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Effect of fish meal supplementation on spatial distribution of lipid microdomains and on the lateral mobility of membrane-bound prostaglandin $F_{2\alpha}$ receptors in bovine corpora lutea

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ABSTRACT

This study examined the effects of fish meal supplementation on spatial distribution of lipid microdomains and lateral mobility of prostaglandin $F_{2\alpha}$ (FP) receptors on cell plasma membranes of the bovine corpus luteum (CL). Beef cows were stratified by BW and randomly assigned to receive a corn gluten meal supplement (n = 4) or fish meal supplement (n = 4) for 60 d to allow incorporation of fish meal–derived omega-3 fatty acids into luteal tissue. Ovaries bearing the CL were surgically removed between days 10 to 12 after estrus corresponding to approximately day 60 of supplementation. A 200-mg sample of luteal tissue was analyzed for fatty acid content using gas-liquid chromatography (GLC). The remaining tissue was enzymatically digested with collagenase to dissociate individual cells from the tissue. Cells were cultured to determine the effects of dietary supplementation on lipid microdomains and lateral mobility of FP receptors. Luteal tissue collected from fish meal–supplemented cows had increased omega-3 fatty acids content ($P < 0.05$). Lipid microdomain total fluorescent intensity was decreased in dissociated luteal cells from fish meal–supplemented cows ($P < 0.05$). Micro and macro diffusion coefficients of FP receptors were greater for cells obtained from fish meal–supplemented cows ($P < 0.05$). In addition, compartment diameter of domains was larger, whereas resident time was shorter for receptors from cells obtained from fish meal–supplemented cows ($P < 0.05$). Data indicate that dietary supplementation with fish meal increases omega-3 fatty acid content in luteal tissue causing disruption of lipid microdomains. This disruption leads to increased lateral mobility of the FP receptor, increased compartment sizes, and decreased resident time, which may influence prostaglandin signaling in the bovine CL.

1. Introduction

Luteinizing hormone from the anterior pituitary gland causes the release of the ovum from the follicle and differentiation of the theca and granulosa cells into small and large luteal steroidogenic cells, respectively [1–3]. Luteal steroidogenic cells secrete progesterone, which is essential for early pregnancy in mammals. In the nonpregnant cow, uterine prostaglandin (PG) $F_{2\alpha}$ is released late in the estrous cycle in a series of 5 to 8 pulses, causing regression of the corpus luteum (CL) allowing for return to estrus [4–6]. Prostaglandin $F_{2\alpha}$ (FP) receptors are heterotrimeric G-protein coupled receptors located on

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steroidogenic luteal cells of the CL [7]. Binding of $PGF_{2\alpha}$ to its receptor triggers a complex intracellular signaling cascade to initiate regression of the CL. In the pregnant female, the conceptus inhibits uterine synthesis and release of $PGF_{2\alpha}$, preventing regression of the CL, allowing for continued secretion of progesterone, and establishment of pregnancy [8]. Loss of pregnancy can occur when a viable conceptus fails to adequately regulate $PGF_{2\alpha}$ secretion, leading to regression of the CL [9,10]. Therefore, diminishing or altering sensitivity of luteal cells to $PGF_{2\alpha}$ may prevent regression of the CL during early pregnancy.

The plasma membrane of mammalian cells is composed of a lipid bilayer, which is highly dynamic. It also contains unique regions called lipid microdomains, ranging in size from 10 to 200 nm in diameter that are high in cholesterol and sphingolipids [11,12]. These domains are well-organized, which cluster to form larger, ordered platforms. The order, or spatial distribution of these domains, favors specific protein-protein interactions, resulting in the activation of signaling cascades [13]. There are 2 distinct lipid microdomain structures associated with plasma membranes–linear domains referred to as lipid rafts and invaginated domains referred to as caveolae. These microdomains have been postulated to regulate vesicle trafficking/sorting, endocytosis of membrane-bound proteins, cholesterol homeostasis, and serve as platforms to facilitate colocalization of intracellular signaling proteins during agonist-induced signal transduction [11]. Numerous studies have shown that ligand-bound membrane receptors often coalesce into lipid microdomains following addition of an agonist resulting in the activation of intracellular signaling pathways [14–16]. In addition, the disruption of lipid microdomains, either by beta-methyl cyclodextrin (β-MCD) or inhibiting transcription of specific microdomain structural proteins, has been shown to exert major effects on G-protein–coupled receptor signaling. This disruption has been reported to effect the localization, trafficking, and signaling of the G-alpha subunit [17]. Therefore, altering lipid microdomain structure on the plasma membrane of luteal cells may reduce $PGF_{2\alpha}$ signaling in bovine CL.

