ULTRASONOGRAPHIC PREGNANCY DIAGNOSIS IN RED DEER

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1. INTRODUCTION

A simple, accurate and cost-effective method of pregnancy detection has application for both intensive deer production and for research purposes. Tests have included:

1.1 Abdominal palpation

The foetus can often be palpated in late pregnancy but this is an unreliable technique and is difficult in hinds which tense abdominal muscles.

1.2 Udder development

The rate of udder development in late pregnancy varies considerably between hinds. Development usually occurs only in the last month of pregnancy and may be delayed to within a week or so of parturition.

1.3 Rectal palpation

This technique can be used in the larger species of deer, e.g. elk and large elk-X red hinds.

1.4 X-rays

Verme (1962) described X-ray determination of pregnancy in white-tailed deer but indicated that accuracy could be achieved only after 85 days gestation when ossification of foetal skeleton was sufficient.

1.5 Blood progesterone

Progesterone content of serial blood samples are useful for determination of pregnancy in deer (Asher, 1985). However, these require repeat blood collection and hormone assays.

1.6 Serum oestrone sulphate

Barrell and Boss (1989) reported elevated serum oestrone sulphate as a supporting sign of pregnancy, but variation in levels makes interpretation of results difficult.

1.7 Pregnancy-specific protein

Fennessy *et al* (1986) first reported detection of a pregnancy-specific protein in deer. This subject is discussed in a preceding paper in this Proceedings (Beatson)

1.8 Ultrasound

In the early 1980's A-mode ultrasonography was used with some success in deer, but no objective studies were performed to determine its accuracy and the optimum timing for its use. This form of ultrasonography has been superseded by B-mode real-time ultrasonography using rectal probes. The result of preliminary investigations and establishment of age prediction equations 42-110 days pregnancy have been recorded elsewhere (Bingham *et al* 1988; Bingham *et al* 1989; Wilson and Bingham 1989).

2. APPLICATIONS OF PREGNANCY DIAGNOSIS IN FARMED DEER.

2.1 Clinical investigation of poor reproductive performance

Poor calving percentages may be associated with conception failures due to stag and/or hind effects or management, or alternatively embryonic or foetal loss. Very early pregnancy diagnosis is a vital tool in the investigation of such problems.

2.2 Calving management

For optimum feed management during calving it is desirable to have all hinds in a group calving within a short period. Ultrasound examination has got the ability to distinguish early from late calving hinds using the equations of Bingham *et al* (1988). This technique has got particular relevance to the application of advanced calving technology (Wilson, 1989) to avoid management problems associated with an extended calving period.

2.3 Confirmation of artificial insemination/embryo transfer success

The age of foetus can be determined accurately using ultrasound to confirm the success or failure of ET or AI. This removes any uncertainly as to parentage, which can result if gestation length alone is used as the determinant in the event that AI or ET was unsuccessful and natural mating occurred one cycle later. This is particularly the case with Wapiti cross-breds which have a variable gestation length.

2.4 Detection of non-pregnant hinds

As deer farming becomes more intensive and individual animal values have reached relatively low levels, there are advantages for the farmer in detecting non-pregnant hinds which can be sent to slaughter rather than consume valuable winter feed. Early pregnancy detection is desired for this.

2.5 To check the "non-rutting" stag

Farmers occasionally become suspicious that a stag is not mating hinds. A method of early pregnancy detection, e.g. less than 18 days, could confirm the necessity to change sires if the stag was not working properly.

3. ULTRASOUND

3.1 Ultrasound pregnancy detection in deer

Mulley *et al* (1987) describes transabdominal ultrasonography for the detection of pregnancy in fallow deer using a 3.5 MHz transducer. The fallow does were restrained manually in a vertical position to expose the relative hairless area around the udder for application of the probe. The foetus was clearly visible at 50-105 days gestation and accuracy of pregnancy detection and non-pregnancy detection was 100%.

The only description in the literature of rectal real-time ultrasonography in deer is by Bingham *et al* (1988 and 1989) using a 5 MHz rectal transducer. These authors studied the development of the foetus and uterine structures from 42 days pregnancy, and established age prediction equations for 11 parameters from 42 to 128 days of pregnancy.

3.2 Limitations of ultrasonography for pregnancy detection in deer

The major limitation appears to be that for very early pregnancy detection. Unlike cows and mares, it is not possible to manually locate the uterus and associated structures in order to accurately direct the ultrasound beam. It is therefore also more difficult in deer to identify the non-pregnant uterus using ultrasound. Bingham *et al* (1988) reported success in distinguishing 30 day pregnancies, but to date no studies appear to have been undertaken in deer to determine the accuracy of pregnancy detection less than 40 days gestation. In cows the vesicle may be observed as early as 11 days gestation with a 5MHz transducer (Curran *et al* 1986), and at 9 days gestation using a 7.5MHz transducer (Boyd *et al* 1988). For mares the vesicle has been found at day 10 with a 5MHz transducer (McKinnon and Squires 1986). In ewes Buckrell (1988) reported that the vesicle can be imaged with accuracy by 20-23 days pregnancy with a 5MHz transducer.

Early pregnancy detection is the subject of a later section of this paper.

3.1 Principles of ultrasonography

3.3.1 Properties of ultrasound

Like audible sound waves, ultrasound waves have a longitudinal wavelength (λ), a frequency (Hz) and a velocity. In typical soft tissues the propagation velocity for ultrasound is 1540 metres/second, with a wavelength of less that 1 mm.

