DEVELOPMENT OF THE APPENDICULAR SKELETON OF ZEBU CATTLE IN NIGERIA

By

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DECLARATION

This is to certify that I carried out the work reported in this thesis in the Department of Veterinary Anatomy under the supervision of Professor H. W. Leipold and Professor S. M. Dennis.

The work of other investigators is acknowledged and referred to. No part of this thesis has been submitted elsewhere for a degree or diploma.

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DEDICATION

To my Parents, My wife, Yemisi, my children, Folashade and Kunle Ojo and Professor H. W. Leipold, my first advisor, and friend.

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ABSTRACT

INTRODUCTION

"His bones are as strong as pieces of brass;
his bones are like bars of iron"

Old Testament

Although cattle have been utilized extensively as experimental animals for economic reasons such as milk and beef production, their use for embryological studies has received little attention. The relative ease of obtaining embryos from abattoirs and an accurate method of ageing embryos and fetuses from known stages of pregnancy has made it possible to use cattle for embryological research.

Few studies have been undertaken on the nature and cause of congenital defects in cattle. Review of the literature reveals that one-third of the bovine congenital defects involve the skeletal system, either the entire skeleton or localized regions. Congenital defects of the appendicular skeleton in man are regarded as significant and are carefully documented while those in cattle with few exceptions, are sparsely documented.

Although developmental studies of mammals have been a major area for the past 75 years, there is little information on

skeletal development of limbs particularly in cattle. Most studies on these important structures are confined to man, but some information is available on horses, a few small mammals, and amphibians. Because of this lack of knowledge and as a basis for pathogenetic studies of congenital defects of the bovine appendicular skeleton, the present study was undertaken in Zebu embryos and fetuses to determine changes occurring in developing limbs and at what stage of embryonic and fetal development these changes occur.

The objectives of the study were to determine:

1. Incidence of pregnancy wastage in Zebu cows at slaughter.
2. Parameters for ageing Zebu embryos and fetuses.
3. External features in the developing Zebu fetus.
4. Development of ossification centers in the Zebu appendicular skeleton.
5. Composition of the humerus in the developing Zebu fetus.

PART 1

REVIEW OF LITERATURE

Ageing Bovine Embryos and Fetuses

Prenatal ageing of cattle has been recorded for over a hundred years. Fetal length as a basis has been investigated by several workers (Gurlt, 1860; Bergmann, 1922; Winters et al., 1942; Paimans, 1927). Paimans (1927) considered fetal length correlated better with age than weight.

Bergmann (1922) stated that by examining hair eruption, weighing the fetus and uterus, measuring the amount of fetal fluids, including a description of certain parts of the maternal genital tract, and the length of the fetus, it was possible to determine the age within a one month.

Investigations carried out by Winters et al., (1942) included a study of development of various organs and measurement of length and breadth of the head and extremities. They used X-rays to determine the presence of ossified bone and developing teeth. Ossification was first seen at 59 days and tooth development at 110 days. They found that bone measurements correlated better with age than fetal length.

Sweet et al., (1948) included other fetal proportions in their investigations and concluded that none gave a sure basis for determining fetal age.

Postma (1947) found the best correlation between fetal age and length up to age 32 ± 4 weeks. Hair eruption and development were studied, and when considered together with several other parameters, the error could be reduced to 1-2 weeks.

Maneely (1952), in a general survey, stressed the need for a standard technique for ageing and with Asdell (1946), was skeptic about breed differences relative to age in young fetuses.

Individual variation in amount of fetal fluids was found to be too great to give any useful information (Jakobsen 1958). The same author (1959) demonstrated a significant correlation between age and the circumference and diameter of the fetal head. These measurements cannot, however, be carried out on mummified fetuses and in these cases he proposed measuring extremities "with ossified foundation" and registering hair eruption as supplementary parameters (Jakobsen 1962).

Gotze (1960) summed up studies in the horse and stated an error of ± 2 - 4 weeks.

Rexroad et al., (1974) recorded crown-rump measurements on 229 purebred Holstein-Friesian fetuses of known gestation age and produced a graph of the regression of crown-rump length

for the day of gestation. He concluded that a single regression line of 34 to 270 days of gestation had significantly greater error of estimate than regression lines for two different age categories, 34 to 92 days and 98 to 270 days. Furthermore, he believed the regression of day on crown-rump length could be utilized for estimating fetal age, e.g., in the study of slaughterhouse material.

Hirt (1969) used the number of somites present to estimate fetuses in days 24 and 26 to combat criticism of crown-rump measurement varying with embryonic configuration, especially at 24 to 26 days of gestation age.

Gjesdal (1969) believed X-ray examination of the fetal head was the best basis for determining fetal age after comparing it with metacarpal and metatarsal measurements. X-ray photographs of tooth development, sequence of skeletal ossification, and the sequence of body hair development and eruption. He also believed that, by using the four parameters mentioned, age of a fetus could be determined within $\pm$ 4-5 days.

Embryology of Bone

Cartilage is formed by mesenchyme cells which begin to proliferate, acquire a rounded shape (chondroblasts), and form a compact, cell-rich tissue known as precartilage. The intercellular spaces of precartilage contain collagenous fibers embedded in a homogenous basophilic substance, the so-called ground substance with further development, the intercellular material or the cartilage matrix, becomes so voluminous that the cells are pushed apart. According to the type of matrix, three different kinds of cartilage are distinguishable: (1) hyaline cartilage found on articular surfaces of bones in synovial joints and contains an intercellular substance with fine collagenous fibrils embedded in a large amount of ground substance; (2) fibrous cartilage found in intervertebral discs and contains many heavy fibers embedded in a lesser amount of ground substance; and (3) elastic cartilage containing branching yellow elastic fibers in addition to collagenous fibers.

Bone Formation

A. Membranous Ossification

In areas where membranous bones are formed, mesenchyme cells proliferate and gradually become irregularly polyhedral or cuboidal. The newly-formed cells osteoblasts have a basophilic cytoplasm and an eccentrically located nucleus (Bourne, 1956).

The osteoblasts are initially arranged in irregular fashion; later they are arranged in rows and secrete an acidophilic collagenous material known as osteoid. This material gives the tissue a membranous appearance and this type of ossification is called membranous ossification. At some distance from the osteoblasts, the osteoid is transformed into bone matrix which subsequently calcifies. Calcification is believed to result from release of the enzyme phosphatase which is produced by osteoblasts (Gardner, 1956). The newly-formed bone is always separated from the osteoblasts by a thin layer of osteoid. A few osteoblasts, however, become trapped in the bone itself and form the bone cells or osteocytes. With time, a number of needle-like bone spicules are formed which progressively radiate from the primary ossification centers toward the periphery.

When ossification of the primary center is developing, the entire primordium is surrounded by dense mesenchyme which forms

the periosteum. On the inner surface of this layer, the mesenchymal cells differentiate into osteoblasts, which deposit parallel bony plates on the surface of the primary ossification center. These parallel plates of bone formed by the periosteum are known as periosteal or compact bone.

With each new layer of bone added to the surface, some osteoblasts are trapped to become osteocytes. These cells are located in small lacunae and are in contact with each other by means of small canals, the canaliculi. Osteoblasts so lost are continously replaced by new ones formed by the periosteum.

In postnatal life membranous bones, as found in the cranium, enlarge by apposition of new layers on the outer surface and by simultaneous osteoclastic resorption from the inside (Hancox, 1956).

B. Endochondral Ossification

By the 7th week of development, long bones of the extremities are represented by hyaline cartilage "models" or anlages surrounded by a layer of dense vascular mesenchyme. This mesenchyme initially forms the perichondrium but later develops into the periosteum.

Soon a vascular bud invades the center of the cartilage

model and around the erosion sites the cartilage cells respond in such a manner that eventually four zones can be recognized. Each zone represents a specific stage in the formation of the endochondral bone tissue: (1) a zone of active growth region in which the cartilage cells have abundant mitoses; (2) a zone of cell hypertrophy; (3) a zone where cells die and the intercullar matrix becomes impregnated with calcium salts; and (4) a zone of invading capillaries which occupy the lacunae left by the dead cartilage cells.

Accompanying the invading capillaries are specialized mesenchymal cells, the osteoclasts, which break down the calcified matrix, and cause the initial small lacunae to fuse. Other specialized mesenchyme cells, the osteoblasts, arrange themselves along the remaining calcified cartilaginous spicules. This process, known as endochondral ossification, gives rise to bone-coated cartilage spicules (mixed spicules).

Shortly after their formation, a great number of the centrally located spicules are broken down by osteoclasts, leaving only the lateral ones intact to reinforce the walls of the bone. As a result of this resorption, a broad marrow appears in the center of the bone.

From the primary ossification center in the shaft or diaphysis of the bone, endochondral ossification progresses gradually towards the ends of the cartilaginous model. Although the cartilage cells close to the marrow cavity are swollen, those farther away are small and crowded and multiply actively, thus forming a region of active growth at each end of the marrow cavity.

At birth, the diaphysis of the bone is usually completely ossified but the two extremities known as epiphyses, are still cartilaginous. Shortly thereafter, however, ossification centers arise in the epiphyses, and the endochondral ossification proceeds in a manner similar to that in the diaphysis. Finally, the epiphyses are composed of spongy bone covered by a cartilaginous shell.

A cartilage plate remains temporarily between the diaphyseal and epiphyseal ossification centers. This plate, known as the epiphyseal plate, plays an important role in the vertical growth of the bone. On both sides of the epiphyseal plate endochondral ossification proceeds rapidly on the diaphyseal side and very slowly on the epiphyseal side. When the bone has acquired its full length, the epiphyseal plates disappear and the epiphyses unite with the diaphysis.

In long bones, an epiphyseal plate is found at each extremity. In smaller bones, such as phalanges, an epiphyseal plate is present only at one extremity, and in irregular bones, such as vertebrae, one or more primary centers of ossification and usually several secondary centers are found (Leblond, 1956).

Whereas growth in length takes place at the epiphyseal plates, growth in thickness is more complicated. In the straight part of the shaft, the bone grows by deposition of new layers of periosteal bone on the outer surface, and by simultaneous osteoclastic resorption on the inside or endosteal surface. As a result, the outer as well as, the inner diameter of the shaft increases. In the epiphyseal region, growth in thickness is caused by a radial expansion of the cartilaginous shell and by progressive endochondral ossification on the inside. Since the increase in size in the epiphyseal region is greater than that in the shaft, the two extremities of the shaft become funnel-shaped. In these regions, growth in thickness is entirely different as new bone is deposited on the endosteal surface, while resorption takes place on the outer surface (Pritchard, 1956).

Osteogenesis

Historical

Throughout the ages, the origin of bone has been and still is one of the most fascinating problems facing not only biologists, but also philosophers and even theologians. However, the scientific study of bone, both as a tissue and as an organ, did not begin until Compton Havers in 1691 described the canals in bone which bear his name. The next important contribution was made by Stephen Hales (1727), when he published the experiment with bone markings which demonstrated that the long bones grow in length only at their extremities. Next followed the outstanding work of Henry Duhamel de Monceau. In 1742 he postulated that bone growth was similar to that of a tree and considered that bone formation depended on the periosteum. In placing a circle of silver wire subperiosteally against the cortex of a living animal, he performed the experiment which, even more than that of Hales, may be considered the basis of experimental osteology. Subsequently, he found the ring embedded in the shaft of the bone and concluded that long bones grow in thickness by apposition. Unfortunately he thought this to be the only genesis of bone. Duhamel's

theory was kept alive during the 19th century and remained almost unchallenged until MacEwen published his masterpiece in 1912, in which, probably for the first time, aseptic procedures were employed.

Contrary to the opinion of Duhamel, Von Haller (1763) and John Hunter (1835) attributed a prominent part in the process of bone formation to the blood vessels. It was unfortunate that neither seemed to have used experimental methods to support their views on the vascular origin of bone. Endochondral ossification in growing bones was apparently not mentioned until 1836 when Miaschar made the first substantial reference to this basic mechanism of the growth process. The earliest complete account of the growth plate was that of Todd and Bowman (1845), which was soon followed by the pains-taking study by Sharpey (1848), the notes and references of Leidy (1849), and the extensive study of Tomes and De Morgan (1853). However, it was not until 1855, when Muller published his now classic work on the mechanism of epiphyseal cartilage calcification, that the first full account of the basic mechanism of growth was published. In the twenty years following publication of Muller's work, the detailed anatomy and function of the epiphyseal cartilage became nearly as well-known

as they are today. In some respects such as those referring to the part played by the vasculature adjacent to the epiphyseal cartilage, authors of past generations seem to have collected more information than is usually found today. The main reason for this is probably the rarity, in present day studies, of vascular perfusion before sectioning for microscopic examination, a technique which was well-known to the workers of the 19th century.

A remarkable step forward was the first reference to the bone-forming cells by Goodsir and Goodsir (1845), followed by Virchow's description (1853) of osteoid or calcifiable matrix elaborated by osteogenetic cells. In 1864, Gegenbaur coined the now universally accepted name of osteoblast and finally, MacEwen (1912) demolished what remained of Duhamel's theory of the exclusiveness of the periosteum as bone forming tissue.

Meanwhile, great interest was shown in the origin of the osteoblast and it was almost impossible to find a writer on osteology who did not air his views on the matter. Some authors thought the osteoblast was derived from cartilage cells (Kolliker, 1853; Muller, 1858; Waldeyer, 1865; Gegenbaur, 1867). Several workers believed that the osteogenetic cells originated from white blood cells (Wolff, 1870; Schaffer, 1888; Kazzander,

1900; Jerusalem, 1901; Danis and Doxinel, 1965). However, the most widely held belief has been that the osteoblast occurs by metaplasia of mesenchymal or connective-tissue cells. Among the large number of supporters of this view only a few will be mentioned (Renaut, 1886; Klotz, 1916; Maximow and Bloom, 1957). Leniche and Policard (1926) thought that osteoblasts were simple unspecific fibrocytes, and Pritchard (1956) considered, as did the great majority of contemporary authors, that the osteoblast had characteristics almost identical with those of connective tissue fibroblasts.

In the past, some observers suspected that osteoblasts might originate from the lining of the marrow vessels or that, at least, bone formation was closely dependent on the presence of blood vessels. Minot (1898) gave the first accurate description of vascular sinusoids in several organs but did not describe the bone sinusoids until 1912. Kolliker (1835) mentioned them but gave no account of their characteristics.

In Minot's concept, the sinusoid is a large capillary with a single cell wall. These vascular lining cells were considered by Aschoff (1902, 1924), to be histiocytes, and Maximow (1928) pointed out the considerable potential of these cells for metaplasia and phagocytosis, and it is this characteristic that

forms the basis of the reticulo-endothelial system of Aschoff, which has been related to immunity and other defense mechanisms.

Nature and Origin of the Chondroblast and Osteoblast

The growth, maturation and decay of the mammalian skeleton may be ascribed in the final analysis to the behavior of two cells: the chondroblast and osteoblast, both related members of the mesenchymal family of tissue cells.

A. Chondroblast

The origin and characteristics of the chondroblast are better known than those of the osteoblast and consequently, subject to less debate. It is recognized that the chondroblast results from adaptation of a mesenchymal tissue cell to certain conditions accompanying local ischemia. The chondrocyte can survive ischemia within its immediate vicinity without losing its reproductive capacity, provided regular intermittent compression helps the progression of tissue fluids to and from the cell. The adaptation of this cell to ischemic conditions is such that a rich blood flow in its neighborhood may seriously impair its survival. Anerobic glycolysis allows chondroblasts to survive and multiply in the absence of oxygen.

One of the most important characteristics of the chondrocyte is its ability to reproduce under conditions which would inhibit the reproductive capacity of other cells requiring a richer supply of transudates from blood.

The chondroblast forms a matrix of collagen and mucopolysaccharides which, under certain conditions, becomes calcified. The collagen fibrils close to the cell are the finest and often arranged radially to the chondrocyte (Zelander, 1959).

The greatest weakness of the chondrocyte is its lack of cytoplasmic processes to link one with another, which explains why, as soon as calcification of the matrix takes place, the cells become walled in, degenerate and die. Ultrastructure studies of chondrocytes revealed short cytoplasmic processes that penetrate the cell capsule but do not connect with processes of other cells (Zelander, 1959). Under optimum local conditions some cartilage cells may remain capable of active division for an unspecified period of time.

The fact that the chondrocyte is almost a vegetative cell explains its unique capacity to withstand long periods of fluid stagnation. Precisely how long a chondrocyte may remain alive after being totally cut off from its nutrients is unknown. The difficulty is due to lack of a reliable technique to detect cell

death before signs of decompsition are present. Trueta (1953) considers about 150 hours are required to kill a chondrocyte when its normal nutrients are not available.

B. Osteoblast

Any cell with the property of being able to lay down a matrix which calcifies, without dying in the process, is an osteoblast. While not differing basically from the chondroblast in its ability to produce, or better retain, cytoplasmic processes which link each cell to its neighbor, the osteoblast becomes an osteocyte and remains alive following calcification of the matrix. Leidy (1849) noted that bone canaliculi formed during calcification as he found no trace of canaliculi on the uncalcified side of bone cells. The intercellular expansions run inside the canalicular system which, it is believed, allows passage of nutrients round coll expansions. In summary, an osteocyte is really an osteoblast trapped by calcified matrix.

Vascular Role in Osteogenesis

By whatever method the osteogenetic process is studied, the proximity of blood vessels to areas of bone formation is obvious. However, there is no clear understanding of the real

vascular role in osteogenesis. The more tempting assumption is that bone formation requires a rich supply of metabolities which could explain the increased vascularity in the vicinity. Von Eggeling (1911) working with long bones of amphibia, found that the early cartilaginous anlage began to ossify from the perichondrium and was replaced by a rough-fibered laminated bone. Later, vascular buds eroded their way into this shell of preliminary bone and around the newly-formed vascular channels, the Haversian system of concentric lamellae, thus placing the vascular system as the basis of osteogenesis. MacEwen (1912) repeatedly expressed his supposition that an intimate connection existed between blood vessels and osteogenesis. The following are quotations from his work: "It was also obvious that the bone cells were deposited abundantly where the capillaries were numerous. -- Some of the capillary vessels became surrounded by bone formation, their lumen being gradually narrowed by the ingrowth of osteoblasts so as to resemble "Haversian Canals" Herzog (1922), in his study of vascular cells in pathology, considered that endothelial cells might be transformed into histocytes, smooth muscle, or osteoblasts. Keith (1927) supported the view that the cells which assumed a bone forming role were derived from

the capillary system, endothelium or reticulo-endothelium. He also believed that the first condition leading to bone formation was the presence of a substance or tissue which, in some way, stimulated the neighboring capillaries.

Osteoblasts in Woven and Lamella Bone

Two types of bone are generally described: woven, fibrous, rough provisional bone and lamellar, Haversian mature bone.

A. Woven Bone

This occurs as a preliminary stage to Haversian and lamellar bone in the early calcification of the anlage early in fracture healing and on occasions, in heterotopic ossification such as myositis ossificans or ossification of renal tissue. Sacerdotti and Frattin (1902) found that by tying the renal vessels on one side in young rabbits, calcification of the necrotic tissue occurred and that bone-- woven bone-- appeared adjacent to the calcium deposits three months later. Liek (1908) reported that he was able to obtain bone 16 to 20 days after ligating the renal artery when the kidney was wrapped in omentum to secure a free circulation. Since then considerable efforts have been made to determine the

nature of the stimulus for ossification but so far they have been unsuccessful.

Osteoblasts in woven bone vary in shape and size and are irregularly located to form a mosaic structure within the calcified matrix. Collagen is laid down without any predominant direction to form a dense texture which explains why this type of bone is called fibrous or rough. The resulting calcification occurs in a scattered, irregular manner. Blood vessels are found in the neighborhood but not necessarily closely attached to the bone. Woven bone contains acid mucopolysaccharides even when included in trabeculae of lamellar bone. Osteoblasts cannot be demonstrated in lamellar bone except in cement lines (Curran and Collins, 1957).

B. Lamellar Bone

In 1845, Todd and Bowman made the observation that while several types of bone can be recognized, only one could be called true bone substance and that this laid within a network of blood vessels. Nevertheless, the way in which true bone differs from other types of bone has not been made clear. However, it may be assumed that true bone has a bone marrow. It seems probable that the early authors believed in the existence of two types of bones, one of vascular origin and the

other unrelated, at least directly, to the vascular system.

Osteoblasts appear in rows of strikingly uniform, flat or cuboidal cells, closely and regularly applied against a rigid wall of calcified tissue of varying nature such as bone trabecula, Haversian bone, calcified cartilage, etc. The collagen fibers are laid down parallel to the rigid wall against which the osteoblast rests. The fact that osteoblasts in lamellar bone line up against a rigid wall has been recognized since Robin (1849) pointed out that calcification is a prerequisite to osteogenesis. Barth (1895) postulated that calcium is a stimulus to bone formation. These views were supported by other workers and were summarized by Wells (1910-11), who concurred with the belief that preliminary calcification, even in necrotic tissue, is required for osteoblasts to appear. He thought the calcium stimulus was not chemical but tactile.

To sum up, the osteoblasts of both woven and lamellar bone have two features in common: ability to lay down a calcificable matrix and to form a syncytium with other osteogenetic cells. They differ, however, in their relationship to blood vessels. Woven bone is formed away from the vessel walls whereas lamellar and Haversian bone occurs in close proximity to blood vessels.

Function of the Osteoblast

While the origin of the bone-forming cell was still being discussed, its function was better understood. After Virchow (1853) described the calcifiable bone matrix and called it osteoid, the ability of osteoblasts to lay down a matrix of collagen and mucopolysaccharides was suspected and later, firmly established.

The cell-dependence of matrix is such that once the cell is dead, the matrix may remain unchanged forever, provided there are no circulating fluids in its neighborhood. Isaacs *et al.*, (1963) found no appreciable changes in the collagen of fossilized bone 200 million years old.

Very little is known of what stimulates osteoblasts to form bone matrix. Neither is it clear how osteoblasts are directed by the parathyroid or thyroid to form new bone. From what is known at present, it appears probable that, in certain circumstances, bone cells in their osteogenetic phase lay down calcifiable matrix and form bone, whereas under another set of conditions, it reverses its constructive activity into one of bone demolition.

Cytoplasmic Processes

It has been stated already that cytoplasmic processes are

responsible for survival of osteoblasts once their matrix has calcified. Consequently, a marrow cell becomes an active osteoblast after it has established a powerful web, anchoring its processes like a spider on a rigid wall of calcified tissue. This is what gives active osteoblasts their cuboidal or polygonal shape, in contrast to the flattened appearance of inactive osteoblasts. Once stablized by its processes, the cell lays down the collagen - mucopolysaccharide matrix. If the processes only connected the osteoblast to the rigid wall and to neighboring osteoblasts, its life would be ephemeral as impregnation of the matrix by hydroxyapatite crystals would have caused osteoblastic necrosis as it does to cells of the hypertrophic layer of growth cartilage. To prevent this, the osteoblast needs to be connected with the cells preceding it in the chain of cell division. By linkage of the protoplasmic processes, a syncytium of at least three cells, osteocyte-osteoblast-precursor cell, is formed. When the cytoplasmic connections are damaged as in micro-fractures occurring in osteoporotic bone, osteocytes beyond the crack die.

In histological preparations, shrinkage of cells is such that not even the processes of osteocytes are preserved, which explains why workers like Maximow and Bloom (1957) and

Goldhaber (1961) believed that cytoplasmic processes penetrated bone canaliculi and do not precede them. Ham (1957) thought that bone canaliculi were empty, the cell expansions having retracted after the canaliculi formed.

Mechanism of Bone Resorption

Bone resorption and bone removal were mentioned by John Hunter but it was not until Tomes and de Morgan (1853) referred to remodelling that interest in the mechanism of bone resorption was aroused and has not abated since.

Robin (1849) apparently was the first to distinguish the multinuclear bone cells now named osteoclasts from the giant cell of the bone marrow. Bassini (1872) gave one of the earliest accounts of bone resorption and considered the osteoclast responsible. Finally, Kolliker (1873) gave the name of osteoclast to the polynuclear cell which appears close to areas of bone resorption and thought it was responsible for bone removal. Kolliker (1853) thought that osteoblasts and osteoclasts were genetically-related, a view held also by Howell (1890) who thought that the osteoclast was formed by fusion of osteoblasts. Arey (1920) studied the possible origin of osteoclasts and concluded that they must arise from old osteoblasts and osteocytes coalescing because of their

syncytial arrangement. He also believed that, in early stages, osteoclasts might arise from mesenchymal or reticular cells of the marrow. These multinuclear cells appeared when bone matrix was altered so that crystals of hydroxyapatite melted away. Findings of Trueta (1953) coincided with those of Arey. Microphotographs have been obtained showing osteocytes still connected by their cytoplasmic expansions to adjacent osteoclasts. With electron microscopy and labelling of marrow cells by tritiated thymidine, ample evidence in favor of multicellularity of the osteoclast has been collected.

Creiser et al., (1958) found rapid bone resorption in rabbits by limb immobilization, either by partial resection of the tendon calcaneus or by a plaster cast. At the time of greater bone resorption, bone marrow vessels were extremely dilatated which suggested that immobilization caused changes in the intraosseous circulation and in the local pH and O_2-CO_2 ratio that would affect bone cells. Later work favored the view that immobilization, like parathormone, causes an increase in intracellular citric acid and possibly other acids of the Krebs cycle (Walker, 1961). Lehman et al. (1959) found that a substantial increase in citric acid tended to break the phosphorus-calcium association and favored demineralization. Under altered local conditions, osteocytes secrete the pro-

teolytic enzymes isolated by Vaes (1964), such as collaginases, which act on the vulnerable matrix. The changed O_2-CO_2 relationship helps to elaborate and release collagenolytic enzymes such as the lysosomes which Woods and Nichols (1963) suggested may be contained in subcellular organelles. Belanger and Migikovsky (1963) found protease activity was related to the presence of more mature osteocytes. It is known that parathormone enhances the proteolytic activity of osteocytes.

That the activity of bone cells is, in some way, related to the amount of available oxygen was demonstrated by Goldhaber (1961) when he caused bone resorption in tissue culture by keeping the oxygen fluid concentration at 20 per cent or more. He thought, that this high oxygen concentration stimulated osteoclasts into attacking bone but did not consider the possibility that the osteoclast in his illustration was not penetrating bone by sending processes into it, but was actually coalescing with and incorporating another osteocyte. These findings suggested that the bone cell, in losing its reproductive capacity under unfavorable environmental influences, acquired the ability to elaborate matrix degrading substances. These local factors are closely associated with vascular changes.

It was found by use of tritiated thymidine that nuclei of osteoclasts belonged to different cells since one nucleus may become labelled, indicating that it alone entered the prophase of cell division (Tonna, 1960; Young, 1962). This would never happen if all the nuclei were part of a single cell.

Current Concepts of Bone Osteogenesis

Osteogenesis begins in the center of the shaft of long bones (Fell, 1925). Noback (1943) described three periods in morphogenesis: (1) period of differentiation during which ossification centers appear (influenced by sex, heritable tendencies, nutrition, species, pathology, and parity; the general order of appearance is diaphyses of the two proximal segments of the extremities, thorax, hand, pelvis, and foot); (2) period of proliferation which occurs at a constant rate and does not reach definite proportions until birth; and (3) period of construction during which each bone is re-organized and grows in length. The time of appearance may not be regular as Noback (1944) found some time asymmetry in appearance of bilateral ossification.

Among the earlier studies of skeletal development of the bovine fetus were those of Retterer (1884), Lesbre (1897), Popowa (1913), and Bruni (1933). These workers based their observations on dissection or histological examination of a small number of specimens of unspecified breeds. Kupfer and Schinz (1923), using a relatively limited number of specimens, made the first radiographic study of the bovine appendicular skeleton. Estimated ages of their specimens ranged from six weeks to about nine months. They recorded appearance of many of the primary and secondary centers of ossification of the appendicular skeleton but did not include the ossa-coxarum. No indication of breed or type of cattle was given. Later, Winter et al., (1942) in a general study of the development of the prenatal bovine, included a section on X-ray studies giving a brief account of the radiographic appearance of the bovine fetus. Their specimens - one per given age- were fetuses of 59, 70, 90, 100, 120, 130, 170, 185, and 200 days, respectively, from grade cows of mixed breeding. They recorded some of the primary centers of ossification present in the appendicular skeleton in these age groups. Fedrigo (1957), in a combined radiographic and dissection study, used seven fetuses with ages said to have been calculated from the

date of fertilization. The ages, however, were given rather broadly in months, the youngest being three months. Although neither breed nor method of fetal recovery was specified, the author reported the number and times of appearance of the primary and secondary centers of ossification but did not include any reference to the transient elements of the developing bovine appendicular skeleton. Patton et al. (1966) investigated prenatal skeletal development in dwarf bovine fetuses aged 60 and 90 days. Abrosimova (1958) studied six fetuses of the Kholmogor breed, aged three, five and nine months. As none of these earlier studies presented a complete survey of the prenatal bovine appendicular skeleton, Lindsay (1969a) collected 650 pregnant bovine uteri from the Glasgow abattoir. These specimens were predominantly from Ayrshire cattle, but included some Friesian, Irish cross cattle, Shorthorns and a few Aberdeen Angus and Galloway. His investigation was made into number, times of appearance and chronological order of the loci of ossification in the prenatal and neonatal bovine appendicular skeleton including the ossa coxarum by histological, radiographical and staining by alizarin red S methods. Loci were identified in general at earlier estimated ages than previously recorded. The study included loci of the

transient elements and recorded for the first time, a locus for the clavicle. All specimens used in Lindsay's study had a cephalic index within 48-60.

