氏　　名	VU VAN HAI
授与した学位	博　士
専攻分野の名称	農学
学位授与番号	博乙第４４３１号
学位授与の日付	平成２６年　９月３０日
学位授与の要件	博士の学位論文提出者 （学位規則第５条第２項該当）
学位論文の題目	Study on luteolytic mechanisms in cattle: Regulation of antioxidant enzymes by prostaglandin $F_{2\alpha}$ and reactive oxygen species （ウシ黄体退行機構に関する研究: プロスタグランデイン $F_{2\alpha}$ および活性酸素種による抗酸化酵素の調節）
論文審査委員	准教授　アコスタ アヤラ トマス　　教授　奥田　潔　　教授　国枝　哲夫

学位論文内容の要旨

This study aimed to clarify the roles of antioxidant enzymes in regulating the luteolytic action of prostaglandin $F_{2\alpha}$ (PGF) and reactive oxygen species (ROS). For that purpose, we examined 1) the dynamic changes of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in bovine corpus luteum (CL) at different stages of the estrous cycle and during luteolysis induced by PGF administration *in vivo* and 2) the dynamic relationship between PGF and ROS and its possible role in regulating antioxidant enzymes in bovine CL using cultured bovine luteal cells (LSCs) *in vitro*.

1) During the estrous cycle, SOD1 protein expression was greater in the developing and mid-luteal stages than in the early, late and regressing-luteal stages. Total SOD activity gradually increased from the early to late-luteal stages and then decreased to the lowest level at the regressing-luteal stage. Catalase protein and the activities of CAT and GPx increased from the early to mid-luteal stage, and then decreased at the regressing-luteal stage. The levels of GPx1 protein were lower in the regressing-luteal stage than in other stages. Immunohistochemical examination also revealed the expression of CAT and GPx1 protein in bovine CL tissue. During PGF-induced luteolysis, injection of a luteolytic dose of PGF increased luteal SOD1 protein expression, total SOD activity, GPx1 protein expression and GPx activity at 2 h but suppressed them at 24 h. Catalase activity decreased at 24 h. These results provide evidence for the protective role of antioxidant enzyme in maintaining CL function during early to late luteal stage in bovine CL. A decrease in these antioxidant enzymes protein and their activities during regressing luteal stage as well as during structural luteolysis induced by PGF suggests that ROS elevation during luteolysis induces cell demise to complete the luteolytic action of PGF. 2) Hydrogen peroxide (H_2O_2) stimulated PGF biosynthesis at 2 and 24 h in a dose- and time-dependent manner. Prostaglandin $F_{2\alpha}$, in turn, induced ROS production. Prostaglandin $F_{2\alpha}$ and H_2O_2 increased SOD1 protein expression and total SOD activity, GPx1 protein and GPx activity at 2 h but suppressed them at 24 h. Catalase protein expression and activity did not change at 2 h but they were suppressed at 24 h by PGF and H_2O_2. These findings confirmed that 1) LSCs are targets of the luteolytic action of PGF and 2) PGF, interacting with ROS, induced luteolysis by suppressing antioxidant enzymes in LSCs during structural luteolysis but not during functional luteolysis.

The overall results demonstrate that PGF through its interaction with ROS regulates the expressions and activities of the antioxidant enzymes SOD, CAT and GPx, in bovine CL, more specifically in LSCs, suggesting that these enzymes are involved in the mechanism of action of PGF in bovine CL. The down-regulation of these proteins and their activities during structural luteolysis could enhance the accumulation of reactive oxygen species, which would result in both increasing luteal PGF production and oxidative stress, to complete the CL regression in cattle.

論文審査結果の要旨

This study aimed to clarify the roles of antioxidant enzymes in regulating the luteolytic action of prostaglandin $F_{2\alpha}$ (PGF) and reactive oxygen species (ROS). For that purpose, we examined 1) the dynamic changes of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in bovine corpus luteum (CL) at different stages of the estrous cycle and during luteolysis induced by PGF administration *in vivo* and 2) the dynamic relationship between PGF and ROS and its possible role in regulating antioxidant enzymes in bovine CL using cultured bovine luteal cells (LSCs) *in vitro*.

In vivo study: During the estrous cycle, SOD1 protein expression was greater in the developing and mid-luteal stages than in the early, late and regressing-luteal stages. Total SOD activity gradually increased from the early to late-luteal stages and then decreased to the lowest level at the regressing-luteal stage. Catalase protein and the activities of CAT and GPx increased from the early to mid-luteal stage, and then decreased at the regressing-luteal stage. The levels of GPx1 protein were lower in the regressing-luteal stage than in other stages. Immunohistochemical examination also revealed the expression of CAT and GPx1 protein in bovine CL tissue. During PGF-induced luteolysis, injection of a luteolytic dose of PGF increased luteal SOD1 protein expression, total SOD activity, GPx1 protein expression and GPx activity at 2 h but suppressed them at 24 h. Catalase activity decreased at 24 h.

In vitro study: Hydrogen peroxide (H_2O_2) stimulated PGF biosynthesis at 2 and 24 h in a dose- and time-dependent manner. Prostaglandin $F_{2\alpha}$, in turn, induced ROS production. Prostaglandin $F_{2\alpha}$ and H_2O_2 increased SOD1 protein expression and total SOD activity, GPx1 protein and GPx activity at 2 h but suppressed them at 24 h. CAT protein expression and activity did not change at 2 h but they were suppressed at 24 h by PGF and H_2O_2.

The overall results demonstrate that PGF through its interaction with ROS regulates the expressions and activities of the antioxidant enzymes SOD, CAT and GPx, in bovine CL, more specifically in LSCs, suggesting that these enzymes are involved in the mechanism of action of PGF in bovine CL. The down-regulation of these proteins and their activities during structural luteolysis could enhance the accumulation of reactive oxygen species, which would result in both increasing luteal PGF production and oxidative stress, to complete the CL regression in cattle.

The results of the present study are relevant for understanding the luteolytic mechanisms and its regulation in cattle and fulfill the requirement to obtain the PhD degree in Agriculture.