences

563 that are significantly higher than intact females are shown in green and those that are lower

564 than intact females are in red. Endpoints that do not significantly differ from intact females are

565 in gray. The numerical fold difference relative to intact females is indicated by the bar size

566 according to the legend at the top of the figure. Male urinary physiology is different from female

567 at baseline. The female voiding parameters affected by exogenous testosterone include many

568 of the same parameters that distinguish male from female voiding function.

569 **Table 1. Formulas for Calculated Cystometry Parameters**

570 **Table 2. Male and Female C57BL/6J Mouse Baseline Urinary Voiding Characteristics**

571 **Table 3. Impact of Age on VSA Characteristics**

572 **Table 4. Impact of Castration on Male Urinary Physiology**

573 **Table 5. Impact of Finasteride on Male Urinary Physiology**

574 **Table 6. Impact of Finasteride on Female Urinary Physiology**

575 **Table 7. Impact of Genetic Prostatic Luminal Cell Ablation on Male Urinary Physiology**

576 **Table 8. Impact of Testosterone on Female Urinary Physiology**

577 **Table 9. Impact of Testosterone on Intact Male Urinary Physiology**

578 **Table 10. Impact of Testosterone on Castrate Male Urinary Physiology**

579

Table 1. Formulas for calculated Cystometry Parameters

CMG Parameter	Abbreviation	Units	Description
Baseline Pressure	BP	$mmHg$	*lowest pressure of trace immediately after void; also defines starting point of new void (see figure 1, BP)*
Number of Non-Voiding Contractions	nNVC	$(no\ units)$	*Number of spikes in pressure prior to voiding that did not result in urine excretion (see figure 1, NVC)*
Threshold Pressure	TP	$mmHg$	*Pressure at start of urination (see figure 1, TP)*
Peak Void Pressure	PVP	$mmHg$	*Greatest pressure achieved during void(see figure 1, PVP)*
Normalized Peak Void Pressure	NPVP	$mmHg$	$PVP - BP$
Normalized Threshold Pressure	NTP	$mmHg$	$TP - BP$
Void Duration	VD	min	$(time\ at\ BP\ of\ void_{(n+1)})$ $- (time\ at\ TP\ of\ void_n)$
Voided Volume	VV	mL	$(mass\ of\ urine\ trace\ after\ void) -$ $(mass\ of\ urine\ trace\ prior\ to\ void)$
Intervoid Interval	IVI	min	$(time\ at\ PVP\ of\ void_{(n+1)})$ $- (time\ at\ PVP\ of\ void_n)$
Infusion Rate	IR	mL/min	*rate at which the pump infuses saline*
Infusion Duration	ID	min	$TP - BP$
Infused Volume	IV	mL	$IR \times ID$
Efficiency	E	%	$\dfrac{VV}{IV}$
Compliance	C	$mL/mmHg$	$\dfrac{IV}{(TP - BP)}$
Volume Flow Rate	VFR	mL/min	$\dfrac{VV}{VD}$
Mass Based Flow Rate	MBFR	mg/min	*slope of urine mass trace during active voiding*

Table 2. An Update to Male and Female Baseline Urination Characteristics

Gross Assessment			Values are: Average ± SEM
	Intact Male (n = 13)	Intact Female (n = 7)	p-value
Body Mass (g)	**24.45 ± 0.49**	**18.13 ± 0.40**	**<0.0001**
Bladder			
Mass (% Body Mass)	0.15 ± 0.01 (n=6)	0.13 ± 0.01	0.1639
Volume (mm^3/g Body Mass)	39.86 ± 7.46	22.72 ± 5.41	0.1366

