Bovine Prenatal Development:

A Comparative Study of Ultrasonography and Radiography techniques

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ABSTRACT

This study was undertaken with the aim of assessing the use of high frequency transducer (7.5 MHz) and a quality ultrasound scanner in the study of in-uterine bovine fetal development i.e. bovine fetal age determination and the ultrasonographic anatomy of the gross tissues and organs of the developing bovine fetus, particularly the stomach compartments, between gestational age 45-90 days. Additionally, ultrasonography was compared to radiography and bone staining techniques, in the study of fetal osteogenesis.

The simple regression, polynomial and logarithmic mathematical models were used to examine and analyse in depth, the growth rate and patterns of crown rump length, biparietal diameter and trunk diameter. Each model was tested for fitness of the raw data, precision to predict age and ability to describe the various characteristics of the growth curve of fetal parameters.

On average, crown rump length had the fastest growth rate and the least variance, while, trunk diameter had the slowest growth rate and the highest variance of the three fetal parameters analysed. The best mathematical model, out of the three, for crown rump length, in terms of fitness of the raw data and precision to determine fetal age, was the

logarithmic model. The linear model was good at giving an overall impression of the average growth rate and steepness of the growth curve.

The model with the best fit of the raw data of biparietal diameter and the most precise in determining fetal age was the polynomial model.

No major difference in the growth rate and size was noted between the width and depth of the trunk diameter and between sizes of trunk diameter taken at the level of the umbilicus and the stomach

This study did not find any major difference of statistical significance between growth curves of embryo transfer fetuses and normal fetuses.

The linear model was the simplest of the three models assessed; deriving the age prediction equation and identifying the features of growth that the coefficients represented was easier than with the other two models. It could also be used to ascertain the average growth rate during a given period. However, it could only be used in linear growth curves, usually present in early gestation and not non-linear characteristics seen in a typical growth pattern of later pregnancy.

The polynomial model had three coefficients, and hence is more complex to interpret and derive age prediction equations, compared to the other two models. It is, therefore, not, in most cases, a good model to use for the purpose of estimating age. However, it was very good at defining and describing the extent and direction of both linear and curvature features of the growth curves. It did not, however, specify the points at which these curvings begin and end on the growth curve.

The two coefficients of logarithmic model are relatively easy to interpret and to derive age prediction equation. It was the most ideal for the purpose of estimating age using fetal dimensions which have typical curvilinear or sigmoid growth curves, because it was able to transform non-linear raw data into linear and has uniform variations and hence, the least coefficient of variation .

No difference, in precision, in determining age of in-uterine bovine fetus between transducers with higher frequency and (7.5 MHz) and those of lower frequencies was found (3.5 and 5.0 MHz.), based on early bovine fetal development.

The improvement of precision in fetal aging and a clearer understanding of growth patterns may depend, among other things, on the use of an appropriate age prediction model, at a given age and not so much on the use of transducers of higher frequencies.

Sonographic images of almost all the major fetal structures, like the brain, orbit, maxilla, mandible, heart, blood vessels, stomach, hind and forelimbs, were identified by Day 45 of gestation.

Sonographic images of the stomach could be identified by Day 39 of gestation, although it was not possible to recognise the differentiation of the stomach compartments until after Day 53 of gestation. The first compartment of the stomach identifiable sonographically was the reticulo-rumen. Differentiation of the rumen into its various sacs became apparent by Day 60 of gestation.

Differentiation of the omasum first appeared as a round hyperechogenic structure by Day 53 of pregnancy. The laminae omasi appeared one week later. Other structures of the omasum identified included the esophageal groove and omasi sulci by Day 70 of gestation.

The most difficult stomach compartment to identify was the abomasum, because of the close resemblance of its images and its proximity to the intestines.

The reticulum was imaged by Day 76 of pregnancy and structures scanned immediate to it were; the liver and diaphragm, cranially, the vestibule of the rumen, caudally, and the omasum on the right .

The sensitivity of ultrasonography and radiography techniques in detecting the earliest time and chronological order of appearance of the loci of ossification were found to be the same. Using ultrasonography the first signs of ossification of the scapula and os coxae appeared by the beginning of the seventh week while those of the humerus, femur, ulna/radius, tibia, metacarpus and metatarsus appeared between the begining and the middle of the seventh week of gestation, about ten (10) days earlier than radiography. The first phalanx to show signs of ossification was the distal phalanx of digits three (3) and four (4) at Day 60 of pregnancy, followed by the proximal phalanx about Day 69 of gestation and lastly, the middle phalanx about Day 74 of gestation. The diaphyseal locus of the transient element, the fibula, was observed, radiographically, by Day 64.5 of gestation while that of the metapodial 5 was identified in fetuses of Days 69.2 and 74 of gestation. No transient elements, seen in radiography, were observed in ultrasonographic images of the distal limbs.

X-ray images of fetuses treated with heavy metals, had better clarity and detail than those of ultrasonography, particularly in the early stages of development.

Food for thought

' O Lord, how manifold are thy works!
in wisdom hast thou made them all:
the earth is full of thy riches. '

Psalm 104 24

Dedicated

to my ,

wife, Ennie H. Mowa,

daughter, Nonde Mowa

sons, Shoma Mowa, Mowa Chishimba Mowa

and mum, Lydia Chishimba

In sweet memory

of my late father, Mr.Mowa S Chishimba.

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CHAPTERS

DECLARATION.

The work presented in this dissertation was carried out by the undersigned, personally.

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Chapter 1.

Introduction.

1.1. General introduction.

Diagnostic ultrasound has now been in use in veterinary reproduction for the past twenty eight (28) years, according to a bibliography by Lamb (Lamb,1988.). Since then, the application of this technique in reproduction and other areas within the veterinary profession has greatly diversified. Tremendous technological advances in the equipment have also been achieved over the years resulting in more detailed and better quality images than before.

In recent times, there has been a number of studies done in bovine theriogenology using scanners and transducers of various frequencies. However, there seem to have been few detailed studies carried out on bovine prenatal aging and development. Most of the studies done on these topics have used transducers of frequencies between 3.5 to 5.0 MHz (Kahn,1989,1990, White and others,1985, Hanzen and Delsaux,1987.). A limited number of studies using a transducer of 7.5 MHz frequency have been reported on the early embryological stages of bovine prenatal development (Omran,1989, de Moura,1993). No detailed work has been published to date, on bovine prenatal aging and general development in the fetal stage using a 7.5 MHz transducer.

The present study was undertaken with the aim of assessing the use of a 7.5 MHz scanner and transducer in studying the general development and aging of bovine conceptuses, in the fetal stage. Additionally, the performance of diagnostic ultrasonography in studying skeletal development of the developing fetus was compared to radiography and bone staining techniques.

1.1.2. Historical review.

The conventional methods of studying bovine prenatal development in the past involved postmortem or abattoir specimens or conceptuses recovered from uteri by induced abortion or at parturition. In the very early years the most common method of study was based on dissections and later, on histological examination of dead fetuses (Boyd, 1974.). The bone staining technique of Alizarin red S.was in use as early as the sixteenth century to study skeletal development. Two hundred (200) years later, in 1895, X-rays were discovered by Roentgen, culminating in the development of radiology. The above techniques have greatly been used to date, to increase our knowledge of prenatal development in many species. However, they can not be used to study the dynamic intrauterine development and other characteristics of the developing fetus, a knowledge which may help the practitioner make appropriate decisions when faced with gynaecological problems. A detailed and complete knowledge of prenatal life establishes a working platform for the study of various factors causing significant deviations from normal development (Winter and others, 1942.). This knowledge in addition, enables one to distinguish normal from abnormal variations. Recently, intrarectal B-mode two dimensional ultrasonography has been demonstrated to be a reliable technique to detect, assess and monitor intrauterine development of the viable embryo. (Curran and others,1986 and Omran,1989.)

1.2.1. Bone staining

Alizarin red stain, one of the earliest type of bone staining technique used to study the skeletal system in the past, was originally devised from experiments using the roots of a herbaceous climbing plant (with yellowish flowers) called madder. The roots of this plant have a strong red dye. Its major ingredient, alizarin red, has a high affinity for calcium ions. In the earlier experiments, the plant was fed to animals as a vegetable root (*Rubia tinctorum .*). In later experiments, the dye was extracted from the roots and combined with glucose as Ruberythric acid (Boyd,1974).

Over the years, the early procedures of alizarin red S.have been modified and a number of new procedures have been developed. Additionally, a small number of new bone staining techniques have been developed (Lundvall,1905, Burdi, 1965, Dingerkus and Uhler, 1977 and Williamson, 1941). Only a selected number of these procedures have been briefly mentioned here.

Most current procedures of alizarin red S.have basically four (4) major steps namely fixation, clearing, staining and preservation (the order and time taken for each step varies.).

1.2.2. Radiography.

The veterinary profession received the first official notice about the discovery of X-rays a year after it was discovered by Professor Roentgen of Wurzburg in 1895, according to a review by Williamson (1978). Within the same year of the announcement, Professors Patton and Duncan published the first veterinary radiograph of an equine foot. As early as the 1920s major technological advances to the equipment were beginning to be seen (Williamson,1978). However, despite this, it was not until the 1950s that veterinary radiology began to play a regular and significant role in veterinary practice and research (Kealy,1992).

Dollar in 1896, wrote the first most practical and scientific article in both medical and veterinary radiology, giving full details of its applications. (Williamson,1978)

Among the early radiographic studies of fetal development are those of Bade at the turn of this century. Later, Kupfer and Schinz in 1923, published the first radiographic study of the bovine appendicular skeleton. In the years after Kupfer and Schinz's publications, the number of radiographic studies on bovine fetal skeleton steadily increased. Among the key ones are those of Fedrigo (1957), Winters and others (1942), and more recently Lindsay (1969) and Gjesdal (1969). One of the most detailed study of appendicular osteogenesis in the bovine fetus is that by Lindsay (1969).

When radiographs of early fetuses are taken, there is a poor contrast between the surrounding soft tissues and the skeletal elements (O'Rahilly and Meyer,1956). This problem of poor radiopacity can be resolved by impregnating the skeletal element with heavy metal salts like silver nitrate, as described by Hodges (1953) and Boyd (1974). The metal salts partially replace the calcium within the skeletal tissues (O'Rahilly and Meyer, 1956).

Among the three conventional methods of detecting osseous tissues, the histological method is the most critical one. No difference was observed in sensitivity of detecting onset of ossification, between alizarin red S.and radiography (when young specimens are impregnated with heavy metal.) (O'Rahilly and Meyer,1956).

1.2.3. Ultrasonography.

The principles of ultrasound were first developed and applied during World War 2 to detect submarines (Omran,1989). Two years later, after the end of World War 2, it was first used in the medical field as a diagnostic aid in human obstetrics (King, 1973). In animals it was first used to evaluate fat and lean proportions of meat in the United States of America (Temple and others, 1956). The first published work on the application of ultrasound as a diagnostic aid in Veterinary Medicine was in 1966 by Lindahl. This pioneer work in reproduction was first performed on sheep. Following Lindahl's publication in 1966, other workers, later extended the technique to other species: cattle (Fraser and others,1971),horses (Fraser and others,1971), dog (Helper,1970) sheep (Keane, 1969) and primates (O'Grady and others,1978). These early workers used either A-mode ultrasound (Lindahl,1966, Koch and Rubin,1969) or Doppler ultrasound (Fraser and others,1967,1971,1973, Lindahl,1969, and Helper,1970). Major technological advances in ultrasound equipment have been achieved from the time it was first devised as a diagnostic aid. As a result of these advancements, better and clearer ultrasound images can now be realised. In the past 14 years there has been a steady increase in the use of diagnostic ultrasonography in the veterinary field, particularly in the area of reproduction.

Fraser in 1971, was one of the first workers to report on the use of diagnostic ultrasound in bovine reproduction. He used a Doppler ultrasound unit to diagnose pregnancy, and also to estimate the age of the developing fetus by noting the variations of the fetal pulse rate with advancing fetal age. He noted a negative correlation between these two. From these observations he established a relationship between fetal pulse rate and fetal age. With the development of B-mode two dimensional diagnostic ultrasonography in recent years, the application of diagnostic ultrasound in bovine reproduction has greatly been broadened. Some of the specific uses of this technique in bovine theriogenolgy include:

a). Detection of pregnancy. (Badtram and others,1991, Boyd and others,1990, Chaffaux and others,1986, Hanzen and Delsaux,1987, Kastelic and others, 1989, Pierson and Ginther,1984, Reeves and others,1984, Totey and others,1991, Yamaga and Too,1984).

b). Study of prenatal development (Curran and others,1986 a, b, Kahn,1989, Kastelic and other,1991).

c). Assessment of fetal number (Davies and Haibel,1993, Dobson and others,1993.)

d). Determination of fetal gender (Curran,1992, Curran and Ginther,1989, 1991, Curran and others, 1989, de Moura,1993, Muller and Wittkowski,1986).

e). Study of normal appearance of reproductive tract and ovaries (Boyd and Omran,1991, Fissore and others,1986, Omran,1989, Pieterse and others,1990, Pierson and Ginther,1987, 1988, Quirk and others,1986).

f). Diagnosis of certain pathological conditions such as luteal and follicular ovarian cysts (Edmondson and others,1986, Farin and others,1990, 1992, Fissore and others,1986, Sawamukai and others,1988), endometritis, pyometra, macerated and mummified fetus, mucometra and hydrometra (Fissore and others, 1986.).

g). Monitoring embryonic mortality (Chaffaux and others, 1986, Semambo and others,1992.)

h). Prediction of calving date (Wright and others,1988.)

i). Estimation of gestational / fetal age (Curran and others, 1986, Kahn,1989,1990, White and others,1985).

1.3. Scientific review.

1.3.1. Radiography and Bone staining: fetal osteogenesis.

Both limbs of the body (fore and hindlimbs.) embryologically originate from the lateral plate somatic mesoderm with the exception of the voluntary muscles. The limb buds are formed by the aggregation of the lateral plate somatic mesoderm under the surface of the ectoderm on four sites. By day 24 of gestation the forelimb buds can be seen while those of the hindlimb forms 2 days later. At the distal end of the limb buds an apical ectodermal ridge forms and persist until the digital mesenchyme is formed (Noden and De Lahunta,1985). The mesenchyme of the limb buds later subdivide into two morphologically distinct populations namely;

i) Proximal population : which later develops into the proximal skeleton of the limbs and,

ii) Distal population : which develops into the distal skeleton of the limbs.

Ossification activity in the appendicular skeleton of bovine prenatal development during the first six weeks is normally low. Between seven (7) and eleven (11) weeks activity was seen to increase markedly in the diaphyses of the proximal parts of both limbs. From eleven (11) to twenty three (23) weeks of gestation the level of ossification dropped substantially. The intensity of ossification increased again between twenty four (24) and thirty four weeks (34) (Lindsay,1969).

The first sign of ossification in the fore limb, according to Lindsay (1969) , was observed between forty (40) and forty two (42) days after gestation in the clavicle, followed by the diaphysis of the humerus at fifty (50) days. The diaphysis of the scapula, radius and ulna ossified two days later at fifty two (52) days. The third major group in the forelimb to ossify was the metacarpals 2,3,4,5 and the distal phalanges at fifty eight (58) days. The last parts of the fore limb to ossify before 90 days of gestation were the proximal and middle phalanges. Gjesdal's observations (1969) of the onset of ossification in the appendicular skeleton, published in the same year as that of Lindsay were generally ten (10) days later than those of Lindsay. This difference in observations between the two workers was probably due to the different techniques used. Lindsay used histology, alizarin and radiography (with silver nitrate impregnation on very young fetuses.) while Gjesdal only used radiography.The radiographic study of Winters and others (1942) reported seeing the onset of ossification of humerus, ulna and radius at fifty nine (59) days after gestation and the phalanges at ninety (90). The first signs of ossification in the appendicular skeleton were observed at eighty (80) days after gestation according to Richardson and others (1990).

Three main periods of ossification before 90 days were observed in the hind limbs as described by Lindsay (1969) . These were :

a) 50 days : diaphysis of femur.

b) 52 days : diaphyses of tibia and fibula

c) 58 days : os coxae, metatarsal 2, 3, 4, 5 and distal phalanx.

d) 68, 75, and 88 days : proximal phalanx, middle phalanx and fibular tarsal bone (calcaneus), respectively.

Gjesdal 's observations of ossification in the hindlimb, as in the forelimb, were again, generally 10 days later than those of Lindsay. The femur and tibia were seen to ossify at 59 days, while the phalanx and fibular (calcaneus) tarsal bones were seen to have ossified at 90 and 100 days of gestation by Winters and others (1942). Fedrigo's (1957) first identification of any locus of ossification was at 90 days or three months while Kupfer and Schinzs' (1923) observations of onset of ossifications were generally, approximately, closer to those of Lindsay (1969).

1.3.2 Ultrasonography: bovine prenatal development and aging.

Recent studies have revealed the complex pattern of normal bovine prenatal growth and how different intrinsic and extrinsic factors of the fetus, dam, and the environment affect and modify the growth process (Richardson.and others, 1990, Holland,1992.). Different fetal organs and tissues grow at different rates and different times during prenatal life (Richardson, and others, 1991). Richardson divides organ and tissue development into early, intermediate and late maturing groups based on the rate and time at which they develop, while, Winters and others (1942), divide the prenatal period into three major developmental periods, namely : ovum, embryonic and fetal, based upon the amount of development and some of the more critical moments in the individual's life.

The period from conception or fertilisation until implantation is known as the period of the ovum. The average duration of the ovum period is twelve (12) days. The conceptus retains basically, a spherical shape or form during this period of development.

The second period, the embryonic period, begins after the attachment of the conceptus to the uterus, at about twelve (12) days of gestation, until about Day 45 of gestation. The embryonic period is the time when the major tissues and organs, and their systems are formed. It is also a time when the body shape of the individual undergoes a series of successive changes, like the C-shape by 26 days of age and the more definite points of flexure at Day 30 of gestation.

The third and final stage of prenatal development, the fetal period, is mainly a period of growth and minor details of differentiation. Dennis (1969) further subdivides the fetal period into three stages, namely: early (45-120 days of gestation.), middle (120-180 days of gestation.) and late fetal stages (180-260 days of gestation.). The most rapid growth in size occurs during the late fetal period. The present study is based on the early fetal period (45-90 days of gestation).

There is a considerable amount of literature on the development of different tissues and organs of the bovine conceptus. Winters and others (1942) give a brief, but valuable, account of the early development of some tissues and organs, like the stomach, head, heart, brain, eyes, limbs e.t.c. Lindsay (1969), Lindsay and others, (1973), Gjesdal (1969), Kupfer and Schinz (1923) and Richardson and others (1990) provide data on the development of bovine fetal skeletal system. Turner (1952) studies the growth of the mammary glands. Winqvist (1954) gives an account of the morphology of the blood and the hemopoetic organs .Lyne and Heldeman (1959) provide a definite developmental pattern of the skin and hair. Guerra-

Pereira (1977) gives a detailed account of cerebellar growth and development. Warner (1958), Lambert (1948), Becker and others (1950, 1963) and Kano and others (1980) have all provided valuable data on fetal stomach development and differentiation. Inomata and others (1981) studied the development of the external genitalia.

By Day 22 of gestation, according to Winters and others (1942), the development of the midgut in bovine fetuses, is underway while the primordia of bovine stomach appears as a spindle-shaped structure by Day 28 of gestation. [Warner (1958), Zimmer (1900), Lambert (1948), Committee on Bovine Reproductive Nomenclature (1972)] The four compartments of the adult ruminant stomach, rumen, reticulum, omasum and abomasum, develop from the spindle-shaped structure (Lambert, 1948). Sonographic images of the digestive tube appear as non-echogenic spaces surrounded by echogenic looplike structures by Day 33 of gestation, according to a recent study (Omran,1989). In another similar study, using a transducer of a lower frequency (5.0 MHz), the images of the stomach were first observed at Day 40 of gestation.

Warner (1958) gave one of the most detailed accounts of the gross development and differentiation of the bovine stomach. According to this report, differences between the bovine stomach and that of other mammals begin to appear by Day 35 of gestation and it resembles an adult organ by Day 64 of gestation.

The fundus, a primordia of the rumen and the reticulum, appears by Day 36 of pregnancy, and three days later, it subdivides into the rumen and the reticulum (Warner, 1958).

The rumen differentiates into the cranial and caudal sac at Day 43 and by Day 58 of pregnancy it further differentiates into the dorsal and ventral blind sacs (Warner, 1958). The rumen is the most predominant structure of the fetal stomach during the first four (4) months of development, when its net weight is about three (3) times the weight of the abomasum. The rumen is outgrown by the abomasum after six (6) months of development. [(Becker (1950, 1963), Kano (1981) and Lambert (1948)] A recent study provides a brief description of the ultrasonographic appearance of a differentiated rumen in the late fetal stage (Kahn, 1989). No detailed account to date, however, has been published on the ultrasonographic appearance of a differentiated rumen.

The first signs of differentiation of the reticulum are observed as a swelling on the great curvature, caudal ventral to the rumen by Day 39 of pregnancy and four (4) days later, it is seen to have shifted to the left and cranial part of the rumen (Warner, 1958). The appearance of the reticulum has not been yet recorded using ultrasonography.

The omasum appears as a bulb-like swelling of the lesser curvature, ventral to the reticulum by Day 36 of gestation, while, a week later, the first laminae omasi are observed The omasal sulci appears by Day 64 of pregnancy (Warner, 1958). A recent study gives a brief account of the ultrasonic appearance of the laminae omasi in the late fetal stage (Kahn,1989.). However, no study has a description of the ultrasonic appearance of the laminae omasi in the early and middle period of the fetal stage.

Signs of abomasal differentiation are first observed by Day 39 of gestation and about a week later, the primary longitudinal folds appear (Warner, 1958). The ultrasonic appearance of the abomasum has not been mentioned in the veterinary literature, todate.

It is clear from the review of previous studies, that there is limited data on the ultrasonic anatomy of the stomach and thus, there is need to study the ultrasonic appearance of the differentiated bovine fetal stomach in greater detail.

Since the work of Gurltz (1860), the measurements of body length have been used to estimate age. Other criteria which have been used to determine age include body weight and time-sequence of hair development, pigmentation, ossification and tooth formation (Committee on bovine reproduction nomenclature, 1972, Gjesdal, 1969).

A simple, accurate and cost-effective assessment of the pregnant dam, fetal development and age determination will help improve and maintain high levels of reproductive efficiency in the cattle industry. A number of different techniques have been in use for some time to detect and or monitor pregnancy, for example, rectal palpation, blood progesterone estimates, pregnancy specific protein determination, radiography and many more. The common practical limitation, however, has been in the failure of these techniques to accurately determine fetal age with unknown mating date .

In the past, before the advent of diagnostic ultrasonography, age determination of a developing bovine fetus, of a pregnancy with unknown mating date, could in reality only be achieved, by rectal palpation. Pregnancy in heifers, can be detected after 36 to 42 days after mating and in cows after 42 to 49 days (Roberts, 1971). The other conventional method for determining fetal age before rectal palpation was developed, could only be performed on abbattior or aborted fetuses by measuring lengths and diameters of various body parts and organs of the dead fetuses. The most common parameter which was used to estimate the age of the fetus was crown rump length ie the linear or curved distance between the crown of the head or the occiput of the head and the caudal edge of the perineum or rump of the fetus. The conventional method was reasonably accurate in estimating the age of fetuses. Its major limitation, however, was (and still is) that it could not be performed without endangering the life of the dam or sacrificing the life of the developing conceptus. Age determination using rectal palpation by an experienced person can only be achieved, with reasonable accuracy, after the gestational periods given above i.e. 36 to 42 days in heifers and 42 to 49 days in cows after mating. In the earlier years, the range of figures or variation of age determination was so wide that the data had little value (Maneely,1952). Later studies lacked a standard measuring technique, until Nichols (1944) demonstrated the use of certain measuring devices on embryos greater than 100 mm in length. As more data on the development of the bovine fetus emerged, the accuracy of estimating age progressively improved. Gjesdal (1969), using time sequence of tooth formation to estimate age at 96 days, was able to reduce the error to 4 days. Variables like breed, nutritional level and number of previous pregnancies of the cow, as well as sex and the number of fetuses are thought to affect the growth rate (Committee on bovine reproduction nomenclature,1972).

All the studies described so far, were based on data collected from dead fetuses. Recently, the relationship between intrauterine fetal measurements of a live fetus and gestational age, using ultrasonography, have been established (White and others, 1985). This technique has greatly improved the precision of estimating age more than any other conventional method, based on body measurements.

With the development of diagnostic ultrasonography, it is now possible to estimate, with high accuracy, the age of the developing fetus as early as 20 days, within a much shorter period and in a non-invasive way. This was not possible with the other earlier methods (White and others, 1985).

Diagnostic ultrasonography has proved to be a useful tool in estimating fetal ages not only in bovine but in a number of different species including the human e.g. goats (Haibel, 1988), canine (England and others, 1990). The parameters suitable for fetal estimation vary in different species and in some cases, at different developmental stages.

White and others (1985) using a real-time ultrasonic scanner equipped with a 3.5 MHz rectal transducer established for the first time in the bovine species the relationship between fetal age and size by measuring fetal lengths and diameters of various fetal body parts and organs . Crown rump length was found to provide the most precise estimate, while uterine diameter was the least effective. Head length and diameters of trunk, head and nose were found to be intermediate in their precision.

Kahn (1989) using a scanner with both sector and linear array transducers of 3.5 to 5.0 MHz frequencies, investigated the suitability of twenty five different fetal parameters for estimating fetal age in the bovine. The parameters which were found to have the highest correlation were crown-rump length and lengths / diameters of femur, metacarpal, tibia, scapula, ischium, eye, brain case, trunk and umbilical cord. Crown-rump length was found to be accessible or within the range of the scanning field in the first three months. The growth rate was seen to increase from 1.4 mm per day in the first two months to 2.2 mm per day by the end of the second month of gestation. During the third month it increased at the rate of 2.5 to 3.0 mm / day reaching a linear length of 12 cm at the end of the third month. Crown-rump length was found to have the least variation and the highest correlation. The hind and fore limbs were found to have the same growth rate and measurements. At day 90 of gestational age their lengths were between 12-16 mm while, at 180 days of gestation they increased to between 55-65 mm. The metacarpal was found to be the most accessible part of the forelimb. The rest of the limb parts in both limbs were only accessible up to seven months of gestational age. The growth rate of the trunk was found to be between 0.9 mm per day, after 70 days of gestation. The largest diameter between 60 and 70 days of gestation was 20 to 30 mm. The umbilical cord was within easy access in the first seven (7) months of gestation with diameters ranging between 5-10 mm in the third month and increasing to 50 mm in the seventh month of gestation.

This study will assess the use of a 7.5 MHz transducer which is a higher frequency than used in previous studies on age determination .

1.4. Objective.

One of the main advantages of a 7.5 MHz transducer used with a good quality scanner over other transducers with lower frequencies is that it provides better definition. The high level of axial resolution (0.5 x 1 mm) makes it possible to determine the presence of small structures, observe the presence of fluid and differentiate between tissue textures of various structures (Boyd and others,1991). It is anticipated that because of the increased resolution of the 7.5 MHz transducer, details of fetal structures and organs would be much clearer than would be possible with the transducers of lower frequencies. Thus a 7.5 MHz transducer was used in this study to compare and contrast the results produced in the past trials with transducers of frequencies less than 7.5 MHz.

The objective of the present work was to assess the use of a good quality scanner with a 7.5 MHz linear transducer in studying:

a) correlations between bovine fetal measurements and age,

b) sonographic anatomy of bovine fetal stomach compartment and other structures and organs of the body, and their chronological sequence of appearance between gestational ages of 40 to 90 days.

c) the performance of diagnostic ultrasonography in studying the number, times of appearance, and chronological order of appearance of the centres of ossification of the prenatal appendicular skeleton, compared to radiography and bone staining.

1.5. Interpretation and artifacts of ultrasound.

The principles of diagnostic ultrasonography are based on the ability of the sound beam to pass through different body tissues, of varying densities, at differing speeds, and the ability of certain tissues to reflect back some of the rays of the sound beam. Some of the reflected rays of the beam (acoustic echoes.) return to the transducer and are processed and converted into electronic signals thus allowing the source of echoes to be displayed as images on the monitor/ television screen as images. There are now a substantial number of reviews dealing with the principles and physics of ultrasound (Ginther,1986, Omran,1989, Pierson and others, 1988, Rantanen and Ewing,1981).

A thorough knowledge of the technology and the skill to interpret ultrasound images correctly are of utmost importance in diagnostic ultrasonography. The sound beam emitted from the transducer into the body, interacts with various body tissues in different ways depending on their densities, shape, contents and the nature of their surfaces. As a general statement, the sound beam undergoes continuous modification as it passes through soft tissues of different acoustic impedance. This is called 'attenuation' which is defined, in simple terms, as a gradual weakening of the sound beam as it journeys through the tissues. Some of the images which appear on the screen do not correspond with the tissues being scanned. Such images are called artifacts.

There are two (2) main types of artifacts based on causation.These are:

(i). Engineering artifacts or electronic noise (Ginther,1986) and
(ii). 'Biological' artifacts caused by sound beam-tissue interaction .

(i). Engineering artifacts. These are mainly caused by engineering flaws and/or electronic noise. They are rarely encountered if the scanner has been adjusted and tuned properly. They are, therefore, of little significance.

ii). **<u>'Biological' artifacts.</u>** This group comprises the most frequently encountered artifacts and therefore constitutes the most important of the two main groups .There are basically five (5) major types of artifacts under this group:

a). Enhancement or through-transmission artifacts are artifacts which appear as columns of comparatively brighter echoes located immediately distal to fluid-filled structures. (Figure 1.1.). They are caused by sound beams passing through a media with minimal attenuation. As a result such sound beams have higher amplitudes compared to other beams of the same depth on each side.

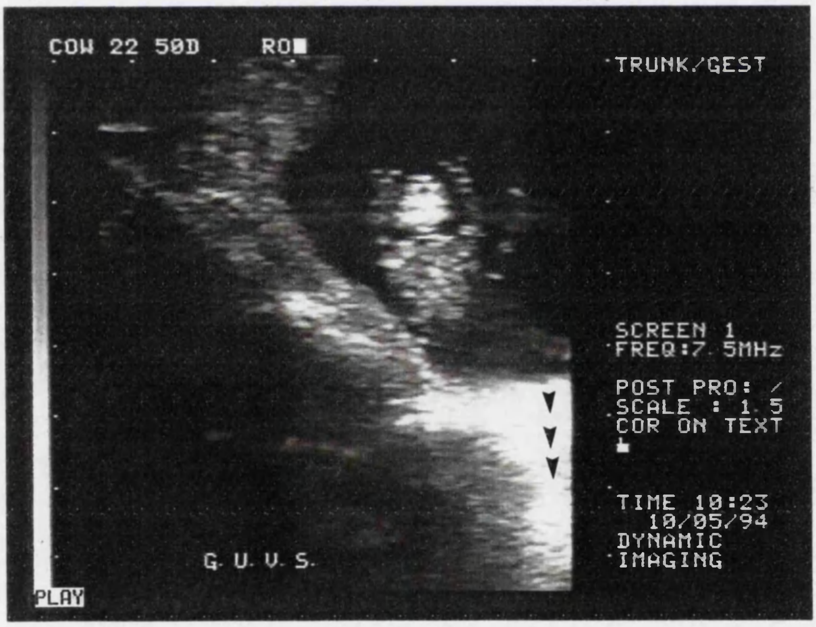


Figure 1.1. Example of enhancement or through-transmission artifacts from a bovine fetus. (arrows)

The enhancement artifact is used in diagnostic ultrasonography to diagnose fluid-filled structures which lie proximal to it. (Figure 1.1.). However, it may be confused with normal body structures.

b). Reverberation artifacts are produced between two smooth surfaced interfaces of markedly different acoustic impedance. The sound echoes oscillate between the two interfaces until they get depleted by attenuation. The artifacts are readily identified. The images created are equidistant from each other, gradually diminish in intensity and are oriented parallel to the reflective interface. (Ginther, 1986)

c). Shadow artifacts are caused by total deviation or blockage of the sound beam resulting in little or no further propagation of the beam in the body tissues (Figure 1.2.). The tissue distal to the acoustic barrier appears dark. Shadowing can be caused by dense tissues or objects, reflection and refraction. Dense objects cause shadowing by largely reflecting most of the beams back to the transducers. A few beams are absorbed by the reflecting structures. Reflection causes shadowing when a sound beam strikes on the side of a smooth curved structure while shadowing in refraction results when the speed of the beam is different on two sides of the smooth surface.

d). Specular reflection artifacts are formed when the sound beams are reflected on interfaces whose surfaces are smooth, wider than the beam and parallel to the transducer (Figure 1.3.). The beam's angle of incidence is equal to the angle of reflection. The major portion of the sound beam is however, propagated past the interface.

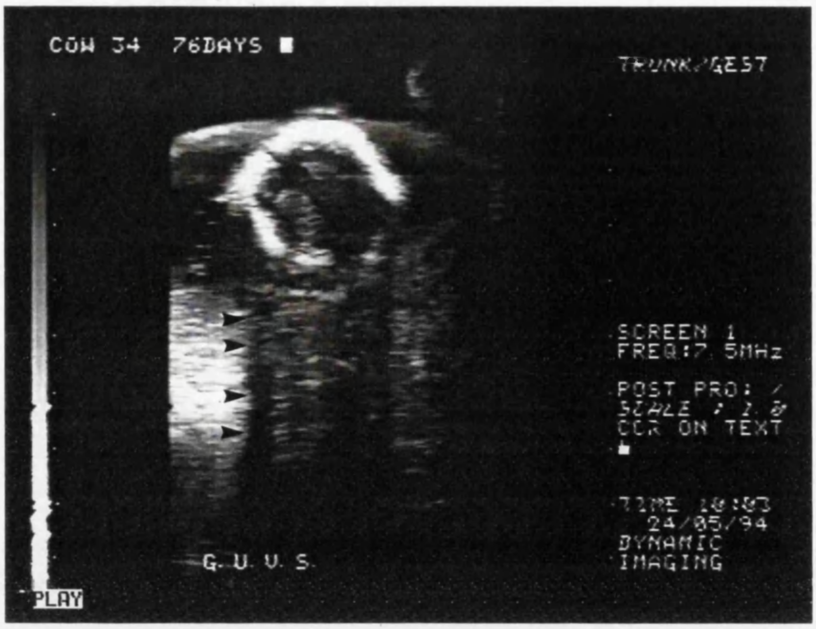


Figure 1.2. Example of shadow artifact from a bovine fetus (arrows).

Figure 1.3. Example of specular reflection artifacts from a bovine fetus (arrows).

e). Non specular or diffuse reflection artifacts are generated when the sound beam strikes on interfaces whose surfaces are rough or irregular and narrower than the sound beams. The angle of incidence in this case, unlike, specular reflections, does not affect the intensity of the artifact (Figure 1.4). The reflected rays are redirected in many different directions. This phenomenon is called scatter. The rays which are redirected back to the sound beam source or transducer are called backscatter. They form a very small percentage of the total redirected rays and are of about 1% amplitude of the specular echo. Gray-scale imaging fully makes use of the scatter phenomenon of non-specular reflections. (Ginther, 1986).

