USMANU DANFODIYO UNIVERSITY, SOKOTO
(POSTGRADUATE SCHOOL)

# RADIOGRAPHIC MONITORING OF GROWTH PLATE APPEARANCE AND CLOSURE OF RADIUS AND ULNA BONES IN NIGERIAN INDIGENOUS DOGS

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# DEDICATION

This work is a dedicated to God the Almighty for making this day a reality, and to my husband Mr Christopher Eyoto Oviawe and also to my children for their support, care and encouragement.

# CERTIFICATION

This Dissertation by Ekaete Ime Oviawe (Adm No. 14210809002) has met the requirements for the award of the Degree of Master of Veterinary Surgery (MVS), of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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| | |
|---|---|
| AC | Articular cartilage |
| AP | Anconeal process |
| Cm | Centimeter |
| CT | Computed tomography |
| DR | Distal radius |
| DSC-W710 | Digital Sony camera |
| DU | Distal ulna |
| EC | Epiphyseal cartilage |
| GP-SOC | Growth plate of secondary ossification center |
| GI | Gastro intestinal |
| IM | Intramuscular |
| Kg | Kilogram |
| kV | kilovolt |
| mAS | Miliampere per second |
| Mm | Millimeter |
| MR | Mid radius |
| MRI | Magnetic resonance imaging, |

| | |
|---|---|
| MU | Mid Ulna |
| NPC | National Population Commission |
| PR | Proximal radius |
| PU | Proximal ulna |
| RD | Radial diameter |
| SCOAP | Separate center of ossification of the anconeal process |
| SOC | Secondary ossification center |
| TSOC | Time of appearance of secondary ossification center |
| UD | Ulna diameter |
| VTH | Veterinary Teaching Hospital |

# ABSTRACT

Growth plate is radiographically identified as a radiolucent gap between epiphysis and metaphysis of a bone where longitudinal growth occurs. A total of 16 (8 males and 8 females) Nigerian indigenous dogs from 3 litters were monitored by radiography at weekly interval from week 1 to 9 and thereafter at 4 weeks interval from week 12 to 48. Each dog was radiographed nineteen times on the right fore limb. The results obtained showed that the growth plate of the distal epiphysis of the radius was the first to appear at week 2 in some dogs and at 3 weeks in others, the proximal epiphysis of the radius appeared second at 5 weeks, the growth plate of the distal epiphysis of the ulna appeared at 6 weeks and the proximal epiphysis of the ulna appeared at 8 weeks in all the dogs. The proximal ulna growth plate was the first to fuse at 24 weeks followed by the proximal radius at 28 weeks, then the distal ulna at 32 weeks while the distal radius was the last to fuse at 36 weeks in most of the dogs. At week 1, the ulna diameter was larger than the radius, but became the same at 32 weeks after which the ulna became much smaller than the radius at week 48. Therefore, the appearance of all the growth plates and secondary ossification centers of the radius and ulna bones in Nigerian local dogs were post-natal and fused at 40 weeks.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 STUDY BACKGROUND

Dog is a mammal, *Canis lupus familaris,* that has been domesticated and selectively bred for ages (Lescureux and Linnell, 2014) for various behaviors such as sensory capabilities, and physical attributes (Dewey and Bhagat, 2002). Their sexual maturity begins at about 6 to 12 months of age for both male and female (Dewey and Bhagat, 2002). Feldman and Nelson (1996) indicated that dogs are described as very young adults at the time of their growth plate closure. They are the oldest domesticated animals and their long association with humans has made them to become uniquely attuned to human behavior (Konrad, 2002: Berns *et al.,* 2012) as well as grow on a diet that is rich in starch which would be inadequate for other canid species (Axelsson *et al.,* 2013).

Dogs are used by people to perform many functions like hunting, herding, pulling loads, protection, assisting police and military, companionship and more recently, aiding handicapped persons. This influence on human society has given them the pet name "man's best friend” (Berns *et al.,* 2012). In breeding circles, a male canine is referred to as a dog, while a female is called a bitch. A group of offspring is called a litter which is generally called puppies until they are about a year old, the father of a litter is called the sire, and the mother is called the dam. The process of birth is whelping derived from the Old English word whelp (Gould, 1978).

It was documented by *Adám (2007) that* dog is not native to Africa and was introduced at an unknown period in the past. It is likely that there have been multiple introductions from different sources, although the only race found in Northeastern Nigeria is what Epstein (1971) called the “pariah dog” which is often referred to as “mongrel,” meaning mixed breed which explains that the mongrel dog is not the result of breeding or product of artificial selection intentionally created by humans but rather developed by natural selection, without planned intervention by humans which can also be called Nigerian local dog (Coppinger, 2001). Modern dog breeds show more variation in size, appearance, and behavior than any other domestic animal (Dewey and Bhagat, 2002). Dogs are predators and scavengers, and like many other predatory mammals, the dog has powerful muscles, fused wrist bones, a cardiovascular system that supports both racing and strength, and teeth for catching and tearing. Dogs are highly variable in height and weight. The smallest known adult dog is the Yorkshire Terrier, that has 6.3 cm at the shoulder, 9.5 cm in length along the head and body, and weighed only 113 grams. The largest known dog is an English Mastiff which weighed 155.6 kg and was 250 cm from the snout to the tail, while the tallest dog is a Great Dane that stands 106.7 cm at the shoulder (Guinness World Records, 2011).

Physical methods for age estimation such as dentition are not accurate from 6 months and above because of the nature of their feeding (bones) and the canine teeth are not usually considered in aging because it is difficult to determine completed eruption in many domestic dogs as the alveolar bone around the canine teeth is prominent and obscures the enamel-cementum junction (Arnall, 1960).

Monitoring the radius and ulna bones is necessary in veterinary radiology as it provides a guide to surgeons on the choice of surgical procedures to be performed in the growth plate region considering the time at which the growth plate and secondary ossification centers of these bones appears, reach their proper anatomical shape and finally fuse.

Bone age is a way of describing the degree of maturation of dog bones as they grow from puppy and finish growth as young adults. The bones of the skeleton change in size and shape as the animal mature. The "bone age" of a dog is the age at which a dog reaches its stage of bone maturation. At birth, only the metaphysis and diaphysis of the "long bones" are present (primary ossification enters)(Hochberg, 2002).

Secondary ossification centers (SOCs) appearafter the primary ossification centers and the appearance of the radiopaque area at the epiphyseal region on radiographs for the first time is considered as the appearance of secondary ossification center (Myo *et al.*,2016). In some breeds of dogs, the SOCs are cartilages and are not seen radiographically. As the dog grows older, the SOCs become calcified and appear on the radiograph, separated by a layer of invisible cartilage where most of the growth occurs (Oestreich, 2010). The development of secondary ossification follows a pattern of the 3 stages of development as reported by Jikken *et al.* (1980). As sex steroid levels rise during puberty, bone maturation accelerates. The remaining cartilaginous portions of the SOCs become thinner and finally obliterates. As these cartilaginous zones become obliterated, the growth plates are said to be "closed" and no further lengthening of the bones will occur (Oestreich, 2010; Gaskin and Kalm, 2011). Bone age assessment using radiograph is an important clinical tool in the area of orthopaedics, especially in relation to surgical procedures and growth disorders (Harris, 1978: Gilsanz and Ratib, 2005).

The identification and observation of any structure in the body can be deduced from its radiographic appearance. Normal radiographic structure is well-informed from evaluating a large number of normal studies (Morgan and MacMillan, 2000). This is particularly true for recognizing the normal anatomical structures that occur at different times in an animal.

Radius and ulna are long bones of the fore limbs; each of these bones is made up of proximal and distal epiphyses and a diaphysis. In the young, each of the epiphyses is separated by the growing cartilage called the growth plate while at maturity, the growth plate ceases to grow and the epiphyses fuse with the metaphysis to become a single bone as both share in the bony replacement of the growth plate (Bryan and Gerald, 2005). The bones are parallel to each other and the ends of the bones are enlarged and have smooth surfaces which are covered by a layer of hyaline cartilage which enters into the formation of the joints (Olsson and Ekman, 2002).

The endochondral ossification regions of the long bones are the growth plates which exist until the postnatal growth is completed and the bone ossifies after the process of postnatal growth.Growth plates(GP)are specialized cartilages extending longitudinally between the epiphysis and the metaphysis of immature long bones, working in harmony with each other and providing longitudinal bone growth but at maturity the growth plate fuses with the metaphysis to become a single bone and longitudinal bone growth ceases (Brighton, 1978: Bryan and Gerald, 2005).Long bones are made up of two epiphyses and a diaphysis and these epiphyses appear, develop and finally fuse with their diaphysis at different times in different bones (Chapman, 1965). At young age, each end of the diaphysis is separated by a metaphyseal growth plate called the growth plate. At maturity, this growth plate fuses with the metaphysis to become one bone (Bryan and Gerald, 2005). Small and medium-size dogs mature at about seven months to one year of age, while large and giant-breed dogs might not be fully grown until they are eighteen months to two years old (Van Ballenberghe and Mech, 1975). When growth plates are open, threaded pins, screws, tension band wires or plates that cross the growth plate should not be used because trauma may lead to premature closure of the growth plate which can lead to different degrees of limb deformity and length discrepancy as a result of growth plate arrest (Shapiro and Forriol, 2005).

Anconeal process is a piece of bony protrusion which forms part of the ulna bone at the back of the elbow joint. In very young animals this part of the ulna is still cartilage and bone is laid down from within its own ossification center (Bryan and Gerald, 2005). Sometimes, the anconeal process does not fuse on to the main body of the ulna, in which case it forms a separate bone and is called an ununited anconeal process (Olsson, 1983). Ideally, the anconeal process of the ulna articulates with the caudal intercondylar surface of the humerus and fits into the supratrochlear fossa when the joint is fully extended. The trochlear notch of the ulna articulates with the trochlear of the humerus. Distal to the trochlear notch are two prominences called the medial and lateral coronoid processes of the ulna bone. The medial process is larger and is located more distal than the lateral (Berzon and Quick, 1980; Fox *et al.,* 1983).

Radiologic imaging can effectively be used in monitoring the development of radius and ulna bones through determination of the ossification centers and growth plate (Aytekin,

1993) which will assist surgeons in knowing the accurate age of a dog at which certain procedures can be performed at the growth plate region, identify conditions which accelerate or delay growth plate closure, detecting growth plate closure defect and also in differentiating between the normal and the abnormal radius and ulna bone (Aslanbey, 2002).

## 1.2 STATEMENT OF THE PROBLEM

Many researches have been conducted on the ossification centers of other breeds of dog(Jikken*et al.*, 1980:Dirsko and Pfeil 2009: Charjan, *et al.,* 2014*:* Myo*et al.,* 2016)but limited information on the radius and ulna bones of Nigerian Indigenous Dogs.

There is paucity of information on the time of appearance and closure of growth plates of radius and ulna bones of Nigerian Indigenous Dogs.

There is paucity of information on the changes in diameter of the radius and ulna bones or variations in growth length of male and female dogs as the animals mature.

There is lack of information on the radiographic appearance of the growth plates of radius and ulna bones as it relates to the age of Nigerian Indigenous Dogs, which is important when treating clinical conditions affecting these extremities (Brinker *et al.*,1983).

## 1.3 JUSTIFICATION OF THE RESEARCH

Most of the fractures involving the articular surfaces of the radius and ulna are usually seen in skeletally immature animals (Skaggs *et al.,* 1973). For accurate anatomical reduction of the fragments, open reduction and internal fixation is usually indicated and knowing the normal appearance of these regions at that age is important especially when both limbs of the animal are affected.

Premature closure of the proximal or distal growth plate of the radius or ulna bone may result in serious shortening of the forearm and an increase in the radio-humeral joint space with consequent elbow instability.

This study will enable veterinarians evaluate how fast or slowly a dog's skeleton is maturing, which can help them diagnose conditions that delay or accelerate physical growth and development.

Thus there is the need to study and document the time of appearance and closure of the growth plate of these bones in Nigerian Indigenous Dogs.

## 1.4 RESEARCH HYPOTHESIS:

Null Hypothesis ($H_{01}$): The time of appearance and closure of the growth plates of radius and ulna bones in Nigerian indigenous dogs is the same with other breeds of dogs.

Alternative hypothesis ($H_{A1}$): The time of appearance and closure of the growth plates of radius and ulna bones in Nigerian indigenous dogs different from other breeds of dogs.

Null Hypothesis ($H_{02}$): The length and diameter of radius and ulna bone is the same in the male and female dogs as the GP closes.

Alternative hypothesis ($H_{A2}$): The length and diameter of radius and ulna bone is different among the male and female dogs as the GP closes.

## 1.5 AIM AND OBJECTIVES OF THE STUDY

The main aim of this study is to evaluate radiographically the growth plate appearance and the closure time in the radius and ulna bones in Nigerian Indigenous Dogs as the animal matures.

The specific objectives of the study are:

1. To determine the age of appearance and development of secondary ossification centers to a point where they reach their normal anatomical shape in the radius and ulna bones.
2. To determine the age of appearance, sequential thickness and closure time of the proximal and distal growth plates of radius and ulna bones as the animal matures.
3. To determine the variations in the length and diameter of the radius and ulna bones in male and female dogs.

# CHAPTER TWO

## 2.0 LITERATUREREVIEW

## 2.1 NIGERIAN INDIGENOUS DOGS

Nigerian indigenous dog is known as a Nigerian mongrel. It is also called mixed-breed meaning a dog that is not an outcome of breeding. Sometimes it is called a mutt (Morris and Desmond, 2008). Estimates place the number of mixed-breed dogs at 150 million in the world (Morris and Desmond, 2008). Unlike mixed-breeds, crossbred dogs are often the product of artificial selection intentionally created by humans, whereas the term "mongrel" specifically refers to dogs that develop by natural selection, without planned intervention of human being. Extensive genetic studies embarked on during the 2010s indicated that dogs diverged from an extinct wolf-like canid in Eurasia 40,000 years ago (Skoglund*et al.,* 2015). Being the oldest domesticated animal, their long association with people has allowed dogs to be uniquely attuned to human behavior (Berns*et al.*,2012) , as well as thrive on a starch-rich diet which would be inadequate for other canid species Axelsson*et al.*, 2013).

### 2.1.1 IMPORTANCE OF DOGS

Domestic dogs inherited behaviors like biting, from their wolf ancestors, which makes them hunters. Their sophisticated forms of social understanding and communication accounts for their playfulness, trainability and ability to fit into human homes and social situations, and these qualities have given dogs a relationship with humans that has allowed them to become one of the most successful species on this earth today (Miklósi, 2007). They also perform several other functions like herding livestock (Williams, 2007), pulling loads, rodent control (Dewey and Bhagat, 2002), protection, assisting police and military, companionship, and, more recently, aiding handicapped individuals. This impact on human society has given them the nickname "man's best friend" (Berns *et al.*, 2012). In some part of the world and in some cultures, dogs are also a source of meat for human consumption (Simoons, 1994: Ahmed, 2004).

From the 1980s, there have been changes in the role of the pet dog, such as the increased role of dogs in the emotional support of their human guardians. People and dogs have become increasingly integrated and implicated in each other's lives as part of the family (Haraway, 2003), to the point where pet dogs actively shape the way families and homes operate (Power, 2008), as they are primarily functional in acting as a guard, children's playmate, or walking companion. Dogs can easily be trained to exhibit other behaviors such as barking, jumping up, digging, rolling in dung, fighting, dancing and dog yoga (Power, 2008). Service dogs such as guide dogs, utility dogs, assistance dogs, hearing dogs, and psychological therapy dogs provide assistance to individuals with physical or mental disabilities.

