REAL-TIME ULTRASONIC SCANNING FOR ESTIMATION OF FOETAL AGE IN FARMED RED DEER

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1. INTRODUCTION

Real-time ultrasonography using a rectally placed transducer is a non-invasive technique which allows the visualisation of the in utero foetus and associated structures. While this technique has been in use for pregnancy diagnosis in farmed deer for a number of years, there have been no objective studies to evaluate its accuracy or to evaluate the optimum time for pregnancy diagnosis. Further, there have been no studies to evaluate the use of this technique in estimating foetal age in red deer.

The only published report of the use of ultrasonography for pregnancy diagnosis in deer was by Mulley et al (1987) on fallow deer. These authors used a 3.5 MHz transducer by the transabdominal route, placing the transducer anterior to the udder on the ventral surface of the abdomen while the deer were held in an upright position. A 100% accuracy of diagnosis of both pregnancy and non-pregnancy was reported when scanning was performed in June, July and September.

This paper reports a study designed to:

i) Determine the accuracy of real-time ultrasonography for the diagnosis of pregnancy in red deer.

ii) Make measurements of the uterus, placentomes, foetal sacs and foetus in order to chart the growth and development of the foetus and associated structures.

2. MATERIALS AND METHODS

2.1 Animals

Two groups of hinds were used. The first ("trial") group consisted of 29 mixed age red hinds mated to a 4-year-old stag. Observations were undertaken from 7.00 a.m. to 6.00 p.m. daily for a three week period from April 1 to determine mating or oestrus dates.

The second ("test") group group consisted of 9 yearling hinds mated separately from the experimental mob. No mating or oestrus observations were undertaken.

2.2 Ultrasound scanning

Hinds were restrained in a hydraulic crush for scanning. Scanning was performed by rectal insertion of a transducer on a rigid extender, connected to a "Concept" real-time ultrasound scanning machine. A 5 MHz linear transducer was used.

The first scan was undertaken May 28 1987, at which time the earliest pregnancy was 42 days. Scanning was thereafter performed at approximately weekly intervals, with the last scan taken August 13, at which time the maximum foetal age was 127 days. The scan of each hind from both the "trial" and "test" groups was recorded on videotape to enable observations and measurements to be taken in the laboratory.

Hinds were scanned on each occasion in random order and recordings were made at each occasion without prior knowledge of stage of pregnancy.

2.3 Pregnancy diagnosis

The definitive criteria upon which pregnancy diagnosis was based were:

i) Presence of one or more placentomes
ii) Presence of a foetus or part thereof
iii) Presence of foetal membranes

If one or more of these criteria were met the animal was diagnosed as pregnant.

Pregnancy status was confirmed ultimately by recording calving dates.

2.4 Parameters measured

The video tapes containing a record of the examination of each hind at each date were played back through a television monitor. A conversion factor for measurements had been previously determined using the electronic

calipers of the ultrasound machine. Measurements of parameters were taken only where appropriate, i.e. when components were considered to be orientated to allow accurate maximum measurements to be made. These were determined by a single frame video progression whereby the tape was stopped at the appropriate time to measure that structure.

The principal measurements taken were:

i. Uterine diameter: the greatest vertical diameter of the uterine horn which contained the foetus;

ii. Amniotic sac: both the largest and smallest diameters of the amniotic sac were measured;

iii. Crown rump length: straight line measurement from the top of the skull to the caudal edge of the perineum (see figure 1).

iv. Head length: from the caudal part of the skull to the end of the rostrum (see figure 1);

v. Nose length: rostral to the orbit to the end of the rostrum (see figure 1);

vi. Head diameter: measured in the transverse plane on a horizontal axis (see figure 1);

vii. Chest depth: the vertical distance between the spinal column and the caudal end of the sternum (see figure 1).

viii. Chest diameter: measured as the horizontal distance between the lateral extremities of the rib cage (see figure 1);

ix. Placentome diameter: the largest diameter of the largest placentome;

x. Placentome base apex length: distance from the uterine wall to the free edge of the largest placentome.