Figure 1.4. Example of non-specular or diffuse reflection artifacts from a bovine fetus (circle).

Chapter 2.

Ultrasonography:

Fetal development and aging.

2.1. Materials.

2.1.1. Animals.

Eighty one (81) clinically normal healthy Friesian diary cows and heifers with body weights ranging from 450 to 600 kgs and ages between 2 and 5 years, respectively, were used for scanning between 40-90 days after insemination. Twenty five (25) of the animals, belonged to the University of Glasgow Veterinary School's home farm. The rest, 56, were embryo transfer fetuses and belonged to Genus Breeding Research Station. The embryos were tranfered to the recepient cows at Day 7 after insemination. All the animals were housed during the test period in covered courts with free access to open yards

2.1.2. Equipment .

a). Monitor/Scanner. A portable B-mode (B for brightness.) real-time two dimensional high quality scanner was used (Figure 2.1.1). (Concept LC , Dynamic Imaging, Livingstone, Scotland).

The scanner had the following in-built facilities with a key board;

(1). Freeze frame mechanism,

(11). Magnification and zoom mode,

(111). Image storage memory with recall and,

(1V). an internal electronic calliper for measuring parameters.

b).Transducers. Linear rectal transducers of frequencies 5.0 and 7.5 MHz were used in this study. The lateral and axial resolutions of the 7.5 MHz transducer were 1.0 mm and 0.5 mm, respectively. This transducer was used as long as the fetus remained within range for fetal measurements and observations so that as much detail of the developing fetus could be seen. The 5.0MHz transducer was only used in individual cases where the fetus could not be reached adequately with the 7.5 MHz transducer.

Table 2.1: Total number of measurements per fetal parameter. (no.of cows 46.)

Fetal parameter	Total number of measurements. (edited)
1. Crown rump length.	41
2. Biparietal diameter.	66
3. Transabdominal :	
. At level of umbilicus	30
. At level of stomach	134
4. Transthoracic.	12
5. Umbilical diameter.	27

c). Recording devices. All scans at the farm were recorded on a video cassette recorder using VHS video tapes. The tapes were replayed later in the laboratory on a Panasonic monitor/player (Model number A.G-500-B , Matsushita.). The images on the video tapes were selected and printed on a thermal printer (Sony U P 811) (Figure 2.1.1) for further examination. Final prints for publication were selected and printed using a Sony colour printer.

<u>Figure 2.1.1</u> : The ultrasound scanner (concept L.C., Dynamic imaging, Livingstone, Scotland.) with the 7.5 MHz transducer. (Top picture) and the video player (left) and thermal printer (Sony UP 811) (right). (Bottom picture).


DYNAMIC
IMAGING
CONCEPT LC


Panasonic
VHS
SONY

2.2. Method .

2.2.1. Training.

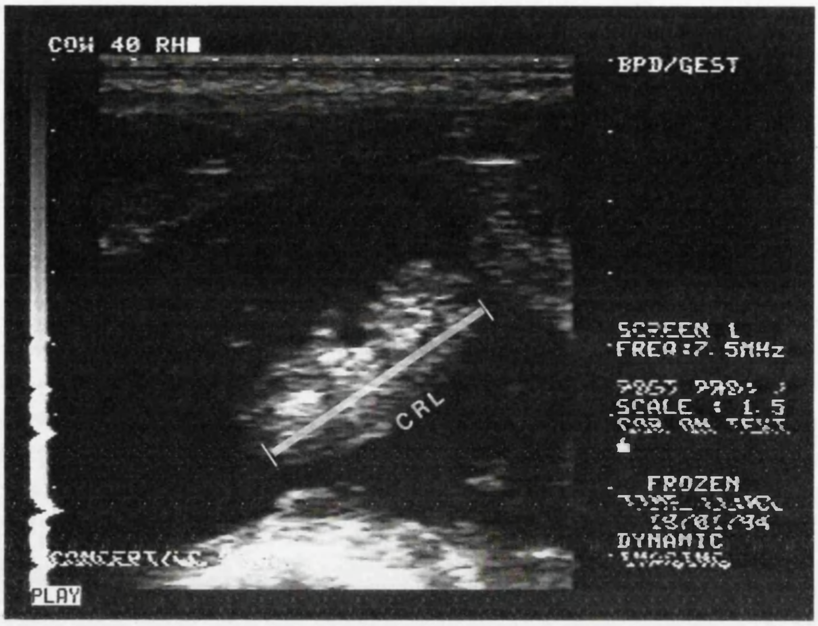
Initially, about five (5) bovine pregnant uteri in the range of 40-90 days of gestational age were scanned in a water bath, in order to learn the technique of ultrasonography. These uteri were obtained from the abattoir. Later, after gaining some experience in water bath scanning, a number of live pregnant cows were scanned in order to perfect the skill. Having gained some experience the study was commenced.

2.2.2. Clinical application

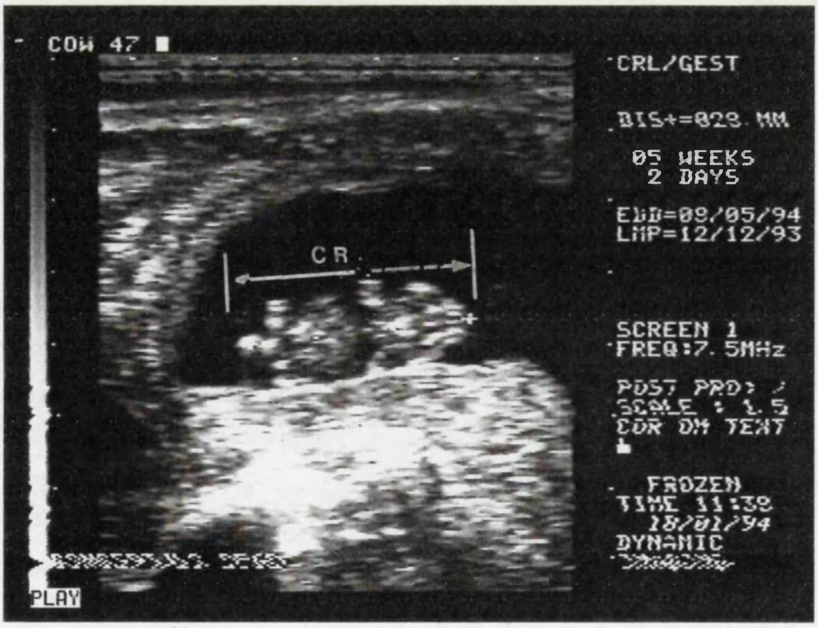
Cows of the Freisian breed, from the Veterinary School's home farm were divided in three (3) groups. The first group had four (4) cows and the second and third group had six (6) and ten (10) cows, respectively. Each group was scanned once a week for seven (7) consecutive weeks beginning with the first group. The rest of the animals (from Genus farm), which were predominantly Friesian, were scanned in larger groups of various gestational ages, once a month for three (3) consecutive months.

The cows were securely restrained in a chute and then individually examined while standing in a crush with the scanner placed on the same side as the examiner's free hand i.e. in this case on the left side of the examiner. The rectalling hand was first lubricated before emptying the rectum in order to facilitate easy penetration through the anal sphincter and prevent injury to the delicate mucous membrane of the cow's rectum. In order to cut down on time spent looking for the structures, the exact orientation and position of the reproductive tract was initially identified by

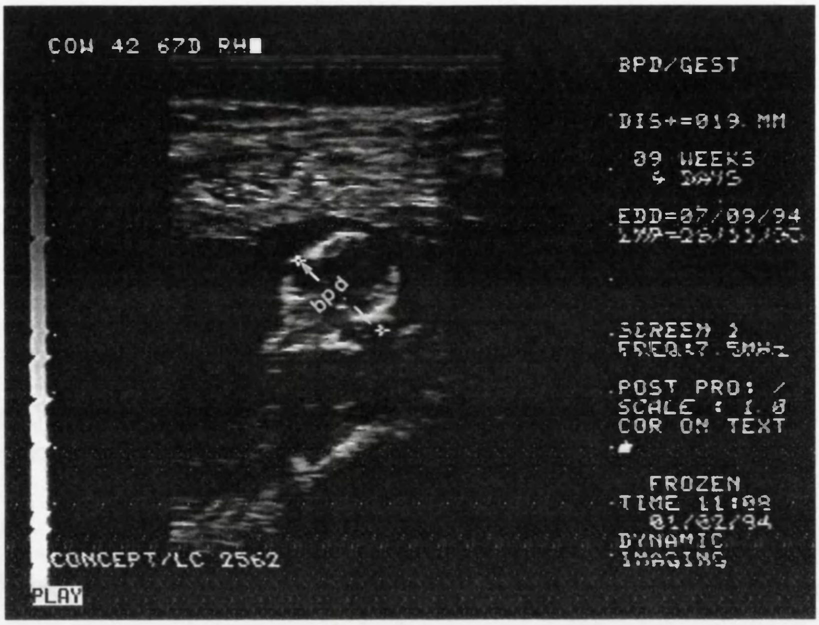
<u>Figure 2.1.2</u>. Sonographic images of the fetus showing the different fetal parameters and their respective planes.



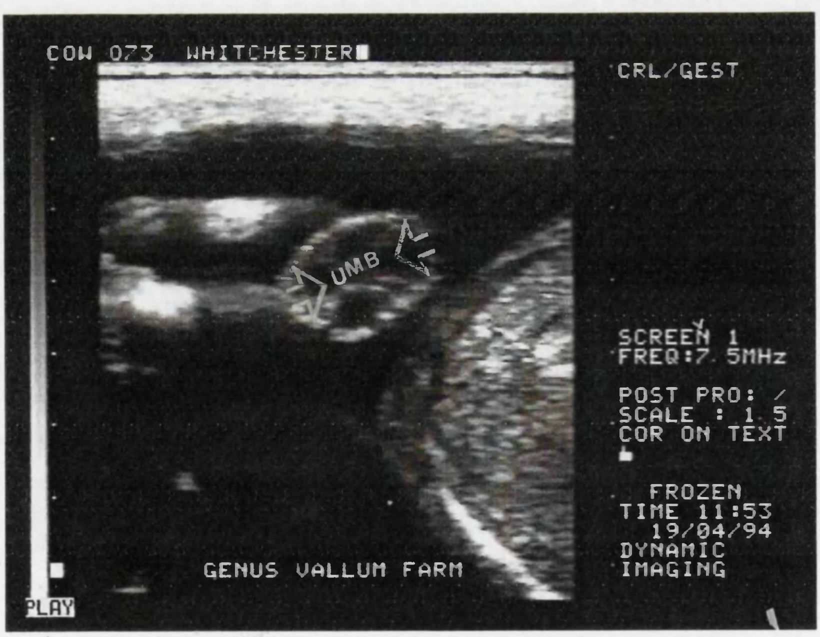
(a) Crown rump length of a 49 days old fetus taken in a longitudinal plane.



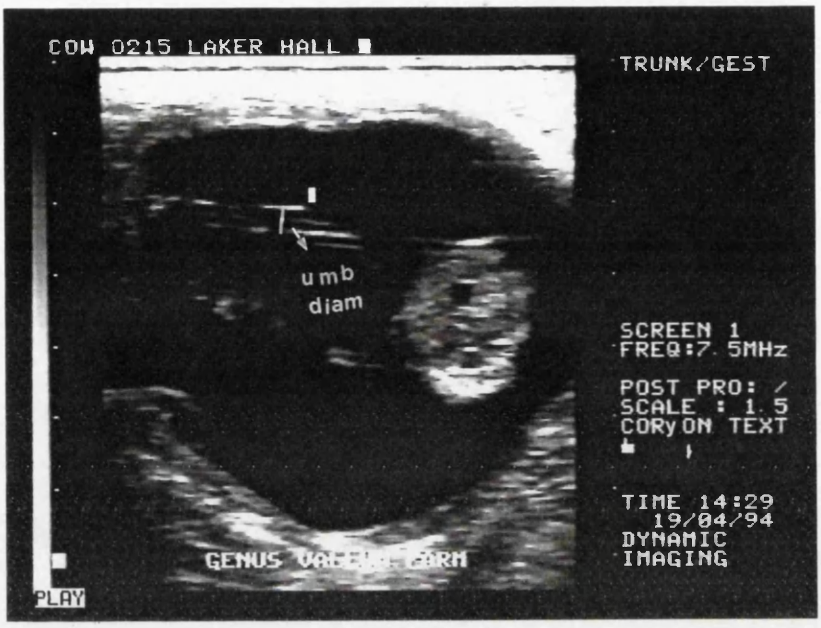
(b) Crown rump length of a 47 days old fetus taken in a dorsal plane.



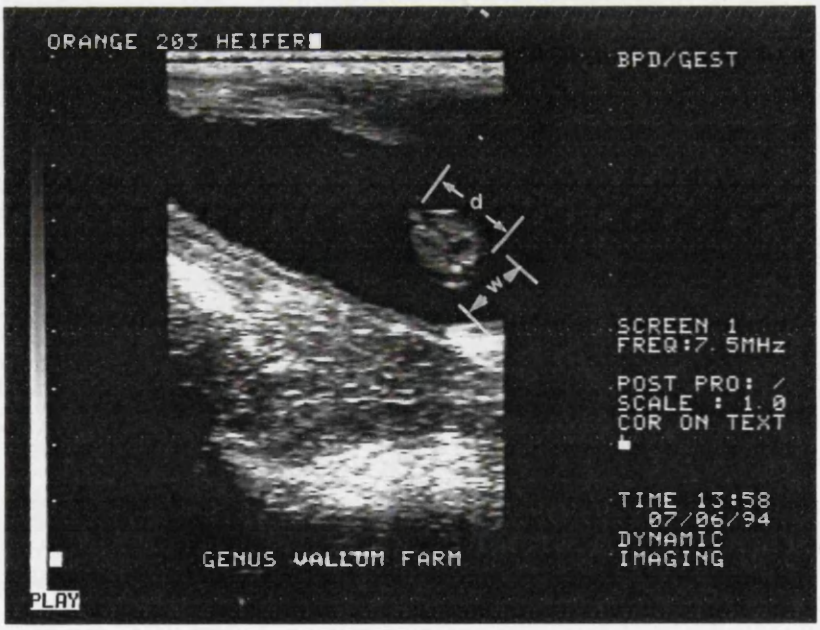
(c) Biparietal diameter of a 67 days old fetus taken in a transversal plane.



(d) A cross section image of the umbilicus of a fetus 110 days of age.

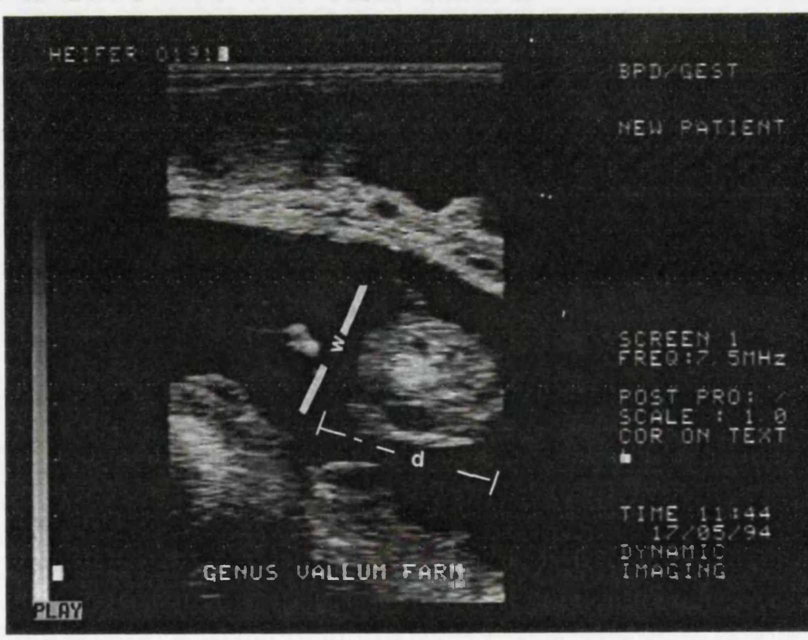


(e) A longitudinal image of the umbilicus of a fetus 62 days after insemination.

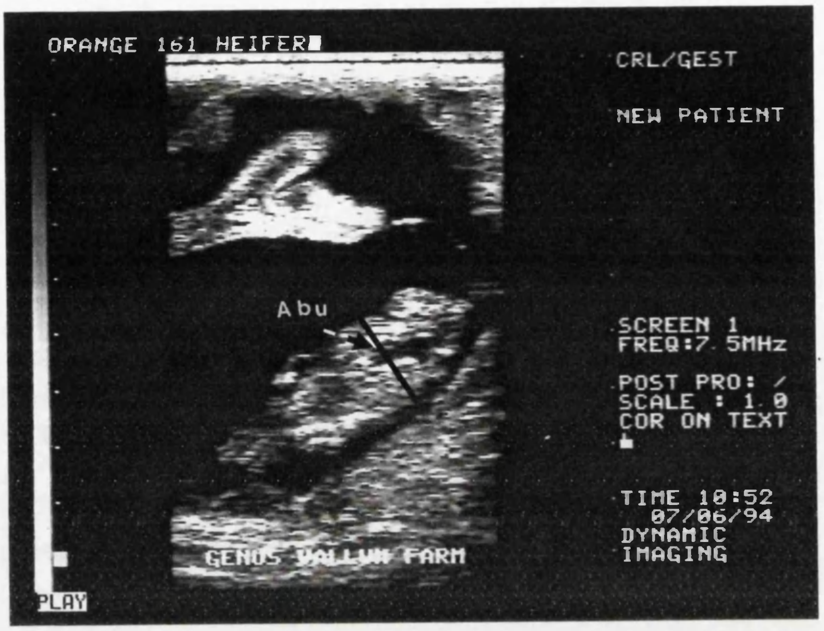


(f) Cross section images of a transthoracic width and depth of a 48 days old fetus.

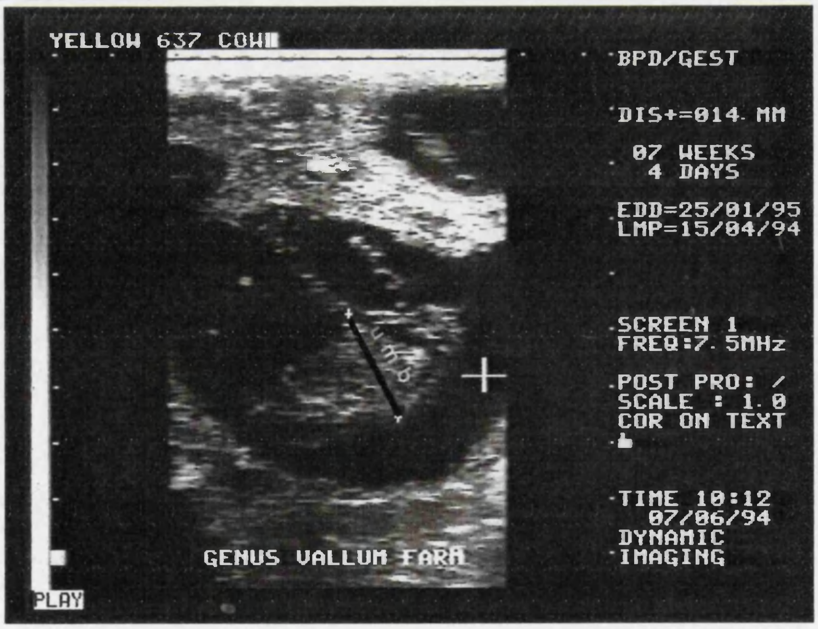
(g) Longitudinal plane images of a 47 days old fetus showing the measurements of transthoracic depth. (arrows)



(h) Cross section images of a transabdominal width (w) and depth (d) of a 62 days old fetus taken across the stomach/liver.



(i) Dorsal section images of a transabdominal depth of a 62 days old fetus taken across the umbilicus.



j) Longittudinal section images of a transabdominal width and depth of 61 days old fetus taken across the umbilicus.

brief gentle palpation through the rectal wall before insertion of the transducer into the rectum.

The transducer was then inserted into the rectum, carried in the palm of the examiner's hand with the transducer face directed over the reproductive tract. The examination procedure followed was as described by Boyd and others (1988). The right ovary was routinely scanned first and examined for the presence of a corpus luteum in order to establish the gravid horn. If no corpus luteum was detected on the right ovary then the left ovary was also scanned. The echo-texture and the size of the corpus luteum were noted. Pregnancy was found in the ipsilateral horn i.e. the horn adjacent to the ovary with the corpus luteum. The scanning was then concentrated on the pregnant horn.

Upon locating the fetus in the pregnant horn the fetus was examined in as many different scan planes as possible. The most common scan planes used were dorsal, longitudinal and transverse.

The scans of all cows were recorded on video tape for further examination and measurements in the laboratory. Measurements taken at the farm were done by using an integral electronic calliper. Only good images where fetal structures were symmetrical and appropriately oriented were measured. In addition, the typical ultrasonographic anatomy, appearance, body size and chronological sequence of development of various fetal organs and body parts at different developmental stages were closely examined and recorded.

The following fetal parameters were measured from fetal images:

a).Crown-rump length. The linear measurement of the developing fetal mass from the top of the skull at the level immediately dorsal to the orbit to the caudal edge of the perineum. (Rump.) (Figure 2.1.2. a, b)

b).Biparietal diameter. The maximum diameter of the skull or the head diameter between the parietal bones. (Figure 2.1.2.c)

c).Umbilical diameter. The maximum diameter of the umbilicus in cross or longitudinal sections within the amnion. (Figure 2.1.2.d, e.)

d).Trunk measurements:

i). Transthoracic :

-<u>Width.</u> The maximum horizontal linear measurement between the two sides of the thoracic cavity taken in a transverse or dorsal plane at the caudal end of the thoracic cavity and/or at the level of maximum heart beat. (Figure 2.1.2.f.)

-<u>Depth.</u> The distance between the dorsal and ventral parts of the thoracic cavity at the level of maximum heart beat. (Figure 2.1.2. g)

ii). Transabdominal :

-<u>Width.</u> The maximum horizontal linear measurement at the level of the stomach and liver taken in a dorsal or transverse section . (Figure 2.1.2. h.)

- <u>Depth.</u> The maximum vertical distance taken at the level of the stomach / liver in a longitudinal plane. (Figure 2.1.2.i.)

Three different mathematical models were assessed in explaining data obtained from the three different fetal parameters namely simple regression, polynomial and logarithmic models.

These models were of the following forms:

(a) Simple regression model had two coefficients and was of the form **$y=a+bx$** where **'y'** represented the predicted fetal dimension, **'a'** was the minimum or initial fetal dimension, **'b'** represented the average daily growth rate or the slope and **'x'** represented gestational age.

(b) The polynomial model had three different coefficients and was of the form **$y=a+b_1x+b_2x^2$**. 'y' was fetal dimension, **'a'** was minimum fetal dimension, **'b_1'** was the linear component of the growth curve, **'x'** was gestational age and **'b_2'** represented the curvature component of the growth curve.

(c) The logarithmic model had only two coefficients and was of the form **$\log y=\log a+bx$**, where **'y'** was the logarithmic number of fetal dimension, **'a'** represented the logarithmic value of the intercept, **'b'** indicated the proportion of growth rate between a given gestational interval and **'x'** was the gestational age.

The model which had the least coefficient of variation (cv), highest adjusted correlation of determination [R^2 (adj)], and the best fit based on residual pattern (r.p) was chosen for fitting growth curves.

All values of the coefficients of the three different models for each fetal parameter were compared to each other and tested by student's t-test. All major statistical tests and analysis were done using the minitab statistical programme.

2.3. Results.

2.3.1. Fetal aging.

All coefficients and correlation of determinations for the three models were found to be significant ($p < 0.05$) Confidence intervals for the age prediction equations were,however, not calculated because of the small sample size used in the study. In all the growth curves of fetal age, size is shown as dependent on age while in age prediction equations, size measurements are the independent variable while age is the dependent variable.

Five different fetal parameters were measured by the internal callipers of the scanner between 40-90 days of gestational age. However, only the measurements of three, out of five, fetal parameters were used in the final analysis of the data namely biparietal diameter, transabdominal diameter and crown rump length. The measurements of the limbs and umbilical diameter were not used in the final analysis of the data because there were not enough measurements taken. Out of a total of 360 scans performed on 81cows about 11% were either discarded for obvious errors or were not recorded due to some technical problems.

Crown rump length. Although some parts of the fetus lay within penetration depth of the ultrasound beam up to the end of the third month, the entire fetus could only fit the viewing field of the ultrasound beam up to the end of the second month of gestation (Day 62).

The overall growth rate of the fetus in length, between 40-62 days of gestational age, based on the regression coefficient of linear regression, was 1.97 mm/day. The initial growth rate between day 40-50 was 1.5 mm/day and later increased to 2.2 mm/day between 50-60 days of gestation. The largest linear measurement at the end of the two months was 67 mm (Figure 2.1.1 a) while the minimum length measured was 17 mm at the beginning of the study (Day 39).

Table 2.2 shows the various statistical indicators of the three different models ie adjusted correlation of determination [R^2(adj)], coefficient of variation (cv), degree of freedom (d.f) and residual patterns (r. p)

The logarithmic model had a slightly higher correlation of determination than the polynomial model, while, that of the regression model was the lowest. The distribution of crown rump length residuals as a function of gestational age were the same for the three models (Figure 2.1.3)

Table 2.2 Values of coefficient for Crown rump length

Model	r^2 (%)	cv	d f	r p
Regression (mm)	91.1	0.12	13	+
Logarithm (cm)	94.2	0.08	13	+
Polynomial (mm)	92.5	0.15	13	+

The logarithmic model best fitted the data, on average, based on the values of the coefficients shown in table 2.2.

Biparietal diameter

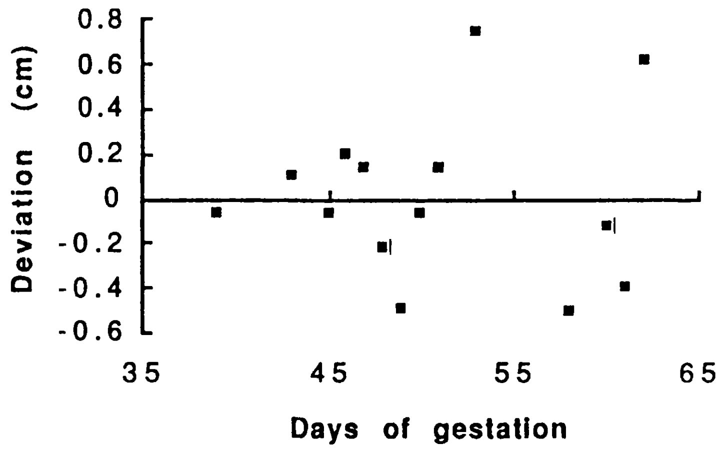
The biparietal diameter was the second most accessible parameter to scan next to trunk diameter during the period of study (40-90 days of gestation). The average growth of biparietal diameter during the period of study was 0.43 mm/day, as expressed by the regression coefficient of the least square regression. The largest diameter measured at day 97 of gestational age was 33 mm. The difference in average growth rate between embryo transfer fetuses and non-embryo transfer fetuses was 0.1 mm/day

Correlation of determination of the logarithmic model was slightly lower than those of the other two models while the distribution of the residuals as a function of age was not patternless, as shown in Figure 2.1.4 c. There was a marked similarity between the correlation of determinations of polynomial and regression models. The plots of residuals of these two models formed patternless scatter graphs. (Figure.2.1.4 a,b)

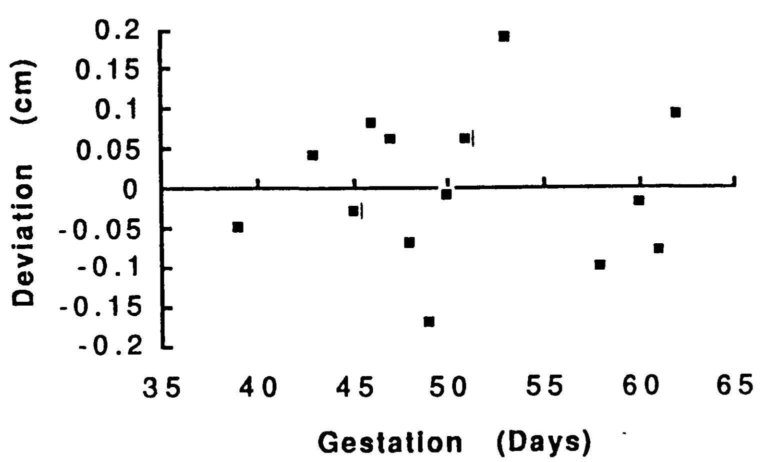
The model which best fitted the raw data was the polynomial model. There was no difference observed between polynomial and regression models, as shown in Table 2.3. However, since the growth curve of the biparietal diameter was not linear, but curvilinear, a linear regression could not provide the best fit without first transforming the raw data into a linear plot .

Trunk diameter measurements were subdivided in two

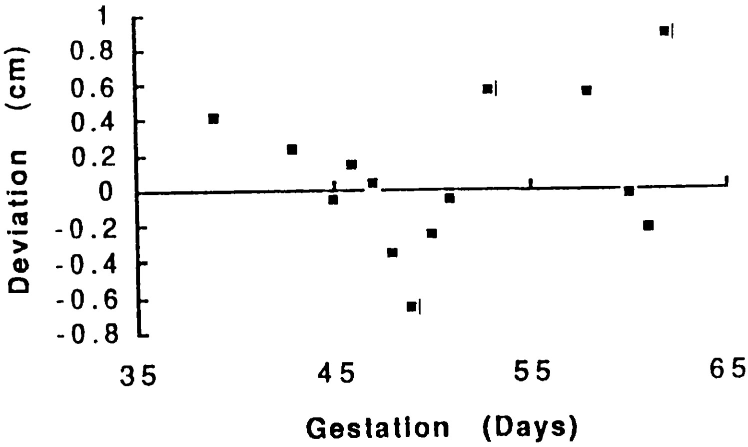
Figure 2.1.3. Crown rump length distribution of residuals as a function of gestational age for the three models: (a) Polynomial (poly.) (b) Linear regression (s.reg.) (c) Logarithm. (Log)


0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Deviation (cm)
35
45
55
65
Days of gestation

(a) poly.


0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
Deviation (cm)
35
40
45
50
55
60
65
Gestation (Days)

(b) s.reg.

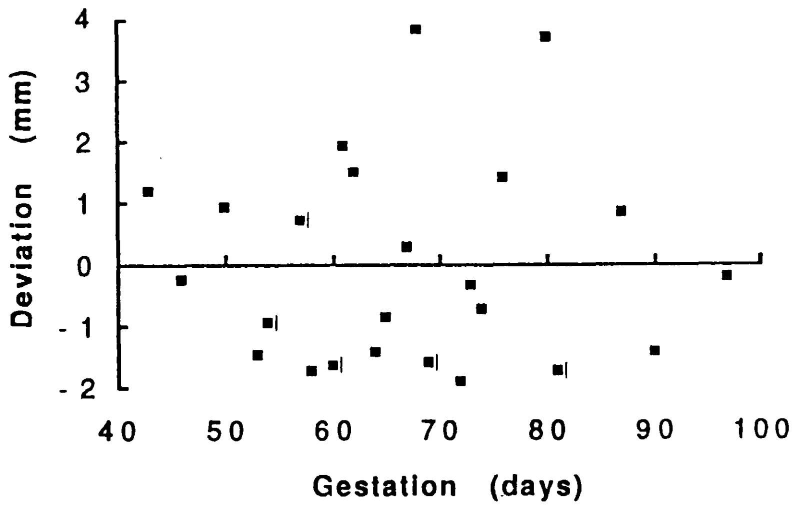


(c) Log

Table 2.3. Value of coefficients for Biparietal diameter

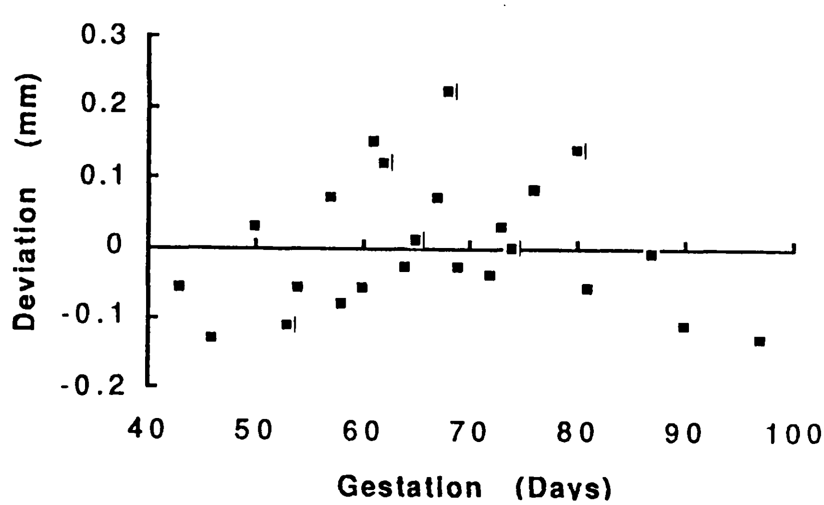
Model	r^2 (%)	cv	df	rp
Regression (mm)	92.4	0.08	23	+
Logarithm (mm)	91.2	0.14	23	?
Polynomial (mm)	91.2	0.11	23	+

Figure 2.1.4. Biparietal diameter distribution of residuals as a function of gestational age (days) for the models: (a) Regression (s.reg.) (b) Polynomial (pol.) (c) Logarithm (log)



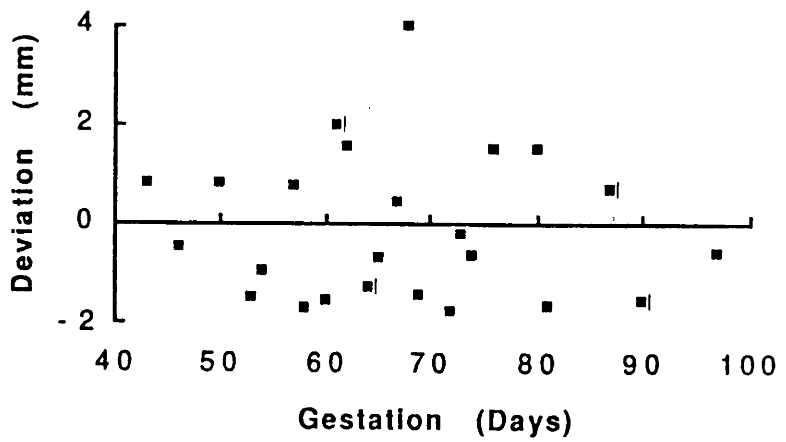
Deviation (mm)
4
3
2
1
0
- 1
- 2
40
50
60
70
80
90
100
Gestation (days)

(a) reg



Deviation (mm)
0.3
0.2
0.1
0
-0.1
-0.2
40
50
60
70
80
90
100
Gestation (Days)

(b) pol



(c) log

different sub-groups, as indicated in the method above, namely transthoracic and transabdominal (ie at the level of the stomach/liver and umbilicus.) (Figure 2.12.). These groups were further subdivided into depth and width measurements. Transthoracic measurements were not included in the final analysis of the data because the number of measurements taken during the period of investigation was not enough for the purpose of analysis.

