Role of Zinc Insufficiency in Bovine Granulosa Cell Viability

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Due to the reproductive similarities between cows and women, and the naturally occurring hyperandrogenism that occurs in subsets of beef and dairy cattle herds, cows are an emerging and promising animal model for the study of polycystic ovary syndrome (PCOS). Recently, serum zinc levels have been found to be abnormal in women with PCOS, with some observing high serum zinc in comparison to healthy controls, but most studies observing low serum zinc in women with PCOS. Despite the robust knowledge on the effects of zinc insufficiency on ovarian function in other animal models, such as mice, the effect of zinc insufficiency on the bovine ovary is limited. To begin to investigate this, we hypothesized that zinc insufficiency would decrease cell viability in bovine mural granulosa cells.

Plated bovine mural granulosa cells were incubated with differing levels of the transition metal chelator, N,N,N',N'-tetrakis (2-pyridymethyl)ethylenediamine (TPEN, 2.5, 3.5, 5, and 10 µM) for 24 hours. Cell viability was measured with an MTT assay. A moderate decrease in cell viability was seen at 3.5 µM TPEN, while a severe decrease was seen at 5 and 10 µM TPEN ($n = 4$; $p<0.05$; one-way ANOVA). This decrease in cell viability was rescued with the addition of 10 and 20 µM $ZnSO_4$, confirming no off-target effects of TPEN ($n=4$; $p<0.05$; one-way ANOVA). Because TPEN has a high affinity for not only zinc, but also copper, iron, and manganese, chelators specific for those micronutrients were used to determine if the above-mentioned decrease in cell viability in response to TPEN seen is zinc mediated (iron chelator = 10 µM 2,2'-Dipyridyl; copper chelator = 10 µM Bathocuproinedisulfonic acid disodium salt; manganese chelator = 10 µM Sodium-4-aminosalicyate dihydrate). None of these other chelators demonstrated a decrease in cell viability compared to the vehicle control, confirming zinc insufficiency is responsible for the decrease in cell viability in response to TPEN ($n=4$; $p>0.05$; one-way ANOVA). These results demonstrate that zinc is an essential micronutrient for bovine mural granulosa cells, a cell type whose proliferation is essential for a growing follicle.