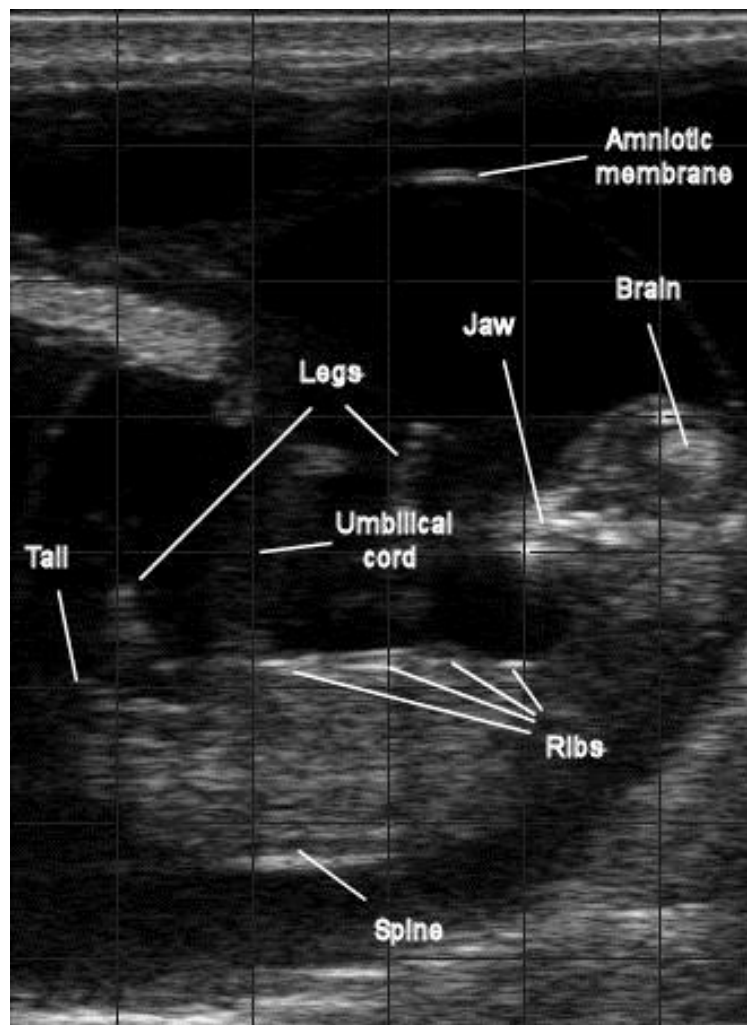


# Foetal Ageing of Farmed Red Deer: A Manual for Scanning Operators

Ian Scott and Geoff Asher, AgResearch



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## **Introduction to foetal ageing**

### **What is foetal ageing?**

Foetal ageing is the assignment of an estimated age of a foetus (in days) at the time of ultrasound scanning within the first trimester (within the first 2-3 months) of pregnancy. During this period the foetus undergoes a uniform growth and development pathway that can be easily visualised through real-time ultrasonography. With practice and regular calibration, ultrasound scanners can assess foetal age from the real-time images of the foetus and associated tissues of pregnancy.

### **What is the value of foetal ageing?**

An assessment of foetal age at time of scanning provides a valuable piece of information on the hind: an estimate of the date she conceived. This is calculated simply by subtracting the foetal age (in days) from the date of scanning. In the case of stud herds, if entered for the hind in question into DEERSelect, DEERSelect can rank her on her early conceiving/calving potential and assign an estimated birth date to her progeny.

In the case of commercial herds, this information can be used by producers to manage calving groups by assigning hinds to groups based on expected calving date.

The data can also be used to check the success of artificial insemination ('AI') by enabling a producer to determine whether AI carried out on a particular day was successful, and whether particular sire stags have performed in their duties during the rut, by identifying which stag out of a sequence (where records were kept of stag movements) actually served the hind.

### **Assigning conception date of hinds in DEERSelect**

This will become increasingly important for genetic selection programmes for two reasons:

- (1) There is a genetic basis to reproductive seasonality in deer; conception date in hinds is moderately heritable. It is therefore possible to select our breeding herds for an earlier calving season to better match pasture growth with feed demand by deer. The only reliable phenotypic measure of this trait is conception date based on foetal ageing for each breeding hind. This requires that hinds are joined early (i.e. before 10 March) so that they can express their true phenotype (i.e. hinds may cycle early but if stags are not present the hinds will not show evidence of this trait).
- (2) Birth date – which is more reliably estimated from estimated conception date than attempted physical monitoring of birthing - is valuable for accurate determination of breeding values for traits such as growth rate. Where a birth date is estimated without reference to a conception date for a progeny crop, calculated growth rates related to weaning date favourably bias the calves born earlier than the estimated date and unfavourably bias the calves born later than the estimated birthing date. Conception date profiling of hinds by foetal ageing will provide a more reliable proxy birth date for each calf, such that other DEERSelect data on the calf will be more reliable as a whole. While this may

not be 100% accurate (because there is a degree of natural variation in gestation length), it is a considerable improvement over the present situation of assigning a standard birth date.

### **Conception date profiling of the herd for calving management**

Commercial farms can benefit from improved management of the breeding herd over calving through the use of conception date data. The ability to draft pre-calving hinds into management groups based on expected calving dates (calculated simply by adding 234 days to the estimated conception date for red deer and 240 days for red x wapiti hybrids) facilitates improved feed management by enabling rotational grazing of hind/calf mobs. Delayed set stocking of later calving hinds allows pasture harvesting options and optimisation of feed quality for this mob. Separation of early and late calving hind mobs improves calf survival. A farmer planning precise rotational grazing will know when a mob of hinds and calves can safely be moved between paddocks if a small range of calving dates applies to the hind mob. Foetal ageing at scanning provides the necessary data for the farmer to make these decisions.

### **Checking the success of AI programmes**

Although ultrasound scanning does not influence the success of AI programmes, it does give an early measure of success and facilitates future herd management (such as drafting of non-conceiving hinds). All deer AI programmes involve the artificial synchronisation of hind oestrus (heat) for fixed-time insemination. Hinds that fail to conceive to AI will generally return to oestrus and be mated to the back-up stag 18-21 days later. This time interval between AI conception and follow-up mating is crucial to the use of ultrasound scanning to assess the effectiveness of the AI. Scanning is normally performed 40-60 days after AI, with success of AI being based on the presence of an appropriately aged foetus. Hinds conceiving to back-up stags will either be not detectably pregnant (i.e. if pregnant the pregnancy is too young to detect by ultrasound: <20 days) or there will be a very clear age gap of about 20 days.

### **How accurate is foetal ageing?**

With practice, good scanning operators will achieve an accuracy of +/- 5 days of actual conception date. However, this accuracy is dependent on the timing of scanning; it can realistically be achieved when the foetus is between 30 and 70 days of age (late April to early June). Before this period, early stage pregnancies can be difficult to detect and the actual foetus is easily overlooked. After this period, foetal growth and development becomes more variable and it also becomes difficult to acquire suitable images of diagnostic features. Under rectal scanning, larger foetuses often drop below the field of view after 70-80 days. Within the first 70-80 days of gestation there is little effect of foetal genotype on the rate of foetal development, therefore no adjustments are required for wapiti-type animals.

### **What training is required by scanning operators to do accurate foetal ageing?**

The deer farming industry is well serviced by commercial ultrasound scanner operators undertaking routine pregnancy diagnostics. In most cases, additional training and practice will enable most existing operators to accurately perform foetal ageing. The purpose of this manual is to assist scanning operators in assigning foetal ages through calibration of their observations with images collected from known-age foetuses. As foetal ageing is conducted early in pregnancy, it is most likely

to be commercially adopted by operators using rectal ultrasound equipment rather than by those that use flank scanners.

### **How much will it cost compared to normal pregnancy diagnoses?**

It is important to negotiate the costs of foetal ageing according to need. Accurate assignment (i.e. +/- 5 days) for purposes of recording data on DEERSelect (i.e. hind conception date and calf birth date) takes longer than a simple pregnancy diagnosis and will likely cost more. However, less accurate assessments for purposes of assigning hinds to 'early' and 'late' calving groups can generally be performed more quickly and at lower cost.

### **Tips for foetal ageing**

1. Try to organise the scanning date to occur during the optimal window of between 30-80 days of pregnancy. This will usually mean scanning mid-May to early June.
2. Diagnosis of non-pregnant hinds is most accurate from 30 days after stag removal (all 'empty' hinds should be rescanned 20-30 days later).
3. Age foetuses into 5-day groupings (as presented in this manual) rather than trying to assign a specific age to each foetus. At best, operator accuracy is  $\pm 5$  days anyway.
4. Placentome size varies enormously, even within an individual hind, so don't rely on this for ageing. Try to visualise at least part of the foetus.
5. Sometimes it is not possible to obtain a clear image of the foetus (e.g. due to faeces or gas). In such case remove the probe from the rectum, clean faecal matter off the probe, apply more lubricant and try again. **Do not apply excessive downward force to the probe as this may damage the rectum.**

## About this Manual

### Image generation

This manual is a pictorial presentation of actual images of known-age red deer fetuses from 20 to 80 days of age.<sup>1</sup> The images were obtained using a BCF Easi-Scan real time scanner fitted with a 5 MHz linear array rectal transducer. Mode selection was set at 'Ovary/Early Pregnancy' and the grid display was set at 1 cm.

Identical images are presented adjacently with and without annotation.

An attempt has been made to collate 'typical' freeze images for each age group. However, real time ultrasound imaging is 'dynamic', with the 2-dimensional plane of the ultrasound transducer slicing quickly through the conceptus to create a mind's-eye view of the 3-D structures. The 'art' of foetal ageing lies in a rapid determination of this 3-D image through which its size, shape, and other morphological landmarks can together be treated to indicate age. This is further complicated by the particular orientation of the foetus as it is being scanned: is it in cross-section, longitudinal section or oblique section (see below for definitions).

Competency in foetal ageing improves with practice as one starts to develop the mental picture of the 3-D image from what can be seen on the screen. An experienced operator can make an accurate judgement of foetal age within 10 seconds of locating the foetus or other diagnostic structures. This manual, therefore, is designed to help operators calibrate their assessments.

