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Growth Rate of Transgenic Pigs and Size of Pig Hearts for Xenotransplantation to Cynomolgus Monkey

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ABSTRACT

To compensate for the critical shortage of human organs for allotransplantation, xenotransplantation studies using genetically modified pigs are being performed in Korea. Two types of pigs that are used are α1,3-galactosyltransferase gene knockout (GalT KO) pigs and GalT KO+hCD46 (human complement regulatory protein) pigs. The present study measured the gestation time, birth weight, daily growth rate, and heart weight of both kinds of transgenic minipigs. The gestation period for both types of pigs was 117～119 days. There was no difference in the body weight of GalT KO (−/+) and GalT KO (−/−) piglets, but GalT KO+hCD46 ($-^{hCD46+}/+$) pigs were significantly heavier at birth than were GalT KO+hCD46 ($-^{hCD46+}/-^{hCD46+}$) pigs. During the first 10 weeks of life, the daily weight gain of GalT KO+hCD46 ($-^{hCD46+}/-^{CD46+}$) piglets, which are considered the optimal type for xenotransplantation, was 0.19 kg. The weight of hearts from GalT KO piglets up to two months of age was affected more by body weight than by age. Transgenic pigs showed no differences in gestation period or reproductive ability compared with normal pigs. These results comprise basic data that may be used in xenotransplantation studies and transgenic animal production in Korea.

(Key words : pig, α1,3-galactosyltransferase gene knockout (GalT KO), body weight, transgenic, human complement regulatory protein)

INTRODUCTION

Transplanting organs from pigs is receiving attention as a solution to the critical shortage of organs for transplantation in humans. However, when pig organs are transplanted to primates, they are rapidly rejected by hyperacute rejection and acute humoral xenograft rejection responses. Both of these immune responses are triggered by xenoreactive natural antibodies directed against a specific epitope (the galactose α1,3 galactose carbohydrate epitope, GalT) on the porcine vascular endothelium (Shimizu and Yamada, 2010). Recently, an α galactosyltransferase gene knockout (GalT KO) pig and a GalT KO+hCD46 (membrane cofactor protein, MCP; human complement regulatory protein) pig were produced by genetic modification to solve this problem. CD46 is known to inhibit complement activation on host cells (Wang *et al.*, 2000). GalT KO and GalT KO+hCD46 pigs have been developed in Korea and used for xenotransplantation to nonhuman primates (Ann *et al.*, 2011; Im *et al.*, 2012; Hwang *et al.*, 2013; Ko *et al.*, 2013). However, anatomical, biological and nutritional information about them that may be utilized in xenotransplantation research is very limited.

Minipigs are smaller than commercial pigs; their small body size helps reduce rearing and breeding costs and makes them easier to handle during medical research (Köhn *et al.*, 2007). The birth weight of normal pigs has important long-term effects on growth, as well as an immediate influence on the piglet's survival prospects. Normal piglets that are born weighing over 1.2 kg have definite survival advantages over littermates that weigh less than 1 kg.

The purpose of the present study was to aid domestic xenotransplantation research by generating basic anatomical, biological, and nutritional information about transgenic minipigs, including their gestation period, litter size, weight at birth, heart weight gain, and growth rate.

MATERIALS AND METHODS

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1. Animals

Two types of transgenic pigs were used: α1,3 galactosyltransferase knockout (GalT KO) pigs (n=20) and GalT KO+hCD46 pigs (n=9), aged up to 10 weeks. The GalT KO and GalT KO+hCD46 pig strains were the result of crossbreeding Landrace with Chicago minipigs and Massachusetts General Hospital miniature pigs, respectively. The offspring of GalT KO pigs and GalT KO+hCD46 pigs were produced by mating, as shown in Table 1. The animal husbandry procedures followed the animal experiment ethics guidelines of the National Institute of Animal Science, RDA.

2. Gestation Period, Litter Size and Body Weight of Transgenic Piglet of Transgenic Pig

The gestation period of transgenic pigs was calculated from the day of mating to the delivery date. This experiment used four replicates. The body weights of GalT KO+hCD46 pigs were measured at one-week intervals from birth to 10 weeks of age. These body weights were compared with those of non-transgenic pigs and heterozygous transgenic pigs.

