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Review

Contribution of *in vitro* systems to preservation and utilization of porcine genetic resources

Kazuhiro Kikuchi [a,b,*], Hiroyuki Kaneko [a], Michiko Nakai [a], Tamas Somfai [c], Naomi Kashiwazaki [d], Takashi Nagai [e]

[a] Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan
[b] The United Graduate School of Veterinary Science, Yamaguchi University, Yamaguchi, Yamaguchi, Japan
[c] Institute of Livestock and Grassland Science, Tsukuba, Ibaraki, Japan
[d] Graduate School of Veterinary Science, Azabu University, Sagamihara, Kanagawa, Japan
[e] National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan

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A B S T R A C T

Historically, the conservation or preservation of mammalian genetic resources, especially farm animals, has been conducted under *in situ* conditions by maintaining living individuals as "livestock." However, systems for laboratory *in vitro* embryo production using gametes such as spermatozoa and oocytes are now available, in addition to *ex situ* preservation methods for mammalian genetic resources. One of these methods is the cryopreservation of gametes, embryos, and gonadal tissues. In pigs, freezing of sperm is the most reliable and well-established method for this purpose. On the other hand, cryopreservation of female gametes (oocytes) and gonadal tissues—usually by vitrification—has been associated with very low efficacies. Recently, in our laboratory, some research themes related to this issue have been pursued. We have been focusing on advances in porcine *in vitro* embryo production systems, and here, we introduce recent data on the vitrification of porcine immature oocytes and gonadal tissues followed by their xenografting into host mice to produce gametes.

1. Update of recent developments in porcine *in vitro* embryo production

Progress in porcine *in vitro* embryo production (IVP) has been delayed in comparison with other species such as mice and cattle. One breakthrough in this technology has been the success of *in vitro* fertilization (IVF), especially after *in vitro* maturation (IVM), because a large quantity of ovaries for laboratory work can be obtained at slaughterhouses. These conditions have enabled the establishment and development of other assisted reproductive technologies in pigs.

1.1. Development of IVP

The first instances of IVF using *in vivo* matured oocytes used freshly ejaculated [1] and frozen-thawed epididymal sperm [2]. Later, the *in vitro* developmental competence and viability of porcine IVM and IVF oocytes to the blastocyst stage were first confirmed and reported [3]. Furthermore, live piglets were produced from IVM and IVF embryos after *in vitro* culture (IVC) to the 2- to 4-cell stages [3,4]. Since then, some laboratories have succeeded in producing piglets from embryos cleaved at the 2- to 4-cell stages after IVM, IVF, and IVC for 24 to 36 hours [5]. Viable

* Corresponding author. Tel.: +81 29-838-7447; fax: +81-29-838-7106.
E-mail address: kiku@affrc.go.jp (K. Kikuchi).

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piglets were also generated after transfer of IVP embryos at the blastocyst stage [6,7]. Our work has demonstrated the importance of an energy source (pyruvate and lactate) at the early stage (the first 2 days of IVC) for production of good-quality blastocysts [7]. On the other hand, a chemically defined IVC medium is considered to be advantageous because it is useful for basic research to evaluate the need for small amounts of specific substances and also for embryo transfer to avoid pathogen contamination from additives derived from the animal source. Initially, *in vivo*–derived embryos cultured in chemically defined IVC medium [8], and thereafter IVP embryos generated using chemically defined IVM, IVF, and IVC media, and transferred surgically [9,10] or nonsurgically [11], have resulted in the birth of piglets.

Over the last few years, IVC procedures have been improved, but IVM and IVF systems still have a number of unsolved problems, including (1) imbalance of nuclear and cytoplasmic maturation and (2) polyspermy (reviewed by Nagai et al. [12] and Grupen [13]). Both these phenomena cause abnormal ploidy in IVP embryos, potentially resulting in loss of embryos after their transfer to recipients. Thus, it is important to achieve normality in IVP embryos after IVM and IVF to develop to healthy piglets.

1.2. Advanced technologies using IVP

The basic IVP procedure for piglet production has been of fundamental importance in studies of embryo freezing/vitrification (at the pronucleus stage [14], the 4- to 8-cell stage [15], the morula stage [16], and the blastocyst stage [17]; Fig. 1), the culture of embryos cloned by somatic cell nuclear transfer (culture to the blastocyst stage [18] and vitrification at the morula [19] and blastocyst [20] stages), the development of handmade cloning [21–23], and intracytoplasmic sperm injection (ICSI)–related technologies [24–27].