### Orientation of the image

The term '*longitudinal*' refers to an image that is obtained by slicing the foetus from cranial (head) to caudal (tail) and may be:

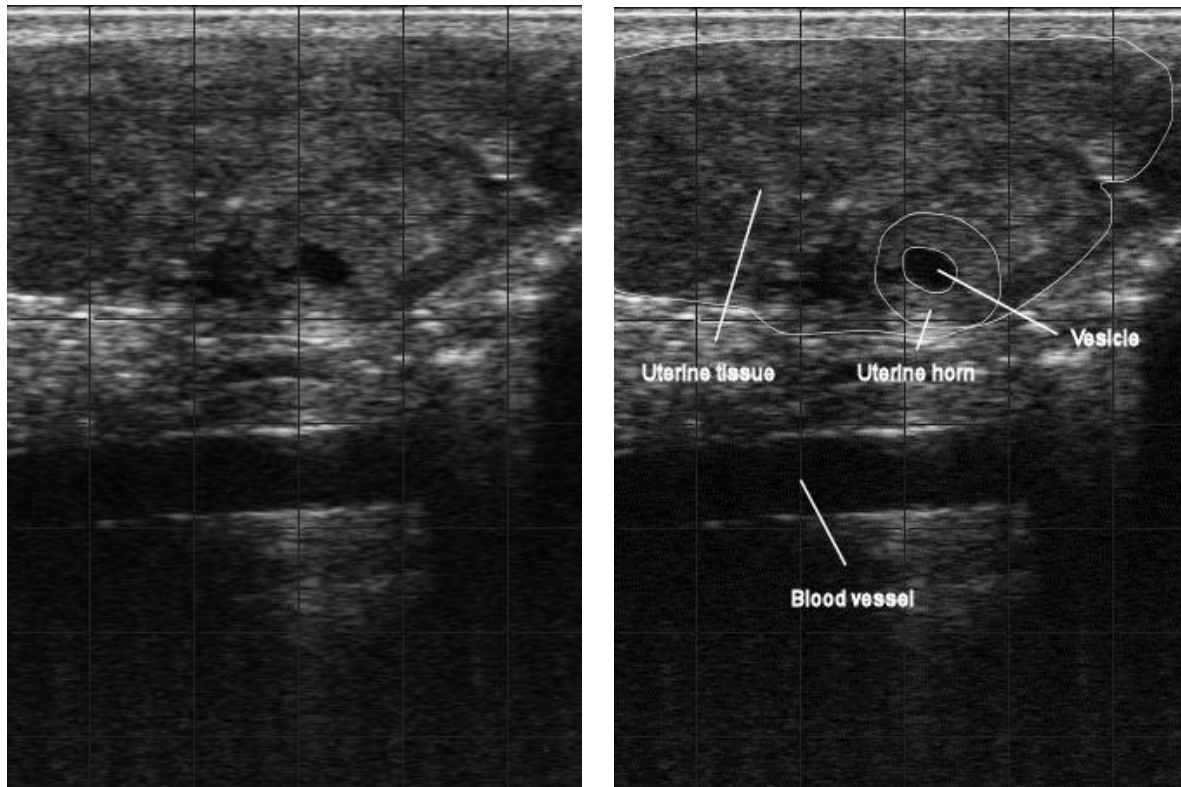
1. Sagittal – a longitudinal plane that slices the foetus from ventral to dorsal (belly to spine) dividing the body into right and left halves.
2. Dorso-ventral – a longitudinal plane that slices the foetus from side to side (laterally) dividing the body into lower (ventral) and upper (dorsal) halves.
3. Oblique – a longitudinal plane that slices through the foetus at an angle.

The term '*cross-section*' refers to an image that divides the body into cranial and caudal (head and tail) portions (i.e. it is perpendicular to the longitudinal plane). A cross-section may also be at an oblique angle.

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<sup>1</sup> Since within the first 70-80 days of gestation there is little effect of foetal genotype on the rate of foetal development, no adjustments are required for wapiti-type animals.

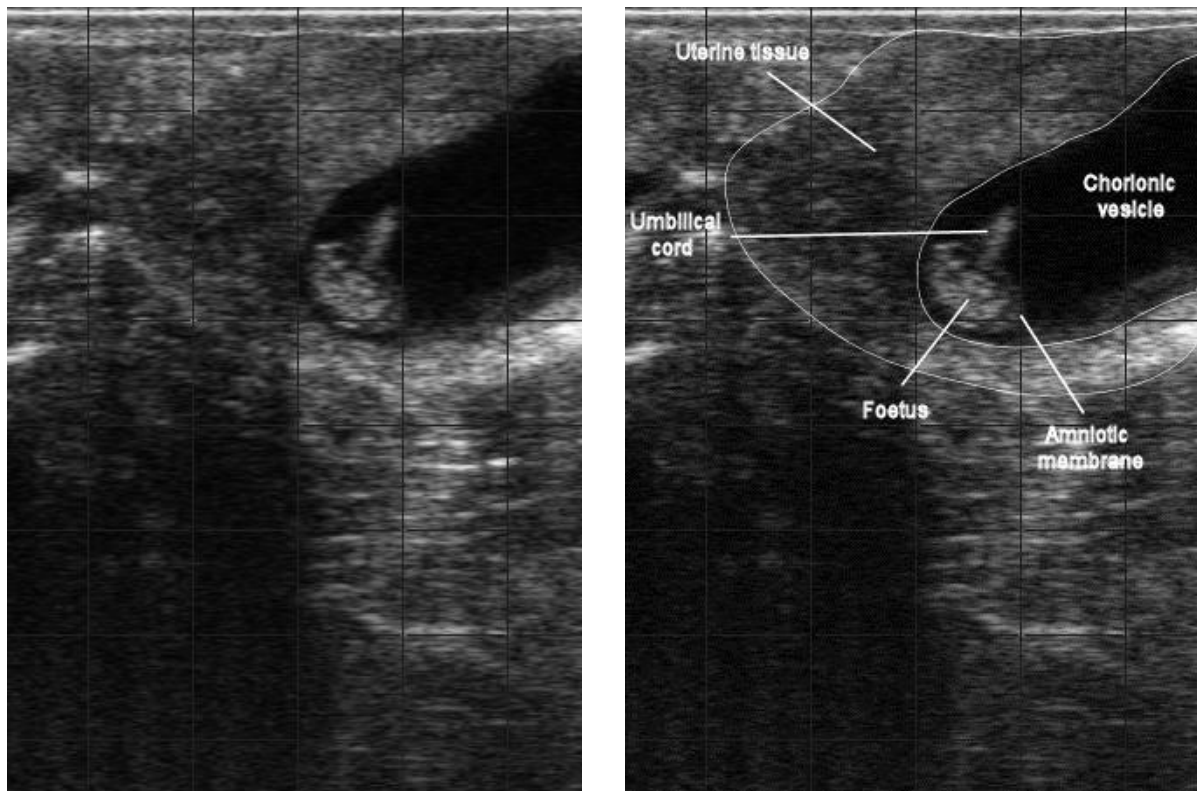
## Day 20



**Figure 1.** Chorionic vesicle of Day 20 pregnancy

Day 20 is approximately the time when the first visible signs of pregnancy can be detected by ultrasound. However, assessment of foetal age at this stage is unreliable as the appearance of the fluid-filled vesicle can be easily overlooked or confused with other structures such as ovarian follicles (see later images). The embryo itself is not visible. Diagnosis of pregnancy at this stage should be considered as tentative and it would be preferable for the hind to be scanned again 20-30 days later.

## Day 30



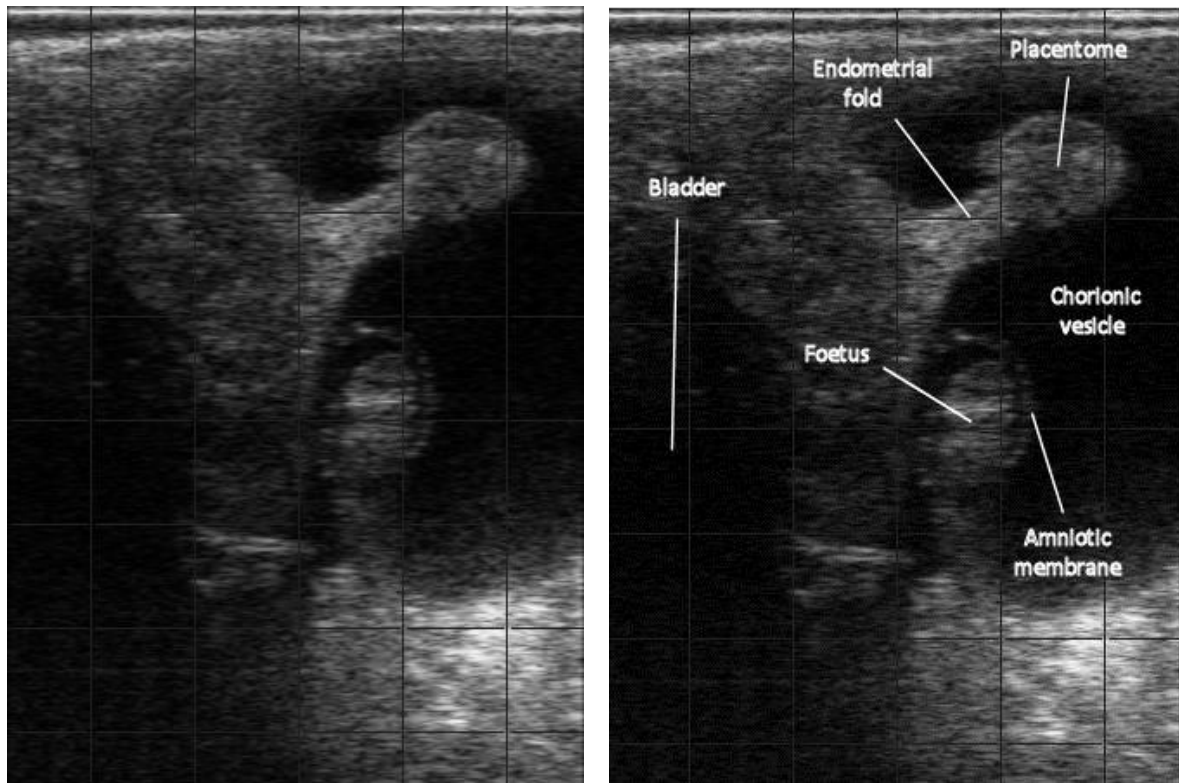
**Figure 2.** Sagittal plane of Day 30 foetus

Day 30 represents the stage when a positive pregnancy diagnosis and a foetal age estimate can be reliably made. The foetus has firmly attached to the uterine wall via the umbilical cord and is clearly surrounded by the amniotic membrane. The foetus, which is about 10-12 mm long and shaped like a comma, has a rapid heartbeat. There is limited differentiation of the head and torso, and limb buds are generally not discernible.

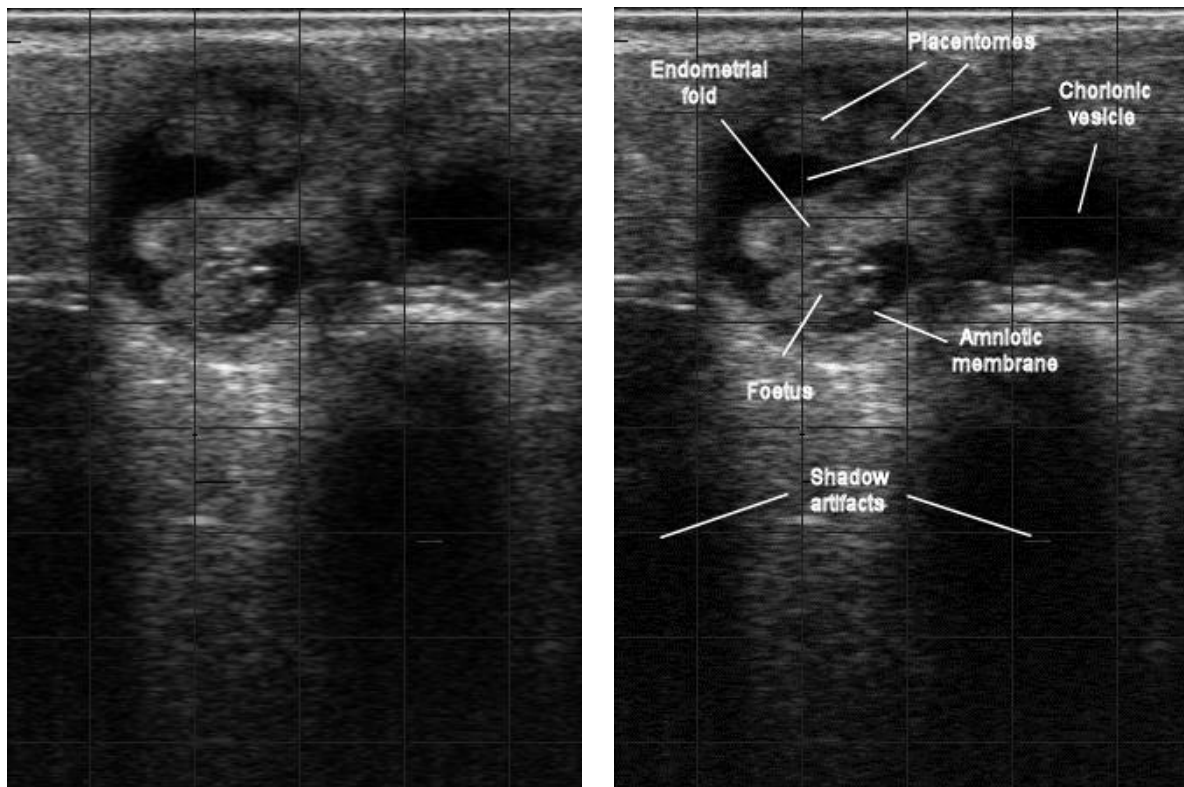
Landmarks: Size and shape of the foetus.