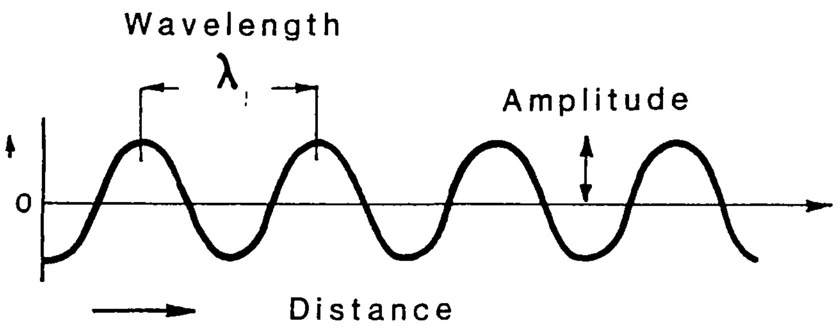


Fig 1 Properties of a typical longitudinal wave Each sound wave has a characteristic wavelength (λ), frequency, and velocity The amplitude represents the intensity of the sound (Herring, D S and Bjornton, G 1985)

Short bursts of ultrasound are emitted into the tissue. They travel at a constant speed until they meet a reflecting surface where a portion of the sound beam is reflected back to the transducer while the rest of the beam continues sending back echoes at all reflecting surface. A reflecting surface is an interface between two tissues with different acoustic impedance.

Acoustic impedance (z) = density (p) x velocity of sound in that tissue (c) (Herring and Bjornton, 1985).

The reflected wave is directly proportional to the difference in acoustic impedance across the tissue interface. The greater the difference the greater the reflected energy; e.g. soft tissue to air as in the lungs or gas bowel, and soft tissue to bone. Reflection between soft tissue, e.g. abdominal contents, is less and therefore tissue differentiation is more difficult (fig 2).

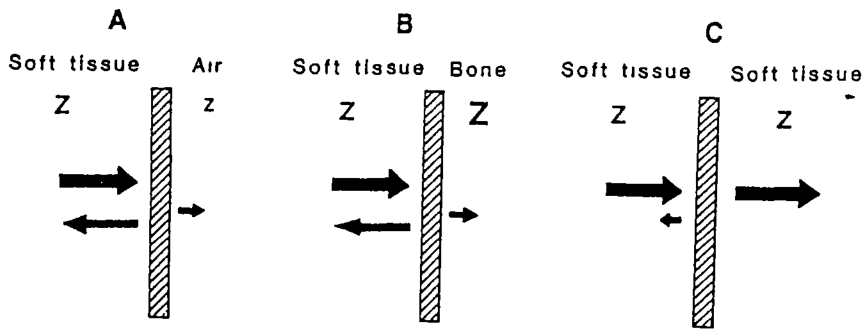


Fig 2 Interaction of ultrasound at various interfaces The *arrows* represent pulses of sound (Herring, D S. and Bjornton, G , 1985)

The best images of structures and boundaries usually come from mirror-like reflections. This requires that the ultrasound beam is perpendicular to the reflecting surface.

As ultrasound waves pass through tissues they are <u>attenuated</u> by two phenomena.

(a) Absorption - waves lose energy in the form of heat. This loss of energy limits the depth to which the ultrasound waves can penetrate. A 3.5 MHz transducer penetrates to the maximum depth of 200-250 mm, a 5 MHz transducer penetrates 100-120 mm, and a 7.5 MHz transducer to 30-50 mm.

(b) Scattering - when a small particle intercepts part of the ultrasound save only a small portion of ultrasound waves are reflected back to the transducer. The remainder of the waves are reflected in non-perpendicular directions

3.3.2 Production of ultrasound waves

In diagnostic ultrasound the sound waves are produced from a vibrating object, a transducer, made of a piezo-electric material. The vibrating frequency is determined by the thickness of the transducer, with thinner transducers producing higher frequencies The wave is produced by applying a high voltage electrical pulse of a few microseconds to the crystal.

The transducer receives reflected sound waves (fig. 3) which are converted to a set of rhythmic voltages which are modified within the machine, and ultimately produce an image on a cathode ray tube as dots of light on a black background. The amplitude of the echo determines the brightness of the dots.

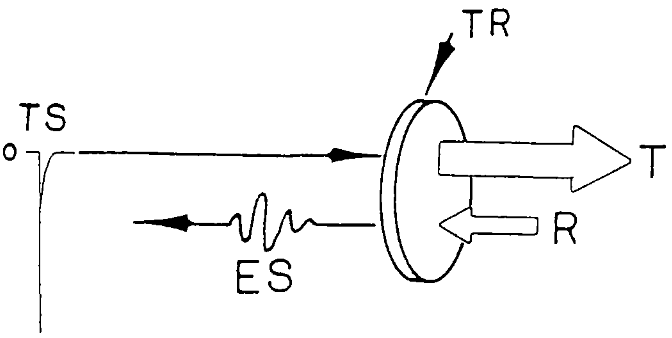


Fig 3 The transducer as an interface The transducer (TR) converts energy into electrical and mechanical forms A transmit signal (TS) causes the transducer to vibrate and transmit ultrasound into the tissue (T) The reflection (R) compresses the transducer and produces an echo signal (ES) for amplification and procession (From Powis, R L , 1986)

The image on the screen results from displaying all the echo signals from all the interfaces at the same time (fig. 4).

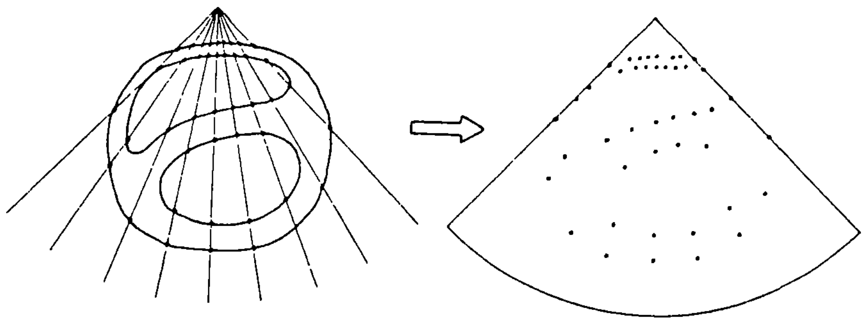


Fig. 4 Forming a two-dimensional image The moving beam on the left receives echoes from the tissue interfaces On screen, to the right, the sonograph moves the B-mode trace to the same spatial positions, creating a cross-sectional image of the interfaces (Powis, R L , 1986)

To produce a succession of images the ultrasound beam must be moved. This is done either mechanically with the transducer moving, or electronically, where the transducer is stationary but the beam redirected using electronics (Powis, 1986). When the beam is swept in the form of an arc it is called a sector scan, but the rectal transducers used in deer produce a rectangular shaped image and these are referred to as linear array scans.

