Progesterone Receptor Component (PGRMC) 1 and 2 Regulate Primordial Follicle Formation. Agnes Asubonteng, Melissa Pepling

The ovarian reserve consisting of primordial follicles at the time of birth defines fertility in most female mammals. Each primordial follicle consists of one oocyte surrounded by a layer of granulosa cells. These oocytes start off as primordial germ cells which divide mitotically but with incomplete cytokinesis so that cells are connected by intercellular bridges, forming cysts. The germ cells enter meiosis and progress through prophase 1 to the diplotene substage. Next, cysts break apart, releasing individual oocytes which are each then surrounded by a layer of granulosa cells forming a primordial follicle containing a diplotene arrested oocyte. The mechanisms controlling these processes remain unclear. Our model is that normally maternal progesterone (P4) prevents premature cyst breakdown and primordial follicle formation. Yet, how the developing ovary receives the P4 signals is unknown. Though P4 signals through both nuclear and membrane receptors, studies have demonstrated active P4-membrane signaling during cyst breakdown. Therefore, our work explores whether the P4 signals are received through P4 membrane receptors, progesterone receptor component (PGRMC) 1 and 2. We also ask whether PGRMC 1 and 2 have roles independent of P4 signaling. Here we used antibodies against PGRMC1 and 2 in whole mount immunohistochemistry to determine if both receptors are present prior to and during primordial follicle formation. Ovaries were harvested at 15.5 and 17.5 days post coitum (dpc) as well as postnatal day (PND). Our results showed PGRMC1 and 2 expression in both oocytes and somatic cells before birth and gradually becoming localized in the cytoplasm of oocytes over time. Furthermore, we used an organ culture system which is a well-established technique that permits us to directly observe the roles of PGRMC1 and 2 on primordial follicle similar to in vivo. Ovaries were collected at 17.5 dpc and PND 1, placed in culture, and treated with control media, with IgG or 20ug/ml of PGRMC 2 function blocking antibody for 4 to 5 days. Our results showed a significant decrease in the percent single oocytes, our measure of primordial follicle formation in PND 1 cultured PGRMC 2 ovaries compared to the control. There was no significant change in the percent single oocytes of 17.5 dpc cultured PGRMC 2 ovaries compare to the control. These results suggest PGRMC 2 may either function independently after birth or requires P4 binding prior to birth to prevent cyst breakdown. PND 1 ovaries were also treated with control media or both PGRMC 1 and 2 function blocking antibodies together, for 5 days to test whether PGRMC 1 and 2 work together to prevent cyst breakdown. There was no significant change in the percent single oocytes of both control and PGRMC 1 and 2 treated ovaries. Our findings suggest a role for PGRMC 2 in the establishment of the ovarian reserve. Currently, we are also testing the role of PGRMC 1 alone during primordial follicle formation.