

SPIDER LAMB SYNDROME
HEREDITARY CHONDRODYSPLASIA
A CONGENITAL SKELETAL DEFECT IN SHEEP

BY

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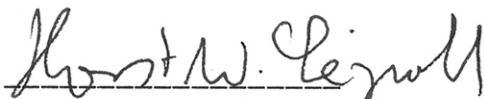
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DEDICATION

To a very special person in my life, my wife
Karla, and our children Dustin, Ryan, Amanda, and
Amy.

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INTRODUCTION

During the early 1980's and possibly the late 1970's reports of skeletal deformities in Suffolk and Suffolk cross lambs began surfacing. Sheep producers and Veterinary practitioners were hearing descriptive terms such as spider, spider lambs, corkscrew lambs, monkey lambs, crooked-legged lambs, and bent lambs. These descriptive terms were used to describe the appearance of affected lambs. In personal communications with the late Dr. Ben Huff of Oregon (veterinary practitioner and purebred Suffolk breeder) he indicated that he had observed this condition or a similar condition in two flocks during the 1975 and 1976 lambing seasons. In private practice at Geneva, Nebraska, we observed our first case during the 1981 lambing season. The next spider lamb was observed during the 1984 lambing season. Work was initiated to define this condition. Review of the veterinary literature and producer publications (in early 1984), did not reveal documentation of the spider lamb syndrome. The present spider lamb research project was initiated in early 1985.

Research revealed that the spider lamb syndrome is confined to Suffolk and Suffolk cross lambs, without confirmed cases outside of Suffolk breeding. The incidence of the spider lamb syndrome appeared to make a significant increase during the 1981 to 1985 period.

The condition is manifested primarily by abnormalities of axial and appendicular skeleton. Increased height, fineness of bone, reduced muscling, and angular deformities provided a spider-like appearance to affected lambs. No official scientific name has been established to describe the syndrome. As afflicted lambs mature they develop severe degenerative joint disease.

The study was designed to evaluate and describe the congenital syndrome in Suffolk lambs referred to as spider lamb and degenerative joint disease. Findings reported here attempt to classify and characterize the syndrome in relation to previously reported congenital defects in sheep.

LITERATURE REVIEW

BACKGROUND INFORMATION

In January 1981, a purebred Suffolk ram lamb twelve weeks of age was presented to the Geneva, Nebraska Veterinary Clinic. It had spinal curvature (kyphoscoliosis), upward deviation of the sternum (pectus excavatum) and angular deformities of the front legs. The distal radi and ulni were rotated medially, the carpi were touching and the feet were splayed out .

A second Suffolk lamb was presented in the summer of 1984. The clinical findings were similar to those of the lamb evaluated in January 1981.

The lambs met the description of the spider lamb syndrome, a term used by Suffolk breeders to describe the condition. Computerized literature searches were initiated to generate references to the syndrome. No citations were found using AGRICOLA, CAB-ABSTRACTS, Federal Research in Progress and CRIS. Searches of the lay journals and literature were also nonproductive. Dr. G F Kennedy, private veterinary practitioner and purebred Suffolk breeder, had written an editorial in the Suffolk Banner 71-72 on the spider lamb syndrome. The National Suffolk Association was approached for support to investigate the condition. Dr. H W Leipold agreed to serve as a major professor for a dissertation on this subject.

EVALUATING AND UNDERSTANDING CONGENITAL MALFORMATIONS

Knowledge of the embryology and principles of development are essential to understanding congenital malformations. Included are all abnormalities arising during prenatal development, regardless of the cause; inherited, induced or spontaneous. In this study several anatomical anomalies whose pathogenesis occurs during development are described. Some conditions are not manifest at birth, but emerge later as the animal becomes ambulatory and more active. Goals include identification, diagnosis, and possible treatment.

Embryology of Bone and Cartilage

GERM LAYERS⁴

Early in development all vertebrate embryos form three layers or sheets of cells, from which all tissues and organs in the emerging fetus will develop. The outer or uppermost layer is the ectoderm, which will form the epidermis, neural tissues, and some of the skeletal and connective tissues of the head. The deepest, innermost layer is the endoderm, which will form the lining of the digestive tract, respiratory system and those organs associated with digestion. Between the two is the mesoderm consisting of a more loosely arranged population of cells. This germ layer will form most of the muscles and skeletal

tissues, the urogenital system, and the heart and blood vessels of the animals.⁴

TISSUE TYPES⁴

Connective tissues are derived from mesoderm in the trunk, and both mesoderm and ectoderm in the head. This category includes cartilage and bone tissues, which are formed by the processes of chondrogenesis and osteogenesis. Mesenchymal cells that become chondroblasts produce type II collagen fibers and a ground substance whose proteoglycan contents varies depending upon the type of cartilage being formed. Hyaline cartilage is most abundant in the embryo and is often produced in areas of the body where bone will replace cartilage.

Mesenchymal cells that differentiate into osteoblasts produce a mixture of fibers and ground substance called osteoid in which calcium and phosphate are precipitated in the process of ossification. Intramembraneous bone is laid down in mesenchyme without a cartilaginous precursor. The clavicle and roofing bones of the skull are examples. Endochondral bone is produced within and around a hyaline cartilagenous model. The appendicular skeleton, vertebrae and bones forming the floor of the braincase are formed by endochondral ossification.⁴

BONE: ENDOCHONDRAL OSSIFICATION^{2,4}

Endochondral ossification entails the proliferation, maturation, degeneration, and modeling of cartilage. The physis is the center of endochondral ossification and is referred to as the growth plate. Cartilage remains as an articular surface and as a disc or plate between the bone forming in the epiphysial center and the bone forming in the diaphysial center. There are five zones of cartilage cells identifiable within the growth plate: resting chondrocytes, proliferative chondrocytes, maturing chondrocytes, hypertrophied chondrocytes, and calcified cartilage.

The zone of resting or non-dividing chondrocytes is adjacent to the epiphysis end-plate. Their primary function seems to be to connect the growth plate to the epiphysis.

The zone of proliferative chondrocytes is characterized by stacks of thin, wedge-shaped cells that are actively dividing. This zone is responsible for elongation as well as adding new cells to replace those lost to metaphysial bone replacement.

The zone of maturing chondrocytes is the region in which several cellular changes occur. Cell and lacunar size increase progressively and lacunae become more rounded than in the proliferative and resting zones. They tend to stack up in columns.

The zone of hypertrophied chondrocytes is a narrow zone next to the maturation zone. The cells are large and the intercellular substance is progressively reduced. This zone is the weakest region of the growth plates.

The zone of calcified cartilage consists of cells, the matrix of which undergoes calcification. This calcification is associated with matrix vesicle and is confined generally to the longitudinal septa. In some species portions or all of the transverse septa may be mineralized.²

Most long bones have at least three centers of ossification. The primary ossification center forms the diaphysis whereas proximal and distal ossification centers form the proximal and distal epiphyses. These are referred to as secondary ossification centers. The morphological features and timing sequence of the events of the secondary ossification center are a variation of the events of the diaphyseal ossification center.

DIAPHYSEAL GROWTH^{2,4}

The growth in length is primarily a function of the growth plate. Although some length is achieved before the formation of the growth plate, the greatest amount of length results from the interstitial growth of the growth plate cartilage.

The continual adding of bone by osteoblastic activity of the periosteum increases the diameter of the diaphysis, which is accompanied by an increase in the diameter of the marrow cavity.

EPIPHYSEAL GROWTH²

The epiphysis of a bone is, by definition, a part of a long bone that develops from an ossification center distinct from the diaphysis, and is separated by the physis. In the adult, an epiphysis consists of bone covered by articular cartilage. In the developing animal, three distinct cartilaginous zones are apparent within the epiphysis. The future articular cartilage has a deep positioned mitotically active zone. An acidophilic cartilage zone is underlaid by the growth cartilage that feeds chondrocytes into the endochondral ossification process of the epiphysis. The ends of the long bones generally are larger than the diaphysis. The epiphyses and metaphyses have a distinct flare. After the formation of the epiphyseal end plate, the growth of the epiphysis occurs independently of the events of the diaphyseal ossification center.²

CARTILAGE^{2,3,5}

Cartilage consists of cells embedded within an amorphous, gel-like substance. The cells of cartilage secrete various substances and become embedded in their own

secretory products. The secretory products of chondrocytes form a firm but resilient matrix. These qualities suit cartilage to its functional role in weight bearing, movement, and organ integrity.²

The toughness and rigidity of cartilage enable it to withstand shearing and contribute to a "friction-free" surface at the articular surfaces of bones. Cartilage provides a resilient form of support and has a crucial role as a precursor or model for the embryonic development and subsequent growth of many bones.³

All cartilage develops from mesenchymal tissue. At the site of chondrogenesis, mesenchymal cells round out and proliferate to form closely packaged groups. At this stage of differentiation the cells have transformed into chondroblasts, and the tissue is called precartilage. Subsequently the chondroblasts begin to synthesize and secrete matrix into the extracellular space, entrapping themselves in lacunae, which become further separated by the formation of additional matrix. The cells completing this sequence have been transformed into mature cartilage cells or chondrocytes.³

Many of the individual chondrocytes within lacunae divide and may create lacunae packed with a mass of cells or cell nest. Each cell nest is a clone of the first chondrocyte in the lacuna. The progeny eventually become

partitioned by the secretion of matrix, resulting in the expansion of the cartilage from within, a process known as interstitial growth.

As the matrix ages it becomes increasingly rigid. Interstitial growth declines rapidly with age because the matrix is no longer able to expand. Further growth proceeds by the addition of cartilage on the existing outer surfaces beneath the perichondrium in a sequence referred to as appositional growth. Appositional growth occurs when the undifferentiated cells of the inner chondrogenic layer of perichondrium proliferate and differentiate into chondroblasts that deposit matrix around themselves.

In early embryonic growth, appositional and interstitial cartilage growth occur together. Later growth is primarily appositional. At maturity, the chondrogenic activity of the perichondrium becomes dormant and is stimulated by injury.^{3,5}

CARTILAGE ZONES OF GROWTH^{2,6-14}

The cellular component of this growth may be divided into four zones as discussed by Kassebaum.⁶ Zone one is referred to as a tangential or gliding zone consisting of elongated cells with their long axes parallel to the surface within a less developed proteoglycan matrix.

Zone two is a transitional zone where randomly distributed chondrocytes are round to ovoid and it is the

most metabolically active layer of cartilage. Electron microscopy of chondrocytes demonstrated extensive networks of round endoplasmic reticulum, dilated cisternae, vacuoles and Golgi apparatus, suggesting protein synthesis and matrix production.⁷ Studies have demonstrated that chondrocytes synthesize macromolecular proteoglycan and collagen component portions intracellularly; then rapidly extruded into the surrounding matrix.^{8,9,10} The secretions surround the chondrocytes which become isolated in lacunae. Chondroblasts maturing into chondrocytes undergo cellular hypertrophy and enlargement of the flattened lacunae to an ovoid angular shape.

Zone three is a radial zone. The cells are lined up in short, irregular columns which become longer and the cells larger in the deeper part of the cartilage. Clusters of chondrocytes in cell nests occur. These are the cell nests of interstitial growth discussed above.

Zone four is a calcified zone which contains matrix and cells heavily encrusted with hydroxyapatite. Zone four is separated from the radial zone by an irregular bluish line, the "tidemark" and on its deep surface the zone merges with the end-plate of the underlying bone.¹¹

The matrical components of cartilage consist of fibers and ground substance. The most prominent fiber being the

fine collagen fiber, while the primary constituents of the ground substance are the gel-like proteoglycans.^{2,12,13}

The gel-like matrix is composed of proteoglycans with a protein core and a large number of attached glycosaminoglycan (GAGs) chains of chondroitin sulfate.² Chondroitin-6 sulfate is the principal glycosaminoglycan, accounting for 45 to 75% of the sugar component of hyaline cartilage. In immature articular cartilage, chondroitin-4-sulfate is more prevalent, and decreases in concentration with age. A third type keratin sulfate increases with age. Chondroitin-4-sulfate seems to be related to ossification, whereas condroitin-6-sulfate is mainly associated with the maintenance of articular surfaces.¹⁴ The proteoglycan aggregates perform three significant functions in cartilage: stability of the matrix, volume definition of the matrix, and compression properties of the matrix.

Stability of the matrix is achieved by the chemical linkage of proteoglycans to hyaluronic acid and collagenous fibers. This ensures that the proper spatial orientation of the matrical constituents is achieved. Proper spatial orientation is essential for the proper binding of interstitial fluid. These macromolecules define spatial volumes by entrapping the surrounding interstitial fluid. The proteoglycans can absorb solvent volumes that are as much as 50 times their dry weight. This property

contributes significantly to the volume definition of the tissue. Defective glycosaminoglycan and proteoglycan synthesis, secretion, and aggregation are associated with a decreased tissue volume because of the decreased ability to absorb water. Bone length and shape are dependent upon proper growth and replacement of cartilage during endochondral ossification. Alterations in bone developments, as seen in chondrodystrophy have been related to the synthesis of defective proteoglycans.

The absorption of water by proteoglycan aggregates imports a vast hydrodynamic domain to them. Proteoglycan aggregates are reversibly resistant to compressive forces. Subjecting proteoglycan aggregates to centrifugal force decreases their volume in proportion to the applied force. Upon removal of the force, these macromolecules return to their original volume. These substances are polyanions, as the molecular domain of each proteoglycan decreases, the charge density and constraints on mobility within the molecule increases. These factors, coupled with the noncompressable property of water, account for the compressive properties of cartilagenous tissues. Weight-bearing on articular surfaces is based on this physical characteristic. Loss of or inappropriate synthesis and aggregation of these compounds or their constituents decrease the hydrodynamic domain of the remaining

proteoglycan aggregates. This decreases the amount of water contained within the cartilagenous matrix and increases its compressibility. Decreasing the ability to resist compression increases the possibility of damage to the tissue. Alteration to the tissue in this manner may be a contributing factor in the development of degenerative joint disease.²

COLLAGEN AS A COMPONENT OF CARTILAGE¹⁵⁻²⁰

The cartilage collagen component is metabolically active, but turnover occurs at a slower rate than ground substance.¹⁵. Cartilage collagens which are categorized as type II, differs from type I collagen of skin or bone. The difference is the number and type of chains found in helical arrangement.^{16,17,18} Type II collagen chains differ from those of type I principally in the increased quantity of hydroxylysine and excessive glycosylation.^{17,18}

Collagen fibers assume an arcade-like arrangement in articular cartilage spanning three architectural zones. The deepest zone, found adjacent to subchondral bone, help to secure cartilage to bone. This radial zone incorporates collagen fibers oriented perpendicular to the subchondral bone. An intermediate zone assures a haphazard formation, similar to randomly arranged zone two chondrocytes. The most superficial layer lies parallel to the articular

surface in the tangential zone. This arcade formation aids cartilage compressibility and elasticity.^{19,20}

Articular Cartilage^{5,21}

Articular cartilage is hyaline cartilage but differs from other hyaline cartilage in that it is devoid of a perichondrium. Articular cartilage lacks direct vascularity, lymphatics, and nerves. Nutrients must pass through a double diffusion barrier in order to reach the cells. In mature adults, all nutrients must first diffuse out of the synovial vascular plexus, transverse the synovial membrane to enter synovial fluid, and pass through the dense hyaline matrix of cartilage to reach the chondrocytes. In immature animals, nutrient diffusion from underlying bone vasculature to the basilar layer of cartilage can occur, in addition to nutrition from synovial fluid.⁵ The source of nutrients is the plexuses of vessels in the subchondral cancellous tissue and vessels of the joint capsule.²¹

Exercise plays an integral role in pumping nutrient-rich synovial fluid through cartilagenous matrix to the chondrocytes. The rate-limiting feature of nutrient diffusion into articular cartilage cartilagenous matrix pore size. Even low molecular weight proteins diffuse slowly across this barrier. Exercise facilitates this through the compressibility of cartilage under pressure

allowing it to act like a sponge. During pressure fluid is expressed and when pressure is released, fluid is reabsorbed. The continued repeating impact cycles favor local penetration by molecules otherwise considered too large to make their way unaided into the dense structure of normal cartilage. Research demonstrates that without exercise cells atrophy, lacunae merge to form cysts, surface integrity is lost and disintegration of the articular cartilage occurs.²¹

MAINTENANCE, REPAIR AND RESPONSE OF ARTICULAR CARTILAGE TO INJURY^{6,22-27}

Maintenance and repair is dependent on the ability of chondrocytes to replicate.⁶ Cell replication is evident in young animals, but even then it is not uniform. This becomes more apparent as the animal reaches maturity. The mitotic index decreases and only one zone remains, located above the zone of vascular invasion in the deeper layers of cartilage.^{22,23} At maturity hyaline cartilage undergoes varying degrees of mineralization and mitotic activity ceases. It has not been determined whether this decrease in mitotic activity is reversible under certain circumstances. Studies have shown that interruption of DNA synthesis was due to inhibition of the replicator apparatus rather than damage to the DNA structure or change to the DNA synthesis sequence.^{6,24} Under the right circumstances chondrocytes can

replicate even in mature animals, but it is a limited response.

Research indicates that injuries associated with the cartilage of mature animals didn't repair completely. The prominent limiting factor is a significant reduction of inflammatory components within the avascular cartilage. Repair seems to show no relationship to size of lesion and histologic examination only reveals minor changes in the amount of cellular matrical components present.^{6,25} There is an increase in mitotic activity following a superficial injury, but it falls back to normal levels one week post-injury. In rabbits, partial thickness defects showed no significant repair response even in young animals. In deeper lesions involving subchondral vasculature, researchers have noted an inflammatory response. It is initially filled by fibrous tissue that becomes chondrified to form a fibrocartilaginous mass.^{26,27}

STAINING CHARACTERISTICS OF CARTILAGE²

The presence of sulfate groups on the compounds imparts an acidic nature to the matrix. As such, the ground substance of cartilage is basophilic and stains appropriately with routine stains (hematoxylin and eosin = H&E). The matrix of most cartilage does not stain uniformly. Young cartilage may be acidophilic, whereas the matrix of the perilacunar margin (capsular or territorial matrix) of old

cartilage stains intensely basophilic. The region of the matrix between individual cells or group of cells, the interterritorial matrix, stains less intensely basophilic than the territorial matrix. The staining properties relate to the number of sulfate and carboxylate groups (basophilic components) and the acidophilic nature of the collagen. In young cartilage with fewer sulfate groups, the acidophilia of the collagen dominates the staining. In the remainder of cartilage, the basophilic components dominate the stain reaction.

The metachromatic staining properties of cartilaginous matrical materials are dependent upon the constituent glycosaminoglycans. Metachromatic stains such as toluidine blue are useful to demonstrate these substances. Magnesium chloride extinction coupled with alcian blue staining adds valuable information about the distribution of different types of glycosaminoglycans in the matrices.²

DEGENERATIVE DISEASE OF JOINTS WITH PRIMARY EMPHASIS ON CARTILAGE CHANGES^{1, 6, 19, 28-36}

Degenerative articular diseases are common disorders that affect mainly and most importantly the articulations of the limbs. They are known as degenerative arthritis and osteoarthritis, but Jubb et al¹ prefer to use the term arthropathy to separate what is primarily a degenerative change from arthritis, which is primarily inflammatory.

Nevertheless, because of the limited range of reactions inherent in articular and synovial tissues, many of the gross morphologic changes seen in chronic arthropathy may also occur in chronic arthritis, and in the end stage joint the differentiation of primary inflammatory disease from a degenerative one may be arbitrary.

Degenerative joint diseases are classified as primary or secondary. Secondary arthropathies result from known diseases of nutritional, traumatic, developmental, or other cause. Virtually any insult that produces structural injury to joint cartilage or subchondral bone or that is associated with prolonged or repetitive abnormal joint function will ultimately produce degenerative joint disease.

Primary arthropathies are, by definition, of unknown cause, but development of the lesions is well documented and generally occurs in a predictable sequence. Generally it is attributed to trauma, constant wear on a joint surface as described above. One theory is that trauma to articular surfaces allows an increased amount of synovial fluid to penetrate the articular matrix. The hyaluronidase fraction begins to break down the proteoglycan matrix.²⁸ Stress on the now weakened cartilage induces cracks and fissures, which in turn allows more synovial fluid to enter.²⁹

A second theory indicates indirect breakdown is associated with factors, which control the chondrocyte's

ability to produce matrix-degrading enzymes. Research has further shown that live synovial tissue was capable of reducing cartilage matrix when they were cultured together in the same compartment, even though they were not in direct contact with the live cartilage, furthermore there was no decrease in the dead cartilage matrix.^{6,30} Other researchers have shown that the increase in matrix degradation was seen only in the pericellular area of cultured cartilage, near the cell membranes.^{6,31} It has been demonstrated with in vitro studies of cultured articular cartilage containing mononuclear cell supernates from blood, was also associated with degradation of proteoglycans and collagen.^{6,32}

Chondromalacia is an early sign of weakened cartilage matrix due to proteoglycan loss.³³ Grossly it appears as yellowish articular surface. This altered matrix is incapable of supporting the collagen framework and weight bearing stress compounds the problem. Proteoglycan loss and tearing of superficial collagen fibers leads to fraying of the surface with formation of multiple vertical clefts, referred to as fibrillation.^{6,33}

The first microscopic change seen as the cartilage softens is its ability to stain with Hematoxylin and Eosin. There is a decrease in affinity for stain evidenced by a pale pink color with a concurrent loss of cellularity. Clusters or clones of chondrocytes (evidence of local replication) may be seen but are ineffective in filling the defect.^{6,33}

Continued fraying of the surface with the formation of vertical clefts with more severe fissuring extending into deeper cartilage zones without penetrating the subchondral bone but erosion may continue to subchondral bone, where mechanical forces and synovial fluid act to polish its surface, by a process termed eburnation.^{19,34}

In the early stages research has revealed an increase in glycosaminoglycan production but changes resemble those of immature cartilage (more chondroitin-4-sulfate, and less keratin sulfate).^{6,35} This sequence of events resembles those of immature cartilage. Typically cartilage produces Type II collagen while 90% of the collagen found in bone and skin is Type I collagen. Analysis of degenerative cartilage has shown there is a disproportionate amount of Type I collagen.^{6,36}

In summation according to Jubb, Kennedy and Palmer, degenerative arthropathy, primary and secondary, is common in most domestic species, with the possible exceptions of sheep and cats.¹

ABNORMALITIES OF DEVELOPMENT

Developmental errors, which are expressed in the skeleton, may be primary abnormalities of bone, cartilage, or primitive mesenchyme. They may be genetic or primitive mesenchyme. Errors may be genetic or conditioned by the environment, in either case the abnormalities may be local or systemic. It is seldom feasible in domestic species to

classify skeletal anomalies as osseous, cartilagenous, or mesenchymal in nature.

In some disorders the entire skeleton may be involved; such is the case in some of the chondrodysplasias, in which the defect is in the preparatory cartilage, and in some of the congenital osteodysplasias, in which the defect is in the formation of the definitive bone. The defect may be localized to certain regions or certain bones producing local chondrodysplastic changes. Abnormal division of cartilagenous precursors, failure of normal division of cartilagenous precursors, and the development of accessory centers of ossification provide a variety of abnormalities of size and shape. It seems to be a general rule that, of the bewildering variety of malformations, there are basic patterns to which are added relatively minor anomalous variations.

Congenital malformations or congenital defects are those abnormalities present at birth that result from errors arising during development. Adaptational deformities of the skeleton are seen as embryonic bones that are capable of a considerable degree of self-determination of their form, as revealed in organ cultures. The final molding, of shape and internal architecture, which begins in the fetus, is self serving to the demands of function and is directed by pressure and tension. Any

deviation in normal stresses, whether in strength or direction, alters the shape and architecture of bones. As a general rule, abnormal stresses on a young, growing bone change both its shape and its structure, but abnormal stresses on a matrix bone influence the internal reorganization of the structural elements rather than lead to alterations of shape. The underlying principle of adaptation to function is known as Wolff's law of transformation.

VERTEBRAL MALFORMATIONS⁴

Most vertebral malformations result in a grossly abnormal shape of the vertebral column. A lateral deviation is called scoliosis. Deviation of the column in the sagittal plane is called kyphosis if vertebrae are fixed in flexed posture and lordosis if they are fixed in an extended posture. Usually in these conditions the vertebral canal is distorted and the spinal cord compressed, resulting in paresis and ataxia in the limbs caudal to the site of the lesion. Except in severe cases, these clinical signs usually do not appear until a few months after birth. An abnormal twisting of the cervical vertebral column is called torticollis (twisted neck), popularly termed wry neck.

Many vertebral malformations preferentially occur at a particular axial level and with greater frequency in

certain species or breeds. Along with vertebral malformations you can also see malformations of the sternum and ribs. Chondrosternal depression (pectus excavatum) is a funnel-like cavitation of the ventral thorax in which the mid to distal, cartilagenous parts of the ribs curve dorsally and attach to a dorsally-displaced sternum.⁴

SPIDER LAMB SYNDROME^{1,38-67}

Some skeletal abnormalities seen in the spider syndrome have been observed in association with certain toxic plants, chemicals, and viruses. A diet deficient in vitamin D or phosphorous can lead to the classical skeletal deformities of rickets. Unlike the spider syndrome, rickets does not usually develop in utero. A proper diet can often reverse rachitic lesions.¹ A similar condition, "bent-limb syndrome," may develop in confined lambs fed excessive dietary iron, which inhibits the synthesis of vitamin D.³⁷

There were a number of articles in the veterinary literature referred to as "bent-limb syndrome," but there are three or four separate syndromes with separate etiologies. Hidiroglou et al³⁸ described a syndrome with front limb deformities in lambs that was grossly similar but histologic features were different from "bowie-leg" as described by Fitch³⁹ and "bent-leg" as described by Clark et al.⁴⁰ These conditions as well as those discussed by

Palmer⁴¹ and Sykes et al⁴² were all nutrition associated problems.

