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Short Communication

Successful surgical correction of anal atresia in a transgenic cloned piglet

Gab Sang Lee[1], Hye Soo Kim[1], So Hyun Lee[1], Dae Yong Kim[3,4], Kang Moon Seo[5], Sang-Hwan Hyun[6], Sung Keun Kang[1,3,4], Byeong Chun Lee[1,3,4,*], Woo Suk Hwang[1,2,3]

[1]*Departments of Theriogenology and Biotechnology,* [4]*Pathology, and* [5]*Surgery, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*
[2]*School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*
[3]*Xenotransplantation Research Center, Seoul National University Hospital, Seoul 110-744, Korea*
[6]*Department of Veterinary Biotechnology, College of Veterinary Medicine, Chungbuk National University, Cheongju 361-763, Korea*

Inbred strains of pig become indispensable for a wide range of biological studies. In biomedical science, it is generally accepted that somatic cell nuclear transfer (SCNT) technology with inbreed strain of pig is essential for xenotransplantation. In this study, we observed the anal atresia in a cloned pig which was derived from fetal fibroblast of inbreed miniature pig. A presumptive anal site of the cloned pig was excised and the rectum was sutured to apposed skin for treatment. This cloned piglet seemed to be normal with healthy status after surgery. This report can be useful for the treatment of anal atresia of cloned piglets.

Key words: anal atresia, cloned pig, miniature pig, nuclear transfer, transgenic pig

Successful somatic cell nuclear transfer (SCNT) of cultured cells, which was demonstrated in cattle [16], has provided an alternative for obtaining genetically modified pigs. McCreath *et al.* [11] reported the first success of obtaining gene-targeting sheep by using gene-targeted fibroblasts as a source of donor nuclei for SCNT. Since the birth of the first piglet from SCNT was reported in 2000 [14], tremendous progress has been made. Successful productions of pig resulting from random genetic modification *in vitro* followed by SCNT [5], as well as those with a specific modification (knock out) have been reported [9] by several groups in a short period. The production of cloned transgenic pigs is now in the transition from investigation to practical applications.

Anal atresia is a rarely recognized congenital disorder in the neonatal piglets, the incidence being estimated to be 0.1 to 1.0% [1]. A fistula develops in affected piglets between anorectum and urethra and between anorectum and vagina, respectively. Congenital absence of the anus causes a build up of feces and consequent distension of the abdomen. The condition almost is invariably fatal within 2 to 3 days in males [12]. This report describes the successful treatment of anal atresia in a transgenic cloned piglet. It is known to be not worth attempting surgical repair local field because death invariably ensues without correct surgery and intensive care.

The SCNT and embryo transfer (ET): SCNT and ET were described in our previous study [4,9].

Treatment of anal atresia: For local anesthesia, 1 ml of 2% lidocaine solution (Xylocaine; Astra, Japan) was administrated around presumptive anal region site. After, sufficient evacuation of the bowel, the rectal wall was apposed to the skin by interrupted sutures [6]. With assisting by pressure on the abdominal wall, gentle traction was used to draw the rectum caudal to the anus. Enrofloxacin 0.5 mg/kg/b.w. (Baytril; Bayer, Korea) was injected IM and surgery site is hip-bathed with 10% povidone-iodine up to 7 days post surgery (Fig. 1).

The piglet was nursied, and hydration status was assessed as normal. Antimicrobial and fluid therapies were continued for 7 days. The cloned piglet was seen to defecate normally. The transgenic cloned pig has been healthy and growing well without a history of complications for one year following surgery.

Since surgical correction, the cloned piglet seemed to be healthy and normal. No abdominal distension and difficulty in defecation were noted. To our knowledge, this is the first case of the anal atresia in cloned piglet. There are many reports on the congenital abnormalities of cloned mice and cattles [3,17]; however such data are sparse in cloned pigs [15]. We could not exclude any possibility that the anal atresia is directly related to SCNT since anal atresia occurs in normal piglet [12]. In the other hand, a previous study suggested that the atresia ani was genetically influenced by 2 major loci, 1 autosomal recessive and 1 autosomal

*Corresponding author
Tel: +82-2-880-1269; Fax: +82-2-884-1902
E-mail: bclee@snu.ac.kr

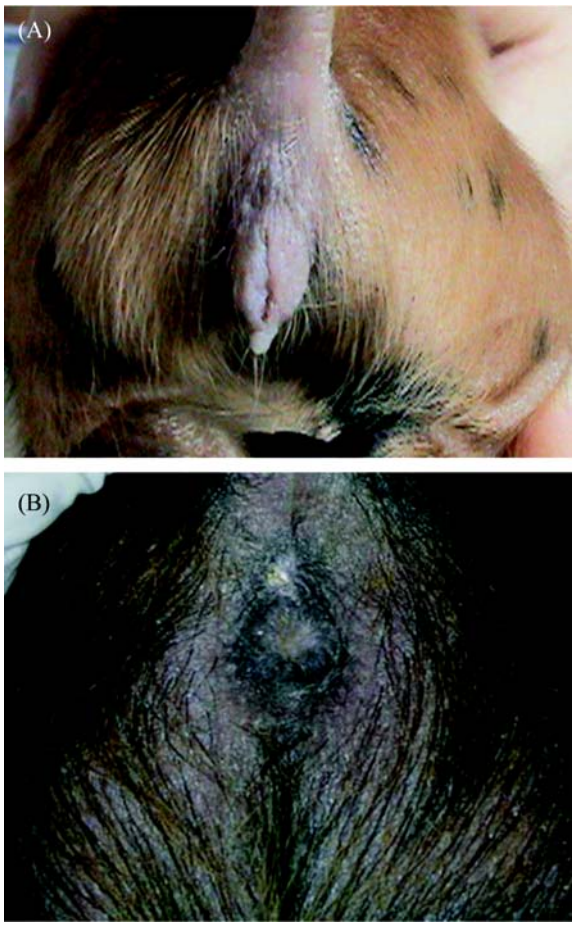

Fig. 1. The anal atresia in female cloned transgenic piglet before (A) and 40 days after surgical correction (B).

dominant [13]. Also the abnormality is known to be related with at least one or more recessive factor [2]. Additionally, mice that are homozygous for a targeted disruption of *Gli2* encoding a Shh-responsive transcription factor shows the imperforate anus and recto-urethral fistula [8]. In this study, the donor cells which provided a nuclear to SCNT embryos might have the high potential incidence for atresia ani, because this inbreeded miniature pigs are maintained for about 30 years [7]. In the previous study, anal atresia was observed in cloned pigs, but it is rarely seen in other reports related with pig somatic cell cloning [18]. We could not distinguish the reason of this malformation between the high incidence of original miniature pig line before inbreed and the genetic depression of inbreed strain. However, for xenotransplantation, pig inbred stocks for genetic modification to minimize graft rejection in the host would be advantageous over genetically diverse outbreed individuals. Therefore, the correction of abnormality in cloned pig is necessary. Also the screening of genetic defects or genetic uniformity is suggested before SCNT.

In that sense, it is critical that more numerous cases needed to be documented or investigated to evaluate incidence and prevalence of congenital abnormalities in cloned piglets and compare those data to normally born piglets. This study shows the usefulness of aggressive surgical approach to keep the piglet alive. Cloned pigs must be cared and treated because they have have invaluable potentials.

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