RECTAL ULTRASONOGRAPHIC INVESTIGATIONS OF PREGNANCY IN FARMED RED DEER (*Cervus elaphus*).

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ABSTRACT

This study describes the ultrasonographic appearance of the non-pregnant reproductive tract, and the pregnant uterus and conceptus from 6 to 211 days after mating, with an emphasis on early pregnancy from 6 to 42 days. In addition, 13 foetal age estimation equations were computed from measurements of foetal, placental and maternal dimensions.

Mating dates were recorded for thirty seven red deer hinds two years of age or older. From immediately prior to the breeding season and at approximately weekly intervals from the commencement of mating to the end of gestation, rectal ultrasonographic scans were taken using a 5 MHz linear transducer while deer were held in a restraining device. Scans were recorded on video for later measurements and analyses.

The vagina and cervix were visible, with the lumen appearing as a continuous or intermittent white line, respectively. The non-pregnant uterus was observed in most cases and was immediately anterior to the bladder. Structures resembling the ovaries were observed only occasionally.

By seven days gestation, a 5 mm vesicle might be observed, and by day 14, oedema of uterine horns was apparent. A comma-shaped foetal mass 6 mm long, foetal membranes and formation of placentomes could be observed at day 24. The heart beat was observed at day 28 when the foetus was 10 mm long. Limb buds were observed at day 31, and by day 37 the head with nose and eyes was clearly distinguishable. Foetal movements were first observed on day 42. Elongation of the neck and the echogenicity of the ribs were observable by day 51 and 52, respectively. By day 58, the long bones were echogenic, and the individual vertebrae were clearly seen by day 59. The bladder and stomach were distinguished by day 62. From day 102, movements of foetal eyes could be observed. From day 114, the placentomes developed a mushroom shape, and some were attached to the endometrium only by a stem. After 150 days gestation, pregnancy could only be detected by viewing the presence of placentomes or foetal extremities in a fluid filled sac.

Measurements of crown-rump, head length, head diameter, nose length, eye diameter, neck diameter, chest diameter, chest depth, umbilical cord diameter, amnion sac length and width, placentome diameter and uterine diameter, were recorded from appropriate scans and growth regression equations were computed. Age estimation equations were computed by transposing the regressions of foetal, placental or uterine dimension on age with age as the independent variable, to equations with age as the dependent variable. All equations were significant ($P < 0.001$). Each dimension was measurable over a defined period of pregnancy. The earliest dimension measurable was uterine diameter but these measurements were variable and no longer feasible after 45 days of pregnancy. Placentomes could be measured from 24 days gestation, but this dimension was also variable. The most accurate estimation of foetal age would be by measurement of the length of the amniotic sac between 37 and 56 days. Measurement of crown-rump length from 24 to 59 days, and head length from 42 to 84 days would also allow accurate estimates of foetal age. Accurate foetal ageing was not possible beyond approximately 150 days gestation.

The sensitivity of rectal ultrasonography pregnancy for testing in red deer hinds was 35% prior to 20 days, 71% between 21 and 30 days, 98% between 31 and 40 days, 100% between 41 and 130 days, and for pregnancies of 131 days or more, the sensitivity was 95%. The reliability of a positive test was 100% between 41 and 130 days.

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LITERATURE REVIEW.

1.1. INTRODUCTION.

In 1947, the use of ultrasound began in human medicine as a diagnostic aid in obstetrics. Subsequently, almost no part of the human body has escaped sonographic examination (Cartee, 1980). However, it was not until 1979 that its use in animals was developed (Rantanen, 1985).

Until recently, real-time B-mode ultrasound examinations were made transabdominally in women. This required a greater depth of penetration for the sound waves and for this purpose a 3.5 MHz transducer was used. More recently, transducers of higher frequency (5 MHz, 7.5 MHz) have been used vaginally for examination of pregnancy in women. Concurrently, high frequency rectal transducers have become available to veterinarians (Boyd *et al.*, 1988) giving the advantage of a higher definition of the image and consequently the possibility of an earlier diagnosis of gestation. Ultrasound imaging technology provides rapid, non invasive visualisation of internal organs. Motile structures, previously only detectable in the static state at necropsy, by surgical removal or by X-ray, may now be visualized in the living animal.

Advantages of ultrasonography for pregnancy examination are that it is safe, easy to apply, and the presentation of the results is immediate.

Deer farming is a growing activity in New Zealand and elsewhere. After twenty years of practice, the deer industry needs genetic and reproductive improvements. Ultrasonography and pregnancy testing have an important role to play. Diagnosis of pregnancy and foetal ageing may be used for clinical investigation of poor fertility, to optimise feed management during gestation and at calving, for detection of non-pregnant hinds which can be slaughtered, and for confirmation of artificial insemination or embryo transfer success. It is also a tool which can be used to check the fertility of a valuable sire early in the mating season.

1.2. PRINCIPLES OF ULTRASONOGRAPHY.

1.2.1. PROPERTIES OF ULTRASOUND.

Ultrasound is a mechanical wave of compressions and rarefactions within a medium, and has wave length, frequency and velocity.

The wavelength (λ) is the distance from two similar points on the given wave. The frequency, referred to as hertz (Hz), is the number of cycles or wavelengths occurring per second. Audible sound varies from 20 to 20,000 Hz, whereas diagnostic ultrasound uses frequencies of 2 to 10 megahertz (MHz).

The velocity is described by Herring and Bjorntorn (1985) as:

Velocity = Frequency x Wavelength

As the frequency of ultrasound waves increases, the wavelength decreases. In typical soft tissues, the average propagation velocity for ultrasound is 1540 meters per second. This means that the ultrasound wavelength for typical diagnostic ultrasound is less than 1 mm. (Powis, 1986).

1.2.2. INTERACTION OF ULTRASOUND WITH TISSUES.

1.2.2.1. REFLECTION.

As ultrasound is emitted into the patient, it travels at a constant speed until it meets a reflecting surface, which is an interface between two media with different acoustic impedance. At the reflecting surface, a portion of the sound beam is reflected back to the transducer and the remainder continues through tissues, sending back echoes at all reflecting surfaces. The strength of this reflection is directly proportional to the difference in acoustic impedance across the interface (Powis, 1986).

Each tissue has a characteristic acoustic impedance:

$$Z = P \times C$$

where P is the density of the tissue, and C the velocity of sound through that tissue.

As the sound beam travels at approximately 1540 m/sec in soft tissues, the only variable that contributes to the difference in acoustic impedance from one soft tissue to another is its density (Herring and Bjornton, 1985). For example, the interface between a soft tissue and either bone or air, will be highly reflective and will result in a well defined line on the screen, whereas the border between two soft tissues will reflect few waves and will be poorly distinguishable on the screen.

If an interface is large compared with the ultrasonic wavelength, then a mirror-like reflection, called specular reflection, occurs at the boundary. If an interface is smaller than a wavelength, then reflection occurs through scattering, the waves being reflected in all directions. The same process lets us see a shaft of light passing through dust-laden air. In general, the brightest echo signals on a display will come from specular reflections, while the softer echo signals will come from scattering.

The best images of structures and boundaries are produced from mirror-like reflections, which require that the ultrasound beam be perpendicular to the reflecting surface. Thus, complete depiction of an organ will require images from different angles (Powis, 1986).

1.2.2.2. ATTENUATION.

Attenuation, which is a distance and frequency-dependent loss of energy, is a combination of absorption, reflection and scattering.

Ultrasound waves loose energy to the carrying medium in the form of heat. This energy loss is called absorption and causes an ultrasonic wave to become weaker as it travels through tissues. This steady loss in wave energy limits how far ultrasound can penetrate into tissues, which in turn limits the tissue depth that can be portrayed in an image. Higher ultrasound frequencies are absorbed faster than lower frequencies. As a result, lower frequencies penetrate deeper and, therefore, image deeper (Powis, 1986).

Scattering involves a small particle intercepting a portion of the ultrasound wave and radiating it in any direction. Only a small portion of the scattered energy is reflected back to the transducer. The rest is lost, consequently reducing the energy of the ultrasound wave. This is the same process that limits penetration of a light beam through fog.

Attenuation is expressed in decibels (dB), and is a comparison between the intensity of a wave at a point within the tissue and at its starting point.

Some typical values for different tissues are (Powis, 1986):

Liver	1.05 dB/cm/MHz
Muscle	1.8
Blood	0.18
Fat	0.63

At an average rate of 1 dB/cm/MHz, a 5 MHz sound beam will be attenuated 40 dB at 4 cm, remembering that the sound beam actually travels 8 cm when striking a reflecting surface that is 4 cm from the transducer.

1.3. TECHNOLOGY OF ULTRASONOGRAPHY.

1.3.1. SIGNAL PROCESSING.

This subject is reviewed by Powis (1986). The following gives only a general introduction to the scanner mechanism.

The transmitter delivers a voltage to the transducer that excites it into vibration. The transmitter is designed to match transducer properties, so it is unwise to use transducers from one scanner with another machine of a different make.

The transmit-receive switch. Because tissue echoes are generally weak, the electrical echo signals are usually in the millivolt and microvolt range. The scanner must amplify these signals but, at the same time, the radio frequency amplifier (or receiver) cannot accept the several hundred volts of the transmitter. The transmit-receive switch protects the receiver from these high voltages. The transmit-receive switch sets the maximum signal that can enter the receiver, but also the smallest, and so influences how deep tissues can be imaged.

The receiver (or radio frequency amplifier) amplifies the signals that pass through the transmit-receive switch.

Time Gain Compensation. Equal acoustic interfaces located at different distances from the scan head will return unequal echoes, because attenuation increases with the distance. Equalising the echoes from different distances requires application of a low gain close to the transducer, and an increasing gain for the deeper echo signals, in order to produce an image that presents the same qualities for equivalent tissues at different depths.

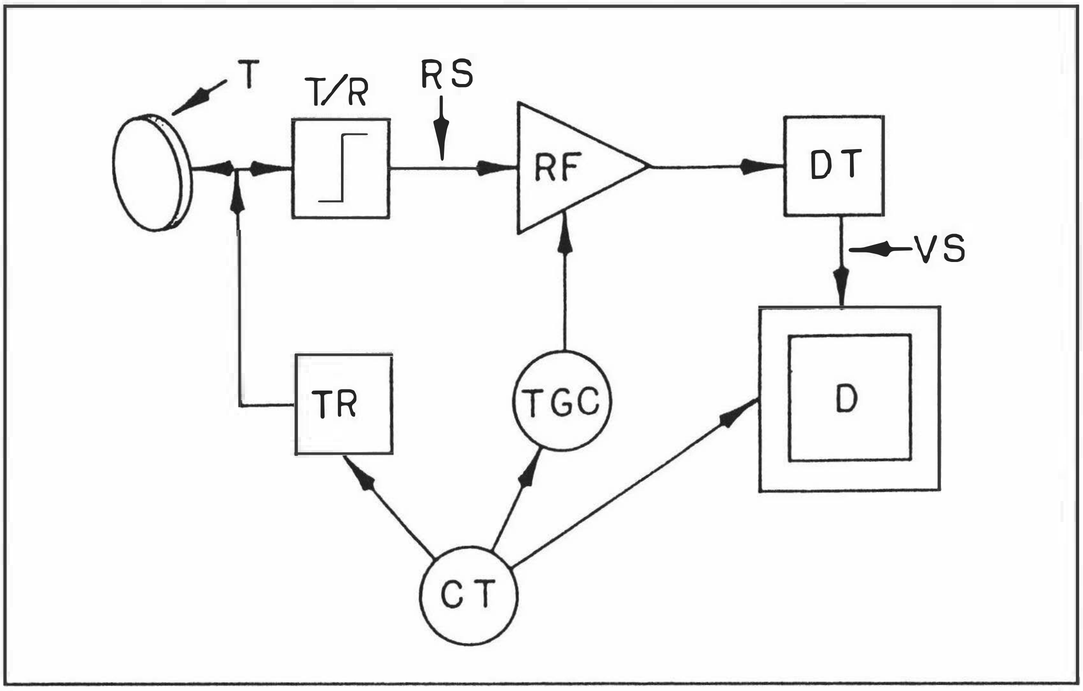


Figure 1.1. The organisation of an **echosonography** (from Powis, 1986).

T, transducer; T/R, transmit-receive switch; RS, radio frequency signals; RF, radio frequency amplifier; DT, amplitude detector; VS, video signal; D, display; TR, transmitter; TGC, time-gain compensation curve generator; CT, central timer.

1.3.2. THE TRANSDUCER.

Mechanical waves come from vibrating objects. In diagnostic ultrasound, the vibrating object is a transducer which changes electrical to mechanical energy and vice versa. An ultrasound transducer is made of a piezo-electric (i.e. pressure-electric) material. The vibrating frequency is determined by the thickness of the transducer: the thinner the transducer, the higher its vibrating frequency.

Vibration is accomplished by applying a high voltage electrical pulse of few microseconds, from a circuit called the transmitter. This shocks the transducer into vibration.

An ultrasound system both produces and receives ultrasound: the reflected ultrasound produces a rhythmic compression in the transducer which is converted into a set of rhythmic voltages which are received by the electronic components within the ultrasound machine.

An ultrasound beam is often focused to improve the ability to depict small structures within the tissues. Focusing ultrasound from a transducer follows the same rules as focusing light using a lens. In general, the best system resolution will occur within the focal zone. Outside the focal zone, the resolution is poorer. Typically, any transducer has both a fixed frequency and a fixed focal zone position (Powis, 1986).

Transducers are normally set into a protective housing, a scan head, that prevents physical damage to the fragile structure. It also prevents any transmitter voltage from reaching the patient's body, and provides a convenient way of holding and manipulating the transducer.

1.3.3. DISPLAY FORMATS.

There are three basic display formats:

- **A-mode**: consisting of vertical peaks along an horizontal axis. The height of the peak corresponds to the amplitude of the echo (Herring and Bjornton, 1985). Although this display shows the location of echo sources along the axis of the ultrasound beam, it does not show anatomical structures (Powis, 1986). This form of ultrasonography was used in echoencephalography but is now rarely included in most modern ultrasound machines (Herring and Bjornton, 1985).

- **B-mode** or brightness mode: returning echoes are displayed on a cathodic ray tube as dots of lights on a black background. The amplitude of the echo determines the brightness of the dots (Herring and Bjornton, 1985).
  A two-dimensional image results from scanning the body with the transducer and linking the position of the B-mode trace on display to the position of the transducer beam in space. The image on the screen results from displaying all the echo signals from all the interfaces at the same time, and allows anatomical structures to be visualised (Powis, 1986).

- **M-mode** tracings are formed when an oscilloscope beam is swept across the screen or by recording the image on moving recording paper.
  This form of image display is devoted exclusively to echocardiography, to follow the dynamics of the heart beating (Herring and Bjornton, 1985).

1.3.4. MOVEMENTS OF THE ULTRASOUND BEAM.

There are two methods of moving the ultrasound beam:

- **Mechanical** scanners move a single transducer, or several, in rotation to scan the ultrasound beam in many successive arrays.

- **Electronic** scanners move the beam using electronics with no moving parts (Powis, 1986). The image quality and the price are higher with this method.

1.3.5. TYPES OF SCANNING FIELD.

As described by Herring and Bjornton (1985), there are two forms of scanning fields produced by transducers:

- **Sector** scans have a triangular shape with the peak of the triangle being at the transducer. Sector scans allow ultrasound waves to go through a small "window" to image the organs, e.g. to image the lungs and heart through the ribs.
Sector scans are formed by sweeping the beam in the form of an arc, by rotating the crystals around an axis, by rocking them back and forth through the desired arc, or by electronic means.

Rotating scanheads are still used on many mobile and portable machines available today. They provide a small acoustic "window" and good image quality, but they are sometimes difficult to place owing to the emission of the sound beam from the side of the scan head rather than from the end.

A single wobbling transducer is usually of the end-fire variety and therefore provides excellent patient contact and easy access.

Electronic sector scanners provide the best images. A micro computer causes sequential firing of the crystals.

- **Linear** array refers to the alignment of 64-120 single small transducers that are electronically fired in a given sequence (Herring and Bjornton, 1985). The resulting image is rectangular, and therefore the contact surface is large, which gives a better view of the anatomical structures close to the transducer. The resolution obtained with a linear array is never as good as this one of an electronic sector scanner, but the cost of the equipment is less. Because the linear array scanheads need a longer contact surface, they sometimes do not fit the body contour but are suitable for rectal ultrasonography as used for horses, cattle and deer.

1.3.6. REAL-TIME ULTRASONOGRAPHY.

The images displayed in a B-mode scan are formed rapidly and are presented in sequence, so that organ movement will be viewed in real-time. The speed of image formation is limited by the pulse repetition frequency produced by the transducer. Microsecond pulses are followed by millisecond pauses for echo reception. Echoes from one pulse should not be allowed to interfere with echoes from a succeeding pulse. This limits the maximum depth of penetration to the distance travelled by the wave in 1 millisecond. Where the velocity of the ultrasound in soft tissues is 1540 m/sec, the distance travelled in 1 millisecond is 154 cm, i.e., a tissue depth of 77 cm (Herring and Bjornton, 1985).

For practical purposes, depending on the tissue attenuation, the maximum scan depth with the lowest transducer frequency available (3.5 MHz) is 20-25 cm. A 5 MHz transducer will scan to 10-12 cm tissue depth, and a 7.5 MHz to 3-5 cm depth.

1.4. REAL-TIME ULTRASOUND IMAGE QUALITIES.

1.4.1. AXIAL RESOLUTION.

Axial resolution is the ability to image two closely apposed interfaces transected by the ultrasound beam. The smaller the axial resolution (in terms of mm), the better the image (Herring and Bjornton, 1985). The longer the transducer wavelength, the poorer the axial resolution (Powis, 1986). Crystals which vibrate at low frequencies (3.5 MHz) are large (about 13-19 mm), while crystals which vibrate at high frequencies (7.5 MHz) are small (about 6 mm). A 5 MHz transducer has a resolution of 2 mm, while a 3.5 MHz transducer has a resolution of only 6 mm (Ginther and Pierson, 1984-a). To change frequencies, additional scanheads must be purchased, although some portable mechanical sector scanners have scanheads with multiple crystals of different frequencies, usually 3.5, 5, and 7.5 MHz.

There is an inherent ability to focus the sector scanner crystals more accurately than the linear array which means a sector scanner will have better resolution at a given tissue depth than a linear array transducer at the same frequency (Rantanen, 1985).

1.4.2. LATERAL RESOLUTION.

Lateral resolution is the ability to distinguish between two echo-forming surfaces lying side by side in relationship to the sound beam. Lateral resolution is determined by the beam width and the size of the transducer face. It is best at, or close to, the focal point (Herring and Bjornton, 1985).

1.4.3. BALANCE BETWEEN RESOLUTION AND PENETRATION.

Resolution can be improved by using higher transducer frequencies, although higher frequencies result in poorer penetration (Powis, 1986), (see section 1.2.1.2.2.).

A 5 MHz transducer provides an appropriate balance between resolution and penetration for using in rectal examination of the broodmare, cow, and hind.

1.4.4. FRAME RATE.

The moving images of a real-time ultrasound scanner are formed on the screen by a succession of different pictures, with a certain frame rate. As the frame rate increases, the quality of the moving image improves . The frame rate of any real-time system is limited by the depth of the field of view and by the number of lines-of-sight that go into making the image. The deeper the display, the slower the frame rate. The greater the number of lines-of-sight, the slower the frame rate (Powis,1986).

1.5. ARTEFACTS.

Artefacts are caused by sound beam properties, transducer qualities, instrument adjustment and scan techniques. Some artefacts such as acoustic shadowing and distant enhancement, can be helpful in making an accurate diagnosis, while others, such as reverberation and mirror images, cause confusion.

1.5.1. ACOUSTIC SHADOWS

Acoustic Shadows are caused by the diminished transmission of sound due to attenuation and/or massive reflection of the sound beam at an acoustic interface such as soft tissue:gas or soft tissue:bone. The most common acoustic shadows of clinical significance are those caused by cystic, renal and biliary calculi (Herring and Bjornton, 1985). This artefact is less common in images of the reproductive tract because of the relative lack of tissues with high density. However, a notable exception is the occurrence of shadowing distal to foetal bones. Sometimes, however, remnants of foetal bones are thin and porous and do not cause shadowing. In a large foetus, the centres of ossification will also cause shadows as the formation of bones progresses. In cattle, the plicae circulares of the cervix may occasionally cause shadow artefacts.

A form of shadowing may be seen when the path of the sound beam is obstructed by bowel gas, lack of intimate contact between the transducer and rectal wall, or the presence of faecal material on the face of the probe (Pierson *et al.*, 1988).

1.5.2. REFRACTION

The portion of the sound beam which strikes the side of the curved boundary of a structure at less than 90^0 may bend or refract, causing a shadowing, or lack of echo formation, beyond the site of refraction. Refraction artefacts are especially common in images of the ovary as the beam encounters fluid filled follicles, or in images of spherical embryonic vesicles such as observed in horses (Pierson *et al.*, 1988).

1.5.3. ENHANCED THROUGH-TRANSMISSION

As the sound beam passes through a relatively homogeneous medium such as urine or bile, less attenuation takes place than in the surrounding echogenic areas. When the sound beam strikes the far wall of a cystic structure, the echoes appear brighter than the surroundings (Herring and Bjornton, 1985). In the reproduction system, this artefact is especially common beneath images of follicles and embryonic vesicles. The intensity of the echoes resulting from through-transmission can be reduced by proper adjustment of the gain control, making the artefact less pronounced (Pierson *et al.*, 1988).

1.5.4. SPECULAR REFLECTION

The portion of the beam which strikes the upper and lower surface of a fluid filled spherical structure may produce a highly echogenic reflection. This specular reflection is present on both the upper (nearest to the transducer) and lower surfaces of the image of the structure. The reflection on the lower surface, however, may be obscured by enhanced through-transmission. The specular reflection on small equine embryonic vesicles (3-6 mm diameter) are a decided aid in locating the vesicle (Pierson *et al.*, 1988). However, the specular reflections are not as readily visualized in non-spherical embryonic vesicles of cattle and deer.

1.5.5. REVERBERATION

This artefact appears as highly echoic parallel lines recurring at regular intervals. They are caused by gas/fluid interfaces and occur when large returning echoes produce a further echo from the transducer face, which bounces back and forth between the reflecting surface and the transducer. Reverberation artefacts are commonly seen during intrarectal examination of the reproductive tract because of gas-filled segments of intestine beneath the areas of interest.

Reverberation artefacts are equidistant, gradually diminish in intensity, and are parallel to the reflective interface. Reverberations may sometimes be diminished by adjustment of the gain controls or by manually adjusting the organs thus altering the relationship with the underlying bowel (Pierson *et al.*, 1988).

1.5.6. MIRROR-IMAGE ARTEFACT

Mirror-image artefact occurs at highly reflective interfaces and is caused by multiple internal reverberations, so that the returning echoes reach the transducer with a time delay and are registered on the image as being beyond highly echogenic interfaces in the path of the beam. This artefact can occur during echocardiography where two beating hearts are imaged at the same time because of the highly echogenic interface between the pericardium and the lungs (Herring and Bjornton, 1985). Mirror-image of the bladder may also be seen during rectal examination.

1.6. SAFETY OF ULTRASOUND.

Portions of the sound beam are absorbed by the tissue and create heat. There is no doubt that cells can be permanently damaged by continuous exposure to these high intensity sound waves. However, the transducer only emits bursts of ultrasound 1/1000 of the time it is operating, for the remainder of the time, it is in the receiving mode. This factor, along with the fact that no proof of harmful biological effects directly related to diagnostic levels of ultrasound has been reported, indicate the safety of diagnostic ultrasound methods (Herring and Bjornton, 1985). Furthermore, Scherboom and Taverne (1984) demonstrated that the procedure of real-time scanning does not affect uterine electrical activity, and McKinnon and Squires (1988) showed that frequent manipulation with a 3.5 MHz transducer within the mare's rectum was not detrimental to continuing pregnancy. In humans, no deleterious effects of ultrasonography have been reported (Sikov and Hildebrand, 1979), and in deer, Bingham *et al.*(1990) reported no loss of conceptus after repeated ultrasound examinations during pregnancy.

1.7. RECORDING THE IMAGE.

A visual image correlates with the tissues being examined and should be recorded as part of the animal's medical record. Ultrasound images, like radiographs, are legally a part of the examination documentation and should be maintained (Rantanen, 1985 and 1986). The methods of recording are Polaroid photographs, multiformat cameras giving black and white prints or slides, and videotape.

Polaroid images are relatively easy to take during the examination, and most instruments have pre-focused cameras as purchase options. However, polaroids prints are expensive and of poor quality.

The multiformat cameras are the most expensive to purchase but provide a reasonable cost per image. These are best suited for academic institutions or large referral hospitals with a large patient load.

Videotape recordings of examinations are ideal in most private or academic situations. The tape can be replayed either on a television monitor, or, if a "video in" connection is available, on the scanner screen. Still photographs can be made from the videotape. Some of the portable ultrasound systems have a direct "video out" connection. Points of reference, such as date and tag number, should be marked on the image.

1.8. APPLICATION OF ULTRASONOGRAPHY FOR EXAMINATION OF THE FEMALE REPRODUCTIVE TRACT.

1.8.1. HUMAN.

1.8.1.1. DESCRIPTION OF THE HUMAN CONCEPTUS BY ULTRASONOGRAPHY.

Approximately 3 weeks after conception, an intrauterine sac can be identified using a 7.5 MHz vaginal probe (Bernascheek *et al.*, 1988). One to two weeks later, the embryo and heart beat can be observed (Nyberg *et al.*, 1986). From the same time and for the subsequent 2 to 4 weeks, the yolk sac can be seen as a small cystic structure adjacent to the foetus (Sauerbrei *et al.*, 1980). Over the next few weeks, foetal anatomy gradually becomes more distinct, with the observation of head, trunk and foetal limbs. By the 8th week after conception, an intracranial echogenic area is observed, with a midline falx, and most of the organs are recognisable (Sanders *et al.*, 1985).

During the first trimester of pregnancy, or embryonic period, the main ultrasonographic uses are for the observation of cardiac activity which establishes foetal viability, counting the number of foetuses, monitoring of the foetal head, limb and trunk growth, and distinction of the yolk sac and umbilical cord from the foetus. Visualisation of anatomy in detail to diagnose foetal anomalies is done from 12 weeks post-conception (Sanders *et al.*, 1985).

1.8.1.2. ACCURACY OF PREGNANCY TESTING.

In human, the diagnosis of pregnancy is usually confirmed by the presence of human chorionic gonadotrophin (hCG) detectable in serum or urine, 7 to 9 days after ovulation, before ultrasonography is able to detect pregnancy. Therefore, ultrasonography is not used for pregnancy testing *per se* but rather to monitor the evolution of the conceptus (Chartier *et al.*, 1979).

1.8.1.3. ESTIMATION OF FOETAL AGE.

Before the advent of ultrasound, the gestational age had to be established by a combination of menstrual history (estimation ± 2.5 weeks, 95% confidence interval) and physical examination after 28 weeks (estimation ± 4-6 weeks, 95% confidence interval).

With ultrasonography, crown-rump length and gestational sac measurements have become the primary means of establishing gestational age during the first trimester of pregnancy, with an estimation ± 5-7 days and ± 7 days, respectively, 95% confidence interval. In the second and third trimester, head biparietal diameter is the more commonly made measurement (estimation at 95% confidence interval ± 5-7 days for the second trimester, and ± 4 weeks during the third trimester), but head circumference, abdominal diameter, abdominal circumference and femur length can also be measured (Kurtz and Needleman, 1988).

1.8.2. CATTLE.

The reproductive tracts of all domestic ruminants are similar. The main difference between the reproductive tracts of cows and hinds is their size. Rectal ultrasonographic examination of cows is done by handling the probe in the rectum; manual palpation of the organs can assist direction of the probe to the desired organ. Therefore, the cow is the preferred animal to use to establish an understanding of the ultrasonographic imaging of the female reproductive tract of ruminants.

Rectal ultrasonography in cattle has been used for the following purposes:

- investigation of follicular or luteal status of cows (Pierson and Ginther, 1984-a; Pierson and Ginther, 1988).
- detection of ovulation (Pierson and Ginther, 1984-a).
- Detection of pregnancy (Pierson and Ginther, 1984-b; White *et al.*, 1985; Chaffaux *et al.*, 1986; Boyd *et al.*, 1988).
- evaluation of embryonic development and age of the conceptus (Chaffaux *et al.*, 1982; Pierson and Ginther, 1984 b; White *et al.*, 1985; Curran *et al.*, 1986 a and b; Kastelic *et al.*, 1988; Wright *et al.*, 1988).
- prediction of the sex of the foetus (Muller and Wittkowsky, 1986).
- to guide an endometrial biopsy instrument to areas of particular interest within the uterus (Pierson *et al.*, 1988).
- location and sampling of intraluminal fluid (Pierson *et al.*, 1988).
- visualization of the deposition of various amounts of semen or physiological saline (Pierson *et al.*, 1988).
- guided puncture and aspiration of follicles (Pierson *et al.*, 1988).
- herd management: ultrasonography may aid in identifying the appropriate animals for luteolysis using prostaglandins (Pierson and Ginther, 1984-a).

- estimation of the number of follicles to evaluate the response to superovulation regimes (Pierson and Ginther, 1988).
- counting corpora lutea on superovulated ovaries (Pierson and Ginther, 1988).
- investigation of pathological conditions: e.g., metritis, pyometra, cystic ovarian conditions, ovarian abscesses, neoplasms, detection of macerated or mummified foetus (Pierson and Ginther, 1988).
- evaluation of uterine morphologic and echotexture changes during the oestrous cycle (Pierson and Ginther, 1988).

1.8.2.1. OVARIAN MORPHOLOGY.

The cow's ovaries can be systematically scanned after location by palpation. Images of the ovary are primarily composed of follicles and corpora lutea.

Follicular fluid is non echogenic, therefore the follicles appear as black, roughly circumscribed areas on the ultrasound image (Pierson and Ginther, 1984 a). Pierson and Ginther (1988) attributed the irregular follicular shapes to compression between adjacent follicles or between a follicle and a luteal structure or stroma. Some of the apposed walls of similar sized adjacent follicles are straight, while some are too thin to be detected by the 5 MHz transducer (Pierson and Ginther, 1988). Follicles can usually be distinguished by a defined, relatively smooth outline (Pierson and Ginther, 1988) compared with the cavity in a corpus luteum which tends to have a less spherical appearance and is surrounded by a border of luteinized tissue (Pierson and Ginther, 1984). Refraction artefact may obscure the follicular outline parallel to the direction of the ultrasound beams, so, gain controls and the brightness and contrast of the video display monitor must be properly adjusted to produce sharp follicular outlines.

Ovulation is detected by the disappearance of the follicle and subsequent formation of a luteal gland (Pierson and Ginther, 1988). Usually the site of ovulation is visible, with the apposed walls of the collapsed follicle often distinguished from the surrounding stroma (Pierson and Ginther, 1988).

The bovine corpus luteum has an echostructure differing from that of ovarian stroma. A well defined border is visible approximately 3 days after ovulation with a 5 MHz transducer. Pierson and Ginther (1988) usually identified the corpus luteum throughout the interovulatory period, but sometimes it was not detectable on days 0 to 2. In some cases, the corresponding corpus albicans can be identified until 3 or more days after the second ovulation.

Some corpora lutea contain centrally located irregular and sometimes multi-locular fluid filled areas ranging from 2 to 22 mm diameter. The cavities are apparently transient structures, first detected 5 days after ovulation, which are observed for only a few days, then decrease in diameter and condense into a dense fibrin-like core. The presence of the cavity corresponds to the period of maximum size of the corpus luteum. There are sometimes aggregations of echogenic matter, 3 to 6 mm diameter within the cavities which were revealed at autopsy to be collections of haemolysed red blood cells (Pierson and Ginther, 1988).

1.8.2.2. UTERINE MORPHOLOGY.

Ultrasonographically visible characteristics of the uterus including thickness of the uterine body, increased vascularity and oedema, and accumulation of intrauterine, intracervical and intravaginal fluids, are influenced by the stage of the oestrous cycle in the cow (Pierson and Ginther, 1987). Increased thickness of the uterine body, intrauterine fluid, ultrasonographic texture indicative of a more loosely organised tissue, and change in shape of the uterus, are signs of oestrus and impending ovulation. A greater number of cross sections, homogeneous appearance, and a more highly coiled shape of the uterine horns are signs of dioestrus (Pierson and Ginther, 1987 and 1988).

1.8.2.3. THE BOVINE CONCEPTUS.

Ageing the bovine conceptus can be based on gross appearance (Chang, 1952), mating date (Winters *et al.*, 1942; Greenstein and Foley, 1958), oestrus date (Betteridge *et al.*, 1980), and ovulation date as determined by transrectal palpation or ultrasonography (Pierson and Ginther, 1984-b).

The morula is formed by day 5 or 6 (Hamilton and Laing, 1946) and subsequently, the ovum begins to hollow to become a blastocyst with the development of an inner cell mass at one edge. By day 8, the blastocyst begins to elongate. The "germ layer" begins to develop from about 14 days and produces the embryo (Winters *et al.*, 1942). The amnion is usually complete by day 18, implantation begins as early as day 20, and the allantois is well developed by day 23 (Peters and Ball, 1986).

Many of these features may be visualised by ultrasound using a 7.5 MHz transducer (Boyd *et al.*, 1988). For example the allantois, an echogenic membranous line 6 mm diameter, encircling and attaching to the embryo, with specular reflection on its dorsal and ventral borders, was observed at day 23.

Conceptus fluid is uniformly clear on the ultrasound image, whereas, endometritic or pyometritic intrauterine fluid contains echogenic particles (Kastelic *et al.*, 1988).

The location of the corpus luteum is a useful indicator of the horn the conceptus would occupy. Furthermore, the ipsi-lateral horn lumen appears non echogenic due to the presence of intrauterine fluid which persists at least 5 days after ovulation (Boyd *et al.*, 1988).

The following signs of pregnancy are observed chronologically in cattle:

Day 9: A discrete non echogenic 2 mm diameter embryonic vesicle was observed with a 7.5 Mhz transducer (Boyd *et al.*,1988).

Day 11: A 2.8 mm vesicle was first observed at 11 days with a 5 MHz transducer by Curran *et al.*(1986-a).

Day 13: A 2 mm echogenic structure resembling a comma of 2 mm, within the vesicle, was recognised as being the embryo, with a 7.5 MHZ transducer (Boyd *et al.*, 1988). However, the same structure was imaged only at day 20 with a 5 MHz transducer (Curran *et al.*(1986-a).

Day 14: The vesicle extends throughout the uterine horns. During elongation, the vesicle remains at a diameter of 2 mm throughout its length until day 19 when it expands to a localised enlargement of 4 mm about the embryo (Curran *et al.*, 1986-a). The vesicle diameter subsequently increases rapidly, reaching 25 mm by day 24 (Boyd *et al.*, 1988).

Day 20: Paradoxically, Curran *et al.*(1986-a) detected the heart beat with a 5 MHz transducer at 20 days, earlier than Boyd *et al.*(1988), at 22 days using a 7.5 MHz transducer. The heart rate was 188 beats/min at day 20, decreasing to approximately 145-150 beats/min by day 26, remaining constant until the end of the experiment on day 60 (Curran *et al.*, 1986-a).

Day 23: The allantois was first observed using a 7.5 MHz transducer (Boyd *et al.*, 1988).

Day 24: The limb buds become recognisable at the extremities of the elongated embryo, at days 24-26, with a 7.5 MHz transducer (Boyd *et al.*, 1988), or at day 29 with a 5 MHz transducer (Curran *et al.*, 1986-b).

Day 29: The allantois and the association of the chorio-allantois are discernable with a 7.5 MHz transducer, and the amniotic sac is visible as a faint echogenic membrane around the embryo (Boyd *et al.*, 1988). At this stage, the spinal column is first detected with a 5 MHz transducer (Curran *et al.*, 1986-b).

Day 30: Round non echogenic structures, presumed to be the optic areas, were detected on the head of the embryo (Curran *et al.*,1986-b).

The length of the embryo increased from 3.5 mm on day 20, to 66 mm on day 60. It was initially a small echogenic line on day 20, but by day 22-30, it had assumed a prominent C-shape, resulting from the cephalic and caudal flexure as well as general curvature of the back. By days 29-39, the neck was straight and the head was raised, giving the embryo an L-shape (Curran *et al.*, 1986), and by day 33, the cranial, thoracic and abdominal regions were differentiated so that by day 35 the foetal form was discernible (Boyd *et al.*, 1988).

1.8.2.4. ACCURACY OF ULTRASONOGRAPHIC PREGNANCY TESTING.

White *et al.*(1985) obtained an 98.3% overall accuracy, i.e., accuracy of positive plus negative diagnosis, for 179 pregnancy diagnoses, 92-202 days after service, using a 3.5 MHz rectal transducer. Curran *et al.*(1986), using a 5 MHz rectal transducer, detected the embryonic vesicle (positive diagnosis) in all of 19 heifers 17 days pregnant. Failure to find a vesicle in heifers in which the vesicle was previously detected, occurred in less than 1% of scans, and these on days 14-17 when the vesicles were elongated but had not yet formed a bulge.

Chaffaux *et al.*(1986), from 309 scans on 113 dairy cows with a 3.5 MHz transducer, recorded a reliability of positive diagnosis (n pregnant/n tested pregnant) before 30 days of pregnancy of less than 45%. The reliability of a negative diagnosis was 80% for the same period. These authors report that reliability of positive and negative diagnoses was near 100% from 45 days and 40 days onwards, respectively. The accuracy of positive diagnoses (n tested pregnant/n pregnant) was 100% from 40 days onwards, but it required a minimum of 45 days to correctly identify nearly all non-pregnant animals.

Hughes and Davies (1989) observed that the accuracy of pregnancy detection was inversely proportional to the age of the cow. Total accuracy was obtained from 4 weeks gestation using a 3.5 MHz transducer on 2 year old animals, but only at 5 weeks for the 3-7 year old, and at 6 weeks for 8 year old or older cows.

1.8.2.5. ESTIMATION OF GESTATIONAL AGE.

White *et al.*(1985) established regression relationships between gestational age (independent variable) and six uterine and foetal dimensions (dependent variable), between 20 and 140 days of gestation. All regressions were exponential. Crown-rump length provided the most precise estimate of gestational age (sd = 4.5 days) and uterine diameter the least (sd = 12.6 days). Head length and diameters of trunk, head and nose were of intermediate precision. Trunk diameter was the measurement most frequently made, while crown-rump length was the least frequent. Until about 140 days of gestation it is usually possible to measure at least one foetal dimension, but after that, the foetus descends beyond the range of ultrasound penetration.

Wright *et al.*(1988) investigated the precision and accuracy for prediction of the calving date on 300 cows between 35 and 125 days of gestation using four of the age-size relationships of White *et al.*(1985). However, results were probably biased because they used uterine diameter, which gives the poorest accuracy, while omitting use of the crown-rump length which gives the best accuracy. The mean difference between the actual and predicted calving dates, was 0.9 days (sd = 9.0 days), with 80.5% of cows calving within 10 days of the predicted date, 67.5% within 7 days, and 38.7% within 3 days. The difference between actual and predicted calving dates will always be compounded by variation in gestation length (sd = 5 days in cattle).

More recently, Hughes and Davies (1989) established a relationship between foetal crown-rump length and age of pregnancy, but the age result was recorded in weeks and the standard deviation was not presented, thus allowing only poor precision for foetal age prediction.

1.8.2.6. SEXING OF THE BOVINE FOETUS.

The male bovine, like the sheep or pig foetus, but unlike the horse foetus, has an early descent of testes (Cox, 1987; Arthur *et al.*, 1989). At about day 60, the foetal scrotal swellings are nearing their final shape. The processus vaginalis extends into the scrotum at day 102 to 105 and the testicles are descended at about 90-130 days (Gier and Marion, 1970).

Bovine female foetuses have mammary glands of 0.6 to 3 mm in diameter, from about 80-130 days (Pavaux, 1981).

Muller and Wittkowski (1986), using a variable frequency transducer (3.5 to 5 MHz) obtained good visualization of the scrotum or mammary glands as early as 70-120 days post-insemination. The vulvar swellings could not always be visualised. The accuracy of sexing 82 pregnant cows was 94%, 57 to 120 days post insemination. However it should be difficult to image a 0.6-3 mm mammary gland with 3.5 or 5 MHz transducer which has a minimum resolution of 6 and 2 mm respectively. No other reports of foetal sexing appear in the literature for comparison.

1.8.3. SHEEP AND GOAT.

Size precludes manual palpation of the reproductive organs of small ruminants. Therefore the technique is different to that used in cattle but may involve rectal or transabdominal scanning.

For rectal scanning, after a fast of 12 hours, ewes are lightly restrained in the standing position. The transducer, on an extender and well lubricated, is inserted in the rectum, centred over the bladder, then rotated 45^0 to the left and to the right to scan the pelvic region. If the uterus can not be imaged, the transducer is inserted further over the pelvic brim (Buckrell *et al.*, 1986).

For transabdominal scanning before 100 days, the scanning is done in the inguinal region with the ewe while standing or tipped. Good transducer contact requires coupling gel and may necessitate removal of the oily-lanolin based material often found in that region. Beyond day 100, a 3.5 MHz head is needed to penetrate to the depth required to image the foetus. In advanced gestation, scanning is best done from side to side on the abdomen of the tipped ewe after removing the fleece, 20-40 cm above the udder. Only a portion of the near term foetus will be imaged and it is then better to rely on indicators such as rib shadows or heart beat. The scanning of the ovaries of either ewes or does was not possible (Buckrell, 1988).

1.8.3.1. PREGNANCY DIAGNOSIS.

Ultrasonographic pregnancy diagnosis of ewes and does has been reviewed by Buckrell (1988).

Early diagnosis (days 20-35), requires a 5 MHz transducer, rectally or transabdominally. Fluid can regularly be imaged rectally by days 20-23. By day 26-28, the foetus and foetal heart beat can be imaged in most, and in all by day 30. Placentomes are routinely found by day 26-28. At 30 days, uterine fluid, placentomes, amnion, foetus and heart beat, can be observed by abdominal scanning.

Does are often less cooperative than ewes when rectal probes are inserted, but lack of fat and multiple foetuses make a diagnosis accurate when using a 5 MHz abdominal transducer at 25-30 days.

Routine pregnancy diagnosis (day 35-50), is done on standing ewes in the inguinal region. Day 45-50 appears to be the optimum time to make a diagnosis of pregnancy using either a 5 MHz or 3.5 MHz transducer to image uterine fluid, placentomes, foetus and foetal movements.

Pregnancy detection in late gestation (Day 100 to term), is accomplished with a 3.5 MHz transducer on the clipped ventral abdomen.

Foetal numbers may be counted in up-ended ewes, clipped 20 cm above the udder using either a 5 MHz transducer (days 40 to 50), or a 3.5 MHz head (days 50-100). It could be difficult to distinguish twins from triplets or quads.

1.8.3.2. ACCURACY OF ULTRASOUND PREGNANCY TESTING.

Using a 3.5 MHz transducer in the flank region, Fowler and Wilkins (1984) reported 95% sensitivity from 40 to 50 days, and 99% sensitivity beyond 50 days.

Using a 5 MHz rectal probe between 25 and 50 days pregnancy, Buckrell et al.(1986) reported 87% sensitivity and 96% specificity.

1.8.3.3. FOETAL AGEING.

Haibel (1988) recorded the biparietal diameter of dairy breed goat foetuses from day 40 to 105, using a 5 MHz linear-array transducer transabdominally. The age prediction equation was linear. Linear dimensions in sheep embryos and early foetuses are quite uniform, regardless of breed, sex (Stephenson and Lambourne, 1960), or litter size (Winters and Feuffel, 1936). Gross differences between individual foetuses due to these factors are manifest primarily in the last trimester when biparietal measurements become more variable (Haibel, 1988).

1.8.3.4. DIAGNOSIS OF REPRODUCTIVE DISEASES.

Infections of the uterus, foetal death and mummification or maceration, can be diagnosed by scanning (Buckrell, 1988).

1.8.3.5. MATERNAL-FOETAL INTERACTIONS.

Ultrasound scanning combined with electromyography on 67-120 day pregnant ewes and does, showed both a passive change of position of the foetus during an episode of myometrial electrical activity, and foetal twitches, trembling, rolls and breathing movements (Scherboom and Taverne, 1984). Of significance to the work undertaken for this thesis, is that a prolonged period of uterine electrical activity influenced the foetus in two ways. First, during a uterine contraction, an increase of the bilateral diameter of the foetal chest has been noticed, as a result of the foetus being bent causing compression of the abdominal cavity and widening of the thoracic cavity. Secondly, foetal body movements seem to be reduced during uterine activity.

1.8.4. HORSES.

The first use of real-time ultrasound for investigation of pregnancy in broodmare practice was reported from France (Palmer and Driancourt, 1980), and its use in this species has expanded significantly since then. Thus, experience and knowledge of ultrasonography for pregnancy testing which has been applied to many species evolved from early studies on the mare.

1.8.4.1. OVARIES.

The equine ovary is sometimes difficult to find and may need to be manually located and repositioned to allow scanning. Small follicles (2 or 3 mm) require a 5 MHz transducer, since the minimum diameter observable with a 3.5 MHz transducer is 6 mm (Ginther and Pierson, 1984-a).

There are several potential applications of ovarian ultrasonographic examinations in mares (McKinnon and Squires, 1986-b; Squires and McKinnon,1986-b). These include estimation of the stage of the oestrous cycle, assessment of preovulatory follicles, prediction and determination of ovulation, examination of the corpus luteum, and diagnosis of ovarian abnormalities such as multiple preovulatory follicles, luteinized unruptured follicles, prolonged maintenance of the corpus luteum, ovarian neoplasia, periovarian cysts. Occasional small follicles (2-5 mm) and the absence of an ultrasonographically visible corpus luteum is suggestive of an anoestrous condition while the presence of multiple, large follicles is characteristic of transitional mares prior to their first ovulation of the year (McKinnon *et al.*, 1986-b). The selective accelerated growth of the follicle that is destined to ovulate begins 5 or 6 days before ovulation (Ginther and Pierson, 1984-a). Ovulation can be predicted within a 24 hour period for most mares (McKinnon *et al.*, 1986-b). Because the equine corpus luteum forms inside the ovary and does not protrude above the surface of the ovary as in cattle, it is easier to visualize the corpus luteum with ultrasonography than it is to palpate the corpus luteum per rectum (Squires and McKinnon, 1986-b).

1.8.4.2. UTERUS.

During anoestrus, the cross section of the uterine horns and uterine body is often flat and irregular. During oestrus, the uterine horns are well rounded and both horns and body have an internal pattern of alternating echogenic and non echogenic areas (McKinnon and Squires, 1986-a) due to the development of endometrial folds. The echogenic areas may be attributable to reflection from tissue dense portions of the folds, and the non echogenic areas are probably due to oedematous portions of the folds or to fluid between the folds (Ginther and Pierson, 1984-b).

When the mare is pregnant or in dioestrus, endometrial folds are less distinct or undiscernible and the echo structure is more homogeneous. When scanning the uterine body, the uterine lumen is often identified by an hyperechogenic white line which is the apposition of the endometrial surfaces.

Ultrasonographic appearance of the pregnant uterus is often identical to that of dioestrus with the exception that, after day 16, the endometrial folds again appear but are not as prominent as during oestrus (McKinnon and Squires, 1986-a).

During an oestrous cycle, the endometrial fold pattern develops gradually over a period of several days, reaches a peak 2 or 3 days before ovulation and then recedes, so that by day 0 or day 1 the uterus returns to the homogeneous condition characteristic of dioestrus. Ginther and Pierson (1984-b) suggested that study of the folding may help determine the optimal time for breeding.

Several pathological conditions can be studied by ultrasound. The two most common forms of uterine pathology detected by ultrasound are uterine fluid and uterine cysts. Less commonly, foetal remnants, iatrogenic debris and neoplasia are recorded. The ejaculate can also be visualised within the uterine lumen (McKinnon *et al.*, 1986-a).

Although the prominence of endometrial folds during oestrus and occasional small fluid accumulations of less than 3 mm should not be considered pathological, it is important to detect abnormal uterine fluid accumulation to be able to administer therapy. Thereafter, the volume and quality of fluid detected at ultrasound can be used to monitor efficacy of treatment (McKinnon *et al.*, 1986-a).

Rectal ultrasonography can detect urine pooling in the vaginal fornix, which may, on occasion, result in severe or chronic endometritis with concomitant infertility (Thornbury, 1975; Brown *et al.*, 1978). The ultrasound characteristics of urine in the bladder vary with diet. In mares fed grass hay, urine is often non or only slightly echogenic, while urine in mares fed alfalfa hay more commonly have an echogenic urine, perhaps due to increased mucus or calcium carbonate production (McKinnon *et al.*, 1986-a).

Ultrasound is also a valuable tool for monitoring uterine involution and to determine the prognosis for conception during the first post-partum oestrus.

There are three distinct morphological types of uterine cystic enlargements recognisable by ultrasonography; single or multiple uterine cysts, commonly pedunculated; transmural cysts, usually small; multiple, flattened, compartmentalized cystic structures. However, if prominent endometrial folds are present, the cystic structures are commonly obscured. All these cystic enlargements are attributable to lymphatic blockage (McKinnon *et al.*, 1986-a). The presence of single uterine cysts may easily be mistaken for an early pregnancy, but could be differentiated from a conceptus by their size and failure to develop when visualized on serial ultrasound scans (Torbeck, 1986).

1.8.4.3. THE CONCEPTUS.

Unlike the embryo of ruminants, the early equine conceptus is highly mobile within the uterine lumen (Ginther, 1983-a), moving between the two uterine horns and uterine body. Transuterine movements occur at intervals of less than 2 to 4 hours, but mobility begins to decrease by day 15, and after day 17, transuterine migration is no longer detected (Ginther, 1983-b).

Early detection of an embryonic vesicle requires a high frequency (5 MHz) transducer. The ultrasonographic imaging of the equine embryonic development was described by McKinnon and Squires (1988). The embryonic vesicle may be found for the first time at day 10. It is then a 2-3 mm spherical non echogenic area. Frequently ultrasonographic images of a 10-14 day vesicle will have a bright echogenic line on the dorsal and ventral poles with respect to the transducer, due to specular reflection.

Before 17 days, the yolk sac, which constitutes in horses the early embryonic vesicle, is spherical in shape, and because of the mobility it may be found anywhere within the uterine lumen. After 17 days, the shape of the yolk sac becomes irregular, and the conceptus is fixed at the caudal portion of one of the uterine horns, near the bifurcation.

The embryo, observed on the ventral aspect of the vesicle, is first detected ultrasonographically at day 20-25. The heart beat is commonly detected at day 22. Surprisingly, McKinnon and Squires (1988) recorded the mean size of a 25-day embryo to be 1.76 cm (sd = 0.59 cm). This is large if it is considered that 20-25 days is the period of first detection of the embryo. However, those dimensions were recorded with a 3.5 MHz transducer and there was no description of the foetus or the way the measurements were taken.

The growth of the allantois, first detected on day 24, ventral to the foetus, and the contraction of the yolk sac, dorsal to the foetus, result in the embryo moving from the ventral to the dorsal aspect of the vesicle. After day 40, the yolk sac has degenerated and the umbilical cord elongates from the dorsal pole, allowing the foetus to gravitate back to the ventral floor where it is seen in dorsal recumbency from day 50 onwards (McKinnon and Squires, 1988).

Surprisingly, the amniotic sac, although fairly obvious in ruminants, is never mentioned in the literature for horses.

1.8.4.4. ACCURACY OF RECTAL ULTRASOUND PREGNANCY TESTING.

The accuracy of pregnancy testing with a 3.5 MHz transducer ranged from 97.4 to 100% and was similar at all stages of gestation reported (15 to 50 days) (McKinnon and Squires, 1988).

1.8.4.5. ESTIMATION OF GESTATIONAL AGE.

McKinnon *et al.*(1988) report a relatively imprecise relationship between the diameter of the embryonic vesicle or the length of the foetus, and gestational age, between 15 and 50 days, using a 3.5 MHz transducer. To determine the age of the foetus after 100 days of gestation, measurement of foetal eye size, calculated from the sum of width plus length, was made with a 5 MHz transducer.

1.8.4.6. DIAGNOSIS OF TWINS.

Mares suspected of conceiving twins should be scanned as early as possible (11 days post ovulation) as twinning is a major cause of abortion in mares. The incidence of spontaneous twin ovulations is approximately 16%, whereas the production of live twin foals is only 1-2% (Squires and McKinnon, 1986-a). Wood and Ginther (1984) demonstrated that a considerable number of twin pregnancies are reduced to one during the mobility phase. Seventy percent of twin embryos become fixed in the same uterine horn. Ginther (1984) showed that the incidence of embryo reduction was much greater for unilateral fixation. After 40 days the twins are less likely to reduce to a singleton but are more likely to undergo abortion.

Generally, diagnosis of twins is easier during the mobility phase because the vesicle is then round and the individual conceptuses can be detected. The most difficult diagnosis is when the twin vesicles are unilaterally fixed since the apposition between the two vesicles is sometimes faint and difficult to discern ultrasonographically. In some cases the only distinguishing characteristic is an oversized vesicle. In addition, the wall of apposition between two vesicles could be confused with the division between the yolk and allantoic sac in a singleton. Another potential problem is confusion with multiple uterine cysts. Later in gestation, i.e. 45 days, careful scanning may identify two umbilical cords (Squires and McKinnon, 1986-a).

1.8.5. DEER.

1.8.5.1. ULTRASONOGRAPHIC TECHNIQUE.

There are 40 species of deer worldwide, and about 200 sub-species (Whitehead, 1972). Deer commonly farmed in New Zealand are (smallest to largest), fallow (*Dama dama*), red (*Cervus elaphus*) and elk (*cervus elaphus canadensis*). Depending on the size of the animal, the ultrasonographic technique may be different. Fallow deer are small enough for transabdominal ultrasonic imaging (Mulley *et al.*, 1987), although rectal ultrasonography is now considered easier and more accurate for early pregnancy diagnosis (English A.W. and Asher G., pers. comm.). In elk deer, ultrasonography may be done in association with manual rectal palpation, as in cows or mares, although the rectum is tight and good restraining devices must be available (Wilson P., pers. comm.).

Fennessy *et al.*(1986) referred briefly to rectal ultrasonography in red deer, but the first detailed description of diagnostic ultrasonography in deer was by Mulley *et al.*(1987). Those authors described transabdominal pregnancy testing of fallow deer, from 7 days of gestation onwards, using a 3.5 MHz mechanical sector transducer. Scanning was performed within 60 seconds of restraining the fallow doe upright, to expose the inguinal area, and blind-folded. Pregnancy was detected easily only after 50 days. Around 50 days, the foetus was visible in whole or in part and foetal movements were common. At 105 days gestation, various sections of foetal anatomy and placentomes were recognised, although not described. The first detailed reports of the method for ultrasonography in red deer were by Bingham *et al.*(1988, 1990) and Wilson and Bingham (1990). Those authors described rectal ultrasonography and its use for foetal ageing.