Two other bent limb defects described in the literature should be mentioned. Pond et al⁴³ reported a thoracic limb defect of unknown etiology in a group of Rambouillet, Finn, and Dorset cross ewes. Millot at the Insitut Pasteur, Paris, France reported a very similar condition that they called bent-limb disease of lambs. They were able to show that it was a simple autosomal recessive gene linked to the OL-Z minor lymphocyte antigen locus and the I locus involved in the expression of R and O antigens in sheep.⁴⁴

Locoweeds (Oxytropis and Astragalus) are known to cause limb deformities in utero.⁴⁵ Parbendazole has been implicated as a cause of limb deformities in sheep.⁴⁶ Affected sheep have abnormalities similar to those of spider lambs including interventricular septal defects. Parbendazole, however, may also induce microphthalmia, atresia ani, spinal bifida, and costal fusion.

Certain viruses cause congenital defects in sheep.⁴⁷ Infections with bluetongue⁴⁸ or Akabane⁴⁹⁻⁵² virus may result in arthrogryposis and hydranencephaly. Border disease can cause abnormalities of the nervous system, skin, fleece, and skeleton. Arthrogryposis lambs has been

confused with spider lambs for several years and is still seen today.

Following is a discussion of the overall incidence of congenital defects in lambs with special emphasis on skeletal defects. Dennis,⁵³⁻⁵⁴ Dennis and Leipold⁵⁵⁻⁵⁶ and Saperstein, Leipold and Dennis⁵⁷ published a complete and concise listing of congenital defects in sheep.

The frequency of occurrence of congenital defects in sheep is difficult to assess, despite several extensive surveys of perinatal lamb mortality in the literature, generalizations remains difficult because population samples were not selected by random process and thus reported defects are largely peculiar to a breed, geographic location, or environmental conditions. Each body system is susceptible to congenital defects; the principal system involved in sheep is the musculoskeletal system, especially the front legs and cranio-facial region.⁵³⁻⁵⁷

Jubb et al¹ state that the most frequent malformations of the limbs in animals are the localized chondrodysplasias. Although the whole limb may be involved, the defect is usually confined to the distal bones, producing some degree of dwarfism. Morphologic analysis of chondrodysplasia is facilitated by understanding the above discussion on cartilage growth; in

that cartilage grows by both interstitial and by superficial apposition. Chondrodysplasias can be present without the classical dwarf syndrome. This depends on which types of growth is most severely affected and to the extent that growth plates in the long bone are affected.

There are few reports in the literature of chondrodysplasias in lambs. Duffell et al reported a chondrodysplasia in lambs where 27 of 110 lambs born in a flock were affected with some degree of dwarfism.⁵⁸ Wray and Matheison reported a case in South County Cheviot sheep which described as an achondroplastic syndrome.⁵⁹ The principal features of the condition were achondrodysplasia of the head with protruding eyes, short ears and tail. The forelimbs were shortened and the forehooves were replaced by an area of hard skin at the end of the limb. The hindhooves were reduced in size and abnormal in shape.

Researchers have also reported hereditary dysplasias of collagen in sheep,^{60,61} but the main defect observed was a skin defect in Type I collagen. Hartley and Kater⁶² reported on the incidence of spinal bifida and arthrogryposis type skeletal defects in New Zealand sheep.

Arthrogryposis was the most common defect which producers confused the spider syndrome with, during the present study.⁶⁹

Anthrogryposis literally means crooked joint. It is a congenital defect characterized by muscle contracture and extension or flexion of joints, with associated angular deformities of the limbs, kyphoscoliosis of the vertebral columns, hydro to hydranencephaly and a round domed cranium.

Dennis and Leipold^{55,56} described anthrogryposis in several publications as one of the more common congenital defects found in sheep. Hartley and Kater⁶² described it in a study of New Zealand sheep under the title of central nervous system diseases. Rook⁶³ listed it as the number one congenital skeletal defect in a recent study of neonatal lamb mortality in Michigan and Ohio.

Leipold, Greene, Huston and Guffy⁶⁴ conducted a study on bovine congenital defects in which arthrogryposis and associated defects was the principal topic. They reported that arthrogryposis is characterized by congenital contracture of the joints of one or more extremities, while the most frequently encountered skeletal defects involved the axial skeleton: Kyphoscoliosis, scoliosis, and spinal bifida. The next most frequent skeletal defect was palatoschisis. A similar study was done by Hartley et al⁶⁵ in New South Wales.

Arthrogryposis has been associated with Akabane virus infection by Hartley et al⁶⁶ and with bluetongue virus by

Richard, et al⁶⁷ but in many cases the etiologic agent is unknown. In outbreaks in Nebraska and Texas in 1986 there was a pattern of occurrence that incriminated arthropod vectors, but no etiologic agent was isolated.⁶⁸⁻⁷⁰

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**SPIDER LAMB SYNDROME,
HEREDITARY CHONDRODYSPLASIA,
A CONGENITAL SKELETAL DEFECT IN SHEEP**

INTRODUCTION

Recently a new syndrome has been observed in Suffolk and Suffolk cross lambs consisting of abnormalities in the axial and appendicular skeleton, plus developing a severe degenerative joint disease as animals mature.

25,26,33a,33b,43,69,70 The pathological findings on 65 lambs are presented in this paper.

MATERIALS AND METHODS

Experimental Animals

Due to the apparent widespread incidence of this syndrome in Suffolk lambs, the National Suffolk Sheep Association provided financial assistance for this study.

In 1985, producers, on a voluntary basis began to submit lambs with skeletal abnormalities to the Congenital Defects Laboratory at Kansas State University (KSUCDL). One hundred-ten lambs were received from eight states (Table 6). Sixty-five were diagnosed as being spider lambs, 28 were diagnosed as being the arthrogroposis-hydranencephaly syndrome (AGH), five were spider lamb suspects and twelve did not fit either syndrome.

Clinical Examination

Upon arrival at the KSUCDL live lambs were photographed and given a complete physical examination including; body temperature, stethoscopic examination of the thorax and

abdomen, and palpation of the entire muscular-skeletal system.

Blood samples were drawn for routine cell counts and serum chemistries on 10 lambs. White blood cells were cultured to examine chromosomes on seven lambs. (APT-6) Live lambs were then euthanatized with intravenous T-61 solution.

GROSS NECROPSY-RADIOLOGY HISTOPATHOLOGY

Following euthanasia, 36 of the 65 spider lambs were radiographed. At necropsy each lamb was evaluated for gross lesions and specimens for microscopic examination were taken including the following: central nervous system (entire brain and spinal cord), endocrine glands, heart, aorta, skeletal muscle, spleen, lymph nodes, liver, kidney, lung, all fore stomachs and the abosamum, small and large intestines, articular cartilage and bone. Soft tissue were fixed in 10% buffered neutral formalin (BNF) for 24 hours, and processed routinely in an autotechnicon, cut 6 microns thick, and stained with hematoxylin and eosin (H&E).

(APT-7) Aortas were stained with three additional stains; Trichrome, Periodic Acid Schiff (PAS), and Van Gieson.

(APT-10-12-13)

Skeletal Evaluations.

Bone sections were cut 2-6 mm thick and fixed in 10% BNF for 10 days. They were washed in tap water for three to

four hours, drained, and placed in 8% sulfasalicylic acid (100 gram in 1250 milliliters of distilled water) for 10 days or until decalcification was complete. Bone specimens were then processed routinely, cut 6 microns thick, and stained with H&E, Alcian Blue (AB), Azure A Eosinate (AAE), Gomori's One Step Trichrome (Tri), and Lillie's Allochrome (LA). The above procedures were repeated on five control lambs.

Skeletal specimens were collected from 36 affected lambs at necropsy. They were first evaluated grossly following the form (APT-4) listed in the appendix under title "Necropsy Examination". The following criteria were used: 1.) front limb abnormalities present (FLA) and extent of involvement. (Table-4) 2.) back limb abnormalities (BLA) and extent of involvement (Table-3) 3.) vertebral abnormalities (VA) and extent of involvement (Table-2) 4.) sternal abnormalities (SA) extent of involvement (Table-1) 5.) cranial-facial abnormalities (CFA) and more specifically Roman-nose (RN) or nasal septal deviation (NSD). (Table-1)

All skeletal muscles were removed and all skeletal bones were sectioned longitudinally. The criteria established for gross examination were used to examine the cut surfaces reported on the form listed in the appendix (APT-4). A (C) was used to designate cut surface and (G) was used to designate gross examination.

RESULTS

Clinical Findings

Sixty-five affected lambs were presented; five were dead on arrival, no data is listed under clinical findings for (R88-37 to R88-42 excluding R88-39).

All live lambs appeared to be in good health based on visual observations of movement and eating patterns. Several had deficiencies in movement due to severe skeletal deformities, but remained bright and alert. Rectal temperatures ranged from 101.2F to 103.2F, which is in normal range for sheep. The lungs, heart, and abdomen were auscultated. Three had moist rales, suggesting pneumonia, this was not born out on the necropsy exam, two had decreased intestinal and ruminal motility of undetermined origin.

Hematological Findings and Serum Chemistries

Hematological and serum chemistries were all within normal limits. Skeletal and muscle enzymes were in the high normal range. Personal communications with researchers at North Dakota State University (Alstadt), Michigan State University (Rook, Krehbiel and Henneman), and the University of Illinois at Urbana (Thomas) indicated their hematological and serum chemistries findings were similar.

Bacteriological and viral Findings

Clinical examinations indicated healthy animals, and

bacterial and viral examinations were limited to five lambs. Isolation attempts were made for bovine viral diarrhea, blue tongue, and Akabane virus, chlamydia, campylobacter, and routine bacterial cultures. Attempts at isolating bacterial or viral pathogens were negative.

MACROSCOPIC FINDINGS

Sixty-five lambs were clinically examined and necropsied (APT3,5). Sixty were presented alive and five were dead on arrival. Ten were verified in February and March 1988 , but were not included in this study. Thirty-seven were females and 28 were males. Statistically these findings are within the normal 1:1 male:female ratio indicating no sex predilection.

All soft tissues were within normal limits on gross examination. All organs and organ systems examined, excluding muscle and skeletal systems, had no gross abnormalities; including the central nervous system. Several lambs appeared to have a decreased muscle mass, suggesting disuse atrophy.

Significant changes were observed in the skeletal systems. The gross skeletal changes were placed in five general classes; front leg abnormalities (FLA), back leg abnormalities (BLA), vertebral abnormalities (VA), sternal abnormalities (SA), and cranio-facial abnormalities (CFA).

Utilizing these classifications, two forms were used; 1.) **Live Examination** (APT-2) 2.) **Necropsy Examination** (APT-4). Figures 1 through 6 illustrate confirmed spider lambs with specific abnormalities and the extent to which the condition was expressed in each lamb.

Front Limb Abnormalities

Front limb abnormalities when present were classified as negative (normal); 1+, 2+, or 3+ depending upon the severity of the abnormality.

The specific abnormalities observed in the forelimbs included extreme long leggedness as compared to normal flock siblings of similar age. Spider lambs were fine boned with minimal muscling and with angular deformities of varying degrees. The angular deformities ranged from slight deviations of the carpal joint to severe angular deformities in which the carpal joint would have nearly a right angle appearance. Many lambs had angular deformities so severe that they could not stand.

In severely affected animals, abnormalities were readily apparent at birth, but some developed several weeks later. The typical "knock-kneed" or medial (varus) deviation was often accompanied by medial rotation of the distal radius and twisting of the carpus. The lower limb was frequently directed outward forcing the carpus even more medially as they grew.

Degenerative changes that accompanied this anomaly included malformed carpal and digital bones with exostoses and arthridites of the shoulder, elbow, carpal and interdigital joints.

Several animals appeared normal at birth but developed front limb abnormalities several weeks following birth. Some of these lambs developed severe angular deformities while others developed only slight abnormalities.

Three lambs had a lateral deviation of the shoulder joint with lateral deviation of the distal scapula and proximal humerus. Seventeen were classified as 1+, 19 were 2+, and 27 were 3+. Two animals did not have front limb abnormalities. With increased age there were increased numbers of lambs showing angular limb deformities. See Table 4.

Rear Limb Abnormalities

Rear limb abnormalities were classified by the same format as used for front limbs. In general, the older the lamb, the more pronounced the angular limb deformities became. Rear limb abnormalities were not as frequent as the front limb abnormalities. There was a much higher percentage of negative and 1+ lambs than the front limb abnormalities.

When present, angular deformities in the rear limbs appeared similar to the forelimbs, even though rear limb

deformities were seen less frequently. (See Table 3) When the angular deformity was present, the tarsus was displaced medially due to a lateral deviation or bow in the distal tibia. In some lambs it was also angled anteriorly in addition to the lateral deviation of the tarsus.

Vertebral Abnormalities

Spinal column abnormalities were observed in 37 (57%) of the lambs examined while alive. Changes noted included kyphosis, lordosis, and scoliosis frequently and some lambs had a combination. The most common change was kyphoscoliosis.

Classifications and photographs of spider lambs illustrating each class of lesions are presented in Figure 13. On live examination vertebral abnormalities were not always detected whereas upon necropsy examination they were readily observed. All suspect lambs necropsied (100%) had some degree of vertebral abnormalities. There was no definite age group distribution of vertebral abnormalities as seen in the limb abnormalities. Some lesions were more noticeable on the cut surface than on the uncut bone. (See Table 2)

Macroscopically the lesion appeared to be excess cartilage and at times accompanied by some bone abnormalities. There was decreased thickness of the articular cartilage of the atlanto-occipital joint seen as

an apparent thinning of the articular cartilage at the lateral aspects of the cranial surface of the atlas. Microscopic and macroscopic findings were those of a chondrodysplasia appearing as an excess of cartilage in the affected vertebrae.

Sternal Abnormalities

The most common sternal defect was "pectus excavatum" (PE); this is a very narrow chest, usually a funnel chest, with an upward deviation caudally. Sternal abnormalities were most severe in lambs having severe vertebral changes. Please see Figure 6.

Cranio-Facial Abnormalities

Twenty-four lambs had cranio-facial abnormalities. The heads of affected lambs, when viewed from the lateral aspect, appeared short and convex between the poll and nostrils. This was commonly referred to as a "Roman Nose". Several lambs had a marked curvature of the nasal septum and nares when viewed antero-dorsally (nasal septal deviation).

Radiographic Findings

Skeletal radiographic examinations (SRE) examinations were completed on 22 affected lambs and five normal (control lambs). The sternum and elbows were radiographed on 20 additional affected lambs. The remaining 23 affected lambs had one elbow radiographed (OEO).

A consistent radiographic pattern emerged in the

affected lambs with a variation observed in the degree of the involvement. The radiographic pattern observed was abnormal endochondral ossification. The elbows, sternum, and scapulas were the areas having the most abnormal endochondral ossification. Secondary ossification centers especially physes, subchondral areas, and cuboidal bones were frequently involved. As affected lambs matured, there was an increase in angular deformities of the long bones and lesions of degenerative joint disease occurred. See Figures 8,9,10

Lesions that were observed in all affected lambs (100%) were radiographic changes in the olecranons and anconeal processes of the ulna. This lesion was present in very young affected lambs without clinically visible deformities. In lateral radiographs of the olecranon there were multiple islands of ossification, without uniform direction of pattern. The sternum which is composed mostly of cartilage at birth, contained multiple irregular islands of bone and cartilage.

The scapulas were incompletely ossified in the supraglenoid region with stippled mottling of the cartilage components. As they matured multiple exostoses occurred along with lesions of degenerative joint disease developed.

The long bones of the limbs were typically longer with a narrower cortex than those of the controls. In different

joints, and with varying degrees of expression a number of changes occurred:

- 1.) loss of joint congruity,
- 2.) widened,
- 3.) flared,
- 4.) irregularly margined physes,
and
- 5.) angular deformities.

The cuboidal bones of the sternum and often the thoracic vertebrae had multiple islands or sites of bone deposition throughout the cartilage without uniform direction or pattern. Asymmetrical and abnormally shaped sternebrae and thoracic vertebrae, as well as, fractures of the lateral processes of the vertebrae and spontaneous rib fractures were also observed.

MICROSCOPIC FINDINGS

Hematoxylin and Eosin Staining (APT-7)

Light microscope examinations were done on the soft tissues of 40 lambs. The specimens were stained with hematoxylin and eosin. Soft tissue abnormalities were not observed. In lambs with a clinical reduction in muscle mass changes were not significant.

The physes of the long bones had defective endochondral ossification with marked variability. Clinically normal

appearing bones frequently had marked changes. Some bones having marked angular deformities had physes considered within normal limits.

Cells of proliferating and maturing cartilaginous zones were disorganized (Figures 18,19) Physes with mild lesions had more proliferating chondrocytes with less hypertrophic maturation and defective ossification due to cartilagenous retention. The most afflicted cartilagenous growth plates were especially disorganized in the proliferating chondrocyte regions. Chondrocytes failed to align properly to form normal appearing rows of chondrocytes.

In older afflicted lambs a degenerative arthropathy was usually evident. The microscopic appearance of the articular lesions varied in severity increasing with age. Earliest developing lesions included:

- 1.) focal chondromalacia with loss of chondrocytes in the superficial tangential layer,
- 2.) interrupted articular surfaces with cartilagenous erosions.

Articular surface erosions progressed to hemorrhagic areas. The hemorrhagic areas organized followed by developing granulation tissue. Subchondral bone thickened and became sclerotic.

SPECIAL STAINS

Alcian Blue Staining (APT-8)

The growth plates of control lambs had normal dye accumulation around the chondrocytes in the territorial matrix with decreasing intensity progressing toward the zone of maturation. The articular cartilage stained intensely blue inward from the articular surface to the zone of resting cartilage. The blue dye was distributed evenly around the chondrocytes in the territorial matrix. Staining intensity did not increase until approaching the maturing hypertrophic zone.

The growth plates of affected lambs varied in the alcian blue staining intensity in relation to the severity of the lesions. Growth plates from the least severely affected lambs were stained intensely blue. In the resting cartilagenous zone, the stain intensity was evenly distributed throughout the territorial and inter-territorial matrix. In the proliferating zone the stain intensity resembled that observed in the territorial region. The proliferating zone progressed into the disorganized maturing hypertrophic zone where it still retained the intensity of stain observed in the more normal territorial zone.

Where the articular cartilage had mild erosion defects, the intense staining extended from the superficial tangential zone, deep into the resting and proliferating zones. Areas with erosions were poorly stained, the

superficial chondrones lacked the intensity of staining observed in control lambs. Bordering the edge of large defects, the stain was light in intensity and evenly distributed in the territorial and inter-territorial matrix.

Epiphyseal plates adjacent to severely affected articular cartilage stained poorly with little retention of dyes. The disorganized hypertrophic zone likewise was stained poorly.

Gomori's One-Step Trichrome Staining (APT-10)

In the growth plates of control lambs, the blue-green dye readily stained the inter-territorial matrix of the resting cartilage while proliferating, maturing and calcifying zones were poorly stained. The growth plates of afflicted lambs retained very little stain giving it a light blue-gray appearance, with a margin of intense staining bordering the territorial matrix of chondrocytes in the hypertrophic and calcifying zones.

Articular cartilage of controls stained intensely in the inter-territorial matrix in the tangential and proliferating zones. Resting cartilage below the tangential zone was lightly stained. Staining intensity increased towards the proliferating zone. The hypertrophic and calcifying zones were very lightly stained while the territorial penetrations of the hypertrophic chondrocytes took on a gray-blue intensity. The articular cartilage of

afflicted animals retained the blue-green stain intensity in the tangential zone and inter-territorial matrix of the hypertrophic and calcifying zones. The resting and proliferating zones were lightly stained.

Lillie's allochrome staining (APT-11)

Growth plates of control lambs stained a deep purple, which progressed into the inter-territorial matrix and resting cartilage. The territorial matrix stained bluish-gray. The purple staining intensity decreased with proliferation and maturation of chondrocytes. Test lamb cartilages were stained more eosinophilic throughout the entire growth plate.

Articular cartilages of control lambs were lightly stained blue in the tangential, immediate resting zones and inter-territorial matrix. The deeper resting, proliferating, maturing, and calcifying zones were intensely stained purple. Articular cartilage of afflicted lambs stained blue from the tangential zone to the hypertrophic zones where staining intensity varied from light purple to eosinophilic appearance, similar to growth plates.

Azure - A - Eosinate Staining (APT-9)

Control lamb growth plates, articular cartilages and tangential zones were poorly stained. The remaining zones stained light purple to a pink intensity. Afflicted lamb articular cartilages and growth plates were stained a light

purple with some narrow irregular concentrations of the dye.

Flock Observations

Studies were permitted in several flocks. The findings suggested that the spider lamb syndrome is an inherited condition and was present only in Suffolk or Suffolk cross sheep.

Afflicted lambs have occurred in sets of twins with one lamb affected and the sibling normal. In herds practicing intensive inbreeding the incidence of spider lambs appeared to increase sharply. In flocks with multiple breeds of sheep, afflicted lambs were observed only in animals which were of Suffolk breeding. Parents of afflicted lambs were phenotypically normal suggesting a possible recessive genetic trait.

Controlled Breeding Trial

A flock of Suffolk sheep was established to conduct a breeding trial. Twenty-three phenotypically normal suffolk ewes were selected which had a history of birthing spider lambs. A Suffolk ram was selected from a line which had produced several confirmed spider lambs. The ram selected had never sired a spider lamb. The ram's dam and sire had produced spider lambs. The ram was allowed to run with the ewes for 60 days from August 1 to October 1, 1986. Twenty-one ewes lambled during January and February 1987. Thirty-four lambs were born of which 27 were normal and

seven were spider lambs confirmed in the KSUCDL. Using the Chi-square examination, the data above supports the theory that the spider lamb syndrome is inherited and follows a simple autosomal recessive pattern with a significance greater than $p=.01$. See Table 5.

DISCUSSION

During the past three years over 100 lambs with skeletal disease abnormalities have been necropsied which were brought to the KSUCDL. Some lambs came from major breeds other than Suffolk. A consistent pattern of skeletal abnormalities were observed in Suffolk and suffolk cross lambs. Skeletal abnormalities were also observed in other breeds; however, radiographic examination and morphologic studies have not revealed the same type of cartilage growth disorder observed in affected Suffolk lambs. This cartilage growth disorder has also been noted by several other research groups. 25,26,33a,33b,37,47,52,55-63,68-72,74-78 Several clinical reports from major universities discuss similar changes.

The following criteria has been established to properly diagnose a lamb with a skeletal abnormality as a spider lamb.

- 1.) Conformational abnormalities, narrow chest, shallow body, increased leg to body ratio;

abnormalities of the vertebrae, sternebrae, nose and legs.

- 2.) The demonstration of radiographic abnormal bone growth in one or more areas of the skeleton.
- 3.) The absence of soft tissue lesions.
- 4.) The presence of abnormal articular cartilage in lambs that are more than three months of age.

Not every cartilagenous and boney change observed in spider lambs is present in the individual lamb.

Skeletal deformities in non-Suffolk lambs may be confused with the Spider Lamb Syndrome. 14,15,24,65 Unique differences from other established skeletal disease syndromes are related to leg abnormalities. 11-13,17-19,24, 46,53,54,72,79 Spider lambs typically have an angular limb deformity with the carpus in, feet out and the tarsus in, feet out. A number of non-Suffolk and Suffolk lambs have been observed with marked backward flexure of the forelegs and forward overextension of the rear legs. This condition is referred to as arthrogryposis, a congenital defect characterized by muscle contracture and extension or flexion of the limbs. Many of these lambs have a very severe kyphoscoliosis involving the entire spinal column. On examination of the brains from lambs having arthrogryposis a severe hydrocephalus with little or no functional brain tissue (hydranencephaly) is usually evident. Arthrogryposis

is a different syndrome from the spider lamb syndrome.^{20-23, 30,34,51,64,82}

Clinically, spider lambs assume two distinct phenotypic developments. 1.) Lambs that are grossly abnormal at birth, and 2.) Grossly normal lambs at birth developing the spider conformation at 30 to 90 days. Radiographically, both groups have similar cartilage defects present at birth. This helps to substantiate a congenital rather than acquired defect and strengthens the hereditary theory.

The most consistent lesion observed in spider lambs appeared grossly to be the presence of excess amounts of cartilage. In many cases the cartilagenous change was accompanied by some bone development abnormalities and degenerative joint disease^{2,6,8,10,31,41,49,66.}

No structural differences in chondrocytes in affected and non-affected areas of cartilage was observed with the light microscope.^{4,39,40,48,80} The lack of orderly maturation of chondrocytes in the affected physes is not unique and may be due to one of several genetic abnormalities. Vanek et al⁷⁴ suggests that the fundamental disturbance in the Spider Lamb Syndrome is a delayed maturation of the growth plates. Wilsman et al⁸¹ believes there is a functional heterogeneity among the cells. This latter theory would explain the regional differences.

The gross and microscopic changes discussed in the

present study indicate that spider lamb syndrome is a type of chondrodysplasia,^{17,18,79} having many similarities to Marfan's syndrome in humans. Rapid growth seems to be correlated with some disorganization of the physes^{27,50}. A growth disturbance of this type could cause the changes seen in the spider lamb physes. Afflicted lambs appear to be longer, taller, with faster cartilage and bone development than occurs in normal Suffolk lambs. Growth plates of the more rapidly growing domestic pig compared to that of the feral pig⁹ have very similar developmental changes as those in spider lambs. The physes of rapidly growing broilers when compared to those of leghorns, which grow at a slower rate likewise have some similar changes.