3. Organ Weight

The hearts of 7 GalT KO (−/−) piglets (less than 2 months old) were surgically removed under inhalation anesthesia (Kim *et al.*, 2013). The GalT KO pig hearts were perfused with 200 mL of histidine tryptophan-ketoglutarate solution infused via the aortic root. The pericardium was removed and all great vessels were bisected at their points of entry to the heart. Chambers were opened and all blood clots were washed away several times. Finally, each heart was weighed using an electronic balance. By comparison, a nonhuman primate commonly used for xenotransplantation is the cynomolgus monkey (*Macaca fascicularis*, Orient Genia, Inc, Gyeonggi-do, Republic of Korea), which weighs 4～7 kg and is ～60 months old when organs are harvested. In order for the organ size to be similar to this size, GalT KO (−/−) piglets were selected that weighed less than 7 kg.

4. Statistical Analysis

Mean ± standard deviation (S.D.) were calculated for the data. The data from the two groups were compared using the Student *t*-test with Excel and $p<0.05$.

RESULTS AND DISCUSSION

1. Gestation Period and Litter Size of Transgenic Pigs

As shown in Table 1, the GalT KO pigs (117 ± 2.7 days) and the GalT KO+hCD46 pigs (119 ± 9.3 days) had a similar gestation period. The litter size of transgenic pigs was 5.0 ± 3.2 (GalT KO) and 5.8 ± 1.3 (GalT KO+hCD46) piglets, respectively. The gestation period of a commercial pig and minipig is known to be 114 days (Bode *et al.*, 2010); therefore, the two transgenic pigs showed a normal gestation period. Few studies have reported the litter size of transgenic pigs, and additional research on this topic is needed.

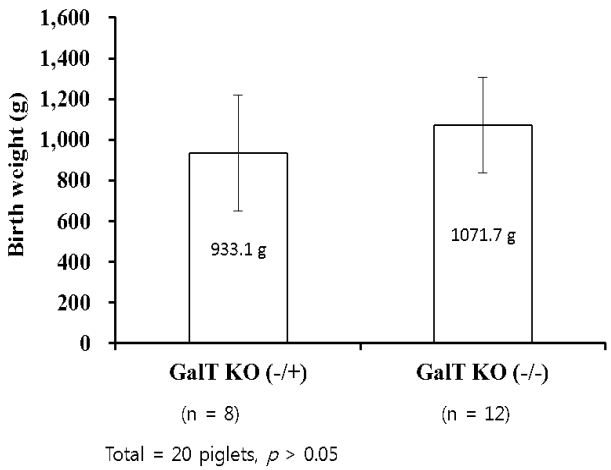
2. Birth Weights of Transgenic Piglets

The birth weights of transgenic piglets produced for xeno-

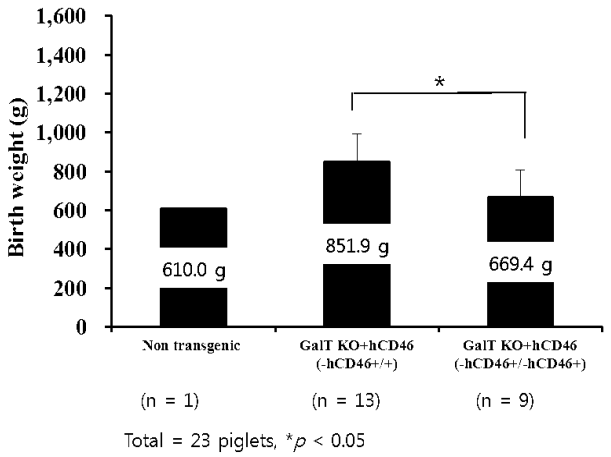
Table 1. The length of the gestation period and litter size in transgenic GalT KO pigs and GalT KO+hCD46 pigs (with four replicates)

Transgenic pig breeding	Gestation period	Litter size	Remarks
GalT KO (−/−) ♂ × GalT KO (−/+)♀	115	9	Multiparous sow
GalT KO (−/−) ♂ × GalT KO (−/+)♀	116	2	Multiparous sow
GalT KO (−/−) ♂ × GalT KO (−/+)♀	121	6	Multiparous sow
GalT KO (−/−) ♂ × GalT KO (−/−)♀	116	3	Primiparous sow
Mean ± S.D.	117 ± 2.7	5.0 ± 3.2	
GalT KO+hCD46 ($-^{hCD46+}$/+)♂ × GalT KO+hCD46 ($-^{hCD46+}$/+)♀	114	6	Multiparous sow
GalT KO+hCD46 ($-^{hCD46+}$/+)♂ × GalT KO+hCD46 ($-^{hCD46+}$/+)♀	133	6	Primiparous sow
GalT KO+hCD46 ($-^{hCD46+}$/+)♂ × GalT KO+hCD46 ($-^{hCD46+}$/+)♀	114	7	Multiparous sow
GalT KO+hCD46 ($-^{hCD46+}$/+)♂ × GalT KO+hCD46 ($-^{hCD46+}$/+)♀	115	4	Multiparous sow
Mean ± S.D.	119 ± 9.3	5.8 ± 1.3	

