学位論文内容の要旨

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学位論文題名

Study on the effect of *Asparagus officinalis* stem extract on the induction of molecular chaperone and cellular function of bovine granulosa cells

（牛顆粒膜細胞への分子シャペロン誘導と細胞機能発現に及ぼすアスパラガス茎抽出物の作用）

The defense response to stress from the outside is very important for maintaining the functions of cells, tissues, organs and whole bodies, and therefore lives have various defense mechanisms. It is well known that heat stress (HS) is one of major stresses that causes harmful effects to the cells, tissues, and organs with affecting gene expressions protein function and increasing reactive oxygen species (ROS). In contrast, cells also have defensive response mechanisms to protect against such stresses. Heat shock protein 70 (HSP70) is a well-known protein induced by heat shock and acts as a molecular chaperone to protect the cell functions against stressed conditions. Recently, a standardized extract of *Asparagus officinalis* stem (EAS) has been found to induce heat stress-independent induction of HSP70 in several somatic cells in relation with regulating redox balance. However, the effects of EAS on the function of reproductive cells remain unknown. This study was conducted to investigate the effect of *Asparagus officinalis* stem extract on the induction of molecular chaperone and cellular functions in relation to intracellular redox status in bovine granulosa cells (GC).

1. Effect of EAS on the induction of HSP70, intracellular redox balance and cell functions in bovine granulosa cells

I investigated the effect of EAS on HSP70 induction and oxidative redox balance in cultured bovine GC. EAS significantly increased *HSP70* expression under non- HS condition. In contrast, expression of *HSP27* and *HSP90* were not affected. Besides, EAS decreased ROS generation and DNA damage with increased glutathione (GSH) synthesis both under non- and HS conditions. Moreover, EAS synergistically increased HSP70 and *heat shock factor (HSF)1* in GC with HS. HSP70 inhibitor significantly increased ROS, decreased GSH, and decreased HSF1, nuclear *factor erythroid 2-related factor 2 (NRF2),* and *Kelch-like ECH-associated protein 1 (KEAP1)* in the presence of

EAS. Thus, EAS was found to improve HSP70-mediated redox balance by non-HS condition in bovine GC, and also act as synergistic effect with HS.

2. Effect of EAS on steroidogenesis in bovine granulosa cells

P4 is a well-known steroid hormone and has a key role in ovarian function. HS is known to decrease P4 synthesis in the corpus luteum (CL) in the ovary by causing imbalance of redox status. HS significantly decreased P4 levels, expressions of *steroidogenic acute regulatory protein (STAR), 3β-hydroxysteroid dehydrogenase (3β-HSD) and* mitochondrial membrane activity. In contrast, EAS significantly increased P4 level with increase in the expressions *STAR, 3β-HSD*, mitochondrial membrane activity and lipid droplet both under non- and HS conditions. Combination of EAS and HS drastically increased P4 levels compared with that EAS treatment under non-HS condition. Furthermore, inhibition of HSP70 significantly reduced the EAS-induced P4 synthesis, mitochondrial function and lipid droplets. These results suggeste that increase in P4 synthesis by EAS is mediated by activation STAR and 3β-HSD pathway together with improvement mitochondrial and lipid metabolism through HSP70-mediated redox balance and chaperone function in bovine GC.

3. Effect of EAS on the cell viability after cryopreservation

Little is known about the effect of pre-freezing EAS treatment on post-thaw viability and redox status under stressed condition. Thus, I investigated the effect of EAS on the post-thaw viability of bovine GC in relation to the redox balance with HS treatment. HS significantly decreased the post-thaw viability of GC, however EAS significantly increased the post-thaw viability of HS treated GC. Furthermore, increased ROS levels by HS were significantly decreased by EAS pretreatment. GSH levels were increased by EAS pretreatment. These results suggest EAS affects the post-thaw viability of HS bovine GC by improving molecular chaperones and the redox status.

Overall study suggests the beneficial effect of EAS on cellular functions of bovine GC by regulating intracellular redox status. Moreover, synergistic effect of EAS and HS on HSP70 production can contribute to improvement of P4 synthesis and cellular survival ability.