Trunk diameter.

The trunk of the fetus lay within the field and penetration range of the ultrasound beam during the entire period of study.

Transabdominal diameter (stomach/liver)

Width. The measurements of this diameter could be taken either in transverse or dorsal planes. (Figure 2.1.2.h, i). The average growth rate between day 40-90 of gestional age was 0.57 mm/day.

Depth. The measurements of depth were taken either in a longitudinal, or cross-sectional plane at the level of the stomach or liver. (Figure 2.1.2 h,i).

The mean growth rate of depth during the period of investigation was 0.54 mm/day. The growth rate at the beginning of the investigation was about 0.4 mm /day.

Table 2.4 Value of coefficients of Transabdominal diameter.

Model	r^2 (%)	cv	d f	r p
Regression	94.3	0.09	33	-
Polynomial	95.6	0.12	33	+
Logarithm	92.5	0.15	33	+

Table 2.5 Relationship of fetal parameters to age of gestation as expressed by Simple regression model.

Fetal parameter (y) (mm)	Equation	Time interval (days)	n
1. Crown rump length.	$y = 1.97x - 64$	39-67	14
2. Biparietal diameter.	$y = 0.421x - 7.49$	41-97	24
3. Trunk diameter.	$y = 0.570x - 14.8$	39-97	34

When transabdominal depth was measured from an oblique plane the value was erroneous ie exaggerated.

Combined (width and depth)

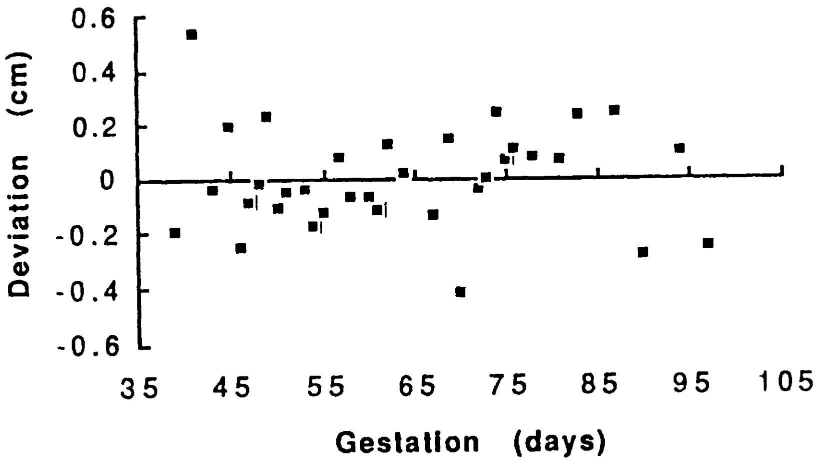
The overall growth rate of width and depth of transabdominal diameter, measured at the level of the stomach or liver, was 0.554 mm/day over a period of 40-90 days of gestation as expressed by the regression coefficient. The maximum and minimum diameters were 42 mm and 8 mm at Days 94 and 39 of gestation, respectively. The growth rate in the first 10 days of the study was about 0.8 mm/day. It later dropped to about 0.4 mm/day by the end of the second month of pregnancy and reverted to the initial growth rate by Day 83 of gestation.

Transabdominal diameter. (At the level of umbilicus)

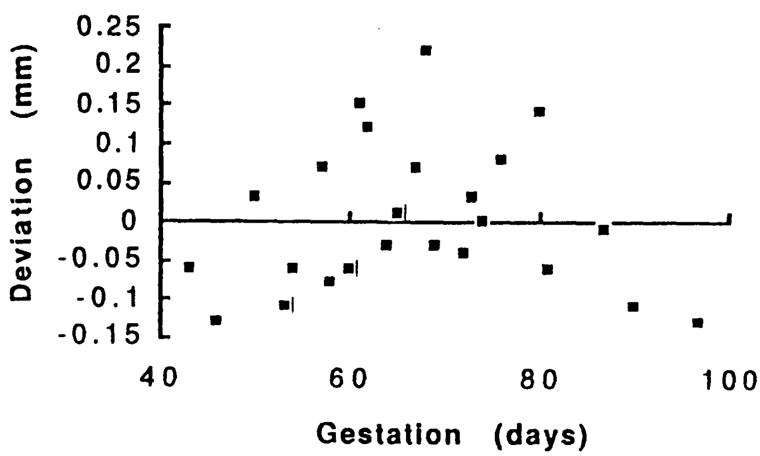
Width. The width in this group increased from 13 mm to 42 mm between Day 42 to 83 of gestation. The mean growth rate during the same period was about 0.725 mm/day.

Figure 2.1.5 Transabdominal diameter distribution of residuals as a function of gestational age for mathematical models:

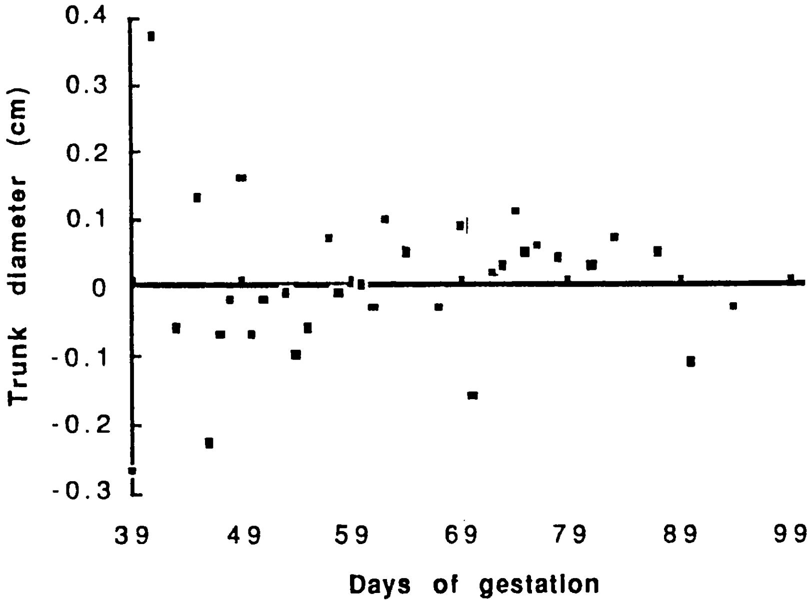
(a) Simple regression (s.reg.) (b) polynomial (poly.)
(c) logarithmic. (log)


0.6
0.4
0.2
0
-0.2
-0.4
-0.6
Deviation (cm)
35
45
55
65
75
85
95
105
Gestation (days)

(a) s.reg


0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
Deviation (mm)
40
60
80
100
Gestation (days)

(b) poly



(c) log

Depth. The maximum measurement of the depth was 33.5 mm at day 81 of gestation while the overall growth rate was 0.40 mm/day during the period of study.

Combined (Width,depth).

The average growth rate of combined width and depth of transabdominal diameter, measured at the level of umbilicus, was 0.56 mm/day, during the period of investigation. The maximum and minimum diameters were 42 mm and 13 mm at Days 83 and 46 of gestation, respectively.

No difference was noted between the measurements of transabdominal diameter width and depth and also between those taken at the level of the stomach/liver and at the level of the umbilicus ($p<0.05$). The overall combined growth rate of the trunk diameter as expressed by the regression coefficient of least square regression was 0.564 mm/day, between Day 40-90 of gestational age.

Table 2.4 shows the values of coefficients of transabdominal diameter. The polynomial model had the highest correlation of determination while the logarithmic model had the lowest correlation of determination. The logarithmic model had the lowest coefficient of variation. In addition it was only the logarithmic model that had a patternless scatter graph of the distribution of residuals as a function of age (Figure 2.1.5 .)

The model that had the best fit among the three, based on the values of coefficients as shown in Table 2.4 and residual analysis (Figure 2.1.5.), was the logarithmic model.

No difference in trunk diameter growth rate was noted between embryo transfer fetuses and non embryo transfer fetuses during the period of study, 40-90 days of gestation ($p<0.05$).

Table 2.6 Relationship of fetal parameters to age of gestation as expressed by logarithmic model.

Fetal parameter (y) (cm)	Equation	Time interval (days)	n
1. Crown rump length.	$\log y = 0.0539x - 1.52$	39-67	14
2. Biparietal diameter.	$\log y = 0.0215x - 0.758$	41-97	24
3. Trunk diameter.	$\log y = 0.0261x - 0.980$	39-97	34

2.3.2 Fetal organogenesis.

Head: By Day 39 of gestation, the general outline of the head and the spinal cord was well defined. Structures which could be seen within the head by Day 39 of gestation include the brain case, orbit, maxilla and mandible. Around Day 41 of pregnancy, the cranium was seen as a hyper reflective structure (Figure 2.1.6) and by 55-60 days of gestation, most bones of the head could be observed.(Figures 2.1.6. b - e)

Additionally, the sonographic imaging of the lens first appeared as an oval structure by Day 53 of pregnancy. In paramedian or transversal planes the nasal cavity, orbit, brain case and hard plate were sonographically visible on Day 55 and 60 of gestation, respectively. (Figure 2.1.6.d.)

Table 2.7 Relationship of fetal parameters to age of gestation as expressed by polynomial model.

Fetal parameter (y) (mm)	Equation	Time interval (days)	n
1. Crown rump length.	$y = 47.5-2.43x+0.0426x^2$	39-67	14
2. Biparietal diameter.	$y = -6.51+0.387x$	41-97	24
3. Trunk diameter.	$y = 3.73-0.016x+0.00439x^2$	39-97	34

Table 2.8 Logarithmic prediction equations of fetal age.

Fetal parameter. (x) (cm)	Equation
1. Crown rump length.	28.20 + 18.55 log nx
2. Biparietal diameter.	35.26 + 46.51 log nx
3. Trunk diameter.	37.69 + 38.46 log nx

Thorax: The heart was the most prominent structure within the thorax up to the beginning of the second month of gestation. By Day 39 of gestation, the pulsations of the heart beat were very pronounced. The early general appearance of the heart was diffused and hypoechoic. (Figure 2.1.7 a.). The outline of the heart and its interior partition became clearer by Day 53 of gestation, in transverse or longitudinal planes. However, the external outline of the heart and the general characteristic anatomy of the interior partition, became more recognisable after Day 70 of gestation (Figure 2.1.7 b..). The interior chambers of the heart appeared near anechoic and were divided by an echogenic wall. (Figure 2.1.7.a.). The outer covering of the heart, the pericardium, could also be seen during the same period. (Figure 2.1.7. a). Blood vessels proximal to the heart had appeared by Day 45 of gestation. About a month later, the major blood vessels of the heart could be differentiated, and were identified as aorta and vena cava by following their course to and from the heart (Figure 2.1.7. b). Some of the major branches of the vena cava and aorta in the trunk of the body, became very clear by Day 70-76 of pregnancy (Figure 2.1.7. d).

The sonographic image of the lung appeared granular and hypoechoic and became identifiable by Day 55-60 of pregnancy. (Figure 2.1.9. k.). Images of the tracheal bifurcation and bronchioles became recognisable by Day 73 of gestation. The outline of the diaphragm became identifiable by Day 73 of gestation.

Abdomen: The liver and the stomach were the most prominent structures of the abdominal cavity. (Figure 2.1.9. g, h, i, j, k). The stomach, together with the umbilical cord, could be identified sonographically by Day 39 of gestation while, images of the liver, which appeared in a moderately hypoechoic and granular form, became identifiable ten days later. Blood vessels of the liver appeared within the liver as small circular anechoic images around the same period. The major blood vessels of the liver were, at a later stage, identified by following their course. (Figure 2.1.7. c, d, e and f.). For instance, the course of the viteline vessel to and from the liver, up to the umbilical vessels, could be followed by holding the long axis of the transducer across the long axis of the fetus and moving it in a backward and forward direction, in a transversal plane. The sonographic texture of the umbilical cord, by Day 39 of pregnancy, was grayish and lacked any structures within it. However, around the second month of gestation, longitudinal planes of the umbilical cord revealed two blood vessels while transverse planes showed four blood vessels. .

The stomach had appeared and was recognisable by Day 39 of gestation. Around Day 53 of pregnancy, the first signs of stomach differentiation were sonographically recognisable (Figure 2.1.9.g.) and three days later, the reticulo-rumen could be distinguished from the omasum. (Figure 2.1.9.h.). It was difficult at this stage, to identify the reticulum until after Day 74 of gestation. The rumen was easily identified because of its relatively large size, lateral and cranial position within the abdomen and its distinguished anechoic appearance (Figure 2.1.9.n.). The omasum was the second largest compartment and occupied a central cranial position, on the right side of the rumen.(Figure 2.1.9.h.)

The rumen, as earlier stated, was the most prominent and largest stomach compartment throughout the period of study. It had an irregular shape and could easily be identified in dorsal, transverse or saggital planes (Figure 2.1.9.h, k, l, q). Cranial to the rumen lay the reticulum (Figure 2.1.9 y (i).) and the abomasum lay to the right (Figure 2.1.9 x (v)), in a ventro-lateral position. The reticulum could only be differentiated from the rumen around Day 74 of gestation in a dorsal or longitudinal plane. The differentiation of the rumen was identified sonographically around Day 60 of gestation. Later, by Day 74 of gestation, the dorsal and ventral blind sacs with the corresponding caudal pillar were observed in a transverse plane, at the most caudal part of the rumen. (Figure 2.1.9.x (v))

The images of the omasum were first seen around Day 53 of gestation, as a circular hyper-reflective structure, as described earlier (Figure 2.1.9 h). Later, around Day 55-60 of gestation, the lumen of the omasum could be recognised. The size of the lumen was seen to increase as gestation advanced. The walls of the omasum were thick and hyper reflective, and thus were easily recognised (Figure 2.1.9 r). The laminae omasi could be seen by Day 70 of gestation. Later, after Day 70 it was possible to recognise the omasal sulci of the omasum.

The abomasum was the most difficult stomach compartment to identify. It lay caudal to the omasum, cranial to the intestine and lateral ventral to the rumen. It became more recognisable by Day 74 of gestation as an irregularly shaped anechoic structure. Transversal planes of the abomasum after Day 80 revealed the primary longitudinal folds which appeared as thin echogenic lines directed to the centre of the lumen of the abomasum.

The intestines, in the early stages appeared as a bright or echogenic roundish mass, to the right side of the abdomen and immediately caudal to the abomasum. The umbilical cord lay caudal to the intestine. By Day 53 of gestation, loop-like spaces within the intestinal mass had appeared.

The liver, like the lung, had a granular sonographic image. However, images of the liver appeared brighter or more echogenic than the lung and in addition had blood vessels running through it. The difference in echogenecity was due to the difference in tissue density. The liver has denser tissues than the lungs. The liver was seen to occupy much of the cranial right portion of the abdomen throughout the period of study. (Figure 2.1.9 g, h, i, q e.t.c.).

The kidneys were first observed around Day 61 of gestation, in a transverse plane as hypoechogenic structures, oval in shape, on both sides of the abdomen, at the level of the umbilical cord and dorsal to the intestine and the blind sacs of the rumen (Figure x (i)). It was not possible at this stage of development to differentiate sonographically, the cortex of the kidney from the medulla.

Pelvic region: Around Day 47, the genital tubercle (the primordium of the penis in male and clitoris in female) was first seen in a dorsal ventral section, as a bilobular echogenic structure, at the level of the hindlimb and between the umbilical cord, cranially, and the tail, caudally. (Figure 2.1.9. c). It was not possible to diagnose the sex of the fetus until after Day 55 of gestation. The scrotum was first seen around Day 76 of gestation in a transverse plane of the pelvic limb and appeared as two echogenic oval-shaped structures. The bladder and the urachus were recognisable at Day 57 of gestation, at the level of the umbilical cord.

Appendicular skeleton : Ultrasonic images of the limbs were identifiable by Day 39 of gestation. At all fetal ages, images of the limbs were hyperechoic in appearance. In the early stages of development, these images appeared as bright circular spots, in dorsal plane (Figure 2.2.1. b). Later, by Day 53 of gestation, the images of the limb began to resemble the adult form of the limb (Figure 2.2.1. c).

Table 3.2 shows the chronological order and earliest time of appearance of the loci of ossification in the appendicular skeleton of the bovine fetus as identified by ultrasonography, in the present study

The scapula and the os coxae were the earliest osseous elements of the appendicular primordia to appear at the beginning of the seventh week. Images of the scapula were first seen on the cranial part of the trunk, in longitudinal plane, as a hyperechogenic triangular structure against the background of the images of trunk structures (Figure 2.2.1. a). In the early stages, the image of the scapula bone was better seen in a longitudinal plane than in the other planes (Compare figure 2.2.1. a and b). Generally stating, images of the scapula, in the early stages of development, were more difficult to scan and identify than the other bones of the limb, distal to the scapula.

Sonographic images of the humerus and the femur could be identified by Day 47 of gestation (Figure 2.2.1, a). Like the scapula and the os coxae, images of the humerus and the femur in the early stages of development, were more difficult to scan when compared to the bones of the limb distal to them. The adult forms of the diaphyses of the humerus and femur could be imaged by Day 87 of gestation.

By Day 53 of pregnancy, ultrasonic images of the radius/ulna and tibia had appeared (Figure 2.2.1, d). It was not possible, by Day 53 of pregnancy, to differentiate images of ulna and radius until after Day73 of gestation (Figure 2.2.1, o). The images of these two bones, ulna and radius, appeared as one fused structure. Unlike the limb bones proximal to the radius/ulna and tibia, the radius/ulna were easier to identify because they were not over shadowed by images of trunk structure.

The metacarpal and metatarsal bones had appeared, sonographically, by the middle of the seventh-week (7) of gestation (Figure 2.2.1, d). In the early stages the images of diaphyses of these bones appeared as discrete segments and by Day 70 of gestation, the images of most diaphyses of the limbs could be seen in full or complete length.

The loci of ossification of the phalanges of digits 3 and 4 began to appear, sonographically, at the begining of the second month of pregnancy (Figure 2.2.1, e). By the first week of the second month of pregnancy, all the phalanges of digits 3 and 4 had appeared (Figure 2.2.1, g, i, k). However, images of the distal phalanx appeared brighter than those of middle and proximal phalanges. The first phalanx to show signs of increased ossification, indicated as an increase in echogenicity, was the distal phalanges by Day 60 of gestation (Figure 2.2.1,e). About a week later

(69 days), images of the proximal phalanges appeared more echogenic, indicating increased activity of ossification (Figure 2.2.1, g, k). The last phalanx to show signs of increased activity in ossification was the middle phalanx at Day 74 of gestation (Figure 2.2.1, p).

At about Day 50 of gestation, three images of structures, at the distal ends of the fore and hindlimbs could be imaged in the longitudinal plane (Figure 2.2.1,c). These three images, located at the sites of the phalanges of the digits, were hypoechogenic and of irregular form. They were believed to be primordia of the phalanges, formed just before the commencement of increased activity of ossification.

Images of all the three phalanges were best seen in the longitudinal plane. Only the distal phalanx was seen in some transverse planes (Figure 2.1.1, e).

No transient element was identified by ultrasound in all the fetuses used in the study between ages 45-90 Days of gestation .

2.4. Discussion and conclusion .

The present study has been the first to assess the use of a 7.5 MHz frequency transducer in determining bovine fetal age between Day 40-90 of gestation. Previous studies have demonstrated the advantage such transducers with higher frequency have over those with lower frequencies (Boyd and others, 1990, Omran,1989, deMoura,1993). Boyd and others (1990) using such transducers reported to have achieved 100% accuracy in pregnancy diagnosis by Day 17 of pregnancy, earlier than in previous studies using transducers of lower frequencies (Tarverne and others 1985, Chaffaux and others, 1986, Kastelic and others, 1988.). By Day 55 of gestation deMoura (1993) recently reported to have achieved a 100% accuracy in determining the sex of the developing fetuses as compared to Day 59-68 of gestation earlier reported. (Curran,1992). This improvement was attributed to the high power of resolution that such transducers have, which resulted in improved image quality

The findings of the present study have shown that transducers with a frequency of 7.5 MHz can be used to measure most of the fetal dimensions up to the third month of gestation. The earliest sonographic study to estimate bovine fetal age using a transducer of lower frequency (3.5MHz) was able to measure most fetal parameters up to Day 140 of gestation (White and others,1985). A more recent study using a transducer of 5.0 MHz frequency reported that the head, thorax, abdominal and pelvis of the fetuses were readily accessible for transrectal sonography during the first 4 months. Although transducers of lower frequencies have lower resolution power, hence poor image quality, they, nonetheless, have a better or deeper penetration range and therefore are able to access fetal dimensions in more advanced pregnancies than transducers with higher frequencies (Boyd and others,1990). In the present study parts of the fetuses, in a few cases, lay within the penetration range up to Day 100 of gestational age. However, in such cases the viewing field of the ultrasound beam was too small to accommodate the parameter, making it difficult to measure the parameter accurately. Trunk and biparietal diameters on the average, could fit in the viewing field of the screen up to 90 days of gestation compared to Day 62 for crown rump length. Trunk diameter was the most frequently measured fetal dimension while crown rump length was the least measured fetal dimension in the present study. These observations are similar to those observed in previous reports (Kahn,1989).

The time taken to locate and obtain an appropriate section for the different fetal parameters varied. More time was generally required to locate and get good sections in the early stages of the study. This was partly due to the inexperience of the author in the initial stage. Initially, the time taken to measure all the parameters was ten minutes per cow. Later the time taken, was greatly reduced.

Crown rump length.

The average growth rate of the crown rump length based on the slope of the linear regression equation between day 39 and 62 was 1.97 mm/day (Table.2.5.). The rate of growth in the initial stage was sluggish compared to the later weeks. As a result of the rapid change in growth a curvilinear growth pattern with a steep slope was formed. Such a growth pattern of the crown rump length corresponded with what other workers have reported (White and others,1985, Kahn,1989, Richardson and others,1990, Evans and Sack,1973.). Crown rump length growth curves of the earliest studies were, however, not curvilinear but concavilinear, due, probably, to the lack of standardised measuring techniques (Maneely,1952).

Of the three different fetal parameters analysed in this study, crown rump length had the steepest slope ie the fastest growth rate as expressed by linear regression, and the highest correlation of determination by the logarithmic model .These findings are compatible with the previous studies (White and others,1985, Richardson,1990, Kahn,1989). The linear regression of the crown rump length,however, had the lowest correlation of determination and highest variation among the regression models of the three fetal parameters . This was probably due to the early appearance of the flex points on the growth curve of crown rump length as compared with the other two parameters whose flex points appeared later. Linear regressions tend to perform better in linear or close to linear, growth curves. Nevertheless, more than 90% of the variability in crown rump length could be accounted for in all the three different mathematical models as indicated by the values of the correlation of determination (Table2.2.).

The average growth rate of crown rump length in this study perfectly matched that of Kahn's (1989) ($p<0.05$). According to Kahn, the average daily growth rate of fetal length increased from 1.4 mm/day at the beginning of month 2 of gestation to 2.2 mm/day at the end, as compared to 1.4 mm/day and 1.97 mm/day during the same period in the present study. However, the values of coefficients or constants of the polynomial models of the second degree between this study and Kahn's study could not be compared and had a significant difference ($p<0.05$). Once again this was due to the differences in gestational ranges used. The present study was based on the early stages of pregnancy before the appearance of the curving on the growth curves

The values of the logarithmic equation of fetal length in this study and White and others (1985) closely corresponded (Tables 2.6 and 2.8.) even though there was a difference of about 30 days in the age range between these studies. However, White's age prediction equation derived or converted from the regression of the logarithmic model could not be matched to the present study. The expected age prediction equation of crown rump length based on their first equation could probably have been of the form :

$$\textbf{age=22.0+20.7}\ \log_n x \text{ as opposed to } \textbf{age=27.5+16.73}\ \log_n x$$

Although the logarithmic model had an overall advantage over the other two models, all the three models had fitted the raw data of fetal length as expressed by the residual pattern. This was the only fetal parameter where all the three models fitted the raw data based on residual patterns.

The level of variations of crown rump length measurements in the present study and other similar sonographic studies, were found to be significantly lower than studies based on the conventional techniques. A recent conventional study on age estimation reduced the variation by 80% (Holland and others,1992). However, the degree of variation reported in that study is still greater than most studies using ultrasonography (White and others, 1985).

Biparietal diameter

The overall growth rate of biparietal diameter based on the mean values of the regression coefficient was much less than the crown rump length (Table 2.5.) The change in growth rate was lower for biparietal diameter than for crown rump length. Between Days 46-60 of gestation the growth rate was about 0.4 mm/day, increasing to about 0.7 mm/day between Day 62-72 and finally to 0.9 mm/day between Day 73-81 of gestation. On average the growth rate increased at the rate of 0.3 mm/day every ten days between Days 40-90 of gestation. The overall growth rate of head diameter in the present study as expressed by the regression slopes approximated with a recent study whose slope was 0.523 (Kahn,1989). However, there was a difference between the value of the intercepts or coefficients (a_o). This is because the regression equation of the other report was based on braincase measurements which have smaller diameters compared to biparietal diameter, hence the difference in the value of the intercepts. ($p<0.05$). Kahn prefers braincase measurements to head diameter because, according to him, head diameter and length seem more ambiguous and difficult to measure. A recent publication on human fetuses reported that the diameter and circumference of head measurements are affected by changes in head shape and that since head size is likely to be determined by both paternal and maternal genes heads of various sizes could result, some of which might be too large (Rossavik and others,1987). Such observations were not noticed in this study.

The logarithmic model was found to have an overall advantage over the other two models based on coefficient of variation and correlation of determination. However, the findings of the present study question the use of the logarithmic model to fit head diameter data between Days 40-90 of gestation, based on residual analysis and residual pattern (Table 2.3). Only the polynomial and linear regression models fitted the raw data of head diameter. One of the early sonographic studies to establish the relationship of fetal measurements and age in cattle used the logarithmic model to fit biparietal diameter raw data. (White and others,1985). It is not clearly stated in their report whether the fitness of the model was tested by residual analysis. The logarithmic model perhaps makes a good fit beyond Day 90 of gestation. There is a need to verify the fitness of logarithmic model on biparietal diameter beyond Day 90 of gestation using residual analysis.

The linear regression and polynomial models have a good fit for the head diameter data between Days 40-90 of gestation based on residual patterns. It should be stated, however, that, the pattern of head diameter growth curves change from near linear form to curvilinear beyond Day 90 of gestation (White and others,1985, Kahn,1989). Therefore, after this change in the growth curve, linear regression model may not have a good fit to the raw data of head diameter without first transforming the data.

The present study revealed that there was no difference in measurements between the head diameter of embryo transfer fetuses and non-embryo transfer fetuses. ($p<0.05$)

Trunk diameter

The findings of the present study indicate no difference between the measurements of transabdominal depth and width. Also, no difference was observed between measurements of trunk diameter measured at the level of the fetal liver/stomach and those measured at the level of the umbilicus. ($p<0.05$)

Among the three fetal parameters considered in this study, trunk diameter had the slowest overall growth rate. (Table 2.5.) The initial growth rate of trunk diameter between Days 40 and about Day 60 of gestation was faster than that observed between Day 60-72. The growth rate increased again after Day 73 of gestation. This unusual growth pattern of trunk diameter could be attributed to its close relationship to the fetal liver which during this period is undergoing rapid increase in size and weight. The reports of Blin and others (1963) and Rossavik and others (1987) support these findings. The bovine fetal liver increases in weight from about 0.06 grams at 4 weeks of gestation, to 1.0 gram at 8 weeks of gestation, an increase of about 1600% (Blin and others,1963). Later, after about the second month of gestation the growth rate of the liver declines for a while. In human fetuses, the trunk size as measured at the level of the fetal liver, is sensitive to changes in nutrition because of its close proximity to fetal liver size (Rossavik and others,1987). The resultant growth curve formed from such a growth pattern of trunk diameter is of a more complex form. The overall growth of trunk diameter between Day 40-90 of gestation was about 0.5 mm/day as expressed by the regression coefficient while the largest diameter was 18-25 mm between Day 60-70. After Day 70 of gestation the growth rate was 0.6-0.7 mm/day and by day 97 of gestation the largest diameter of the trunk was 42 mm. Trunk diameter exceeded the scanning field of the ultrasound transducer at about Day 97 of gestation having reached 43 mm. The present findings were quite similar to those observed in a recent report (Kahn,1989). The largest diameter observed between Days 60-70 was 20-30 mm and the increase in growth after Day 70 was about 0.9 mm per day.

Only the logarithmic model out of the three models considered, has a good fit of trunk diameter between Day 40-90 of gestation based on residual patterns, according to the findings of the present study. The failure of the linear regression model to fit the data of trunk diameter could be attributed to the complex form of the growth curve caused by the rapid increase of fetal liver size during early pregnancy, as described earlier. However, the failure of the polynomial model to fit trunk diameter data between Day 40-90 could not be explained. A recent study, however, used a polynomial model to fit data of trunk diameter (Kahn,1989). It was not indicated in that report whether the model's fitness was tested by residual analysis.

The coefficients of the logarithmic model in the present study correspond very closely with those previously reported. (White and others,1985) ($p<0.05$) Coefficient **a**, in the present study was 0.258x compared to 0.243x in the earlier study, while the second constant, **b**, in this study was -0.956 compared to -0.8735 in the other report. It was however, difficult to test the significance of these similarities, statistically.

The present study found no differences in trunk diameter growth rates and measurements between embryo transfer fetuses and non-embryo transfer fetuses (Days 40-90 of gestation.)

The linear model was the simplest of the three models assessed; deriving the age prediction equation and identifying the features of growth that the coefficients represent was easier than with the other two models. It could also be used to ascertain the average growth rate during a given period. However, it can only be used in linear growth curves usually present in early gestation and not non-linear characteristics seen in a typical growth pattern seen later in pregnancy.

The polynomial model, on the other hand, is identified by three coefficients, and hence is more complex to interpret and derive an age prediction equation, compared to the rest. For this reason it is not, in most cases, a good model to use for the purpose of estimating age. However, it is very good at defining and describing the extent and direction of both linear and curvature features of the growth curves. It does not, however, specify the points at which these curvings begin and end on the growth curve.It could be used to describe the extent and general direction of raw data.

The logarithmic model, like the linear regression model, involves the estimation of only two coefficients, which are relatively easy to interpret and to derive age prediction equation. It is the most ideal to use for the purpose of estimating age using a fetal dimension which has a typical curvilinear or sigmoid growth curve. This is because it is able to transform non-linear raw data into linear and because of this it has uniform variations and hence the least coefficient of variation. The low coefficient of variation will give values that differ only slightly among different cattle populations. Since this model transforms the raw data from non-linear to linear it can be used to describe data with curvatures on the growth curves.

To the authors' knowledge this is the first study to assess in detail, the suitability of different mathematical models for estimating age and describing some of the growth features of raw data.

The values of coefficients in the present study resembled very closely those of previous studies which used transducers of lower frequencies. (White and others,1985, Kahn,1989). However, it was not possible, statistically, to conclusively state whether such a similarity was significant or not because of the difference in age range, a lack of the raw data from the previous studies and the small sample size used in the present study. Future studies using significantly large sample sizes and similar age range could verify the findings of the present study. All previous studies using regression to analyse data, including the present study, have, due probably to practical limitations, taken more than one measurement from the same cows. This however, is not desirable and is a violation of one of the basic assumptions of regression. Future studies using regression for handling data should,where possible, avoid making more than one measurement from any single animal in order to obtain more valuable results.

If the findings of the present study are statistically significant, it seems that the improvement of precision in fetal aging and a clearer understanding of growth patterns may depend, among other things, on the use of an appropriate age prediction model at a given age and not so much on the use of transducers of higher frequencies. In the species like canine and human (England and others, 1990) models with multiple fetal parameters have been used to improve the precision of age prediction. However, coefficients of such models are more difficult to interpret and relate the characteristics of growth that they represent.

It is possible that, as more models are tested and developed, separate relationship of breeds, various nutritional levels etc. will be established. In the human, a number of models have been tested to estimate age, describe the raw data and reflect certain biological phenomenons of human fetal growth (Todros and others,1987, Rossavik and others,1987). Preliminary data from fetuses of mothers on drugs show that the biparietal diameter growth curve is consistently lower compared to the normal one while that of diabetic mothers has a later flex point and therefore a longer phase of rapid growth (Todros and others,1987).

The present study further demonstrated that ultrasonography can be used successfully to observe gross structures of most organs and tissues of the developing bovine fetus. The clarity of the images of organs or tissues depended primarily, on the size (Minimal size in this case was any dimension above the resolution factor of a transducer with 7.5 MHz frequency i.e. 0.5 x 1 mm.) and the density of the particular organ/s, relative to the densities of the organs immediate to them/it. For instance, fluid-filled structures, like the eye, stomach, heart, blood vessels and bladder, which are surrounded by solid organs or tissues were easier to identify than those which were surrounded by tissues or organs of, almost, the same density. e.g. rumen and reticulum, cranial bones, liver and lungs, abomasum and intestines. Generally stating, the contrast in appearance between sonographic images of most organs and tissues was poor or absent in the early stages of development. The poor sonographic contrast between the organs, during the early stages of gestation , made it difficult, or impossible in some cases, to see or identify most of the organs. The predominant tissue type in the early stage of fetal and embryonic development is the low density, jelly-like mesenchyme which, according to the present author, is responsible for the poor contrast between the images of the organs. After Day 50, the contrast between the developing organs significantly improved. Therefore, it became relatively easier to identify the images of most organs.