The latest study using magnetic resonance imaging (MRI) to humans and dogs together proved that dogs have same response to voices and use the same parts of the brain as humans to do so. This gives dogs the ability to recognize emotional human sounds, making them friendly social pets to humans (McNicholas and Collis, 2006).

### 2.1.2 PUBLIC HEALTH SIGNIFICANCE

Earlier studies have shown that people who keep pet dogs or cats exhibit better mental and physical health than those who do not, making fewer visits to the doctor and being less likely to be on medication than non-guardians (Headey, 1999). Research has, however, pointed to significantly less absence from school through sickness among children who live with pets (McNicholas and Collis, 2006). In one study, new guardians reported a highly significant reduction in minor health problems during the first month following pet acquisition, and this effect was sustained in those with dogs through to the end of the study (McNicholas and Collis, 2006). In addition, people with pet dogs do more physical exercise than those with cats and those without pets. The results provide evidence that keeping pets may have positive effects on human health and behavior. Pet guardianship has also been associated with increased coronary artery disease survival, with human guardians being significantly less likely to die within one year of an acute myocardial infarction than those who did not own dogs (Friedman and Thomas, 1995). The health benefits of dogs can result from contact with dogs in general, and not solely from having dogs as pets. For example, when in the presence of a pet dog, people show reductions in cardiovascular, behavioral, and psychological indicators of anxiety (Headey, 1999). Other health benefits are gained from exposure to immune-stimulating microorganisms, which, according to the hygiene hypothesis, can protect against allergies and autoimmune diseases. The benefits of contact with a dog also include social support, as dogs are able to not only provide companionship and social support themselves, but also to act as facilitators of social interactions between humans (Wilson, 1991). One study indicated that wheelchair users experience more positive social interactions with strangers when they are accompanied by a dog than when they are not (McNicholas and Collis, 2006).

The practice of using dogs and other animals as a part of therapy dates back to the late 18th century, when animals were introduced into mental institutions to help socialize patients with mental disorders (Kruger and Serpell, 2006). Animal-assisted intervention research has shown that animal-assisted therapy with a dog can increase social behaviors, such as smiling and laughing, among people with Alzheimer's disease (Batson*et al.*, 1998). One study demonstrated that children with behavior disorders who participated in an education program with dogs and other animals showed increased attendance, increased knowledge and skill objectives, and decreased antisocial and violent behavior compared to those who were not in an animal-assisted program (Katcher and Wilkins, 2006).

Medical detection dogs are capable of detecting diseases by sniffing a person directly or samples of urine or other specimens. Dogs can detect odour in one part per trillion, as their brain's olfactory cortex is (relative to total brain size) 40 times larger than humans. Dogs may have as many as 300 million odour receptors in their nose, while humans may have only 5 million (Wilson, 1991). Each dog is trained specifically for the detection of single disease from the blood glucose level indicative of diabetes or cancer. To train a cancer dog requires 6 months. A Labrador Retriever called Daisy has detected 551 cancer patients with an accuracy of 93 percent and received the Blue Cross (for pets) Medal for her life-saving skills (Wilson, 1991).

## 2.2 INDICATIONS FOR BONE SURGERY IN DOGS

Bone surgeries can also be called osteotomy which is an elective surgical procedure in which bones are cut in an attempt to correct abnormality that has resulted from trauma or disease. Radial and ulnar osteotomy is a technique needed to treat abnormalities of the canine forelimb mainly mal-unions and forelimb deviations resulting from growth plate abnormalities. This treatment allows for correction in six different planes: valgus or varus, flexion or extension, internal or external rotation, lengthening or shortening, medial or lateral displacement, or dorsal or ventral displacement (Newton,1974).

Osteotomy is used for several specific indications such as variation in growth of paired bones, eccentric epiphysiodesis, diaphyseal angulation due to malunion fractures or growth anomalies, torsional deformity, limb length discrepancy, and correction of disease whereby an osteotomy of normal bone may correct a disease condition (Rudy, 1975: Newton and Nunamaker1984).

### 2.2.1 Variation in growth of paired bones

Trauma, disease, or genetic predisposition may lead to the premature closure of the growth plate in the radius and ulna or the tibia and fibula. If the unaffected bone continues to lengthen while the damaged bone lengthens at a slower rate, the normal bone will be forced to bow away from the shortened bone. Osteotomy is necessary to straighten the deformity and return the limb to a normal function and appearance (Rudy, 1975).

### 2.2.2 Eccentric epiphysiodesis

Trauma to a portion of a growth plate may result in a selective slowing or cessation of growth in only that part. Continued growth from the unaffected side results in a deformity at the epiphysis. Such an injury commonly occurs in trauma to the lateral side of the distal radial physis or trauma to the lateral side of the distal tibialphysis. Such an injury may occur to any physeal plate. Osteotomy through the site of maximal deformity is necessary to return the bone to normal anatomical position and should result in improved function in the involved limb (Newton and Nunamaker1984).

### 2.2.3 Diaphyseal angulation due to malunion

Long-bone fractures that have healed without reduction or bones in which fixation was removed prematurely may heal in angular, rotational, or shortened positions that are nonfunctional. Such bones may be osteotomized, realigned, and allowed to unite in proper alignment. Improved limb function will invariably result (Rudy, 1975).

### 2.2.4 Torsional deformities

Torsional abnormalities occur most often as a result of fracture misalignment or genetic disease. Miniature breed dogs with medial patellar luxation may demonstrate proximal tibial torsion as the primary deformity leading to patellar luxation. Osteotomy to derotate the entire proximal tibia or only the tibia tuberosity may be necessary to restore more normal function to the affected limb (Newton and Nunamaker1984).

### 2.2.5 Limb length discrepancy

Trauma or disease to growing physes may result in gross shortening of a long bone or pair of long bones. While dogs and cats have the ability to adapt to some bone shortening, eventually the shortening may become so severe as to render the affected limb useless. In such instances osteotomy may be performed and the bone ends forcibly distracted mechanically to restore sufficient bone length to allow limb use. Such a procedure can also be useful to force paired bones to lengthen at a similar rate (Newton, 1974).

### 2.2.6 Correction of disease

Corrective osteotomy in the very young animal who is affected by hip dysplasia may result in the formation of a deep congruent acetabulum and allow the animal to continue to mature without the disease becoming more severe. In many instances the effects of the disease process have been effectively eliminated (Newton and Nunamaker 1984).

Osteotomy to correct disease can be successful only if performed early, before the secondary changes of the disease occur. Osteotomy of bony prominences is a common procedure used to facilitate surgical exposure of bones or joints. When employed for this purpose the same precautions must be adhered to as when using osteotomy to correct abnormality (Rudy, 1975).

## 2.3 SURGICAL METHODS IN DOGS

Surgical methods performed in dogs fall into three broad categories: orthopaedics (bones, joints), soft tissue surgery (skin, body cavities, cardiovascular system, gastro intestinal/urogenital/respiratory tracts), and neurosurgery. Advanced surgical techniques such as joint replacement (total hip, knee and elbow replacement), fracture repair, stabilization of cranial cruciate ligament deficiency, oncologic (cancer) surgery, herniated disc treatment, complicated gastrointestinal or urogenital procedures, kidney transplant, skin grafts, complicated wound management, minimally invasive procedures (arthroscopy, laparoscopy, thoracoscopy), are performed by Veterinary Surgeons.

Surgical procedures performed in the bones of dogs are: ruptured anterior cruciate ligament repair, hip dysplasia, femoral head osteotomy, triple pelvic osteotomy, hip replacement, leg amputation, bone fracture repair, arthroscopy, medial patellar luxation and anterior patellar luxation (Newton, 1974).

## 2.4 SURGICAL INTERVENTIONS IN RADIUS AND ULNA BONES

The main categories of surgical interventions are external fixation, percutaneous pinning, open reduction and internal fixation, and the insertion of bone scaffolding materials (Riser and Shirer, 1965).

## 2.5 AGE ESTIMATION

Age can be estimated usingvariation of dental, skeletal, and sexual maturity among the domestic dogs (Schmeling*et al.,* 2007)**.** There have been disagreements as to whether there are breed specific differences of the age of attainment of dental, skeletal, and sexual maturity. Many authors have reported breed specific differences of the timing of tooth eruption. Schmeling, *et al*. (2007), reported delayed completed eruption of the upper deciduous canine teeth in the fox terrier compared to Basenji, Beagle, Cocker spaniel, and Shetland sheepdog. Kauhala and Helle (1990), noted that the permanent teeth erupt several months earlier in large breeds compared to smaller terriers. Medium sized breeds (e.g., setters) are in an intermediate position. Similar statements was made by Nagorsen*et al.* (1988).

An animal’s age is a critical part of its history. Age-based expectations about growth plate conformation, strength, mobility, and stress tolerance can vary widely between younger and older animals. Failure to consider age in surgical management can result in devastating consequences (Schmeling*et al.,* 2007). The age of a patient affects the thought processes concerning every aspect of veterinary medicine, including how animals are restrained, surgical management options, diagnostic differentials are evaluated, treatment modalities are considered, and anesthetics and drug doses are selected; age also affects an animal's adaptability and life expectancy as well as euthanasia decisions (Kauhala and Helle, 1990). Veterinarians in private practice or humane shelters are called on daily to determine the age of animals with unknown histories or to examine animals in which stated ages are incorrect (Kauhala and Helle, 1990).

Methods of age estimation vary and include: variation of dental, skeletal maturity, sexual maturity (Salmeri*et al.*, 1991) and ocular reflections (Purkinje-Sanson images) within domestic animals (Ekesten and Torrång, 1995). The ocular age estimation in dogs older than 4 years of age, is more than twice as accurate as is the dental method of estimating age (Dapson and Irland, 1972):  This technique can be learned quickly and does not require special equipment (Kealy*et al.,* 2011).

## 2.6 RADIOGRAPH

When a body part is interposed between the path of X-rays and an X-ray film, its shadow is formed. This shadow on the film is processed to obtain a permanent record in the form of a radiograph (Singh and Singh 2001). Radiological imaging is an effective method to demonstrate the time of appearance and positions of ossification centers which are very important in deciding whether there is normal or abnormal development of the bones (Todhunter *et al.,*1997).

### 2.6.1 Film processing

X-ray films are universally processed in standard vertical tanks which are commercially available in 9, 10, 13 and 22 liters capacity. The material used for this tank is either stainless steel or plastic and four tanks are required (for developing, rinsing, fixing and washing) for film processing (Singh and Singh2001).

## 2.7 RADIOGRAPHIC VIEWS

An ideal radiographic detail is essential for accurate assessment of the development of radius and ulna bones. Radiographs are usually taken in lateral, sternal recumbency or dorsal recumbency of the animal with vertical or horizontal beam (Sirois *et al.*, 2010). In dealing with the radius and ulna bones, cranio-caudal view with the animal on sternal recumbency or mediolateral (lateral) views with the animal on lateral recumbency are usually the standard views taken. The standard view documented by Douglas*et al.* (1987) for visualization of most structures of the radius and ulna bone is the mediolateral (lateral) extended projection. There is also the flexed mediolateral projection of the elbow which allows for a better visualization of structures like the anconeal process. However, this view results in superimposition of several clinicaly significant structures which cannot be avoided (Douglas *et al.*, 1987)

Radiograph of the radius and ulna bones can be achieved by using table-top techniques, non-grid exposures, collimating to the radius and ulna bones, centering the primary beam on the diaphysis of the radius and ulna bones, using detail-intensifying screens of the cassette with low exposure factors (Guthrie *et al.*, 1992). In addition, Sirois*etal.* (2010)documented that the standard cranio-caudal and mediolateral projections have been improved by several additional projections like mediolateral extended and flexed mediolateral projections in an effort to highlight definite anatomical sites or pathological disorders.

| | |
|---|---|
| <br>Cranio-caudal projection | <br>Flexed mediolateral projection. |

**Plate 2.1**Different positioning of the dog for radius and ulna radiographs

**Plate 2.2:**Mediolateral extended radiograph of the elbow of a dog. Key: 1 = distal diaphysis of the humerus; 2 = humeral condyle with superimposed epicondyles; 3 = anconeal process of the ulna superimposed over the medial epicondyle of the humerus; 4 = tuber olecranon; 5 = medial coronoid process of the ulna superimposed over the radial head; 6 = lateral coronoid process of the ulna; 7 = cranial aspect of the head of the radius; 8 = proximal diaphysis of the radius; 9 = proximal diaphysis of the ulna.

(Sirois *et al.*,2010).

**Plate 2.3**: Flexed mediolateral projection of the elbow of a dog. Key: 1 = distal diaphysis of the humerus; 2 = caudal margin of the medial epicondyle of the humerus; 3 = humeral condyle with superimposed epicondyles (not visualized); 4 = radial head; 5 = medial coronoid process of the ulna; 6 = anconeal process of the ulna; 7 = tuber olecranon; 8 = proximal diaphysis of the ulna; 9 = proximal diaphysis of the radius; 10 = nutrient canal of the radius along the caudal interosseous border.

(Sirois *et al.*,2010).

**Plate 2.4**: Caudocranial radiograph of the elbow of a dog. Key: 1 = lateral epicondyle of the humerus; 2 = medial epicondyle of the humerus; 3 = trochlea (articular surface of the humerus opposite the articular surface of the medial coronoid process of the ulna); 4 = olecranon of the ulna superimposed over the midportion of the humeral condyle; 5 = capitulum (articular surface of the humerus opposite the articular surface of the radial head); 6 = lateral aspect of the radial head; 7 = medial coronoid process of the ulna.

(Sirois*et al.*,2010).

## 2.8 DEVELOPMENT (EMBRYOLOGY) OF BONE

Ossification, also known as osteogenesis, is the process by which bones are formed in the body. The appearance of radio opaque area at the appropriate anatomical locations on radiographs for the first time was considered as the appearance of ossification center (Hare, 1961). The frame of an embryo is composed of either fibrous membranes or hyaline cartilage which is shaped like bones and provides the medium for ossification. They appear as early as 28 days after conception in dogs (Summerlee, 2002) and continues till the dog matures. Two types of bone formation are known to occur which include intramembranous ossification and endochondral ossification.

These two kinds of ossification do not lead to changes in the structure of matured bones but rather specify different methods of bone formation. Both mechanisms involve the replacement of a pre-existing connective tissue with bone (Shapiro, 1987).

The first stage in the development of bone is the migration of mesenchymal cells (embryonic connective tissue cells) into the region where bone formation is about to begin (Coulsen and Lewis, 2002). These cells increase in number and size. In some skeletal structures where capillaries are lacking they become chondroblasts which will be responsible for formation of cartilage. The osteoblasts will form bone tissue by intramembranous or endochondral ossification (Ferrara *et al.*, 2003).

### 2.8.1 Intramembranous ossification

This type of bone formation occurs on flat bones of the roof of the skull, parts of the mandible, and probably part of the clavicles (Ferrara *et al.,* 2003).

The basics of the process of formation of intramembranous ossification shape according to Mackie *et al.* (2008) are as follows:

1. Osteoblasts which are formed from mesenchymal cells cluster in the fibrous membrane and are called a center of ossification.

2. The osteoblasts then secrete intercellular substances partly composed of collagenous fibers that form a framework on which calcium salts are quickly deposited by a process called calcification.