Void Spot Assay			
	Intact Male (n = 9)	Intact Female (n = 7)	p-value
Count	32.11 ± 12.67	22.86 ± 1.16	0.4890
Total Area (cm^2)	**11.49 ± 3.17**	**23.84 ± 2.13**	**0.0089**
Percent area in center	15.78 ± 9.97	2.48 ± 1.48	0.0524^Δ
Percent area in corners	61.14 ± 13.26	65.12 ± 9.69	0.8220
Spots of a certain size (area)			
0 - 0.1 cm^2	28.11 ± 11.30	16.29 ± 0.68	0.9740
0.1 - 0.25 cm^2	1.00 ± 0.47	1.57 ± 0.43	0.2940
0.25 - 0.5 cm^2	0.67 ± 0.33	0.86 ± 0.14	0.2550
0.5 - 1 cm^2	0.67 ± 0.37	0.43 ± 0.20	>0.9999
1 - 2 cm^2	0.33 ± 0.33	0.29 ± 0.18	0.5500
2 - 3 cm^2	0.44 ± 0.34	0.29 ± 0.29	0.8500
3 - 4 cm^2	0.11 ± 0.11	0 ± 0	>0.9999
4+ cm^2	**0.78 ± 0.22**	**3.14 ± 0.26**	**<0.0010**

Cystometry			
	Intact Male (n = 6)	Intact Female (n = 7)	p-value
Void Duration	**0.64 ± 0.08**	**0.41 ± 0.02**	**0.0252**
Intervoid Interval	5.10 ± 1.18	3.50 ± 0.46	0.2430
Baseline Pressure	3.61 ± 0.31	2.68 ± 0.81	0.2800
Normalized Threshold Pressure	**3.35 ± 0.32**	**9.36 ± 1.16**	**<0.0001**
Normalized Peak Void Pressure	**18.37 ± 2.06**	**25.35 ± 1.11**	**0.0160**
Non-voiding contractions	2.26 ± 0.72	2.4 ± 0.61	0.8840
Voided Volume (x 10^{-2})	7.60 ± 1.74	4.72 ± 0.51	0.1660
Compliance (x 10^{-2})	**1.92 ± 0.49**	**0.52 ± 0.10**	**0.0025**
Volume Flow Rate (x 10^{-3})	1.98 ± 0.30	1.96 ± 0.21	0.9390
Mass Based Flow Rate	23.70 ± 4.09	16.80 ± 3.97	0.2530
Efficiency (%)	96.40 ± 3.98	104.20 ± 4.24	0.2080

Table 3. Impact of Age on VSA Characteristics

Void Spot Assay							Values are: Average ± SEM
	6-week-old	**7-week-old**	**8-week-old**	**9-week-old**	**10-week-old**	**12-week-old**	**14-week-old**
Count	8.33 ± 0.88	11.97 ± 1.50	13.20 ± 1.66	32.10 ± 12.70	53.45 ± 13.66^{A,B}	65.90 ± 20.43^{A,B,C}	75.10 ± 19.38^{A,B,C}
Total Area (cm^2)	12.68 ± 1.07	17.34 ± 1.22	21.89 ± 2.28^A	11.50 ± 3.17	25.91 ± 1.88^{A,B,D}	31.69 ± 3.08^{A,B,D}	21.01 ± 1.70
Percent area in center	5.26 ± 3.32	2.41 ± 0.69	6.31 ± 2.57	15.80 ± 9.97	10.74 ± 2.51^{A,B}	11.38 ± 3.95^A	14.08 ± 4.08^{A,B}
Percent area in corners	69.67 ± 5.94	59.22 ± 4.30	57.87 ± 7.45	61.10 ± 13.30	48.17 ± 6.24	47.64 ± 7.06	51.56 ± 8.19
Spots of a certain size (area)							
0 - 0.1 cm^2	5.37 ± 0.65	7.57 ± 1.17	8.10 ± 1.29	28.10 ± 11.30	35.45 ± 9.90^{A,B}	44.20 ± 15.56^{A,B,C}	50.20 ± 14.15^{A,B,C}
0.1 - 0.25 cm^2	1.10 ± 0.23	1.80 ± 0.35	1.55 ± 0.39	1.00 ± 0.47	11.95 ± 4.40^{A,C,D}	14.30 ± 4.47^{A,B,C,D}	16.20 ± 7.13^{A,B,C,D}
0.25 - 0.5 cm^2	0.23 ± 0.11	0.37 ± 0.16	1.00 ± 0.26	0.67 ± 0.33	2.25 ± 0.38^{A,B}	3.00 ± 0.75^{A,B}	3.30 ± 1.29^{A,B}
0.5 - 1 cm^2	0.20 ± 0.07	0.47 ± 0.16	0.45 ± 0.14	0.67 ± 0.37	0.75 ± 0.20	1.20 ± 0.29^A	1.60 ± 0.40^A
1 - 2 cm^2	0.07 ± 0.05	0.23 ± 0.16	0.15 ± 0.11	0.33 ± 0.33	0.50 ± 0.17	0.50 ± 0.17	1.10 ± 0.35^{A,B,C}
2 - 3 cm^2	0 ± 0	0.07 ± 0.05	0.05 ± 0.05	0.44 ± 0.34	0.25 ± 0.10	0.10 ± 0.10	0.80 ± 0.36^A
3 - 4 cm^2	0.03 ± 0.03	0.07 ± 0.05	0.05 ± 0.05	0.11 ± 0.11	0.15 ± 0.08	0.10 ± 0.10	0.60 ± 0.22^{A,B,C}
4+ cm^2	1.33 ± 0.12	1.40 ± 0.14	1.85 ± 0.21	0.78 ± 0.22	2.15 ± 0.22^D	2.50 ± 0.27^{A,D}	1.30 ± 0.21