## Day 35



**Figure 3.** Sagittal plane of Day 35 fetus

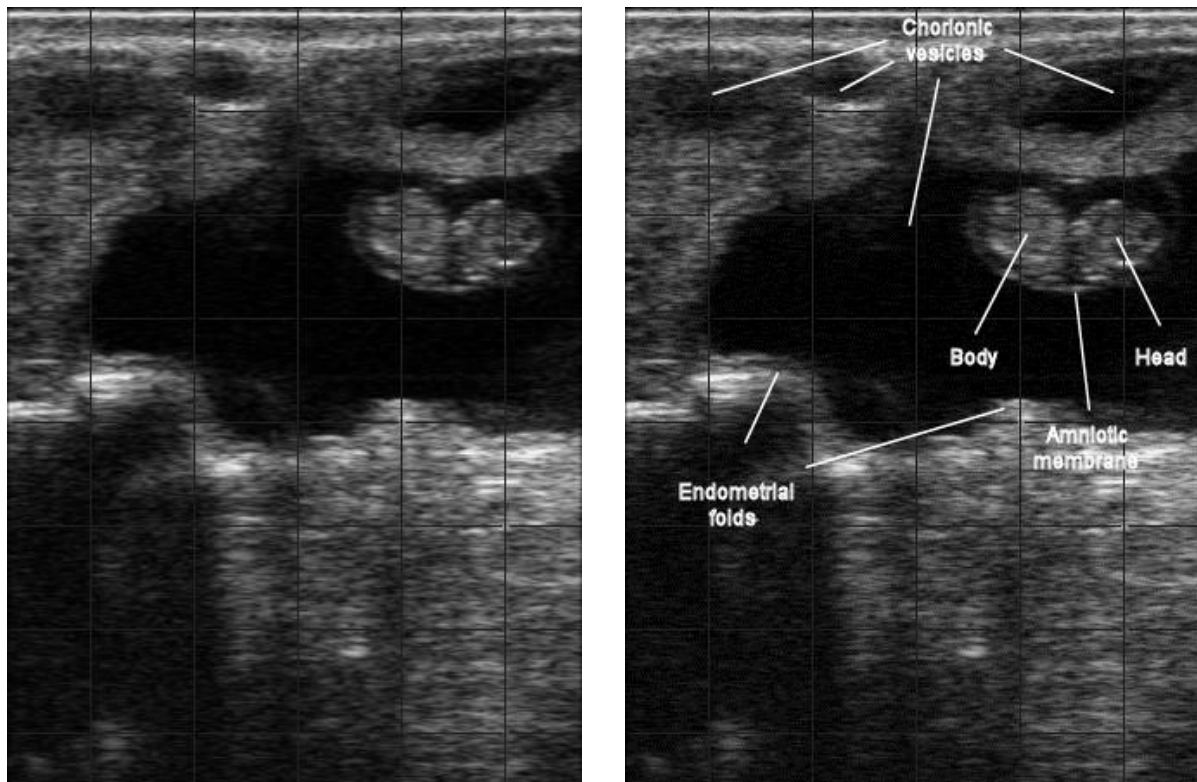


**Figure 4.** Cross-section plane (slightly oblique) of Day 35 fetus

The foetus is about 15mm long. While the head has become enlarged and rounded, there is no clear differentiation of head structures (such as nose or eyes). Limb buds are often visible in dorso-ventral scanning planes. There is often complex convolution of the chorionic vesicle appearing at this stage, necessitating a careful scan through the entire uterus to locate the foetus. Placentomes (maternal/foetal contact structures) start to become obvious at this stage, but their size, number and stage of development are highly variable during early pregnancy, negating their usefulness in foetal ageing.

Landmarks: Size and shape of the foetus.

## Day 40



**Figure 5.** Sagittal plane of Day 40 foetus

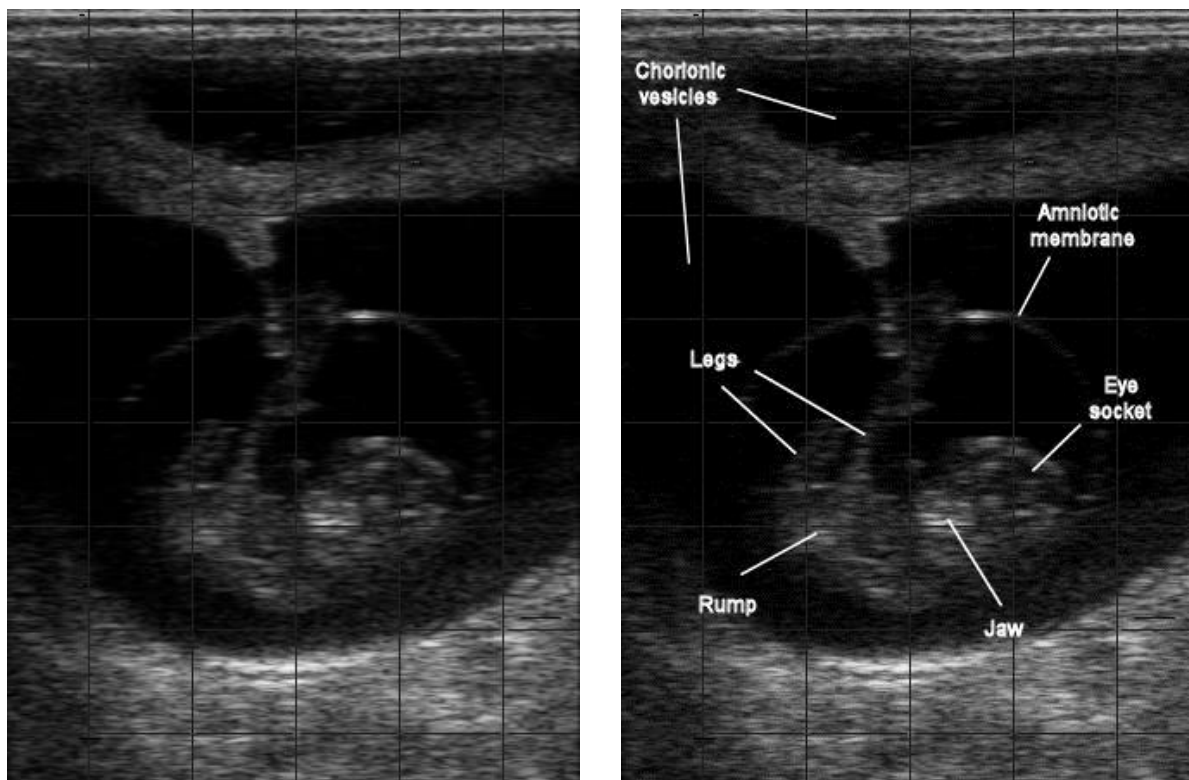
The foetus is about 20mm long. Facial structures on the head start to become discernible. Limb buds are clear in dorso-ventral view. At this stage it is fairly easy to detect a heart-beat.

Landmarks: Size and shape of the foetus.