The collagen stains used in this study suggest a possible collagen change. Troyer et al⁷¹ reporting on spider lamb tissues found no evidence of type III collagen in the cortical bone, and observed that the electrophoretic migration of alpha 1 and alpha 2 chains were normal. This finding does not rule out the possibility of abnormalities in cross-linking, which in some respects resembles Marfan's Syndrome or a collagen abnormality in the physeal cartilage rather than cortical bone. Troyer et al⁷¹ also found no ultrastructural differences in type II fibrils in the affected and non-affected physes. This finding would not rule out specific biochemical differences in synthesis

and/or post translational modifications. Troyer et al reported that there are a number of minor cartilage collagens including 1 alpha, 2 alpha, 3 alpha, type IX, and type X, and one of these could be structurally defective.^{3, 7, 16,19,29,32,35,38,42,44,45} They further noted that ruthenium red staining did not indicate major differences in distribution in affected and normal growth plates of connective tissue macromolecules such as the proteoglycans.²²

Similar phenotypic skeletal abnormalities including arachnodactyly and kyphoscoliosis are in spider lamb syndrome and marfan's syndrome in man. In Marfan's syndrome, cardiovascular and ocular abnormalities are present.^{1,36,73} The occurrence of associated non-skeletal features in the spider lamb syndrome did not include aortic aneurysm or ectopia lentis. However, there was a trunk ectomorphysm and muscle atrophy similar to the findings in Marfan's syndrome. Special stains used on spider lamb aortas did not detect abnormalities. The eyes of the spider lambs were considered normal.

Patients with the Marfan's syndrome have recently been reported to have a 20-25 amino acid insertion in the proalpha-2 chain near the collagenase cleavage site. Since the alpha-2-chain in Troyer et al⁷¹ studies migrated normally, it would appear to be a different mechanism than

the change observed in Marfan's syndrome. Type I collagen abnormality is frequently observed in the Marfan Syndrome.^{36,42} It is possible that in some instances the basic defect in these two species is similar in the discussed syndromes. The occurrence of angular limb abnormalities seen in the Spider Syndrome is a consequence of the fact that ambulation occurs much earlier in sheep than man. Some inherent weakness of the spider lamb affected growth plates could predispose it to structural change.

SUMMARY

Sixty-five lambs confirmed as spider lambs were morphologically evaluated and the findings compared to five normal control lambs. Primary abnormalities were confined to the muscular skeletal system and most were in the limbs. At present there is no official scientific name for this syndrome in lambs. Affected lambs are referred to as "spider lambs", "spiders", "monkey lambs", "crooked-legged", or "corkscrew lambs".

Clinically the afflicted lambs were within normal range for pulse, temperature, and respiratory rate. The most striking phenotypic changes were those of extremely tall, long-legged lambs when compared to normal flock mates and siblings of similar age.

On gross examination the angular limb deformities included medial displacement of carpus and tarsus with lateral displacement of the feet. Severity of limb deformities progressed with age. Kyphoscoliosis was observed in all afflicted lambs at necropsy, but was not always apparent on the live lambs, a prominent Roman nose or wry nose was principal finding in living lambs. The sternum was narrow with an upward deviation posteriorly (pectus excavatum).

Significant abnormalities were not observed on blood count and serum chemistries. Skeletal and muscle enzymes were on the high side of normal.

Radiographic evaluations were made. A consistent radiographic pattern emerged in afflicted lambs varying only in degrees of involvement.⁷⁸ Abnormal endochondral ossification was observed and was most consistently present in the elbows, especially the anconeal process, scapula and sternum. Separate unorganized islands of ossification in specific subchondral regions with loss of joint congruity, widened, flared, irregularly margined physes led to angular limb deformities.

On histopathological evaluation all soft tissues were essentially normal. Endochondral ossification was disrupted. There were regions in the affected physes lacking the distinctive progressive maturation appearance.

Excess cartilage was evident, most notably in the physes, cuboidal bones (sternebrae and vertebrae), the olecranon and anconeal processes of the elbow.

The spider lamb syndrome is believed to be a specific chondrodysplastic syndrome in sheep and current research suggests that it is due to a simple autosomal recessive mode of inheritance.^{5,59,67,78}

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DATA SUMMARIES

TABLE 1

CRANIAL FACIAL ABNORMALITIES									
CLINICAL AND GROSS NECROPSY EXAMINATIONS									
ABNORMALITY CLASSIFICATION	NEG	FN	1+	2+	3+	NSD	1+	2+	3+
LAMBS AFFLICTED	41	19	14	6	3	5	4	1	0
NOTES: 1. LIVE AND NECROPSY EXAMINATIONS WERE THE SAME 2. 19-ROMAN NOSE WITHOUT NASAL SEPTAL DEFECT 3. 1-NASAL SEPTAL DEFECT WITHOUT ROMAN NOSE 4. 4-WITH BOTH ROMAN NOSE AND NASAL SEPTAL DEFECT 5. THERE WAS EQUAL DISTRIBUTION AMONG AGE GROUPS									

STERNAL ABNORMALITIES				
CLINICAL EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1+	2+	3+
LAMBS AFFLICTED	40	12	9	4
NECROPSY EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1+	2+	3+
GROSS EXAMINATION	34	12	14	4
CUT SURFACE EXAMINATION	25	22	13	5

NOTES: 1. STERNAL ABNORMALITIES ARE MORE READILY VISUALIZED ON NECROPSY EXAMINATION 2. THERE WAS AN EQUAL DISTRIBUTION AMONG AGE GROUPS

DATA SUMMARIES

TABLE 2

VERTEBRAL ABNORMALITIES					
CLINICAL EXAMINATION					
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +	4 +
LAMBS AFFLICTED	28	13	12	8	4
NECROPSY EXAMINATION					
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +	4 +
GROSS EXAMINATION	0	21	12	16	7
CUT SURFACE EXAMINATION	2	22	22	14	5

NOTES:

1. NO SIGNIFICANT DIFFERENCE BETWEEN GROSS AND CUT SURFACE EXAMINATIONS
2. EQUAL DISTRIBUTION AMONG AGE GROUPS
3. VERTEBRAL ABNORMALITIES ARE MORE READILY VISUALIZED ON NECROPSY EXAMINATIONS (43% INCREASE OVER CLINICAL EXAMINATIONS)

DATA SUMMARIES

TABLE 3

BACKLIMB ABNORMALITIES				
CLINICAL EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +
LAMBS AFFLICTED	13	22	18	12
NECROPSY EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +
GROSS EXAMINATION	11	25	19	10
CUT SURFACE EXAMINATION	32	31	2	0

ABNORMALITY CLASSIFICATION--AGE DISTRIBUTION	
1 +	--18 OF 22 WERE OVER THREE WEEKS OF AGE
2 +	--A) 17 OF 18 WERE OVER 3 WEEKS OF AGE --B) 14 OF 18 WERE OVER 6 WEEKS OF AGE
3 +	--10 OF 12 WERE OVER 12 WEEKS OF AGE

NOTES:

1. NO DIFFERENCE BETWEEN CLINICAL AND NECROPSY EXAMINATIONS
2. LESIONS ARE HARDER TO IDENTIFY ON CUT SURFACE EXAMINATIONS
3. ANGULAR LIMB DEFORMITIES INCREASE IN SEVERITY AS LAMBS INCREASE IN SIZE AND AGE

DATA SUMMARIES

TABLE 4

FRONT LIMB ABNORMALITIES				
CLINICAL EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +
LAMBS AFFLICTED	2	17	19	27
NECROPSY EXAMINATION				
ABNORMALITY CLASSIFICATION	NEG	1 +	2 +	3 +
GROSS EXAMINATION	2	17	20	26
CUT SURFACE EXAMINATION	0	1	23	41

ABNORMALITY CLASSIFICATION---AGE DISTRIBUTION	
NEG	--THE ONLY NEGATIVES WERE UNDER 4 WEEKS OF AGE
1 +	--11 OF 17 WERE LESS THAN 7 WEEKS OF AGE
2 +	--9 OF 19 WERE OVER 7 WEEKS OF AGE
3 +	--17 OF 27 WERE OVER 17 WEEKS OF AGE --ONLY 3 OF 27 WERE UNDER 7 WEEKS OF AGE

NOTES:

1. SEVERITY OF FRONT LEG ABNORMALITIES INCREASES WITH AGE
2. THERE IS A DRAMATIC DIFFERENCE BETWEEN GROSS NECROPSY EXAMINATIONS AND CUT SURFACE EXAMINATIONS

DATA SUMMARIES

TABLE 5

SPIDER LAMB SYNDROME CONTROLLED BREEDING TRIAL		
ANIMALS USED IN BREEDING TRIAL		
1-RAM--REGISTERED SUFFOLK RAM FROM KNOWN CARRIER DAM AND SIRE		
23-EWES--REGISTERED SUFFOLK EWES WHICH HAD ALL PRODUCED SPIDER LAMBS-CONFIRMED BY THE KSUCDL		
NOTE: ALL ANIMALS USED IN THE BREEDING TRIAL WERE PHENOTYPICALLY NORMAL		
EWES BRED		23
EWES LAMBED		21
TOTAL LAMBS BORN		34
SPIDER LAMBS PRODUCED		7
NORMAL LAMBS PRODUCED		27
CHI-SQUARE EXAMINATION OF DATA		
	SPIDER LAMBS	NORMAL LAMBS
OBSERVED(O)	7	27
EXPECTED(E)	8.5	25.5
DIFFERENCE(D)	-1.5	1.5
DXD	2.25	2.25
DXD/E	0.26	.088
TABLE VALUE FOR P AT 0.01 LEVEL= 6.635--P=.352		

DATA SUMMARIES

TABLE 6

SPIDER LAMB ORIGINS	
NUMBER OF AFFLICTED LAMBS EXAMINED	6 5
NUMBER OF DIFFERENT SHEEP FLOCKS EXAMINED	2 1
NUMBER OF STATES FROM WHICH AFFLICTED LAMBS ORIGINATED	8

STATE	NUMBER OF LAMBS
MISSOURI	2 2
NEBRASKA	1 7
KANSAS	1 1
COLORADO	6
IOWA	4
WISCONSIN	2
WASHINGTON	2
ILLINOIS	1

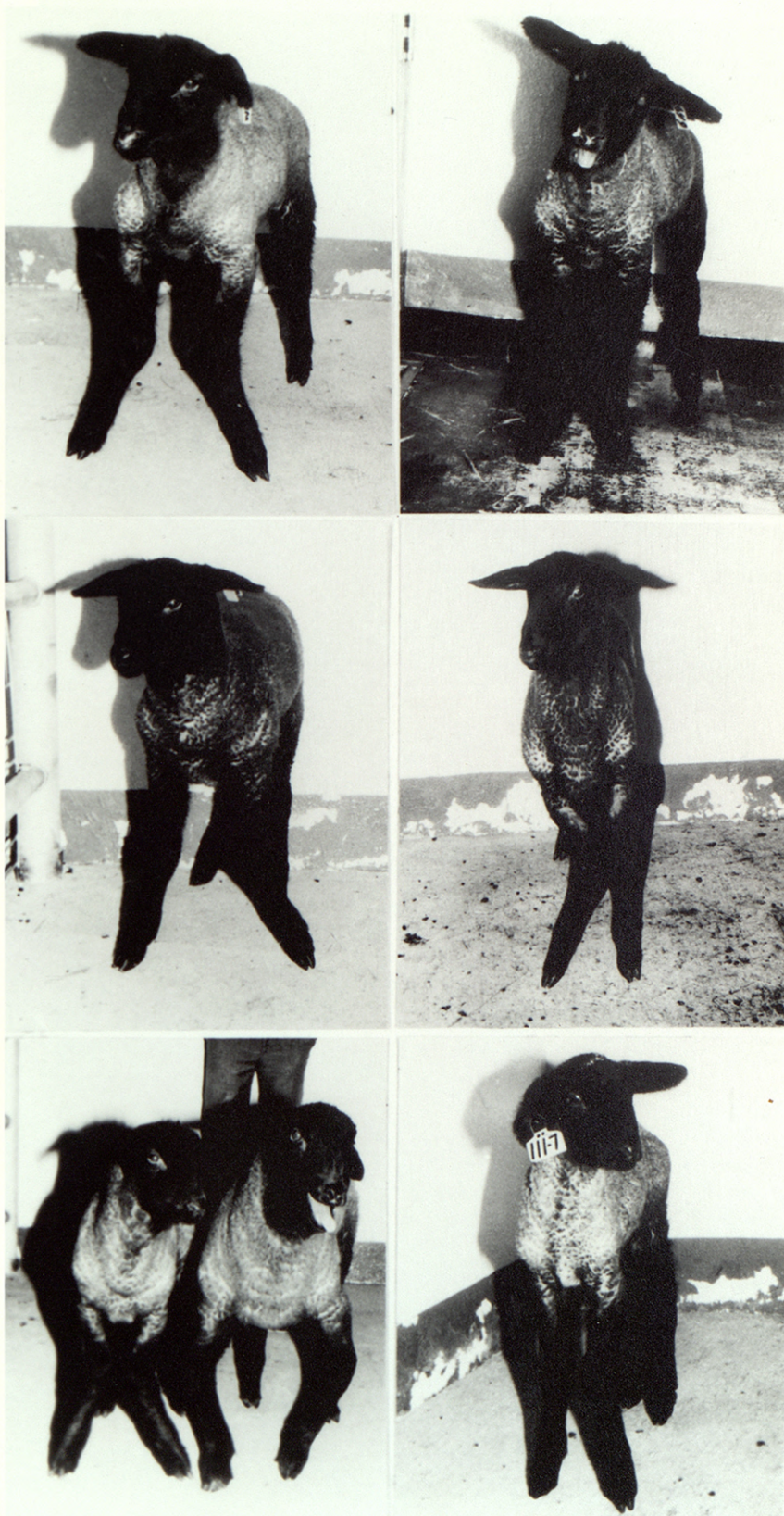
A	B
C	D
E	F

FIGURE-1: SPIDER LAMB SYNDROME

SPIDER LAMBS WITH VARYING DEGREES
OF FRONT LIMB ABNORMALITIES
(ALL ARE UNDER 4 WEEKS OF AGE)

- A. ABNORMAL ANGULATION OF THE CARPAL JOINT AND PHALANGES
- B. ABNORMAL ANGULATION OF THE SHOULDER JOINT WITH DECREASED ANGULATION DISTAL TO THE SHOULDER
- C. INCREASED ANGULATION OF THE LEFT CARPAL JOINT
- D. NARROW CHEST AND ABNORMAL ANGULATION OF THE FRONT LIMBS
- E. COMPARISON OF VALGUS AND VARUS CHANGES IN THE CARPAL JOINTS(ABNORMAL ANGULATION OF THE CARPAL JOINTS)
- F. NARROW CHEST AND ABNORMAL ANGULATION OF THE FRONT LIMBS

Figure 1



A	B
C	D
E	F

FIGURE-2: SPIDER LAMB SYNDROME

SPIDER LAMBS WITH VARYING DEGREES
OF VERTEBRAL AND REAR LIMB
INVOLVEMENT(ALL UNDER 4 WEEKS)

- A. KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- B. KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- C. KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- D. KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- E. ABNORMAL ANGULATION OF THE TARSAL
JOINTS AND PHALANGEA JOINTS
- F. ABNORMAL ANGULATION OF THE TARSAL
JOINTS AND PHALANGEA JOINTS

Figure 2



A	B
C	D
E	F

FIGURE-3: SPIDER LAMB SYNDROME

SPIDER LAMBS ILLUSTRATING VARYING DEGREES OF VERTEBRAL, FRONT LIMB AND CRANIAL FACIAL ABNORMALITIES (ALL ARE 5-8 WEEKS OF AGE)

- A. ABNORMAL ANGULATION OF THE CARPAL AND PHALANGEAL JOINTS
- B. ABNORMAL ANGULATION OF THE CARPAL AND PHALANGEAL JOINTS
- C. CRANIAL FACIAL DEVIATION
- D. KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- E. ABNORMAL ANGULATION OF THE SHOULDERS
KYPHOSCOLIOSIS-THORACIC VERTEBRAE
- F. ABNORMAL ANGULATION OF THE SHOULDERS

Figure 3

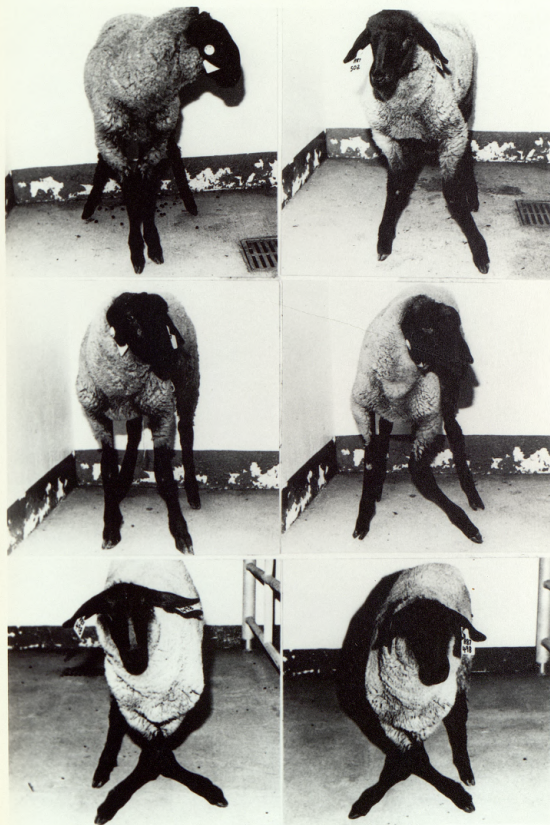


A	B
C	D
E	F

FIGURE-4: SPIDER LAMB SYNDROME
(OVER 4 MONTHS OF AGE)

- A. NARROW CHEST, ROMAN NOSE, ABNORMAL ANGULATION OF THE CARPAL JOINTS WITH A DECREASED ANGULATION OF THE FRONT LIMB DISTAL TO THE CARPAL JOINT
- B. FRONT LIMBS 2+ ABNORMALITY CLASSIFICATION
- C. FRONT LIMBS 1+ ABNORMALITY CLASSIFICATION
- D. FRONT LIMBS 3+ ABNORMALITY CLASSIFICATION
- E. FRONT LIMBS 4+ ABNORMALITY CLASSIFICATION
- F. FRONT LIMBS 4+ ABNORMALITY CLASSIFICATION

Figure 4



A	B
C	D
E	F

FIGURE-5: SPIDER LAMB SYNDROME

ALL LAMBS ARE SHOWING ABNORMAL
ANGULATION OF THE REAR LIMBS
(OVER 4 MONTHS OF AGE)

- A. REAR LIMB SHOWING A 1+ ABNORMALITY
- B. REAR LIMB SHOWING A 2+ ABNORMALITY
- C. REAR LIMB SHOWING A 2+ ABNORMALITY
- D. REAR LIMB SHOWING A 3+ ABNORMALITY
- E. REAR LIMB SHOWING A 4+ ABNORMALITY
- F. REAR LIMB SHOWING A 4+ ABNORMALITY

Figure 5



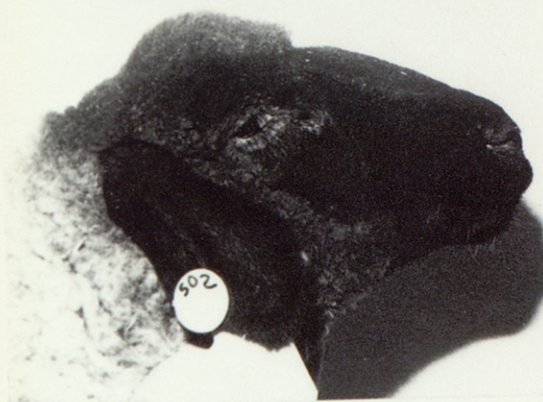
A	B
C	D
E	F

FIGURE-6: SPIDER LAMB SYNDROME

**LAMBS ILLUSTRATING VARYING DEGREES
OF CRANIAL FACIAL ABNORMALITIES**

- A.** NORMAL LAMB-NORMAL NOSE
- B.** SPIDER LAMB-NORMAL NOSE
- C.** SPIDER LAMB WITH A 1+ ROMAN NOSE
- D.** SPIDER LAMB WITH A 2+ ROMAN NOSE
- E.** SPIDER LAMB WITH A 3+ ROMAN NOSE
- F.** SPIDER LAMB WITH A 3+ ROMAN NOSE

Figure 6



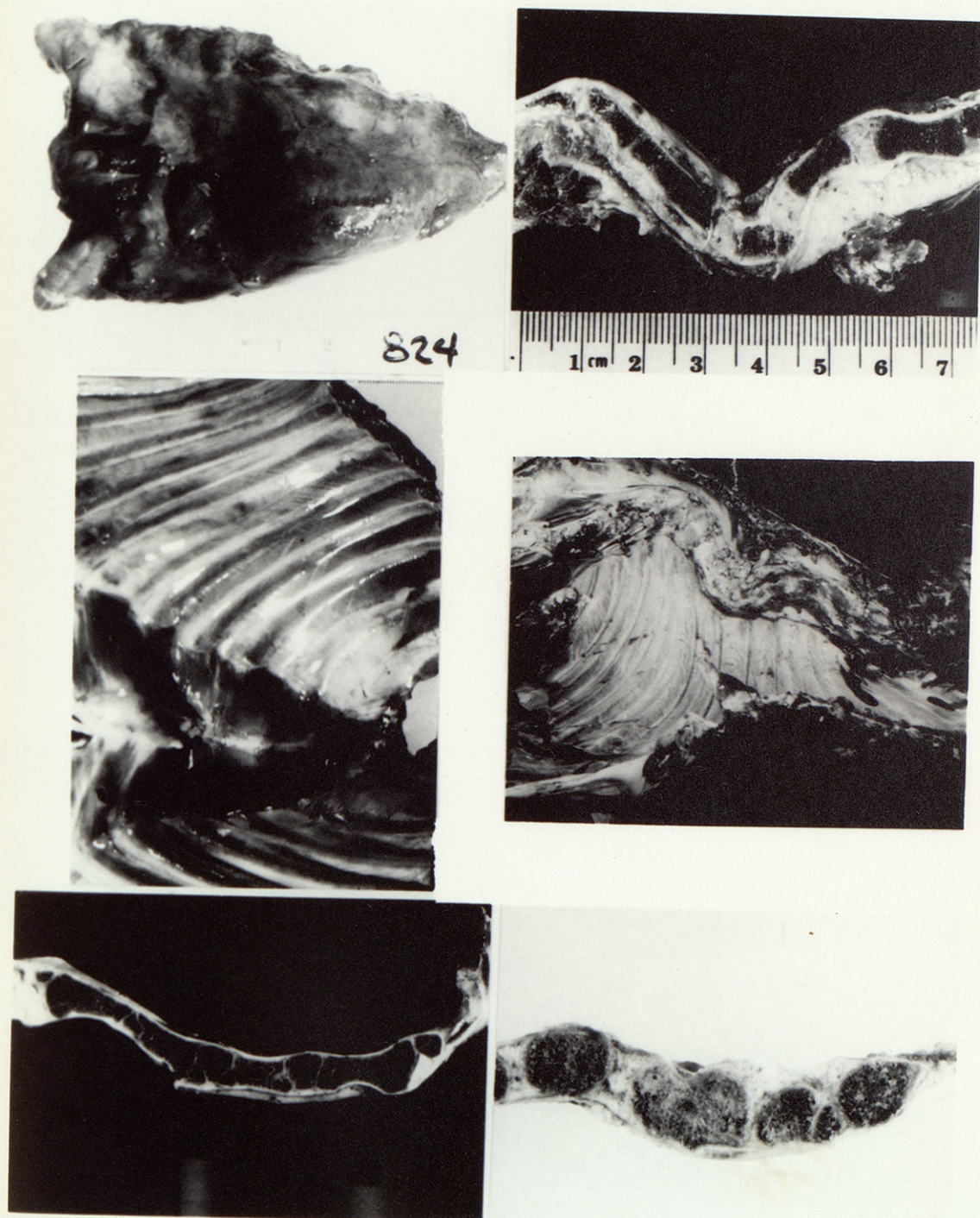
A	B
C	D
E	F

FIGURE-7: SPIDER LAMB SYNDROME

LAMBS ILLUSTRATING VARYING DEGREES
STERNAL AND VERTEBRAL INVOLVEMENT

- A. STERNUM-PECTUS EXCAVATUM
- B. STERNUM-PECTUS EXCAVATUM-CUT SURFACE
- C. STERNUM-PECTUS EXCAVATUM-WITH RIBS
- D. KYPHOSCOLIOSIS-THORACIC VERTEBRAE WITH RIBS
- E. STERNUM ILLUSTRATING IRREGULAR SIZE AND SHAPE
OF AFFECTED STERNEBRAE
- F. STERNUM ILLUSTRATING IRREGULAR SIZE AND SHAPE
OF AFFECTED STERNEBRAE