3. When a cluster of osteoblasts is completely surrounded by the calcified matrix, it is called trabeculae. As trabeculae form in nearby ossification centers, they fuse into the open latticework characteristic of spongy bone. With the formation of successive layers of bone, some osteoblasts become trapped in the lacunae and lose their ability to form bone and are called osteocytes (Mackie *et al.,* 2008).

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5. The spaces between the trabeculae fill with red marrow. The original connective tissue that surrounds the growing mass of bone then becomes the periosteum. The ossified area has now become true spongy bone (Mackie *et al.*, 2008).

6. Eventually, the surface layers of the spongy bone will be reconstructed into compact bone. Much of this newly formed bone will be destroyed and reconstructed so the bone may reach its final adult size and shape (Bryan and Gerald, 2005).

### 2.8.2 Endochondral ossification

This is the replacement of cartilage by bone. Most bones of the body, including parts of the skull are formed in this way although this type of ossification is best observed in long bones (Mackie *et al.,* 2008).

The basics of the process of formation of endochondral ossification are as follows:

1. Early in embryonic life, a cartilage model or template of the future bone is laid down which is covered by a membrane called the perchondrium (Ferrara and Gerber, 2001).

2. Midway along the shaft of this model a blood vessel penetrates the perichondium, stimulating cells in the internal layer of the perichondrium to enlarge and become osteoblasts (Mackie *et al.,* 2008).

3. The osteoblasts begin to form a collar of compact bone around the middle of the diaphysis of the cartilage model. Once the perichondium starts to form bone, it is called the periosteum (Bryan and Gerald, 2005).

4. Simultaneously with the appearance of the bone collar and the penetration of blood vessels changes occur in the cartilage in the center of the diaphysis called the primary ossification center, the cartilage cells hypertrophy probably because they accumulate glycogen for energy and produce enzymes that catalyze future chemical reactions (Bryan and Gerald, 2005)..

5. When the hypertrophied cells burst, there is a change in extracellular pH to a more alkaline pH causing the intercellular substance to become calcified, with minerals deposited within it. Once the cartilage becomes calcified, nutritive materials required by the cartilage cells can no longer diffuse through the intercellular substance leading to the death of the cartilage cells (Mackie *et al.*, 2008).

6. This intercellular substance begins to degenerate leaving large cavities in the cartilage model. The blood vessels grow along the spaces where cartilage cells were previously located and enlarge the cavities further. Gradually, these spaces in the middle of the shaft join with each other, and the marrow cavity is formed (Bryan and Gerald, 2005).

7. As these developmental changes are occurring, the osteoblasts of the periosteum deposit successive layers of bone on the outer surface so that the collar thickens, becomes

thickest in the diaphysis. The cartilage model continues to grow at its ends, steadily increasing its length (Mackie *et al.,* 2008).

8. Eventually, blood vessels enter the epiphyses and secondary ossification centers appear in the epiphyses and also lay down spongy bone. In the radius and ulna bones secondary ossification centers develop in the proximal epiphyses soon after birth while the other centers develop in the distal epiphyses later (Mackie *et al.,* 2008).

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9. After the secondary ossification centers have been formed, bone tissue has completely replaced the cartilage except a dark line which remains as a plate between the epiphysis and diaphysis, called the epiphyseal plate (Bryan and Gerald, 2005).

10. The epiphyseal plate allows the diaphysis of the bone to increase in length until maturity. As the animal grows, cartilage cells are produced by mitosis on the epiphyseal side of the plate which are then destroyed and the cartilage is replaced by bone on the diaphyseal side of the plate. In this way, the thickness of the epiphyseal plate remains fairly constant, but the bone on the metaphyseal side increases in length (Bryan and Gerald, 2005).

11. Growth in diameter occurs along with growth in length. In this process, the bone lining of the marrow cavity is destroyed so that the cavity increases in diameter. At the same time, osteoblasts from the periosteum add new osseous tissue around the outer surface of the bone. Initially, diaphyseal and epiphyseal ossification produces only spongy bone. Later by reconstruction, the outer region of spongy bone is reorganized into compact bone (Ferrara and Gerber, 2001).

Any disturbance of endochondral ossification may lead to abnormal skeletal development and maturation (Shapiro and Forriol, 2005: Mackie *et al.*, 2008).

## 2.9 IMAGING OF RADIUS AND ULNA STRUCTURES

Radiography is the main diagnostic imaging for the assessment of musculoskeletal development and disorders and can provide the morphologic description of bone which can lead to the establishment of a conclusive or differential diagnosis (Kraft and Gavin, 2001; Latorre*et al.,*2006). Much has changed in the aspect of imaging of body structures, its development and their functions. These newer modalities are referred to as "advanced imaging techniques" and they include computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (Singh and Singh 2001: Latorre*et al.,*2006).

## 2.10 DEVELOPMENTAL FEATURES OF THE RADIUS AND ULNA BONES IN IMMATURE DOGS

Radius and ulna are long bones in the forearm which run parallel to each other and extend from the elbow (where they join with the humerus and the radius joins to the ulna at the radial notch) to the carpus where the radius forms a joint with the ulna bone (Olsson and Ekman, 2002).

The radius and ulna, together with the carpus, grow as a separate unit, and normal development depends upon a synchronization of the growth of these bones. The radius and ulna individually have two growth plates, and each plate grows at a different rate (Riser and Shirer, 1965).

### 2.10.1 Radius

The longitudinal growth of the radius follows the pattern of most long bones and expands by endochondral conversion of cartilage to bone (Dirsko and Pfeil, 2009). On the radiograph, the epiphyses, growth plates, metaphyses, diaphysis and nutrient foramen are easily identified (Riser and Shirer 1965). At birth, the epiphyses of the radius are composed of cartilage, and are not visible radiographically until they are about 12 to 14 days of age when the mineralized epiphyseal nucleus (secondary ossification center) appears radiographically; osteogenesis can also be seen histologically. The entire outline of both epiphyses becomes visible on radiograph before four weeks of age (Dirsko and Pfeil, 2009).

The growth plates are composed of cartilage and appear radiographically as a radiolucent lines where bone length is added (Conzemius*et al.*, 1994). The proximal radius is conPlated by one secondary ossification center. During active ossification, the cartilage cells proliferate, line up in columns, mature, hypertrophy, calcify, and disintegrate, leaving a straight strip or core of noncellular calcified cartilage matrix on which new bone is deposited by the osteoblasts. These newly formed cores of bone are called primary trabeculae (Dirsko and Pfeil, 2009). The newly mineralized primary trabeculae become the metaphyses which are situated adjacent to the growth plate and appear radiopaque. These primary trabeculae are replaced by secondary, and then tertiary trabeculae, which finally form the cortex of the diaphysis. The bone at the growth plate is very wide while the shaft narrows at the cut-back zone as it tubulates into the diaphysis (Newton, 1974).

Under normal conditions of the radius, 70% of the growth takes place at the distal metaphysis, while 30% occurs in the proximal area. The amount of growth at each end can be accurately determined from a radiograph by locating the nutrient foramen and then measuring the percentage of bone present on either side of this vessel (Dirsko and Pfeil, 2009).

In a growing bone, an estimate of the degree of radiopacity present in the metaphysis at each growth plate and the size of the expanded metaphysis also serve as guides to the amount of growth activity that is taking place, e.g., the distal end of the radius during growth shows greater density, and the metaphysis is larger in diameter than the proximal metaphyseal area (Newton, 1974).

### 2.10.2 Ulna

Anatomically, the ulna is relatively different from the radius in the sense that the distal epiphysis is long and pointed with a cone shaped growth plate which grows more actively than that of the distal radius (Dirsko and Pfeil, 2009). At birth, the distal epiphysis is composed of cartilage and its nucleus ossifies and appears radiographically from two to three weeks after birth (which is after the appearance of the radial epiphyses). The epiphyseal apex ossifies even later than does the proximal epiphysis. The proximal ulnar growth plate stretches across the tip of the olecranon which is relatively inactive, and contributes little to ulnar length (Riser and Shirer, 1965).

Carlson *et al.*(1972) documented that accelerated growth is accomplished by the development of anatomical features: the conical shape of the distal growth plate, and the increased diameter of the entire ulnar shaft. The conical shape of the ulnar plate increases the growth surface area by 1.5 times over a flat area of the same diameter (Clayton-Jones and Vaughan, 1970). The diameter of the ulna during growth in the giant breeds increases, apparently to accommodate the added enchondral bone production; it diminishes in diameter as growth slows. The ulna at four to five months of age commonly is as much as 50% larger in diameter than the radius; at seven to nine months, the two bones are about the same size (Lau *et al.*, 2015). It has been documented by Newton (1974) that longitudinal growth from the proximal (olecranon) growth plate has been restricted to 15% of the total length, while the distal growth plate provided the remaining 85%. From this, it becomes evident that the distal ulna must grow at least 15% faster than the distal radial companion if the two bones are to maintain their proper length (Dirsko and Pfeil, 2009).

The epiphysis is a secondary ossification center in the hyaline cartilage forming the joint surfaces at the proximal and distal ends of the bones. Growth of the epiphysis arises from two areas: the vascular reserve zone cartilage, which is responsible for growth of the epiphysis toward the joint (Boskey, 2008), and the epiphyseal plate, which is responsible for growth in bone length (Olsson and Ekman, 2002). The time of appearance of ossification center has been documented for dogs of between 25-35kg by Dirsko and Pfeil (2009) to be variable for anconeal process, 3-4 months for olecranon and distal epiphysis of the ulna and prenatal for the proximal and distal epiphysis of the radius.

In adult life, when remodeling and resorption are completed, and the growth plates have closed and the metaphyseal bulging has disappeared, the ulnar diameter is one half that of the radius. Most of the excess bone is absorbed from the lateral side. The distal ulna represents the fixed point around which the radius and the carpals act (Logan and Lindau, 2008).

## 2.11 ANCONEAL PROCESS

The anconeal process of the ulna articulates with the caudal intercondylar surface of the humerus and fits into the supratrochlear fossa when the joint is fully extended (Fox *et al.*, 1983). The trochlear notch of the ulna articulates with the trochlear of the humerus and distal to the trochlear notch are two prominences called the medial and lateral coronoid processes. The medial process is larger and is located more distal than the lateral (Berzon and Quick, 1980; Fox *et al.,* 1983). The proximal ulna, in most instances, has two ossification centers; the olecranon and the anconeal process, which can be identified radiographically, as one secondary ossification center called the olecranon apophysis (Morgan and MacMillan,2000).

Fusion time of anconeal process to the ulna has been documented in the literature for some breeds of dogs which include: 16 to 20 weeks in German shepherd and other large breed dogs (Mitten and Hoefle, 1978; Turner *et al.,* 1998) and 14 to 15 weeks in the greyhound (Van Sickle, 1966). If the anconeal process is not radiographically united at 20 weeks of age, spontaneous union will not occur and the condition is called ununited anconeal process (Mitten and Hoefle, 1978).

Ossification centers of the anconeal process appear at 12 to 14 weeks and might develop as a direct extension of the diaphysis of ulna or as a separate center of ossification (Guthrie*et al.,* 1992), and at this time, the cartilaginous medial coronoid process begins to ossify from base to its tip and has no separate center of ossification (Olsson, 1983; Dirsko and Pfeil, 2009). The ossification of the coronoid process and the fusion of the anconealprocess are completed by approximately 16 to 22 weeks (Olsson, 1983), and it is expected that at 14 weeks of age, all the secondary ossification centers should have reached their proper anatomical shape although, not yet fused with the diaphysis (Lau *et al.,* 2015).

However, Frazho*et al.* (2010) documented that most breeds of dog do not have a separate center of ossification of the anconeal process (SCOAP). Examples of these dogs include the Bernese Mountain dog, Rottweiler, Mastiff, St. Bernard and Newfoundland. (Janutta and Distl, 2008: Frazho*et al.,* 2010,) while those that have a separate center of ossification include the German Shepherd dogs, Greyhound, Pit Bull mix, Doberman Pinscher, Golden Retriever, and Labrador Retriever mix (Frazho*et al.,* 2010). In German shepherd and Greyhound dogs, fusion of a SCOAP to the anconeal process was reported to occur at 20 weeks of age while in the Bernese mountain dog and English mastiffs, SCOAP fusion was documented to be between 16 and 23 weeks of age (Janutta and Distl, 2008).

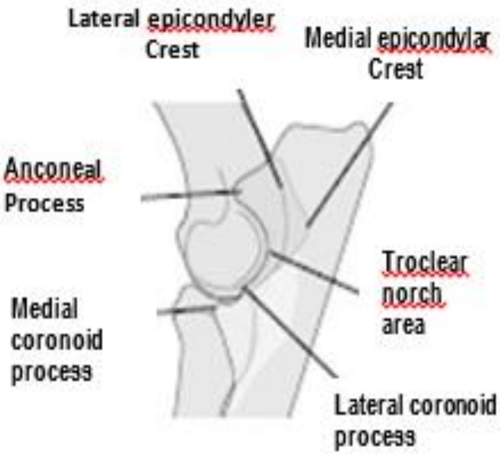
| Radiograph Representation | Diagrammatic Representation |
|---|---|
|  |  |

**PLATE 2.5:** Mediolateral view of proximal radius and ulna of a 5 year old Doberman (Barr, and Kirberger, 1983).

## 2.12 OSSIFICATION CENTERS

Ossification is the calcification of tissues to bonelike mass involving the normal process by which bones are formed (Summerlee, 2002). Two different processes are involved which include the primary and secondary ossification centers; both processes replace previous supportive tissue. Formation of secondary ossification centers begins at each end of a long bone with infiltration by blood vessels. The development of cartilage enables them to get larger (Dirsko and Pfeil, 2009). The shaft of a long bone is a compact bone with bone marrow inside it while the ends are made of spongy bone covered by a thin shell of compact bone. The process of ossification is developed in two different ways which are: the endochondral and intramembranous. The endochondral ossification is characterized by the formation of bone from growth cartilage, while the intramembranous ossification is characterized by the formation of bone from a mesenchymal structure, as occurs with the flat bones of the skull (Ferrara *et al.,* 2003). Primary and secondary ossification centers appear at different times in long bones of the body and develop from endochondral ossification. All the long bones follow this pattern of development except the flat bones which develops by intramembraneous ossification (Ballabriga,2000). Jikken *et al.* (1980) reported that development of secondary ossification center (SOC) consisted of three biological stages which were: the first stage which was considered to account for the appearance of the secondary ossification centers and their accelerated development at early period, while the second stage corresponded to the subsequent gradual development. The third stage represented a period when the ossification centers reached a complete or almost complete union to metaphysis.

Complete ossification of the coronoid process can be seen radiographically at the age of 20 to 22 weeks (Olsson, 1983). In some breeds of dogs, a separate center of ossification may be present in the anconeal process, which unites with the olecranon between the ages of 4 to 6 months. The head of the radius ossifies at 3 to 5 weeks of age. The olecranon ossifies at 7 to 9 weeks (Morgan and MacMillan, 2000). The proximal radio-ulnar joint allows the rotation of the forearm (Hermanson and Evans, 1993).

## 2.13 DESCRIPTION OF GROWTH PLATE

Growth plates can also be called physes, epiphyseal plate, metaphyseal growth plate, epiphyseal cartilage and epiphyseal disk. They are endochondral ossification areas of long bones. These plates ossify after the postnatal growth process (Aslanbey, 2002). Specialized tissues extending longitudinally between the epiphysis and metaphysis of immature long bones, working in harmony with each other and providing longitudinal bone growth are called growth plates (Brighton, 1978). It is seen as a narrow radiotranslucent line extending completely across the bone (Douglas *et al.,* 1987).

