Double knockout of *NR5A1* and *NR5A2* in the mouse ovary disrupts follicle development and ovulation

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The two orphan receptors *Nr5a1* (Steroidogenic factor 1; SF-1) and *Nr5a2* (Liver receptor homolog receptor-1; LRH-1) are essential for ovarian function. Previous studies have shown that LRH-1 regulates multiple processes necessary for ovulation, while SF-1 plays roles in follicle assembly and subsequent development. Both receptors bind to the same motif sequence to regulate gene transcription, and studies indicate that they act on both common and distinct gene targets. Individual depletion of SF-1 or LRH-1 suggests there is little compensation of one for the other in the ovary. To further understand the roles of these receptors, we established a model of conditional depletion of both *SF-1* and *LRH-1,* using Cre recombinase under the control of the *Amhr2* promoter (*Amhr2Cre; Nr5a1 f/f; Nr5a2 f/f;* dKO). In the resultant mouse strain, the LRH-1 and SF-1 signals were abolished in granulosa cells of dKO ovaries, while SF-1 persisted in cells of the theca and the ovarian stroma. Tabulation of the follicle populations at postnatal day (PND) 4 revealed that the ovarian reserve was reduced, in that there were five-fold fewer primordial follicles in the dKO ovaries compared to controls, indicating an effect on follicle assembly. The primary follicle population was similarly disrupted at PND4, with an approximate threefold reduction in dKO ovaries relative to control, demonstrating an effect on the activation of primordial follicles. This was further in evidence at PND13, where the population of secondary follicles was half of that in control ovaries. In adult (five months of age) dKO mice, the primordial follicle population was entirely depleted, with near depletion of primary and secondary follicles. As some antral follicles persisted at five months, we sought to determine whether antral follicles in dKO mice were capable of ovulation. Immature (25-28 days of age) dKO and control mice were treated with 5IU eCG followed by 5IU hCG. The control mice displayed a mean of 39 ± 5 ovulations, while only a few of the dKO mice ovulated <3 oocytes. Analysis of transcript abundance by qPCR in dKO and control ovaries following gonadotropin treatment indicated that concurrent depletion of LRH1 and SF-1 dysregulated genes associated with steroidogenesis, extracellular matrix formation, and cell proliferation, among others. We conclude that the combination of depletion of LRH-1 and SF-1 in mouse granulosa cells has profound negative effects on follicle assembly, follicle activation, further follicle development, and ovulation.

Supported by PJC 166020 from the Canadian Institutes of Health Research to BDM and NG.