

Effect of Gene Related to Stress on *In Vitro* Maturation, Fertilization and Development in Porcine Fresh/Frozen-Thawed Oocytes

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The gene, which are related to stresses, are known as effects on reproductive ability in pigs. This study was carried out to investigate the effects of gene related to stress on *in vitro* maturation, fertilization and development in porcine fresh/frozen-thawed oocytes. The porcine cumulus oocyte complexes(COCs) were divided into four groups according to whether they were: (1) matured the fresh COCs; (2) cryopreserved the COCs, then matured; (3) matured and fertilized the fresh COCs; (4) Cryopreserved the COCs, then matured and fertilized. Maturation of porcine COCs was accomplished by incubation in NCSU23 medium. The cryo-preservation method of immatured oocytes was Open Pulled Straws(OPS) developed by Vajta *et al.* Oocytes were stained by Acetic-Orcein method, observed the stage and penetration of oocytes under the microscope. As a basis for future studies on the role DNA in porcine development, we have isolated and characterized DNA from porcine ovaries. DNA extracting by extraction solution was analyzed using RAPD(Random Amplified Polymorphic DNA) -PCR(Polymerase Chain Reaction), and PCR-SSCP(Single Strand Conformational Polymorphism) method. RAPD-PCR method amplify DNA using single primers of arbitrary sequences UBC No. 5, 9, 24, 60, different appearance and size of DNA band was showed in the photograph. PCR-SSCP method confirm the polymorphism of gene showed 3 types of AA, AB and BB, the rates of BB genotype was lowest. The rates of MII stage oocytes increased when genotype was AA. The penetration and polyspermy rates did not show a significant difference according to the genotypes of ovaries. These results obtained from the experiment indicate that the different genotypes affect *in vitro* maturation, fertilization and development in porcine fresh/frozen-thawed oocytes.

Key words) *Porcine oocytes, cryopreservation, stress gene, RAPD-PCR, PCR-SSCP*