^{A}-Significant difference from 6-week-old

^{B}-Significant difference from 7-week-old

^{C}-Significant difference from 8-week-old

^{D}-Significant difference from 9-week-old

Table 4. Impact of Castration on Male Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Intact Male (n = 13)	Castrate Male (n = 14)	p-value
Body Mass (g)	24.45 ± 0.49	21.51 ± 0.41	<0.0001
Bladder			
Mass (% Body Mass)	0.15 ± 0.01 (n=6)	0.12 ± 0.00 (n=6)	0.0190
Volume (mm^3/g Body Mass)	39.86 ± 7.46	18.04 ± 3.13	0.0041
Prostate Mass (% Body Mass)	0.20 ± 0.01	0.03 ± 0.00	<0.0001
Anterior Mass (% Body Mass)	0.09 ± 0.00	0.02 ± 0.00	<0.0001
Ventral Mass (% Body Mass)	0.04 ± 0.00	0.01 ± 0.00	<0.0001
Dorsal Mass (% Body Mass)	0.04 ± 0.00	0.01 ± 0.00	<0.0001
Lateral Mass (% Body Mass)	0.01 ± 0.00	0.00 ± 0.00	0.0003
Seminal Vesicle Mass (% Body Mass)	0.62 ± 0.04	0.04 ± 0.00	<0.0001

Void Spot Assay			
	Intact Male (n = 9)	Castrate Male (n = 11)	p-value
Count	32.10 ± 12.70	42.00 ± 6.10	0.1025
Total Area (cm^2)	11.50 ± 3.17	20.50 ± 2.31	0.0301
Percent area in center	15.80 ± 9.97	2.34 ± 0.666	0.1567
Percent area in corners	61.10 ± 13.30	77.40 ± 3.96	0.2177
Spots of a certain size (area)			
0 - 0.1 cm^2	28.10 ± 11.30	38.10 ± 5.84	0.1472
0.1 - 0.25 cm^2	1.00 ± 0.47	1.36 ± 0.43	0.5651
0.25 - 0.5 cm^2	0.67 ± 0.33	0.64 ± 0.15	0.6534
0.5 - 1 cm^2	0.67 ± 0.37	0.46 ± 0.21	0.9591
1 - 2 cm^2	0.33 ± 0.33	0.18 ± 0.12	>0.9999
2 - 3 cm^2	0.44 ± 0.34	0 ± 0	0.1895
3 - 4 cm^2	0.11 ± 0.11	0 ± 0	0.4500
4+ cm^2	0.78 ± 0.22	1.27 ± 0.14	0.1328

Cystometry			
	Intact Male (n = 6-7)	Castrate Male (n = 7-8)	p-value
Void Duration	0.64 ± 0.08	0.42 ± 0.03	0.0311
Intervoid Interval	5.10 ± 1.18	2.77 ± 0.12	0.0968^{Δ}
Baseline Pressure	3.61 ± 0.31	2.21 ± 0.26	0.0040
Normalized Threshold Pressure	3.35 ± 0.33	4.75 ± 0.73	0.1087
Normalized Peak Void Pressure	18.40 ± 2.06	17.20 ± 0.75	0.6145
Non-voiding contractions	2.26 ± 0.72	1.46 ± 0.25	0.3254
Voided Volume ($\times 10^{-2}$)	7.60 ± 1.74	4.05 ± 0.11	0.0977^{Δ}
Compliance ($\times 10^{-2}$)	1.92 ± 0.49	0.89 ± 0.20	0.0359
Volume Flow Rate ($\times 10^{-3}$)	1.98 ± 0.30	1.69 ± 0.16	0.3871
Mass Based Flow Rate	23.70 ± 4.09	23.4 ± 3.17	0.9452
Efficiency (%)	96.40 ± 3.98	96.8 ± 3.306	0.9381

