（学位第３号様式）

学 位 論 文 要 旨

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題　目	Studies on Establishment of Effective *In Vitro* Fertilization System of *In Vitro*-Matured Pig Oocytes （ブタ体外成熟卵子の効率的な体外受精系の確立に関する研究）

ABSTRACT: In pigs, immature oocytes released from ovarian follicles can resume meiosis and reach metaphase II stage in culture, and the matured oocytes are penetrable *in vitro* by fresh and frozen-thawed spermatozoa. However, few normal fertilized oocytes are obtained under conventional systems because of the high incidence of polyspermy. This was one of the most prominent obstacles to producing pig blastocysts from immature oocytes by *in vitro* maturation, fertilization and culture. This study was carried out to establish the effective *in vitro* fertilization system of *in vitro*-matured pig oocytes.

In the first experiment, *in vitro*-matured oocytes were co-cultured with fresh spermatozoa collected from Clawn miniature pigs in a 200 μL PCR tube which was rotated at 1 rpm to overcome the high incidence of polyspermy. Some oocytes were inseminated in the same tube without rotation as controls. The rate of total penetrated oocytes was not affected by the presence or absence of rotation. However, the presence of rotation prevented polyspermic penetration and increased monospermic penetration. These results indicated that the rotation method for *in vitro* fertilization is useful for producing normal fertilized pig oocytes *in vitro*.

Polyphenols (PFs) extracted from green tea, known to possess the powerfully potent anti-oxidants, have been reported to be effective in preservation of mammalian cells from various species and isolated organs. Therefore, it was tested whether treatment with PFs prior to freezing procedure might also be effective for maintaining the integrity of frozen-thawed boar spermatozoa in the second experiment. Ejaculates were diluted in Mulberry III medium containing various concentrations of PFs (0, 0.01, 0.05, 0.1, and 0.2% [w/v]) and then stored at 15°C overnight. The semen samples were processed, using the straw freezing procedure, and then frozen in liquid nitrogen. After rapid thawing at 40°C, the spermatozoa were subjected to several assays to evaluate semen quality. Spermatozoa stored in the medium containing 0.01% PFs exhibited significantly higher degrees of post-thawed viability and acrosomal integrity than those stored in the absence of PFs. However, no change in the mitochondrial activity was noted between the two groups. Addition of 0.01% PFs to the storage solution of spermatozoa prevented polyspermic penetration in oocytes inseminated using the rotation method, resulting in an increased blastocyst formation rate after *in vitro* culture. These results indicated that preincubation with 0.01% PFs prior to freezing procedure exerts a protective effect on boar spermatozoa by preventing injuries associated with freezing-thawing and this method is useful for producing normal fertilized pig oocytes that can develop to the blastocyst stage *in vitro*.

In conclusion, a novel *in vitro* fertilization system for *in vitro*-matured pig oocytes has been established. This system will contribute to increase the efficacy of pig blastocyst production *in vitro*.