Luteinizing Hormone Stimulation of Germ-Free and Conventionalized Female Mice Reveals Microbiome-Dependent Effect on Ovarian Steroidogenesis and Ovulation

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Germ-free female mice have decreased litter sizes and increased pregnancy intervals compared to conventionally raised mice, suggesting that the microbiome affects their ovulation and/or oocyte quality. However, little is known about how the absence of a microbiome affects ovulatory stimulation and oocyte maturation. The hypothesis of this study was that female mice lacking a microbiome have reduced fertility due to alterations in the ovarian response to LH stimulation. To test this hypothesis, we utilized two strains of mice, C57BL6/J (B6) and C3H/HeN (C3H). Mice from each strain were devoid of all microbiota (GF; B6-GF n=10, C3H-GF n=8) or conventionalized via oral gavage with fecal microbiota from conventionally reared mice upon reaching mature body weight (CVZ; B6-CVZ n=9, C3H-CVZ n=8). All mice were fed normal rodent chow until 14-16 weeks of age. Females were stimulated to ovulate with 5 IU PMSG and 5 IU hCG. Following hCG injection, only 44% of B6-GF females ovulated, compared to 100% of B6-CVZ females ($P<0.01$). There was no difference in ovulation between C3H-CVZ (87.5%) and C3H-GF (100%). Additionally, the number of oocytes ovulated was significantly lower ($P<0.05$) in B6-GF (11±2.2) compared to B6-CVZ (17±1.2), while there was no difference between C3H-CVZ (13.7±2.5) and C3H-GF (11.0±1.3). We then assessed steroidogenesis in response to ovulatory stimulation. Blood serum was obtained from all females to determine post-ovulatory circulating estradiol (E2) and progesterone (P4) concentrations. We detected a 5-fold increase ($P<0.01$) in E2 concentration in C3H-GF compared to C3H-CVZ. However, E2 was not different between B6-CVZ and B6-GF. Conversely, progesterone (P4) tended to be decreased ($P=0.09$) in B6-GF compared to B6-CVZ, while there was no difference between C3H groups. The E2/P4 ratio was significantly increased ($P<0.05$) in B6-GF compared to B6-CVZ and tended to be increased ($P=0.06$) in C3H-GF compared to C3H-CVZ females. Consistent with these changes in circulating steroids, the mRNA abundance of steroidogenic enzymes within whole ovaries was also altered. The abundance of *Star,* which moves cholesterol into the mitochondria, was increased ($P<0.01$) in B6-GF compared to B6-CVZ, but not in C3H ovaries. Conversely, *Hsd3b*, which converts pregnenolone to P4, tended to be increased ($P=0.09$) in C3H-GF compared to C3H-CVZ, but not between B6-GF and B6-CVZ females. *Cyp17a1*, which converts progesterone to androgens, was significantly increased ($P<0.01$) in B6-GF compared to B6 CVZ, while there was a tendency ($P=0.08$) for decreased *Cyp17a1* in C3H-GF compared to C3H-CVZ. Lastly, *Cyp19a1*, which aromatizes androgens to estrogens, was significantly decreased ($P<0.05$) in C3H-GF compared to C3H-CVZ, but not in B6. To determine whether the lack of a microbiome affected oocyte maturation and developmental competence, we assessed spindle structure and performed qPCR to quantify markers of cumulus expansion. There were no differences in the percentage of normal spindles between B6 (CVZ, 83%; GF, 71%) or C3H (CVZ, 73%; GF, 78%) experimental groups. There was also no difference in the expression of *Has2*, *Tnfaip6*, or *Ptx3* in cumulus cells from GF and CVZ mice from either strain. However, there was a significant decrease ($p<0.01$) in the abundance of *Ptgs2* in cumulus cells from B6-GF compared to B6-CVZ, but not C3H-GF and C3H-CVZ. Taken together, these data suggest the microbiome does not affect nuclear maturation. Rather, it may be essential for LH responsiveness of somatic cells and the physiological process of ovulation.