The adult bovine sternum is described in the standard veterinary textbooks as consisting invariably of seven sternebrae. Little information on the prenatal bovine sternum is available. Montane and Bourdelle (1917) stated that each of the seven sternebrae develops from two lateral loci of ossification, which unite with one another in series from before backwards. Sisson and Grossman (1959) stated that most of the seven sternebrae develop from two lateral centers. Cottereau (1952), in a study of the ossification of the lumbar vertebrae of the calf, recorded that in a fetal calf about one and a half months old (estimated age), the sternum had a longitudinal fissure and at two months, the sternal fissure was closed. Winters et al., (1942) in their X-ray study of the prenatal bovine, noted that at an estimated age of 90 days, the sternebrae had ossified to some extent. Lindsay (1969b) in his study of neonatal and postnatal cattle found that at about 50 days of gestation, the bovine sternum originated as bilateral cores of cartilage which fused to form a median, continuous cartilaginous precursor. This synchondrosis occurred

in a cranio-caudal direction and was completed about day 65 of prenatal life. Ossification commenced by day 75 and typically the locus for the seventh sternal segment appeared first and that the first sternal segment appeared last. Exception to this order of appearance involved mainly the sixth locus in the six fetuses of Type I in which this locus was much smaller than the others and was therefore possibly the last to appear. Typically, the loci were unpaired and median in position. In 120 neonatal calves aged one to eight weeks, a similar variation in number of loci was found and again, the three sternal types were recognized. In only one calf of Type 3 a sub-type was identified, i.e. sub-type 3 a/b with absence of the fifth locus and coalescence of loci six and seven. It was observed in these calves that, although comparable sterna did not vary noticeably in length, the loci of Type I were the smallest and those of Type 3 the largest.

In 124 specimens from cattle age 18-30 months and over, the same three types occurred as in the prenatal and neonatal groups. Morphologically sub-type 2, in which there was no evidence of coalescence and loci five or six absent, was identified. Sub-type 2 b was identified in those specimens in which coalescence of loci six and seven was deduced to have

occurred because of the marked increase in length of the appropriate segment. As expected, in the specimens of 30 months and over, the percentage incidence of the three sternal types decreased as the sternal segments became increasingly synostosed until in cattle of four years and older, all sternebrae, except the first, may be synostosed.

Limited studies of the ossification program of the prenatal and neonatal bovine vertebral column are recorded. Winters et al. (1942) in their X-ray studies made brief reference to the ossification of the prenatal bovine axial skeleton. Sisson and Grossman (1959) give a brief general descritpion of development of a vertebra and noted that in the horse and ox, the body and arch are fused at birth or unite soon after, whereas in the pig, sheep and dog a neurocentral synchondrosis persists during the first few months of postnatal life. Gjesdal (1969) in a table indicating ossification sequence based upon X-rayed fetuses, indicated age at which some ossification was noted in the vertebral regions and in the dorsal spinous processes of thoracic vertebrae. Age of fetuses studied varied between 55 and 145 days. Cottereau (1952) used gross dissection, injection and histological methods and confined his ossification studies to the lumbar vertebrae

Pituitary

The early influence of the pituitary gland on growth was demonstrated by removing the pituitary primordium from chick embryos at 33 to 38 hours of incubation, which inhibited growth by retarding differentiation of the thyroid glands (Fugo, 1938). Atwell (1926) found that the pituitary gland of chickens produced a thyrotrophic hormone probably earlier than the 11th day of incubation.

In the human embryo, the first pituitary acidophil cells are present from the third month when the epithelium of the cells of Rathke's pouch actively proliferates, while the basophilic cells appear later (Atwell, 1926). Dandy and Reichert (1938) demonstrated by radiographs of hypophysectomized puppies that bony growth and maturation (i.e. epiphyseal union) were markedly retarded by pituitary removal. Dwarfism and failure of epiphyseal union were the main findings in a human skeleton in which a destructive lesion of the pituitary gland was found at autopsy (Erdheim, 1916). On the other hand, repeated clinical observations of pituitary hyperfunction in human giantism were substantiated experimentally when Evans and Long (1922) induced excessive growth in intact rats by administering crude anterior lobe extracts of the pituitary gland.

Other classic reports have been cited in reviews (Simpson et al. 1950; Asling et al., 1951; McLean and Urist, 1955).

Growth Hormone

All endocrine glands have the ability to influence each other's activity in various and occasionally opposite ways, but without any doubt the hormone secreted by the anterior pituitary gland, known as H.G.H., somatotrophic hormone or somatrophin, does more so than any other.

Marie in 1886 described acromegaly, and four years later Massalongs (1892) described, for the first time, hyperactivity of the pituitary gland. Since then, numerous reports have appeared describing the activity of somatotrophic hormone and culminated in isolation of growth hormone free of impurities by Hi and Evans in 1933. Growth hormone is one of eight hormones isolated from the anterior hypophysis. The work of Evans and his collaborators on the effect of somatotrophic hormone on growth was incorportated in a book on growth and gonad stimulation by the anterior hyphophysis (1933) which is a classic in this area.

The action of somatotrophin on growth is now fairly well understood even if some pecularities of activity or target cells

need further clarification. Ample experimental evidence is available on the changes that growth hormone withdrawal causes in epiphyseal cartilage but, so far, nothing on the effect of removal of the anterior hypophysis on the vasculature adjacent to growth cartilage (Trueta, 1953). It is well established that changes caused in young laboratory animals by hypophysectomy occur mainly in the germinal and proliferative zones, consequently cell columns become much shorter than normal following mitotic inhibition and reduction of cell size. Concurrently, vascularity of the metaphyseal side of the growth cartilage is arrested (Trueta, 1953).

A peculiarity of growth hormone, which is not satisfactorily explained, is that in tissue culture it does not cause any appreciable effect upon its apparent target, the rate of cell division being approximately the same as when the hormone is withdrawn. This phenomenon has been noted by many investigators since Fell (1955) first reported it. Another important activity of growth hormone is related to carbohydrate metabolism. Houssay (1947) found prolonged administration of the hormone caused hyperglycemia and glycosemia; this effect may explain, at least, some changes in growth cartilage, since enchondral ossification is intimately related to glycogen metabolism.

Thyroid Gland

By the 10th week, the human embryo has the thyroglossal outgrowth and by the 12th week, definite follicles appear. The full activity of the thyroid gland occurs between the fourth and fifth month (Fox, 1908).

The function of the thyroid gland in the mammalian embryo is not well understood even though it begins secreting early in fetal life. In the pig fetus at 50 days, less than half its gestational life, iodine is already detectable. On occasions, cretinism is recognizable *in utero*. To retardation of skeletal development, Wilkins (1957) added epiphyseal dysgenesis or "stippling" of developing epiphyseal centers to the features to be expected with hypothyroidism. Permanent brain damage has been observed in fetal hypothyroidism.

A function hitherto unsuspected has recently been assigned to the thyroid. The hormone calcitonin, described by Copp (1961-62) as being produced by the parathyroid glands has been shown by Hirsch *et al.*, (1963) and Foster *et al.*, (1965) to be produced by the thyroid.

Parathyroid Gland

In the human embryo by the fifth week, the parathyroids

have well-differentiated "water clear" cells which suggests early activity but no definite evidence is available as to their function, at this stage, in initiating ossification which begins in the human embryo before the end of the seventh week.

The lack of parathyroid hormone causes severe metabolic changes; phosphorus secretion in urine decreases as serum phosphorus level rises, and calcium secretion in urine decreases as does serum calcium level. Hyperparathyroidism causes the same four metabolic changes, but in the opposite direction. Albright and Reifenstein (1948) believed that serum phosphorus fell and rose as serum calcium rose and fell in order to keep some solubility product constant. They thought that the mechanism of action was as follows: parathyroid hormone affects phosphate dissolution in body fluids and this increases renal excretion of phosphate resulting in hypophosphatemia; the body fluids become less saturated with calcium and phosphorus resulting in increased resorption of calcium-phosphate salts from bone, and serum calcium increases while serum phosphate decreases. Subsequently, increased renal excretion of calcium occurs, which tends to further cause undersaturation of body fluids, and easily brings about further

removal of bone calcium. Albright and Reifenstein (1948) suggested that this vicious circle was accompanied by another of osteoblastic activity. By removal of minerals, bones become weaker and become subject to more changes by stresses and strains.

Whatever the mechanism by which parathormone works, there is little doubt that its function is to regulate calcium metabolism and the calcium level in blood, which are essential for normal cardiac function, neuromuscular excitability, blood coagulation, and cell membrane permeability. The information of calcium level in the blood is carried to the parathyroids and the thyroid. When the blood with the secreted hormones, calcitonin (thyroid) and parathormone (parathyroid), reaches the row of osteogenic cells via the bone vessels, the cells act either by building or by demolishing bone.

The role of parathormone, first reported by MacCallum and Voeglin (1909), has been repeatedly studied, but its mechanism of action is still controversial. It is widely believed that parathromone acts directly on bone, and this is in keeping with the fact that it causes bone lysis in tissue cultures and induces staining changes around lacunae of osteocytes far away from the surface of lamellae, indicating bone resportion

(Heller-Steinber, 1951; Gailland, 1961). Newman and Newman (1958) believed that parathormone action on bone making the crystals melt away is closely related to the increase in intracellular citric acid. A substantial increase in citric acid tends to break the phosphorus - calcium association and favors demineralization (Walker, 1961). Proteolytic enzymes, such as collagenases, act easily upon vulnerable matrix (Vaes, 1965).

Gonads

Despite controversial views it seems likely that fetal testes secrete androgens and that they may control development of the genital tract of mammals. So far, there is no evidence that the mammalian fetal ovary secretes any hormones.

The sexual dimorphism of the skeleton is, in most aspects, genetically determined (Todd, 1933). However, certain features become accentuated with appearance of sex hormones. Many observations illustrate the interaction of genetic and hormonal factors in skeletal development: sex differences in structure of the human pelvis are present from the fifth month of gestation (Fehling, 1876) and gradually increase thereafter; centers of ossification are better developed in girls than in boys (Hill, 1939); and epiphyseal closure follows essentially a

genetic pattern, but again in adolescent boys, epiphyseal closure and skeletal age, in general, are more closely related to sexual development (Francis, 1940).

Skeletal Effects of Ovarian Deficiency

Skeletal changes have been studied in cases of primary ovarian deficiency as occurs spontaneously in freemartin cattle and in humans with ovarian agenesis or hypoplasia, and follow-up therapeutic or experimental ovariectomy. The bones are delicate and thinner than usual, the epiphyseal growth zones and cranial sutures close later than normal, and apperance of centers of ossification are delayed. The pelvic diameters are shortened, horns continue to grow beyond their normal length, and decreased stature with disproportionately long extremities are observed. In adults, ovarian deficiency fails to exert noticeable skeletal effects (Kaijser, 1949). Proof that ovarian deficiency is the cause of post-menopausal osteoporosis is still lacking (Anderson, 1950).

Skeletal Effects of Estrogen

Bone metabolism is intimately related to that of minerals.

Therefore, investigations of skeletal effects of steroids have been concerned with their action on mineral metabolism.

Serum Calcium

The levels of serum calcium in untreated animals vary considerably according to species, age, sex, degree of maturity, and generally on the amount of estrogen circulating in the blood. Some of the discrepancies reported can be explained by difference in methods of sampling blood and calcium determination and in particular, pooling of blood may fail to disclose significant individual differences. Most investigators did not distinguish between non-ultrafiltrable and ultrafiltrable fractions of serum calcium. However, in pigeons estrogens increase primarily in the non-ultrafiltrable fraction (McDonald et al., 1945). In mammals, serum calcium responds to estrogen less consistently and less conspicuously than in birds. Slight and temporary rises as well as deadlines in the levels of serum calcium have been reported. Young animals react more vigorously to estrogen than older individuals (Day et al., 1941).

Data on the effects of estrogen on calcium and phosphorus are few and contradictory. The negative calcium and phosphorus balance prevailing in older individuals reverted to positive

after administration of estrogen (Albright et al., 1941), while in prepuberal girls estrogen caused the normally positive balance to become negative (Johnston, 1941).

The conflicting views on normal osteogenesis are reflected in different interpretations of the effects of steroids on formation and resorption of bone. Various answers have been given to questions like the following: Is the excessive bone seen after administration of estrogen identical with ordinary bone and is it similarly formed? Specifically, do estrogens cause deposition of calcium carbonate or bicalcium phosphate in uncalcified matrix, and do they promote conversion of the latter into tricalcium phosphate? Is the increase in bone caused by estrogens relative or absolute, and is this a primary or secondary effect? The available data are often difficult to integrate, not only because of divergence of material tested, but because different investigators used different methods of standardization.

Estrogens increase the percentage of total bone ash in animals of various species. However, overmineralization is not uniform throught the skeleton, and some bones such as the os illi and symphysis pubis may actually undergo demineralization (Gardner, 1940). Radioactive isotope studies confirmed that the turnover of skeletal phosphorus was intensified after estrogen

administration and closely followed that of calcium (Dallemagne, 1950).

The chemical nature of mineral deposition under the influence of estrogen has not been unequivocally established. The low Ca/P ratio in the bone suggested early deposition of bicalcium phosphate which was supposed to be speedily converted into tricalcium phosphate (Ettori et al., 1942). However, X-ray diffraction studies failed to demonstrate the pattern of bicalcium phosphate (Dallemagne, 1950), and therefore, the role of this mineral in estrogen - induced osteogenesis remains in doubt.

The significance of changes induced by estrogen in bone ash may have been generally overrated, while major changes in the non-asheous components of bone were disregarded. In young rats receiving estrogen, the water content and non-asheous solids of bone decreased progressively. If, however, the values were corrected for body weight, the proportion of asheous to non-asheous material was the same as in non-injected animals (Lippman et al., 1942). The effect of estrogen manifests itself in decreased formation of organic matrix, and the apparent increase in the ash content constitutes concentration of ash within a smaller volume of organic matrix

rather than an absolute increase (Silberberg and Silberberg, 1956). This interpretation appears to be the only one that links the biochemical changes in long bones with inhibition of linear growth by estrogens.

The close relationship of overmineralization of bone and inhibition of linear growth is also indicated by the decreased effectiveness of estrogen in adult animals. In old mice or young hypophysectomized mice given estrogen, bone ash increased less than in growing mice treated correspondingly (Gardner and Clouet, 1944, 1945). Overmineralization is, to a large extent, due to the concentration of ash in a decreased volume of organic matrix. Some of the excessive bone ash can be accounted for by decreased resorption of cortical bone and by secondary accretion of bone.

Gross changes

Estrogen exerts three major effects on skeletal structure: inhibition of linear growth, accelerated development, and ageing and condensation of bone (Silberberg and Silberberg, 1956). In addition, estrogen may cause pubic relaxation. In growing individuals of all species investigated, estrogen inhibits growth of long and flat bones (Lenz, 1913). Accelerated

development is indicated by hastening epiphyseal closure and by premature appearance of ossification centers.

Histologic changes

Growth of cartilage is inhibited, while hyalinization and calcification of the ground substance are intensified; erosion by capillaries of the provisional cartilage is delayed, and the latter persists longer than normal (Silberberg and Silberberg, 1956). Provided the cartilage is not too badly damaged, growth may be resumed after cessation of treatment, or even under continued treatment, and the excessive spongiosa may also be resorbed. In articular cartilage, estrogens render the ground substance resistant to breakdown and liquefaction, and thus may counteract development of spontaneous osteoarthritis and joint lesions induced by anterior hypophyseal hormone (Silberberg and Silberberg, 1946).

There are some differences in the response of avian and mammalian bones to estrogen. In birds, the increased medullary deposition of bone originates in the diaphysis, while in mammals the process begins in the metaphysis of the long bones. The tissue changes are complex and variegated (Silberberg and Silberberg, 1946; Salomon et al., 1947). Therefore, it is

understandable that many investigators stressed various details of some observations and minimized others. The evaluation of static microscopic pictures are rendered particularly difficult by the rapid succession and interaction of osteogenic and osteolytic processes. Emphasis has been placed on osteoblastic proliferation, on damage to bone marrow by secondary deposition of bone, and on inhibition of resorption of provisional cartilage and bone followed by accretion of osseous substance.

While estrogen may increase the number of osteoblasts, it is doubtful whether this is due to direct hormonal stimulation or whether it is secondary to hormonal changes in the bone marrow. Such early changes consist of hyperemia, stimulation of hematopoiesis, and subsequent deposition of collagenous fibrils and osteoid. The latter undergoes calcification to form trabeculae. The newly-formed spicules contain delicate doubly refractile fibrils which are less serrated and regular than those of pre-existent bone. The new bone does not form lamelae and is not arranged according to mechanical stresses as in ordinary bone (Benoit et al., 1945).

In adult and old animals, there is no consistent effect of estrogen on bone (Silberberg and Silberberg, 1941). In young animals treated with estrogen, the spongiosa of flat

bones, such as calvaria, vertebrae, and mandibles, may undergo osteosclerotic changes (Sternberg, 1896).

Because of its condensing effect on bone, estrogen has been used for treating osteogenesis imperfecta. Decreased fragility was noted during the period of treatment (Ropes at al., 1946). No objective evidence has yet been put forward to demonstrate a beneficial effect of estrogen on the bone changes of menopausal osteoporisis (Anderson, 1950) and its effects on callus is equivocal (Hills and Weinberg, 1941).

Estrogen cause gradual replacement of the symphyseal cartilage by connective tissue (Sternberg, 1896). This process in pelvic relaxation is distinguished from the effects of relaxin, which rapidly disintergrates symphyseal cartilage.

Factors Modifying the Skeletal Effects of Estrogen

The effects of estrogen vary considerably with the conditions under which the hormone is tested. The modifying factors may be exogenous and related to the hormone itself, such as the type of estrogen, the duration of treatment, and mode of administration; or the modifiers may be endogenous due to species, strain, sex, age, and local conditions within individual bones (Silberberg and Silberberg, 1941). Of the

numerous natural and synthetic steroids, the benzoic and propionic esters of estradiol as well as estrone and diethylstilbesterol elicit the most vigorous skeletal response (Urist et al., 1950). The relative potencies of these steroids are difficult to establish because their effects vary, not only in degree, but also in time of appearance and duration. Steroids dissolved in sesame oil are the most stable (Barnicot, 1951). A given amount is usually more effective if administered in divided doses than if given at one time. Estrogen have been administered orally, intraperitoneally, subcutaneously, intramuscularly, percutaneously, topically, and by implantation. However, the rate of absorption, esterification and excretion are more important in determining results than the estrogen's mode of application (Folley, 1942). The amount of bone deposited is usually proportionate to the metabolized amount of estrogen.

1. Age

Skeletal susceptibility to estrogen decreases with age (Zondek, 1936; Landauer and Zondek, 1944; Silberberg and Silberberg, 1946; Salomon et al., 1947). However, growth inhibition is less marked in neonatal than in prepuberal animals.

This may be due to higher growth potential of cartilage of neonates and thus harder to suppress than that of older animals, which grow at a slower rate. Aged cartilage cannot respond to further estrogenic activation.

2. Species

While exogenous estrogen inhibits growth in young animals of all species, species differences exist in regard to bone reactivity. The osteogenic response of pigeons and ducks exceeds that of chickens; of mammalian bones, those of mice react most vigorously to estrogen. These differences may be due to species differences in metabolic degradation of estrogen or tissue susceptibility.

3. Sex

Sex difference in skeletal response to estrogen, particularly conspicuous in mice, may be partly explained by the higher physiological bone age of growing females compared to that of males of the same age. Male mice of most strains react more vigorously to estrogen than females. However, in C_3H strain mice estrogen exerted a more marked reaction in the bones of females than males. This is considered to be due to

antagonism between exogenous estrogen and endogenous androgen present in the male. Numerous experiments (Landauer et al., 1941; Budy et al., 1952) have failed to establish unequivocally the existence of androgen-estrogen antagonism relative to the skeleton.

Response of individual bones

The susceptibility of individual bones to estrogen decreases in the following order: lower femur, upper tibia, upper femur, humerus, and cubitus (Urist et al., 1948). The rule that the younger a bone is physiologically at the beginning of treatment, the more vigorous its reaction, applies also to individual bones. The os ilii is remarkable in that it actually undergoes resorption under estrogenic influence (Gardner, 1936, Urist et al., 1950).

Mechanism of Action of Estrogen

One of the major questions regarding the mode of estrogenic action on bone is, whether or not the hormone acts on the skeleton directly or indirectly through mediation of other endocrine glands. Several possibilities have been investigated.

1. Role of Hypophysis

Changes in structure and function of the anterior hypophysis following administration of estrogen suggests that it exerts its skeletal effects by inactivating the former. This is plausible since both estrogen and hypophysectomy inhibit linear growth. However, the microscopic changes in the epiphyseal cartilage in the two conditions are distinctive: hypophysectomy depresses growth, development, and ossification of the provisional cartilage but estrogen intensifies ossification and regressive changes. The latter changes affect the cartilage so severely that they may inhibit local growth. Estrogen , however, may act on both serum calcium and bone in the absence of the hypophysis (Gardner and Clovet, 1945; Riddle and McDonald, 1945). The failure of estrogen to elicit skeletal effects in hypophysectomized animals (Kibrick et al., 1942) does not necessarily indicate an intermediary function of the hypophysis. Following hypophysectomy, growth of cartilage is maximally depressed so that further inhibition by estrogen is impossible. Similarly, bone formation is at a minimum following hypophysectomy so that there is little or no substratum for estrogen to act on. In conclusion, while it is not disproven that estrogenic growth inhibition may, at least,

be partly mediated through the hypophysis, the possibility is slight.

2. Role of Parathyroids

Since the skeletal effects of estrogens can be observed in the absence of parathyroids (Riddle and McDonald, 1945) the latter may be disregarded as a factor essential for estrogenic action on bone. However, in some species, estrogen might stimulate the parathyroids (Riddle et al., 1945), and parathyroid hormone may contribute to the peculiar combination of formation and resorption of the resultant bone.

3. Role of Nutritional Factors

Estrogen did not accelerate repletion of body calcium following calcium depletion (Duckworth and Ellinger, 1950). The hormone exerted its effects on bone of animals kept on a vitamin D deficient (Segaloff and Cahill, 1943) or low calcium diet (Benoit and Calvert, 1947). A low protein diet (Gardner and Mahan, 1946) did not alter the skeletal response to estrogen, and in guinea pigs, estrogen was capable of acting in the absence of vitamin C, while at the same time retarding development of acute scorbutic lesions (Silberberg and Silberberg,

1948). Estrogen also exerted its effects on long bones with their nerve supply severed (Armstrong et al., 1945).

Based on the observations described above, four major theories have been advanced regarding the mode of action of estrogen on the skeleton:

(1) Estrogen stimulates primarily osteoblastic growth. This view presupposes a primary anabolic effect of estrogen on osteoblasts (Gardner and Pfeiffer, 1943). However, there is no supporting evidence for an anabolic effect being exerted by estrogen on any mesenchymal tissues.

The remaining three hypotheses agree on a primary injurious effect being exerted on mesenchyme by estrogen, either on cells or the ground substance, or both.

(2) Estrogen induces hyalinization of the ground substance. This change interferes with linear growth, vascularity, and resorptive processes during formation of new osseous tissue. The ensuing condensation of bone is primarily due to decreased resorption, and to a limited degree, deposition of new material (Silberberg and Silberberg, 1939, 1943).

(3) Estrogen causes osteoclasis and osteolysis associated with or followed by excessive medullary bone formation (Benoit and Calvert, 1943).

(4) Estrogen injures reticulum cells of the bone marrow, and the subsequent excessive bone formation constitutes a result of cicatrization (Landauer and Zondek, 1944; Urist et al., 1948). This last theory is more specific than the former in regards to the injurious effects of estrogen, which are supposed to be directed against cells rather than matrix. The use of the term cicatrization for changes taking place in the bone marrow is unfortunate as it denotes a permanent pathological change.

In conclusion, more new factual material is needed to establish the basic skeletal reactions caused by estrogen. Probably the effect will turn out to be related to that exerted by estrogens on connective tissue in general.

Skeletal Effects on Progesterone

The effect of progesterone on calcium metabolism and on bone are not conspicuous and are of interest only because of their relation to the action of other hormones. Progesterone has no influence on ultimate body size (SoSa-Gallardo and Arias, 1941). It was unable to prevent the estrogen- induced changes in the bones of mice (Gardner, 1936). In young guinea pigs, progesterone slightly stimulated epiphyseal growth and

temporarily retarded regressive changes and ossification of cartilage (Silberberg and Silberberg, 1941). The effect resembles that seen at early stages following ovariectomy and may be due to inhibition of endogeneous steroid that hasten skeletal development.

Skeletal Effects of Testosterone

In the growing skeleton, effects of castration manifest themselves in the tubular and flat bones and in such secondary sexual characteristics as horn and antlers. Skeletal development is retarded and more so, the earlier in life castration is performed (Tandler and Selheim, 1913). There is delay in epiphyseal closure followed by a prolonged growth period at subnormal rate, and hence a moderate degree of stunting. The castrate skeleton has a disporportionate increase in the length of those bones whose growth zones are open at the time of castration. Castrate skeletons, as a whole, are delicate and sometimes porotic (Hammond, 1932).

Microscopically, the growth zones of long bones of castrates contain an increased number of columbar cartilage cells, while hypertrophy of these cells is delayed. Regressive changes and replacement of provisional cartilage by bone are

retarded (Silberberg and Silberberg, 1946).

In adult individuals, deficiency of male sex hormones is supposed to give rise to osteoporosis and in old age, this is believed to become accentuated due to declining production of androgens (Heller and Shipley, 1951). There is, however, in aged patients overlapping effects of androgen deficiency with those of adrenal cortical steroids so that definite conclusions should be withheld until adequate experimental data are available.

Testosterone in large doses suppresses proliferation of cartilage, but intensifies hypertrophy, hyalinization, calcification, and ossification (Silberberg and Silberberg, 1956). The hormone had no effect on bone repair when tested strictly under control conditions (Armstrong, 1942).

Like estrogen, the skeletal effects of testosterone are subject to a number of modifying influences. Among these factors, dose is important since it determines whether or not growth will be stimulated. Furthermore, susceptibility to testosterone changes with physiological age of the skeleton; a maximum response is elicited shortly before puberty, while newborn and adult individuals have a decreased reaction or none at all (Silberberg and Silberberg, 1956). Female mice

respond more vigorously to the age-promoting action of testosterone than males (Silberberg, and Silberberg, 1956). This may be due to the relatively old physiological age of females as compared with males of corresponding age. In the physiologically older female, the hormone merely accentuates a prevailing trend, while in the physiologically younger male, the steroid has to overcome such growth capacities still present.

Skeletal Effects of Adrenal Cortical Hormones

In adrenalectomized rats, growth of epiphyseal cartilage is arrested (Ingalls and Hayes, 1941). This effect is apparently non-specific and caused by decreased food consumption. In adrenalectomized animals maintained with chlorides and desoxycorticosterone, the long bones are somewhat enlarged. This slight growth stimulation supposedly results from elimination of growth inhibitors present in the adrenal cortex.

1. Transplant of Adrenals

In mice, cortical secretions formed by grafted adrenals counteracted the growth processes in articular cartilage and thus decreased the incidence of degenerative joint disease

caused by anterior hypophyseal homeografts (Silberberg et al., 1954).

2. 17-Ketosteroids

The output of 17-ketosteroids is increased under pathological conditions associated with adrenal cortical hyperplasia or benign or malignant cortical neoplasms (Hurxthal and Musulin, 1953). The skeletal effects exerted by these ketosteroids are identical with those by 17-ketosteroids of testicular origin.

3. Mineralocorticoids

Administration of desoxycorticosterone or similar mineralocorticoids slightly inhibited body growth of chicken embryos (Stock et al., 1951).

4. Glucocorticoids

Cortisone and presumably other steroids of this group are ineffective until the eight or tenth day of chick incubation (Karnofsky et al., 1950). In young rodents, cortisone inhibited linear growth (Wells and Kendall, 1940) by interfering with proliferation of the epiphyseal cartilage and with production of cartilaginous matrix. The primary spicules were

poorly ossified, inadequately resorbed, and may persist for an abnormally long period (Follis, 1951). In accordance with the retarding effect of cortisone on growth of connective tissue, callus formation is delayed (Blunt et al., 1950).

Cortisone also increases the incidence of malformations, in particular cleft palate in newborn mice whose dams were treated with high doses of hormone during pregnancy (Fraser and Fainstat, 1951). It has been suggested that cleft palate may result from regressive changes and resorption of bone by cortisone.

Experimental data are either absent or scarce regarding the effects of cortisone on adult bone. Glucocorticoids have been implicated as a cause of senile osteoporosis (Albright, 1947) and of osteoporosis associated with Cushing's syndrome (Cushing, 1932). Cushing syndrome is characterized by overproduction of glucocorticoids. The anti-anabolic glucocorticoids are supposed to prevent formation of osseous matrix, and thus shift the balance between formation and resorption of bone in favor of the latter. However, few microscopic studies carried out on bones of patients with Cushing's syndrome revealed neither decreased osteogenesis nor increased osteolysis (Follis, 1950). Evidence is accumulating that

adrenal cortical steroids produce major changes in the ground substance and fibrils of ordinary connective tissue (Miner and Hechter, 1953), and it may be only a question of time before such changes can be demonstrated in cartilage and bone.

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INTRODUCTION

A source of wastage well-recognized by abattoir personnel but perhaps overlooked by stockowners, animal scientists and veterinarians is slaughter of pregnant cows. The wastage is of concern to the federal and state governments but the incidence is unknown. The relationship of pregnancy and ovarian status of slaughtered cows to age, season and carcass weight are also unknown.

During collection of Zebu embryos and fetuses for studying development of the appendicular skeleton, records were kept of pregnancy wastage encountered in slaughter slabs around Zaria. Reported here are the results of these observations.

MATERIALS AND METHODS

Location and Time: The study was conducted at slaughter slabs in Samaru, Sabon Gari, and Zaria City from September, 1975 to November, 1976. This period included the four seasons; pre-dry (October - December), dry (January - March), pre-rain (April - May) and rain or wet (June - September).

Cows: Cows were randomly selected during daily visits (Monday to Friday) to the three slaughter slabs and the breed recorded. The cows originated from Sokoto, Katsina, Kano, Bornu, and Zaria.

Age: Age was estimated by examining the incisor teeth (Sisson and Grossman, 1959). The cows were divided into three age groups: young-- temporary teeth to 6-tooth (less than 3.5 years); mature -- full-mouth with little wear (3.5 to 7 years); and old-moderate to excessive wear (greater than 7 years).

Pregnancy Status: Pregnancy was assessed visually and by palpation of the exposed uterus after slaughter. Cows were recorded as non-pregnant or pregnant in first, second or third trimester. Uteri were dissected to confirm early pregnancies. The stage of pregnancy was estimated by fetal total contour length (nose to tip of the tail) and weight according to criteria developed from known aged Zebu embryos and fetuses removed by Caesarian section (Part III).