transplantation were measured (Fig. 1). In general, fetuses or offspring produced by somatic cell nuclear transfer (SCNT) or *in vitro* fertilization and culture are larger than normal, resulting in large offspring syndrome in some cases (Hill, 2014). The analysis of birth weight was conducted in order to reveal whether genetic modification affected the health of the piglets of two types of transgenic pigs. Among the offspring of GalT KO pigs, there was no significant difference between GalT KO (−/+) pigs (933.1 ± 284.8 g) and GalT KO (−/+) pigs (1,071.7 ± 234.2 g). However, among the offspring of GalT KO+hCD46 pigs, there was a significant difference between GalT KO+hCD46 ($-^{hCD46+}$/+) pigs (851.9 ± 139.7 g) and GalT KO+hCD46 ($-^{hCD46+}/-^{hCD46+}$) pigs (669.4 ± 137.3 g). The GalT KO+hCD46 pigs produced only one nontransgenic offspring of normal size, and it was not included in the analysis because of the small sample size number. The results differed presumably according to breed and strain differences and the difference in the inserted genes, especially the expression level of hCD46 as an inhibitor of human complement activation. The average birth weight of the Göttingen minipig is known to be 460 g. The GalT KO pigs were originally developed by crossbreeding Landrace pigs, which have a general pig body type, so the GalT KO pig showed less of a difference in birth weight due to genetic modification than did the GalT KO+hCD46 pig.



(A) Offspring of GalT KO pigs



(B) Offspring of GalT KO+hCD46 pigs

Fig. 1. Birth weights of transgenic piglets.

3. Growth Rate of Transgenic Pigs

GalT KO+hCD46 ($-^{hCD46+}/-^{hCD46+}$) pigs are known to present minimal hyperacute and acute rejection reactions (Mohiuddin *et al.*, 2012, 2014); therefore, they are considered the optimal animals for xenotransplantation in Korea. After the heart of a GalT KO+hCD46 pig was transferred to a monkey, the monkey survived for an additional 8 months (Mohiuddin *et al.*, 2012). Xenotransplantation of the heart of a GalT KO+hCD46+thrombomodulin (TBM) pig to a human also resulted in recipient survival exceeding 1 year (Mohiuddin *et al.*, 2014).

In order to obtain a heart of the correct size for xenotransplantation, it is important to know whether the growth rate of organs in the GalT KO+hCD46 pigs depends on their body weight. As shown in Fig. 2, although GalT KO+hCD46 ($-^{hCD46+}/-^{hCD46+}$) pigs had low birth weights (0.71 ± 0.16 kg, n=8), their growth rates were normal. They reached 10 ± 1.53 kg at two months of age. Daily live weight gain of GalT KO+hCD46 pigs from birth to 10 weeks was 0.19 kg/day. Accor- ding to aprevious study, the daily live weight gain of a large white pig containing inserted CD45 was 0.49 kg/day at 10 weeks, no different than that of commercially raised healthy pigs of the same age (Tucker *et al.*, 2002). The low daily weight gain value in the present result probably reflects differences in breed (mini pig vs. commercial pig) and in the inserted genes. On the basis of these results, the appropriate transplant age of GalT KO+hCD46 ($-^{hCD46+}/-^{hCD46+}$) pigs is 4 ~7 weeks, the age at which they did not exceed the body weight of the cyno- molgus monkey (~60 months old) used for xenotransplantation (4~7 kg). In the pig-to-monkey model, the cardiac xenograft was performed using the heterotopic cardiac xenograft method; therefore, the body weight of the pig was a very important factor.