## Day 45



**Figure 6.** Dorso-ventral plane (slightly oblique) of Day 45 foetus.

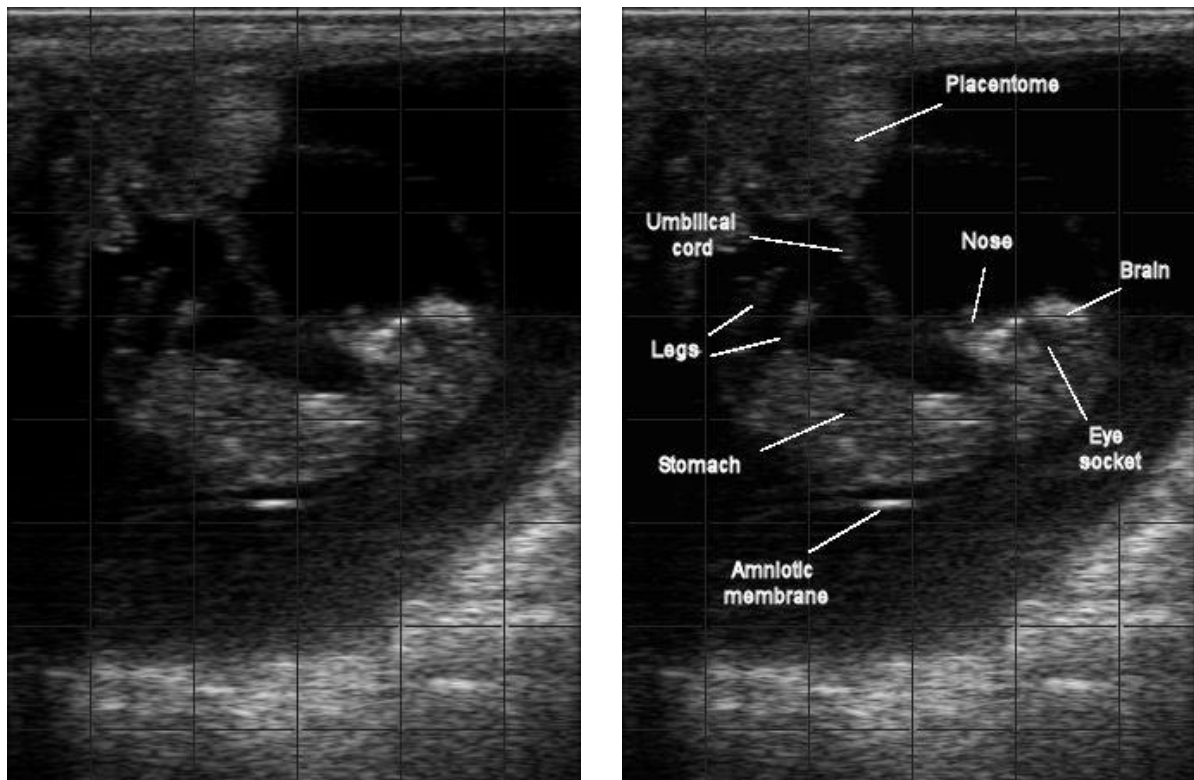


**Figure 7.** Oblique sagittal plane of Day 45 foetus

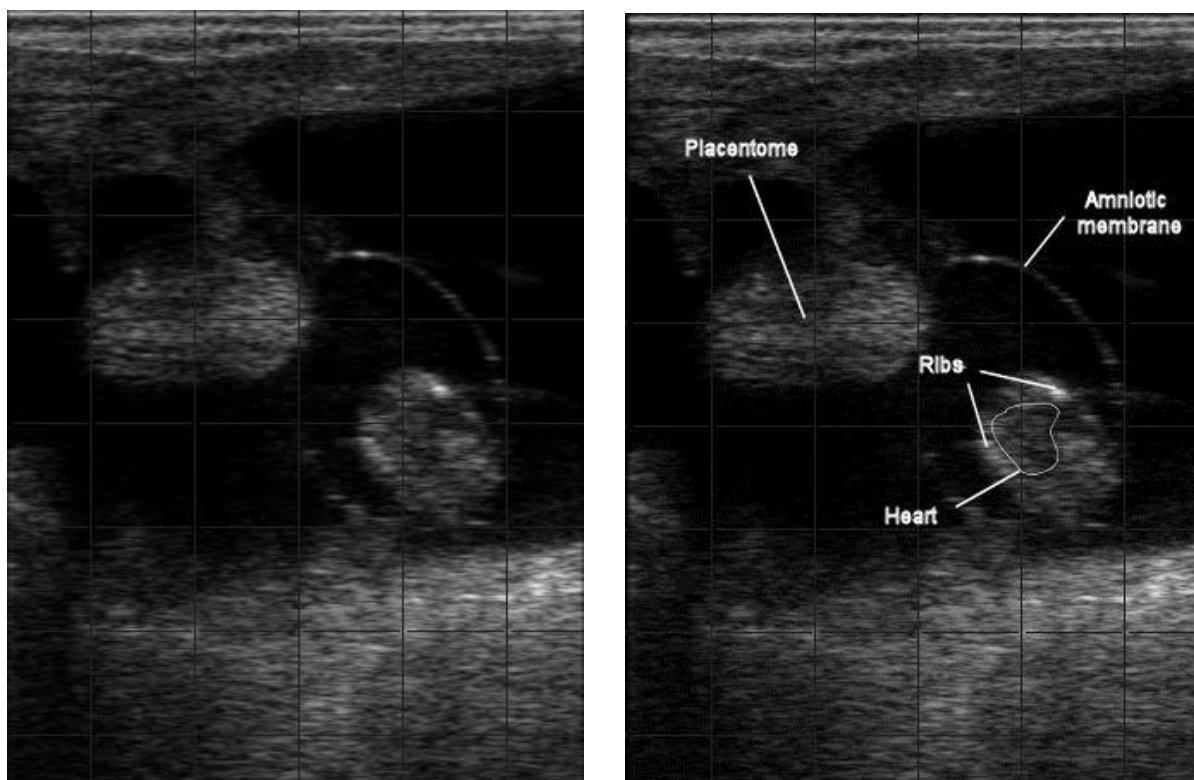
This is one of the most reliable diagnostic stages for pregnancy diagnosis due to the shape and size of the foetus. The foetus is about 30-35mm long, with clear signs of differentiation of the limbs, tail and head. The eye socket and jaw are often clearly visible, with the overall shape of the head starting to resemble that of a deer (although still very rounded). A heart-beat is easily detected from Day 45 onwards.

Landmarks: Size and shape of the foetus, and particularly the shape of the foetal head.

## Day 50



**Figure 8.** Oblique sagittal plane of Day 50 fetus

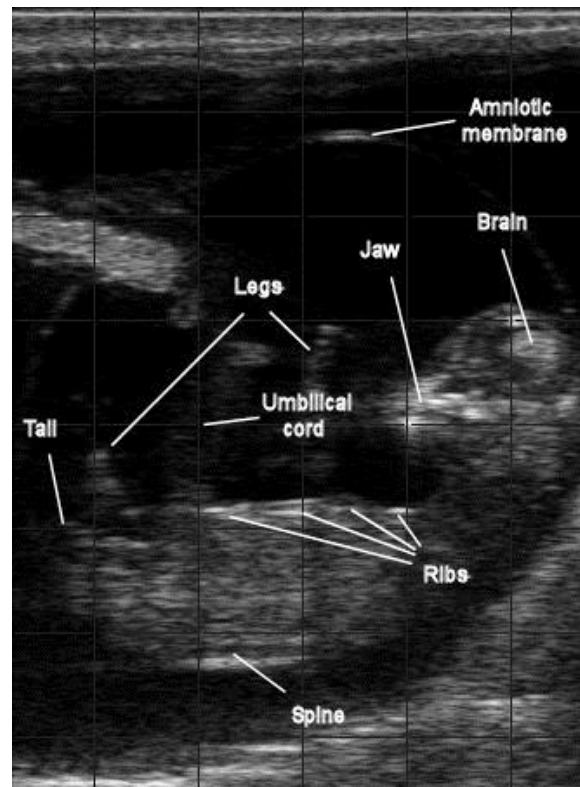


**Figure 9.** Cross-section plane through chest of Day 50 fetus

The foetus is 40-45mm long. There is clear elongation of the snout and differentiation of the eye socket. The developing brain is often discernible in both sagittal and cross-sectional images. Also, differentiation of some internal organs can be seen within the torso. There is often some calcification of rib bones appearing in cross-sectional images.

Landmarks: Foetal head shape and size; some calcification of ribs.

## Day 55



**Figure 10.** Sagittal plane of Day 55 fetus



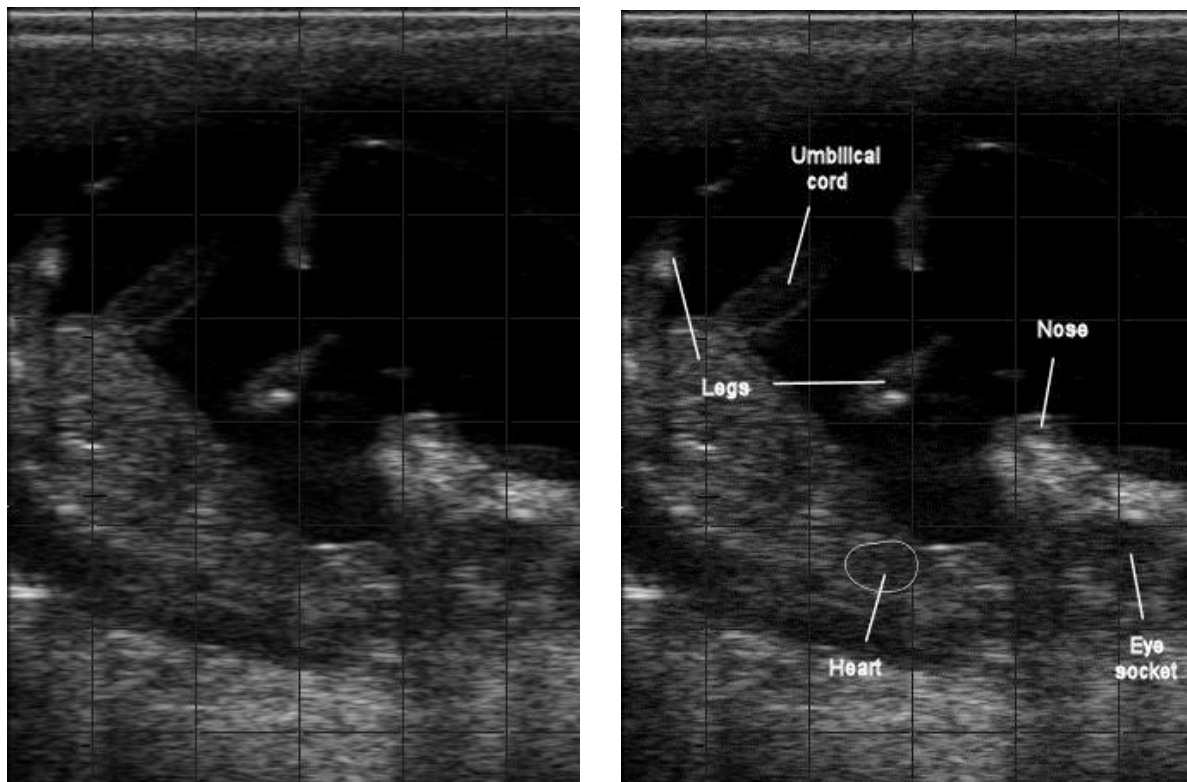
**Figure 11.** Oblique sagittal plane of Day 55 fetus



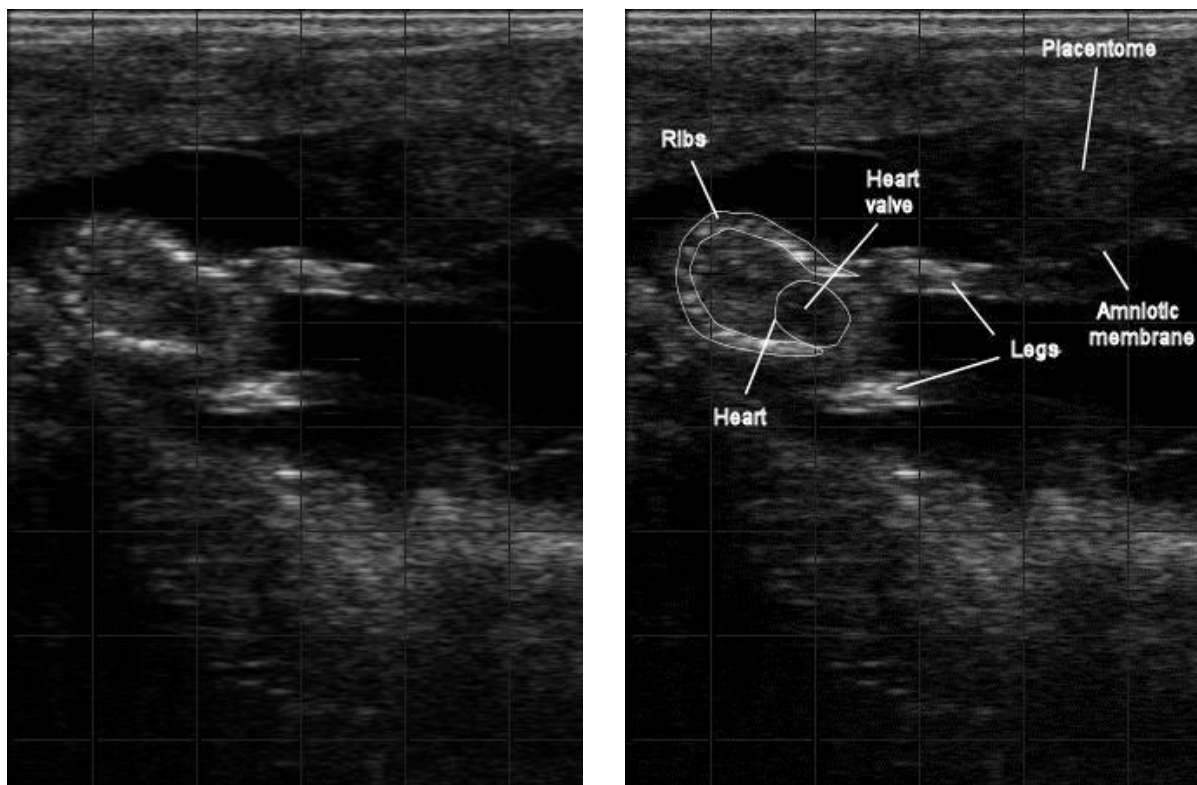
The foetus is about 60mm long and the foetal head is about 20mm long from snout to posterior cranium. There is clear evidence of progressive calcification of jaw/nasal bones, the cranium, the ribs and the spine. The brain is also clearly differentiated on many images. Internal organs are often clearly delineated. At this stage the foetus often fully occupies the width of the scanner screen if in sagittal section.