3.3.3 Resolution

The qualities required for the image are axial and lateral resolution.

Axial resolution is a measure of the ability to show two interfaces when they are closely separated along the axis of the ultrasound beam. The smaller the axial resolution the better the image. The longer the wavelength, the poorer the axial resolution. Therefore for maximum axial resolution high-frequency transducers are preferred.

Lateral resolution refers to the ability to distinguish between two echo-forming surfaces lying side by side in parallel with the sound beam. Therefore lateral resolution will be determined by the beam width and the size of the transducer face. It is best at the focal zone of the transducer.

3.3.4 Artifacts

Artifacts may be due to sound beam properties, transducer quality, instrument adjustment or scan techniques. Some artifacts, such as acoustic shadowing and distance enhancement, can improve the diagnostic accuracy. Others, such as reverberation and mirror images, can cause confusion.

The commonly encountered artifacts are:

(a) Acoustic shadows - these are caused by the diminished transmission of sound due to attenuation and/or reflection of the sound beam at acoustic interfaces such as soft tissue-gas or bone (Herring and Bjornton, 1985). In a large foetus the centres of ossification will cause shadows, as almost all sound waves are reflected and therefore structures deeper than the bone will not be imaged. Other forms of shadowing may occur when the path of the sound beam is obstructed by bowel gas, or if there is lack of intimate contact between the transducer and rectal wall, and when faecal material has adhered to the transducer.

(b) Refraction - the portion of the sound wave which strikes the side of the curved boundary of a structure at less than 90° may refract, causing a shadowing or lack or echo formation beyond the site of refraction. Refraction artifacts are common in images of the ovary, as the beam encounters fluid-filled follicles or the sides of spherical embryonic vesicles (Pierson *et al* 1988).

(c) Enhanced-through-transmission - as the sound beam passes through a relatively homogenous medium such as urine, bile or fluid of ovarian follicles or embryonic vesicles, less attenuation takes place than in the surrounding echogenic areas. When the sound beam strikes the far wall of the cystic structure, the echoes appear brighter than the surrounding structures (Herring and Bjornton, 1985).

(d) 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 on the ultrasound image of the structure. This specular reflection is present on both the upper (i.e. nearest the transducer) and lower surfaces of the image of the structure. Specular reflection can be a considerable aid in some instances, e.g. examination of small vesicles (3-6 mm diam.).

(e) Reverberation - these artifacts are commonly seen during intrarectal examination of the reproductive tract, because gas-filled segments of the intestine reflect sound waves both back to the transducer and also off the near and far surfaces of the gas-filled structure. Reverberation artifact has three distinguishable characteristics:

- they are equidistant;
- they gradually diminish in intensity;
- and are parallel to the reflective interface (Pierson *et al* 1988).

(f) Mirror-image artifacts

These occur at highly reflective interfaces and are caused by multiple internal reverberations, so that returning echoes reach the transducer with a time delay and are registered on the image as being beyond the highly echogenic interface. This artifact is common during echocardiography because of the highly echogenic interface between the pericardium and the lungs. Two beating hearts are imaged at the same time (Herring and Bjornton 1985).

3.4 Recording the image

For many purposes ultrasound images should be recorded as part of the animal's medical record. Veterinarians should consider this when certifying pregnancy for valuable animals. Multiformat cameras are available for this purpose, but the simplest method of image recording is by the use of a polaroid camera. These are usually standard optional extras on most ultrasound machines. A disadvantage is that polaroid photograph quality is not particularly good. Many machines have video outlets and therefore the entire scan can be stored. This is the preferred technique for most research purposes, as subsequent viewing and measurements and recordings can be made remote from the animal and with the time to take accurate records.

Still photographs can be taken either from the ultrasound screen or from video screen subsequently.

4. ANATOMY OF THE FEMALE REPRODUCTIVE TRACT OF RED DEER

A study was undertaken to determine the anatomical characteristics of the reproductive tract of mixed-age red deer in order to assist *in vivo* scanning (see fig. 5).

4.1 Ovary

The ovaries of mature red deer are surrounded by the free part of the uterine horn. They have a kidney shape of 19 mm length (17-25 mm), a width of 10.4 mm (7-15 mm), and a depth of 7.5 mm (6-10 mm).

4.2 Uterus

The uterus is divided into two horns only 20 mm from the cervix. The proximal part of the uterine horns have a length of 88 mm (65-130 mm) and an external diameter of 20 mm. The horns are united by the intercornual ligament and have a common peritoneal covering. The free or distal part of the uterine horns which have a length of 80 mm (60-115 mm) curve ventrolaterally.

Internally usually four caruncles in each horn can be observed. These are spread regularly along the length of the horn. The caruncles have an elongated shape of approximately 8 mm width and approximately 30-40 mm length. The caruncle in the distal end of the uterine horn is often smaller (5 mm length). Only in few hinds are more than four caruncles observed.

4.3 Cervix

The cervix has a length of 60 mm (50-70 mm) and an average external diameter of 23 mm. Internally the cervix has four to six annular rings with the widest being near the vagina (10-15 mm), and the shortest (2-5 mm) close to the uterus.

4.4 Vagina

The vagina in mixed age hinds has a mean length of 210 mm (180-225 mm). The opening of the urethra is situated on the ventral wall 50-80 mm from the vestibule. The mucosa is shaped with longitudinal plicae. These are particularly concentrated along the floor of the vagina and particularly around the urethral opening.

