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Case Report

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Normal bone healing in three cloned dogs with long bone fractures

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Abstract: A number of mammals have been cloned, including cats and dogs. The purpose of this report was to study the bone fracture and bone fracture healing of three cloned dogs. The three cloned dogs, aged 3 months to 6 months, received separate surgeries. Clinical and radiological examination of each of the three cloned dogs revealed the fracture of a femur, a tibial tuberosity, and a humerus, respectively. Each dog had undergone surgical reduction and stabilization of their individual bone fracture with an intramedullary pin, a screw, and/or a wire. No abnormalities were observed during the follow-up period. The dogs showed normal callus formation and bone remodeling. Among the cases, the tibial tuberosity fracture was caused due to a breed-related fracture in a cloned greyhound. There was no evidence that these fractures were the result of cloning. Moreover, there were no complications including a delayed union, nonunion, malunion, or osteomyelitis during the healing process. All three cloned dogs showed normal bone fracture healing. To the author's knowledge, this is the first report about the bone healing of cloned dogs. Results show that long bone fractures in cloned dogs heal in the same manner as normal dogs.

Key words: Cloned dog, long bone fracture, bone fracture healing, surgical reduction

1. Introduction

The cloning method that is termed somatic cell nuclear transfer is the insertion of the nucleus of a donor somatic cell into an unfertilized and enucleated recipient oocyte. Animal cloning technology had a significant breakthrough when successful nuclear transfer was reported using somatic cells derived from adult tissue in sheep (1). After the first cloned mammal, a number of mammalian species have been cloned (2). Until the first successfully cloned dog was reported (3), dogs were considered significantly difficult to clone due to species-specific characteristics of canine reproductive physiology as compared to other mammals (4). Successful cloning of various mammals has established the potential for somatic cell nuclear transfer to preserve several species (5–7).

Several phenotypic abnormalities such as abnormal legs and a short face in piglets have been reported (8). However, the history of dog cloning is short compared to other species. There are very few reports associated with cloned dogs and, to date, there was no report about bone fracture and bone fracture healing in cloned dogs.

Fracture of the femur is the most common in the dog and cat, accounting for almost half of all long bone fractures (9). It was reported that about 45% of long bone fractures were femoral fractures and 26% were tibial fractures (10). Another study reported that approximately 10% of all limb fractures represented humeral fractures, of which 20% were condylar fractures (11).

The purpose of this report was to study the healing of long bone fractures among three cloned dogs.

2. Case history

Three cloned dogs aged 3 months to 6 months with complaints of lameness had been presented to the veterinary medical center of Chungbuk National University. All dogs were from the Sooam Biotech Research Center, Korea. Each dog was a different breed, namely a Tibetan mastiff, a greyhound, and a beagle.

Case 1 was a 6-month-old cloned female Tibetan mastiff weighing 11 kg that had complaints of lameness and pain in the left hind limb. On clinical examination, the left femur was found swollen. A radiograph of the affected limb showed an oblique comminuted fracture of the left femur (Figure 1). The dog was sedated, and the surgical site was shaved and disinfected. Acepromazine (Sedaject, Samwoo Pharmacy, Korea; 0.05 mg/kg SC), atropine

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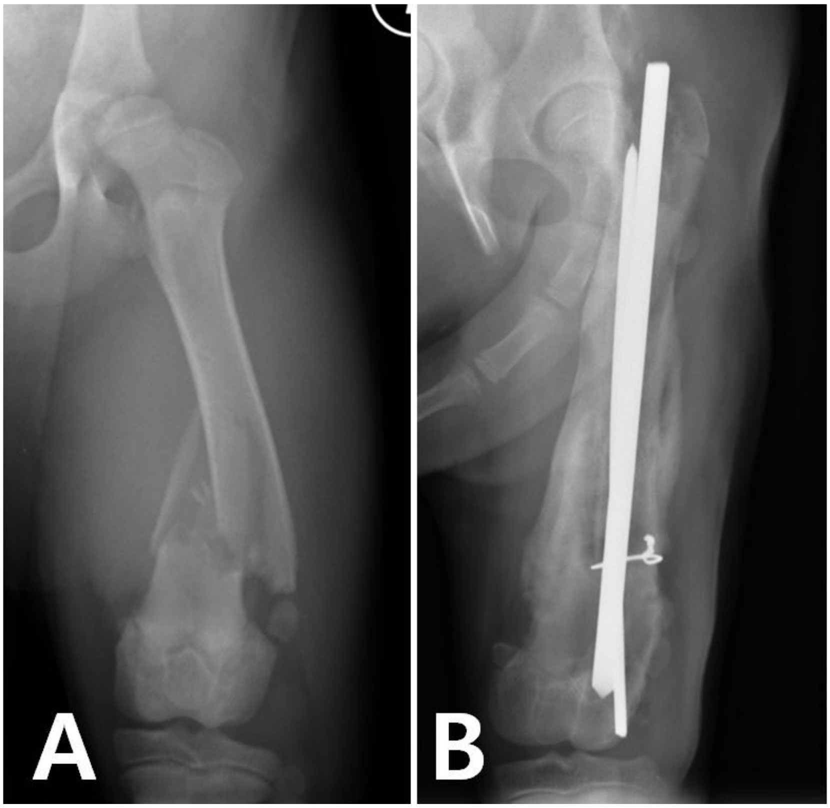