Landmarks: Size and early calcification of the head.

## Day 60



**Figure 12.** Sagittal plane (slightly oblique) of Day 60 fetus

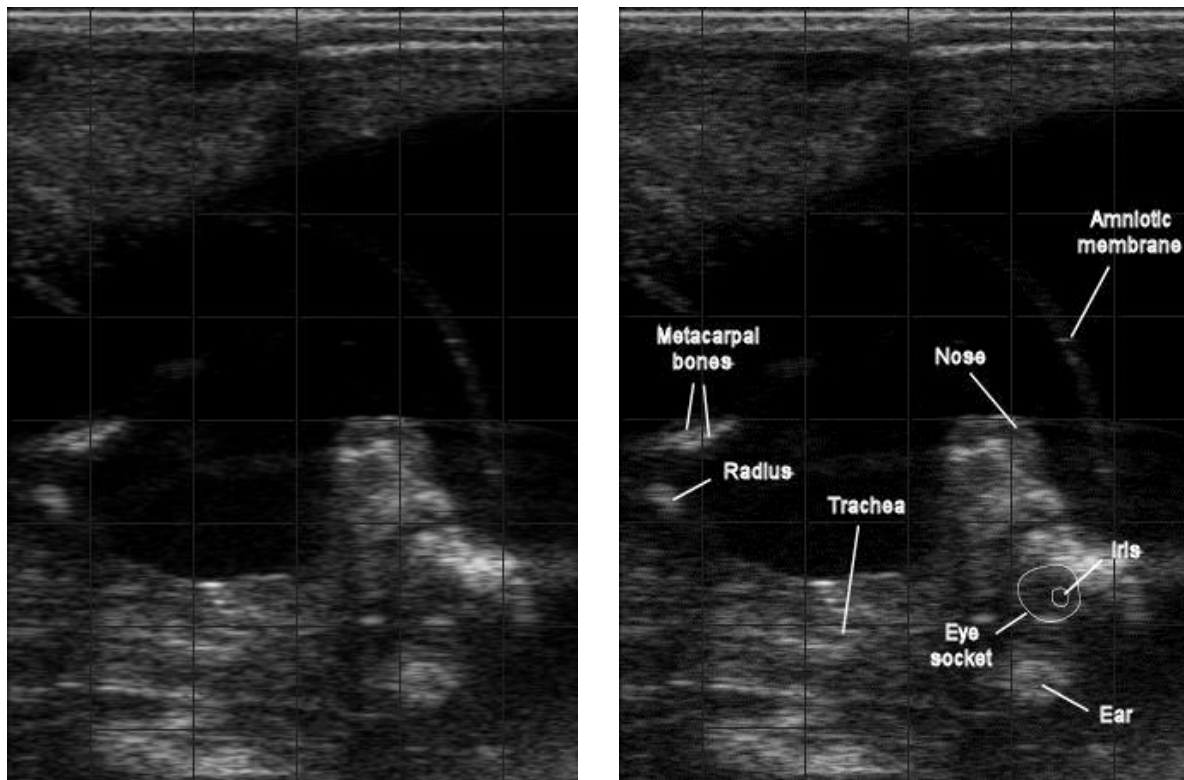


**Figure 13.** Cross-section plane through chest of Day 60 fetus

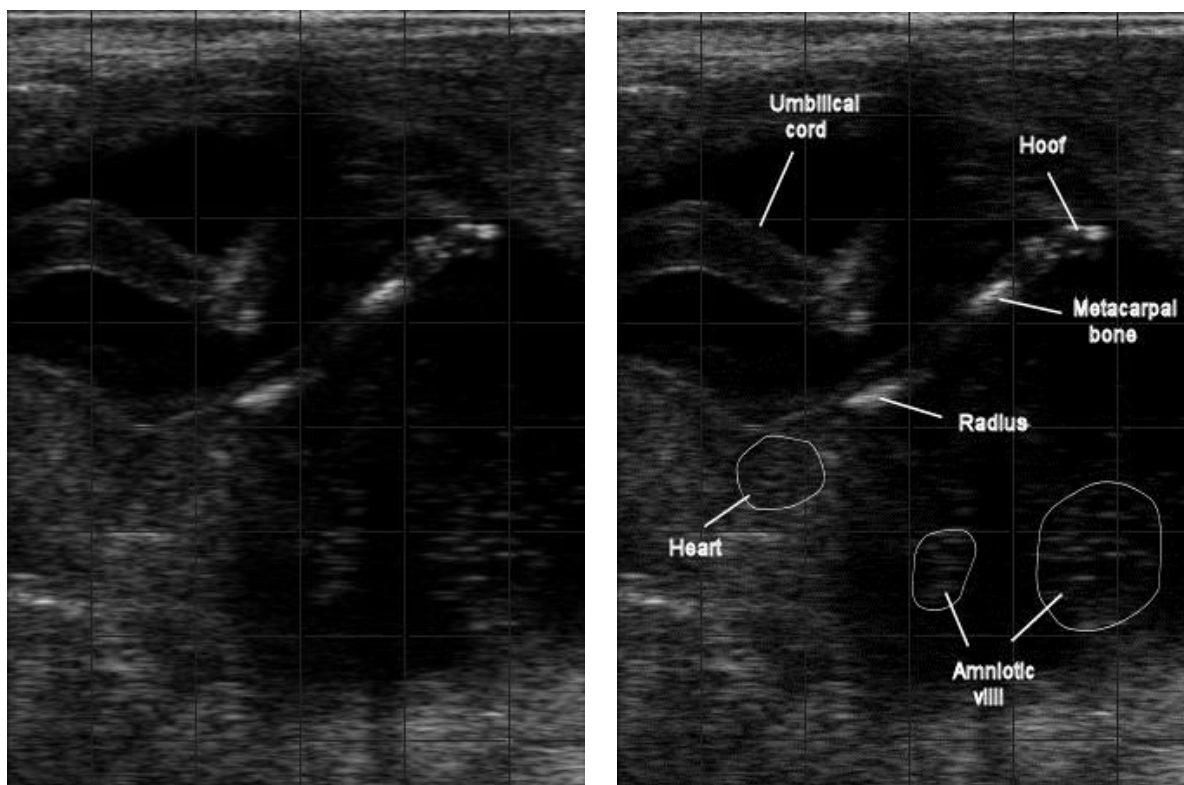
The foetus is about 70-75mm long and the foetal head is about 30mm long. It is becoming increasingly difficult to get a whole longitudinal image of the foetus on the screen. The head and limbs are probably the most diagnostic feature from Day 60. The head shows considerable calcification, particularly around the jaw and eye region. The long bones of the elongating limbs show the first signs of calcification. Cross-sectional images through the torso clearly display calcified ribs.

Landmarks: Size and calcification of the head; early signs of calcification of the limb long bones.