5. ULTRASOUND EXAMINATION OF THE NON-PREGNANT REPRODUCTIVE TRACT IN RED DEER

In most deer the non-pregnant uterus could not be imaged on the ultrasound screen. This was probably because without fluid in the lumen the acoustic impedance of uterine tissue is similar to surrounding tissues and therefore there is very little reflection of ultrasound waves at tissue boundaries. In some instances the uterus appears as a slightly darker area surrounded by intestine, but distinguished from the latter by the lack of intestinal motility which is manifest by moving particles on the ultrasound screen.

The vagina appears immediately dorsal to the bladder and is characterised by a horizontal white line along the interface between the rectum and the dorsal wall of the vagina. A horizontal white continuous line arises from the apposition of the vaginal lumen mucus surfaces. Occasionally a black area is observed at the same level as the second white line and this appears to be fluid, probably mucus from the vagina.

The cervix may be observed on the anterior border of the bladder and is continuous with the vagina. It can be distinguished by its discontinuous central white line which arises as a result of the series of annular rings which close the cervix (fig. 5).

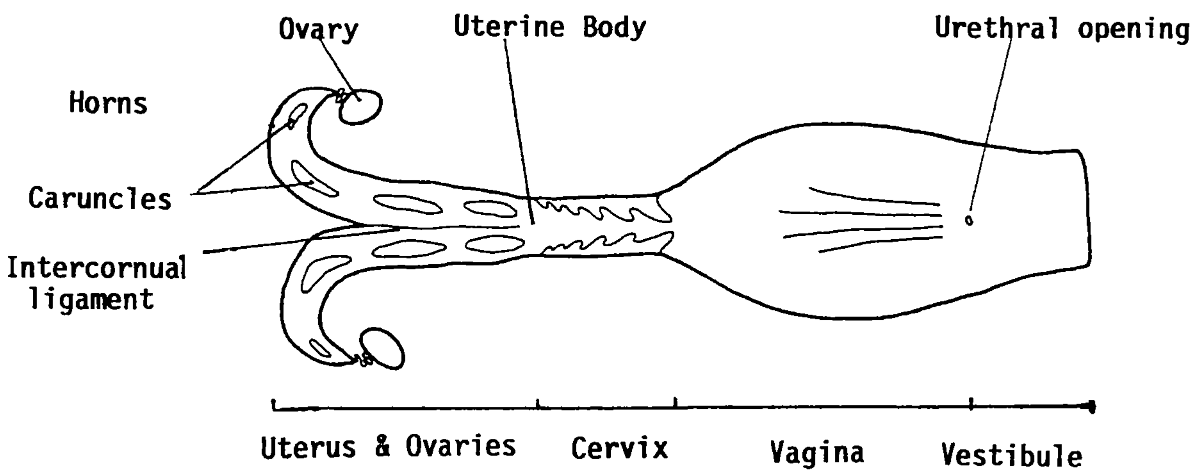


Fig. 5 **Sagittal section of the female reproductive tract of Red Deer**

6. ULTRASOUND EXAMINATION OF THE EARLY PREGNANT RED HIND

An earlier report (Bingham *et al* 1988) indicated that pregnancy could be diagnosed accurately from 40 days and reported observations of pregnancies as early as 30 days gestation. However, data from deer less than 40 days pregnant was scant, so a further study has been implemented to investigate in detail the very early signs of pregnancy in red deer.

The pregnancy of 40 mixed age hinds, 35 with known mating dates, has been investigated, using a 5MHz rectal transducer, from day 7 to day 42 of gestation. Two other hinds were diagnosed non pregnant.

The following signs of gestation were observed:

- By day 7, a vesicle of 5mm may be seen in the uterine mass in some pregnancies (Fig. 6A).
- From day 14 the uterus may be readily visible, "crown-like" in appearance, due to oedema in the horns. This "crowned uterus" (Fig. 6B) can be seen from the 2nd week until about 1 month of gestation, disappearing progressively as the horns become filled by the developing vesicle.
- By day 24, a comma shaped body of 6mm within the vesicle is assumed to be the foetus.
- From day 24, a foetal membrane appears as a highly reflective or dotted line surrounding the foetus. From the same day, placetomes of 5mm are seen on the uterine wall, growing to very variable widths ranging between 10 to 25mm at 40 days.
- By day 28, a rapid heart beat confirms the presence of a foetus which is now approximately 10mm.
- At 31 days, four bright spots indicate the formation of the legs.
- By day 37, the head can be distinguished from the body, the nose and the formation of eyes can be observed.
- The foetus has his own movements from day 42.
- Depending on the direction of the probe, the elongated cranial end of the bladder can be seen sometimes as dissociated from the bladder itself (Fig 6H) and may be confused with a vesicle of early pregnancy. To avoid this confusion, the probe must be moved about to eventually detect the connection between the "false vesicle" and the bladder. Furthermore, you must be sure that the vesicle is part of the uterus which appears as a grey static mass.
- At 30 days of gestation, the fluid filled uterus could be confused with an iliac blood vessel (Fig. 6I) that is observed upon directing the probe slightly toward to the right or left side of the pubis. It is worthwhile to stop moving the probe when the structure is located to ensure the absence of a slow pulse on your real time ultrasound image.