Figure 1. Radiographic view of left femoral comminuted fracture of the Tibetan mastiff in which IM pins were applied. A- Preoperative radiographic view, B- radiography 35 days after surgery.

sulfate (Atropin Sulfate Daewon, Daewon Pharmacy, Korea; 0.05 mg/kg SC), and cefazolin (Cefazol, Hangook Chorus Pharm, Korea; 30 mg/kg IV) were administered as premedication. Following this, general anesthesia was induced using propofol (Pofol, Jeil Pharmacy, Korea; 7 mg/kg IV) and maintained with isoflurane (Aerane, Ilsung Pharmacy, Korea; 2%–2.5%). Intramedullary pinning and hemicerclage wiring techniques were applied to stabilize the fracture; 4.8 mm and 3.0 mm diameter Kirschner wires and 1.0 mm diameter surgical wires were used for fracture stabilization. Robert-Jones bandages were applied for 3 weeks.

In case 2, a 6-month-old, 14.8 kg intact cloned male greyhound was referred for acute nonweight-bearing lameness with pain in the right pelvic limb. A radiograph showed the avulsion of the whole tibial tuberosity physeal fracture (Figure 2). The principal clinical signs were swelling, pain, and lameness of the affected limb. Metoclopramide (Makcool Ju, Jeil Pharm, Korea; 1 mg/kg IV) and cefazolin were administered as premedication. After sedation, anesthesia was induced with propofol and maintained with isoflurane. To fix the fragments of tibial tuberosity to the tibia and to prevent the lifting of the fragment with contraction of the quadriceps femoris muscle, two Kirschner wires were inserted and also a tension band was placed. After completing the surgery, the affected limb was bandaged with Robert-Jones bandages for 3 weeks.

Case 3 was a 3-month-old intact cloned female beagle weighing 6.4 kg with multiple fractures caused by a fall. The animal was in left recumbency and the right elbow joint was swollen. A radiograph revealed a right humeral condylar fracture (Figure 3), bilateral sacroiliac luxation, and ischial fracture. Prior to surgery, the dog was premedicated with cefazolin. Propofol was injected to induce anesthesia and maintained with isoflurane and vecuronium (Vecron, Myung Moon Pharm, Korea). Surgery was performed to correct the sacroiliac luxation using two 2.0 mm cortical screws, and to fix the humeral condylar fracture, two Kirschner wires and a cortical screw were applied. Fracture of the ischium was not repaired and bandages were applied to the forelimb for 3 weeks.

The laboratory tests were not remarkable except for alkaline phosphatase level, which was elevated in all three dogs. After surgeries were completed, the three dogs were given tramadol (Tamadol, Dongkwang Pharmacy, Korea;