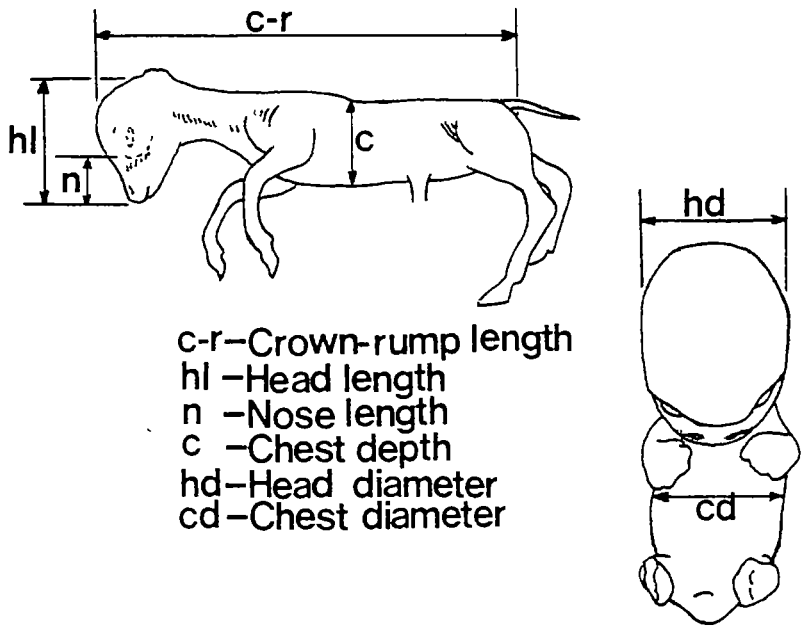

Figure 1. Principal foetal dimensions recorded from the ultrasonograms.

2.5 Analysis of Data

The relationships between foetal age and the measurements recorded for each parameter were derived by quadratic regression analysis using the least squares principle. As the ultimate objective was to provide a means of estimating foetal age; equations to predict this were derived using the size measurement as the independent variable.

In addition, data was charted to indicate the age of appearance and/or disappearance of the parameters measured, thus producing a chronological record of the appearance of both the external and internal features visible during pregnancy.

2.6 Estimation of foetal age of "test" deer

Parameters measured at each scanning period for each "test" deer were used to assess foetal age. These parameters were then averaged for each deer at each scanning period to obtain the most accurate foetal age estimate at that scan. These age estimates were subsequently correlated with actual age measurements determined from records of actual calving dates, and regression analysis applied.

2.7 Pregnancy diagnosis before 42 days

All pregnancies were clearly obvious at the commencement of the above study. A repeat study was undertaken the following year. Ultrasound scans were performed as early as April 20 and again on May 10, on 35 mixed age hinds with mating and oestrous behaviour observed from April 1. Appearance of the uterus and contents were noted and foetal mass length measured.

3. RESULTS AND DISCUSSION

3.1 Accuracy of diagnosis

Of the trial deer, on only one of 256 occasions was a deer which failed to produce an offspring diagnosed pregnant (i.e. observations between 40-130 days of pregnancy). That deer was diagnosed pregnant early in gestation, but subsequent scans proved negative. A likely explanation is that foetal reabsorption had occurred.

On seven occasions pregnant deer were diagnosed as non-pregnant. Four of these scans were on the last scanning occasion (Aug 13) and the negative diagnoses probably were due to descent of uterine and foetal structures beyond the range of a 5 MHz rectally placed transducer.

In the hands of experienced operators the accuracy of pregnancy diagnosis from 30-130 days gestation using ultrasound should be 100%. This is supported by data of Mulley et al (1987) for fallow deer. First diagnosis of pregnancy at 30 days gestation is somewhat later than possible diagnosis in horses and cattle. The reason probably is that in the larger species the uterus can be palpated manually and the rectal probe manipulated directly over the identified structures. This is not possible in red deer using the rigid extended rectal probe.