Figure 7

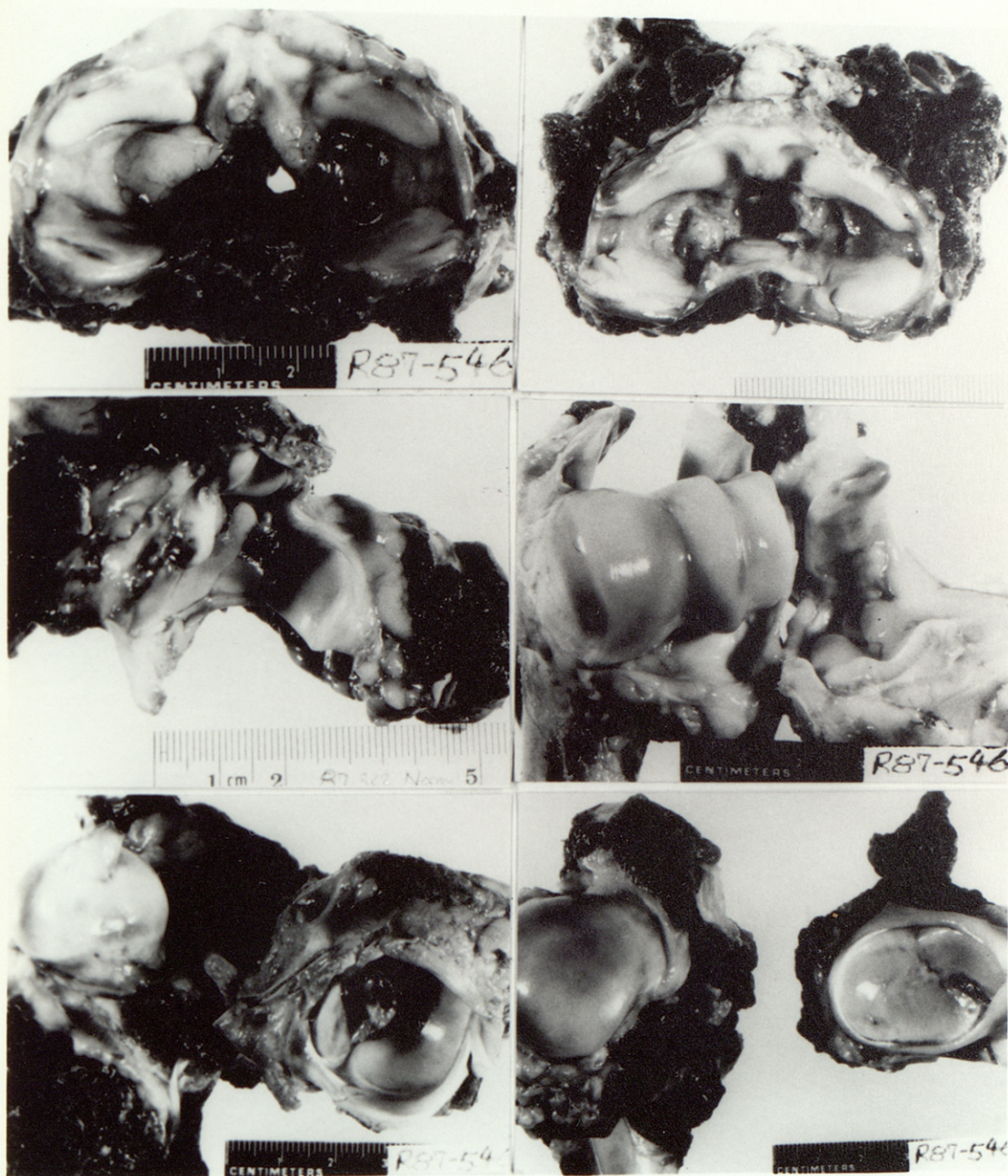


A	B
C	D
E	F

FIGURE-8: NORMAL CONTROL LAMBS

- A. ATLAS
- B. ATLAS
- C. ELBOW
- D. ELBOW
- E. SHOULDER
- F. SHOULDER

Figure 8



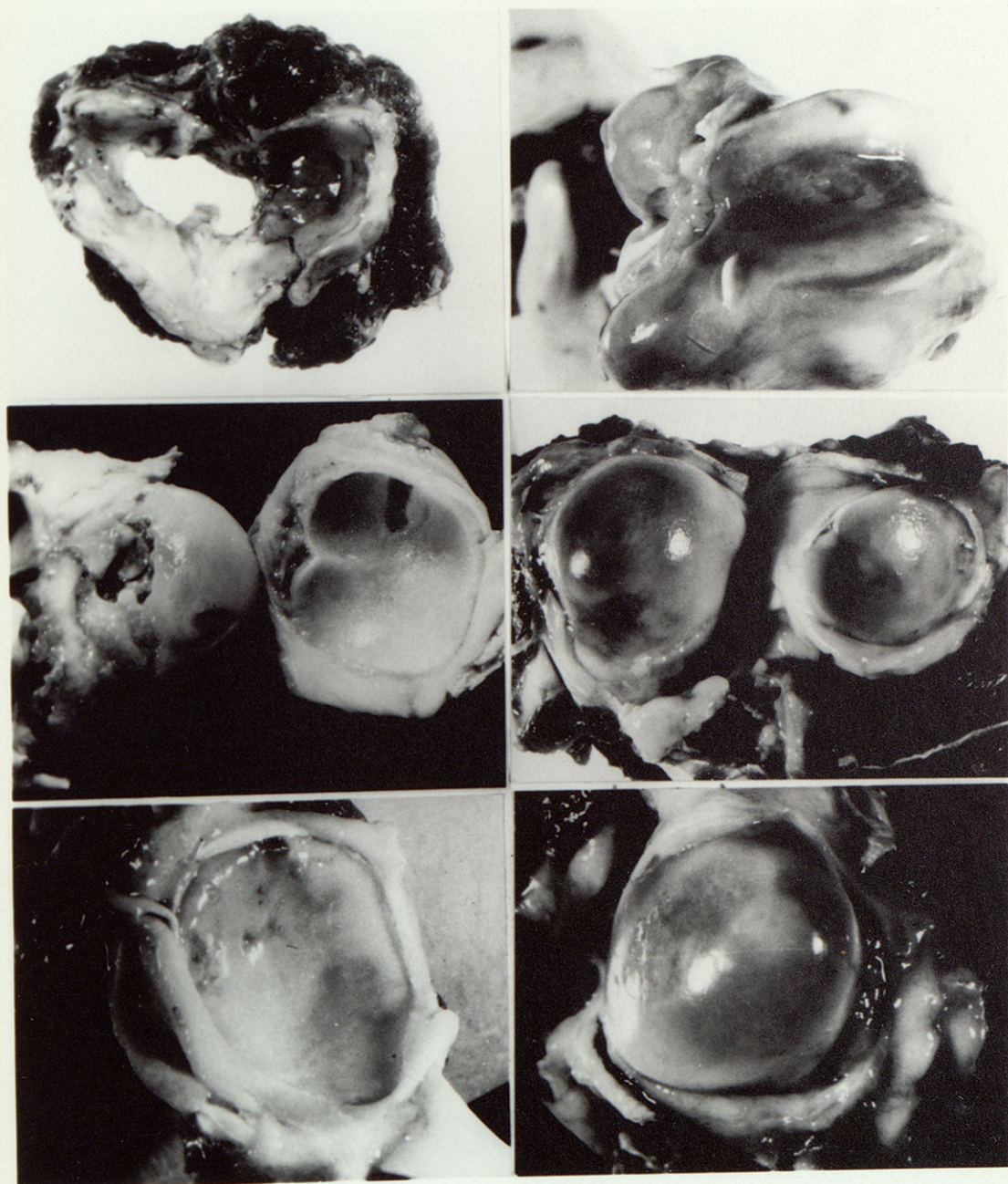
A	B
C	D
E	F

FIGURE-9: SPIDER LAMB SYNDROME

CARTILAGE EROSIONS

- A. ATLAS
- B. CARPUS-TROCHLEAR GROOVE ON DISTAL HUMERUS
- C. SHOULDER
- D. SHOULDER
- E. SHOULDER-SCAPULA
- F. SHOULDER-PROXIMAL HUMERUS

Figure 9



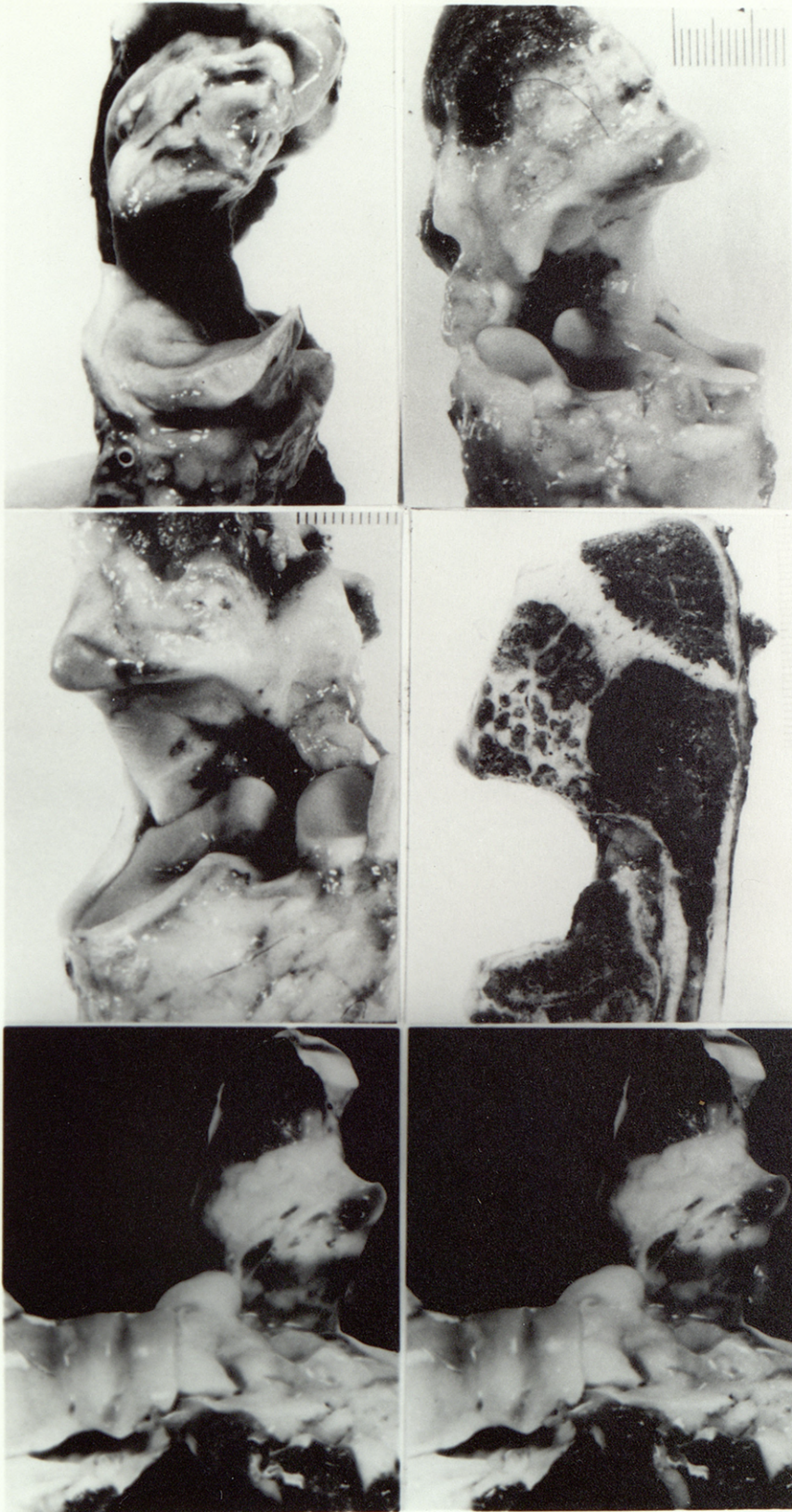
A	B
C	D
E	F

FIGURE-10: SPIDER LAMB SYNDROME

TIBIAL-HUMERAL ARTICULATION
ILLUSTRATING VARIOUS STAGES
OF DEGENERATIVE JOINT DISEASE

- A. PROXIMAL ULNA WITH CARTILAGE EROSIONS ON THE ARTICULAR SURFACE
- B. SEVERE CARTILAGE EROSIONS WITH EBURNATION
- C. PROXIMAL ULNA WITH CARTILAGE EROSIONS ON THE ARTICULAR SURFACE
- D. CUT SURFACE OF THE PROXIMAL ULNA WITH IRREGULAR ISLANDS OF OSSIFICATION OF THE CARTILAGE IN THE ANCONAL PROCESS
- E. DISTAL TROCHLEA OF THE HUMERUS AND ARTICULAR SURFACE OF THE PROXIMAL ULNA (RIGHT ELBOW)
- F. DISTAL TROCHLEA OF THE HUMERUS AND ARTICULAR SURFACE OF THE PROXIMAL ULNA (LEFT ELBOW)

Figure 10



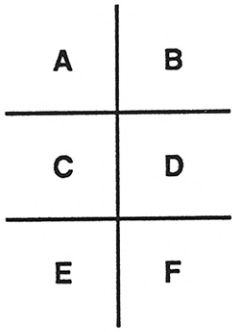
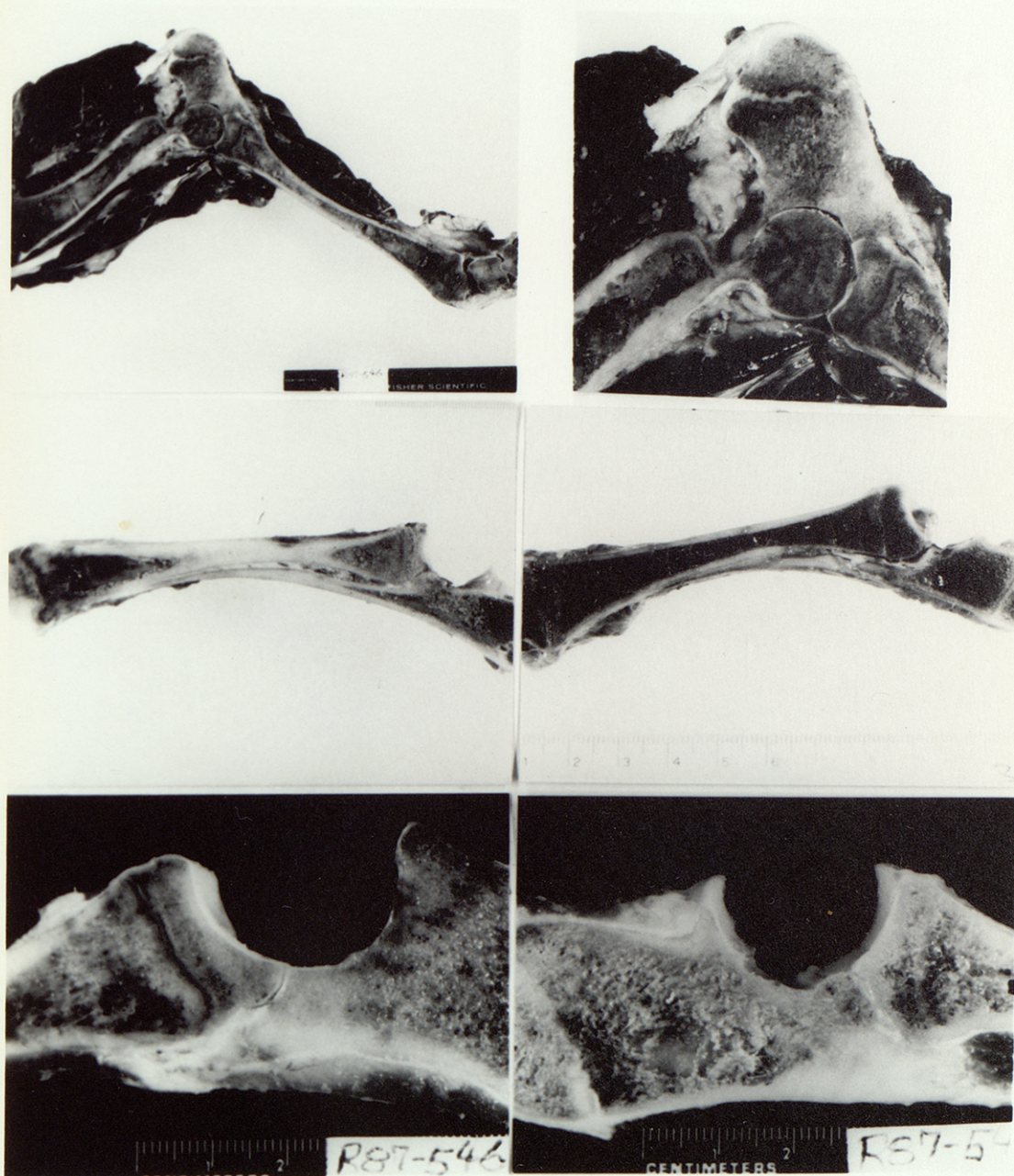


FIGURE-11: NORMAL LAMB
 CUT SURFACE

- A. HUMERUS, ULNA, CARPUS
- B. HUMERAL-ULNAR ARTICULATION
- C. RIGHT ULNA
- D. LEFT ULNA
- E. PROXIMAL ULNA AND ANCONEAL PROCESS
- F. PROXIMAL ULNA AND ANCONEAL PROCESS

Figure 11



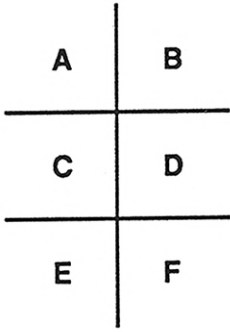
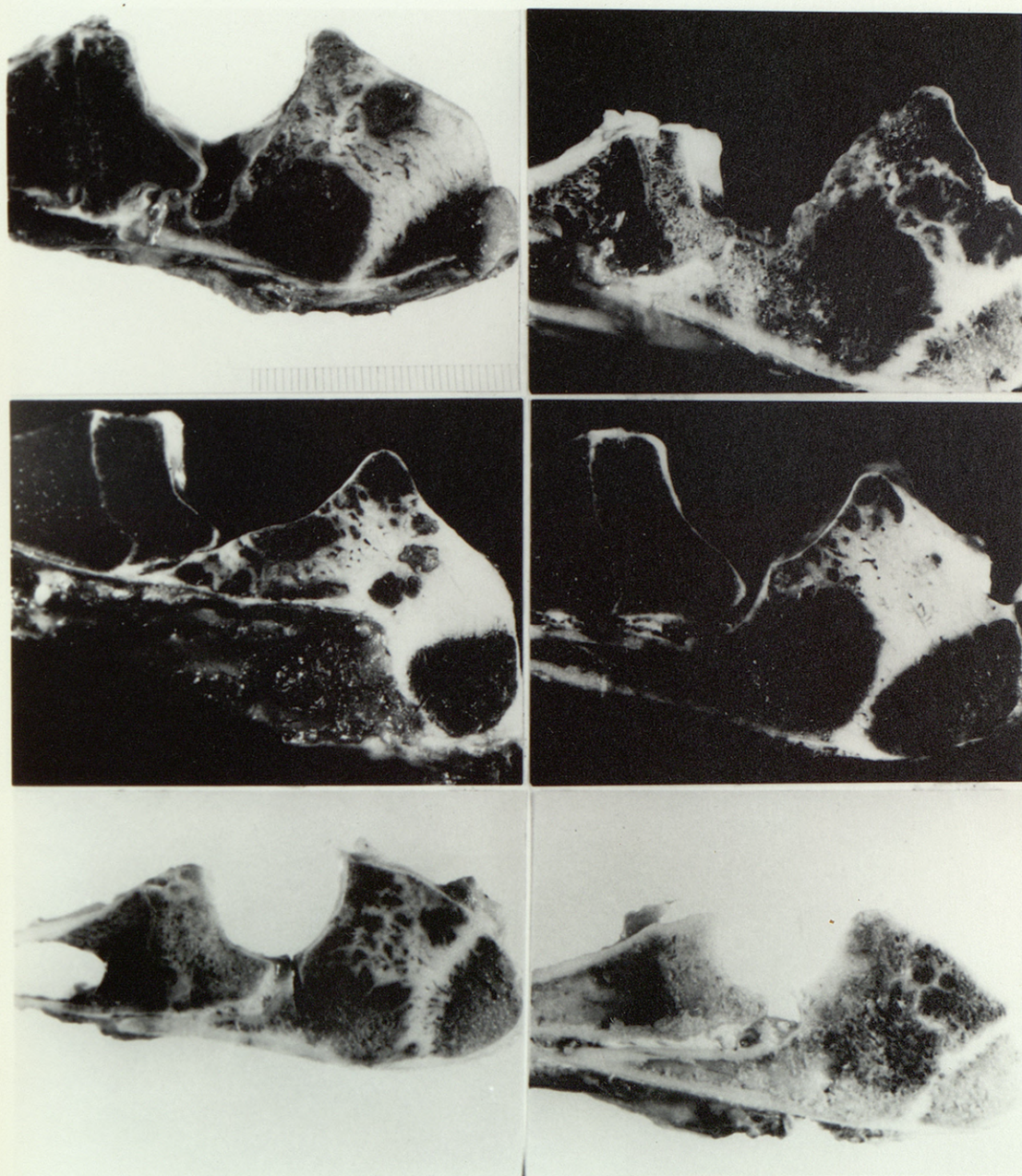


FIGURE-12: SPIDER LAMB SYNDROME

CUT SURFACE OF THE PROXIMAL ULNA
ANCONAL PROCESS

- A. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS
- B. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS
- C. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS
- D. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS
- E. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS
- F. IRREGULAR ISLANDS OF OSSIFICATIONS OF CARTILAGE IN THE ANCONAL PROCESS

Figure 12

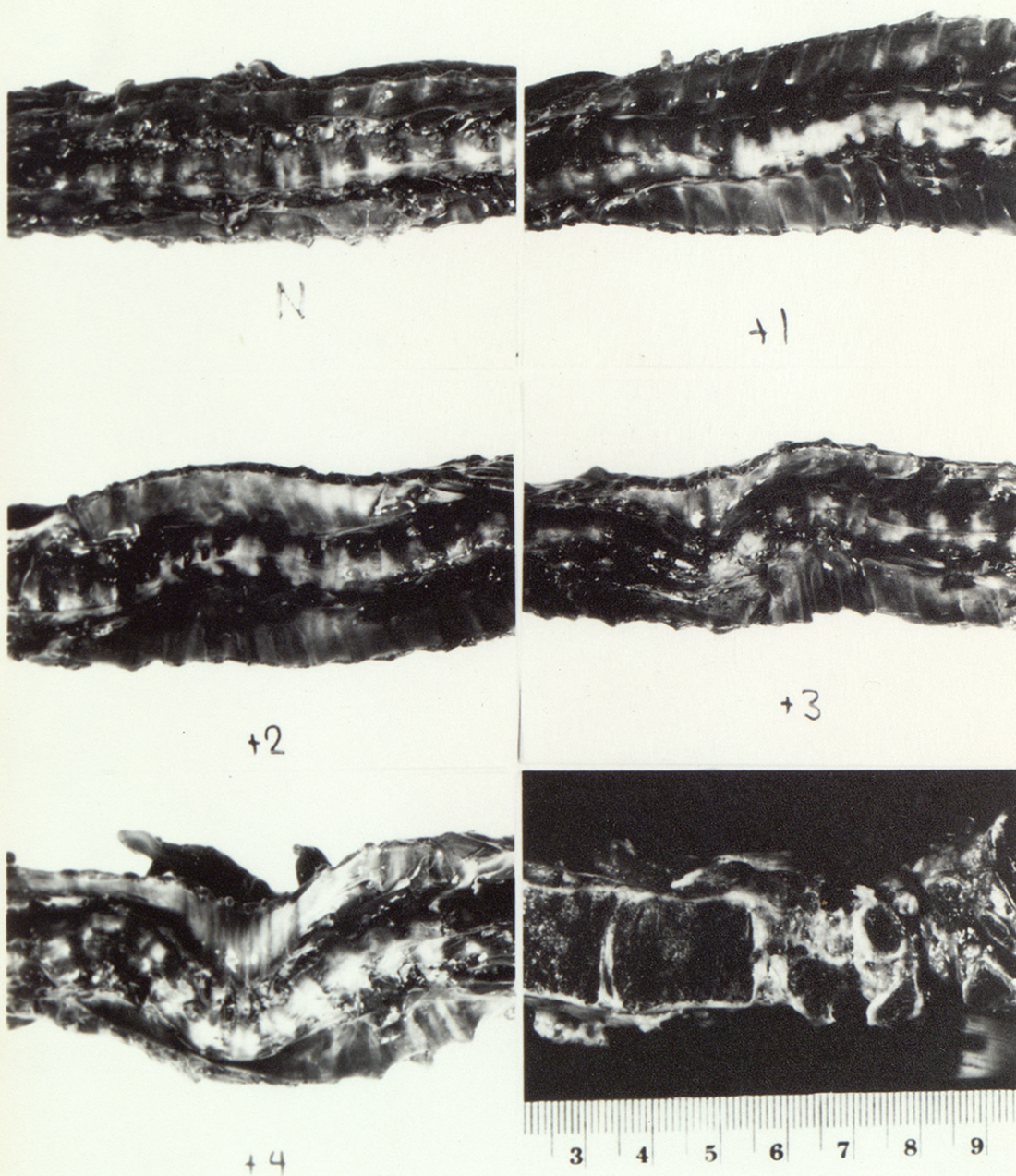


A	B
C	D
E	F

FIGURE-13: THORACIC VERTEBRAE

- A. NORMAL
- B. 1+ ABNORMALITY-SCOLIOSIS
- C. 2+ ABNORMALITY-KYPHOSCOLIOSIS
- D. 3+ ABNORMALITY-KYPHOSCOLIOSIS
- E. 4+ ABNORMALITY-KYPHOSCOLIOSIS
- F. CUT SURFACE-THORACIC VERTEBRAE WITH
IRREGULAR ISLANDS OF OSSIFICATION
AND COMPRESSED IRREGULAR VERTEBRAE

