Theriogenology 91 (2017) 154–162

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Production of sperm from porcine fetal testicular tissue after cryopreservation and grafting into nude mice

Hiroyuki Kaneko [a, *, 1], Kazuhiro Kikuchi [a, b, **, 1], Nguyen Thi Men [a], Michiko Nakai [a], Junko Noguchi [a], Naomi Kashiwazaki [c], Junya Ito [c]

[a] Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Kannondai 2-1-2, Tsukuba, Ibaraki, 305-8602, Japan
[b] The United Graduate School of Veterinary Science, Yoshida 1677-1, Yamaguchi University, Yamaguchi, 753-8515, Japan
[c] Laboratory of Animal Reproduction, School of Veterinary Medicine, Azabu University, Sagamihara, Kanagawa, Japan

ARTICLE INFO

Article history:
Received 8 August 2016
Received in revised form
29 December 2016
Accepted 29 December 2016
Available online 30 December 2016

Keywords:
Fetal testis
Cryopreservation
Xenografting
Testis development
ICSI
Pig

ABSTRACT

A major goal of testicular xenografting is to salvage germ cells from immature animals that cannot be used for reproduction and generate their offspring. In this study, we investigated whether porcine fetal testicular tissue would acquire the ability to produce sperm with full developmental competence after they had been cryopreserved and grafted into nude mice. Testicular fragments from fetuses at 35, 55 and 90 days postartificial insemination (dpi) were vitrified and stored in liquid nitrogen. Immediately after warming, testicular fragments at each fetal stage were transplanted under the back skin of castrated nude mice (Crlj:CD1-*Foxn1*^{nu}) (35-, 55- and 90-dpi groups, respectively) (day 0 = grafting). Before grafting, the testicular fragments contained seminiferous cords consisting of only gonocytes and Sertoli cells. Histological analyses of the testicular grafts revealed that the differentiation of seminiferous tubules was largely dependent on the time after grafting, and not on donor age. On day 180 in each group, 10–20% of the total number of tubule/cord cross-sections examined had germ cells that had progressed beyond the spermatogonial stage. Fewer than 5% of tubule cross-sections contained elongated spermatids or sperm. Between days 360 and 420, tubule differentiation advanced further, until more than 45% of the tubule cross-sections contained elongated spermatids or sperm. Sperm were recovered for the first time from a single mouse in the 55-dpi group on day 180, although on days 360–420 sperm were recovered from most mice in all of the groups. Serum concentrations of inhibin and testosterone in host mice in all of the groups were higher than those in castrated mice that had received no testicular grafts. Single sperm collected from mice in each group on day 300 or later were injected into individual *in vitro*-matured oocytes, and these sperm-injected oocytes were transferred to the oviducts of 2 or 3 estrus-synchronized recipient gilts. None of the recipients in any of the groups produced piglets. The present results clearly indicate that porcine fetal testes during the gestational period acquire endocrine and exocrine functions after being cryopreserved and grafted into nude mice. However, the ability of xenogeneic sperm derived from fetal testis to generate piglets was not confirmed in the present study.
© 2017 Elsevier Inc. All rights reserved.

1. Introduction

Since pigs closely resemble humans in terms of anatomy and physiology, several genetically modified pig strains have been developed as human disease models [1,2]. However, some model pigs produced by cloning, such as hemophilia A [3] and immunodeficient [4] pigs, often die in the early neonatal period. Fetal and neonatal testes could be an important genetic resource for these genetically modified cloned pigs if their germ cells could be appropriately stored and salvaged for generation of offspring. Such trials may be justified, as it has been found that, in cloned mice, phenotypic abnormality caused by epigenetic errors is not transferred to the next generation [5,6]. Xenografting of testicular tissue into immunodeficient mice, described for the first time by Honaramooz et al. [7], makes it possible to recover sperm from testis of

* Corresponding author.
** Corresponding author. Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Kannondai 2-1-2, Tsukuba, Ibaraki, 305-8602, Japan.
E-mail addresses: kaneko@affrc.go.jp (H. Kaneko), kiku@affrc.go.jp (K. Kikuchi).
[1] H. Kaneko and K. Kikuchi contributed equally to this study.

http://dx.doi.org/10.1016/j.theriogenology.2016.12.036
0093-691X/© 2017 Elsevier Inc. All rights reserved.

neonatal or juvenile animals. Reports published so far have indicated that donor testicular tissues after cryopreservation have the ability to initiate spermatogenesis in host mice: their germ cells reached the spermatocyte (human [8,9] and primate [10]), spermatid (pig [11,12] and sheep [13]) or sperm (rabbit [14] and pig [7,15,16]), although the results appeared to depend on the time after testis grafting. The ability of xenogeneic sperm to generate offspring has been demonstrated by intracytoplasmic sperm injection (ICSI) using sperm from cryopreserved testicular tissues of neonatal rabbits [14] and pigs [16,17]. The reproductive ability of offspring produced using such xenogeneic sperm has also been confirmed [18,19]. In comparison to testes from neonatal or young donors that have been used in the studies mentioned above, a limited number of trials have been conducted on fetal testis. When fresh bovine testicular tissue from mid-gestational-age fetuses was grafted into nude mice and retained in them for a long period to allow completion of development, pachytene-stage spermatocytes appeared in the seminiferous tubules at 10 months after grafting [20]. On the other hand, nude mice harboring fresh human fetal testicular tissue have been used to evaluate the effects of exposure to environmental chemicals on testicular function [21,22]: the retention time (3–5 months) was too short for human fetal grafts to attain full testis development. Thus, it remains to be determined whether fetal testis of large mammalian species, especially after cryopreservation, can produce sperm with full developmental competence.