Table 5. Impact of Finasteride on Male Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Males w/ Oil (n = 6)	Males w/ Fin (n = 9)	p-value
Body Mass (g)	24.67 ± 0.36	23.53 ± 0.73	0.2562
Bladder			
Mass (% Body Mass)	0.13 ± 0.01	0.12 ± 0.01	0.3701
Volume (mm^3/g Body Mass)	33.71 ± 9.25	41.35 ± 6.61	0.5016
Prostate Mass (% Body Mass)	**0.21 ± 0.01**	**0.14 ± 0.01**	**0.0009**
Anterior Mass (% Body Mass)	**0.11 ± 0.01**	**0.07 ± 0.00**	**0.0004**
Ventral Mass (% Body Mass)	**0.05 ± 0.01**	**0.04 ± 0.00**	**0.0467**
Dorsal Mass (% Body Mass)	0.03 ± 0.00	0.03 ± 0.00	0.3465
Lateral Mass (% Body Mass)	**0.02 ± 0.00**	**0.01 ± 0.00**	**0.0292**
Seminal Vesicle Mass (% Body Mass)	**0.72 ± 0.03**	**0.33 ± 0.03**	**<0.0001**

Void Spot Assay			
	Males w/ Oil (n = 7)	Males w/ Fin (n = 9)	p-value
Count	23.20 ± 4.92	24.10 ± 10.10	0.1692
Total Area (cm^2)	17.00 ± 4.33	18.40 ± 4.41	0.8209
Percent area in center	8.82 ± 7.54	12.10 ± 11.00	0.8845
Percent area in corners	48.90 ± 10.90	58.50 ± 13.30	0.3610
Spots of a certain size (dia.)			
0 - 0.1 cm	19.90 ± 4.36	21.00 ± 9.29	0.8658
0.1 - 0.25 cm	0.89 ± 0.35	1.22 ± 0.64	>0.9999
0.25 - 0.5 cm	0.89 ± 0.31	0.22 ± 0.15	0.1312
0.5 - 1 cm	0.11 ± 0.11	0.22 ± 0.22	>0.9999
1 - 2 cm	0.11 ± 0.11	0 ± 0	>0.9999
2 - 3 cm	0.11 ± 0.11	0.11 ± 0.11	>0.9999
3 - 4 cm	0.11 ± 0.11	0 ± 0	>0.9999
4+ cm	1.11 ± 0.31	1.33 ± 0.33	0.8013

Cystometry			
	Males w/ Oil (n = 9)	Males w/ Fin (n = 9)	p-value
Void Duration	0.63 ± 0.07	0.70 ± 0.13	0.8371
Intervoid Interval	**3.35 ± 0.44**	**4.74 ± 0.42**	**0.0394**
Baseline Pressure	3.96 ± 0.16	3.13 ± 0.51	0.1590
Normalized Threshold Pressure	3.64 ± 0.67	3.65 ± 0.56	0.9965
Normalized Peak Void Pressure	13.00 ± 0.99	15.90 ± 1.74	0.2011
Non-voiding contractions	1.21 ± 0.22	2.33 ± 0.53	0.0755^{Δ}
Voided Volume (x 10^{-2})	**4.24 ± 0.69**	**6.44 ± 0.47**	**0.0168**
Compliance (x 10^{-2})	1.24 ± 0.22	1.89 ± 0.31	0.1280
Volume Flow Rate (x 10^{-3})	1.31 ± 0.27	1.87 ± 0.23	0.1377
Mass Based Flow Rate	22.80 ± 3.63	17.10 ± 1.74	0.1497
Efficiency (%)	108.00 ± 20.40	110.00 ± 6.85	0.8371

