学 位 論 文 の 要 旨

論文題目　Mechanisms by which Bacterial and Viral Pathogens cause Ovarian dysfunction.

（病原体感染による卵巣機能不全メカニズムに関する研究）

広島大学大学院生物圏科学研究科
Bioresource Science 専攻
学生番号　D186658
氏　　名　Adedeji Olufemi Adetunji

Summary

Infertility is a major cause for concern especially in parts of the world that place a high value on childbearing. Besides, sub-fertility or infertility often causes heavy losses to breeders and dairy farmers as they require the animals to get pregnant for milk production and herd expansion. Infectious diseases of bacteria or viral origin induces inflammatory responses from the host, however, the mechanism by which they impact negatively on reproductive health is unknown. These studies aim to determine the deleterious effect of infectious diseases on ovarian functioning at a cellular and molecular level using the mouse model approach.

1. **Study on the effect of LPS on overall ovarian health and Epigenetic dynamics of the *Lhcgr* and *Cyp19a1* gene promoter region**

Lipopolysaccharide (LPS) is an endotoxin and a component of the cell membrane of gram-negative bacteria, and it can pass through the protective physical barriers and tight junctions of the basement membrane. Apart from having been isolated from the genital tract of animals suffering from uterine damage and ovarian dysfunction, LPS action affects the epigenetic signature of genes regulating and inducing a cycle of methylation in the DNA around the promoter region. In Experiment 1, it was hypothesized that bacterial infection changes the DNA methylation status of the *Lhcgr* and *Cyp19a1* promoter regions. To clear this hypothesis, granulosa cells and ovaries were collected from immature mice treated with eCG or with eCG and LPS injection intraperitoneally. More so, granulosa cells were cultured in DMEM/F12 with

or without supplement and the addition of LPS (0.1 and 1 μg/ml LPS). Likewise, the expression of DNMT1 in granulosa cells of mice treated with LPS was investigated (*in vivo* and *in vitro*) and the epigenetic dynamics of LPS on DNA methylation was studied. In the results, Normal large antral follicles were observed in ovaries obtained from eCG and LPS coinjected mice, and the morphology of the ovaries was similar to that observed in the control group (eCG-injected mice). These antral follicles were not deemed atretic because few TUNEL-positive cells were observed. However, the granulosa cells of large antral follicles did not acquire the ability to respond to hCG stimulation. Also. the number of ovulated oocytes was significantly lower in LPS-injected mice after superovulation compared to mice that were not exposed to LPS. It was also observed that the low reactivity was caused by the limited expression of the *LHCGR* gene, which encodes the LH receptor in granulosa cells as well as an LPS-induced increase in the level of Dnmt1 expression both *in vivo* and *in vitro*. The methylation rate of the *LHCGR* promoter region was significantly higher in granulosa cells obtained from the LPS treatment group compared with the control group. Together, these findings demonstrated that the decrease in the expression of *Lhcgr* and Cyp19a1 as well as the induction of chemokines is due to bacterial infection/LPS which leads to ovarian follicular cysts in humans and animals. More so, the decrease in the induction of *Lhcgr* and *Cyp19a1* due to LPS exposure is a result of the epigenetic regulatory action of LPS.

2. Study on the roles of Toll-like receptor 7/8 in mouse ovary

Viral particles and TLR7/8 agonists (Resiquimod) are detected by the host through Pattern Recognition Receptors (PPRs) and they induce type 1 interferon (IFN) and pro-inflammatory cytokines. The presence of receptors for specific pathogen allows for entry and transfer of elements across the host plasma membrane. In experiment 2, it was hypothesized that TLR7/8 are expressed in mouse ovaries and TLR7/8 agonist (R848) causes a negative impact on the structure and functioning of the ovary. To clear this hypothesis, the mechanism by which TLR7/8 causes ovarian dysfunction was studied by injecting TLR7/8 agonist (R848) to eCG-

superovulated immature mice and culturing granulosa cells in DMEM/F12 medium with and without R848 (1 and 10nM dosage). It was observed that R848 increased the production of cytokine and chemokines in the spleen of mice. Also, in mice treated with R848, the number of matured oocytes and fertilization rate was reduced. More so, R848 was capable of causing a dramatic increase in genes associated with progesterone receptor and epidermal growth factors such as *Areg, Ereg*, *Cyp11a1*, *Star*, *PgR*, *Snap25* in both *in vivo* and *in vivo* studies. For the first time, R848 was reported to prompt the luteinization of follicles. It was also established that *ACE2* and *CD163* which are receptors for SARS-CoV-2 and PRRS are expressed in mouse ovaries. Although TLR7/8 and ACE2 receptor was expressed in the different time points, it was highest at 8h point which demonstrates that the influence of viruses is highest during the ovulatory phase. In addition, the injection of only R848 to superovulated mice caused the luteinization of follicles and a dose-dependent increase in progesterone synthesis when granulosa cells were cultured *in vitro*. These suggests that R848 prompts the irreversible transition to a luteal cell phenotype without inducing ovulation by causing a decline in cell cycle activators and an increase in cell cycle inhibitors culminating in luteinization. Also, the presence of receptors for SARS-CoV-2 virus and PRRS in mouse also provides novel opportunities for understanding the mechanism of action of these viruses.

3. Conclusion

In conclusion, the present study demonstrated that the decrease in the induction of *Lhcgr* and *Cyp19a1* due to LPS is a result of the epigenetic regulatory action of LPS. The immunological response of the host to LPS or bacterial infection prevents the preovulatory follicles from responding to the LH surge, which is required for ovulation. Moreover, ovarian dysfunction and polycystic ovaries, as well as other PIDs that are characterized by bacterial infection in humans and animals, are closely connected to the methylation of the *Lhcgr* promoter region. In addition, R848 suppresses oocyte maturation and fertilization and prompts the irreversible transition to a luteal cell phenotype without inducing ovulation by causing a decline in cell cycle activators

and an increase in cell cycle inhibitors culminating in luteinization. This study provides a novel contribution to the field of mammalian ovarian biology. It is also essential to understanding the etiology of ovulation disorder and the mechanism of action of infectious diseases in a bid to improve the efficacy and safety of ART in animal management systems and in developing therapeutic treatment.