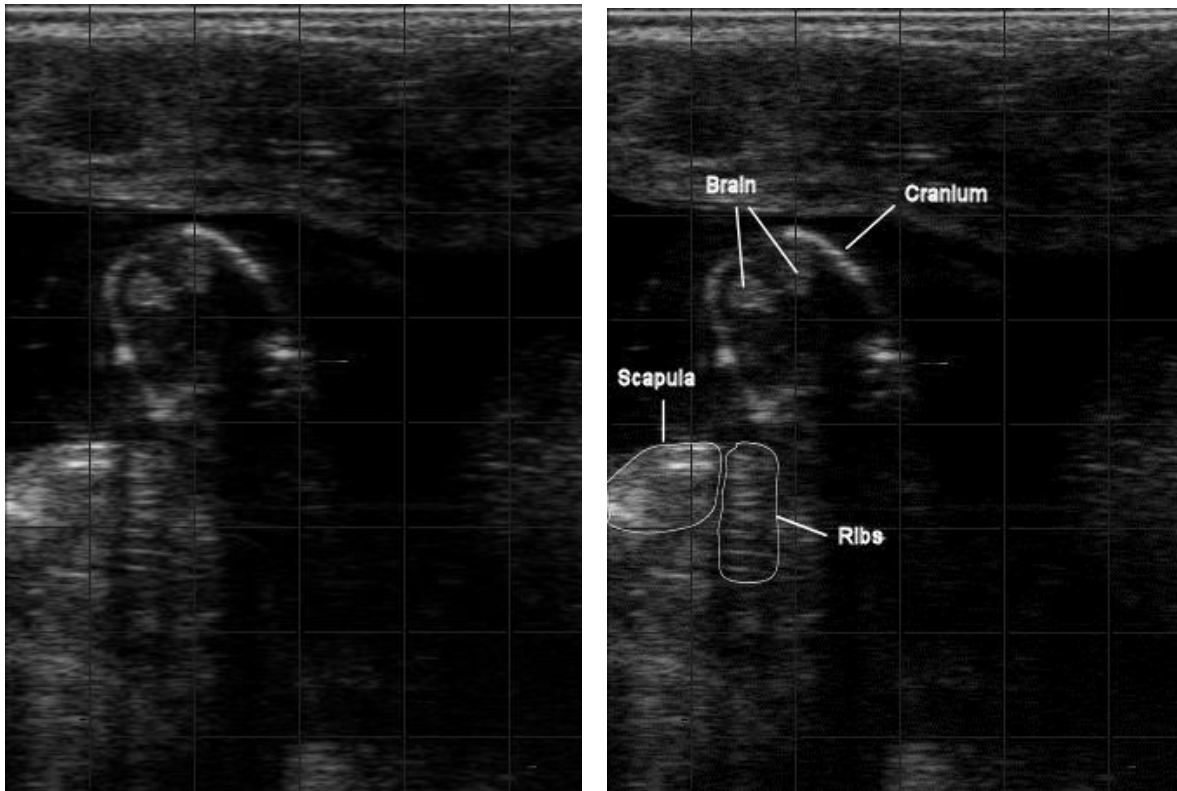
## Day 65



**Figure 14.** Sagittal plane of head and neck region of Day 65 fetus



**Figure 15.** Oblique sagittal plane of fore-limb and chest of Day 65 fetus



**Figure 16.** Dorso-ventral plane through the skull of Day 65 foetus

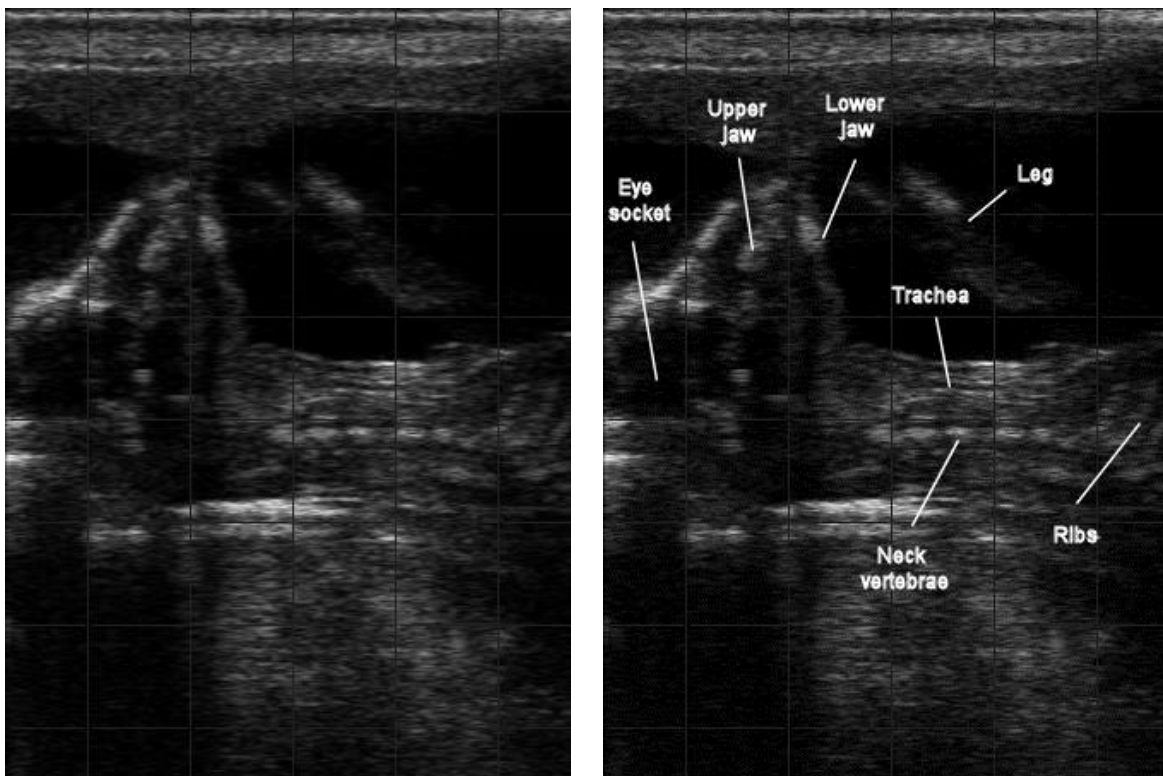
A whole longitudinal foetal image is not possible, but the head and limbs provide the key diagnostic features from Day 65 onwards. The head is 35-40mm long and extensively calcified, including the cranium when viewed in cross-section. The limbs show considerable elongation and progressive calcification. The hooves are often clearly visible.

Landmarks: Size and calcification of the head; elongation and calcification of the limbs.

**Day 70**



**Figure 17.** Sagittal plane through torso of Day 70 fetus.

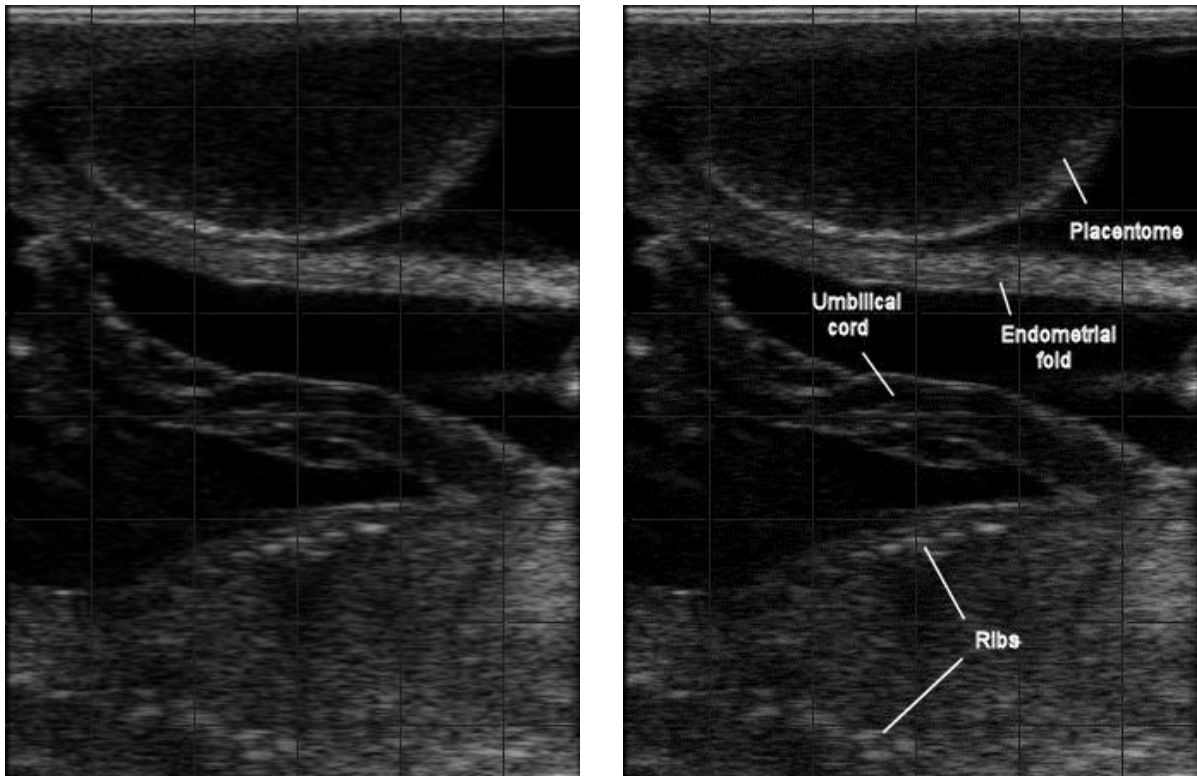


**Figure 18.** Sagittal plane through neck and head of Day 70 fetus

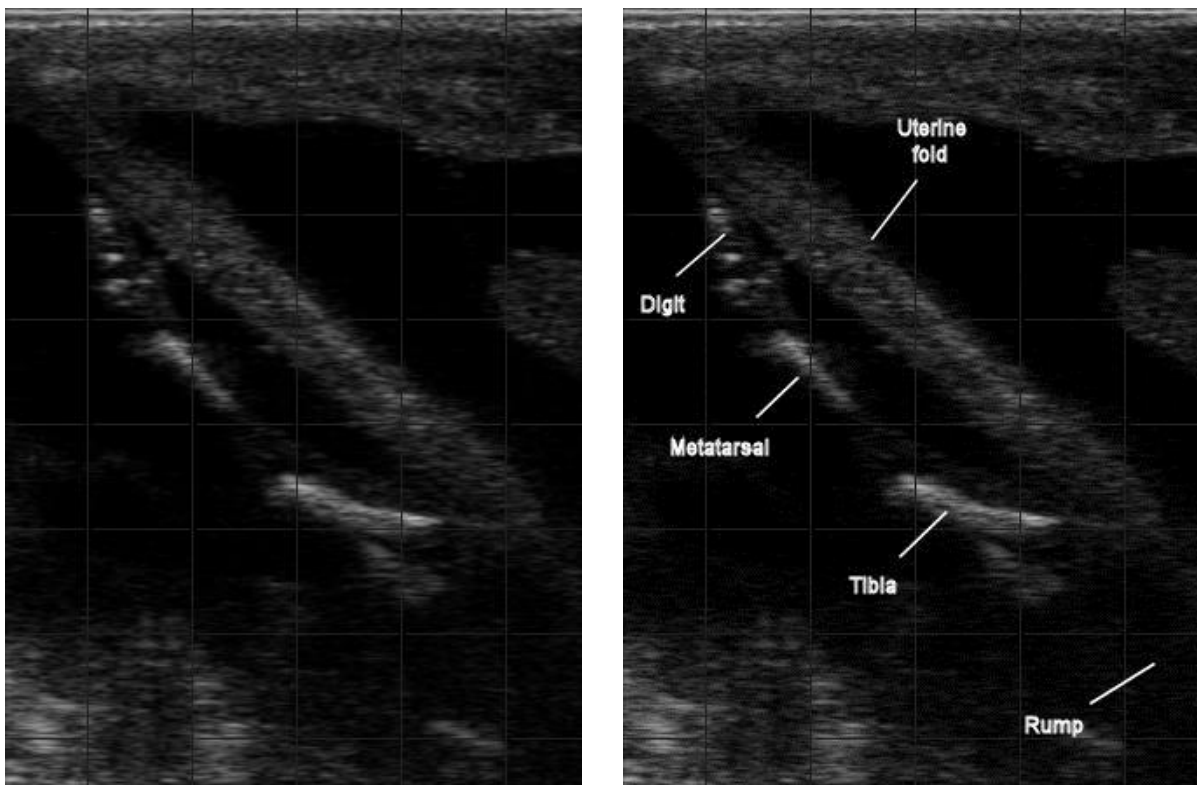
The head is about 45mm long, with very clear differentiation of components of the calcified skull. Spine, rib and long bones are clearly visible. Calcification of the limb long bones about 50% complete.

Landmarks: Size and calcification of the head; clear delineation of the bones in the skull and neck.

