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Research Article

A Comparative Study on Reproductive Performance of Large White Boars Cloned by Somatic Cell Nuclear Transfer and Growth Performance of Their Progeny

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Abstract

The success of cloning animal paves a new way to increase livestock production and conserve genetic resources. After the success of first cloned pig production, a large number of research reports related to cloned pig have been published. However, a few researches into reproductive ability of cloned pig were conducted. The evaluation of reproductive performance of cloning pig is of great importance in practical application for breeding and reproduction. The purpose of present study was to compare reproductive performance of cloned Large White boars with non-clones. First, cloned pigs were produced by somatic cell nuclear transfer (SCNT) technique. After confirmation of cloning success, two groups (clones versus non-clones) were created for a comparison. The semen quality and reproductive performance of pigs derived from SCNT were tested. Growth performance of their progeny was also compared. The results showed that there were no significant differences in semen quality and reproductive performance between clones and non-clones. The parameters related to growth performance such as weight and average daily gain were similar in both groups. To sum up, no remarkable differences were observed between clones and non-clones, indicating that SCNT technology could be applied not only to increase swine production but also to preserve and spread superior Large White pig species.

Keywords

Somatic Cell Nuclear Transfer, Cloning, Reproductive Performance, Large White Boar

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1. Introduction

After the first cloned sheep, Dolly was born in 1996, somatic cell nuclear transfer (SCNT) technique has regarded as a promising technology in preserving and spreading superior livestock species [1]. With the introduction of this technology to various mammals, the first cloned pig was successively produced in 2000 [2]. The success of pig cloning provided a new opportunity to increase swine production and conserve genetic resources. However, current development of SCNT is facing several challenges such as low cloning efficiency, embryo abnormalities and shorter lifespan [3-5]. Therefore, extensive studies have been carried out to investigate genetic, physiological, biochemical and epigenetic mechanisms related to SCNT [6-8]. Although notable results have been published through such researches so far, limited data are available in terms of reproductive performance of cloned boars [3, 9, 10].

To assess the reproductive performance is of great importance in practical applications for breeding and reproduction. Reproductive ability is the essential nature of life. It could be difficult to maintain and spread cloning animals if they exhibited poor reproductive performance. Several abnormal changes observed in cloning animal may infer the changes in reproductive performance. Therefore, to evaluate whether the reproductive performance of cloning pig is in the range of normal or not is necessary to investigate.

In this context, the present study was designed to evaluate reproductive performance of cloned large white boars. Semen quality and reproductive performance of cloning pig were investigated. Growth performance of their progeny was also compared. This experimental data will be valuable in livestock practice to evaluate reproductive ability of cloned pigs derived from SCNT.

2. Materials and Methods

2.1. Preparation of Cloned Pigs

Cloned pigs were produced according to previous method [11, 12]. Briefly, ear fibroblasts were isolated from a superior purebred Large White boar. The donor nuclei of obtained fibroblasts were injected into the enucleated matured oocytes. Then, the reconstructed embryos were transferred into Fallopian tube of recipient gilts. The microsatellite analysis was performed for the assessment of cloning success. Four swine DNA microsatellite markers (SW911, SW240, SW857, SO178) on different pig chromosomes were used in this experiment. Growth performance of cloned pigs was compared to the control (standard Large White Boar) for 8 months after birth. After 15 months, reproductive performance of cloning boars was evaluated.

2.2. Experimental Animal

Two groups, namely cloned (n=3) and non-cloned Large White boars (n=3) were compared in this study. Each group also included 15 gilts for the assessment of reproductive performance. All pigs were housed in the same conditions and fed the identical manufactured feed.

2.3. Semen Quality Evaluation Assay

Semen quality was evaluated according to the previous method [13]. Briefly, semen samples from the cloned and non-cloned boars were collected by the gloved-hand technique. The semen quality was tested immediately after collection by phase-contrast microscope. Sperm concentration, sperm motility, percentage of abnormal sperm was counted by hemocytometer.

2.4. Reproductive Performance Test

Reproductive performance of cloned and non-cloned boars was performed by the previous method [9]. The litter size, number of live piglets, the survival to weaning were compared.

2.5. Growth Performance of Piglet

The growth performance of cloned piglets and non-cloned piglets up to weaning was compared according to the previous method [9]. In this study, 187 progeny of the clones and 194 progeny of the non-clones were investigated. The other conditions such as daily feed intake, feed composition, water accessibility, temperature and housing condition were the same. Weight at birth, weight at weaning and average daily weight gain were studied.

2.6. Statistical Analysis

The normality distribution of each experiment result was evaluated by Shaprio-Wilk's test. After confirmation of the normal distribution, independent Student's t-test and one-way analysis of variance (ANOVA) were applied for the statistical analysis. For the parameters not following the normal distribution, non-parametric test (Mann-Whitney-Wilcoxon test and Kruskal-Wallis test) were used to evaluate the differences. Survival analysis was applied for censored data of cloned pigs. Differences were considered significant at $P<0.05$. All data in this study were expressed as mean±standard deviation. The experimental data were processed by R program and Microsoft Office Excel 2021.