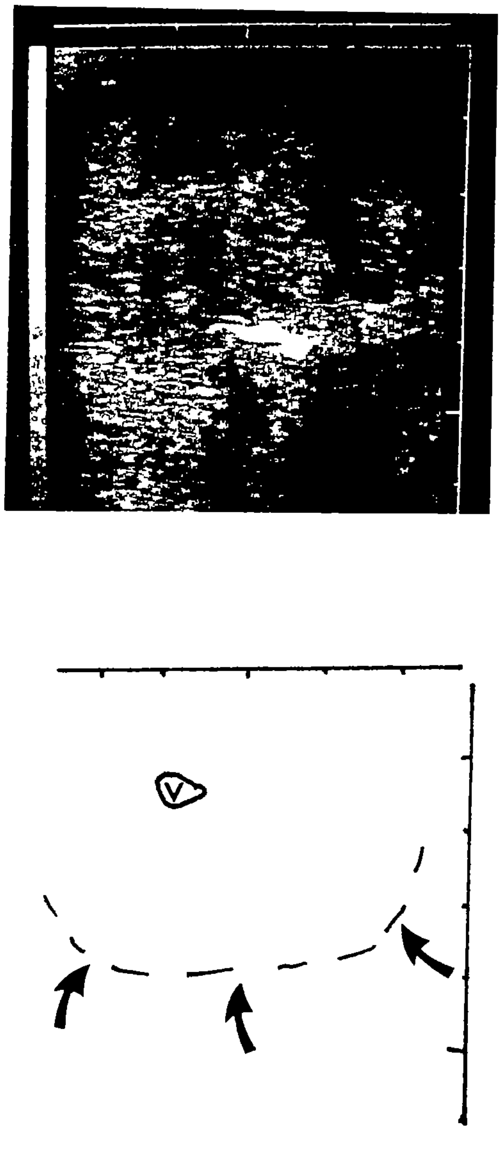


Fig. 6-A: 7 days gestation

v = vesicle

arrows = uterine mass

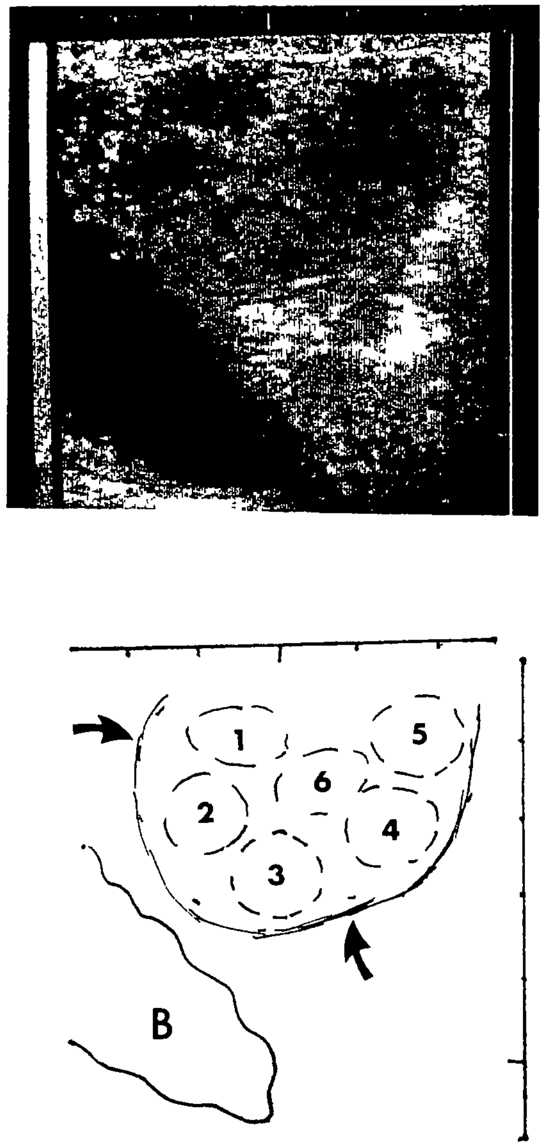
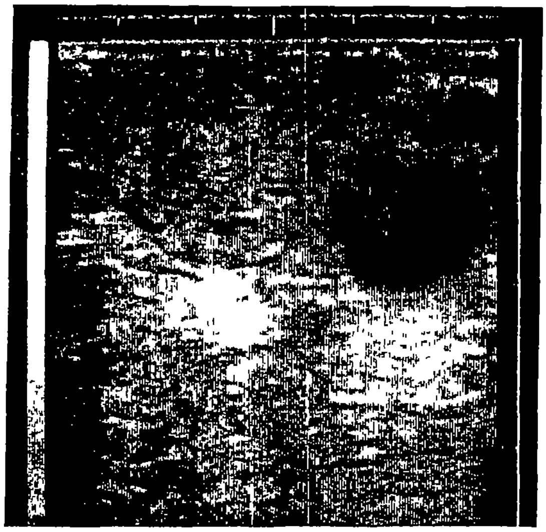


Fig. 6-B: 21 days gestation

"crownlike" uterus (arrows) with six section of oedematous areas (1 -> 6).



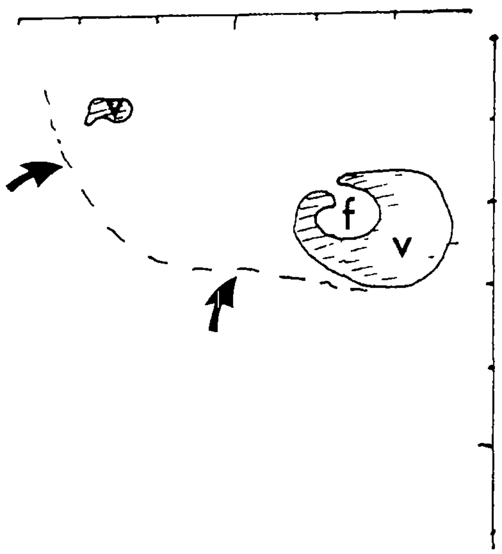


Fig. 6-C: **30 days gestation**

foetus (f) within the vesicle (v)

