pISSN 1598-298X / eISSN 2384-0749
J Vet Clin 35(5) : 226-228 (2018)
http://dx.doi.org/10.17555/jvc.2018.10.35.5.226

Journal of Veterinary Clinics
www.ksvc.or.kr

Umbilical Hernia and Repair in a Transgenic Male Cloned Pig

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(Received: July 06, 2017 / Accepted: August 08, 2018)

Abstract : We generated a transgenic male cloned pig which was derived from fibroblast of white Yucatan miniature pig. After 2 weeks of birth, umbilical hernia which was not easily reduced was identified. Considering the usefulness of cloned pig, surgical treatment for umbilical hernia correction was performed and a cloned pig has been maintained healthy. This is the first report and can be useful for the treatments of umbilical hernia of cloned piglets.

Key words **:** umbilical hernia, surgical correction, somatic cell nuclear transfer, male cloned pig.

Introduction

For obtaining genetically engineered pigs, somatic cell nuclear transfer (SCNT) has been providing tremendous progress since the production of the first cloned pig (11). Especially due to value in biomedical research of pigs, this technique with gene editing is widely performed for production of genetically engineered pigs for xenotransplantation and disease models (1,10).

In pigs, umbilical hernia is one of the most common defects. The prevalence of umbilical hernia ranges from 0.13% to 2.25% depending on the breeds (14). Due to the risk of ulceration or strangulation of intestine, herniated pigs are slaughtered at a young age (14). It often leads to poor animal welfare and economic loss. This report describes a cloned piglet with an umbilical cord hernia and the successful clinical treatment.

Case

To produce cloned piglet, SCNT was performed as our previous study (4). Briefly, fibroblasts from Yucatan miniature male pig were isolated. Then, a single fibroblast was electrically fused with enucleated porcine oocyte. A total of 238 reconstructed embryos were electrically activated, cultured *in vitro* for 1-2 days. Among 238 embryos, 104 embryos of 2 cells stage above and 124 embryos of one cell stage were transported to the experiment farm in a portable incubator at 37°C in 2 hours. In a previous study, both-side deposition transfer had a significantly higher delivery rate (74% vs. 44%) and mean litter size (6.1 ± 0.7 vs. 4.2 ± 0.6) than the one-side transfer (13). Therefore, cloned embryos were loaded into a sterilized Tomcat catheter and transferred into both sides of oviduct. Pregnancy was identified with ultrasonography and one piglet with no gross structural abnormality was delivered by Cesarean section. The protocol for animal use was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-151019-4) in accordance with the Guide for the Care and Use of Laboratory Animals of Seoul National University.

During the first 2 weeks, piglet was healthy and housed alone in intensive care unit. After that, umbilical hernia appeared. Two days before the operation, hernia sac palpation was performed daily (Fig 1). We found that contents could not be easily reduced and the piglet showed signs of pain or distress during manipulation. At the age of 20 days old weighting 1470 g, operation was performed. A piglet was starved for 8 hrs prior to surgery, sedation was induced with ketamine 5 mg/kg and xylazine 3 mg/kg, administered by intramuscular injection in the neck area. Isoflurane was used via a face mask to maintain anesthetic status. In dorsal recumbency, the hernia has not been manually reduced, surgical repair such upon open herniorrhaphy was conducted by aseptic preparation. Because cloned piglet is a male, the prepuce and preputial opening are located in the umbilical area. Therefore, V shaped incision is made through the skin that the two incisions could be met in anterior portion of the preputial orifice. Under the incision, the intestine is herniated through the hernia ring (Fig 2A). The prepuce and preputial sac are reflected to one side. The hernia ring was detected and the herniated intestine was inserted through the hernia ring (Fig 2B). Finally, hernia ring was exposed and closed by overlapping sutures with absorbable material (Ethicon Inc., Somerville, NJ, USA) (Fig 2C). To prevent an abscess on the peritoneal portion of the hernia sac and remove dead space, several mattress sutures between muscle layer and fascia were performed. The excessive skin was removed and subcutaneous tissues were sutured by continuous suture for better apposition (Fig 2D).

After the surgery, the piglet had a normal status of hydration and was able to defecate and urinate himself. Amoxicillin 10 mg/kg and clavulanic acid 1.5 mg/kg (Kuhnil Pharmaceutical Co. Ltd., Korea) was applied orally. Wound dressing was performed daily with 10% povidone iodine for 14 days after surgery (Fig 1C, D).