Figure 13

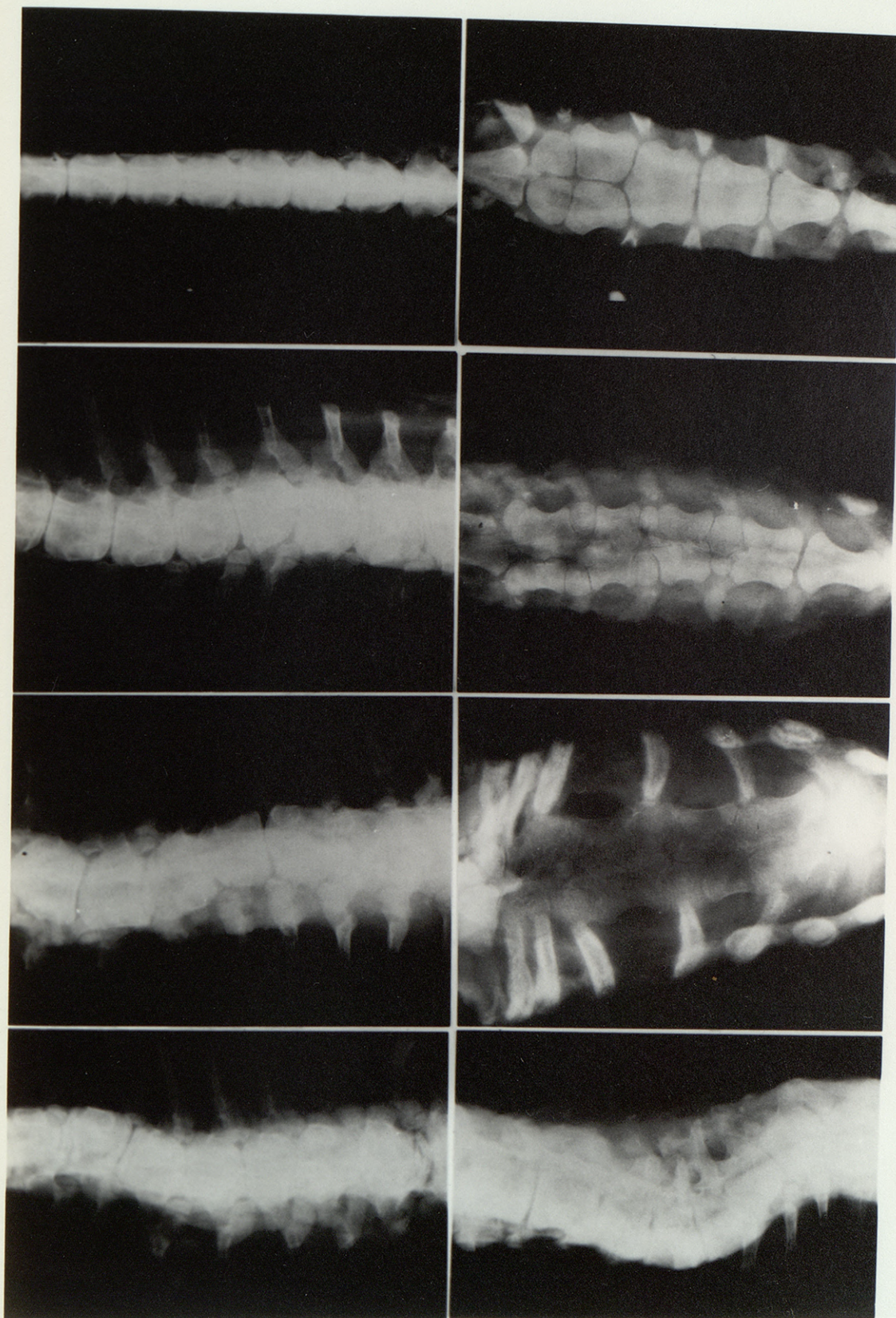


A	B
C	D
E	F
G	H

FIGURE-14: RADIOGRAPHS-THORACIC VERTEBRAE AND STERNEBRAE

- A. NORMAL THORACIC VERTEBRAE
- B. NORMAL STERNEBRAE
- C. 1+ THOACIC VERTEBRAE-KYPHOSIS
- D. IRREGULAR ISLANDS OF OSSIFICATIONS AND NON-SYMMETRICAL STERNEBRAE
- E. 2+ THORACIC VERTEBRAE-KYPHOSCOLIOSIS
IRREGULAR OSSIFICATION
- F. IRREGULAR ISLANDS OF OSSIFICATIONS AND NON-SYMMETRICAL STERNEBRAE
- G. 3+ THORACIC VERTEBRAE-KYPHOSCOLIOSIS
- H. 4+ THORACIC VERTEBRAE-KYPHOSCOLIOSIS

Figure 14

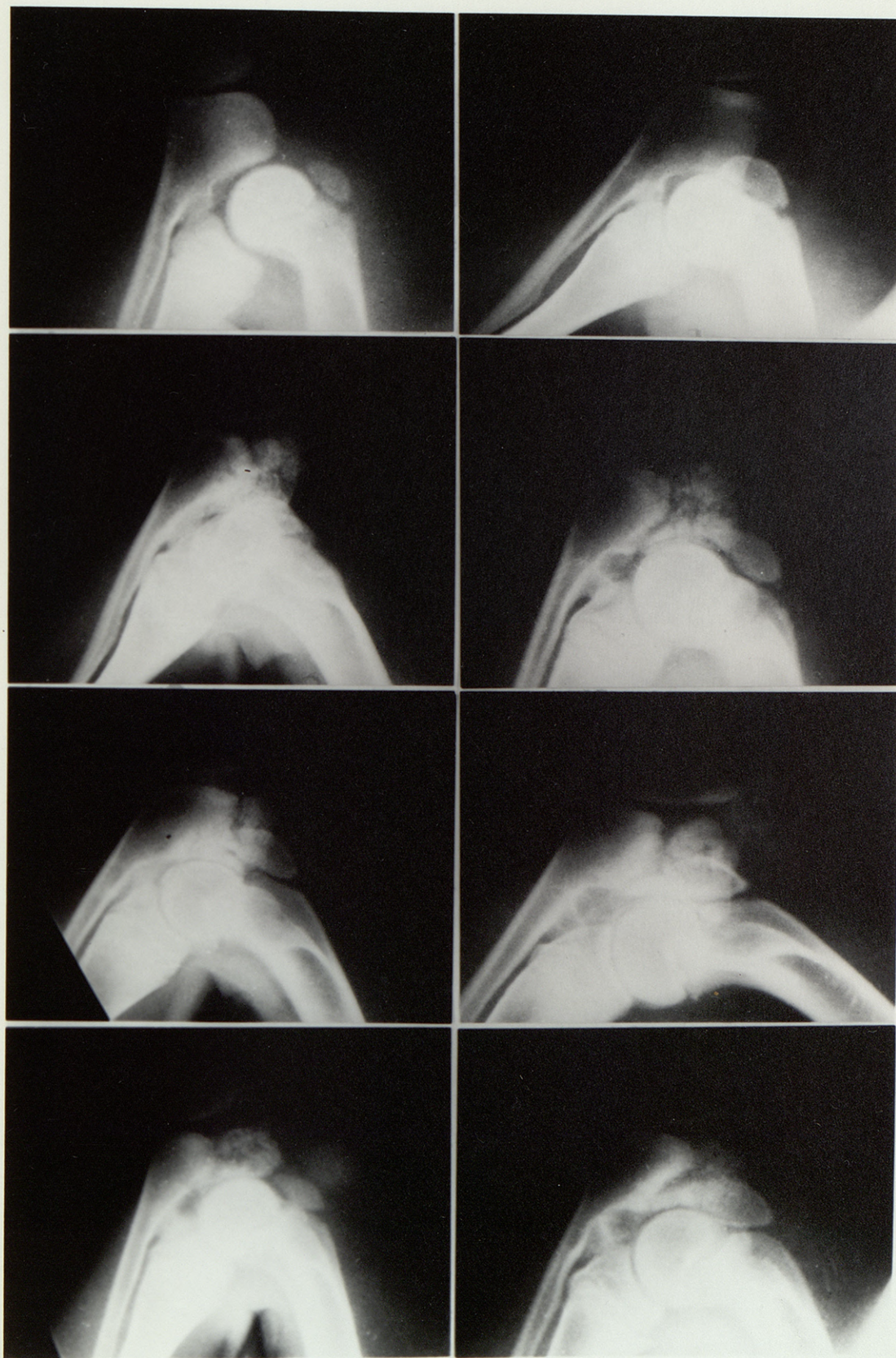


A	B
C	D
E	F
G	H

FIGURE-15: RADIOGRAPHS-ELBOW

- A. NORMAL-1 WEEK OF AGE
- B. NORMAL-9 WEEKS OF AGE
- C. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
1-WEEK OF AGE
- D. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
9-WEEKS OF AGE
- E. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
1-WEEK OF AGE
- F. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
9-WEEKS OF AGE
- G. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
1-WEEK OF AGE
- H. IRREGULAR OSSIFICATION OF THE ANCONIAL PROCESS
9-WEEKS OF AGE

Figure 15

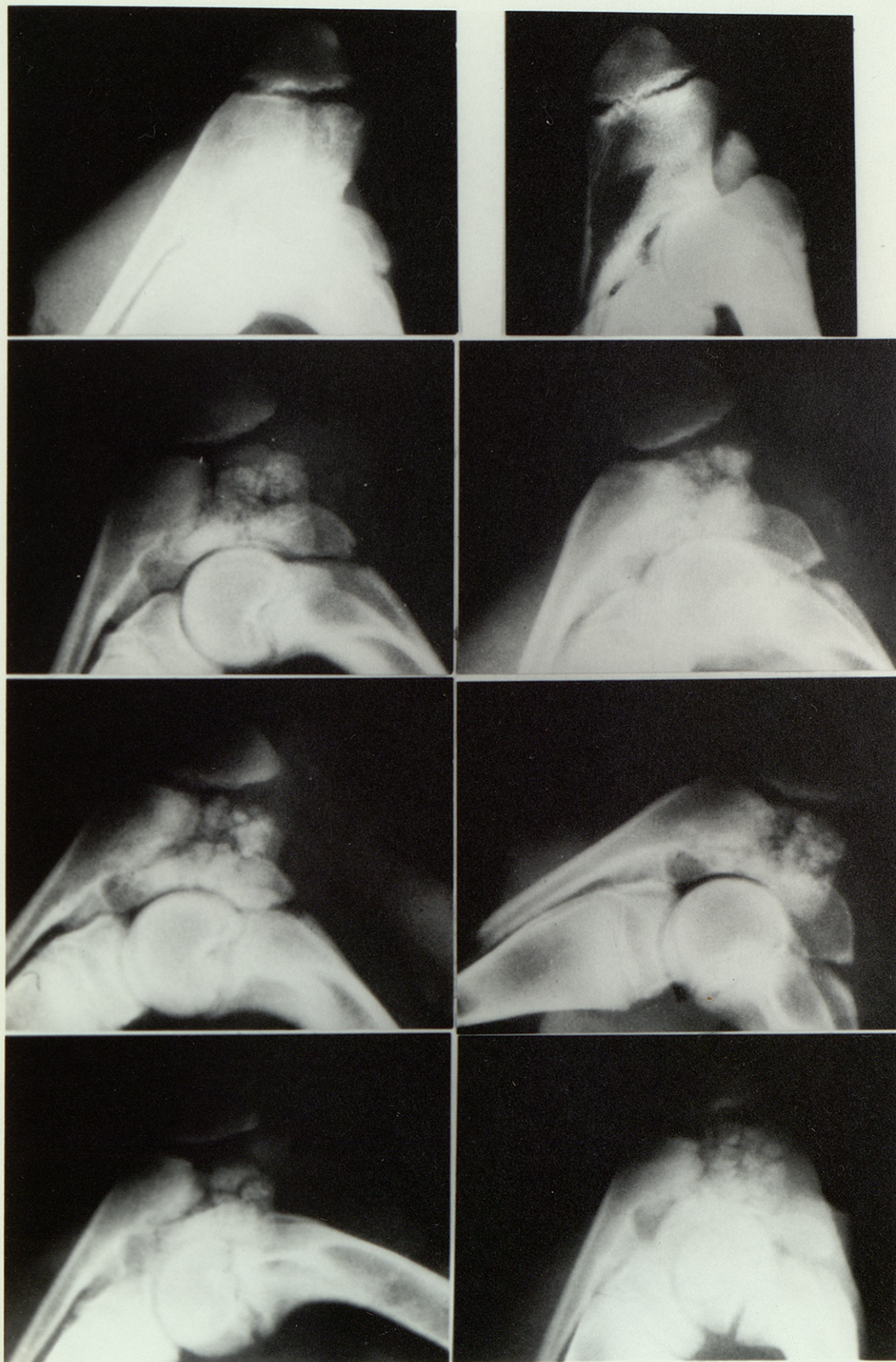


A	B
C	D
E	F
G	H

FIGURE-16: RADIOGRAPHS ELBOW

- A. NORMAL
18-WEEKS OF AGE
- B. NORMAL
36-WEEKS OF AGE
- C. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
18-WEEKS OF AGE
- D. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
36-WEEKS OF AGE
- E. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
18-WEEKS OF AGE
- F. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
36-WEEKS OF AGE
- G. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
18-WEEKS OF AGE
- H. IRREGULAR OSSIFICATION OF ANCONIAL PROCESS
36-WEEKS OF AGE

Figure 16



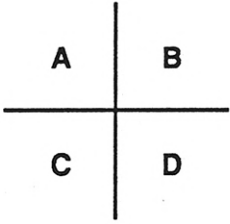


FIGURE-17: RADIOGRAPHS ELBOW

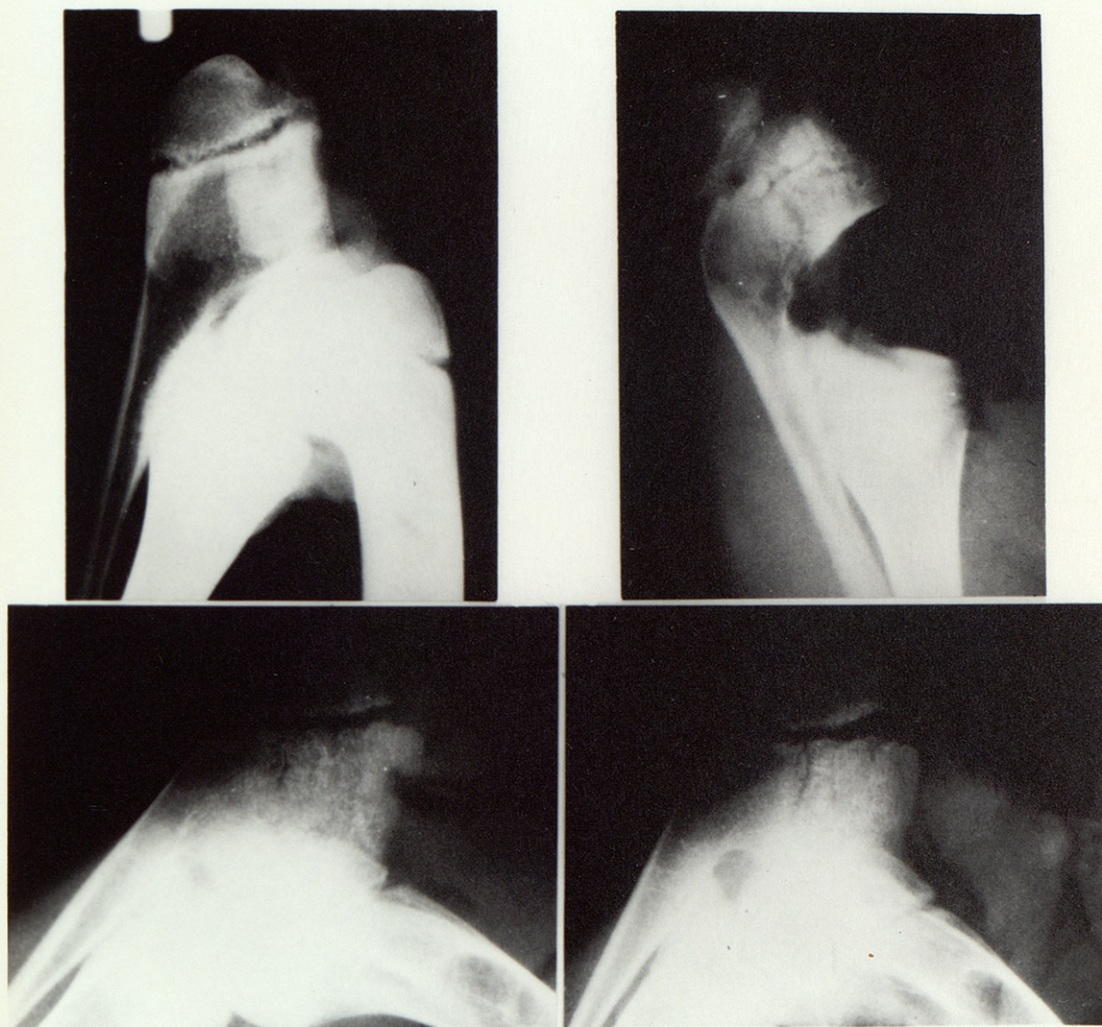
- A. NORMAL
54-WEEKS OF AGE

- B. IRREGULKAR ISLANDS OF OSSIFICATION
 OF THE ANCONEAL PROCESS
54-WEEKS OF AGE

- C. IRREGULKAR ISLANDS OF OSSIFICATION
 OF THE ANCONEAL PROCESS
54-WEEKS OF AGE

- D. IRREGULKAR ISLANDS OF OSSIFICATION
 OF THE ANCONEAL PROCESS
54-WEEKS OF AGE

Figure 17



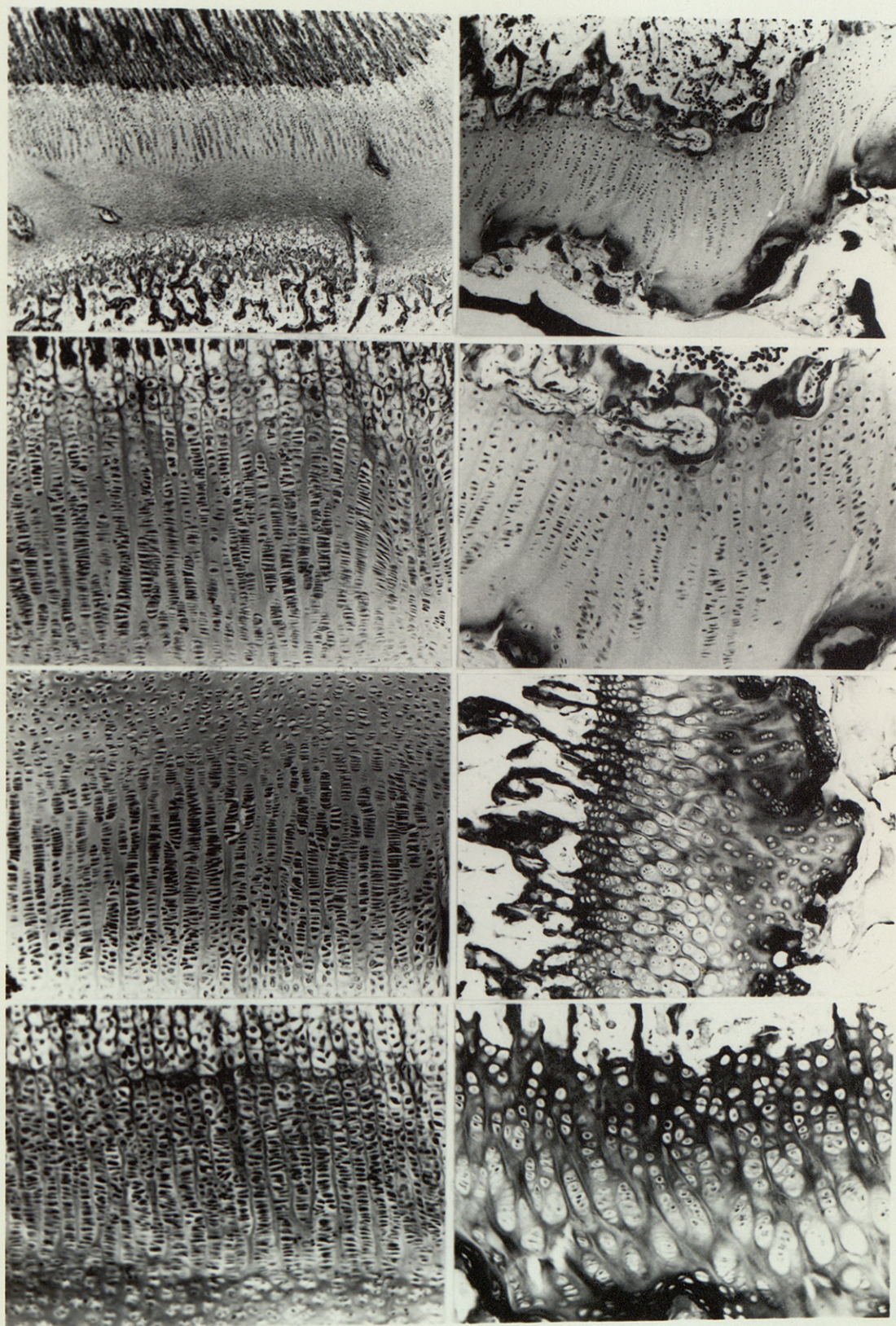
A	B
C	D
E	F
G	H

FIGURE-18: NORMAL CONTROLS AND SPIDER LAMB HISTOPATHOLOGY SLIDES OF THE DISTAL PHYSIS OF THE RADIUS

NOTE: THE REGULAR ORGANIZED APPEARANCE OF THE NORMAL CONTROL VERSUS THE IRREGULAR SEVERLY DISORGANIZED APPEARANCE OF THE SPIDER LAMB PHYSI

- A. NORMAL-LOW POWER
- B. SPIDER LAMB-LOW POWER
- C. NORMAL-HIGH POWER
- D. SPIDER LAMB-HIGH POWER
- E. NORMAL-HIGH POWER
- F. SPIDER LAMB-HIGH POWER
- G. NORMAL-HIGH POWER
- H. SPIDER LAMB-HIGH POWER

Figure 18

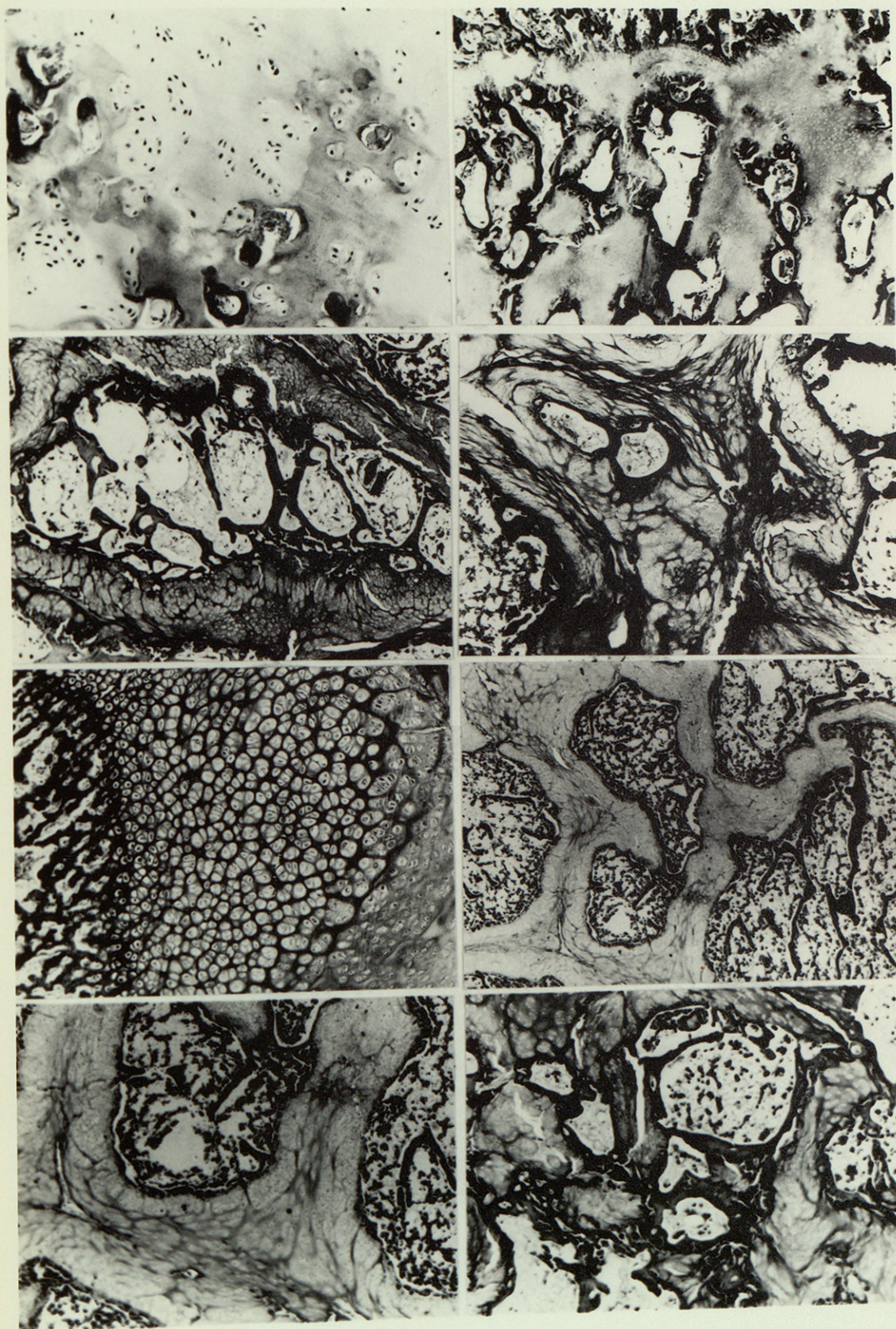


A	B
C	D
E	F
G	H

FIGURE-19: SPIDER LAMB HISTOPATHOLOGY SLIDES ILLUSTRATING IRREGULAR OSSIFICATION OF THE ANCONEAL PROCESS AT VARYING LEVELS OF MAGNIFICATION FROM LOW POWER TO HIGH POWER

- A. LOW POWER
- B. LOW POWER
- C. LOW POWER
- D. LOW POWER
- E. HIGH POWER
- F. HIGH POWER
- G. HIGH POWER
- H. HIGH POWER

Figure 19



Arthrogryposis-Hydranencephaly Syndrome
in Sheep

INTRODUCTION

Arthrogryposis is defined as persistent flexure or contracture of a joint.^{3,22,33,39,46} Arthrogryposis implies neither a pathologic entity nor a specific etiology.²⁹

Arthrogryposis-hydranencephaly (AGH) syndrome in lambs is characterized by congenital fixed flexion or extension of one or more joints of the extremities and hydranencephaly, associated with neurogenic skeletal muscle atrophy. It was documented in the literature by Ballantyne in 1904¹ and in cattle by Blood in 1956⁵.