The growth plate closure (cessation of the longitudinal bone growth) occurred relatively early in the life of rabbits, cats and dogs while the closure of growth plates occurred later in the life of non- human and human primates (Rauch, 2005).

The age of closure of the growth plate has been reported to vary according to animal's breed and species as well as the physiological conditions, disease, cross-breeding, nutritional conditions, and the management system which is complex and vary among bones as studied by Kilborn *et al.*(2002). At puberty, the bone growth stop and the growth plate closes while the appositional bone growthcontinues which leads to changes in the shape of the bone (Rauch, 2005). Human and non-human primates, cows and sheep are considered adults at the age when the growth plate closure occurs (Johnson, 1994) while the findings of Hillyer (1994) and Feldman and Nelson (1996) indicated that rabbits, dogs and cats are described as very young adults at the time of growth plate closure.

When the growth plate is open, threaded pins, screws, tension band wires, or plates that span the epiphyseal plate should not be used (Lau *et al.,* 2015). If they must be used, they should be removed immediately following union in order to avoid closure of the epiphyseal plate and deformity (Denny and Gibbs 1980).

### 2.13.1 Development of growth plates

Endochondral ossification is responsible for the early bone development from cartilage in-utero and the longitudinal growth of long bones from the growth plate. The plate's chondrocytes undergo constant division by mitosis. These daughter cells pile facing the epiphysis while the older cells are pushed towards the diaphysis (Mackie *et al.,*2008). As the older chondrocytes deteriorate, osteoblasts ossify the remains to form new bone. In adult animals, an increase in the level of estrogen, in both females and males, leads to increased cell death of chondrocytes in the growth plate (*Zhong et al.,*2011). Reduction of chondrocytes due to cell death leads to less ossification and growth slows down and later stops when the entire cartilage has become substituted by bone, leaving only a thin epiphyseal scar which later disappears as the animal grows older. Once the adult stage is reached, the only way to manipulate height is modifying bone length via distraction osteogenesis (Dirsko and Pfeil, 2009).

Failures of chondrogenesis leading to cessation of growth is commonly as a result of trauma to the plate, occasioned by a fracture or crush injury, or of the interruption of the vascular supply to the germinal cells (Stefan *et al.,* 2011). Diseases such as, rickets, osteomyelitis and endocrine disorders make the plate more vulnerable to injury and predispose the bone to epiphyseal separation (Aytekin, 1993). Early ossification of metaphyseal cartilage induces the arrest of longitudinal bone growth. After the closure, only the transverse bone growth occurs (Yilmaz, 1999).

The sequence of growth plate closure has been reported to be constant among domestic dog breeds (Salmeri *et al.,* 1991) but whether the absolute timing of closure of the growth plates is different among domestic dog breeds has been a matter of debate. Sumner-Smith (1970) found that there are no differences among Poodle, Greyhound, and German shepherd, whereas (Salmeri *et al.,* 1991) reported differences of the timing of growth plate closure among breeds. Some degree of variation has been reported among specimens of the same breed (Kealy *et al.*, 2011) even among litter mates in the red fox (Harris, 1978) the wolf (Van Ballenberghe and Mech, 1975), and the domestic dog (Mackie *et al.*,2008). Age at growth plate closure is further expected to depend on nutritional conditions (Cupps, 1991) and age at time of castration of puppies (Salmeri *et al.*, 1991). The influence of sex on growth plate closure was reported to be not significant (Salmeri *et al.*, 1991), whereas only one study argued that there is some variation due to sex (Smith and Allcock, 1960).

Among breeds of domestic dogs, females of small breeds are supposed to attain sexual maturity earlier than females of larger breeds (Cupps, 1991), although this correlation has also been debated (Johnson, 1994).The age at sexual maturity may also be affected by line and breed dependent genetic factors, cross-breeding, diet, and housing conditions (Cupps, 1991: England, 2013).

## 2.14 CLINICAL SIGNIFICANCE OF GROWTH PLATES

Defects in the development and continued division of growth plates can lead to growth disorders. The most common defect is achondroplasia, where there is a defect in cartilage formation. Achondroplasia is the most common cause of dwarfism. Another is the Salter–Harris fractures, which are fractures involving growth plates and hence tend to interfere with growth and height of long bones (Ramadan and Vaughan, 1979). Most premature closure of the distal ulnar growth plate usually occurs in injured dogs with an average age of less than four months (Skaggs *et al.*, 1973).

Injury of the growth plate is a specific problem in traumatology and can cause limb deformity and length discrepancy as a result of growth arrest. It is actually a specific problem in the field of pediatric traumatology. It can lead to different degrees of limb deformity and length discrepancy as a result of growth arrest. Treatment of growth plate injuries is challenging. The most serious complication is the growth disturbance with length deficiency and axis deviation. Iatrogenic injuries of the growth plate following surgical treatment should absolutely be avoided. However, surgical manipulation of the growth plate is sometimes necessary for the fixation of certain growth plate fractures (Stefan, *et al.*, 2011).

### 2.14.1 Growth plate closure time and contribution to the overall growth

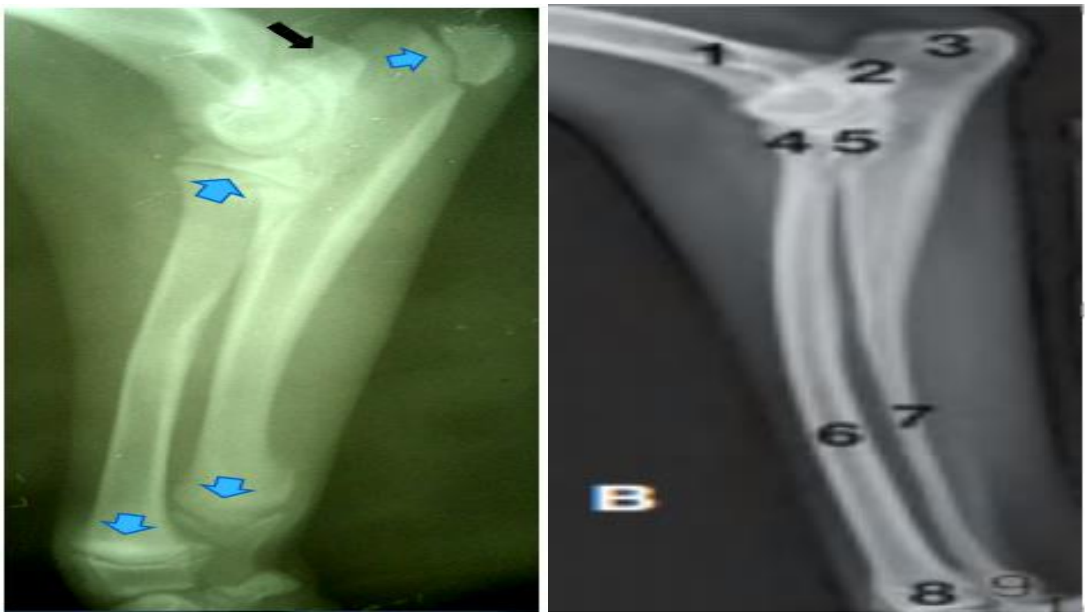
In dogs, major growth occurs between the ages of 3 and 6 months (Carrig, 1983). Most dogs achieve 90% of their adult size by the end of 9 months (Newton, 1974). Most growth plates close between 4 and 12 months of age, depending on the anatomic site and breed of dog. However, the growth plates of some giant-breed dogs may not close until 15- 18 months of age. The time frame of growth plate closure in the front limbs of the average dog has been documented to be 5 - 8 months for proximal ulna, 6 - 11 months for distal ulna, 5 to 9 months for proximal radius and 6 to 11 months for distal radius (Summerlee, 2002). However, it was documented that epiphyseal closure occurs earlier in smaller animals (Hare, 1961).

Studies have evaluated the amount that each epiphyseal plate contributes to total growth of canine long bones to be 40% for proximal radius and 60 % to the distal radius, 0-15% by the proximal ulna and 80-100 % for distal ulna (Shapiro, 1987: Mackie *et al.,*2008).

## 2.15 RADIUS AND ULNA BONES IN A MATURE DOG

The proximal radius is formed by an oval and concave radial head, which articulates with the humeral capitellum. The metaphyseal area tapers slightly to become the flattened radial diaphysis (Lau *et al.,* 2015). The diaphysis is uniformly shaped, flattened cranial-caudally, and curves slightly as it moves from a lateral position at the elbow to a medial position at the carpus. Distally the metaphysis enlarges and enters the distal epiphysis which has a concave articular surface that sits upon the radial carpal bone. A medial pointed prominence, the styloid process, serves as proximal attachment of the medial collateral ligament (Dirsko and Pfeil, 2009).

The proximal ulna is formed by a large bony process (the olecranon), which serves as the insertion of the triceps muscles. The articular surface, termed the trochlear notch, articulates with the humeral trochlea of the medial condyle. The proximal trochlear notch is formed by the anconeal process, while the distal trochlear notch ends in the coronoid process (Dirsko and Pfeil, 2009). The ulna tapers below the articular surface and curves cranially and the diaphysis continues to taper along its length, which begins medially at the elbow and ends laterally at the carpus (Fox *et al.*, 1983). The distal process, the styloid process, is the proximal attachment of the lateral collateral ligament of the carpus. The medullary canal of the radius is usually uniform in size and much wider medial-laterally than cranial caudally. The ulnar medullary cavity is wide proximally and tapers along its entire length. In some small dogs it may be very small or nonexistent (Dirsko and Pfeil, 2009).



Young dog (14 weeks) Matured dog (2.5 years)

**Plate 2.6:** Radiographic images of the right radius and ulna bones of a young and matured dog.

Dog positioned for a Medio lateral radiograph of the radius, ulna and elbow joint in young and adult dogs. (B) Radiograph of an antebrachium with no abnormalities Key: 1 = distal diaphysis of the humerus; 2 = anconealsprocess of the ulna superimposed over the medial epicondyle of the humerus; 3 = tuber olecranon; 4 = radial head and proximal epiphysis; 5 = medial coronoid process of the ulna superimposed over the radial head; 6 = proximal diaphysis of the radius; 7= proximal diaphysis of the ulna; 8 = distal radial epiphysis; 9 = styloid process of the distal ulna (Lau *et al.*, 2015).

## 2.16 NORMAL VASCULARISATION OF BONE

A sufficient blood supply is essential for a bone to carry out its normal physiological function. This blood supply is derived from three basic sources; the afferent vascular system, the intermediate vascular system of compact bone and the efferent vascular system (Miller, 1964). The afferent system carries arterial blood and consists of the principal nutrient artery, the metaphyseal arteries and the periosteal arterioles at muscle attachments. The periosteal arterioles are minor components of the afferent system and supply the outer layer of the cortex in the vicinity of firm fascial or muscle attachments (Hermanson and Evans, 1993). The vessels in compact bones are intermediate between the afferent and efferent systems and function as the vascular lattice where critical exchange between the blood and surrounding living tissue occurs. This system consists of the critical canals of Havers and Volkmann and the minute canaliculi which convey nutrients to the osteocytes (Brinker *et al.*,1997). Venous drainage (the efferent system) of cortical bone takes place at the periosteal surface. Blood flow through the cortex is essentially centrifugal from medulla to the periosteum.

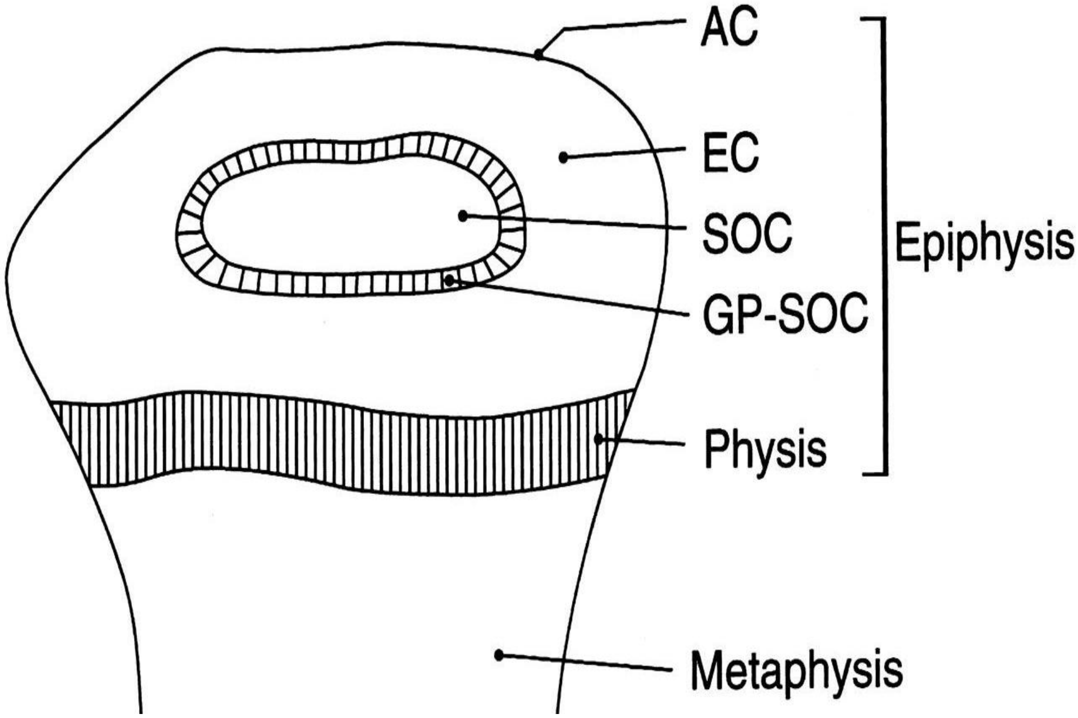


**Plate 2.7** The components of the developing end of a long bone. The epiphysis is composed of the articular cartilage (AC), the epiphyseal cartilage (EC), and the physis (growth plate). The secondary ossification center (SOC) formed by the endochondral mechanism within the epiphyseal cartilage. It is completely surrounded in the earlier phases of development by another growth plate, the growth plate of the secondary ossification center (GP-SOC), which is responsible for the circumferential growth of the secondary center.

(Rivas *et al.*,2002)

# CHAPTER THREE

## 3.1 MATERIALS AND METHODS

### 3.1.1 STUDY AREA

The study was carried out in Sokoto Metropolis. Sokoto state is geographically located in the north western part of Nigeria between longitude 11°30° to 13°50° east and latitude 4° to 6°40° north. The state shares borders with Niger Republic to the North, Kebbi State to the South, and Zamfara State to the East. It covers a total land area of about 32,000 square kilometers with an estimated human population of 3,696,999 million (National Population Commission, NPC, 2006).

## 3.2 EXPERIMENTAL PREPARATION

A total of sixteen (8 males, 8 females) apparently healthy Nigerian local puppies, weighing 0.3kg (week 1) to 14kg (week 48), obtained from three different liters were used for the present study. They were physically examined to ascertain their health status; each puppy was dewormed and vaccinated against rabies, leptospirosis, parvovirus enteritis, hepatitis and canine distemper and parainfluenza virus. The dogs were maintained on daily ration composing of jollof rice, gari with soup and beans. Clean drinking water was provided *ad libitum*.