1.8.5.2. ACCURACY OF PREGNANCY DETECTION.

Bingham *et al.*(1988, 1990), using a 5 MHz linear transducer for rectal examination of red deer, obtained a 97.6% accuracy of pregnancy diagnosis from day 40 to day 132 of gestation, or a 99.3% accuracy when the period was 40 to 117 days. The specificity of the test (number of non pregnant hinds tested non pregnant/number of non pregnant hinds) was 96.5%, while the sensitivity (number of pregnant hinds tested pregnant/ number of pregnant hinds) was 100%. However, the same operators later reported 100% accuracy on 162 hinds, 30 to 110 days pregnant (Wilson and Bingham, 1990).

Mulley *et al.* (1987) using a 3.5 MHz transducer for transabdominal scanning on fallow deer, reported all of the positive pregnancy diagnosis (54/60) were pregnant, and in all cases of non detection, the does were non pregnant. However, this did not prove the accuracy of the technique as the results were from the same does scanned sequentially.

1.8.5.3. FOETAL AGEING.

Bingham *et al.*(1988) reported for the first time in deer the relationship between foetal or uterine dimensions and the advancement of the gestation. Eleven age prediction equations were computed (Bingham *et al.*, 1988, 1990) from the measurement of uterine diameter (42-76 days), largest and smallest diameters of the amniotic sac (42-68 and 42-112 days, respectively), crown-rump length (42-66 days), head length (42-123 days), head diameter (42-121 days), nose length (42-68 days), chest depth (42-116 days), chest diameter (42-94 days), placentome width (42-127 days) and placentome base-apex (42-128 days). These equations allow foetal age prediction between 42 and 128 days of gestation.

Wilson and Bingham (1990) tested the accuracy of foetal age estimation on commercial farms with the age prediction equations established by Bingham *et al.*(1988 and 1990) with a 5 MHz linear rectal transducer. Foetal age was calculated from the mean of the age estimations derived from each dimension measured on individual deer between 44 and 110 days gestation. The mean error of calving date prediction in 132 eighteen-month-old deer was +0.97 day. The estimates of individual calving date were all within 13 days of the actual recorded calving date, with 92.5% of hinds calving within 10 days of the predicted value. This result was in accordance with the different standard deviations of the equations used, ranging from 2.9 to 13.5 days, enhanced by the natural variation in length of the gestation of red deer which is 233 days + or - 7 days (Wilson P.R., pers. comm.). Although of acceptable accuracy, a more accurate method would have been to weight the importance of each dimension, or to ignore the less accurate dimensions (uterus diameter, placentome) referring to only one or two of the more accurate dimensions (crown-rump, amnion length...), rather than calculation from the mean of the estimations given from a number of dimensions measured. Estimates using dimensions giving equations with the largest errors should be used only when the operator is unable to obtain better measurements.

White *et al.*(1989) also presented foetal age estimation equations for red hinds, from the measurement of foetal head diameter and trunk diameter, during the gestational period 35 to 150 days. A 5 MHz transrectal linear transducer was used from 30 to 50 days pregnancy, while a 3.5 MHz transabdominal sector transducer was used from 50-150 days. Uterine lumen diameter and cotyledons could be measured, although the authors did not use them for age estimation because of the variability of the measurements. Examination of standard deviations show that the equations reported by White *et al.*(1989) are less accurate than those produced by Bingham *et al.*(1988). It should be noticed that the abstract given by White *et al.*(1989) contains an error (the standard deviation of the growth equations are given in days instead of cm) which may confuse the reader as far as the precision of his equations are concerned. However, the equations of White *et al.*(1989) give the ability to predict calving dates until 150 days gestation with an acceptable accuracy (sd = 8.5-9.8 days) while equations presented by Bingham *et al.*(1990) were useful only until 128 days gestation.

There is a discrepancy up to 12 days when using the equations of Bingham *et al.*(1990) or those of White *et al.*(1989) to predict the foetal age. The equations of the latter give lower results. This may be due to a different technique, or a different management of the data (linear versus quadratic regressions).

1.8.6. SOUTH AMERICAN CAMELIDS.

There are six species of camelids in the world. In South America, the llama (*Lama glama*) and alpaca (*Lama pacos*) are domesticated, while the Guanaco (*Lama guanicoe*) and the vicuna (*Lama vicugna*) are still wild species. In the old world, the bactrian camel (*Camelus bactrianus*) is found in Asia, while the dromedary camel (*Camelus dromediarus*) is found in Africa (Simpson, 1945). New Zealand has a particular interest in alpacas, which produce a very soft fibre, finer than that of Merino sheep and of higher price (De

Vore, 1984).

Pregnancy can be detected by lack of oestrus behaviour, palpation, hormonal assay, doppler, ultrasound scanning or radiology. Fowler (1989) advises to use transrectal ultrasonography for pregnancies of less than 120 days; later in gestation, transabdominal scanning might be of more use, as sometimes the foetus is found in a very anterior position, ventral to the stomach. It is important to know that lamoids are not as susceptible to rectal trauma as the mare, but are more sensitive than the cow.

As with deer, the different species of South American camelids vary in size, and rectal ultrasonography will be performed with manual direction of the probe on the larger species, but with a rigid extension for the smaller species. The foetal crown-rump length progresses uniformly for the first three trimesters, but growth rate increases during the last three months. For rectal palpation or ultrasonography it is of some interest to know that the relative quantity of placental fluids is much less in lamoids than in other livestock species (Fowler, 1989).

Very little is known about pregnancy detection by ultrasonography, and even less is known about ultrasonographic detection of reproductive diseases or malformations, although infertility is the main problem in South American camelids (Sumar, 1983). Ultrasonography would provide a valuable tool for investigation of reproductive problems in these species.

1.9. RED DEER REPRODUCTIVE ANATOMY.

Requirements for an accurate interpretation of ultrasound images include an understanding of ultrasound technology as discussed, and a knowledge of the anatomy of the organs to be imaged.

1.9.1. UTERUS.

1.9.1.1. NON-PREGNANT UTERUS.

The only reference in the literature appears to be that of Thome (1980). The following is summarised from that publication.

The uterus of the non pregnant hind is almost completely in the pelvic cavity. The uterus appears similar to that of other ruminants, being divided into two horns closely united at their bifurcation by the ligamentum intercornuale. The uterine body and cervix rest on the bladder. The horns have a total length of 74-135 mm when yearling hinds, 121-312 mm for older hinds. The horns lie parallel and close to one another at their posterior part, for a distance of 38-75 mm in yearlings, and 55-175 mm in older hinds. At the ligamentum intercornuale, the horns separate from one another and curve ventro-laterally, forming an almost closed circular bend in yearlings, but only a flat loop in older hinds. The diameter of the horn, measured from the ligamentum intercornuale, was on average 11-18 mm, respectively, for yearlings and adult hinds. At their distal extremity, the horns are in continuation with the fallopian tubes. The uterus is suspended by the mesometrium which is formed by the parietal peritoneum originating from the abdominal and pelvic cavity. The uterine wall is composed of an inner mucous membrane, the endometrium, which is surrounded by an outer layer of smooth muscles, the myometrium. The endometrium of yearling non pregnant hinds has a smooth surface, while in older hinds the endometrium is thicker and folded.

Cervids are classified as oligocotyledonary. Caruncles, the maternal component of placentation, are few in number compared with cattle. Four or 5 variable shaped elongated caruncles lie adjacent to the mesometrial attachment of each horn. The central caruncles in each horn are often the largest (up to 34 mm long x 14 mm wide x 13 mm high), while those lying closest to the extremities of the horns are small, the distal end of the horn being generally free of caruncles. The uterine body is short and is merely the connection between the two horns. The cervix is thick-walled and presents a slightly conical external shape, larger at the posterior end. The cervical canal contains 5 to 8 circular folds. These folds are mainly rings, but spiral and sickle shaped folds occur. Posterior folds are generally more developed.

1.9.1.2. PREGNANT UTERUS.

At the beginning of pregnancy, the pregnant horn increases in size more than the non pregnant horn. This was clearly noticeable at day 34 and became more pronounced towards day 55 (McMahon, 1989). At approximately the third month of pregnancy, the ligamentum intercornuale becomes loose, and the distinction between the two horns becomes less discrete. From 5-6 months, the pregnant uterus extended cranially to the first lumbar vertebra and it had contact with the ventral and dorsal rumen sac (Thome, 1980). The uterine weight, excluding the conceptus, was observed to decrease from 13 to 34 days before increasing in weight from 48 to 55 days gestation when observations were concluded (McMahon, 1989). While no explanation of the early weight decrease was given, the latter weight increase was attributed to hyperplasia and hypertrophy of the myometrium, stimulated by the rapid growth of the foetus (McMahon, 1989). However, the weight gain could have been partly attributable to endometrial hyperplasia, producing folds, from approximately the first to third months pregnancy (Thome, 1980). Thome (1980) described the endometrial folds as mucous membranes with a height up to 8 mm, in the intercaruncular region during the first month. These were rapidly replaced by other larger folds which stretched in a three quarter arc in the lumen of the uterine horn, from dorsolateral, to medial and ventrolateral. Also, numerous other membranous folds of various lengths were found running longitudinally along the horns. Until day 55, which was the last day of the observations, the caruncles were reported growing in width and height, but not in length, and only in the pregnant horn (McMahon, 1989). However, the caruncles were found to have increased in length up to 80 mm, in width up to 112 mm, and in height up to 61 mm, one or two months before parturition (Thome, 1980). At the beginning of pregnancy, the caruncle was connected to the endometrium over its entire area, but from approximately the fourth month, the attachment was restricted to the middle third or to one of the caruncle poles. From 5-6 months gestation, all caruncles had simply a stem-like contact with the endometrium, with a stalk of 30 mm. Accessory caruncles, about 5 mm diameter, appeared during the second month of pregnancy approximately. These grew to about 15 mm diameter at approximately 4 months, and were found isolated on the lateral wall of the uterine horn (Thome, 1980).

Implantation occurs in apposition, adhesion and attachment phases. McMahon (1989) found the apposition phase had occurred by days 27-34. However, given that McMahon's observations were only at 7 day intervals, it could be suggested that if apposition was sometimes found at day 27, the starting of the process could have been any time between 21 and 27 days. The adhesion phase was observed by 34-41 days, and attachment occurred from day 41 onwards.

Single or multiple cysts of 2-5 mm diameter, created by blockages of lymph drainage, were observed on both the exterior of the uterus and on the endometrium, in pregnant or non pregnant hinds (McMahon, 1989).

1.9.2. EMBRYO AND FOETUS.

There have been few reports in the literature describing the uterus and the developing conceptus in red deer.

The embryo is generally found in the posterior third of either uterine horn, near the second caruncle (Thome, 1980; McMahon, 1989). Transuterine migration was found in

25% of the pregnancies (McMahon, 1989) although the number of observations (3/12) was small.

At day 13 the embryo is a spherical blastocyst; between day 13 and 20, gastrulation begins, and by day 27, that process is complete. The embryo develops a C-shape, and limb buds become apparent by day 34. By day 48, the separation between the phalanges can be seen. It was found that muscle development, particularly myocardium, had already commenced at 27 days (McMahon, 1989). From day 72, the first day of observation in the experiment of Wenham *et al.*(1986), radiographic studies of foetal skeletal growth detected a long neck characteristic of the species with the cervical vertebrae being longer than the other vertebrae. At the same time, the majority of ossification centres were present, except for the carpus, tarsus and phalanges. It was also found that the bones of the hind limbs grew faster than those of the forelimbs, and the metacarpus and metatarsus were growing faster than the proximal limb bones (Wenham *et al.*, 1986). During the first 55 days of pregnancy, the crown-rump length of the foetus was shown to follow a linear increase in respect to time (McMahon, 1989). Around the seventh month of gestation, the amniotic sac and the foetus reached to the area of the corpus uteri (Thome, 1980).

1.9.3. FOETAL MEMBRANES AND FLUIDS.

The blastocyst hatches from the zona pellucida at about day 13, after which the trophoblast extends and reaches the extremities of both horns by day 27 (McMahon, 1989). The resultant membrane forms a freely mobile strong straw-stalk shaped sac, the chorion (Thome, 1980).

There are four membranous foetal sacs: the chorion, amnion, allantois, and yolk sac. The embryo is entirely contained within the fluid filled amnion, totally enclosed by the chorion. Occupying the space between the amnion and the chorion is the fluid filled allantois which keeps the chorion, and therefore the uterus, fully distended (Chaplin, 1977). The yolk sac is small and supports blood cell production by the foetal liver (King et al, 1982; McMahon, 1989).

The allantois reached 20% of the length of the chorion by day 27, and by day 34, the chorion was completely filled by the allantois, forming the allanto-chorion, while the yolk sac was in regression (McMahon, 1989). From about the third month of gestation, Thome (1980) found hippomanes which are oval structures consisting of aggregations of secretions from endometrial glands, suspended in the allantoic fluid and connected to the inner surface of the allantois by short stems. In late pregnancy, the hippomanes reached the size 30 x 20 x 6 mm. Probably because his experiment stopped at 55 days, McMahon (1989) did not find hippomanes in the allantois Discrete circumscribed oval chorionic plaques appear facing the caruncles. Villi arise from those areas, which then become a cotyledon. When the villi penetrate and become established into the caruncular crypts, a placentome is formed (Hamilton et al, 1960). Thome (1980) described the presence of white bands on the allanto-chorion (30 x 140 mm) but could not clarify the function of these depositions. However, McMahon (1989) suggested they might be formed by the expansion of the areolae, described by Hamilton *et al.*(1960) as differentiated surfaces facing the endometrial glands. Their role might be for nutrient intake from the uterine milk.

McMahon (1989) saw plaques of unknown function on the exterior surface of the amniotic sac, and, from about 2-3 months pregnancy, Thome (1980) found numerous membranous growths on the inner surface of the amnion and on the umbilical cord. Their shape was variable as clubs, rings, bands or villi, and they increased considerably in number and size to reach 20 mm length at approximately the fourth month of gestation. The amniotic fluid increased noticeably in viscosity during gestation (Adam *et al.*, 1988-b).

1.9.4. OVARY.

The left and right ovary of a non pregnant hind are identical in weight. The weight of the ovulating ovary does not differ from the weight of the non-ovulating ovary from 13-55 days gestation (McMahon, 1989). However, the corpus luteum increases significantly in size from early to late gestation, from 9.8 to 12.3 mm (Kelly and Challies, 1978). Accessory corpora lutea are histologically identical to, but half the size of the primary corpus luteum, and are believed to be formed early in the breeding season and to persist until parturition (Kelly and Challies, 1978).

Except for those studies of the corpus luteum, there is no detailed anatomical description of the red deer ovary in the literature.

1.10. DETECTION OF OESTRUS.

Oestrus can be detected by continual observation of reproductive behaviour as described by Dudley (1983) and Veltman (1985). Alternatively, vasectomized stags fitted with harnesses and crayons may be used (Guinness *et al.*, 1971; Kelly *et al.*, 1985; Asher *et al.*, 1988-a). Crayon marks are mainly produced during low mounts, but where the mating, which is very brief and with few contacts (Veltman, 1985), is without preliminary low mounts, crayon signs may be absent. Consequently, the effectiveness of harnesses for detection of oestrus in red deer is highly variable, contrary to the 90% detection rate reported in fallow deer using this technique (Asher, 1985). The fallow buck exhibits a higher low mount/service ratio and wallows less than the red stag, keeping the crayon usable for a longer period (Asher *et al.*, 1990).

Spermatozoa in a vaginal swabs detected recent matings (Kelly *et al.*, 1985), but involved a lot of handling and is not a practical technique for oestrus detection.

Use of rectal ultrasonography associated with palpation is highly effective for oestrus detection in cows and mares (see section 1.2.7.1.1), and would probably be reliable as well in larger deer species as elk, but is not practical in red deer due to the size of the anus and rectum, preventing consistent location of the ovary.

Because of the difficulties encountered with oestrus detection, hormonal oestrus synchronisation is used for artificial breeding programmes in this species.

1.11. DETECTION OF PREGNANCY IN DEER.

Pregnancy diagnosis early in gestation is more important for investigation of infertility and for management purposes than is pregnancy diagnosis later in gestation.

High plasma progesterone concentrations may indicate conception if found in two samples taken at a 14 day interval, thus avoiding confusion with the luteal phase of a non pregnant hind. This method requires double handling and laboratory work, and, from trials on fallow deer, it gives an overestimate of conception rate by 10% (Asher *et al.*, 1988-b), possibly because of the presence of luteal cysts or persistent corpora lutea.

A pregnancy-specific protein (PSP) dependent on the existence of a viable embryo was detected in human serum 48 hours after fertilization (Rolfe, 1982). Similarly, PSP was detected 4 to 6 hours after fertile mating in mice (Morton *et al.*, 1976, quoted by Rolfe, 1982) and within 24 hours in sheep (Morton *et al.*, 1979). Detection of PSP provides a means of monitoring embryonic development during the pre-implantation and early post-implantation period, and in humans allowed the discovery that only 30% of all conceptions survive to birth. Thus, this test could be useful for studying early embryonic deaths in farmed animals,in addition to pregnancy diagnosis. A pregnancy-specific protein

(PSP-B) was isolated from bovine placental membranes (Ivani *et al.*, 1984) and is reported as accurate for pregnancy detection in cattle, goats, sheep, and red deer hinds where it was first detected 24 days after mating, but more consistently 30-32 days after mating (Fennessy *et al.*, 1986).

Rectal ultrasound scanning is the most appropriate method for pregnancy diagnosis of large numbers of animals. It is accurate and rapid from 30 to 130 days pregnancy, and has the advantage of giving a direct answer, while being relatively economical. This technique is described in detail in section 1.8.5.1.

Late pregnancy can be diagnosed by observing abdominal distension. The diagnosis would be improved if accompanied by weighing the hind twice at a few months interval with the last weighing one or two months before the predicted calving date (Wilson, pers. comm.). Abdominal palpation or ballotment is unreliable and requires abdominal wall relaxation. It is therefore difficult. Udder development occurs mostly during the last month of gestation (Adam *et al.*, 1988-a), but variable between hinds and sometimes delayed to within a week of parturition. Even when developed, the udder is still relatively small, possibly reflecting the low volume-high solids milk production characteristic of the red deer (Arman *et al.*, 1974). The red deer hind anus and rectum is too small to allow manual rectal palpation, although this can be undertaken on elk and larger red-elk hybrids (Wilson, pers. comm.).

1.12. FEMALE RED DEER REPRODUCTIVE CYCLE.

1.12.1. REPRODUCTIVE CYCLE AND SEASONALITY.

Of the 40 species of deer living from the polar regions to the tropics, 24, including red deer, are adapted to cold or temperate climates and 16, including rusa deer, are adapted to tropical climates (Whitehead, 1972).

Most species of deer are seasonal breeders. Births are usually confined to when conditions best favour survival of the offspring, i.e. spring or early summer. For the more northern species, such as moose and reindeer, the birth season may be restricted to 1-2 weeks, while for the more tropical species the season tends to be longer and more variable. Differences in seasonal patterns between species from the tropics and those from temperate and cold regions reflect genetic differences. Fletcher (1974) reported that when a temperate species such as the red deer is moved to more equatorial latitudes, it continues to show a clearly defined calving season in early summer, with no increase of the length of the calving season. Translocation from one hemisphere to the other results in a shift of calving season by 6 months. In parallel to these observations, the findings of Lincoln (1971) showed that spermatogenesis and testosterone secretion in the stag was initiated at the period of the summer solstice. Indeed, the only aspect of photoperiod which is common to all latitudes is the timing of the solstices and equinoxes. Tropical species continue to produce offspring over an extended season when taken to colder climates, and, even after many years in captivity, they fail to change their breeding pattern (Lincoln, 1985, adapted from Zuckerman, 1953), suggesting that seasonality has a genetic rather than environmental causality.

Social organisation during the rut varies between species, depending on whether females tend to be solitary or gregarious. In most species, dominant males mate with several females either by tending individual females over the period of oestrus (e.g. roe deer, reindeer, whitetailed deer) or by monopolising a harem as in red, fallow, sika, and Pere David's deer . For temperate species, the timing of the rut is organised to allow for differences in gestation length: the larger species with the longest gestation length rut earliest (Lincoln, 1985), although the exception is the roe deer which is a small species yet has a very early rut in late summer. Roe deer have a delayed implantation (Short and Hay, 1966) probably to allow both the rutting season and calving to take place at a favourable time of year. For the red deer in Scotland (Lincoln and Guinness, 1973; Guinness *et al.*, 1978) the majority of conceptions occur in October, and the calves were born in late May and early June. In the South Hemisphere, in New Zealand, conceptions are mainly in April. Thus the mean calving date is in late November (Kelly and Moore, 1977; Asher and Adam, 1985). Stags begin rutting behaviour and establish harems a few weeks before the hinds start to come into oestrus. The majority of hinds conceived at their first oestrus of the season (Guinness *et al.*, 1971). However, there appears to be a stag effect on conception rates. At Massey University, conception rates to first oestrus have been seen ranging from 30% to 100% when different stags were used (Wilson, pers. comm.).

Under farm conditions, a red hind conceives for the first time when about 16 months of age. As suggested by Hamilton and Blaxter (1980), the timing of puberty is more related to weight than age, but is seasonal, equating to the breeding season in adults. A liveweight of 60 kg was demonstrated to be the minimum for yearlings to conceive (Kelly and Moore, 1977), and 94% of hinds weighing 85 kg will conceive (Hamilton and Blaxter, 1980).

1.12.2. REPRODUCTIVE ENDOCRINOLOGY.

1.12.2.1. OESTROUS CYCLE.

Red deer hinds are seasonally polyoestrous, with 18-day oestrous cycles commencing in autumn. If conception does not occur, it is possible for a hind to have up to eight oestrous cycles, with each oestrus lasting 12-24 hours (Guinness *et al.*, 1971; Kelly and Moore, 1977). There is a gradual increase in oestrous cycle length, evident with later cycles (Guinness *et al.*, 1971; Asher, 1985).

The hormonal pattern associated with luteal regression, follicular growth, oestrus and ovulation in domestic ruminants was described in a review by Henderson and McNatty (1988): Prostaglandin F2α from the uterus causes regression of the corpus luteum, associated with a fall of plasma progesterone and an increase of the pulse frequency of LH released from the pituitary. LH stimulates the production of androgen and oestradiol 17β by the developing ovarian follicle, leading to oestrous behaviour.

The cyclicity of plasma progesterone concentrations during the breeding cycle of red hinds was clarified recently by Jopson *et al.*(1990). Anoestrus was characterised in red hinds by low levels of plasma progesterone (less than 0.2 ng/ml). The start of the breeding season was indicated by a short period (6-11 days) of elevated plasma progesterone (up to 0.9 ng/ml) immediately followed by the first oestrus. The moderate progesterone elevation preceding the first oestrus was considered to be the result of a "silent oestrus", also reported in other deer species (Thomas and Cowan, 1975; Harder and Moorhead, 1980; Asher, 1985). The first oestrus was coincident with low progesterone levels (mean = 0.2 ng/ml). Two to three days later, progesterone increases and reaches a peak level of 2.2-3.1 ng/ml, 14-16 days after oestrus. Thereafter concentrations fall within 3 days to the low level of progesterone characteristic of oestrus (Jopson *et al.*, 1990). Other authors also report plasma progesterone profiles in deer resembling those of other species (Adam and Atkinson, 1984; Adam *et al.*, 1985). However, Kelly *et al.*(1985) and Webster and Barrell (1985) found variable and sometimes high levels of progesterone during oestrus in red deer, and Kelly *et al.*(1985) did not report pre-oestrus progesterone elevation at all. It is probable that the hormonal assay employed by Kelly *et al.*(1985) was less sensitive than that of Jopson *et al.*(1990), and therefore, did not allow detection of the slight pre-oestrus increase. It has been postulated that elevated plasma progesterone levels at oestrus might be due to the persistence of a corpus luteum or to luteal cysts (Asher *et al.*, 1988-b). Furthermore, experimental results from Jopson *et al.*(1990) indicated that the adrenal gland might be a significant source of progesterone in the red deer hind. Thus stress, resulting in adrenal steroid secretion, might confuse results.

A luteinizing hormone (LH) peak of more than 10 ng/ml was recorded in 5 of 14 occurrences of oestrus in red deer hinds using daily blood samples (Kelly *et al.*,1985). These authors concluded that the preovulatory rise of LH in red hinds had a duration of 24 hours. However, as blood samples were taken daily, allowing detection of only 35% of LH peaks, it appears probable that LH actually rises for a period of less than 24 hours.

A peak of oestradiol observed on and the day before oestrus was variable with a recorded maximum of 148 pg/ml. One function of these high concentrations of oestradiol may be to counter the negative behavioral effects of elevated levels of progesterone observed by some authors during oestrus (Kelly *et al.*, 1985). However, Asher *et al.*(1986), who observed no elevation of progesterone at oestrus in work based in fallow deer, suggested that the role of oestradiol in stimulating the preovulatory surge of LH and the expression of oestrous behaviour was equivocal.

1.12.2.2. PREGNANCY.

Red deer hinds mate in autumn and calve the following summer with a gestation period of 231 days (sd = 4.5) on the Isle of Rhum (Guinness *et al.*, 1971), or 233 days in New Zealand (Kelly and Moore, 1977) with a range of ±7 days from Massey University records (Wilson P., pers. comm.).

Plasma progesterone concentrations of the pregnant hind reach a peak value of 3-5 ng/ml, and remain relatively constant thorough pregnancy (Kelly *et al.*, 1982; Adam *et al.*, 1985) until 200 days of gestation, when concentrations decline gradually to reach basal levels after parturition. Kelly *et al.*(1982) suggested that luteal tissue is the major source of progesterone in pregnant red deer. In addition to the larger primary corpus luteum, an accessory corpus luteum was often present (Guinness *et al.*, 1971; Kelly and Challies, 1978). However, pregnancy was maintained adequately without the accessory luteal body, thus its role seems obscure (Kelly and Challies, 1978). Its presence was not detectable from blood progesterone values (Adam *et al.*, 1985).

Oestradiol concentrations decline from the mating day, reaching 5-10 pg/ml around 160-190 days of gestation, then increase to about 35 pg/ml just before parturition (Kelly *et al.*,1982). The prepartum rise in oestradiol concomitant with a decline in progesterone was similar to that recorded for other polyoestrous ungulates (Heap *et al.*, 1973) and probably indicates a role in the initiation of parturition.

Prolactin levels decline to undetectable levels 14 days after mating and thereafter remain low for the next 100 days. However, plasma prolactin appears to be more closely related to photoperiod than to the reproductive status of the animal. Indeed, within the assay sensitivity, prolactin was undetectable in the blood during winter season, and at peak level during summer (Kelly *et al.*, 1982).

LH declined to basal or undetectable levels about 14 days after mating and thereafter remained low or undetectable (Kelly *et al.*, 1982).

1.12.2.3. SYNCHRONISATION OF THE OESTROUS CYCLE.

Oestrus synchronization can be achieved either by shortening the luteal phase by administration of a luteolysin i.e. prostaglandins, or by simulating the activity of the corpus luteum through administration of progestagens.

Prostaglandins have a luteolytic effect on the corpus luteum, but only when it is active, i.e. from 9 days after ovulation. Therefore, it is necessary to inject twice at a 10-day interval to induce luteolysis followed by ovulation (Fennessy *et al.*, 1989-a), and synchronization programmes are therefore limited to the period after the onset of the natural rut (Asher *et al.*,1990).

Progesterone treatments mimic the natural pattern of the oestrous cycle, and the rapid drop in progesterone levels after intravaginal sponges or CIDR (Controlled Internal Drug Release) device removal, leads to oestrus 48-78 hours later in red hinds, with the LH peak occurring at about 48 hours. Assuming a constant interval between LH peak and ovulation, Fennessy *et al.*(1989-a) suggested ovulation occurs at about 72 hours after progesterone device withdrawal. There was a high rate of loss of intravaginal sponges (Kelly *et al.*, 1982) while the retention rate of the CIDRs was high (98-100%) (Asher *et al.*, 1988-a). The device originally designed for sheep (CIDR-S, 9 or 12% progesterone) is generally used for red hinds, and left in place for about 14 days before withdrawal. To mimic the natural level of progesterone, an additional CIDR, either two devices initially or alternatively, replacing the first CIDR generally 3-5 days before progesterone withdrawal, is theoretically necessary (Jopson *et al.*, 1990). However, Fennessy *et al.*(1987) and Fennessy and Mackintosh (1988) noticed little effect in doing so. Pregnant mares' serum gonadotrophin (PMSG), having FSH and LH activity, is administered routinely at or near the time of CIDR withdrawal. This improves the proportion of deer ovulating, particularly prior to the onset of the normal breeding season (Fisher *et al.*,1986), overcomes the reduction of ovulation due to the stress of handling (Bringans, 1989-a), and improves synchronisation of ovulation from the time of CIDR withdrawal (Fennessy *et al.*,1989-a). PMSG should be used at a low level (200-250 UI) to reduce the risk of multiple ovulation (Fennessy *et al.*,1989-a).

1.12.2.4. SUPEROVULATION.

Superovulation may be induced after CIDR-progesterone priming, followed by drugs having FSH action. The use of PMSG and some less refined preparations of FSH gave inconsistent results (Bringans, 1989-a), due to their high and variable LH/FSH ratio. Fennessy *et al.*(1989-b) showed improved superovulation rates and quality of embryos with preparations having more specific FSH activity.

1.12.2.5. ADVANCEMENT OF THE BREEDING SEASON.

The advancement of the breeding season in New Zealand would result in a better match of feed demand with feed supply (Wilson, 1989), and more time would be available to obtain a good carcass weight when venison prices are high. In terms of live weight, the advantage of an earlier birth date was shown by Fisher *et al.*(1989) to be, in January, on average an extra 0.44 kg/day born earlier, and in March, at weaning, 0.3 kg/day earlier.

The techniques used for advancing the onset of oestrus are, hormonal induction of ovulation, hind:hind and stag:hind interactions, improvement of nutrition, early weaning, artificial light-dark manipulation, melatonin treatments (Wilson, 1989).

1.12.2.5.1. Induction of ovulation.

Induction of ovulation by synchronisation of the oestrous cycle (see section 1.12.2.3) prior to the natural breeding season, was investigated in yearlings and non lactating hinds (Adam *et al.*, 1985; Fisher *et al.*, 1986; Moore and Cowie, 1986; Duckworth and Barrel, 1988) and in lactating hinds (Fisher *et al.*, 1989). Advancement of oestrus was up to 3 weeks before the onset of the natural breeding season with lactating hinds (Fisher *et al.*, 1989), and up to 6 weeks in yearlings and non lactating hinds (Adam et al, 1985; Fisher *et al.*, 1986; Moore and Cowie, 1986). However, poor fertility resulted. An explanation could be that induced ovulations were silent, as observed in sheep (Rodway and Swift, 1985). Furthermore, stag rutting behaviour and/or fertility might not be sufficiently developed before the onset of the breeding season. However, stags could mate successfully as early as mid to late February, with hinds calving early in October (Moore and Cowie, 1986). A melatonin-treated stag could be employed for hinds induced into early oestrus (Fisher *et al.*(1989).

1.12.2.5.2. Hind:hind, stag:hind interactions.

Interactions between animals can cause advancement and synchronisation of oestrus. McComb (1987) provided the first evidence that male vocalizations can affect the timing of ovulation in female red deer, showing that oestrus was synchronised earlier in the natural breeding season when hinds were exposed to play-back roaring prior to introduction of the stag (6 days difference in mean conception date), or when they were in the presence of a vasectomized stag prior to introduction of a fertile stag. The latter effect of vocalization combined with pheromones and male behaviour resulted in an advancement of mean calving date by 8 days. Wilson *et al.*(1988) and Fennessy and Fisher (1988) reported earlier oestrus for control hinds in-contact with melatonin treated hinds. This may have been a hind effect combined with a stag effect as the treated hinds were run with a stag, although Fisher *et al.* (1990) did not recognise the existence of hind effect. From an experiment involving melatonin treated stags, Wilson (1990) showed a stag effect, with the advancement of median calving dates of 8 and 10 days in two trials.

1.12.2.5.3. Hind weight.

The hind body weight has an effect on the onset of the breeding season. Mitchell and Lincoln (1973) report that hinds in good condition were first to breed at the start of the mating season, while Hamilton and Blaxter (1980) quantified the relationship to show that oestrus is advanced one day for every 4 kg increase in body weight. Nutrition also has a role in determining the length of lactational infertility, through the effect of suckling frequency (Loudon *et al.*, 1983). Consequently, early weaning should reduce the lactational infertility period.

1.12.2.5.4. Photoperiod manipulations.

Suttie and Simpson (1985) demonstrated the involvement of photoperiod in deer seasonality, and Webster and Barrell (1985) showed the effects of reduced photoperiod were mediated by melatonin. Melatonin influences the production of gonadotrophic hormones, LH and FSH, thus leading to seasonal changes in the fertility of both sexes (Lincoln, 1985).

Investigations of artificially shortened daily photoperiod advanced the calving season by one month, accompanied by early moulting of the summer pelage (Webster and Barrell, 1985).

The effect of shortened daily photoperiod can be mimicked by supplementary melatonin given either by injections (Webster and Barrel, 1985), feeding (Adam and Atkinson, 1984; Nowak *et al.*, 1985; Fennessy *et al.*, 1986; Adam *et al.*,1986; Adam *et al.*, 1989), or subcutaneous implants (Fennessy *et al.*, 1986; Fisher and Fennessy, 1987; Fennessy and Fisher, 1988; Wilson *et al.*, 1988; Wilson, 1990; Asher, 1990). Subcutaneous implants are more practical than injections or feeding, and are designed for commercial purpose. Studies in farmed red deer (Wilson, 1990) showed that melatonin should be administrated to hinds with a 45 day two treatments starting in December, using 18 mg of melatonin. Adam *et al.* (1989) demonstrated the possibility of using melatonin treatment on pregnant and lactating hinds without any effect on the onset or maintenance of lactation. However, this may be a matter of treatment starting date. Low prolactin levels, induced by melatonin, appears to have no effect on maintenance of lactation, but it is expected that if given to late pregnant hinds it may compromise initiation of lactation. It is usually not practical to handle and yard lactating hinds because of the risk of mismothering and of injuries to fawns (Wilson, 1990). While melatonin treatments produce earlier matings, the end of the breeding season may still remain the same. Although, the extended breeding season results in a higher conception rate from increased mating opportunities (Wilson, 1990).

1.13. MANIPULATION OF REPRODUCTION IN DEER.

The following is a brief resume of artificial insemination (AI) and embryo transfer (ET), and is included because one of the important applications of ultrasonography, which is the primary subject of this thesis, is in confirming pregnancy to AI and ET procedures, and distinguishing them from subsequent matings.

When predicting the calving dates of artificially bred animals, one should remember that the length of gestation is decided by the foetus rather than the hind. Thus, if a red hind was inseminated with elk semen, the length of the gestation will be between 233 days (red component) and 260 days (elk component). A red hind carrying a pure elk embryo will have a gestation length of circum 260 days (Bringans, 1987).

1.13.1. ARTIFICIAL INSEMINATION.

Artificial insemination is used in New Zealand to aid the distribution of genetic material, to increase the impact of particularly desirable sires, and for hybridisation between species that do not interbreed freely, e.g. red deer x Pere David deer. Artificial insemination is also a popular means for international transfer of genetic material without many of the risks of live animal transfer, or the high cost involved.

1.13.1.1. SEMEN COLLECTION.

There are two alternatives for semen collection: electro-ejaculation or natural service in an artificial vagina. Both methods are limited to the season of sexual activity (Shackell, 1989). While electro-ejaculation is the most commonly used method, it has inherent risks due to anaesthesia (Fennessy and Mackintosh, 1988), and the semen quality is unpredictable (Shackell, 1989). There is now a preference for the artificial vagina method (Krzywinski and Jaczewski, 1978) and further research is being conducted (Jabbour and Asher, 1990). After collection, semen is evaluated as described by Shackell (1989), extended according to Fennessy *et al.*(1987), and frozen in straws each containing approximately 50 million sperm with the expectation of a minimum of 50% post-thaw motility, giving about 25 million live sperm at insemination (Fennessy *et al.*, 1987).

1.13.1.2. INSEMINATION.

Insemination per vaginam results in deposition of semen in the vagina, cervix or uterus, although the latter site is only reached in 25% of deer due to the difficulties in penetrating the cervical rings (Bowen, 1989). With single intravaginal/intracervical insemination, average conception of 40% or less were reported, respectively, by Fennessy and Mackintosh (1988) and Asher (1990). There were no significant differences when single inseminations were performed at various intervals (36-68 hours) after CIDR removal (Asher *et al.*, 1990), but the conception rate was about 50% with double inseminations performed at 44 and 68 hours after CIDR withdrawal (Fennessy *et al.*, 1987; Asher *et al.*, 1990), suggesting relatively poor synchrony of oestrus in red deer hinds. However, Bowen (1989) found no difference in conception rate between a single insemination at 48 hours after CIDR withdrawal or a double insemination at 48 and 60 hours, although precise data were not given to explain this result.

Laparoscopic intrauterine inseminations are used routinely for commercial purposes, with variable but better results than with intravaginal insemination. Conception rates of 25-75% with frozen semen, and 40-75% success with fresh semen are reported (Bowen, 1989). An advantage of the laparoscopic technique is the visualization of the uterine horns, allowing confirmation of oestrus if they appear turgid and firm. On the other hand, laparoscopy involves anaesthesia and its inherent risks.

1.13.2. EMBRYO TRANSFER.

Embryo transfer is potentially useful to increase the utilisation of female genetic material, resulting in an increase in selection pressure on the population. Furthermore, for both disease prevention and economic reasons, it is better to import or export embryos rather than live animals. In this instance, the animal is born in the importing country and does not have to adapt to new conditions (Bringans, 1989-b).

1.13.2.1. EMBRYO COLLECTION.

Embryo collection was undertaken 8 days after CIDR removal (Bringans, 1987), i.e. 6 days after oestrus. At this stage the embryos should be found in the uterine horn associated with the ovary containing the corpus luteum. However, in one case, Dixon (1986) found embryos in the oviduct, 6 days post oestrus. While elk or larger red x elk hybrids could be flushed per vaginam (Dixon, 1986), a surgical approach has been undertaken with red hinds (Fennessy *et al.*, 1989-b; Bringans, 1989-a,b). Bringans (1989-a) reported an unexplained highly variable rate of embryo recovery (38-90%). Clembuterol, a smooth muscles relaxant, was found to improve recovery rate (Fennessy *et al.*, 1989-b). Nine days after embryo collection, donors should be injected with prostaglandins to abort any non-recovered embryos. However, 3% of hinds produce offspring, even after prostaglandins. Only singletons were born (Bringans, 1989-a).

1.13.2.2. EMBRYO IMPLANTATION.

Recipient hinds must be synchronised for oestrus. Laparoscopic examination ensures the presence of an adequate corpus luteum before performing the laparotomy for a surgical implantation (Bringans, 1989-a). The ipsilateral horn is exteriorised and the embryo injected (Dixon, 1986). Fresh embryo implantation led to a 60-80% pregnancy rate, while frozen embryo implantation resulted in a 55-66% pregnancy rate (Bringans, 1989-a).

1.14. CONCLUSION, AND PURPOSE OF THE PRESENT STUDY.

Ultrasonographic investigations require an understanding of the ultrasound technique, its current use in domestic animals, and a good knowledge of the anatomy of the organs to be imaged. This review reports the anatomy of the female deer reproductive tract and ultrasound applications for investigation of the female reproductive tract of different species, giving an idea of the direction that could be taken in the present research project.

This study was designed to complement other studies on ultrasonography already undertaken in deer (Bingham, 1987), and targeted particularly the gestation periods before 42 days and after 128 days. It was also designed to study the periods before 30 days and after 150 days gestation to complement the studies on foetal age estimation reported by White *et al.*(1989) and Bingham *et al.*(1990). In addition, the study set out to provide a description of the evolution of the conceptus and foetal ageing equations from 6 to 211 days after mating, with the aim of improving both the descriptions of pregnancy and the precision of foetal age equations reported before, and to introduce new dimensions for foetal ageing to improve the accuracy of foetal age estimation for research or practical purposes.

CHAPTER 2

MATERIALS AND METHODS.

2.1. ANIMALS AND MANAGEMENT.

This study was conducted at the Massey University Deer Research Unit during 1989, on a herd of 44 farmed red deer hinds aged 16 months or more. A description of the hinds with their year of birth, weight at the beginning of the mating season, mating and calving dates, is given in table 2.I.

The older hinds were captured from the feral environment prior to 1979. All hinds aged less than ten years were born on the farm. The mob was run on 0.5-2 hectare paddocks. The pasture composition was a mixture of ryegrass and white clover.

The hinds were weaned in the first week of March. A 3-year-old red deer stag was joined to the herd on March 22, and replaced by a 5-year-old red deer stag on April 26. The stag was retrieved on May 20.

Table 2.I. Description of the hinds and recorded matings and calvings.

Tag number	Year of birth	Weight (kg) 22/3/89	Mating dates, and number of copulations ()	Calving date and calve sex ()	Gestation length
1	-1979	99	15/4/89 (2)	03/12/89 (M)	232
3	-1979	95	03/04/89 (2)	Not identified	-
6	-1979	110	04/04/89 (1)	24/11/89 (F)	234
11	1980	92	03/04/89 (1)	26/11/89 (F)	237
12	1980	94	Not observed	10/12/89 (M)	-
14	1979	92	04/04/89 (2)	Not identified	-
15	1981	89	Not observed	30/11/89 (M)	237
19	1981	99	05/04/89 (2)	27/11/89 (M)	236
21	1982	91	07/04/89 (1)	30/11/89 (F)	237
38	1982	92	02/04/89 (1) 03/04/89 (1)	21/11/89 (F)	233
43	1983	84	10/04/89 (3) 20/04/89 (2)	Non Pregnant	
44(#)	1983	96	02/04/89 (1)	24/11/89 (M)	236
45	1983	105	08/04/89 (1)	Not identified	-
46	1983	94	Not observed	02/12/89	-
47	1983	109	03/04/89 (1)	Not identified	-
48	1983	100	12/04/89 (1)	01/12/89 (F)	233
52	1984	102	Not observed	30/11/89	-
53	1984	108	03/04/89 (3)	21/11/89 (F)	232
54	1984	100	Not observed	23/11/89 (F)	-
58	1985	106	11/04/89 (2)	25/11/89 (F)	228
59	1985	117	02/04/89 (1)	22/11/89 (F)	234
62	1985	104	02/04/89 (1)	23/11/89 (M)	235
66	1985	101	11/04/89 (1)	28/11/89 (F)	231
67	1985	100	12/04/89 (2)	05/12/89 (F)	237
69	1985	91	09/04/89 (1)	Not identified	-
74	1986	98	02/04/89 (1)	17/11/89 (M)	229
75	1986	86	10/04/89 (1)	28/11/89 (M)	232
79	1986	96	10/04/89 (2)	29/11/89 (M)	233
80	1986	107	12/04/89 (2)	01/12/89 (F)	233
83	1986	103	03/04/89 (3)	23/11/89 (M)	234 *
84	1986	84	09/04/89 (3)	28/11/89 (M)	233
86	1986	99	Not observed	20/11/89 (F)	-
87	1987	97	16/04/89 (2)	04/12/89 (M)	232
88	1987	117	12/04/89 (2)	07/12/89 (F)	239
90	1987	100	14/04/89 (1)	Not identified	-
93	1987	104	12/04/89 (3)	01/12/89 (M)	233
94	1987	85	12/04/89 (1)	Not identified	-
95	1987	105	13/04/89 (1)	24/11/89 (F)	225 *
104	1987	98	24/04/89 (2)	Not identified	-
106	1987	104	12/04/89 (1)	Not identified	-
107	1987	89	09/04/89 (1)	01/12/89 (F)	236
109	1987	95	02/04/89 (1)	Not identified	-
0	1987	98	16/04/89 (1)	23/11/89 (M)	221 *
128	1988	Not Recorded	Not observed	Not identified	-

Mean recorded gestation length was 233 days.

Note: Only deer with observed oestrus were used for this study.

(#) = this hind was rejected from the study because of difficulties in handling.

* Calf died.

2.2. OESTRUS DETECTION.

The hinds were observed daily, from March 28 to May 5, principally by the author, but with assistance of six volunteer undergraduate students. Continuous observations, from a caravan parked in the paddock in a strategic viewing position , were made with 50x7 binoculars and a telescope (Bisley Deluxe 10x-40x, D = 40 mm), between sunrise, around 6 am, and sunset, around 7 pm.

Sexual behaviour and matings were recorded (Table 3.I), according to the description given by Veltman (1985) comprising the followings:

- Sniffing: stag's olfactory investigation of the hind.
- Short chase: the stag chases the female for less than 5 metres, loosing interest thereafter.
- Long chase: the stag sustains the chase, eventually zig-zaging between the other hinds.
- Low mount: the stag mounts the hind but does not copulate.
- Hind mounts: the hind mounts another hind or the stag.
- Copulation or high mount.

The time of observation of each action and the tag number of the hind were recorded. The number of times each sexual action was observed before a mating, the time of first appearance of low mount and hind mounts stag, and the time at which the following mating takes place were recorded (see table 3.I. later).

The logical succession of sexual behaviour and subsequent calving date were used to confirm a mating date, where actual matings were not observed.

The oestrous period was defined as a period of sequential sexual activity involving at least one long chase, low mount or hind on stag, leading to a mating. Accessory periods of sexual activity were defined as sexual activity taking place outside of the oestrus period and including at least two long chases, one hind mounting stag and/or one low mount, but without copulation. Occasional or sporadic exhibitions of sexual behaviour such as short chase, only one long chase, or/and hind mounting another hind, were not considered as part of an oestrous or accessory sexual behaviour.

2.3. ULTRASOUND SCANNING.

2.3.1. SCANNING SCHEDULE.

An ultrasonographic examination of the non pregnant reproductive tract was made by scanning the whole herd on March 22. Subsequently, scans were undertaken at approximately weekly intervals from April 19 to October 30, as presented later in table 3.IV.

2.3.2. HANDLING AND RESTRAINT.

The herd was mustered 20 minutes before scanning and kept in an open yard. Groups of 4 to 5 hinds were introduced to a dark room of approximately 2 x 2 meters, and individually pushed into an hydraulic crush (*Nu-mac, Mac Ewans Machinery Limited, New Zealand*) giving easy access to the anal area.

Two persons were sufficient to handle and scan the hinds. However, time efficiency was increased when three persons were working; one being in the dark room for handling, one at the restraint controls, and one performing ultrasonographic examinations.

After completion of the scanning, hinds were returned to pasture and, when still in the breeding season, the stag rejoined immediately.

2.3.3. ULTRASOUND MACHINE.

A portable Aloka echo camera (model SSD-210DX II, Aloka Co.,Ltd) with an electronic 5 MHz linear rectal transducer (Intra-operate probe, UST-5810 I-5), commonly seen in equine practice, was used (fig.2.1). The only adaptation necessary was a rigid extension to enable moving the scanhead without rectal manual palpation.

The ultrasound machine was sited on a shelf, out of reach from the hinds, although the screen could be easily watched by the operator (fig.2.2).

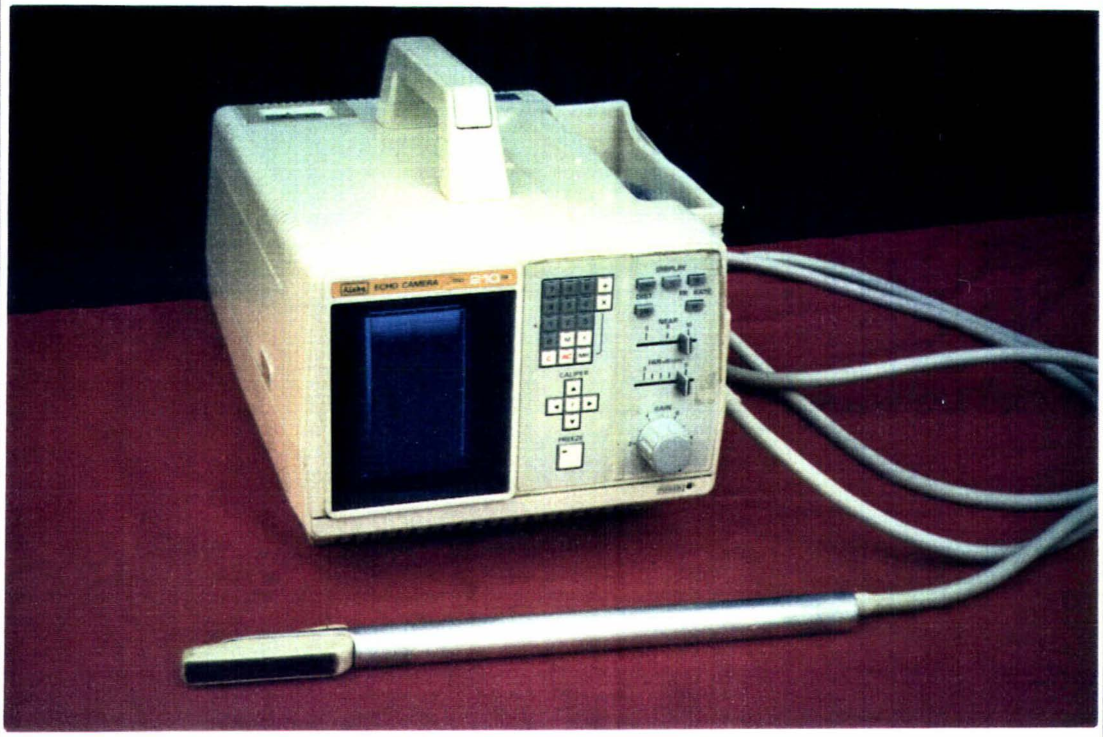


Figure 2.1. Portable ultrasound machine with its probe and extension.

Figure 2.2. Demonstration of the restraint and ultrasound scanning method.

2.3.4. SCANNING METHOD.

For rectal scanning, the operator stood behind the hind while it was restrained in the crush, although slightly on the side to avoid possible kicking. Methyl cellulose lubricating gel was employed to assist insertion of the probe into the rectum. The scanhead, held by the extender and orientated downwards, was introduced 10 to 40 cm into the rectum. Looking at the scanner screen, the operator followed the progression of the probe within the rectum.

In non pregnant or early pregnant hinds, the first organ usually encountered was the bladder, a fluid filled non echogenic organ lying on the pelvic floor. The bladder was the landmark to orientate the search of the uterus (see section 3.2).

In late pregnancy, the fluid filled uterus was often the first organ to be viewed.

To obtain a good depiction of the reproductive tract and the conceptus, the probe was moved within the rectum on horizontal and vertical planes, and by rotation.

Abdominal scanning with a 3 MHz transducer was undertaken on a few animals after 50 days gestation. The hinds were restrained manually in a standing position while the operator scanned the abdomen at the udder area.

2.3.5. IMAGE RECORDING.

The date and hind tag number were entered on the ultrasonograph screen. A video cassette recorder (National, NV-G15) was connected to the video output of the scanner, and each scan was recorded on video tapes (Panasonic 180), giving a total recorded time of 33 hours. The inbuilt frame-freeze and the electronic calliper, functions of the ultrasound machine, were not used at the time of scanning which was therefore relatively rapid, from 5 seconds to 5 minutes. The tapes were played back later on the scanner screen, through the connection of a second video player/recorder with frame by frame capabilities (National AG-6200) to the video input plug of the ultrasound machine. The description of the developing conceptus and measurements were made during the play-back.

Initially, polaroid prints were taken with a camera directly fixed on the ultrasound screen. The poor quality of the prints soon precluded its use, and it was replaced by a 35 mm single lens reflex camera (Olympus, OM2N; Lens 50 mm macro). The camera was settled on a tripod in front of the ultrasound screen, and 100 ASA black and white print films (Kodak, T/MAX 100) were used.

2.3.6. DESCRIPTION OF THE UTERUS AND FOETAL DEVELOPMENT.

A file was established for each hind, with or without known mating date, and description of the ultrasonographic image of the conceptus or uterus was recorded. Helped by the frame by frame function of the video player, black and white pictures of the most characteristic features were taken. A chronological description of ultrasonographic imaging was established from the hinds with known mating dates.

2.3.7. DETERMINATION OF PREGNANCY STATUS.

A positive or negative pregnancy diagnosis was made for each scan: positive, when foetal fluids or at least one feature of pregnancy could be observed as described in section 3.3; negative, when none of the pregnancy signs were observed (see section 3.2).

2.3.8. FOETAL, PLACENTAL AND UTERINE MEASUREMENTS.

The conceptus was ultrasonographically imaged from different planes to allow measurements of the maximum number of dimensions for each scan. The play-back was at low speeds or frame by frame, to capture the image showing the best orientation for a given dimension. It was then frozen with the "video pause", and measurements were taken from the screen with a vernier calliper. Measurements were corrected for the magnification factor of the real-time image.

The following 13 foetal, placental and maternal dimensions were measured (see fig.2.3):

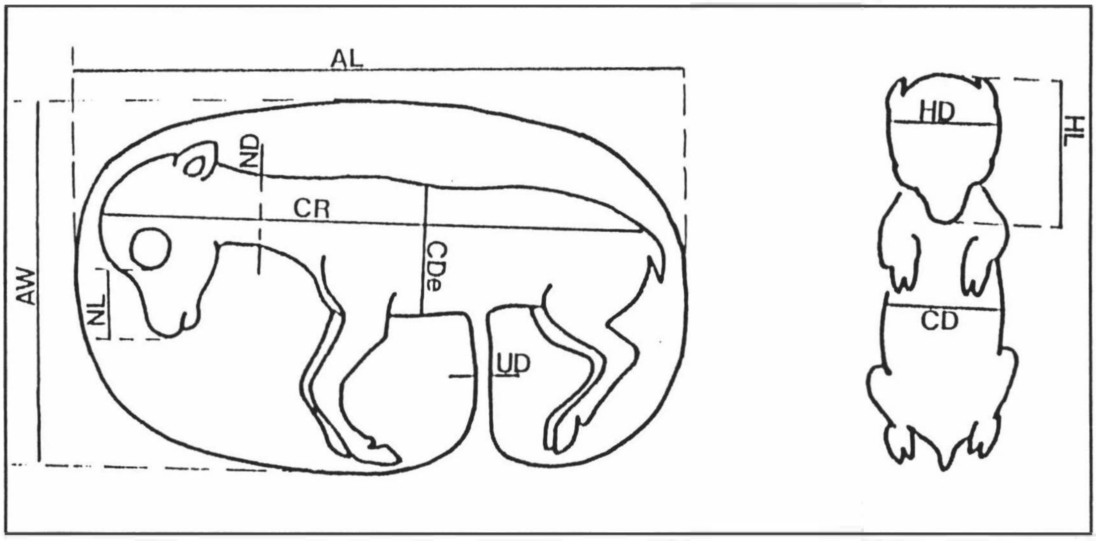


Figure 2.3. Foetal dimensions.

