

# Effect of Ovariectomy (OVX) on Uropathogenic E.coli (UPEC) Clearance in the Urothelially Restricted Estrogen Receptor Beta Overexpressing Transgenic Mouse



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#### Introduction

Urinary tract infection (UTI) is common in females. After menopause, the rates of UTI increase, and there is a greater probability that the UTI will become recurrent. Antibiotics are routinely used to treat UTIs, however the overuse of antibiotics can lead to unintended consequences such as antibiotic resistance and changes in host microbiome. Therefore, given the prevalence, methods and strategies other than antibiotics for the management of UTIs need to be explored further. The urothelial layer of the bladder is crucial to protection against UTI's, and a better understanding of host defense at the urothelial level is needed. Our lab created a transgenic mouse model that overexpresses estrogen receptor  $\beta$  (ER $\beta$ ) restricted to the bladder urothelium (uER $\beta$ -OE+). When inoculated with uropathogenic E. coli (UPEC), the normal estrous uER $\beta$ -OE+ female mice cleared UPEC significantly faster than two control groups (uER $\beta$ -OE- and wildtype C57BL/6), providing evidence that urothelial ER $\beta$  is important for defense against UTIs. We sought to determine whether estrogen is necessary in female uER $\beta$ -OE+ mice to clear UPEC infection more rapidly than negative litter mates and wild type controls.

#### Study design, materials and methods

Bilateral ovariectomies (OVX) were performed to eliminate the presence of estrogen. Surgeries were done on 3 groups of female 5-7 month old female mice via dorsal vertical midline incision on the same day. The presence of ovaries was confirmed by random pathology. The three groups include: (1)  $\mu$ ER $\beta$ -OE+ (n=6), (2) negative littermates  $\mu$ ER $\beta$ -OE- (n=5), and (3) wild type (WT) (n=6). The mice were given 4 weeks of recovery after OVX was performed. This time period was chosen because it allowed appropriate time for recovery from surgery and more importantly for elimination of estrogen. Thereafter, the three groups of mice were inoculated transurethrally with 2x10<sup>7</sup> CFU UPEC (UTI89). Urine was collected on 4 consecutive days for cultures to measure bacterial count. On the fourth day, the mice were sacrificed and the bladder and kidneys were homogenized for culture to measure the bacterial count.

#### Results

After inoculation of UPEC, there were no difference in the clearance of bacteria in the 3 cohorts of the animals (Figure 1). There was no difference in CFU between uER $\beta$ -OE+ vs. uER $\beta$ -OE-, uER $\beta$ -OE+ vs. WT and uER $\beta$ -OE- vs. WT of days 1, 2, 3 and 4. Also, the bladder and kidney CFU were not different between groups on day 4 (Figure 2).

### **Interpretation of Results**

Estrogen appears to be necessary for the  $ER\beta$ 's protective effect at the urothelial level against UTI. Furthermore, this may be the first step in elucidating pathophysiologic mechanisms as to why postmenopausal females are prone to urinary tract infections compared to pre-menopausal females. Four weeks after OVX, UPEC clearance rates were similar between the uERβ-OE+ and controls. Conversely, in prior experiments, we found that pre-menopausal uERβ-OE+ mice cleared UPEC infections significantly faster compared to two control groups. A caveat is that his experiment utilized a lover UPEC bacterial count inoculation (2x10<sup>7</sup> CFU) compared to our prior experiment where a higher load (2x10<sup>8</sup> CFU) was used.

#### Conclusion

Removal of ovaries abrogated urothelial ER $\beta$ 's protective effect against UTI in a murine model. Future directions include studying the difference of bacterial clearance based on the CFU inoculated as well as further exploration downstream pathways activated by ER $\beta$  activation.



Figure 1- Geometric mean +/- standard deviation of daily UPEC bacterial CFU counts. There is no significant difference between groups on any day (ER+ vs. ER-, ER+ vs. WT, ER- vs WT; P > 0.05 for days 1-4)



Figure 2 - Geometric mean +/- standard deviation of bladder and kidney day 4 CFU counts. There is no significant difference between groups for either organ. (ER+ vs. ER-, ER+ vs. WT, ER- vs WT; P > 0.05 Bladder and kidney)