Hartley and Kater¹⁹ reported AGH in New Zealand in lambs in 1962. Dennis reported AGH in a purebred flock of purebred Southdown flock in Western Australia in the 1963-1965 lambing season.⁹

Nobel et al³⁰ reported AGH in lambs, calves and goats in Israel during the 1969 and 1970 lambing season. In the early 1970s Hughes et al²³ reported AGH in a necropsy survey of 4,408 newly born dead lambs at the University of Melbourne. In 1971 Erchanbrack and Price¹⁷ reported AGH in USDA research flock of Rambouillet at Dubois, Idaho. Dennis further reported AGH in Western Australia in a perinatal lamb mortality study in 1974.¹² Also in 1974 Hartley and Haughey²¹ reported AGH in lambs in New South Wales. Later in 1974 Dennis published a summarized survey of congenital

defects indicating that AGH was one of the more common congenital defects reported in the literature.¹⁴

In 1975 Saperstein et al⁴⁰ reported AGH in a review of congenital defects in sheep. Inaba et al²⁴ in 1975 reported on an epizootic abortion, premature birth, stillbirths, and congenital AGH in cattle, goats and sheep in Japan caused by Akabane virus. In Australia, Parsonson³² confirmed Inaba's findings by experimental inoculation of Akabane virus in pregnant ewes.

In 1979 Dennis and Leipold¹⁶ published a review on congenital and inherited defects in sheep and stated that AGH is a common ovine congenital defect. Dennis and Leipold¹⁵ further reported in 1986 that the incidence of congenital and inherited defects in sheep ranged from 0.2 to 2.0% of all lambs born based on studies in Australia, New Zealand and the United States.

Congenital defects affecting the central nervous system and joints have been reported for bluetongue virus^{18,20,28,31,34,42,47}, bovine viral diarrhea virus^{2,45}, certain toxic chemicals or drugs^{41,43}, and certain toxic plants^{4,8,25,26,27}.

Researchers have confirmed Akabane virus as a cause of the AGH syndrome in Japan²⁴ and Australia³² but has not been reported in the United States. Recently Cache Valley

virus has been implicated in AGH outbreaks in the United States⁴⁹.

Following a request to producers and veterinary practitioners for lambs with musculo-skeletal defects, 110 phenotypically abnormal lambs were presented to the Congenital Defects Laboratory at Kansas State University (KSUCDL). These lambs were from eight states in the midwest.

Sixty-five of these lambs were confirmed as spider lambs, five were spider lamb suspects, 28 were AGH lambs and 12 were found not to fit either the spider lamb or AGH syndromes.

This report deals with the 28 lambs with arthrogryposis to provide a better description for the arthrogryposis-hydranencephaly syndrome.

MATERIALS AND METHODS

Experimental Animals

A total of 110 lambs were submitted to the KSUCDL as part of ongoing research on the spider lamb syndrome. Of the 110 lambs, 28 had arthrogryposis.

The 28 lambs with arthrogryposis were from 15 flocks. Six of the lambs were purebred Suffolk, 12 were Suffolk crosses, six were Rambouillet, four were Hampshire.

Lambs with arthrogryposis were all dead upon arrival at the KSUCDL. Histories provided indicate that four of the lambs were alive at birth but only survived for a few minutes.

NECROPSY-RADIOLOGY-HISTOPATHOLOGY

All lambs with arthrogryposis were examined and photographed prior to necropsy. Each lamb was evaluated grossly and 12 lambs radiographed (lateral of each detached limb, lateral of the trunk without legs, and dorsal-ventral of the thoracic vertebrae). Radiographs were taken of the entire skeletal system of several lambs.

Histopathology

Tissues for histopathological examination were taken from all lambs except those that had been frozen prior to submission. Tissues including central nervous system (entire brain and spinal cord), endocrine glands (adrenal, thyroid, and parathyroid in some when found), heart, skeletal muscle, spleen, lymph nodes, liver, kidney, lungs, rumen, reticulum, omasum, abomasum and small and large intestines. Specimens were also taken from articular cartilage and bones. All tissues except bone were fixed in 10% buffered neutral formalin (BNF) for at least 24 hours before trimming, processed routinely in an autotechnicon,

cut 6 microns thick, and stained with H&E. Bone specimens were placed in 10% BNF and fixed for 10 days before decalcification. The bone was washed in tap water for three hours, drained, and placed in 8% sulfasalicylic acid for 10 days or until decalcification was complete. The bone was processed routinely, cut 6 microns thick, and stained with H&E, alcian blue, azure A eosinate, Gomorri's one step trichrome, and Lillies allochrome. Similar procedures were performed on tissues from five control lambs.

VIRAL AND BACTERIAL CULTURES

Samples from 12 lambs representing three farms were submitted for viral isolation by the KSU Diagnostic Laboratory. Attempts were made to isolate the bovine viral diarrhea, bluetongue, Akabane and Cache Valley viruses.

Attempts were made to culture chlamydia, campylobacter, and other bacterial pathogens using routine laboratory techniques. The KSU Veterinary Diagnostic Laboratory carried out all the procedures for viral and bacterial isolations.

RESULTS

Soft Tissues and Skeletal Muscle

Upon gross examination all soft tissues appeared to be

within normal limits with exceptions of the skeletal muscle and central nervous systems. The skeletal muscle was reduced in mass, pale and often resembled fatty connective tissue in color and consistency. Microscopic examination of the skeletal muscle revealed very small and poorly developed myocytes and replacement of the myocytes with cells resembling lipocytes.

Central Nervous System

The cerebral hemispheres were almost completely replaced by fluid filled sacs (hydranencephaly) in all 28 lambs. The meninges were present and contained the cerebrospinal fluid. Figure 20 is a series of photographs of AGH lambs.

The anterior brain stem (basal ganglions, hippocampus, thalamic bulges and choroid plexuses) was readily recognizable upon gross and microscopic examination. The anterior brain stem was frequently reduced in mass when compared to the control lambs.

The cerebellum was normal upon gross and microscopic examination. In some cases the cerebellum was displaced caudally with mild coning of the vermis into the foramen magnum (Arnold-Chiari like deformity).

Spinal cord changes varied from severe hydromyelia to an absence of gross changes. The spinal cord varied greatly

in diameter depending on the severity of changes in the vertebrae. Vertebral alterations ranged from severe to essentially normal appearance.

Appendicular Skeletal System

The appendicular skeletal system was grossly altered in all 28 lambs. Front limbs were frequently crossed-over the sternum, or reflected straight back along the thorax, while the rear limbs were reflected over the abdomen or aligned straight forward along the abdomen and thorax.

The appendicular joints were aligned in distorted positions and the joints were arthrogryptic. The scapulo-humeral (shoulder), humero-radial (elbow), femoro-radial (knee), coxo-femoral (hip) and femoro-tibial (hock) joints were fixed in flexion and the phalanges were fixed in extension. Not all joints were arthrogryptic.

Axial Skeletal System

Axial skeletal changes noted were associated with the vertebral column. Torticollis was evident in all 28 lambs. Vertebral changes consisted of kyphosis, scoliosis, lordosis, torticollis or a combination of the above were observed in most afflicted lambs. Either alone or in combination kyphosis, scoliosis, and lordosis were found in 26 of the 28 lambs. Figure 20 is a collection of

photographs of lambs with AGH.

The most common changes in the skull was brachygnathia inferior. Brachygnathia inferior was present in 24 of the 28 lambs. Some of the lambs with hydrocephalus, porencephaly and hydranencephaly had an associated "domed-head" appearance.

Bacteriological and Viral Isolation Findings

Viral isolation on the 12 lambs submitted for bovine viral diarrhea, bluetongue, Akabane, and Cache Valley were negative. Attempts to isolate chlamydia, campylobacter, and other bacterial pathogens from tissues of the same 12 lambs were also negative.

Radiographic Findings

Detailed radiographic examinations were made on 12 affected lambs and five normal lambs. All radiographs appeared within normal limits other than the bony skeleton. Soft tissues were within normal limits. Some twisting of the arthrogryphotic joints that were grossly evaluated were also abnormal in the radiographs.

MICROSCOPIC FINDINGS:HEMATOXYLIN AND EOSIN STAINING

Under the light microscope the endocrine glands (adrenal, thyroid, and parathyroid when found), heart,

spleen, lymph nodes, liver, kidney, lungs, rumen, reticulum, omasum, abomasum and small and large intestines were within normal limits. Significant microscopic changes of the bone and articular cartilage were not found. Most tissues had autolytic changes including lighter staining of the tissues. All tissues studied tended to be lighter stained than those from control lambs which were euthanatized immediately before collection of the tissues.

Changes in the brain and spinal cord were consistent with the gross findings. Changes observed included mild hydrocephaly and/or porencephaly. Severe loss of neurons were in areas of hydranencephaly. The major finding in hydrancephaly was absence of tissue with the remaining tissues being normal with the exception of sparsity of neurons (neuronal aplasia).

Spinal cord changes ranged from normal to severe hydromyelia. Absence or greatly reduced numbers of neurons was a constant finding in the more severely affected lambs. The neurons that were observed were normal in size and in staining affinity.

Microscopic changes noted in the skeletal muscle included the following: very small and poorly developed myocytes (myocyte hypoplasia); nuclei were reduced in numbers but of normal size; cross striations were conspicuous by their absence; some myocytes contained

multiple, variable sized lipid-like vacuoles (fatty change) which resembled lipocytes; some areas of skeletal muscle were replaced by cells resembling fibroblasts with collagen deposition. The more severely affected animals had the most severe microscopic changes. See Figures 22 and 23.

HEMATOLOGICAL FINDINGS AND SERUM CHEMISTRIES

Hematological evaluations, and serum chemistries are not available since all arthrogryptic lambs had been dead for several hours when presented to the KSUCDL.

FLOCK HISTORIES AND OBSERVATIONS

In the flocks examined the problem occurred over a four to eight week period early in the lambing season followed by a short break in lambing, then ending with the lambing of normal lambs. Findings did not incriminate toxic plants or nutritional deficiency. No pathogens were isolated from samples that were submitted to the KSUVDL.

DISCUSSION

Based on the results of the present study and findings cited by other researchers, it is evident that several criteria must be met to classify lambs as having the o4?0 AGH syndrome: 1.) they must have a typical grotesque outward appearance with the limbs and vertebral column distorted and

immobilized; 2.) they must be some degree of hydrocephalus, porencephaly or hydranencephaly with the secondary muscular changes associated with the lack of innervation (hypoplasia of muscles). There can be a wide degree of variance in severity of lesions from animal to animal.

Hartley and Kater¹⁹ (1962) reported AGH in New Zealand in lambs. They based their report on 1,700 brains and 100 spinal cords. Their description of the arthrogrypotic lambs did not include gross or microscopic changes in the skeletal system or soft tissues.

Dennis¹⁰ (1972) reported AGH in a purebred Southdown flock in Western Australia in the 1963-1965 lambing season.

Nobel et al³⁰ reported AGH in lambs, calves and goats in Israel during the 1969 and 1970 lambing season. They reported on the pathological changes of the arthrogryposis-hydranencephaly syndrome encountered in 82 calves, 24 lambs and 10 kids. Their description of changes in skeletal muscle were similar to the findings in this study.

In the early 1970s Hughes et al²³ reported AGH in a necropsy survey of 4,408 newly born dead lambs at the University of Melbourne. They studied 79 flocks over two lambing seasons. Malformations considered sufficient to impair viability was observed in 80 lambs. Agnathia was

observed in 25 lambs and was the commonest congenital lethal malformation. Developmental defects of the muscular and skeletal systems, central nervous system, cardiovascular system and imperforate intestinal tract were the next most common malformations.

Dennis and Leipold¹¹ reported 74 of 401 malformed lambs examined during a 3-year period had agnathia. In 1971 Erchanbrack and Price¹⁷ reported AGH in an USDA research flock of Rambouillet at Dubois, Idaho. Over a 15 year period, 21,031 Rambouillet lambs. Thirty-six congenital defects were detected.

Dennis reported AGH in Western Australia in a perinatal lamb mortality study in 1974.¹²

In New South Wales (1974) Hartley and Haughey²¹ reported 20 cases of micrencephaly in flocks of Merino and crossbred ewes. The micrencephaly was associated with hydrocephalus, cavitation of the central white matter of the cerebral hemispheres, arthrogryposis and cerebella agenesis. In one flock five of 12 dams of affected lambs had mucosal disease antibodies. The mucosal disease virus was thought to be the cause of the arthrogryposis.

Later in 1974 Dennis published a survey of congenital defects of sheep in Australia and indicated that arthrogryposis was the 14th most common congenital defects reported in the literature.¹³ In the same year Dennis o4?0

reported the results of a questionnaire and examination of dead lambs and placentae submitted by sheep farmers during the 1963-1965 lambing season and estimated the rate of perinatal lamb losses was 15%. Common congenital defects were agnathia, atresia ani, cardiac defects, micrognathia, arthrogryposis, hernias, embryonic duplications, limb defects, hypospadias, hydrocephalus, meningocoel and perosomus elumbis.

In 1975 Saperstein et al⁴⁰ reported AGH in a review of congenital defects in sheep.

Inaba et al²⁴ in 1975 reported on an epizootic abortion, premature birth, stillbirths, and congenital AGH in cattle, goats and sheep in Japan caused by Akabane virus. No specific description of the disease in sheep was discussed. Vertical infection was demonstrated in the experimental infections of pregnant cows, sheep and goats with the OBE-1 strain of Akabane virus isolated from the fetus of a sentinel pregnant cow, and AH was experimentally produced in a calf and a kid. The results of the experimental infection in sheep were not included in the article.

In Australia, Parsonson³² (1975) confirmed Inaba's findings by experimental inoculation of Akabane virus in pregnant ewes. Congenitally malformed lambs were only obtained from four of a group of 13 ewes inoculated between

30 and 36 days of gestation. The four ewes that gave birth to abnormal lambs had twins. One lamb of each set of twins was abnormal, and the four abnormal lambs two were still born and two were alive. The two dead fetuses had AG/HE, with scoliosis, kyphosis, and brachygnathia, and removal of the calvaria revealed a lack of the main areas of the brain with the resulting space being filled with a blood stained liquid. One live lamb had a small head, showed slight incoordination and at autopsy was found to be micrencephalic, the other lamb was found at autopsy to have porencephaly. One picture of an arthrogryptic lamb was shown. The findings of gross lesions in their study was similar to the AGH lambs in the present study. Researchers have confirmed Akabane virus as a cause of the AGH syndrome in Japan and Australia but has not been reported in the United States.

In 1979 Dennis and Leipold¹⁶ published a review article on congenital and inherited defects in sheep and stated that arthrogryposis is a common ovine congenital defect. They further stated that it is a syndrome of diverse etiology; recessive¹⁷, viral,^{21,24,32} phytoterato gen²⁵ and unknown^{13,23}

The specific descriptions for the changes seen with bluetongue virus,^{7,18,31,42,47} bovine viral diarrhea virus,⁴⁵ certain toxic chemicals or drugs (parben

dazole),^{41,43} and certain toxic plants⁴ do not fit the definition of AGH. Some necropsy findings fit part of the AGH syndrome in that arthrogryposis may be found but that hydranencephaly is absent in the above viral or toxin induced diseases.

Recently Cache Valley virus has been implicated in AGH outbreaks in the United States.^{37,38,49} The description of the changes seen with the Cache Valley virus fit the AGH lesions in the current study very well.

Rook et al at Michigan State University conducted a lamb neonatal mortality study in 1986 and stated that AGH and hydranencephaly were the most common congenital defects seen along with the spider lamb syndrome.³⁵⁻³⁷ Rook also mentioned that there appeared to be no sex or breed predisposition.

Several AGH lambs examined came from Nebraska near the USDA Meat and Animal Research Center (MARC) at Clay Center, Nebraska.⁴⁴ An outbreak of the AGH syndrome in a group of early lambing ewes had been reported earlier from this immediate area⁴⁸ Discussions with Dr. Ross³⁸ at MARC indicated that they had not isolated a causal agent in AGH lambs born at the MARC. Their studies included serum samples from several ewes from the early lambing flock in which they observed AGH lambs. All ewes producing AGH lambs had positive titers for the Cache Valley virus. Similar

titer findings have been reported by Dr. E. Colli son and Shang-Ing Chuy at Texas A & M University⁶ and by Dr. Rook at Michigan State University^{35,37}. A paper was presented at the 1987 World Veterinary Congress in Montreal by Doctors John Edwards, Charles Livingstone and Robert Crandell incriminating the Cache Valley virus in an outbreak of AGH in the research flock at San Angelo, Texas.⁴⁹ Serologies from the flocks that were involved in the present study were not available for similar studies.

In the present study the etiological cause of the cases presented was not established. Histories made available suggested toxic plants or products were not causal factors. Attempts to isolate a causal viral or bacterial agent were not productive. Histories provided indicated that the AGH lambs appeared in the early lambing flocks where ewes were bred before the first killing frost. These findings suggest a possible insect-borne agent, similar to Akabane virus. No causal agent was established during the present study. The Cache Valley virus is a likely candidate but was not isolated in this study.

SUMMARY

R Data was collected on 28 AGH lambs and compared to five normal lambs. All AGH lambs were born with limb and brain deformities. Most were stillborn.

On gross examination all appendicular joints appeared fixed, (fused or unmovable) with most being fixed in a flexed position while the phalanges were fixed in an extended position. The vertebral columns were often severely twisted with kyphoscoliosis. Brachygnathia inferior was frequently present. When the cranium was opened either hydrocephalus, porencephaly or hydranencephaly was evident and in some there was very little cerebrum present. The brain stem and cerebellum were easily identified. The spinal cords had variable changes ranging from slight to an extensive reduction in diameter with mild to severe hydromyelia.

The syndrome was defined and differentiated from the spider lamb syndrome. The cause of the AGH was not established.

Complete flock histories were provided with cases submitted. Toxic plants and/or other toxin products were not incriminated. Based upon weather bureau data for the states concerned, no lambs were born from ewes bred after the first killing frost. This would suggest an arthropod borne viral disease such as Cache Valley virus could be a factor in the AGH syndrome.

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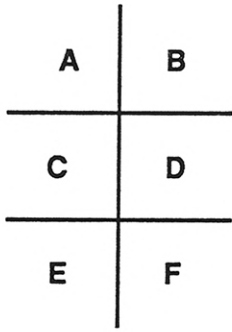
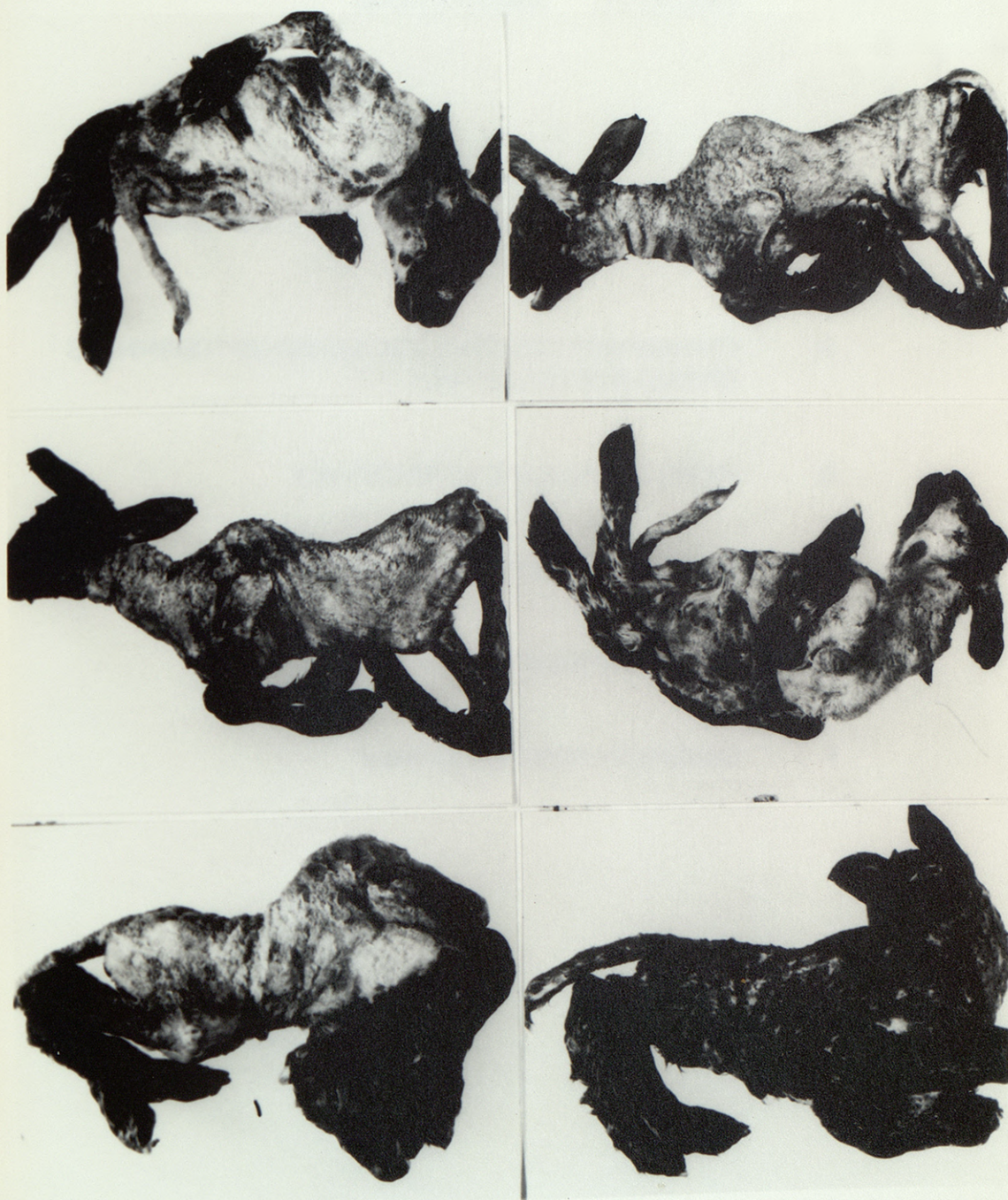


FIGURE-20: AGH-SYNDROME

ARTHROGRYPOSIS LAMBS

- A. CROSSBRED LAMB
- B. CROSSBRED LAMB
- C. HAMPSHIRE LAMB
- D. CROSSBRED LAMB
- E. CROSSBRED LAMB
- F. SUFFOLK LAMB

Figure 20

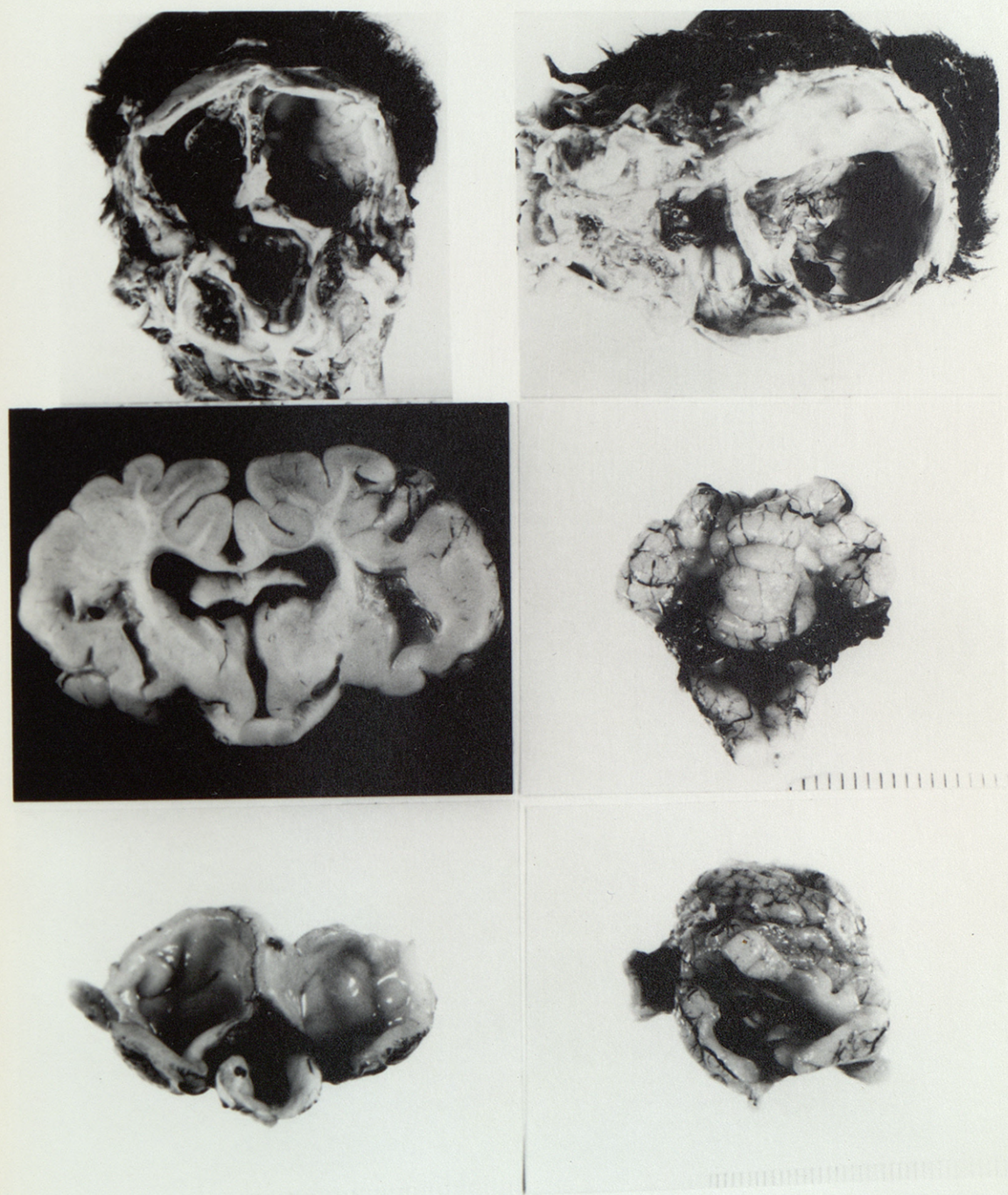


A	B
C	D
E	F

FIGURE-21: AGH-SYNDROME

- A.** CRANIUM WITH CEREBRAL CORTEX AND LEPTOMENINGES
SEVERE HYDRANENCEPHALY
- B.** CRANIUM WITH CEREBRAL CORTEX AND LEPTOMENINGES
SEVERE HYDRANENCEPHALY
- C.** HYDROCEPHALUS AND PORENCEPHALY
- D.** CEREBELLUM-HYDRANENCEPHALY
- E.** CEREBRUM-HYDRANENCEPHALY
- F.** CEREBRUM-HYDRANENCEPHALY