The diagnosis of non-pregnancy cannot be as accurate as the diagnosis of pregnancy and therefore veterinarians would be advised to repeat observations at a later date where non-pregnancy is suspected at the first scan. The predominant reason for lack of accuracy is placement of the uterus. It is the observation of the authors that, particularly in older hinds, the uterus descends into the abdominal cavity earlier, and if the rumen is full

the uterus is displaced toward the right abdomen. Such displacement appears greater if deer are scanned in a free-standing posture. It would appear that restraint in crush systems tends to displace the uterus more dorsally and therefore more accessible to ultrasound waves.

3.2 Estimate of foetal age

The age prediction equations for each principal measurement are given in Table 1. All correlation coefficients were highly significant($p < 0.001$). The standard error of the regression given for each equation provides a relative indication of the accuracy of the equations in predicting foetal age.

Note: No extrapolation using the equations should be made beyond the recorded data, the limits of which are given in Table 1.

The age prediction equations provide a precise estimation of foetal age, but an approximate estimate can be obtained by simple inspection of Table 2, which is a chronology of the ultrasonic appearance of various parameters observed.

Figures 1 - 6 show some of the characteristics measured at selected ages of development.

3.3 Estimate of foetal age of "test" deer

The regression of predicted vs. actual calving dates of 8 hinds with unknown mating was significant ($p < 0.01$) indicating that the estimation of foetal age in deer with unknown mating date is indeed an accurateprocess. In practice prediction of calving date in the 8 test deer varied from actual calving date by 1, 1, 1, 2, 4, 5, 9 and 11 days, respectively.

It should be noted, however, that the accuracy of estimate of foetal age depends on the variables measured and the error in measurement of each of those variables; e.g. early age estimating using crown rump lengths will be the most accurate (see the range of errors presented in Table 1). For practical purposes, if a number of parameters can be measured at an individual scan and the mean of all age estimates for those parameters is taken, the errors tend to cancel. Therefore the final estimate will be more

TABLE 1 **Age prediction equations from the principal linear measurements on foetal age from sequential scans from 42 to 127 days of pregnancy**

Parameters	Age Prediction Equation	S.E. of Regression	Range of days for which equation is valid	Range of measurements (mm) of parameters for which equation is valid
Crown Rump	$Age = 31.418 + 0.589x - 0.002x^2$	2.9	42 - 66	23 - 70
Nose length	$Age = 38.358 + 2.193x - 0.009x^2$	6.6	42 - 68	5 - 42
Chest Depth	$Age = 28.242 + 2.276x - 0.014x^2$	4.7	42 - 116	6 - 61
Chest Diameter	$Age = 36.543 + 1.806x - 0.008x^2$	5.0	42 - 94	4 - 36
Foetal sac - long diameter	$Age = 29.342 + 0.675x - 0.003x^2$	4.1	42 - 68	23 - 68
Foetal sac - short diameter	$Age = 24.171 + 1.461x - 0.015x^2$	5.3	42 - 112	14 - 54
Head Length	$Age = 38.101 + 1.035x + 0.002x^2$	5.8	42 - 123	5 - 65
Head Diameter	$Age = 43.877 + 1.501x$	6.0	42 - 121	4 - 54
Uterine Diameter	$Age = 38.507 + 0.439x - 0.001x^2$	7.8	42 - 76	27 - 69
Placentome B-A	$Age = 13.604 + 4.1x - 0.048x^2$	11.5	42 - 128	7 - 46
Placentome Width	$Age = 4.551 + 2.469x - 0.015x^2$	13.5	42 - 127	12 - 75