arrows = uterine mass

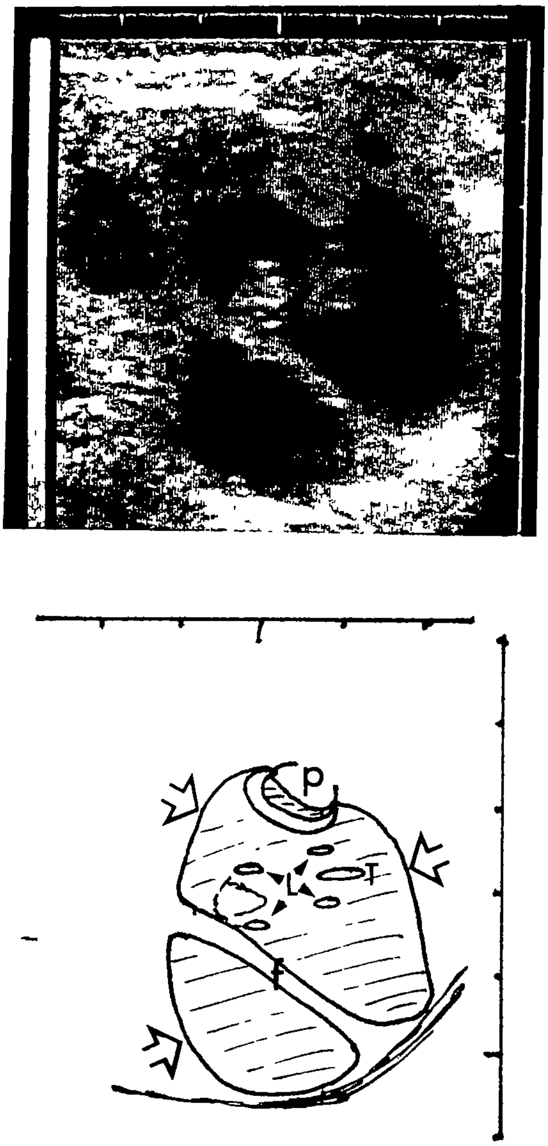


Fig. 6-D: 38 days gestation

foetus + limbs (l) + tail (t)

f = endometrial fold

p = placentome

arrows = vesicle

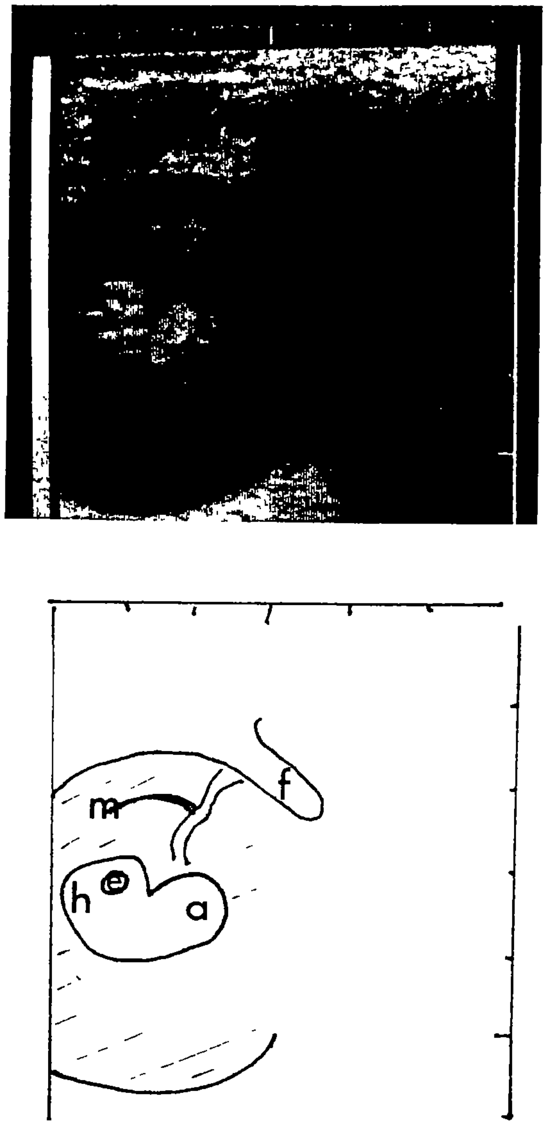


Fig. 6-E: 38 days gestation

Depending on the direction of the probe, a different view of the foetus (compare with Fig. 6-D)

m = foetal membrane

e = eye

h = head

a = abdominal mass

f = endometrial fold

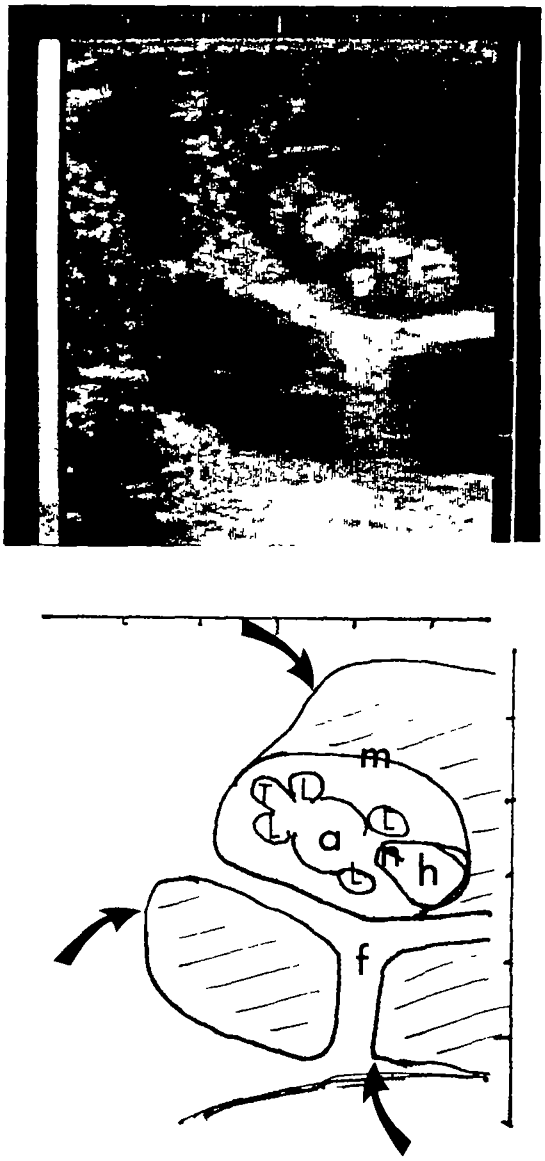


Fig. 6-F: 45 days

vesicle (arrows) - endometrial fold (f)

- foetal membrane (m)

foetal head (h) - nose (n)

abdominal mass (a) - limbs (l)

tail (t)