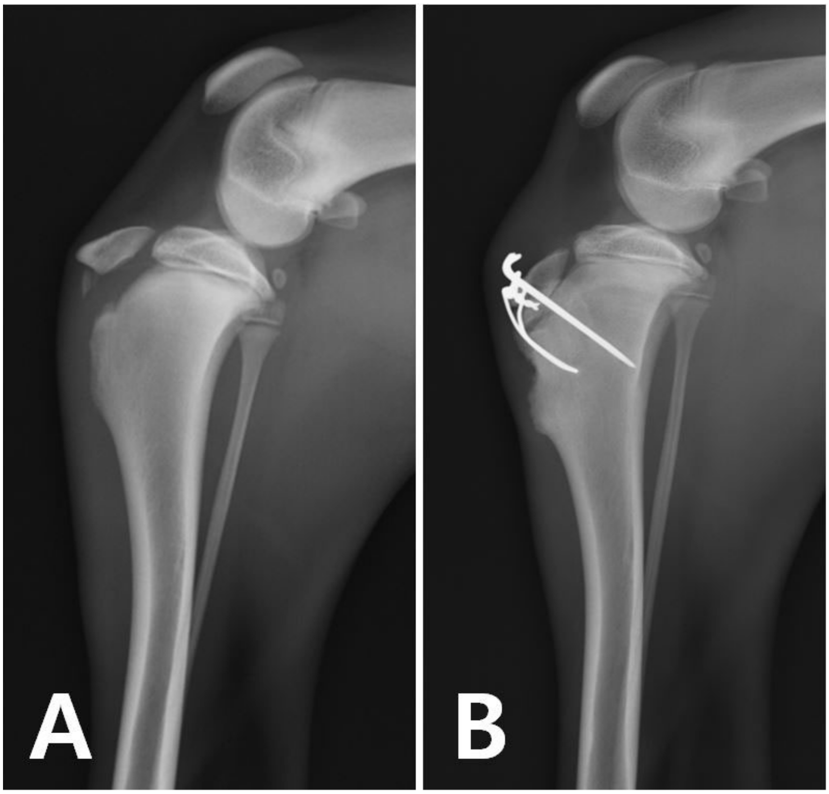

Figure 2. Radiographic view of stifle joint showing the avulsion fracture of tibial tuberosity in greyhound dog. A- Preoperative radiographic view, B- radiography 28 days after surgery.

4 mg/kg) and cefazolin (30 mg/kg) intravenously for 7 postoperative days.

After the surgical correction, results for all three dogs were evaluated by clinical and radiological examinations. In case 1, radiographic tests were taken at 14 days after surgery and showed rough and solid periosteal reactions and massive callus formation around the fracture site, which had gradually reduced 34 days after surgery. The dog gradually became able to bear its weight on the affected limb while walking 4 weeks after surgery. The continuity of the cortical bone of the fracture site was observed in a radiograph (Figure 1).

In case 2, the dog had undergone a routine follow-up 4 weeks after surgery and the radiological examination showed a rough and solid periosteal reaction on the tibial tubercle (Figure 2). The Kirschner wires were removed 2 months after surgery. No abnormalities such as stiffness, lameness, malunion, nonunion, or osteomyelitis were observed during a second follow-up 3 months after the first surgery.

In case 3, the beagle could stand up and walk with assistance 7 days after surgery. Radiographs at 16 days after surgery showed a solid periosteal reaction in the humeral condyle.

Ten weeks after surgery another follow-up radiograph was taken, which showed bone remodeling and continuity of the cortical bone at the fracture site in the right elbow (Figure 3). The sacroiliac joints were aligned and the dog was fully weight-bearing on the hind limb. The range of motion in the right elbow had improved but had not recovered completely.

3. Results and discussion

The aim of the surgical correction of a fracture in a young animal is to protect the growth plate for the purpose of aiding in the early return to usual function (12,13). Fracture healing will be shorter if anatomical alignment is achieved (14). The bone healing time is variable and affected by age, breed, location and configuration of fractures, degree of soft tissue injury, existence of bone defects, and type of fixation applied. The current study included dogs aged 3 to 6 months. In the three cases, the healing process was quite satisfactory and there were no abnormalities such as delayed union or nonunion observed.

Comminuted fractures of the femur are the most difficult to maintain stability following a surgical reduction. If the bone fragments are small, neither semi nor cerclage wires will be able to maintain the suitable auxiliary

