Conservation and utilization of pig resources by xenografting to mice

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Abstract: In vitro fertilization of *in vitro* matured oocytes in pigs has become the most popular method of studying gametogenesis and embryogenesis in this species. In addition, because of recent advances in *in vitro* culture of those embryos, *in vitro* embryo production now enables us to generate viable embryos as successfully as for *in vivo*-derived embryos and with less cost and in less time. These technologies contribute not only to developments in reproductive physiology and agriculture but also to conservation of porcine genetic resources. Until now, conservation of pig genetic recourses has been limited by cryopreservation of sperm and, in some cases, *in vivo*-derived embryos. These aspects may decrease the general activates in *ex situ* conservation (gene banking) in this species. In recent years, however, unique technologies such as xenografting of gonadal (testicular and ovarian) tissue into immunodeficient experimental animals, which are combined with *in vitro* embryo production technologies, have been developed to help conservation and utilization of gamete resources. We have already shown fertilizing ability and developmental ability of porcine embryos from gametes grown in mice. Here, we discuss the possibility of conservation and utilization of pig resources by xenografting to mice.

Xenografting of testicular tissues into immunodeficient mice

The possibility of spermatogenesis by the transplantation of germ cells into mouse testis has been suggested in mice (Brinster and Avarbock 1994; Brinster and Zimmermann 1994) and pigs (Honaramooz et al. 2002a). In these cases, germ cells, including spermatogonial stem cells, were injected directly into the seminiferous tubules. Spermatozoa from the introduced cells would be expected to be produced in the ejaculate. However, this technique requires special skills for the injection and also accurate separation procedures to select the spermatozoa derived from the introduced cells. Although germ cell transplantation into mice has produced complete donor-derived spermatogenesis in rodents, it has not yet been succeed in other mammalian species. Xenografting of testicular tissues can be performed into immunodeficient mice heterotopically, such as under the skin of the back (Honaramooz et al., 2002b). They succeeded in the production of mammalian sperm in testicular tissues grafted into nude mice. After several years, successful embryo production by using these sperm cells from xenografted testicular tissues has been reported but limited to rhesus monkeys (Honaramooz et al., 2004) and pigs (Honaramooz et al., 2008, Nakai et al., 2009). In our previous study (Nakai et al., 2009), blastocysts could be produced after intra-cytoplasmic sperm injection (ICSI). The rate and quality were recorded 24.9% and 41.9 cells, respectively, which were comparable to those from *in vitro* fertilization [25.3% and 48.7 cells, respectively (Kikuchi et al. 2002)]. Recently, we have also reported the successful live piglet production when the embryos just after the ICSI were transferred to the recipients (Nakai et al., 2010). Although the efficacy of piglet production remains low, the results suggest the possibility of producing embryos with developmental potential by using porcine spermatozoa differentiated from the gonocytes within the xenografts. In combination with cryopreservation of testis tissue, this procedure will be one of useful methods for conservation of male genetic recourses.

Xenografting of Ovarian tissues into immunodeficient mice

Primordial follicles act as stores of ovarian follicles and are potential resources of oocytes for medical, agricultural and zoological purposes. Ovarian grafting is a potential method of maturing the oocytes in the primordial follicles (primordial oocytes) of large mammals. Cross-species ovarian grafting (xenografting) seems to be more advantageous for the multiplication and conservation of domestic or endangered animals. Mouse oocytes that grow within ovarian tissue xenografted to nude rats acquire the ability to generate pups (Snow et al. 2002). To date, ovarian tissues could be prepared from some species phylogenetically distant from mice (including pigs: Kaneko et al. 2003; Kagawa et al. 2005), and then been xenografted into immunodeficient mice. To our knowledge, only our previous studies, in which neonatal pig ovarian tissues were xenografted, had proven that primordial oocytes can develop in the host mice and acquire *in vitro*-fertilizing ability (Kaneko et al. 2003; Kikuchi et al. 2006) and also that viable embryos can be generated after *in vitro* fertilization (Kaneko et al. 2006). We further treated host mice with gonadotropin (eCG or FSH) resulting in acceleration of follicular growth and embryonic development of fertilized oocytes compared with those in control mice (Kaneko et al., 2006). However, it is also suggested that those oocytes derived from primordial follicles, even after *in vivo* growth and maturation, cytoplast with full developmental ability seems to be one possible method for improving the ability. Fusion of an ooplasmic fragment(s) prepared by 'Centri-Fusion' method (Fahrudin et al. 2007) to the oocytes obtained from xenografts may be the other possibility. In combination with cryopreservation of ovarian tissue, this procedure will be one of useful methods for conservation of female genetic recourses.

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Effects of NGF and BDNF on proliferation of bovine granulosa cells

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Abstract: Effects of BDNF and NGF concentrations on proliferation of bovine granulosa cells were studied. Supplementation of DMEM cultured media with BDNF at the concentration of 20 μg/L or NGF at the concentration of 5μg/L caused a significant increase in number of granulosa cells after cultrued for 48h, indicating that NGF and BDNF can promote in vitro proliferation of bovine granulosa cells.

Key words: BDNF; NGF; bovine; granulosa cells

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