CR - **Length of the foetus:** the greatest length of the embryonic mass during early pregnancy when the body parts are not yet recognisable, or the crown-rump length when the body parts are differentiated and the measurement can be taken from the top of the skull to the tail.

HD - **Head diameter:** the biparietal diameter of the skull.

HL - **Head length:** distance between the caudal part of the skull and the extremity of the nose.

- **Eye diameter:** the greatest diameter of the eye ball.

NL - **Nose length:** distance from the anterior edge of the ocular orbit to the extremity of the nose.

ND - **Neck diameter:** distance from the dorsal to the ventral edge of the neck, perpendicular to the longitudinal axis, half way between the head and the thorax.

CD - **Chest diameter:** greatest distance between the lateral walls of the rib cage.

CDe - **Chest depth:** perpendicular distance between the spine and the caudal end of the sternum.

UD - **Umbilical cord diameter:** the diameter of a cross section of the umbilical cord within the amnion.

AL - **Amnion length:** greatest diameter of the amniotic sac, parallel to the longitudinal axis of the foetus.

AW - **Amnion width:** smallest diameter of the amniotic sac, perpendicular to the longitudinal axis of the foetus.

- **Placentome:** the greatest diameter of the largest placentome found.

- **Uterine lumen diameter:** the greatest diameter of the fluid filled uterine lumen.

2.4. ANATOMY AND IN VITRO ULTRASONOGRAPHY.

Seven non-pregnant mixed age red deer reproductive tracts, and a pregnant uterus from a fallow and a red deer, were obtained from the deer slaughter place.

Anatomical features and dimensions were recorded from the non-pregnant uteri. The pregnant reproductive tracts were first suspended in water and scanned, and measurements of the foetal components were made. The uteri were then dissected to confirm and explain the ultrasonographic images.

2.5. STATISTICAL METHODS.

2.5.1. OESTROUS BEHAVIOUR.

Duration of oestrus (table 3.II) was calculated as the time between the first and the last appearance of any stereotyped sexual behaviour.

Table 3.II. presents the number and the time of first appearance of sexual actions such as low mounts and hind mounting stag, before mating. Only data from the oestrous periods are used for these calculations.

The correlations between hind age and hind weight, hind age and mating date, and hind weight and mating date, were established through a linear regression model.

The number of matings per day and the minimum mating intervals were calculated.

2.5.2. PREGNANCY DIAGNOSIS.

The efficiency of ultrasonography for pregnancy diagnosis was established using two standard criteria, accuracy and reliability, as described by Buckrell *et al.*(1986) and Chaffaux *et al.*(1986).

Accuracy evaluates the magnitude of correct results. It has three components:

Overall accuracy (%) = (n correct test results/n tested) x 100
Sensitivity (%) = (n pregnant animals testing pregnant/ n pregnant) x 100
Specificity (%) = (n non pregnant animals testing non pregnant/n non pregnant) x 100

The reliability, also referred to as the predictive value, is the probability that a diagnosis will later be found to be correct. It has two components:

Reliability of **a negative test** (%) = (n non-pregnant/n animals tested non-pregnant) x 100
Reliability of **a positive test** (%) = (n pregnant/n animals tested pregnant) x 100

2.5.3. FOETAL AGEING.

Data of foetal, placental or uterine dimensions were analysed by least squares regression analysis with dimensions (mm) as the dependent variable, and days as the independent variable. Linear, exponential, multiplicative, quadratic, and cubic equations were computed, and the model giving the lowest standard deviation and the best correlation was chosen as the most appropriate for describing the conceptus growth pattern.

Foetal-ageing equations were computed by transposing the axes such that the dimension became the independent variable and the days of gestation the dependent variable. Comparisons were made between regression models as above and the most appropriate was chosen. However, when two models, including a linear, gave similar correlation and standard deviation, the linear regression was preferred because it allows easier interpretation and rapid prediction in the field.

In addition to computation of equations, the data were presented graphically with 95% confidence interval, to permit simple rapid extrapolation of age of the pregnancy from measurements of foetal, placental or uterine dimensions.

A three dimensional histogram of the frequency of the measurement, in relation to the stage of pregnancy and the magnitude of the measurement, was produced for each dimension. These histograms describe pictorially the appropriate limits of gestational age, and magnitude of measurement, for which each foetal ageing equation should be used. These limits may be function of foetal development as the foetus descends into the abdominal cavity, or of the limited capability of the ultrasound machine in measuring dimensions of more than 60 mm.

CHAPTER 3

RESULTS

3.1. OESTROUS BEHAVIOUR.

The chronological appearance of sexual behaviour is presented in table 3.I., and oestrous behaviour analyses are presented in table 3.II. Observations are summarised:

- an average of 1.6 matings (range 1-3) per oestrus were observed.
- an average of 4.9 low mounts (range 0-76) were observed before a mating.
- an average of 2.2 hind mounts stag (range 0-13) were observed before mating.
- Low mounting began 5.7 min before mating (range 1-345 min).
- Hind mounting stag began 20.5 min before mating (range 1-916 min).
- The duration of oestrous behaviour averaged 23 h 45 min (range 1-111 h).

Twenty one hinds showed between one to five accessory periods of sexual activity. Table 3.III indicates the intervals between these periods of sterile sexual activity and copulation.

Linear regressions of hind weight on hind age, mating date on hind age, mating date on hind weight, showed an absence of correlation between these variables:

x = Age	y = Weight	$r^2 = 0.0037$
x = Age	y = Mating date	$r^2 = 0.1947$
x = Weight	y = Mating date	$r^2 = 0.0031$

Only the first stag introduced was seen performing matings, and this for a period of only 23 days, from April 2-24. The sequential number of matings/day was: 6, 11, 3, 2, 0, 1, 1, 5, 6, 3, 12, 1, 1, 2, 3, 0, 0, 0, 2, 0, 0, 0, 2. The minimum interval between two matings was 5 minutes.

Table 3.I. Observation of Oestrous behaviour and matings.

This table gives the chronological daily order of six observed stereotyped sexual behaviours. The numbers indicate the number of times the observation was made for a given day, and before each mating. The numbers in brackets indicate the time of first observation of a particular action before a mating, and the time of mating.

The duration of oestrus behaviour was defined as a period of daily sexual activity involving at least one long chase, low mount or hind mounting stag, leading to a mating. The oestrous behaviour period is depicted in this table between two * * * * lines or between * * * * and ——— lines. The times of first and last observation of sexual activity during this period are indicated in the last column (oestrous behaviour period) in relation to the day of observation.

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind [1]	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
1	03/04	3						
	* * * *							
	15/04	12	5	5 (08.44)		5 (08.44)	1 (09.34)	07.27
		2	7	1 (13.33)		2 (13.32)	1 (13.34)	
		1	3					
	16/04		1					07.25
3	29/03	1						
	* * * *							
	03/04						1 (06.26)	06.26
		2		2 (13.18)			1 (13.19)	13.19
	* * * *							
	22/04	1						
6	30/03		1					
	31/03	1						
	01/04	1			1u			
	* * * *							
	03/04	1	2					17.26
	04/04	2		3 (07.07)		1 (7.05)	1 (7.08)	
		1	1					15.00
	* * * *							
	05/04				1u			
	12/04				1u			
	13/04				1u			
	17/04				1u			
11	02/04	3	2			9 (16.26)		12.07
	03/04			1 (07.42)		1 (07.42)	1 (07.50)	
					4o			
		1			1o			09.57
14	03/04	1						
	* * * *							
	04/04	3	2	7 (10.51)			1 (10.57)	09.03
		1	5	9 (14.14)			1 (15.09)	
		2	2					17.44
	* * * *							
	14/04	1						
	16/04				1o			
19	02/04	1						
	* * * *							
	04/04	2	1					12.43
	05/04	8	14	2 (13.37)		2 (10.36)	1 (13.38)	
		1	2	5 (15.34)			1 (15.35)	
	06/04		1					16.07
	* * * *							
	16/04				1u			

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
21	30/03	1						
	31/04	1						
	03/04	2	1					
	05/04	1						
	06/04	1						
	07/04	10	5			1 (12.22)	1 (12.34)	06.36
		2	5					18.04
	11/04	1						
38	31/03		1					
	02/04	1	13	5 (13.52)			1 (15.00)	10.34
		1						
	03/04			1 (11.25)	1o		1 (11.26)	
		1	1					18.14
	04/04	1						
	09/04				1o			
	12/04				1o, 1u			
	16/04				1o			
	17/04				1o	2		
	28/04				1o			
43	01/04		1					
	02/04	1				1		
	03/04	2						
	04/04	1						
	09/04	1						
	10/04	6	2	12 (07.22)		9 (07.20)	1 (08.27)	06.35
		2	3	21 (12.23)		2 (12.32)	1 (12.56)	
				6 (16.00)			1 (16.15)	
				1				18.25
	20/04	14	6	17 (09.37)		14 (08.05)	1 (11.31)	06.35
				27 (14.30)		14 (14.32)	1 (17.50)	17.50
44	30/03				1o			
	02/04	7	19	2 (16.17)	1o	2 (15.06)	1 (17.24)	15.06
								17.24
	04/04				1o			
	12/04				1o			
45	29/03	4						
	31/03				1u			
	01/04	2			1u	1		
	03/04				1u			
	05/04	1						
	06/04	2						
	07/04	17	15					09.15
	08/04	15	8	2 (11.15)		3 (11.04)	1 (11.20)	
		1						14.53
47	03/04			2 (11.24)	10 o,3 u	5 (11.20)	1 (15.51)	09.04
	04/04	1						11.29
	09/04				1u			
	12/04				2o			

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
48	28/03	8	1					
	29/03	2	2					
	30/03	1						
	02/04				1u			
	03/04	1						
	04/04	8	4					
	05/04	1	1					
	06/04	3						
	08/04	1				1		
	10/04	1						
	11/04		1					18.00
	12/04			1 (10.35)	3o, 2u		1 (16.20)	
		1			2u			17.30
	24/04					1		
53	29/03	7	8					
	30/03				1u			
	01/04	2						
	03/04						1 (06.48)	06.48
		4	10	1 (12.00)	1o	4 (10.40)	1 (12.01)	
			4	2 (15.36)		3 (15.35)	1 (15.38)	15.38
	15/04		1		1u			
58	01/04				1o			
	05/04	1	2					
	09/04	1						
	10/04	1						
	11/04	2	8	2 (14.56)			1 (14.59)	06.34
			1	6 (16.17)		5 (16.17)	1 (16.23)	18.02
59	01/04	1						
	02/04	4	1	2 (18.32)		4 (16.06)	1 (18.46)	07.46
	03/04			1	16u, 3o	4		12.00
	05/04		1		1u			
	11/04				1u			
	12/04				2u			
	14/04				1u			
	20/04				1u			
62	02/04	3	1	2 (09.17)		2 (10.35)	1 (10.36)	06.57
								10.36
	04/04	1						
	13/04				1o			
66	01/04	1						
	03/04			1		2		
	04/04	2						
	10/04	4	4					10.13
	11/04	1		2 (13.10)		1 (13.10)	1 (13.15)	13.13

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
67	04/04	2	1					
	05/04	2						
	06/04	1						
	10/04	1	1					
	11/04	1						
	******	******	******	******	******	******	******	******
	12/04		1	1 (11.55)			1 (11.56)	11.55
		1	1	1 (15.22)			1 (15.23)	
	13/04	2	1					14.50
	******	******	******	******	******	******	******	******
	18/04				1o			
69	29/03	4	1					
	******	******	******	******	******	******	******	******
	08/04	6	9					06.55 [2]
	09/04	1		76 (08.40)	1o, 1u	9 (09.08)		
	******	******	******	******	******	******	******	******
	11/04	1						
	17/04				1u			
74	01/04	1	1			6 (16.20)		16.20
	02/04		3	1 (09.24)			1 (09.25)	09.25
	******	******	******	******	******	******	******	******
	03/04				1u			
	04/04		1		1u			
	05/04		1		1o			
	24/04					1		
	28/04				1u			
75	28/03	2						
	29/03	2				2		
	30/03	1				1		
	02/04	1				4		
	08/04	1						
	******	******	******	******	******	******	******	******
	09/04	2				3 (16.14)		10.34
	10/04	4	3		1o		1 (16.02)	16.02
	******	******	******	******	******	******	******	******
	11/04				1o			
	12/04				1s			
	13/04					1		
	22/04					2		
79	28/03	6						
	29/03		2					
	******	******	******	******	******	******	******	******
	09/04	2	1					12.48
	10/04	4	1				1 (08.21)	
			2	1 (12.31)	1u	3 (12.32)	1 (12.33)	
			1					15.55
80	03/04	2	2					
	04/04	2						
	05/04	2	2					
	09/04				1o			
	******	******	******	******	******	******	******	******
	12/04	7	7		2o	6 (06.47)	1 (13.38)	06.47
		3	2	2 (14.55)			1 (14.56)	
			2		1s			17.40

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on Hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
83	01/04					1		
********	********	********	********	********	********	********	********	********
	03/04			6 (06.20)		1 (06.46)	1 (07.09)	06.20
				5 (07.43)		1 (09.08)	1 (09.10)	
				7 (16.06)	2o, 1u	10 (12.49)	1 (16.22)	16.22
84	30/03		2					
	31/03	1						
	01/04					3		
	07/04	1						
	08/04	2						
********	********	********	********	********	********	********	********	********
	09/04	10	25	1 (12.21)		1 (11.30)	1 (12.23)	06.45
			3	7 (14.10)		5 (14.11)	1 (14.32)	
		1		4 (17.21)		1 (17.20)	1 (17.24)	17.24
87	28/03	1						
	31/03					1		
	03/04	1	1					
	13/04	1						
	15/04	2						
********	********	********	********	********	********	********	********	********
	16/04	7	17	3 (09.00)		13 (08.11)	1 (09.54)	07.00
		1	11	2 (14.42)		1 (14.38)	1 (14.43)	
		2						
	17/04	1	1					12.07
88	30/03	2		1				
	01/04	2						
	02/04	1						
	04/04	1						
	10/04	3						
********	********	********	********	********	********	********	********	********
	11/04	4	2					06.33
	12/04			1 (06.50)	1o, 4u	8 (06.34)	1 (06.55)	
		1	1		3u		1 (10.05)	
		1	2		1u, 1o, 1s			18.00
********	********	********	********	********	********	********	********	********
	13/04	1						
	18/04				1o			
	20/04			1	1o			
	23/04				1o			
90	30/03				1o	2		
	01/04	2			1u			
	02/04			2				
	05/04	1						
	06/04		1					
	08/04					2		
********	********	********	********	********	********	********	********	********
	11/04					2 (16.15)		16.15
	12/04				1u, 9o	1		
	13/04	3	1					
	14/04	5	5	2 (16.02)		2 (14.32)	1 (16.03)	
		2						
	15/04					1		
	16/04					1 (07.19)		07.19
********	********	********	********	********	********	********	********	********
	18/04					3		
	22/04				1o	2		
	23/04				1u			
	28/04				1o			
	04/05				1o			

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
93	28/03	2						
	29/03	1						
	01/04				1o			
	02/04		2					
	04/04	1	3					
	06/04	1						
	08/04	1						
	12/04		1	1 (12.02)		1 (11.59)	1 (12.07)	11.59
		2	1	1 (13.30)	1s	1 (13.31)	1 (13.33)	
					1o		1 (16.57)	16.57
	16/04					1		
	28/04				1u			
	04/05				1u			
94	29/03	2						
	30/03	3	2					
	05/04	2						
	12/04	1	3				1 (17.12)	15.50
		1						18.03
95	02/04		1					
	04/04	1						
	11/04	1						
	12/04	1						
	13/04	11	3	9 (11.51)		2 (11.48)	1 (11.59)	06.55
		5	1					14.26
104	04/04			1				
	09/04	2						
	13/04					1		
	15/04	1				1		
	16/04	1	1					
	18/04				1u			
	21/04	3						
	22/04		1					12.37
	23/04	9	4					
	24/04	8	5	1 (13.10)			1 (13.11)	
		5	1	14 (15.52)		3 (13.50)	1 (16.29)	
	25/04	2	1					15.54
106	28/03	1						
	30/03	3						
	31/03					5		
	01/04				1u			
	03/04				4o			
	04/04	1	1					
	09/04				1u			
	10/04		1					
	11/04	2						
	12/04	1		5 (11.09)	2o		1 (11.12)	09.03
					1s			16.30

Hind N°	Date	Number of short chases	Number of long chases	Number of low mounts (h)	Number of hind on hind	Number of hind on stag (h)	Number of matings (h)	Beginning and end of oestrus
107	30/03	2	3					
	31/03	7	6					
	01/04	14	16					
	********							
	08/04	6	3					06.27
	09/04			2 (08.43)			1 (08.44)	08.44
	********							
	11/04	2				2		
109	02/04			2 (12.36)			1 (12.37)	12.36
		1						13.10
	********							
	10/04	1			1u			
	11/04				1u			
	16/04		1					
0	06/04	3	1					
	10/04		1					
	13/04	1						
	14/04	2						
	********							
	15/04	6	2					10.39
	16/04	9	2			2 (17.41)	1 (17.54)	
	17/04	6	9					17.20
	********							
	25/04	1						

(1): o = hind mounts another hind.
u = hind is mounted by another hind.
s = hind mounted between two other hinds.

(2): Mating was not observed for 69. However, oestrous behaviour was precise enough to assume a mating in the evening.

Table 3.II. Summary of oestrous behaviour statistics.

Hind N°	Mating N°	Number of "low mounts" for each mating	Time from first low mount to mating (min)	Number of "hind on stag" actions for each mating	Time from first hind on stag mounts to mating (min)	Duration of oestrous behaviour (hours) *
1	1	5	50′	5	50′	
	2	1	1′	2	2′	24
3	1	-	-	-	-	
	2	2	1′	-	-	7
6	1	3	1′	1	3′	22
11	1	1	8′	10	916′	46
14	1	7	6′	-	-	
	2	9	55′	-	-	9
19	1	2	1′	2	2′	
	2	5	1′	-	-	51
21	1	-	-	1	12′	12
38	1	5	68′	-	-	
	2	1	1′	-	-	32
43	1	12	65′	9	67′	
	2	21	33′	2	24′	
	3	6	15′	-	-	12
	1	17	114′	14	206′	
	2	27	200′	14	198′	11
44	1	2	67′	2	78′	3
45	1	2	5′	3	16′	30
47	1	2	267′	5	271′	26
48	1	1	345′	-	-	24
53	1	-	-	-	-	
	2	1	1′	4	81′	
	3	2	2′	3	3′	9
58	1	2	3′	-	-	
	2	6	6′	5	6′	12
59	1	2	14′	4	160′	28
62	1	2	79′	2	1′	4
66	1	2	5′	1	5′	27
67	1	1	1′	-	-	
	2	1	1′	-	-	27
69	1	76	? (1)	9	? (1)	? (1)

Hind N°	Mating N°	Number of low mounts per mating	Time from first low mount to mating	Number of hind on stag mounts per mating	Time from first hind on stag mounts to mating (min)	Duration of oestrous behaviour (hours) *
74	1	1	1'	-	-	17
75	1	-	-	-	-	31
79	1	-	-	-	-	
	2	1	2'	3	1'	24
80	1	-	-	6	411'	
	2	2	1'	-	-	11
83	1	6	49'	1	23	
	2	5	87'	1	2'	
	3	7	16'	10	213'	10
84	1	1	2'	1	53'	
	2	7	22'	5	21'	
	3	4	3'	1	4'	11
87	1	3	54'	13	103'	
	2	2	1'	1	5'	29
88	1	1	5'	8	21'	
	2	-	-	-	-	36
90	1	2	1'	2	91'	111
93	1	1	5'	1	8'	
	2	1	3'	1	2'	
	3	-	-	-	-	5
94	1	-	-	-	-	3
95	1	9	8'	2	11'	8
104	1	1	1'	-	-	
	2	14	37'	3	159'	75
106	1	5	3'	-	-	8
107	1	2	1'	-	-	27
109	1	2	1'	-	-	1
0	1	-	-	2	13'	55
Mean	1.6 matings/ oestrus	4.9/mating	5.7'	2.2/mating	20.5'	23 hours 45'
Range	1-3	0-76	1-345'	0-13	1-916'	1-111 hours

* rounded up.

Table 3.III. Description of accessory sexual activity periods.

Hind N°	Days from copulation	Hind N°	Days from copulation
38	+15	83	-2
43	-8	84	-10 -8
45	-7	87	-16
48	-14 -8 -4 +12	88	-13 +10
53	-5 +12	90	-15 -12 -6 +4 +8
58	-6	93	-10 -8 +4
66	-8	94	-13
74	+22	104	-20 -11 -9
75	-12 -8 +3 +12	106	-12 +2
79	-12	107	-9
80	-9 -7		

Mean interval -9.7 days before mating (range 2-20 days).
+9.2 days after mating (range 2-22 days).

3.2. ANATOMY OF THE FEMALE REPRODUCTIVE TRACT.

Anatomical observations from the dissection of 7 non-pregnant uteri were in accordance with the findings of Thome (1980) and McMahon (1989). The vagina had an average length of 208 mm (range 180-225 mm). The urethral opening was situated 150 mm (range 120-170 mm) from the cervix. The cervix was 60 mm (range 50-70 mm) long, 23 mm diameter, and normally contained 6 (range 4-6) internal rings, 2 to 15 mm long. The uterine body was consistently 20 mm long, and 21 mm (range 20-23 mm) diameter. Proximally, the horns lie parallel to each other for 88 mm (range 65-130 mm) with a diameter of 19 mm (range 15-20 mm). Distally, the horns were free for another 77 mm (range 50-115 mm), their diameter diminishing gradually towards the continuation with the fallopian tube.

Ovaries were bean-shaped, with average dimensions of 19 (range 15-22) x 10 (range 7-15) x 7.5 (range 6-9) mm. No significant size difference was identified between the right and left ovary.

Of particular interest for the present study was the location of the uterus in relation to the bladder (fig.3.1). Without rectal palpation, the bladder is the ultrasonographic landmark for location of the uterus (see section 3.3).

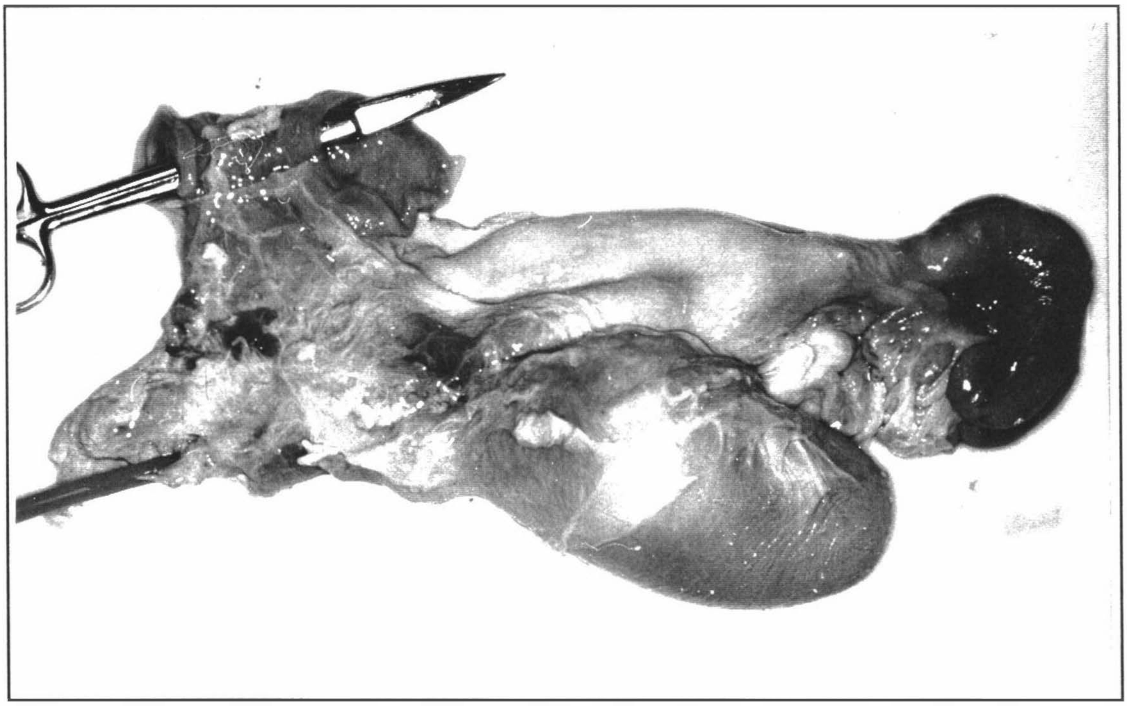


Figure 3.1. The anatomical relationship between uterus, bladder and rectum. The scissors indicate the rectum; the bladder is filled with water.

The ultrasonographic observation of pregnant uteri in water showed that measurements using the ultrasound machine were consistent with measurements made from the dissected uteri, thus confirming the validity of measurements at the same time as confirming anatomical features detected by ultrasonography. For example, the echogenic appearance of limb buds, tail and eye was observed. The red hind was approximately 35 days pregnant, while the fallow doe was 60 days pregnant, according to red deer hind foetal data.

3.3. SCANNING METHOD

The abdominal scanning technique was rejected, because of the danger to the operators, the stress on the animal which may react to physical restraint, and the risk to the equipment. Furthermore, the image quality was inadequate for accurate measurements.

Rectal scanning was easier to undertake, particularly when animals were mechanically restrained in a crush, and image quality was better. Therefore, rectal scanning with a 5 MHz transducer was the method used for this study.

3.4. ULTRASONOGRAPHIC OBSERVATIONS.

In order to locate the uterus and ensure correct orientation of the probe, the first organ to view is the bladder. The bladder presents variable shapes depending on the degree of repletion. It is usually elongated and surrounded by a typical thick folded wall (fig.3.2). Even after urination, the bladder still contains some fluid and is therefore almost always possible to visualise. The vagina was sometimes seen lying dorsal to the bladder, characterised by an elongated grey area with a central echogenic line, which is the apposition of the vaginal mucous membranes (fig.3.3). A few times the cervix was detected lying dorso-cranial to the bladder, and characterised by an interrupted central echogenic line, representing the cervical rings, in continuation with the vagina.

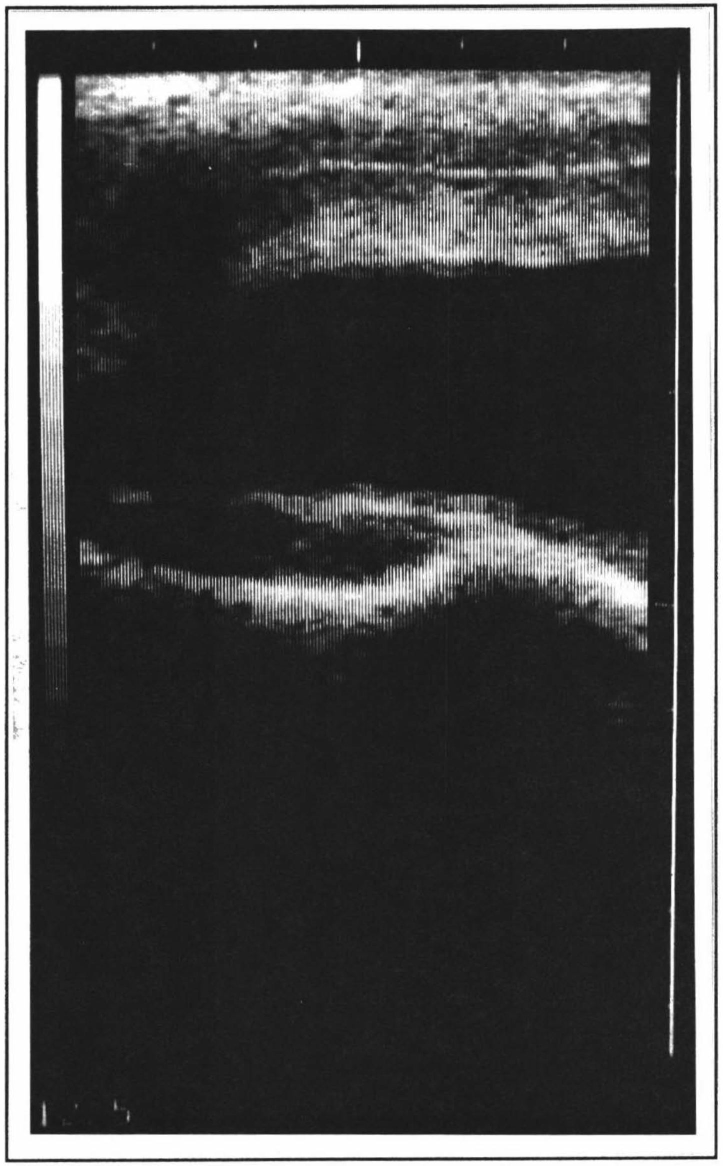


Figure 3.3.

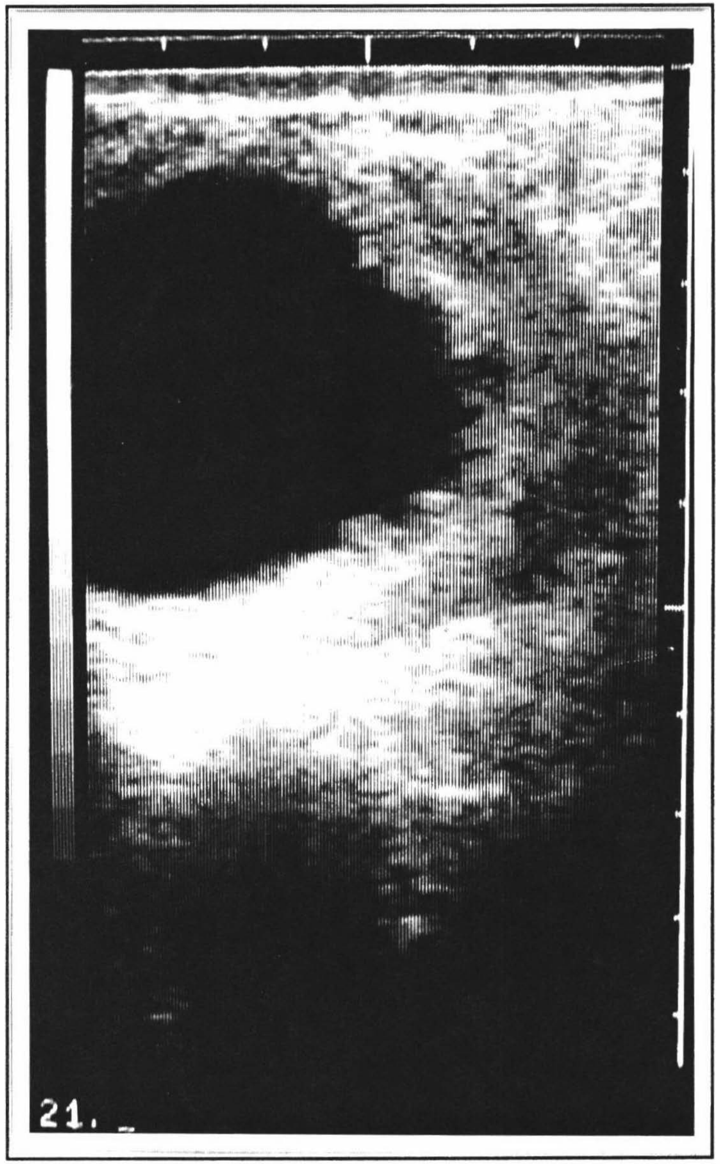


Figure 3.2.

Figure 3.2. **Bladder.**

The bladder appears as a non echogenic area, surrounded by a typical thick folded wall.

Figure 3.3. **Vagina.**

The vagina, characterised by an elongated grey area with a central echogenic line which is the apposition of the vaginal mucous membranes, lies dorsal to the bladder.

3.5. NON-PREGNANT UTERUS.

The non-pregnant uterus was found dorso-cranial to the bladder. It was regularly found in a central position, although it was sometimes displaced laterally. Thus the probe needed to be rotated or moved in vertical or horizontal planes to find it.

The uterus was recognised as a grey mass, round or polygonal with smoothed angles, 30-50 mm diameter (fig.3.4). It was obscured sometimes by the interposition of intestines between the probe and the reproductive organs. Intestinal peristaltism, visualised as movements of faecal particles, was often helpful in distinguishing digestive organs from the uterine mass which was static.

To reach a diagnosis of non pregnancy, the uterine mass was scanned in as many planes as possible by the manipulation of the rectal probe. A diagnosis of non pregnancy was given only when the uterus appeared as a grey static mass without any fluid filled non echogenic areas.

3.6. DESCRIPTION OF FOETAL, PLACENTAL, AND UTERINE DEVELOPMENT.

Fluid in the uterus was a consistent sign throughout gestation, first appearing at 7 days, represented by a small fluid filled chorionic vesicle (fig.3.5).

In early pregnancy, until approximately 30 days gestation, the uterus was similar in shape and size to the non pregnant uterus, and, therefore, was found by the technique described in sections 3.1 and 3.2. At this early stage of gestation, it might take some time to locate it, and there was a high risk of missing the vesicle (see section 3.4).

As pregnancy progressed, foetal fluids expanded and the distended uterus was more easily detected as a non echogenic area. From 28 days, the heart beat was the beacon to spot the foetus and gave an assurance of its viability.

Placentome formations (fig.3.8) were observed from 24 days onwards. They first appeared as 3 layers of different echogenicity; an area of little echogenicity between two echogenic areas, the caruncle and cotyledon (fig.3.9). As placental growth progressed, placentomes showed more uniform echogenicity. Later in pregnancy, from 114 days onwards, the placentomes appeared hollowed (fig. 3.10) as they evolved a mushroom shape (fig.3.11), sometimes attached to the endometrium only by a stem (fig.3.12). From 114 days, accessory placentomes appeared (fig.3.13).

From 100 days onwards, the foetus became heavier and descended from the pelvic cavity where it became difficult to observe with a rectal probe, and, when observed, the foetus exceeded the dimensions of the scanner screen. Thus foetal parts were often difficult to recognise. The fore feet (fig.3.33) and the head (fig.3.34) or nose were most commonly scanned, although the enlarging and more numerous placentomes sometimes obscured the foetus. As it descended into the abdomen, the foetal weight stretched the uterine wall which sometimes collapsed, leading to a virtually non-existent lumen, thus no sign of pregnancy.

Female and male foetal characteristics were not observed.

The following features were observed chronologically during pregnancy. Each feature is recorded on the first day it was observed, and the advancement of gestation was calculated from observed mating date.

Days of pregnancy	Observation
7	- The first sign of pregnancy imaged ultrasonographically was the fluid filled chorionic vesicle, 5 mm diameter, located within the uterine mass (fig.3.5).
14-35	- The uterus appeared with darker patches in a rosette pattern (fig.3.6). This appearance is assumed to be the result of oedema of the uterine horns. It was seen from the second week until about one month of gestation, disappearing progressively as the horns become filled by the developing chorionic vesicle (fig.3.7).
24	- A comma shaped mass, 6 mm long, within the chorionic vesicle, was believed to be the embryo. - A foetal membrane was observed within the fluid filled vesicle as a highly reflective or sometimes dotted line (fig.3.14). It was assumed to be either the amniotic or the allantoic membrane. - Formation of placentomes was first observed on the uterine wall, with the apposition of the cotyledon and the caruncle (fig.3.8).

28	- A rapid heart beat confirmed the presence of the foetus which was then approximately 10 mm (fig.3.15).
31	- The formation of the limb buds appeared as echogenic spots (fig.3.16).
32	- The eyes were distinguished as highly reflective structures (fig.3.17).
36	- The umbilical cord was clearly defined (fig.3.18).
37	- The head had a triangular shape, enhanced by the echogenicity of the nose, and was easily distinguished from the body (fig.3.19).
38	- The heart cavity and valves movements were observed. - The spinal cord was echogenic.
42	- Spinal flexion, head and legs movements were observed. - The skull cavity was observable (fig.3.20).
45	- The pelvic bones became visible.
51	- The amnion, which was previously kidney-shaped (fig.3.21), assumed a less regular shape as the amniotic membrane waved along with fluid movements (fig.3.22). - An elongated pupil was seen in the centre of the iris (fig.3.23). - The elongation of the neck gave more amplitude to foetal movements (fig.3.24).
52	- The ribs became echogenic (fig.3.25). - The nasal passages were distinguished.
58	- The long bones became echogenic (fig.3.26).
59	- Individual vertebrae were clearly seen (fig.3.27).

62 - Non echogenic vesicles within the abdomen distinguished internal organs such as the bladder and the stomach (fig.3.28).

67 - Numerous white spots were observed in the amnion (fig.3.29).

77 - The tongue was distinguished when the foetus opened its mouth.

80 - The trachea was seen as a lumenous non echogenic organ (fig.3.30).

87 - Blood vessels were visible at their connection to the heart, as non echogenic tubular shaped organs, in continuity with the non echogenic heart chambers (fig.3.31).

93 - The aorta and its pulse was seen running parallel to the spine.

102 - The eyes (fig.3.32) had their own movements.

114 - The placentomes developed a mushroom shape (fig.3.11). Some were almost free in the allanto-chorion, attached to the endometrium only through a stem (fig.3.12). Accessory placentomes of 5 to 15 mm appeared (fig.3.13).

Figure 3.5.

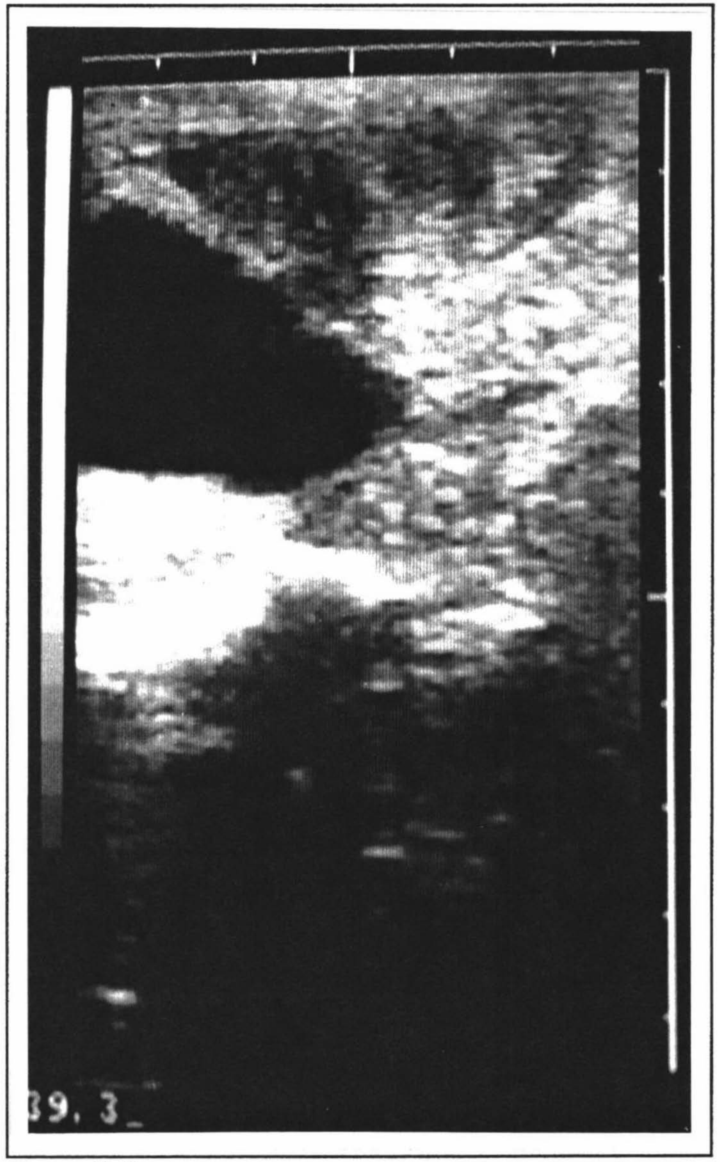


Figure 3.4.

Figure 3.4. Non-pregnant uterus.

The uterus is imaged as a grey mass, round or polygonal with smoothed angles, dorso-cranial to the bladder.

Figure 3.5. 7 days gestation; chorionic vesicle.

The chorionic vesicle, a 5 mm diameter non echogenic area located within the uterine mass, is the first sign of pregnancy imaged by rectal ultrasonography.

Figure 3.7.

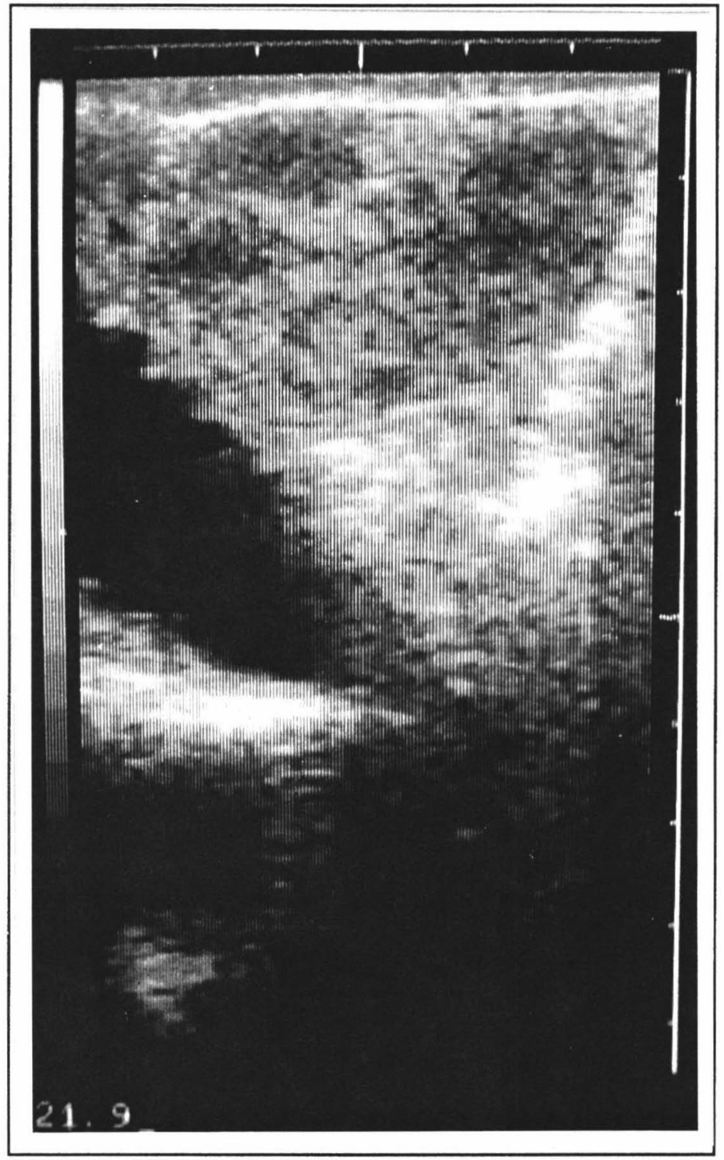


Figure 3.6.

Figure 3.6. **Day 21; oedema of the uterus.**

The "rosette" appearance of the uterus, presumably caused by oedema of the horns, was seen from the second week until about 1 month of pregnancy.

Figure 3.7. **Day 37; extension of the chorionic vesicle.**

The rosette pattern of the uterus disappear progressively as the horns become filled by the developing chorionic vesicle.

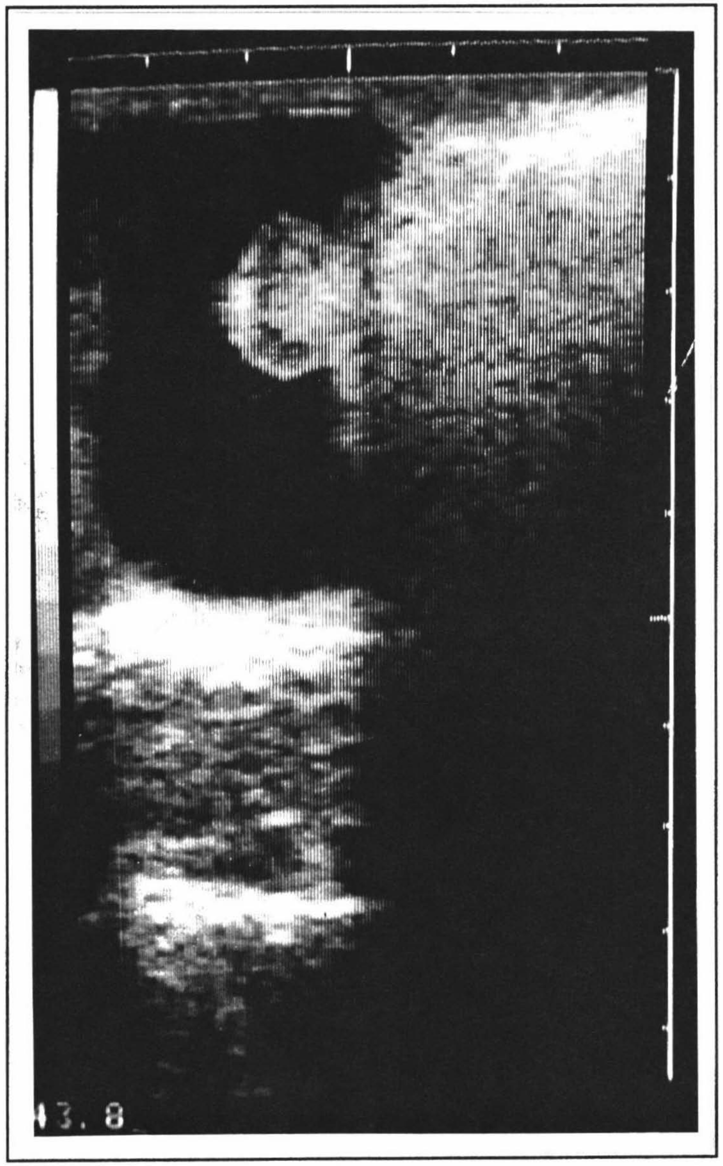


Figure 3.9.

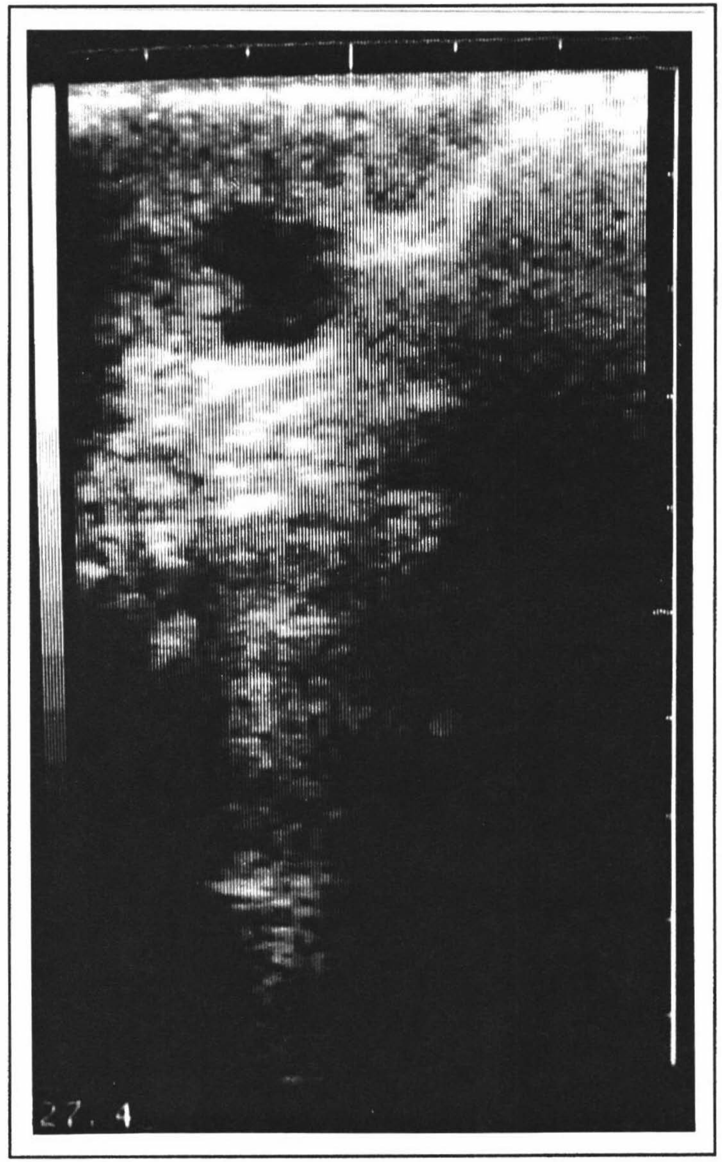


Figure 3.8.

Figure 3.8. **Day 24; Placentome formation.**

Apposition of the caruncle and cotyledon.

Figure 3.9. **Day 38; placentome.**

The placentome is characterised by 3 layers. The caruncle and cotyledon are echogenic, and separated by an oedematous zone presenting less echogenicity.

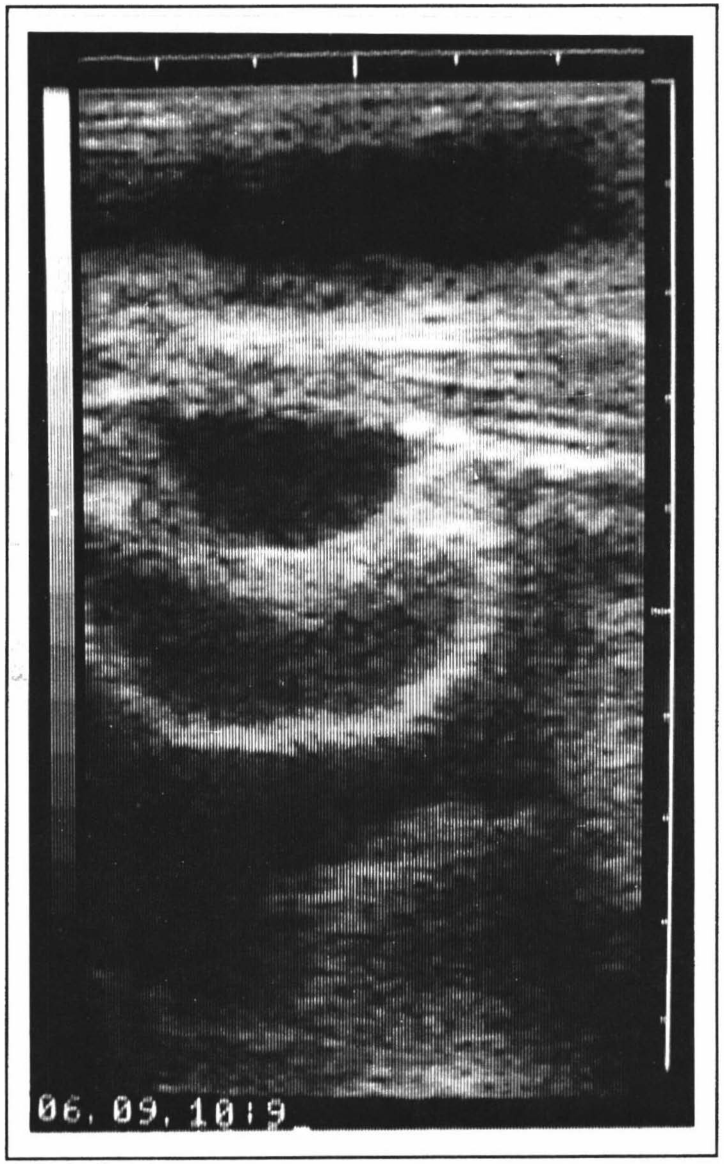


Figure 3.11.

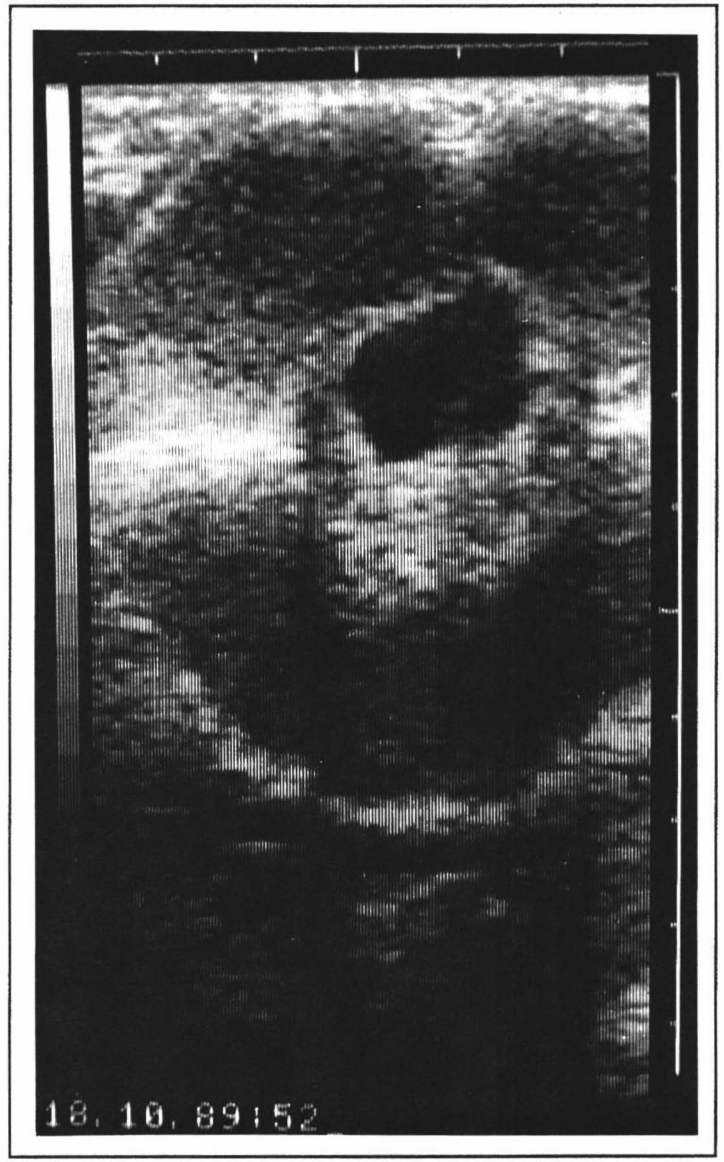


Figure 3.10.

Figure 3.10. Day 189; placentome.

As pregnancy progresses, placentomes show more uniform echogenicity, and, from 114 days onwards, the placentomes hollow.

Figure 3.11. Day 157; placentome.

At the beginning of pregnancy, the placentome was connected to the endometrium over its entire area, but from approximately 114 days gestation, the attachment is restricted to the middle third, giving a mushroom-like appearance.

