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Review Article

Radiographic Specification of Skeletal Maturation in Donkeys: Defining the Ossification Time of Donkey Growth Plates for Preventing Irreparable Damage

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Abstract

There is no doubt that the donkey, as a drafting animal, plays a critical role in the developing communities for both agriculture production and riding purposes. Therefore, to maintain healthy skeleton in these animals, the understanding of radiographic anatomy of ossification centers and growth plates (locations, types and closure times), need to be advanced. It is imperative that an appropriate maturation point is reached to avoid early overexertion of the donkey and the resulting irreparable damage. It is therefore the aim of this review is to highlight the importance of expanding the limited dataset on epiphyseal growth plate maturation and begin to define its ossification times in the donkey. Moreover, it has been recently proposed that the receptors of gonadal sex hormones including estrogen, androgen as well as pituitary sex hormones including luteinizing hormone and prolactin are localized in human and animal growth plates. This indicates that these hormones actively participate in the chondroregulation mechanisms at the ossification growth plates. A connection with that, we noticed that the more primitive stem cells as well as hematopoietic stem cells and mesenechymal stem cells express these receptors as well. Therefore, it would be very interesting to address the link between sex hormones and ossification time of growth plates, as well as the sharing activities of these stem cells during the ossification process.

Key words: Apophysis, donkey, epiphyseal closure, growth plate, ossification center, hock, stifle

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INTRODUCTION

It is well known that the ossification centers are potential indicators for the skeletal maturity in both human and animals (Fukuda and Matsuoka, 1979; O'Connor *et al*., 2013; Ogden *et al*., 1981). Each ossification center consists originally of hyaline cartilage and is interpreted by a radiographer as a radiolucent line bordered by slightly higher radiopacities (Von Pfeil and DeCamp, 2009). Next, with increase in age, this cartilage undergoes ossification and is accompanied by decreases in their radiolucency; this process is named endochondral ossification (Kim *et al*., 2001; Von Pfeil and DeCamp, 2009).

Therefore, from the practical point of view, the study of ossification centers is of great importance, not only in the determination of age and hence acquired information about the starting time for pushing animals into work or training, but also for understanding both normal and abnormal developmental orthopedic diseases such as osteochondritis dissecans (OCD) and physitis (Strand *et al*., 2007; Lepeule *et al*., 2011).

In support of this, many studies proposed the timing rate of some epiphyseal and apophyseal ossifications centers in horses (Myers and Emmerson, 1966; Banks, 1972; Brown and MacCallum, 1975; Fretz *et al*., 1984; Smith *et al*., 1991; Koskinen and Katila, 1997; Vulcano *et al*., 1997; Uhlhorn *et al*., 2000; Strand *et al*., 2007), goat (Genccelep *et al*., 2012), dogs (Fukuda and Matsuoka, 1980), cats (Vazquez-Auton *et al.,* 1993), black beef cattle (Oishi *et al*., 1996), monkey (Hofmann *et al*., 2007), deer (Flinn *et al*., 2013) and mice (Fukuda and Matsuoka, 1980).

However, there are very few studies concerning the epiphyseal closure times in donkeys (Ahmed, 2005; Behery *et al*., 2007; Samy *et al*., 2007). The aim of this review is therefore to gather the information in the context of ossification time rate in donkeys and to compare this data with information previously documented in horses.

Why skeletal maturity is important: Because ossification centers at younger ages are composed of cartilage, it makes these points weaker. Pushing the animals into extra hard work at these ages, results in many distortion and conformational problems in these growth plates (Shapiro, 1987; Soeta *et al*., 2000). In this context, understanding the skeletal maturity in horses, mules or donkeys, could aid in capturing the proper age of maturity.

For instance, bone maturity is slow in Arabian horses and donkeys (Myers and Emmerson, 1966; Ahmed, 2005; Behery *et al*., 2007; Samy *et al*., 2007). Based on that, these latter animals should not be used for riding before three to four years old and any hard work should be avoided until they are at least five to six years old, in spite of these animals appearing physically mature at ages less than 3 years old (Brown and MacCallum, 1975; Ogden *et al*., 1981; Samy *et al*., 2007; Tavana and Peighambarzadeh, 2014).

In addition, premature ossification of growth plates or uneven maturation results from certain practices including unbalanced hoof trimming, leading to conformational defects such as open knees and knock-knees. Moreover, the proper radiographic assessment of the epiphyseal closure times of young animal skeletons allows veterinarians to determine whether there are delayed or premature closure defects (Mason and Bourke, 1973; Salter and Harris, 2001).

Types of the growth plates in equines: Bone formation in immature animals is fulfilled by either intramembranous ossification or endochondral ossification. In the case of intramembranous ossification, the bone is formed by differentiation of the connective tissue cells into bone cells and this mechanism occurs mainly in flat bones (e.g., cranium and scapula) where it leads to increases in bone width. While in case of endochondral ossification, the bone is formed by transformation of chondrogenic cells into bone cells, where it causes increases in bone length in addition to the shaping of articular surfaces (Summerlee, 2002). The latter process is responsible for structuring the three long bone regions: the epiphysis, metaphysis and diaphysis (Boskey, 2002) (Fig. 1).

