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Radiographic Evaluation of Limb Bone Development in Miniature Porcine

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Epiphyseal development in the long bones was studied radiographically in minipigs. Radiographs of the proximal and distal epiphyses of humerus, radius, ulna, femur and tibia were obtained at 4, 8, 12, 20, 40, 48, 96 and 144 weeks of age in total 58 minipigs. The assessment of maturity process was made in accordance with the criteria proposed by Owada and Sutow. The secondary ossification centers developed rapidly from 4 weeks of age to 40 weeks of age, and gradually thereafter until 96 weeks of age. The earliest epiphyseal fusion was apparent in the proximal radius, proximal and distal femur at 96 weeks of age. The complete fusion of the epiphyseal line in the long bones was evident on 144 weeks of age and was observed in most long bones such as the proximal humerus, the proximal and distal ulna and the distal radius, and the proximal tibia in minipigs.

Key words – Ossification, epiphyseal closure, minipigs, radiography

Introduction

Understanding of normal morphological development of bones is extremely important to diagnose the skeletal disease and evaluate chronological age from the degree of ossification in growing animals. Normal epiphyseal developments of various bones have been standardized in human beings [10], dogs [6], cats [12], horses [8], and other various animals [4,9]. This normal skeletal developmental process based on the endochonral ossification provides general characteristics of immature bone maturity, physeal closure times, and growth velocity of bones. Recently, minipigs which have high genetic and physiologic similarities to humans [15], have been developed as human disease models. Minipigs have focused on a useful animal model due to the various possibility of practical application specific bone diseases such as osteochondrosis, osteoporosis, and employement of experimental study to further investigate etiology and pathogenesis of human skeletal diseases instead of the porcine because of reduced size and more easier handing [14]. However, there have been no reports on precise epiphyseal development and ossification in minipigs. The purpose of this study is to judge the ossification process and development degree of bone in the healthy and growing minipigs and then to establish the standard at each age.

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Materials and Methods

Animals

Total fifty eight experimental minipigs at 4, 8, 12, 20, 40, 48, 96 and 144 weeks of age were used in this study (Table 1). All animals obtained from the PWG Genetics Korea were raised under strict SPF barrier system and microbiologically well defined conditions. The minipigs were individually housed indoors in cages, fed dry pig food, and provided with water *ad libitum*.

Methods

A standard radiographic examination of minipigs was performed on the long bones including the forelimb such as humerus, radius and ulna, and the hindlimb such as femur and tibia by two projections, a lateral and craniocaudal views. General sedation was induced with an intramuscular injection of tiletamine plus zolazepam (Zoletil®, Virvac, Korea), at a dose of 4.0~8.8 ml/kg five to ten minutes before the procedures for all examined minipigs except for 4 and 8 week-age minipigs [5,7]. Ossification process (Table 2) at each age was evaluated on the basis of the classification (grade 0 to 10) described by Owada and Sutow [2,10]. To investigate the development process of secondary ossification centers, the images of the proximal

Table 1. Number of examined minipigs at various ages

Week	4	8	12	20	40	48	96	144
No	11	8	9	13	8	6	2	1

Table 2. Classification method by Owada and Sutow

Grade	Radiographic findings about criteria
0	No appearance of an epiphysis
1	Appearance of a small round epiphysis
2	Developing ossification center
3	Beginning typical difference of an epiphysis
4	Smaller width of an epiphysis than that of a metaphysis
5	Equalization of both width of an epiphysis and metaphysis
6	Capping and narrowing of gap between an epiphysis and metaphysis
7	Partial fusion of an epiphysis with a metaphysis
8	Development of epiphyseal fusion (over half)
9	Completion of epiphyseal fusion (remaining clear epiphyseal line)
10	Almost or perfect fusion

and distal epiphysis of long bones on the film were scrutinized. Changes in epiphyseal gradings obtained from the mean grade value in various ages were used for estimating epiphyseal development and ossification.

Results

Changes in grading values at all epiphyseal sites of secondary ossification in long bones are shown in Fig. 1 and Fig. 2. The curve showing the maturation process expressed in Fig. 1 and 2 has been plotted out of the mean values in each age group. Epiphyseal gradings increased sharply at birth to 10 months of age but revealed little changes after 10 months of age to 2 years old in all epiphyses. The complete fusion of all epiphyses was found after three years old.

