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Biological Sciences

"Evaluating the Effects of Bile Acids on Steroidogenesis and Reproductive Success of Dairy Cows"

Abstract

Bile acids are cholesterol metabolites that are best known for their role in the digestion of fats in the small intestine. Recently, two specific bile acid receptors were identified. The first bile acid receptor is a nuclear receptor (FXR) and the second bile acid receptor identified is a transmembrane G-protein coupled receptor (TGR5). These receptors are expressed in reproductive tissues and bile acid signaling has been found to also play a role in endocrine function including steroid hormone production. In addition, the bile acid profile in follicular fluid of women undergoing fertility treatment is associated with improved developmental capacity of the oocytes. Our preliminary data shows that bile acid levels differ between cows with high versus low fertility. Cows with low fertility also have impaired steroid hormone production. Taken together, these results suggest that bile acids play a role in influencing the reproductive success of dairy cows. The fertility of dairy cows is a critical factor for the success of the dairy industry, but overall fertility of dairy cows is poor.

The primary aim of this project is to determine how bile acid signaling affects steroidogenesis and oocyte developmental potential. We will conduct in vitro culture of follicle wall cells with different bile acids and specific bile acid receptor agonists to determine its effects on androgen and estrogen production, which are the two hormones that are found to be lower in ovarian follicles of cows that fail to ovulate. Concentrations of these steroid hormones in culture media will be determined by ELISA and the relative abundance of mRNA transcripts of key steroidogenic enzymes such as StAR, CYP11A1, CYP17A1, and CYP19A1 in follicle wall cells will be compared between bile acid supplemented cells and controls. We will also supplement in vitro fertilized bovine embryos in culture to determine if bile acid supplementation improved developmental competence. By elucidating the effects of bile acids on steroidogenic enzymes and oocyte development, we will better understand the role and mechanisms of bile acid signaling on gonadal function, which may lead to the identification of therapeutic targets to maximize fertility of dairy cows.

Biographical Sketch:

I am currently a junior in the College of Arts and Sciences, studying Biological Sciences.

I have been involved in research since the summer after my freshman year, when I joined the Davisson Lab to help conduct research in preeclampsia. Through investigating the mechanisms of periimplantation and its role in placental defects, I became interested in reproductive research, particularly at the molecular level. I continued to develop my interest through a summer internship with the March of Dimes Prematurity Research Center at the University of Pennsylvania under Dr. Simmons. The chance to conduct research under leading physician scientists allowed me to observe the intersection of science and medicine, which has inspired me to pursue an M.D./Ph.D. after graduation in the hopes of both advancing basic science and partaking in its real life applications through patient care. I currently work as a research assistant in the Cheong Lab at the College of Veterinary Medicine, where I continue to learn about various aspects of reproduction, fertility, and uterine environment in bovine and equine models.

On campus, I am heavily involved in the organization Science Olympiad at Cornell, which consists of a group of undergraduate students who plan and host annual science competitions that bring hundreds of middle and high students from across the country to compete. In addition, I also volunteer at various local regional competitions across New York State, which culminates in the state competition each year in March. Outside of Science Olympiad, I have been involved with several publications, including RICE Magazine, *The Research Paper,* and *Stethoscoop.* I also write for an online college mentorship blog, sharing stories, insights, and advice about the college application process with high schoolers and parents.

Statement of Purpose:

Please describe your project in detail: explain the significance of your research and your reasons for wishing to engage in it, and tell us where you will carry out this research and what resources you will use. Discuss briefly how your project relates to existing research in your field on the same subject. Be sure to include a bibliography.

The dairy industry is the largest agricultural industry in upstate New York and dairy products are an essential, low-cost, and high nutritional value food source, especially for children. Dairy production depends on successful reproduction of dairy cows, but overall dairy cow fertility has declined in the past five decades as amount of milk produced per cow has increased (1). Ovarian follicular activity in cattle ceases in late pregnancy and resumes in normal cows within a week after calving. A common cause of infertility is the delayed resumption of ovarian cyclicity postpartum, which decreases the likelihood of pregnancy (2). Up to 60% of dairy cows experience anovulation early postpartum, while up to 20% of cows continue to experience anovulation 50 days postpartum, when rebreeding typically begins (3). Taken together, anovulation is a common disease of dairy cows that impairs fertility and lowers the productivity of dairy farms across the world.