3. Result and Discussion

3.1. Cloned Pig Production and Growth Performance of Cloning Pig

In this experiment, a total of 2137 reconstructed embryos

were transferred to 30 recipients. Cloning efficiency was still low in this experiment, similar to previous reports [14-16]. Three recipient gilts became pregnant and eleven pigs were obtained. Among newborn piglets, only three pigs were survived to maturity and they were all male. The genetic identity of cloned piglets was confirmed by four swine DNA microsatellite markers. Qualitative indices such as the shape of head, mouth and ear, skin color and leg posture were identical to the donor Large White boar. From this result, it can be concluded that ear fibroblast of Large White boar could be used as a donor cell for SCNT. Quantitative parameters such as body weight, average daily gain and body length were compared to the control. The data are presented in Table 1.

As shown in Table 1, cloned pig showed overall better growth performance than control, indicating that superior Large White boar was worthy of being a donor pig.

Table 1. *Comparative parameters of cloned large white boars and control.*

Groups	Weight at birth (kg)	Weight at weaning (kg)	Weight at 10 month	Average daily gain	Body length (cm)	Circumstance of chest (cm)	Back-fat thickness (mm)
Clones	1.4 ±0.5[a]	7.4 ±0.7[a]	124.2 ±2.3[a]	0.41 ±0.17[a]	114.5 ±1.6[a]	107.7 ±0.5[a]	13.28 ±0.04[a]
Control	1.5 ±0.3[a]	6.9 ±0.4[b]	118.1 ±1.8[b]	0.39 ±0.11[b]	112.7 ±2.5[a]	106.2 ±0.3[b]	13.50 ±0.07[a]

Note: Different superscripts in the same row mean significant difference ($P<0.05$).

3.2. Semen Quality Assay

Several parameters related to semen quality were described in Table 2. As can be seen in Table 2, no remarkable differences were observed between cloned and non-cloned group. In previous report [9], the percent of abnormal sperm of cloning boar was higher than normal boar. The researcher explained that it could be the result from the use of young cloned boar as an experimental animal. In this experiment, we used 15 month-cloning large white boar as an experimental animal and did not observe any differences, indicating that the semen quality of cloned boar was in the normal range.

Table 2. *Semen quality parameters in cloned and non-cloned boars.*

Group	Ejaculates (ml)	Sperm motility (%)	Sperm concentration ($\times 10^8$/ml)	Sperm pH	Percent of abnormal sperm (%)
Clones	142.4 ±2.7	73.4 ±1.6	2.2 ±0.23	7.3 ±0.1	3.2 ±0.3
Non-clones	139.2 ±2.5	70.9 ±1.5	2.1 ±0.21	7.3 ±0.1	3.0 ±0.5

Note: each experiment was repeated 12 times. Data were shown in mean ±standard.

3.3. Reproductive Performance

Table 3 depicts comparative result of reproductive performance. As shown in Table 3, there were no significant differences between the tested parameters, indicating that reproductive performance was similar to normal pig.

Table 3. *Reproductive performance of cloned and non-cloned boars.*

Groups	Litter sizes	Live piglets born	Survival to weaning
Clones	9.3 ±1.2	8.7 ±1.3	8.2 ±1.0
Non-clones	9.5 ±1.3	9.0 ±1.2	8.4 ±1.6

3.4. Growth Performance of Progeny

Three growth parameters, namely weight at birth, weight at weaning and average daily weight gain were presented in Table 4. As can be seen, there were no notable differences between growth parameters. This result refers that progeny of cloned boar could grow within a normal range.

Table 4. *Growth performance of piglets from clones and non-clones.*

Groups	Weight at birth (kg)	Weight at weaning (kg)	Average daily weight gain (kg)
Clones	1.5 ±0.1	9.5 ±0.2	1.3 ±0.2
Non-clones	1.3 ±0.2	9.2 ±0.3	1.3 ±0.4

4. Conclusion

The present study showed that reproductive performance of cloned large white boar was similar to non-cloned boars. This result will provide more information in SCNT in terms of preserving and spreading superior pig species.

Abbreviations

SCNT Somatic Cell Nuclear Transfer

Author Contributions

Chang-Gon Sin: Conceptualization, Writing – original draft
Su-Chol Rim: Conceptualization, Writing – original draft
Chang-Sin Rim: Methodology, Resources
Ry-Chol Kim: Methodology, Resources
Kwang-Il To: Project administration, Supervision
Chol-Min Kim: Investigation, Validation
Won-Ju Hwang: Investigation, Resources
Chong-Nam So: Investigation, Validation
Hyok Ri: Investigation, Visualization
Myong-Il Ra: Investigation, Visualization
Hyok-Won Kim: Investigation, Software

Conflicts of Interest

The authors declare no conflicts of interest.

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