Elevated Methylglyoxal Concentrations Induces Cellular Senescence in Endometrial Cells in Dairy Cows

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Excessive sugar in the body of dairy cows due to high grain rations may bind to proteins and accelerate the production of advanced glycation end products (AGEs). Methylglyoxal (MGO) is a precursor of AGEs that induces neurodegenerative diseases and vascular endothelial damage via intracellular reactive oxygen species (ROS) production. Reactive oxygen species induce DNA damage and promote cellular senescence by arresting cell cycle via elevated expression of cyclin-dependent kinase inhibitors p16 and p21. Senescent cells secrete inflammatory cytokines and chemokines, called a senescence-associated secretory phenotype (SASP) which causes inflammation. Based on these findings, we hypothesized that increased blood MGO in dairy cows causes cellular senescence via ROS production in the endometrial cells, resulting in reduced fertility. Initially, blood MGO concentrations in beef and dairy cows were compared. Blood MGO concentrations in Holstein dairy cows (n=20, fed corn silage) were significantly higher ($p < 0.05$) compared with Japanese Black beef cows (n=20, fed grass silage). Subsequently, we examined the effects of MGO on bovine endometrial cells and its mechanism. Methylglyoxal was added to bovine endometrial cells (passage 2) cultured at 38°C and 5% CO_2 in the air at 0, 0.1, and 1 mM, respectively. Reactive oxygen species production was measured 6 h after MGO treatment. The cell proliferation rate, DNA damage level, senescence-associated β-galactosidase (SA β-gal)-positive cell rate, and *p21* mRNA expression levels were measured 12 h after MGO treatment. IL-8 secretion was measured 12 h after MGO treatment followed by 24 h of culture in a normal medium. Reactive oxygen species production after 1 mM MGO treatment significantly increased ($p < 0.05$) compared to the control. The expression level of γH2AX protein (a DNA damage marker) after 1 mM MGO treatment was significantly higher ($p < 0.05$) than that of the control. The cell proliferation rate was significantly decreased ($p < 0.05$) by 1 mM MGO treatment compared

to the control, and the ratio of SA-β gal positive cells was significantly increased ($p < 0.05$) compared to the control. *p21* mRNA expression significantly increased ($p < 0.05$) after 1 mM MGO treatment compared to the control. These results suggest that MGO causes DNA damage via increased ROS production and induces cellular senescence in bovine endometrial cells. Furthermore, IL-8 production tended to increase ($p < 0.1$) in bovine endometrial cells after 1 mM MGO treatment compared to the control, suggesting that cytokines secreted by MGO-induced senescent cells may cause a decrease in uterine function, resulting in reduced fertility in dairy cows.