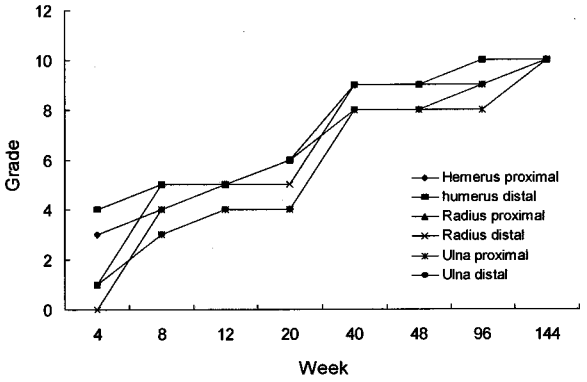

Fig. 1. Maturity process of the secondary ossification center at the forelimb in minipigs.

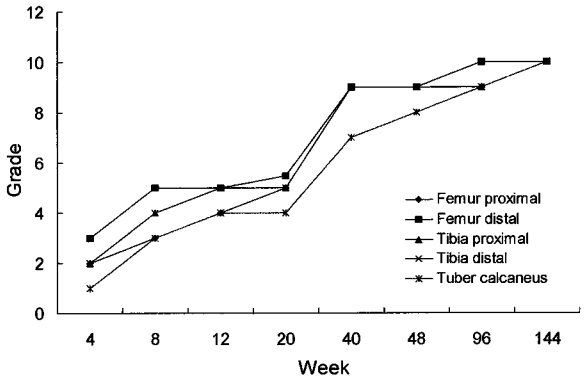

Fig. 2. Maturity process of the secondary ossification center at the hindlimb in minipigs.

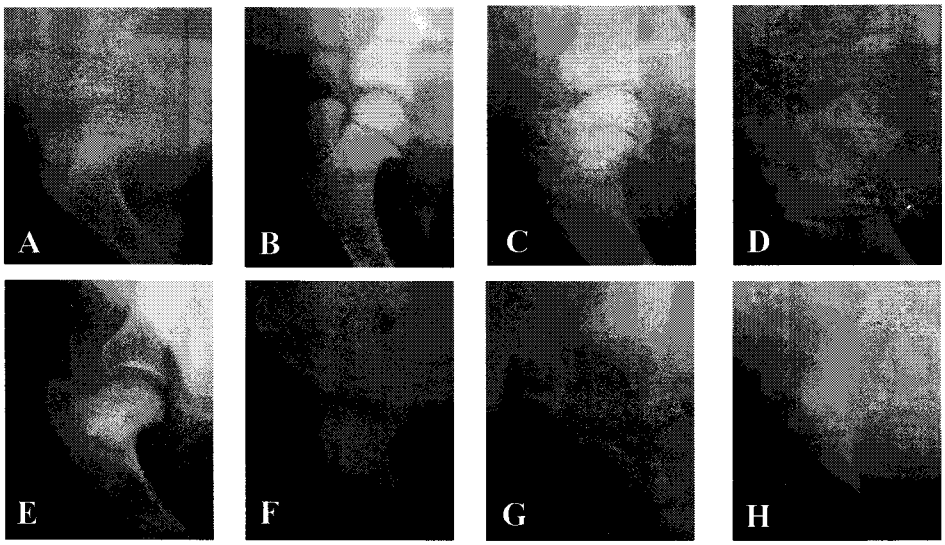

Fig. 3. Radiographs of ossification process in the proximal humerus at 4 (grade 3), 8 (grade 4), 12 (grade 5), 20 (grade 6), 40 (grade 8), 48 (grade 8), 96 (grade 9) and 144 (grade 10) weeks of age.

Epiphyseal development of the humerus

Separate ossification centers of the humeral head and greater tubercle were identified at 4 weeks of age (Fig. 3A and 3B). The humeral head and greater tubercle ossification centers enlarged gradually at 12 weeks of age (Fig. 3C). They united with each other but remained ununited with the body of the humerus (Fig. 3D and 3E). The proximal humeral epiphysis began to fuse caudally at 48 weeks of age (Fig. 3F). Closure of the epiphyseal line completed at 144 weeks of age (Fig. 3H). Epiphyseal fusion of the distal humerus was present at 96 weeks of age earlier than the proximal one (Fig. 4G).

