

## **Active Testosterone Treatment Impairs In Vitro Fertilization (IVF) Outcomes in a Female Mouse Model for Gender-Affirming Testosterone with Improvement in Outcomes Following Cessation of Testosterone Treatment**

Amanda Schwartz. *University of Michigan*

Amanda R. Schwartz<sup>1</sup>, Min Xu<sup>1</sup>, Vasantha Padmanabhan<sup>1</sup>, Ariella Shikanov<sup>1</sup>, Molly B. Moravek<sup>1</sup>.

<sup>1</sup>University of Michigan, Ann Arbor, Mich.

**Introduction:** The impact of testosterone (T) on reproductive potential is poorly understood. Masculinizing hormones have demonstrated mixed effects on ovarian histology and data on assisted reproductive outcomes are limited to small case series. There is consensus among major medical societies to encourage fertility preservation counseling prior to initiation of gender-affirming hormone care, however, it is uncertain whether a break in T treatment prior to undergoing oocyte cryopreservation is beneficial. We hypothesized that T treatment would not have an impact on IVF outcomes.

**Methods:** C57BL/6N female mice were implanted with silastic tubing with either 10 mg testosterone enanthate in ethanol (n = 20) or ethanol alone (n = 18) at 10 weeks of age. Four groups of C57BL/6N female mice were used in this study: 1) current T implant 2) current sham implant 3) T cessation 4) control cessation. Biweekly blood samples and vaginal cytology were undertaken to monitor T levels and estrous cycles. For groups 1 and 2, mice were stimulated twelve weeks following implantation. For groups 3 and 4, implants were removed after twelve weeks and mice were stimulated two weeks after explantation. All mice were stimulated with 0.2 mL intraperitoneal CARD HyperOva followed 48 hours later by 7.5 IU intraperitoneal human chorionic gonadotropin (hCG). Oocytes were collected 14 hours after hCG injection and fertilized in vitro with sperm from 12-week-old B6D2F1/J male mice. 2-cell embryos were transferred into the oviducts of pseudopregnant recipient females. The study was powered to detect a 25% difference in 2-cell embryos. Data were analyzed using Chi-squared and unpaired t-tests with Prism 9.0.

**Results:** All T treated mice ceased cycling within one week of implantation and developed clitoromegaly. The current T implant group had fewer total oocytes retrieved (17.00 vs 36.00;  $p < 0.0001$ ), mature oocytes (13.00 vs 28.10;  $p = 0.0002$ ) and 2-cell embryos (12.78 vs 26.90;  $p = 0.0007$ ) as compared to the current sham implant group. There was no significant difference in maturity (76.47% vs 78.06%;  $p = 0.6937$ ) or fertilization rate (75.16% vs 74.72%;  $p = 0.9161$ ). Pseudopregnant females who had 2-cell embryos transferred from current T implant mice were less likely to have a live birth than those with transfers from current sham implant mice (25.00% vs 80.00%;  $p = 0.0196$ ). In contrast, comparison of IVF outcomes in the T cessation group with corresponding control cessation group revealed no significant differences between total oocytes (22.30 vs 30.75;  $p = 0.0708$ ), mature oocytes (20.80 vs 27.75;  $p = 0.1288$ ), or 2-cell embryos (20.40 vs 27.38;  $p = 0.104$ ).

**Conclusion:** In a mouse model of gender-affirming testosterone, active T treatment negatively impacted IVF outcomes. Improved outcomes following cessation of T treatment support reversibility of T impact on reproductive potential.

Presentation Type: Oral Session

Presentation Date: Monday, June 13

Presentation Time: 11 AM-12:30 PM

Location: B405