In European pigs, sexual differentiation of gonads occurs around 30 days postcoitum (dpc): male gonads start to form seminiferous cords by 26 dpc [23,24], while egg nests appear in the female gonads from 32 to 33 dpc [24]. The number of germ cells and length of seminiferous cords in the testis gradually increase from 42 dpc onwards [25]. At approximately 60 dpc, the testis begins to descend from its ventromedial position in the mesonephros, passing the inguinal canal at about 85 dpc and finally reaching the bottom of the scrotum shortly after birth (testis descent) [23].

In the present study, to plan for future trials aimed at utilization of fetal testis for production of progeny of several gene-modified cloned pigs, we examined whether wild-type porcine testicular tissue acquires complete spermatogenic potential after being cryopreserved and grafted into nude mice. We then injected sperm recovered from the recipient mice into *in vitro*-matured porcine oocytes and assessed the full-term fetal development of the fertilized oocytes after transfer to estrus-synchronized recipients. We chose three different fetal ages based on the stages of fetal testis development described previously [23,24]: 35 days postartificial insemination (dpi) soon after morphological differentiation of fetal gonads, 55 dpi just before the beginning of testis descent, and 90 dpi when the testis had reached the neck of the scrotum.

2. Materials and methods

All chemicals were purchased from the Sigma-Aldrich Corporation (St. Louis, MO, USA), unless otherwise indicated.

2.1. Preparation of testicular fragments

Protocols for the use of animals were approved by the Animal Care Committee of the Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Tsukuba, Japan. Female crossbreeds (Landrace × Large White) were artificially inseminated with fresh Duroc semen at the Institute of Livestock and Grassland Science, NARO, Tsukuba, Japan. Fetuses were collected from 7 pregnant sows at 35 days postartificial insemination (dpi), 7 sows at 55 dpi and 6 sows at 90 dpi, and transferred to the laboratory at 25 °C within 30 min. Gonads of fetuses at 35 and 55 dpi were gently separated from the mesonephros using a pair of fine forceps under a stereomicroscope with an illuminating device. The sex of fetuses at these ages was judged by examining the external genitalia and presence of a vein on the gonadal surface (testicular surface vein). From 90-dpi fetuses, testes were collected by pulling their spermatic cords from the side of the abdominal cavity. An outline of the experiment is shown in Fig. 1. Thirty-three fetal donors at 35 dpi, 33 donors at 55 dpi and 30 donors at 90 dpi were collected for the experiment. Two donors were selected from 2 litters at each fetal age and their testes were subjected to histological examination after cryopreservation: testes from the remaining donors were used for cryopreservation and xenografting. Testes were minced into fragments measuring approximately 1.5 × 1.5 × 1.5 mm in saline supplemented with 668 units/mL penicillin G potassium and 0.2 mg/mL streptomycin sulfate at 25 °C [26]. Before mincing, the capsules of testes at 55 and 90 dpi were gently removed, but those at 35 dpi could not be removed since definitive capsules had not formed on the surface of the testes at this stage.

2.2. Vitrification and storage of testicular tissue

Immediately after mincing, testicular fragments were vitrified according to the method described by Dinnyes et al. [27] and Somfai et al. [28], with some modifications [16]. Briefly, fewer than 35 fragments were washed as a group two times in 2 mL of base solution at room temperature (BS: modified North Carolina State University (NCSU)-37 solution [29] without glucose but supplemented with 0.17 mM sodium pyruvate, 2.73 mM sodium lactate, 4 mg/mL bovine serum albumin, and 50 mM β-mercaptoethanol) (IVC-PyrLac [30]) supplemented further with 20 mM HEPES (Dojindo, Kumamoto, Japan), and then transferred to 500 μl of equilibration solution (BS supplemented with 4% ethylene glycol (EG)) in four-well dishes (Nunclon Multidishes; Thermo Fisher Scientific, Waltham, NA, USA) and incubated for 15 min.

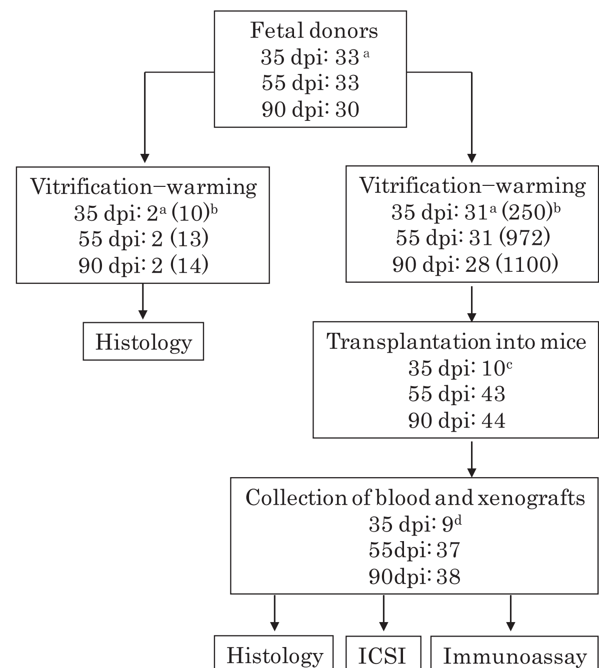

Fig. 1. Outline of the experimental design. [a]Number of fetal donors used. [b]Number of testicular fragments prepared from respective donors. [c]Number of mice used for testicular transplantation. [d]Number of mice that survived and from which blood and porcine testicular xenografts were collected. dpi; days postartificial insemination.

Download English Version:

https://daneshyari.com/en/article/5523491

Download Persian Version:

https://daneshyari.com/article/5523491

Daneshyari.com