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Effects of Gamisoyosan on *In vitro* Fertilization and Ovulation in Stress-induced Mice by Foot Shock

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It has been generally accepted that Gamisoyosan (GSS) is a useful prescription for treating insomnia, dysmenorrhea, infertility and to improve stress induced by stressors. The aim of this study was to clarify whether GSS improved reproductive dysfunction caused by stress in mice. Mice were suffered stress by foot shock for a week, followed by administration of GSS at 500 mg/kg body weight per day during a week. Thereafter, changes of body weight, ovulation rate, *in vitro* and *in vivo* fertilization and embryonic development were measured. GSS markedly increased the body weight of mice with stress, but not normal mice. GSS had significant effects on the ovulation rate, both the *in vitro* and *in vivo* fertilization rates and embryonic develoment. These results suggest that GSS can improve in the reproductive dysfunctions caused by stress.

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The E Subunit of Vacuolar-type H+-ATPase *(vha-8)* is Essential for Embryogenesis and Yolk Transfer in *C. elegans*

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Vacuolar H+-ATPases (V-ATPases) are ATP-dependent proton pumps composed of peripheral V1 sector and membrane-bound V0 sector. It is localized at membranes of intracellular acidic organelles and plasma membranes of various cell types. By virtue of its regulation in acidification, V-ATPase is required for many intracellular processes such as receptor-mediated endocytosis and protein sorting. We have characterized the E subunit of V-ATPase in *C. elegans*. This subunit is one of the most well conserved subunits sharing approximately 57% identity with the human homologue, ATP6E. Green fluorescent protein (GFP) and whole-mount immunostaining analyses showed that V-ATPase E subunit (vha-8) abundantly expresses in the H-shaped excretory cell, consistent with the expression patterns observed for other V-ATPase subunits. RNA mediated interference targeted to *vha-8* resulted in embryonic and larval lethality indicating that *vha-8* is essential during early developmental processes. Recently, we have isolated a deletion mutant of *vha-8*, which shows embryonic lethality. We are currently characterizing this mutant in order to elucidate the functional roles of V-ATPase during early embryogenesis as well as in receptor-mediated endocytosis.

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Isolation and Characterization of Deletion Mutant of Cytosolic Aconitase in *C. elegans*

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Cytosolic aconitase converts citrate into isocitrate and regulates iron concentration in the cytosol. In mammals, cytosolic aconitase is known as iron regulation protein (IRP), because it controls the expression of ferritin, which is an iron binding protein. IRP normally represses the translation of ferritin mRNA by binding to its 5'UTR. In high cytosolic iron, IRP is released from ferritin mRNA so the translation of the ferritin is increased. Previously, C. elegans cytosolic aconitase (ACO-1) has been cloned and characterized (ref. Brett L. Gourley et al (2002) J. Biol. Chem.). Interestingly, this study reported that *C. elegans* cytosolic aconitase (ACO-1) does not bind to ferritin mRNA (*ftn-1, 2*). Nevertheless, the expression level of *aco-1* and *ftn-1, 2*is increased when worms were cultivated at high iron concentration. In addition, the life span of N2 was reduced where they grow on the iron-supplemented plates. We have recently isolated a deletion mutant of *aco-1 (jh131)*. It contains a 1.5Kb deletion, which covers from the 950bp upstream of the first exon to the part of third exon. Although it appears to be a null mutant, nut homozygote shows normal development suggesting *aco-1*may not be essential for development. We are currently characterizing the detailed phenotypes of this mutant to study the function of cytosolic aconitase.

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Patial Characterization of Allatostatin cDNA and Expression of Allatostatin in Central Nervous System from the Japanese Oak Silkworm *Antheraea yamamai*

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The allatostatins are a familyof peptides in the invertebrate, which are expressed in the CNS and gut of the insect species. They affect numerous physiolosical processes in-cluding the inhibition of juvenile hormone biosynthesis, inhibition of ovarian ecdysteroid biosynthesis, and inhibition of vitellogenin release from the fat body in insects. The allatostatin peptide family currently comprises over 60 different members, identified 10 species, representing four insect orders. These peptides, which are generally referred to as allatostatin-like, contain the conserved amidated C-terminal pentapeptide sequence YXFGL-NH2, and appear to be ubiquitous in insects. There is considerable variation in length and N-terminal amino acid sequence among individual peptides within and between different insect species. In this study we charaterized allatostatin gene in the CNS and identified allatostatin-producing neurons in the CNS of the Japanese Oak Silkworm *Antheraea yamamai* using a polyclonal antiserum to Dip-AST.