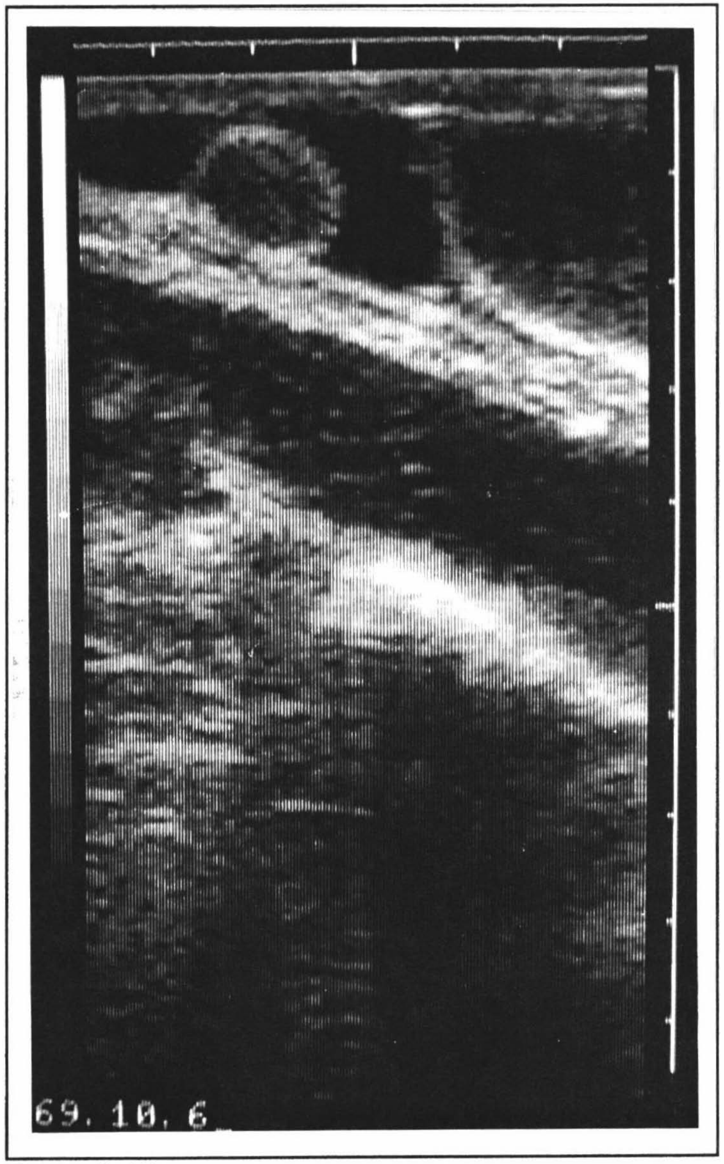


Figure 3.13.

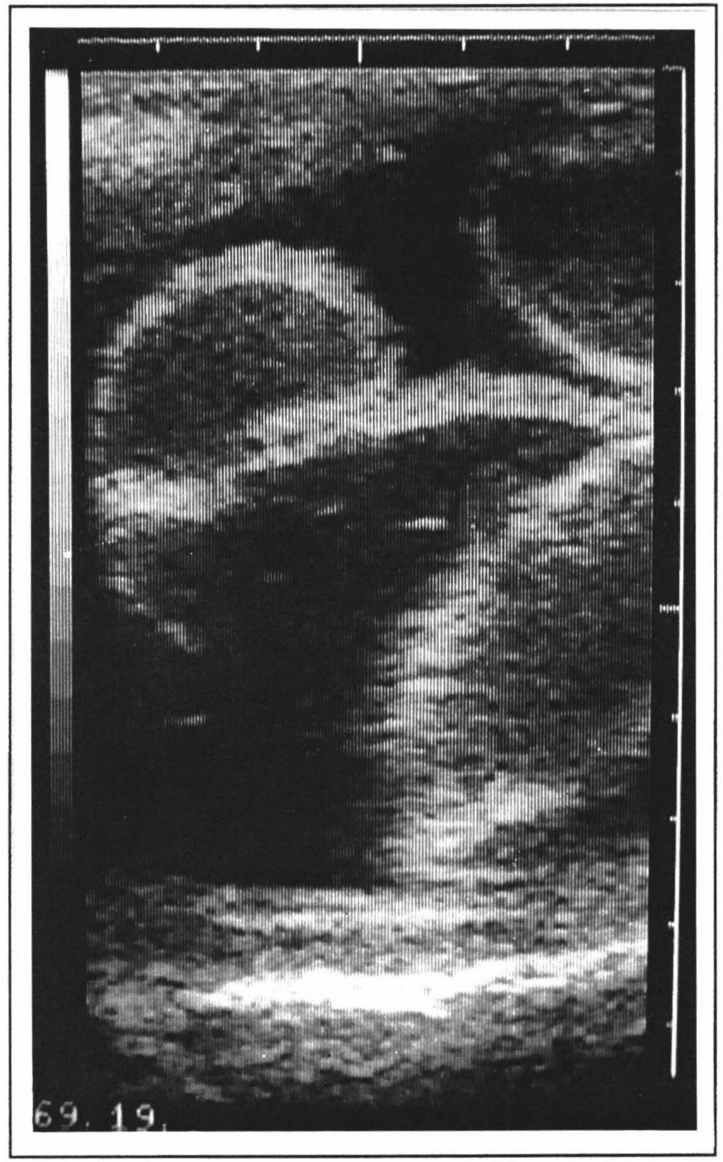


Figure 3.12.

Figure 3.12. **Day 157; placentome.**

The placentome is sometimes attached to the endometrium only with a stem.

Figure 3.13. **Day 147; accessory placentome.**

Placentomes, 5 to 15 mm diameter, appear between the large placentomes from 114 days pregnancy.

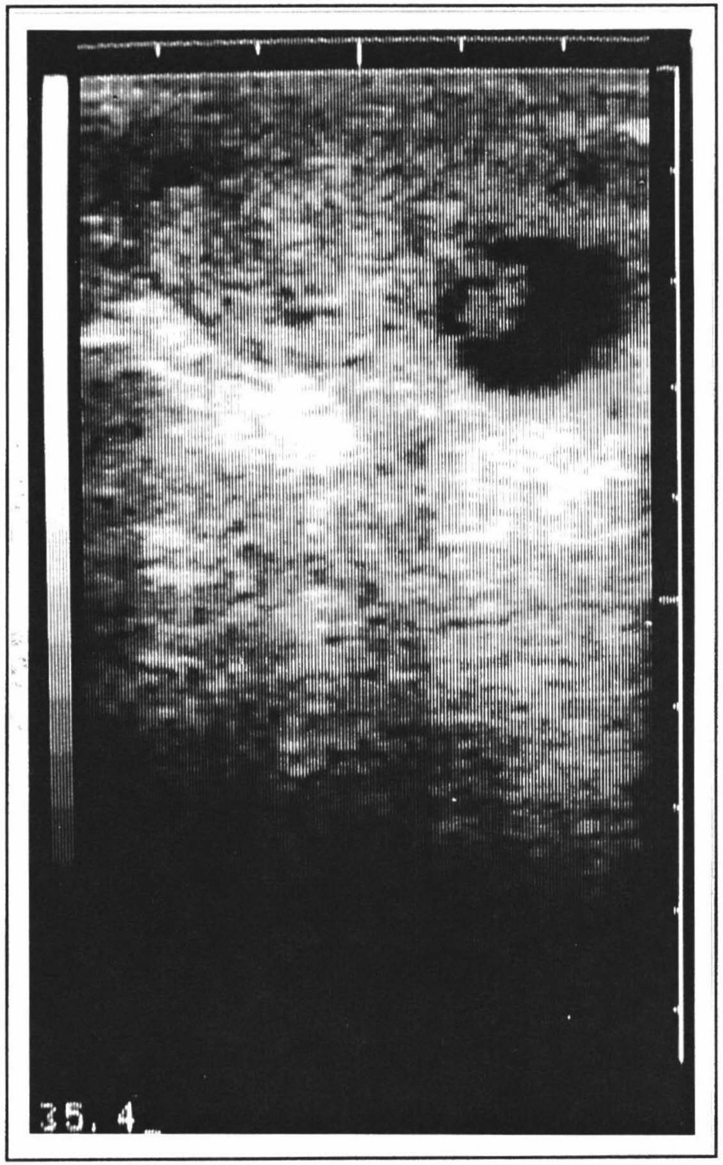


Figure 3.15.

Figure 3.14.

Figure 3.14. Day 24; first appearance of a foetal membrane.

A foetal membrane, probably the allantois, was observed within the chorionic vesicle as a highly reflective or dotted line.

Figure 3.15. Day 28; embryo.

Characteristic appearance of the embryo.

Although not seen in this figure, the heart beat is detected in real time.

Figure 3.17.

Figure 3.16.

Figure 3.16. Day 38; limb buds and tail.

The formation of the limb buds and tail appear by ultrasonography as echogenic spots on this coronal section of a foetus. The head is distinguished from the body by an increased echogenicity of the nose.

Figure 3.17. Day 38; foetal eyes.

The eyes are distinguished as highly reflective structures on this sagittal section of a foetus.

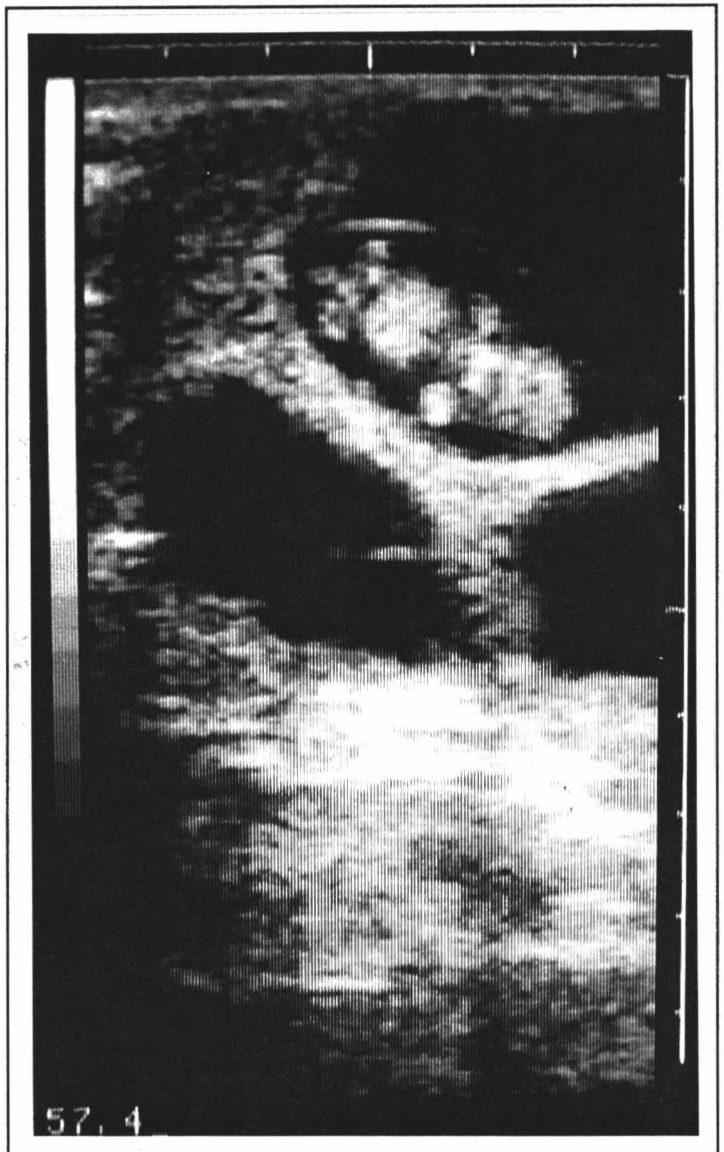


Figure 3.19.

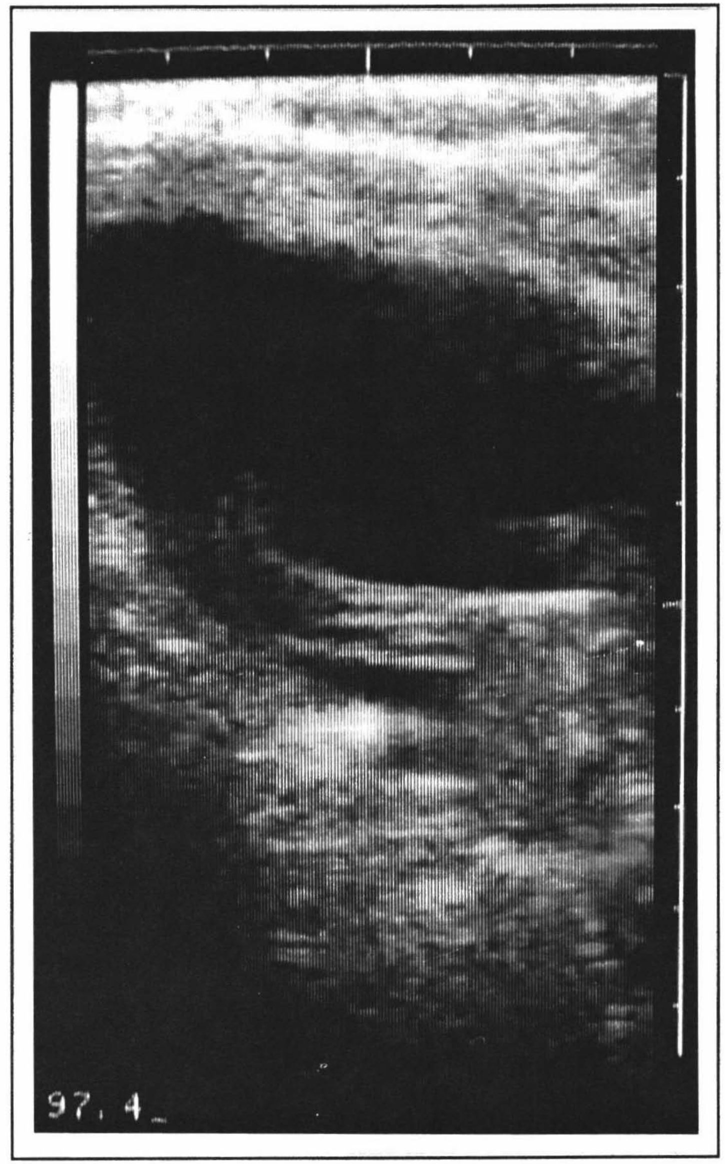


Figure 3.18.

Figure 3.18. Day 73; umbilical cord.

The umbilical cord is seen on a large proportion of scans. It shows foetal pulse movements.

Figure 3.19. Day 45; foetus.

The foetal parts (head, nose, abdomen, legs, tail) are readily distinguished. From 42 days onwards, spinal flexion, head and legs movements are observed.

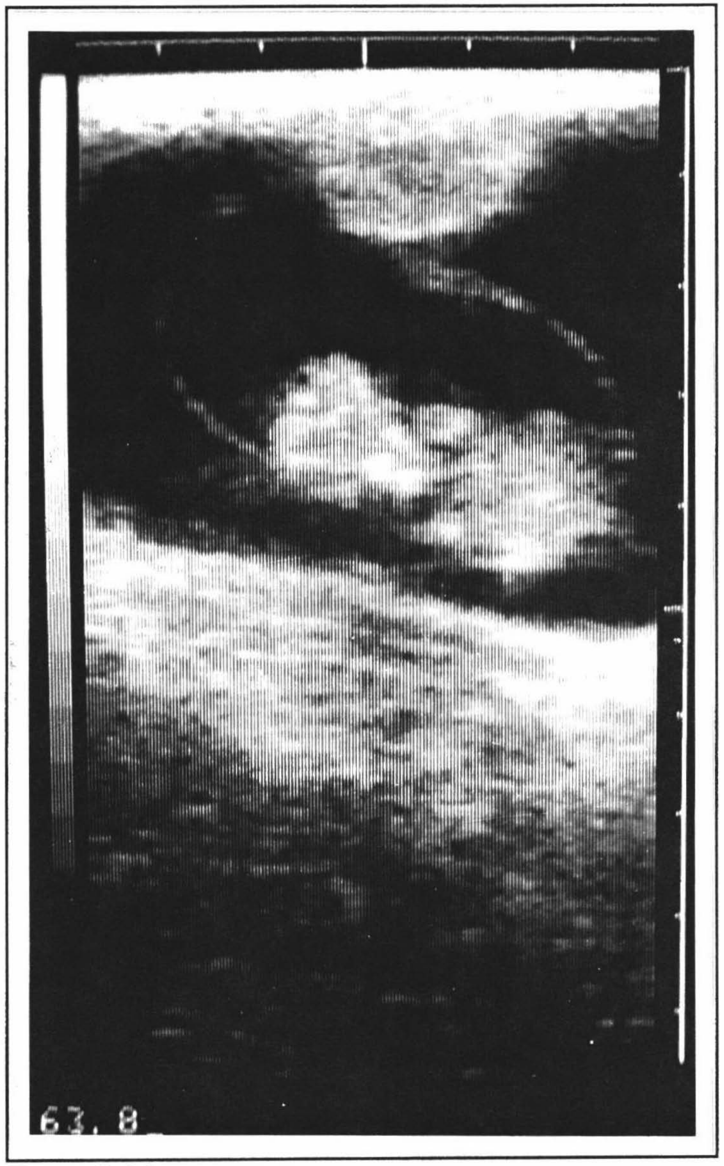


Figure 3.21.

Figure 3.20.

Figure 3.20. Day 80; skull.

The coronal section of the skull shows the left and right cerebral hemispheres.

Figure 3.21. Day 52; amnion.

The amniotic sac has a regular shape and surrounds the foetus.

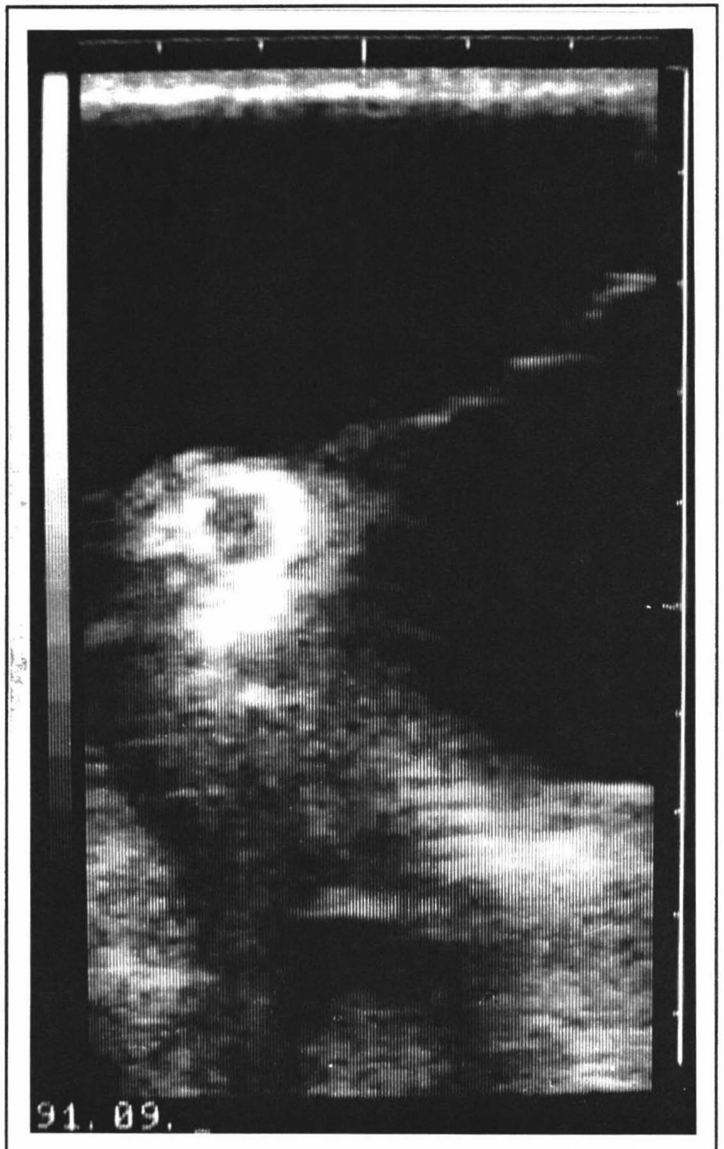


Figure 3.23.

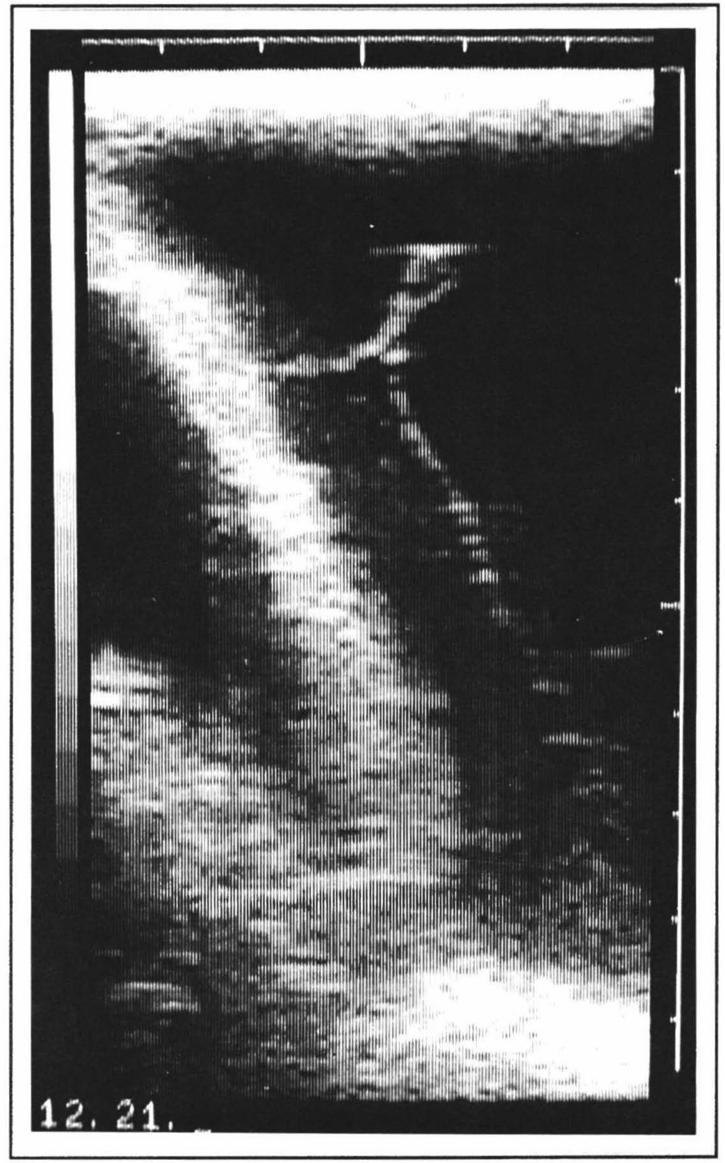


Figure 3.22.

Figure 3.22. Day 98; amniotic membrane.

In late gestation, the amniotic sac does not show a regular shape any more, and the amniotic membrane waves along with fluid movements.

Figure 3.23. Day 73; eye and pupil.

The elongated pupil is seen in the centre of the iris.

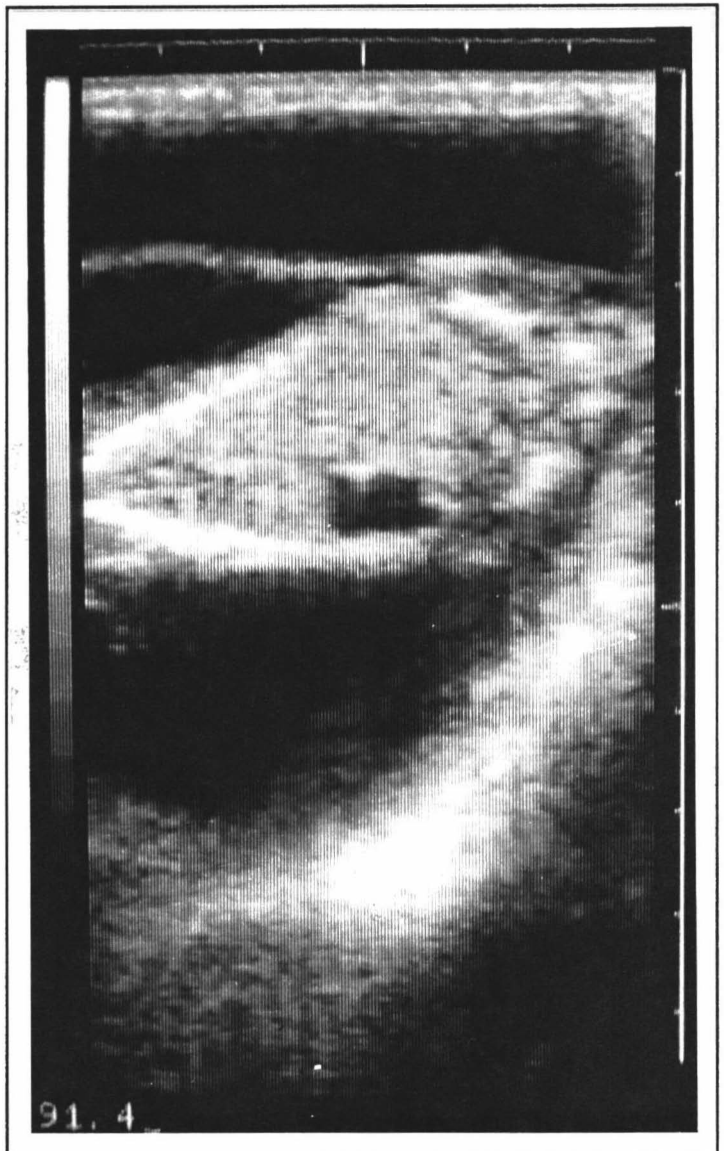


Figure 3.25.

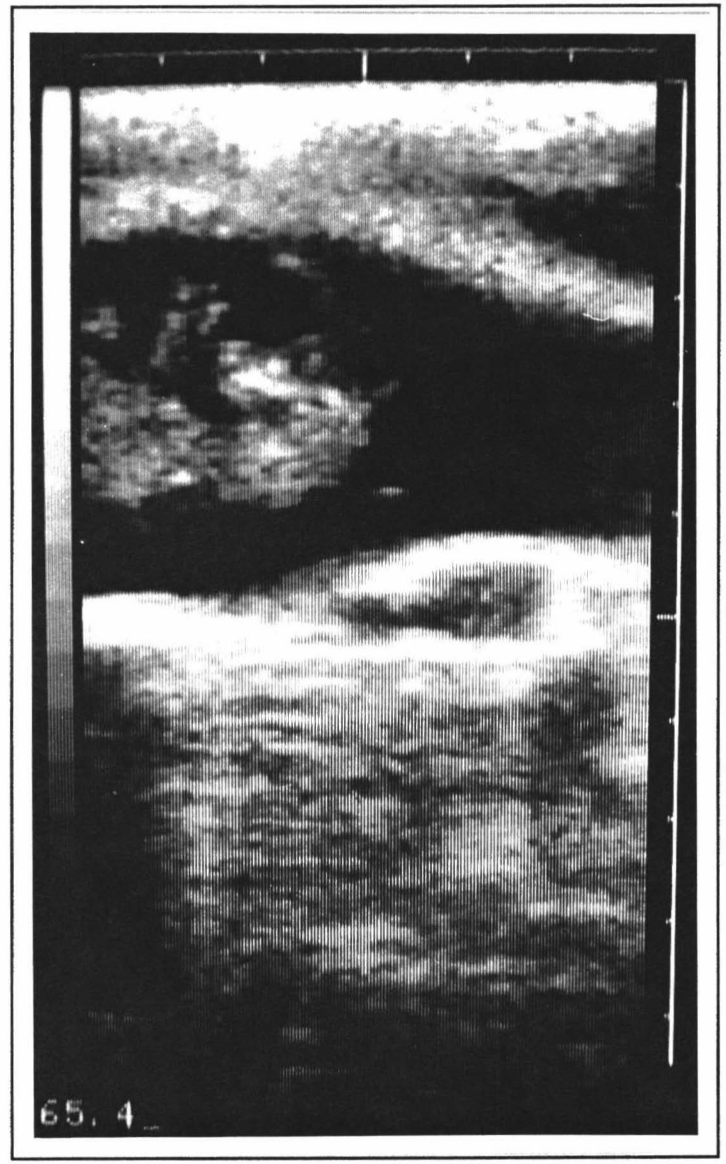


Figure 3.24.

Figure 3.24. **Day 56; elongation of the neck.**

The length of the neck, characteristic of the species, gives more amplitude to foetal movements. Thus, the crown-rump length becomes more variable.

Figure 3.25. **Day 71; chest.**

The coronal section of the thorax shows echogenicity of the ribs.

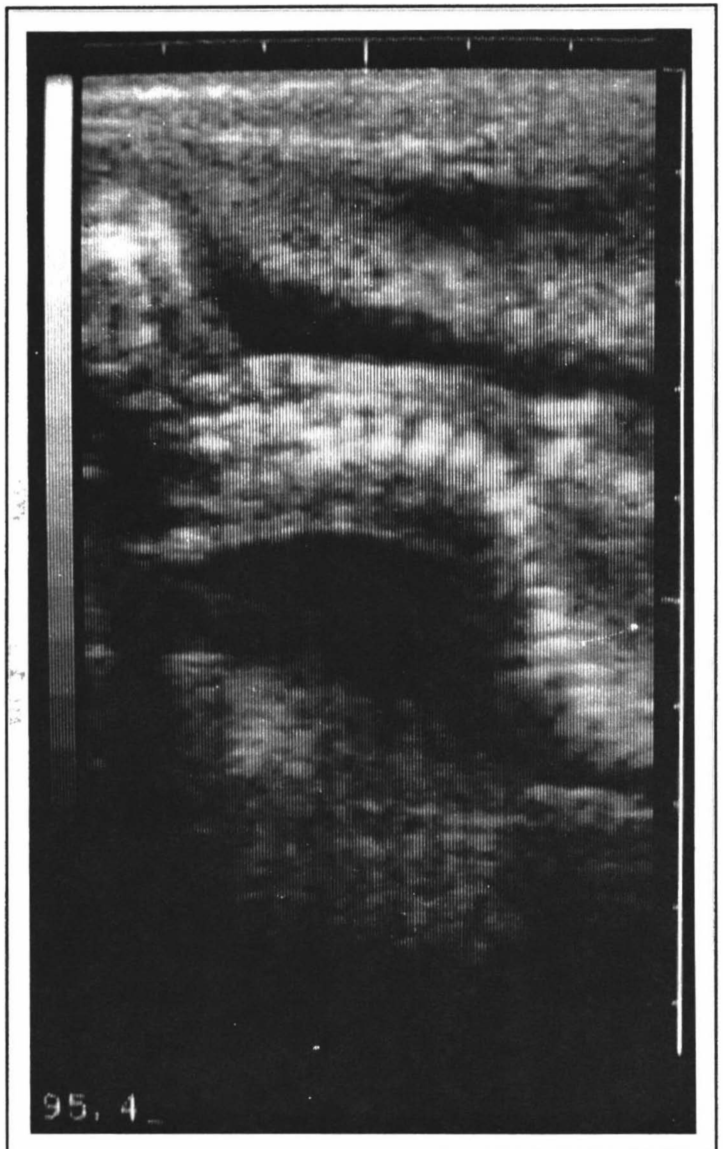


Figure 3.27.

Figure 3.26.

Figure 3.26. **Day 73; long bones.**

This axial section of the foetus, at the foetal pelvic level, shows the echogenicity of metatarsal bones.

Figure 3.27. **Day 65; vertebrae.**

A sagittal section of neck and upper chest shows the cervical and thoracic vertebrae.

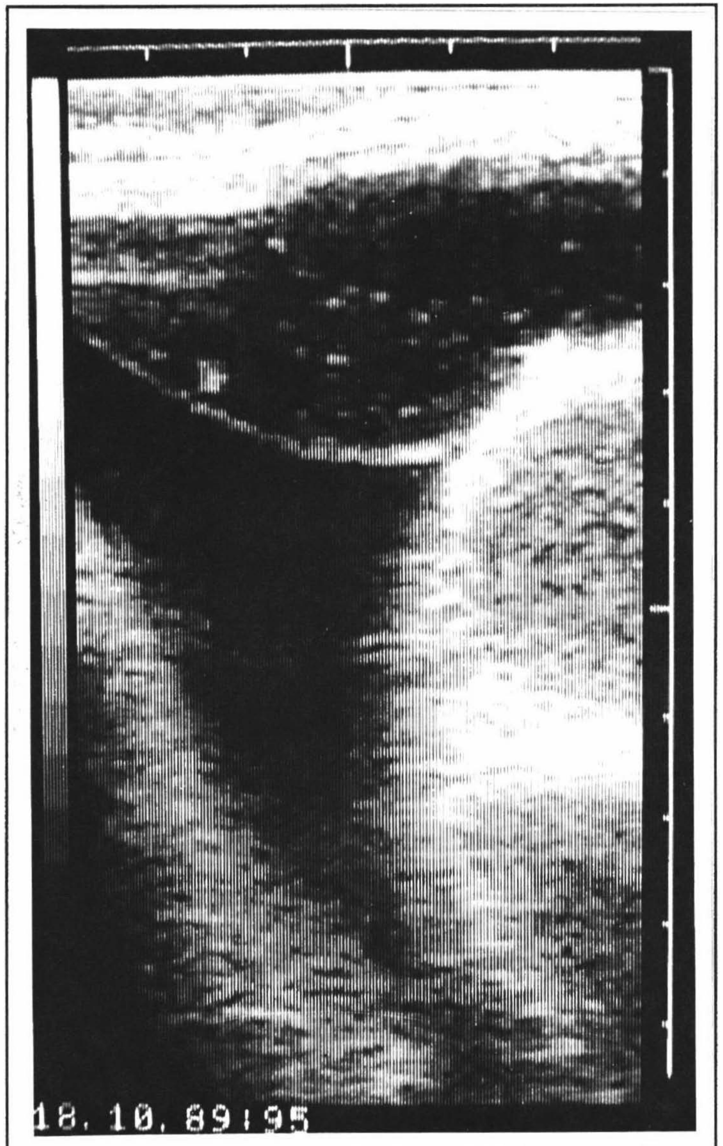


Figure 3.29.

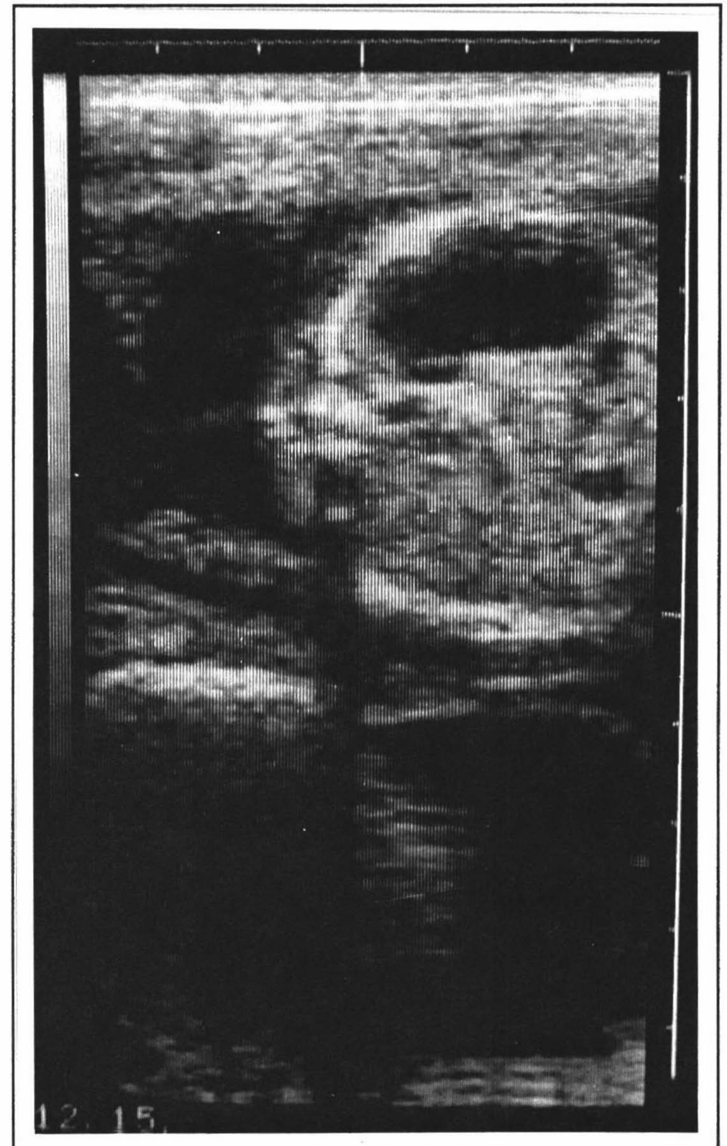


Figure 3.28.

Figure 3.28. Day 97; axial section of the foetus showing the stomach.

The fluid filled organ seen as a non-echogenic area in the abdomen is the stomach, dilated by swallowed foetal fluid.

Figure 3.29. Day 188; amniotic villi.

From 67 days onwards, numerous echogenic spots are observed in the amniotic sac, caused by membranous villi stretching from the inner surface of the amniotic membrane. These villi help the ultrasound operator to distinguish the amniotic sac from the allanto-chorion which is uniformly non-echogenic.

Figure 3.31.

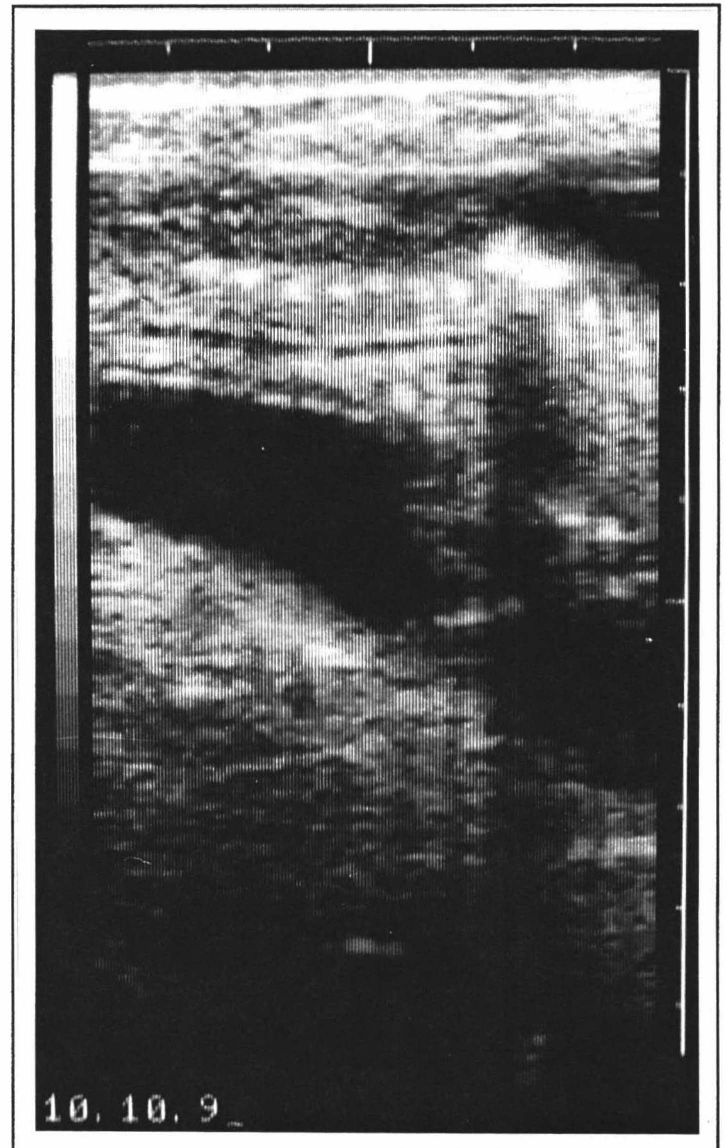


Figure 3.30.

Figure 3.30. **Day 80; trachea.**

The trachea is observed with a non echogenic lumen.

Figure 3.31. **Day 93; heart chambers.**

The coronal section of the heart shows the inter-ventricular and inter-auricular walls and the valves.

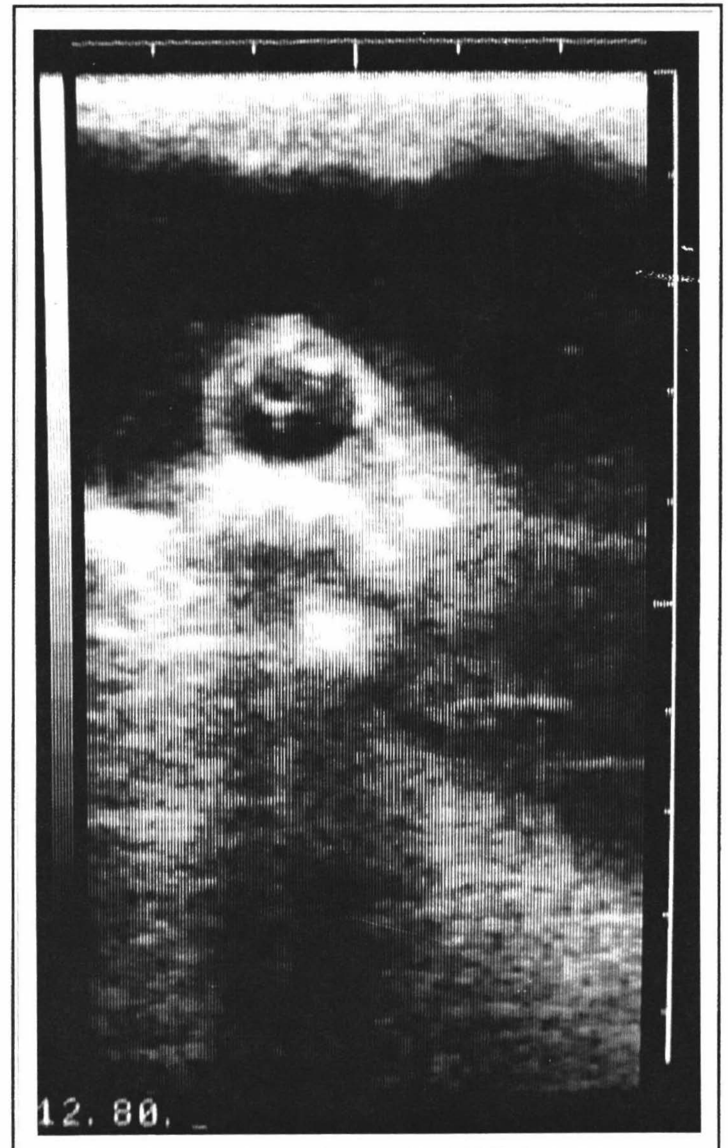


Figure 3.32.

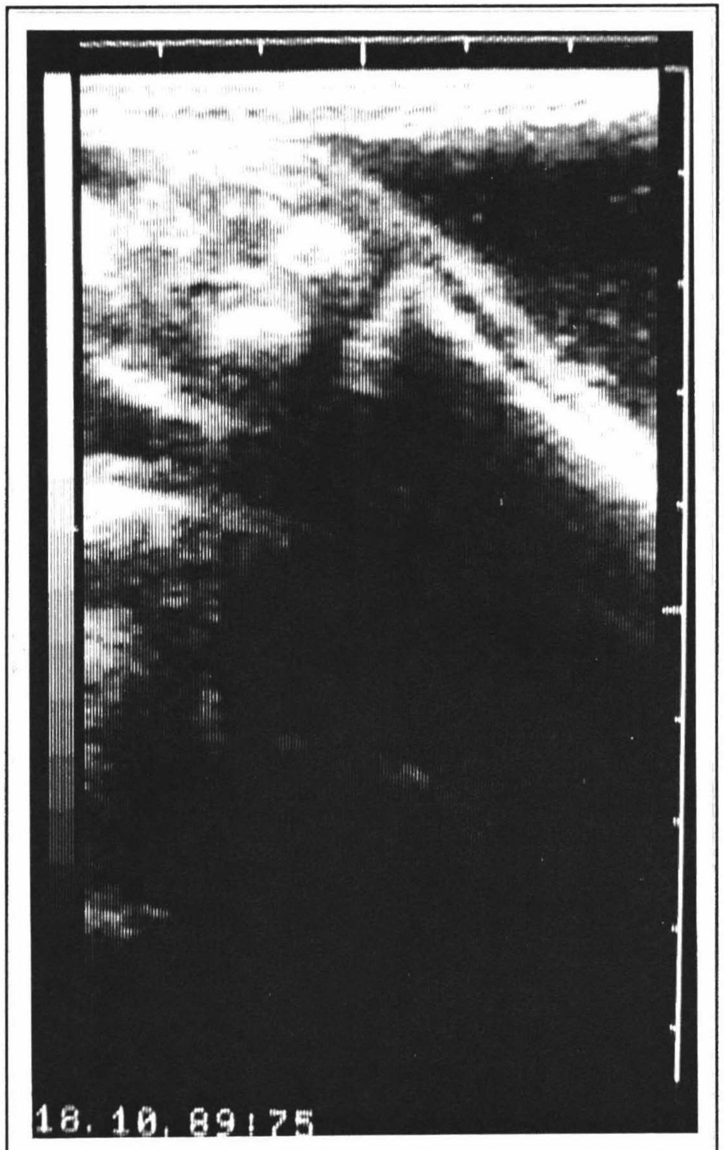


Figure 3.33.

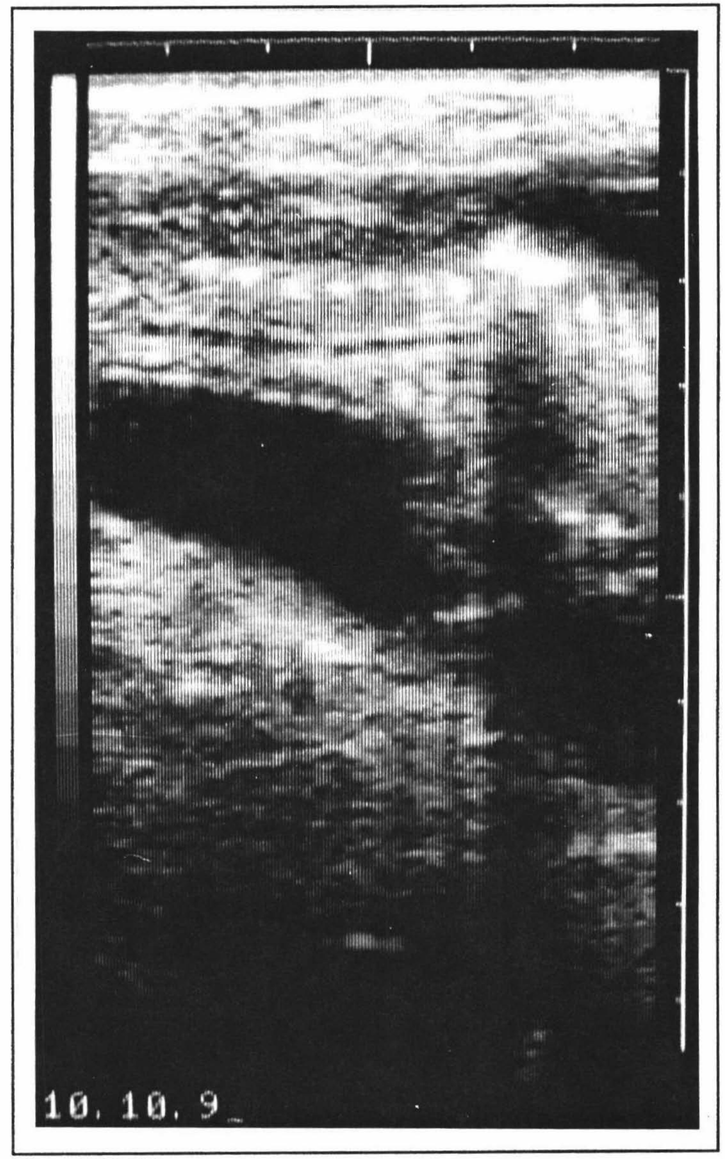
Figure 3.28. Day 97; axial section of the foetus showing the stomach.

The fluid filled organ seen as a non-echogenic area in the abdomen is the stomach, dilated by swallowed foetal fluid.

Figure 3.29. Day 188; amniotic villi.

From 67 days onwards, numerous echogenic spots are observed in the amniotic sac, caused by membranous villi stretching from the inner surface of the amniotic membrane. These villi help the ultrasound operator to distinguish the amniotic sac from the allanto-chorion which is uniformly non-echogenic.

Figure 3.31.



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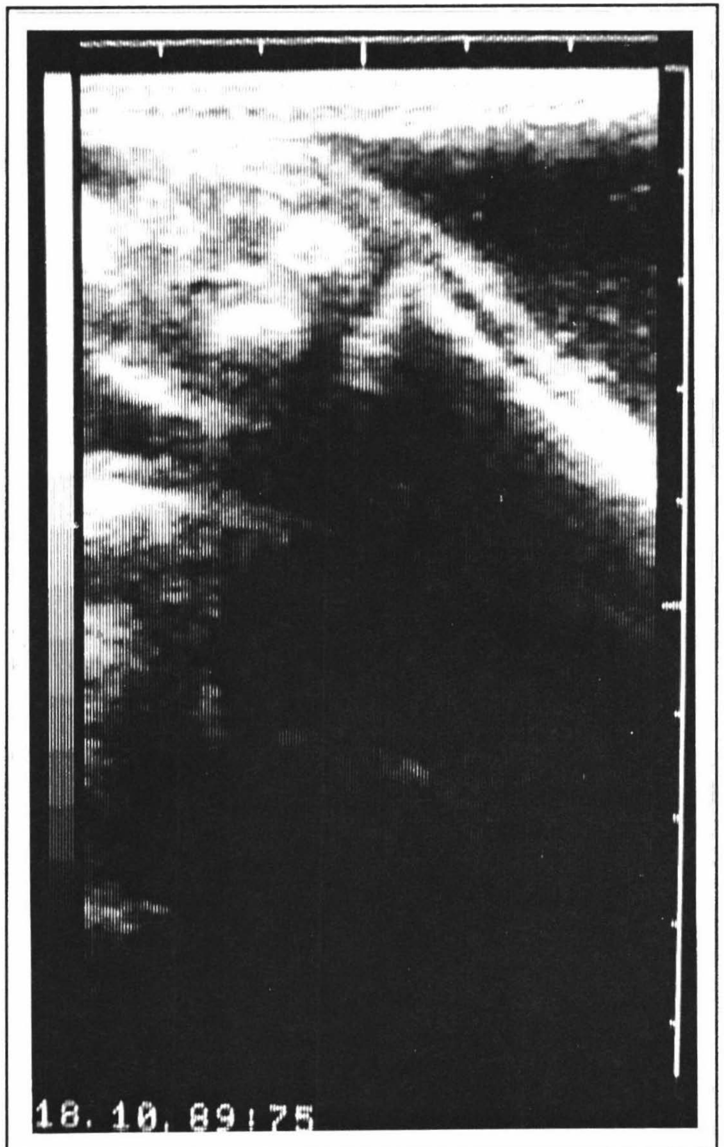

Figure 3.33.

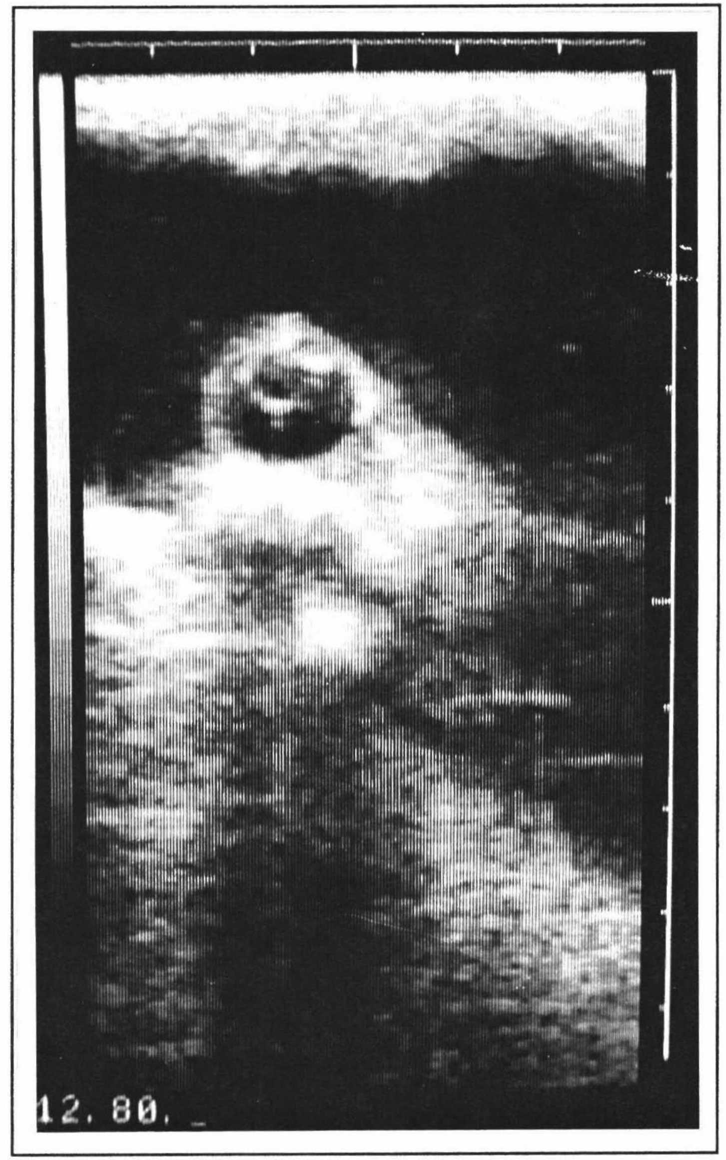

Figure 3.32.

Figure 3.32. **Day 93; eye.**

At this stage, the eyes have their own movements.

Figure 3.33. **Day 191; anterior foot.**

In late pregnancy, when the foetus becomes heavier and has fallen off the pelvic cavity, it becomes more difficult to view the foetus. However, due to the position of the foetus, the anterior feet and the head (see fig.3.34) are still imaged regularly. Here, The metacarpus and digits are distinguished.