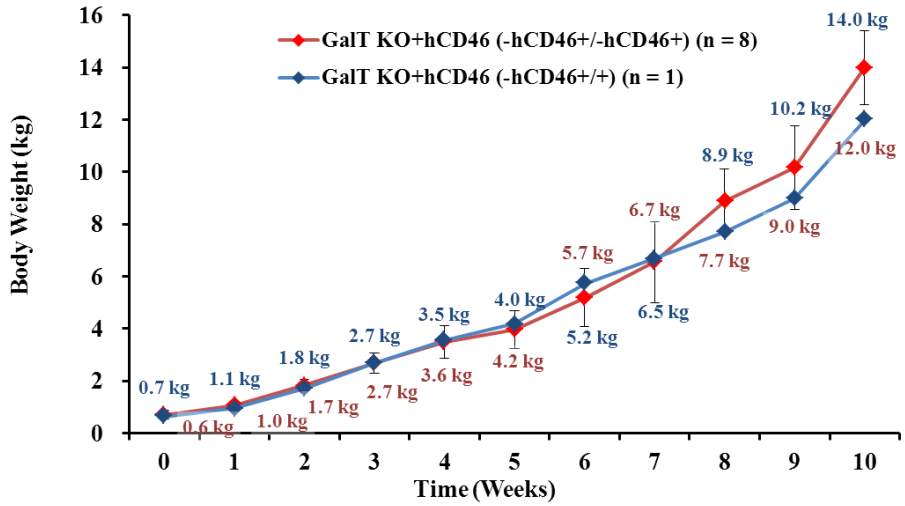


Fig. 2. Growth rate in GalT KO+hCD46 piglets. The weights of 9 piglets were measured at 1-week intervals from birth to 10 weeks.

4. Weight of Hearts from Transgenic Pigs

The genetically modified pigs in the present study were GalT KO pigs and were used for the actual organ transplantation to a non-human primate model. The genetic type of all GalT KO pigs used for xenotransplantation is homozygous [GalT KO (−/−)] and the heart was used when the pig was less than two months old so that it would fit the size of the implanted monkey (body weight 4～7 kg). Heart weight before xenotransplantation from the pig to the monkey is shown in Fig. 3. Previous studies reported that the heart weight of pigs was directly affected by body weight rather than by age (Soin *et al.*, 2000; Tucker *et al.*, 2004), and the results of the present study were consistent with these reports. At 31 days and 45 days of age, GalT KO piglets weighed 6.5 kg (41.6 g heart weight) and 6.3 kg (44 g heart weight), respectively (Fig. 4). During the first 10 weeks of life, the heart weight of GalT KO piglets ranged from 25.6 g to 44 g. This information may be used to time transplantation in order to synchronize the organ size between the donor and the recipient.

The present study revealed basic anatomical and biological information about transgenic pigs developed for xenotransplantation in Korea. This information includes the physiological characteristics of transgenic animals, which may be used to determine the most suitable age of transgenic piglets for successful xenotransplantation from pigs to nonhuman primates.

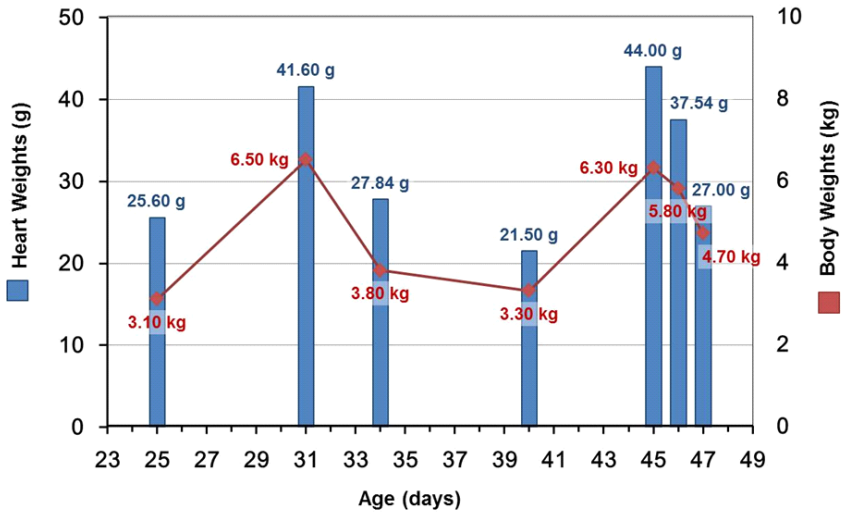


Fig. 3. Correlation between heart weight and body weight in GalT KO (—/—) piglets (*n*=7).

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