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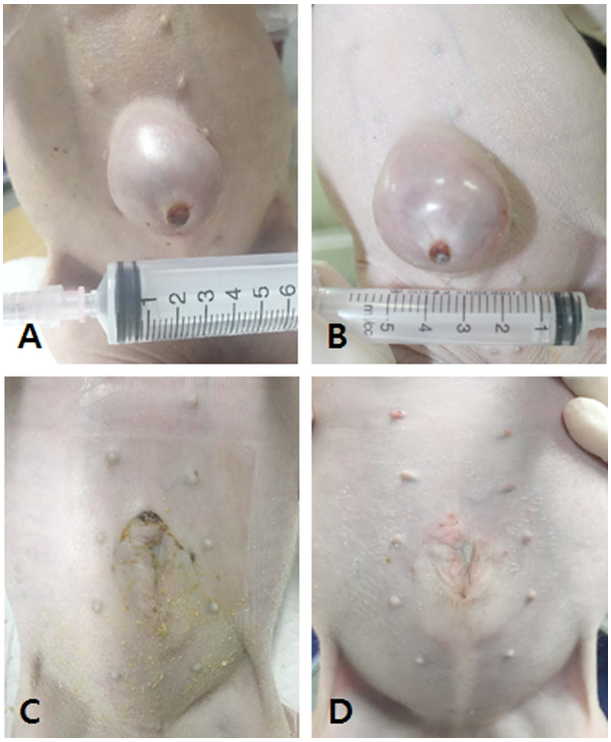

Fig 1. The umbilical hernia in male cloned piglet before (A, 2 days; B, 1 day) and after (C, 8 days; D, 15 days) surgical correction.

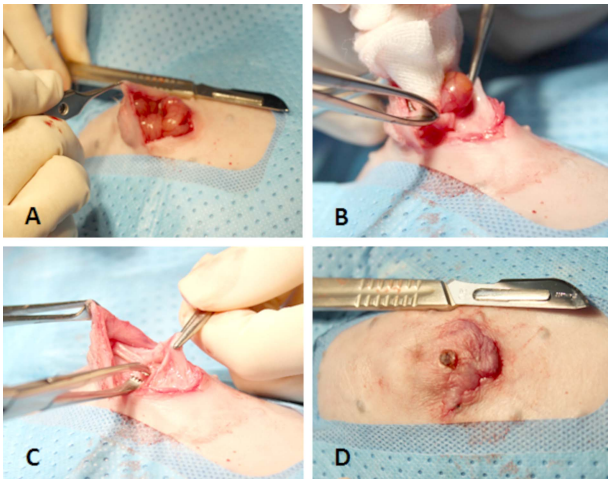

Fig 2. Surgical correction of umbilical hernia. The V shaped incision line was designed appropriately for male piglet, not to incise herniated intestine (A). Gently and careful reduction of visceral contents performed with aseptic gauze (B). The hernia ring was identified and sutured (C). Skin was opposed by continuous subcutaneous suture (D).

Discussion

In porcine, umbilical hernia is commonly seen between 9 and 14 weeks of age and is not fatal despite the lack of treatment (14). However, the present case indicated that umbilical hernia in a cloned piglet could appear just 2 weeks after birth. In veterinary treatment for farm animals with umbilical hernia, various non-invasive methods have been described including topical application of concentrated nitric acid and commercially-available rubber bands called elastrators. These methods are suitable only for easily reducible hernia, but not for strangulated or complicated cases (15). If the hernia ring persists for more than 2 to 3 weeks, surgical intervention is recommended (12). Therefore, the present case tried to correct umbilical hernia by aggressive surgical approach. For successful repair, careful surgical approach for preventing the unintended puncture of herniated intestine contents.

To our knowledge, this is the first report of the umbilical hernia correction in a male cloned piglet. Recently, it has been reported that congenital abnormalities including anal atresia, dimorphic facial appearance and intrapancreatic splenic tissue could occur in cloned pigs (2,5,7). Although the adult pig who provide donor cells did not show the umbilical hernia during growth (data not shown), we could not exclude any possibility that the umbilical hernia in transgenic cloned pigs is directly related to SCNT. It has been reported that absent phenotypic and gross abnormality in the G2 (donor cell providing pig) could reappeared in the G3 clones (2). Until now, 32 susceptible loci have been detected by genome-wide scans with microsatellite markers (3). Recently, it has been reported that the pathogenesis of porcine umbilical hernia is affected by copy number variations in Duroc, Landrace and Yorkshire breeds (8). Furthermore, *C57BL/6* genetic background mice that fail to express fibulin 3 develop multiple large hernias (9) and transgenic mice for insulin like factor 3 develop inguinal hernias (6). We could not find the reason of the umbilical hernia in cloned pig. Further studies involving larger numbers of herniated cloned pigs of different ages and sex are needed.

Conclusions

In conclusion, we produced a male cloned pig without umbilical hernia. However, umbilical hernia with not easily reduced contents in hernia sac was appeared. Although surgical intervention was performed with the risk of death due to the anesthesia in young age, lastly it became healthy after treatment. By aggressive surgical correction, we could maintain a healthy valuable transgenic male cloned pig.

Acknowledgment

This work was supported by National Research Foundation (#2015R1C1A2A01054373; 2016M3A9B6903410), the Brain Korea 21 PLUS Program for Creative Veterinary Science Research, and the Research Institute for Veterinary Science.

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