Saturated Fatty Acids are Crucial Components in Follicular Fluid for Ensuring Endocrine Regulation of Estradiol Production in Bovine Ovary

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Ovarian granulosa cells (GCs) have evolutionarily conserved steroidogenic functions mainly controlled by the consecutive actions of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Estradiol (E2) synthesized by GCs is essential for the feedback regulation of gonadotropin production, uterine extracellular modeling, and the manifestation of estrous symptoms in dairy animals such as cows and buffaloes. Metabolic conditions like postpartum negative energy balance in animals and diseases like diabetes and obesity in humans are characterized by increased concentrations of long-chain non-esterified fatty acids (NEFA) in follicular fluid and are associated with female subfertility. Oleic acid (OA; C18:1), palmitic acid (PA; C16:0), and stearic acid (SA; C18:0) are the predominant fatty acids in follicular fluid and constitute up to 70% of the NEFA fraction under healthy conditions. However, their concentrations are further elevated in postpartum animals due to negative energy balance-induced fat mobilization. Earlier studies in GCs indicate that unsaturated fatty acids (UFAs) downregulate and saturated fatty acids (SFAs) upregulate estradiol production. However, it was unclear whether pituitary gonadotropins induce accumulation of NEFA in the follicular fluid since FSH induces and LH inhibits estradiol production in the ovary. To address this, we have performed gas chromatography analysis of follicular fluid, which revealed no differential accumulation of NEFAs between pre- and post-luteinizing hormone surge follicles. We, therefore, wondered how estradiol production is regulated in the physiological context, as UFAs and SFAs are mutually present in the follicular fluid despite their opposite effects on E2 production. We thus performed in vitro primary GC cultures with palmitate, palmitoleate, stearate, oleate, linoleate, and alpha-linolenate (ALA), representing >80% of the NEFA fraction in the follicular fluid, and analyzed 62 different cell culture conditions to understand the regulation of estradiol biosynthesis. Our analyses show that UFAs (OA and ALA) downregulate GC's E2 production by strongly downregulating the expression of aromatase and gonadotropin receptors FSHR and LHCGR. This suggests that UFAs could hamper the function and also the gonadotropin signaling in GCs. Surprisingly, cosupplementation of SFAs (PA and/or SA) with UFAs (OA or ALA) alleviated the inhibitory effects of UFAs by rescuing E2 production, expression of gonadotropin receptors and aromatase. Next we analyzed the underpinning signaling pathways responsible for the regulation of E2 production upon NEFA treatments. Transcriptome data of oleic acid–treated GCs indicated that UFAs induce the ERK1/2 and AKT signaling pathways. Western blot validation showed that UFAs activate and SFAs inhibit the ERK1/2 and AKT pathways in GCs. Interestingly, cosupplementation of SFAs and UFAs did not induce phosphorylation of ERK1/2 and AKT proteins, suggesting a possible role of these pathways in UFA-dependent downregulation of E2 production. Next, we inhibited these pathways using chemical inhibitors PD98059 and LY294002. Attenuation of ERK1/2 phosphorylation resulted in elevation of E2 levels along with the expression of genes downregulated by UFAs, indicating SFAs-induced rescue of E2 production is mediated via inhibition of ERK1/2 signaling. ERK1/2 mRNA knockdown assay further confirmed that ERK1/2 is indeed a negative regulator of estradiol production in GCs. Overall, our results demonstrate for the first time that SFAs are vital components of the follicular fluid, without which gonadotropin signaling and the ovarian cycle would probably be

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