## 3.3 ANIMAL PREPARATION FOR EXPOSURE

At week 1, animals were placed on the right lateral recumbency on the x-ray table for radiography without sedation and the weight of each dog was taken before each radiograph.From 4 weeks to 48 weeks, Xylazine hydrochloride (0.2mg/kg) (VMD Arendonk, Belgium®) and Atropine (0.04mg/kg) (8-Methyl-8-azabicyclo 3.2.1, Atropen®) combination were administered intramuscularly to achieve sedation (Fossum, 2002).

**Plate 3.1:** Weighing of 4 weeks old experimental puppy before exposure.

**Plate 3.2:** Weighingof 36 weeks old experimental dogs before exposure.

## 3.4 METHODOLOGY

Radiographic exposures were scheduled at weekly interval till 9 weeks and thereafter at every four weeks interval till 48 weeks (Myo*et al.,* 2016).Puppies were radiographed using Power mobile x-ray machine (LX-8 MOB) installed at Veterinary radiology unit of Usmanu Danfodiyo University Sokoto.

## 3.5 EXPERIMENTAL PROCEDURE

### 3.5.1 Setting the x-ray machine for exposure

Each cassette (Dr Goos Suprema® Germany, size 35x43cm and 24x30cm) was loaded with an X-ray film (AGFA DT2B India and FUJI Japan Tokyo, size 35x43cm and 24x30cm) in the dark room and brought into the x-ray room for radiography where they were placed on the x-ray table. Accurate settings ofbetween 50-60 kV, 8-12mASwere used for this study (Plate 3.3) (Singh and Singh, 2001). These settings were influenced by the thickness of the limbs of each animal and the concentration of the chemicals at the time of processing the films. At week 1 to week 9, a setting of 50kV and 8mAS was used and maintained because of the small density of the limbs which increased as the animal grew older.

**Plate 3.3:**The x-ray room and machine setting of the exposure factors

### 3.5.2 Positioning for exposure

Medio-lateral radiographs of the right fore limb was used throughout the research. The right limb was placed on the loaded cassette containing the x-ray film (Plate 3.4) on the x-ray table and the light beam was collimated with the primary beam focused on the diaphysis of the radius and ulna bone. The left limb was retracted caudally and tied to the table while the head and neck were slightly extended out of the primary beam (Plate 3.4) (Douglas *et al.,* 1987). All radiographs were taken at a film-focal distance of 100 cm (Armbrust, 2009).

**Plate 3.4:** Positioning and collimation of light during radiography

## 3.6 PROCESSING OF THE EXPOSED RADIOGRAPH

After taking the radiograph, the cassette was takeninto the dark room for identification and processing. The identification was done with the help of a pencil to temporarily identify the animal at one angle of the film after which it was fixed onto an adequate size hanger before it was manually processed as described by Singh and Singh, (2001).After the drying of the film, each film was permanently marked with a sellotape at one angle of the film.

## 3.7 EVALUATION OF RADIOGRAPHIC STRUCTURES

The structures evaluated were: olecranon, anconeal process, proximal and distal epiphyses and growth plates of the radius and ulna bone, the medial coronoid process that is superimposed on the radial head, diameter of radius and ulna bone. The X-ray films were viewed using an X-ray film illuminator (Techmel and Techmel Texas U.S.A) and the radiographic images were captured using a digital Sony camera (DSC-W710) and transferred to a computer system (Myo *et al.,* 2016).

Monitoring the appearance and disappearance of the growth plate of radius and ulna bones was achieved with the aid of an illuminator (Techmel and Techmel Texas U.S.A), electronic digital caliper (Raider®) RDDC 706 model to the nearest 0.01mm modifications and ruler in cm.

Radiographic parameters observed from the x-ray films included:

I. The time of appearance and development of secondary ossification centers of radius and ulna bones.

II. Presence or absence of a separate center of ossification for the medial and lateral coronoid processes and the anconeal process.
III. The time of appearance and closure of the proximal and distal growth plates of radius and ulna.
IV. The thickness of the growth plate at the proximal and distal radius and ulna bones as the animal matures.
V. The growth in length and diameter of the radius and ulna bones as the animal matures.

The thickness of the growth plate was measured using an electronic digital caliper (Raider®) (Plate 3.5) in mm until the growth plate closed and the bone became a single bone. The time of fusion was noted and recorded, whilethe length of radius and ulna was measured using a transparent ruler (Plate 3.6) in cm for 48 weeks.

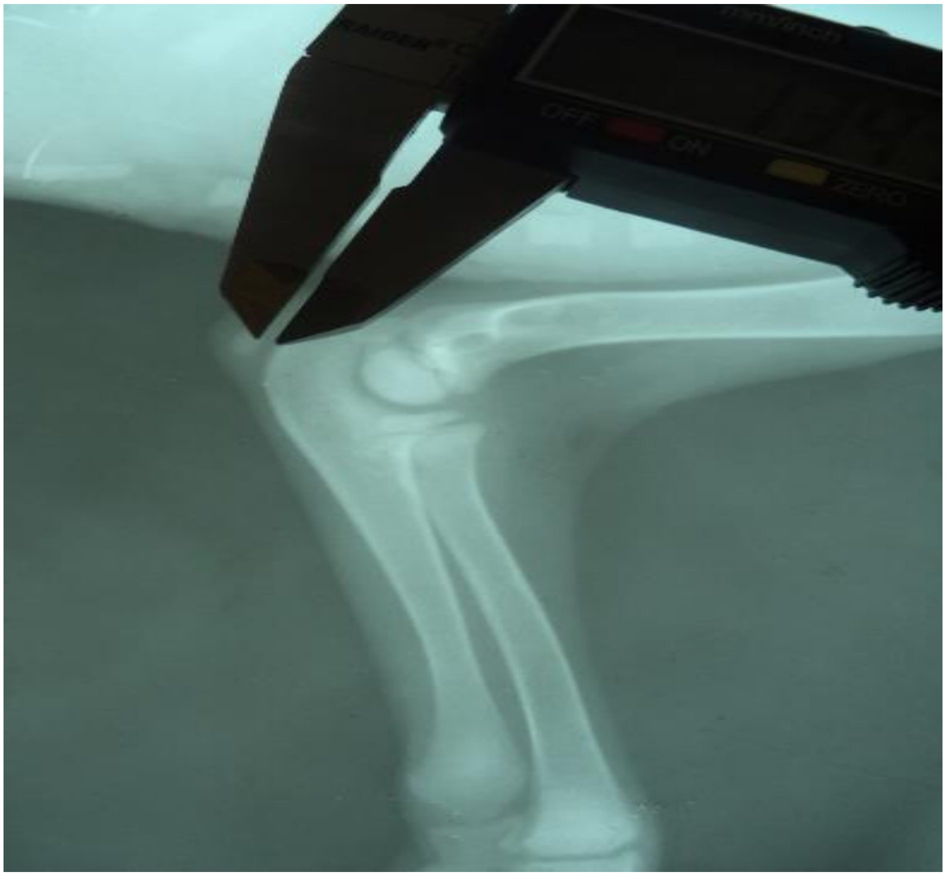


**Plate 3.5:** Measurement of the thickness of growth plate in mm using electronic digital caliper (Raider®).

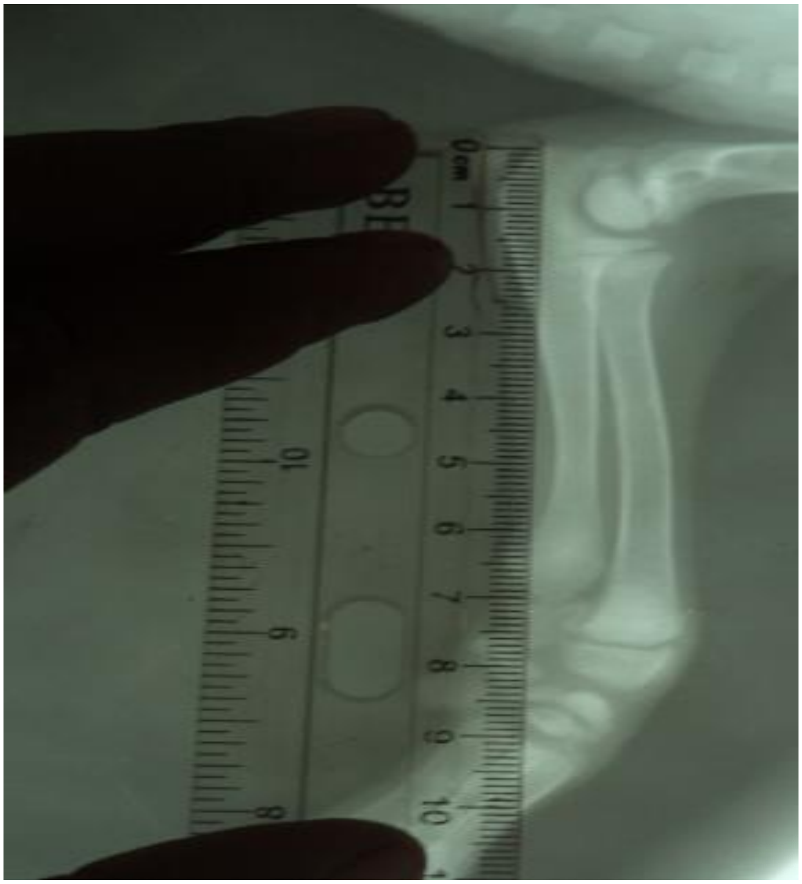


**Plate 3.6:** Measurement of the length of the bones in cm using ruler.

## 3.8 DIAMETER OF RADIUS AND ULNA BONE

The diameter of radius and ulna bones were determined by placing a vernier caliper on the radiograph which was placed on the illuminator. For the radial bone, the proximal measurement was obtained by placing the vernier caliper at the proximal metaphysis, below the point at which the medial coronoid process touched the radius. The middle diameter was obtained by measuring at the point between the proximal and distal metaphysis. The distal diameter of the radius was obtained by measuring at the point just proximal to the distal metaphysis which was measured using Vernier caliper.

For the ulna, the proximal diameter was obtained by measuring at the point just distal to the coronoid process. The middle diameter was obtained by measuring at the point between the proximal and distal metaphyses while the distal diameter of the ulna was obtained by measuring at the point proximal to the distal metaphysis. All the measurement of the proximal radius (PR), middle radius (MR) and distal radius (DR), as well as the proximal ulna (PU), middle ulna (MU) and distal ulna (DU) of radius and ulna were added and divided by 3 to get the radial (RD) and ulnar diameter (UD) (Myo *et al.*, 2016).

**RD** = PR+MR+DR /3, **UD** =PU+MU+DU/3

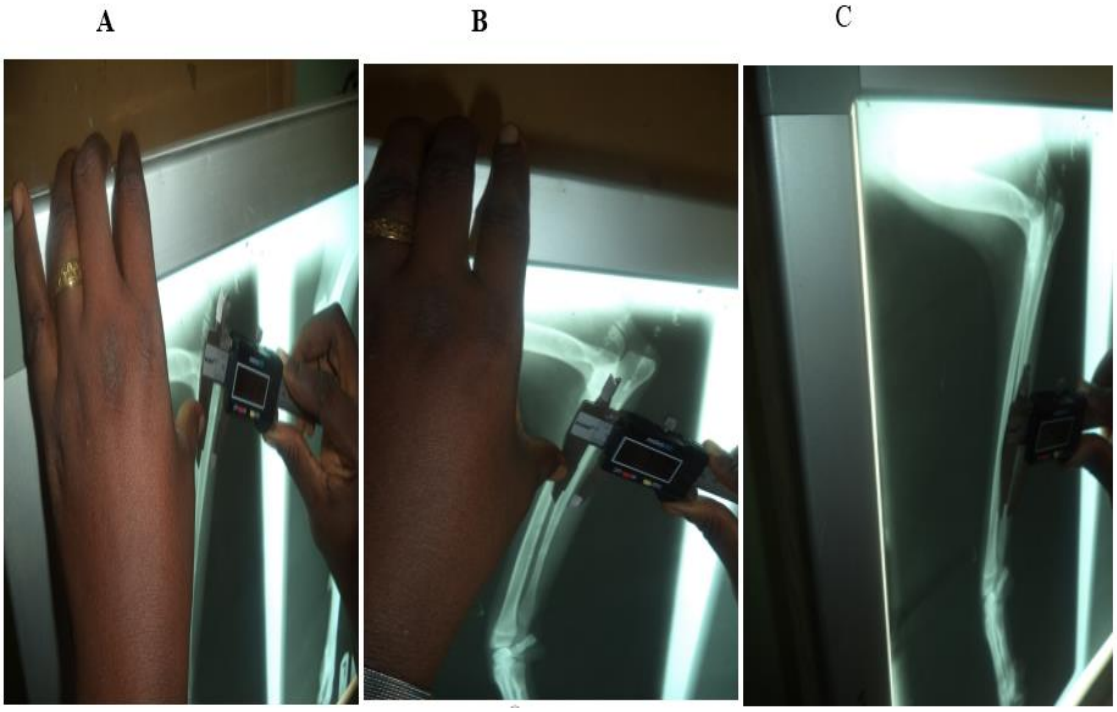


**Plate 3.7:**Measurement of the diameter of radius and ulna bone by measuring the proximal (A), middle (B) and distal segment of the ulna bone (C).

## 3.9 DATA ANALYSIS

Data obtained from this study were presented in pictures and tables formatfor descriptive analysis.

Independent sample *t*-test was used to compare the male and the female growth length and diameter at 4 weeks interval, using Invivo Stat Statistical software version 3.0.0.0, 2008-2015.

Variables were compared for statistical significance at a probability of 5% ($P \leq 0.05$) with a confidence interval of 95%.

# CHAPTER FOUR

## 4.0 RESULTS

## 4.1 RADIOGRAPHIC FINDINGS:

### 4.1.1 Radiographic appearance of secondary ossification center (SOC) of radius and ulna bones

In this study, at week 1, medial and lateral coronoid processes of the ulna bone were already present in all the dogs (Plate 4.1). At week 2, the SOC of the distal epiphysis of the radius appeared as a small ovoid structure which was present in 12 dogs (6 males and 6 female) (Plate4.2) and absent in 4 dogs which eventually appeared at 3 weeks in all the dogs (Plate 4.3). At weeks 5, the SOC of the proximal epiphysis of the radius appeared in all the dogs (Plate4.5). At week 6, the SOC of the distal epiphysis of the ulna appeared in all the dogs (Plate 4.6). At weeks 8, the SOC of the proximal ulna epiphysis (olecranon) appeared (Plate4.7), (Table 4.1).