NOTE: THESE EQUATIONS MUST NOT BE USED FOR AGE PREDICTIONS OUTSIDE THE AGE OR MEASUREMENT LIMITS PRESENTED IN THIS TABLE

TABLE2 Chronology of the ultrasound appearance of the developing foetus

Parameters (mm)	Age (days) 40	50	60	70	80	90	100	110	120
Crown rump length	-	36	62	-	-	-	-	-	-
Amniotic sac -									
- longest diameter	-	37	64	-	-	-	-	-	-
- shortest diameter	-	24	47	-	-	-	-	-	-
Chest depth	-	11	15	21	28	35	43	54	-
Head length	-	12	21	30	38	47	54	60	-
Head diameter	-	5	11	18	24	31	37	44	51
Nose length	-	5	10	15	21	26	33	39	46
Uterine Diameter	-	30	58	58	-	-	-	-	-
Placentome width	-	21	27	33	41	49	62	82	82
Placentome base-apex length	-	10	14	17	22	28	38	43	38
Parameters ( )									
Foetus visible	+	+	+	+	+	+	+	P+	P+
Placentomes visible	+	+	+	+	+	+	+	+	+
Heart beat seen	+	+	+	+	+	+	+	+	+
Body parts discernable	-	+	+	+	+	+	+	+	+
Rostral skull hyperechoic	-	+	+	+	+	+	+	+	+
Remainder of skull hyperechoic	-	+	+	+	+	+	+	+	+
Vertebrae visible	-	-	+	+	+	+	+	+	+
Sternum visible	-	-	+	+	+	+	+	+	+
Ribs visible	-	-	+	+	+	+	+	+	+
Limbs visible	-	-	+	+	+	+	+	+	+
Foetus moves	-	-	+	+	+	+	+	+	+
Limb bones distinct	-	-	+	+	+	+	+	+	+
Neck elongated	-	-	-	+	+	+	+	+	+
Heart chambers visible	-	-	-	+	+	+	+	+	+
Intestinal mass visible	-	-	-	+	+	+	+	+	+
Urachus visible	-	-	-	+	+	+	+	+	+
Diaphragm visible	-	-	-	+	+	+	+	+	+
Accessory claws visible	-	-	-	+	+	+	+	+	+
Liver visible	-	-	-	+	+	+	+	+	+
Amniotic membrane flaccid	-	-	-	+	+	+	+	+	+
Hypoechoic particles	-	-	-	-	-	+	+	+	+
Trachea and carotids visible	-	-	-	-	-	+	+	+	+
Aorta visible	-	-	-	-	-	+	+	+	+

accurate than if one parameter alone could be measured. More parameters from each scan can be measured early in pregnancy because as pregnancy advances the foetus descends beyond the range of the ultrasound probe. Beyond approximately 80-90 days usually only placentomes can be measured. Estimates based on only placentome measurements have the greatest error (see Table 1). Bearing these factors in mind it would appear the most accurate age estimates will be achieved between 42 and approximately 66 days of pregnancy.

While there was a significant regression of predicted vs actual calving dates, it must be remembered that the age prediction equations have been established from known mating date data. Gestation length in red deer averages 233 days, but the range is from 226-240 days, i.e. 7 days (unpublished observations, Massey University). Therefore the errors of predicted vs. actual calving dates are a compound of both age estimate and gestation length variables. There is no way of avoiding these errors. The range of errors observed in the "test" group (1-11 days) falls within the predicted range based on these compounded variables.

3.4 Foetal mass length 29 - 40 days

Observations indicate that pregnancy can be observed with accuracy as early as 29 days. The criteria at this stage is to view a fluid-filled cavity traversed by a band of tissue which represents the section through the coiled uterine horn, and a foetal mass which is usually situated close to that band. The measurements of foetal mass length (Table 3) can be used to estimate foetal age with reasonable accuracy since growth at this stage is very rapid.

Early pregnancy must not be confused with fluid-filled loops of bowel. Therefore pregnancy should not be diagnosed unless a foetal mass, membranes or placentomes are observed.

TABLE 3 Foetal mass length observed in red deer from days 29-42 of gestation

Days pregnant (range)	No. observations	Mean foetal length (mm)
29	2	8.5 (7-10)
30	1	11
31	1	8
32	-	-
33	1	12
34	3	12.3 (11-14)
35	1	12
36	3	13.6 (9-17)
37	6	13.3 (12-18)
38	2	17 (14-20)
39	-	
40	1	18
41	1	22

4. GENERAL DISCUSSION

Practical uses for foetal aging

This technique has practical application for research purposes where an accurate estimate of foetal age is necessary for a number of reasons, or to confirm pregnancy at a given mating.