There are two main types of ossification centers: primary and secondary ossification centers. During prenatal life, the diaphysis is developed from the primary ossification center consisting of a mixture of pluripotent mesenchymal stem cells, calcifying chondrocytes and surrounding thin sheet of cancellous bone (Boskey, 2002). Concerning postnatal life, a recent study evidenced that there are highly specific pluripotent mesenchymal stem cells, so called skeletal stem cells, located in growth plates and during the growth period these cells form osteoblasts and chondrocytes at the area of growth plates (Chan *et al*., 2015). Regarding the secondary ossification center, pressure epiphyses (at the ends of long bones) and traction epiphyses (apophyses) are considered the secondary ossification centers (Fig. 1). Apophyses are located at the point of insertion or origin of muscles (e.g., tuber-calcis, dorsal tibia tuberosity, lateral and medial tibia malleoli).

The epiphysis is growing from the vascular reserve cartilage zone (which is responsible for the growth of the epiphysis toward articular surfaces) and the epiphyseal growth plate (physis), which is composed from hyaline cartilage

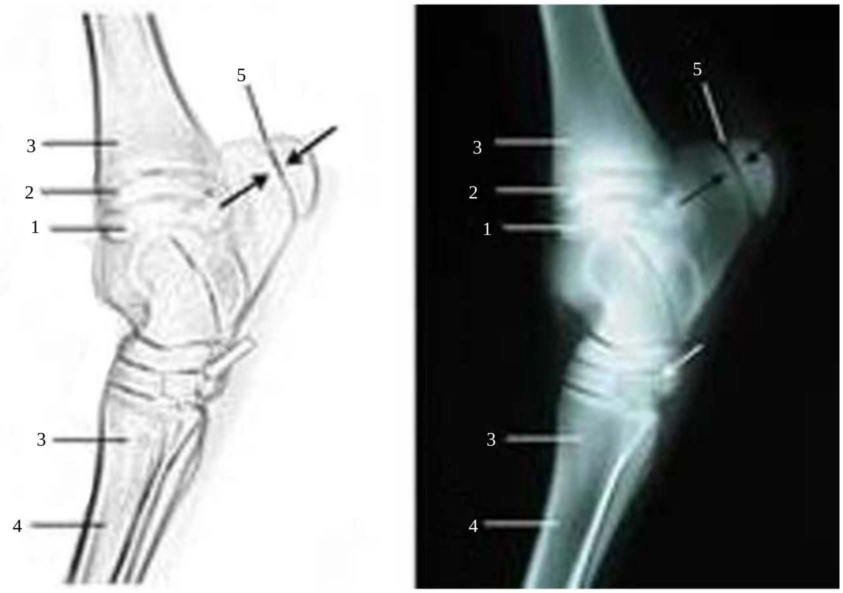

Fig. 1: Mediolateral radiograph of normal hock joint of a one month-old donkey foal. Note a clear fusion surface of both tuber-calcis and calcaneus proper (black arrows) and fused first and second tarsal bones (white arrow). The epiphyseal and apophyseal growth plates are visible as a clear radiolucent line between the epiphysis-metaphysis (2) and tubercle-main bone (5), respectively (Ahmed, 2005). (1) Pressure epiphysis (epiphysis), (2) Epiphyseal growth plate (physis), (3) Metaphysis, (4) Diaphysis (shaft) and (5) Traction epiphysis (apophysis)

between the epiphysis and the metaphysis, responsible mainly for growing the bone in length. The third part "metaphysis" is present between diaphysis and epiphysis. The metaphysis is developed from the contacting zones of the growth plate (Shapiro, 1987; Salter and Harris, 2001; Olsson and Ekman, 2002).

Cartilaginous, fibrous and bony parts are the main compositions of the growth plate (Brighton, 1987; Burdan *et al*., 2009). The cartilaginous part consists of three major zones: The reserve, proliferative and hypertrophic zones. The latter zone is subdivided into maturation, degeneration, primary spongiosa and secondary spongiosa subzones (Abad *et al*., 2002). This order of zones is directed from epiphysis toward metaphysis. The fibrous part surrounds the cartilaginous part and "looks like a ring" for supporting the physis and protecting it from shear stresses (Deppermann *et al*., 1989). The bony parts are located on either side (dorsal and ventral) of the growth plate (Shapiro, 1987; Soeta *et al*., 2000; Von Pfeil *et al*., 2009).

Growth plates ossification times in donkeys: Donkeys, as drafting animals, participate actively in developing communities for agriculture production and riding purposes. The number of donkeys in the world has reached approximately 44 million (Tavana and Peighambarzadeh, 2014). For this reason and from our view, greater understanding of the skeleton of the donkey is necessary, especially in context of growth plate closure times, since there is no data currently available. Our previous results provided beneficial information in that context (Ahmed, 2005). Table 1 and 2 shows the approximate closure times of stifle and hock joints of donkeys from our study and compares this data with those previously published in horses.