The ovarian status of non-pregnant cows was determined by examining both ovaries for presence of corpora lutea and developing follicles and was recorded as active or anestrus.

Carcass Weight: Cold carcass weight ("hot" or recently slaughtered weight less three per cent) was recorded whenever possible.

Herd History: Owners were interviewed to determine the main reasons for culling cows for slaughter. Questions included: lactation status prior to slaughter, whether calves had been weaned within previous one to six months, was rectal palpation used as an aid for culling, and grazing conditions in the state of origin at the time of culling.

Data Processing and Statistical Analysis: Herd and individual cow data was recorded on punch cards and analysed by computer. Differences in carcass weight between age, stage of pregnancy and ovarian activity were assessed by student's "t" test.

RESULTS

Of the 7,941 cows examined, 4,041 (50.9% were pregnant when slaughtered. The incidence, maternal age, stage of pregnancy, and seasonal relationship are given in Tables 1-3.

The breeds involved were Rahaji (65%), Bunaji (30%), and Wadara (5%).

The approximate age of 6,324 of the cows was determined. The age distribution was: 2-tooth 18 (0.3%), 4-tooth 23 (0.4%), 6-tooth 402 (6.3%), full-mouth without wear 992 (15.7%), full-

mouth with some wear 3,006 (47.5%), incisors well-worn 1,345 (21.3%), and incisors worn to the gums 538 (8.5%). Composite grouping revealed that 7.0 per cent of the cows were young, 63.2 per cent mature, and 29.8 per cent old.

Pregnant Cows

Age: Relationship of age to pregnancy (Table 2) was highly significant between young, mature, and aged cows ($P < 0.005$), and highly significant between mature plus aged and young cows ($P < 0.005$).

Season and Stage of Pregnancy: The percentage of pregnant cows slaughtered did not vary significantly with the seasons (Table 1). The relationship of season to stage of pregnancy was not significant (Table 3). Differences between the three stages of pregnancy at slaughter were highly significant ($P < 0.005$); 67 per cent of the cows were in the second trimester. The highest percentage in the first, second, and third trimesters were found in pre-dry, pre-rain and rain seasons, respectively; the lowest percentage in pre-rain, pre-dry, and dry seasons, respectively.

Carcass Weight: The relationship of cold carcass weight to stage of pregnancy, ovarian activity in non-pregnant cows, and

age are given in Table 6. There was little difference in carcass weight between the three age groups and the stage of pregnancy.

Non-Pregnant Cows

The relationship of age and season to ovarian status is given in Tables 4 and 5.

There was no significant difference in ovarian status and age. The incidence of anestrus increased with age.

Ovarian activity differed significantly between seasons ($P < 0.05$), being highest during the rain season. Differences between rain season and the three drier seasons were highly significant ($P < 0.01$). The incidence of anestrus was highest during the dry season and lowest during the rain ($P < 0.01$).

Anestrus cows and non-pregnant cows with active ovaries had the lowest mean (135 $\pm$ 41 kg) and the highest mean (170 $\pm$ 43 kg) carcass weights, respectively (Table 6); the differences being significant ($P < 0.01$). When age was considered together with pregnancy and ovarian status, aged anestrus cows had the lowest carcass weight (127 $\pm$ 28 kg) and the mature cows with active ovaries had the highest (180 $\pm$ 47 kg).

The relationship of carcass weight to age of 4,229 cows is outlined in Fig. 1.

Herd History: Interviews with the cattle owners were unsatisfactory as the great majority were buyers who had little or no history of the herd or area of origin. None had cows pregnancy diagnosed prior to slaughter or knew of rectal palpation being used as an aid for culling from the herds from which they purchased their cattle.

DISCUSSION

Daily collection of fetuses quickly confirmed a problem of pregnancy wastage in cattle. That almost 75 per cent of the pregnant cows slaughtered were in the second and third trimesters is serious because these pregnancies could have been simply and readily detected by rectal palpation prior to sale and/or slaughter. Detection and retention of pregnant cows by the original owners or by female recovery schemes would contribute considerably towards meeting the meat production target for Nigeria. It is recommended that this source of wastage of urgently needed meat be reduced by pregnancy diagnosis being made readily available by the appropriate authorities.

The magnitude of the problem of pregnancy wastage in cattle

in Nigeria may be extrapolated from this study. The total number of cattle slaughtered annually at the three slaughter slabs of Zaria is approximately 24,000. Allowing for half being females, then half or 6,000 would be pregnant. It is reasonable to deduct 2,000 for aged cows and other inescapable cullings. This leaves an annual wastage of 4,000 fetuses. This figure may not appear large but when multiplied by expected similar results from other slaughter establishments around the Federation, the potential wastage is a problem for major concern and positive action.

The finding that the maximum percentage of cows pregnant in the first, second and third trimester occurred during October-December, January-March, and June-September, respectively, is in general agreement with previous findings in the northern states of Nigeria, that conceptions are highest during July and August (Osori, 1976). Jochle (1972) found that most Zebu cows in the Mexican Gulf coast tended to conceive during the rainy season (June-October). That less pregnant cows were slaughtered during the third trimester suggests that owners and buyers were able to detect heavily pregnancy cows by visual appraisal.

With regards to carcass weight in relation to age <u>per se</u>, the finding that carcass weight appeared to be highest in cows

four to six years-old differed with the results of Brinks et al. (1962) in the U.S.A. who found that the weight of Hereford range cows nursing calves increased up to eight years of age. The obvious decrease in carcass weight after maturity of cows in the present study is probably due to breed difference, poorer management and harsher environmental conditions.

A higher pregnancy rate in cattle in good condition is expected (Donaldson et al., 1967); therefore, the apparent increase in carcass weight with advancing pregnancy during the dry and pre-rain season, when pastures were scarce and of declining quality, was unexpected. It was anticipated that cows slaughtered during the last trimester of pregnancy would have had decreased carcass weights.

Possibly the carcass weight differences associated with pregnancy were attributable to the lactation status, which was not recorded. However, in one extensive study in the southern U.S.A., lactation was not necessarily associated with decreased body weight (Fitzhugh et al., 1967); in fact, most cows were found to gain weight from parturition to weaning and then lost weight from weaning to parturition.

Herd history was relatively meaningless as the owners were recent buyers of the cattle. Some owners were co-operative and

others not, and others refused to supply any information without payment.

Finally, it should be emphasised that studies involving slaughter establishments in Nigeria are difficult for a number of reasons: attitude of slaughter personnel; methods of payment of slaughtermen by by-products and certain organs (often one of the specimens required); method of slaughter; difficulty of arranging co-operation with all concerned; difficulty in obtaining and maintaining co-operation for even a short period of time; specimens increase in price until prohibitive; inability to examine parts of the carcass, for example, udder and at times, teeth; what you can do and obtain one day you may not be able to the next; continual fear that you may not be able to complete collecting the minimum number of specimens required for the study, etc. In this study, the lactation status of each cow could not be observed because the method of slaughter and degree of co-operation; the age was determined by examining the incisors in 79.6 per cent of the cows, and carcass weight was obtained in only slightly more than half the cows examined (53.3%). The above difficulties were constant handicaps in this study and these must be overcome before studies involving slaughter house specimens can become routine

and meaningful to animal health and production in Nigeria.

SUMMARY

Of 7,941 Zebu cows examined at three Zaria slaughter slabs, 4,041 (50.9%) were pregnant when slaughtered. The breeds involved were Rahaji (65%), Bunaji (30%), and Wadara (5%). The age distribution was estimated by teeth examination to be young -- 7 per cent (less than 3.5 years); mature -- 63.2 per cent (3.5 to 7 years), and old -- 29.8 per cent (over 7 years).

Relationship of age to pregnancy was highly significant between the three age groups and between mature plus aged and young ($P < 0.005$). The relationship of season to the stage of pregnancy was not significant. The differences between the three stages of pregnancy at which cows were slaughtered were highly significant ($P < 0.005$); the majority (67%) being in the second semester. There was little difference in carcass weight between the three age groups and the stages of pregnancy.

Ovarian activity was highest during the rain season and anestrus highest during the dry season ($P < 0.01$). Anestrus cows and non-pregnant cows with active ovaries had the lowest mean

(135 ± 41 kg) and the highest mean (170 ± 43 kg) carcass weights, respectively. Aged anestrus cows had the lowest carcass weight (127 ± 28 kg). and mature cows with active ovaries had the highest (180 ± 47 kg).

The magnitude of the pregnancy wastage problem in cattle in Nigeria was discussed and it was recommended that pregnancy diagnosis per rectum be made available prior to culling or slaughter in order to reduce this major source of meat loss.

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Table 1: Incidence of pregnancy in Zebu cows slaughtered in Zaria between September, 1975 and November, 1976.

Season	Cows			
	No. Pregnant	No. Empty	Total Examined	% Pregnant
Pre-dry	902	1,381	2,283	39.5
Dry	1,259	812	2,071	60.8
Pre-rain	1,078	811	1,889	57.1
Rain	802	896	1,698	47.2
Total	4,041	3,900	7,941	51.1

Table 2: Relationship of age to stage of pregnancy of Zebu cows slaughtered in Zaria between September, 1975 to November, 1976.

Age Group*	Stage of Pregnancy						Pregnant cows examined	
	1 - 3 mths		4 - 6 mths		7 - 9 mths			
	No.	%	No.	%	No.	%	No.	%
Young	153	19.4	594	75.4	41	5.2	788	19.5
Mature	498	25.9	1,342	69.9	81	4.2	1,921	47.5
Old	399	30.0	772	57.9	161	12.1	1,332	33.0
Total	1,050	26.0	2,708	67.0	283	7.0	4,041	

* Young (< 3.5 yrs); Mature (3.5-7 yrs); and Old (> 7 yrs).

Table 3: Relationship of season to stage of pregnancy in Zebu cows slaughtered in Zaria between September, 1975 to November, 1976.

Season	Stage of Pregnancy						No. pregnant cows examined
	1-3 mths		4-6 mths		7-9 mths		
	No.	%	No.	%	No.	%	
Pre-dry	336	37.3	486	53.9	80	8.8	902
Dry	304	24.2	918	72.9	37	2.9	1,259
Pre-rain	168	15.6	865	80.2	45	4.2	1,078
Rain	242	30.2	439	54.7	121	15.1	802
Total	1,050	26	2,708	67	283	7	4,041

Table 4: Relationship of age to ovarian activity in non-pregnant Zebu cows slaughtered in Zaria between September, 1975 and November, 1976.

Age Group*	Ovarian Status				No. empty cows examined
	Active		Anestrus		
	No.	%	No.	%	
Young	355	71	145	29	500
Mature	1,687	74	593	26	2,280
Old	661	59	459	41	1,120
Total	1,703	68	1,197	32	3,900

* Young (<3.5 yrs)
Mature (3.5-7 yrs)
Old (>7 yrs)

Table 5: Seasonal relationship of ovarian activity in non-pregnant Zebu cows slaughtered in Zaria between September, 1975 and November, 1976.

Season	Ovarian Status				No. empty cows examined
	Active		Anestrus		
	No.	%	No.	%	
Pre-dry	1,166	62	215	38	1,381
Dry	456	56	356	44	812
Pre-rain	567	70	244	30	811
Rain	783	87	113	13	896
Total	2,972	70	928	30	3,900

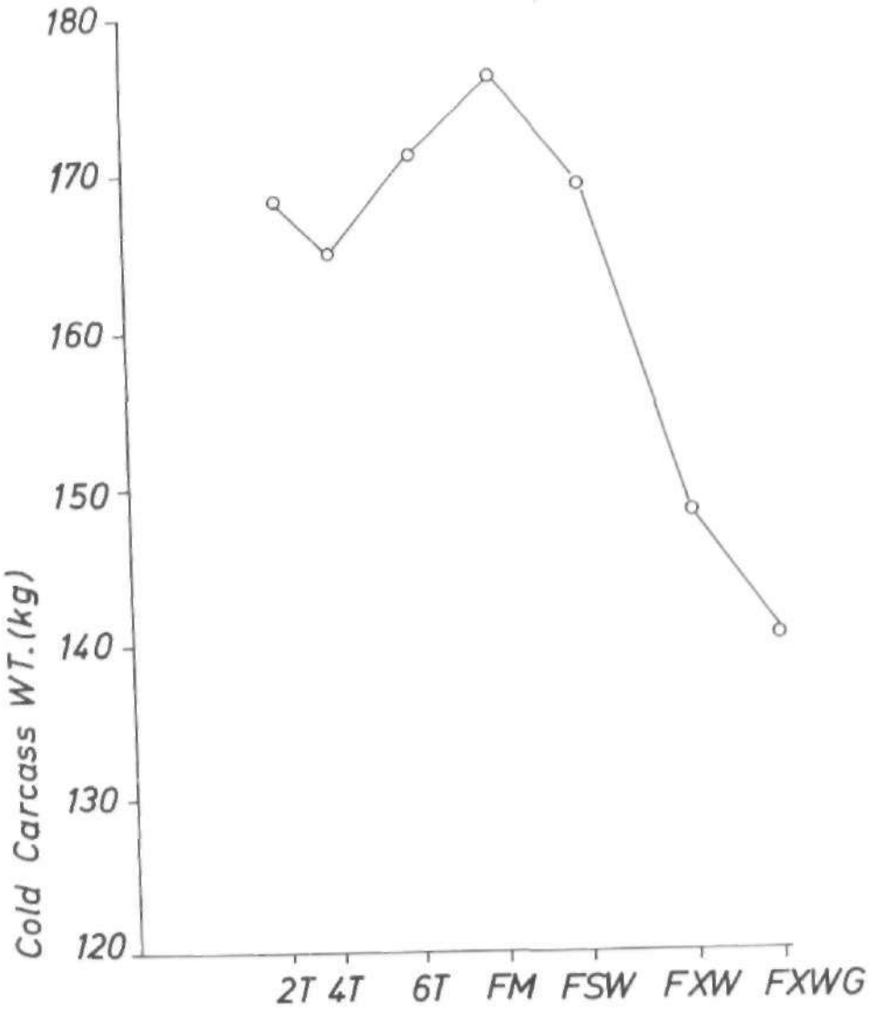
Table 6: Relationship of cold carcass weight to age, stage of pregnancy and ovarian activity in Zebu cows slaughtered in Zaria between September, 1975 and November, 1976.

Age Group*	Stage of Pregnancy						Non-Pregnant Cows					
	1-3 mths		4-6 mths-		7-9 mths		Active Ovaries		Anestrus		Cows examined	
	No.	Wt.(kg)	No.	Wt.(kg)	No.	Wt.(kg)	No.	Wt.(kg)	No.	Wt.(kg)	No.	Wt.(kg)
Young	88	158±32	171	168±34	43	176±35	186	169±38	72	136±42	574	164±38
Mature	431	164±38	781	167±36	211	173±36	538	180±47	185	144±50	2,187	169±43
Old	280	148±31	548	151±30	144	150±28	310	152±32	179	127±28	1,468	147±31
Total	799	157±36	1,500	161±35	398	165±35	1,034	170±43**	436	135±41	4,229	161±40

* Young (<3.5 yrs); Mature (3.5-7 yrs); and Old (>7 yrs)

** P 0.01

Fig. 1. Relationship of carcass weight to age of 4,229 slaughtered Zebu cows.



2T – 6T = 2 to 6 teeth erupted respectively
FM = Full mouth
FSW = Full mouth with some wear
FXW : FXWG = Excessive wear: Worn to gum respectively

PART III

AGE DETERMINATION IN PRENATAL ZEBU CATTLE OF NORTHERN NIGERIA

INTRODUCTION

It is remarkable that there appears to be no literature on ageing Bos indicus fetuses. In general, data concerning accurate ageing of Bos taurus embryos and fetuses are sparse (Bergmann, 1922; Winters et al., 1942; Nichols, 1944; Swett et al., 1948). Nichols (1944) and Swett et al. (1948) reported weights and body lengths of bovine fetuses, and Bergmann (1922) and Winters et al., (1942) included various measurements in addition to weight of developing fetuses. By studying the available literature, Jakobsen (1957) derived a method for estimating fetal age from weight, length and maturation signs. By correlating fetal weight, body length and head circumference Thomsen (1975) derived a mathematical method for calculating fetal age.

In recent years, attempts have been made to put this important aspect of comparative embryology on a sounder basis but owing to shortage of suitable specimens much remains to be done (Gjesdal, 1969; Mancely, 1952; Rexroad et al., 1974; Thomsen,1975). There are several reasons for the shortage including economic and humanitarian. Also, when specimens are available, the service dates are usually not and although

internal parasites and sprayed with pfizona[2] for ticks and then routinely throughout the experimental period. The cattle were identified by ear-tags. They were kept in a large paddock and were taken out to graze daily between 8.00 a.m. and 2.00 p.m. During the dry season they were supplemented with hay and 1 kg guinea-corn per head daily. As the aim was to maintain the herd under husbandry conditions similar to those of Fulani herdsmen, feed was kept simple and the cattle fed sufficiently to supply minimal body protein requirements.

The bulls ran with the cows. The cows were carefully watched for signs of estrus which were recorded.

Fetuses

Twenty fetuses were collected by Caesarian section from August, 1975 to December 1976: two per gestational month plus an extra one for both the fourth and fifth month. Fetal age was determined from 6.00 p.m. on the day of service to the time Caesarian section was performed. All pregnancies were confirmed by rectal palpation.

Fetuses were recovered by Caesarian section via a flank or midline incision under local anesthesia with 2 per cent

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[2] Pfizer Products Ltd., P.M.B. 111, Ikeja, Nigeria.

Versicaine[3] and the umbilical vessels tied off.

Fetal Measurements

Fetuses were weighed (WT) to the nearest 10 mg with a Mettler balance[4] after excess fluid was removed by paper towels. Each fetus was stretched out on a flat tray so that the nasal bones were at right angles to the long axis of the body. The following fetal measurements to the nearest mm were recorded: forehead - rump (FR), cranial length and width, cephalic index (CI), total body contour length (TCL), length of femur (LF), and length of humerus (LH).

Criteria for these fetal measurements were (Fig. 1):

FR - Most anterior part of the frontal bone to the space between the last sacral and first coccygeal vertebrae.

CI - Calculated from cranial length (nose to base of the occipital bone) and cranial width (zygomatic arch to zygomatic arch).

TCL - From nose to tip of the tail.

LF - Length from greater trochanter to lateral condyle of

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[3] Lignocaine hydrochloride. May and Baker Ltd., Dagenham, Essex, England.

[4] Models P163N and P10N. Mettler Instrument A.B., Zurich, Switzerland.

dissected femur.

LH - Length from head to lateral condyle of dissected humerus.

Sex, breed and season of collection of the 20 fetuses and the 250 slaughter fetuses were also recorded.

Statistical Analysis

The data collected were recorded on punch cards and analysed by computer using regression , standard least square analyses, and estimate of 95 per cent tolerance limit for level of significance ($P < 0.05$).

RESULTS

Data of fetal measurements, sex and season of the 20 Rahaji fetuses of known age recovered by Caesarian section are summarised in Table 1. The correlation coefficients of the seven variables were significant ($P < 0.05$) except cephalic index (Appendix, Fig. 1) which was negatively correlated with the other variables ($P < 0.05$).

Correlations were linear between log WT and log FR, log TCL,

log LF, long LH and Log Age (Figs. 2-6). Regression lines were from standard least square analyses.

These correlations (Appendix, Table 1) can be expressed by:

$$Y = X^{b}.q \qquad \text{(i)}$$

where b is the regression coefficient from the corresponding log - log plot ($\log Y = b.\log X + \log q$) and q is a constant. Applying this regression equation to a growing Zebu fetus when Y is a three-dimensional measure (e.g. WT) and X is a one-dimensional measure (e.g. length), b can be anticipated to have a value of 3.

In this study, $b = 2.67 \pm 0.08$ which closely agreed with expectation. Applying this to the relationship between log WT and log TCL (Fig. 3), the following equation emerged:

$$\log WT = -2.4286 + 2.6746 \log TCL \qquad \text{(ii)}$$

With the other variables, the following equations were similarly derived.

$$\log WT = -3.1498 + 2.6319 \log FR \qquad \text{(iii)}$$

$$\log WT = -1.3962 + 3.1372 \log CL \qquad \text{(iv)}$$

$$\log WT = -4.6276 + 1.3379 \log LF \qquad \text{(v)}$$

$$\log WT = -3.1959 + 2.4993 \log LH \qquad \text{(vi)}$$

$$\log WT = -2.4994 + 3.1959 \log Age \qquad \text{(vii)}$$

The validity of these expressions was tested with the 20 Rahaji fetuses of known age. The regression equation for body-weight and total body contour length was the most accurate for predicting body weight ($P < 0.05$). Regression equation for body weight and fetal age was best for estimating age of a Zebu fetus of known weight ($P < 0.01$).

There was little change in the cephalic index of the growing Rahaji fetus except for a distinct peak at three months (Fig. 7).

There was good correlation between FR and age (Fig. 8) in the developing Rahaji fetus ($P < 0.05$). From this correlation a scale was made for estimating gestational age (Fig. 9).

No significant differences were found for the seven variables due to sex or breed when ageing the 250 Zebu fetuses obtained at slaughter (Appendix, Tables 2-18). A significant increase ($P < 0.05$) was observed in fetal weight and length of femur and humerus during the wet season (Appendix, Table 11).

DISCUSSION

Prenatal development of farm animals, with the exception

of the pig (Marrable and Ashdown, 1967), has received little attention. A more complete knowledge of this phase of intrauterine animal existence is desirable not only to study normal variations in development but also to establish a basis for studying factors causing deviations from normal development. Before the effects of nutrition, genetics and teratogens on production of abnormal, dead or weakened neonates can be completely understood, a standard of normal development must be established. Otherwise it might be difficult to determine the nature, pathogenesis and importance of any developmental change.

From an economic standpoint, beef cattle raised for market spend a good portion of their lives in utero, nearly one-third. If more were known about the prenatal phase of their existence, perhaps some of the problems that arise during extrauterine life could be more easily explained.

Thomsen (1975) observed linear correlations between log maximum head circumference, log body length and log weight and derived mathematical expressions concerning gestational age of Bos taurus fetuses. He found these relationships applicable for re-examining data from earlier work by Bergmann (1922), Nichols (1944), and Jakobsen (1959). Bergmann (1922) measured body length from the mouth to tuber coxael, Nichols (1944) from crown

to rump, and Thomsen (1975) from nose to tailroot, and Jakobsen (1959) defined maximum head circumference. Both Bergmann and Thomsen used slaughter fetuses of unknown gestational age. The least square analyses of the Zebu fetuses compared favourably with Thomsen's findings allowing for breed differences except for cephalic index.

It is surprising that no statistical difference was observed in the three breeds of Zebu cattle studied regarding the variables especially weight. Asdell (1946) stated that breed has a definite effect on the birth weight. As the three Zebu breeds were similar in size and conformation, breed differences had little effect during gestation.

Although work on other domestic animals has revealed conflicting observations on the effect of sex on weight and body length measurements (Wrathall et al., 1974), no sex differences were observed in the fetal variables studied. The increase in weight observed during the wet season can be explained by better climatic conditions and adequate good quality forage.

The relative value of body length and weight to age is worth considering. Increase in length is due to increase of cartilage and bone in a particular direction and tends to be

more uniform than increase in weight over a similar period of time. Irregular increase in weight has been attributed to differential growth rates of various organs and tissues (Wallace, 1945). He found in sheep that organs such as kidney, liver, lungs, urinary bladder, and brain made a greater proportion of their growth earlier in gestation than other organs. Apparently similar work has not been done in cattle.

The aim of this study was to examine easily measurable fetal data which could correlate significantly with age and from these relationships derive a method for calculating the age of Zebu fetuses for both experimental and field use. The regression equation for body weight and body length (TCL) was found to be the most accurate for predicting fetal weight and that body weight and fetal age was the best for estimating age of Zebu fetuses of known weight. From the non-log correlation between body length (FR) and age of developing Rahaji fetuses, a simple scale was devised for estimating gestational age of fetuses in the field.

SUMMARY

Data on seven easily measured variables (WT, FR, TCL, LF,

LH, CI and Age) from 20 Rahaji embryos/fetuses recovered by Caesarian section and from 250 embryos/fetuses collected from slaughtered Rahaji, Bunaji and Wadara cows are presented. Data from the 20 Rahaji embryos/fetuses were used for establishing a normal growth curve and linear correlations for ageing the 250 slaughter fetuses. The correlations between the variables were significant except for the cephalic index ($P < 0.05$). The regression equation for body weight and body length (TCL) was found to be the most accurate for calculating fetal weight and that body weight and fetal age was the best for estimating age of Zebu fetuses of known weight. From the non-log correlation between body length (FR) and age of developing Rahaji fetuses, a simple scale was devised for estimating fetal age in the field. No significant differences for sex or breed but a significant increase was found in body weight and length of femur and humerus during the wet season ($P < 0.05$).

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Table 1: Fetal variables of 20 known age fetuses of Rahaji breed in Nigeria.

No.	WT	FR(cm)	TCL(cm)	LH(dm)	LF(cm)	CI(cm)	Season	Sex	Age (mths
1	3.00g	2.50	3.80	0.03	0.04	3.5	Wet	M	1
2	1.55	2.49	3.15	0.02	0.03	4.0	Dry	F	1
3	15.00	8.60	10.50	0.80	0.85	3.6	Dry	F	2
4	15.40	8.80	10.80	1.00	1.00	2.9	Wet	F	2
5	110.00	15.30	20.60	1.80	2.00	4.7	Wet	M	3
6	115.00	14.98	19.40	1.80	2.10	4.8	Dry	F	3
7	475.00	24.01	35.50	3.00	3.30	2.6	Dry	M	4
8	492.00	24.50	39.00	3.00	3.20	2.8	Wet	F	4
9	478.00	24.00	35.50	3.00	3.40	2.6	Dry	F	4
10	1.050kg	34.95	50.00	4.20	8.50	2.2	Dry	M	5
11	1.100	35.00	49.50	4.30	8.50	3.7	Wet	M	5
12	1.105	35.20	50.00	6.50	8.50	2.3	Wet	F	5
13	3.185	48.20	73.50	7.50	9.10	2.9	Dry	F	6
14	3.200	48.50	72.00	7.50	9.00	2.8	Wet	F	6
15	9.500	63.50	86.00	8.60	11.00	2.9	Wet	M	7
16	9.200	63.01	84.75	8.80	10.60	2.9	Dry	F	7
17	12.205	80.20	105.00	13.00	10.80	3.1	Wet	M	8
18	12.000	81.00	105.50	13.10	11.00	3.1	Dry	M	8
19	20.500	99.00	117.60	17.50	16.20	3.0	Wet	M	9
20	22.200	97.50	115.50	17.00	17.50	2.9	Dry	F	9

Fig. 1: Diagram illustrating landmarks for fetal measurements.

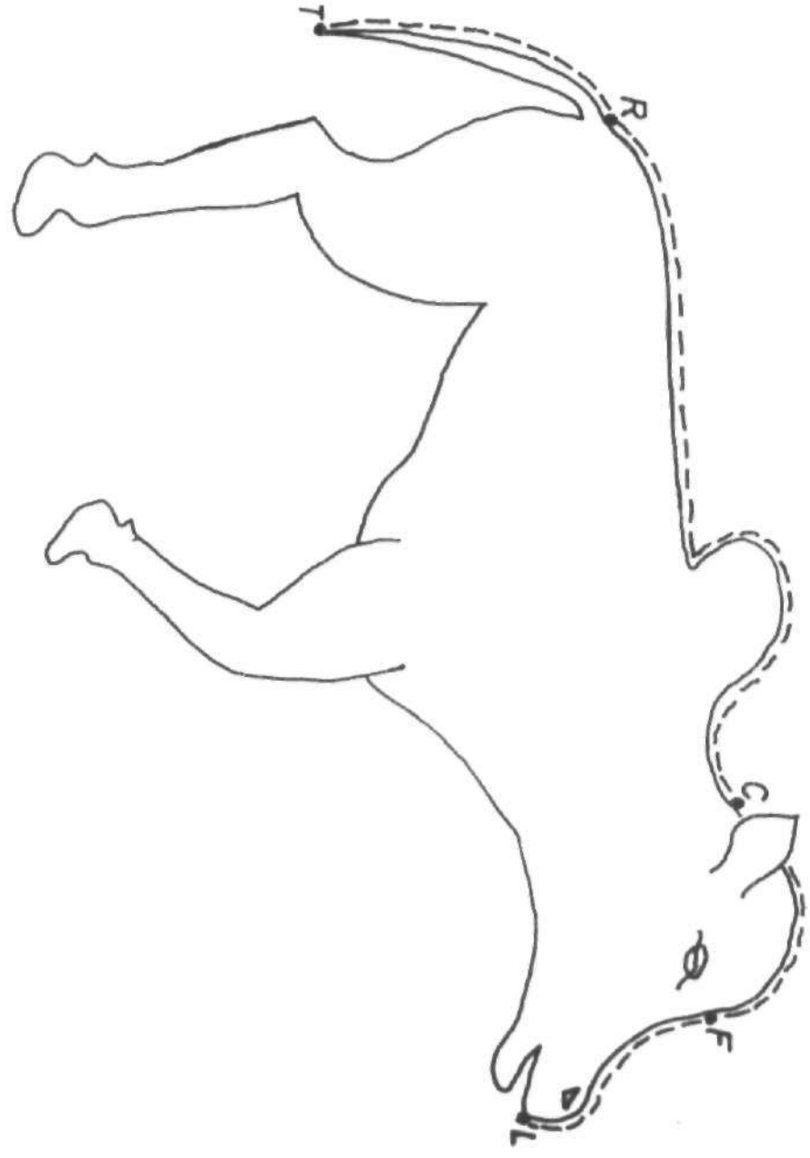

R
C
F
TL

Fig. 2: Log of weight (W) plotted against log of forehead-rump body length (FR).