By Day 45 of gestation tissues and organs like the brain, orbit, maxilla, mandible, heart, blood vessels, spinal cord, vertebrae, liver, stomach, forelimb, hindlimb and umbilical cord could be identified. This finding is in accord with previous studies carried out on bovine prenatal development (Omran, 1989, Winters and others,1942, Warner, 1958, Kahn, 1990.). According to a recent sonographic study, the eye and the stomach could be identified around Day 40 of gestation, while, the spinal column showed initial signs of ossification at five (5) weeks of pregnancy. (Kahn, 1989). In another similar study, the embryonic heartbeat was seen as a pulsatile movement on Day 19 of gestation and eight (8) days later, the umbilical cord was first noticed as a narrow short echogenic structure. Between Days 31 and 34 of pregnancy, the oropharynx, optic vesicle, abdominal organs, brain, mandible, maxilla, aortic arch and dorsal aorta could be recognised from the rest of the embryonic body (Omran, 1989). However, the time of appearance of a number of organs and tissues reported in past studies on bovine prenatal development using conventional methods were slightly earlier than those using ultrasonography. For instance, the oropharynx, optic vesicles, dorsal aorta and aortic arch loop, to mention a few, in Winters and others' study (1942) were reported to have appeared not less than ten days earlier than that of the most precise sonographic study (Omran, 1989.). In another study using conventional methods by Warner (1958) regional differentiation of the stomach chamber was well underway by Day 28 of gestation compared to Day 40 of gestation when the stomach became visible in a sonographic study (Kahn, 1990)

In the present study, the stomach became visible by Day 39 of gestation and by Day 53 of gestation signs of its differentiation were beginning to be apparent. The size of the fluid-filled anechoic reticulorumen had substantially increased by Day 55 of pregnancy and was the most easily recognisable stomach compartment by its nearly anechoic appearance. It was not possible at this early stage to differentiate the rumen from the reticulum until Day76 of gestation. However, the differentiation of the rumen into the cranial and caudal sacs and later, into dorsal and ventral blind sacs became recognisable by Days 60 and 74, respectively. Differentiation of the omasum became visible on Day 53 of gestation as a bright prominent echogenic circular structure while the laminae omasi were first seen about a fortnight later after the first signs of differentiation. The abomasum was not as easily recognisable as the rest of the stomach compartments, probably, because of its close resemblance in appearance and its close proximity to the intestines.

The present work is the first in the literature, to study, sonographically, the development of bovine fetal stomach to such a depth.

The position, echotexture, shape and size of the differentiated stomach compartments observed, sonographically, in the present study correspond with the observations made on dissections performed by the author on bovine fetus between Days 50 and 100 of gestation and to previous studies (Warner, 1958, Lambert, 1948, Kano, 1981.). Warner describes the appearance of the omasum as a bulb-like swelling at Day 36 of gestation which later, changes its position from caudal to cranial and on the right side of the rumen. This description is, generally stating, in accord with the present findings. However, the laminae omasi, of the omasum and differentiation of the rumen into caudal, cranial and later, blind sacs could only be recognised after Day 70 of gestation, in the present study, compared to Days 43 and 58 of gestation respectively, as was earlier reported (Warner, 1958).

The present study was able to follow the full course of the viteline vessels, for the first time, sonographically, from Day 60 of gestation. Observations made by the author on the dissected bovine fetus corresponded with the sonographic findings.

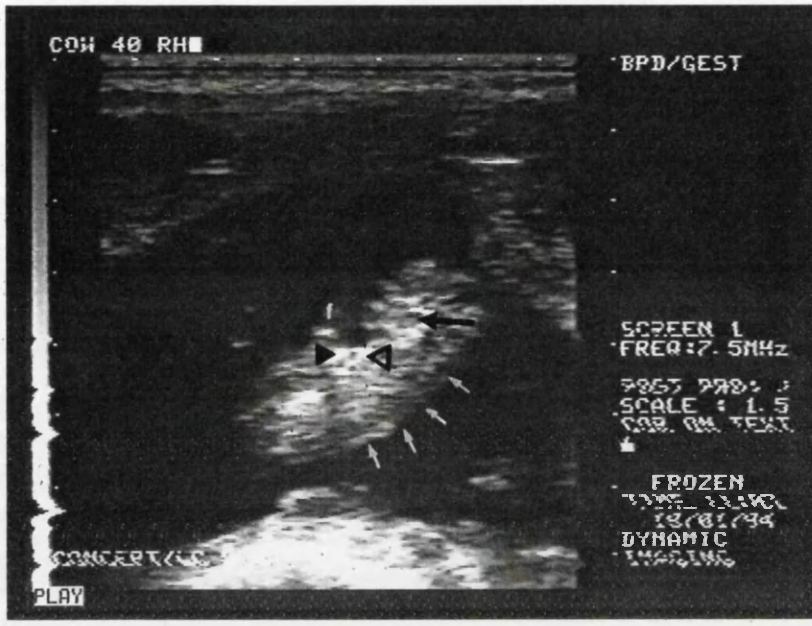
The kidneys in the present study were recognisable much earlier than previously reported (Kahn,1990). However, the medulla and the cortex of the kidney were not identified in the present study, probably, because the echotexture of the two parts of the kidney, were at the time, the same.

The longitudinal plane of the umbilical cord showed two vessels, while, the transverse plane revealed four on Day 60 of gestation. This observation is in accord with past studies, although, the observation was made earlier than had been previously reported. (Kahn, 1990)

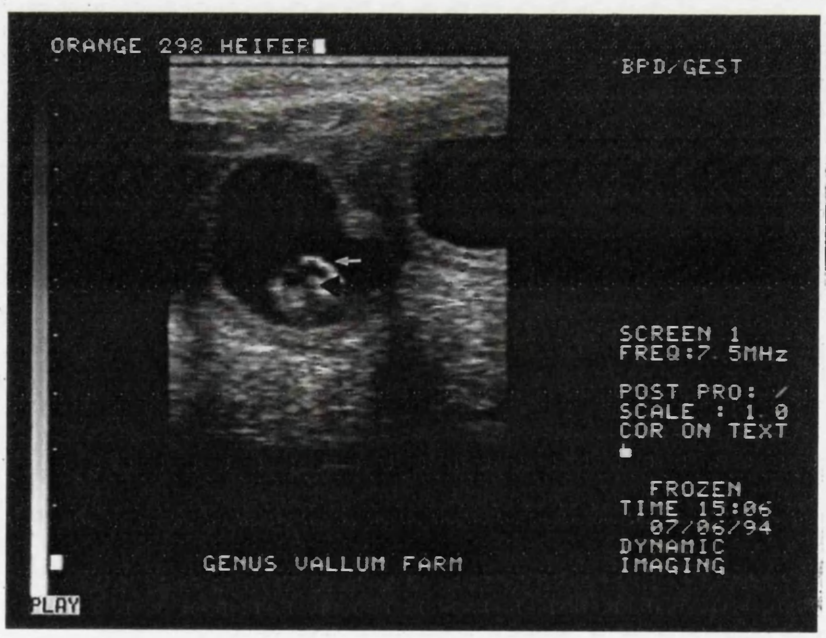
The bones of the cranium became more echogenic by Day 41 of gestation. Embryological studies have revealed that the ossification of the bones of the cranium begins between Days 55-60 of pregnancy, two weeks later than the present findings (Committee on bovine reproductive nomenclature, 1972.).

A transverse plane of the head at the level of the orbit on Day 60 revealed the cavity of the oropharynx, the two orbits of the eyes, brain case, nasal cavity and the hard plate. The lens of the eye in the bovine fetus develops around Day 50 to 60 of gestation, according to an earlier study. (Bistner and others, 1973.). The finding of the present study is in accord with Bistner and others.

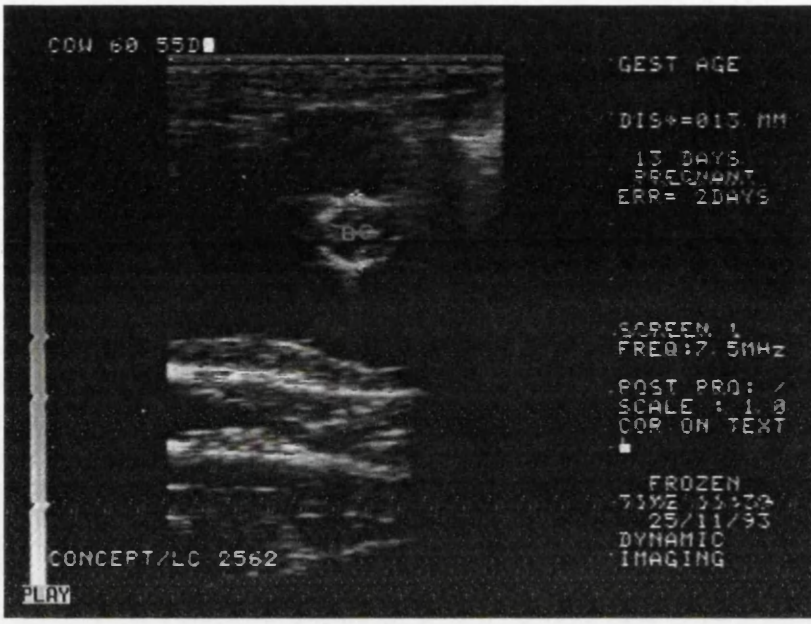
Figure 2.1.6. Ultrasonic appearance of bovine fetal head at different developmental stages.



a. Longitudinal ultrasound scan of a fetus, 47 days after insemination. Note the forelimb (f), humerus (short shaded arrow), scapula (unshaded arrow), optic vesicle (long shaded arrow) and spinal cord (white arrows)



b. Transverse ultrasound scan of the cranium of a fetus 41 days after insemination. Arrows indicate the cranium showing signs of ossification (white) and the brain tissue (black).

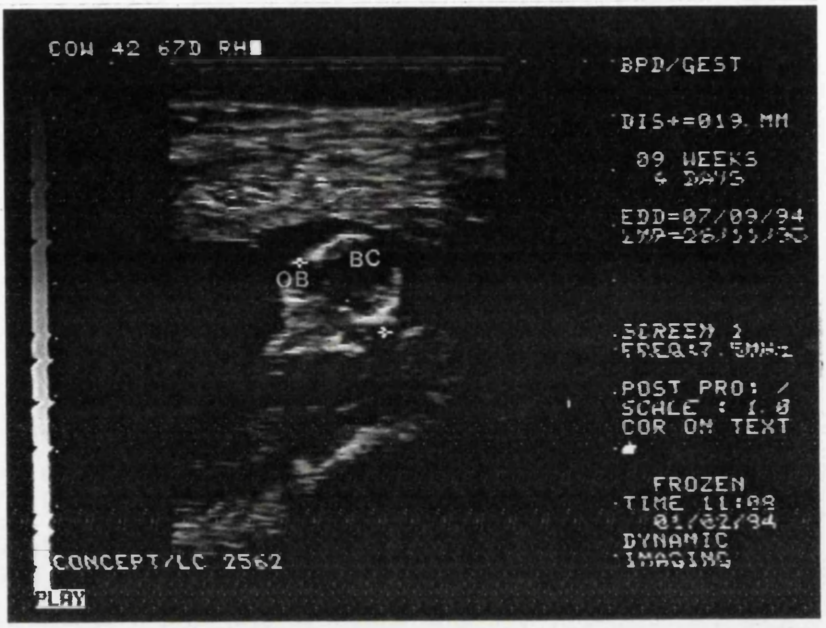


c. Oblique transverse ultrasound scan of the cranium of a fetus 55 days after insemination. BC = brain cavity.

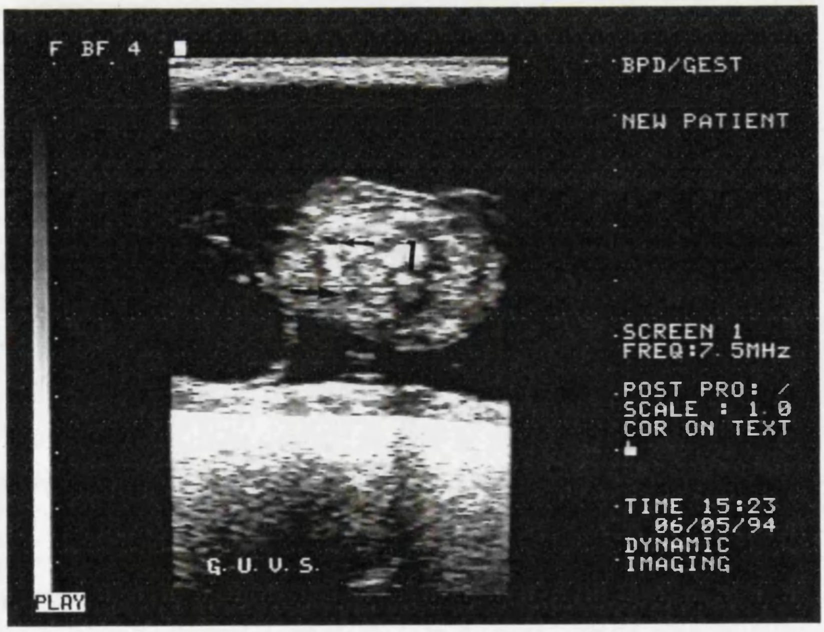
d. Transverse ultrasound scan of the head at the level of the eye, 60 days after insemination. orbt = orbit, N. cav = nasal cavity and o. cav = oral cavity.

e. Transverse ultrasound scan of the head at the level of the cranium, , 60 days after insemination. cr = cranium.

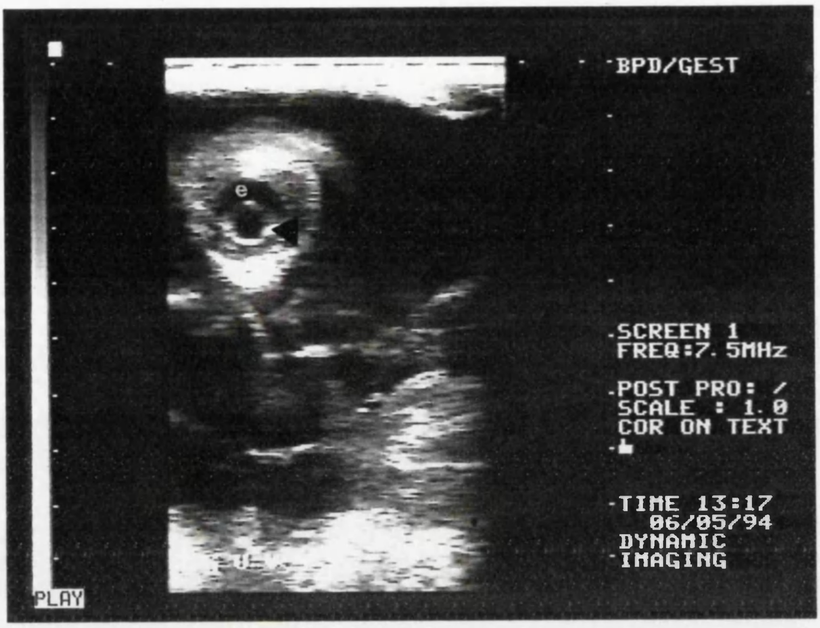
f. sagittal ultrasound scan of the head, 62 days after insemination. Note the nasal conchae (big white arrow) seen within the nasal cavity, oral cavity (small white arrow) and mandible (unshaded arrow).



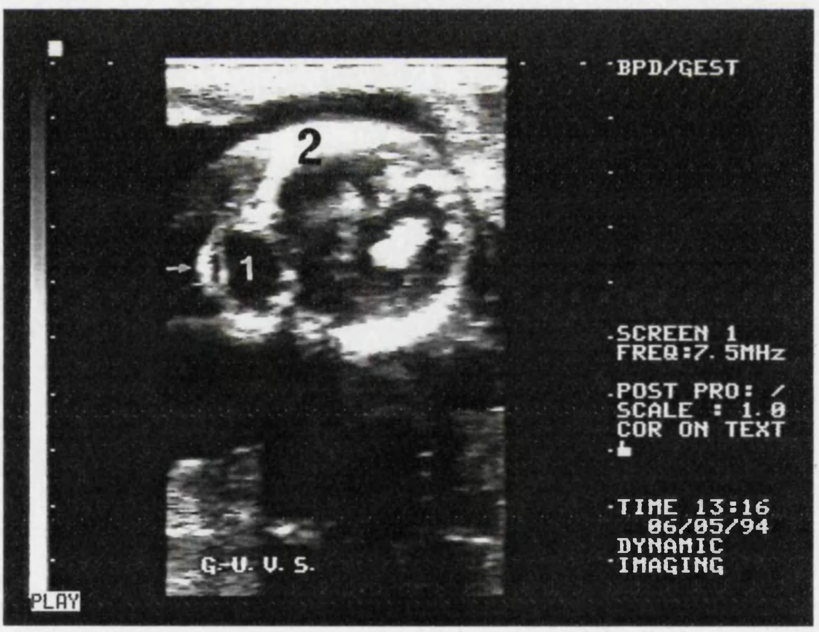
g. Dorsal ultrasound scan of the head, 67 days after insemination. Note the brain cavity (bc) and orbit (ob)



h. Transverse ultrasound scan of the neck, 103 days after insemination. Note the cervical vertebrate (1), trachea (small black arrow) and jugular vein (big black arrow)

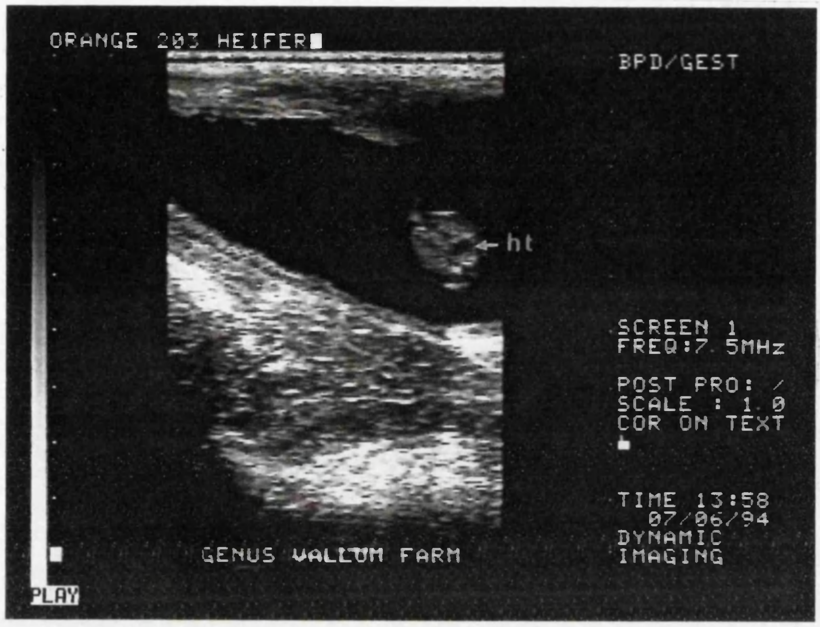


i. Ultrasound scan of the eye (e), estimated age of 110 days. Note the iris (arrow).



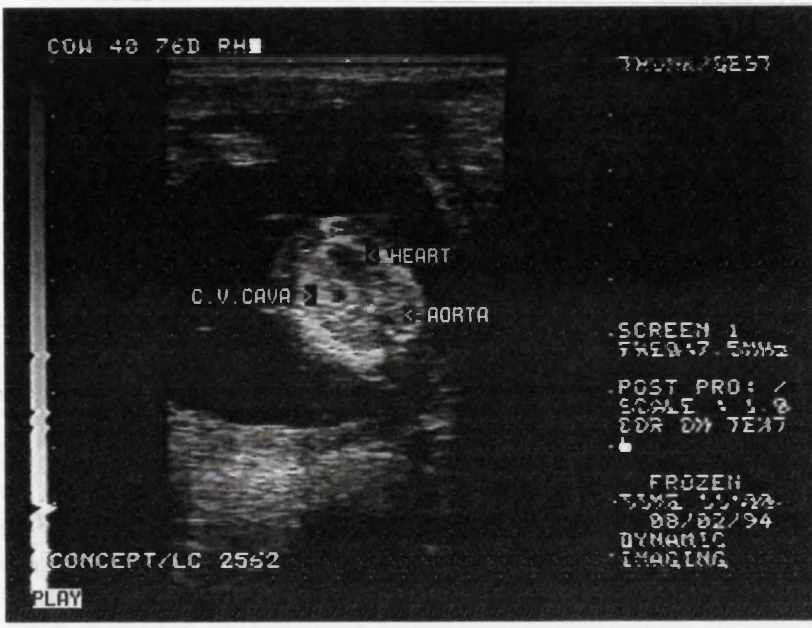
j. Oblique transverse ultrasound scan of the head at the level of the orbit, 103 days after insemination. Note the eye (1), lens (arrow) and brain case (2).

Figure 2.1.7. Ultrasonic appearance of the major structures of the thoracic cavity and some major blood vessels of the bovine fetus at different developmental stages.

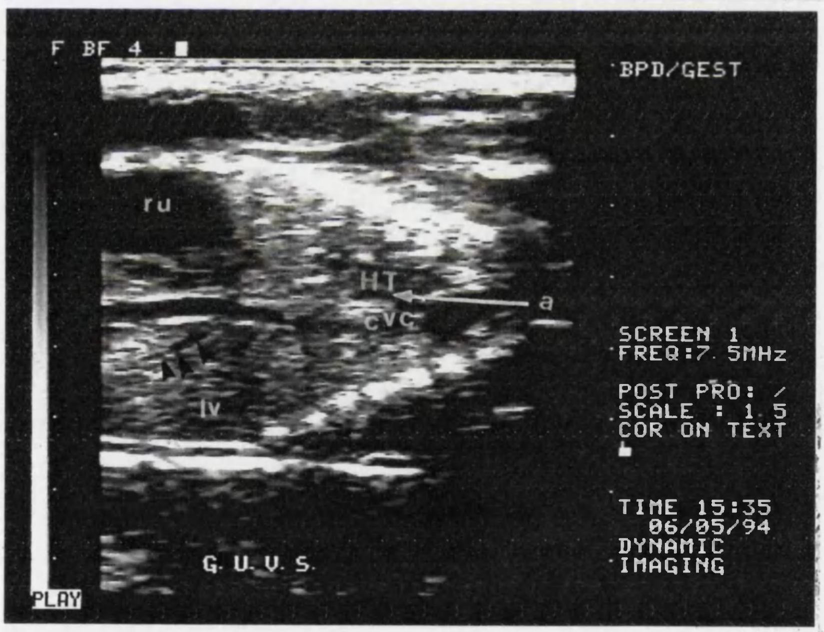


a. Transverse ultrasound scan of the thorax at the level of the heart. (ht), 48 days after insemination.

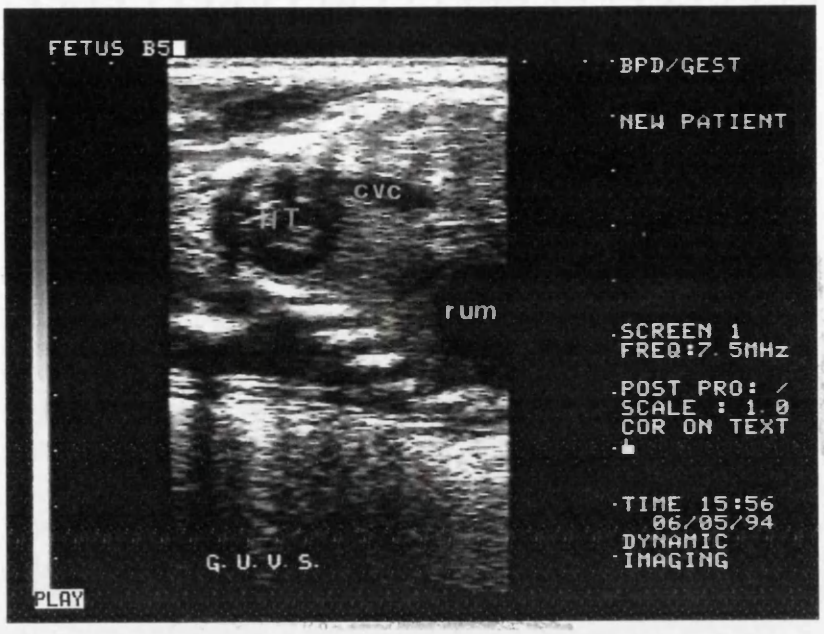
b. Transverse ultrasound scan of the thorax at the level of the heart, 76 days after insemination. Note the two interior chambers of the heart divided by the interventricular septum (ivs), i.e. left ventricle (L. vent).



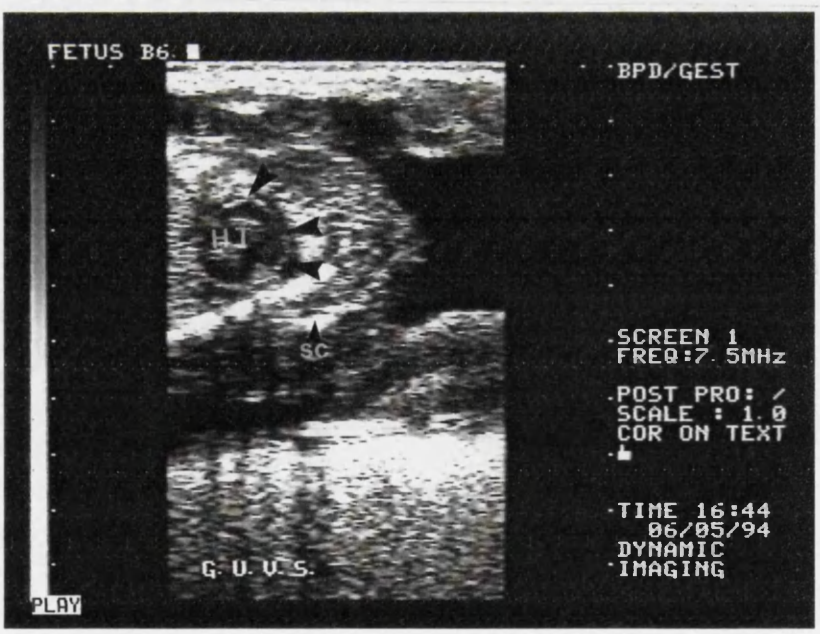
c. Transverse ultrasound scan of the thorax at the level of the caudal part of the heart, 76 days after insemination. Note the caudal vena cava (c.v.cava) entering the base of the heart. (heart) and the aorta, dorsal to the heart and the caudal vena cava.



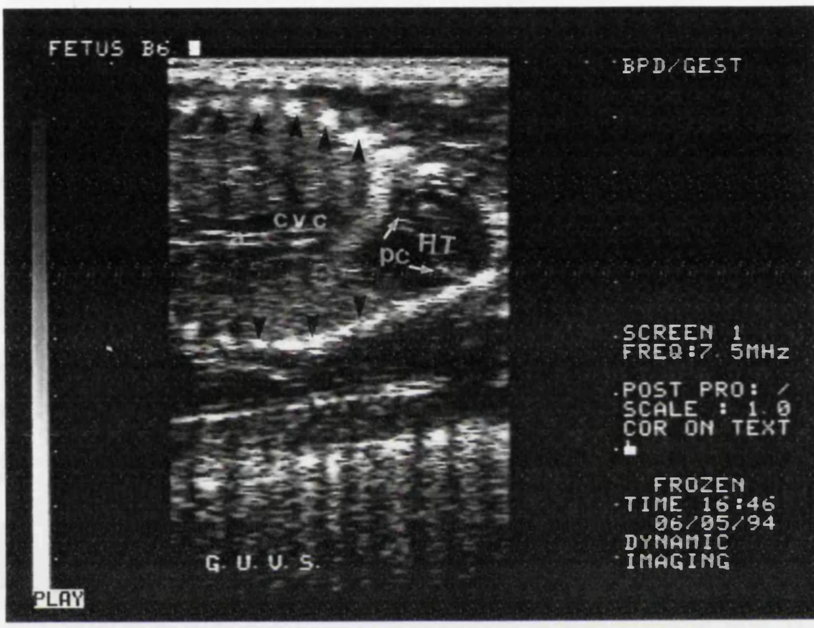
d. Dorsal ultrasound scan of the thorax and the abdomen of estimated age 103 days . Note the caudal vena cava (c.v.c.) pushing its way through the liver (lv), before emptying into the heart (HT). Note also the blood vessel of the liver joining the caudal vena cava (black arrows). Other structures seen include aorta (a) and rumen (ru)



e. Dorsal ultrasound scan of the thorax and the abdomen of estimated age 103 days . Note the rumen (rum) and the caudal vena cava (c.v.c.) just entering the heart (HT).

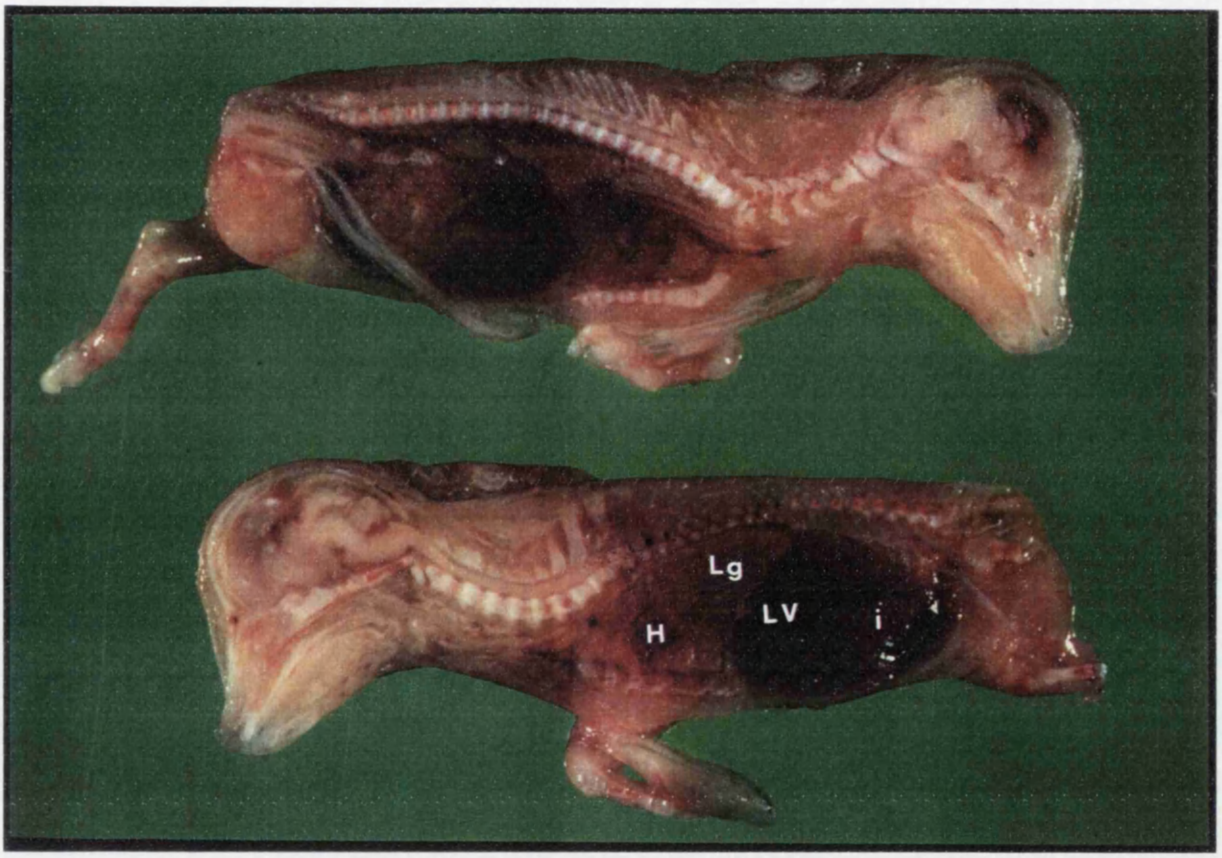


f. Dorsal ultrasound scan of the thorax of a fetus of estimated age 115 days . Note the pericardium (big black arrows), heart (HT) and the scapula (sc)

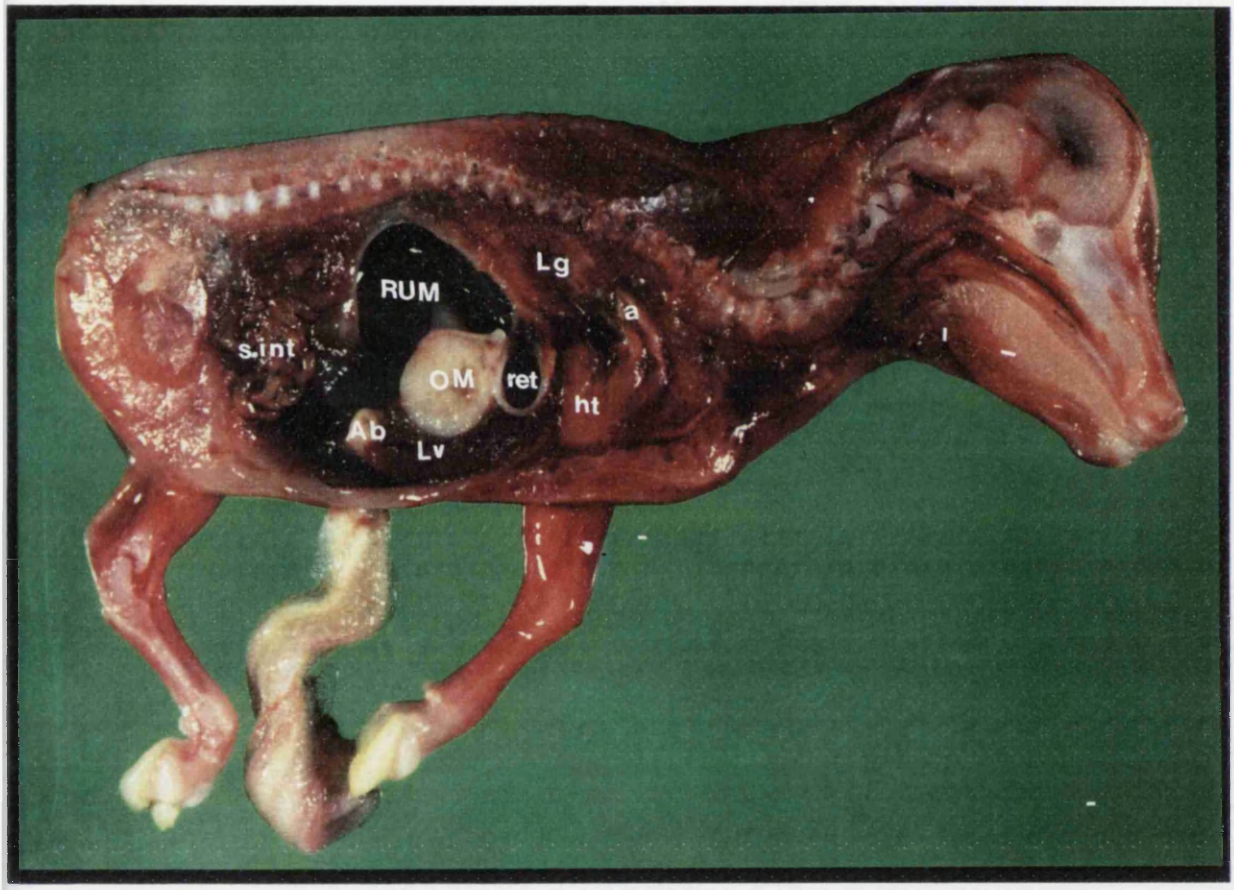


g. Dorsal ultrasound scan of the thorax and the abdomen of a fetus of estimated age 115 days . Note the pericardium (pc), heart (HT), caudal vena cava, aorta (a) and the ribs (black arrows).

