Ovarian Infusion of Anti-Müllerian Hormone Does Not improve Fertility Outcomes in Reproductively Aged Rhesus Macaques

Adam J. Krieg[1,2], Kang Wang[1,2], Shally Wolf[1], Emily Mishler[1], Fernanda C. Burch[1], Nadine Piekarski[1], Kaya L. Burd[1], Carol Hanna[1], Mary B. Zelinski[1,2], Jing Xu[1,2,3].

1. Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR, USA
2. Department of Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR,
3. Current address: Department of Biology and Chemistry, Liberty University, Lynchburg, VA, USA

Anti-Müllerian Hormone (AMH) plays an important role in the regulation of follicle growth in mammalian ovaries. At the mechanistic level, expression of AMH by the growing follicle pool is essential for suppressing the activation of primordial follicles and maintaining follicle reserve in rodent models. In non-human primate models, AMH has been shown to promote the growth of secondary and pre-antral follicles *in vitro* and *in vivo*, but suppresses the development of antral follicles. Expression of AMH by the growing follicle pool in humans is used as a proxy for follicle reserve, guiding therapeutic approaches for infertility patients. These observations then raise the question: Can manipulation of AMH in humans with low follicle reserve increase the pool of growing follicles, thereby increasing the total number of follicles available for retrieval? In order to answer this question, reproductively aged female rhesus macaques (N=5, range of 17-21 years of age, roughly equivalent to 40-45 yo women) underwent ovarian catheterization in a cross-over longitudinal design to directly infuse ovaries with recombinant human AMH (N=2, 25 ng/hr) or PBS (N=3, 0.25 µl/hr) for 60 days. Animals underwent controlled ovarian stimulation (COS), and ultra-sound guided follicle aspiration for *in vitro* fertilization (IVF). Animals then underwent 2 menstrual cycles of recovery with saline infusion, followed by a final 60-day infusion of either PBS (N = 2) or AMH (N =3), ending with a final COS-IVF cycle. Overall oocyte yield, maturation, fertilization rate, embryo cleavage rate, and blastocyst formation rate were quantified. Levels of serum estradiol, progesterone, and AMH levels were measured in every cycle. Mean values for each parameter from each treatment group were compared using Wilcoxon matched paired tests. Aggregating the COS and embryo data by treatment group demonstrated no significant differences in the overall numbers of oocytes retrieved, the numbers and proportions of MII oocytes, the number and ratios of fertilized oocytes formed, and the numbers and ratios of cleavage stage embryos. Although not statistically significant (P=0.125), the blastocyst rate with PBS treatment was over 2 times higher than with AMH treatment (59.23% ± 24.86% vs 25% ± 35.36). Additionally, although there were no notable differences in estradiol or progesterone for the COS cycles, there was a trend (P = 0.15, One-Way ANOVA) for decreased serum AMH when animals (N=4) were treated with recombinant AMH compared to PBS or saline infusions (2.60 ±4.24 ng/mL vs. 8.12 ± 6.12 ng/mL vs 7.7 ± 5.4 ng/mL), suggesting negative feedback signaling. The results of these ongoing studies suggest that exogenous administration of AMH to the primate ovary neither increases the total number nor overall quality of the oocytes, and may inhibit blastocyst formation. Further study in nonhuman primates is required to determine if altering the dose or timing of AMH administration can improve fertility outcomes in reproductively aged women.

This work is supported by NIH R01HD082208 (JX and AJK) and NIH P51OD011092 (ONPRC Core grant), with excellent support from the ONPRC Surgical Services Unit and the Endocrine Technologies Core.