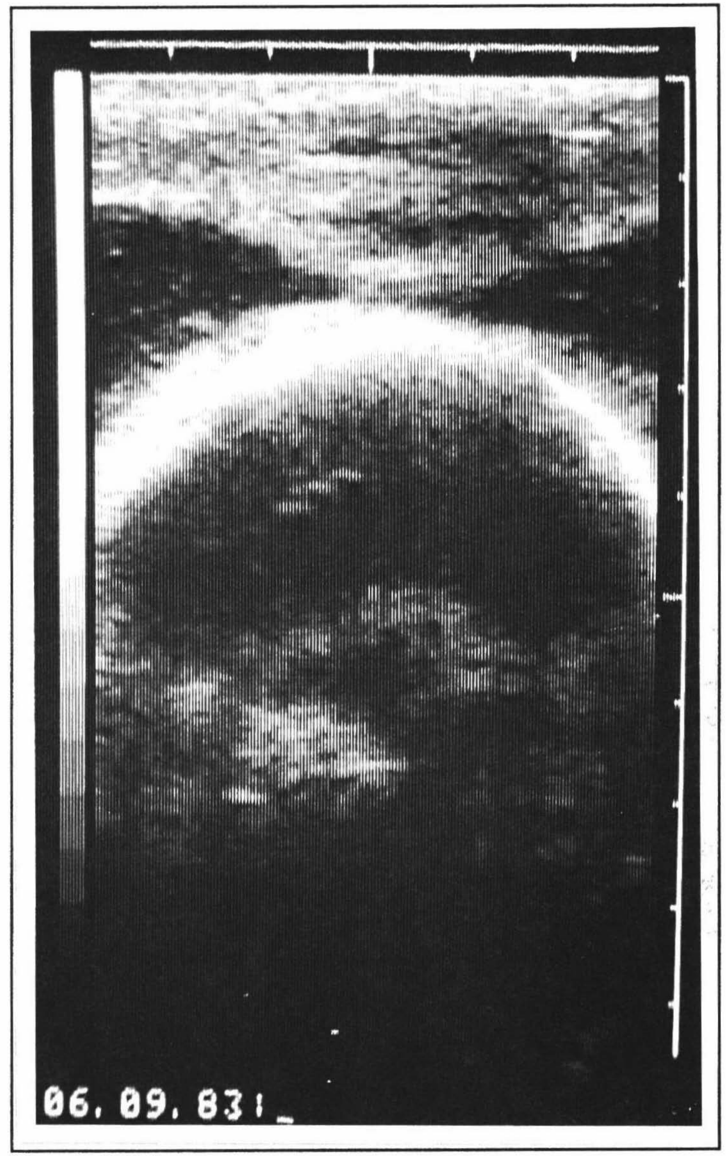

Figure 3.34.

Figure 3.34. Day 156; skull.

In late pregnancy only parts of the foetus can be imaged, due to the length of the transducer. As the foetus generally lies in a anterior presentation in the uterus, the foetal parts most commonly imaged are the anterior feet (see fig.3.33) and the head. Here, the foetal part scanned is the top of the skull.

Table 3.IV. Schedule of scanning for each deer giving pregnancy diagnosis on that date.

Tag N°	19/04	26/04	04/05	10/05	17/05	24/05	31/05	07/06	14/06	21/06	28/06	14/07	26/07	08/08	25/08 [5]	06/09	18/10	30/10
1	/	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	/	+
3	+	-	+	+	+	+	+	+	+	+	+	+	+	/	/	/	/	/
6	-	+	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+	+
11	-	-	+	+	+	+	+	+	+	+	+	+	+	+	/	-	+	/
12 [1]	/	/	-	-	-	/	/	/	/	/	/	/	/	/	/	/	/	/
14	+	/	+	+	+	+	+	+	+	+	+	+	+	/	/	+	+	+
15	/	/	+	-	+	+	+	+	+	+	+	+	+	/	+	+	+	+
19	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/	/
21	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/
38	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/	+
43 [2]	-	/	-	-	-	-	-	-	-	-	-	-	-	-	/	/	/	/
44 [3]	/	/	/	/	+	/	/	/	/	/	/	/	/	/	/	/	/	/
45	-	-	+	+	+	+	+	+	+	+	+	/ [4]	/	/	/	/	/	/
46 [3]	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
47	+	+	+	+	+	+	+	+	/	+	+	+	/	/	/	+	/	/
48	/	+	-	+	+	+	+	+	+	+	+	+	+	+	/	+	/	+
52	/	/	+	+	+	+	/	+	+	+	+	+	+	+	+	+	+	+
53	-	-	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+	+
54	/	/	+	+	+	+	+	+	+	+	+	+	/	/	/	/	+	/
58	/	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
59	-	-	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+	/
62	-	+	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+	-
66	/	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	/
67	+	-	+	+	+	+	+	+	/	+	/	+	+	+	/	+	/	/

Tag N°	19/04	26/04	04/05	10/05	17/05	24/05	31/05	07/06	14/06	21/06	28/06	14/07	26/07	08/08	25/08	06/09	18/10	30/10
69	/	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
74	+	+	+	+	+	+	+	+	+	+	+	+	+	/	-	+	/	/
75	/	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	/
79	/	+	+	+	+	+	+	+	+	+	+	/	+	+	+	+	/	/
80	/	-	+	+	+	+	+	+	+	+	+	+	+	+	+	/	/	/
83	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
84	/	-	/	+	+	+	+	+	+	+	+	+	+	/	/	+	+	+
86	/	/	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+	/
87	/	+	-	+	+	+	+	+	+	+	+	+	+	+	/	+	/	/
88	/	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
90	/	-	-	+	+	+	+	+	+	+	+	+	+	+	/	+	+	+
93	-	/	-	+	+	+	/	/	/	/	/	+	+	+	+	+	/	+
94	/	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
95	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
104	/	/	+	+	+	+	+	+	/	/	/	+	+	+	/	+	/	/
106	-	-	-	+	+	+	+	+	+	/	+	/	+	/	+	+	/	/
107	-	-	-	+	-	+	+	+	+	+	+	+	/	/	/	/	/	+
109	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
128	/	/	+	+	+	/	+	+	+	+	+	/	/	+	/	/	/	/
0	/	-	-	+	+	+	+	+	+	+	+	+	+	+	+	/	+	+

N.B.: All hinds, except 43, were pregnant.

(1): not often scanned because of poor health.
(2): uterus presented unusual less echogenic areas.
(3): not often scanned because of handling difficulties.
(4): sent to the slaughter.
(5): technical problems did not allow the reading of most scans on that date.

\+ positive pregnancy diagnosis
\- negative pregnancy diagnosis
/ hind was not scanned

Table 3.V. **Pregnancy diagnosis results for each hind at different stages after mating**

	Days after mating						
TAG N°	**6-10**	**11-20**	**21-30**	**31-40**	**41-100**	**101-130**	**131-2**
1 (P)	/	+-	+	+ +	‡ + + + +	+ +	+ + +
3 (P)	/	+	-	+ +	‡ ‡ + + +	+ +	/
6 (P)	/	-	+ +	+	‡ ‡ + + +	+ + +	+ + +
11 (P)	/	-	-	+ +	‡ ‡ + + +	+ + +	-+
14 (P)	/	+	+	+	‡ ‡ + + +	+ +	+ + +
19 (P)	/	+	-+	+	‡ ‡ + + +	+ + +	+ +
21 (P)	/	-+	+	+ +	‡ ‡ + + +	+ +	+ + +
38 (P)	/	-	+	+ +	‡ ‡ + + +	+ + +	+ + +
45 (P)	/	--	+	+ +	‡ + + + +	/	/
47 (P)	/	+	+	+ +	‡ + + + +	+	+
48 (P)	/	+	-+	+	‡ ‡ + + +	+ +	+ +
53 (P)	/	-	-	+ +	‡ ‡ + + +	+ + +	+ + +
58 (P)	/	+	+ +	+	‡ ‡ + + +	+ +	+-+ +
59 (P)	/	-	-	+ +	‡ ‡ + + +	+ + +	+ +
62 (P)	/	-	+	+ +	‡ ‡ + + +	+ + +	+ +-
66 (P)	/	-	+ +	+	‡ ‡ + + +	+ +	+ + +
67 (P)	+	-	+ +	+	‡ + + + +	+	+
69 (P)	/	-	+	+ +	‡ ‡ + + +	+ +	+ + +
74 (P)	/	+	+	+ +	‡ ‡ + + +	+ +	-+
75 (P)	/	+	--	+	‡ ‡ + + +	+ +	+ + +
79 (P)	/	+	+ +	+	‡ + + + +	+ +	+ +
80 (P)	/	-	+ +	+	‡ ‡ + + +	+ +	+
83 (P)	/	-	+	+ +	‡ ‡ + + +	+ + +	+ + +
84 (P)	/	-	/	+ +	‡ ‡ + + +	+	+ + +
87 (P)	+	-	+	+ +	‡ + + + +	+ +	+
88 (P)	/	+	-+	+	‡ ‡ + + +	+ +	+ + +
90 (P)	/	--	+	+ +	‡ + + + +	+ +	+ + +
93 (P)	-	/	-+	+	+ +	+ +	+ + +
94 (P)	/	-	--	+	‡ ‡ + + +	+ +	+ + +
95 (P)	-	-	+ +	+	‡ ‡ + + +	+ +	+ + +
104 (P)	+	+	+ +	+	+ + +	+	+
106 (P)	-	-	-+	+	+ + + + +	+	+ +
107 (P)	-	-	-	+-	‡ ‡ + + +	/	+
109 (P)	/	-	+	+ +	‡ ‡ + + +	+ + +	+ + +
0 (P)	-	-	+	+ +	‡ + + + +	+ +	+ + +
43 (NP)	-	/	--	-	-------	--	/

+ = diagnosed pregnant; - = diagnosed non pregnant; / = not scanned during this period.
(P) = pregnant; (NP) = non pregnant.

Table 3.VI

Accuracy of pregnancy diagnosis at different stages after mating.

	Days after mating				
Accuracy	6-20	21-30	31-40	41-130	131-211
Sensitivity	35%	71%	98%	100%	95%
Specificity[1]	100%	100%	100%	100%	/
*Reliability of positive test	287%	140%	102%	100%	105%
*Reliability of negative test	3%	33%	50%	100%	/
Number of observations	47	51	55	305	87

Total number of observations: 545

Overall accuracy: 91%

(1) calculated from only one hind.

* > 100% means underestimation.
< 100% means overestimation.

3.7. ACCURACY OF PREGNANCY DIAGNOSIS.

The dates of scanning and individual pregnancy testing results are presented in table 3.IV.

The accuracy of pregnancy diagnosis was found to vary at different stages of gestation. The individual pregnancy test results in relation to the stage of gestation are presented in table 3.V. From these data, the accuracy of pregnancy diagnoses was calculated using the equations described in section 2.4.1, and is presented in table 3.VI. Rectal ultrasonography under-estimates pregnancy before 40 days and after 130 days gestation, while non-pregnancy is over-estimated before 40 days. No data were obtained for the reliability of a negative test after 130 days. The sensitivity of the test was 100% between 41 and 130 days, but was 35% between 6 and 20 days, 71% from 21 to 30 days, 98% from 31 to 40 days, and 95% between 131 and 211 days. Specificity, calculated from only one hind with known mating but which did not fawn, was 100%.

3.8. GROWTH AND FOETAL AGEING CURVES.

Equations of the regressions describing the growth of foetal, uterine and placental dimensions throughout pregnancy, with their correlation coefficient, standard deviation, probability level, and the number of observations for each dimension, are presented in table 3.VII.

Regression equations used for age estimation, with their correlation coefficient, standard deviation, probability level, and the range of validity for each dimension, are presented in table 3.VIII.

For the purpose of presentation for this thesis, the data for each dimension is illustrated by three graphs: a three dimensioned histogram of the frequency of the measurement depending on the stage of gestation and the size of the dimension, and regression graphs for growth and for gestational age estimation.

Foetal age may be determined either mathematically using the appropriate age estimation equation, or by direct interpolation from the age estimation graph.

3.8.1. CROWN-RUMP LENGTH.

Crown-rump length was measured between 24 and 59 days when the foetal length was 6 to 61 mm (table 3.VIII). The histogram shows that measurements were made more often during the period 30-50 days, when the length was 10-30 mm (fig.3.35).

An exponential model best represented the evolution of the crown-rump length (fig.3.36; table 3.VII), while the most appropriate model for foetal age estimation was linear after transformation of the measurement to natural logarithm (fig.3.37; table 3.VIII).

3.8.2. HEAD DIAMETER.

Head diameter was measured between 42 and 128 days, when the head was 8 to 39 mm (table 3.VIII). The histogram shows that measurements were made more often between 50 and 80 days, when the head diameter was 10-30 mm (fig.3.38).

A linear model was most appropriate for both growth (fig.3.39; table 3.VII), and foetal age estimation equations (fig.3.40; table 3.VIII).

3.8.3. HEAD LENGTH.

Head length was measured between 42 and 84 days, when the length was 8 to 46 mm (table 3.VIII). The histogram shows that measurements were made mostly between 50 and 60 days, when the length was 10-20 mm (fig.3.41).

A linear model was most appropriate both for head length growth (fig.3.42; table 3.VII), and for foetal age estimation equations (fig.3.43; table 3.VIII).

3.8.4. EYE DIAMETER.

Diameter of the eye was measured between 57 and 156 days when the eye was 3 to 25 mm (table 3.VIII). The histogram shows that measurements were made mostly between 100 and 110 days, when the eye was 15 to 20 mm in diameter (fig.3.44).

A linear model was most appropriate for both growth (fig.3.45; table 3.VII) and foetal age estimation equations (fig.3.46; table 3.VIII).

3.8.5. NOSE LENGTH.

Length of the nose was measured from day 47 to day 120 when the length was 3 to 39 mm (table 3.VIII). The frequency of measurement was higher between 50 and

80 days when the nose was 3 to 20 mm (fig.3.47).

A linear model was most appropriate for both growth (fig.3.48; table 3.VII) and foetal age estimation equations (fig.3.49; table 3.VIII).

3.8.6. NECK DIAMETER.

The diameter of the neck was measured between 71 and 120 days when the neck was 14 to 26 mm (table 3.VIII). The frequency of the measurement was highest between 71 and 110 days when the neck was more than 14 mm diameter (fig.3.50).

A multiplicative model best fitted the growth curve (fig.3.51; table 3.VII), and a linear model was most appropriate for foetal age estimation (fig.3.52; table 3.VIII).

3.8.7. CHEST DIAMETER.

The diameter of the chest was measured between 45 and 127 days when the chest was 10 to 58 mm (table 3.VIII). Measurements were taken most frequently between 70 and 80 days when the diameter was 20 to 30 mm (fig. 3.53).

A multiplicative model best described the growth pattern (fig.3.54; table 3.VII), while a linear model was most appropriate for foetal age estimation (fig.3.55; table 3.VIII).

3.8.8. CHEST DEPTH.

Chest depth was measured between 51 and 93 days when the depth was 15 to 49 mm (table 3.VIII). The measurements were taken with a regular frequency throughout the period (fig.3.56).

A multiplicative model best described the evolution of chest depth (fig.3.57; table 3.VII), while a linear model was chosen for foetal age estimation (fig.3.58; table 3.VIII).

3.8.9. UMBILICAL CORD DIAMETER.

The diameter of the umbilical cord was measured from 33 to 192 days when the umbilical cord was 3 to 27 mm (table 3.VIII). Measurements were taken most frequently between 60 to 80 days when the diameter was 5 to 10 mm (fig.3.59).

A multiplicative model best described the evolution of umbilical cord diameter (fig.3.60; table 3.VII), while a linear model was chosen for foetal age estimation (fig.3.61; table 3.VIII).

3.8.10. AMNION LENGTH.

The length of the amniotic sac was measured from 37 to 56 days when it was 16 to 55 mm long (table 3.VIII). However, the measurements were concentrated between 40 and 50 days when the amnion was 20 to 30 mm long (fig.3.62).

A multiplicative model best described the evolution of the amnion length (fig.3.63; table 3.VII), while a linear model was chosen for foetal age estimation (fig.3.64; table 3.VIII).

3.8.11. AMNION WIDTH.

The width of the amnion was measured from 38 to 63 days when the amniotic sac was 9 to 31 mm wide (table 3.VIII). Measurements were taken on a regular basis from 40 to 60 days (fig.3.65).

A linear model best fitted both the evolution of the amniotic sac width (fig.3.66; table 3.VII), and the foetal age estimation equation (fig.3.67; table 3.VIII).

3.8.12. PLACENTOME DIAMETER.

Placentome diameter was measured from 24 to 211 days when the placentome was 5 to 88 mm (see fig.3.69). Two peaks of measurement frequency were observed, the first between 30 and 40 days, the second between 100 and 110 days (fig.3.68).

A quadratic model best represented the growth of the placentomes (fig.3.69; table 3.VII). The age estimation regression was limited to the period 24-52 days when the placentomes were 5 to 32 mm. A linear model best fitted the data for foetal age estimation (fig.3.70; table 3.VIII).

3.8.13. UTERINE LUMEN DIAMETER.

The diameter of the uterine lumen was measured from 7 to 45 days when the lumen was 4 to 60 mm (table 3.VIII). However, the measurements were concentrated between 10 and 30 days when the uterus was 4 to 20 mm (fig.3.71).

An exponential model best described the evolution of the uterine lumen (fig.3.72; table 3.VII), while a linear model was chosen for foetal ageing (fig.3.73; table 3.VIII).

3.9. GESTATION LENGTH.

The mean gestation length, calculated from recorded matings and calvings, was 233 days, ranging from 221 to 239 days (table 2.I).

Table 3.VII
Regression equations describing the growth of foetal and uterine dimensions measured during the study.

Dimension	Equation	r^2 %	sd (mm)	Probability level(1)	Number of observations
Crown-rump (CR)	CR = exp (0.54 + 0.059 x DAYS)	93.1	3.8	I and S: $p < 0.00001$	114
Head diameter (HD)	HD = -7.1 + 0.37 DAYS	89.6	2.4	I and S: $p < 0.00001$	113
Head length (HL)	HL = -31.3 + 0.91 DAYS	95.3	2.1	I and S: $p < 0.00001$	54
Eye diameter (ED)	ED = -7.2 + 0.22 DAYS	82.0	2.1	I and S: $p < 0.00001$	69
Nose length (NL)	NL = -13.8 + 0.39 DAYS	93.7	1.9	I and S: $p < 0.00001$	24
Neck diameter (ND)	$ND = 0.125 \times DAYS^{1.126}$	72.8	1.9	I: $p < 0.017$ S: $p < 0.0001$	18
Chest diameter (CD)	$CD = 0.027 \times DAYS^{1.565}$	93.6	2.5	I and S: $p < 0.00001$	39
Chest depth (CDe)	$CDe = 0.037 \times DAYS^{1.521}$	94.7	2.4	I and S: $p < 0.00001$	19
Umbilical cord diameter (UD)	$UD = 0.065 \times DAYS^{1.129}$	75.6	1.6	I and S: $p < 0.00001$	125
Amnion length (AL)	$AL = 0.002 \times DAYS^{2.521}$	92.3	3.0	I and S: $p < 0.00001$	30
Amnion width (AW)	AW = -11.1 + 0.65 DAYS	65.9	2.7	I: $p < 0.004$ S: $p < 0.00001$	42
Placentome (PL): 24-211 days	$PL = -5.6 + 16.0\ DAYS - 11.02\ (DAYS)^2$	81.9	8.7	I and 2 coefficients: $p < 0.0001$	129
Uterine lumen diameter (UT)	UT = exp (1.22 + 0.057 DAYS)	43.5	12.5	I and S: $p < 0.00001$	64

(1): I = intercept; S = slope.

Table 3.VIII
Regression equations for gestational age estimation, and range of validity.

Dimension (x)	Age prediction equation	r^2 %	sd (days)	Probability level[1]	Range of validity mm	Days
Crown-rump	DAYS = -5.7 + 15.75 ln* x	93.1	2.2	I and S: $p < 0.00001$	6-61	24-59
Head diameter	DAYS = 25.1 + 2.43 x	89.6	6.1	I and S: $p < 0.00001$	8-39	42-128
Head length	DAYS = 35.5 + 1.04 x	95.3	2.3	I and S: $p < 0.00001$	8-46	42-84
Eye diameter	DAYS = 44.4 + 3.64 x	82.0	8.6	I and S: $p < 0.00001$	3-25	57-156
Nose length	DAYS = 37.3 + 2.37 x	93.7	4.8	I and S: $p < 0.00001$	3-39	47-120
Neck diameter	DAYS = 30.7 + 2.97 x	72.5	7.1	I: $p < 0.005$ S: $p < 0.0001$	14-26	71-120
Chest diameter	DAYS = 32.4 + 1.75 x	92.6	4.8	I and S: $p < 0.00001$	10-58	45-127
Chest depth	DAYS = 29.5 + 1.70 x	93.2	4.5	I and S: $p < 0.00001$	15-49	51-93
Umbilical cord diameter	DAYS = 29.4 + 5.31 x	73.8	11.5	I and S: $p < 0.00001$	3-27	33-192
Amnion length	DAYS = 29.2 + 0.51 x	91.1	1.7	I and S: $p < 0.00001$	16-55	37-56
Amnion width	DAYS = 27.6 + x	65.9	3.4	I and S: $p < 0.00001$	9-31	38-63
Placentome (to 55 days)[2]	DAYS = 23 + 0.94 x	64.7	4.7	I and S: $p < 0.00001$	5-32	24-52
Uterine lumen diameter	DAYS = 19.7 + 0.34 x	37.2	6.9	I and S: $p < 0.00001$	4-60	7-45

(1): I = intercept; S = slope.
(2): Only the 43 observations made under 55 days pregnancy were taken in account for this equation.
* Natural logarithm.

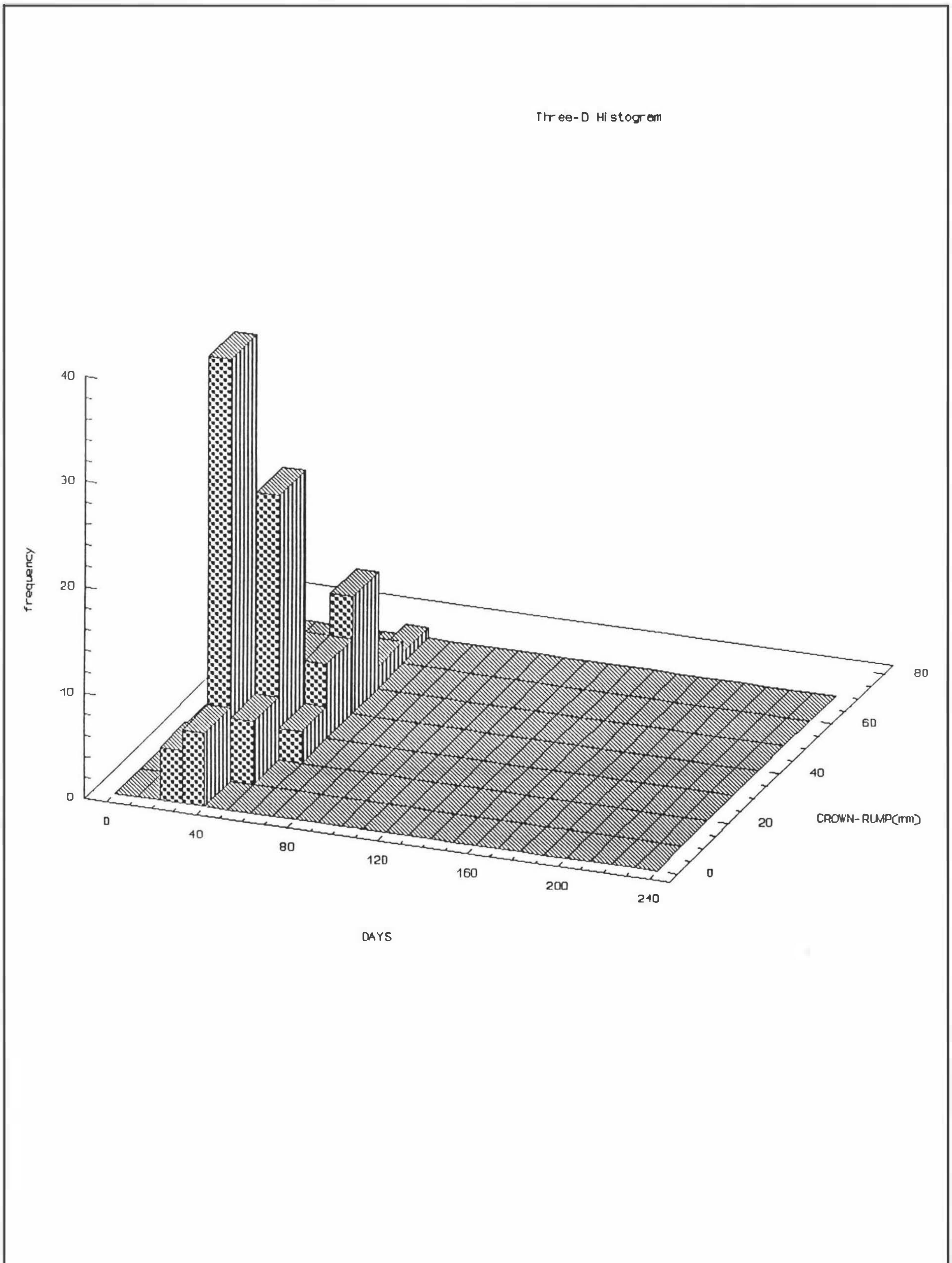

Figure 3.35. Histogram of crown-rump length measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

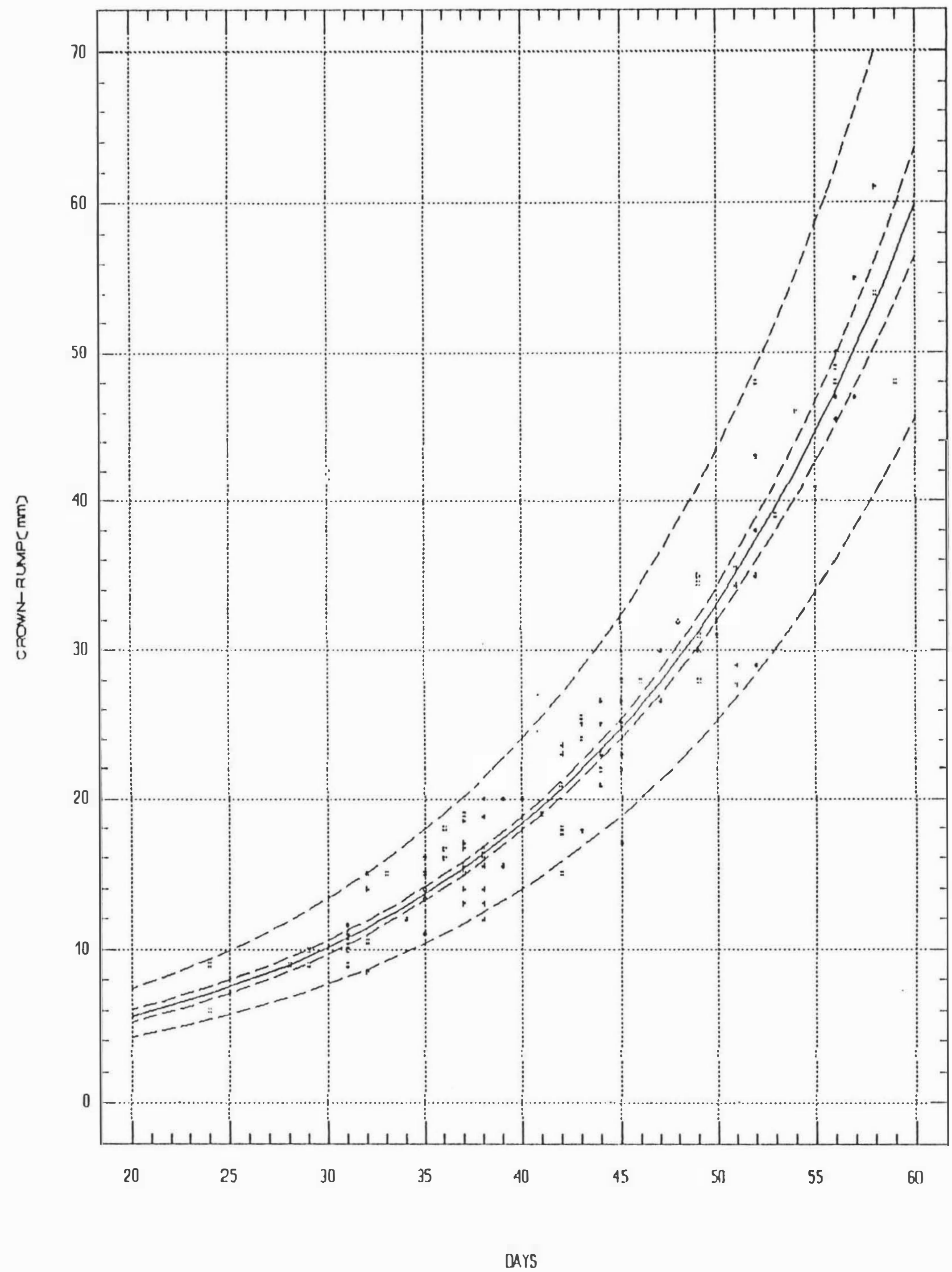

Figure 3.36. Crown-rump growth.
Scattergram of crown-rump length with foetal age and the best fit regression for the data (95% confidence interval).

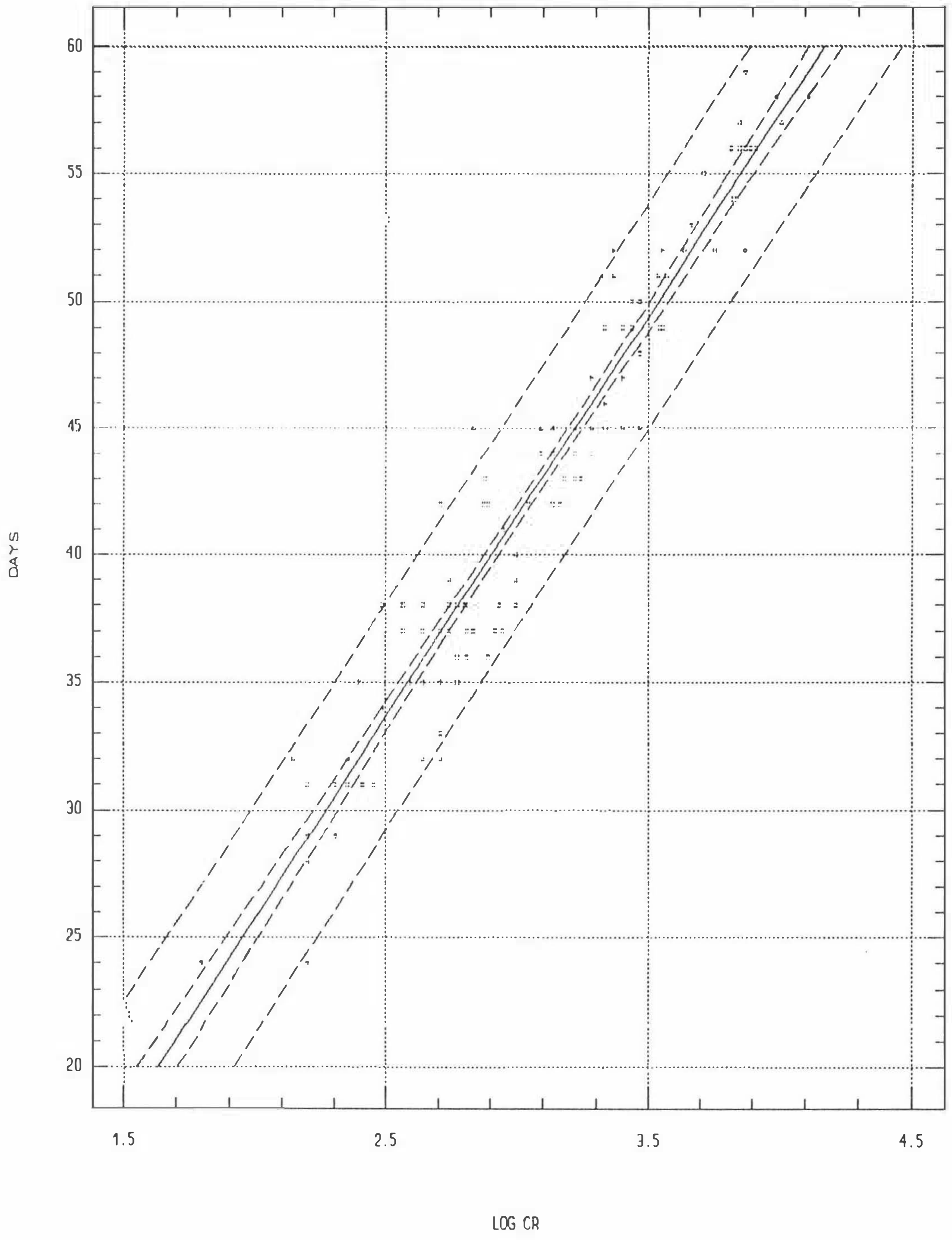

Figure 3.37. Age estimation using crown-rump length.
Scattergram of age on crown-rump and the regression used for foetal age estimation (95% confidence interval).

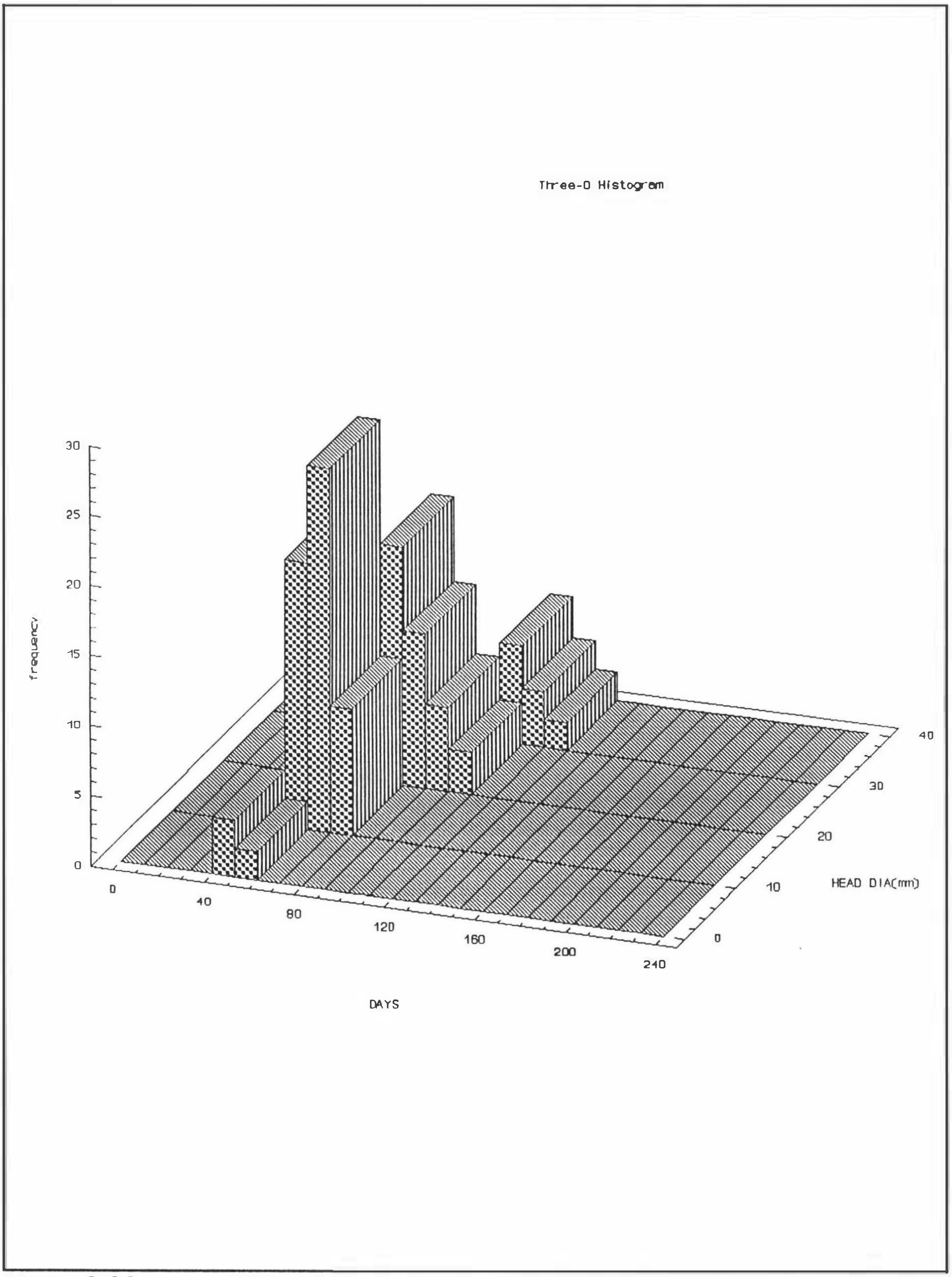

Figure 3.38. Histogram of head diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

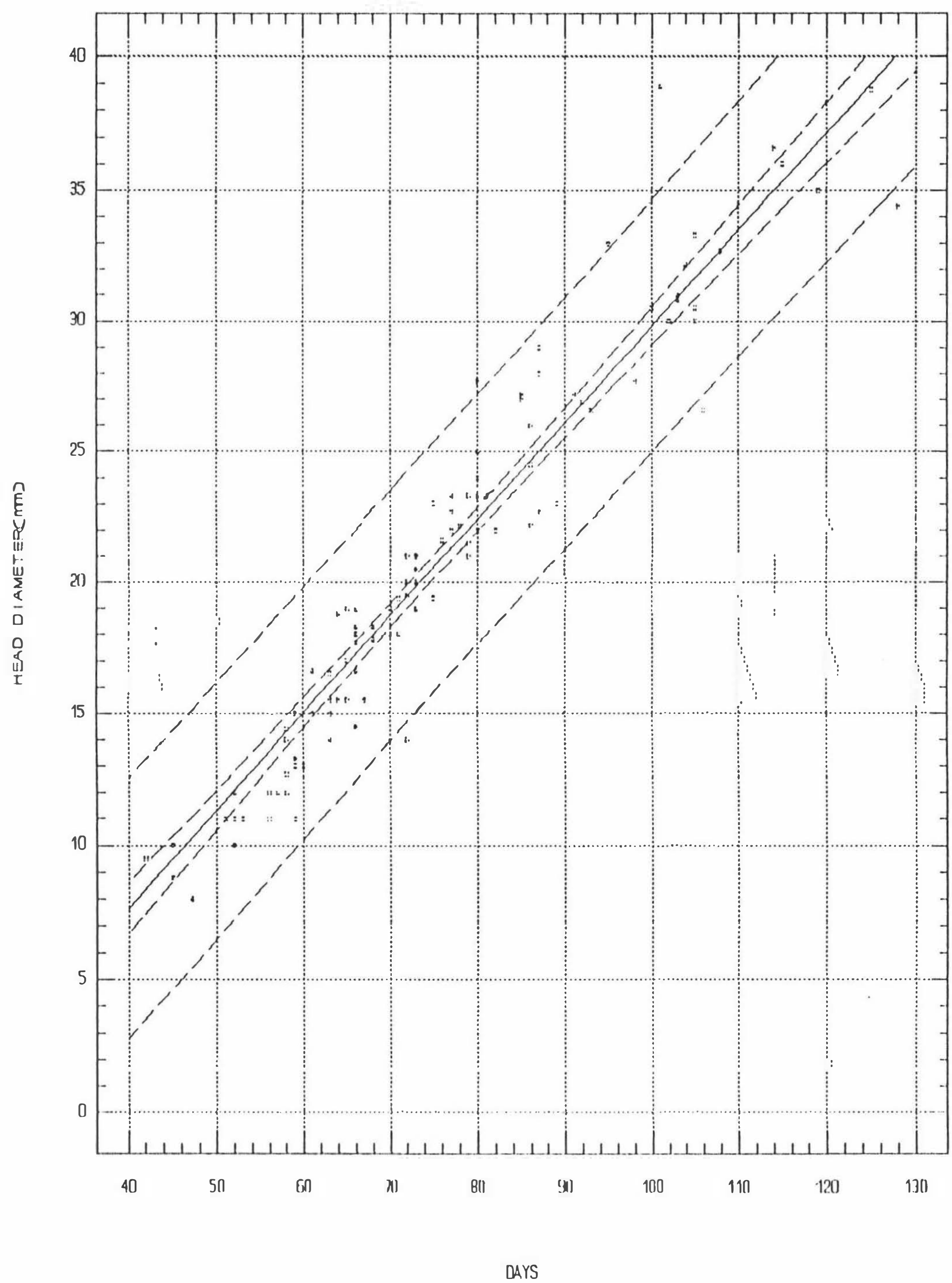

Figure 3.39. Head diameter growth.
Scattergram of head diameter with foetal age, and the best fit regression for the data (95% confidence interval).

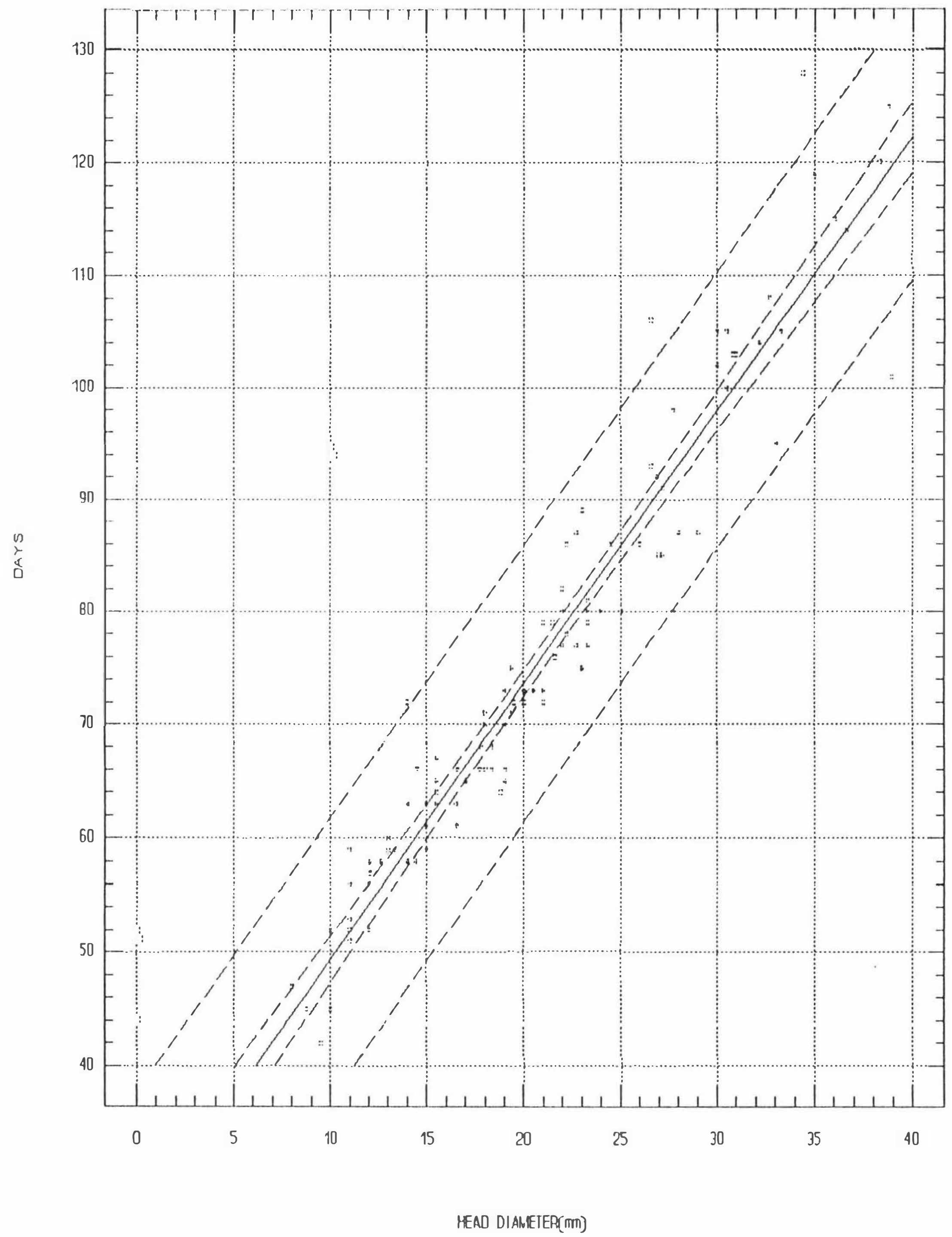

Figure 3.40. Age estimation using head diameter.
Scattergram of age on head diameter and the regression used for foetal age estimation (95% confidence interval).

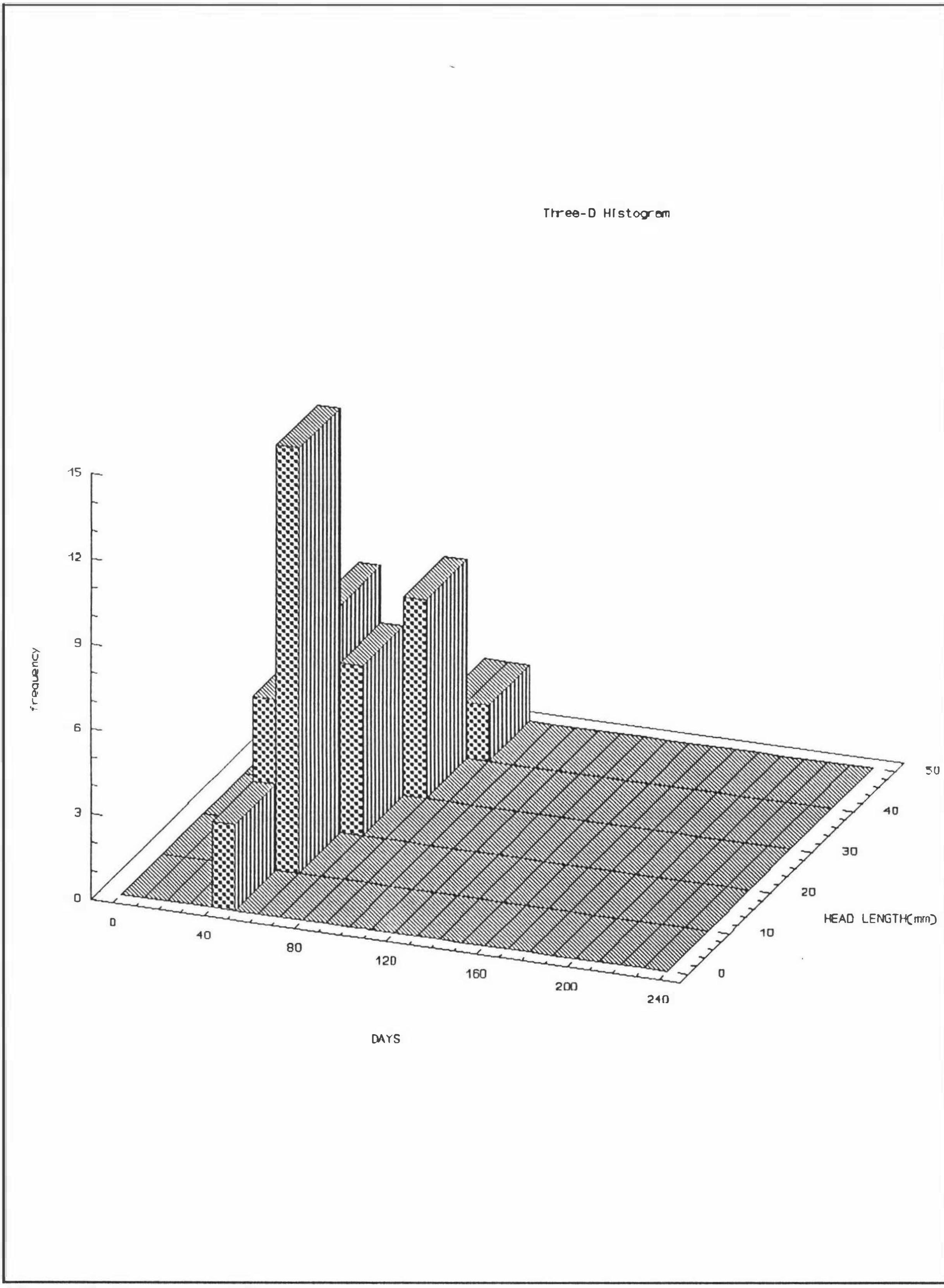

Figure 3.41. Histogram of head length measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

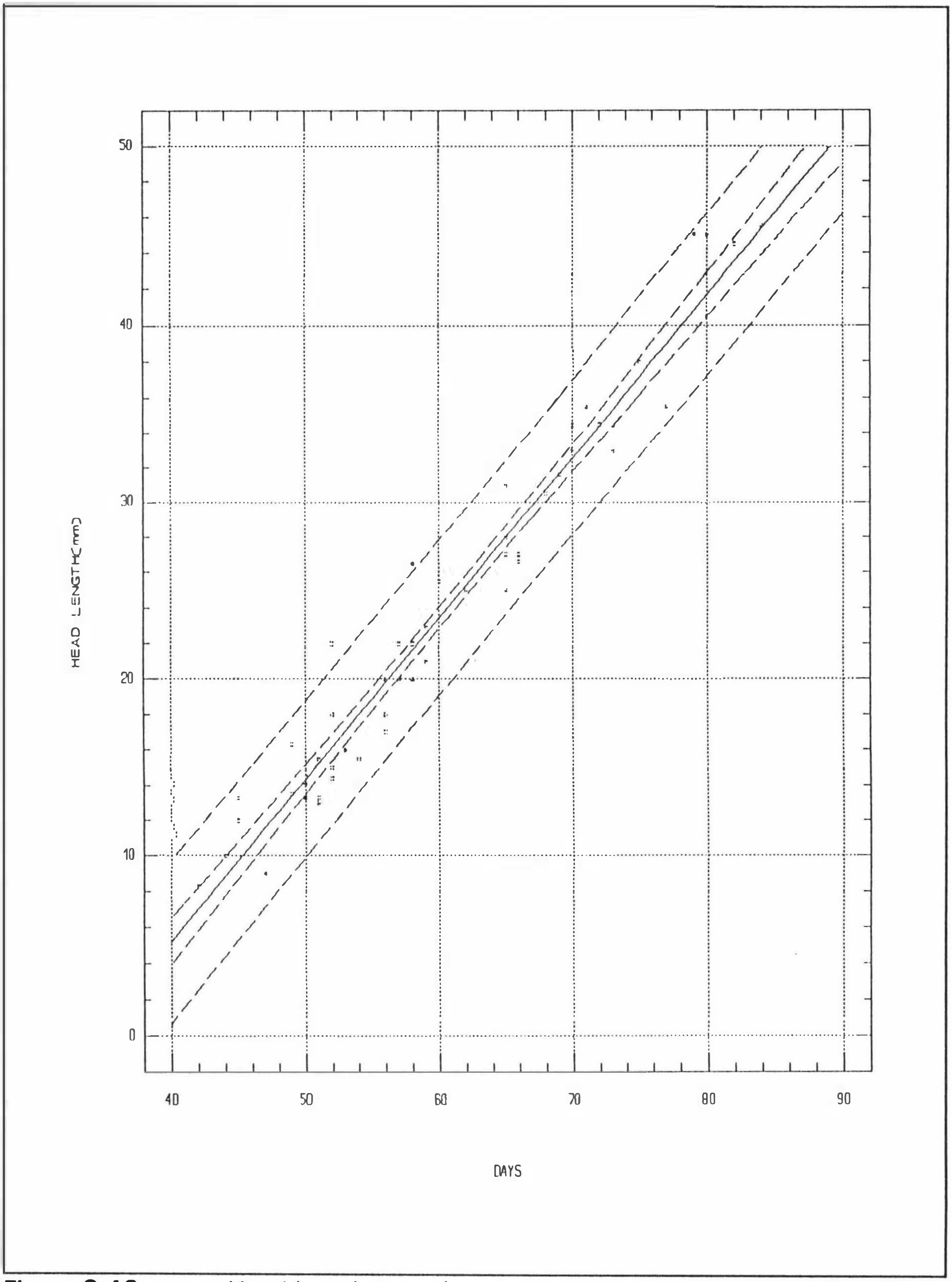

Figure 3.42. Head length growth.
Scattergram of head length with foetal age, and the best fit regression for that data (95% confidence interval).

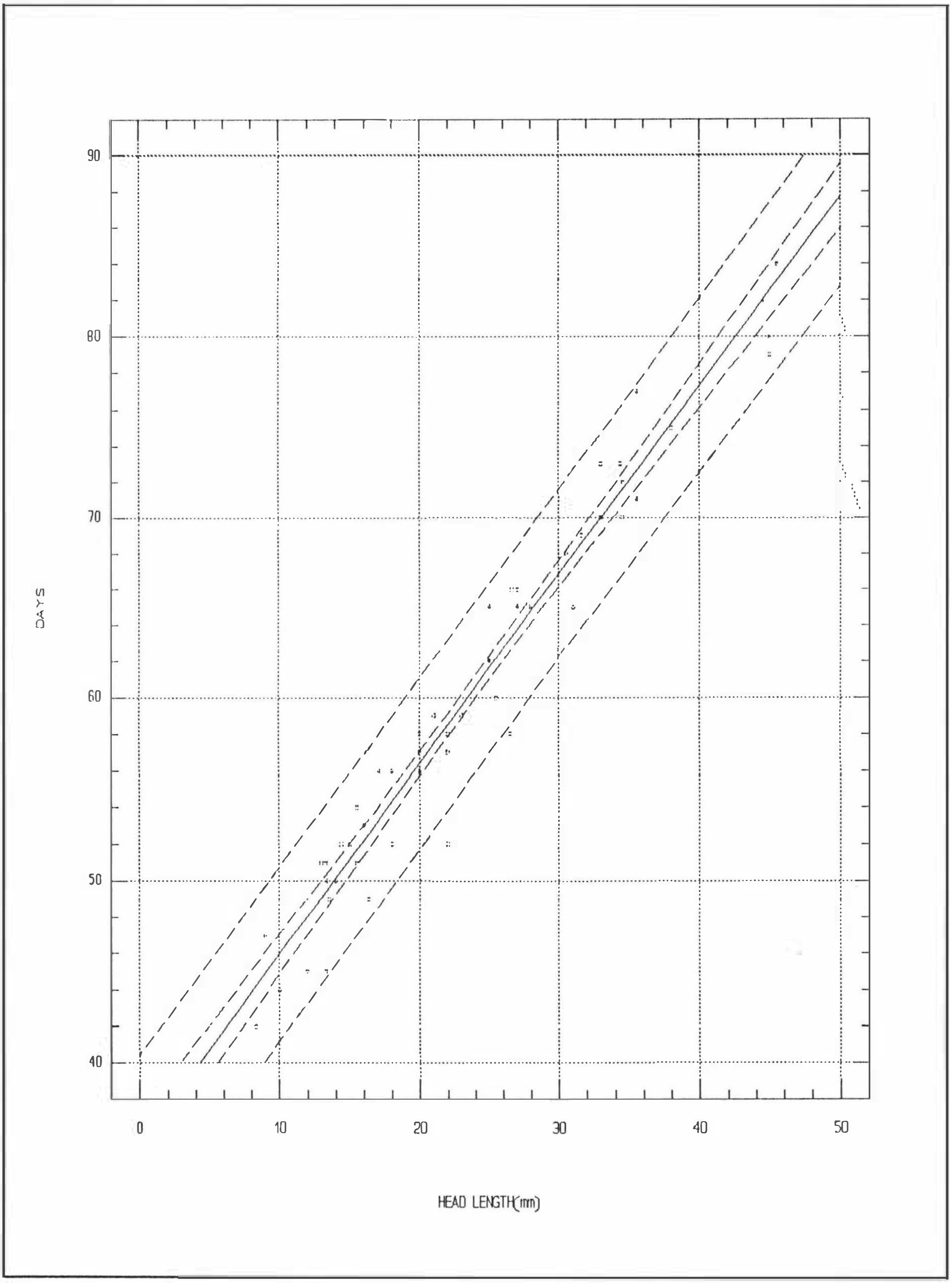

Figure 3.43. Age estimation using head length.
Scattergram of age on head length and the regression used for foetal age estimation (95% confidence interval).

