Utilization of Porcine Testicular Tissues after Cryopreservation and Grafting into Nude Mice

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In pigs, freezing of sperm is the most reliable and well-established method for the conservation or preservation of genetic resources. On the other hand, cryopreservation of female gametes (oocytes) and gonadal (testicular and ovarian) tissues usually by vitrification has been conducted, however, resulted in very low efficacies. Recently, our laboratory conducted some research themes related to these issues. In this session, we introduce recent progress on the vitrification of testicular tissues for producing sperm cells and finally living piglets.

One approach for inducing spermatogenesis in isolated testicular tissues is ectopic (into another site in the body) grafting into immunodeficient host animals (xenografting). Ectopic xenografting has been reported in mammals including pigs (Kaneko et al. 2008, Nakai et al. 2009, Nakai et al. 2010). It is preferable to graft testicular tissue under the skin of the back of commercially available severe combined immunodeficiency mice (nude mice). A series of studies has been conducted in our laboratory (Kaneko et al. 2008, Nakai et al. 2009, Nakai et al. 2010, Kikuchi et al. 2011) to evaluate whether boar spermatogonia can develop into sperm in testicular tissues grafted into nude mice, and whether live piglets can be produced after intracytoplasmic sperm injection (ICSI) into oocytes. We firstly confirmed the oocytes developed into blastocysts; of which quality is similar to that of the oocytes from prepubertal gilts after in vitro maturation/fertilization (Kikuchi et al. 2002, Nakai et al. 2009). When the oocytes at the pronuclear stage were transferred to oviducts of estrous-synchronized recipients, we were able to obtain both male and female piglets (Nakai et al. 2010), which showed normal reproductive abilities when developed to the adulthood (Kaneko et al. 2012).

For more advanced utilization of this technique, we have investigated for the possibility of vitrification of testicular tissue fragments before xenografting. This method enables long term storage in liquid nitrogen of the tissue and the production of sperm whenever the need arises. Sperm can be obtained after the recovery from the tissues and were applied for ICSI, and the oocytes were transferred to recipients. We could obtain live male and female piglets (Kaneko et al. 2013). Both the male and female pigs showed normal reproductive abilities (Kaneko et al. 2014). It can be suggested that cryopreservation of testicular tissues is one of the conservation methods for boar genetic resources.

Key words: Pig, Cryopreservation, Vitrification, testicular tissue, Gene bank

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