2. Preservation of unfertilized oocytes and gonadal tissue

The main type of genetic material for routine cryopreservation by gene banking is spermatozoa, that is, so-called semen cryobanking. Banking of other materials such as oocytes or early embryos has been considered

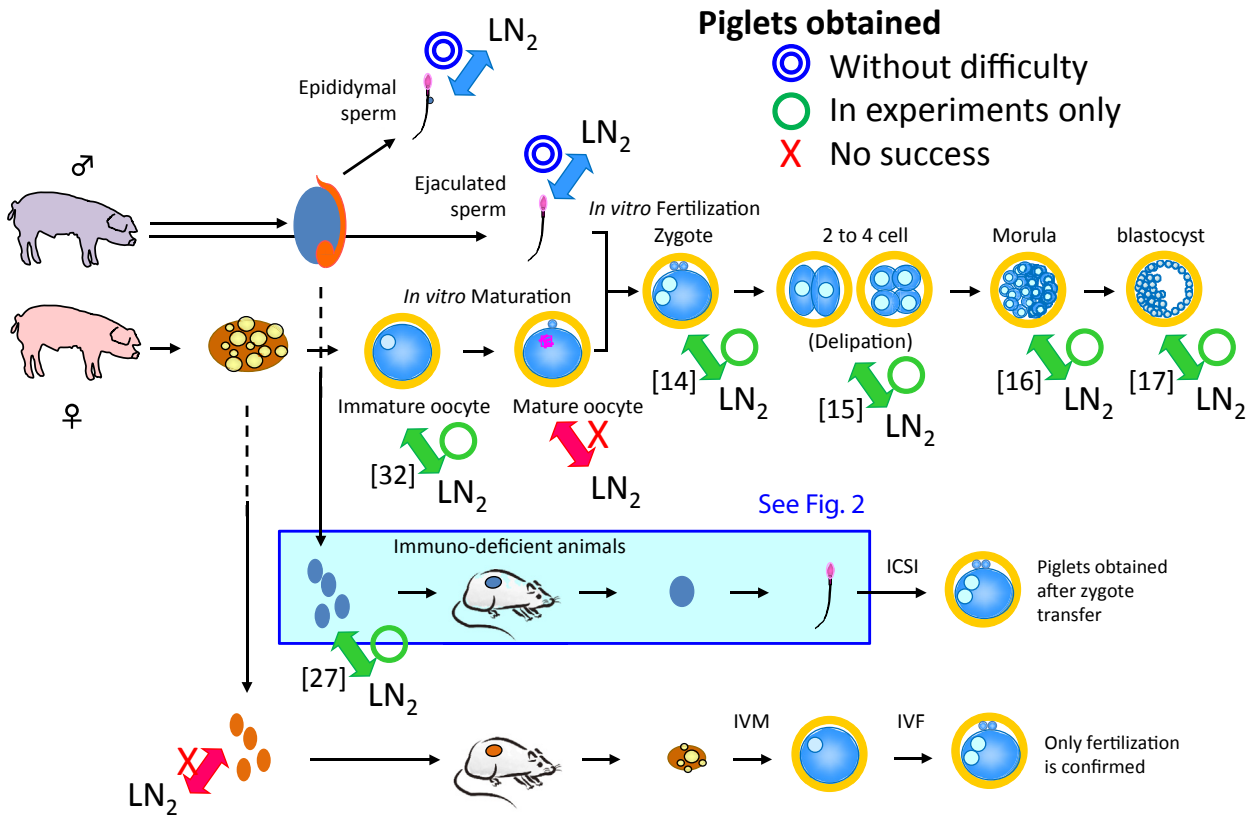

Fig. 1. Status of the procedures used to cryopreserve porcine gonadal tissue, gametes, and embryos in liquid nitrogen (LN_2). This approach can be divided into three groups on the basis of difficulty for piglet production. The most difficult approach is to produce offspring in pigs by cryopreservation of matured oocytes and xenografting of ovarian tissues to mice. Sperm obtained from vitrified and warmed testicular tissue (xenografts) support piglet production after intracytoplasmic sperm injection (ICSI), whereas oocytes obtained from vitrified and warmed ovarian tissue and then matured *in vitro* (IVM) only support *in vitro* fertilization (IVF) and the formation of pronuclei (no further embryonic development has been achieved). Numbers in the brackets indicate reference numbers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)