Epiphyseal development of the radius and ulna

The proximal and distal radial and ulnar epiphyses had immature and small shape at 4 weeks of age (Fig. 4A and 5A). The epiphyseal line of the proximal and distal radius fused at 40 weeks of age (Fig. 4E and 5E). The closures of the proximal and distal radius were found at 96 and 144 weeks of age, respectively (Fig. 4G and 5H). The epiphyseal line of the proximal and distal ulna fused at 96 weeks of age (Fig. 4G and 5G) and closed at 144 weeks of age (Fig. 4H and 5H).

Epiphyseal development of the femur

The ossification center of the femoral head and greater trochanter was visible at 4 weeks of age (Fig. 6A). The wavy shape of the epiphyseal line in the distal femur was observed at 4 weeks of age (Fig. 7A). The proximal and distal femoral epiphyses were united rapidly from 8 to 40 weeks of age (Fig. 6B to 6E and Fig. 7B to 6E). Complete epiphyseal closure at the proximal and distal femur was

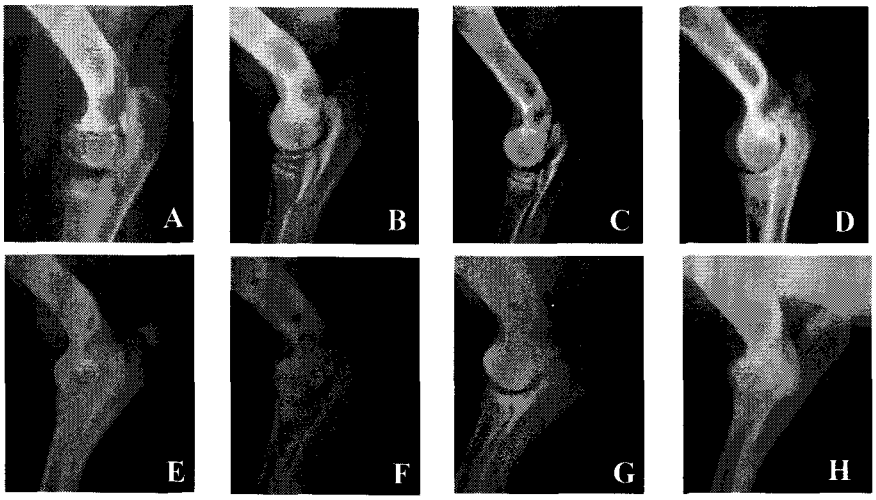

Fig. 4. Radiographs of ossification process in the distal humerus, proximal radius and proximal ulna.

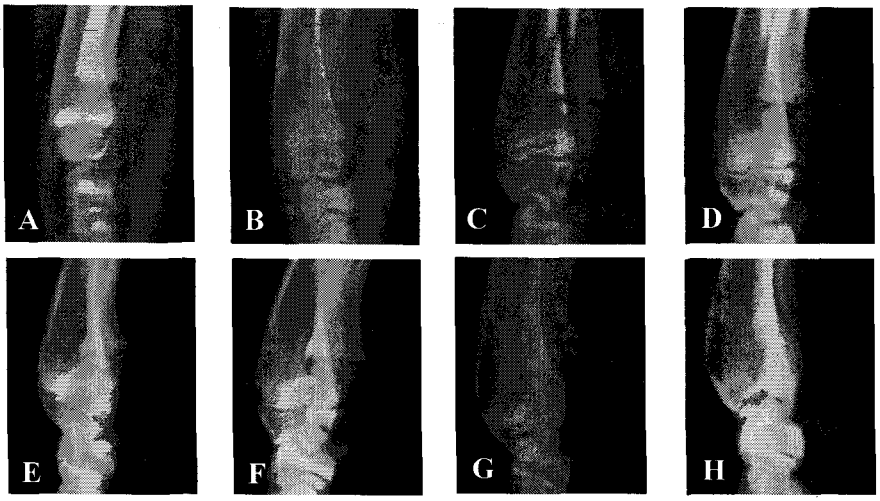

Fig. 5. Radiographs of ossification process in the distal radius and distal ulna.