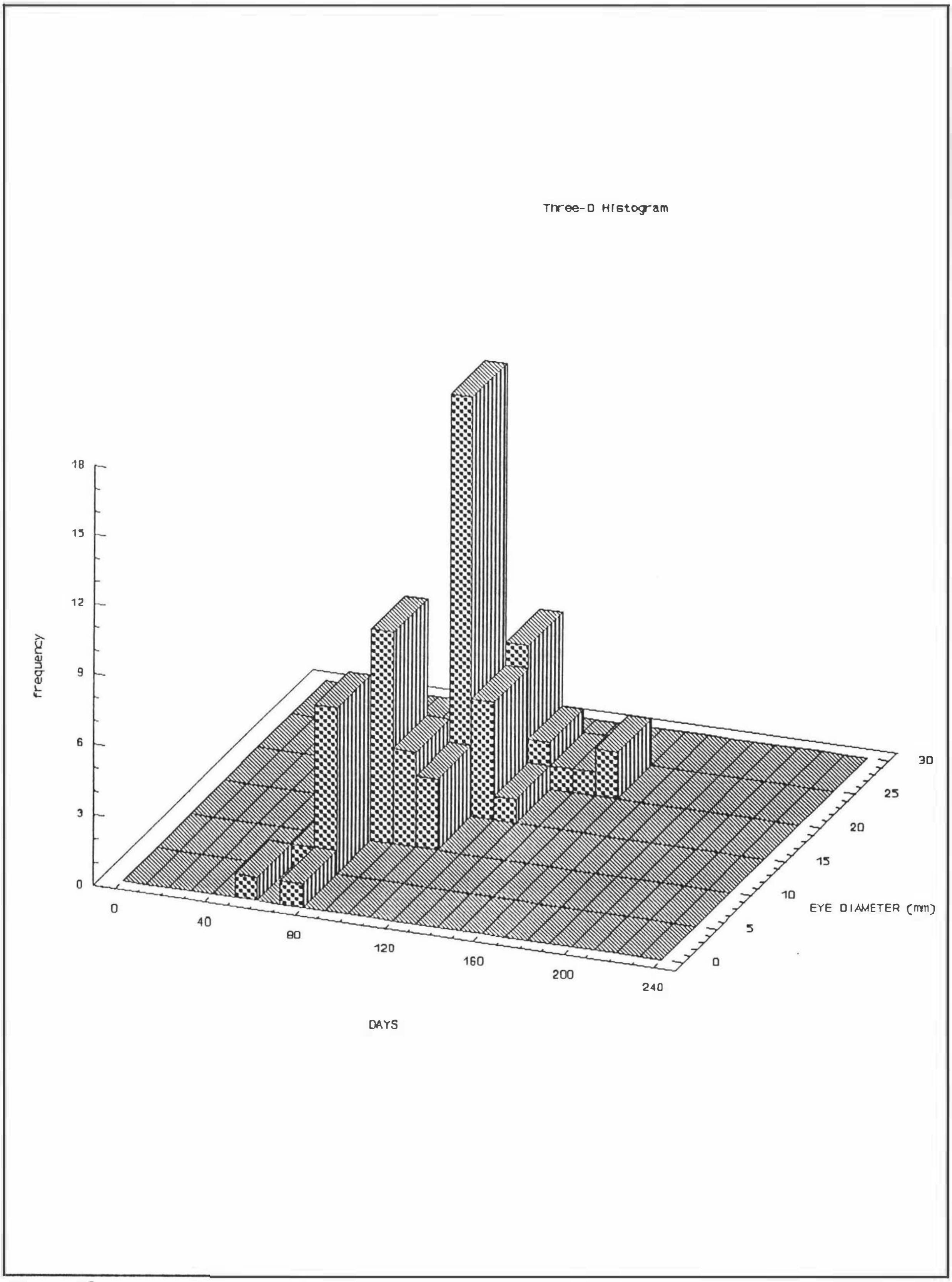

Figure 3.44. Histogram of eye diameter measurements showing the frequency of measurements by foetal age and magnitude of the measurement.

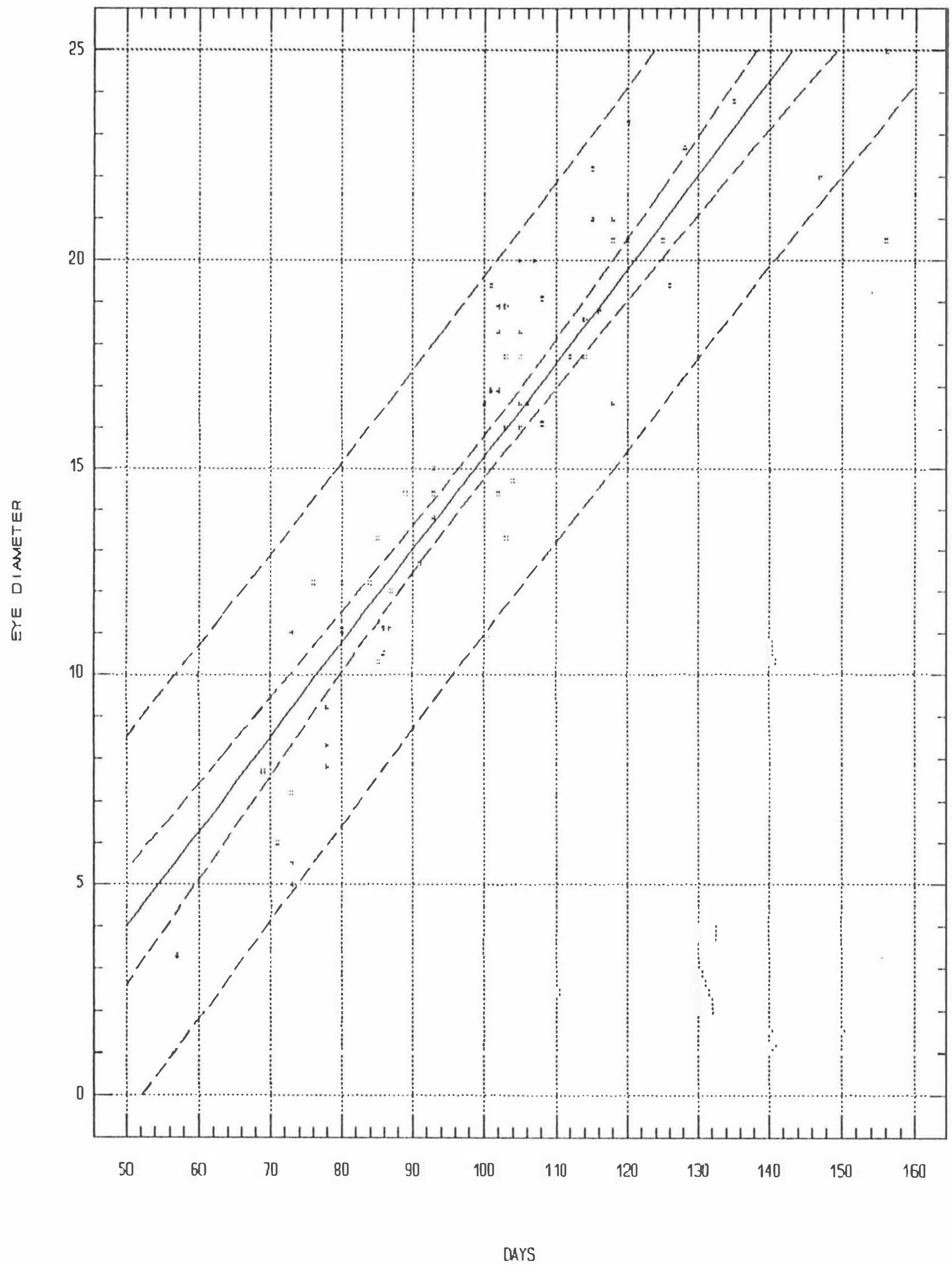

Figure 3.45. Eye diameter growth.
Scattergram of eye diameter length with foetal age and the best fit regression for that data (95% confidence interval).

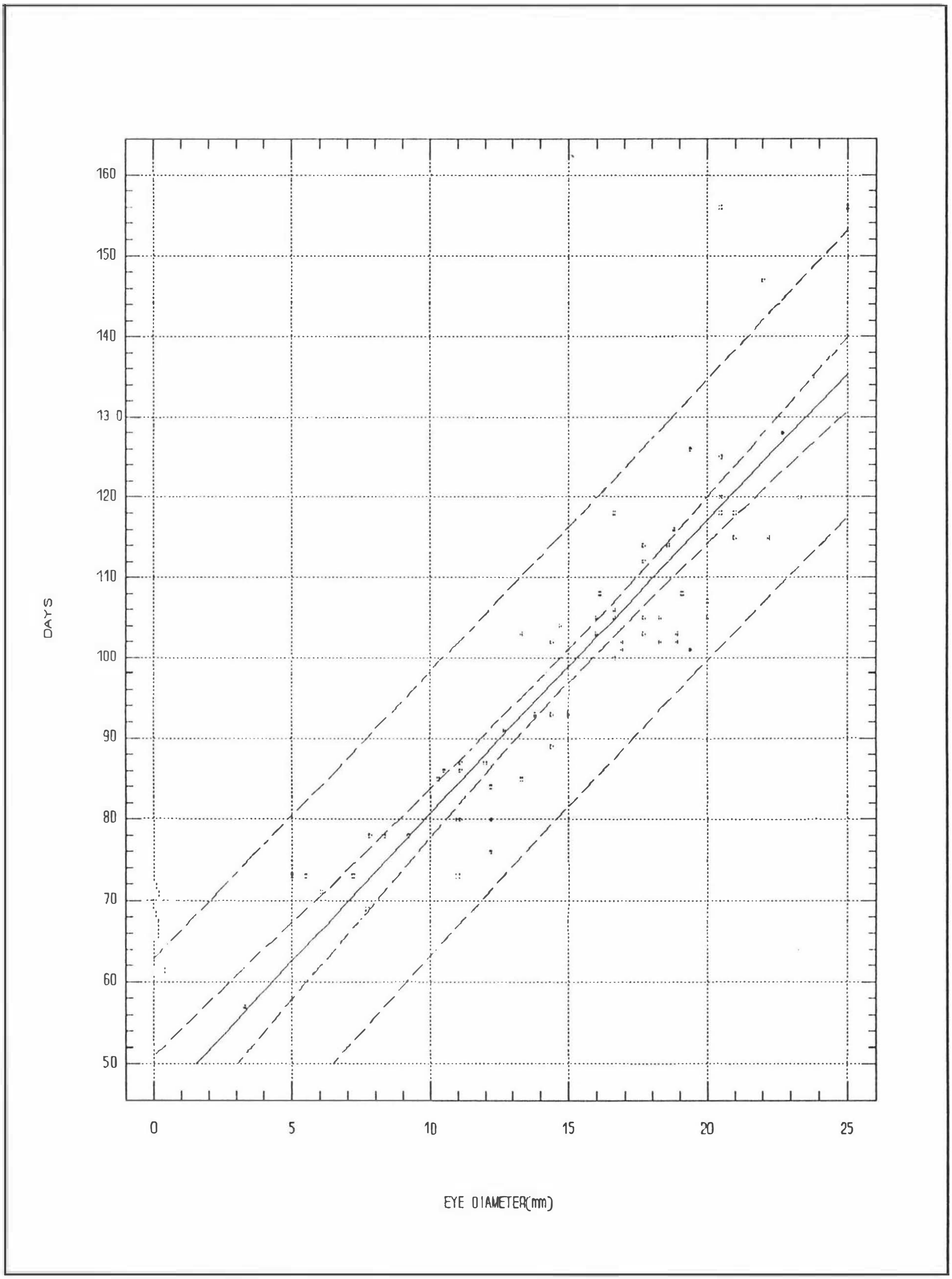

Figure 3.46. Age estimation using eye diameter.
Scattergram of age on eye diameter, and the regression used for foetal age estimation (95% confidence interval).

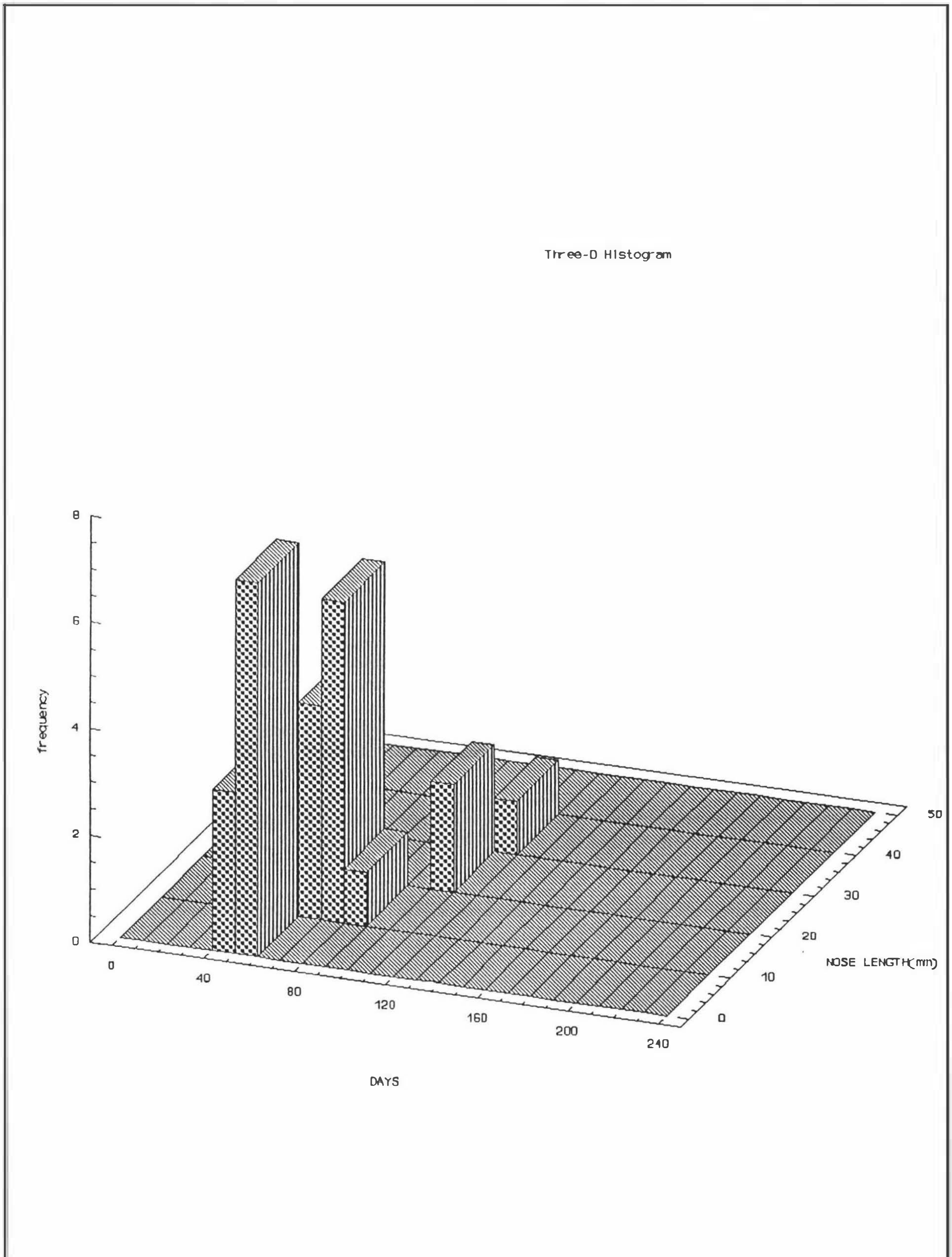

Figure 3.47. Histogram of nose length measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

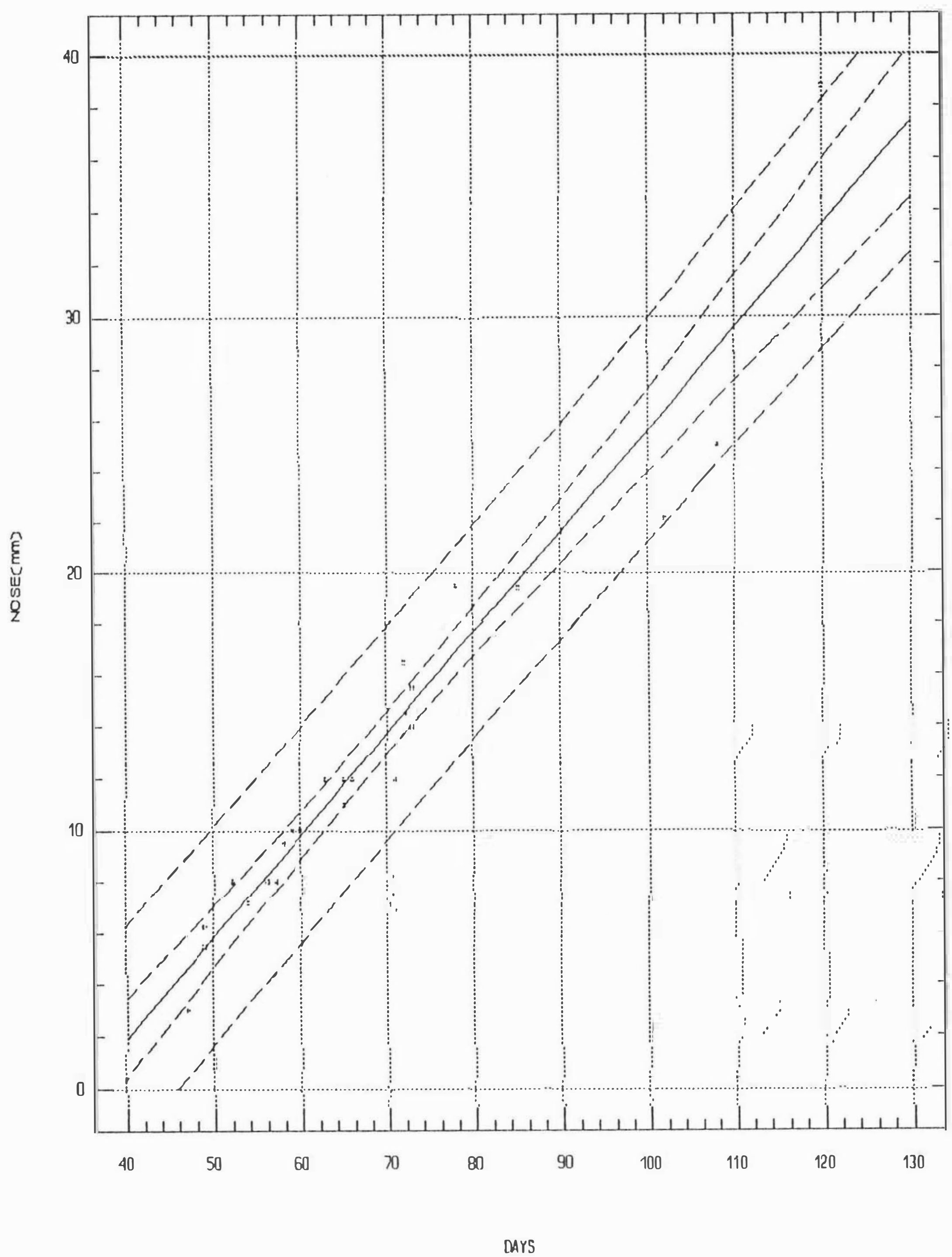

Figure 3.48. Nose length growth.
Scattergram of nose length with foetal age, and the best fit regression for that data (95% confidence interval).

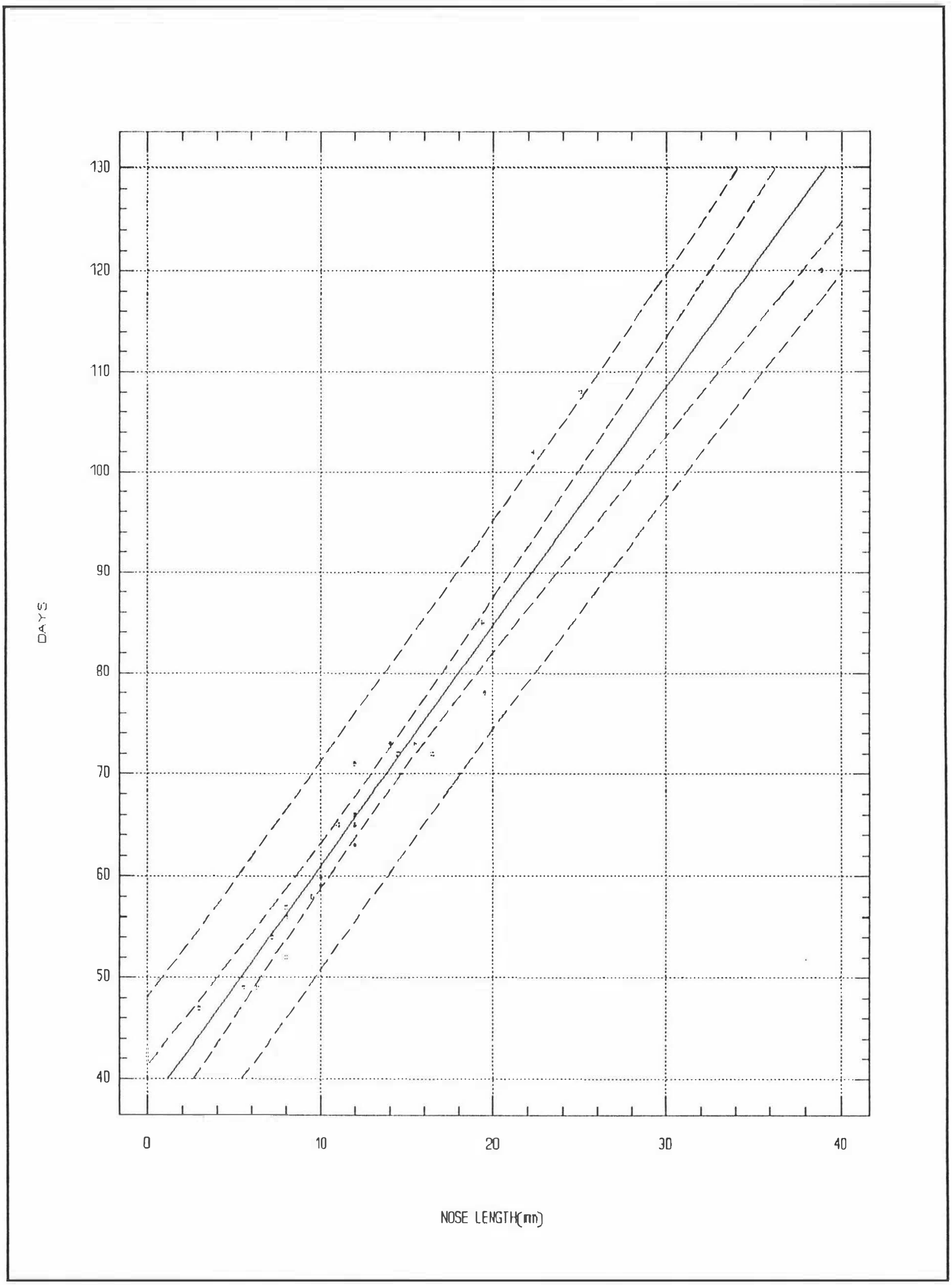

Figure 3.49. Age estimation using nose length.
Scattergram of age on nose length, and the regression used for foetal age estimation (95% confidence interval).

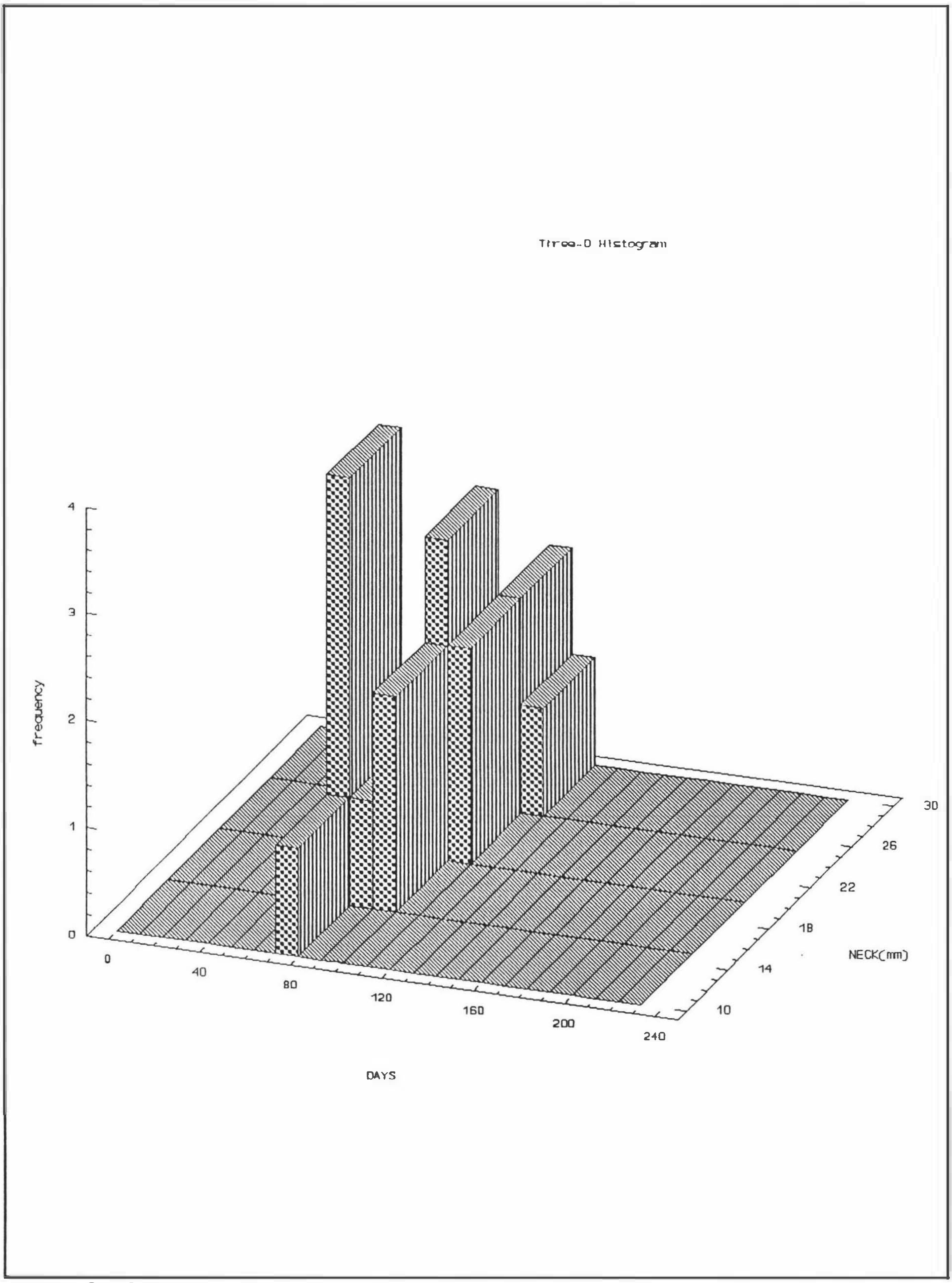

Figure 3.50. Histogram of neck diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

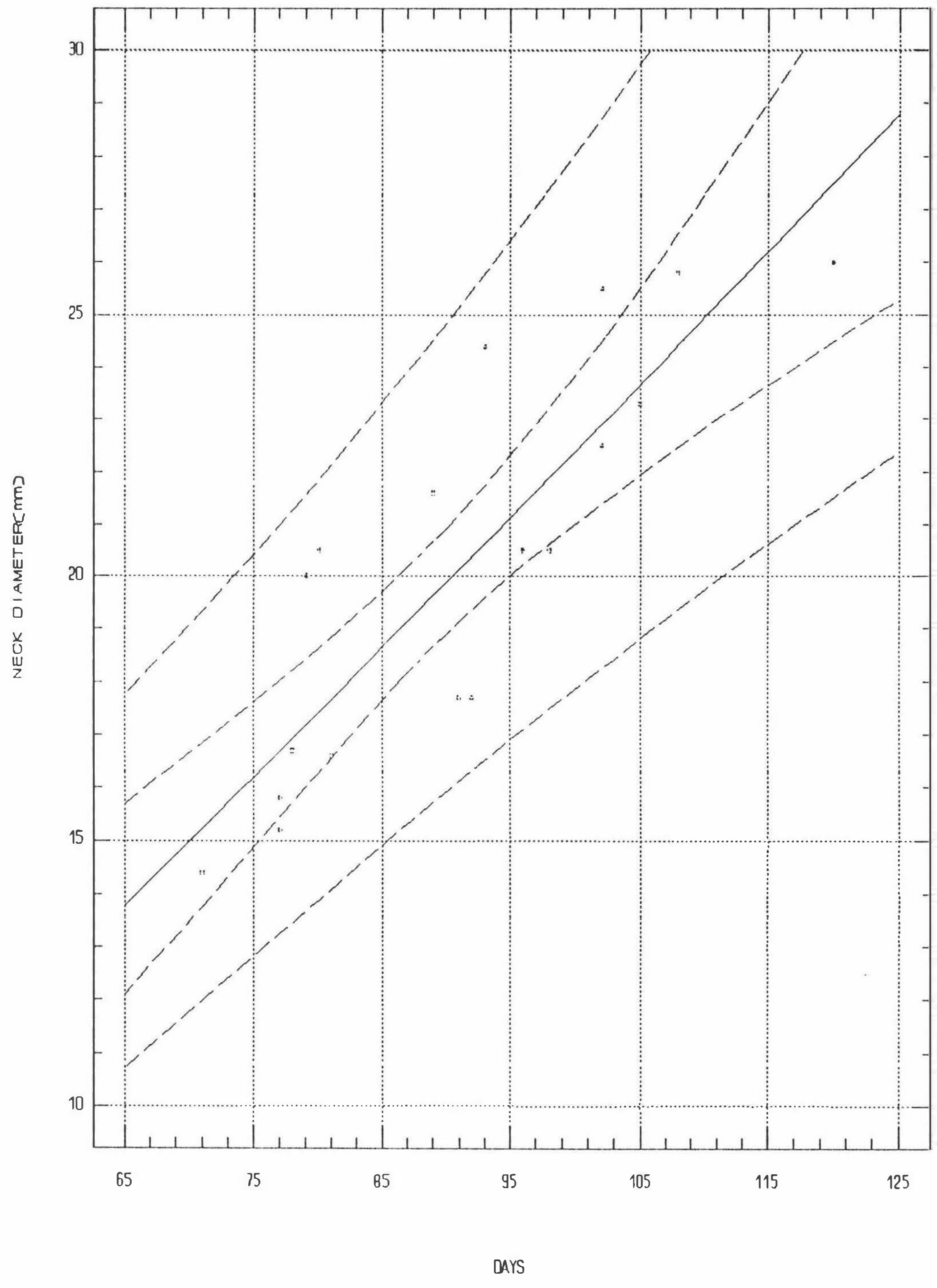

Figure 3.51. Neck diameter growth.
Scattergram of neck diameter with foetal age, and the best fit regression for that data (95% confidence interval).

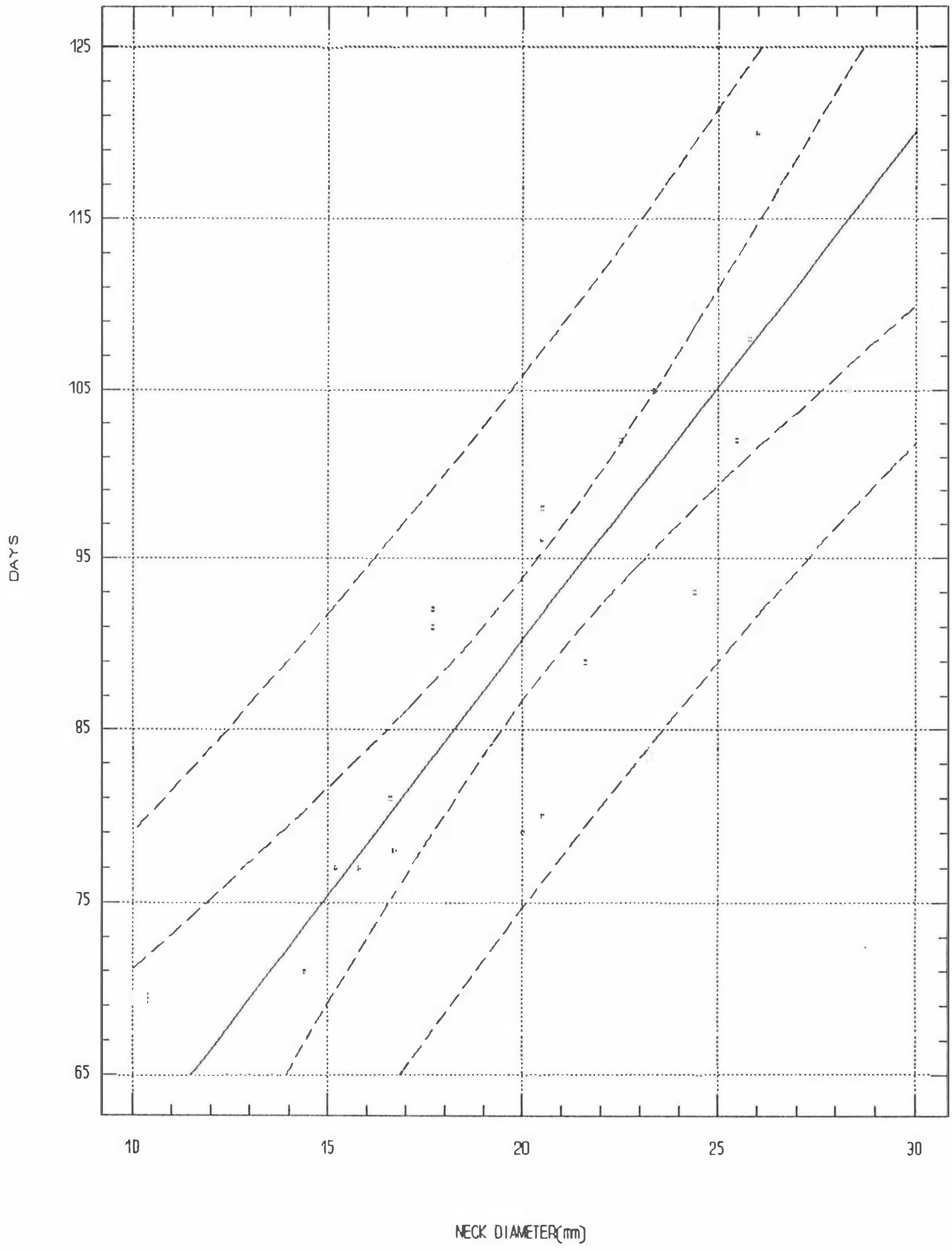

Figure 3.52. Age estimation using neck diameter.
Scattergram of age on neck diameter, and the regression used for foetal age estimation (95% confidence interval).

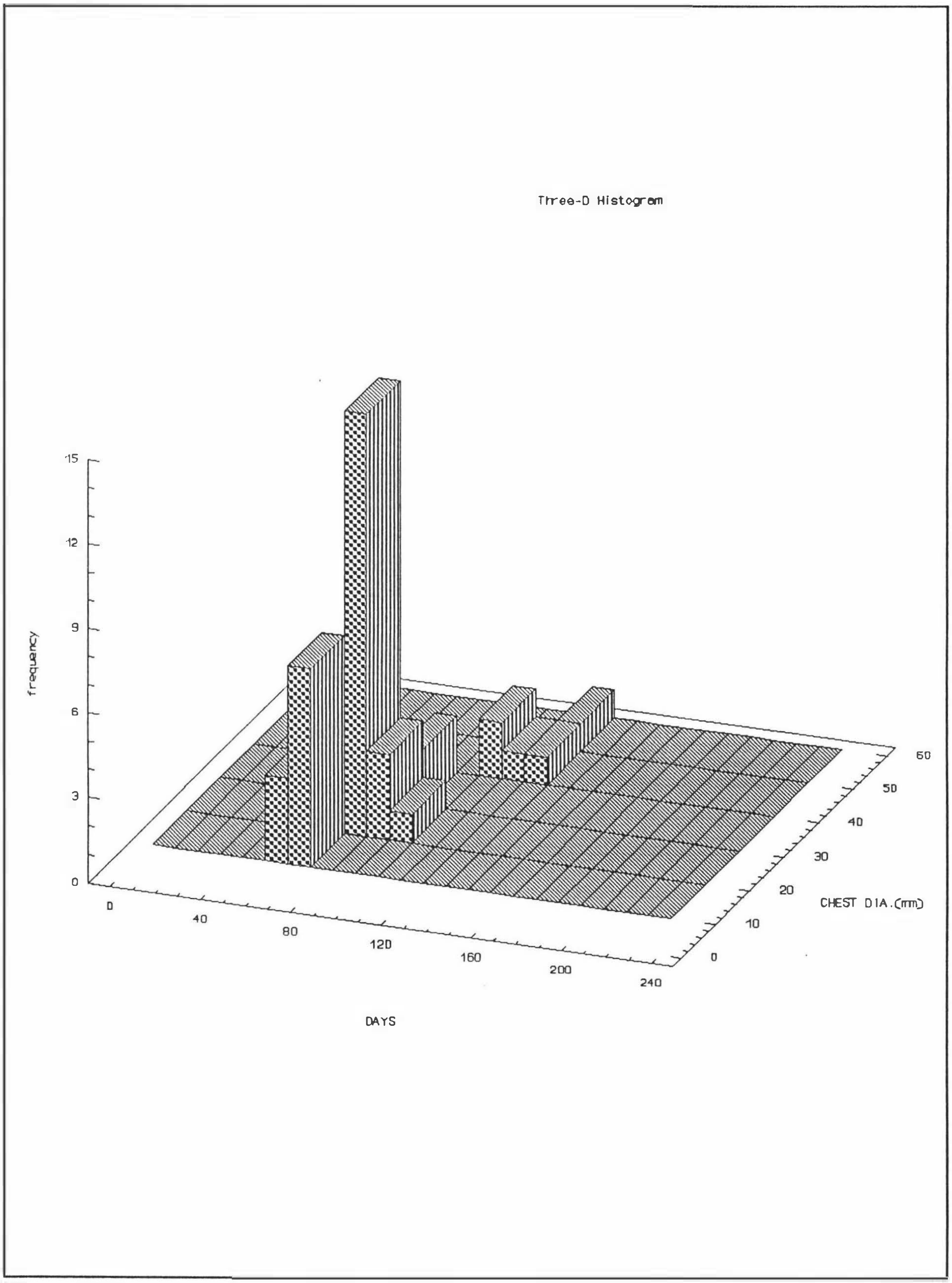

Figure 3.53. Histogram of chest diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

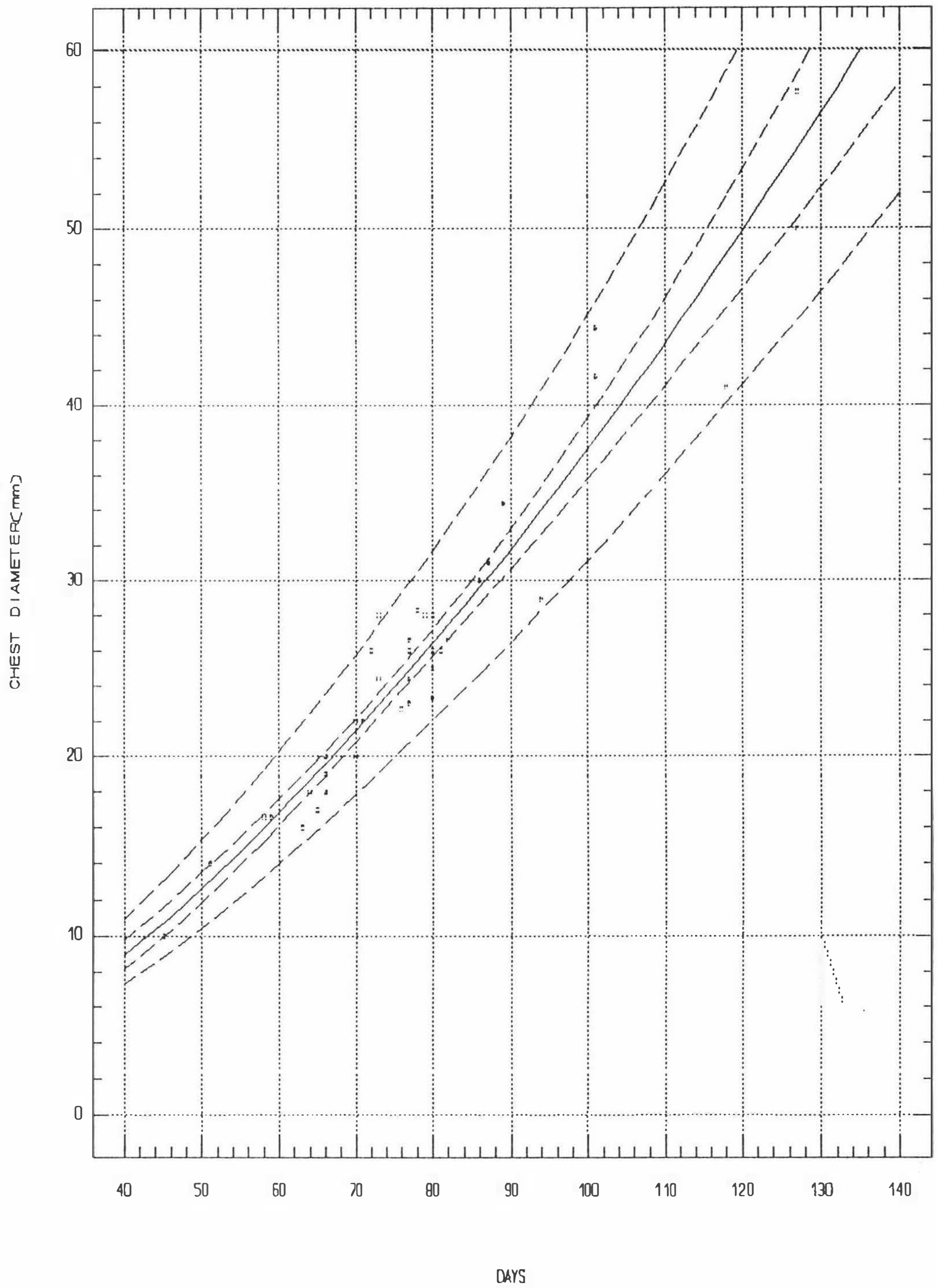

Figure 3.54. Chest diameter growth.
Scattergram of chest diameter with foetal age and the best fit regression for that data (95% confidence interval).

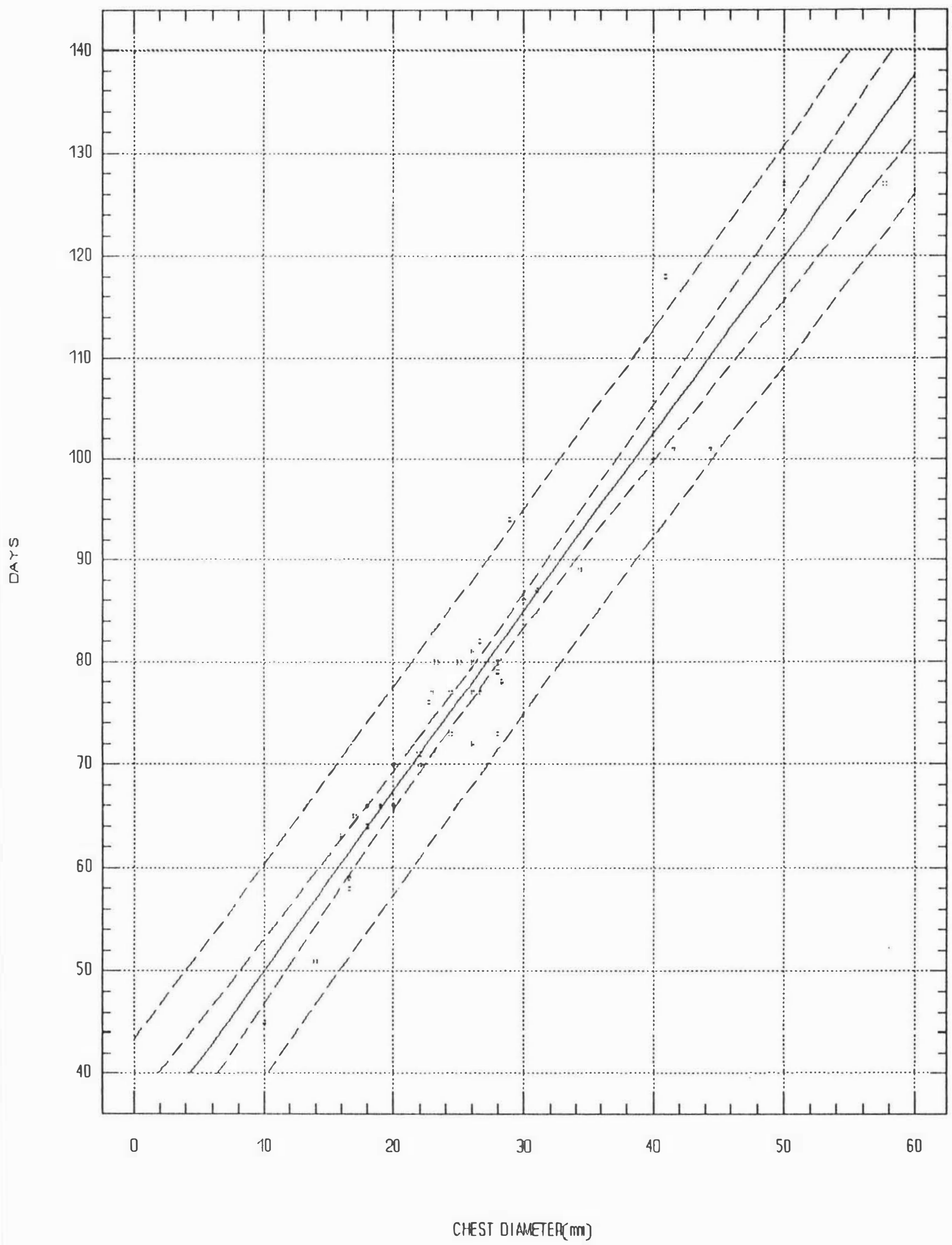

Figure 3.55. Age estimation using chest diameter.
Scattergram of age on chest diameter, and the regression used for foetal age estimation (95% confidence interval).

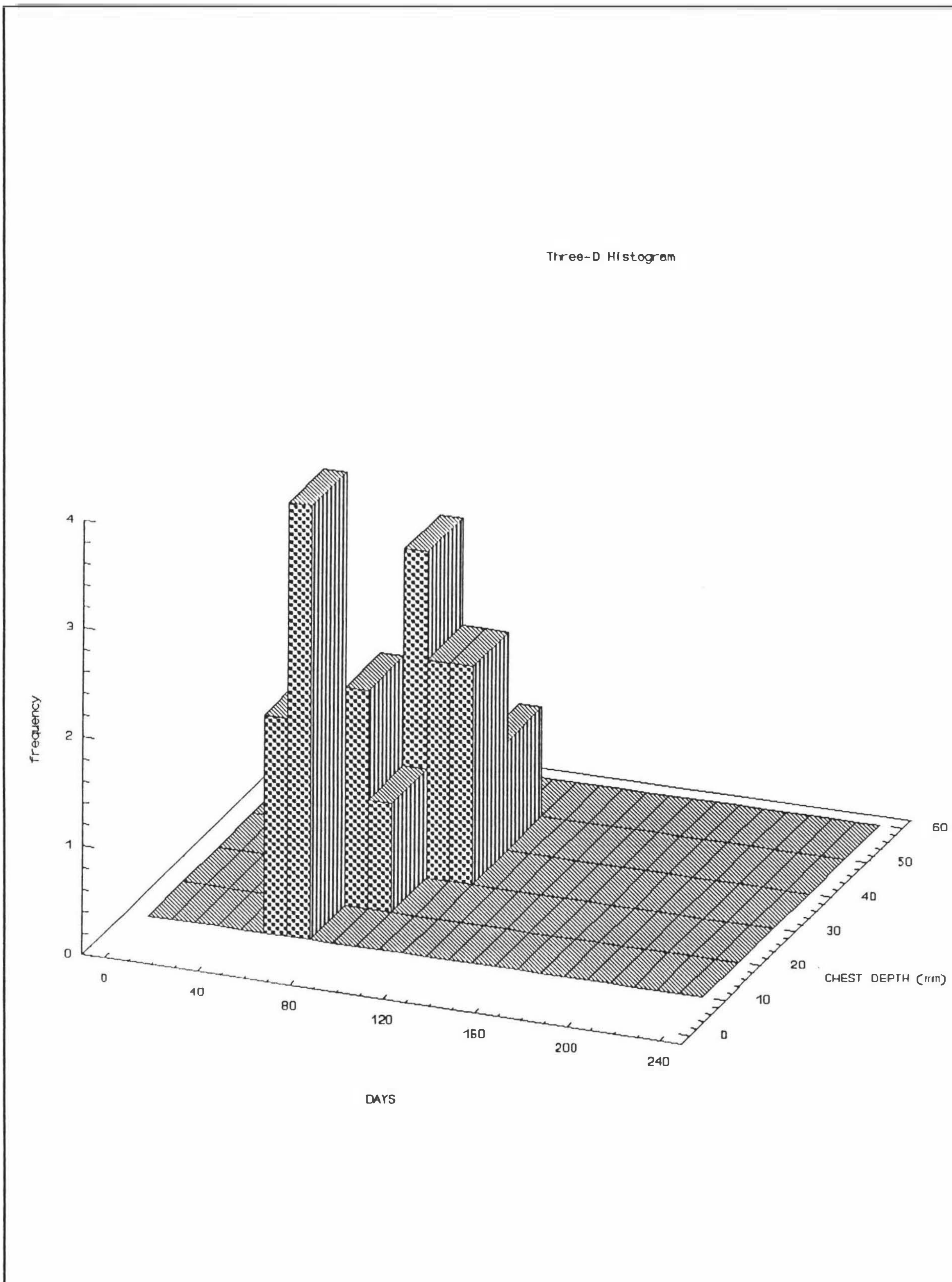

Figure 3.56. Histogram of chest depth measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

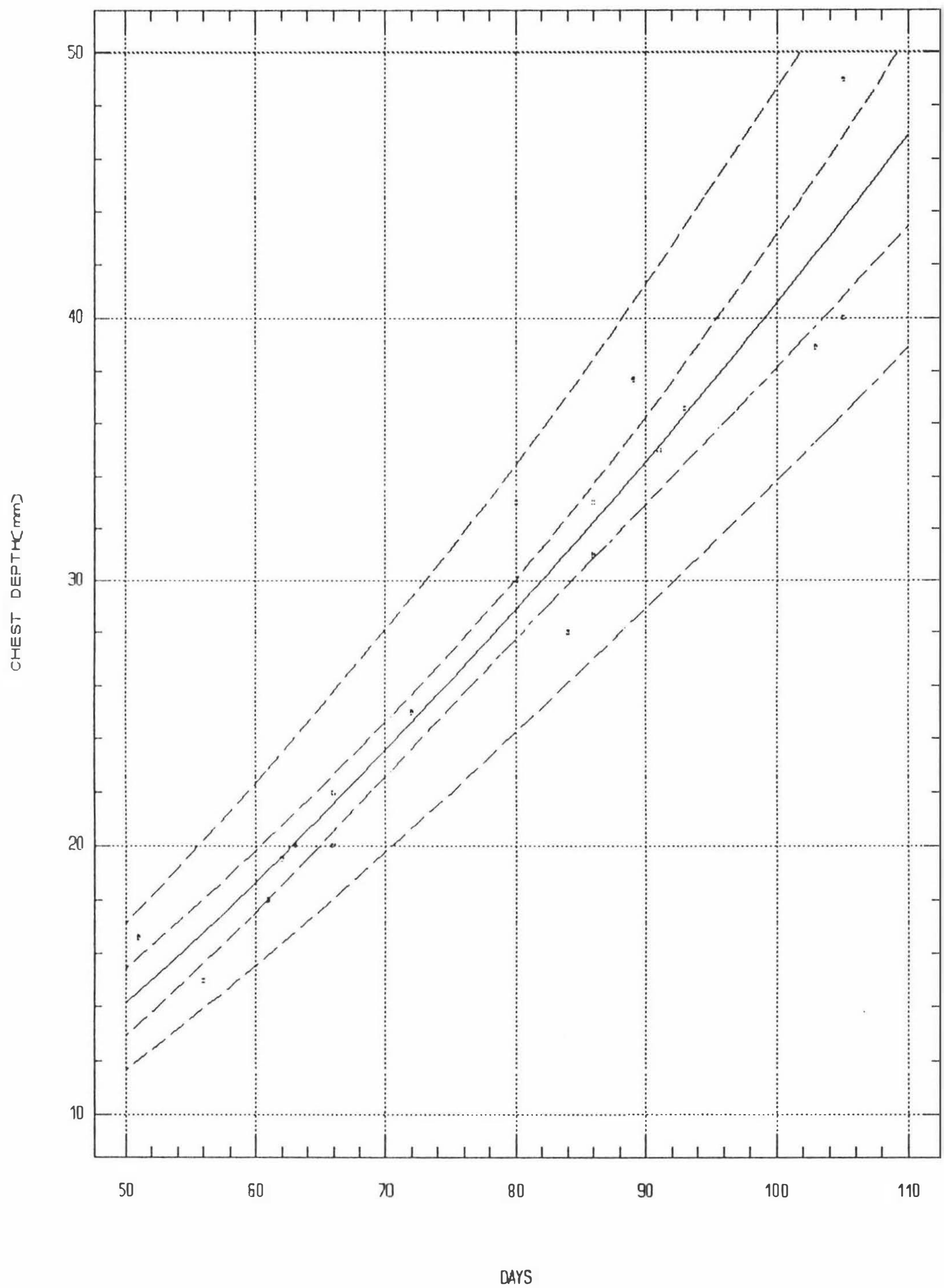

Figure 3.57. Chest depth growth.
Scattergram of chest depth with foetal age, and the best fit regression for that data (95% confidence interval).