Figure 21

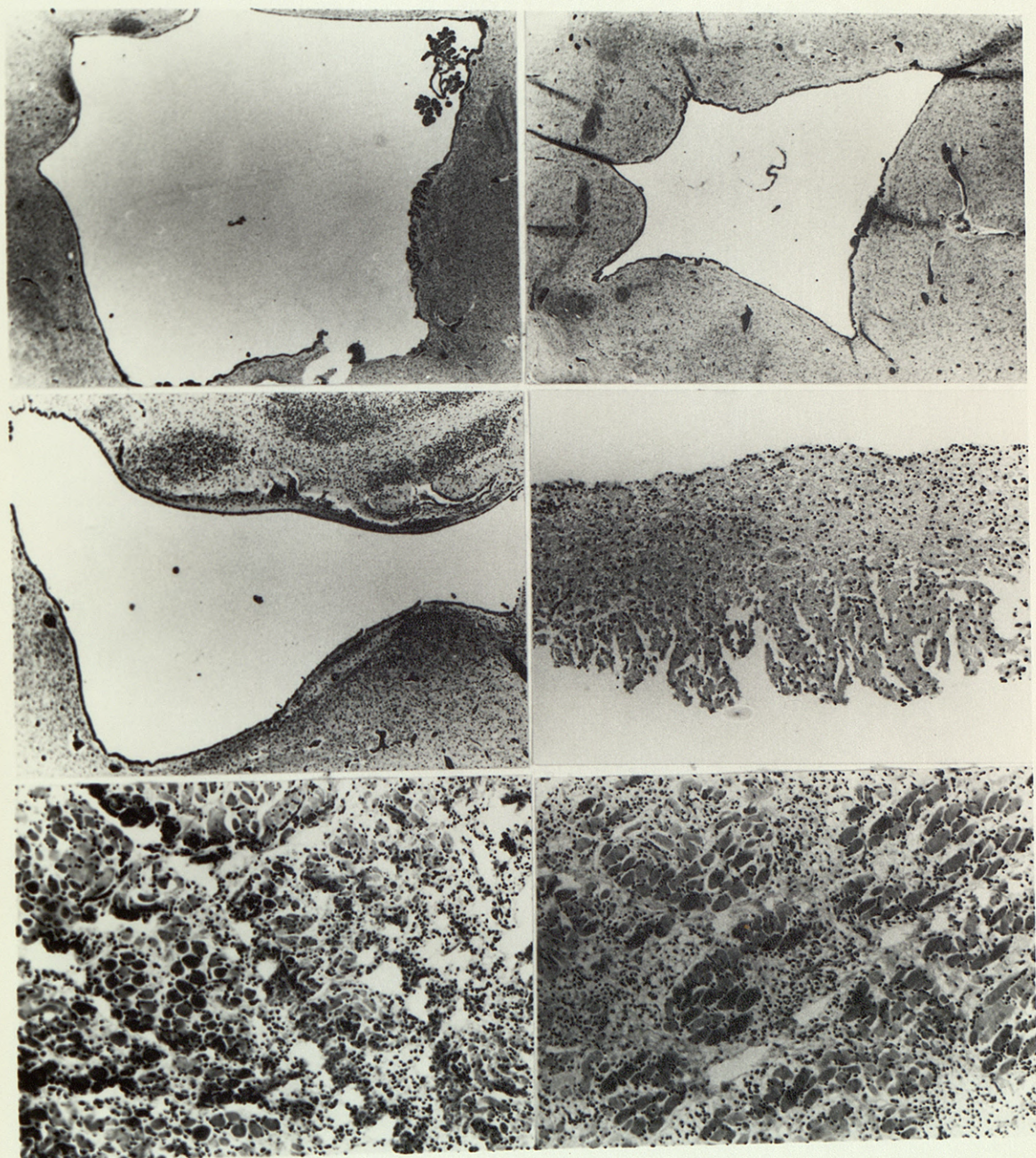


A	B
C	D
E	F

FIGURE-22 AGH-SYNDROME

- A. CEREBRUM DILATED VENTRICLE 100X HE-STAIN
- B. CEREBRUM DILATED VENTRICLE 100X HE-STAIN
- C. CEREBRUM DILATED VENTRICLE 100X HE-STAIN
- D. CEREBRUM-CORTEX ONLY 6MM THICK ASSOCIATED WITH SEVERE HYDRANENCEPHALY
- E. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE 100X HE-STAIN
- F. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE 100X HE-STAIN

Figure 22

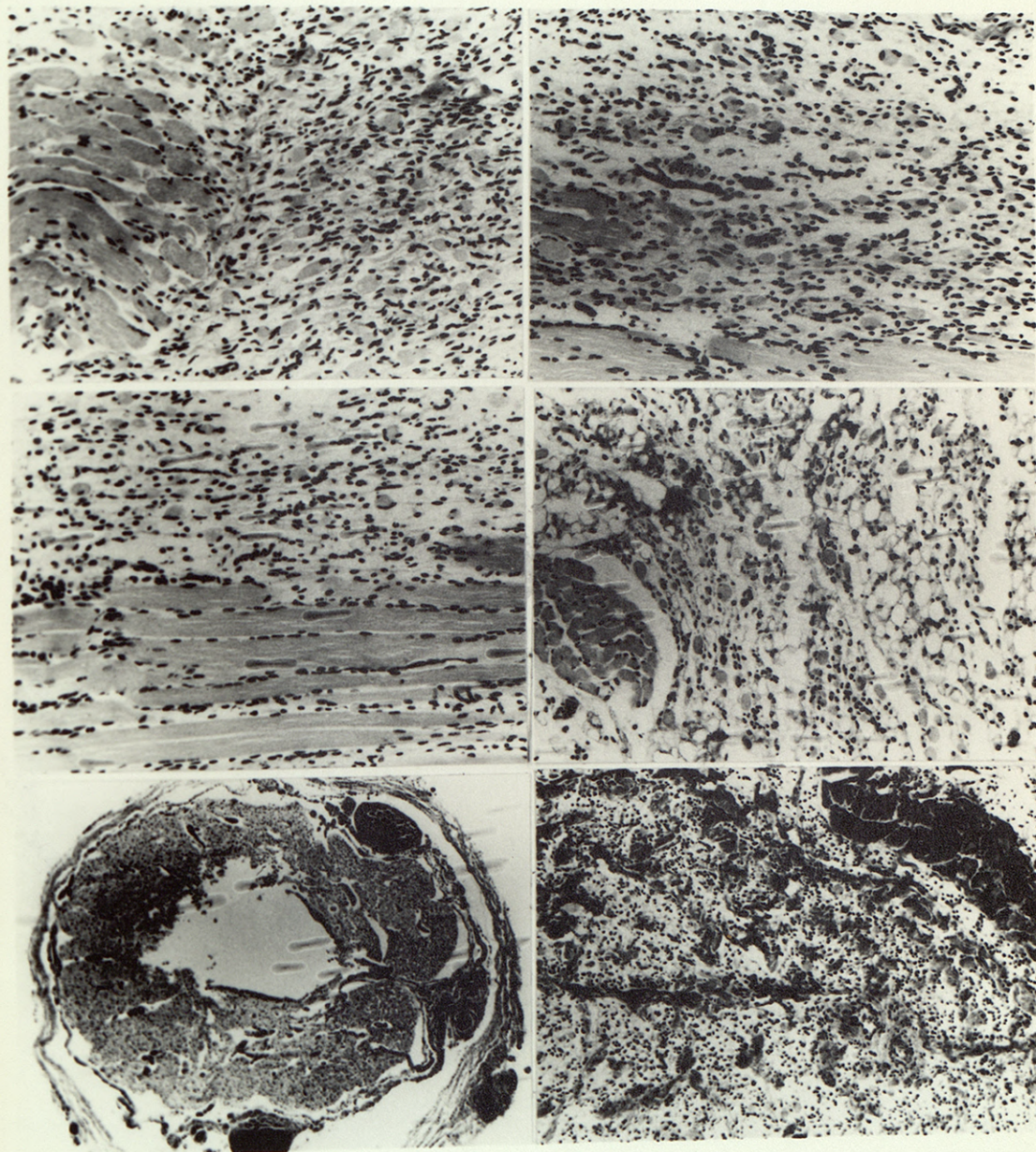


A	B
C	D
E	F

FIGURE-23 AGH-SYNDROME

- A. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE
100X HE-STAIN
- B. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE
100X HE-STAIN
- C. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE
200X HE-STAIN
- D. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE
200X HE-STAIN
- E. SPINAL CORD HYDROMYELIA 10X HE-STAIN
- F. MUSCLE FIBERS REPLACED WITH CONNECTIVE TISSUE
100X HE-STAIN

Figure 23



GENERAL DISCUSSION

General Discussion

During the course of this research it became evident that there are two major types of skeletal deformities in newborn lambs in the United States. This observation essentially confirms what was reported in the December 1987 National Wool Growers Association Journal. Within the sheep industry, skeletal deformities are minor contributors to overall lamb mortality. Producers and veterinarians rarely recognize a problem until it becomes repetitive in significant numbers to produce a pattern that is recognizable.

It soon became evident that producers and veterinarians were grouping the two deformities discussed under one name, Spider Lamb. This report attempts to define the two syndromes; 1.) Spider Lamb Syndrome and, 2.) AGH-Syndrome (Arthrogryposis hydranencephaly) as was presented in section two (II) and three (III).

The two syndromes are very different and do not appear to have a common etiology. The pathogenesis of the two conditions has not been clearly defined. There remains a significant amount of research yet to be done on both syndromes.

Major etiological problems concerning these two syndromes;1.) If Cache Valley virus is involved, how is it transmitted: 2.) Why is it so difficult to reproduce the AGH-Syndrome in the laboratory: 3.) Is it due to more than one causative agent. If the Spider Lamb Syndrome is genetic in origin following a simple autosomal recessive pattern, why are there specific flocks that do not fit this pattern: 4.) Is there DNA change that could be identified with DNA probe: 5.) Are there any blood group antigens associated with the spider gene locus such as seen in the autosomal recessive-bentleg syndrome in the Charmere sheep: 6.) Initial serum and tissue screening of spider lambs in this study, performed by Dr. Prieur at Washington State University, were low for beta-galactosidase: Could this be an indication of a lysosomal storage disease. The syndromes have been well defined, but there are still many unanswered questions concerning "Etiology and Pathogenesis".

APPENDIX

ABBREVIATIONS USED IN DISSERTATION TABLES

G = GROSS EXAMINATION

C = CUT SURFACE EXAMINATION

GC = GROSS AND CUT TOGETHER

K = KYPHOSIS

S = SCOLIOSIS

KS = KYPHOSCOLIOSIS

PE = PECTUS EXCAVATUM

ACE = ATLAS CARTILAGE EROSIONS

RN = ROMAN NOSE

NSD = NASAL SEPTAL DEVISTION

FLA = FRONT LIMB ABNORMALITY

BLA = BACK LIMB ABNORMALITY

VA = VERTEBRAL ABNORMALITY

SA = STERNAL ABNORMALITY

- = NEG = NEGATIVE

CFA = CRANIAL FACIAL ABNORMALITY

D.J.D. = DEGENERATIVE JOINT DISEASE

CRE = COMPLETE RADIOGRAPHIC EXAM

OEO = ONE ELBOW ONLY

LIVE EXAMINATION

FLA	☐	NEG	☐	1 +	☐	2 +	☐	3 +
BLA	☐	NEG	☐	1 +	☐	2 +	☐	3 +
VA	☐	NEG	☐	1 +	☐	2 +	☐	3 +
SA	☐	NEG	☐	1 +	☐	2 +	☐	3 +
CFA	☐	NEG	RN	☐	1 +	☐	2 +	☐
			NSD	☐	1 +	☐	2 +	☐

FRONT LIMB DESCRIPTION

BACK LIMB DESCRIPTION

VERTEBRAE

STERNEBRAE

NOTES:

LIVE EXAMINATION				
IDENTIFICATION NUMBER	RESEARCH NUMBER	FRONT LIMBS	BACK LIMBS	VERTEBRAE
1	R86-330	NEG	2 +	NEG
2	R86-336	NEG	NEG	4+KS
3	R86-371	3 +	2 +	NEG
4	R86-439	1 +	1 +	NEG
5	R86-440	1 +	1 +	2+KS
6	R86-441	2 +	1 +	NEG
7	R86-707	4 +	3 +	NEG
8	R86-753	3 +	2 +	NEG
9	R86-754	2 +	1 +	NEG
10	R86-796	1 +	1 +	2+S
11	R86-797	1 +	1 +	2+S
12	R86-8954	2 +	2 +	NEG
13	R86-938	3 +	3 +	1+S
14	R86-939	3 +	3 +	NEG
15	R87-127	3 +	1 +	2+S
16	R87-128	1 +	NEG	4+KS
17	R87-185	2 +	NEG	NEG
18	R87-186	3 +	3 +	3+KS
19	R87-193	2 +	NEG	NEG
20	R87-211	1 +	NEG	NEG
21	R87-212	1 +	NEG	1+S
22	R87-213	3 +	1 +	2+KS

LIVE EXAMINATION				
IDENTIFICATION NUMBER	RESEARCH NUMBER	FRONT LIMBS	BACK LIMBS	VERTEBRAE
23	R87-214	1 +	NEG	2+S
24	R87-223	3 +	2 +	3+KS
25	R87-262	3 +	3 +	2+KS
26	R87-263	3 +	3 +	1+S
27	R87-271	1 +	1 +	1+S
28	R87-314	2 +	1 +	NEG
29	R87-327	3 +	2 +	NEG
30	R87-328	3 +	1 +	NEG
31	R87-329	2 +	2 +	1+KS
32	R87-330	2 +	1 +	NEG
33	R87-333	3 +	2 +	NEG
34	R87-364	3 +	2 +	1+S
35	R87-365	3 +	3 +	2+KS
36	R87-417	2 +	1 +	3+KS
37	R87-443	3 +	3 +	1+S
38	R87-456	3 +	3 +	3+KS
39	R87-457	3 +	2 +	NEG
40	R87-458	3 +	3 +	3+KS
41	R87-474	3 +	1 +	NEG
42	R87-489	3 +	NEG	3+KS
43	R87-490	1 +	3 +	1+S
44	R87-491	3 +	1 +	NEG

LIVE EXAMINATION				
IDENTIFICATION NUMBER	RESEARCH NUMBER	FRONT LIMBS	BACK LIMBS	VERTEBRAE
45	R87-492	3 +	1 +	NEG
46	R87-493	3 ++	NEG	NEG
47	R87-494	1 +	3 ++	NEG
48	R87-495	1 +	NEG	NEG
49	R87-496	2 +	2 +	2+KS
50	R87-497	3 +	2 +	2+KS
51	R87-498	2 +	1 +	1+KS
52	R87-499	1 +	NEG	NEG
53	R87-500	3 +	2 +	2+KS
54	R87-501	2 +	2 +	1+KS
55	R87-502	2 +	2 +	2+KS
56	R88-30	2 +	2 +	1+KS
57	R88-31	2 +	2 +	NEG
58	R88-32	2 +	NEG	NEG
59	R88-33	1 +	1 +	NEG
60	R88-34	1 +	1 +	1+S
61	R88-37	1 +	NEG	1+S
62	R88-39	2 +	1 +	4+KS
63	R88-40	2 +	2 +	3+KS
64	R88-41	2 +	1 +	4+KS
65	R88-42	1 +	1 +	3+KS

LIVE EXAMINATION CONTINUED			
IDENTIFICATION	STERNEBRAE	HEAD	NOTES
1	NEG	NEG	
2	NEG	NEG	
3	1+PE	NEG	
4	1+PE	CFA1+RN	
5	1+PE	NEG	
6	NEG	CFA1+RN	
7	1+PE	CFA1+RN	
8	NEG	NEG	
9	NEG	CFA1+RN	
10	1+PE	NEG	
11	2+PE	NEG	
12	NEG	NEG	
13	1 +	CFA2+RN	
14	NEG	NEG	
15	NEG	NEG	
16	3+PE	NEG	
17	NEG	NEG	
18	2 +	NEG	
19	NEG	NEG	FRACTURE RIGHT TIBIA
20	NEG	NEG	TALL-NARROW CONFORMATION
21	NEG	CFA2+RN	
22	1 +	NEG	

LIVE EXAMINATION CONTINUED			
IDENTIFICATION	STERNEBRAE	HEAD	NOTES
23	NEG	NEG	
24	2 +	CFA3+RN	TAG-233
25	1+PE	NEG	FRACTURE RIGHT TIBIA
26	NEG	NEG	
27	1+PE	NEG	
28	NEG	NEG	
29	NEG	NEG	
30	NEG	NEG	
31	NEG	CFA1+RN	
32	NEG	NEG	
33	NEG	NEG	
34	NEG	NEG	
35	1+PE	NEG	
36	2+PE	NEG	
37	NEG	CFA2+RN	
38	2+PE	CFA3+RN	CONTAGIOUS EXAN. PNEUMONIA
39	1+PE	CFA3+RN1+NSD	CONTAGIOUS EXAN. PNEUMONIA
40	2+PE	NEG	UNDER FENCE BLOAT
41	NEG	CFA2+RN	HEAT STROKE
42	2+PE		FOUND DEAD
43	NEG		
44	NEG		

LIVE EXAMINATION CONTINUED			
IDENTIFICATION	STERNEBRAE	HEAD	NOTES
45	NEG	CFA1+RN	
46	NEG	CFA1+RN	
47	NEG	CFA2+RN	
48	NEG	CFA1+RN	
49	NEG	CFA1+RN	
50	2+PE	CFA1+RN	
51	NEG	CFA1+RN	
52	NEG	CFA1+RN	SHOULDERS OUT
53	NEG	NEG	
54	NEG	CFA1+RN	
55	NEG	NEG	SHOULDERS OUT
56	1+PE	NEG	
57	NEG	CFA1+RN	
58	NEG	NEG	SHOULDERS OUT
59	NEG	NEG	
60	NEG	NEG	
61	NEG	NEG	DROWNED
62	3+PE	CFA2+RN1+NSD	
63	2+PE	NEG	
64	3+PE	NEG	
65	3++PE	NEG	

NECREOPSY EXAMINATION

FLA	G		NEG		1 +		2 +		3 +
	C								
BLA	G		NEG		1 +		2 +		3 +
	C								
VA	G		NEG		1 +		2 +		3 +
	C								
SA	G		NEG		1 +		2 +		3 +
	C								
CFA		NEG	RN		1 +		2 +		3 +
			NSD		1 +		2 +		3 +

NOTES:

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NECROPSY EXAMINATION			
ID #	RESEARCH #	FRONT LIMB	BACK LIMB
1	R85-330	-G, 2 + C	2 + GC
2	R86-336	-G, 3 + C	-G, 1 + C
3	R86-371	2 + G, 3 + C	2 + G, 1 + C
4	R86-439	1 + G, 2 + C	1 + G, -C
5	R86-440	1 + G, 2 + C	1 + GC
6	R86-441	2 + GC	-G, 1 + C
7	R86-707	3 + GC	3 + G, 1 + C
8	R86-753	3 + GC	2 + G, 1 + C
9	R86-754	2 + G, 3 + C	1 + GC
10	R86-796	1 + G,, 3 + C	1 + G, -C
11	R86-797	1 + G, 3 + C	1 + GC
12	R86-895	2 + GC	2 G, -C
13	R86-938	3 + G, 2 + C	2 + G, -C
14	R86-939	3+ G, 2 + C	2 + G, 1 + C
15	R87-127	3 + GC	1 + GC
16	R87-128	1 + G, 3 + C	-GC
17	R87-185	2 + GC	-GC
18	R87-186	3 + GC	3 + G, HC
19	R87-193	2 + GC	-GC
20	R87-211	1 + G, 3 + C	-G, 1 + C
21	R87-212	1 + G, 2 + C	1 + GC
22	R87-213	3 + GC	1 + GC

NECROPSY EXAMINATION			
ID #	RESEARCH #	FRONT LIMB	BACK LIMB
23	R87-214	1 + G, 3 + C	1 + GC
24	R87-223	3 + GC	2 + G, 1 + C
25	R87-262	4 + GC	3 + G, 1 + C
26	R87-263	3 + GC	3 + G, 1 + C
27	R87-271	1 + G, 2 + C	1 + G, -C
28	R87-314	2 + GC	1 + G, -C
29	R87-327	3 + GC	1 + G, -C
30	R87-328	3 + GC	1 + G, -C
31	R87-329	2 + G, 3 + C	2 + G, 1 + C
32	R87-330	2 + G, 3 + C	1 + G, -C
33	R87-333	3 + GC	2 + G, 1 + C
34	R87-364	3 + GC	2 + G, 1 + C
35	R87-365	3 + GC	3 + G, 1 + C
36	R87-417	2 + GC	1 + G, -C
37	R87-443	3 + GC	3 + G, 1 + C
38	R87-456	3 + GC	3 + G, 1 + C
39	R87-475	3 + GC	2 + G,, 1 + C
40	R87-458	3 + GC	3 + G, 1 + C
41	R87-474	3 + GC	1 + G, -C
42	R87-489	3 + GC	1 + GC
43	R87-490	1 + G, 3 + C	3 + G, 1 + C
44	R87-491	3 + GC	1 + G, -C

NECROPSY EXAMINATION			
ID #	RESEARCH #	FRONT LIMB	BACK LIMB
45	R87-492	3 + GC	1 + G, -C
46	R87-493	3 ++ G, 3 + C	neg
47	R87-494	1 + G, 2 + C	3 ++ G, 1 + C
48	R87-495	1 + G, 2 + C	neg
49	R87-496	2 + GC	2 + G, -C
50	R87-497	3 + GC	2 + G, 1 + C
51	R87-498	2 + GC	1 + G, -C
52	R87-499	1 + G,, 2 + C	neg
53	R87-500	3 + GC	2 + G, -C
54	R87-501	2 + GC	2 + G, -C
55	R87-502	2 + GC	2 + G, 1 + C
56	R88-30	2 + GC	2 + G, -C
57	R88-31	2 + G, 3 + C	1 + GC, S
58	R88-32	2 + G, 3 + C	neg
59	R88-33	1 + G, 3 + C	1 + G, -C
60	R88-34	1 + G, 2 + C	1 + G, -C
61	R88-37	1 + G, 2 + C	neg
62	R88-39	2 + G, 3 + C	1 + G, -C
63	R88-40	2 + G, 3 + C	2 + GC
64	R87-41	2 + G, 3 + C	1 + G, -C
65	R88-42	1 + GC	1 + G, -C

NECROPSY EXAMINATION CONTINUED			
ID #	VERTEBRAE	STERNEBRAE	HEAD
1	2 + GC, KS	neg	neg
2	4 + GC, KS	3 + GC, PE	neg
3	1 + GC, K	1 + GC, PE	neg
4	2 + GC, KS	1 + GC, PE	CFA, 1 + RN
5	3 + GC, KS	2 + GC, PE	neg
6	1 + GC, KS	neg	CFA, 1 + RN
7	2 + GC, KS	2 + G, HC	CFA, 1 + RN
8	1 + GC, KS	neg	neg
9	2 + GC, S	neg	CFA, 1 + RN, NSD
10	3 + GC, KS	2 + GC, PE	neg
11	3 + GC, KS	2 + GC, PE	neg
12	1 + G, -C, KS	neg	neg
13	2 + GC, KS	1 + GC, PE	CFA, 2 + RN
14	1 + G, -C, KS	neg	neg
15	3 + GC, KS	1 + G, 2 + C, PE	neg
16	4 + GC, KS	3 + GC, PE	neg
17	1 + GC, KS	-G, 1 + C	neg
18	3 + GC, KS	2 + G, 1 + C, PE	neg
19	1 + GC, KS	1 + GC, PE	neg
20	1+ G, 2 + C, S	neg	neg
21	1 + GC, S	neg	CFA, 2 + RN
22	4 + G, 2 + C, KS	neg	neg