This season, scanning of deer which had been artificially inseminated has been carried out 60 days after insemination. At this stage, those which had not conceived to AI and had conceived to the subsequent oestrus would be 42 days pregnant. Those having not conceived to those ovulations would appear not pregnant but may have conceived at a subsequent oestrus (this can be checked at an appropriate date). At 60 vs 42 days, using knowledge presented in this paper, it is a relatively simple process to distinguish deer conceiving to AI from those conceiving to a later natural mating. This can give the farmer considerable confidence in the outcome of the exercise, and where valuable progeny are expected, the AI pregnant deer can be run as a separate group and given intensive observation during calving.

Experimental work aimed at advancing the onset of calving has shown in field trials (see elsewhere in this Proceedings) that the calving span for treated hinds is considerably lengthened. Ultrasound ageing of foetuses has

an important role in allowing the farmer to distinguish early from late calvers, thereby allowing the herd to be split for grazing management purposes during the spring. For this purpose it may be appropriate to ultrasound the treated and any in-contact deer early in May, simply to gauge whether the hind is pregnant or not, i.e. those pregnant within 30 days would not be detected, therefore those mated during March would be identified and could be managed accordingly at calving.

5. CONCLUSION

This study has shown that it is possible to age foetal development accurately between 30 and 100 days by careful examination of a number of parameters. While video tape recording and playback is necessary to achieve the greatest level of accuracy, the veterinarian in the field can utilise the data presented to a level acceptable for management purposes, such as dividing herds into early and late calving groups.

The accuracy of positive diagnosis of pregnancy is high, but caution must be exercised when diagnosing a deer not pregnant. Repeat examination is advised.

It is intended that this data will be published in full in the New Zealand Veterinary Journal.

6. Acknowledgments

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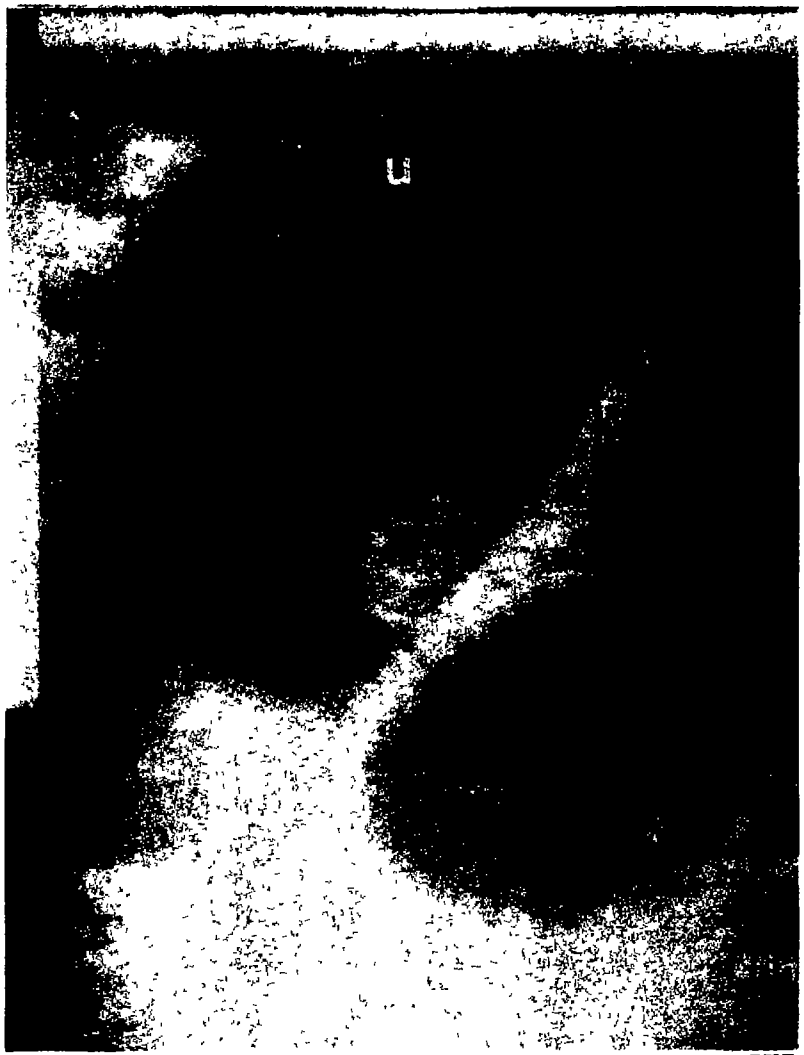

