Elucidation and *in vitro* reconstitution of gonadal somatic cell specification machineries in chicken and mouse

**Summary**

Gonadal somatic cells (GSoCs) support gametogenesis, trigger sex differentiation during embryonic development, and are essential for induced primordial germ cells (PGCLCs) to develop into functional egg or sperm. Identifying and reconstituting the mechanisms underlying the differentiation of GSoCs are important. However, how GSoCs, located at the coelomic mesoderm (CoM) at the ventral part of the mesonephros, are specified in earlier embryos is unclear. We performed lineage tracing and enabled pinpoint gene manipulation in the CoM of chicken embryos. The results clarified that ventral cells of medial CoM are differentiated to GSoCs by Hedgehog–BMP signaling, whereas dorsal cells form the mesonephric capsule. This different outcome is determined by Sonic hedgehog secreted from the endoderm. From these findings, we established a method to reconstitute female GSoCs development from mouse embryonic stem cells. Single-cell RNA-sequencing analysis showed that induced female GSoCs (FOSLCs) differentiate into all subpopulations constituting female gonad (ovary). When FOSLCs are aggregated with PGCLCs to form embryonic gonad, they undergo feminization and form numerous follicles, where oocytes are surrounded by granulosa and theca cells. In addition, fertilized metaphase II oocytes from reconstituted follicles develop into offspring. Our studies identify a method to elucidate and reconstitute gonadogenesis of various animals.