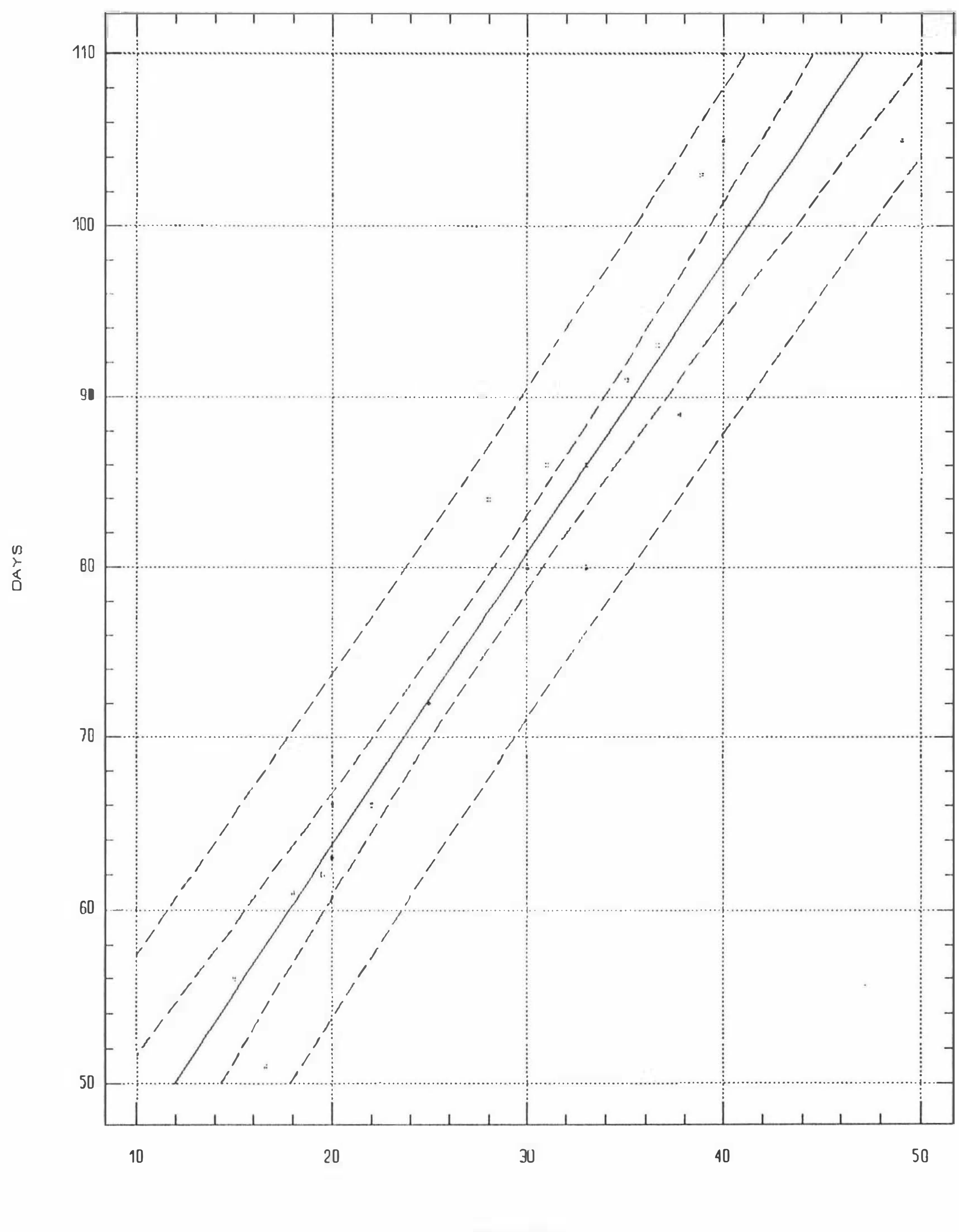

Figure 3.58. Age estimation using chest depth.
Scattergram of age on chest depth, and the regression used for foetal age estimation (95% confidence interval).

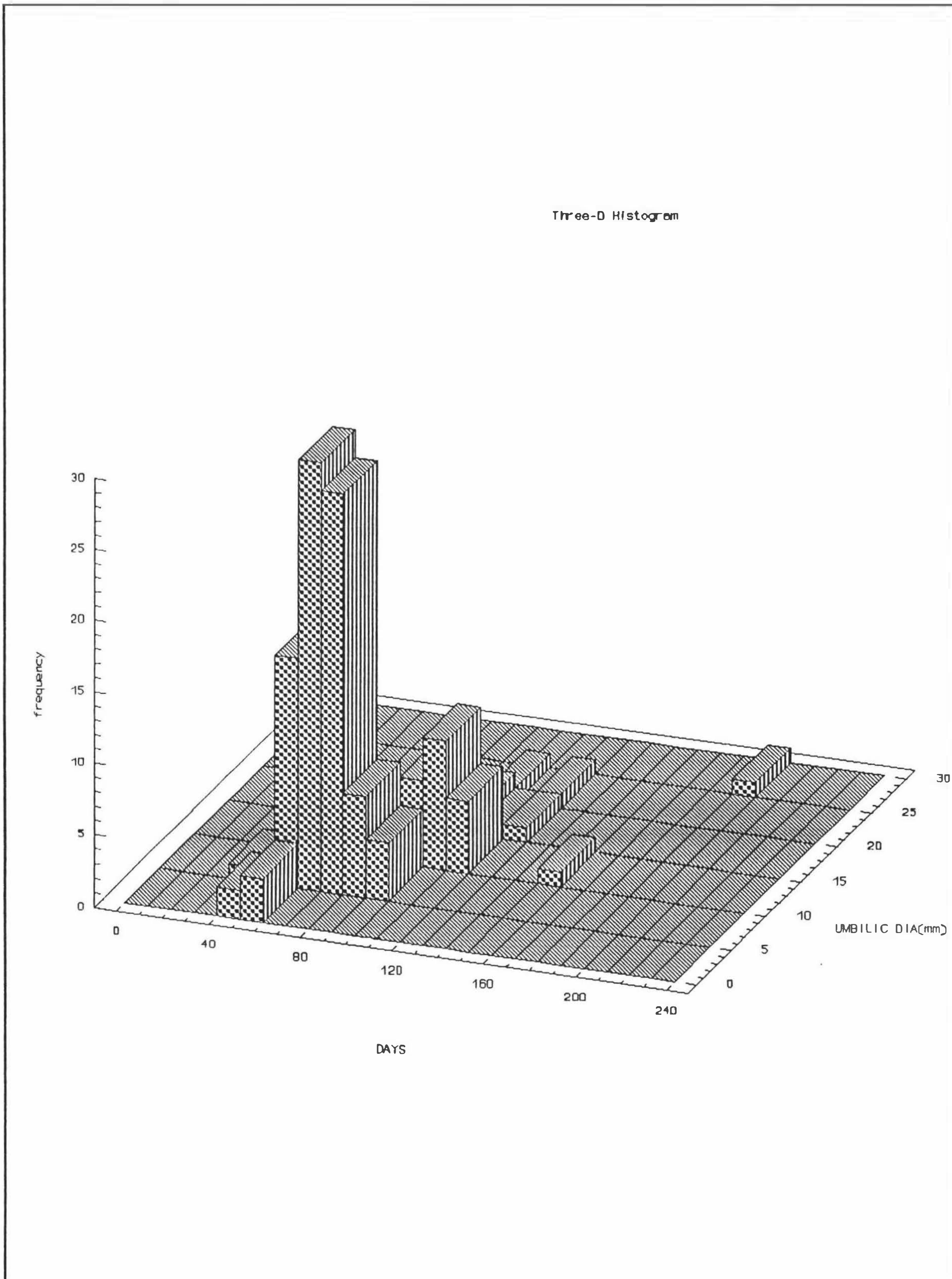

Figure 3.59. Histogram of umbilical cord diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

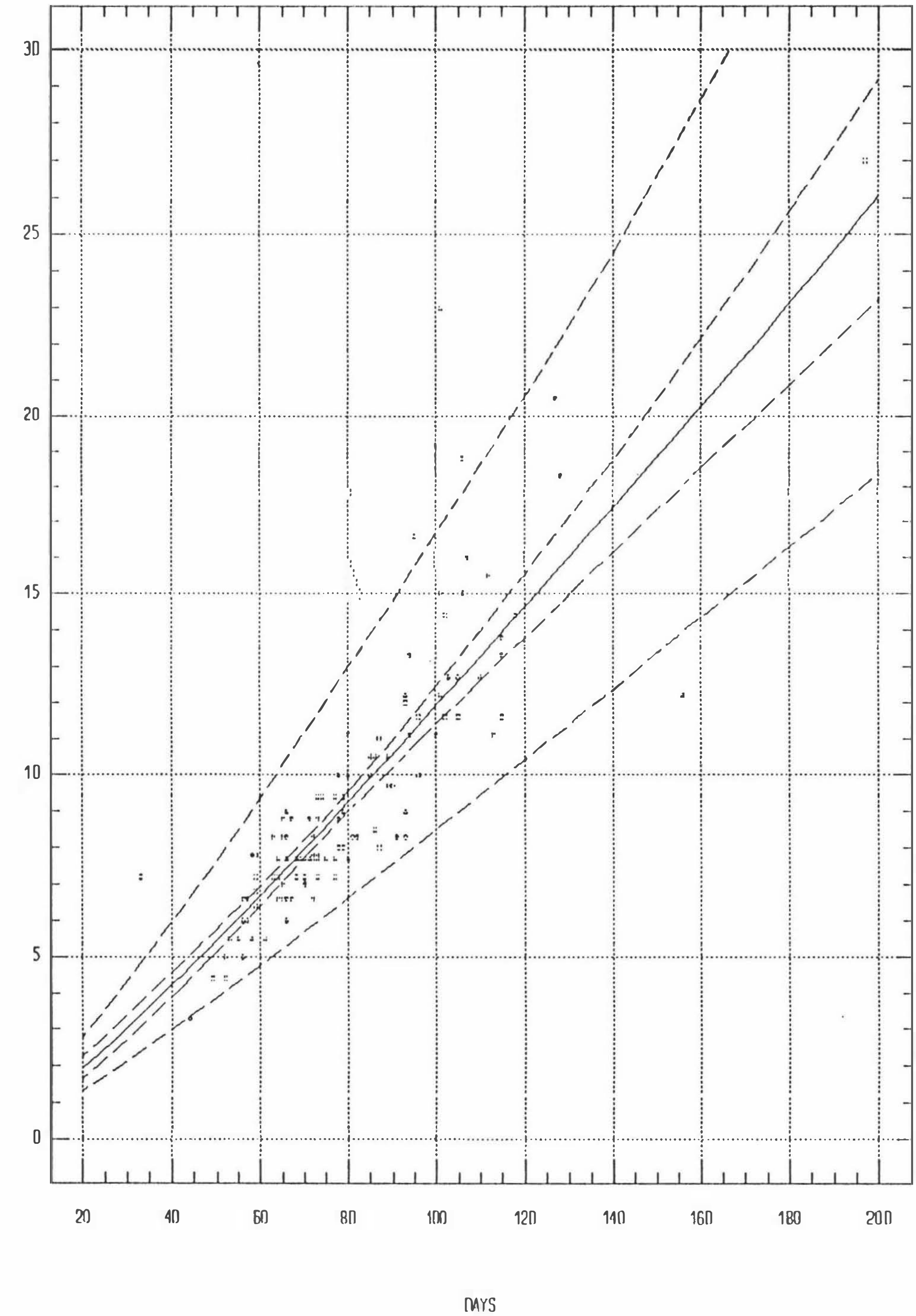

Figure 3.60. Umbilical diameter growth.
Scattergram of umbilical diameter with foetal age, and the best fit regression for that data (95% confidence interval).

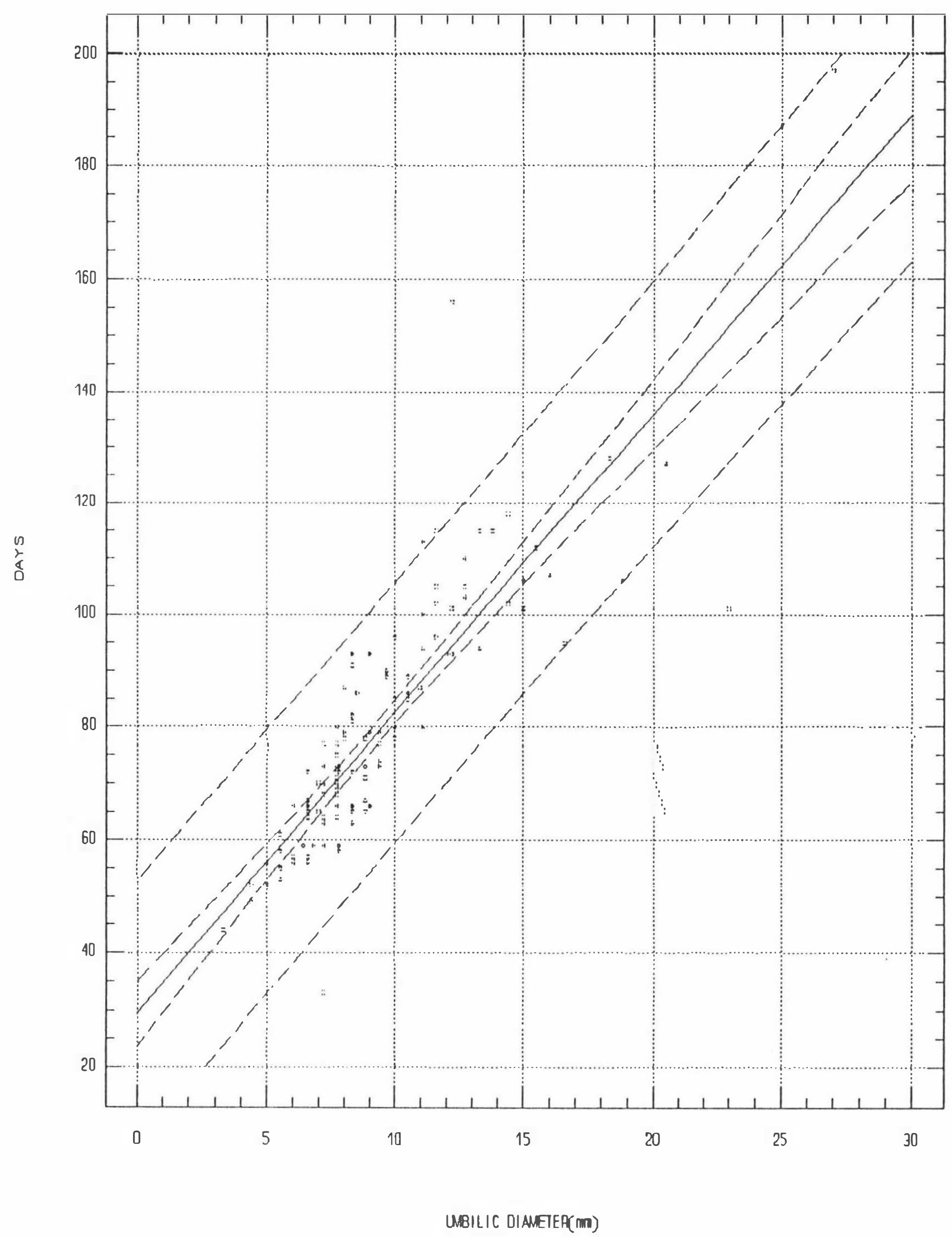

Figure 3.61. Age estimation using umbilical cord.
Scattergram of age on umbilical cord, and the regression used for foetal age estimation (95% confidence interval).

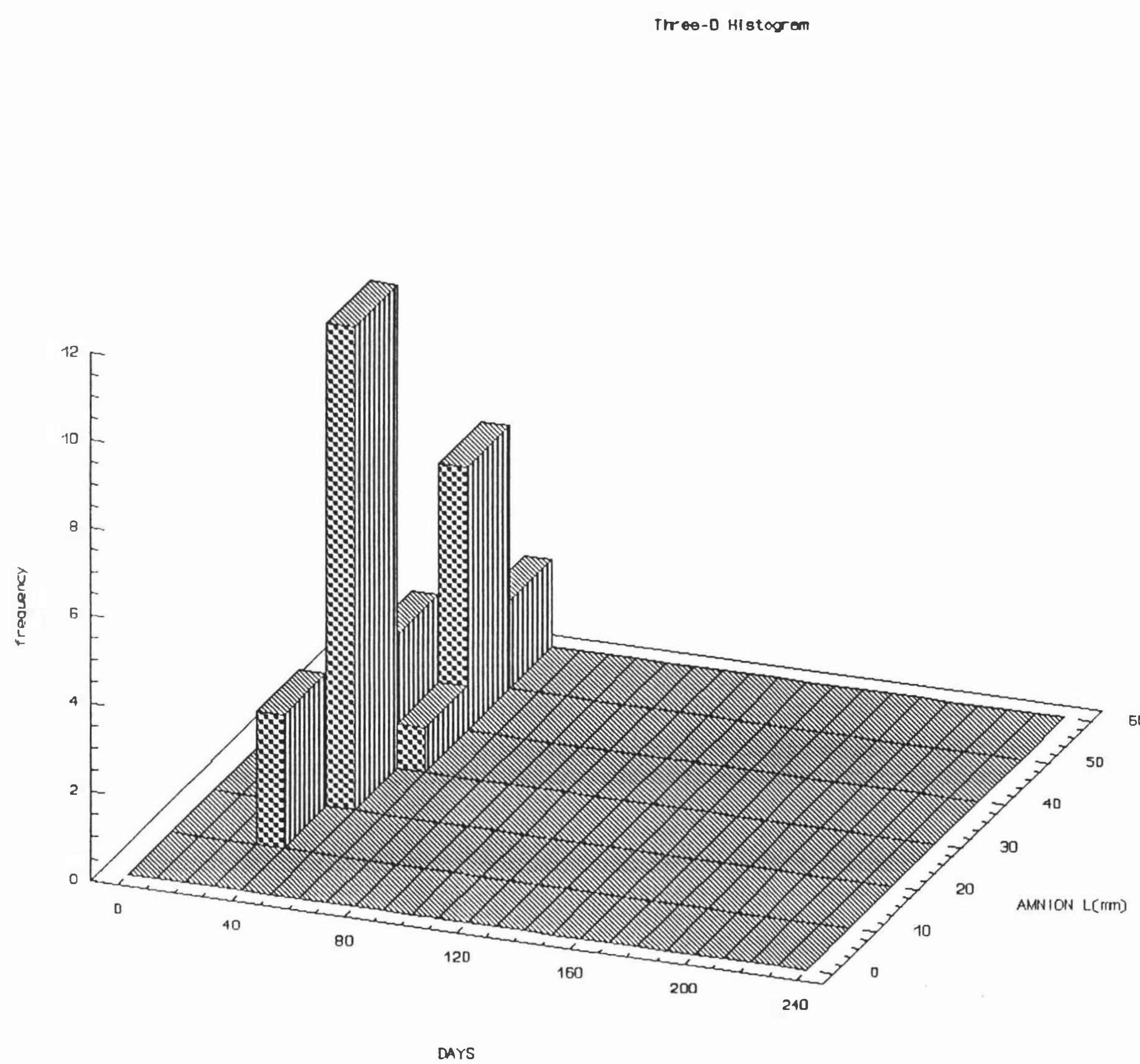

Figure 3.62. Histogram of amnion length measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

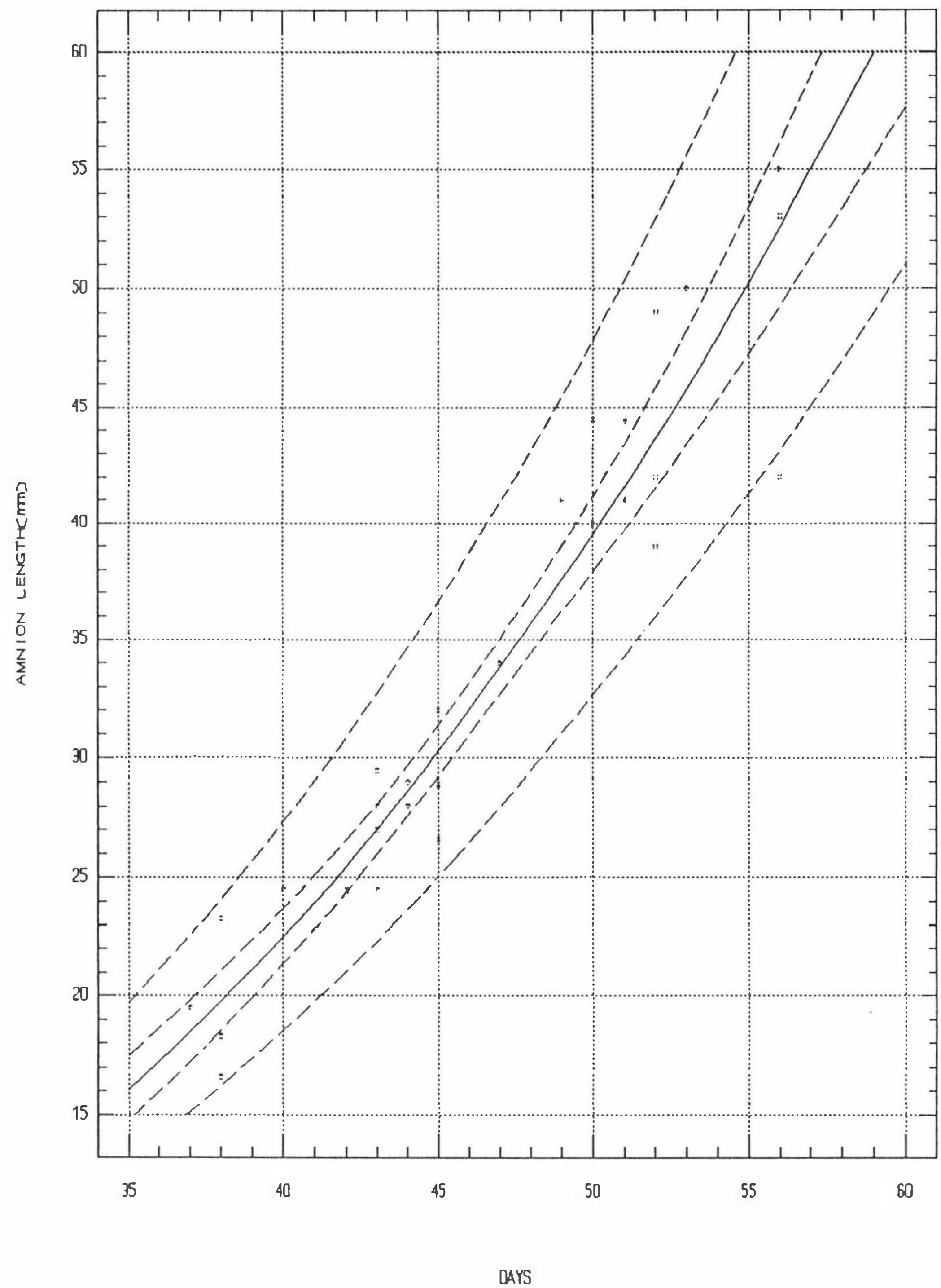

Figure 3.63. Amnion length growth.
Scattergram of amnion length with foetal age, and the best fit regression for that data (95% confidence interval).

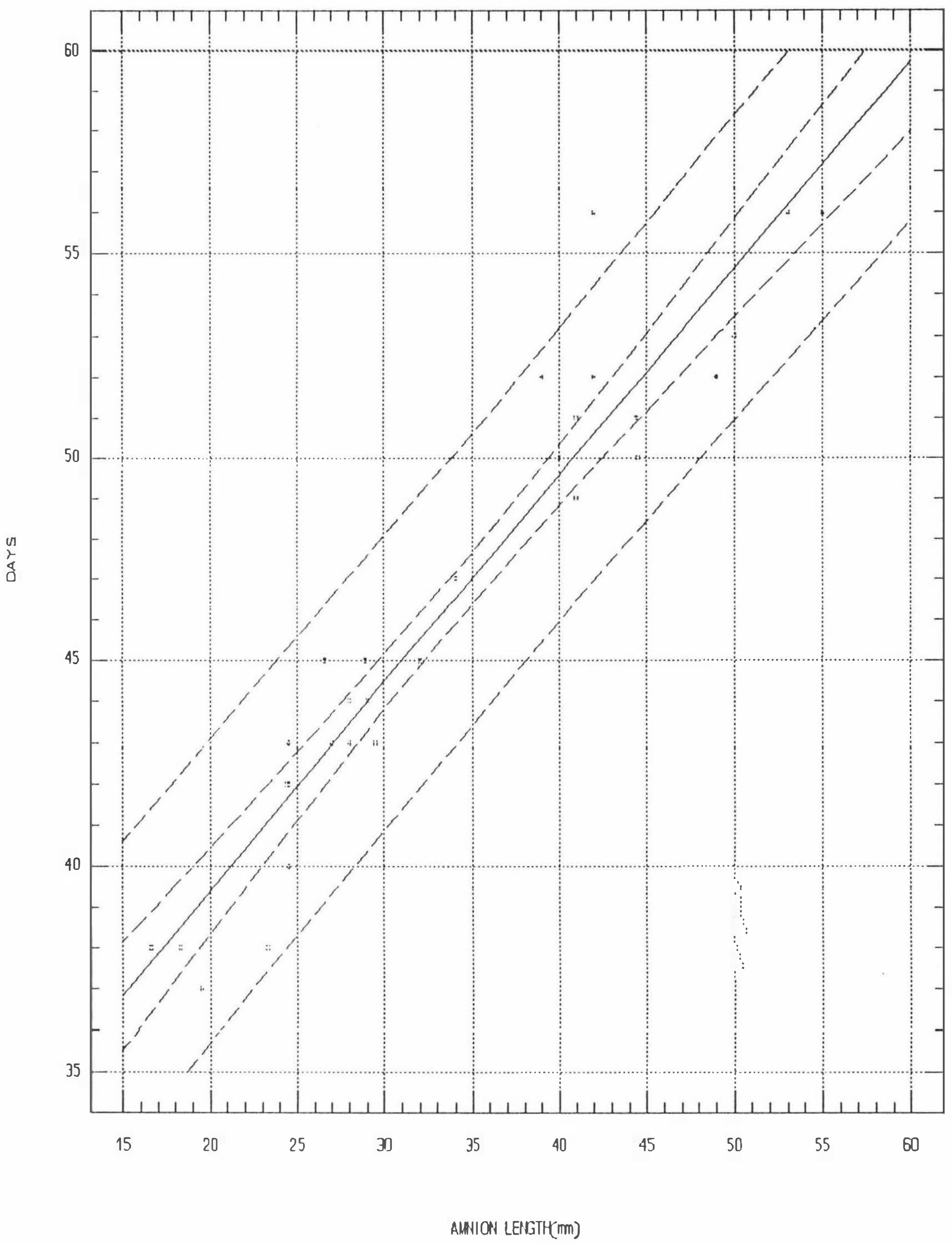

Figure 3.64. Age estimation using amnion length.
Scattergram of age on amnion length, and the regression used for foetal age estimation (95% confidence interval).

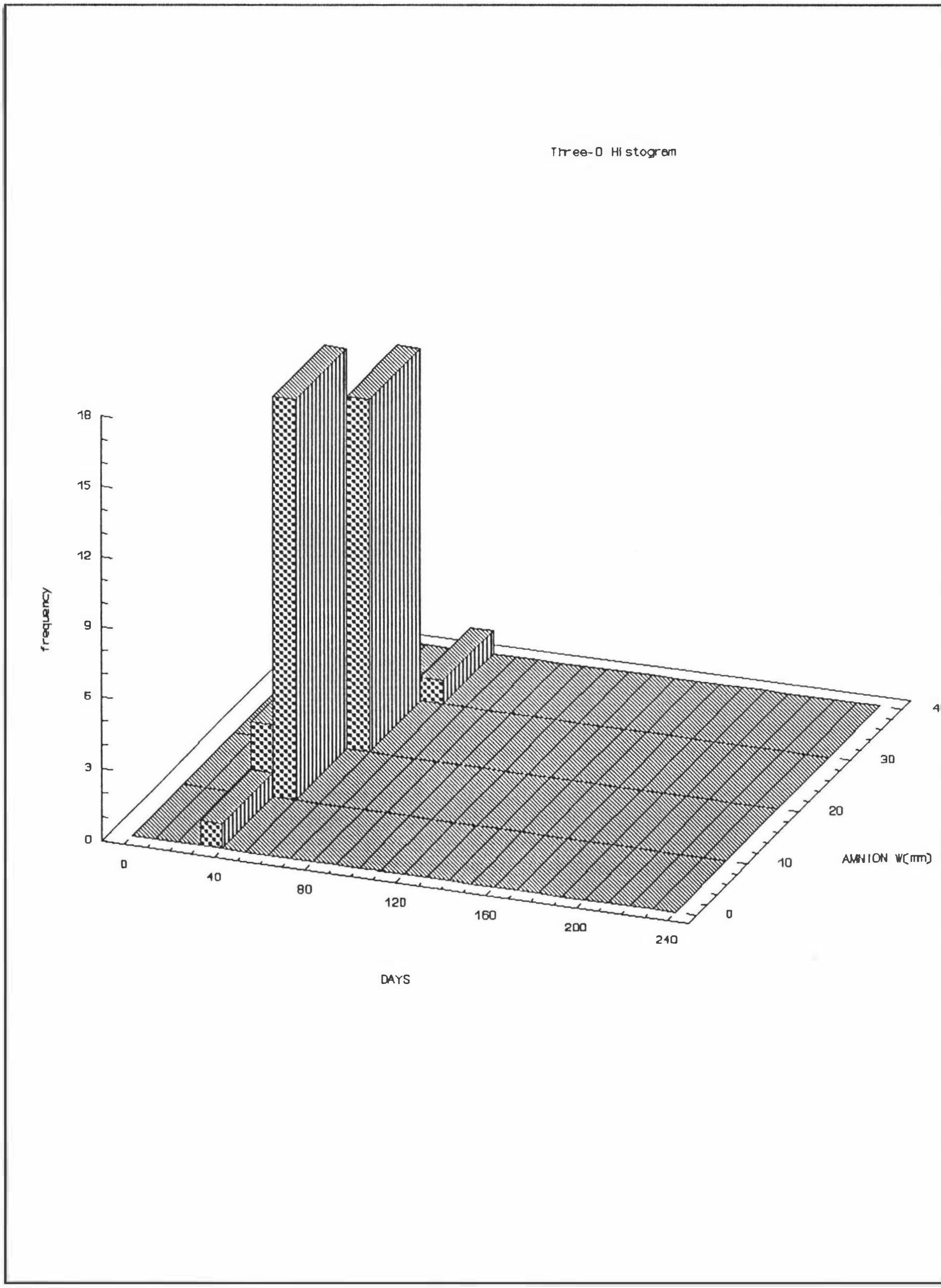

Figure 3.65. Histogram of amnion width measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

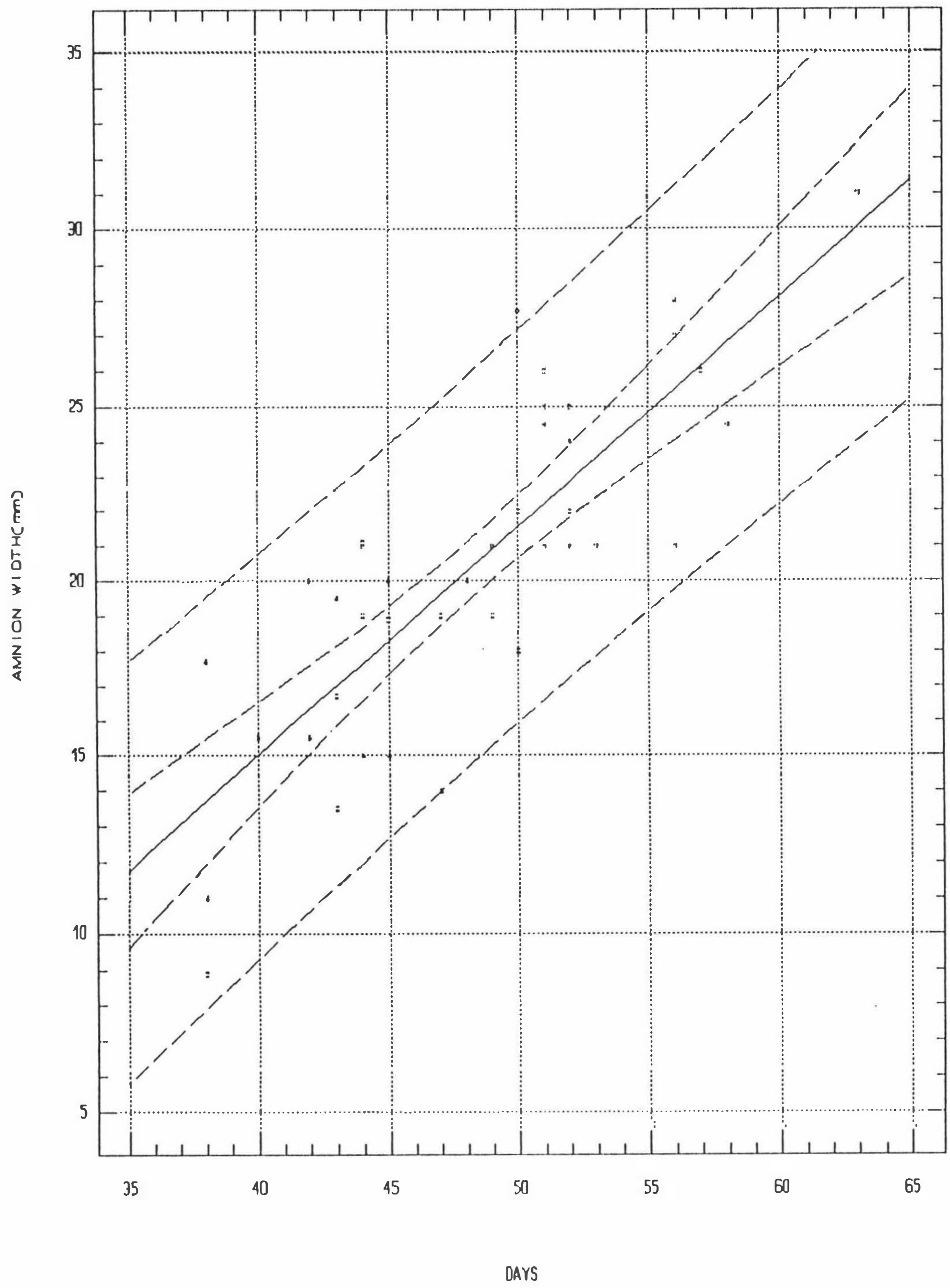

Figure 3.66. Amnion width growth.
Scattergram of amnion width with foetal age, and the best fit regression for that data (95% confidence interval).

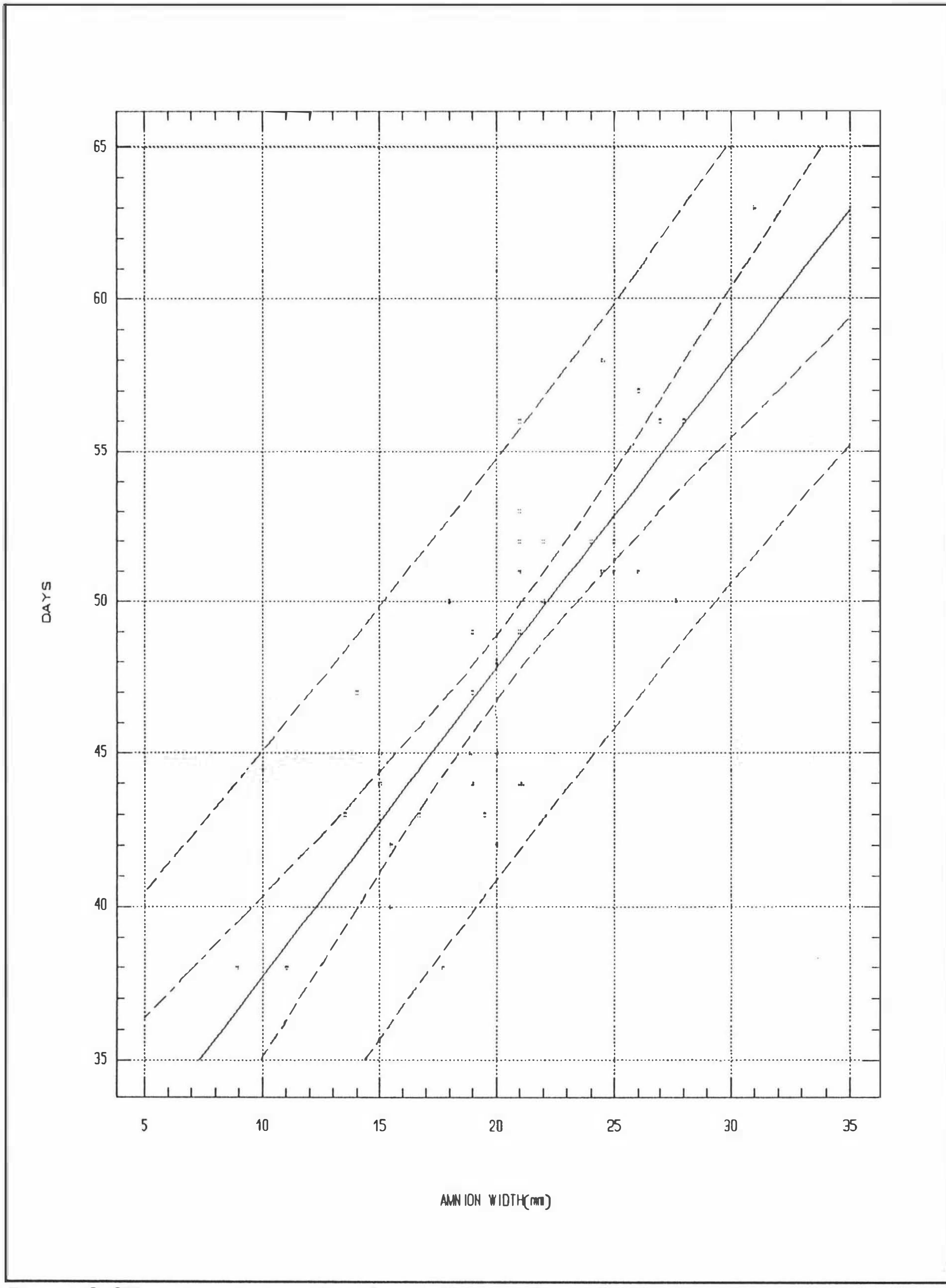

Figure 3.67. Age estimation using amnion width.
Scattergram of age on amnion width, and the regression used for foetal age estimation (95% confidence interval).

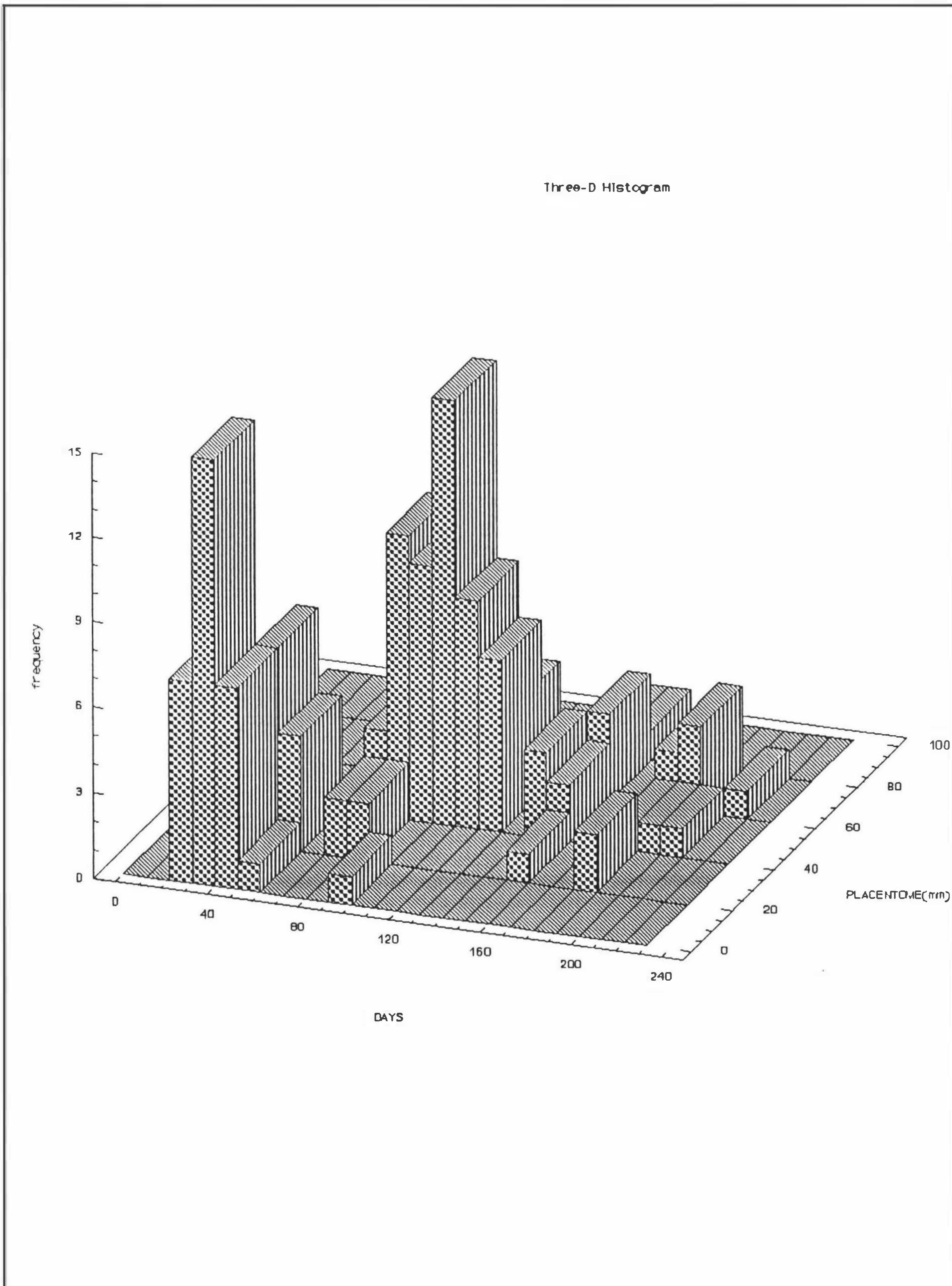

Figure 3.68. Histogram of placentome diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

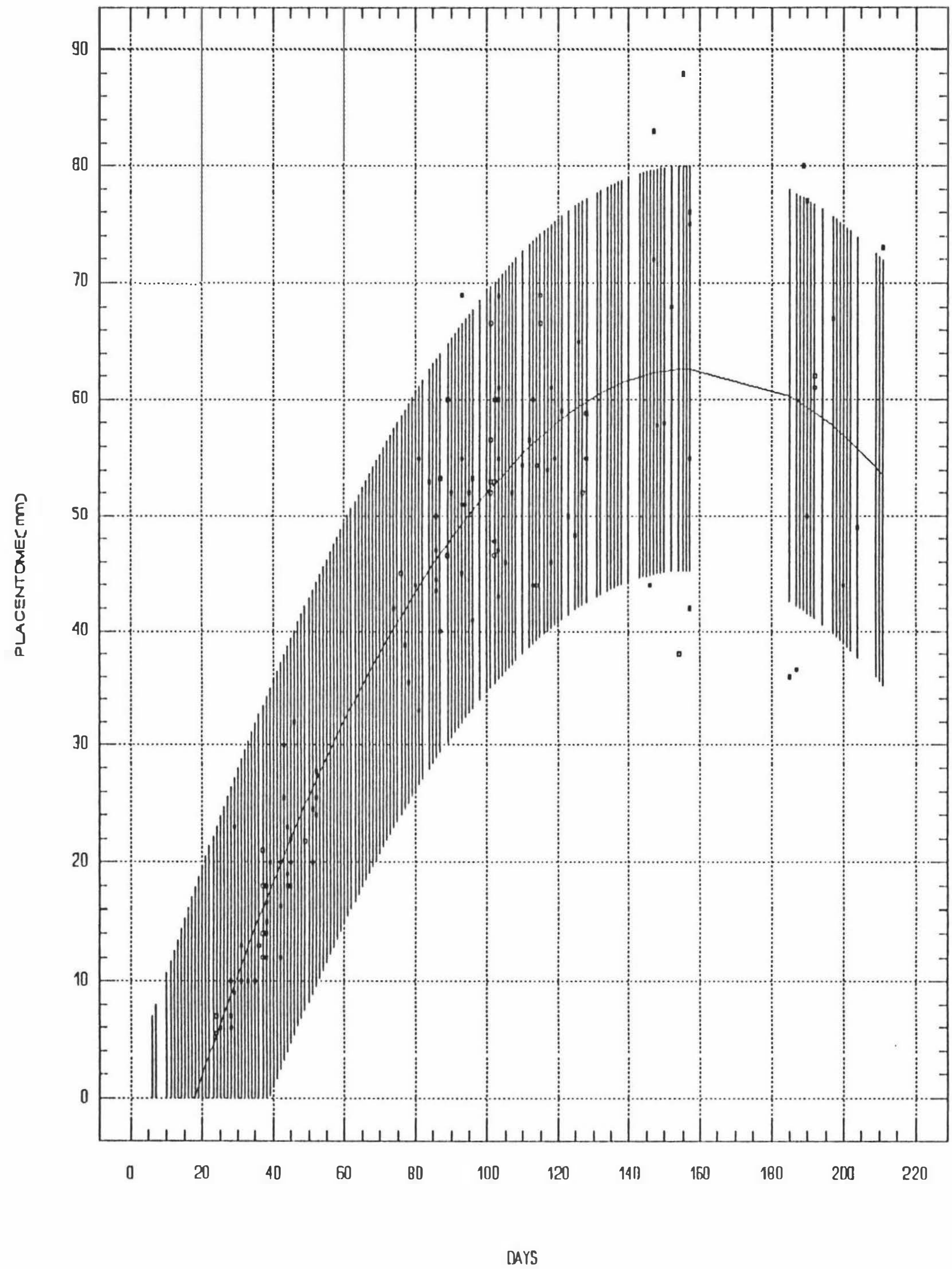

Figure 3.69. Placentome diameter growth.
Scattergram of placentome diameter with foetal age, and the best fit regression for that data (95% confidence interval).

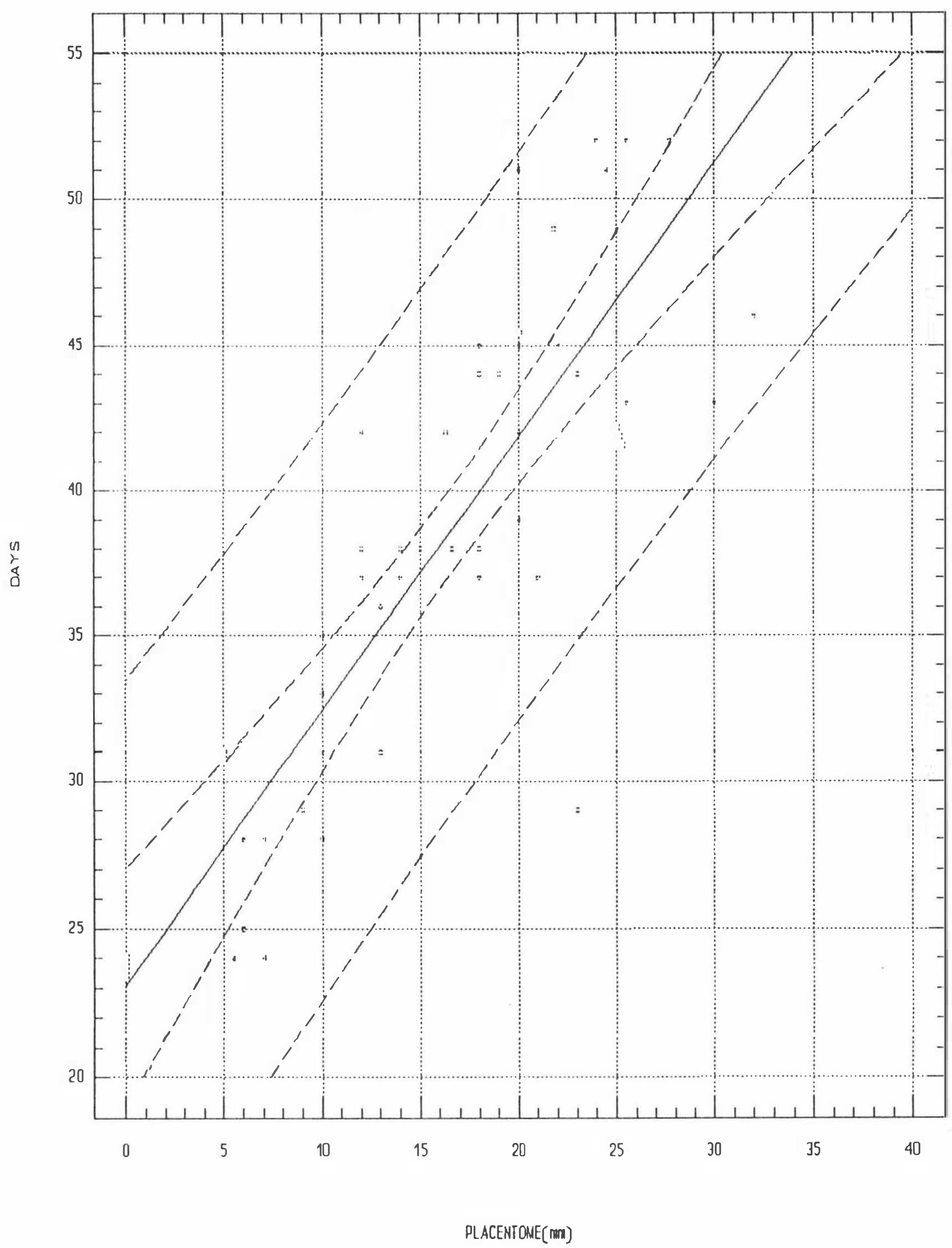

Figure 3.70. Age estimation using placentome diameter. Scattergram of age on placentome diameter, and the regression used for foetal estimation (95% confidence interval).

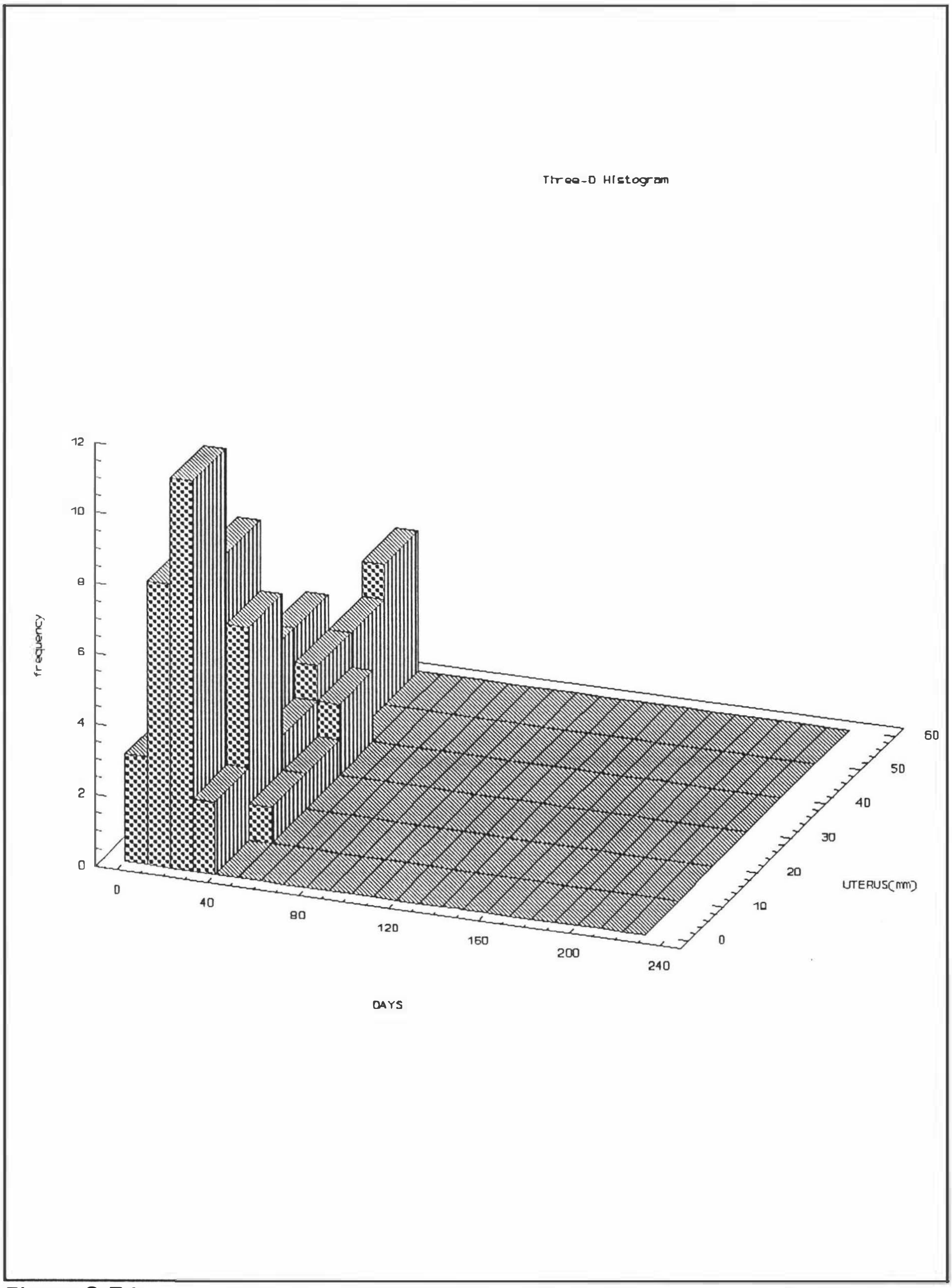

Figure 3.71. Histogram of uterine lumen diameter measurements showing the frequency of measurements by foetal age and magnitude of the dimension.