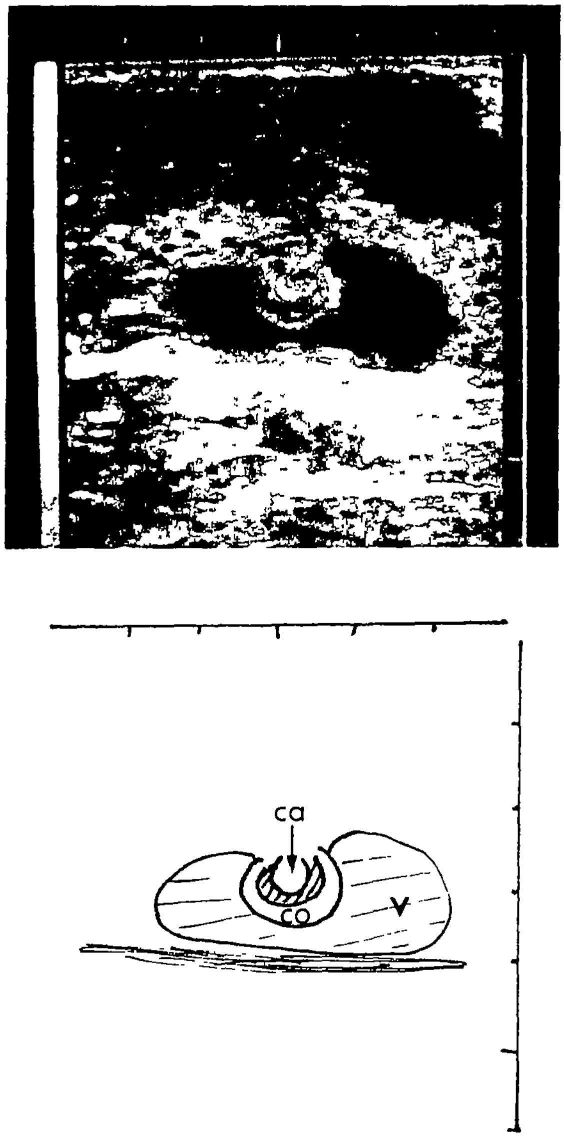


Fig. 6-G: Placentome

37 days, with the caruncle (ca)

and cotyledon (co)

(Placentome = CA + CO)

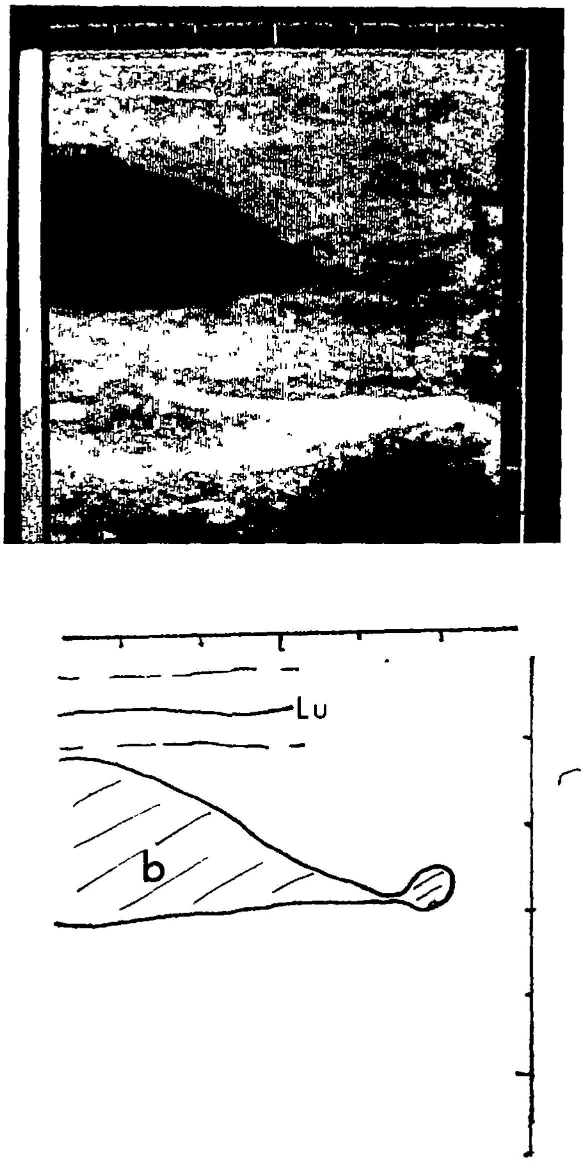


Fig. 6-H: Bladder

False early pregnancy diagnosis may be made if the cranial end of the bladder (b) is mistake for a vesicle.

(**Note:** The lumen of the vagina is seen as a bright line (lu))