Table 6. Impact of Finasteride on Female Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Female w/ Oil (n = 7)	Female w/ Fin (n = 5)	p-value
Body Mass (g)	18.49 ± 0.42	17.50 ± 0.31	0.0953$^{\Delta}$
Bladder			
Mass (% Body Mass)	0.12 ± 0	0.12 ± 0.01	>0.9999
Volume (mm^3/g Body Mass)	17.82 ± 3.88	17.27 ± 3.24	0.9162

Void Spot Assay			
	Female w/ Oil (n = 7)	Female w/ Fin (n = 5)	p-value
Count	23.30 ± 5.73	12.20 ± 3.16	0.1337
Total Area (cm^2)	**22.20 ± 2.72**	**11.70 ± 1.51**	**0.0083**
Percent area in center	0.65 ± 0.25	0.49 ± 0.33	0.1698
Percent area in corners	67.50 ± 12.60	73.70 ± 8.43	0.7023
Spots of a certain size (dia.)			
0 - 0.1 cm	17.90 ± 5.02	8.50 ± 2.68	0.1464
0.1 - 0.25 cm	1.29 ± 0.71	0.50 ± 0.34	0.6166
0.25 - 0.5 cm	0.14 ± 0.14	0.17 ± 0.17	>0.9999
0.5 - 1 cm	0.29 ± 0.18	0.33 ± 0.21	>0.9999
1 - 2 cm	0.29 ± 0.18	0.50 ± 0.34	0.7797
2 - 3 cm	0.14 ± 0.14	0.83 ± 0.31	0.0862$^{\Delta}$
3 - 4 cm	0.71 ± 0.71	0 ± 0	>0.9999
4+ cm	**2.57 ± 0.297**	**1.33 ± 0.21**	**0.0087**

Cystometry			
	Female w/ Oil (n = 7)	Female w/ Fin (n = 5)	p-value
Void Duration	0.51 ± 0.05	0.47 ± 0.03	0.5419
Intervoid Interval	2.90 ± 0.56	2.49 ± 0.30	0.5803
Baseline Pressure	5.70 ± 1.25	4.88 ± 0.83	0.6330
Normalized Threshold Pressure	11.3 ± 1.87	8.22 ± 0.75	0.2212
Normalized Peak Void Pressure	25.2 ± 1.32	21.70 ± 1.38	0.0965$^{\Delta}$
Non-voiding contractions	3.63 ± 0.1.12	1.64 ± 0.47	0.3422
Voided Volume (x 10^{-2})	4.10 ± 0.638	3.72 ± 0.42	0.6595
Compliance (x 10^{-2})	0.29 ± 0.04	0.37 ± 0.06	0.2640
Volume Flow Rate (x 10^{-3})	1.39 ± 0.20	1.37 ± 0.22	0.9335
Mass Based Flow Rate	17.90 ± 9.02	7.99 ± 1.10	0.4346
Efficiency (%)	111.00 ± 4.55	114.00 ± 6.44	0.7246

Table 7. Impact of Genetic Prostatic Luminal Cell Ablation on Male Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Pbsn4$^{cre/+}$; R26R$^{Td/+}$ (n = 8)	Pbsn4$^{cre/+}$; R26R$^{Dta/+}$ (n = 4)	p-value
Body Mass (g)	25.58 ± 0.59	24.35 ± 0.80	0.1414
Bladder			
Mass (% Body Mass)	0.15 ± 0.01	0.14 ± 0.00	0.5442
Volume (mm^3/g Body Mass)	35.52 ± 6.16	36.98 ± 10.11	0.8992
Prostate Mass (% Body Mass)	0.22 ± 0.02	0.20 ± 0.01	0.3434
Anterior Mass (% Body Mass)	0.11 ± 0.00	0.12 ± 0.01	0.4629
Ventral Mass (% Body Mass)	0.05 ± 0.01	0.06 ± 0.00	0.6707
Dorsal Mass (% Body Mass)	**0.05 ± 0.00**	**0.03 ± 0.00**	**0.0222**
Lateral Mass (% Body Mass)	**0.01 ± 0.00**	**0.00 ± 0.00**	**0.0242**
Seminal Vesicle Mass (% Body Mass)	0.76 ± 0.04	0.72 ± 0.03	0.5431