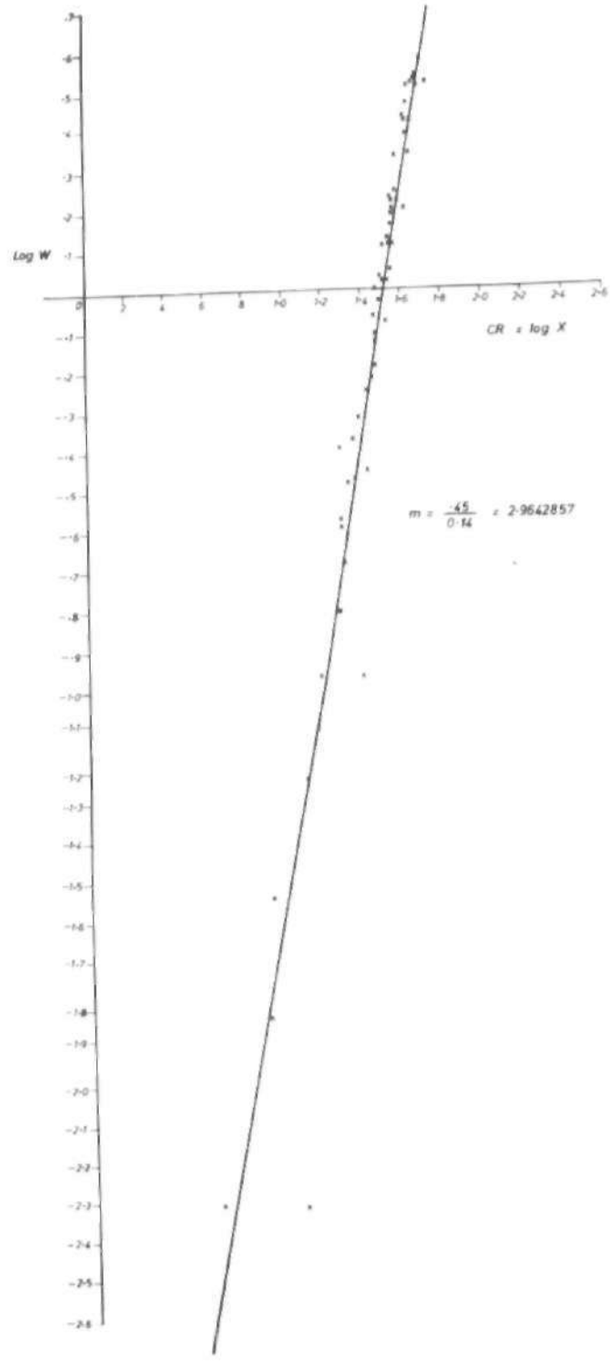
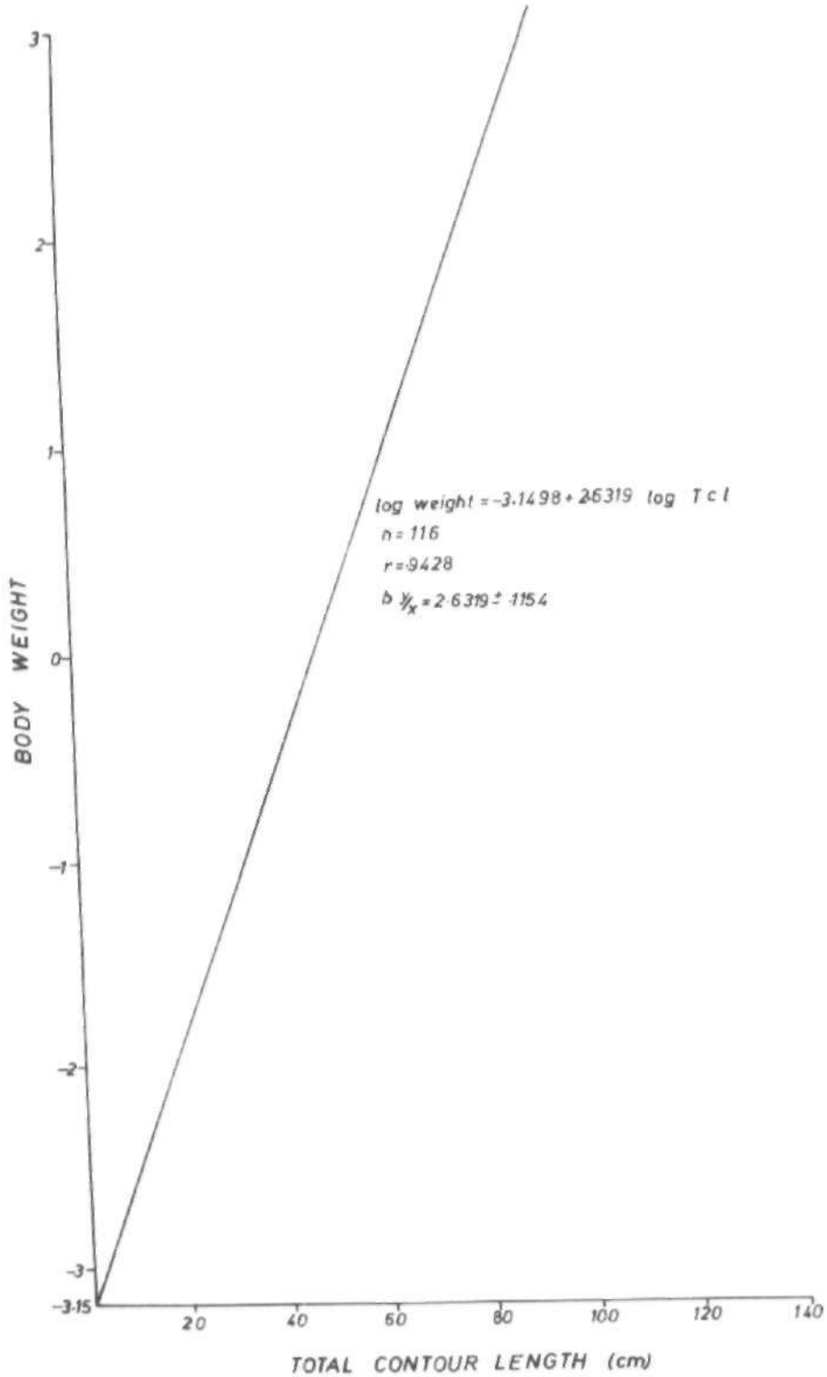
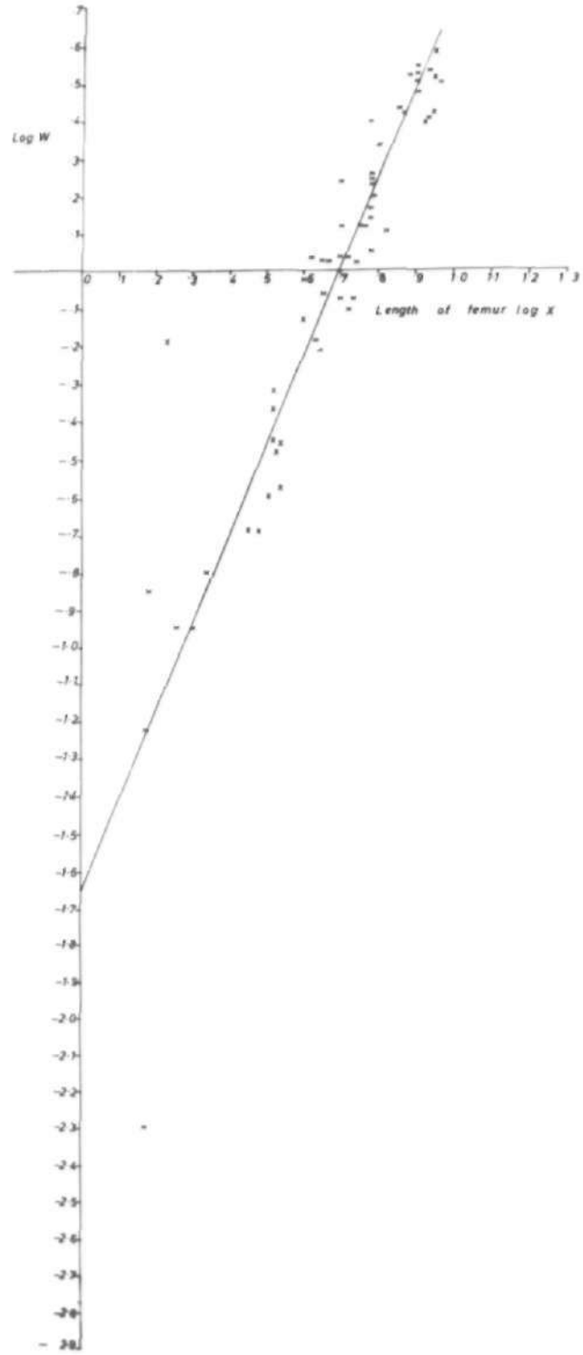

Log W
CR = log X
m = -45 / 0·14 = 2·9642857

Fig. 3. Log of weight (W) plotted against log of total body length (TCL).


log weight = -3.1498 + 2.6319 log T c l
n = 116
r = .9428
b y/x = 2.6319 ± .1154
BODY WEIGHT
3
2
1
0
-1
-2
-3
-3.15
20
40
60
80
100
120
140
TOTAL CONTOUR LENGTH (cm)


Log W
Length of femur log X

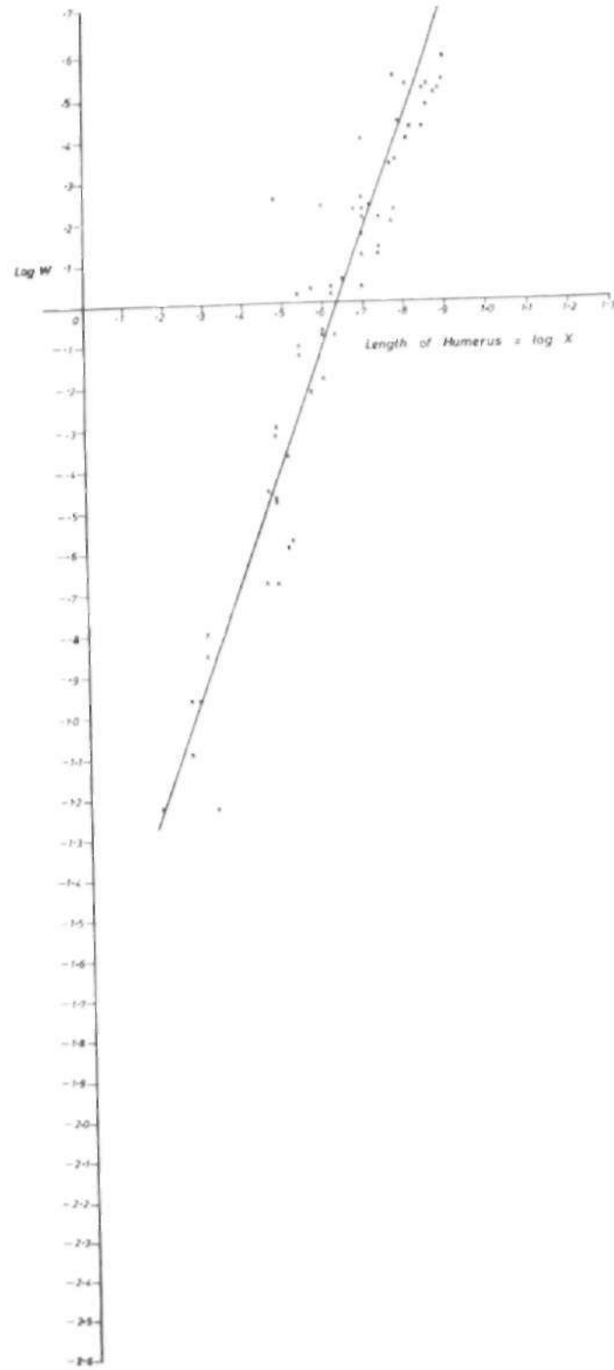

Log W
Length of Humerus = log X

Fig. 6. Log of weight (W) plotted against log age.

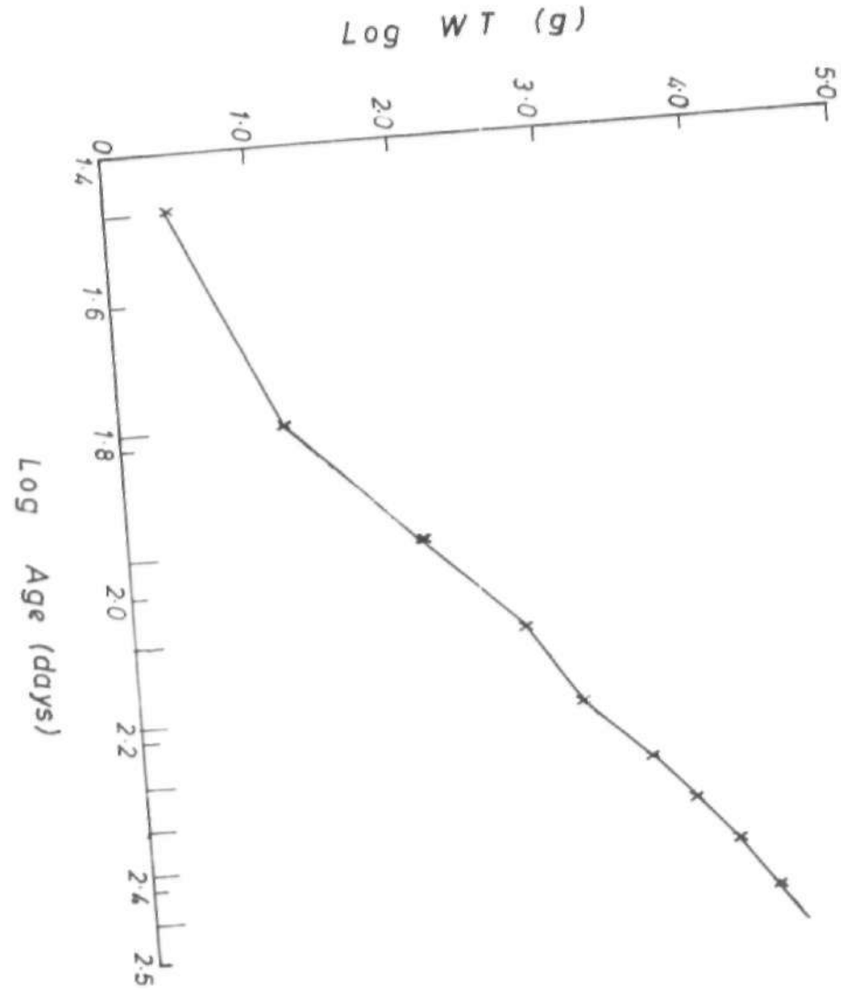

Log WT (g)
0
1·0
2·0
3·0
4·0
5·0
Log Age (days)
1·4
1·6
1·8
2·0
2·2
2·4
2·5

Fig. 7. Cephalic index (CI) plotted against fetal age.

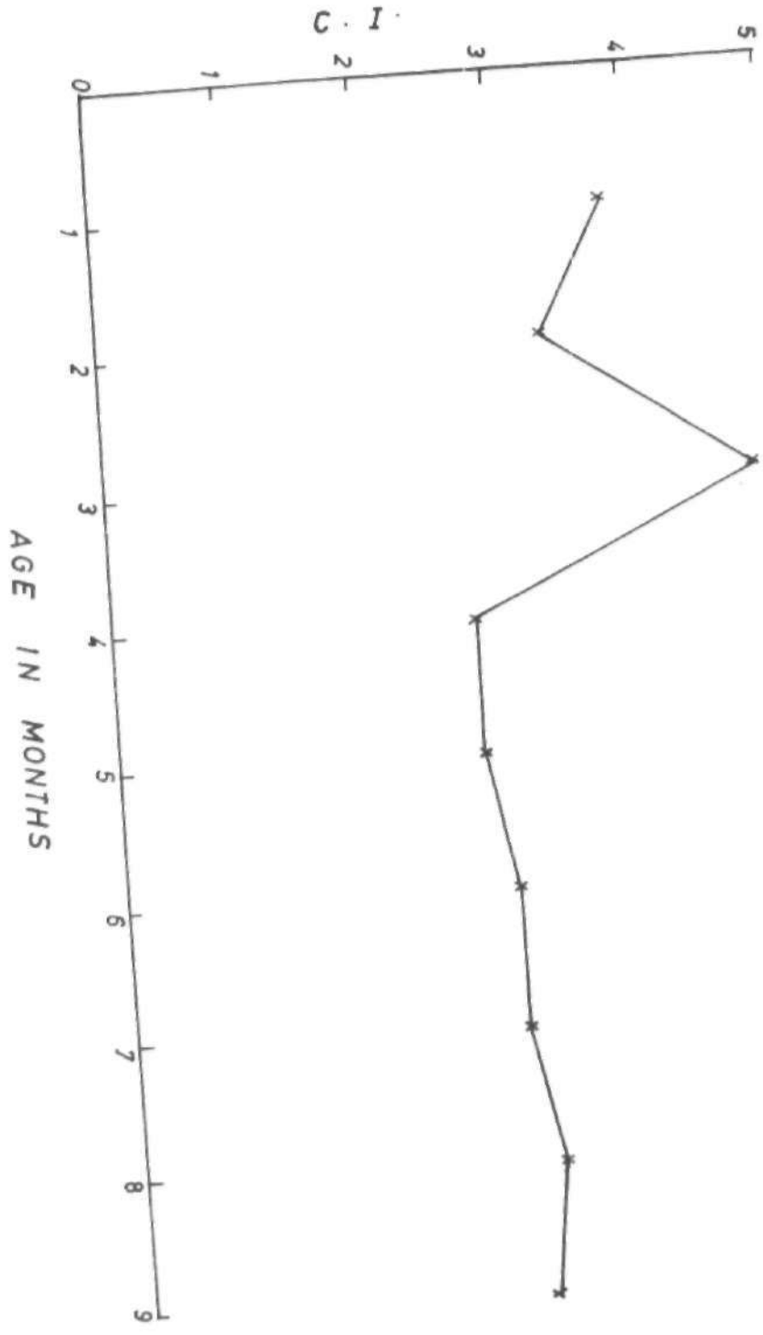

C. I.
0
1
2
3
4
5
AGE IN MONTHS
1
2
3
4
5
6
7
8
9

Fig. 8. Correlation between forehead-rump length and fetal age.

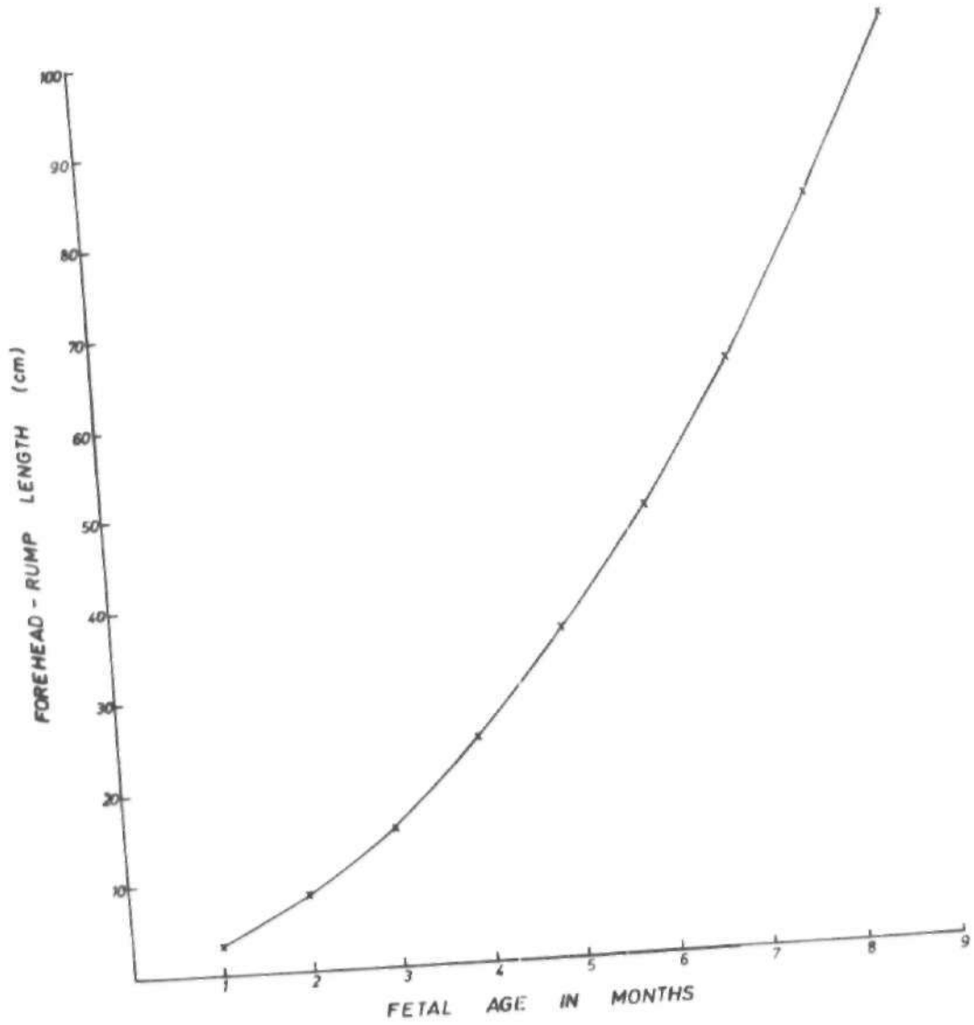
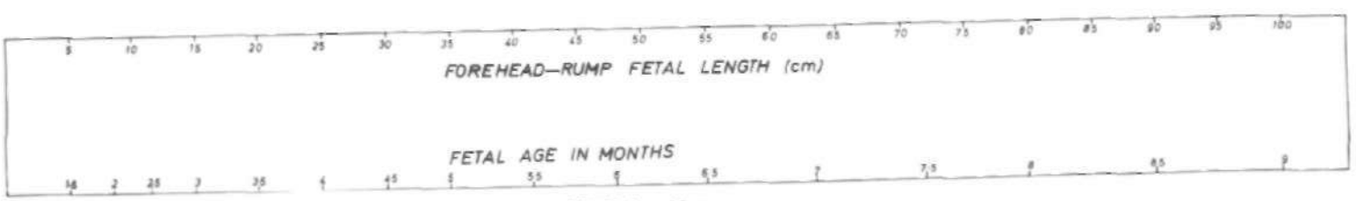

100
90
80
70
60
50
40
30
20
10
FOREHEAD - RUMP LENGTH (cm)
1
2
3
4
5
6
7
8
9
FETAL AGE IN MONTHS

Fig. 9. A scale for estimating the age of developing Zebu fetuses based on forehead-rump length.



SCALE: 1cm = 25cm

PART IV

DEVELOPMENT OF HAIR AND OTHER EXTERNAL FEATURES IN PRENATAL ZEBU CATTLE

INTRODUCTION

Information on hair development in the bovine fetus is restricted to Bos taurus breeds (Bergmann, 1922; Postma, 1947; Goetze, 1960; Jakobsen, 1962).

Bergmann (1922) observed hair growth on the muzzle and around the eyes of bovine fetuses by the end of the fourth month of gestation, and by the middle of the sixth month on the eyelids, at and aroud the base of the horns and on the edges of the ears.

Postma (1947) gave a general account of hair eruption in bovine fetuses. Hair eruption was first observed at 15 weeks on the upper and lower lips and on the eyebrows. The first eyelid cilia (dorsal) were visible intraepithelially at 23 weeks and were erupting during the 24th week. On the tip of the tail and along the edge of the ears hairs were erupting during the 26th week. At the same time hair eruption started around the coronets and below the ventral commissure of the vulva. Small intraepithelial zones of facial hairs and dense hair growth on the inner surface of the ear occurred at 27 weeks. During the 28th week hair eruption was observed over the extremities and around the umbilical region. Short, diffuse

haircoverwas present all over the body at 30 to 33 weeks, and dense and full growth at 35 weeks.

Gotze (1960) summed up the sequence of hair growth reported by other investigators, and stated generally, that age determination based on hair eruption was safer than measurements and weights in "unusual cases".

Jakobsen (1962) described the length of individual hairs on the upper lip of fetuses 122 to 229 days of age, and on the eyelids of fetuses between 194 to 229 days. He observed appearance of hair on the metacarpus and metatarsus in fetuses at 163 and 173 days of age, respectively. He described further hair growth as follows: Hair occurred in the horn region at five to five and half months of age, around the external entrance to the ear at five and half months, around the eyes at six to six and half months, and total hair cover at seven and half months. As a conclusion of his investigations on hair growth and measurements of extremities and head, he stated that hair patterns gave a reliable basis for determining fetal age because they were not influenced by mummification.

Schmaltz (1921) stated that hairs were loosened and lost during maceration.

Reported here are observations of hair development and other

external features of Zebu fetuses collected from local slaughter establishments.

MATERIALS AND METHODS

Fetuses

One hundred and fifty fetuses, 100 to 231 days of age, from slaughtered Rahaji, Bunaji and Wadara cows were used for studying hair development, and all 270 fetuses for investigating the external features (Part III). All gross observations were made within one to four hours postmortem.

Hair Growth

Observations of hair eruption and growth were made of specific areas illustrated in Fig. 1. Intraepithelial hair growth was assessed grossly and microscopically in hematoxylin and eosin stained sections. Records were kept of the first observation of intraepithelial growth and up to 20 mm length after eruption or day 190 of gestation.

Other External Features

Sequential development of the following external features was recorded in developing Zebu embryos and fetuses from day 24 to 230 gestation: limb buds, mammary ridge, olfactory pits, eyes, eyelids, groove between digits, ear pinnae, facial cleft closure, genital tubercle, teats, scrotum, testicular descent, hoof formation, horn buds, teeth eruption, fusion of hard palate, carpal and patella separation, and detection of meconium.

RESULTS

The sequence and rate of hair growth in selected body areas of the developing Zebu fetus is summarized in Table 1. The development sequence of other external features is given in Table 2.

DISCUSSION

Localisation and pattern of hair growth on *Bos taurus*

fetuses has been recommended for use in fetal ageing (Bergmann, 1922; Postma, 1947; Goetze, 1960; Jakobsen, 1962). Lack of similar information in Zebu breeds prompted this study on the appearance of external features including hair growth as a possible adjunct for ageing Zebu fetuses.

The pattern and sequence of hair growth was found to be indecisive as an aid for ageing Zebu fetuses and inferior to the regression equation based on body weight and length (Part III). Hair eruption and sequence of appearance in different regions of the body is easily observed on fresh fetuses but measuring length of individual hairs and differentiating between intraepithelial hair formation and hairs in the process of eruption may present some technical difficulties. This may result in subjective appraisal even with fresh fetuses.

The initial appearance of external body features in Zebu fetuses could serve as a guide especially in evaluating the age of malformed fetuses. The following easily recognisable features should be of value:

Day 25	C-shaped embryo.
34	Facial clefts closed.
50	Eyelids begin to cover eyes.
60	Eyelids closed, hooves forming, horn buds present.

80 Teats present, hooves beginning to cornify.

83 Scrotum present.

150 Hairs on eyes and muzzle.

187 Hairs on coronets.

197 Eyelids separated.

215 Legs covered with hair.

230 Body fully covered with hair.

It is obvious from the results reported here that more observations on hair and other external features of Zebu fetuses are needed before meaningful comparisons can be made with the information published on Bos taurus breeds. Finally, the observations on the 150 fetuses will find use as a rough but quick method for ageing Zebu fetuses in the field.

SUMMARY

The sequence and rate of hair growth was studied in 150 fetuses, 100 to 231 days of age, from slaughtered Rahaji, Bunaji and Wadara cows. Sequential development of the following external features were observed in embryo and fetuses from day 24 to 230 gestation: limb buds, mammary ridge, olfactory pits,

eyes, eyelids, facial cleft closure, intradigital grooves, ear pinnae, genital tubercle, teats, scrotum, testicular descent, hoof formation, horn buds, teeth eruption, fusion of hard palate, carpal and patella separation, and meconium detection. Pattern of hair growth was found to be indecisive as an aid to ageing Zebu fetuses. Initial appearance of certain external features could serve as a rough but quick ageing guide.

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Table 1: Sequence and rate of hair growth in selected body areas of the developing Zebu fetus.

Area*	Hair growth in mm	Gestational Day
1.	Visible intraepithelially	100 - 115
	Eruption	116 - 123
	3 - 5	125 - 140
	6 - 7	146 - 150
	8 - 10	152 - 158
	10 - 12	161 - 174
	12 - 20	174
2.	Visible intraepithelially	100 - 113
	Eruption	115 - 123
	3 - 5	127 - 140
	6 - 7	142 - 150
	8 - 10	152 - 158
	10 - 12	160 - 174
	12 - 20	178
3.	Visible intraepithelially	100 - 113
	Eruption	152 - 168
	1 - 3	173 - 180
	4 - 5	187 - 192
4.	Visible intraepithelially	100 - 142
	Eruption	145 - 152
	1 - 3	158 - 170
	4 - 7	173 - 192

Table 1: Continued

Area*	Hair growth in mm	Gestational Day
5.	Visible intraepithelially	100
	Eruption	113
	2 - 3	123
	3 - 5	130 - 144
	5 - 8	145 - 156
	7 - 10	160 - 169
	10 - 12	170 - 175
	12 - 15	176 - 180
	16 - 20	180 - 187
	20	190
6.	Visible intraepithelially	187
	Eruption	205
7.	Eruption over horn region	135
8.	Eruption	175 - 180
	2 - 3	180 - 185
	3 - 5	185 - 190
9.	Visible intraepithelially	163
	Eruption	170 - 178
	Dense hairs	180
10.	Eruption	160
	2 - 3	165 - 174
	3 - 5	175 - 185

Table 1: Continued

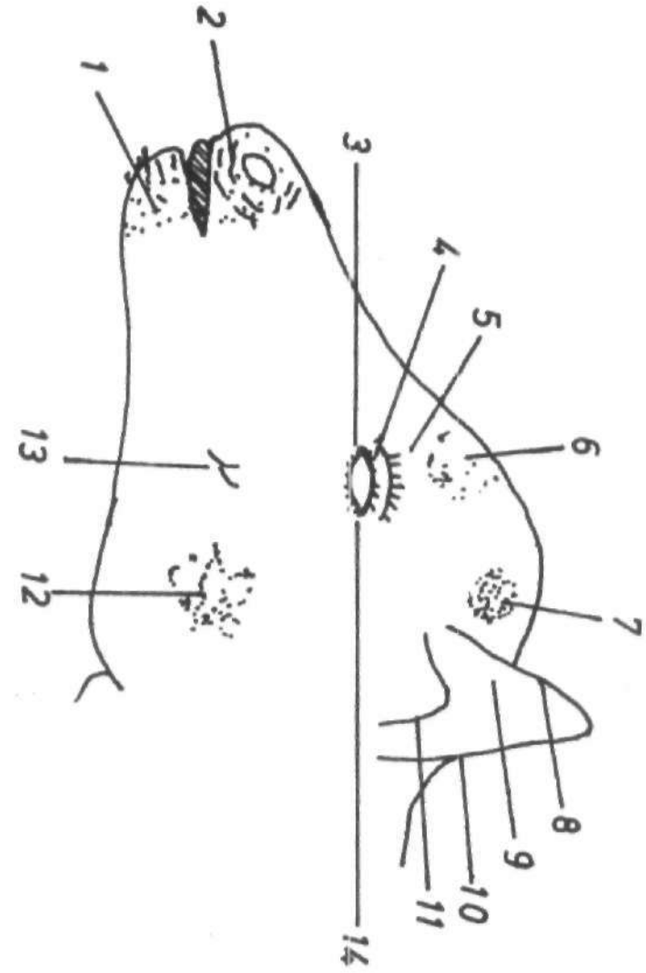
Area*	Hair growth in mm	Gestational Day
11.	Eruption	158
	2 - 3	160 - 165
	3 - 5	170 - 180
	6	190
12.	Visible intraepithelially	200
13.	1 - 2 single hair	
	Eruption	130
	2 - 3	130 - 140
	3 - 5	130 - 140
	6 - 8	140
	8 - 12	170
14.	Eye cleft opening	161
	General body surface	
	Small zones of intraepithially developing hairs	187 - 200
	Zones of hair eruption	195 - 210
	Dense short hair covering the body	210 - 231

* Outlined in Fig. 1.