## Days 75 and Day 80

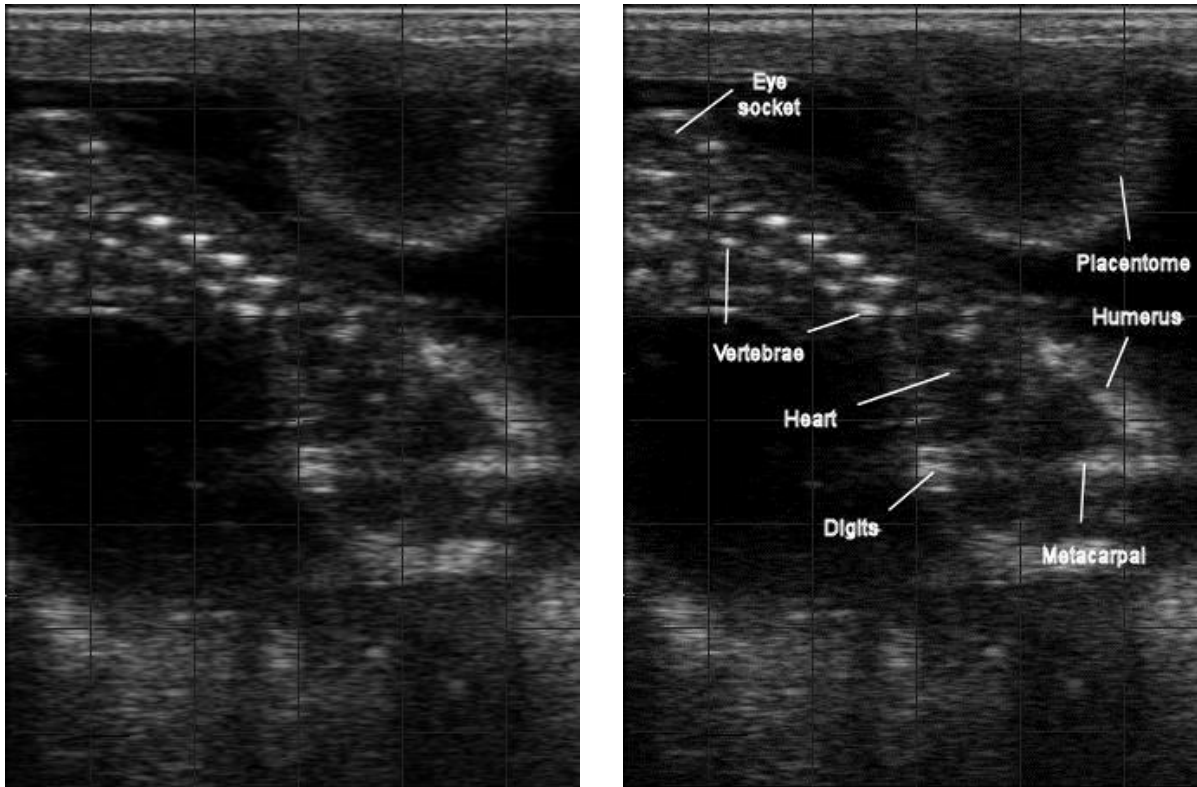


**Figure 19.** Oblique sagittal plane through chest of Day 75 foetus

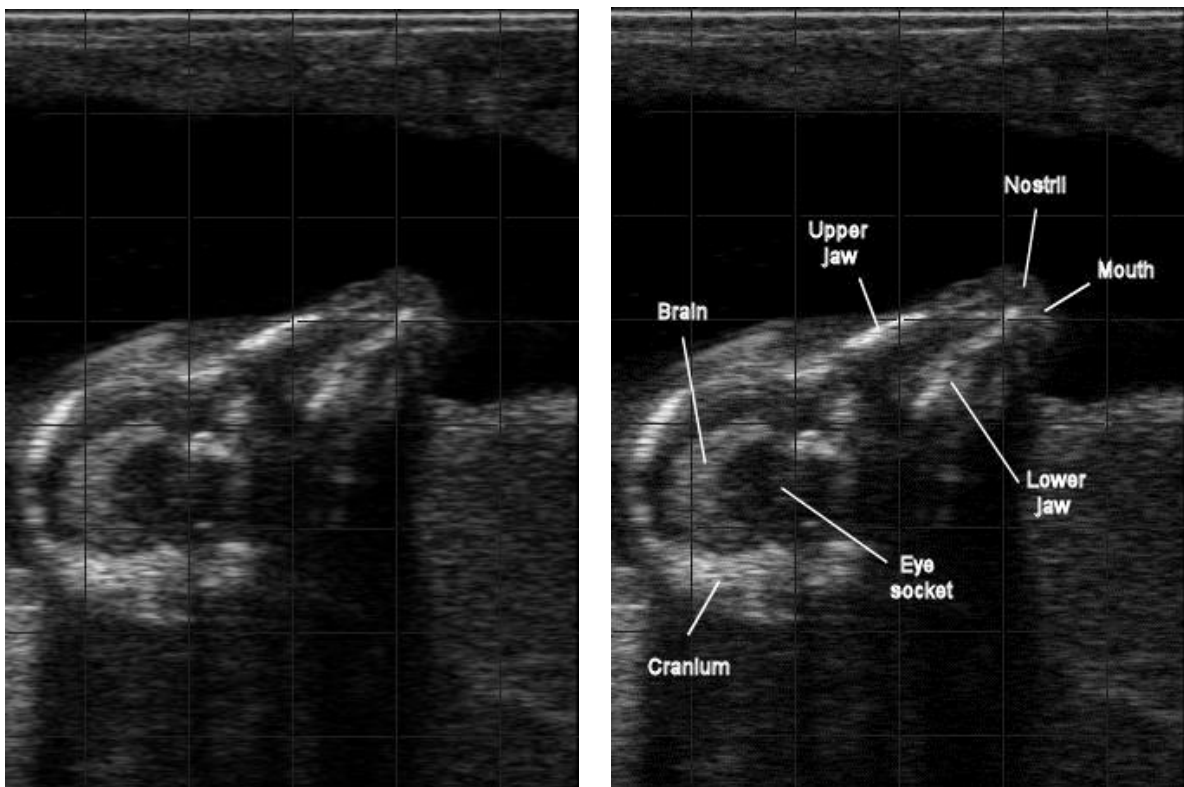


**Figure 20.** Sagittal plane through hind-limb of Day 75 foetus

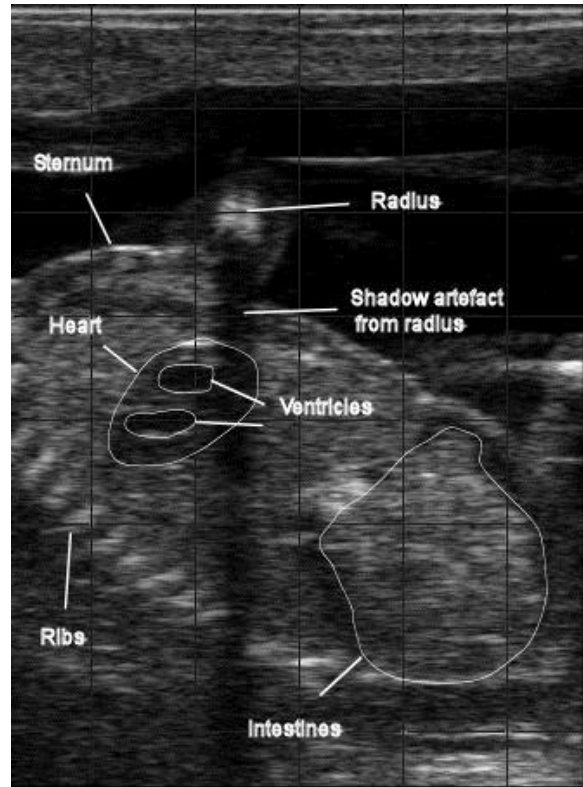
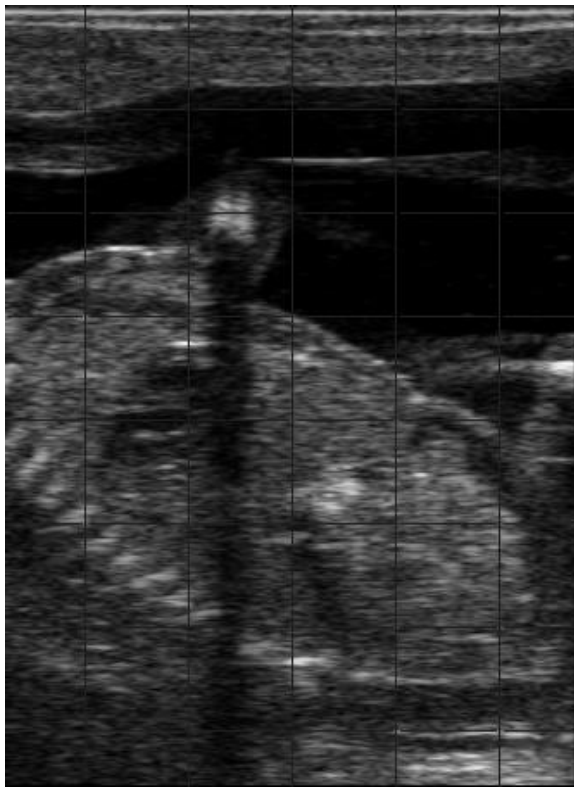




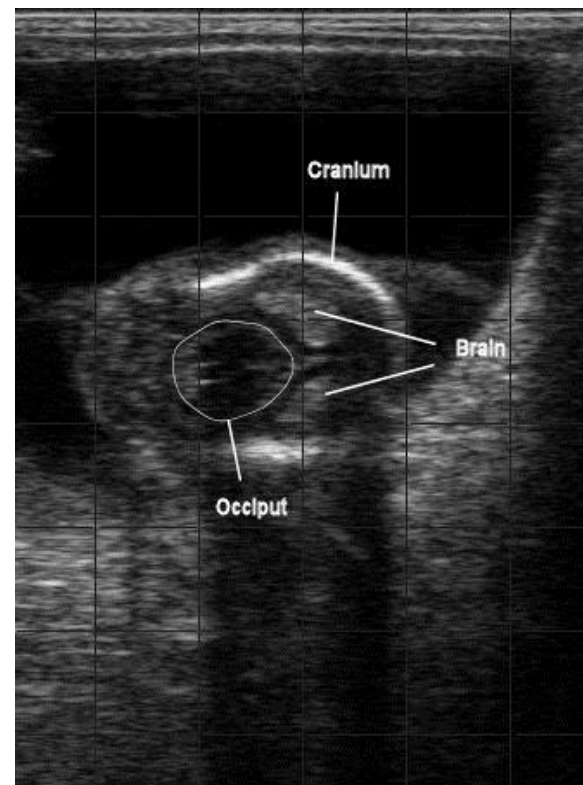
**Figure 21.** Dorso-ventral plane through neck and upper-torso of Day 75 fetus



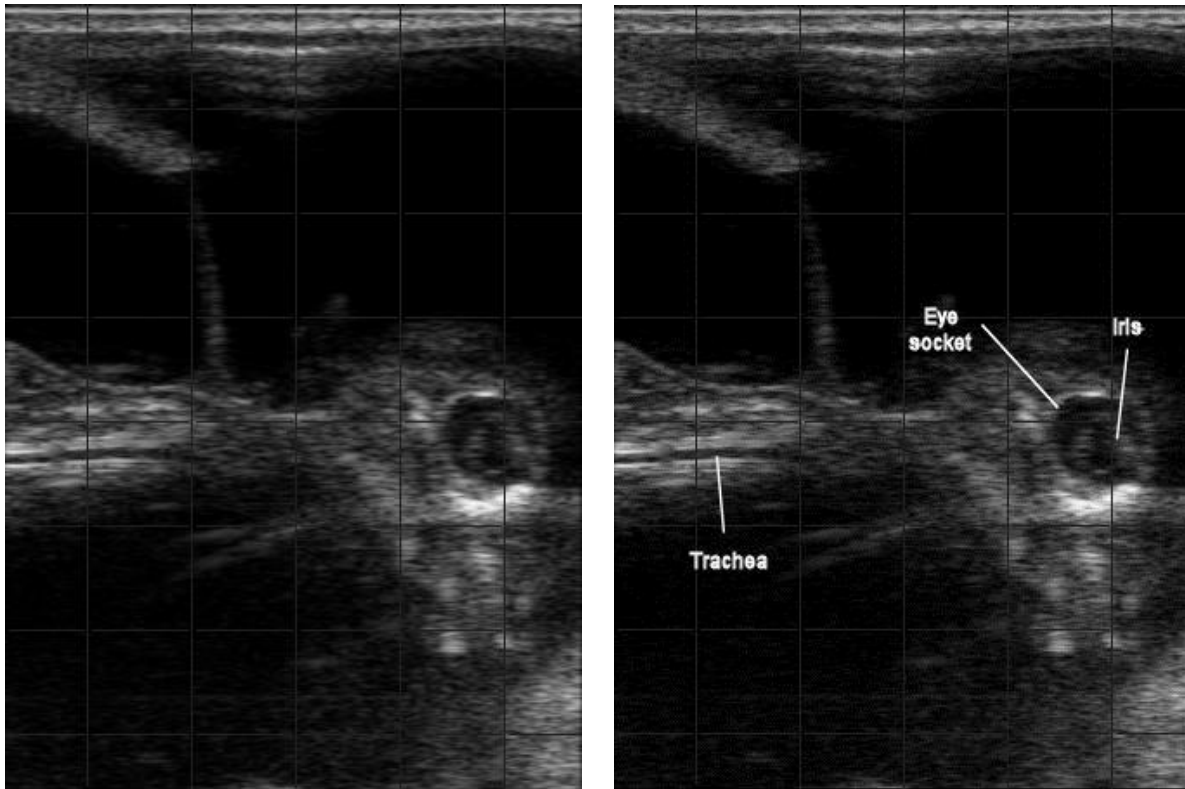
**Figure 22.** Oblique sagittal plane through head of Day 80 fetus