Void Spot Assay			
	Pbsn4$^{cre/+}$; R26R$^{Td/+}$ (n = 8)	Pbsn4$^{cre/+}$; R26R$^{Dta/+}$ (n = 4)	p-value
Count	78.10 ± 30.09	73.00 ± 54.70	0.3677
Total Area (cm^2)	36.60 ± 3.55	30.60 ± 6.96	0.4060
Percent area in center	5.47 ± 2.30	13.50 ± 13.30	0.2141
Percent area in corners	69.40 ± 6.49	56.00 ± 20.50	0.4413
Spots of a certain size (dia.)			
0 - 0.1 cm	67.30 ± 26.50	51.30 ± 36.10	0.3455
0.1 - 0.25 cm	6.00 ± 4.02	14.00 ± 13.70	0.3939
0.25 - 0.5 cm	1.25 ± 0.49	4.50 ± 4.17	0.8990
0.5 - 1 cm	1.00 ± 0.38	1.25 ± 0.95	>0.9999
1 - 2 cm	0 ± 0	0 ± 0	>0.9999
2 - 3 cm	0 ± 0	0 ± 0	>0.9999
3 - 4 cm	0.13 ± 0.13	0.25 ± 0.25	>0.9999
4+ cm	2.50 ± 0.27	1.75 ± 0.25	0.1293

Cystometry			
	Pbsn4$^{cre/+}$; R26R$^{Td/+}$ (n = 8)	Pbsn4$^{cre/+}$; R26R$^{Dta/+}$ (n = 4)	p-value
Void Duration	0.44 ± 0.06	0.44 ± 0.07	0.9972
Intervoid Interval	5.32 ± 0.80	3.76 ± 0.87	0.2546
Baseline Pressure	2.44 ± 0.26	2.34 ± 0.62	0.7471
Normalized Threshold Pressure	5.68 ± 1.59	3.65 ± 0.77	0.4083
Normalized Peak Void Pressure	19.70 ± 3.51	16.70 ± 4.80	0.6277
Non-voiding contractions	6.60 ± 2.06	2.12 ± 0.57	0.0695$^\Delta$
Voided Volume (x 10^{-2})	6.97 ± 1.09	6.14 ± 1.24	0.6720
Compliance (x 10^{-2})	1.81 ± 0.35	1.59 ± 0.452	0.7230
Volume Flow Rate (x 10^{-3})	3.69 ± 1.02	2.21 ± 0.214	0.3574
Mass Based Flow Rate	31.70 ± 2.49	19.90 ± 6.63	0.0672$^\Delta$
Efficiency (%)	107.00 ± 8.21	114.00 ± 12.80	0.6029

Table 8. Impact of Testosterone on Female Urinary Physiology

Gross Assessment

Values are: Average ± SEM

	Intact Female (n = 7)	Female w/ T (n=7)	p-value
Body Mass (g)	**18.13 ± 0.40**	**20.66 ± 0.32**	**0.0004**
Bladder			
Mass (% Body Mass)	0.13 ± 0.01	0.13 ± 0.01	>0.9999
Volume (mm^3/g Body Mass)	22.72 ± 5.41	30.43 ± 5.84 (n=6)	0.3534

Void Spot Assay

	Intact Female (n = 7)	Female w/ T (n=7)	p-value
Count	**22.86 ± 1.16**	**91.00 ± 19.95**	**<0.0001**
Total Area (cm^2)	**23.84 ± 2.13**	**17.82 ± 1.56**	**0.0415**
Percent area in center	2.48 ± 1.48	11.55 ± 4.15	0.0524$^\Delta$
Percent area in corners	65.12 ± 9.69	66.84 ± 9.72	0.9025
Spots of a certain size (area)			
0 - 0.1 cm^2	**16.29 ± 0.68**	**81.00 ± 15.43**	**<0.0001**
0.1 - 0.25 cm^2	1.57 ± 0.43	2.71 ± 1.41	0.9930
0.25 - 0.5 cm^2	0.86 ± 0.14	3.29 ± 2.80	0.4700
0.5 - 1 cm^2	0.43 ± 0.20	1.00 ± 0.53	0.6941
1 - 2 cm^2	0.29 ± 0.18	0.29 ± 0.29	0.7220
2 - 3 cm^2	0.29 ± 0.29	0.29 ± 0.29	>0.9999
3 - 4 cm^2	0 ± 0	0.29 ± 0.18	0.4620
4+ cm^2	**3.14 ± 0.26**	**2.14 ± 0.26**	**0.0189**