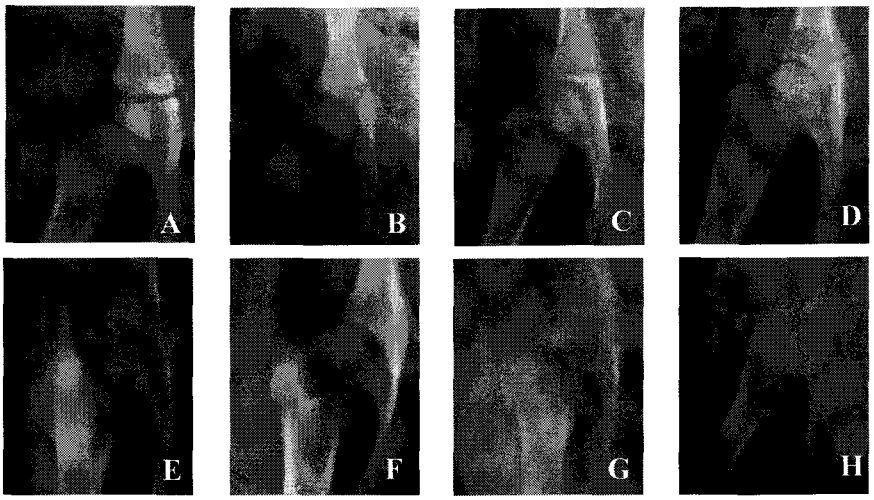

Fig. 6. Radiographs of ossification process in the proximal femur at 4 (grade 2), 8 (grade 3), 12 (grade 4), 20 (grade 5), 40 (grade 9), 48 (grade 9), 96 (grade 9-10) and 144 (grade 10) weeks of age.

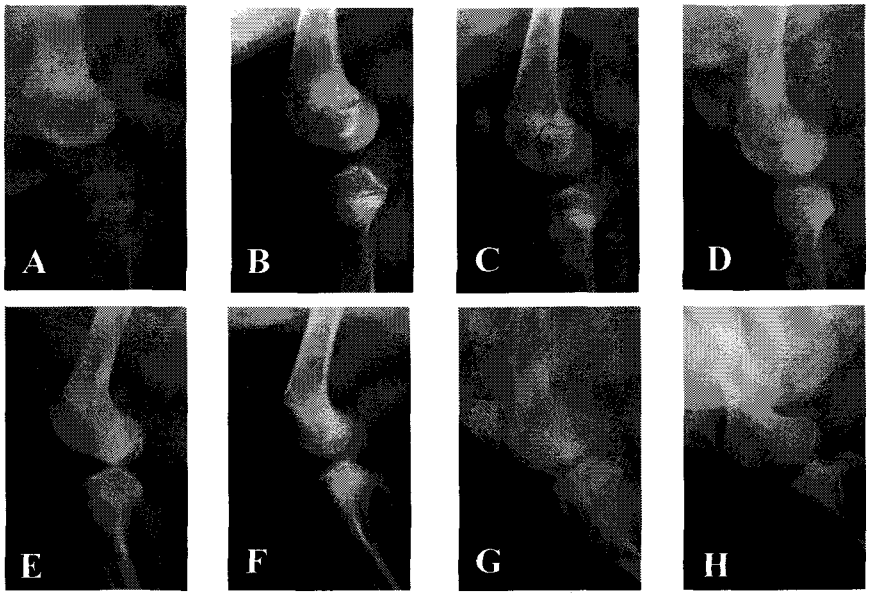

Fig. 7. Radiographs of ossification process in the distal femur and proximal tibia.

observed on the 96 weeks of age (Fig. 6G and 7G).

Epiphyseal development of the proximal tibia and tuber calcaneus

A small and round secondary ossification center of the proximal tibia was apparent at 4 weeks of age (Fig. 7A). The tibial tuberosity ossified and was identified in front of the proximal tibia at 8 weeks of age (Fig. 7B). The proximal tibia and tibial tuberosity had enlarged and fused gradually up to 48 weeks of age (Fig. 7C to F). Fusion between the proximal tibial epiphysis and tibial tuberosity commenced at 96 weeks of age (Fig. 7G) and then finished at 144 weeks of age (Fig. 7H). Fusion and closure of the epiphyseal line in the tuber calcaneus was observed at 40 to 48 weeks of age (Fig. 8E and F) and 144 weeks of age (Fig. 8G), respectively.

