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Genetic polymorphisms, growth performance, hematological parameters, serum enzymes, and reproductive characteristics in phenotypically normal Landrace boars produced by somatic cell nuclear transfer

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ABSTRACT

Understanding the performances of cloned pigs and their offspring is critical to evaluate the practical applications of somatic cell nuclear transfer. In this study, genetic polymorphism, growth performance, hematological parameters, and reproduction characteristics of cloned Landrace boars were compared with those of controls. In addition, the growth performance of clone offspring was also evaluated. A total of 479 reconstructed embryos were transferred to five recipient pigs and resulted in the delivery of 14 piglets (overall cloning of 2.9%) from two litters. Analyses of microsatellite markers and polymorphisms of the specific genes confirmed that the 14 clones were genetically identical to the nuclear donor and maintained the desirable genotypes. Growth performance of five healthy, phenotypically normal cloned boars from one litter and eight of their male offspring did not differ from age, breed, and management-matched controls. Although some significant differences were observed between cloned and control boars in hematological and serum enzymes, most of these parameters were within the normal range. Cloned boars had less ($P < 0.05$) normal sperm in the ejaculated boars than in control boars (71.4% vs. 77.9%, respectively), but sperm production (ejaculate volume, sperm concentration, and total sperm) did not differ between these groups. In addition, use of frozen-thawed semen from cloned boars for insemination produced results that seemed comparable to a control. In conclusion, the present study reported that somatic cell nuclear transfer is effective in reproducing preferred genetic traits and has potential applications to conserve elite bloodlines in a routine pig breeding program.

1. Introduction

Many mammals have been successfully cloned by somatic cell nuclear transfer (SCNT), a technique used for reprogramming a terminally differentiated cell to its totipotent state. In 1989, the first pig was successfully cloned by using undifferentiated embryonic cells as donor nuclei [1]. Thereafter, new protocols were developed to generate cloned pigs by double nuclear transfer [2] and somatic cell microinjection into enucleated oocytes [3]. The efficiency of pig cloning was subsequently improved by modifying activation protocols and culture systems [4–7], increasing the embryo numbers for transfer [8], treating embryos with histone deacetylase inhibitors [9,10], and increasing the duration of donor nucleus exposure to oocyte factors [11]. Pig cloning could be used to preserve invaluable genetic resources or to generate genetically modified pigs for agriculture purposes and regenerative medicine [12].

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Studies on cloned pigs have revealed a higher perinatal mortality and higher rate of malformations compared with the conventional reproduction programs, whereas growth rate, reproductive performance, behavioral traits, and disease incidence are normal throughout the lifetime of surviving clones [8,13,14]. Notably, the variability of some phenotypic characteristics is even higher in cloned pigs derived from a single donor cell line than those from routine breeding programs [15,16]. Despite the variation found in the cloned pigs, similar phenotypic characteristics are shown between progenies of cloned and noncloned pigs [16,17]. Meat composition of the offspring of clones is also not different from that from the control pigs [18], suggesting no discernible risks associated with the consumption of products from the offspring of cloned pigs [16]. However, to date, the information of genotypes and other phenotypic characteristics, including growth and reproductive performance of cloned pigs and their offspring, are still poorly documented. In addition, the performance of cloned pigs and their offspring warrants investigation to assess the practical feasibility of SCNT technology.

In Taiwan, the growth performance index in combination with marker-assisted selection is now the preferred routine to accelerate the improvement of economically important traits in pig breeding program [19–21]. In order to assess the practical application SCNT in breeding programs, polymorphism of genes associated with reproductive performance and meat quality, growth performance, hematological parameters, and semen characteristics of cloned Landrace boars were compared with those of noncloned boars. In addition, the growth performance of clone offspring was also evaluated.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise indicated. The media were prepared as described previously [22].