**Plate 4.1:** Medial and lateral coronoid processes formation in the ulna bone of the dogs at week 1 (arrow).

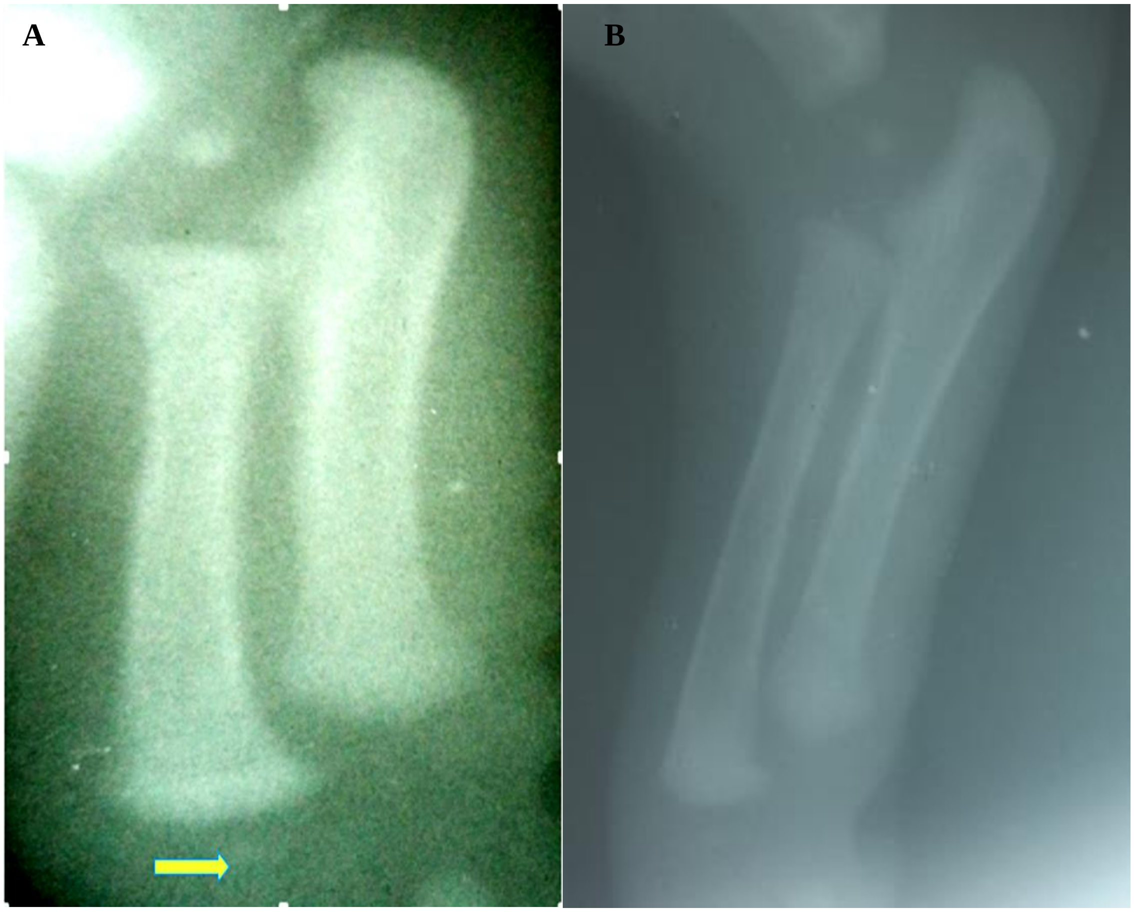


**Plate 4.2:** Formation of the secondary ossification centers of the distal radial epiphysis in 12 dogs (A) and absent in 4 (B) of the dogs at week 2 (arrow).

**Plate 4.3:** Further appearance and development of the secondary ossification center of the distal radius in all the dogs at week 3 (arrows).

**Plate4.4:** Further development of the coronoid process and distal radius of the dogs at week 4.

**Plate 4.5:** Appearance of secondary ossification center of the proximal radius of the dogs at week 5 (arrow).

**Plate 4.6:** Appearance of secondary ossification center of the distal ulna of the dogs at week 6 (arrow).

**Plate 4.7**: Appearance of the secondary ossification center of the proximal ulna of the dogs at week 8 (arrow).

**Table 4.1:** Radiographic appearance of the Secondary Ossification Centers of radius and ulna bones.

| **Bone** | **Ossification center** | **Age of appearance (weeks)** |
|---|---|---|
| Ulna | • Medial coronoid process<br>• Lateral coronoid process<br>• Distal epiphysis<br>• Olecranon<br>• Anconeal process | 1<br>1<br>6<br>8<br>9 |
| Radius | • Distal epiphysis<br>• Proximal epiphysis | 2<br>5 |

### 4.1.2 Presence or absence of a separate center of ossification for the medial and lateral coronoid processes and the anconeal process.

In the present study at week 1, the medial and lateral coronoid processes were already present as a direct extension from the ulna bone while the anconeal process was completely absent from week 1 to 9 but present at week 12 as a direct extension of the proximal ulna diaphysis from the base to the tip of the bone (Plate 4.8).

### 4.1.3 The developmental stages of the secondary ossification centersSOCs

From this study, the development of SOCs followed the pattern of the 3 stages of development which included the first, second and third stage.

The first stage of development of SOCs was 2-3 weeks for distal radial epiphysis (Plate4.2), 5 weeks for proximal radial epiphysis (Plate4.5), 6 weeks for distal ulnar epiphysis (Plate4.6) and 8 weeks for the proximal ulnar epiphysis (Plate4.7).

The second stage of development of SOCs occurred at 2-12 weeks for distal radial epiphysis, 5-12 weeks for proximal radial epiphysis, 6-12 weeks for distal ulnar epiphysis and 8-12 weeks for proximalulnar epiphysis.

The third stage of development was 24 weeksfor proximal ulnar epiphysis (Plate 4.9), 28 weeks for proximal radial epiphysis (Plate4.10), 32 weeks for distal ulnar epiphysis(Plate 4.11) and 36-40weeks for distal radial epiphysis (Plate 4.12 and 4.13).

**Plate 4.8:** Appearance of a fused anconeal process from the proximal ulna and at this age, all the SOCs reached their proper anatomical shape in all the dogs at week 12 (arrows).

## 4.2THE TIME OF APPEARANCE AND CLOSURE OF THE PROXIMAL AND DISTAL GROWTH PLATES OF RADIUS AND ULNA

In the present study, the radiolucent space located between the epiphysis and the metaphysis is the growth plate which was observed after the appearance of secondary ossification centers of each bone. The first growth plate to appear was the distal radius which appeared at 2 weeks (Plate4.2), followed by the proximal radius which appeared at 5 weeks (Plate4.5) then the distal ulna that appeared at 6 weeks (Plate4.6) and finally the proximal ulna that appeared at 8 weeks (Plate4.7).

Early signs of closure of the proximal growth plate of the ulna bone in this study, was radiographically noticed at 20 weeks in 13 dogs (6 males and 7 females) (Table 4.11) although the complete closure of the radiolucent space of the growth plate area of the proximal ulna was observed at 24 weeks (Plate 4.9), then in the proximal radius at 28 weeks (Plate 4.10), followed by the distal ulna at 32 weeks (Plate4.11) and finally the distal radius at 36 weeks (Plate 4.12) (Table 4.2). The time of appearance and closure of the growth plates of the radius and ulna was notdifferent between the male and female dogs.

**Plate 4.9:** Fusion of the proximal ulna growth plate (olecranon) of the dogs at week 24 (arrow).

**Plate 4.10:** Fusion of the proximal radial growth plate of the dogs at week 28 (arrow).

**Plate 4.11:** Fusion of the distal ulna growth plate of the dogs at week 32 (arrow).

**Plate4.12:** Fusion of the distal radial growth plate of the dogs at week 36 (arrow).

**Plate 4.13:** Matured radius and ulna bone with a fused growth plate of the dogs at week 40, only changes in diameter that occur at this point.

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**Table 4.2:**Age of appearance and closure times of the Growth Plates of the radius and ulna bones.

| **Growth plate** | **Age at appearance (Weeks)** | **Age at closure (Weeks)** |
|---|---|---|
| Distal radius | 2 -3 | 32-40 |
| Proximal radius | 4-5 | 24-28 |
| Distal ulna | 5-6 | 28-32 |
| Proximal ulna | 7-8 | 20-24 |

## 4.3 LENGTH OF RADIUS AND ULNA BONES IN MALE AND FEMALE DOGS

The length of radius and ulna bones in male and female is presented on table 4.3. There was no statistical significant difference ($P>0.5$)between the male and female dogs.

Our findings showed that the radius and ulna bones grew concurrently, but the ulna bone was always longer at all times compared to the radial bone to maintain the radio-ulna ratio. Their major growth occurred between 4thto 16thweeks for both radius and ulna bones of males and females and attained maximum height at 32nd weekwith a length of 19.10cm for ulna bone of females and 19.11cm for the maximum ulna height of males at the 40th week. The radial bone of females attained their maximum height (15.75cm) at the 36th week while the males attained maximum height (15.76cm) at the 40th week. Making the females approach their final height earlier than the males. However there was no statistical significant difference ($P>0.05$) observed between the length of radius and ulna bone in the male and female dogs used in the present study.

**Table 4.3:** Mean ± SD of length of radius in male and female dogs observed at 4 weekly intervals.

| Age (weeks) | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males /cm | 4.55 ± 0.05 | 6.86 ± 0.12 | 10.33 ± 0.69 | 13.51 ± 0.31 | 14.84 ± 0.51 | 15.33 ± 0.13 | 15.39 ± 0.08 | 15.66 ± 0.21 | 15.75 ± 0.21 | 15.76 ± 0.20 | 15.76 ± 0.25 | 15.76 ± 0.27 |
| Females/ cm | 4.56 ± 0.13 | 6.88 ± 0.27 | 10.33 ± 0.85 | 13.52 ± 0.13 | 14.71 ±0.44 | 15.37 ± 0.12 | 15.41 ± 0.13 | 15.65 ± 0.21 | 15.75 ± 0.18 | 15.75 ± 0.18 | 15.75 ± 0.13 | 15.75 ± 0.13 |

**P>0.05**

**Table 4.4:** Mean ± SD of length of ulna in males and females dogs observed at 4 weekly intervals.

| Age /weeks | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males/cm | 5.18 ± 0.07 | 8.18 ± 0.26 | 13.31 ± 0.44 | 16.15 ± 0.47 | 17.80 ± 0.69 | 18.43± 0.39 | 18.9 ± 0.93 | 19.09 ± 0.53 | 19.10 ± 0.08 | 19.11 ± 0.05 | 19.11 ± 0.25 | 19.11 ±0.06 |
| Females/cm | 5.16± 0.09 | 8.18 ± 0.37 | 13.23 ± 0.93 | 16.16 ± 0.21 | 17.82 ± 0.64 | 18.44± 0.56 | 18.93 ± 0.09 | 19.10 ± 0.64 | 19.10± 0.05 | 19.10 ± 0.05 | 19.10 ± 0.05 | 19.10±0.05 |

**P>0.05**

## 4.4: THE THICKNESS OF THE GROWTH PLATE OF PROXIMAL AND DISTAL RADIUS AND ULNA BONES IN MALE DOGS.

The mean thickness observed in the present study of the growth plate of proximal and distal radius and ulna bone in male dogs is as presented in the table 4.5.

The measurement of the thickness of the growth plate was documented at 4 weeks interval. In the male dog, at week 1, no growth plate was found. At week 4, the first thickness was measured in the distal radius as 0.91mm. At week 8, all the growth plate of proximal and distal radius and ulna bones were present with the distal ulna having the largest thickness of 1.40mm and proximal radius had the least thickness of 0.56mm. Partial union was observed before the final fusion of each of the growth plate which showed signs of fusion from one aspect of the growth plate like in the proximal ulna at week 20 which finally fused at week 24 (table 4.4), then the proximal radius at week 24 and finally fused at week 28 (table 4.4), then the distal ulna at week 28 and finally fused at week 32 (table 4.4) and finally the distal radius at week 32 and finally fused at 36 weeks for 6 dogs and 40 weeks for 2 male dogs (table 4.4).

**Table 4.5:** Mean thickness of growth plate of radius and ulna bones in mm and time of closure in males.

| **Age/weeks** | **proximal radius/ mm** | **Distal Radius/ mm** | **Proximal Ulna / mm** | **Distal Ulna/ mm** |
|---|---|---|---|---|
| 1 | Not developed | Not developed | Not developed | Not developed |
| 4 | Not developed | 0.91 | Not developed | Not developed |
| 8 | 0.56 | 0.68 | 0.96 | 1.40 |
| 12 | 0.77 | 0.76 | 0.72 | 1.38 |
| 16 | 0.49 | 0.47 | 0.34 | 0.29 |
| 20 | 0.35 | 0.46 | partial union | 0.21 |
| 24 | 0.32 | 0.46 | Fused | partial union |
| 28 | partial union | 0.32 | Fused | Fused |
| 32 | Fused | partial union | Fused | Fused |
| 36 | Fused | (6 fused 2 not) | Fused | Fused |
| 40 | Fused | Fused | Fused | Fused |
| 44 | Fused | Fused | Fused | Fused |
| 48 | Fused | Fused | Fused | Fused |

## 4.5 THE THICKNESS OF THE GROWTH PLATE OF PROXIMAL AND DISTAL RADIUS AND ULNA BONES IN FEMALE DOGS.

In the female dogs, the mean thickness of the growth plate of proximal and distal radius and ulna bone observed in the present study is as shown in table 4.5.

At week 1 the proximal radius and ulna bones were not developed as a result no growth plate was found as well as the distal radius and ulna. At week 4, the first thickness was measured in the distal radius as 0.94mm after the development of its growth plate. At week 8, all the growth plate of proximal and distal radius and ulna bones were present with the distal ulna having the largest width of 1.38mm and proximal radius having the least width of 5.4mm.Partial union was observed before the final fusion of each of the growth plates which showed early signs of fusion from the middle aspect of the growth plate of the proximal ulna at week 20, and finally fused at week 24 (Table 4.4) then the proximal radius at week 24 and finally fused at week 28 (Table 4.4), then the distal ulna at week 28 and finally fused at week 32 (Table 4.4) and finally the distal radius at week 32 and finally fused at 36 weeks for 7 dogs and 40 weeks for 1 female dogs (table 4.4).

**Table 4.6:** Mean thickness of growth plate of radius and ulna bones and time of closure for females.

| Age/weeks | Proximal Radius/ mm | Distal Radius / mm | Proximal Ulna / mm | Distal ulna / mm |
|---|---|---|---|---|
| 1 | Not developed | Not developed | Not developed | Not developed |
| 4 | Not developed | 0.94 | Not developed | Not developed |
| 8 | 0.54 | 0.80 | 1.36 | 1.38 |
| 12 | 0.67 | 0.71 | 0.96 | 1.30 |
| 16 | 0.37 | 0.55 | 0.25 | 0.31 |
| 20 | 0.32 | 0.48 | partial union | 0.22 |
| 24 | 0.31 | 0.41 | Fused | partial union |
| 28 | 0.26 | 0.22 | Fused | Fused |
| 32 | partial union | 0.22 | Fused | Fused |
| 36 | Fused | partial union (7 fused 1 not) | Fused | Fused |
| 40 | Fused | Fused | Fused | Fused |
| 44 | Fused | Fused | Fused | Fused |
| 48 | Fused | Fused | Fused | Fused |

## 4.6 DIAMETER (MM) OF RADIUS AND ULNA BONE IN MALE AND FEMALE DOGS (MEAN ±SD)

The diameter of ulna in male and female dogs is presented in Table 4.7. The diameter was measured at every 4 weeks interval which showed some variations in week 12, 20, 24, 26 and week 30 although no statistical significant difference ($p>0.05$) was observed between the ulna of male and female dogs.

The diameter of the radius in male and female dogs is presented in Table 4.8. The diameter was measured at every 4 weeks interval and showed some variations in week 8, 16, 28and 32 although no statistical significant difference ($p>0.05$) was observed between the radius of male and female dogs.