Discussion

Recently, transgenic minipigs (Micro-pig®, PWG Genetics Korea, Ltd.) which have high genetic and physiologic similarities to humans, have been developed and are widely used as models of human diseases [3]. However, there was little study on the fundamental information of diagnostic

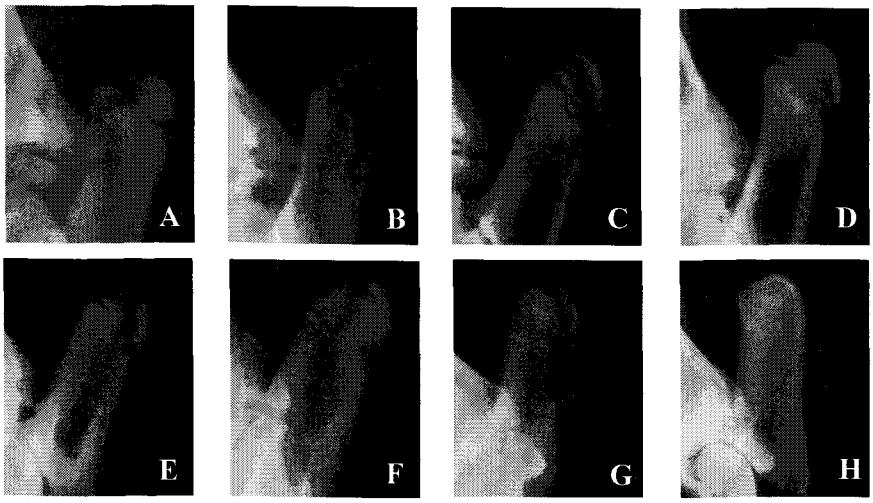

Fig. 8. Radiographs of ossification process in the tuber calcaneus.

imaging including maturity process of the secondary ossification centers in long bones of minipigs using radiography. This study is to provide standardized knowledge which can estimate minipig's age through fusion stage and appearance of the secondary ossification centers in long bones.

Secondary ossification centers are established in the epiphysis of long bones [13]. The function of these centers is similar to the primary centers but the growth is radial rather than longitudinal. Bone growth is under the influence of growth hormone, sex hormone, species, individual characters, nutrition, and mechanical injury [1]. Growth plate closure times are various depending on those factors and their locations. On average, the physis of long bones closes between 8 and 14 months of age in dogs and between 24 and 48 months in horses [13].

In this study, radiographic epiphyseal closure time is about three years old in the proximal humerus, the distal radius, the proximal and distal ulna, the proximal and distal tibia and tuber calcaneus similar to the porcine [11]. While complete fusion time of the distal humerus and proximal radius in the porcine is around one year old, that of the distal humerus and proximal radius in the minipig is around two years old and three years old, respectively. Fusion of the proximal and distal femur in the minipig is finished at two years old compared to the porcine with three years old. In fact, the difference of bone growth between the minipig and conventional pig needs further study.

In summary, growth plate closure is highly relative to age [2], and in particular the rate of maturity process develops fast from at birth to puberty. There is no noticeable difference for radiographic pattern of physeal closure process of minipigs compared to other animals.

Radiographic examination is an ideal method for judgment of skeletal maturity and ossification appearance. However, it is somewhat hard to take X-ray due to a big body conformation and abundant fat accumulation in adult minipigs. The epiphyses of elbow region are superimposed on each other, making it difficult to evaluate their development.

There is some limitation about insufficient examined number of minipigs, the irregular age interval and no clarification of difference in gender. This is, however, the first work to explain the limb bone development and establish radiographic references for assessing skeletal maturity in minipigs.

Acknowledgement

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초록 : 미니돼지의 팔다리 뼈 성장에 대한 방사선학적 평가

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미니돼지의 긴 뼈의 뼈끝(epiphysis) 성장에 대한 연구를 위해 상완골, 요골, 척골, 대퇴골, 경골의 근위 및 원위 뼈끝 부위를 중심으로 방사선 촬영을 실시하였다. 실험에 이용된 미니돼지의 연령대는 4주, 8주, 12주, 20주, 40주, 48주, 96주, 144주령으로 총 58마리였다. 뼈 성장과정에 대한 평가는 Owada & Sutow가 제안한 11가지 기준을 토대로 하였다. 긴 뼈에서 이차뼈발생중심은 4주령에서 40주령까지 빠른 성장을 보였고, 그 이후 96주령까지는 초기보다 느린 성장을 보였다. 가장 빠른 뼈끝 유합시기는 96주령으로 근위 요골, 근위 및 원위 대퇴골에서 관찰되었다. 미니돼지의 일반적인 긴 뼈의 완전한 뼈끝 유합은 144주령에 나타났으며 근위 상완골, 근위 및 원위 척골, 원위 요골, 그리고 근위 경골에서 관찰되었다.