In most dairy cows, the ovaries are able to produce a dominant follicle 7 to 10 days after calving (2). Although similar in physical appearance and growth rate, only about 40% of these follicles successfully ovulate. The key difference between normal cows and cows that fail to ovulate is the impairment of the ovarian follicles of anovulatory cows to produce steroid hormones, specifically androgens and estrogen (4). Estrogen is the most specialized reproductive steroid hormone and is produced by the granulosa cells of the ovarian follicle wall, which converts androgens produced by the theca cells of the ovarian follicle into estrogen by the enzyme aromatase. In normal cows, estrogens produced by the ovary elicit a positive feedback signal to the hypothalamic-pituitary axis to release the luteinizing hormone (LH) surge which induces the ovarian follicle to ovulate. Anovulatory follicles do not produce estrogens and thus the LH surge is not induced (5, 6). The reason for the discrepancy in hormone production by the ovarian follicle wall is currently unknown, although recent discoveries and preliminary data suggest that bile acids may have some influence, given the role they play in reproductive cell signaling and hormone production.

Bile acids are cholesterol metabolites whose main role is to aid in the digestion of fats in the small intestine. Interestingly, recent research has shown bile acid molecules to also function as signaling molecules, with their presence being detected in bovine follicular fluid (7). Concurrently, bile acid receptors have been identified in bovine reproductive tissue, specifically a nuclear receptor (FXR) and a transmembrane G-protein coupled receptor (TGR5) (8,9). Given that total bile acid levels differ between anovulatory and ovulatory cows, with the most fertile cows being those that have the lowest level of bile acids, we hypothesized that bile acids play a role in steroidogenesis and may contribute to the steroidogenic defects seen in anovulatory cows.

To test this hypothesis, we will supplement bovine ovarian follicles *in vitro* with increasing doses (0, 10 and 50 μM) of purified bile acids (cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, ursodeoxycholic acid, and tauroursodeoxycholic acid) or specific bile acid receptor agonists (INT 747, GW4064 and INT 777) for three days. Each condition will be tested on tissues from multiple follicles and repeated over the course of several weeks. Follicular cells will then be extracted and snap frozen for RNA analysis.

To compare mRNA levels of bile acid receptors and steroidogenic enzymes in cells treated with bile acids versus those not treated, total RNA will be isolated using Trizol and then synthesized into cDNA for PCR analysis. Specific probes have been selected to test for the presence and relative abundance of StAR, CYP11A1, CYP17A1, and CYP19A1 (steroidogenic enzymes) and FXR and TGR5 (bile acid receptors). Western blots will also be run to test for corresponding protein levels of the mRNA transcripts of interest.

This research will be conducted at the College of Veterinary Medicine in the Cheong laboratory. The ovaries used in the in vitro studies will be obtained from a slaughterhouse in Pennsylvania where the PI has a standing weekly order for 5 lbs of bovine ovaries. The PI of this project is a fellow of the Cornell Dairy Center of Excellence, which provides a valuable resource for information and help.

This project is part of a larger, overarching project aimed at studying the relationship between bile acids and various aspects of bovine reproductive success. Other projects include investigating the effects of bile acid supplementation on oocyte development, as well as the underlying mechanisms for elevated bile acid levels in dairy cows. These projects will all contribute towards understanding how bile acids are related to fertility in dairy cows, which may eventually be applied to developing therapeutic targets to increase overall dairy fertility, thus boosting the productivity of dairy farms and profitability of the dairy industry. Funding for this project has been approved through the College of Veterinary Medicine Research Grants in Animal Health by the USDA, National Institute of Food and Agriculture, Animal Health and Disease Research Program.

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