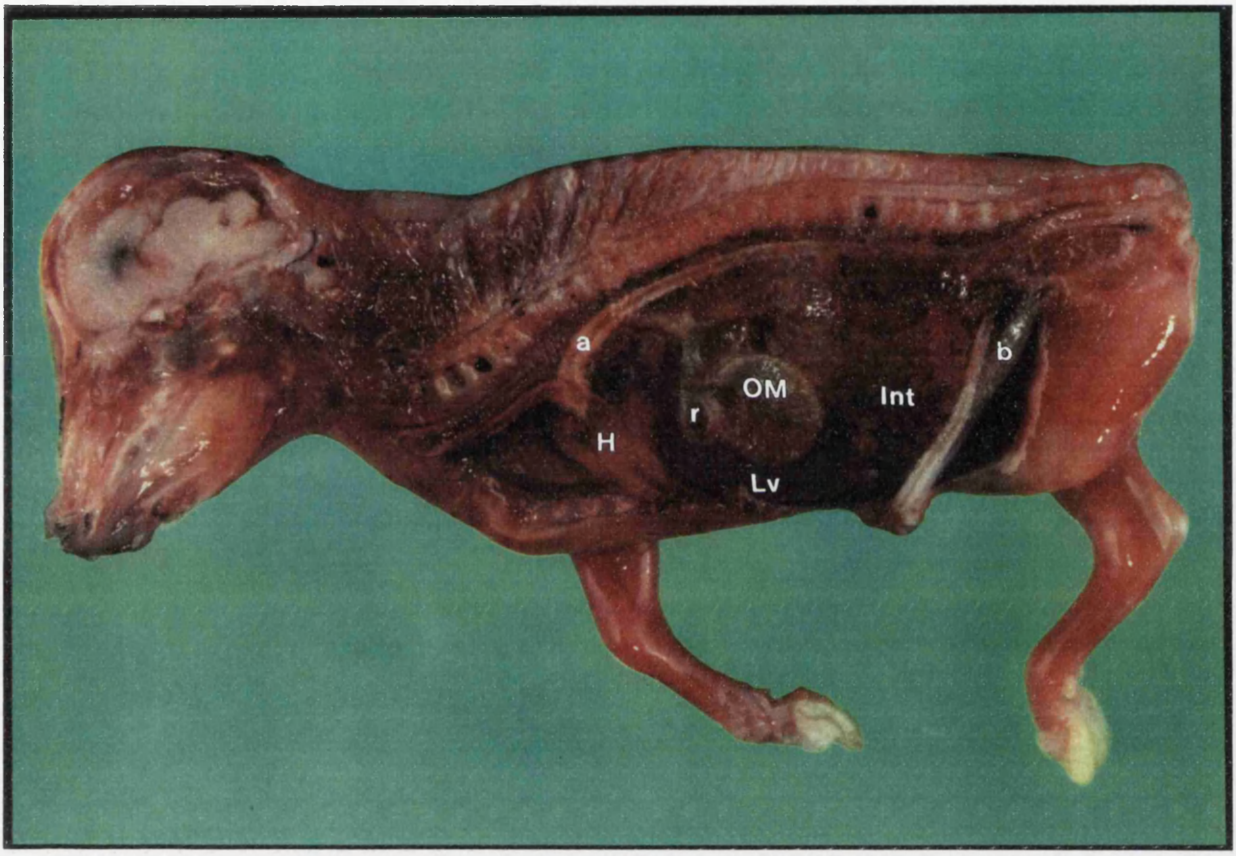
Figure 2.1.8 Longitudinal sections of bovine fetuses.



(a). H = heart, Lg = lung, Lv = liver and i = intestine

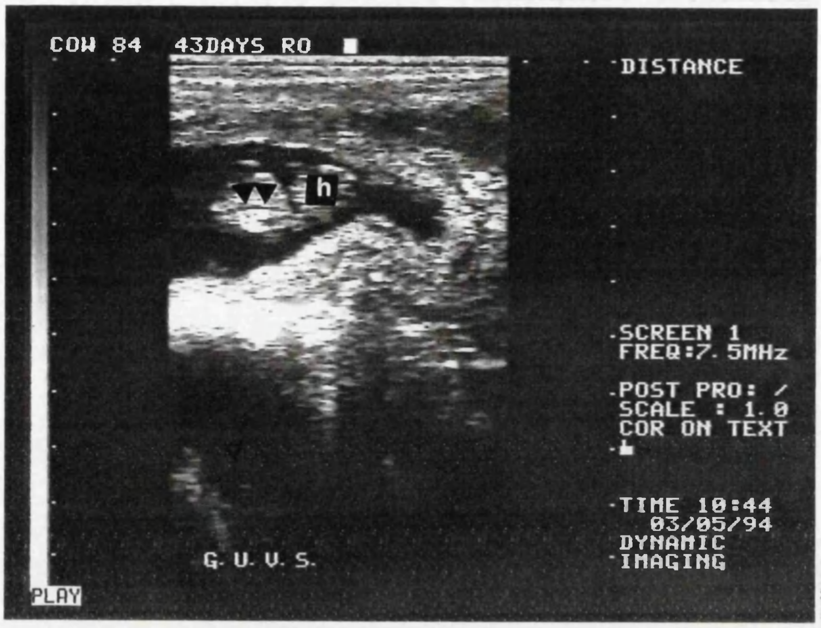


(b). RUM = rumen, lg = lung, s.int = small intestine, Ab = abomasum, Om = omasum, ret = =reticulum, Lv = liver, ht = heart and a = aorta.



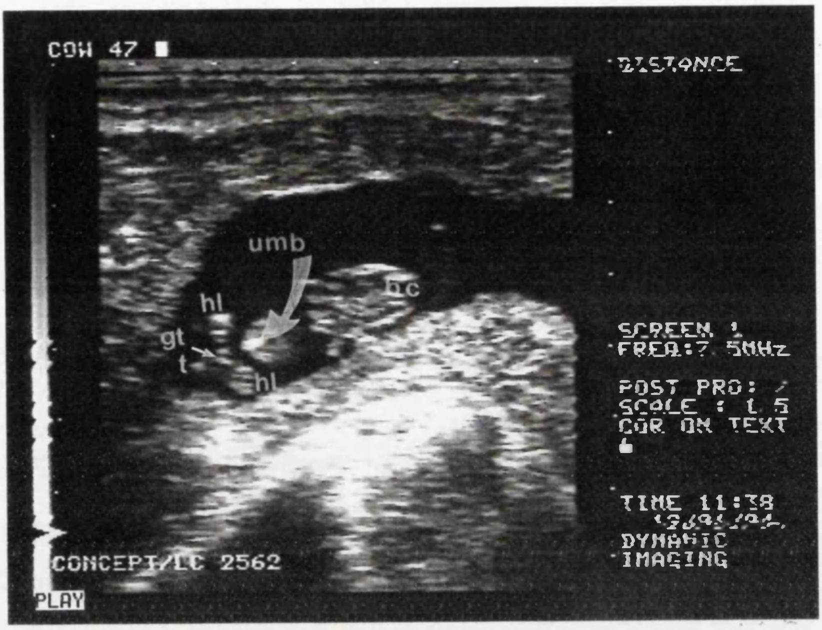
(c). int = intestine, om = omasum, lv = liver, r = =reticulum, bladder, H = heart and a = aorta.

Figure 2.1.9. Ultrasonic appearance of abdominal and pelvic structures of bovine fetus at different developmental stages.

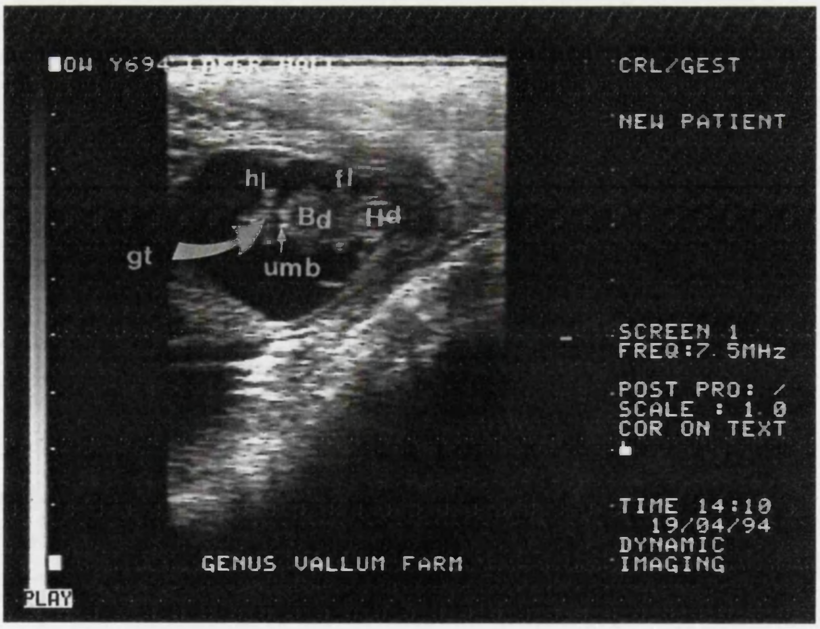


a. 43 days after insemination, h = head, digestive tube (arrows)

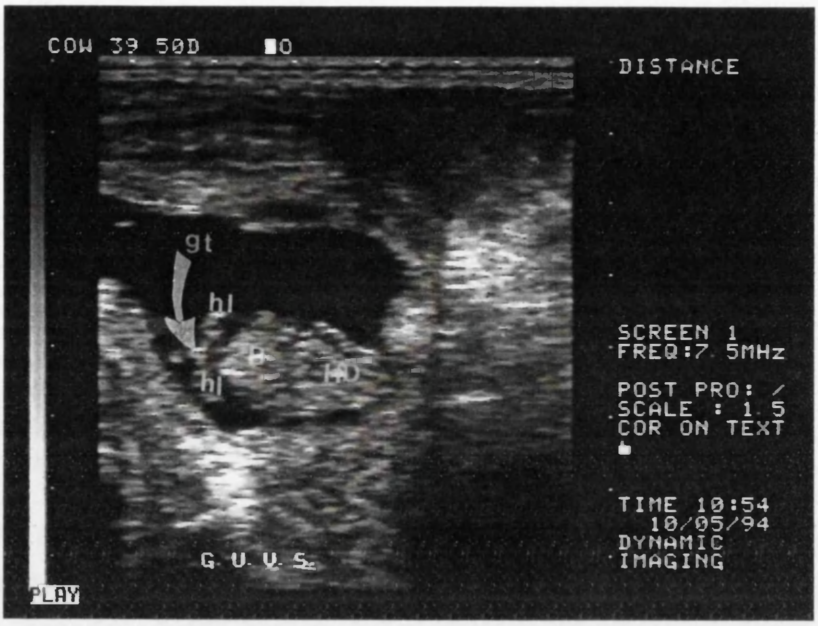
b. 46 days after insemination, br. cav.= brain cavity, st = stomach.



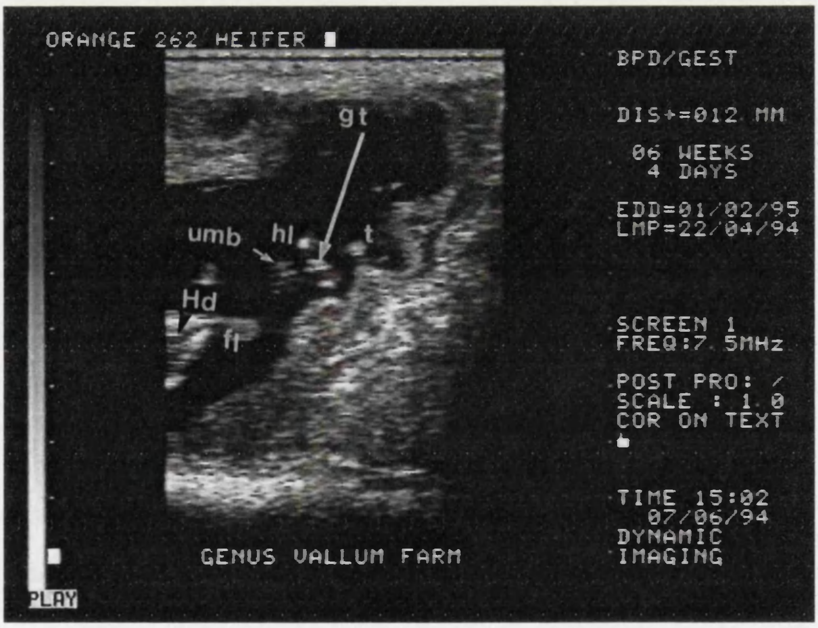
c. 47 days after insemination, bc = brain cavity, umb = umbilicus, hl = hindlimb, gt = genital tubercle and t = tail.



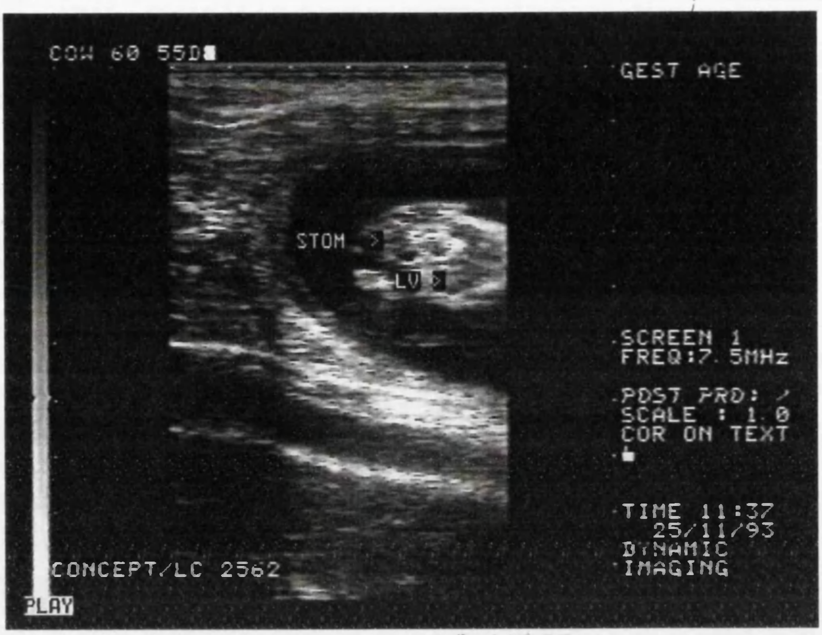
d. 49 days after insemination, Hd = head, fl = forelimb, Bd = body, umb = umbilicus, hl = hindlimb and gt = genital tubercle.



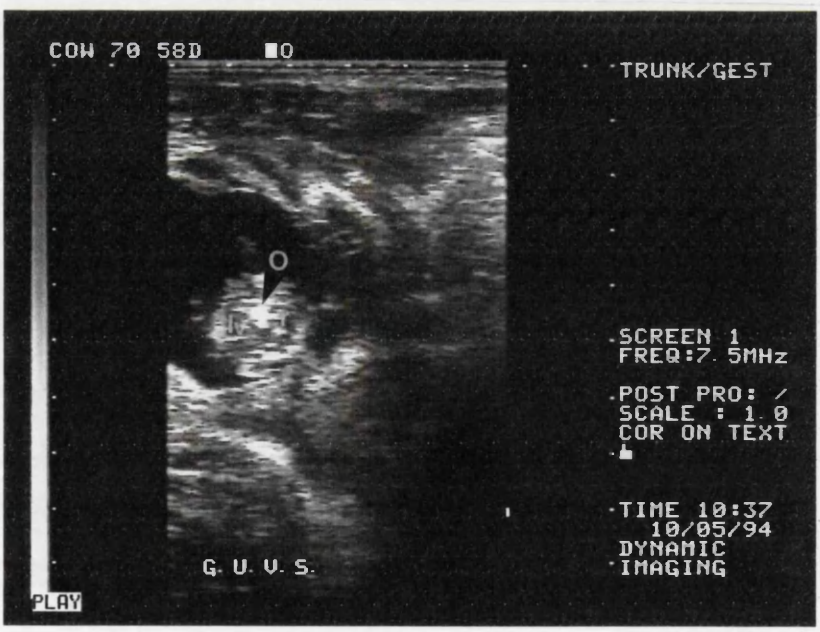
e. 50 days after insemination, Hd = head, B = body, hl = hindlimb and gt= genital tubercle.



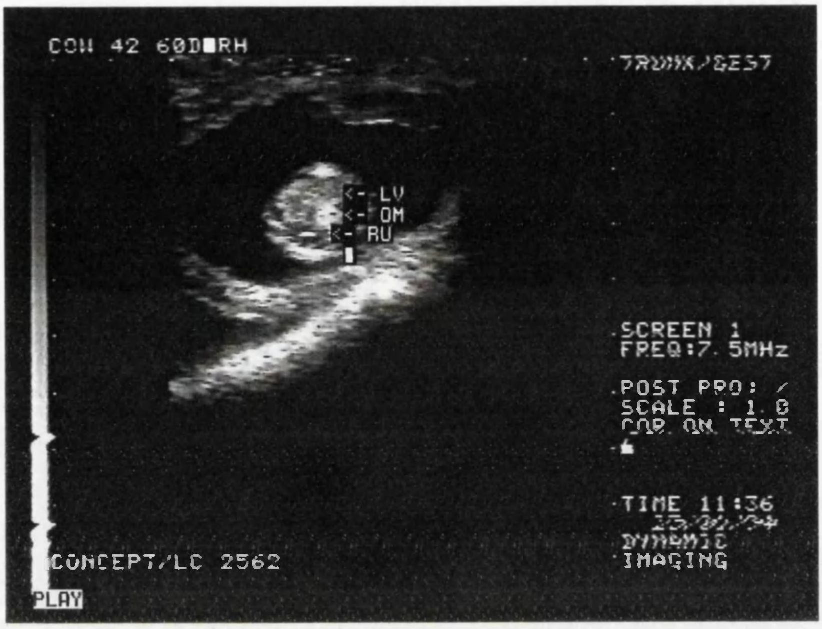
f. Dorsal ultrasound scan of a 50 days old fetus showing the head (Hd), forelimb (fl), umbilicus (umb), hindlimb (hl), genital tubercle (gt) and tail (t)



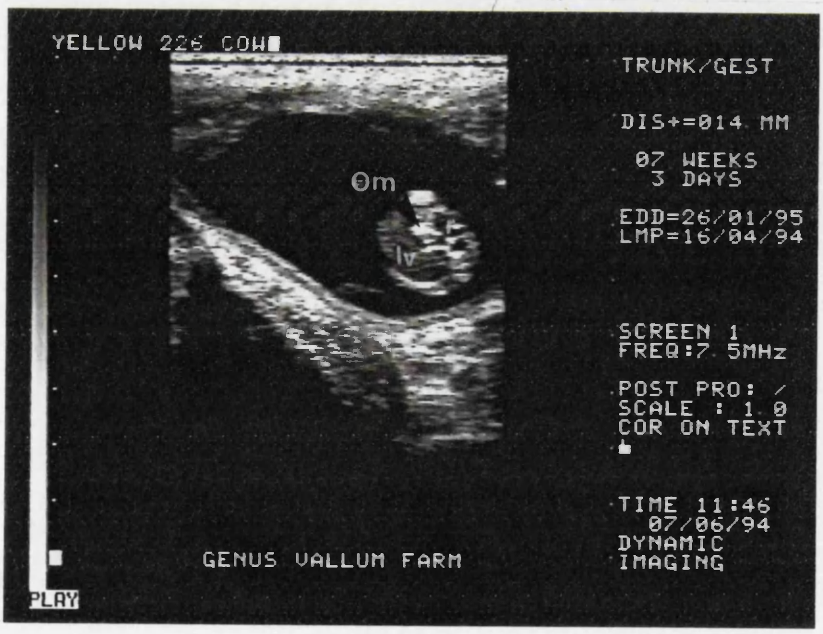
g. Transverse ultrasound scan of the abdomen at the level of the stomach (stom) and liver (lv) (55 days after insemination.)



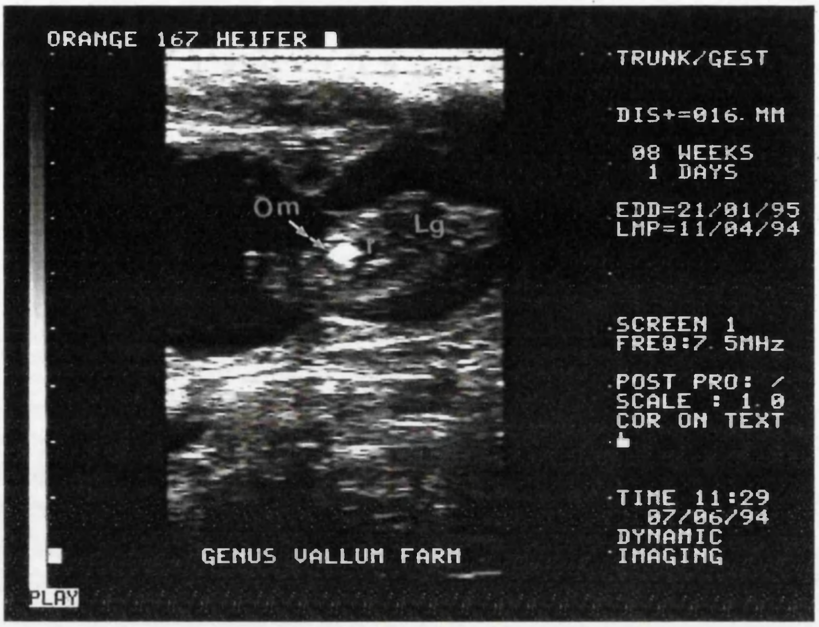
h. Transverse ultrasound scan of the abdomen at the level of the omasum (o), rumen (r) and liver (lv) (58 days after insemination.)



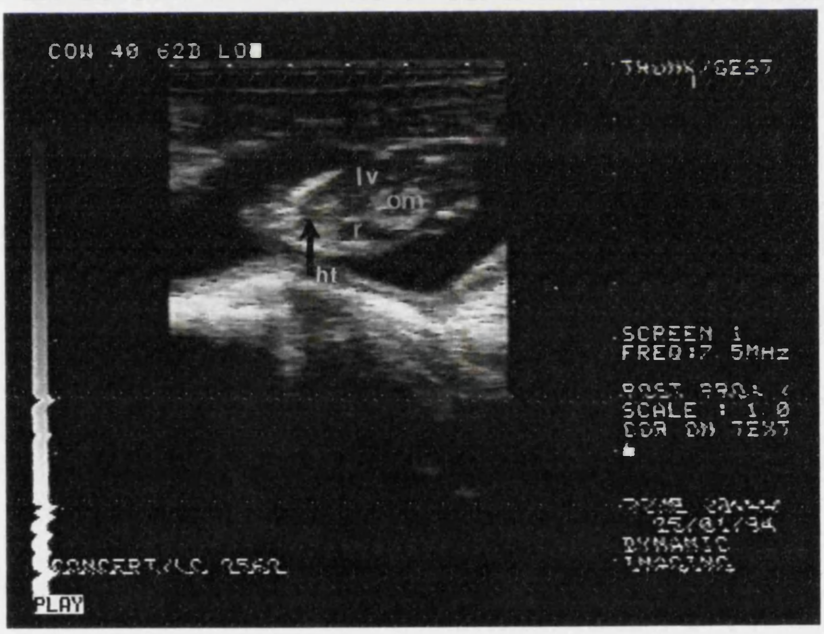
i. Transverse ultrasound scan of the abdomen at the level of the omasum (om), rumen (ru) and liver (lv) (60 days after insemination.)



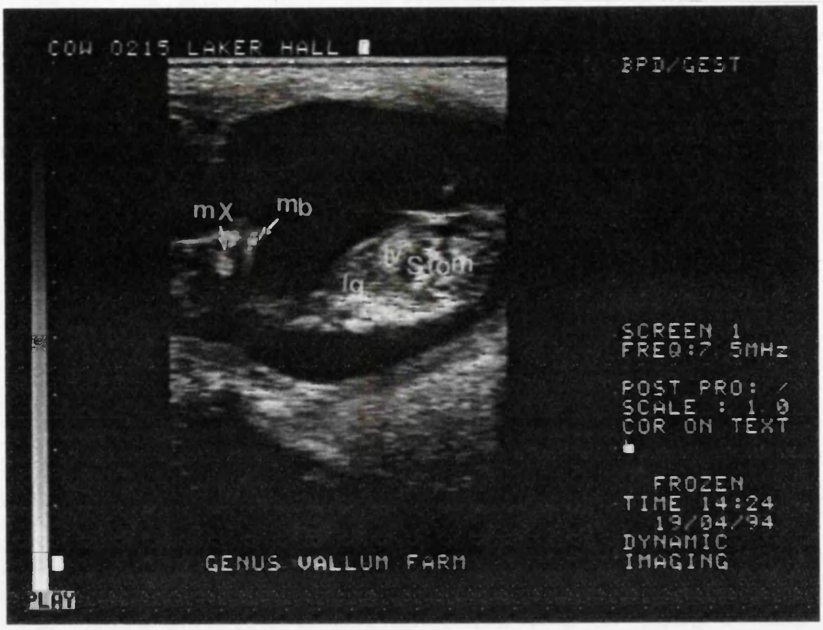
j. Transverse ultrasound scan of the abdomen at the level of the omasum (om), rumen (r) and liver (lv) (61 days after insemination.)



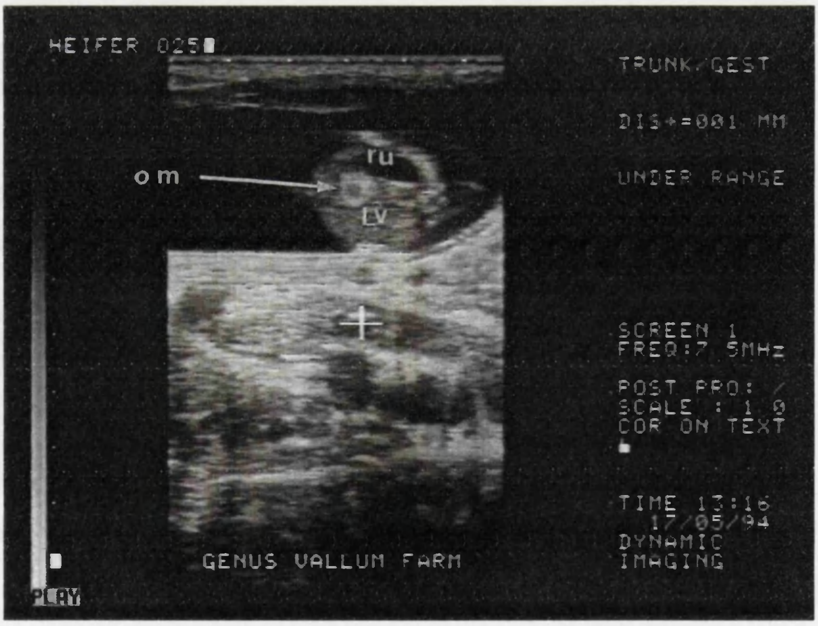
k. Longitudinal ultrasound scan of the trunk showing the lung (lg), rumen (r) and the omasum (om) (61 days after insemination.)



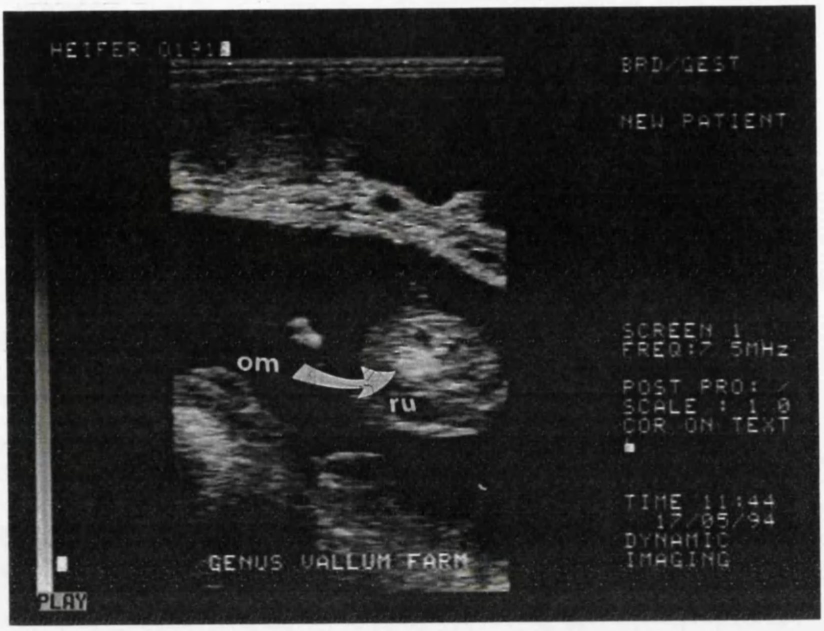
l. Dorsal ultrasound scan of the trunk showing the heart (ht), rumen (r), omasum (om) and liver. (lv). (61 days after insemination.)



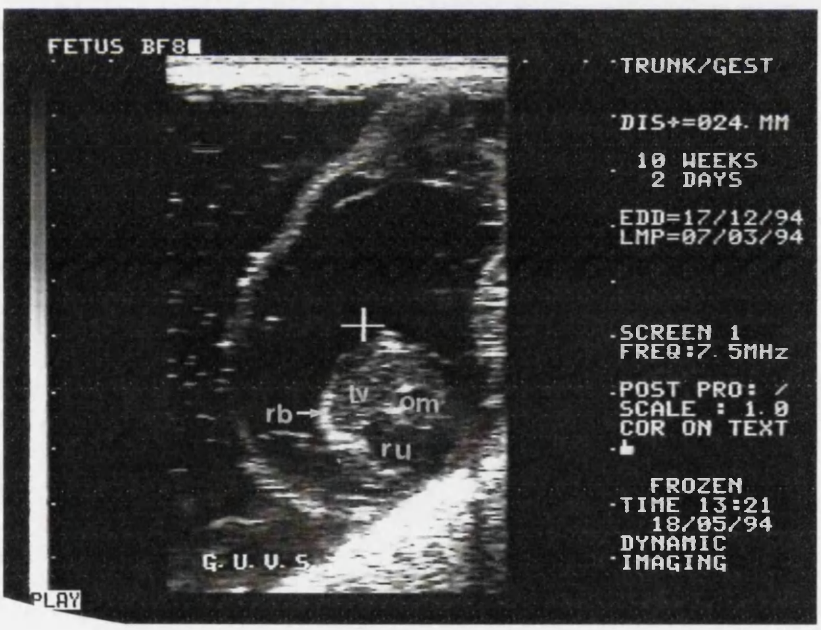
m. Longitudinal ultrasound scan of the fetus showing the maxilla (mx), mandible (mb), lung (lg), liver (lv) and stomach complex (stom). (62 days after insemination.)



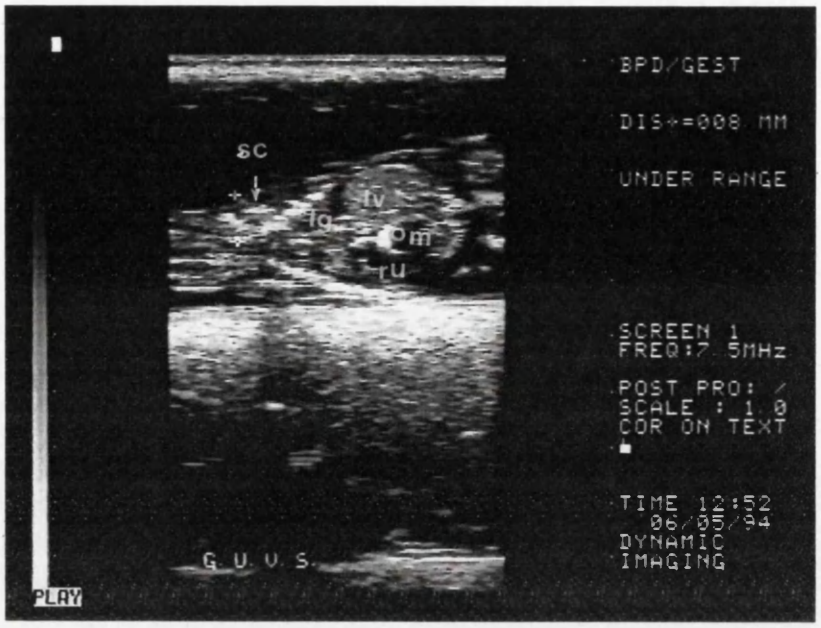
n. Transverse ultrasound scan of the abdomen at the level of the omasum (om), rumen (ru) and liver (lv). (68 days after insemination.)



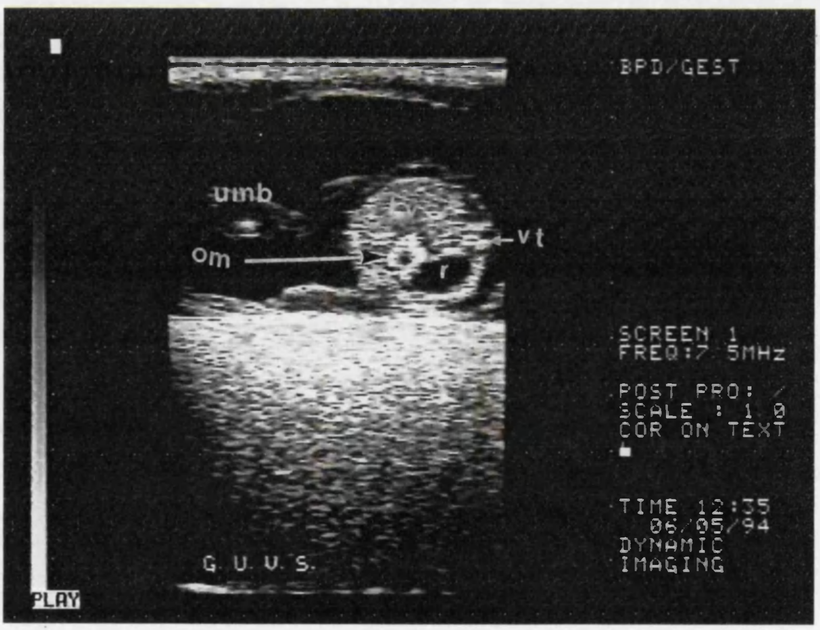
o. Transverse ultrasound scan of the abdomen at the level of the omasum (om) and rumen (ru). (69 days after insemination.)