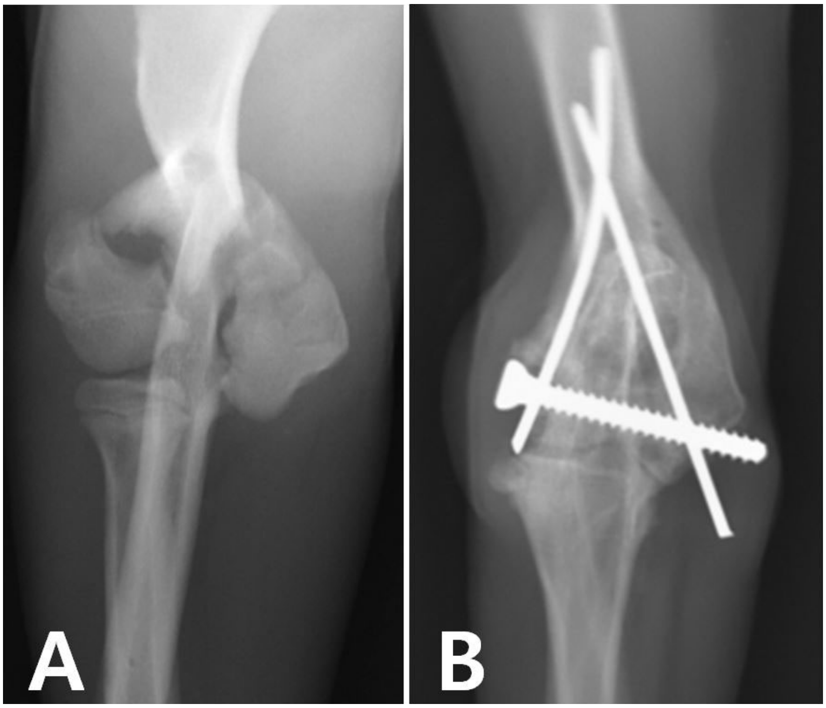

Figure 3. Radiographic view of right elbow joint showing the humeral condylar fracture in a beagle. A- Preoperative radiographic view, B- radiography 78 days after surgery.

support. For a satisfactory union, an intramedullary pin would be the choice of treatment for such fractures especially when the cerclage wires become unstable or when the unstable fractures become more dependent on periosteal blood vessels for bone repair than on nutrition from normally dominant endosteal pathways, which may lead to an unsatisfactory union due to the presence of loose cerclage wires. There was no loss of fixation or failure of any implants in case 1. The cerclage wires did not loosen and callus formation progressed normally. The avulsion of the tibial tubercle is seen mostly in younger animals of less than 10 months of age. Complete avulsion requires an open reduction and internal fixation by using the tension band principles to reform the integrity of the quadriceps complex (15). In case 2, the greyhound showed no abnormalities, no malunion or nonunion, 3 months after surgery, which led to normal fractured bone healing. Among the three cases, case 2 represented a breed-related fracture, which is common in greyhounds (15).

Femoral fractures are usually the result of major trauma, but they can be caused by disease of the bone itself. In the present study, the Tibetan mastiff had no previous history of bone disease that might lead to femoral fracture. Most frequently found complications in femoral fracture healing are delayed union or nonunion, avascular necrosis, secondary osteoarthritis, and cessation of neck growth in young animals, which may lead to subluxation of the hip. Clinical and radiographic evidence of complications are mostly evident within 6 weeks (9). However, in the current study, the Tibetan mastiff did not show such complications both clinically and radiographically, which indicates that normal healing was in progress with IM pins and Kirschner wires.

Internal fixation of the tibial plateau with K-wires, in combination with a tension band wire for the avulsion of the tibial tuberosity, plays an important role in complete healing (16). It was reported that the deformity of the tibial plateau was thought to be caused by an injury to the germinal cells of the proximal tibial epiphysis, premature fusion of the tuberosity to the epiphysis, or a combination of both (17). In the case of the greyhound dog, we did not find these abnormalities during or after healing.

In pups, most humeral fractures are condylar fractures as a result of falling from heights because the extended elbow joint forces the anconeal process to contract the caudal aspect of the supracondylar region of the humerus with violent hyperextension or torsion of the limb; the anconeal process may act as a wedge leading to the separation of the medial and lateral aspects of the humeral condyle (11), which also supports the present study in the case of the beagle. Decreased range of motion is a major complication related to these injuries and early return to function is imperative to ensure a good outcome (18).

There was no evidence that these fractures were the result of cloning and the laboratory results were also similar to those of noncloned dogs with a fracture. Moreover, the three cloned dogs showed normal callus formation and bone remodeling. There were no complications, including delayed union, malunion, nonunion, or osteomyelitis, during the bone fracture healing process. Radiographs also did not show any soft tissue infection at the site of surgery. All these findings suggested that the fracture of long bone in cloned dogs healed in a process similar to that of noncloned dogs.