Table 2: Sequential development of external features in prenatal Zebu cattle.

Gestational age in days	External features
24	Forelimb bud present.
25	Embryo is C-shaped; third branchial arch present.
27	Hindlimb bud and mammary ridge present.
30	Olfactory pits forming optic cup eyes pigmented.
34	Facial clefts closed, groove between forelimb digits appears.
38	Pinna present as ridge, genital tubercle and grooves between hindlimb digits.
50	Eyelids begin to cover the eyes.
56	Hard palate fused.
60	Eyelids closed, hoof formation beginning, horn bud present.
80	Teats present, hoofs begin to cornify.
83	Scrotum present.
111	Tooth eruption begins (Incisor).
150	Hairs around eyes and muzzle.
152	Descent of testes completed.
182	Horn bud covered with hairs.
187	Hairs on coronet.
196	Meconium can be obtained from anus.
197	Eyelids separated.
200	Carpal and patella bones discernible.
215	All four legs covered with hairs.
230	Body fully covered with hair.

Fig. 1. Diagram indicating areas observed for hair eruption and growth.


1
2
3
4
5
6
7
8
9
10
11
12
13
14

PART V

DEVELOPMENT OF OSSIFICATION CENTERS IN THE APPENDICULAR SKELETON OF PRENATAL ZEBU CATTLE

INTRODUCTION

Initial ossification centers in the skeleton during prenatal life have been investigated mainly by three methods: (1) dissection and clearing with or without subsequent staining; (2) radiography with or without metallic intensification of radiopacity; and (3) histological examination of serial sections with or without histochemical procedures (Mall, 1906; Noback and Robertson, 1951). Clearing and staining by Alizarin red S process described by Dawson (1927) was found to be more accurate and reliable than roentgenographic methods (Harman and Saffry, 1934).

The technique used, age of specimens examined, use of postnatal anomalies to interpret prenatal normalities, and inaccurate age determination of embryos and fetuses examined are the basis of most of the controversies in the literature.

Among the earliest studies of skeletal development of the bovine fetuses were those of Retterer (1884), Lesbre (1897), Popowa (1913), and Bruni (1933). These workers based their observations on a small number of specimens from unspecified breeds. The estimated ages of their fetuses ranged from six weeks to about nine months. Later Winters et al. (1942) in a

valuable general study of prenatal bovine development used fetuses from grade cows of mixed breeding. They recorded some of the primary centers of ossification present in the appendicular skeleton of fetuses aged from 59 to 200 days. Lindsay (1969) used 650 bovine fetuses collected from Glasgow abattoir for studying the number, times of appearance and chronological order of loci of ossification in the prenatal and neonatal bovine appendicular skeleton including ossa coxarum. Methods used by Lindsay were histological, radiographical and staining by Alizarin red S. Loci were identified, in general, at earlier estimated ages than previously recorded. The study included loci of transient elements and recorded for the first time a locus for the clavicle. All specimens used in the study had a cephalic index within 48-60. The fetuses were predominantly Ayrshires but included some Friesians, Irish-cross cattle, Shorthorns, and an occasional Aberdeen-Angus or Galloway.

As the above studies were confined to Bos taurus breeds, the primary loci of ossification in the appendicular skeleton of Zebu cattle were investigated by using Alizarin red S staining and the results are reported here.

MATERIALS AND METHODS

Fetuses

Seventy embryos/fetuses were selected from the 270 described in Part III; 34 male and 36 female. Fifty-five fetuses were Rahaji, 10 Bunaji and 5 Wadara. Age of the fetuses collected at slaughter were estimated by the method reported in Part III. For days between the established forehead-rump (FR) lengths, an arbitary scale for daily growth increments was derived by dividing the difference between successive FR lengths by the number of intervening days. Three to four fetuses were studied for each gestational age (Table 1).

Preparation of Fetuses for Staining

Fetuses were skinned, eviscerated, fixed in 70 per cent ethanol for one to two weeks depending on size, and then defatted in acetone for 24 hours. Fetuses and dissected limbs were cleared in 2-10 per cent potassium hydroxide and then bleached in a solution of ethanol and hydrogen peroxide (9 parts of 95% ethanol to 1 part of H_2O_2). Clearing took three to four days for embryos and small fetuses and two to three weeks for near term fetuses.

Alizarin Red Staining

The cleared preparations were stained in 0.1 per cent Alizarin red S dissolved in one per cent potassium hydroxide for 24 to 48 hours until the bones were stained a deep red. The stained preparations were then dehydrated by passing through a graded series of glycerol in one per cent potassium hydroxide; 50 per cent for three days, 70 per cent for two days, 90 per cent for two to three days, and finally 100 per cent glycerol.

RESULTS

The earliest estimated observation of primary loci of ossification in limbs of Zebu fetuses is outlined in Figs. 1 and 2. The forehead-rump measurements and estimated ages of the fetuses are given in (Table 1). Figs. 3 to 8 illustrate some of the Alizarin red S stained fetal preparations.

The chronological order of appearance of loci of ossification in the appendicular skeleton is given in Table 2. Appearance of primary loci of ossification in the pectoral and pelvic limbs are listed in Tables 3 and 4.

During the first six weeks of prenatal life there was little ossification activity. Disappearance of transient elements of the appendicular skeleton is indicated by cross points (Fig. 1 and 2). During the 7th to 11th week of gestation many primary loci of ossification were laid down. From 11th to 23rd week, few new loci appeared but there was considerable growth of existing ones. From 24th to 34th week, new loci appeared and there was intense growth of existing loci. After 238 days gestation, no further primary loci of ossification were observed.

The earliest osseous representative of the appendicular primordia to appear was the clavicles, first identified at about 42 days. In the phalanges of digits three and four, the loci of ossification of the distal phalanges appeared first, followed by the proximal and then the middle phalanges. Loci of ossification of the accessory digits appeared later than those of the weight bearing digits. In the adult Zebu the fibula is represented by a vestigial proximal segment and a separate distal extremity known as the lateral malleolus. During the second month of fetal life, the cartilaginous fibula exhibited a diaphyseal locus of ossification which regressed leaving only a small proximal segment. The distal segment locus

of ossification appeared much later at an estimated age of 220 days. After appearance of proximal sesamoids at estimated age of 238 days, no further loci of primary ossification were observed.

DISCUSSION

More than one hundred years ago the early ossification loci in the bovine were studied vigorously and in a short time, the subject was virtually closed. As there was no recorded information on the pattern of ossification in prenatal Zebu cattle this study was undertaken. Alizarin red S is a specific stain for calcium salts (Cameron, 1930) and as calcium-impregnated osseoid tissue is bone, all prenatal bone is stained red in properly cleared and stained preparations.

The period between day 49 and 77 gestation in prenatal Zebu cattle when many primary centers of ossification are laid down coincides with the period of rapid development described by Winters et al. (1942). They commented that "After the 49th day the length of the fetus and its component parts increase rapidly". In the tarsus a locus of ossification is formed for

both the central and fourth tarsal bones and these bones were not fused until parturition. This observation is in accord with that of Lesbre (1897). In the distal row of carpal bones the second and third carpals, which are fused in the adult, show a single locus of ossification.

Comparison of time of appearance of loci of ossification found in Zebu fetuses approximate closely with observations in Bos taurus breeds by Lindsay (1972) but there were several differences with findings by Hirt (1969). For example, Hirst identified some loci at earlier ages. Unfortunately, he did not include the parameters used for fetal ageing which would have helped in comparing results.

It is generally agreed that weight of bovine fetuses of the same age but of different breeds may differ considerably. Differences in forehead-rump length, however, are not so marked and it is considered that by selecting abattoir specimens with relative forehead-rump lengths, some degree of uniformity may be provided with the Zebu cattle types used in this study.

The factors which determine the time of ossification are obscure. A comparison of tarsal and carpal bones and to some extent long bones suggests size is a factor as larger bones commence to ossify earlier. This however does not account for

the early ossification of the clavicle. Functional and morphological factors may be involved.

Various theories have, from time to time, been advanced to account for the initial appearance of centers of ossification. It has been suggested that the deposition of calcareous salts in the first stages of endochondral ossification is a degenerative change. This was based on the presumption that there was a definite limit to the size which can be attained by tissues such as cartilage which depends on its nutrients by imbibition of fluid from the surrounding blood vessels. When this limit is reached the central cells of the cartilage undergo degeneration. This is accompanied by precipitation of calcareous salts in the cartilaginous matrix, perhaps due to a local increase in the acidity of the tissue fluids. The calcified cartilage is then invaded by surrounding blood vessels and removed like any other foreign-body. It can hardly be maintained, however, that commencement of primary ossification in cartilage is delayed until the latter has increased in size up to its nutrional limits. At the time when they commence to ossify, for instance, the cartilaginous model of a phalanx is incomparably smaller than the cartilaginous model of the femur at birth. On the other hand, the large cartilaginous epiphyses

in cattle are richly permeated with vascular canals sometime before ossification commences, so that there is no reason to suppose that nutrition of the cartilage is in any way defective. It is important to recognise that there is a clear distinction between simple calcification and endochondral ossification. The former may be regarded as a degenerative process related to a poor blood supply and breakdown of tissues but the latter is an active growth process accompanied by a rich vascularization and tissue proliferation. Some bovine cartilage undergoes simple calcification with advancing age without necessarily forming true bone, e.g. thyroid cartilage.

The onset of ossification has been thought to be dependent on mechanical stimuli, particularly tensile forces and friction. Krompecher (1937) presented evidence that in healing of fractures, bone formation was directly promoted by tensional forces. On the other hand, pressure tends at first to initiate cartilage formation and that with persistent pressure, cartilage also becomes replaced by bone. Thoma (1913) considered that practically all bone formation and bone growth, both embryonic and postnatal, are directly dependent on the response of osteogenic tissues to tensile and compression forces. The appearance of centers of ossification in the

membranous bones of the vault of the skull, for example, he speculatively related to the tensile forces set up by the rapidly expanding brain. It was pointed out, however, that in cases of anencephaly (a developmental anomaly characterised by the almost complete absence of the cerebral hemispheres) the membranous bones are still ossified though their final shape is distored by the abnormal conditions of development. It is also the case that the adjacent margins of two skull bones along a sutural line may expand at very different growth rates though presumably they are exposed to the same field of tensile forces (Prichard et al., 1956).

If observation and experiment suggest that, in certain types of ossification, extraneous stimuli provide the conditioning factors, it remains certain that the normal appearance of centers of ossification is, to a large extent, dependent on intrinsic growth factors. This has been demonstrated by self-differentiation of bones in explanted tissues entirely removed from their usual environment. It seems evident that the pattern of the adult skeleton is already mapped out in the somatic mesoderm of the early embryo long before ossification occurs. In other words, development of each element of the typical vertebrate skeleton has an intrinsic morphological basis.

The sequence of appearance of the appendicular ossification centers during prenatal life showed varying degress of conformity to the "sequence" of cranio-caudal development (the so-called law of anterio-posterior development or law of developmental direction). The regional order of appearance of primary ossification centers in Figs. 1 and 2 is good evidence to conclude that the time sequence of appearance of ossification centers of the distal, proximal and middle phalanges did not conform to the law of anterio-posterior development.

The conservatism of skeletal elements in evolution is clearly of great significance to the comparative anatomist for studying phylogenetic history. Ancestral traits may be recorded in the persistence of archaic features which have lost theirfunctional significance. For example, the second and fifth metapodials, as well as the clavicle bones, observed as transient elements in this study.

Some specimens in this study were preserved in 10 per cent formalin and for a long time it was practically impossible to clear them in the ordinary way. Finally in desperation, they were placed in 10 per cent solution of potassium hydroxide and they began to clear at the end of a month. By further treatment, it was found that such embryos could be cleared perfectly, and

if the bones were not decalcified by formalin, excellent preparations were obtained with tendons and other white fibrous tissues tougher than in specimens treated with ethanol alone.

SUMMARY

The number, time of appearance, and chronological order of the primary loci of ossification in limbs of the Zebu fetus were investigated in 70 (Rahaji 55, Bunaji 10, and Wadara 5) fetuses by Alizarin red S staining. The ossification pattern observed was as follows: first six weeks little activity; 7 to 11 weeks - many primary loci laid down; 12-23 weeks - few new loci but considerable growth of existing loci; 24-34 weeks - new loci appear together with intense growth of existing loci; and after 34 weeks no further primary loci.

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Table 1: Forehead-rump lengths and estimated ages of the Zebu fetuses.

Forehead-rump (cm)	Estimated fetal age (days)
4.10	40
4.13	42
5.70	50
5.90	52
8.00	58
10.00	68
11.75	75
12.70	80
14.52	88
22.80	116
40.63	163
43.67	170
49.50	183
52.00	188
53.00	190
58.00	200
59.50	203
61.00	206
62.50	209
66.70	215
72.37	220
79.00	238

Table 2: Chronological order of appearance of primary loci of ossification in the appendicular skeleton of prenatal Zebu cattle by Alizarin red S staining.

Gestational Day	Pectoral Limb	Pelvic Limb
42	1. Clavicle	----------
50	2. Humerus	Femur
52	3. Scapula	Tibia: fibula
52	Radius: ulna	
58	4. Metacarpals; 2,3,4 & 5 distal phalanges of digits 3 & 4	Metatarsals; 2,3,4,&5 distal phalanges of digits 3 & 4
58		Ilium
58	----------	Ischium
68	5. Proximal phalanges of digits 3 & 4	Proximal phalanges of digits 3 & 4
75	6. Middle phalanges of digits 3 & 4	Middle phalanges of digits 3 & 4
88	----------	Fibular tarsal bone
116	----------	Pubis
163	7. Ulnar: fourthcarpal	Tibia: fourthtarsal
183	8. Radial and intermediate carpals	----------
188	9. Second/third carpals	----------
190	----------	Central, second/third tarsals
209	10. Accessory Carpal	----------
238	11. Proximal Sesamoids	Patella, first tarsal
		Proximal sesamoids

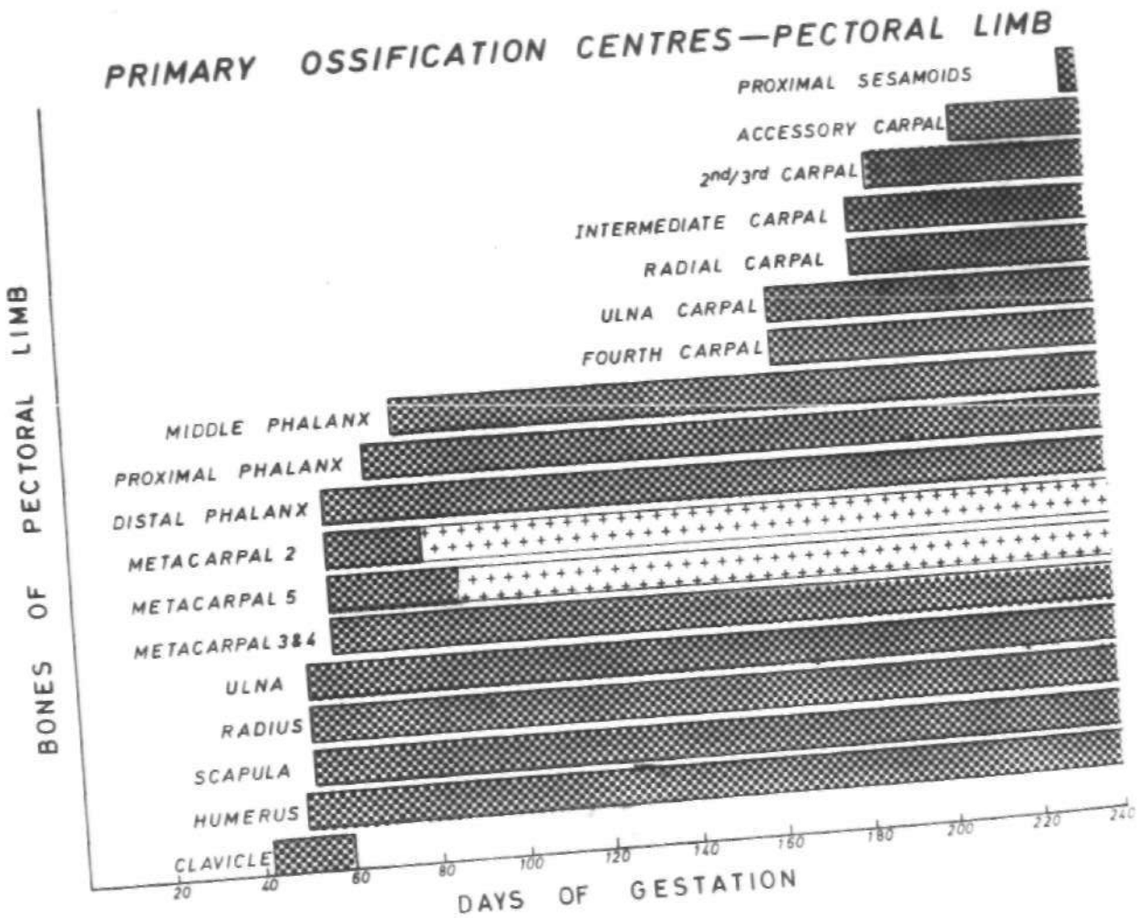
Table 3: Time of appearance of primary loci of ossification in the pectoral limb of prenatal Zebu cattle by Alizarin red S staining.

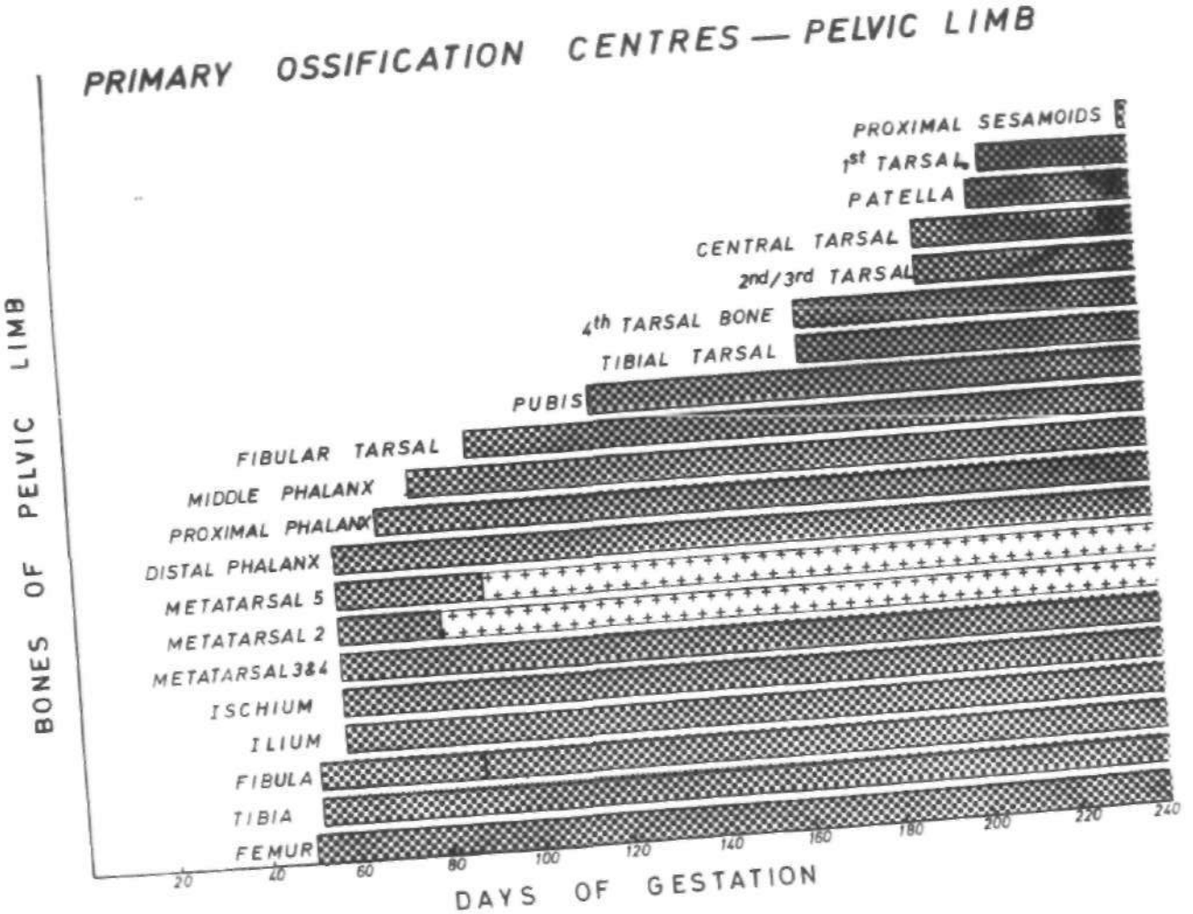
Bone	Day observed
Clavicle	42
Scapula	52
Humerus	50
Radius	52
Ulna	52
Carpal, radial	183
Carpal, intermediate	183
Carpal, ulna	163
Accessory Carpal	209
2/3rd Carpals	188
Fourth Carpal	163
Metacarpals 3 and 4	58
Metacarpal 5	58
Metacarpal 2	58
Proximal phalanx	68
Middle phalanx	75
Distal phalanx	58
Proximal sesamoids	238

Table 4: Time of appearance of primary loci of ossification in the pelvic limb of prenatal Zebu cattle by Alizarin red S staining.

Bone	Day observed
Ilium	58
Ischium	58
Pubis	116
Femur	50
Tibia	52
Fibula	52
Patella	203
Tarsal, fibular	88
Tarsal, tibial	163
Tarsal, central	190
4th tarsal	163
2/3rd tarsal	190
1st tarsal	206
Metatarsals 3 and 4	58
Metatarsal 2	58
Metatarsal 5	58
Proximal phalanx	68
Middle phalanx	75
Distal phalanx	58
Proximal Sesamoids	238

Fig. 1. The primary centers of ossification in the pectoral limb of the developing Zebu fetus detected by Alizarin red S staining.


PRIMARY OSSIFICATION CENTRES—PECTORAL LIMB
BONES OF PECTORAL LIMB
PROXIMAL SESAMOIDS
ACCESSORY CARPAL
2nd/3rd CARPAL
INTERMEDIATE CARPAL
RADIAL CARPAL
ULNA CARPAL
FOURTH CARPAL
MIDDLE PHALANX
PROXIMAL PHALANX
DISTAL PHALANX
METACARPAL 2
METACARPAL 5
METACARPAL 3&4
ULNA
RADIUS
SCAPULA
HUMERUS
CLAVICLE
20
40
60
80
100
120
140
160
180
200
220
240
DAYS OF GESTATION


PRIMARY OSSIFICATION CENTRES — PELVIC LIMB
BONES OF PELVIC LIMB
PROXIMAL SESAMOIDS
1st TARSAL
PATELLA
CENTRAL TARSAL
2nd/3rd TARSAL
4th TARSAL BONE
TIBIAL TARSAL
PUBIS
FIBULAR TARSAL
MIDDLE PHALANX
PROXIMAL PHALANX
DISTAL PHALANX
METATARSAL 5
METATARSAL 2
METATARSAL 3&4
ISCHIUM
ILIUM
FIBULA
TIBIA
FEMUR
20
40
60
80
100
120
140
160
180
200
220
240
DAYS OF GESTATION

Abbreviations used in Figs. 3-8

T = Tibia

Mt = Metacarpal

Pd = Distal phalanx

Ac = Accessory metacarpal

Mc = Metacarpal

Fi = Fibula

Is = Ischium

S = Scapula

H = Humerus

R = Radius

U = Ulna

PP = Proximal Phalanx

F = Femur

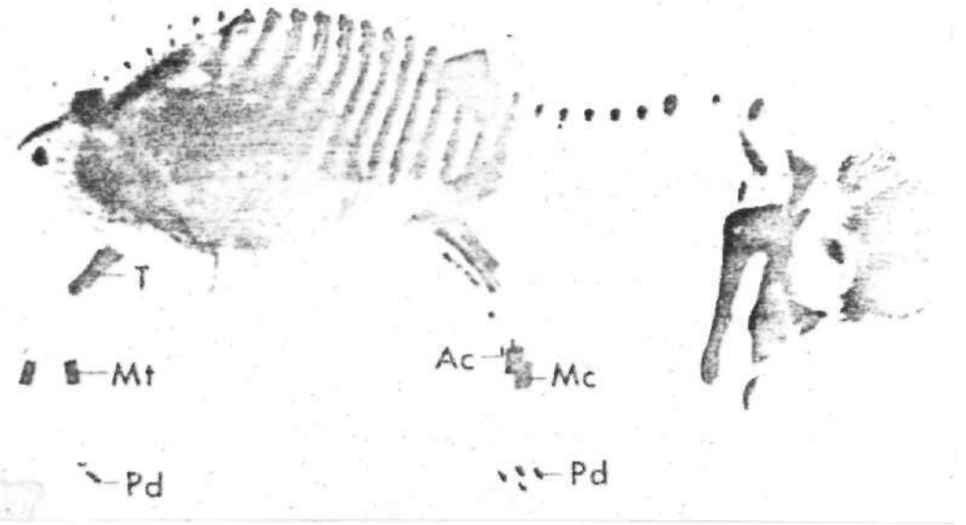
Ca = Calcaneous

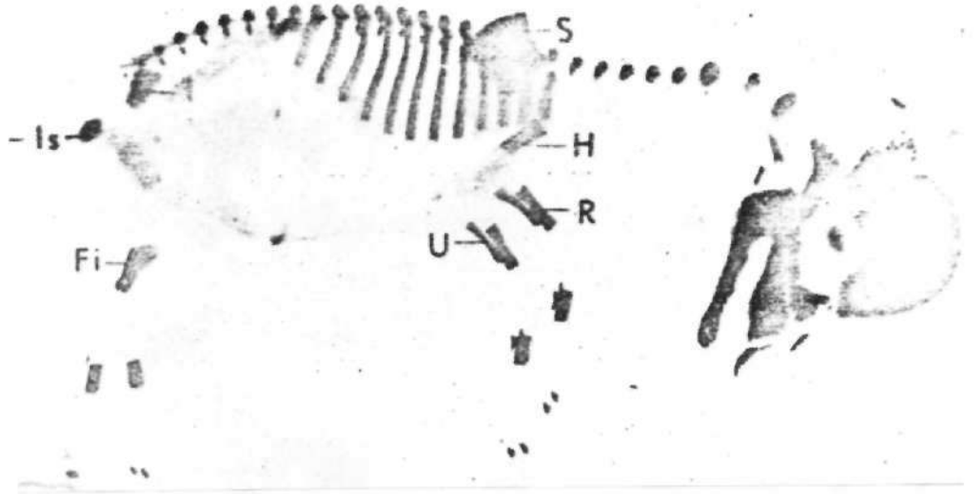
I = Ilium

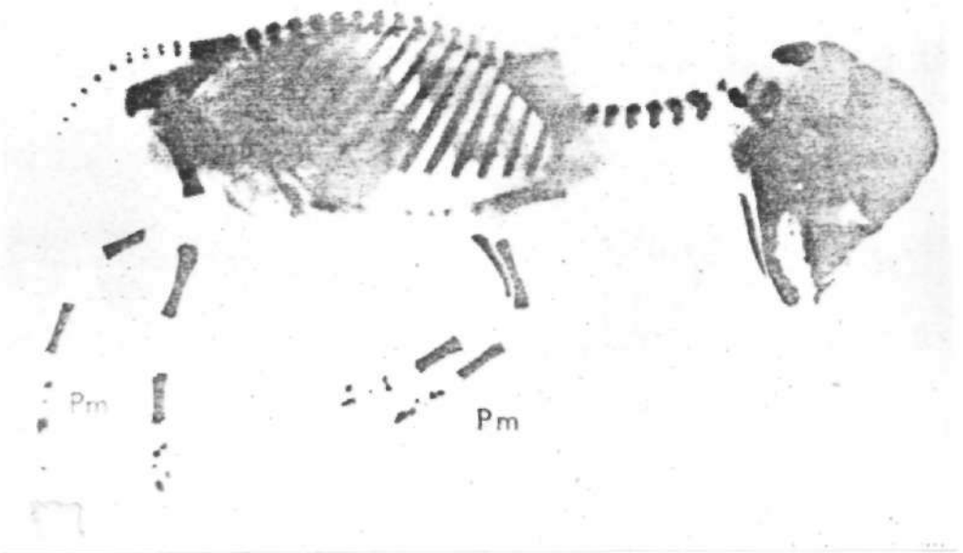
Pm = Middle phalanx

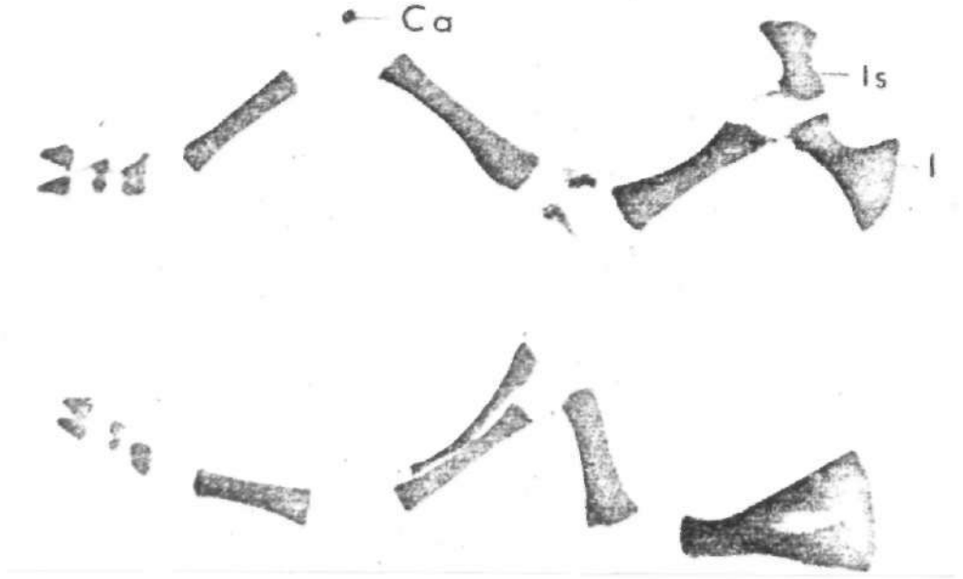
Fig. 3. Alizarin red preparation of a Zebu fetus, estimated age 58 days. The fibula is visible along the posterior border of the tibia and metacarpals 2 and 5 along the posterior border of metacarpals 3 and 4.