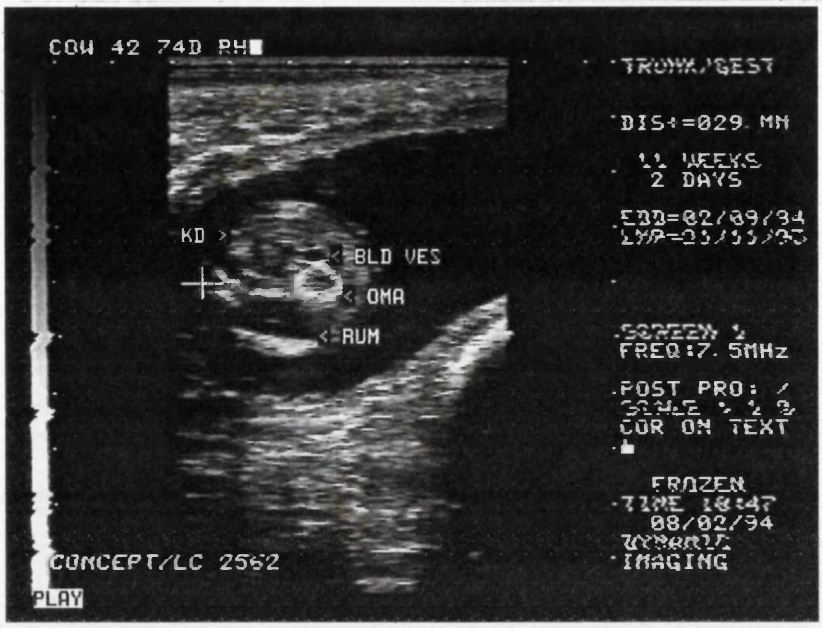
p. Transverse ultrasound scan of the abdomen at the level of the omasum (om), rumen (r) and liver (lv). rib (rb) (69 days after insemination.)



q. Dorsal ultrasound scan of the abdomen and the thorax showing the scapula (sc), lung (lg), omasum (om), rumen (ru) and liver (lv). (73 days after insemination.)

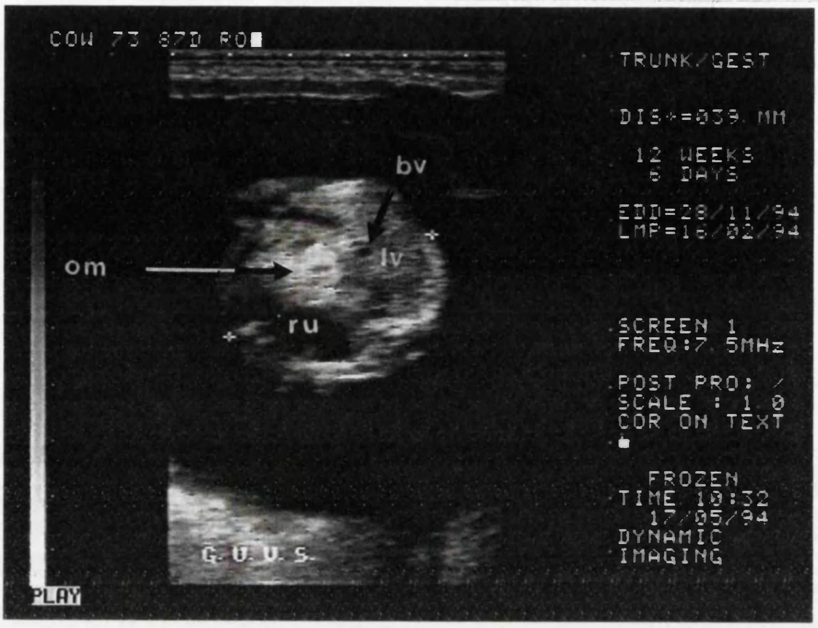


r. Transverse ultrasound scan of the abdomen taken at the level just cranial to the umbilicus (umb). Note the omasum (om), rumen (r), liver (lv). and vertebrate (vt) (73 days after insemination.)

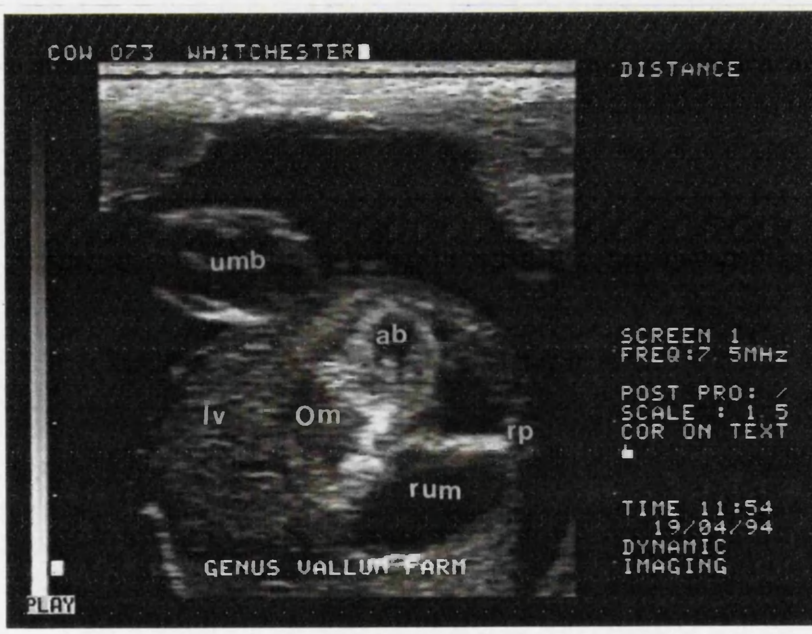


s. Transverse ultrasound scan of the abdomen taken at the level of the kidney (kd). Note the omasum (om), rumen (r), and blood vessel from the liver. (74 days after insemination.)

t. Transverse ultrasound scan of the cranial abdomen . Note the rib, umbilicus, omasum (oma), rumen (ru), liver and blood vessel of the liver. (76 days after insemination.)



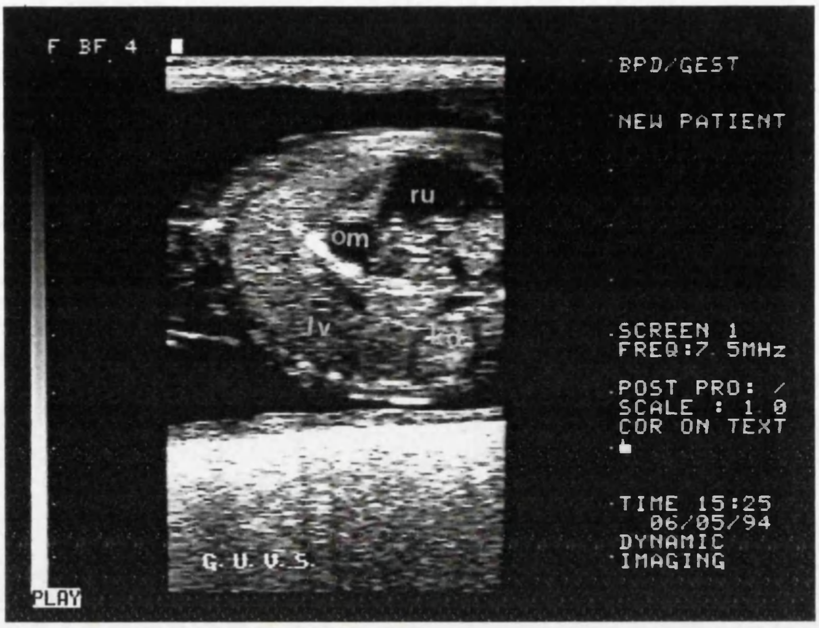
u. Transverse ultrasound scan of the abdomen taken at the level cranial to the umbilicus. Note the omasum (om), rumen (ru), liver (lv) and its blood vessel (bv). (87 days after insemination.)



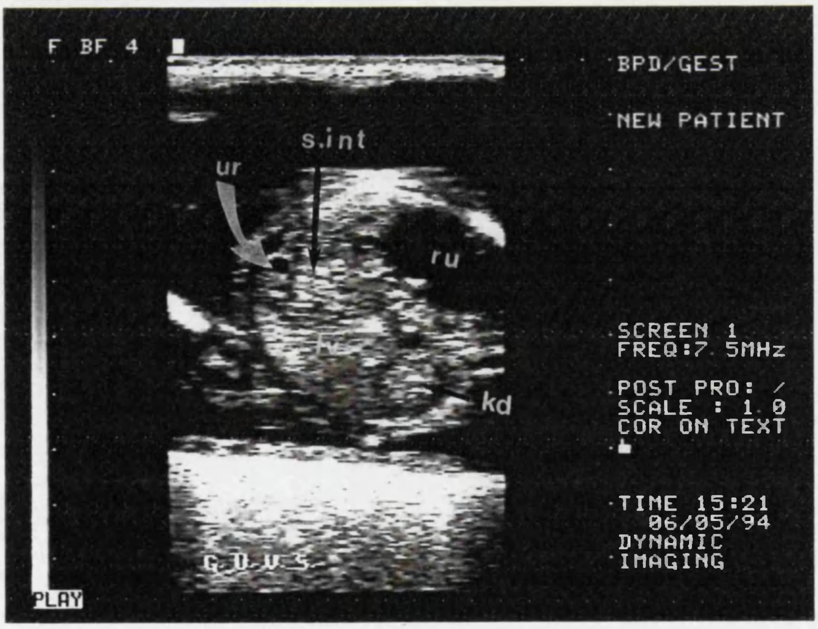
v. Transverse ultrasound scan of the abdomen taken at the level of the umbilicus. (umb). Note the abomasum (ab), omasum (om), blind sacs of the rumen (ru) divided by the ruminal pillae and liver (lv). (101 days after insemination.)

w. 101 days after insemination, cross section of the umbilicus (umb) and the abdomen. Note the four blood vessels within the umbilicus and the small intestine (s.int) in the abdomen.

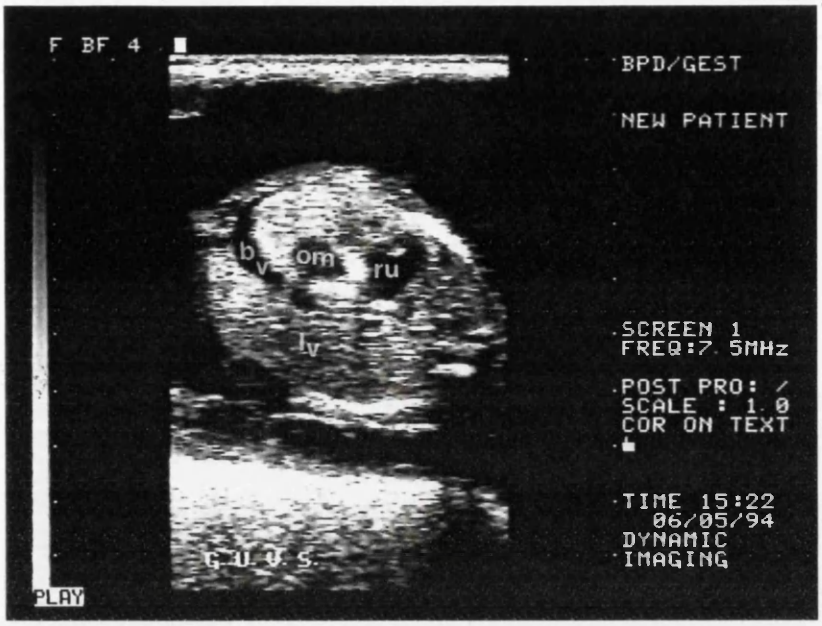
x. Serial transverse scans of the abdomen (7) and one dorsal scan of a fetus of estimated age 103 days.



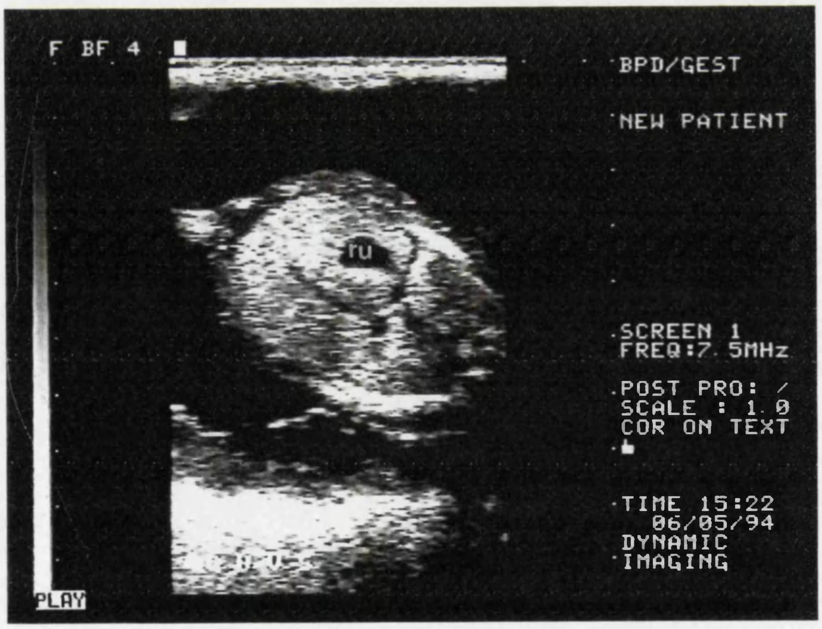
i. ru = rumen, om = omasum, lv = liver, and kd = kidney.



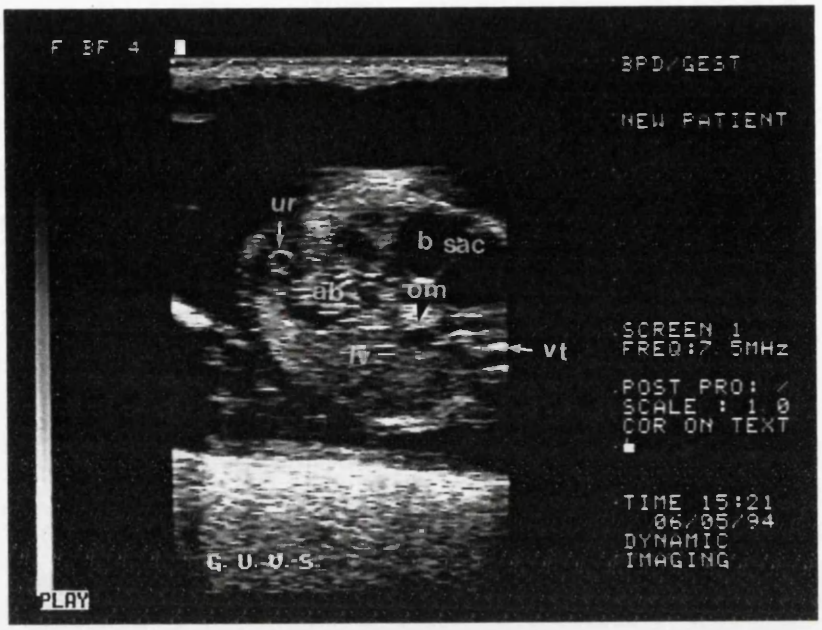
ii. s.int = small intestine, ur = urachus, lv = liver ru = rumen and kd = kidney.



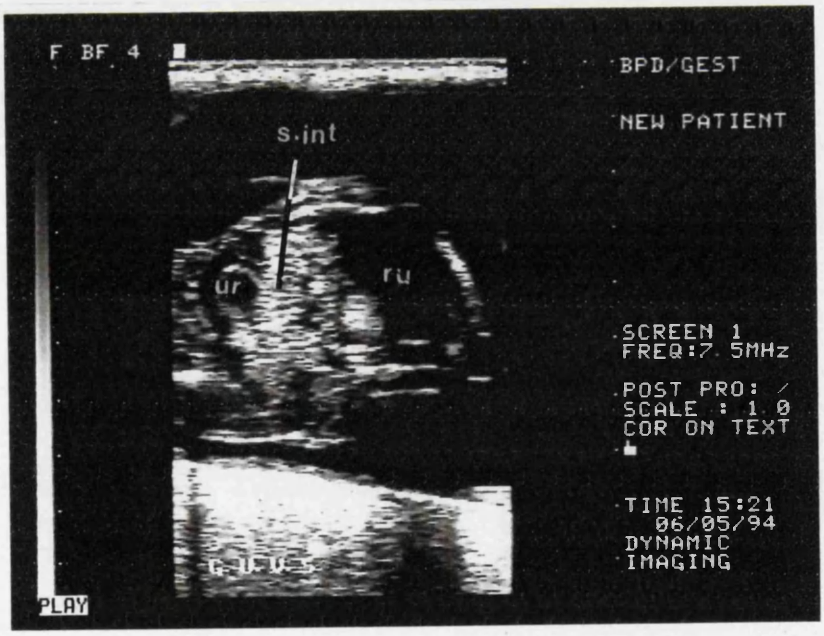
iii. om = omasum, bv = blood vessel running from the liver to the umbilicus, ru = rumen and lv = liver



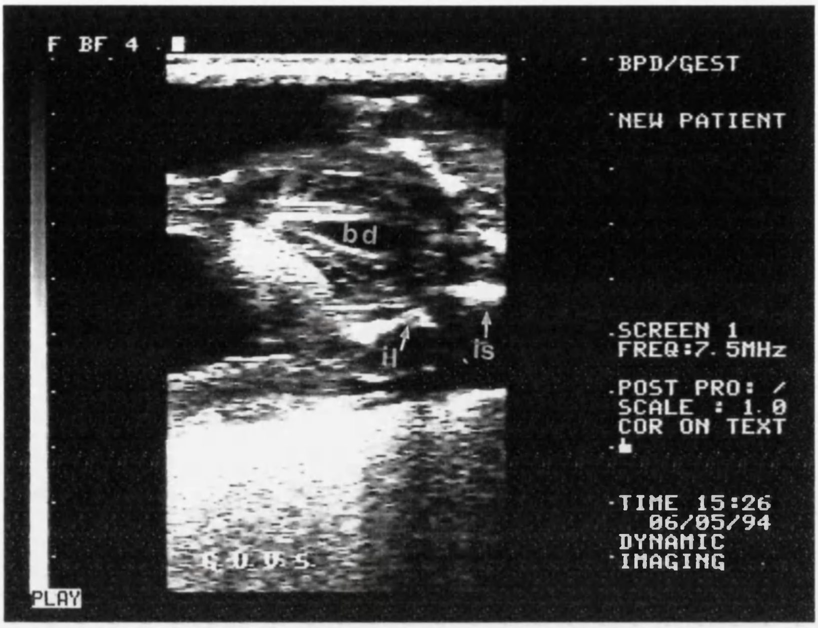
iv. ru = rumen and bv = blood vessel.



v. ur = urachus, ab = abomasum, b.sac =blind sacs of the rumen, om = omasum, lv = liver and vt = vertebrate The scan was taken at the level just caudal to the umbilicus.

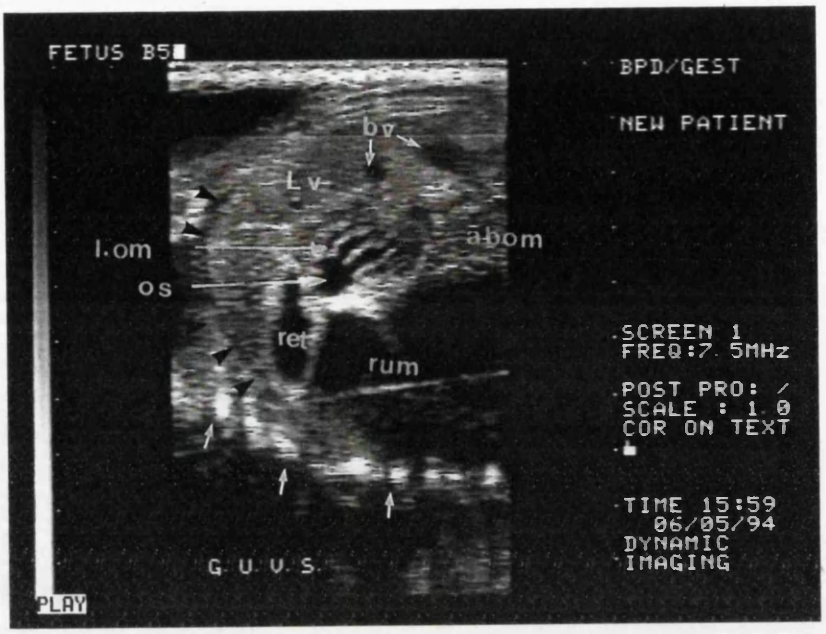


vi. ur = urachus, s.int = small intestine, ru = blind sacs of the rumen. The scan was taken at the level caudal to the umbilicus.

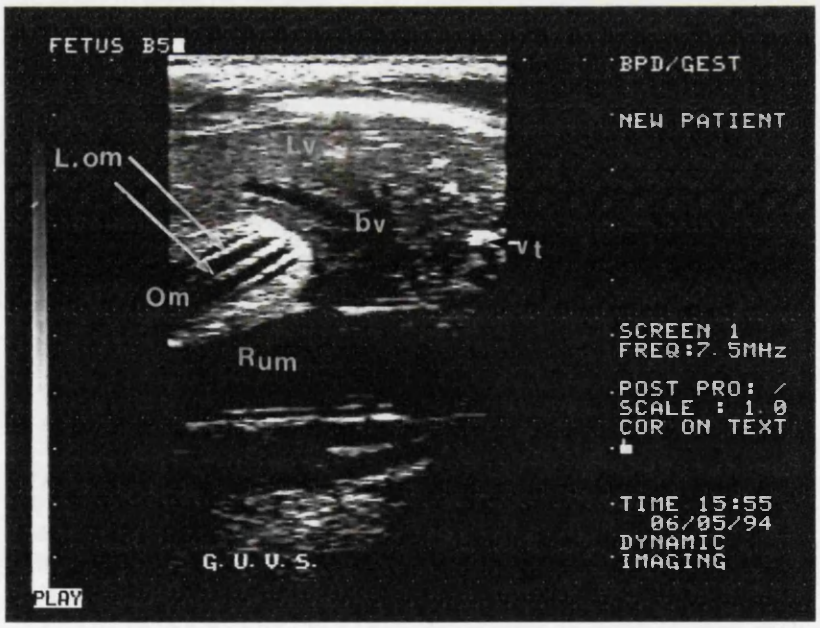


vii. bd = bladder, il = ilium and is = ischium. (Dorsal scan.)

y. Dorsal (i) and Transverse (ii) scans of the trunk of a fetus of estimated age 110 days.

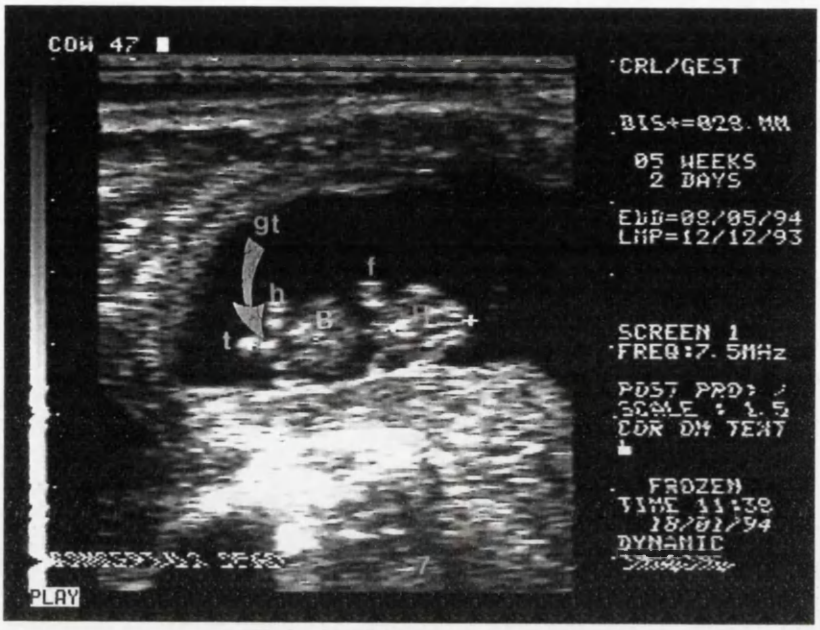


i. lv = liver and and its blood vessels (bv), l. om = lamina omasi, o.s = omasal sulci, ret = reticulum, abom = abomasum, rum = rumen, three white arrows = ribs and black arrows = diaphgram.



ii. L. om = lamina omasal, om = omasum, Rum = rumen, bv = blood vessel, lv = liver, and vt = vertebrates.

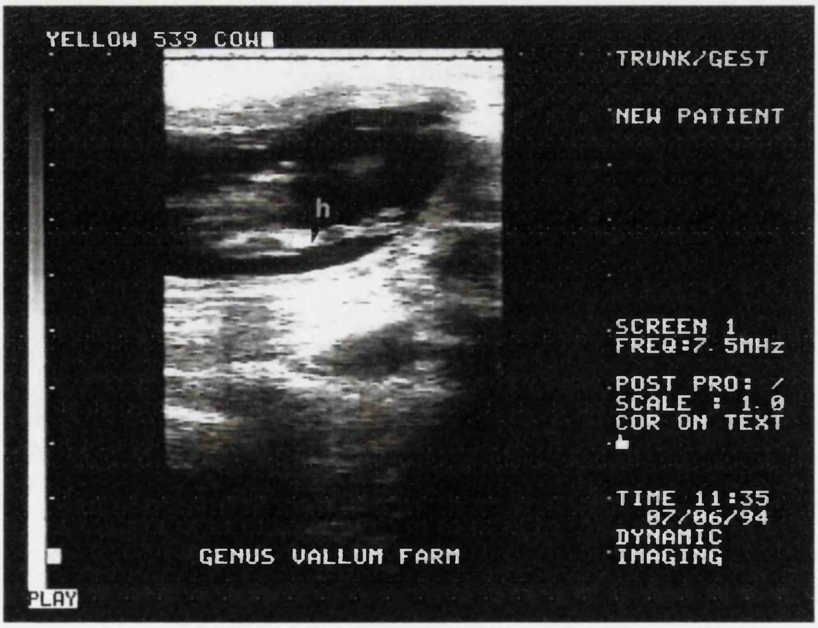
Figure 2.2.1. Ultrasonic appearance the appendicular skeleton of the bovine fetus at different developmental stages.



a. 47 days after insemination, H = head, f = forelimb, B = body, h = hindlimb, t = tail, and gt = genital tubercle.

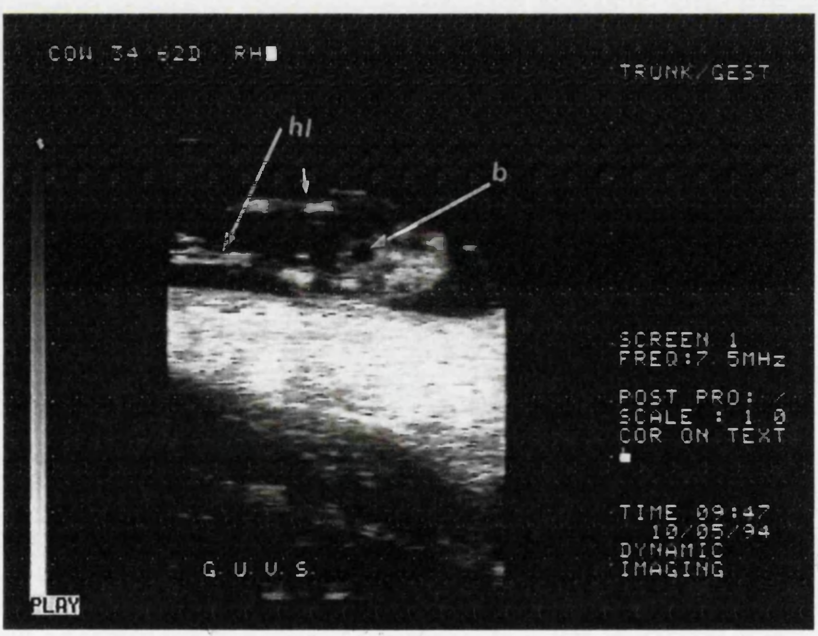
b. 47 days after insemination, bc = brain cavity, sc = scapula, hu = and humerus.

c. Dorsal ultrasound scan of a fetus, 50 days after insemination. H = head, f = forelimb, B= body. Note the parted hooves at the distal end of the forelimb.

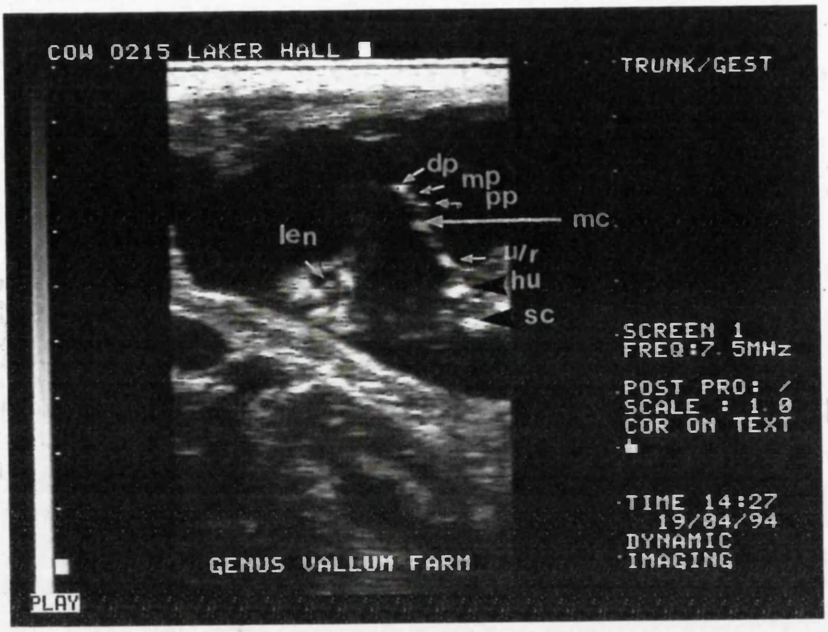


d. Transverse ultrasound scan of the hindlimb (h), 50 days after insemination.

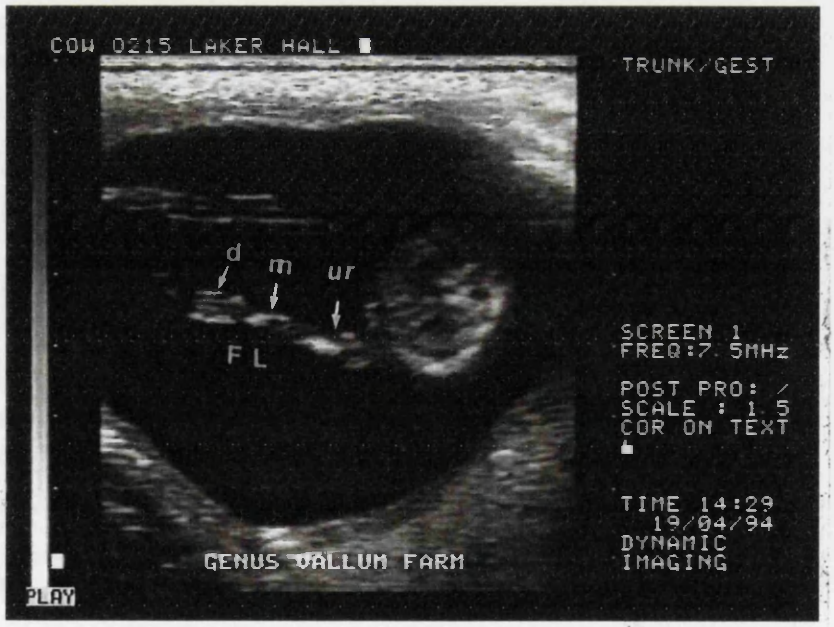
e . Transverse ultrasound scan of the forelimb, 60 days after insemination. Note the distal phalanges. (dig. ph)



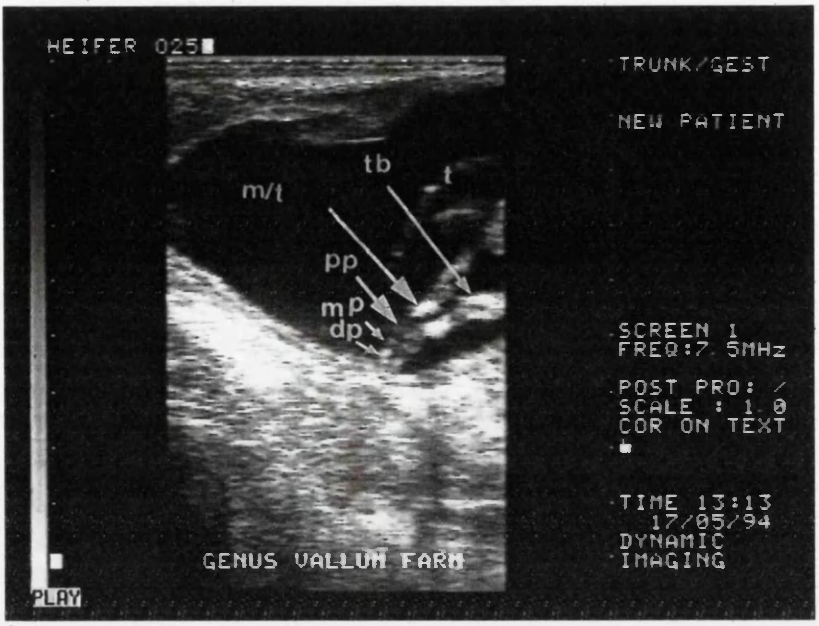
f . Transverse ultrasound scan of the hindlimb (hl), 62 days after insemination. Note the bladder (b)



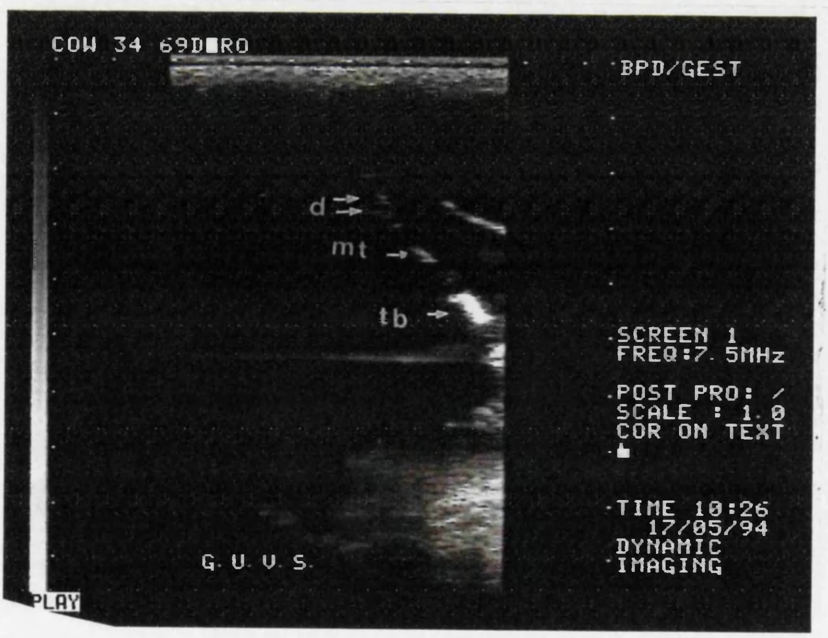
g. Longitudinal ultrasound scan of the fetus, 62 days after insemination. Note the lens (len), distal (dp), middle (mp) and proximal (pp) phalanges , metacarpal (mc), ulna/radius (u/r), humerus (hu) and scapula (sc)



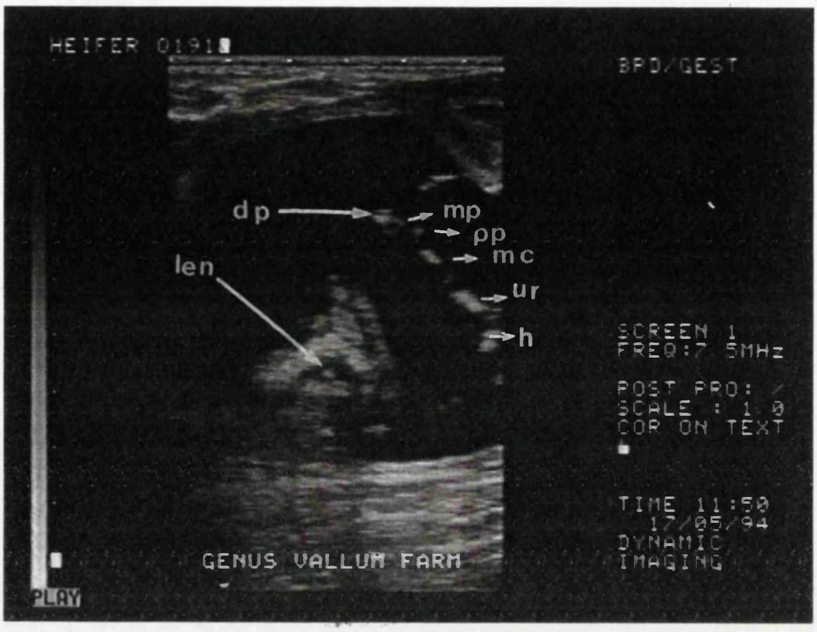
h. Oblique transverse ultrasound scan of the Forelimb (FL), 62 days after insemination. Note the difference in brightness between the skeletal elements of this scan and that of (g) above, although the two images were taken from the same fetus and during the same period.