**Table 4.7:**Mean ± SDof diameter (mm) of ulna bone in male and female dogs.

| Age (weeks) | 1 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | 3.24 ± 0.29 | 4.87 ± 0.20 | 5.10 ± 0.24 | 8.10 ± 0.19 | 8.49 ± 0.18 | 7.79 ± 0.17 | 7.71 ± 0.08 | 7.68 ± 0.10 | 7.62 ± 0.05 | 7.69 ± 0.04 | 8.04 ± 0.08 | 8.61 ± 0.19 | 8.62 ± 0.18 |
| Females | 3.24 ± 0.30 | 4.87 ± 0.15 | 5.10 ± 0.24 | 8.11 ± 0.19 | 8.49 ±0.18 | 7.80 ± 0.16 | 7.72 ± 0.08 | 7.69 ± 0.76 | 7.63 ± 0.63 | 7.69 ±0.04 | 8.05 ± 0.06 | 8.63 ± 0.19 | 8.62 ± 0.19 |

$P>0.05$

**Table 4.8:**Mean ± SDof diameter (mm) of radial bone in male and female dogs.

| Age (weeks) | 1 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | 3.40 ± 0.07 | 5.53 ± 0.13 | 5.57 ± 0.10 | 9.75 ± 0.05 | 9.20 ± 0.24 | 8.19 ± 0.06 | 7.90 ± 0.01 | 7.90 ± 0.07 | 7.61 ± 0.03 | 6.66 ± 0.05 | 6.44 ± 0.09 | 6.34 ± 0.07 | 6.27 ± 0.22 |
| Females | 3.40 ± 0.06 | 5.53 ± 0.12 | 5.56 ± 0.96 | 9.75 ± 0.46 | 9.21 ±0.23 | 8.19 ± 0.64 | 7.90 ± 0.14 | 7.91 ± 0.07 | 7.62 ± 0.02 | 6.66 ± 0.04 | 6.44 ± 0.0.09 | 6.34 ± 0.06 | 6.27 ± 0.21 |

**P>0.05**

# CHAPTER FIVE

## 5.0 DISCUSSION, CONCLUSION, RECOMMENDATIONS AND LIMITATIONS OF THE STUDY

### 5.1 DISCUSSION

Radiographic monitoring revealed that the bones developed normally in all dogs. No orthopedic disorder was observed during the entire experimental period. The exposure in lateral position was better for the evaluation and measurement of radiographic findings. Measurements could not be performed with images taken in cranio-caudal positions, because the ossification centers and growth plates of the radius and ulna bones could not be effectively identified on the films due to the superposition of the ossification centers of the radius and ulna bones. For these reasons, mediolateral radiographs of the right fore limb was used throughout the research.

In our findings, the secondary ossification centers (SOCs)were completely absent at week 1 which is similar to the work reported by Riser and Shirer (1965) who reported that at birth, the SOC of the radius and ulna bones were composed of cartilage and were not visible radiographically but by second week of age, a mineralized ossification center appeared radiographically. It is also in line with the work conducted by Charjan *et al.* (2014) who reported that the distal radius appeared at17.5±2.50 days of age in Pomeranian dog,22.5±3.36 days of age in German shepherd dog and 25±3.17 days of age in non-descript dogs. However, this is contrary to the work reported by Dirsko and Pfeil (2009) who reported the proximal and distal SOCs of the radius to be prenatal while the proximal and distal ulna appear at 12-16 weeks in Terrier dogs. This difference might be due to breedgenetic variations or other factors such as nutrition and management system.

The early and close range of appearance of both the proximal and distal SOCs could be responsible for their accelerated growth.

The time of appearance of the growth plate was the same as the time of appearance of the SOCs. The SOCs appeared first as a radiopaque area proximal and distal to the metaphysis of these bones which increased as the animal grew older while the growth plate was the radiolucent space located between the epiphysis and the metaphysic. Our findings revealed that the appearance of the growth plate and SOC (first stage of development) was 2-3 weeks for the distal radius, 5 weeks for the proximal radius, 6 weeks for the distal ulna and 8 weeks for the proximal ulna,this is similar to the work of Morgan and MacMillan (2000) who reported that the head of the radius ossifies between 3 to 5 weeks of age and the olecranon ossifies between 7 to 9 weeks in Beagle dogs and Myo *et al.* (2016) who reported the distal radius, proximal radius, distal ulna and proximal ulna to be 2 weeks, 4 weeks, 6 weeks, 8 weeks, respectively in local dogs of Myanmar.Contrary to our findings is the work conducted by Dirsko and Pfeil, (2009) who observed the appearance of proximal and distal SOCs of the radius to be prenatal and the proximal and distal SOCs of the ulna to appear at 12-16weeks for Terrier dogs,this can probably be due to genetic variations among the breeds and might probably be the reason for their accelerated growth after their appearance.

The second stage of development in our study for the proximal radius was from 5-12 weeks, for distal radius was 2-12 weeks, for proximal ulna was 8- 12 weeks while distal ulna was 6-12 weeks, and this differs from the work conducted by Lau *et al*. (2015) who reported the time at which the secondary ossification centers reached their normal anatomical position to be 14 weeks for Labrador Retriever dogs.

The time of growth plate closure is the third stage in the development of secondary ossification centers and it is the time of complete fusion of the epiphysis to the metaphysis which was 24 weeks for proximal ulna, 28 weeks for proximal radius, 32 weeks for distal ulna and 36-40 weeks for distal radius. This differs from the work of Dirsko and Pfiel (2009) who reported an average closure time (third stage) of 46 weeks and 49 weeks for proximal ulna and proximal radius respectively and the distal growth plate of the ulna and radius to be 60 weeks in Terrier dogs. This variation in the closure time of the growth plates is probably due to breed variations leading to further growth of the Terrier dog due to their longer time of growth plate closure.

In the study, the ossification center of the anconeal process (AP) appeared as a direct extension of the proximal ulna from the base to the tip which is similar to the appearance reported by Frazho*et al.* (2010) for Bernese Mountain, Rottweiler, Mastiff, St. Bernard and Newfoundland breeds of dog but contrary to the appearance reported by Janutta and Distl, (2008) who reported that the AP developed from a separate ossification center for German Shepherd, Greyhound dogs and English mastiffs andthis could also be attributed to breed genetic variations. The time of ossification of AP in this research was 12 weeks which is similar to the time documented by Guthrie *et al.* (1992) and Turner *et al.* (1998) who reported the appearance of the AP to be 12 to 14 weeks while Van Sickle (1975) also documented a time of 12 ± 1 weeks and added that they might develop as a direct extension of the diaphysis of the ulna bone or as a separate center of ossificationGerman shepherd.

The three stages of development of the secondary ossification centers in this studyfollows the same pattern to the work conducted by Jikken *et al.* (1980) who reported that development of SOCs consisted of three biological stages which were: the first stage which accounted for the appearance of the secondary ossification centers, while the second stage corresponded to the subsequent development. The third stage represented a period where the ossification centers reached a complete union to the metaphysis.

The last growth plate to close in this study for radius and ulna bone was the distal ulna which closed at 40 weeks while the average closure time of the distal growth plate of the ulna and radius was reported to be the last which closed at 60 weeks in Terrier dogs (Dirsko and Pfeil 2009). This result is supported by the report of Van Ballenberghe and Mech (1975) and Cupps (1991) who reported that among domestic breeds of dogs, small breeds attain maturity earlier within seven months to one year while large and giant-breed dogs might not be fully grown until they are fifteen to eighteen months, this could probably be due to breed variations.

Our findings showed no variation in the time of growth plate closure between male and female dogs and similar findings were reported by Sumner-Smith (1970) and Salmeri *et al*. (1991) who documented that sex had no influence on the time of closure of the growth platein mixed-breed dogs whereas Smith and Allcock (1960) documented variations due to sex in Greyhound, which could probably be due to hormonal variations of the male and the female dogs of that breed.The slight delay that was observed in 2 males and 1 female in the growth plate closure of the distal radius in this studyis suspected to be genetic variations within the same breeddue to the nature of their breeding.

In the present study, the length of the radius and ulna bones grew concurrently, but the ulna bone was always longer at all times compared to the radial bone to maintain the radio-ulna ratio. This agrees with the findings of Lau *et al*. (2015) who reported that radius is transiently shortened in relation to the ulna. We also observed that the dogs acquired their greatest height between 4th to 16th week for both radius and ulna bone. This is earlier than the report made by Newton and Nunamaker (1984)who reported that major growth occurs between 12th to 28thweeks depending on the breed. The length of the radial and ulnar bone among the male and female dogs showed no statistically significant difference ($p>0.05$) among the weeks which agrees with the findings of Salmeri*et al*. (1991) who reported no difference in the length of male and female dogs. We also observed that the female reached their final height slightly earlier than the males, having a final height of 19.10cm (32 weeks) for the females and 19.11cm (40 weeks) for the ulna bones of the males. The final height of radial bone was 15.75cm (36 weeks) for females and 15.76cm (40 weeks) for males, which agrees with the findings of Dharmesh *et al.* (2011) who reported that females attain epiphyseal fusion earlier compared to the males. This could probably be due to geographical variations.

We observed from this study that the diameter of the radius from 1st to 28th week was smaller than that of the ulna bone. At 32ndweek, the radius and ulna became similar with a difference of 0.01mm, and finally, the radius was larger than the ulna at 48th week. This is in line with the report documented by Newton (1974), Logan and Lindau (2008) who reported that at early life, the ulna is larger in diameter than the radius, although in the course of development, the ulna diameter becomes equal to that of the radius and later becomes smaller at the closure of the growth plate. In this study, no statistical significant difference (p>0.05) was observed between the diameter of the males and females. The diameter of the radius was observed to be more uniform in size and much wider at the proximal and distal ends while the ulnar medullary cavity was wide proximally and narrowing along its entire length distally. This is in agreement with the report documented by Dirsko and Pfeil (2009) who reported that the medullary cavity of the radius is almost uniform at maturity while the ulna narrows towards its distal endand that in some smaller dogs, the ulna may be very small or nonexistent.

In this study, the difference between the radius and ulna lengths measured every 4 weeks interval was attributed to the bone growth as a result of activation of the growth plates. The variations observed in the time of appearance and closure of SOCs and growth plate closure times may be due to individual genetic variations.

## 5.2 CONCLUSION

1. Theage at appearance of SOCs for distal and proximal radius were 2-3 and 5 weeks while the distal and proximal ulna were 6 and 8 weeks respectively. At 12th week the SOCs reached their proper anatomical shape.
2. The age of appearance of GP was the same as the time of appearance of SOCs and the closure time for proximal and distal radius had a range of 24-28 and 32-40 weeks while the proximal and distal ulna were 20-24 and 28-32 weeks respectively.
3. The thickness of the growth plate was wider at the time of appearance of the GP with:

- 0.94mm for the distal radius in females and 0.91mm for the distal radius of males at 2 weeks.
- 0.54 mm for proximal radius in females and 0.56mm for proximal radius of males at 5 weeks.
- 1.38 mm for distal ulna of females and 1.40 mm for distal ulna of males at 6 weeks.
- 1.46 mm for proximal ulna of females and 0.96mm for proximal ulna of males at 8 weeks.
- These growth plates kept reducing in thickness as the age progressed until it finally fused.

4. No significant difference was observed in the length and diameter of the male and female radius and ulna bones throughout the period of the study.

## 5.3 RECOMMENDATIONS

Further researches should be conducted to monitor the appearance and closure of the growth plate of other long bones in Nigerian Indigenous Dogs.

## 5.4 LIMITATIONS OF THE STUDY

Analytical imaging measuring software like Image was not accessible as at the time of the research.

3D imaging techniques like computed tomography (CT) and magnetic resonance imaging (MRI) were not available.

This research could be conducted along with hormonal profile to know if some hormones may have role in growth plate appearance and closure.