Our previous study was carried out on clinically apparent healthy *Equus africanus asinus* donkeys (n = 66) with ages from 1 day to 4 years old. In all animals, the stifle and hock joints were clinically and radiographically investigated. The following radiographic projections were employed; lateromedial, caudocranial for the stifle joint and lateromedial, mediolateral, dorsoplantar for hock joint. The exposure criteria used for radiography were 66-74 kVp, 1-10 mAs for the stifle joint imaging and 50-58 kVp, 1-6 mAs for tarsal joint imaging. In all radiographs, Focal Film Distance (FFD) 100 cm was used. We found that most of the growth plates of stifle and hock joints become ossified (mature) at approximately 4 years of age (Ahmed, 2005; Behery *et al*., 2007; Samy *et al*., 2007).

Moreover, like horses, donkey foals under six months of age are rarely affected by upward fixation of the patella

Table 1: Approximate closure times of the ossification centers of stifle joint in horses and donkeys, as determined by radiography

	Age at fully ossified (months)	
Bone and ossification centers	Horse †	Donkey ‡
Femur		
Distal epiphysis	n/a	6-9
Distal physis	36 – 42	24-30
Trochlear ridge	n/a	4-6
Tibia		
Proximal epiphysis	n/a	9-12
Proximal physis	36-42	24-30
Cranial tibial apophysis	38.6-40.1	36-42
Intercondylar eminences	n/a	3- 6
Fibula		
Proximal epiphysis	n/a	12-15
Proximal physis	24-36	24-30
Shaft	n/a	24-30
Patella		
Patella ossification center	n/a	6-8

n/a: Not available, †: Banks *et al.* (1969), Smith *et al.* (1991) and Strand *et al.* (2007), ‡: Ahmed (2005)

Table 2: Approximate closure times of the ossification centers of tarsal joint in horses and donkeys

	Age at fully ossified (months)	
Bone and ossification centers	Horse †	Donkey ‡
Tibia		
Distal epiphysis	n/a	9-12
Distal physis	20-24	15-18
Lateral malleolus apophysis	n/a	3-6
Medial malleolus apophysis	n/a	4-6
Talus		
Talus ossification center	n/a	n/a
Calcaneus		
Calcaneus proper	n/a	3-6
Tuber-calcis apophysis	24-36	36-48
Tarsal bones		
Central	n/a	4- 6
First and second	n/a	4-6
Third	n/a	4-6
Fourth	n/a	4-6
Metatarsus		
Proximal epiphysis	Before birth	Before birth
Lateral splint bone		
Proximal physis	n/a	6-8
Medial splint bone		
Proximal physis	n/a	3-6

n/a: Not available, †: Banks *et al.* (1969), Smith *et al.* (1991) and Strand *et al.* (2007), ‡: Ahmed (2005)

because medial and lateral femoral trochlear ridges are approximately of equal height (Ahmed, 2005; Samy *et al.*, 2007). Other recent report proposed that Icelandic horses reach to their full appendicular skeleton maturity at approximately 3 years of age (Strand *et al.*, 2007).

Unlike donkeys, based on other results, Arabian horses reach to complete fusion of their tuber-olecranon, as a last one do fuse, at 26.6-29.7 months of age (Myers and Emmerson, 1966). Further, more interestingly, the most of epiphyseal lines in donkeys fuse initially at the middle third and followed by their plantar and finally the dorsal thirds (Ahmed, 2005).

Future directions: The highly organized process of forming cartilage and after wards its remodeling into ossified tissues is named “endochondral ossification”. There are several well-known biological factors which orchestrate this mechanism *in-situ*, such as bone morphogens (BMP2, BMP4, BMP7), vitamin D3 and chondrocyte growth factor, ect. (Dziewiatkowski, 1954; Kasper *et al.,* 1982; Tsumaki and Yoshikawa, 2005). In addition to these factors, it has also been proposed that the gonadal and pituitary sex hormones (SexHs) share actively in this mechanism. These studies showed, at molecular and proteomic level, the localization of the SexHs receptors in human and animal fetal growth plates (Ben-Hur *et al.,* 1997; Kusec *et al.*, 1998; Nilsson *et al.*, 2003). In so doing, this confirms the regulatory effect of the SexHs during the various stages of endochondral ossification including proliferation, differentiation and remodeling phases. Moreover, other studies revealed that follicle stimulating hormone, prolactin and luteinizing hormone have shown as key players in development and ossification of growth plate as well as bone remodeling (Allan *et al.*, 2010; Seriwatanachai *et al.*, 2012). More recently, our research study exclusively provide evidence that primitive population of AC133+Lin-CD45- very small embryonic-like stem cells as well as AC133+Lin-CD45+ hematopoietic stem cells and mesenchymal stem cells express the functional SexH receptors (Abdelbaset-Ismail *et al.*, 2016). Based on these exciting data, the intriguing orchestration mechanism managed by SexHs of the endochondral ossification timing in donkey and horses, from our point of view, is important to address for better understanding and enhancing their skeletal maturation.

CONCLUSION

The rate of skeletal maturity of donkeys needs to be characterized further to avoid any problems during the maturation period, namely developmental orthopedic diseases, including physitis and OCD and also to provide information to farmers and donkey users about the proper starting age for working and training the donkey like their partner horse.

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