Cystometry

	Intact Female (n = 6)	Female w/ T (n = 5)	p-value
Void Duration	0.41 ± 0.02	0.42 ± 0.02	0.7880
Intervoid Interval	3.50 ± 0.46	3.39 ± 0.56	0.8860
Baseline Pressure	2.68 ± 0.81	2.99 ± 0.75	0.7860
Normalized Threshold Pressure	**9.36 ± 1.16**	**5.13 ± 1.13**	**0.0207**
Normalized Peak Void Pressure	**25.35 ± 1.11**	**17.76 ± 0.89**	**0.0006**
Non-voiding contractions	2.4 ± 0.61	3.68 ± 0.43	0.1700
Voided Volume (x 10^{-2})	4.72 ± 0.51	4.88 ± 0.98	0.8870
Compliance (x 10^{-2})	0.52 ± 0.10	0.83 ± 0.11	0.0714$^\Delta$
Volume Flow Rate (x 10^{-3})	1.96 ± 0.21	1.94 ± 0.34	0.9580
Mass Based Flow Rate	16.80 ± 3.97	32.50 ± 12.30	0.1620
Efficiency (%)	104.20 ± 4.24	111.80 ± 10.91	0.5050

Table 9. Impact of Testosterone on Intact Male Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Intact Male (n = 13)	Male w/ T (n = 8)	p-value
Body Mass (g)	24.45 ± 0.49	24.55 ± 0.56	0.9015
Bladder Volume (mm^3/g Body Mass)	39.86 ± 7.46	48.33 ± 8.91	0.3996
Prostate Mass (% Body Mass)	**0.20 ± 0.01**	**0.27 ± 0.03**	**0.0009**
Anterior Mass (% Body Mass)	**0.09 ± 0.00**	**0.13 ± 0.01**	**<0.0001**
Ventral Mass (% Body Mass)	0.04 ± 0.00	0.05 ± 0.01	0.1341
Dorsal Mass (% Body Mass)	0.04 ± 0.00	0.05 ± 0.01	0.7933
Lateral Mass (% Body Mass)	0.01 ± 0.00	0.03 ± 0.01	0.0829$^\Delta$
Seminal Vesicle Mass (% Body Mass)	0.62 ± 0.04	0.81 ± 0.11	0.4025

Void Spot Assay			
	Intact Male (n = 9)	Male w/ T (n = 8)	p-value
Count	32.10 ± 12.70	83.50 ± 29.49	0.0760$^\Delta$
Total Area (cm^2)	**11.50 ± 3.17**	**24.49 ± 3.41**	**0.0325**
Percent area in center	15.80 ± 9.97	4.26 ± 0.62	0.2043
Percent area in corners	61.10 ± 13.30	67.35 ± 11.98	0.7805
Spots of a certain size (area)			
0 - 0.1 cm^2	28.10 ± 11.30	76.00 ± 27.49	0.0696$^\Delta$
0.1 - 0.25 cm^2	1.00 ± 0.47	2.50 ± 1.26	0.3021
0.25 - 0.5 cm^2	0.67 ± 0.33	1.00 ± 0.41	0.4476
0.5 - 1 cm^2	0.67 ± 0.37	0.50 ± 0.29	>0.9999
1 - 2 cm^2	0.33 ± 0.33	1.00 ± 0.71	0.2028
2 - 3 cm^2	0.44 ± 0.34	0.50 ± 0.50	>0.9999
3 - 4 cm^2	0.11 ± 0.11	0.50 ± 0.50	0.7692
4+ cm^2	0.78 ± 0.22	1.50 ± 0.29	0.2042