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# *APPENDICES*

## *APPENDIX I*

### *Graphs of results*

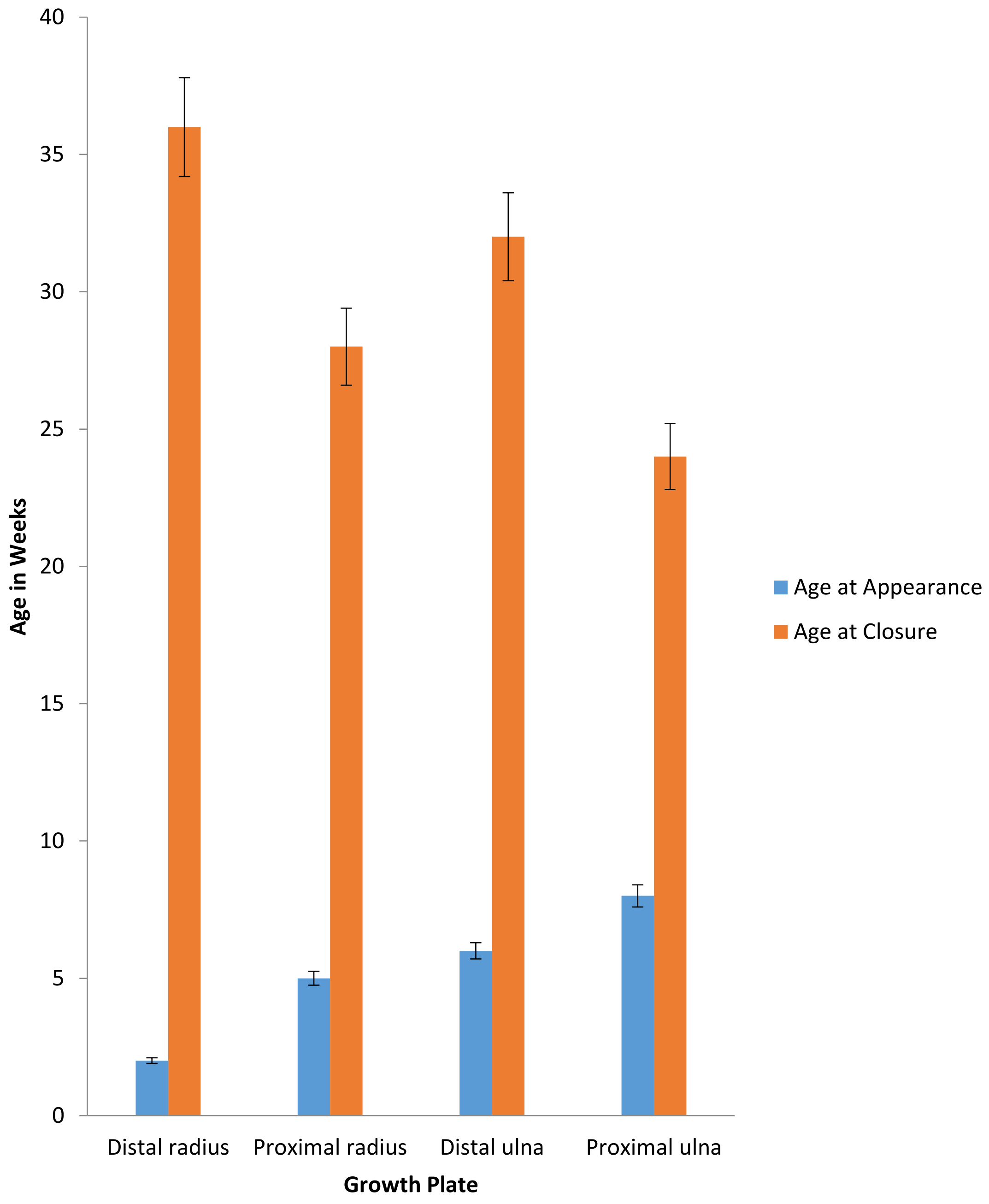

***Figure 1 Age of Appearance and Closure times of the growth plate of Radius and Ulna bones.***

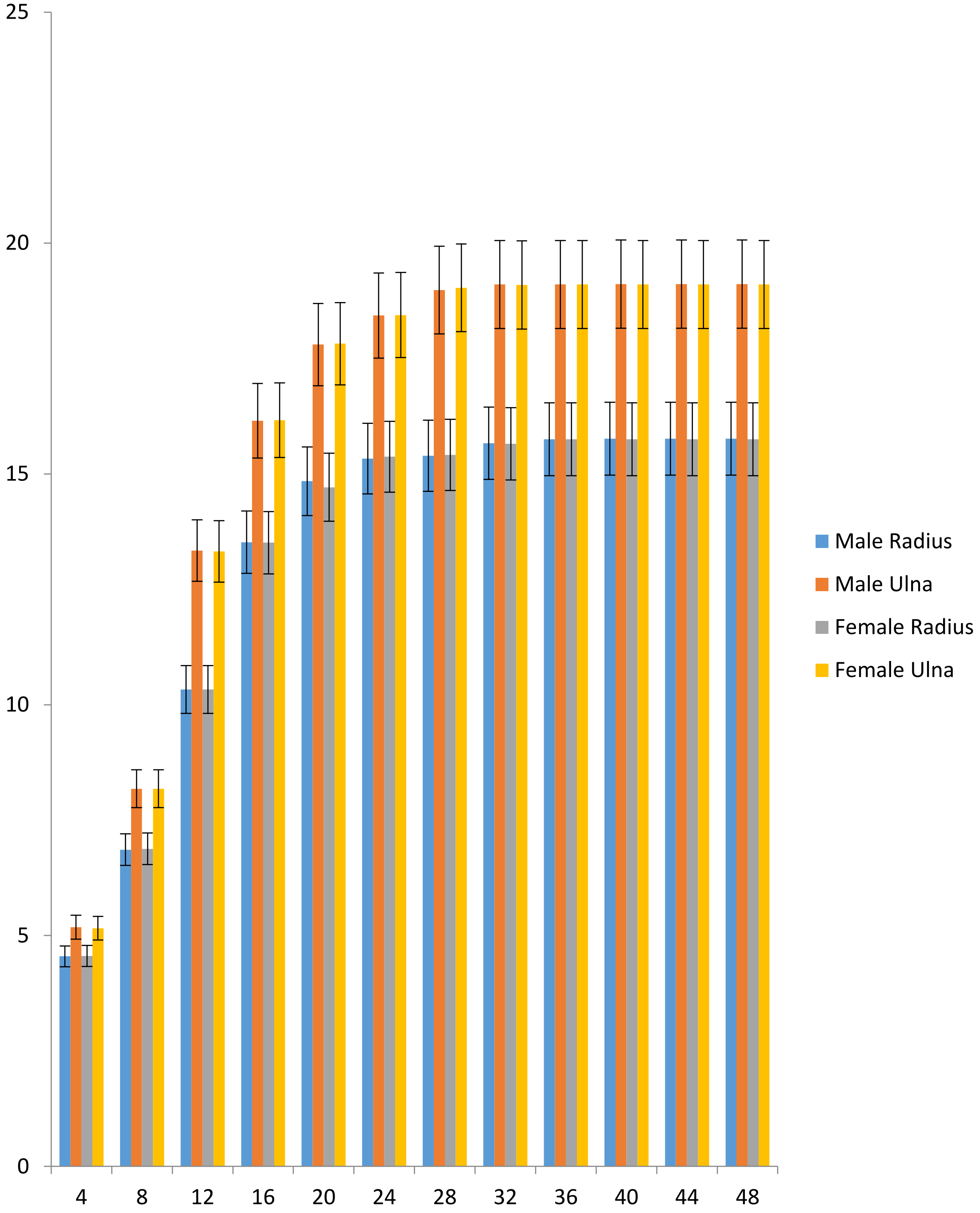

***Figure 2**: Length of Radius and Ulna bones at every four weeks in Male and Female dogs*

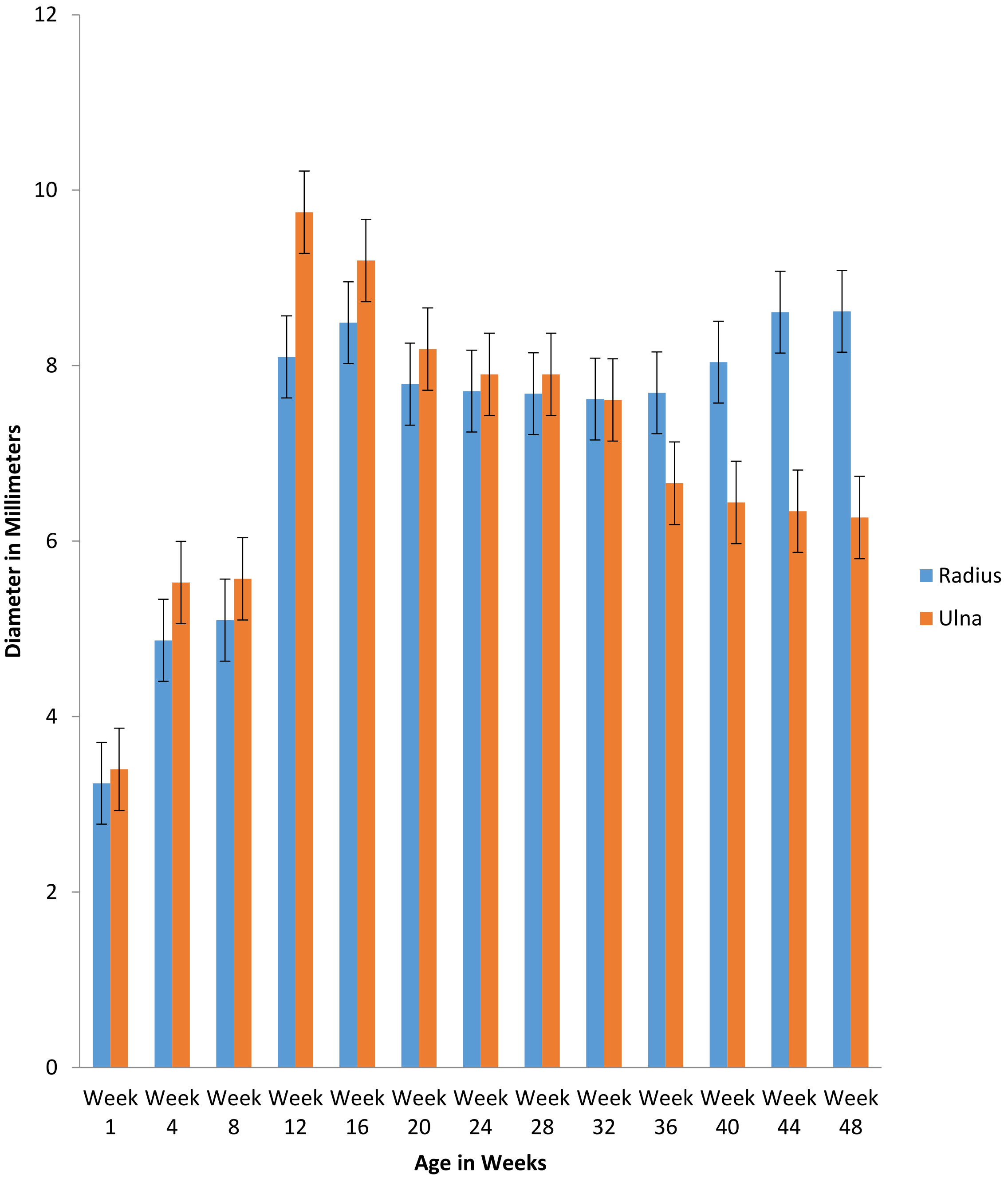

***Figure 3: Diameter of Radius and Ulna bone in mm***

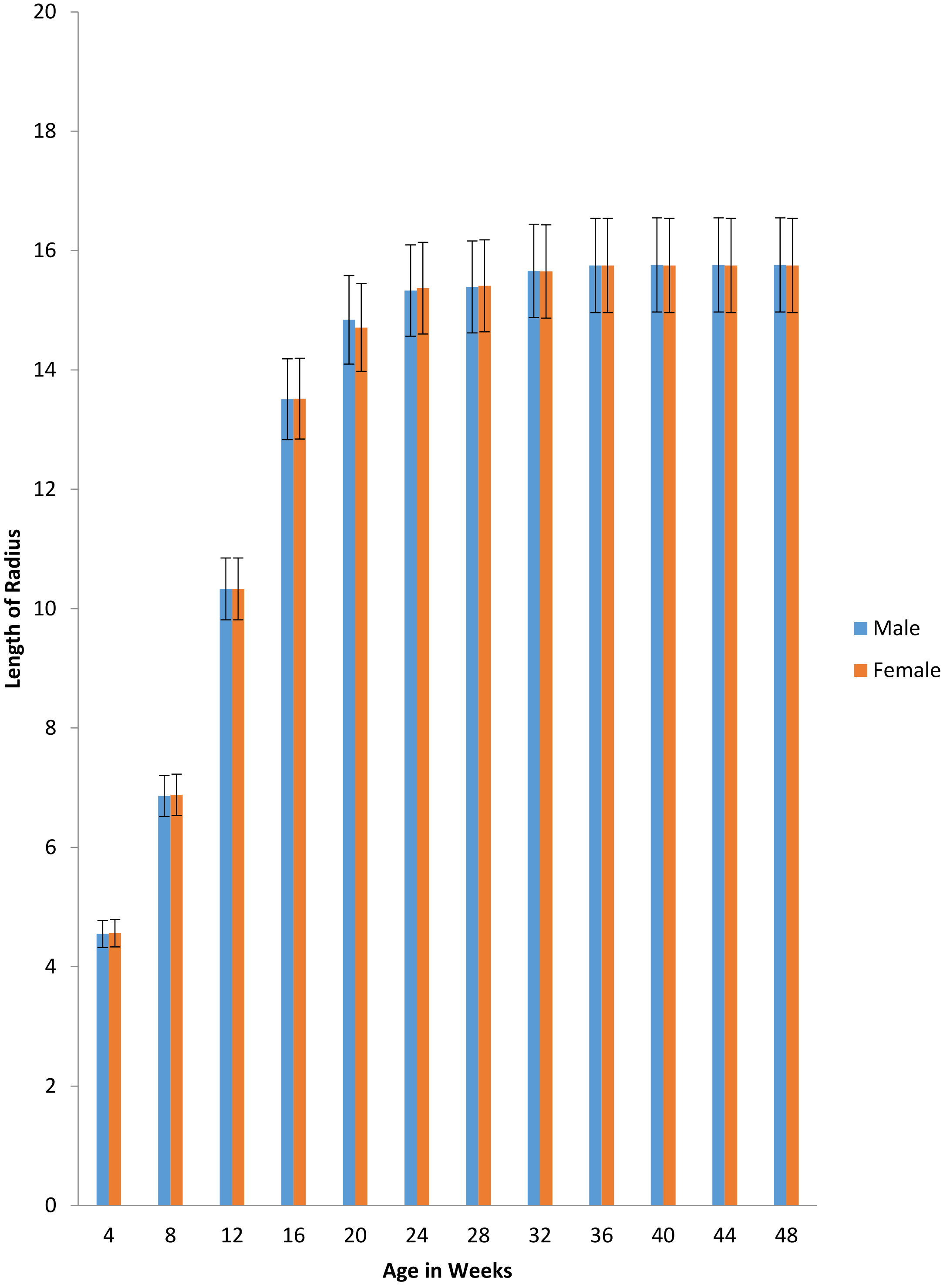

***Figure 4: Mean of length of Radius in Male and Female dogs every four weeks***

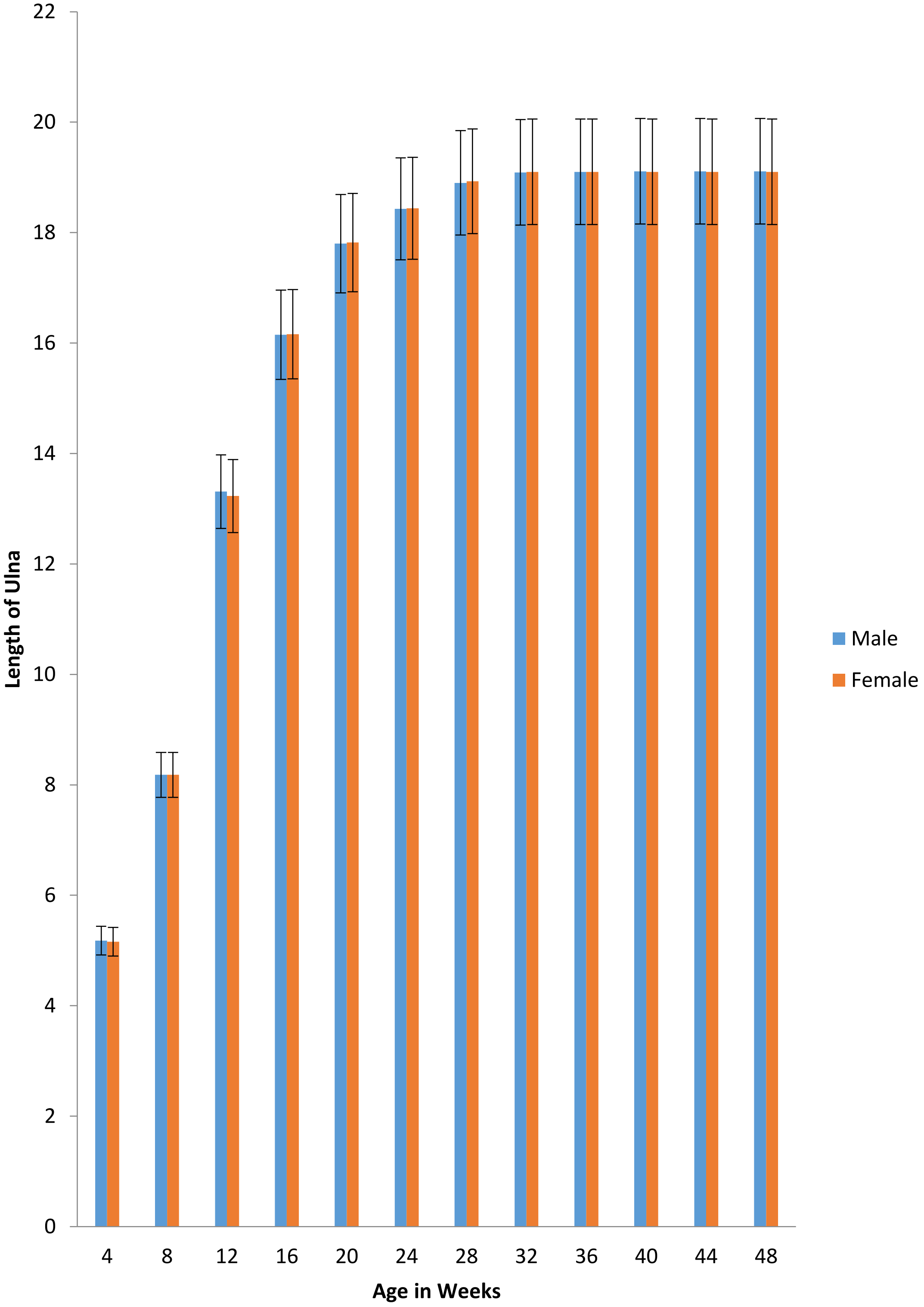

*Figure 5: Mean of length of Ulna in Male and Female dogs every four weeks*