There has been premature aging and degenerative lesions in cloned animals (19). However, in this study, all of the cloned dogs were aged less than 1 year and there was no follow-up information longer than 6 months. Although the three young cloned dogs showed normal bone fracture healing, more studies are necessary about bone fracture occurrence, pattern, and fracture healing in aged cloned dogs.

This report shows that long bone fractures in cloned dogs heal in the usual manner as that of normal dogs.

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References

1. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997; 385: 810-813.
2. Shin T, Kraemer D, Pryor J, Liu L, Rugila J, Howe L, Buck S, Murphy K, Lyons L, Westhusin M. A cat cloned by nuclear transplantation. Nature 2002; 415: 859.
3. Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, Hossein MS, Kim JJ, Kang SK, Schatten G et al. Dogs cloned from adult somatic cells. Nature 2005; 436: 641.
4. Hossein MS, Jeong YW, Park SW, Kim JJ, Lee E, Ko KH, Kim HS, Kim YW, Hyun SH, Shin T et al. Cloning missy: obtaining multiple offspring of a specific canine genotype by somatic cell nuclear transfer. Cloning Stem Cells 2009; 11: 123-130.
5. Kato Y, Tani T, Sotomaru Y, Kurokawa K, Kato J, Doguchi H, Yasue H, Tsunoda Y. Eight calves cloned from somatic cells of a single adult. Science 1998; 282: 2095-2098.
6. Polejaeva IA, Chen SH, Vaught TD, Page RL, Mullins J, Ball S, Dai Y, Boone J, Walker S, Ayares DL et al. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature 2000; 407: 86-90.
7. Li Z, Sun X, Chen J, Liu X, Wisely SM, Zhou Q, Renard JP, Leno GH, Engelhardt JF. Cloned ferrets produced by somatic cell nuclear transfer. Dev Biol 2006; 293: 439-448.
8. Park MR, Cho SK, Lee SY, Choi YJ, Park JY, Kwon DN, Son WJ, Paik SS, Kim T, Han YM et al. A rare and often unrecognized cerebromeningitis and hemodynamic disorder: a major cause of sudden death in somatic cell cloned piglets. Proteomics 2005; 5: 1928-1939.
9. Piermattei DL, Flo GL, DeCamp CE. Handbook of Small Animal Orthopedics and Fracture Repair. 4th ed. Philadelphia, PA, USA: WB Saunders; 2006. pp. 512-561.
10. Harasen G. Common long bone fractures in small animal practice-part 1. Can Vet J 2003; 44: 333-334.
11. Marcellin-Little DJ. Humeral fractures in dogs. Walthum Focus 1998; 8: 2-8.
12. Altunatmaz K, Yücel R. Köpeklerde antebrachiumda karşılaşılan ortopedik lezyonlar ve bunların sağaltımları üzerine klinik çalışmalar. Veteriner Cerrahi Dergisi 1999; 5: 118-126 (in Turkish).
13. Lewis DD, Cross AR, Carmichael S, Anderson MA. Recent advances in external skeleton fixation. J Small Anim Pract 2001; 42: 103-112.
14. Özsoy S, Altunatmaz K. Treatment of extremity fractures in dogs using external fixators with closed reduction and limited open approach. Vet Med 2003; 48: 133-140.
15. Butterworth SJ. Tibia and fibula. In: Coughlan A, Miller A, editors. BSAVA Manual of Small Animal Fracture Repair and Management. Gloucester, UK: BSAVA; 2006. pp. 228-248.
16. Schmokel H, Weber U, Hartmeier G. Salter-II fracture of the proximal tibia with avulsion of the tuberositas tibiae in the dog. Schweiz Arch Tierheilkd 1995; 137: 124-128.
17. Goldsmid S, Johnson KA. Complications of canine tibial tuberosity avulsion fractures. Vet Comp Orthop Traumatol 1991; 4: 54-58.
18. Tomlinson JL. Fractures of the Humerus. In: Slatter DH, editor. Textbook of Small Animal Surgery. 3rd ed. Philadelphia, PA, USA: WB Saunders; 2003. pp. 1905-1918.
19. Jang G, Hong SG, Oh HJ, Kim MK, Park JE, Kim HJ, Kim DY, Lee BC. A cloned toy poodle produced from somatic cells derived from an aged female dog. Theriogenology 2008; 69: 556-563.