Fig. 1. An ultrasonogram of a 42 day old foetus showing how difficult it is to differentiate body parts at this age.

F - Foetus
u - Fluid filled uterus
W - Infold of uterine wall
Arrows - Amniotic membrane

Fig. 2. An ultrasonogram of a 50 day old foetus showing the head, body and limb buds.

F - Foetal body
H - head of foetus
L - Limb buds
UC - Umbilical cord
u - Fuild filled uterine
Arrows - amniotic membrane

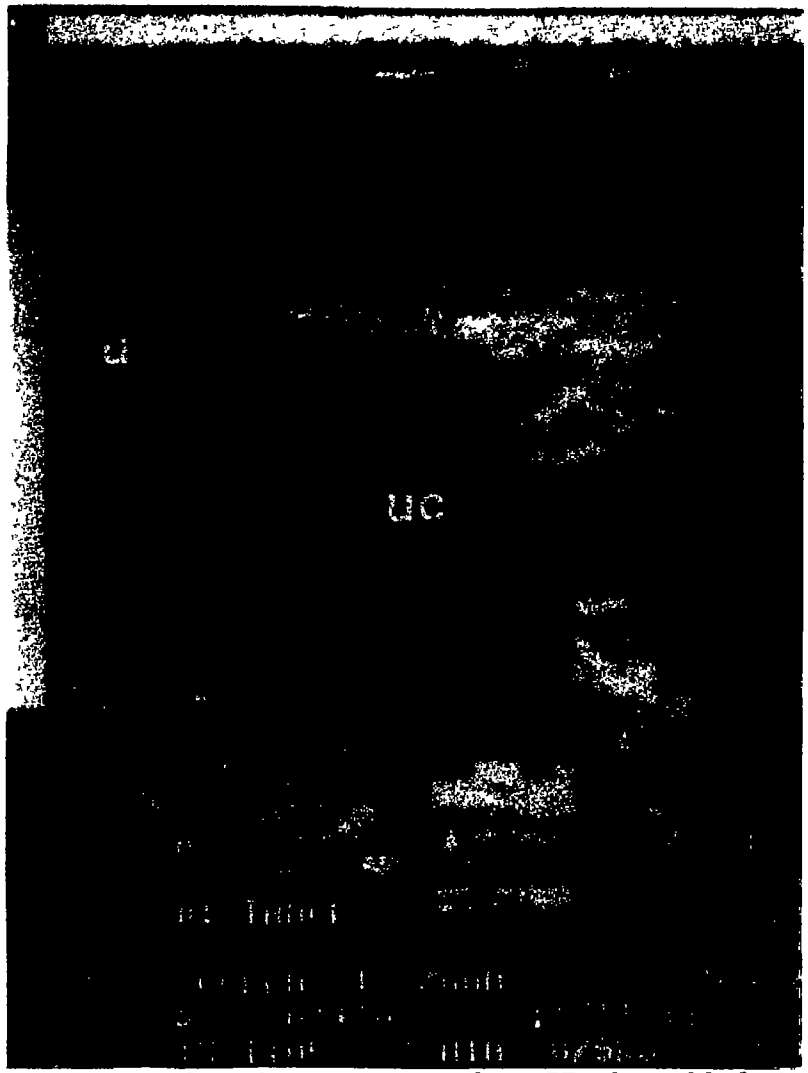

Fig. 3. An ultrasonogram of a 57 day old foetus showing the elongation of the neck.