Long-chain polyunsaturated fatty acids such as omega-3 fatty acids can be incorporated into glycerophospholipids and increase membrane fluidity [18,19]. Changes in membrane order or fluidity have been reported to affect ligand affinity and subsequent ion flux for the acetylcholine receptor [20]. In addition to altering membrane fluidity, these fatty acids have been reported to disrupt lipid microdomain composition, affect mobility of membrane-bound receptors, and decrease cell signaling [21–23]. We recently reported that inclusion of fish meal in the diet of nonlactating beef cows increased blood plasma [24–26] and luteal [26] content of omega-3 fatty acids. Therefore, it is hypothesized that omega-3 fatty acids in fish meal will incorporate into the plasma membrane of luteal cells altering lipid microdomains and lateral mobility of FP receptors in cells of the bovine CL. The objectives of the present study were to examine the effects of fish meal supplementation on (1) plasma and luteal omega-3 fatty acid composition, (2) organization and spatial distribution of lipid microdomains on cells of the bovine CL, and (3) lateral mobility of the membrane-bound FP receptors.

2. Materials and methods

2.1. Animals and tissue collection

All animal procedures described herein were approved by the Colorado State University Institutional Animal Care and Use Committee (Approval # 13-4440A). Beef cows of mixed breeds were purchased at a local sale barn in Fort Collins, Colorado and housed at the Colorado State University Animal Reproduction Biotechnology Laboratory Foothills campus. Reproductive organs were palpated per rectum for presence of gross anatomical abnormalities (cystic follicles) and adhesions. Transrectal ultrasonography was performed on ovaries for presence of CL and uteri for absence of a fetus. Cows with adhesions, cystic follicles, absence of a CL, or pregnant were removed from the study.

Cows were stratified by BW and randomly assigned to receive corn gluten meal ($n = 4$; controls) or fish meal ($n = 4$; SeaLac, Omega Protein). Diets were delivered daily to cows at 2% BW on a dry matter intake basis that met or exceeded NRC requirements [27]. The ration consisted of 95% mixed hay and 5% pelleted supplement of either fish meal or corn gluten meal. Diets were formulated to be isocaloric and isonitrogenous (Tables 1 and 2), and cows were fed for approximately 60 d to allow for adequate time to incorporate omega-3 fatty acids into blood and reproductive tissues in the fish meal–supplemented animals. Cows were housed in a dry lot and were individually penned (3.7×3.0 m) between 6 AM and 10 AM each day to receive supplements and hay. After consumption of rations, cows were then turned out to have ad libitum access to water. Body weights were collected weekly to monitor changes in weight, and diets were adjusted as needed.

Jugular blood samples were collected immediately before supplementation commenced and weekly thereafter to measure changes in plasma fatty acid composition. Samples were collected in 3-mL blood tubes containing 5.4 mg of EDTA (BD Vacutainer, Becton and Dickson Co, Franklin Lakes, NJ) and immediately placed on ice. Samples were centrifuged at $1500 \times g$ for 15 min, after which plasma was then collected and stored at −80°C until GLC analysis.

Cows were administered 25-mg injections of $PGF_{2\alpha}$ (Lutalyse, Pharmacia and Upjohn Co, MI, USA) on day 36 and 50 of the supplementation period to synchronize estrous cycles. Ovaries bearing the CL were surgically removed by standing flank procedure, as previously described [28] between days 10 to 12 after estrus following the second $PGF_{2\alpha}$ injection (approximately day 60 of the supplementation period). After collection, the ovary was placed in 1 × sterile PBS and transported on ice to the laboratory at the University of Northern Colorado. Superficial sterilization of the ovary was performed by immersing into a 70% ethanol solution.

2.2. Cell preparation

Using sterile techniques under a laminar flow hood, the CL was removed from the ovary, and a 200-mg sample of tissue was placed in a 1.7-mL micro centrifuge collection tube and stored at −80°C until GLC analysis. The remaining