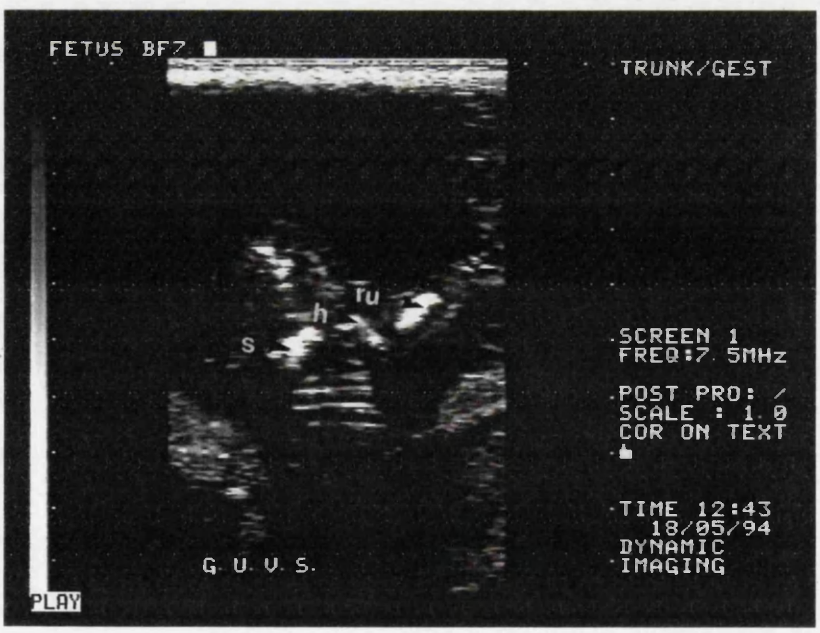
i. Transverse ultrasound scan of the hindlimb, 68 days after insemination. Note the distal (dp), middle (mp) and proximal phalanges (pp), meta tarsal (m/t), tibia (tb) and tail (t).



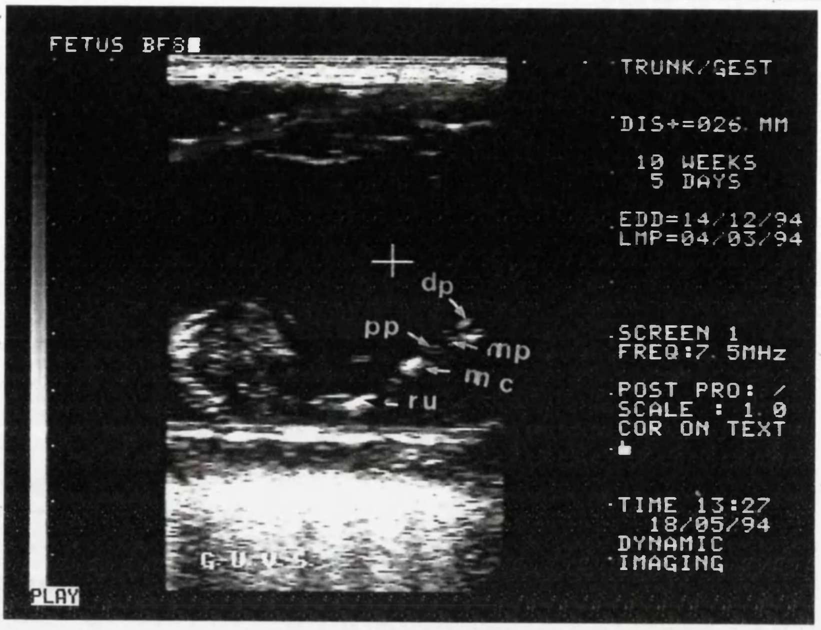
j Transverse ultrasound scan of the hindlimb, 69 days after insemination. Note the digits (d), meta tarsal (m/t) and the tibia (tb).



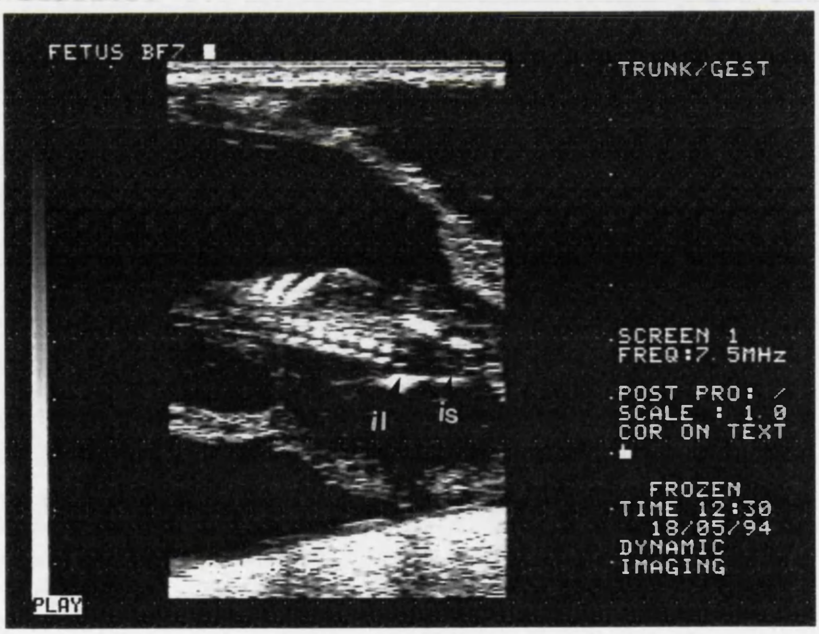
k. 69 days after insemination. Note the lens (len), distal (dp), middle (mp) and proximal (pp) phalanges , metacarpal (mc), ulna/radius (u/r), and humerus (hu).



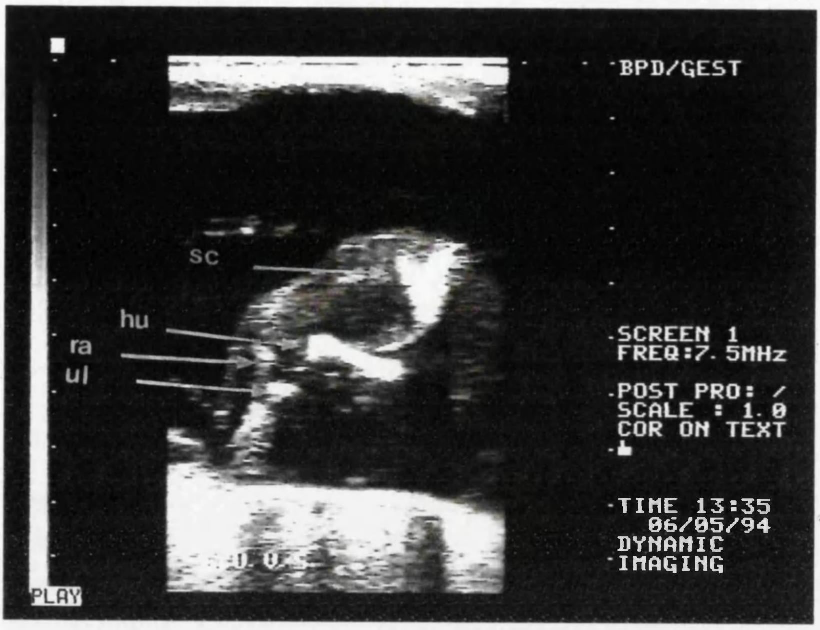
l. 69 days after insemination. Note the ulna/radius (u/r), humerus (h) and scapula (s).



m. Transverse ultrasound scan of the fetus, 69 days after insemination. Note the distal (dp), middle (mp) and proximal (pp) phalanges , metacarpal (mc) and ulna/radius (u/r).



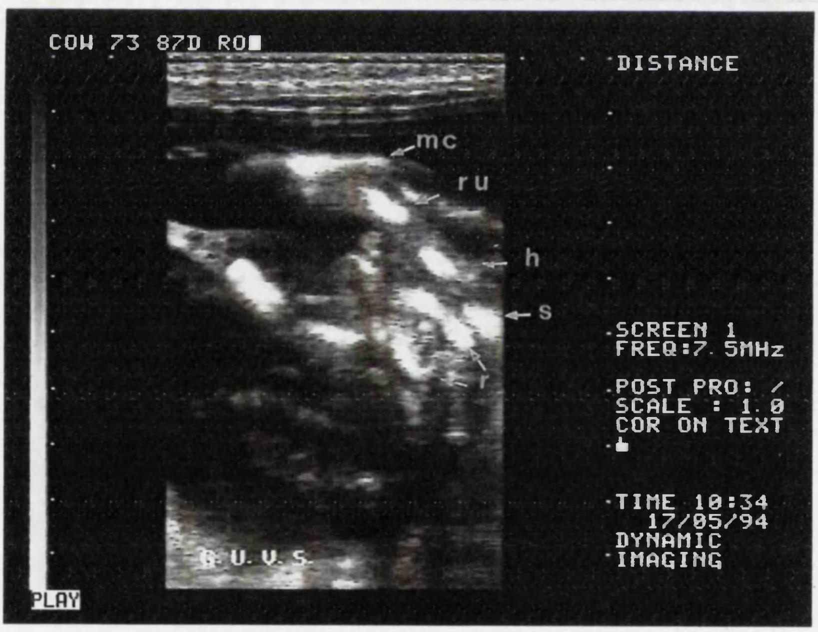
n. Dorsal ultrasound scan of a fetus of estimated age 69 days. il = ilium and is = ischium.



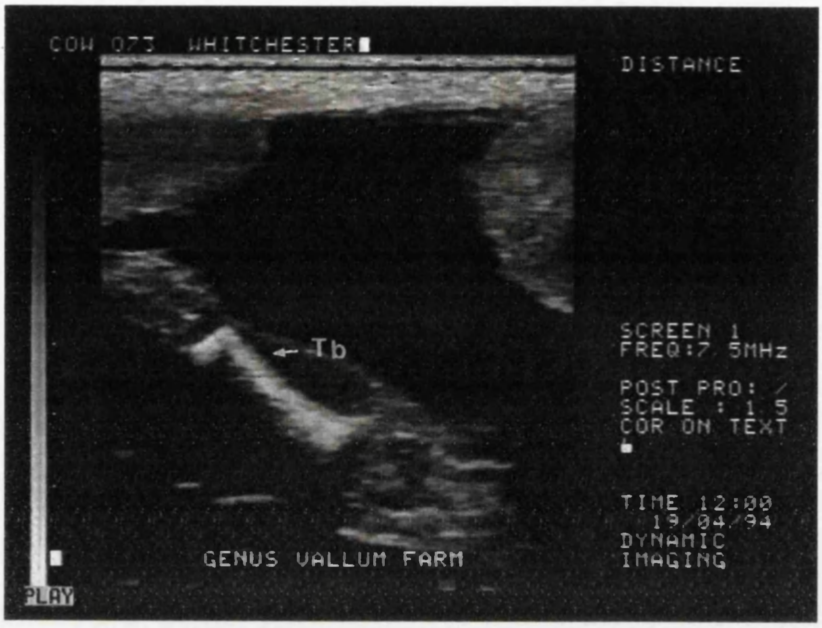
o. Fetus of estimated age 73 days, ul = ulna, ra = radius, hu = humerus and scapula.

p. Transverse ultrasound scan of the fetus, 76 days after insemination. Note the digits (dig), metacarpal (mc), ulna/radius (u/r) and humerus (hum).

q. Transverse ultrasound scan of the fetus, 76 days after insemination. Note the metacarpal (mc), ulna/radius (rd/ul) humerus (hum), scapula (sc) and ribs (rb).



r. Transverse ultrasound scan of the fetus at the level of the heart, 87 days after insemination. Note the metacarpal (mc), ulna/radius (r/u) humerus (h), scapula (s) and ribs (r).



S. 101 days after insemination. Tb = tibia.

Table 2.9. Time and order of appearance of fetal structures based on ultrasonography technique.

Fetal structures	Gestational age (days)
i. HEAD and NECK	
Brain case	By 39
Orbit	By 39
Maxilla	By 39
Mandible	By 39
Cranium	By 41
Lens	By 53
Nasal cavity	55 - 60
Hard palate	55 - 60
ii. THORAX:	
Heart	
Pulsation	By 39
Diffused outline and partition	By 53
Clear outline and partition (Including septum and pericardium)	By 70
Blood vessel (proximal to heart)	

Identifiable	By 45
Differentiation of aorta and v. cava.	70 - 76

Lung

Identiable	55 - 60
Tracheal bifurcation and bronchioles	By 73

Diaphragm outline	By 73

iii. ABDOMEN

Liver

Identifiable	By 49
Blood vessel	By 49

Umbilicus

Identifiable	By 39
Blood vessel	By 60

Stomach (general)

Identifiable	By 39
Signs of differentiation	By 53
Reticulo-rumen identifiable	By 56

Reticulum

Identifiable	By 74

Omasum

General outline identifiable	By 56
Lumen	55 - 60
Laminae omasi	By 70
Omasal sulci	> 70

Abomasum

Identifiable	By 74
Longitudinal folds	By 80

Rumen

Ruminal sacs identifiable	By 60
Blind sacs identifiable	By 74

Intestines

Identifiable	By 53

Kidneys

Identifiable	By 61

iv. PELVIC STRUCTURES

Genital tubercle	By 47
Scrotum	By 76
Bladder and urachus	By 57

Chapter 3

Radiology and Bone staining:Fetal osteogenesis.

3.1. Equipment and materials.

3.1.1. Radiology

a). X-ray machine: Model D-44 (A.E Dean & Co. Ltd. Croydon England.)

b). 0.5% (volumetric) aqueous solution of silver nitrate.

c). 95% alcohol.

d). Film and screen type: Dupont cronex and Quantum Fast datail, respectively.

3.1.2. Alizarin red stain.

The chemicals and procedure used were as described by Kimmel and Trammell (1981) as earlier stated in chapter 1.

a). Staining solution:

Alcian blue 0.14% in 70% ethanol , filtered	2 parts.
Alizarin red S 0.12% in 95% ethanol , filtered	1 part .
Glacial acetic acid	8 parts.
70% Ethanol	50 parts.

b). Acetone.

c). 90% alcohol.

d). 1% Potassium permaganate.(kOH)

e). Glycerin.(pure)

f). Hydrogen Peroxide

3.2. Method.

Ten pregnant bovine uteri (Approximately, 40-90 days.) were collected from Glasgow and Paisley abattoir. The specimens were predominantly of the Freisian breed of cattle. The breeds of the rest of the specimens was not known.

The fetuses in the uteri were first scanned and in a water bath using the same equipment and measurements as described in chapter 2. The fetuses were then removed by incising the uteri and its membranes and ligaturing of the umbilical cord. They were sexed, weighed and measured (c.r.l). Ages were estimated by measuring various fetal dimensions using the computer programme on the scanner. The final figure of the fetal age taken was the average of these measurements. The specimens were within the range of 64.5-115 days of age. The smaller fetuses were hemisected and one half of each specimen was either stained with alizarin red S. or radiographed after treating them with silver nitrate.

3.2.1. Radiology. The specimens were radiographed in dorsal-ventral and lateral projections. Younger fetuses with poor radiological density were first fixed in 95% alcohol for forty-eight hours (48) and then impregnated with 0.5% aqueous solution of silver nitrate for two (2) to three (3) days as described by Hodges (1953), Boyd (1974). The specimens were immersed in silver nitrate just long enough for partial replacement of calcium by the silver. Prolonged immersion of the specimens leads to high silver deposits in the soft tissue as earlier explained in the introduction. The limbs of the larger fetuses were dissected free and radiographed separately. The screen and Ma S range used were QD (Quantum datum) and 3.2-80, respectively. The K.V. varied between 38-48, according to the size or density of the specimen.

3.2.2. Alizarin red S. The specimens were completely eviscerated, rinsed in tap water, and immersed for about 30 seconds in a water bath heated to 70 C. These steps helped to strip off the skin of thelarger specimen more rapidly. The specimens were then stained in the staining solution above for two to three days depending on the size. They were later transferred into 90% alcohol for twelve (12) to twenty- four (24) hours before treating them with 1% KOH for about twenty-four (24) hours. They were washed in increasing concentrations of glycerine in 1% KOH i.e. 1:4, 2:3, 3:2 and 4:1 concentration ratios, respectively. Finally, they were stored in pure glycerine with a drop of hydrogen peroxide to prevent mould .

Cartilage stained blue while the calcified bone stained bright red.

3.3 Results.

Results of bone stained and silver nitrate treated X-ray specimens were the same, hence, the overall results for the two techniques have been presented as one.

The diaphyseal loci of the humerus, radius and femur were almost of the same length between Day 64.5 to 115 of gestational age. However, diameters of the radius and ulna were slightly smaller than those of the humerus especially in older fetuses. The diaphyseal locus of the tibia had the longest length of all the long bones of the appendicular skeleton during the period of study. The long bones had already attained rod-like shapes by Day 64.5 of gestation while the scapula and ilium had almost reached their final definative shape. Initially, there was a marked difference in size between the loci of the individual bones of the os coxae .This difference was gradually reduced as fetal age advanced.

Almost all diaphyseal loci of both the pectoral and pelvic girdle had appeared by Day 64.5 of gestation. There was a low level of activity of ossification between Days 60-70 of pregnancy with almost no increase in length of the already present loci and very few new loci

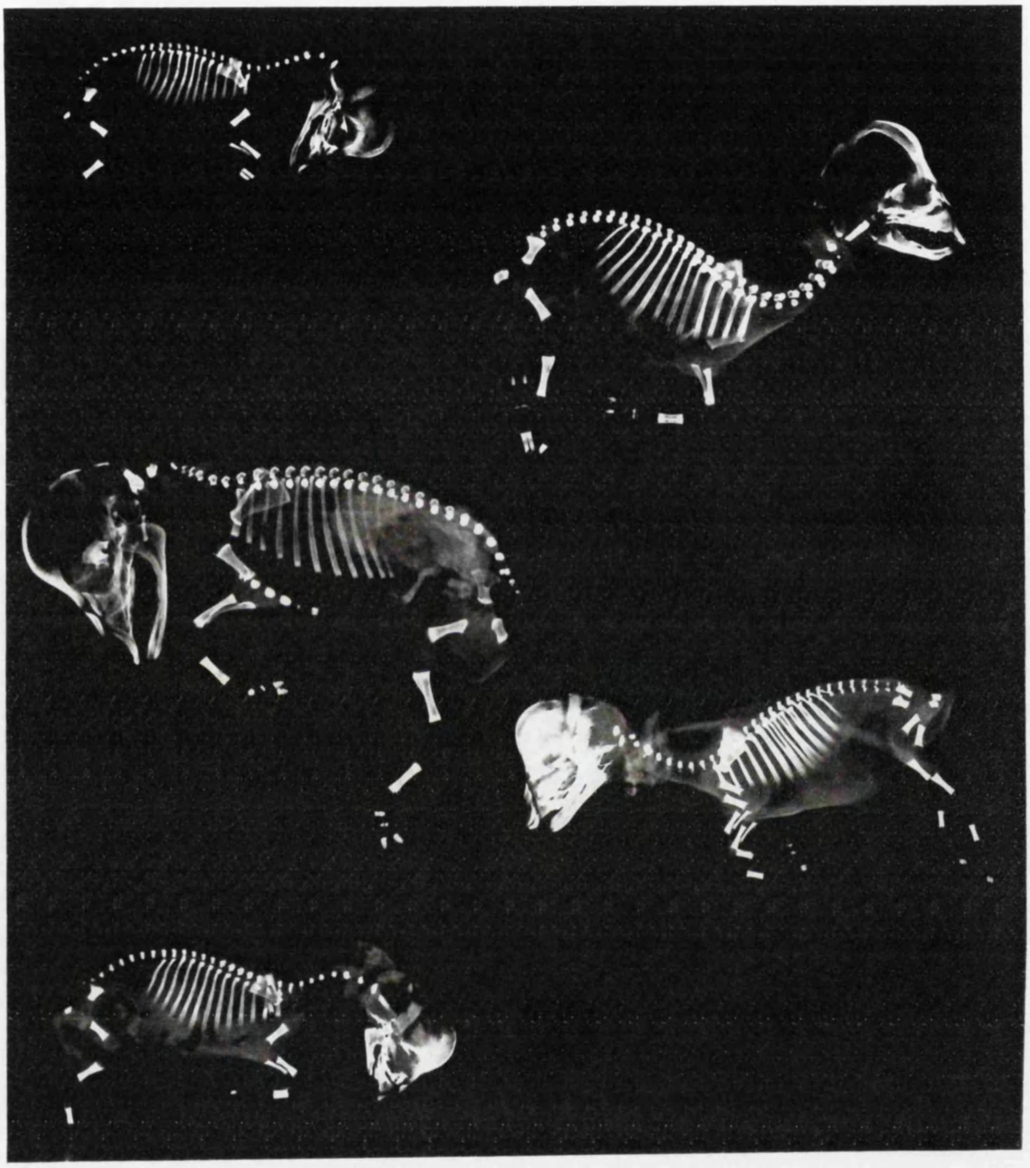


Figure 3.1. X- ray photographs (Silver nitrate preparation.) of Bovine fetuses. (a) 64 days.(b) 74 days (c) 91 days (d) 70 days (e) 69 days. Note fibula (fb) and metapodial 5 (m5)

appearing. By Day 74 of gestation all loci of the three phalanges had been formed. The loci of the distal phalanges of digits 3 and 4 of both limbs appeared first followed by those of the proximal phalanges and lastly the middle phalanges. By Day 64.5 the locus of the distal phalanx had already appeared while the proximal phalanx had already appeared by Day 74 of gestation.

The diaphyseal locus of the first transient element, the fibula, was present by day 64.5 of pregnancy. It was more clearly seen in a Day 74 old fetus. The locus of the other transient element, metapodial 5, was seen in fetuses of gestational age 69.2, 69.8, and 74 days. No other loci of transient elements were observed.

3.4 Discussion and conclusion.

The lengths of most diaphyseal loci of the forelimb and the pelvic girdle were found to be equal. This finding is very much in accord with the measurements reported in a previous study (Gjesdal,1969).

Few studies make mention of the development of the scapula and the os coxae in their reports. Richardson and others (1990) in a fairly detailed study of the number of ossification centres of the appendicular skeleton in relation to age, described all the loci of pectoral and pelvic bones, except those of the scapula and the os coxae. The majority of the other studies only make mention of these bones very briefly. Lindsay (1969) reports to have seen the first signs of ossification in the scapula at Day 52 while at Day 61 the scapula was reported to have been in a diffuse form (Gjesdal, 1969). Winters and others,(1942) in their study on bovine prenatal development report that the scapula attained its final form by Day 90 of gestation compared to less than Day 64.5 in the present study. The present study observed a well defined locus of the scapula as early as Day 64.5 of gestation

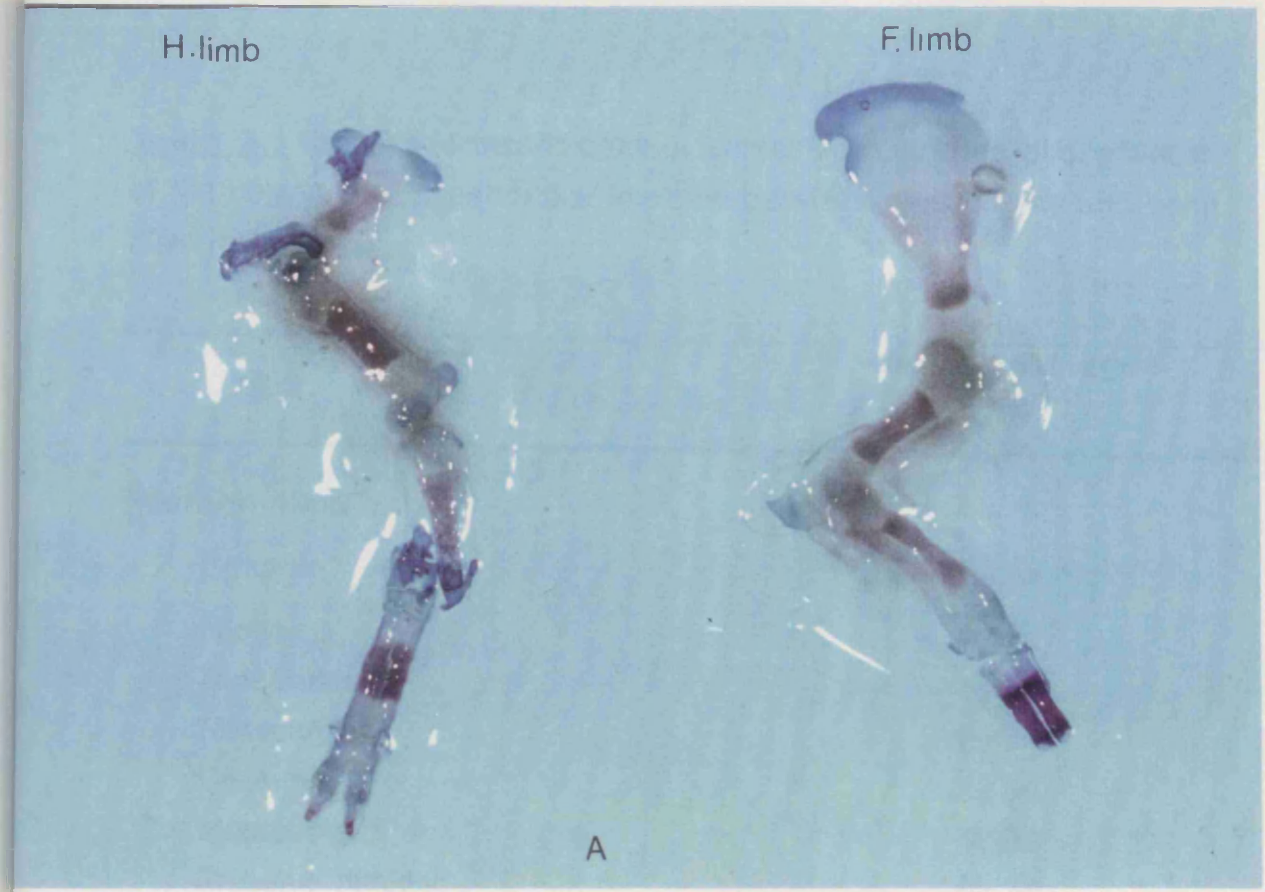
The times of appearance and regression of the transient elements observed in this study approximated with the ones reported in a review report by the committee of bovine nomenclature (Committee on Bovine reproductive nomenclature, 1972.). According to this review, the loci of all the transient elements appeared between Day 50 and 60 and regressed by about Day 90 of gestation. However, the locus of metapodial 2 in the present study could not be seen, probably because of the plane of projection used to radiograph the fetuses'.phalanges. Other similar studies gave a general date of the appearance of phalangeal loci of ossification and their figures were twenty to thirty days more when compared to those indicated in review report of the committee (Winters and others,1942, Gjesdal,1969.)

In general, the times and order of appearance as given by Lindsay were close to the present findings (Lindsay,1969). This study observed that almost all the diaphyseal loci of both the forelimb and the pelvic girdles had appeared by Day 64.5 of gestation and the loci of digits 3 and 4 distal phalanges appeared first by Day 64.5 of gestation, followed by proximal phalanges and lastly, by the middle

Although the analysis of the findings of the present study were limited by the small sample size, the wide gaps and irregular ages between the specimens used, its findings, in general, were found to be very similar to some of the most detailed studies carried out in the past (Lindsay, 1969, Gjesdal,1969, Burt and others,1968, Fedrigo, 1957). The main aim of this study was to form a basis for comparing the performance of radiography and bone staining with the ultrasonography technique in studying the general development of bovine prenatal skeleton, as explained in the introductory chapter.

Tables 3.1 and 3.2 show the chronological order and earliest time of appearance of loci of ossification in the appendicular skeleton of the bovine fetus, as identified by the techniques of radiography and ultrasonography, respectively. The results shown in the two tables, clearly indicate that the onset and order of loci of ossification, as identified by ultrasound and radiography, are similar. However, there were a few slight differences in some of the observations. The ultrasonography technique detected the onset of ossificstion in the proximal parts of the limb (scapula/os coxae to metacarpal/metatarsal) about ten (10) days earlier than radiography, while the clarity and detail of the images of the osseous structures of radiography were better than those of ultrasonography. Transient elements, like the fibula and metapodial five (5) were identified in radiographed fetuses, while in ultrasound images of fetuses of similar ages, they could not be identified. Transient elements could only be identified in radiographed fetuses treated with heavy metals. In general, the external outline and clarity of the osseous structures of the limbs, in the early stages of development, appeared better in radiography than in ultrasonography, until after Day 74 of gestation.

Figure 3.2. Bovine fetuses (a) 64.days.(b) 69 days, Alizarin red S. preparation.


H.limb
F.limb
A

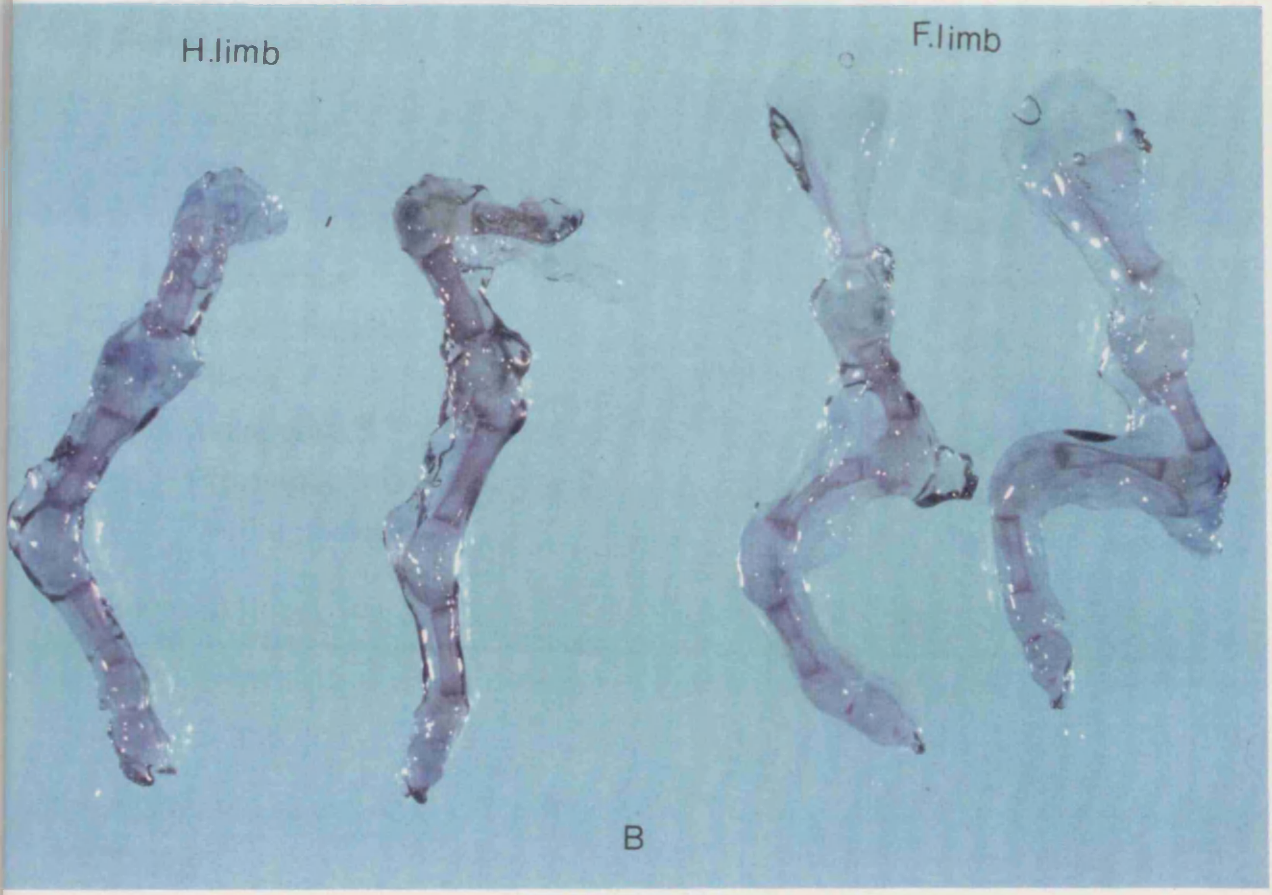

H.limb
F.limb
B

Table 3.1 Estimated time and order of appearance of the loci of ossification of the bovine fetal appendicular skeleton based on radiography and bone stain techniques.

Bone type	Gestational age (days)
Pectoral limb	
Scapula	By 64
Humerus	By 64
Ulna/radius	By 64
Metacarpus	By 64
Distal phalanx	By 64
Metapodial 5	By 69
Proximal phalanx	By 74
Middle phalanx	By 74
Pelvic limb	
Os coxae	By 64
Femur	By 64
Tibia	By 64
Metatarsus	By 64
Distal phalanx	By 64
Fibula	By 64
Metapodial 5	By 69
Proximal phalanx	By 74
Middle phalanx	By 74

Table 3.2 Earliest time and order of appearance of the loci of ossification of the bovine fetal appendicular skeleton based on ultrasonography technique.

Bone type	Gestational age (days)
Pectoral limb	
Scapula	By 47
Humerus	By 47
Ulna/radius	By 49
Metacarpus	By 53
Distal phalanx	By 60
Proximal phalanx	By 69
Middle phalanx	By 74
Pelvic limb	
Os coxae	By 47
Femur	By 47
Tibia	By 49
Metatarsus	By 53
Distal phalanx	By 60
Proximal phalanx	By 69
Middle phalanx	By 74

Chapter 4

General discussion and conclusion.

