Elevated Testosterone Attenuates Vaginal Wall Thickness in Rhesus Macaques

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Rhesus macaques exhibit 28-day menstrual cycles similar to those of women and are excellent animal models for assessing the effect of exogenous steroids on the reproductive tract. This study contrasted the effect of high-dose testosterone (T) therapy, similar to that used for gender-affirming care, with low-dose T, similar to the hyperandrogenemia seen in women at risk for polycystic ovary syndrome (PCOS). Our focus was on vaginal wall thickness, as this is implicated in several disorders including risk for vaginal prolapse. **In Exp.1**, ovariectomized macaques were treated with Silastic capsules releasing estradiol (E) and progesterone (P) to create artificial 28-day menstrual cycles. Similar capsules were employed to administer testosterone (T) to a subset of the monkeys. Vaginal wall samples were collected during the artificial proliferative phases (80-100pg E/ml; n=4) and secretory phases (E plus 5-6 ng/ml P; n=3) and after 28 days of E plus T (serum T =12.2+1.3 ng/ml n=4). **In Exp. 2**, 9 cycling monkeys received Silastic capsules that produced serum levels of 1.18 $\pm$ 0.12 ng T/ml, similar to those reported for women at risk for PCOS and does not disrupt menstrual cycles. Vaginal samples were collected in the proliferative phase (n=4) and in the secretory phase (n=5). **In Exp. 3,** cycling monkeys were treated with a high dose T (5.3+2.1 ng/ml) for 30 days, which suppresses cyclicity. All vaginal samples were embedded in tissue tek OCT, and frozen in liquid propane. Cryosections were analyzed histologically for epithelial thickness, extent of cornification, and immunohistochemistry (IHC) was performed to analyze ERα, PR, AR and Ki-67 (a marker of cell proliferation) expression. ER, PR, and AR staining was quantified by h-score. Ki-67 was quantified by counting positive cells. In the epithelium and subepithelial stroma high expression of ER, PR, and AR was observed, with minimal cyclic changes in tissue/cellular localization. Vaginal wall thickness was maximal (>2 mm) and markedly cornified following E treatment alone/during the follicular phase. Vaginal wall thickness and Ki-67 positive epithelial cells were reduced in the secretory phase ($P<0.05$). Mild hyperandrongenima did not affect vaginal wall thickness, Ki-67 positive cells, or the localization of ER, PR and AR, potentially due to continued menstrual cyclicity during this treatment. In contrast, high dose T (>5ng/ml), which disrupted menstrual cyclicity, resulted in loss of vaginal cornification and a thinning of the epithelium ($P<0.05$). High-dose T also reduced epithelial cell proliferation (Ki-67; $P<0.05$) and reduced the abundance of ER and PR-positive epithelial cells ($P<0.01$). In conclusion, mild hyperandrogenemia, as observed in women with PCOS does not affect vaginal integrity, or steroid receptor expression, whereas high-dose T therapy may result in clinical complications due to vaginal thinning. Supported by NIH OD011092.