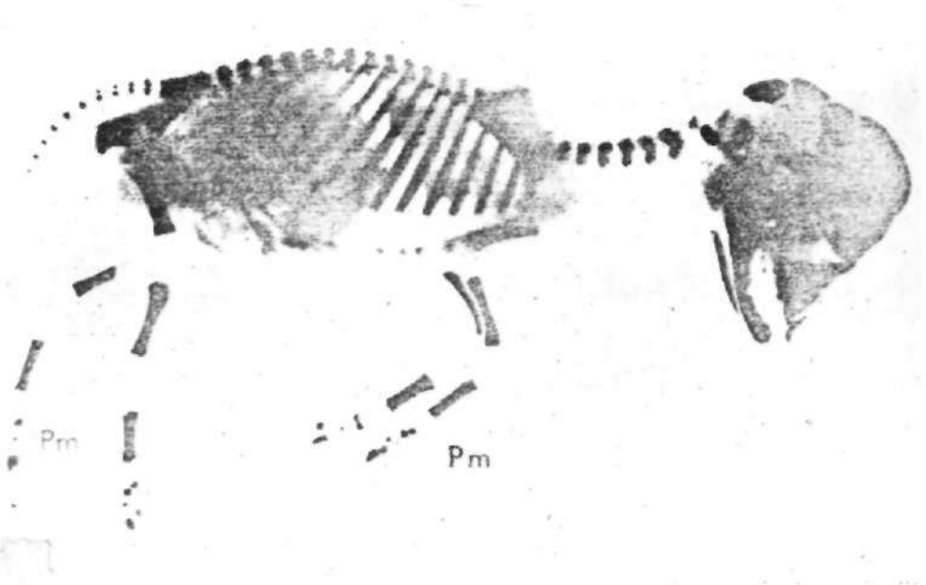
Fig. 4. Alizarin red preparation of a Zebu fetus, estimated age 63 days. The distal phalanges are present in the limbs and the fibula is fused to the tibia.

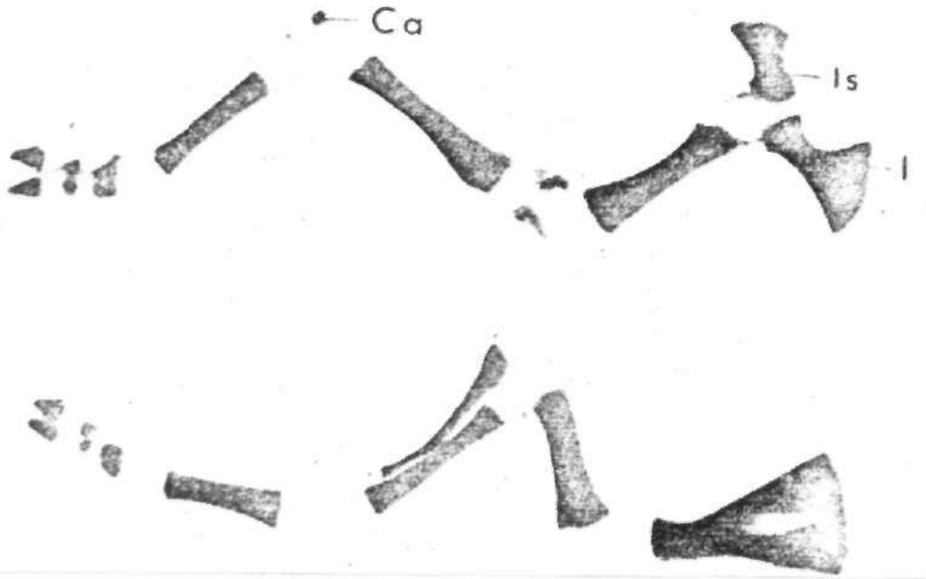

T
Mt
Pd
Ac
Mc
Pd

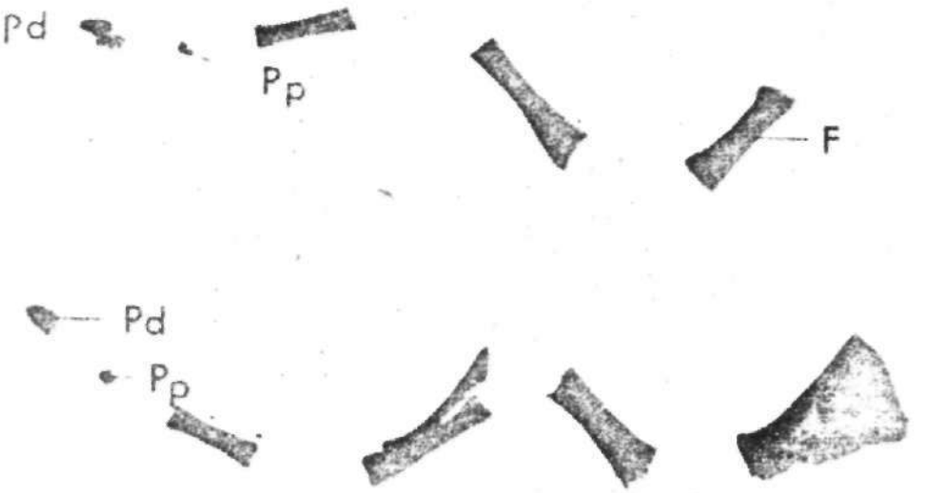

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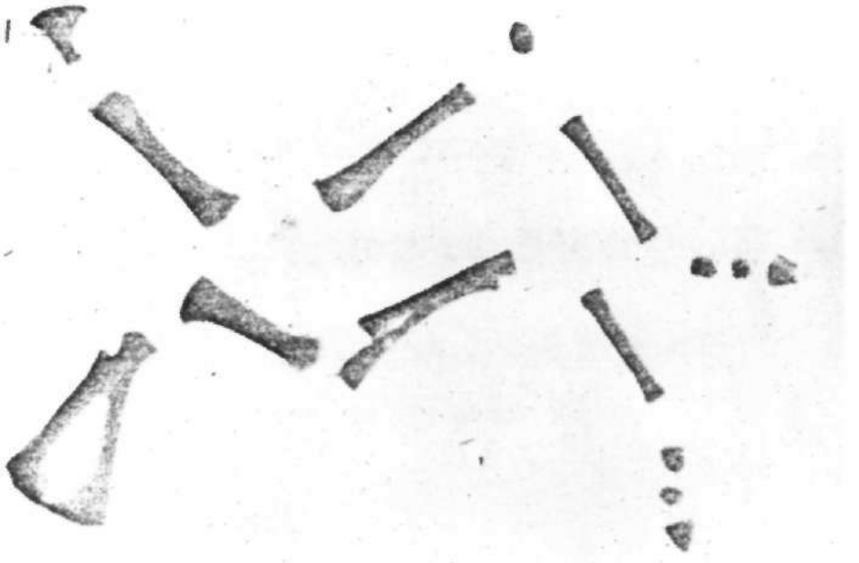


Fig. 5. Alizarin red preparation of a Zebu fetus, estimated age 73 days. The proximal phalanges are present as well as the accessory metacarpal and metatarsal bones.

Fig. 6. Alizarin red preparation of a Zebu fetus, estimated age 75 days. All phalanges are present in both the fore and hindlimbs.

Fig. 7. Alizarin red preparation of a Zebu fetus, estimated age 72 days. The calcaneous is the first tarsal bone to appear. The fifth metacarpal and fifth metatarsal are reduced to small spurs.

Fig. 8. Alizarin red preparation of a Zebu fetus, estimated age 100 days. Tuberosities and processes appear on individual bones as they acquire adult characteristics.

PART VI

RADIOGRAPHIC STUDY OF THE PRIMARY LOCI OF OSSIFICATION IN THE LIMBS OF PRENATAL ZEBU CATTLE

INTRODUCTION

To the extent that progressive differentiation, growth and maturation can be detected, almost any organ system of the body may be used to assess development status. The skeleton, however, offers unique advantages over other systems because of the ease with which its series of developmental changes can be followed quantitively and objectively by radiography. The sequence of skeletal changes is similar for all normal individuals of the species, irrespective of their body size or whether the changes occur quickly or slowly in terms of chronological age. It is not surprising that skeletal radiography is regarded by pediatricians as one of the best means of determining development status in children (Tanner, 1962) or that standards for human skeletal changes have been defined by serial X-ray studies (Acheson, 1966).

In contrast to man, the bovine undergoes a much greater proportion of its skeletal differentiation before birth and therefore, skeletal radiography is more useful during fetal development. Few studies on bovine skeletal development have used radiographic methods and none have attempted to define normal standards for skeletal growth and differentiation (Winters et al., 1942; Fedrigo, 1957; Lindsay, 1969). As no record of

skeletal studies in Zebu cattle was found, a radiographic investigation was undertaken. The objectives were to improve the accuracy of fetal ageing, to devise a method for ageing dead and mummified fetuses, and to compare radiography with Alizarin red S staining for detecting primary loci of ossification.

MATERIALS AND METHODS

Fetuses

Fifty fetuses, aged 40 to 285 days, were selected from the remaining 200 fetuses to correspond as closely as possible to those studied in Part V. Thirty-five fetuses were Rahaji, 10 Bunaji, and 5 Wadara, and 23 were males and 27 were females. Fetal age and the daily growth scale were estimated as described in Part V. Two to three fetuses were studied for each gestation age listed in Table 1.

Radiographic Examination

The right fore and hind limbs were X-rayed[1] with the fetus

[1] Model Roentgen 301, Watson X-ray Company, England.

lying on its left side and by using Agfa-Gevaert standard film. With larger fetuses the limbs were removed and the lateral sides radiographed. Exposure depended on fetal age and body density and was adjusted to give an easy to read high contrast radiographs. The anode to film distance was 90 cm.

The films were developed manually for 5 minutes at 20°C and when dry, they were read in a darkroom using a cold light illuminator. The radiographs were examined carefully for ossification centers in the developing limbs. A locus of ossification was assessed as a spot or area of increased opacity at an appropriate site.

RESULTS

The earliest estimated observation of primary loci of ossification in limbs of Zebu fetuses radiographically is outlined in Figs. 1 and 2. No observation of the clavicle was made by this method. The forehead-rump measurements and estimated fetal ages are given in Table 1. Table 2 lists in chronological order the appearance of the primary loci of ossification detected radiographically. The loci are enumerated

dorso-ventrally in their order of appearance. Figs. 3 to 11 illustrate some of the X-rayed limbs.

No ossification activity was revealed radiographically during the first eight weeks of intrauterine life (Figs. 1-3) but extensive ossification was present by the nineth week. The scapula, humerus, ulna and radius, and femur, fibula and tibia were visible. Practically all bones of the axial group had some ossification by day 70 (Fig. 4). Development had taken place in the third and fourth metacarpals and metatarsals, in the distal phalanges of both limbs, and in the ilium and ischium. No deposition was observed in the carpal or tarsal bones. The third and fourth metacarpal bones, although separate at 70 days, later fuse into a single structure.

At day 82, the middle phalanges (Fig. 5) and pubis have some ossification. The first tarsal bone to reveal any ossification was the fibular tarsal at day 100 (Fig. 6).

In the phalanges of digits three and four of both fore and hind limbs, the loci of ossification of the distal phalanges appear first, followed by the proximals and lastly, the middle phalanges.

Figs. 7-11 illustrate bone development at 120, 130, 170, 185 and 215 days, respectively. Further ossification of the tarsal bones was visible at day 185 and day 200, the carpal and patella

bones were discernible.

DISCUSSION

Deposition of sufficient osteoid to be detected radiographically occurs early in the fetal period. The early deposition is generalised and takes place in both axial and appendicular segments, and is not limited to either cartilaginous or membranous bones. Radiographic techniques for determining the time of appearance of ossification centers are now generally accepted (Lindsay, 1972). Noback and Robertson (1951), however, considered the methods were not as reliable as staining for detecting very early ossification centers and they found errors up to five days in human embryos. Alizarin red S staining was found to be more efficient for determining the early loci of ossification in Zebu fetuses (Part V). The retrogressive changes observed in the formation of the Zebu foot was not detected radiographically.

Hodges (1953) employed heavy metal impregnation to increase radiologic density in fetal pigs bones. Wrathal et al. (1974) observed that impregnation with heavy metallic salts resulted in

cartilaginous precursors taking up the metal and appearing as bone. For this reason, this method was not used in this study. Radiography was useful for determining the extent of ossification in older embryos in which calcification had progressed far enough to render the bone adequately X-ray opaque.

Some degree of decalcification was observed in some of the smaller specimens kept in formalin while awaiting X-ray examination which may have contributed to some of the irregular results. This could have resulted from inadequate buffering of the formalin during preparation. It is recommended that embryos and small fetuses be preserved in 95 per cent ethanol prior to X-raying. Harman and Saffry (1934) observed some decalcification in fetal bones of guinea pigs preserved in Bouin's solution and attributed the decalcification to the picric acid.

In this study with Zebu fetuses the centers of ossification in the limb bones were, in general, detected by radiography several days later than by Alizarin red S staining. Part of this may have been due to the X-ray machine that was used.

SUMMARY

The number, time of appearance, and chronological order of the primary loci of ossification in the developing Zebu limbs were investigated in 50 fetuses (Rahaji 35, Bunaji 10, and Wadara 5) by radiography. No ossification activity was observed during the first eight weeks, by the ninth week a number of primary loci were detected, and no primary loci were observed after 210 days. Under the conditions of this investigation, radiography was found to be less efficient than Alizarin red S staining for detecting the primary loci of ossification in the appendicular skeleton of the Zebu fetus.

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Wrathall, A. E., Bailey, J. and Hebert, C. N. (1974): A radiographic study of development of the appendicular skeleton in fetal pig. Res. Vet. Sci. 17: 154-168.

Table 1. Forehead-rump lengths and estimated fetal ages of the 50 Zebu fetuses.

Forehead-rump (cm)	Estimated fetal age (days)
4.10	40
4.13	42
5.50	49
5.90	52
8.20	59
10.43	70
12.70	80
14.98	90
18.00	100
24.10	120
27.76	130
43.67	170
50.50	185
58.00	200
61.00	206
66.70	215
72.37	220
79.00	238
98.70	285

Table 2: Chronological order of appearance of the primary loci of ossification in the appendicular skeleton of prenatal Zebu cattle by radiography.

Gestational age (days)	Pectoral Limb	Pelvic Limb
59	1. Humerus	Femur
61	2. Scapula	Tibia and Fibula
	Radius and ulna	
70	3. Distal phalanges of digits 3 and 4	Distal phalanges of digits 3 and 4
		Ilium
		Ischium
75	4. Proximal phalanges of digits 3 and 4	Proximal phalanges of digits 3 and 4
82	5. Middle phalanges of digits 3 and 4	Middle phalanges
		Fibular tarsal
		Pubis
61	6. Ulna carpal	Tibia; fourth tarsals
190	7. Radial and intermediate carpals	-------
200	8. Second/third carpals	-------
		Central, second/third tarsals
170	9. Accessory carpal	-------
210	------	Patella, first tarsal

Table 3: Time of appearance of primary loci of ossification in the pectoral limb of prenatal Zebu cattle by radiography.

Bone	Day observed
Scapula	61
Humerus	59
Radius	61
Ulna	61
Carpal, radial	190
Carpal, intermediate	190
Carpal, ulna	215
Accessory Carpal	170
2nd/3rd Carpal	200
Fourth Carpal	170
Metacarpals 3 and 4	70
Proximal phalanx	75
Middle phalanx	82
Distal phalanx	70

Table 4: Time of appearance of primary loci of ossification in the pelvic limb of prenatal Zebu cattle by radiography.

Bone	Day observed
Ilium	70
Ischium	70
Pubis	82
Femur	59
Tibia	61
Fibula	61
Patella	210
Tarsal, fibula	100
Tarsal, tibia	170
Tarsal central	200
4th tarsal	170
2nd/3rd tarsal	215
1st tarsal	215
Metatarsal 3 and 4	70
Proximal phalanx	75
Middle phalanx	82
Distal phalanx	70

Fig. 1. The primary centers of ossification in the pectoral limb of the developing Zebu fetus detected radiographically.

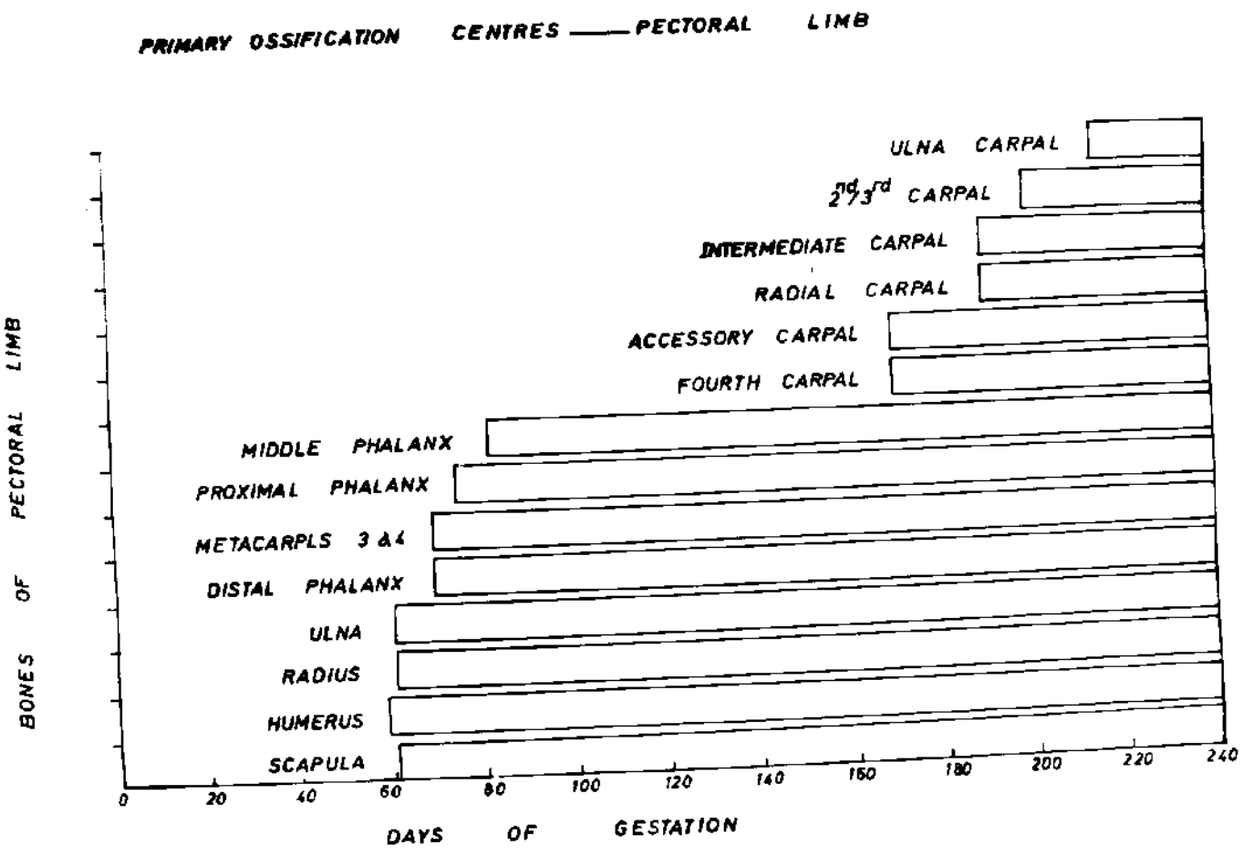

PRIMARY OSSIFICATION CENTRES —— PECTORAL LIMB
BONES OF PECTORAL LIMB
ULNA CARPAL
2nd 3rd CARPAL
INTERMEDIATE CARPAL
RADIAL CARPAL
ACCESSORY CARPAL
FOURTH CARPAL
MIDDLE PHALANX
PROXIMAL PHALANX
METACARPLS 3 & 4
DISTAL PHALANX
ULNA
RADIUS
HUMERUS
SCAPULA
0
20
40
60
80
100
120
140
160
180
200
220
240
DAYS OF GESTATION

Fig. 2. The primary centers of ossification in the pelvic limb of the developing Zebu fetus detected radiographically.

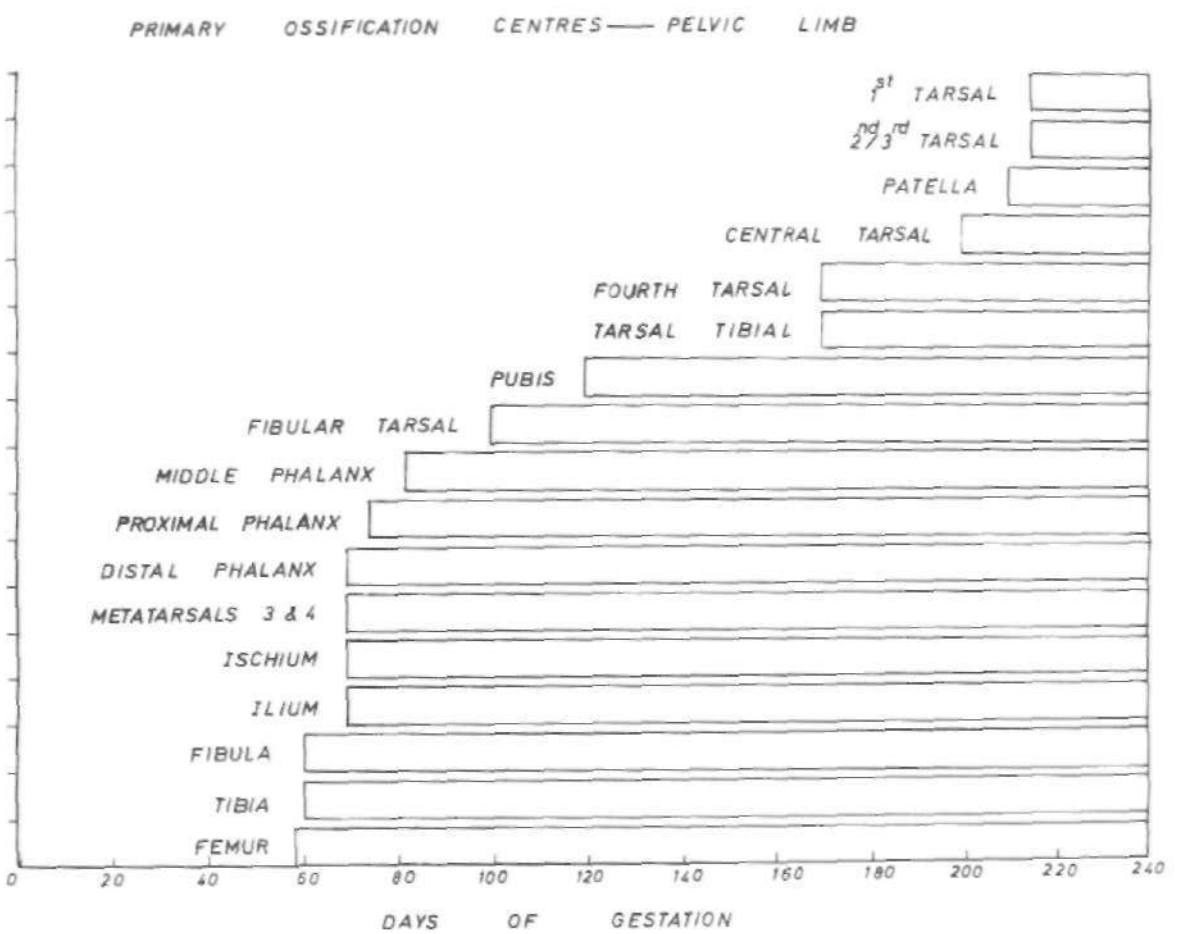

PRIMARY OSSIFICATION CENTRES—— PELVIC LIMB
1st TARSAL
2nd 3rd TARSAL
PATELLA
CENTRAL TARSAL
FOURTH TARSAL
TARSAL TIBIAL
PUBIS
FIBULAR TARSAL
MIDDLE PHALANX
PROXIMAL PHALANX
DISTAL PHALANX
METATARSALS 3 & 4
ISCHIUM
ILIUM
FIBULA
TIBIA
FEMUR
0 20 40 50 80 100 120 140 160 180 200 220 240
DAYS OF GESTATION

Fig. 3. Radiograph of a Zebu fetus, estimated age 52 days, showing no ossification activity.



Fig. 4. Radiograph of a Zebu fetus, estimated age 70 days, showing early ossification of the axial and appendicular skeleton.

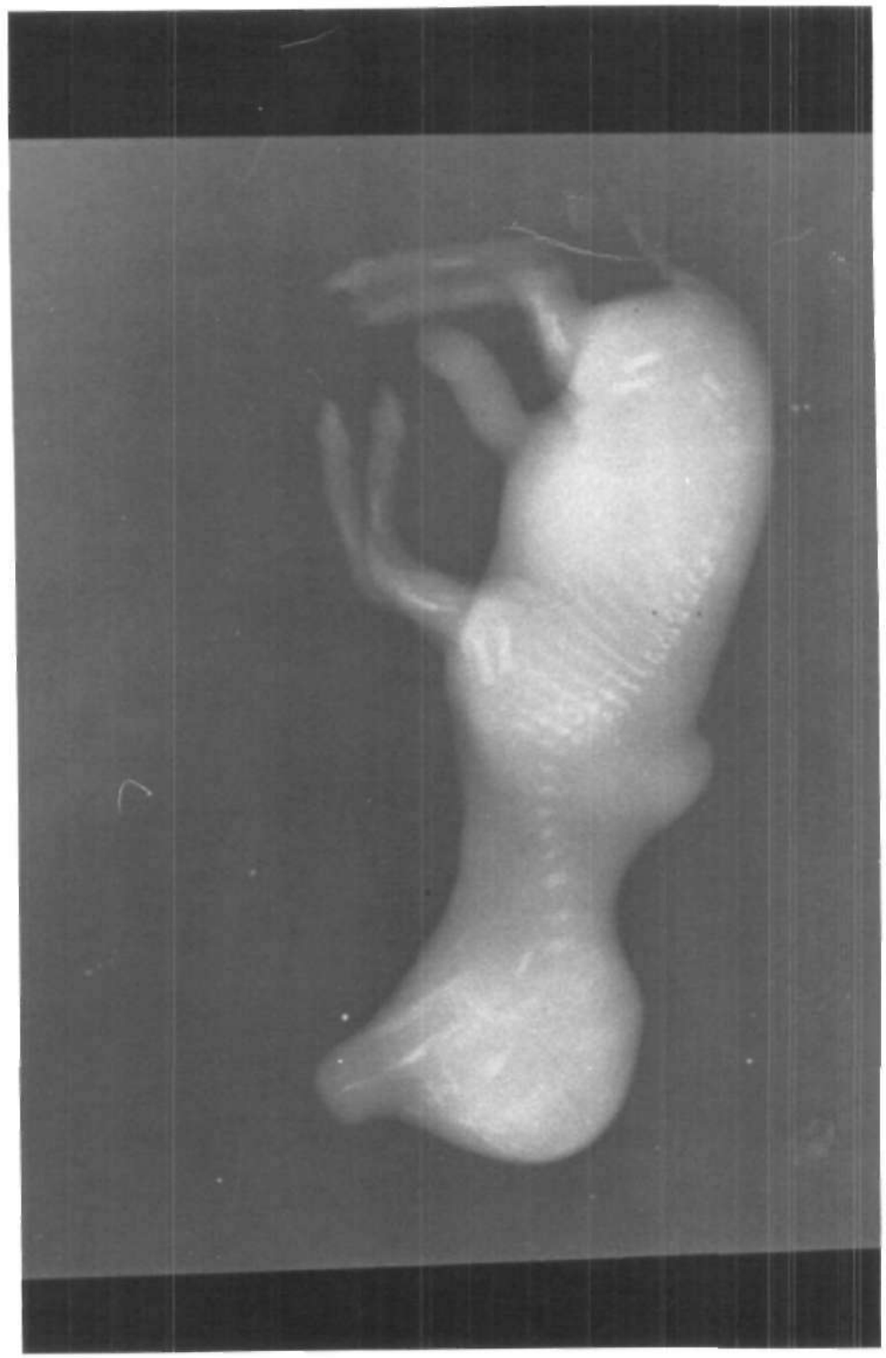


Fig. 5. Radiograph of a Zebu fetus, estimated age 82 days, showing ossification of the pelvis and middle phalanges.