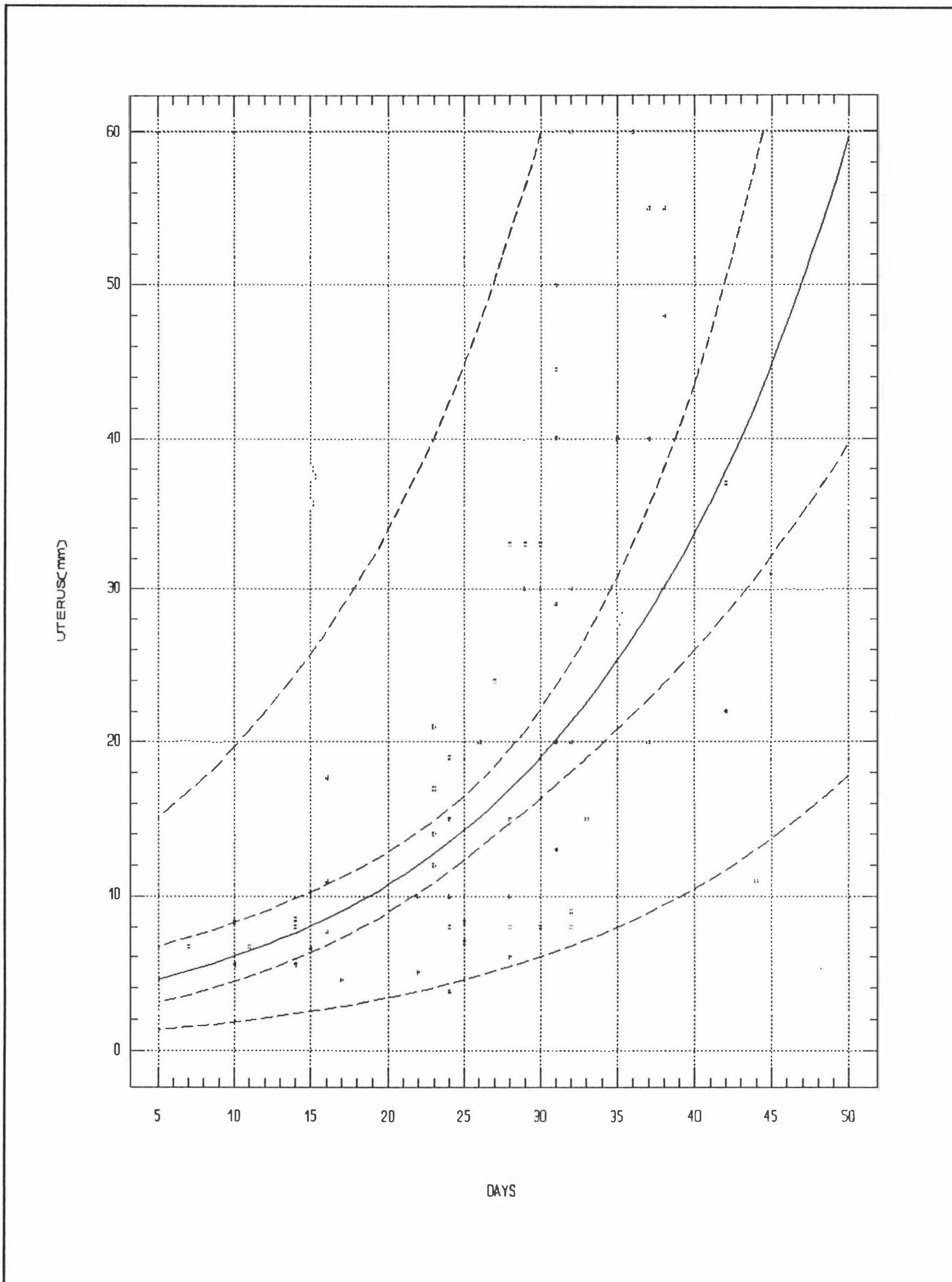

Figure 3.72. Uterine lumen diameter growth.
Scattergram of uterine lumen diameter with foetal age and the best fit regression for that data (95% confidence interval).

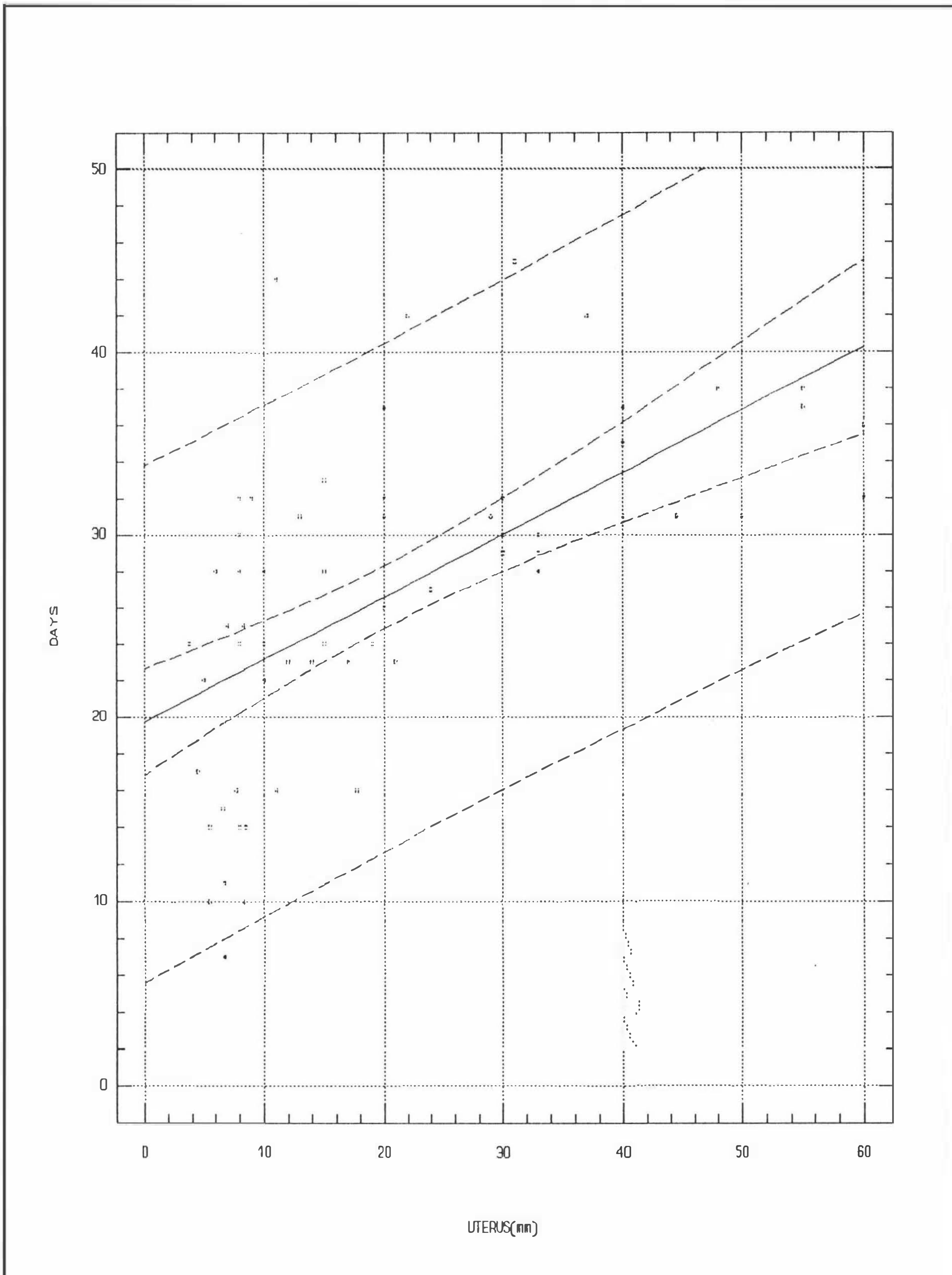

Figure 3.73. Age estimation using uterine diameter.
Scattergram of age on uterine diameter, and the regression used for foetal age estimation (95% confidence interval).

CHAPTER 4

DISCUSSION

4.1. OESTROUS BEHAVIOUR.

4.1.1. ACCESSORY SEXUAL BEHAVIOUR.

Twenty one hinds (57%), showed periods of sexual activity including long chases, hind mounting stag and low mounts, but without copulation (see table 3.I). These observations are consistent with the findings of Jopson *et al.*(1990) who detected elevated plasma progesterone for a 6-11 days period, prior to the first endocrine oestrus. Jopson *et al.*(1990) considered this phenomenon as a silent oestrus. Kelly and Challies (1978) detected an accessory corpus luteum in 46.8 to 58.9% of feral pregnant hinds, from which 1.5% (1/64) had two corpora lutea, one on each ovary. In the present study, 43% (9/21) of the hinds showing accessory sexual activity, had 2 to 5 activity periods, which may coincide with formation of multiple corpora lutea at different times.

The average interval between the period of accessory (non-copulatory) sexual activity and mating was 9.7 days prior to mating (table 3.III), similar to the 6-11 day periods of elevated plasma progesterone observed by Jopson *et al.*(1990). Eight hinds exhibited non-copulatory sexual activity 2 to 22 days after mating, possibly as a result of the formation of accessory corpora lutea of pregnancy as observed by Kelly and Challies (1978).

Thus, the behaviour pattern observed in this study seems to conform with reported physiological and anatomical findings in relation to pre or post-ovulatory ovarian activity. It suggests that the "silent oestrus" is often not silent, but rather sterile. For this reason, the author prefers the term "accessory" sexual behaviour.

In the present study, one hind, N° 90, showed 5 accessory sexual periods, and this was associated with an excessively long oestrous behaviour (see section 4.1.2).

There is no report in the literature of the anatomical observation of 5 accessory corpora lutea. Behavioral, anatomical and physiological studies should be undertaken simultaneously to confirm the relationship between this aspect of sexual behaviour and ovarian activity.

4.1.2. OESTROUS CYCLE.

One hind, N° 43, which did not become pregnant, was mated at two oestrous periods at a 10 day interval (table 3.I). This is not in accordance with the 18 days oestrus cycle length reported by Guinness *et al.*(1971) using hand reared scottish hinds and a vasectomized stag. Kelly and Moore (1977) also reported an oestrous cycle length of 18 days, but did not describe their experimental method.

Data from this study from only one hind does not allow statistical conclusions, and this hind did not become pregnant, suggesting a reproductive problem. Indeed, the cycle shown by hind N° 43, appears similar in length to the pre-oestrus cycle of 9.7 days detected in our study (see section 4.1.1) and might therefore be associated with a small corpus luteum, inefficient to sustain pregnancy. Similarly, Dudley (1983) reported a 10 days oestrus cycle in a hind which also failed to conceive.

The mean duration of behavioral oestrus recorded in this study was 23 h 45 min, which is in agreement with the findings of Guinness *et al.*(1971) and Kelly and Moore (1977) who reported an oestrus of 12-24 h. However, in the present study, the length of oestrous behaviour ranged from 1 to 111 hours. A shorter observed oestrus might indicate either a very quiet oestrus, or a lack of behavioral observation in case of night-time oestrus. Only one hind presented an abnormally long oestrous behaviour of 111 hours, and presented as well an excessive number of accessory sexual behaviour periods (see 4.1.1). No explanation was given for that peculiarity.

4.1.3. COURTSHIP.

A similar courtship pattern to that described by Dudley (1983) and Veltman (1985) was observed in this study, but a statistical description of oestrous behaviour (table 3.II) is limited by the confinement of observations from dawn to dusk.

Sniffing and short chases were not always related to mating, but rather were part of the stag's general investigations of the hind. Sniffing was the most common behaviour. Since sniffing and short chases were not reliable forerunners to the onset of oestrus, these actions were not taken into account in assessing the timing of behavioral oestrus in table 3.I.

While Dudley (1983) and Veltman (1985) did not observe frequently the action of hinds mounting each other, this behaviour was seen in 70% of hinds in this study. This behaviour was seen from 1 to 25 times per hind, and varied from the hind being on the top or under another hind, or sandwiched between two hinds. In contradiction to Guinness et al.(1971) who suggested that homosexual behaviour was seen when two hinds were in oestrus simultaneously, but in accordance with the findings of Veltman (1985), hind/hind mounting was found in this study to be an unreliable indication of the onset of oestrus. It was often observed isolated from any other sexual action, and the place of the hind, on the top or under, appeared to be related to individual preferences. Homosexual behaviour was performed, in this study, in the presence of the rutting stag, which is in contradiction with the suggestion by Dudley (1983) that hind/hind actions may result from the frustration of the hinds in the absence of the stag.

Unlike short chase behaviour, long chases by the stag were observed mainly during the oestrous period, although it was not unusual to see this action at times not related to mating.

Veltman (1985), in the statement that "a hind riding the stag is definitively in oestrus" considered the hind mounting stag action as the ultimate sexual approach before mating. However, data in this study showed this action generally started earlier than low mounts. Furthermore, this action was also observed outside of the oestrous period in 40% of the hinds (see table 3.I). Data in this study showed an average of 2.2 hind mounting stag actions per mating, while Veltman (1985) found 4 hind mounting stag actions/mating. This difference might be due to individual stag or hind and/or environmental influences, although the likely explanation might be that Veltman (1985) employed all mounting actions for this calculation, including those observed in the accessory sexual activity period, prior to copulatory oestrus (see section 4.1.1). Calculations in this study used only the actions during the oestrous period leading to mating.

Low mount, stag on hind, was found to be a better indicator of an imminent mating. This action began, on average, approximately 6 minutes before mating. Only 11% of the hinds were involved in this action without subsequent mating (see table 3.I). An average of 4.9 low mounts/mating, similar to the ratio reported by Veltman (1985), was observed in this study.

Thus, the sequence of behaviour was, in order, sniffing, short chase, long chase, hind mounting stag, low mount, and mating.

Only the observation of copulation could confirm oestrous. However, the observation of hind mounting stag and low mount actions performed on one daylight period can give the date of oestrus if confirmed later by ultrasonographic foetal ageing or by the calving date (see table 3.I).

4.1.4. DAY:NIGHT RATIO OF MATING.

Of a herd of 44 hinds, 36 (82%) were observed mating. One hind (N° 69) was observed going through the different phases of oestrous behaviour, including "hind mounting stag" and "low mount", until dusk. Mating was assumed to have occurred soon after dusk, and this was confirmed later by ultrasonography and the calving date. This finding appears to support the conclusion of Veltman (1985) who concluded that red deer tend to mate more in day time; However, that may not be the case. Indeed, this study recorded intervals up to 20 h 26 min between the first and last mating of a hind during one oestrous period (table 3.I); therefore, the probability of observing at least one mating is high, even if matings are equally frequent by day or night. Guinness et al.(1971), using a raddled vasectomized stag, reported as many matings at night as during the day.

4.1.5. MATING ORDER.

In this study, neither the age of the hinds, nor their weight, could explain the order of mating. Thus, as the social rank is positively correlated with the weight (Veltman, 1985), by deduction, social rank did not influence the order of mating.

This is in accordance with the observations of Guinness *et al.*(1971). Veltman (1985) observed a social rank effect on the order of mating for two years of study, but was unable to show that effect in a third year of observations.

4.1.6. CONCEPTION RATE.

In this study, the conception rate at first oestrus was 97%. Only one hind (N° 43) did not become pregnant, even after 5 matings distributed on 2 oestrous periods. Thus, the conception rate of the stag at first oestrus in fertile hinds can be considered 100%.

However, in this study the order of conception is equivalent to the order of mating, one should remember that a lower hind fertility might postpone the conception time to the next oestrus. Therefore, while the mating order does not appear to be influenced by the hind's weight or age, the conception order is strongly correlated to the body weight. A hind weighing less than 52 kg will not calve, and 94% of the hinds weighing 85 kg will calve successfully. Conception date will be advanced of one day for every 4 kg of additional weight (Hamilton and Blaxter, 1980).

In this study, the hinds weighed 84-117 kg, and are all within the optimum weight range for fertility, which may explain the absence of body weight influence on the conception date.

4.2. GESTATION LENGTH.

The gestation lengths, calculated from the dates of observed mating and calving, ranged from 221 to 239 days, with an average of 233 days. This is consistent with the gestational length reported by Kelly and Moore (1977) in New Zealand, but is 2 days longer than that reported by Guinness *et al.* (1971) from observations on the Isle of Rhum. Although this difference might not be significant, a climatic or/and environmental influence could be suggested.

It might be argued that a gestation of 221 days (hind N° 0) indicates a conception 12 days earlier than the recorded mating date, which would coincide with the timing of accessory sexual behaviour (section 3.1; table 3.III). However, no matings of that or any other hinds were recorded during accessory sexual behaviour periods. Furthermore, foetal measurements during this study indicated a growth pattern corresponding to fertilisation on the day of recorded mating. It should be noted that the calf died, suggesting that this short gestation length was due to premature calving.

4.3. ANATOMY OF THE FEMALE REPRODUCTIVE TRACT.

The reproductive history and age of the seven hinds used for anatomical description of the reproductive tract were not known. Measurements recorded must be interpreted in this context.

There appears to be no precise description of ovarian morphology in the literature.

4.4. ULTRASOUND SCANNING TECHNIQUE.

The transrectal route is the only method for ultrasonographic pregnancy diagnosis before 50 days in red deer, because the uterus is then mainly located in the pelvic cavity and is not able to be viewed by transabdominal scanning.

After 50 days gestation, transabdominal scanning can also be undertaken, as described by Mulley *et al.*(1987), but involves more restraining problems in red deer, due to the size of the animal, and the design of the restraining devices commonly found on farms prevents their use for abdominal scanning. Furthermore, the resulting image quality is poorer, due to the use of a 3 MHz transducer necessary for deep penetration through the abdomen.

Wilson and Bingham (1990) suggested it may be more difficult to orientate the rectal probe than the transabdominal one to achieve the optimum plane for measurements. Experience gained during this study showed that the orientation of transabdominal scanning is limited by the orientation of the skin surface. Furthermore, a 3 MHz transducer gives poorer image resolution than a 5 MHz rectal transducer, leading to poor accuracy of measurement. This is indeed illustrated by the comparison of the results obtained by White *et al.*(1989), who employed rectal ultrasonography before 50 days but transabdominal ultrasonography from 50 days onwards, with those of Bingham *et al.*(1990), who used only rectal ultrasonography. Age estimation equations using head diameter and chest diameter as the dimensions (Bingham *et al.*(1990), had a standard deviation of 6 and 5 days, whereas the equivalent standard deviations of White *et al.*(1989) were 9.8 and 8.5 days.

With good conditions, i.e. restraining devices and hinds accustomed to handling, 45 hinds were scanned per hour, using rectal ultrasonography.

4.5. ULTRASONOGRAPHIC IMAGING OF PREGNANCY.

4.5.1. TIME OF EARLY PREGNANCY DETECTION.

Pregnancy, distinguished by the presence of a vesicle within the uterine mass, was detected as early as 7 days after mating in this study. Reports indicate that pregnancy could be detected at day 10 in mares (McKinnon and Squires, 1988), day 11 in cattle (Curran et al., 1986-a), and day 20 in sheep (Buckrell, 1988), using a similar transducer. The difference between the time of detection of pregnancy in red deer, cattle and horses is probably due to the difference in gestation lengths, and depends also on which fluid filled foetal sac is observed. For example, horses have a regular spherical yolk sac which produces specular reflection enhancing the early detection of the embryonic vesicle (McKinnon and Squires, 1988). Ovine foetal sacs are similar to those of deer and sheep have a shorter gestation length; thus, further studies on early pregnancy in sheep should detect the chorionic vesicle at 7 days or earlier.

4.5.2. OEDEMA OF THE UTERINE HORNS.

A rosette pattern of the uterus, with circular areas of decreased echogenicity, was observed between 14 and 35 days gestation. The less echogenic areas were assumed to be a result of oedema of the horns. In mares, a similar image appeared during oestrus but also from 16 days gestation (McKinnon *et al.*, 1988; McKinnon and Squires, 1986). The rosette appearance of uterine ultrasonographic images of mares during oestrus were attributed to endometrial fold formations, separated by oedema. A similar explanation was given for the uterine images after 16 days gestation, although the endometrial folds were not as prominent as during oestrus. However, the above authors failed to indicate at which stage this feature disappeared, but it might be because the oedema was replaced progressively in prominence by the developing conceptus, as observed in red deer in this study. These changes were simultaneous with oestrogen production during oestrus, and might be associated with increasing tone due to conceptual or ovarian production of oestrogens in early gestation (McKinnon and Squires, 1986).

Therefore, further studies should be undertaken to determine if these signs of oedema occur in oestrous hinds. Meanwhile, caution is needed in assuming that oedema is a definitive sign of pregnancy.

4.5.3. PLACENTOMES.

The beginning of implantation was seen for the first time at 24 days, which is in agreement with the anatomical findings of McMahon (1989) who did not see the apposition of caruncle and cotyledon at 21 days, but did at 27 days gestation. For comparison, the implantation of the bovine conceptus begins as early as day 20 (Peters and Ball, 1986).

The ultrasonographic appearance of the developing placentome recorded in this study, was in accordance with the anatomical findings of Thome (1980) who described mushroom-shaped placentomes and 10 mm accessory placentomes from the fourth month of gestation, and stem-like placental attachment to the endometrium from the fifth month (see section 1.9.1).

4.5.4. FOETAL MEMBRANES AND FLUIDS.

A foetal membrane observed for the first time at day 24 may be either the amniotic or allantoic membrane. The curvature of the echogenic membranous line at that stage, appeared similar to the shape of the amnion observed later. Furthermore, the membrane seen at day 24 often appeared as a dotted line, similar to the appearance given sometimes by the amniotic membrane later in pregnancy. However, from extrapolation of the amnion length and amnion width growth regression curves, the sac apparently formed by this membrane seems too big to be an amniotic sac for a 24 day embryo. Furthermore, the allantois was observed with a 7.5 MHz transducer, for the first time at day 23 in cattle (Boyd *et al.*, 1988), and at day 24 with a 5 MHz transducer in horses (McKinnon and Squires, 1988). In red deer, the allantois completely filled the chorionic sac by day 34, forming the allanto-chorion, closely related to the endometrium, and not discernable from the endometrial surface (McMahon, 1989). It is therefore suggested that the foetal membrane observed at day 24 in red deer in this study was part of the allantoic sac.

As the amnion develops, numerous variable shaped membranous projections appear on its inner surface (Thome, 1980). These growths explain the ultrasonographic observation of white spots in the amnion from approximately 67 days. The snow-like or speckled appearance is a useful feature to distinguish the amniotic sac from the allanto-chorion (fig 3.29) and this assists the location of the foetus. It is not to be considered as a pathological feature.

Although hippomanes were observed by Thome (1980) in the allantoic fluid, these structures were never detected by ultrasonography in this study. This may be due to similar density with the surroundings tissues, or to confusion with placentomes. Indeed, their shape is oval and they are connected to the inner surface of the allantois by a stem, thus closely resembling the placentome shape.

4.5.5. EMBRYO AND FOETAL DEVELOPMENT.

A comma-shaped mass, 6 mm long, observed at 24 days, was assumed to be the embryo. This finding differs slightly from the anatomical observations of McMahon (1989) who described a 5 mm embryo at 27 days. As the conceptus is generally found near the second caudal caruncle (Thome, 1980; McMahon, 1989), the apparent overestimation of the embryo length by ultrasonography in this study might be due to the difficulty at this stage of differentiating the limit between the embryonic mass and the placentome, and/or to the difficulty of differentiating the length of the amniotic sac from the length of the embryo. .

McMahon (1989) described myocardial development from 27 days. The heart beat was observed in this study at 28 days gestation. The limb buds were observed by ultrasonography at 31 days, which is consistent with the anatomical findings of McMahon (1989) who did not find them at 27 days, but described their appearance at 34 days.

It is likely that the present study has allowed a more precise chronological sequence of foetal development since records were achieved from deer at a greater range of days than observations from the study of McMahon (1989) who observed only at 7-day intervals. The anatomical observations of Thome (1980) were undertaken on a wider range of days than McMahon (1989) but the lack of mating dates did not allow a precise chronology.

Wenham *et al.*(1986) studied the foetus from 72 days gestation radiographically, and found the cervical vertebrae longer than the other vertebrae. This characteristic became more important with increasing foetal age, as these vertebrae sustained a higher specific growth rate than the other vertebrae. Elongation of the neck, characteristic of the species, was observed in the present study from 51 days gestation.

From human ultrasonographic observations (Isaacson *et al.*, 1986), it is apparent that the echogenicity of the eye is due to the lens or to a shut eyelid. An elongated pupil was seen from 51 days on ultrasonographic images of the red deer foetus in this study (fig.3.23). The pupil was obviously shaped by the iris.

Internal organs, such as the bladder or the stomach, which may be observed as the foetus develops, appear on the ultrasound screen as non echogenic areas because they are fluid filled (fig.3.28). The liver, being soft tissue, appears in grey shades, difficult to distinguish from surrounding soft tissues. However, the intrahepatic umbilical vein may be seen as a non echogenic tube running from the umbilicus to the liver. Similarly, any blood filled organ, such as the aorta or the heart cavities, show a non echogenic ultrasonographic appearance.

4.6. ACCURACY OF PREGNANCY DIAGNOSIS.

Thirty five percent sensitivity of pregnancy detection before 20 days emphasis the difficulty in finding the small chorionic vesicle of early pregnancy. This difficulty was overcome gradually as the chorionic vesicle expanded and as the foetus and other structures became more prominent, to allow total accuracy of positive diagnosis from 41 to 130 days. Later on, as the foetus descended from the pelvic cavity and stretched the uterus, the signs of pregnancy were sometimes missed and the sensitivity of pregnancy diagnosis decreased to 95%.

As a result of these anatomical changes, the number of pregnancies was underestimated before 41 days and after 130 days, while the reliability of a positive diagnosis was 100% between 41 and 130 days gestation. The number of non-pregnant animals was overestimated before 41 days, but determined with 100% accuracy from 41 to 130 days. No data were available to calculate the reliability of a negative pregnancy diagnosis after 130 days.

In comparison, the sensitivity of pregnancy diagnosis with a similar rectal probe in sheep, was reported by Buckrell *et al.* (1986) to be 87% for the period 25-50 days after breeding. This poor result may be partly due to the experimental design: only the ewes diagnosed not pregnant were scanned on a subsequent occasion.

Unlike for sheep and red deer, the efficacy of rectal ultrasonography in cattle and horses is improved by rectal palpation to locate the uterus and to direct the transducer onto the conceptus. In cattle, the sensitivity of rectal ultrasound pregnancy testing was 100% from 45 days with a 3.5 MHz transducer (Chaffaux *et al.*, 1986), and from 17 days with a 5 MHz transducer (Curran *et al.*, 1986). Hughes and Davies (1989) reported that accuracy of pregnancy diagnosis was inversely proportional to the age of the cow. This finding needs to be investigated in deer. In horses, the sensitivity of the test, using a 3.5 MHz transducer, was 97.4 to 100% and was similar at all stages of gestation from 15 to 50 days (McKinnon and Squires, 1988).

The statistics relative to non-pregnancy reported in this study are of limited significance, as the 13 scans related to confirmed non-pregnancy (see table 3.V) were taken from only one hind (N° 43). This hind was diagnosed non-pregnant at each scan, leading to a specificity of 100%.

Thus, to achieve maximum accuracy from a single scan in red deer, the optimum period to undertake a rectal pregnancy test is 41-130 days after mating when the sensitivity is 100%. However, a skilful operator may undertake the test at any time between 31 and 130 days, with an acceptable sensitivity of 98-100%. Those results confirm earlier studies on red deer, which obtained 97.6% accuracy of pregnancy diagnosis from 40 to 132 days (Bingham *et al.*,1988,1990), and 100% between 30 and 110 days (Wilson and Bingham, 1990). However, for practical and management purposes, it may be optimum to ultrasound a herd before all deer are 30 days pregnant, to identify early pregnant deer, and then to repeat the examination in deer without signs of pregnancy, at a later date.

4.7. CONCEPTUS GROWTH.

In this study, for the period over which measurements could be taken, the length of the foetus and the uterine lumen diameter followed an exponential curve. Head diameter, head length, eye diameter, nose length, amnion width followed a linear progression, while neck diameter, chest diameter, chest depth, umbilical cord diameter, amnion length followed a multiplicative curve. Placentome diameter growth was best described by a quadratic curve.

Crown-rump length and foetal weight are the most frequently used parameters to describe foetal evolution. Crown-rump length of many domestic and laboratory species such as domestic rabbit, rat, mouse, guinea pig, dog, cat, horse, pig, cow and sheep, follows a growth curve similar to the crown-rump growth curve presented in this study (Evans and Sack, 1973). However, it would appear that anatomical observations may not give identical measurements to ultrasonographic observations. This is illustrated by the differences between the crown-rump growth curve presented in this study, and that presented by McMahon (1989). Before 30 days of pregnancy, ultrasonography may not differentiate the embryo itself from the amniotic sac, thus leading to overestimation of the embryo length. Later on, anatomical studies take the measurement of the crown-rump while the foetal body is in extension, but ultrasonographic studies measure the foetus *in situ*, thus leading to underestimation of the length due to the foetal posture in C-shape. An intensive study of ultrasonography combined with sequential slaughter would be needed to investigate and quantify these apparent discrepancies.

Thome (1980) described the growth of red deer foetuses from their weight, and did not use the measurement of crown-rump length.

With its tendency to a downwards inflexion from 120 days gestation, the parabolic curve of placentome development described in the present study, the appearance of smaller accessory placentomes, leading to greater variation in measurements, and a decrease of average placentome diameter. Placentome growth curves (width and base-apex) presented by Bingham *et al.* (1990) follow the same pattern.

White et al.(1989) used a linear model for both head diameter and trunk diameter of red deer foetuses, while Bingham et al.(1990) chose quadratic regression curves to describe each dimension. The choice of different models to describe a feature of growth does not mean significant variations in the evolution of the foetus, but rather is influenced by the number of data available, the length of gestation covered, and the statistical techniques used by the authors.

4.8. FOETAL AGEING.

Because rectal ultrasonography in red deer is not directed by rectal palpation, it is difficult to orientate the probe for optimum measurement of a feature. Therefore, the probability of imaging at least one dimension will increase with the number of dimensions available to the operator. That is why foetal ageing is described in this study through 13 dimensions. Some of these dimensions, such as amnion length, crown-rump and head length, give an acceptable accuracy of foetal age estimation, while others, such as uterus diameter, placentome diameter or umbilical cord diameter are less accurate but are sometimes the only features the operator is able to image.

When more than one dimension are measured, the more reliable should be chosen for age estimation. However, if the dimensions measured present a similar accuracy of age estimation, an average of the estimations should be calculated.

4.8.1. CROWN-RUMP.

In this study, crown-rump measurement was not often made before 28 days pregnancy because of the difficulty in locating the embryo. From 28 days, the heart beat facilitated the detection of the foetus, and thereafter, its length was measured frequently. Eventually, the foetus became difficult to fit entirely on the "Aloka" ultrasonograph screen, which is 60 mm wide, limiting the crown-rump measurements to about 60 days gestation. From 51 days, the elongation of the neck, characteristic of the species, allowed rolling and extension of the head, thus introducing measurement errors due to the posture of the foetus. For this reason, it was important to take the measurement only when the foetus was in a standard position (see fig.2.3).

With a standard deviation for foetal age prediction of ± 2.2 days, crown-rump measurement allows accurate foetal age estimation. While the most appropriate equation for foetal age estimation from crown-rump measurement in this study was logarithmic, Bingham et *al.*(1990) computed a quadratic regression equation. Both equations give identical predictions between 42 and 59 days, with a similar standard deviation. Furthermore, both equations are complementary, with the present study giving earlier predictions (from 24 days), while Bingham *et al.*(1990), using an ultrasound machine with the capability of a wider scan, covered a later period (until 66 days).

In cattle, a logarithmic regression of crown-rump length produced the most precise estimate of gestational age (White et al., 1985). In horses, the relationship between crown-rump and stage of gestation was given by McKinnon *et al.*(1988), although no age estimation equation was presented. In humans, the crown-rump length is the most commonly measured foetal estimation dimension during the first trimester of pregnancy (Blum and Kurtz, 1990).

4.8.2. HEAD DIAMETER.

While the head was recognised at 37 days, it was 42 days before the outline was readily distinguishable and the biparietal diameter measurable. After 128 days, the foetus descended from the pelvic cavity, and the skull, if observed, was only partially visible, preventing measurement of the biparietal diameter.

Age prediction from the head diameter was best achieved by a linear model. The same model was employed by Bingham et *al.*(1990) and White et *al.*(1989). However, while the present study obtained a standard deviation similar to that calculated by Bingham *et al.*(1990), the slope was quite different, giving identical results when HD = 20 mm, but with a difference of + or - 10 days when HD = 10 or 30 mm, respectively. The precise plane chosen for the measurement is probably involved in these errors. Indeed the diameter varies considerably at different cross sections of the skull, and the globular eyes, when not recognised as such, might be included in a measurement, leading to overestimation of the actual head diameter. Such variation resulted in a standard deviation of 6 days in both the present study and in the study of Bingham *et al.*(1990).

The age estimation equations of White *et al.*(1989) gave a standard deviation of 9.8 days.

In cattle (White *et al.*, 1985) and goats (Haibel, 1988) head diameter age estimation equations also followed a linear model. In humans, the biparietal diameter is the most commonly used foetal ageing dimension during the second and third trimester of pregnancy (Graham and Sanders, 1985).

4.8.3. HEAD LENGTH.

As for head diameter records, head length was not measured before 42 days. Difficulty was experienced in orientating the probe parallel to the head axis as a result of increasing flexibility of the neck. Care was taken to ensure a standard position of the head by checking that the axis was longitudinal through the nose. This resulted in a satisfactory standard deviation ($\pm$2.3 days), while the standard deviation of the equation of Bingham *et al.*(1990) was $\pm$5.8 days. However, data in the latter study covered the period 42-128 days while data from this study was from 42 to 84 days.

Head length was also used for foetal ageing in cattle (White *et al.*, 1985).

4.8.4. EYE DIAMETER.

Eye diameter was measured to allow ageing of foetuses in late pregnancy when the anterior part of the foetal body is the most common part seen. While the eyes were distinguished as highly reflective structures on day 32, the orbits were not readily visible to allow measurement before day 57.

Measurement of a globular structure is prone to error as a lesser diameter might be mistaken for the greater if careful scanning is not undertaken. This error is compounded further by the shape of the orbit being not entirely spherical. Consequently, the accuracy of foetal age estimation based on eye orbit diameter would be quite low, as the standard deviation is 8.6 days.

In horses, eye size was calculated from the sum of width plus length (McKinnon *et al.*(1988). In humans, the eye size was not recorded as such, but the interorbital distance, i.e. distance between the lateral walls of the orbits, was used for foetal age estimation in those instances where a satisfactory biparietal diameter could not be obtained (Graham and Sanders, 1985).

4.8.5. NOSE LENGTH.

The nose was distinguishable by day 37, although the head conformation did not allow accurate measurement of the nose length until 47 days gestation.

The accuracy of age estimation based on nose length was moderate, with a standard deviation of ±4.8 days. The equation of Bingham *et al.*(1990) had a standard deviation of ±6.6 days. Age estimates at low nose length measurements gave similar age predictions using the equations of Bingham *et al.*(1990) or those derived in this study. However, a discrepancy arose in late gestation when up to 20 days difference appeared. This could be explained by the increasing difficulty in orientating the probe parallel to the nose axis. Furthermore it is sometimes difficult to assess the limits of the nose length at its orbital end if the eyes are not imaged.

Nose length was also used for foetal age estimation in cattle (White *et al.*, 1985).

4.8.6. NECK DIAMETER.

The neck was elongated on day 51 but it was only from day 71, when the length allowed it to be completely extended, that measurements were taken.

The accuracy for age estimation using neck diameter measurements was low (sd = ±7.1 days). This is probably due to individual differences, and to the shape of the neck which is not spherical but rather oval.

The neck diameter was not measured in other species.

4.8.7. CHEST DIAMETER.

This measurement was taken from day 45, although it was easiest from 52 days onwards, when the ribs became echogenic.

The standard deviation of the age estimation equation was similar to that of Bingham *et al.*(1990) (sd = ±6 days), but less than that reported by White *et al.*(1989) (sd = ±8.5 days). The major reason for error was the difficulty of orientation to ensure the appropriate plane for measurement. The equation from this study gives results for age estimation between those of Bingham *et al.*(1990) and White *et al.*(1989). Estimations derived from this study are ±5 days lower than the estimations given by the equations of Bingham *et al.*(1990), and 7 to 20 days higher than the estimations given by the equation of White *et al.*(1989). The technique of measurement was not described in the paper of White *et al.*(1989) and measurement of "trunk diameter" (White *et al.*, 1989) might be different from "chest diameter" as described by Bingham *et al.*(1990) and in the present study.

Trunk diameter was also used in cattle for foetal ageing. In humans, the closest related foetal age estimation dimension is the abdominal diameter (Blum and Kurtz, 1990).

4.8.8. CHEST DEPTH.

The accuracy of age estimation using the equation calculated in this study, was similar to that of Bingham *et al.*(1990) for chest depth.

This dimension was not reported in other species.

4.8.9. UMBILICAL CORD DIAMETER.

The umbilical cord is soft tissue of variable dimension and, therefore, precise measurements were difficult. However, it was seen most of the time and was measurable until 192 days gestation at which time no other dimension could be measured. A standard error of 11.5 days shows the poor accuracy for age estimation using this dimension.

Umbilical cord was not reported for foetal age estimation in other species.

4.8.10. AMNION LENGTH.

The amnion length was measured until day 56. Measurements were not continued past that age because from day 51 a decrease in the amniotic fluid pressure was observed, leading to a more variable amniotic shape, and consequently variable measurements and less precision for the age estimation equation.

Within the period for measurements, amnion length is the best dimension for foetal age estimation (sd = ± 1.7 days). The equation of Bingham *et al.*(1990) had a sd of ± 4.1 days, probably because data were collected until day 68, when the amnion shape was less consistent.

This dimension was not reported for foetal ageing in other species.

4.8.11. AMNION WIDTH.

Similar to amnion length, amnion width was also dependent on the shape of the sac. Furthermore, a kidney shape gives a greater propensity for variable measurements. This is reflected in the standard deviation of the age estimation equation (±3.4 days), compared with ±1.7 days for the equation using amniotic length measurements.

This dimension was not reported for foetal age estimation in other species.

4.8.12. PLACENTOMES.

The placentome growth curve was quadratic with a parabolic shape as a result of the appearance of smaller accessory placentomes in late pregnancy, giving both more variability of measurements and a reduction of the mean dimension of placentomes. Thus, a placentome of more than 35 mm diameter may be characteristic of two different foetal ages, and, therefore useless for predicting calving dates. Consequently, age prediction using placentome measurement must be limited to the period before 55 days when the diameter is less than 32 mm, which gives a standard deviation of ±4.7 days.

On the basis of observations from this study, it is suggested that the equation for foetal age estimation using placentome diameter measurements reported by Bingham *et al.*(1990) is of little value. Those authors noted the high standard error of equation (± 13.5 days) but did not discuss the significance of the quadratic curve, in light of the occurrence of accessory placentomes. Furthermore, Bingham *et al.*(1990) also reported placentome base-apex measurements. Observations from the present study would suggest these measurements are of little relevance for foetal age estimation because of the difficulty in distinguishing base-apex measurements from placentome dimensions, particularly once placentomes develop the mushroom-like appearance late in pregnancy.

Placentome measurements were not reported for foetal age estimation in other species.

4.8.13. UTERINE LUMEN DIAMETER.

Fluid in the uterine lumen is the first sign of pregnancy observed ultrasonographically, and the diameter of the vesicle can be measured, as early as 7 days gestation. The uterine horns have a variable diameter, greatest at the uterine body and smallest at the distal extremity. Furthermore, the conceptus develops in the proximal third of the uterine horn which enlarges first in this area. The errors come from the difficulty in locating the greatest uterine diameter. As a result, data were variable, resulting in a poor correlation of the regression (37.2%) and a relatively high standard deviation for foetal age estimation (sd = ± 6.9 days).

Data of Bingham *et al.*(1990), using uterine diameter for foetal age estimation, produced a quadratic relationship with a correlation of 45%. However, a higher correlation of the regression, despite achieving statistical significance, does not mean the regression line is describes reality. A quadratic model, proposed by Bingham *et al.*(1990), which resulted in a parabolic curve, is not appropriate since in reality uterine diameter continues to enlarge as pregnancy progresses. The apparent error of Bingham *et al.*(1990) may be due to measurements taken as late as 76 days from an ultrasound screen which limited measurements to 70 mm. Consequently, since the largest diameter of the uterus probably exceeded 70 mm, it would appear that measurements taken were not from the largest part of the uterine lumen, but rather from any part which could fit on the screen. This appears to have led to a false impression that uterine growth stops and even decreases as pregnancy progresses. This is clearly not the case. These difficulties highlight the care that must be taken when interpreting both ultrasonographic images and the data that can be recorded.

The fluid-filled vesicle is also the first sign of pregnancy ultrasonographically observed in other species, but it is used for foetal age estimation only in humans during the first trimester of pregnancy (Blum and Kurtz, 1990).

4.9. GENERAL DISCUSSION.

4.9.1. ROLE OF ULTRASONOGRAPHY FOR PREGNANCY TESTING IN RED DEER.

Scanning has two major "raison d'etre": research and farming.

It is a tool for the researcher to follow anatomical evolution *in vivo*, without having to slaughter a large number of animals. The present study often allowed a more accurate chronology of anatomical features than some other studies using sequential slaughter of pregnant hinds (McMahon, 1989).

Scanning can also accelerate progress for some types of experiments on seasonal breeders by predicting results. The reproductive success of an experiment can be easily and cheaply assessed in early pregnancy, allowing planning of future experiments without waiting for the delay caused if mating dates are calculated from calving dates. For example, a field study of advancement of the breeding season using melatonin (Wilson 1991), was assessed by rectal ultrasonography for foetal ageing 3 months after the hinds were joined to the stag, thus giving the results at least 4 months earlier than would have been possible if the researcher had to wait for calving dates.

Time efficiency can be achieved by using ultrasound scanning for foetal ageing instead of observing oestrus behaviour when conception dates are needed. In the present study, approximately 6 weeks of day time observations were needed to record the mating dates in 82% of the hinds. However, 30 days after retrieval of the stag, only 2 to 3 hours are needed to scan, measure some foetal age estimation dimensions, and calculate the estimated conception dates. Furthermore, the conception date is estimated in 100% of the hinds when scanning compared with a lower percentage with day-time observations. However, errors are inherent to the estimation method. Therefore, it can only be used when precise mating dates are not required. The estimation method is very accurate for establishment of mean or median calving dates (Wilson and Bingham, 1990).

A further important use for scanning is to assess experimental artificial insemination or embryo transfer 1 to 2 months after fertilisation (Asher *et al.*, 1990), thus allowing planning and evaluation well in advance in the next breeding season.

For the farmer, ultrasound investigations on a deer herd can be useful for management purposes. Ultrasonography detects the non-pregnant hinds which can be slaughtered prior to the winter season of feed shortage, thus saving valuable feed. The risk of a non-rutting stag is decreased if ultrasound pregnancy testing is used early in the breeding season, thus allowing the farmer either to change the stag if fertility problems are detected, or, if the fertility is acceptable, to keep the same stag during the entire breeding season for genetic reasons. Foetal ageing can help the farmer for feed management during calving, by having all the hinds in the same group calving in a short period. After artificial insemination or embryo transfer, foetal ageing will inform the farmer about the origin of the pregnancy, from the artificial breeding or from the back-up stag.

Real-time ultrasonography allows the operator to image the conceptus and its movements and to assess the viability of the foetus through observation of the heart beat. Furthermore, the evolution of pregnancy can be followed if successive scannings are undertaken. This may be useful on farms with infertility problems, to determine either lack of conception, embryonic loss, or reproductive pathology. In the present study, no embryonic loss was recorded.

In addition to pregnancy diagnosis, rectal ultrasonography allows investigations of pathological reproductive conditions, such as pyometra, foetal mummification, uterine cysts, endometritis and emphysematous foetus.

4.9.2. ADVANTAGES OF THE TECHNIQUE.

Ultrasonography is a non invasive effective method of pregnancy testing in red deer.

It has many advantages over hormonal measurements in that the result is given without delay, and avoids potential problems of laboratory such as mix-ups of the assays or sample loss or breakage. Furthermore, hormonal assays are not always 100% reliable while rectal ultrasonography in red deer was 100% reliable between 40 and 130 days. Asher *et al.*(1990) reported approximately 10% overestimation of the number of pregnancies with plasma progesterone assays. Real-time ultrasonography has also the advantage on hormonal assays by visualising the conceptus allowing assessment of its viability.

Rectal ultrasound is a practical method of pregnancy diagnosis for large herds.

4.9.3. LIMITATIONS OF THE TECHNIQUE.

In red deer, the primary limitation of the technique is the small size of the anus preventing concurrent rectal palpation which allows more accurate placement of the probe as is possible in cattle and horses. Rectal palpation is possible in larger species of deer such as elk, and it is probable that in that species, more precision and accuracy could be achieved, particularly in early pregnancy. This anatomical limitation also prevents some studies from being undertaken, such as monitoring of conceptus transuterine migration before implantation. It also limits the extend of uterine and ovarian scanning.

4.9.4. PRACTICAL APPLICATIONS.

4.9.4.1. PREGNANCY DETECTION.

The best period for pregnancy testing was shown to be from 30 to 130 days when the sensitivity of the test was 98-100% (table 3-VI). Therefore, on farms with natural breeding, pregnancy testing could be undertaken 2 months after the onset of oestrus in hinds. However, for management purposes, the ultrasound pregnancy test can be undertaken earlier, e.g. 1 month after the onset of oestrus, but all hinds which were not diagnosed pregnant should be retested 30 days after the stag was withdrawn.

4.9.4.2. FOETAL AGEING.

Foetal ageing requires the imaging of measurable features. The best dimensions are amnion length (sd = 1.7 days), crown-rump length (sd = 2.2 days) and head length (sd = 2.3 days), which are found respectively at 37-56 days, 24-59 days, and 42-84 days gestation (table 3.VIII).

Therefore, the best period for foetal age estimation is from 28 days, when the heart beat confirms existence of the embryo, to 84 days, beyond which point the number of features measurable decreases and variability of dimensions increases. However, foetal ageing can be undertaken outside these limits, but with less precision.

4.9.4.3. ARTIFICIAL BREEDING.

The assessment of success for artificial insemination or embryo transfer, requires distinction of an embryo due to artificial breeding from one due to conception by a back-up stag at the next oestrous cycle. For this purpose, the best period for rectal ultrasonography is 40-45 days after AI/ET when the foetus is easily detected with its 18-24 mm crown-rump length and the characteristic differentiation of its body parts. At this stage, an embryo due to the back-up stag will be 22-27 days, i.e. a mass of 6-8.5 mm, without any body differentiation or heart beat.

4.9.5. ECONOMICS OF ON-FARM USE OF ULTRASONOGRAPHY.

An economic analysis was made, using a standard 1989 deer farm model, and creating five different situations with normal or reduced fertility, with or without the help of ultrasonographic pregnancy testing (see appendix 1). The partial budgets indicate an economical return on costs when using ultrasonography, particularly for investigation and management of reproductive problems. Early pregnancy testing allows the farmer to decide whether to replace the non-pregnant hinds by new purchased mixed age hinds, or to slaughter the non-pregnant hinds and save some grazing during winter. However, each case is specific, and economics depend mainly on the value of the animals, their reproductive history, and the cost of pregnancy testing.

4.9.6. FUTURE PROSPECTS FOR ULTRASONOGRAPHY FOR REPRODUCTION SYSTEM INVESTIGATIONS IN DOMESTIC LIVESTOCK.

While use of ultrasonography is already well established in horses and some other species, this work substantiated its use for reproductive investigations in red deer.

The future of ultrasonographic pregnancy testing should be in the hands of technicians under veterinary supervision. Indeed, well trained technicians would be as efficient and less expensive than veterinarians for pregnancy testing or prediction of the calving dates. However, veterinary supervision would be necessary to analyse the results and to deal with the reproductive problems of the herd.

The technique may be applied to other farmed animal species. It would be a very useful tool for evaluation of reproduction of South American camelids since they experience a lot of reproductive problems. For example, up to 50% of the embryos are aborted or reabsorbed within the first month of gestation (Sumar, 1983). Ultrasonography will be a useful tool to detect these problems and to investigate ways to improve breeding performance.

Sheep breeding programs could benefit from improvement of ultrasonographic detection of early pregnancy.

Deer breeding programs will develop in the future. Ultrasonography for reproductive investigations will become an increasingly popular tool in commercial herds, provided the cost is kept to an acceptable level.

4.9.7. FURTHER INVESTIGATIONS OF ULTRASONOGRAPHY IN DEER.

This study has expanded the knowledge of ultrasonography for pregnancy testing, and will provide data and information to help the practitioner in using this tool to the best efficiency in farmed deer.

However, further investigations might be undertaken to further improve knowledge of some areas. For example, this study has a limited amount of data for pregnancy prior to 20 days post mating. This study has consistenly identified signs of oedema of the uterine horns in early pregnant deer. However, similar observations arise from oestrous mares suggesting that this may not be a characteristic specific to pregnancy in deer. It is now necessary to study the ultrasonographic appearance of the uterus during the oestrous cycle, and to differentiate the oedema (if any) of oestrus from the oedema of early pregnancy. The recognition of characteristic features of oestrus would help the farmer and the researcher in the detection of oestrus.

Evaluation of ultrasound for infertility investigations needs to be undertaken. Of great interest for the practitioner would be the ability to recognise pathological reproductive conditions. In this study, one hind did not calve, and another was fertile but showed an excessive sexual behaviour. However, except for some intra-uterine fluid in the non-pregnant hind, nothing specific was recognised.

A more complete understanding of ultrasound images would benefit from a study of anatomical description following slaughter concurrent with ultrasonography. This could also help to ultrasonographic recognition of pathological reproductive conditions.

To assess the validity of the foetal age estimation equations computed in the present study, they need to be critically used on commercial farms.

In this study, a 5 MHz was used, but 7.5 MHz transducers are now becoming available for veterinary research purposes. Early pregnancy diagnosis might be improved in the future, with earlier detection of the first sign of pregnancy using higher frequency transducers.

Some amelioration of the technique might enhance ultrasonographic observations of pregnancy, e.g. a longer probe would allow the operator to view and measure uterine, placental and foetal dimensions later in gestation than the 60 mm long probe used in this study allowed, thus giving more precision to foetal age prediction beyond 84 days gestation.

During this study, rectal ultrasound scans of fallow deer and red x elk pregnancies were undertaken by the author but were beyond the scope of this thesis. Dimensions of the conceptus were not measured. Although early pregnancies conceptuses appear similar in red deer, fallow deer and red x elk deer, further investigations with precise measurements should be undertaken in the different species and cross-species of deer.

4.10. CONCLUSION.

This study has described the ultrasonographic appearance of the non-pregnant reproductive tract and of pregnancy in farmed red deer from the 6 to 211 days after mating. It has provided information to allow the ultrasonographer to determine the early stages of pregnancy and to allow estimation of gestational age with accuracy up to approximately 150 days.

Appendix 1.

Cost-benefit analysis

An example of the use of ultrasonographic pregnancy testing on a commercial farm.

This model is based on data for a typical deer breeding herd in 1989 (Farm costs and prices, 1988; MAF farm monitoring report, 1989; Product price, assumptions, 1989). The partial budget is calculated on 1 year, beginning at the time of pregnancy testing, 2 months after the beginning of the breeding season.

When no pregnancy testing is carried out, the non-pregnant hinds are slaughtered at the weaning season. When pregnancy testing is carried out, the non-pregnant hinds are slaughtered immediately at the end of the breeding season.

The calculated numbers of animals are rounded to the closest unit.

Assumptions.

Herd size and characteristics:

Herd:	100 breeding hinds overwintered, 16 yearling hinds, 5 stags.
Pregnancy rate:	94%
Mortality before weaning:	8%
Mortality of adults:	4%
Replacement policy:	Breed own replacements.
Selling policy:	Hinds and stags sold as weaner.

Costs:

Animal health:	$ 12.43 /head/year.
Grazing:	$ 3 /week/head.
Pregnancy testing (40 hinds/hour):	$ 180 /hour or $ 4.5 /hind.

Sale prices:

Venison	$ 6 /kg carcass, $ 360 /animal
Live hind/yearling	$ 360
Females weaner (50 kg)	$ 6 /kg live weight, $ 300 /animal
Males weaner (60 kg)	$ 4 /kg live weight, $ 240 /animal

Models.

First situation. Normal fertility, no pregnancy testing, non-pregnant hinds are slaughtered at weaning and replaced by yearlings.

116 hinds, 109 pregnant, 100 weaner.

50 female weaner:	16 are kept for replacement.	
	34 are sold	10,200
50 male weaner:	sold	12,000
11 hinds	4 = live sale	3,960
	7 NP = slaughter	
Return for 1 year		**$ 26,160**

Second situation. Normal fertility, pregnancy testing, immediate replacement of the non-pregnant hinds by purchased mixed-age hinds. However, those hinds are not pregnancy tested, and only 70% of them were pregnant.

116 hinds, 109 pregnant.
The non-pregnant hinds are replaced with purchased mixed age hinds.
114 pregnant, 105 weaner.

53 female weaner:	16 are kept for replacement	
	37 are sold	11,000
52 male weaner:	sold	12,480
11 hinds	live sale	3,960
7 non-pregnant hinds slaughtered		2,520
7 mixed age hinds purchased		(2,520)
Pregnancy testing (116 animals)		(522)
Return for 1 year		**$ 26,918**

Third situation. Normal fertility, pregnancy testing, non-pregnant hinds are slaughtered and not replaced, grazing is saved.

116 hinds, 109 pregnant, 100 weaner.

50 female weaner:	16 are kept for replacement	
	34 are sold	10,200
50 male weaner:	sold	12,000
11 hinds	live sale	3,960
Save grazing (7 hinds/6 months)		546
Pregnancy testing (116 animals)		(522)
Return for 1 year		**$ 26,184**

Fourth situation. Fertility problem (70% pregnant), no pregnancy testing, 11 non-pregnant hinds are replaced by yearlings at the weaning season, the others are retained.

116 hinds, 81 pregnant, 75 weaner.

38 females weaner:	16 are kept for replacement	
	22 are sold	6,600
37 male weaner:	sold	8,880
11 non-pregnant	slaughter	3,960
Return for 1 year		**$ 19,440**

Fifth situation. Fertility problem (70% pregnant), pregnancy testing, immediate slaughter and replacement of non-pregnant hinds by purchased of mixed-age hinds (70% of them are pregnant); the other non-pregnant hinds are retained.

116 hinds, 81 pregnant.
35 hinds are purchased, 25 are pregnant
106 pregnant, 98 weaner.

49 female weaner	16 are kept for replacement	
	33 are sold	9,900
49 male weaner	sold	11,760
11 hinds	live sale	3,960
35 non-pregnant	slaughter	12,600
35 hinds (replacement)	purchased	(12,600)
Pregnancy testing (116 hinds)		(522)
Return of 1 year		**$ 25,098**

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