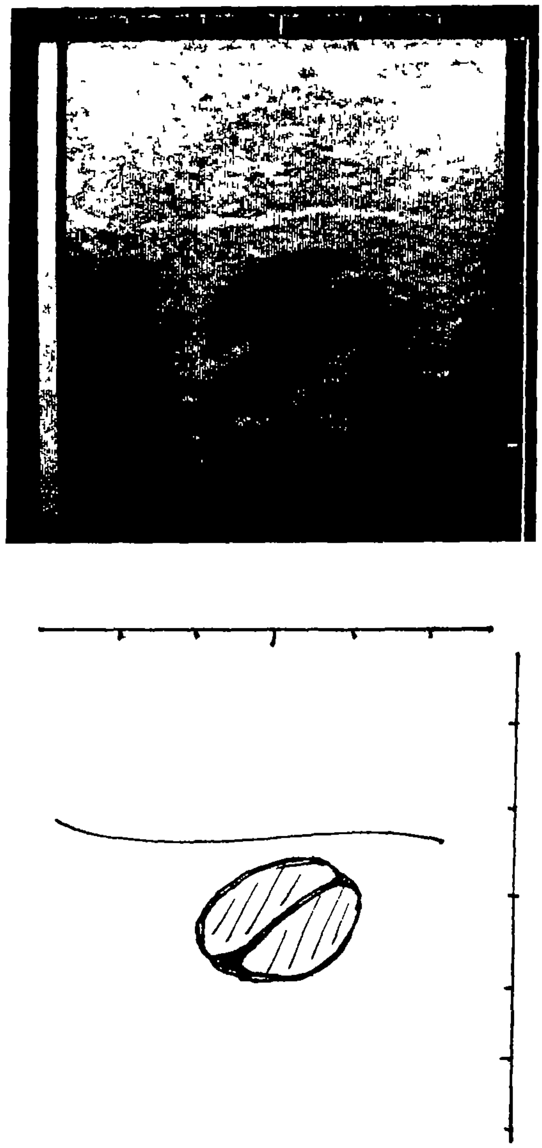


Fig. 6-I: Iliac Blood Vessel

Not to be confused with a 30 days pregnant fluid filled uterus.

Note: Lack of foetal mass.

(Also, on the realtime scan, these vessels are seen to pulse).

Accuracy of pregnancy diagnosis was shown by our data to be as follows:

Days gestation	Accuracy = $\frac{\text{n correct test results}}{\text{n tested}} \times 100$
7 - 20	60%
20 - 30	80%
30 - 42	97%

Therefore for practical purposes, ultrasound pregnancy diagnosis should commence from 30 days gestation.

For ageing the foetus, the optimum period is from 30 to 60 days after mating, allowing enough measurements to be taken as the foetus and the membranes are already well developed, but not yet descended out of reach of the probe.

7. FOETAL AGING

Bingham *et al* (1988) presented quadratic regression equations describing the growth of eleven foetal and associated parameters from 40 to 128 days gestation. In that trial calving dates were predicted for 9 deer with unknown mating dates. On average predictions were 4 days away from actual calving dates.

During 1988,132 red yearling hinds were scanned between May 25 and June 16 and the foetal age prediction equations used to predict individual calving dates. Data is produced here in summary and will be published in full elsewhere (Wilson and Bingham, 1989).

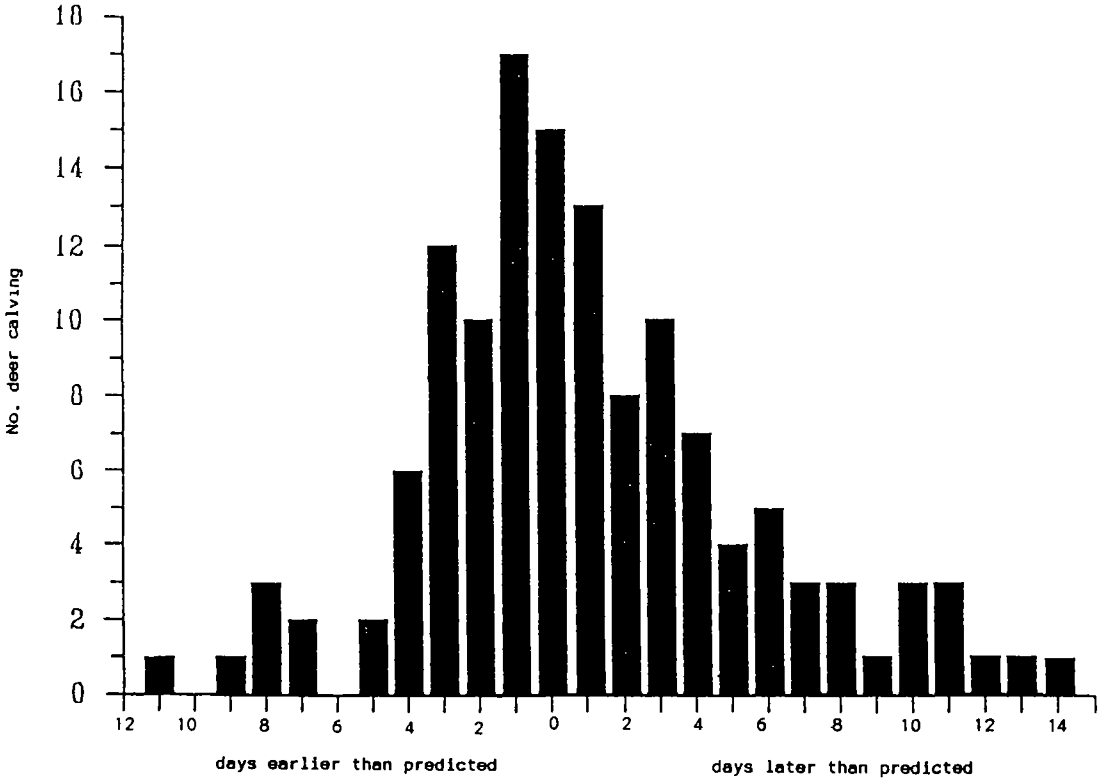
Figure 7 shows the relationship between actual calving date and predicted calving date. On average we were able to predict within 0.97 days of the actual calving dates.

Data indicate that the optimum time for pregnancy detection and foetal aging is between 44 and 60 days pregnancy, where predictions were within 0.4 days of actual calving date, whereas those at 60-80 days were within 0.95 days and those predictions at 80-110 days averaged 4.7 days away from actual calving dates.

The most common number of parameters measurable was two per scan (39%). Three parameters could be measured in 30%, one in 16% and four to six in only 15% of scans. In general more parameters could be measured in the more advanced pregnancies. However, the accuracy of prediction was not dependent on the number of parameters measurable, but depended more on the parameter measurable; e.g. foetal sac dimensions and crown rump provide the most accurate prediction of gestational age, and this is consistent with the errors of regression reported in the paper by Bingham *et al* (1988).

It can be seen, therefore, that foetal age prediction equations are very accurate in practice. Their application is particularly useful for research purposes where conception dates are required, e.g. advanced calving (see Wilson 1989 this Proceedings).

Figure 7: Number of deer calving earlier or later than predicted using foetal age prediction equations.



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