S

Fig. 6. Radiograph of a Zebu fetus, estimated age 100 days, showing early ossification of the fibular tarsal bone.



Fig. 7. Radiograph of a Zebu fetus, estimated age 120 days, showing extensive ossification activity.

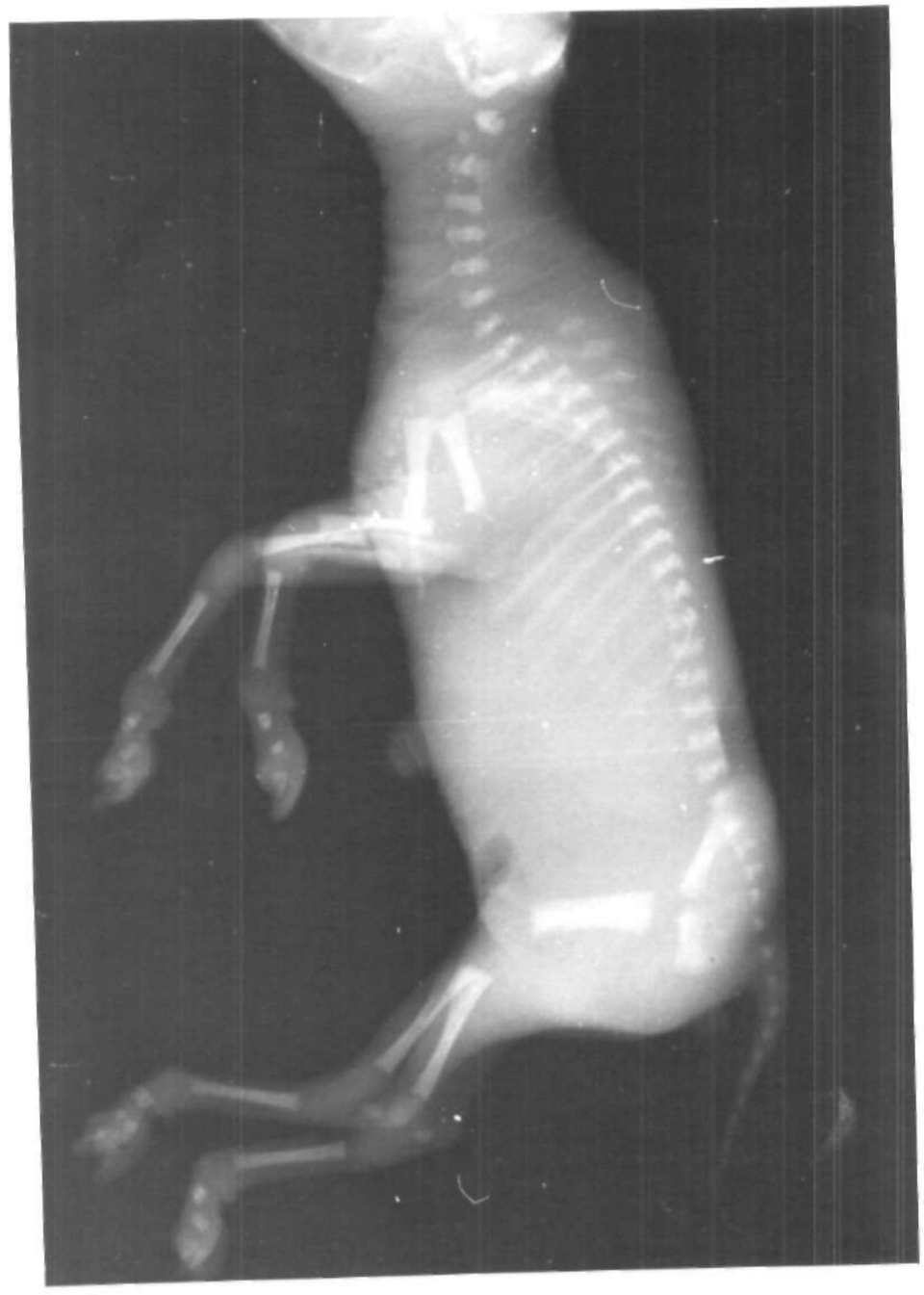


Fig. 8. Radiograph of a Zebu fetus, estimated age 130 days showing further bone formation.


129

Fig. 9. Radiograph of a Zebu fetus, estimated age 170 days, showing active ossification including sternabrae.


I 94

Fig. 10. Radiograph of a Zebu fetus, estimated age 185 days, showing early ossification of the tarsal and carpal bones.

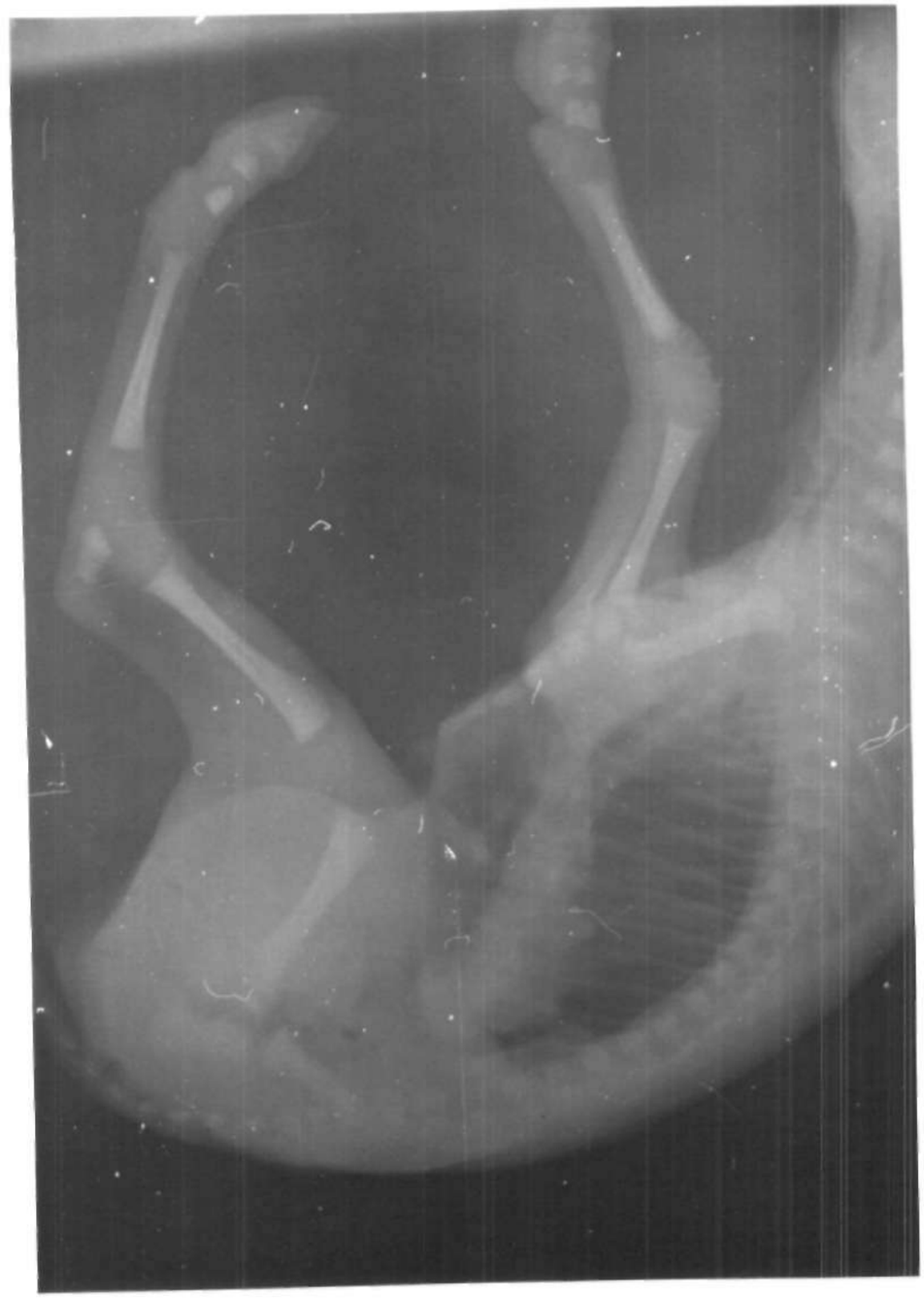


Fig. 11A. Radiograph of a Zebu fetus, estimated age 215 days, showing ossification of the pectoral limb especially the carpal bones.



Fig. 11B. Radiograph of a Zebu fetus, estimated age 215 days, showing ossification of the pelvic limb especially the tarsal bones.



PART VII

COMPOSITION OF THE HUMERUS OF THE DEVELOPING ZEBU FETUS

INTRODUCTION

Adult osseous tissue of man freed from fatty marrow is composed of organic material (mainly protein), water, and minerals (Best and Taylor, 1966). No information was found on the water, ash, organic matter and fat content of fetal bones of cattle.

Reported here is a preliminary study of the composition of the humerus of the developing Zebu fetus.

MATERIALS AND METHODS

Fetuses

Twelve Rahaji fetuses, aged 68 days to two days postpartum at 20 day intervals, were selected from those of known ages in Part III.

Preparation of Humeri

Each humerus was removed, carefully dissected to remove the soft tissues, macerated in water kept just below boiling point by

means of an automatic thermostat to remove any remaining soft tissue. The cleaned and air-dried bones were weighed on a platform balance[1] to the nearest 0.01g.

Chemical Analysis

Water Content: The clean, dissected humeri were dried in an oven at 94°C for three to four days to a constant weight. The difference in weight between air-dried and oven-dried humeri was taken as the water content.

Fat: Fat was extracted from each oven-dried humerus in a soxhlet apparatus[2] with petroleum ether for 24 hours. The extract was dried under vacuum in a clean, weighed residue flask and then weighed. The difference between the two weights being the fat content. The humeri were dried in an oven at 94°C for four to five days until the weights were constant.

Six humeri were subjected to further defatting in acetone for 24 hours to check the efficiency of soxhlet extraction. Little or no difference in bone weights were found after acetone-treated bones were dried and weighed.

Ash: The fat-free humeri were ashed individually in a crucible in a muffle furnace[3] at 600°C for eight hours. Larger humeri were

1 Model P163N, Mettler Instrument A.G., Zurich, Switzerland.

2 Model OV-062, Gallenkamp Co. Ltd., Christopher treet, London E.C.2, England.

3 Type 1500 Furnace, Thermolyne Corporation, Iowa, U.S.A.

sawed in two when required. After cooling, the crucible was weighed and the difference before and after was recorded as the weight of bone ash.

Organic Matter: The difference between the original weight and the sum of the weight for water, fat and ash was regarded as the organic material.

Degree of Calcification

The ossified portion of the diaphysis of each humerus was measured with a transparent plastic ruler to the nearest 0.2 mm.

RESULTS

The composition of the humeri is given in Table 1 and Fig. 1.

The water content of the humerus changed rapidly during fetal development: at 68 days it comprised 95 per cent of the total bone weight, 92 per cent at 88 days, dropped to 75 per cent by day 128, 62 per cent by day 248, and finally to 50 per cent at birth. The decline in water content coincided with increases in organic and inorganic matter (Fig. 1).

The amount of organic material increased from 2.2 per cent at day 68 to 14 per cent by day 128 and rose to 29.55 per cent of the total humeral weight by two days postpartum. The most rapid increases occurred during 68 to 88 days. The amount of inorganic material increased from 1.2 per cent on day 68 to 20 per cent by two days postpartum, the most rapid increase Occurring during 68 to 88 days. Fat content did not increase markedly like other solids. On day 68 the fat content was 1.6 per cent but it dropped below one per cent for the other measuring times. Although considerable fat was extracted from the bones, the relative percentage continued to drop so that by two days after birth fat constituted less than one half of one per cent of the net weight of the humerus.

Paralleling changes in bone composition, the percentage of diaphyseal ossification changed accordingly (Table 2). At 68 days the percentage of total length ossified was 38 and this increased to 80 per cent by 260 days. At birth, diaphyseal ossification was about 82 per cent of the total humeral length, the remainder being cartilage with some bone being produced in the epiphyses.

DISCUSSION

Factors during intrauterine life such as age and nutrition may affect the relative amounts of organic and inorganic materials in developing bones. Changes in the physical and chemical nature of the humerus of the developing Rahaji fetus were evident throughout gestation.

The percentage of water decreases as fetuses grow older. This is in spite of the fact that the absolute amount of body water increases with age. It is clear that the absolute increase in bone water is less rapid than that of other bone constituents, and that bones undergo progressive dehydration as they grow older. This phenomenon was reported by Hammett (1925) and it is a generalised age reaction not peculiar to the osseous system, but involves the nervous system and other organs.

The decrease in water percentage is compensated by an increase in the percentage of ash and organic matter, indicating that they are continuously displacing water from the bones. The most rapid increase in these constituents occurred between 68 to 88 days which signifies that the rate of ossification in the humerus is most likely greatest during this period.

A possible source of error in the dry, fat-free weight

determined in this study is the remains of non-osseous organic material which can be demonstrated easily in the medullary cavity when bones are sawed just before ashing. In small bone samples this has been removed by dissection (Baker, et al., 1946) and in larger samples by a constant boiling aqueous mixture of ethylene diamine in a soxhlet extractor (Williams and Irvine, 1954). None of these methods were applicable in this study since the aim was to ash the whole bone. The effect of the remaining non-osseous organic material on the weight of the dry, fat-free humerus was considered to be negligible.

This study was undertaken as a preliminary to a larger investigation into the chemical differentiation, organic and inorganic, of normal bone of Zebu cattle during prenatal growth.

SUMMARY

The composition of the humerus of the Rahaji fetus was studied at 20 day intervals from day 68 to 2 days postpartum. The water content changed rapidly from 95 per cent at day 68 to 50 per cent at birth. Organic matter increased from 2.2 per cent at day 68 to 28 per cent by 2 days postpartum. Inorganic matter

increased from 1.2 per cent on day 68 to 20 per cent by day 2 postpartum. The most rapid increases in organic and inorganic matter occurred between day 68 to 88, the period of rapid ossification in the humerus. Fat content decreased from 1.6 per cent on day 68 to less than half a per cent at day 2 postpartum.

Increases in diaphyseal ossification coincided with changes in bone composition. At day 68, 38 per cent of the total length of the humerus was ossified; this increased to 82 per cent at birth.

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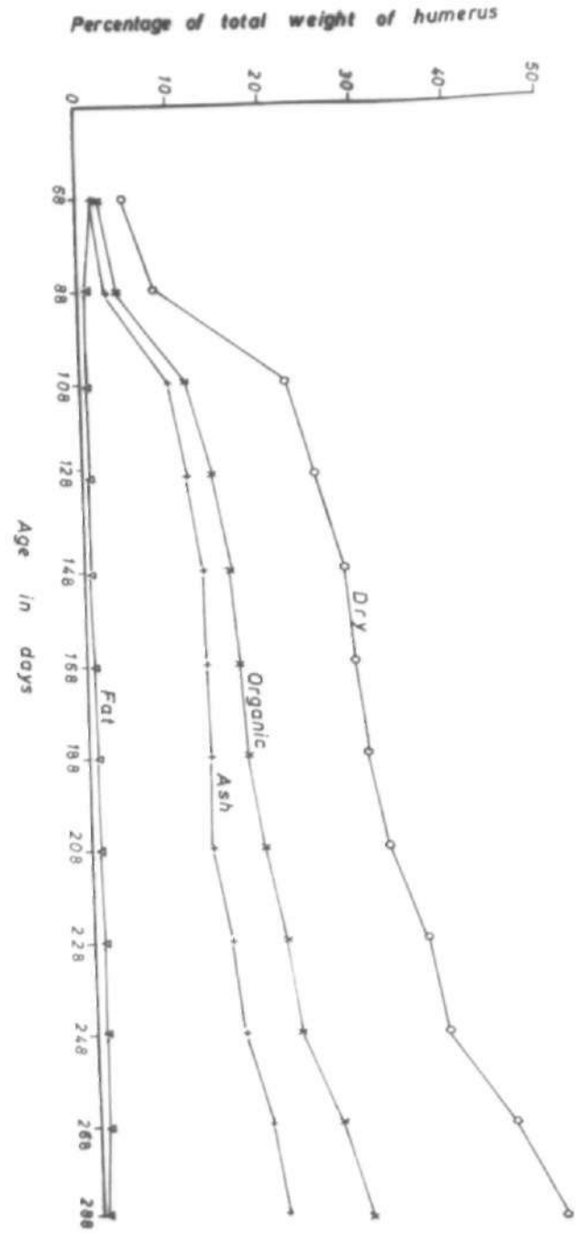
Table 1: Water, fat, ash, and organic content of humeri studied from prenatal Rahaji cattle.

Gestational Day	% Water	% Fat	% Ash	% Organic	Wet Wt.(g)
68	95	1.6	1.20	2.20	0.04
88	92	0.98	3.00	4.02	0.88
108	78	0.60	9.80	11.60	2.80
128	75	0.60	10.40	14.00	5.07
148	72	0.60	11.80	15.60	8.80
168	71	0.60	12.20	16.20	12.50
188	70	0.60	12.40	17.00	19.20
208	68	0.60	12.96	18.44	21.00
228	64	0.60	14.58	20.82	22.20
248	62	0.60	15.40	22.00	23.10
268	55	0.60	18.23	26.17	24.50
288	50	0.45	20.00	29.55	25.60

Table 2: Percentage of ossified humerus length in relation to days of gestation.

Day of Gestation	Length of Humerus in cm.	% Length of Humerus ossified
68	1.2	40
88	1.80	45
108	2,52	48
128	3.10	53
148	4.20	57
168	5.90	60
188	7.60	65
208	8.50	69
228	11.00	74
248	13.20	78
268	16.52	81
288	17.50	82

Fig. 1. Drymatter, fat, organic and inorganic composition of the developing Zebu humerus.


Percentage of total weight of humerus
0
10
20
30
40
50
68
88
108
128
148
168
188
208
228
248
268
288
Age in days
Dry
Organic
Ash
Fat

PART VIII

DEVELOPMENT OF THE APPENDICULAR SKELETON IN ZEBU CATTLE: GENERAL DISCUSSION

Developmental research utilizes a variety of species with diverse and often unique ontogenetic features. Any study concerning embryonic and fetal effects of metabolic or dietary disturbances, teratogenic or mutagenic substances, radiation or viremia or other environmental factors such as temperature and oxygen level or pressure must rely on the normal pattern of development as a basis for comparison. Documentation of normal embryonic and fetal growth serves as a guide for understanding the consequences of harmful influences at various stages of gestation.

The consistency of some fetal measurements varied as criteria for determining growth of the developing Zebu fetus. The standard embryological features observed and their time of appearance were four to five days later than that of Bos taurus breeds. Embryos and fetuses may have the same weight but different age or be in a different stage of development. This was found not only in this study but also by Winters and Feuffel (1936) in a study of fetal sheep.

The various regression equations established in this study provide a basis for ageing Zebu fetuses and hence open the way for further experimental studies with Zebu fetuses. The scale based on fetal length should be useful for estimating fetal age

in the field.

The pattern and sequence of hair growth was found to be indecisive for ageing Zebu fetuses in contrast to earlier recommendations for Bos taurus breeds (Bergmann, 1922; Postma, 1947; Jakobsen, 1962).

The sequential appearance of primary loci of ossification in the appendicular skeleton in the Zebu was detected earlier by Alizarin red S staining compared to radiographic examination.

Pathology, race, sex, nutrition and other factors have been shown to influence the time of the appearance of ossification. To obtain sufficient data to properly evaluate these factors, consolidation of data in the literature should be helpful. However, a short review of literature reveals the difficulty of such consolidation because of omission of vital data and the variety of techniques employed in studying the prenatal skeleton. The age of fetuses examined are often given but the method of calculation or fetal dimensions are frequently omitted. The sex, number, pathology and breed of fetuses examined are also often omitted. In order to obtain an accurate idea concerning the time range of appearance of ossification centers, the absence of centers should be reported.

To accurately consolidate data, account must be taken of the technique employed in observing the time of appearance of

ossification centers. The time difference between the various techniques employed should help in selecting the most accurate technique.

Although the study on the composition of the fetal humerus was only preliminary, it appears to be the only record of bone composition in the bovine fetus. If this is so, then it is obvious that this area needs more intensive investigation.

The reproductive efficiency of cattle indigenous to Nigeria is low (Lamorde and Weinman, 1972; Zemjanis, 1974; Oseri, 1976; Nuru and Dennis, 1976). The annual calf crop in Fulani herds is approximately 40 per cent (range 34.2 to 54.5 per cent). When the high pregnancy wastage detected in this study is taken into consideration, the reproductive efficiency is actually higher - probably an average of 50 per cent. Reducing this pregnancy wastage in slaughter cows would help in meeting the meat production targets in Nigeria. This could be accomplished by pregnancy diagnosis being made available before sale and slaughter by appropriate authorities.

At this point it should be re-emphasised that studies involving slaughter establishments in Nigeria are difficult and unpredictable. It is recommended that appropriate authorities do all they can to facilitate work of livestock personnel in slaughter establishments.

It is hoped that the information gathered in this thesis will provide a solid foundation for further work on both normal and abnormal developmental problems in Zebu cattle. With time and general process of skeletal development as depicted here, much needed studies of the morphogenesis of muscle, nerves and blood vessels of appendages can be undertaken. Effects of radiation and drugs on appendage development have been observed only after gross abnormalities resulted. Part of this is due to inadequate basic information on normal development. The effects of drugs such as thalidomide should be determined as affecting apical ectoderm, blastema, chondrification, ossification, and growth rather than shortening of a limb or fusion of a joint. Also, studies of other species should be made to provide specific information for each experimental animal and by comparison possibly provide answers that were not available during the present study.

The effect of breed on prenatal development was elusive in this study. It would be of interest to know just when differences in rate of development take place, for example, between Holstein-Friesians with calves weighing 70-125 lbs at birth and Zebu breeds with calves weighing 40-70 lbs.

Finally, as a result of this investigation the following studies are planned:

1. Nationwide appraisal of pregnancy wastage in cattle and its effect on the annual meat target.
2. Re-evaluation of the 35 to 40 per cent reproductive efficiency of indigenous cattle in light of the present finding that 50 per cent of cows slaughtered were pregnant.
3. Effect of breed on fetal measurements as well as the effect of sex on weight and body length measurements.
4. More observations on hair and other external features of Zebu fetuses for meaningful comparisons with information published on Bos taurus breeds.
5. Study of secondary loci of ossification occurring prenatally and postnatally as a useful adjunct for ageing neonates, weaners and yearlings whose birth histories are unknown.
6. Chemical differentiation of normal bone of Zebu cattle during prenatal growth.
7. Development of other body organs in prenatal Zebu cattle.

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APPENDIX

AGE DETERMINATION OF ZEBU FETUSES

Tables 1 - 18 and Fig. 1.

Table 1: Correlation table of the six variables in 20 Rahaji fetuses of known age.

	FR	TCL	WT	CI	LF	LH	AGE
FR	1.000	.98606	.9198	-.09699	.9622	.8282	.98611
TCL	.98606	1.00	.9556	-.07452	.9642	.9287	.9734
WT	.9198	.9256	1.000	-.01669	.93496	.9171	.8694
CI	-.09699	-.74452	-.01669	1.00	-.0812	-.1042	-.1113
LF	0.9622	.9642	.93496	-.0812	1.00	.9171	.9304
LH	.9282	.9287	.9171	-.1042	.9171	1.00	.928
AGE	.98611	.9734	.8694	-.1115	.9304	.9228	1.00

Table 2: Fetal measurements from 250 fetuses collected from Rahaji, Bunaji and Wadara cows after slaughter in Zaria.

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
1	F	2.450	44.00	62.50	17.00/7.50	8.50	6.50
2	M	1.050	35.50	50.00	14.50/6.50	5.50	4.20
3	F	0.825	34.00	46.00	12.50/6.00	5.00	4.00
4	M	0.500	25.60	37.00	11.50/4.50	4.00	3.00
5	F	0.006	6.00	8.50	4.00/1.70	1.60	2.10
6	F	0.650	30.00	45.00	13.50/6.00	4.50	4.00
7	M	1.050	34.00	38.70	14.50/5.70	4.60	4.20
8	M	0.560	26.80	37.00	12.50/4.00	3.40	3.00
9	M	0.005	4.50	7.00	3.70/1.10	0.06	0.50
10	F	0.340	23.20	33.00	10.10/4.30	3.50	3.00
11	F	0.925	31.40	47.00	14.40/5.50	5.30	4.20
12	M	1.125	36.50	50.00	15.00/6.00	6.00	4.50
13	M	1.650	37.00	54.00	17.50/5.50	5.20	6.50
14	M	2.650	47.00	64.00	20.00/8.00	9.00	7.00
15	F	0.600	28.50	38.20	12.00/5.00	4.50	3.70
16	F	1.565	37.50	53.00	15.00/7.00	6.00	5.00
17	F	0.210	20.20	27.20	8.30/3.60	2.80	2.80

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
18	M	0.775	29.80	31.70	12.70/4.70	5.20	3.50
19	M	1.575	42.20	59.20	17.00/6.00	6.60	5.50
20	F	2.150	39.00	53.20	17.00/6.50	6.30	5.90
21	M	1.075	34.00	48.60	14.50/5.80	5.00	3.70
22	F	1.300	35.20	54.20	15.30/7.10	5.60	5.50
23	F	0.050	12.00	17.00	6.10/3.00	1.50	2.00
24	F	1.550	37.50	54.00	16.20/7.20	6.20	5.90
25	M	2.925	44.50	66.50	17.60/7.60	8.00	7.20
26	M	0.850	29.4	46.00	14.80/6.00	4.50	4.00
27	M	0.015	8.2	10.8	4.00/1.10	0.80	0.80
28	F	1.450	36.20	56.30	15.00/6.20	6.00	5.00
29	F	3.300	47.00	70.00	17.60/5.5	8.00	6.50
30	F	0.160	19.10	24.50	8.70/4.00	2.20	2.00
31	F	3.185	48.8	73.50	20.10/7.00	9.10	1.50
32	F	1.275	37.00	51.50	15.80/6.20	5.80	7.50
33	F	0.825	33.50	50.70	14.50/5.00	5.40	5.0
34	M	0.210	20.50	30.50	9/3.3	3.00	3.00
35	M	2.175	45.20	62.50	16.5/6.3	6.3	6.00

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
36	M	1.700	39.50	58.60	17/6.8	6.00	5.3
37	M	2.600	44.00	64.20	19.5/7.5	7.4	6.6
38	M	0.140	14.50	21.50	7.5/3.2	1.5	2.0
39	M	0.270	19.50	30.00	9.2/4	3.5	3.3
40	M	1.350	35.50	53.00	15.5/5.3	6	5.5
41	F	1.275	33.70	52.00	15/6	5.00	5.00
42	M	1.785	38.50	55.00	15.3/6	6.00	5
43	F	0.030	8.60	13.00	4.0/1.9	0.9	0.9
44	M	1.650	39.50	55	15.5/6.0	6.0	5
45	M	1.075	32.50	48.30	15/5.2	4.2	5
46	M	0.006	12.70	16.50	6.3/2.0	1.5	1.5
47	F	2.475	43.50	63.00	17/5.2	6.00	5
48	F	3.225	50.00	78.00	20/5.5	9	7
49	M	3.775	53.00	81.50	20/6.5	9	8
50	F	1.050	33.00	48.00	13.5/6.0	4.5	3.5
51	F	3.475	46.00	71.00	19.5/8	8	6
52	M	3.275	50.00	73.00	19.5/6	7.5	7.3
53	M	1.700	36.00	59.00	15/6	5	4

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
54	F	1.025	30.00	46.00	13/4.2	5	4
55	M	0.430	23.00	32.00	11/4.2	3.3	3.2
56	M	0.080	14.50	20.50	7.5/2.5	1.7	1.8
57	M	3.370	49.00	77.00	19.5/7	8.5	8.0
58	M	0.750	30.00	44.40	13.5/4.5	4.0	3.5
59	M	2.657	42.8	62.00	19.1/7.5	7.0	6.2
60	F	1.650	39.00	59.00	16.00/5.6	5.3	4.8
61	M	0.475	24.5	35.5	10.5/4.0	3.3	3.0
62	M	0.110	15.3	20.6	7.0/1.5	2.0	1.8
63	F	0.110	25.2	32.2	7.0/2.5	1.8	1.9
64	M	0.336	21.5	30.00	10.5/4.5	3.4	3.0
65	M	0.260	20.00	30.00	9.0/2.5		3.2
66	M	0.354	27	35.00	11/4.7	3.3	2.9
67	M	3.200	44.5	65.00	17.5/7.2	8.00	7.8
68	M	0.031	10	15.00	4.5/2.1	1.0	1.1
69	M	1.790	35.0	56.00	17/6	5.0	5.2
70	F	0.560	25.7	38.00	12.5/4	3.8	3.5
71	F	0.240	18.5	27.5	9/3.5	2.3	2.3

Table 2: Continued

No.	Sex	WT(cm)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
72	F	2.510	40	61.00	18/6.8	7	6.0
73	M	0.800	29.00	40.00	14/4		
74	F	1.375	37.50	52.00	16.5/15	5.0	5.0
75	F	1.150	35	50	14/5.2	4	4.5
76	M	0.030	10	13	6.0/1.5		
77	F	0.150	18	22	9/2.5	2.2	2.0
78	F	0.310	24.5	29.5	11/4	3.2	3.0
79	M	0.925	30	44	13.5/5	4.2	3.7
80	M	0.190	18.5	28	9/3.5	2.7	2.2
81	M	0.170	19.5	27.0	9.5/3.9	2.3	2.0
82	M	0.175	18.5	28.0	9/3	2.2	2.2
83	M	1.440	34.5	50.5	15.5/6	5.2	4.5
84	M	0.200	19	26.5	9/3.6	2.3	2.0
85	M	3.210	53.00	77.60	19.2/6.5	9	8
86	M	2.860	46.5	68.60	17/13	8	6
87	M	0.990	30.00	45.30	13.5/5	4.2	4.00
88	M	0.289	22.5	31.70	9/3	3.3	2.8
89	F	0.040	11.00	14.50	5.3/1.5	1.3	1.2

