



Title	Unique morpho-functional characteristics of folliculogenesis found in the ovary of cotton rat (<i>Sigmodon hispidus</i>) [an abstract of dissertation and a summary of dissertation review]
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学位論文内容の要旨
Abstract of the dissertation

博士の専攻分野の名称：博士（獣医学）

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学位論文題名
The title of the doctoral dissertation

Unique morpho-functional characteristics of folliculogenesis found in the ovary of cotton rat
(*Sigmodon hispidus*)
(コットンラット (*Sigmodon hispidus*) の卵胞形成にみられる特異な形態機能)

The reproductive characteristics and ovarian development in cotton rats (*Sigmodon hispidus*, CRs) are unclear, although CRs are commonly used as animal models in biomedical research. Moreover, folliculogenesis is a research topic with many unanswered questions in mammals. Most ovarian follicles contain only a single oocyte having a single nucleus in mammals. However, two or more oocytes and nuclei are observed within one follicle and one oocyte respectively in several species, including CR. Our investigation revealed unique morphologies in CR ovaries, particularly in oocytes. CR ovaries at postnatal days (PND) 0, 4, 7 or 4 weeks or 6-8 weeks of age were obtained from the Hokkaido Institute of Public Health (HIS/Hiph) and another inbred strain of CR at 4 weeks of age were obtained from the University of Miyazaki (HIS/Mz). Samples were analyzed by light and transmission electron microscopy.

In Chapter 1, the follicles were developed up to secondary stages in neonatal CR ovaries at PND0 to 7, while double nucleated oocytes (DNOs) were observed at PND0, and multi-oocyte follicles (MOFs) started to develop from PND4. With ages, DNOs were also decreased, but MOFs increase with the progression of the follicle development. Like single oocyte follicles (SOFs), MOFs expressed the oocyte marker, DEAD-box helicase 4 (DDX4), and fewer oocytes were positive TUNEL analysis than ICR mice, and granulosa cells surrounding MOFs and DNOs showed proliferative activity. Moreover, like SOF, mitochondrial clouds (MCs) assembly was

observed throughout the cytoplasm of MOFs and DNOs. These findings indicated that early folliculogenesis events as nest breaking and oocyte vitality rather than proliferation and cell death in each oocyte affect the unique ovarian phenotypes, including MOFs or DNOs.

In Chapter 2, comparison between two strains at 4 weeks of age as the time when the first estrus and spring onset of CR, HIS/Hiph and HIS/Mz, MOFs and DNOs were observed in all types of developing follicles at HIS/Hiph, whereas HIS/Mz had MOFs upto secondary stages and lacked DNOs. MCs assembly was also observed throughout the cytoplasm of all oocytes. However, immunostaining indicated that there was no clear strain differences in the appearance of positive oocytes in MOF or DNOs for the mediators of proliferation (Ki67, PCNA) and cell cycle (p63). These results indicated that the number of follicles and the appearance of MOFs and DNOs depended on the genetic background of CR.

In Chapter 3, the ovaries of CRs (HIS/Hiph, 6-8 weeks old) that had reached the fertile stage were scrutinized. MOFs was found in all follicular stages and DNOs also found except tertiary follicles. MOFs and DNOs were maintained the expression of DDX4 but MCs assembly was polarized in the cytoplasm of oocytes. These results indicated that the characteristic phenotypes of CR ovaries, especially the appearance of MOFs and DNOs, are maintained from the neonatal period to the fertile period.

Therefore, these analysis of unique phenotypes of CR ovary might contribute to the understanding of mammalian folliculogenesis, oocyte formation, and follicular disorders, indicating that CRs might be useful as an animal model in these areas.