Cystometery			
	Intact Male (n = 6-7)	Male w/ T (n = 8)	p-value
Void Duration	0.64 ± 0.08	0.63 ± 0.03	0.9266
Intervoid Interval	5.10 ± 1.18	5.67 ± 0.67	0.6714
Baseline Pressure	3.61 ± 0.31	2.91 ± 0.47	0.2477
Normalized Threshold Pressure	3.35 ± 0.33	3.84 ± 0.55	0.4781
Normalized Peak Void Pressure	18.40 ± 2.06	21.26 ± 1.90	0.3205
Non-voiding contractions	**2.26 ± 0.72**	**5.09 ± 0.37**	**0.0029**
Voided Volume (x 10^{-2})	7.60 ± 1.74	7.56 ± 1.15	0.9874
Compliance (x 10^{-2})	1.92 ± 0.49	2.23 ± 0.35	0.6342
Volume Flow Rate (x 10^{-3})	1.98 ± 0.30	2.08 ± 0.32	0.8395
Mass Based Flow Rate	23.70 ± 4.09	18.26 ± 2.61	0.2621
Efficiency (%)	96.40 ± 3.98	117.30 ± 19.39	0.4630

Table 10. Impact of Testosterone on Castrate Male Urinary Physiology

Gross Assessment			Values are: Average ± SEM
	Castrate Male (n = 14)	Castrate w/ T (n=8)	p-value
Body Mass (g)	21.51 ± 0.41	23.65 ± 0.69	0.0023
Bladder			
Volume (mm^3/g Body Mass)	18.04 ± 3.13	33.57 ± 6.89	0.0192
Prostate Mass (% Body Mass)	0.03 ± 0.00	0.21 ± 0.02	<0.0001
Anterior Mass (% Body Mass)	0.02 ± 0.00	0.10 ± 0.01	<0.0001
Ventral Mass (% Body Mass)	0.01 ± 0.00	0.05 ± 0.01	<0.0001
Dorsal Mass (% Body Mass)	0.01 ± 0.00	0.04 ± 0.00	<0.0001
Lateral Mass (% Body Mass)	0.00 ± 0.00	0.02 ± 0.00	0.0009
Seminal Vesicle Mass (% Body Mass)	0.04 ± 0.00	0.35 ± 0.07	<0.0001

Void Spot Assay			
	Castrate Male (n = 11)	Castrate w/ T (n = 4)	p-value
Count	42.00 ± 6.10	50.30 ± 14.90	0.5462
Total Area (cm^2)	20.50 ± 2.31	22.60 ± 8.62	0.7419
Percent area in center	2.34 ± 0.666	7.35 ± 5.93	0.8645
Percent area in corners	77.40 ± 3.96	72.80 ± 15.30	0.6846
Spots of a certain size (area)			
0 - 0.1 cm^2	38.10 ± 5.84	44.00 ± 13.20	0.6410
0.1 - 0.25 cm^2	1.36 ± 0.43	2.00 ± 0.82	0.5509
0.25 - 0.5 cm^2	0.64 ± 0.15	1.00 ± 1.00	0.5692
0.5 - 1 cm^2	0.46 ± 0.21	0 ± 0	0.3956
1 - 2 cm^2	0.18 ± 0.12	0.25 ± 0.25	>0.9999
2 - 3 cm^2	0 ± 0	0.50 ± 0.50	0.2667
3 - 4 cm^2	0 ± 0	0 ± 0	>0.9999
4+ cm^2	1.27 ± 0.14	2.50 ± 0.87	0.1692

Cystometry			
	Castrate Male (n = 7-8)	Castrate w/ T (n = 8)	p-value
Void Duration	0.42 ± 0.03	0.51 ± 0.04	0.1049
Intervoid Interval	2.77 ± 0.12	3.00 ± 0.45	0.6444
Baseline Pressure	2.21 ± 0.26	2.92 ± 0.61	0.3111
Normalized Threshold Pressure	4.75 ± 0.73	3.32 ± 0.58	0.1437
Normalized Peak Void Pressure	17.20 ± 0.75	16.00 ± 1.70	0.5400
Non-voiding contractions	**1.46 ± 0.25**	**3.58 ± 0.56**	**0.0039**
Voided Volume (x 10^{-2})	4.05 ± 0.11	4.60 ± 0.64	0.4184
Compliance (x 10^{-2})	0.89 ± 0.20	1.34 ± 0.31	0.1840
Volume Flow Rate (x 10^{-3})	1.69 ± 0.16	1.53 ± 0.18	0.5159
Mass Based Flow Rate	23.40 ± 3.17	20.60 ± 4.57	0.6126
Efficiency (%)	96.80 ± 3.31	90.74 ± 5.81	0.3993