This study was undertaken with the aim of assessing the use of a high frequency transducer (7.5 MHz) and quality ultrasound scanner in the study of intra-uterine bovine fetal development.

The present study has demonstrated that valuable information of intra-uterine development of bovine prenatal life can be gained by ultrasonography. Transrectal sonography makes it possible to measure dimensions of the developing fetus and thus, determine the age of the fetus. Sonographic imaging in addition, makes it possible to observe most macroscopic anatomical structures of the developing fetus. This study has also demonstrated that the techniques of ultrasonography and radiography identify loci of ossification of the appendicular skeleton at similar ages of gestation.

Most previous studies on bovine prenatal life were based mainly on dead fetuses obtained either by induced abortion or from abattoirs, until the advent of ultrasonography. (Richardson and others,1990, Winters and others, 1942, White and others,1985.). Undoubtedly, past studies on bovine prenatal life using conventional methods have generated valuable information. However, the use of dead fetuses in conventional studies, from either induced abortion or postmortem surveys, makes it difficult to obtain some of the information of the developing fetus.

The technique of ultrasound makes it possible to study large numbers of intra-uterine fetuses more simply, cheaply, accurately and rapidly than the conventional methods. There are more than eighteen measurable bovine fetal dimensions possible by ultrasound, although, only a few of these have strong associations with fetal aging (Kahn, 1989, White and others, 1985.). Past sonographic studies have established the relationship between some of the fetal parameters and fetal aging using mathematical models like polynomial, linear regression and logarithmic models (White and others, 1985, Kahn, 1989.). However, no study on bovine fetal aging is known to have tested the fitness of the models and described the characteristics of fetal growth that their coefficients represent.

Most of the findings in the present study are in accord with previous studies (White and others, 1985, Kahn, 1989,) However, this study is the first to give a more detailed analysis of the mathematical models ; their fitness, weakness, strength and appropriate use in the study of bovine prenatal development. It also gives a more detailed account of the sonographic appearance of bovine fetal stomach in the early fetal stage.

The first chapter gives a historical and scientific review of the conventional and the sonographic studies on bovine prenatal development, in general. This chapter also gives the principles and interpretations of ultrasonography and their importance. A thorough knowledge and skill to interpret ultrasonography images correctly was emphasised. A lack of knowledge and skill in interpretation will not only make it hard to understand ultrasound images but also lead to the danger of erroneous interpretations and conclusions. The two main types of ultrasonographic artifacts, based on causation, namely Biological and Engineering, are mentioned. The five different types of biological artifacts, which is the most important of the two types of artifacts, are described briefly.

In chapter two , the use of a transducer with a high frequency (7.5 MHz) and a quality scanner to study bovine fetal development is assessed. An attempt was also made to observe and describe the ultrasonic anatomy of the gross tissues and organs of the developing bovine fetus between gestational age 45-90 days. This study was the first to use a transducer of such a frequency (7.5 MHz) to study the development of early bovine fetal development. (45-90 Days of gestation.)

The growth rate and patterns of three fetal parameters, out of five initially attempted, were examined and analysed in details using three different mathematical models. Each model was tested for its fitness of the raw data and its ability to describe the various characteristics of the growth curve of each fetal parameter. The accessibility of each parameter was also examined.

Crown rump length had the fastest growth rate and the least variance while trunk diameter had the slowest growth rate and the highest variance of the three fetal parameters analysed. The 'benchmarks' of crown rump length, in the early stages, were difficult to identify, sonographically. This may lead to discrepancies between different studies. The best mathematical model, out of the three, for crown rump length, in terms of fitness of the raw data and precision to determine fetal age, was found to be the logarithmic model. The linear model was found to be good at giving an overall impression of the average growth rate and steepness of the growth curve.

The biparietal diameter was the most accessible parameter of the three during the period of study. The model with the best fitness of the raw data of biparietal diameter and the most precise in determining fetal age was the polynomial model.

No major difference in the growth rate and size was noted between the width and depth of the trunk diameter and between sizes of trunk diameter taken at the level of the umbilicus and the stomach. However, although the measurements of transthoracic diameter were not analysed and compared to those of transabdominal diameter, the impressions obtained in this study from the raw data of the two diameters seem to suggest that a difference between the two diameters exists.Therefore, this means that measurements of trunk diameter, taken from the thorax for purposes of determining fetal age, will underestimate the age of gestation, unless a separate model of transthoracic diameter is used.

Growth patterns and dimensions of transabdominal diameter during late embryonic and fetal development, are influenced by the rapidly growing liver.

This study did not find any major difference of statistical significance between growth curves of embryo transfer fetuses and the normal fetuses.

The linear model was the simplest of the three models assessed; deriving the age prediction equation and identifying the features of growth that the coefficients represent was easier than with the other two models. It could also be used to ascertain the average growth rate during a given period. However, it can only be used in linear growth curves, usually present in early gestation and not non-linear characteristics seen in a typical growth pattern of later pregnancy.

The polynomial model has three coefficients, and hence is more complex to interpret and derive age prediction equations, compared to the other two models. It is therefore not, in most cases, a good model to use for the purpose of estimating age. However, it is very good at defining and describing the extent and direction of both linear and curvature features of the growth curves. It does not, however, specify the points at which these curvings begin and end on the growth curve

The two coefficients of logarithmic model are ,relatively easy to interpret and to derive age prediction equation. It is the most suitable for the purpose of estimating age using fetal dimensions which have typical curvilinear or sigmoid growth curves, because it is able to transform non-linear raw data into linear and has uniform variations and hence, the least coefficient of variation .

The findings of this study do not suggest any difference in precision in determining the age of intra-uterine bovine fetus between transducers with higher frequency and (7.5 MHz) and those of lower frequencies. (3.5 and 5.0 MHz.) However, since the data, in the present study, was only based on early bovine fatal development and a relatively smaller sample size compared to previous studies using transducers of lower frequencies, it was not possible to conclusively state whether there was any difference of statistical significance. Verification of the present findings in future investigations is necessary. More studies in future, on age prediction mathematical models, are desirable in order to improve the precision of fetal age determination and to describe characteristics of the more complex growth patterns. It is necessary, in all such studies, to test the fitness of the mathematical models to the raw data of fetal parameters.In addition, not more than one measurement of fetal dimension should be taken from any single cow, where possible, in order to have better statistically reliable results. Detailed investigations on intrinsic and extrinsic factors affecting growth rate and patterns, like nutritional levels of the pregnant dam, breed, sex, multiple pregnancy e.t.c., can now be extensively carried out by ultrasonography. In addition, population flex points of fetal growth curves of different breeds, sex, multiple pregnancies e.t.c. should be possible to establish with the right application of appropriate mathematical models.

Fetal organs and tissues with sizes above the resolution factor of the transducer, and with densities different from structures around them, had good and clear sonographic images. e.g. fluid-filled structures like the stomach and the heart. The clarity of images in the early stages of fetal development was poorer than in the later stages, probably, because of the low density and jelly-like predominant tissue type, the mesenchyme, found during embryonic and early fetal development.

Sonographic images of almost all the major fetal structures, like the brain, orbit, maxilla, mandible, heart, blood vessels, stomach, hind and forelimbs, could be identified by Day 45 of gestation. However, details of the images of most organs could only be properly observed in the later stage of fetal development. The gross appearance of embryonic structures is identified much earlier and in more detail, in conventional techniques than in ultrasonography. For instance, oropharynx, optic vesicles, dorsal aorta and aortic arch loop were first seen ten days later by sonographic technique than by conventional technique (Omran, 1989, Winters and others, 1942.) However, they did require sacrifice of the embryos and fetuses.

Although, in general, sonographic images of the stomach could be identified by Day 39 of gestation, it was not possible to recognise the differentiation of the stomach compartment until after Day 53 of gestation. The first compartment of the stomach which was able to be identified, sonographically, was the reticulo-rumen. Differentiation of the rumen into its various sacs became apparent by Day 60 of gestation.

Sonographic images of the differentiated omasum first appeared as a round hyperechoic structure by Days 53 of pregnancy. Images of the laminae omasi appeared about fourteen (14) days later. Other structures of the omasum which were identified sonographically include the esophageal groove and omasi sulci by Day 70 of gestation.

The abomasum was the most difficult stomach compartment to identify, probably because of the close resemblance of its images and its proximity to the intestines.

The reticulum could be imaged in dorsal or sagittal planes by Day 76 of pregnancy. The structures immediate to the reticulum were; the liver and diaphragm, cranially, the vestibule of the rumen,caudally, and the omasum on the right .

The sensitivity of ultrasonographic and radiographic techniques in detecting the earliest time and chronological order of appearance of the loci of ossification were, generally stating, found to be the same. With ultrasonography the first signs of ossification of the scapula and os coxae appeared by the beginning of the seventh week while those of the humerus, femur, ulna/radius, tibia, metacarpus and metatarsus appeared between the begining and the middle of the seventh week of gestation, about ten (10) days earlier than radiography. The first phalanx to show signs of ossification was the distal phalanx of digits three (3) and four (4) at Day 60 of pregnancy, followed by the proximal phalanx at Day 69 of gestation and lastly, the middle phalanx at Day 74 of gestation. The diaphyseal locus of the transient element, the fibula, was observed, radiographically, by Day 64.5 of gestation while that of the metapodial 5 was identified in fetuses of Days 69.2 and 74 of gestation. No transient element was observed in ultrasonographic images.

Although the two techniques, ultrasonography and radiography, had a similar sensitivity in detecting onset and chronological order of appearance of the loci of ossification in the appendicular skeleton, x-ray images of fetuses treated with heavy metals, had better clarity and detail than those of ultrasonography, particularly in the early stages of development.

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Appendix

Bovine fetal growth curves.

Appendix 1.1. Crown rump length as a function of gestational age for the three models: (a) Polynomial (poly.)

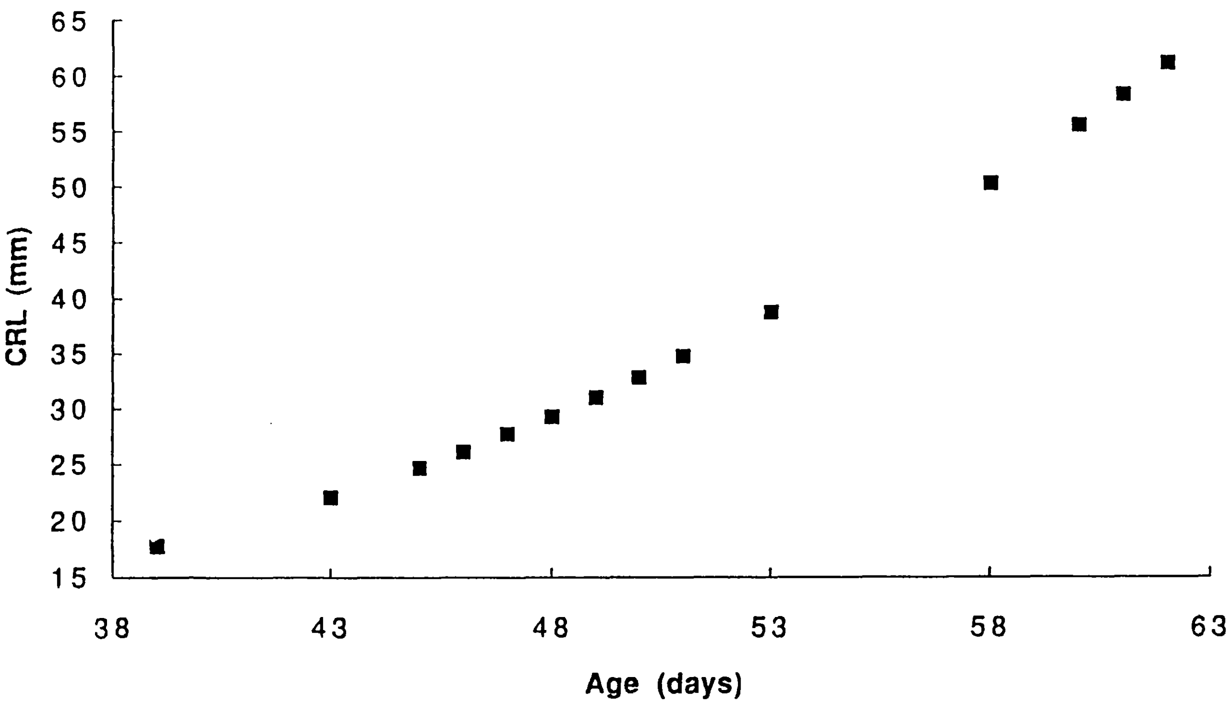
(b) Linear regression (s.reg.)

(c) Logarithm. (Log)

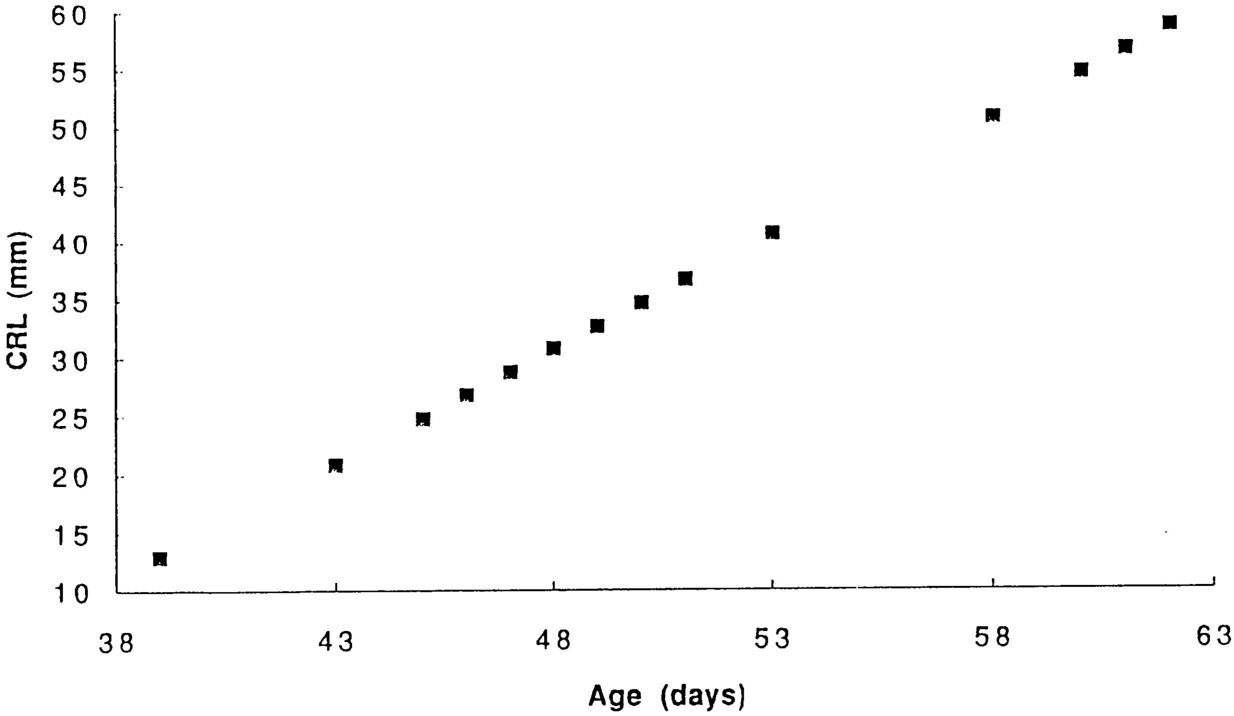
(a) Poly.

(b) s. reg.

CRL poly g. curve 2



CRL linea g. curve 2



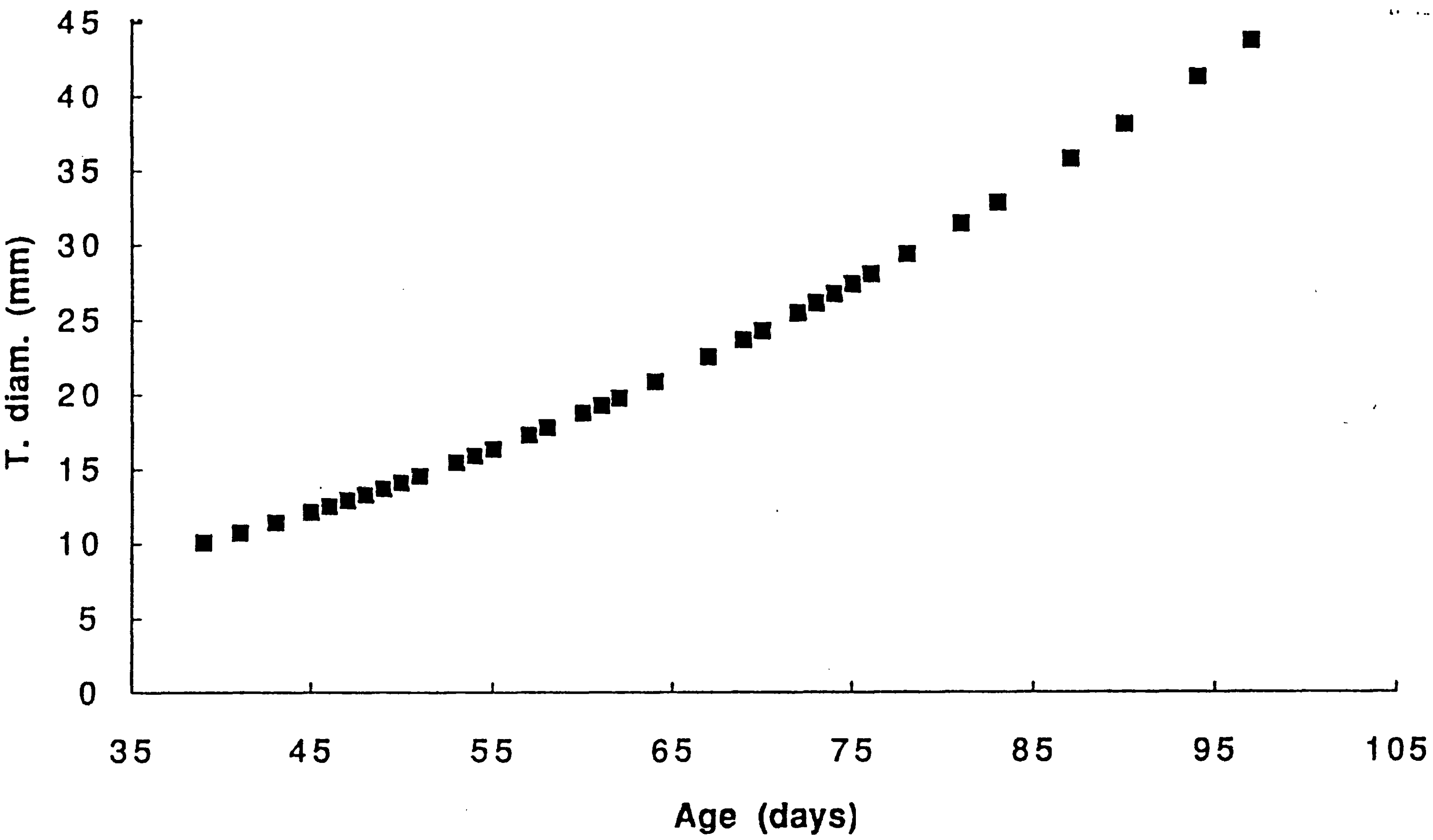
(c) Log.

Appendix 1.2. Transabdominal diameter as a function of gestational age for the three models: (a) Polynomial (poly.) (b) Linear regression (s. reg.) (c) Logarithm. (Log)

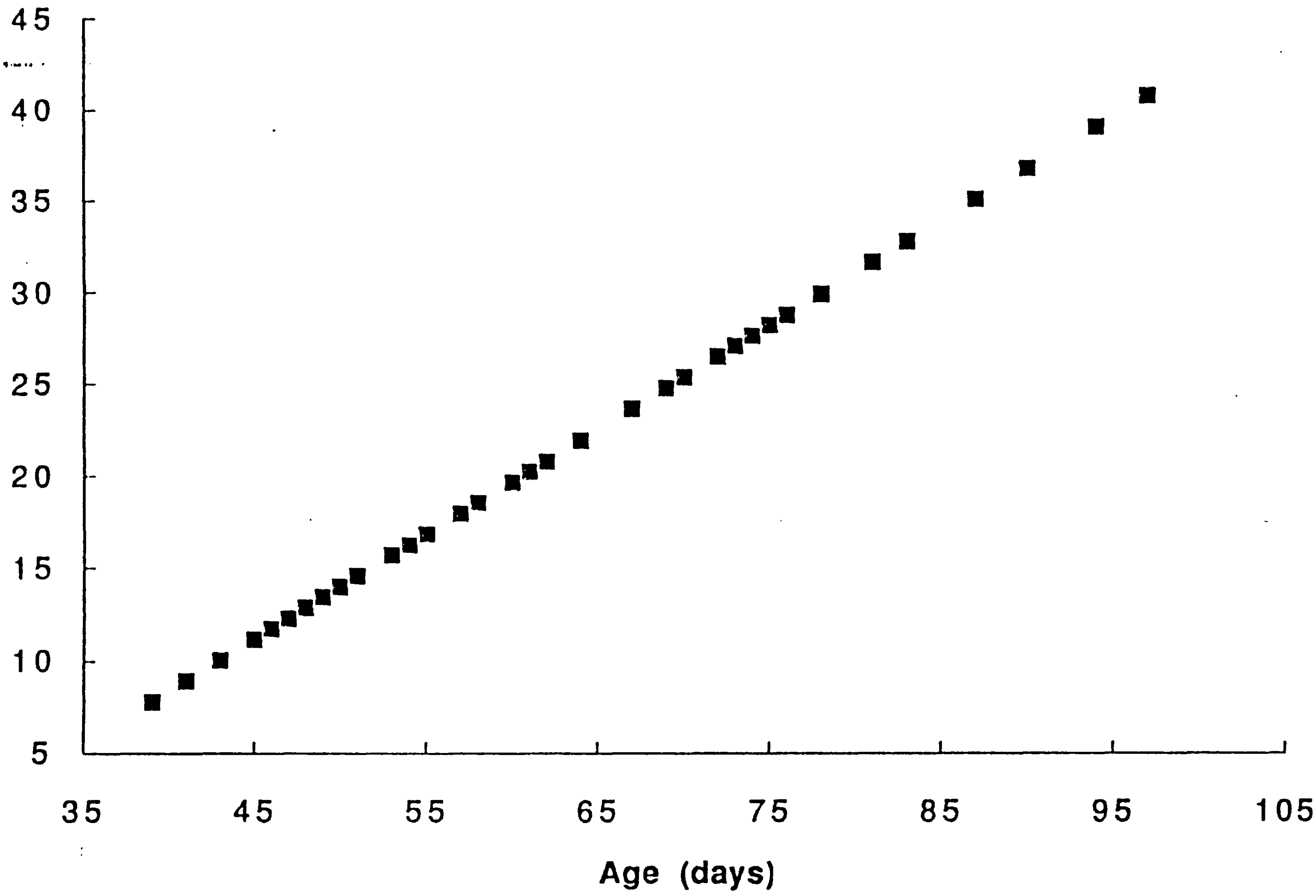
(a) Poly.

(b) s.reg.

T.diam poly growth curve

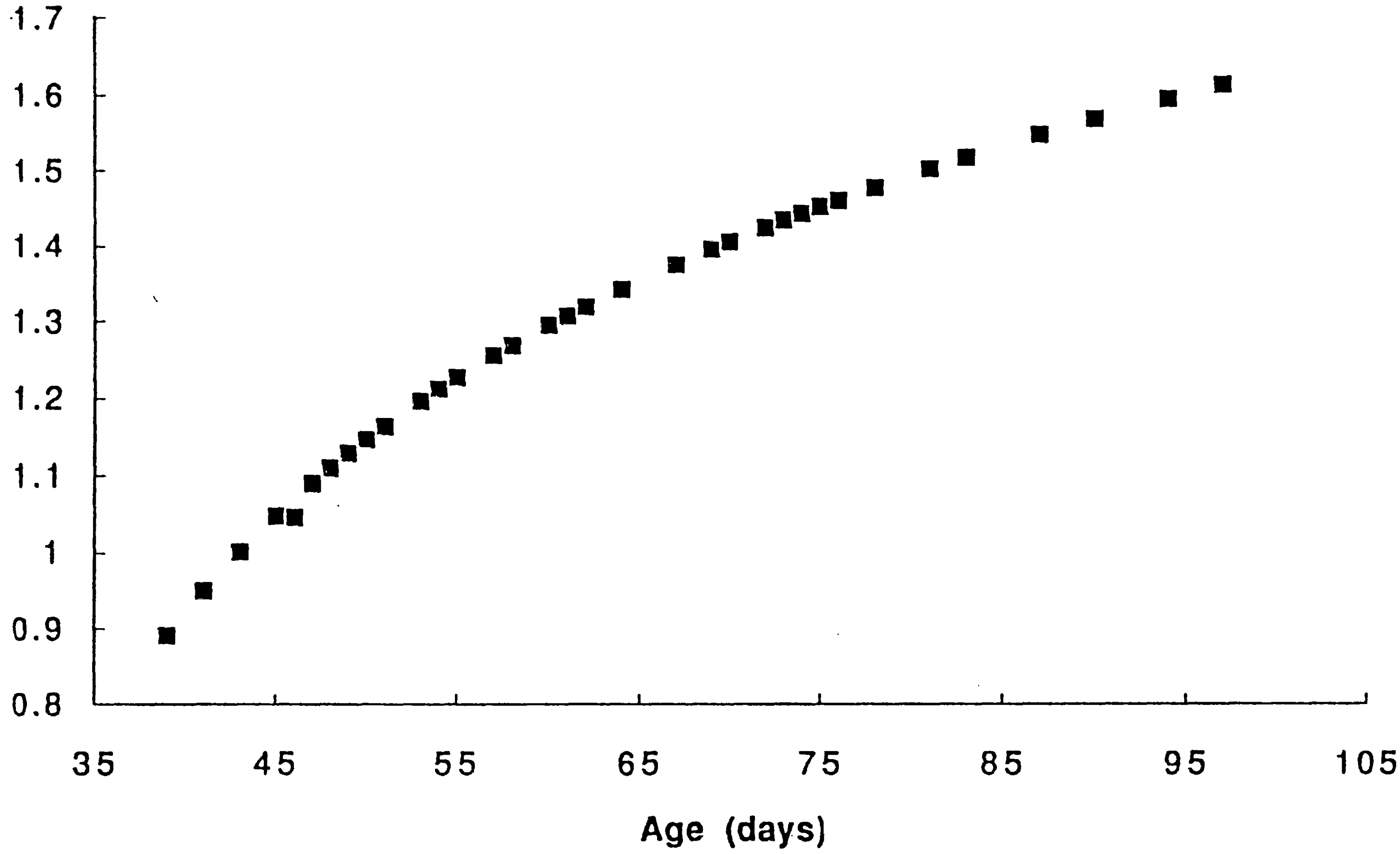


T.diam linear g. curve



(c) Log.

T. diam. log g. curve 2



<u>Appendix</u> 1.3. Biparietal diameter as a function of gestational age for the three models: (a) Polynomial (poly.)

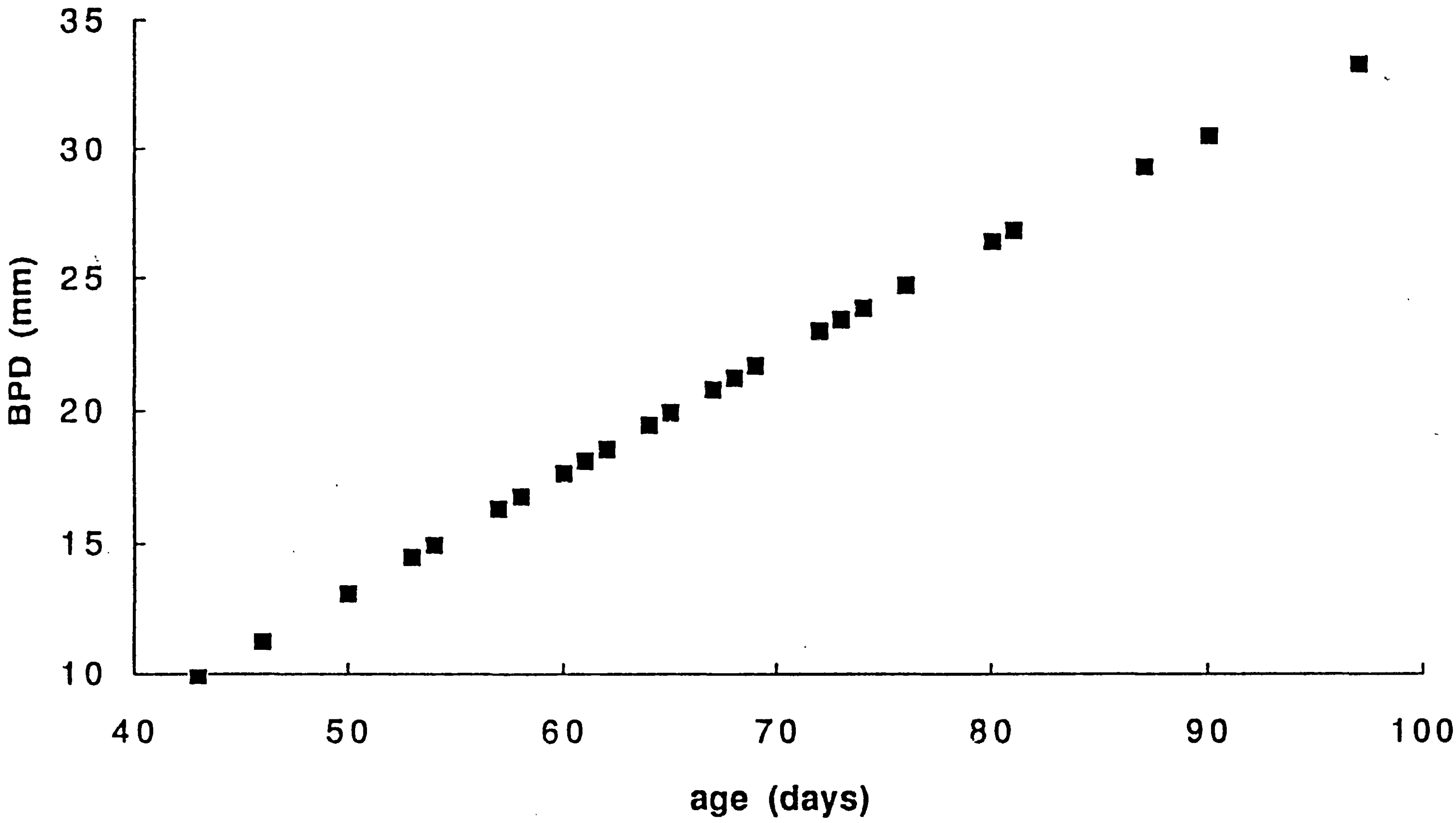
(b) Linear regression (s. reg.)

(c) Logarithm. (Log)

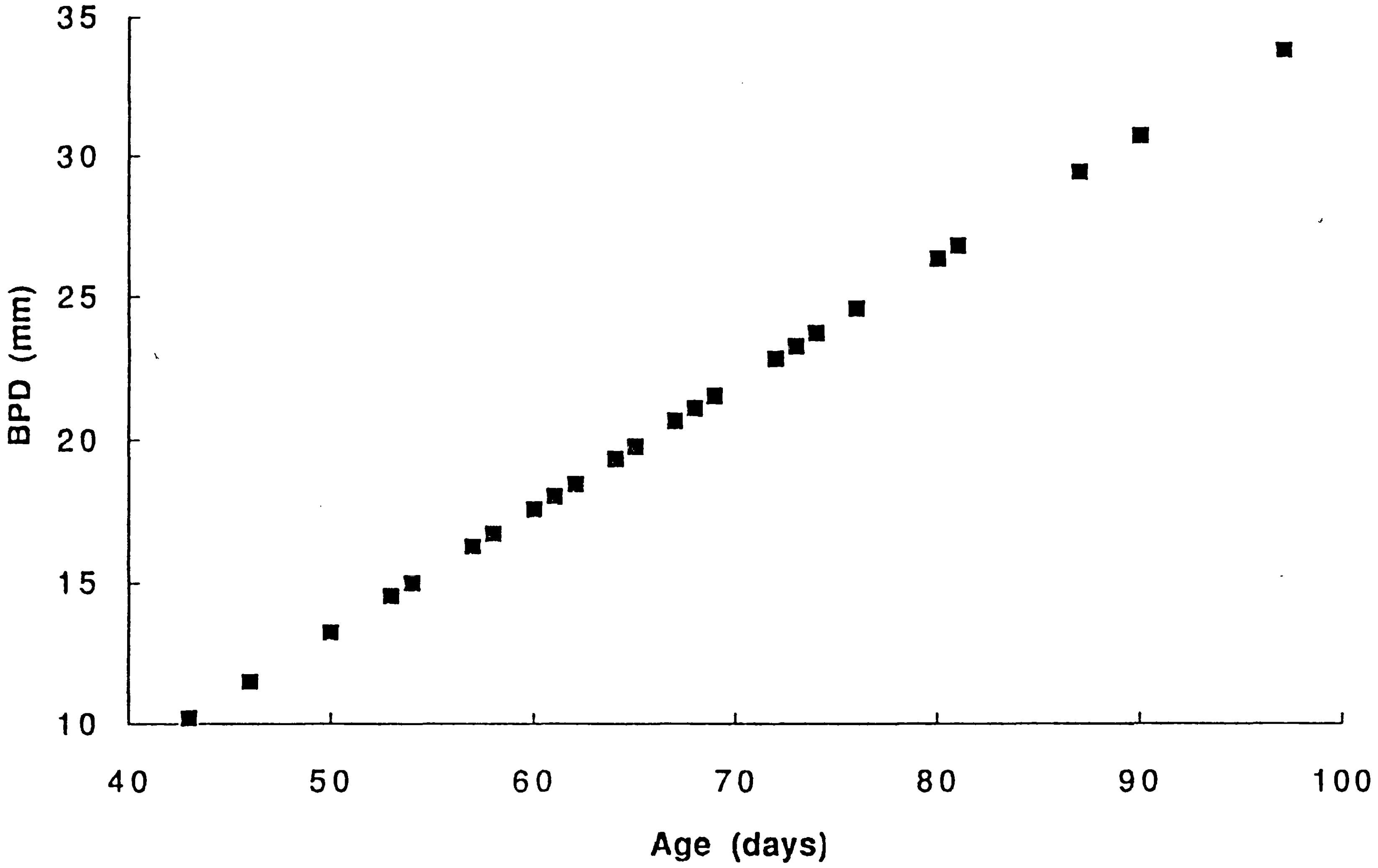
(a) Poly.

(b) s.reg.

BPD poly g curve



BPD linear g.curve



(c) Log.

BPD log g.curve