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
90	M	0.375	25.3	34.70	10/4.5	3.6	3.0
91	F	0.765	33.5	44.50	13/6.5	4.4	3.9
92	M	0.455	30.0	37.50	12/5	4.2	4.0
93	M	0.300	35.5	55.00	14.2/6	4.4	4.2
94	F	3.825	59.5	73.8	20.6/11	9.2	7.3
95	M	0.010	7.00	9.00	3/1		0.6
96	M	0.930	34.00	49.50	15/5.5	5.2	4.5
97	M	0.160	19.00	28.00	9.5/3.2	2.3	2.0
98	M	2.400	49.5	65.7	16.8/10	7.4	6.1
99	M	0.250	21.6	29.7	9.2/5.5	2.8	2.4
100	M	0.049	14.1	18.00	5.6/3.4	1.5	1.3
101	M	0.0027	5.3	7.00	2.8/1.1		.07
102	M	0.180	20.4	29.00	9.3/49	2.8	2.5
103	F	0.730	35.3	46.2	12.3/7.6	4.3	3.8
104	F	1.600	43.6	57.7	16.8/10.6	6.0	5.4
105	F	0.550	29.00	42.50	12/.5/4	3.5	3.3
106	F	0.073	14.5	20.5	6/3	1.7	1.8
107	M	2.787	48.50	69.50	18.50/6	7.5	6.50

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
108	F	0.491	26.00	39.00	11.5/40	3.6	3.3
109	F	0.206	19.2	27.4	9.5/4.6	2.7	2.4
110	F	.250	22.6	27.6	11/6.1	3.3	2.8
111	M	290	23.5	33.2	10.5/4.0	3.1	3.0
112	F	.700	29.2	43.8	12.7/5.6	4.6	4.0
113	F	0.028	11.4	16.8	5.9/2.7	1.3	1.2
114	M	1.275	34.2	52	14.8/8.6	5.2	4.5
115	F	2.500	43.2	67.4	19.6/95	7.2	6.3
116	M	1.175	40.5	57	17/5.5	5.0	5.0
117	M	0.575	29.3	42.3	13.5/5.3	4.0	3.9
118	F	1.500	40.50	58.00	16.0/5.2	5.0	5
119	M	1.300	39.00	54	15.0/5.0	6.0	4.9
120	F	3.625	11.50	17.00	6/2.3	1.4	1.3
121	F	0.190	24.7	30	9.3/3.7	2.8	2.6
122	M	0.140	19.00	24.9	8.3/4	2.2	2.2
123	M	3.175	50.30	72.00	8.3/4.4	7.3	0.4
124	M	0.295	22.5	31.00	9/3	1.2	0.9
125	M	0.190	21.00	29.00	8.8/3.8	2.4	2.2

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
126	M	1.700	42.5	58.2	17.3/7.3	6.0	5.8
127	F	2.100	45.5	63.2	21/7	6.1	5.9
128	M	0.880	31.10	47.00	14.5/5.5	4.4	4.2
129	F	0.100	17.50	24.20	8/3.3	2.1	2.1
130	M	7.885	48.00	70.50	20.4/6.6	7.0	7.0
131	F	1.492	26.50	39.00	12.00/4.30	3.2	2.5
132	F	0.995	33.0	49.5	14.1/5.1	4.5	3.8
133	M	0.195	20.5	29.0	9/4	2.6	2.1
134	M	3.680	49	70.00	18.5/6.5	7.00	6.4
135	F	0.085	16.2	22.00	7.5/3.0	2.0	1.5
136	F	8.240	78.00	114.00	28/11	14.2	12.4
137	M	0.470	27.00	39.00	12/5	3.0	2.8
138	F	1.630	39	57.00	16/6	4.1	3.9
139	M	6.220	98	148	28/11	19.2	15.2
140	F	1.020	32.20	48.00	16.3/6	4.7	4.0
141		1.015	4	7.00	2/1	.09	.06
142	M	3.520	51.2	72.2	22/7	7.4	6.5
143	F	2.020	42.0	61.00	17.7/5.5	6.2	5.8

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
144	F	.078	14.2	21.0	7/3.2	1.2	1.1
145	M	0.700	31.0	44.5	13.5/4.7	4.0	3.5
146	F	0.15	10.00	13.00	4.3/1.5	1.00	1.00
147	F	1.475	47	66	20.5/7.5	8	7.2
148	M	0.700	30	42	12.5/4.5	4	3
149	F	2.850	50.5	70	22.5/6.00	8	6.8
150	M	1.100	35.5	49	15/5	4.8	4.0
151	M	0.250	22.00	28.5	9/3.7	2.6	2.2
152	F	0.250	23.0	33.00	9/3.5	3	2.5
153	M	0.575	34.00	50.00	15.0/5.8	4.7	3.5
154	F	0.105	17.00	24.00	8/3.5	1.9	1.7
155	F	1.200	85	118	25/13½		
156	M	3.300	50	73.00	12.5/8		
157	M	2.675	42	65	20/6.5	6	5.5
158	M	3.500	48.2	73	20/7.00		
159	F	8.200	74	107			
160	F	0.950	31.8	47.50	15/5	4.5	4.0
161	M	2.050	40	57.00	16.8/6.8	6.2	5.0

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
162	M	0.500	25.00	39.00	12.5/4.5	3.5	3.0
163	F	1.725	38.80	58.00	19.00/6.00	6.0	
164	F	0.725	30.20	46.20	14.00/5.00	4.5	4.80
165	F	0.325	25.30	37.00		4.5	4.0
166	M	2.575	40.20	62.00	11.5/4.9	3.2	2.6
167	F	1.225	31.00	47.00	19/7	5.6	4.9
168	M	0.525	26.00	37.5	15.3/6	4.4	4.0
169	M	1.685	38.20	57.20	11.5/5.5	5.0	4.5
170	M	1.500	37.5	50.40	15.2/5.6	4.4	4.1
171	F	1.225	32.10	57.00	14.5/4.5	4.8	4.0
172	F	1.300	36.00	54.50	15/5.5	4.5	4.0
173	F	1.275	36.00	54.50	15/5.5	4.7	4.0
174	M	0.011	8.5	11.5	4/2	0.5	0.40
175	F	2.775	49.20	75.00	26/6.9	5.4	6.0
176	F	1.900	34.00	55.00	18.00/5.5	6.3	5.00
177	M	2.575	40.00	63.00	17.5/6.5	6.5	5.5
178	M	1.700	32.50	49.00	15.00/5.5	4.5	4.00
179	M	1.950	36.00	50.00	14.00/6.5	5.5	5.00

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
180	F	4.040	48.00	58.00	15.5/6.00	5.8	5.3
181	M	2.050	32.4	50.00	14.5/5.5	5.0	4.3
182	M	2.055	39.00	57.00	16.00/7.00	5.5	4.5
183	M	0.725	32.3	46.6	14.1/7.00	4.4	4
184	F	2.000	37.00	55.00	15.3/6.4	5.9	4.8
185	F	2.060	33.00	53.00	16.7/7.3	5.8	4.7
186	F	2.573	42.8	62.8	19.00/7.8	6.4	5.5
187	F	500	25.8	38.5	12.2/5.00	3.8	3.00
188	M	95	11.5	15.6	5.5/2.5	1.2	1.1
189	F	695	27.00	42.00	13/5	4	3.5
190	F	580	25.50	42.00	12.5/5	3.9	3.4
191	F	430	24.30	36.50	11/4.5	3.4	3.00
192	F	455	24.70	37.00	11.2/4.2	3.00	2.5
193	F	1.700	34.10	52.00	15.7/5.6	5.6	4.1
194	M	6.12	6.20	9.001	3.2/1.5	0.60	0.50
195	M	2100.0	42.5	63	17/6.5	6.0	5.1
196	F	775.0	29.00	42.50	13.5/5	4.9	4.1
197	M	425	25.00	38.00	10.3/4.6	3.3	3.00

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
198	F	1.225	33.50	50.00	6.00	6.00	4.5
199	F	150	15.00	21.2	7.5/3.2	2.2	1.9
200	M	1700	38.3	58.00	13.00/6.3	6.00	5.00
201	M	825	29.00	42.50	13/4.5	4.5	3.5
202	F	825	29.00	44.50	13.5/4.0	4.5	4.0
203	F	525	27.00	39.00	12/4.5	4.00	3.3
204	M	225	20.50	29.00	9/3	3.00	2.6
205	F	725	28.50	43.60	14/4.5	4.5	3.8
206	F	25.00	13.00	18.50	6/2	1.8	1.6
207	M	1925	40.20	61.00	17.5/6.5	6.7	5.4
208	F	1475	35.50	53.00	16.5/5.5	5.00	4.6
209	F	1400	36.4	55	17.2/6	5.5	5.00
210	F	850	32.0	46.00	14.5/4.5	4.2	3.7
211	F	3750	54	84	20/7	9.00	8.00
212	M	900	33.20	46.00	14/5.0	4.00	3.5
213	F	3100	30	55	19/6.5	-	-
214	M	160	18.5	23.5	8.5/3.00	2.6	2.4
215	F	900	32.40	48.00	15/5.5	4.8	4.0

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
216	F	1825	40.50	62.00	19/6	6.00	5.7
217	F	220	18.6	28.5	9.4/3.4	2.5	2.3
218	F	210	21.00	28.00	9.3/3.9	2.9	2.6
219	M	1275	35.00	53.00	15.5/5.7	5.5	4.7
220	F	1725	40.8	61.5	16.5/6.5	6.4	5.3
221	M	2900	46.00	70.00	20.0/7.0	7.5	6.8
222	M	2375	46.00	69.00	18/6.5	7.00	5.8
223	M	3650	52	75	20/7.0	8.00	6.5
224	M	1750	46	67.00	18/6.5	7.1	5.8
225	F	650	31.00	44.5	14.2/5.0	4.6	3.5
226	M	1648	39.2	60.00	17/6.3	6.2	5.1
227	M	1765	39.5	58.2	17.3/5.3	6.1	5.9
228	F	750	30.4	44.8	14.00/5.00	4.4	4.0
229	F	700	29.00	42.00	13.9/4.7	4.5	4.1
230	M	1725	42.00	61.00	17.5/7	6.00	5.7
231	M	3.975	49	77	22.50/8.00	8.9	7.7
232	M	675gm	29	41	14/5.0	4.00	3.5
233	F	4.025	51	80	23/7.0	10.20	8.5

Table 2: Continued

No.	Sex	WT(kg)	CR(cm)	TCL(cm)	CI(cm)	LF(cm)	LH(cm)
234	F	4,100	59	89	24/8	11.00	9.00
235	M	10	70	114.0	27/10	13.5	11.00
236	M	12,200	80.00	121.00	26/8.2	11.25	10.8
237	F	13,500	85.50	127.50	26.5/8.3	16.70	13.00
238	F	21,100	90.50	135.00	27/10	17.5	12.8
239	M	20,950	99.00	148.50	29.5/10.3	19.3	15.2
240	M	22,050	97.00	145.50	28/9.5	18.5	14.3
241	F	11,800	75.00	112.50	24/8	13.90	11.60
242	F	20,100	98.20	147.30	28.5/9.5	18.0	13.9
243	M	19,805	95.50	143.25	28/9.3	17.9	12.9
244	F	12,00	81.60	122.40	24.9/8.1	14.8	12.00
245	M	11,500	77.00	115.50	24.2/8	14.00	11.80
246	F	13,497	85.50	127.50	26.3/8.3	16.65	13.2
247	F	21,095	90.30	135.45	27/9.5	17.40	12.7
248	M	21,150	92.90	139.35	27.9/9.3	17.60	12.8
249	M	22,205	99.00	148.40	30/10	19.2	15.3
250	F	21,000	89.50	134.25	27/10	17.20	12.50

WT = Fetal weight
FR = Forehead-rump length
TCL = Total body contour length
CI = Cephalic index
LF = Length of femur
LH = Length of humerus

Table 3: Effect of sex on the variables in 116 fetuses collected after slaughter of Zebu cows.

Variable	Group	No. Cases	Mean	Standard Deviation	Standard Error	F Value	2-Trail Prob	T Value	Degrees of Freedom	Significance*	
	1	69	30.5761	13.664	1.664						
	2	47	30.3531	12.115	1.731	1.26	.395	.09	114	.927	NS
TCL	1	69	43.7731	20.373	2.489						
	2	47	43.6329	17.962	2.566	1.29	.361	.04	114	.969	NS
WT	1	69	1172.9657	1105.705	135.083						
	2	47	1081.8357	1064.071	152.010	1.08	.787	.44	114	.657	NS
CI	1	69	2.5960	.567	.069						
	2	47	2.5688	.489	.070	1.34	.284	.27	114	.788	NS
LF	1	69	4.6284	2.590	.316						
	2	47	4.5245	2.274	.325	1.30	.344	.22	114	.823	NS
LH	1	69	4.0418	1.950	.238	1.19	.521	.23	114	.816	NS
	2	47	3.9592	1.784	.255						
Est. Age	1	69	4.4269	1.285	.157	1.22	.472	-.01	114	.994	NS
	2	47	4.4286	1.163	.166						

1 = Dry season S = Significant F = Variance ratio

2 = Wet season NS = Not significant Est. Age = Estimated age

T = Deviation of estimated mean * (P 0.05)

Table 4: Analysis of variance of WT of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B.G.	2	242778.3818	121389.1909	.102
W.G.	113	135030169.7561	1194957.2545	NS
Total	115	135272948.1379		

Test for Homogeneity of Variances

Cochrans: C = Max. Var./Sum (Ar.) .3577, P = .678 (Approx.)

Bartlett-Box F = .017, P = .899

Max. Variance/Min. Variance = 1.187

D.F. = Degree of freedom; F = Variance ratio

BG = Between groups WG = Within groups

Table 5: Analysis of variance of FR of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B.G.	2	50.6809	25.3404	.149 NS
W.G.	113	19246.1511	170.3199	
Total	115	19296.8320		

Cochrans C = Max. Variance/Sum (Var.) = .4052, P = .253 (Approx.)

Box F = .930, P = .395

Maximum variance/Minimum variance = 1.529

Table 6: Analysis of variance of TCL of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B.G.	2	106.6548	53.3274	.141
W.G.	113	42775.4031	378.434	
Total	115	42882.0579		

Chochrans C = Max. Variance/Sum (Var.) = .4074, P = .239 (Approx.)

Bartlett-Box F = 1.031, P = .357

Maxi. Variance/Minimum Variance = 1.562

Table 7: Analysis of variance of CI of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B. G.	2	1.3549	.6775	2.439
W. G.	113	31.3831	.277	NS
Total	115	32.7381		

Cochrans C = Max. Variance/Sum (Var.) = .3833, P = .417 (Approx.)

Bartlett-Box F = .534, P = .587

Maximum Variance/Minimum Variance = 1.375

Table 8: Analysis of variance of LF of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B. G.	2	.8454	.4273	.070
W. G.	113	690.4975	.1106	NS
Total	115	691.3521		

Cochrans C = Max. Var./Sum (Var.) = .4684, P = .037 (Approx.)

Bartlett-Box F = 2.196, P = .112

Maximum Variance/Minimum Variance = 1.215

Table 9: Analysis of variance of LH of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B. G.	2	3.8177	1.9088	.539
W. G.	113	399.9568	3.5394	
Total	115	403.7745		

Cochrans C = Max. Var./Sum (Var.) = .3733, P = .511 (Approx.)

Bartlett-Box F = .174, P = .840

Maximum Variance/Minimum Variance = 1.215

Table 10: Analysis of variance of Age of slaughter fetuses by breed.

Source	D.F.	Sum of Squares	Mean Squares	F
B.G.	2	.7511	.3755	.245
W.G.	113	173.0806	1.5317	NS
Total	115	173.8317		

Cochrans C = Max. Var./Sum (Var.) = .378, P = .458

Barlett-Box F = 1.036, P = .355

Maximum Variance/Minimum Variance = 1.495

Table 11: Effect of season on the variables of 116 slaughter fetuses.

Variable	Group	No. Cases	Mean	Standard Deviation	Standard Error	F Value	2-Tail Prob	T Value	Degree of Freedom	F
CR	1	69	32.2406	13.138	1.582					
	2	47	27.9000	12.365	1.804	1.73	.669	1.79	114	.076
TCL	1	69	46.5957	19.918	2.398					(NS)
	2	47	39.4830	17.748	2.589	1.26	.409	1.97	114	.051
WT	1	69	1317.7681	1123.583	135.263					(NS)
	2	47	865.2340	974.701	142.175	1.33	.308	2.24	114	.027
										(S)
CI	1	69	2.6319	.473	.057					
	2	47	2.5149	.610	.089	1.66	.057	1.16	114	.248
LF	1	69	5.0478	2.568	.309					(NS)
	2	47	3.9043	2.118	.309	1.49	.167	2.52	114	.013
LH	1	69	4.3232	1.882	.227					(S)
	2	47	3.5426	1.782	.260	1.12	.702	2.24	114	.027
Est. Age	1	69	4.5986	1.237	.149					(S)
	2	47	4.1760	1.187	.173	1.09	.777	1.83	114	.069
										(NS)

1 = Dry season
2 = Wet season

Est. Age = Estimated Age

Table 12: Calculations for analysis of variance of weight of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	1174.1228	1100.5357	145.7695	28.0000	3825.0000	882.1114 - 1466.1342
2	32	1065.3125	1127.0902	199.2433	3.0000	4100.0000	658.9532 - 1471.6718
3	27	1132.4815	1034.4797	199.0857	5.0000	3370.0000	723.2549 - 1541.7080
Total	116	1134.4138			3.0000	4100.0000	
Ungrouped Data			1084.5574	100.6996			934.9473 - 1333.8802
Fixed effects Model			1093.1410	101.4955			933.3327 - 1335.4949
Random " "			56.3717	32.5462			994.3773 - 1274.4603

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

Table 13: Calculations for analysis of variance of FR length of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	44.6860	17.6386	2.3363	13.6000	81.5000	40.0058 - 49.3661
2	32	42.5719	22.0438	3.8968	7.0000	110.5000	34.7242 - 50.6195
3	27	42.8928	19.8928	3.8284	7.0000	77.0000	35.0170 - 50.7656
Total	116	43.7138			7.0000	110.500	
Ungrouped Data			12.9537	19.3103			40.1624 - 47.2652
Fixed effects Model			13.0507	19.4562			40.1349 - 47.2927
Random " "			.8233	1.1517			40.8280 - 46.5796

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

Table 14: Calculations for analysis of variance of TCL of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Maximum	Minimum	95% Conf. interval for Means
1	57	31.1404	11.9051	1.5769	59.5000	8.6000	27.9815 - 34.2992
2	32	30.0187	14.7233	2.6027	73.000	5.3000	24.7104 - 35.3271
3	27	29.6407	13.2855	2.5568	49.5000	4.5000	24.3852 - 34.8963
Total	116	30.4819			73.5000	4.5000	
Ungrouped Data			12.9337	1.2027			28.0995 - 32.8643
Fixed effects Model			13.0507	1.2117			28.1815 - 32.8825
Random " "			.8233	.4753			28.4367 - 32.5271

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

Table 15: Calculations for analysis of variance of cephalic index of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	2.4986	.4911	.0651	1.3100	4.0000	2.3683 - 2.6289
2	32	2.7544	.5758	.1018	1.8900	4.6700	2.5468 - 2.9620
3	27	2.5644	.5405	.1040	1.3600	3.6400	2.3506 - 2.7783
Total	116				1.3100	4.5700	
Ungrouped Data			5336	0495			2.4864 - 2.6826
Fixed effects Model			5270	0489			2.4875 - 2.6814
Random " "			1354	0781			2.2482 - 2.9207

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

Table 16: Calculations for analysis of variance of LF of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	4.6614	2.1847	.2894	.9000	9.9000	4.0817 - - 5.2411
2	32	4.5625	3.0076	.5317	.7000	15.0000	3.4781 - 5.6469
3	27	4.4481	2.3436	.4510	.6000	9.0000	3.5210 - 5.3753
Total	116	4.5845			.600	15.0000	
Ungrouped Data			2.4519	2277			4.1335 - 5.0354
Fixed effects Model			2.4720	.2295			4.1298 - 5.0392
Random " "			.1118	.0645			4.3068 - 4.8621

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

Table 17: Calculations for analysis of variance of LH of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	4.1456	1.8268	2420	.9000	8.0000	3.6609 - 4.4515
2	32	3.7188	1.8533	3294	.6000	9.3000	3.0470 - 4.3530
3	27	4.0556	2.0139	3875	.5000	8.0000	3.2589 - 4.8522
Total	116	4.0069			.5000	8.0000	
Ungrouped Data			1.3738	.1740			3.6623 - 4.3515
Fixed effects Model			1.8813	.1747			3.6608 - 4.3530
Random "	"		.2287	.1321			3.4387 - 4.5751

Group 1 = Rahaji

2 = Bunaji

3 = Wadara

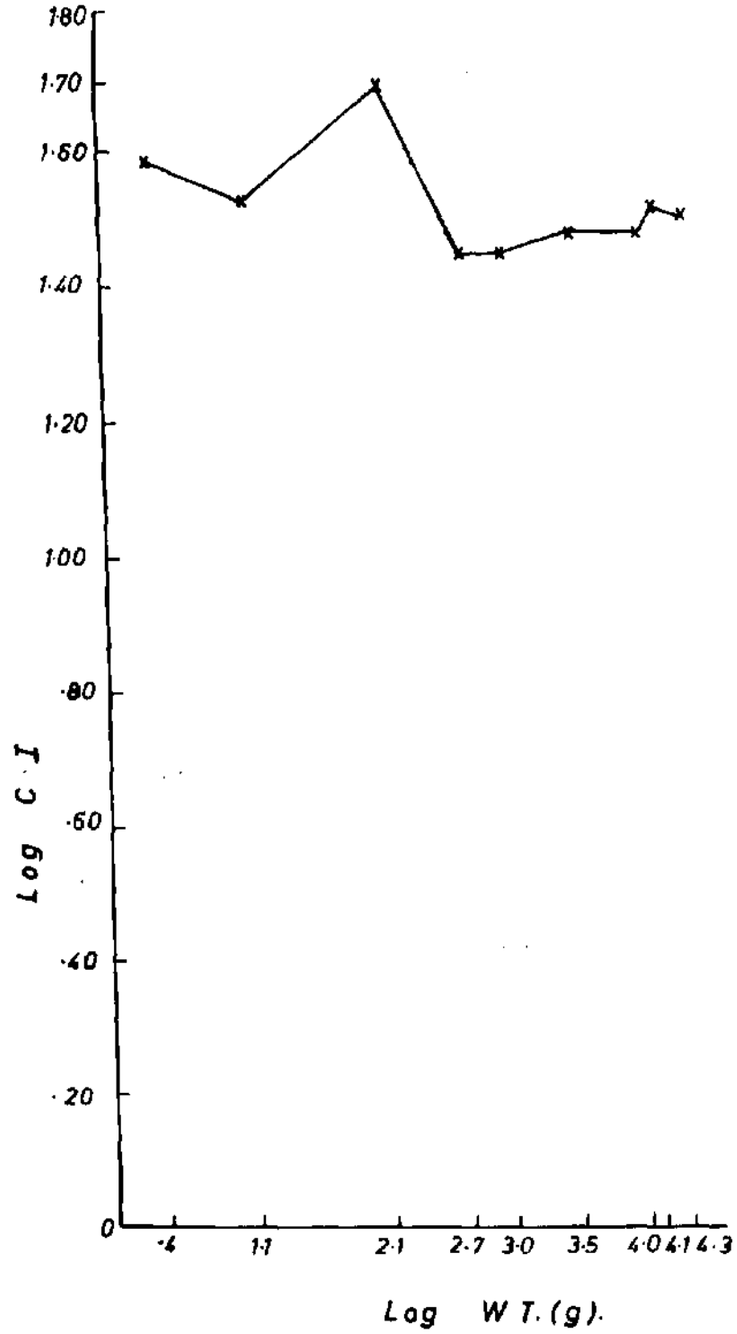
Table 18: Calculations for analysis of variance of estimated age of slaughter fetuses by breed.

Group	No.	Mean	Deviation	Error	Minimum	Maximum	95% Conf. interval for Means
1	57	4.5088	1.1123	.1473	2.0000	6.5000	4.2135 - 4.8039
2	32	4.3625	1.3404	.2369	1.4000	7.5000	3.8792 - 4.8458
3	27	4.3333	1.3601	.2618	1.3000	6.1000	3.7953 - 4.8714
Total	116	4.4276			1.3000	7.5000	
Ungrouped Data			1.2295	.1142			4.2015 - 4.6537
Fixed effects Model			1.2376	.1149			4.1999 - 4.6552
Random " "			.0993	.0573			4.1810 - 4.6742

Group 1 = Rahaji

2 = Bunaji

3 = Wadara


1·80
1·70
1·60
1·40
1·20
1·00
·80
·60
·40
·20
0
Log C.I
·4
1·1
2·1
2·7
3·0
3·5
4·0
4·1
4·3
Log W T. (g).

DEVELOPMENT OF THE APPENDICULAR SKELETON OF ZEBU CATTLE IN NIGERIA

By

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AN ABSTRACT OF A THESIS

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The present study was undertaken in Zebu embryos and fetuses to determine changes occurring in developing limbs from the initial appearance of limb buds through blastema formation, chondrification, and ossification to birth, and to determine at what stage of embryonic and fetal development these changes occur.

Of 7,941 Zebu cows examined at three Zaria slaughter slabs, 4,041 (50.9%) were pregnant when slaughtered. The breeds involved were Rahaji (65%), Bunaji (30%), and Wadara (5%). The age distribution was estimated by teeth examination to be young -- 7 per cent (less than 3.5 years); mature -- 63.2 per cent (3.5 to 7 years), and old -- 29.8 per cent (over 7 years).

Relationship of age to pregnancy was highly significant between the three age groups and between mature plus aged and young ($P<0.005$). The relationship of season to the stage of pregnancy was not significant. The difference between the three stages of pregnancy at which cows were slaughtered were highly significant ($P<0.005$); the majority (67%) being in the second semester. There was little difference in carcass weight between the three age groups and the stages of pregnancy.

Ovarian activity was highest during the rain season and anestrus highest during the dry season ($P<0.01$). Anestrus cows and non-pregnant cows with active ovaries had the lowest mean

(135 ± 41 kg) and the highest mean (170 ± 43 kg) carcass weights, respectively. Aged anestrus cows had the lowest carcass weight (127 ± 28 kg) and mature cows with active ovaries had the highest (180 ± 47 kg).

The magnitude of the pregnancy wastage problem in cattle in Nigeria was discussed and it was recommended that pregnancy diagnosis per rectum be made available prior to culling or slaughter in order to reduce this major source of meat loss.

Data on seven easily measured variables (WT., FR, TCL, LF, LH, CI, and Age) from 20 Rahaji embryos/fetuses recovered by Caesarian section and from 250 embryos/fetuses collected from slaughtered Rahaji, Bunaji and Wadara cows are presented. Data from the 20 Rahaji embryos/fetuses were used for establishing a normal growth curve and linear correlations for ageing the 250 slaughter fetuses. The correlations between the variables were significant except for the cephalic index ($P < 0.05$). The regression equation for body weight and body length (TCL) was found to be the most accurate for calculating fetal weight and that body weight and fetal age was the best for estimating age of Zebu fetuses of known weight. From the non-log correlation between body length (FR) and age of developing Rahaji fetuses, a simple scale was devised for estimating fetal age in the field. No significant differences

for sex or breed but a significant increase was found in body weight and length of femur and humerus during the wet season ($P < 0,05$).

The sequence and rate of hair growth was studied in 150 fetuses, 100 to 231 days of age, from slaughtered Rahaji, Bunaji and Wadara cows. Sequential development of the following external features were observed in embryo and fetuses from day 24 to 230 gestation: limb buds, mammary ridge, olfactory pits, eyes, eyelids, facial cleft closure, intradigital grooves, ear pinnae, genital tubercle, teats, scrotum, testicular descent, hoof formation, horn buds, teeth eruption, fusion of hard palate, carpal and patella separation, and meconium detection. Pattern of hair growth was found to be indecisive as an aid to ageing Zebu fetuses. Initial appearance of certain external features could serve as a rough but quick ageing guide.

The number time of appearance, and chronological order of the primary loci of ossification in limbs were investigated in 70 (Rahaji 55, Bunaji 10, Wadara 5) fetuses by Alizarin red S staining. The ossification pattern observed was as follows: first six weeks little activity; 7 to 11 weeks - many primary loci laid down; 12-23 weeks - new loci appear together with intense growth of existing loci; and after 34 weeks no further primary loci.

The number, time of appearance, and chronological order of the primary loci of ossification in developing limbs were investigated in 50 fetuses (Rahaji 35, Bunaji 10, and Wadara 5) by radiography. No ossification activity was observed during the first eight weeks, by the ninth week a number of primary loci were detected, and no primary loci were observed after 210 days. Under the conditions of this investigation, radiography was found to be less efficient than Alizarin red S staining for detecting the primary loci of ossification in the appendicular skeleton of the Zebu fetus.

The composition of the humerus of the Rahaji fetus was studied at 20 days intervals from day 68 to 2 days postpartum. The water content changed rapidly from 95 per cent at day 68 to 50 per cent at birth. Organic matter increased from 2.2 per cent at day 68 to 28 per cent by 2 days postpartum. Inorganic matter increased from 1.2 per cent on day 68 to 20 per cent by day 2 postpartum. The most rapid increases in organic and inorganic matter occurred between day 68 to 88, the period of rapid ossification in the humerus. Fat content decreased from 1.6 per cent on day 68 to less than half a per cent at day 2 postpartum.

Increases in diaphyseal ossification coincided with changes in bone composition. At day 68, 38 per cent of the total length

of the humerus was ossified; this increased to 82 per cent at birth.

The data obtained on development of the appendicular skeleton in prenatal Zebu cattle will provide a firm basis for further work on normal and abnormal skeletal development and should open the way for studies on the morphogenesis of muscle, nerves and blood vessels of the appendages.