h - Head of foetus
b - Body of foetus
Lb - Limb buds
uc - Umbilical cord
u - Fluid filled uterus
w - Infold of uterine wall

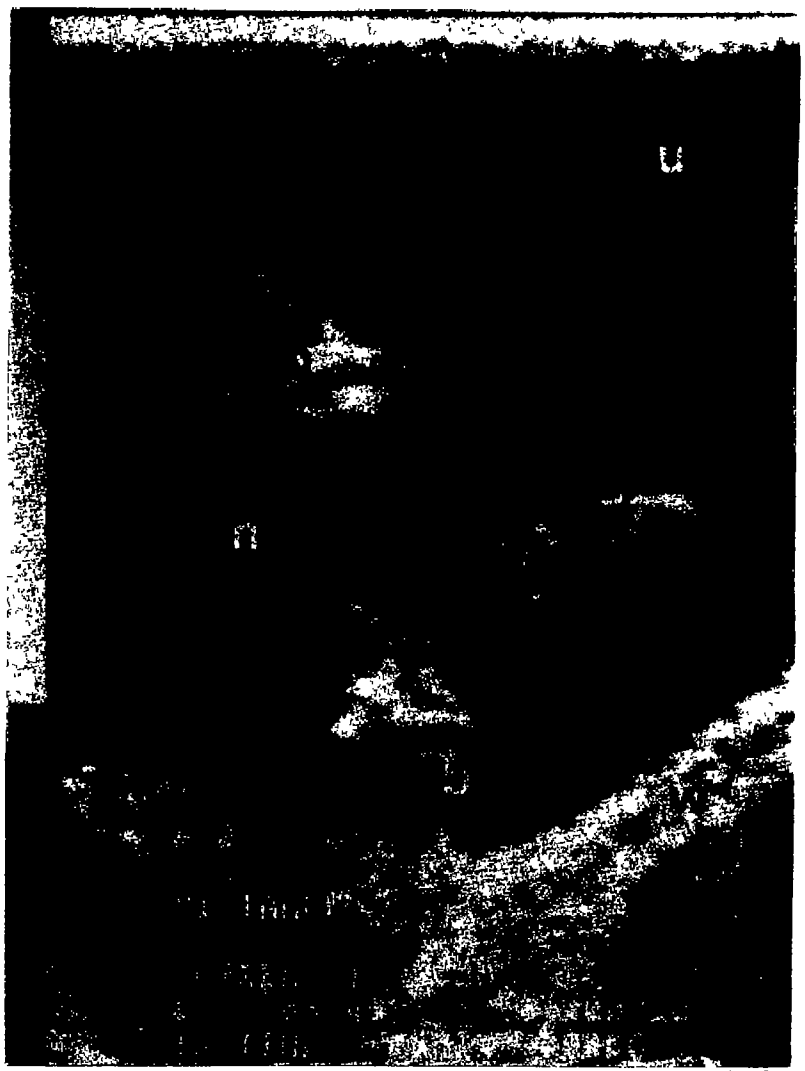

Fig. 4. An ultrasonogram of a 58 day old foetus showing the presence of hyperechoic areas on the muzzle and the elongation of the limb buds to form limbs.

h - Head
n - Elongated neck
b - Body
f - Forelimb
u - Fluid filled uterus
w - Infold of uterine wall

Fig. 5. An ultrasonogram of a 60 day old foetus showing the extension of hyperechoic over the whole head and the presence cranial cavity and heart chambers.

c - Cranial cavity
h - Heart
f - Forelimbs
P - Placentome
Arrows - Amniotic membrane

Fig. 6. An ultrasonogram of a 63 day old f showing the striated appearance of ribs.

R - Ribs
c - Cranial cavity
f - Forelimb
UC - Umbilical cord
Arrows - amniotic membrane