2.2. Superovulation and oocyte collection

The estrous cycle of pubertal crossbred (Landrace, Yorkshire, and Duroc) gilts at 8 to 10 months of age were synchronized by feeding commercial feed mixed with Regumate (containing 0.4% altrenogest; 20 mg/day; Intervet International BV, Boxmeer, the Netherlands) for 15 days. Follicle formation was stimulated by treating gilts with 2000 IU pregnant mare serum gonadotropin (Folligon, Intervet) on Day 16. After 80 hours, 1750 IU hCG (Chorulon, Intervet) was used to induce ovulation. Oocytes were collected surgically by flushing the oviduct with Dulbecco's PBS (Gibco BRL, Gaithersburg, MD, USA) at 44 to 46 hours after hCG treatment. In each replicate of trial, 100 to 150 oocytes were collected from five to six gilts. In total, five replicates of SCNT were performed in this study.

2.3. Preparation of donor cells

Fibroblast cells were obtained by ear biopsy from an elite Landrace boar L277-10. Briefly, after quick rinses in 75% ethanol and Dulbecco's PBS supplemented with penicillin (100 IU/mL) and streptomycin (100 μg/mL), ear tissues were minced into 1- to 2-mm pieces and washed in DMEM with 10% fetal bovine serum (FBS). Five to ten pieces of ear tissues were settled at the bottom of 60-mm cell culture dishes and cultured in 2 mL of DMEM with 10% FBS. The dishes were then placed in a humidified 38.5 °C incubator with 5% CO_2 and the culture medium was changed every other day. The fibroblasts were harvested with 0.05% trypsin-EDTA and passaged at 1:4 split ratios (passage number 1, P1). The fibroblast cells at passages 3 to 5 were then frozen in liquid nitrogen with the medium containing 90% FBS and 10% DMSO. For SCNT, fibroblast cells were thawed and cultured to 80% of confluency. Serum starvation was then used to synchronize the cell cycle of donor cells at G0/G1 phases in DMEM with 0.5% FBS for 3 to 5 days. Donor cells were treated with trypsin and resuspended in DMEM with 0.5% FBS before SCNT.

2.4. Nuclear transfer, activation, and embryo culture

Micromanipulation was performed using a modified protocol based on a previous study [22]. Briefly, cumulus-denuded oocytes were centrifuged at 12,000 × *g* for 10 minutes in an Eppendorf tube with TL-Hepes medium to locate the first polar body. For enucleation, groups of oocytes were transferred into droplets of TL-Hepes containing 5 μg/mL cytochalasin B. A glass needle was prepared to make an incision in the zona pellucida. The polar body and metaphase chromosomes with the appropriate amount of surrounding cytoplasm were then removed by pressing the oocyte with a glass needle. Successful enucleation was confirmed by ultraviolet light exposure of the presumed karyoplasts under a fluorescent microscope after staining with 5 μg/mL Hoechst 33342.

The piezo-driven intracytoplasmic injection was performed by a similar procedure used in mouse cloning [23]. Donor cells were transferred to microdroplets with TL-Hepes containing 10% (wt/vol) polyvinylpyrrolidone before injection. The membrane of donor cells was punctured by gentle aspiration in and out of the injection pipette (~12 μm in diameter). Each donor cell was then injected into an enucleated oocyte through the zona pellucida slit. Reconstructed embryos were cultured in PZM-3 and then returned to a humidified incubator (38.5 °C) with 5% CO_2 for 3 to 4 hours. Activation was then performed by electrical pulses combined with 6-dimethylaminopurine treatment. Briefly, after washing and preincubation for 3 minutes in a 1:1 mixture of activation medium (0.25 M mannitol solution supplemented with 0.01% polyvinyl alcohol, 0.5 mM Hepes, 0.1 mM $CaCl_2 \cdot H_2O$, and 0.1 mM $MgCl_2 \cdot 6H_2O$ with pH 7.2) and TL-Hepes medium with 10% FBS, the reconstructed embryos were transferred to activation medium for 3 minutes at room temperature. Reconstructed embryos were then transferred to the activation medium contained in an electrical chamber. A BTX Electro Cell Manipulator ECM 2001 (Biotechnologies and Experimental Research, Inc., San Diego, CA, USA) was used to deliver an electrical pulse for 10 seconds at 5 V AC followed by a 30-μs DC pulse at 2.2 kV/cm. Following the DC pulse, the reconstructed embryos were washed several times in PZM-3 and then