**Figure 23.** Sagittal plane through torso of Day 80 fetus



**Figure 24.** Dorso-ventral plane through head of Day 80 fetus

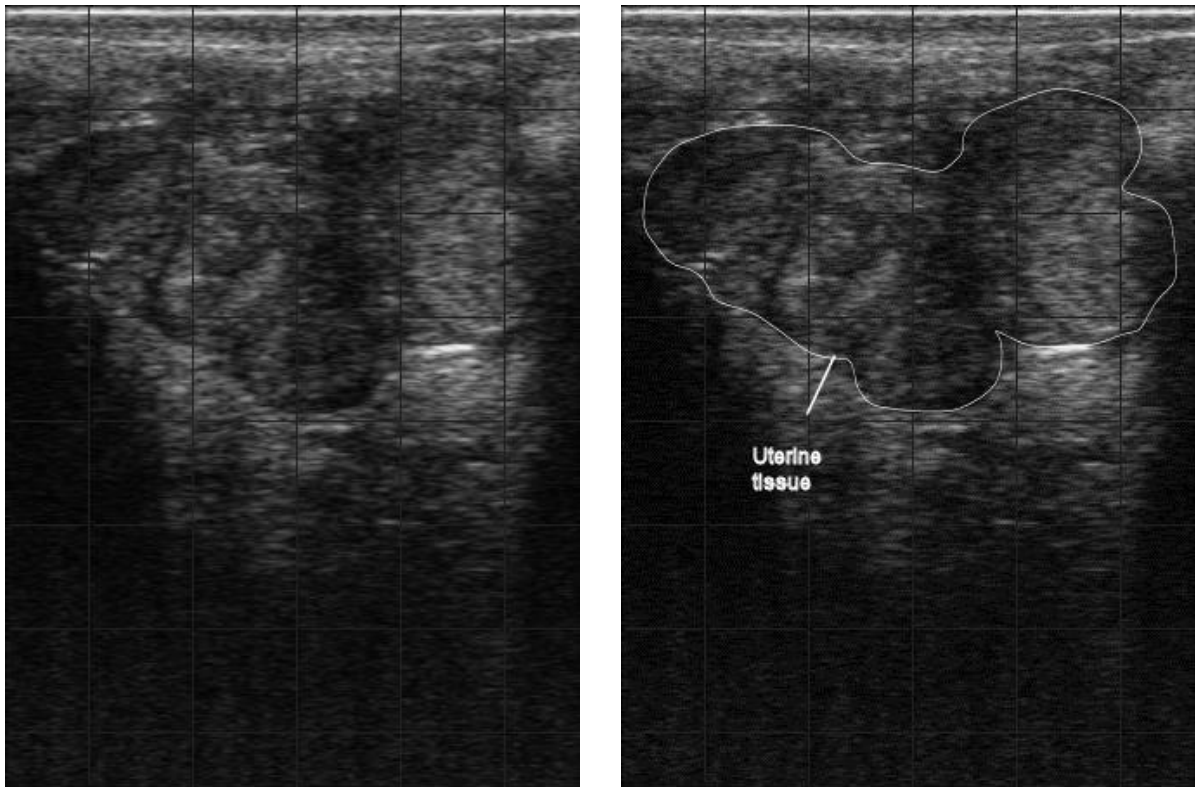


**Figure 25.** Oblique sagittal plane through head and neck of Day 80 foetus

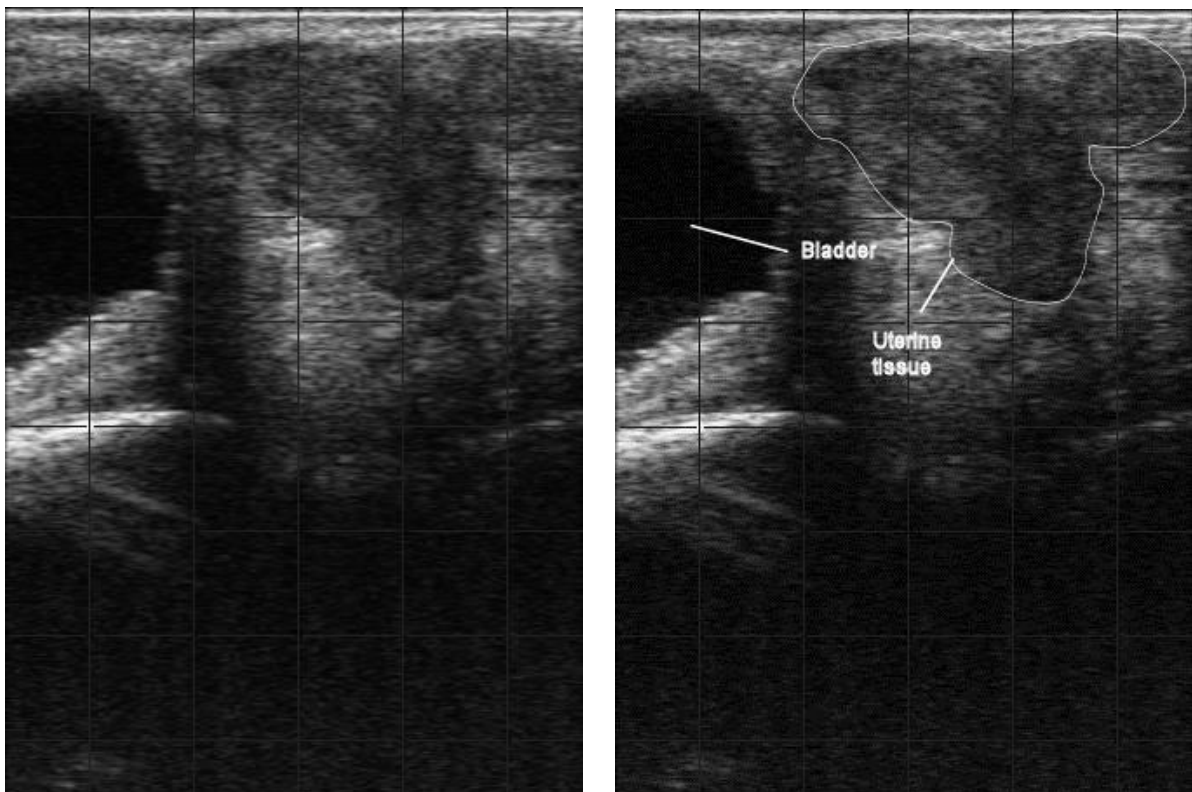
Beyond 75 days, the accuracy of foetal age diagnosis starts to decline due to the general inability to get a clear image of the salient foetal features. Also, later foetal growth is influenced by other factors including foetal genotype and hind nutrition. In many cases the entire conceptus has descended from the inguinal canal and into the abdominal cavity. This places the foetus at the extreme range of ultrasound detection when using a rectal probe. At best, only part of the foetus is visible. Again, the head provides the best diagnostic markers of foetal age, based mainly on size and differentiation of such features as the eye orbit. The limbs are often in view and their size and level of calcification can indicate a foetal age >75 days.

Landmarks: Size of head and delineation of skull features; size and calcification of limbs.

## Non-pregnant



**Figure 26.** Non-gravid uterus (confirmation of non-pregnant state).

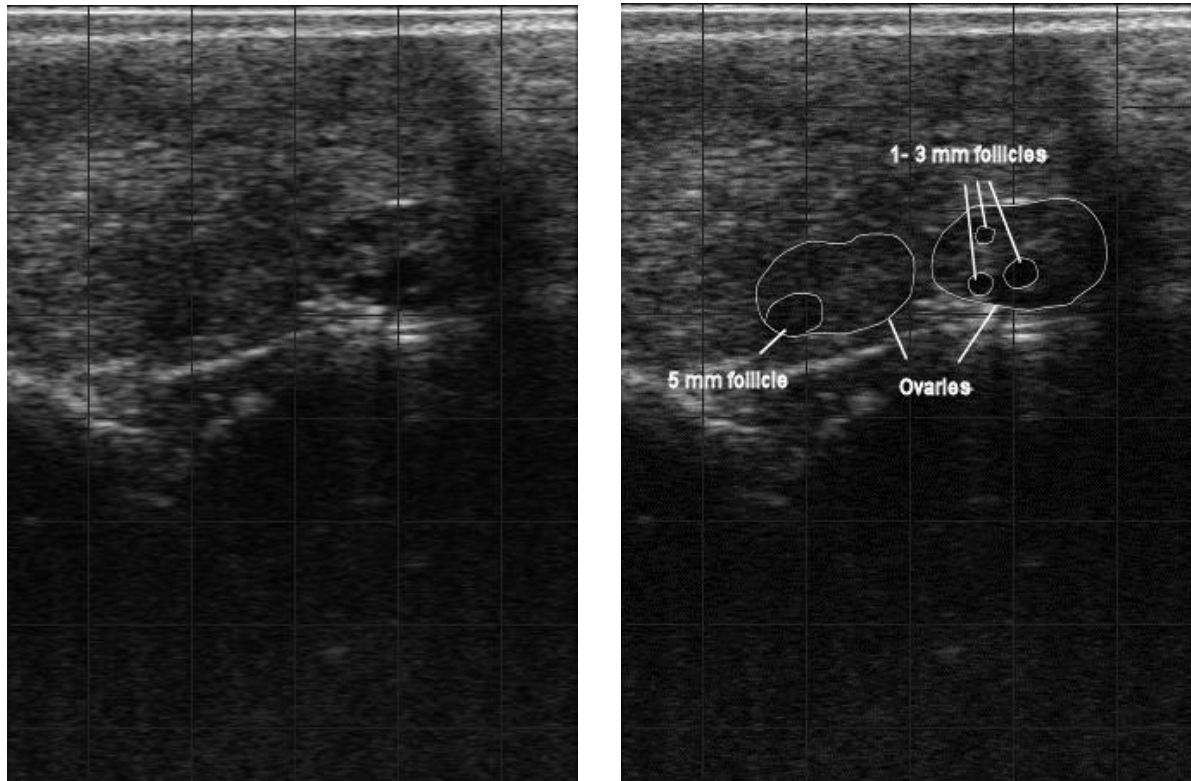


**Figure 27.** Non-gravid uterus anterior to bladder

Sometimes it is difficult to find signs of pregnancy. **However, diagnosis of the non-pregnant state should be based on the appearance of the uterus itself (it should have 'non-gravid' (empty) features) rather than the scanner's inability to visualise a pregnancy.** This will reduce the incidence of false negatives, which may lead to hinds with perfectly good pregnancies being culled.

The non-gravid uterus appears as a tightly coiled mass (convoluted uterus) immediately anterior of the bladder. If this state is visualised more than 30 days after stag removal, then it is a conclusive diagnosis of non-pregnancy. However, if visualised fewer than 30 days after stag removal it is possible that the hind is at a very early stage of pregnancy that is undetectable by ultrasonography. In such case, rescanning 20-30 days later is advised.

## The ovary



**Figure 28.** Ovaries

It is not uncommon to visualise one or both ovaries nestled alongside the uterus. These are spherical organs between 10 and 20 mm in cross-section, and typically contain a number of fluid-filled follicles (which contain individual eggs). Such structures can be mistaken for signs of early pregnancy. However, due to their spherical shape and relatively small size, they cut in and out of the image very quickly. By contrast, fluid-filled vesicles of pregnancy within the uterus are longer, tubular structures which can be tracked through the real-time image.