NECROPSY EXAMINATION CONTINUED			
ID #	VERTEBRAE	STERNEBRAE	HEAD
23	3 + GC, KS	1 + GC, PE	neg
24	4 + G, 2 + C, KS	2 + GC, PE	CFA, 3 + RN
25	3 + GC, KS	2 + GC, PE	neg
26	2 + GC, KS	1 + G, 2 + C, PE	neg
27	2 + GC, KS	1 + GC, PE	neg
28	1 + GC, KS	neg	neg
29	1 + GC, KS	-G, 1 + C	neg
30	2 + G, 1 + C, KS	neg	neg
31	2 + GC, KS	-G, 1 + C	CFA, NSD
32	1 + GC, S	-G, 1 + C	neg
33	2 + GC, KS	-G, 1 + C	neg
34	2 + GC, KS	neg	neg
35	3 + GC, KS	2 + GC, PE	neg
36	3 + GC, KS	2 + GC, PE	neg
37	2 + GC, KS	1 + GC, PE	CFA, 1 + RN
38	4 + G, 1 + C, KS	2 + G, 1 + C, PE	neg
39	2 + G, 1 + C, KS	1 + GC, PE	neg
40	3 + G, 1 + C, KS	2 + G, 1 + C, PE	CFA, 2 + RN
41	1 + GC, KS	neg	CFA, 3 + RN
42	3 + G, 1 + C, KS	2 + GC, PE	CFA, 3 + RN, NSD
43	2 + GC, KS	neg	neg
44	1 + GC, S	neg	CFA, 2 + RN

NECROPSY EXAMINATION CONTINUED			
ID #	VERTEBRAE	STERNEBRAE	HEAD
45	2 + GC, KS	neg	CFA, 1 + RN
46	1 + GC, KS	neg	CFA, 1 + RN
47	1 + GC, KS	neg	CFA, 2 + RN
48	1 + GC, KS	neg	CFA, 1 + RN
49	2 GC, KS	-G, 1 + C, PE	CFA, 1 + RN
50	3 + GC, KS	2 + GC, PE	CFA, 1 + RN
51	2 + GC, KS	-G, 1 C	CFA, 1 + RN
52	1 + GC, S	neg	CFA, 1 + RN
53	3 + GC, KS	1 + GC, PE	neg
54	2 + GC, KS	neg	CFA, 1 + RN
55	3 + GC, KS	neg	neg
56	2 + GC, KS	1 + G, 2 + C, PE	neg
57	1 + GC, S	-G, 1 + C	CFA, 1 + RN, NSD
58	1 + GC, KS	-G, 1 + C	neg
59	1 + GC, KS	neg	neg
60	2 + GC, KS	neg	neg
61	2 + GC, KS	neg	neg
62	4 + GC, KS	3 + GC, PE	CFA, 2 + RN, 1 + NSD
63	3 + GC, KS	2 + GC, PE	neg
64	4 + GC, KS	3 + GC, PE	neg
65	3 + GC, KS	3 + GC, PE	neg

NECROPSY EXAMINATION CONTINUED

ID #	HISTO	HISTO BONES	RAD EXAM	SEX AGE	NOTES
1	+	+	CRE	4WF	
2	+	+	CRE	3WM	
3	+	+	CRE	A-F	DJD
4	+	+	CRE	5WM	
5	+	+	CRE	5WF	
6	+	+	CRE	5WF	
7	+	+	CRE	3WM	DJD, ACE
8	+	+	CRE	3WM	ACE
9	+	+	CRE	A-F	DJD, ACE
10	+	+	CRE	10WF	ACE
11	+	+	CRE	10WM	
12	+	+	CRE	16WM	
13	+	+	OEO	A-F	DJD ++
14	+	+	OEO	A-M	DJD ++
15	+	+	BES	3WF	
16	+	+	CRE	5dM	
17	+	+	BES	3WF	
18	+	+	BES	5WM	
19	+	+	CRE	A-F	DJD, ACE
20	+	-	BES	10WM	DJD, ACE
21	+	-	BES	16WF	DJD, ACE
22	+	-	CRE	16WM	DJD, ACE

NECROPSY EXAMINATION CONTINUED					
ID #	HISTO	HISTO BONES	RAD EXAM	SEX AGE	NOTES
23	+	-	CRE	12WF	DJD, ACE
24	+	-	BES	10WM	DJD, ACE
25	+	-	CRE	A-F	DJD, ACE
26	+	-	BES	A-F	DJD, ACE
27	+	-	BES	3WF	
28	+	-	BES	6WM	ACE
29	+	-	CRE	13WM	ACE
30	+	-	BES	4WM	
31	+	-	BES	6WF	
32	+	-	BES	4WF	
33	+	-	BES	A-F	DJD, ACE
34	-	+	CEO	12WF	ACE
35	+	-	CEO	12WM	ACE
36	-	-	BES	12WM	ACE
37	-	+	CEO	17WM	ACE
38	-	-	CEO	35WF	DJD, ACE
39	-	-	CEO	35WM	DJD, ACE
40	-	-	CEO	35WF	DJD, ACE
41	-	-	CEO	35WF	DJD, ACE
42	-	-	CEO	35WF	DJD
43	+	+	CEO	A-F	DJD
44	-	-	BES	A-F	DJD

NECROPSY EXAMINATION CONTINUED

ID #	HISTO	HISTO BONES	RAD EXAM	SEX AGE	NOTES
45	+	+	OEO	A-M	DJD
46	+	+	BES	A-F	DJD
47	+	+	OEO	A-F	DJD
48	-	-	BES	A-M	DJD
49	+	+	CRE	AA--F	DJD
50	+	+	OEO	A-F	DJD
51	+	+	OEO	A-M	DJD
52	+	+	OEO	A-M	DJD
53	-	+	CRE	A-M	DJD
54	+	-	OEO	A-M	DJD
55	+	+	OEO	A-F	DJD
56	+	+	CRE	4WF	
57	+	+	CRE	7WM	
58	+	+	BES	5WM	
59	+	+	BES	4WM	
60	+	+	BES	4WM	
61	-	-	OEO	2dM	
62	-	-	OEO	1dF	
63	-	-	OEO	1dF	
64	-	-	OEO	1dF	
65	-	-	OEO	1dM	

APT - 6
KARYOTYPING⁴

Karyotyping was carried out in the Cytogenetics Laboratory at Kansas State University by a method described by Eldridge¹ and modified by Leipold and Saperstein² and Johnson³.

The culture medium was prepared as follows.^{1,3} One gram of Ham's nutrient mixture F-10 (GIBCO Laboratories)^a was placed in a 150 ml sterilized glass beaker and 98 ml of sterile, double glass-distilled water and a sterile magnetic stir bar added and the beaker placed on a stir plate. One ml antibiotic-antimycotic solution containing 10,000 units/ml penicillin, 10,000 ug/ml streptomycin and 25 ug/ml Fungizone and 1 ml lyophilized phytohemagglutinin were added to the swirling culture medium. The pH was adjusted using a digital pH meter to 7.0 ± 0.05 by dropwise adding a 10% sodium bicarbonate solution. A sterile disposable 0.22 μ m Nalgene® sterilization filter unit (115 ml)^b was prepared by filtering and discarding 150 ml hot, sterile, double glass-distilled water after which the buffered medium was filtered.

^a Gibco Laboratories, Grand Island, N.Y.

^b Sybron/Nalge Company, Rochester, N.Y.

Six ml of filtered medium was added to a 30 ml sterile glass bottle and a Corning disposable 25 cm² tissue culture flask using a sterile 10 ml glass pipette.³

Using a 3 ml syringe and a 22 gauge needle, 1 ml of blood was transferred to each of the labelled culture containers and gently swirled by hand.³ Each container was stoppered tightly and incubated at 38.5 C. The cultures were gently swirled by hand every hour for the first 6 hours. After 46 to 48 hours incubation, the cultures were removed and 0.05 ml of colchicine prepared from a 1 mg/ml stock solution diluted 1:19 with sterile, double glass-distilled water was added to each container with an Eppendorf pipette. The containers were returned to the incubator at 38.5 C for 2.5 hours.³

The containers were removed from the incubator, gently swirled and the contents transferred to a graduated, labelled, siliconized centrifuge tube and centrifuged at 300 x g for 10 minutes.^{1,3} The supernatant was aspirated to the 1.2 ml level with a Pasteur pipette connected to a water-vacuum system. A 0.-67 M solution of KCl was added, the tube gently agitated by repeatedly drawing up and expelling the solution via a siliconized Pasteur pipette and a rubber bulb. The mixture was left standing for 23 minutes at

room temperature. During the last minutes, 1 ml of fixative (1 part glacial acetic acid to 3 parts absolute reagent grade methyl alcohol freshly mixed and kept in an ice bath) was added, gently mixed but thoroughly with a siliconized pipette and rubber bulb. The resultant blackish mixture was centrifuged at 300 x g for 10 minutes. The supernatant was carefully aspirated to 0.5 cm above the packed cells. the cells were washed thrice with the fixative. During the first wash, the fixative was added to the 5 ml level, gently mixed, centrifuged for 10 minutes at 300 x g, and the supernatant discarded. During the third wash, the cold fixative was added to the 3 ml level, gently mixed, centrifuged for 10 minutes at 300 x g, and the supernatant discarded. The centrifuge tube was gently tapped to mix the cells in the remaining fluid and cold fixative was added to obtain the desired turbidity.^{1,3}

Microscopic glass slides were stored in methyl alcohol solution with a drop of HCl.^{1,3} Before using, the slides were rinsed in distilled water, dried and polished using a Kimwipe.^c Using a siliconized Pasteium pipette, the solution of fixative and harvested cells was mixed and 3-4 drops were splattered onto the polished, labelled slide from a height of 2 feet above the slide. The edge of the slide was touched to a

flame from a Bunsen burner and the alcohol was allowed to burn off. The slide was flicked with the wrist to remove any excess acetic acid and set on end to air dry. The dried slides were examined under phase contrast microscopy to evaluate the chromosomes.^{1,3}

If chromosome spreads were present, the slide was stained in a 1:20 dilution of Giemsa stain buffered to a pH 6.8 for 20 minutes.³ The slides were rinsed in tap water, followed by distilled water, blotted dry in a paper towel and any excess water removed by a Kimwipe.³ The slides were allowed to dry for at least 24 hours before being permanently mounted under a coverslip.³

If chromosome spreads were present, the slide was stained in a freshly prepared saturated orcein solution² (7 g orcein combined with 180 ml acetic acid and stirred until dissolved, 120 mls of boiling double distilled water added and stirred until mixed, then filtered) for 30 minutes. The slides were then dipped in 45% acetic acid until free of excess stain, placed into 1:1 T-butyl for one minute, 100% T-butyl for 1 minute, and finally into three baths of xylene of one

^c Kimberly Clark, Neenah, WI

minute each. The slides were dried and permanently mounted under a coverslip.

Slides with metaphase spreads were selected at random from each lamb counted and visually evaluated from structural and numerical abnormalities. At least 6 chromosome spreads were evaluated for numerical and morphological chromosomal abnormalities, for each animal. No abnormalities were noted.

REFERENCES

1. ELDRIDGE, F.E.: Bovine lymphocyte methods.
Mammalian Chromosome Newsletter 23: 176-180, 1982.
2. LEIPOLD, H.W.; SAPERSTEIN, G.: Rectal and vaginal
construction in Jersey cattle. J Am Vet Med Assoc
166: 231, 1975.
3. JOHNSON, J.E.: Chromosomal Analysis of Congenital
Abnormalities in Cattle, PhD Dissertation, Kansas
State University, Manhattan, Kansas 1985.
4. This section on Karyotyping is an exact copy of the
procedure given to me by the technical staff working
in the Kansas State University Congenital Defects
Laboratory - original author unknown

ROUTINE STAINING WITH H AND E

Ames = Histo - Tek Automatic Slide Stainer

--- Miles Laboratories Inc., Elkhart, Indiana

1.	Xylene	4 sec.
2.	100% Alcohol	2 sec.
3.	50% Alcohol	3 sec.
4.	Harris' Hematoxylin.	4 sec.
5.	Acid Alc	2 sec.
6.	70% Alcohol	2 sec.
7.	Ammonia in Alcohol	2 sec.
8.	95% Alcohol	2 sec.
9.	Eosin Solution	2 sec.
10.	100% Alcohol	2 sec.
11.	100% Alcohol	2 sec.
12.	Xylene	2 sec.
13.	Xylene Resin	4 sec.
14.	4 seconds to drop point.	4 sec.

60 sec.

BACK-UP HAND STAINING PROCEDURE FOR H + E

Solutions:

Harris' Hematoxylin

Eosin (working solution)

Ammonia water. 16 drops ammonia in 400ml
distilled water

Acid Alcohol 3.0ml. conc. HCT to 400ml
50% alcohol

Xylene. .2 min.

Xylene. .2 min.

Xylene. .2 min.

Xylene. .2 min.

100% alcohol. .2 min.

100% alcohol. .2 min.

95% alcohol. .2 min.

80% alcohol. .2 min.

60% alcohol. .2 min.

Dist. water . .2 min.

Tap water.rinse

Acid alc . . Dip to pink color

Tap water. rinse

Ammonia water. . . until blue

Tap water (running) . . . 3 min.

Dist. waterrinse

Eosin sol 15 sec.

Tap waterrinse

80% alcoholrinse

95% alcoholrinse

100% alcohol. . . .3 changes,
2 min. ea.

Xylene.3 changes,
2 min. ea.

Harris' Hematoxylin. .5 min.

HARRIS' HEMATOXYLIN

Hematoxylin crystals.	5.0 gm.
Alcohol, 100%	50.0 gm.
Ammonium, or Potassium alum.	100.0 gm.
Distilled Water	1000.0 ml.
Mercuric oxide.	2.5 gm.

Dissolve the hematoxylin in the alcohol, the alum in the water by the aid of heat. Remove from heat and mix the two solutions. Bring to a boil as rapidly as possible. (Limit this heat to less than one minute and stir often). Remove from heat and add the mercuric oxide slowly. Reheat to a simmer until it becomes dark purple, remove from heat immediately and plunge the vessel into a basin of cold water. Stir until cool. Filter before use.

Most references recommend the addition of glacial acetic acid for use. We follow the Ann Preece manual and leave the acetic acid out.

1% STOCK EOSIN

Eosin (Water Soluble).	1.0 gm.
Distilled water.	100.0 ml.

Dissolve the Eosin in the distilled water and add .25 ml of glacial acetic acid to the solutions.

WORKING EOSIN SOLUTION

1% stock Eosin solution.	100.0 ml.
80% ethyl alcohol.	300.0 ml.
Glacial Acetic Acid.	2.0 ml.

ALCIAN BLUE FOR MUCOSUBSTANCES

FIXATION: 10% Buffered Neutral Formalin

TECHNIQUE: Paraffin sections at 6 microns

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Mordant in 3% acetic acid solution for 3 minutes.
3. Alcian Blue solution for 30 minutes.
4. Wash in running water for 10 minutes.
5. Rinse in distilled water.
6. Counterstain in Nuclear Fast Red for 5 minutes.
7. Wash in running water for 1 minute.
8. Dehydrate in 95% Alcohol, absolute alcohol and clear in xylene.
9. Mount with Permount or Histoclad.

SOLUTIONS:

3% Acetic Acid

Glacial acetic acid. 3.0 ml.
Distilled water. 97.0 ml.

1% Alcian Blue Solution

Alcian Blue 8GX. 1.0 g.
3% glacial acetic acid. 100.0 ml.

Adjust pH to 2.5

Filter and add a few crystals of thymol.

Nuclear Fast Red

Nuclear Fast Red 0.1 g.
Aluminum sulfate 100.0 ml. (5%)

Dissolve .1 g. NFR in 5% solution of 100 ml.
Aluminum sulfate with the aid of heat. Cool,
filter and add grain of thymol as preservative.

Acid mucopolysaccharides stain blue.
Nuclei stain pink.

BUFFERED AZURE EOSINATE METHOD- Modified Nocht's

FIXATION: Zenker's solution or 10% buffered neutral formalin

PROCEDURE:

1. Bring 6 micron thick paraffin sections to water (using 0.5% iodine, 5% sodium thiosulfate sequence for mercuric chloride fixed material.
2. Stain 1 hour in the above staining solution.*
3. Dehydrate in 2-3 changes of acetone.
4. Clear in a 50:50 acetone-xylene mixture.
5. Two changes in xylene.
6. Mount in synthetic resin

* On some tissues, it is advantageous to heat slides to a nearly dry state on a slide warmer (35-40C) prior to immersion in acetone.

STAINING RESULTS:

Nuclei, bacteria, and rickettsiae stain blue.
Mast cell and basophile leukocyte granules stain blue violet.
Cartilage matrix stains reddish violet.
Calcium deposits stain dark blue.
Cytoplasm of surviving cells stain light blue to violet or lavender.
Erythrocytes and hemoglobin stain orange-pink.

SOLUTIONS:

1% Azure Eosinate

Azure A Eosinate. 1.0 g.
Glycerol.50.0 ml.
Methanol, absolute.50.0 ml.
(This stock solution stable for several years.)

*M/10 citric acid - solution subject to bacterial contamination.

21.0 g. citric acid per liter of 25% methanol.
Make fresh for each usage.
1.05 g. citric acid per 50 ml of 25% methanol
(12.5 ml. absolute methanol) 100
.52 g. citric acid per 25 ml. of 25% methanol
(37.5 ml. distilled water) 300

*M/5 disodium phosphate

28.4 g. per liter of 25% methanol
174

- 1.42 g. disodium phosphate per 50 ml of 25% methanol.
 .71 g. disodium phosphate per 25 ml. of 25% methanol.

SOURCE: Biologica Stain Commision 1960, 2nd Edition, pages 45-46.

*For pH tables of these mixtures, see page 253 of source.

Preparation of staining solution: (pH 4.1) x2

1% Azure Eosinate . . .	0.5ml	1.0ml	2.0ml	4.0ml
M/10 citric acid. . . .	1.2ml	2.4ml	4.8ml	9.6ml
M/5 disodium phosphate.	0.8ml	1.6ml	3.2ml	6.4ml
C.P. acetone.	5.0ml	10.0ml	20.0ml	40.0ml
Distilled water	25.0ml	50.0ml	100.0ml	200.0ml

NOTE: The buffer mixture (pH 4.1) may be altered, lower pH levels giving redder effects, higher levels more blue. Zenker fixed materials require pH 4.5 to 5.0; formalin fixed, pH 3.75 to 4.3.

Nitrocellulose sections and celloidized sections require 2 to 2 1/2 hours staining, or 2-3 times as much dye. If necessary to preserve the celloidin coat, substitute isopropanol for acetone in the dehydration and clearing schedule.

NOTE: The small Coplin-jar quantities are used at once and discarded after use.

GOMORI'S ONE STEP TRICHOME METHOD

FIXATION: 10% buffered neutral formalin

TECHNIQUE: Cut paraffin sections at 6 microns

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Place in Bouin's solution in oven at 560F for one

- hour.
3. Wash well in running water or until yellow color disappears.
 4. Stain nuclei with Weigert's iron hematoxylin 10 minutes.
 5. Wash in water.
 6. Trichrome stain for 15 to 20 minutes.
 7. Place in 0.05 glacial acetic water for 2 min.
 8. Rinse in distilled water.
 9. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene-two changes.
 10. Mount with Permount or Histoclad.

STAINING RESULTS:

Muscle fibers stain red.
Collagen stains green (blue if aniline blue is used).
Nuclei stain blue to black.

REFERENCE: AFIP p. 94

SOLUTIONS:

Bouin's solution (check for stock solution)

Picric acid, saturated aqueous solution.	750 ml
37 - 40% Formalin.	250 ml
Glacial acetic acid.	50 ml

TRICHROME STAIN

Ponceau 2R	0.6 g
Light Green SF yellowish	0.3 g
Glacial acetic acid.	1.0 ml
Phosphotungstic acid	0.8 g
Distilled water.	100 ml

LILLIE'S ALLOCHROME PROCEDURE

FIXATION: Any well-fixed tissue may be used.

EMBEDDING: Paraffin. Sections cut at 5 um.

METHOD:

1. Hydrate slides.
2. Oxidize 10 minutes in 1% aqueous periodic acid.
3. Wash 5 minutes in running water to remove excess

traces of the acid.

4. Place in Schiff's reagent for 10 to 15 minutes.
5. Immerse in three separate changes of 0.5% sodium metabisulfite, 2 minutes each change. Wash 10 minutes in running water.
6. Stain 2 minutes in Weigert's iron hematoxylin.
7. Wash 10 minutes in running tap water.
8. Stain 6 minutes in collagen stain.
9. Dehydrate and differentiate in 2 changes each of 95% and absolute alcohol, clear in xylene, and coverslip, using a synthetic mounting medium.

STAINING RESULTS:

Nuclei stain black, gray, or brown.

Cytoplasm and muscle cells stain gray-green to greenish yellow.

Collagen stains blue.

Reticular connective tissue usually stains blue.

Glomerular basement membranes stain red-purple.

SOLUTIONS

1% aqueous periodic acid Schiff reagent.

0.5% sodium metabisulfite.

Weigert's iron hematoxylin.

Collagen stain: 40 mg of methyl blue dissolved in 100 ml of saturated aqueous picric acid.

PERIODIC ACID - SCHIFF (PAS)

FIXATION: 10% buffered neutral formalin.

TECHNIQUE: Paraffin sections at 6 microns; variation for frozen sections.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in periodic acid solution for 5 minutes.
3. Rinse in distilled water
4. Place in Coleman's feulgen for 15 minutes.
(Save solution in refrigerator, cover jar during staining and discard if solution becomes bright pink).
5. Wash in running water 10 minutes to develop pink coloration.
6. Stain in Harris Hematoxylin for 30 seconds.
7. Wash in running water 3 minutes.
8. Dehydrate in two changes each of 95% and 100% ethanol, clear in 2 changes xylene.

STAINING RESULTS:

Nuclei stain blue.

Fungi stain red.

Glycogen, mucin, reticulin, fibrin or thrombi, colloid droplets, hyalin of arteriosclerosis, hyalin deposits, basement membranes stain rose to purplish red.

USE CONTROL SLIDE-----ACID CLEAN GLASS!!!

SOLUTIONS:

NORMAL HYDROCHLORIC ACID

Hydrochloric acid, sp. gr. 1.19. 83.5 ml.

Distilled water. 916.5 ml.

0.5% PERIODIC ACID

Periodic acid. 0.5 g.

Distilled water. 100.0 ml.

COLEMAN'S FEULGEN SOLUTION

Basic fuchsin. 1.0 g.

Hot distilled water. 200.0 ml.

Potassium metabisulfite. 2.0 g.

Normal hydrochloric acid 10.0 ml.

Activated charcoal (Norit) 0.5 g.

Dissolve basic fuchsin in hot distilled water. Bring to boiling point. Cool and add potassium metabisulfite and normal hydrochloric acid. Let bleach 24 hours, then add 0.5 g. activated charcoal. Shake for 1 minute and filter through coarse filter paper. Repeat filtration until solution is colorless. STORE IN REFRIGERATOR.

See source for solution test.

SOURCE: AFIP 3rd edition, pp. 158-159 Modification from original

VAN GIESON'S METHOD FOR COLLAGEN FIBERS

CONTROL SLIDE

FIXATION: Any well fixed tissue

TECHNIQUE: Paraffin sections at 6 microns

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Weigert's hematoxylin for 10 minutes.
3. Wash in distilled water.
4. Van Gieson's solution for 1 to 3 minutes.
5. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene, two changes each.
6. Mount with Permount or Histo-clad.

RESULTS:

Collagen stains red.
Muscle and cornified epithelium stain yellow.
Nuclei stain blue to black.

SOURCE: AFIP 3rd Edition, pages 76-77.

SOLUTIONS:

WEIGERT'S IRON HEMATOXYLIN SOLUTION

SOLUTION A

Hematoxylin crystals.	1.0 g.
Alcohol, 95%.	100.0 ml.

SOLUTION B

Ferric chloride, 29% aqueous.	4.0 ml.
Disstilld water.	95.0 ml.
Hydrochloric acid, conc.	1.0 ml.

WORKING SOLUTION: EQUAL PARTS solutions A and B
LET SOLUTIONS A AND B SET FOR APPROXIMATELY TWO
WEEKS BEFORE USE.

VAN GIESON'S SOLUTION

Acid fuchsin, 1% aqueous	2.5 ml.
Picric acid, saturated aqueous	97.5 ml.

SPIDER LAMB SYNDROME
HEREDITARY CHONDRODYSPLASIA
A CONGENITAL SKELETAL DEFECT IN SHEEP

BY

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the
requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Pathology
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1989

Spider lamb, a lay term, identifies a pathologic syndrome in which congenital skeletal deformities are present. Initial findings were made in the late 1970's with the incidence increasing in the 1980's. It was a syndrome first reported in the veterinary literature in 1985. It was reported only in Suffolk and Suffolk cross lambs.

The present study was initiated to make a detailed evaluation of the spider lamb syndrome. Emphasis was placed on cataloging and categorizing specific defects present and separating the syndrome from other lamb deformities. Five main areas are affected in spider lambs:

- 1.) Thoracic vertebrae- kyphoscoliosis,
- 2.) Sternebrae- pectus excavatum,
- 3.) Front limbs- angular deformities,
- 4.) Rear limbs- angular deformities,
- 5.) Cranio-facial- Roman nose and septal deviation.

Two distinct entities evolved; 1.) lambs that were grossly abnormal at birth, and 2.) apparently normal lambs at birth which develop the spider conformation at 4-6 weeks of age.

Radiographicly both groups have similar chondrodysplastic lesions. The sternebrae, olecranons (especially the anconeal process) and thoracic vertebrae all have cartilage defects containing multiple irregular islands of ossification.

The following criteria was developed for diagnosing the chondrodysplasia/spider lamb syndrome:

- 1.) Conformation and abnormalities; narrow chest, shallow body, increased leg to body ration; vertebrae, sternebrae, and leg deformities.
- 2.) Radiographic evidence of abnormal bone growth in one or more areas of the skeleton.
- 3.) Absence of soft tissue lesions.
- 4.) The presence of abnormal articular cartilage in lambs more than three months of age.

Geneologic studies suggested a genetic basis for the syndrome. Available data suggests a simple autosomal recessive pattern.

As part of this study, a number of lambs with the arthrogryposis-hydranencephaly syndrome were submitted and these were also scientifically evaluated. They were really shown to be two distinct and separate syndromes.