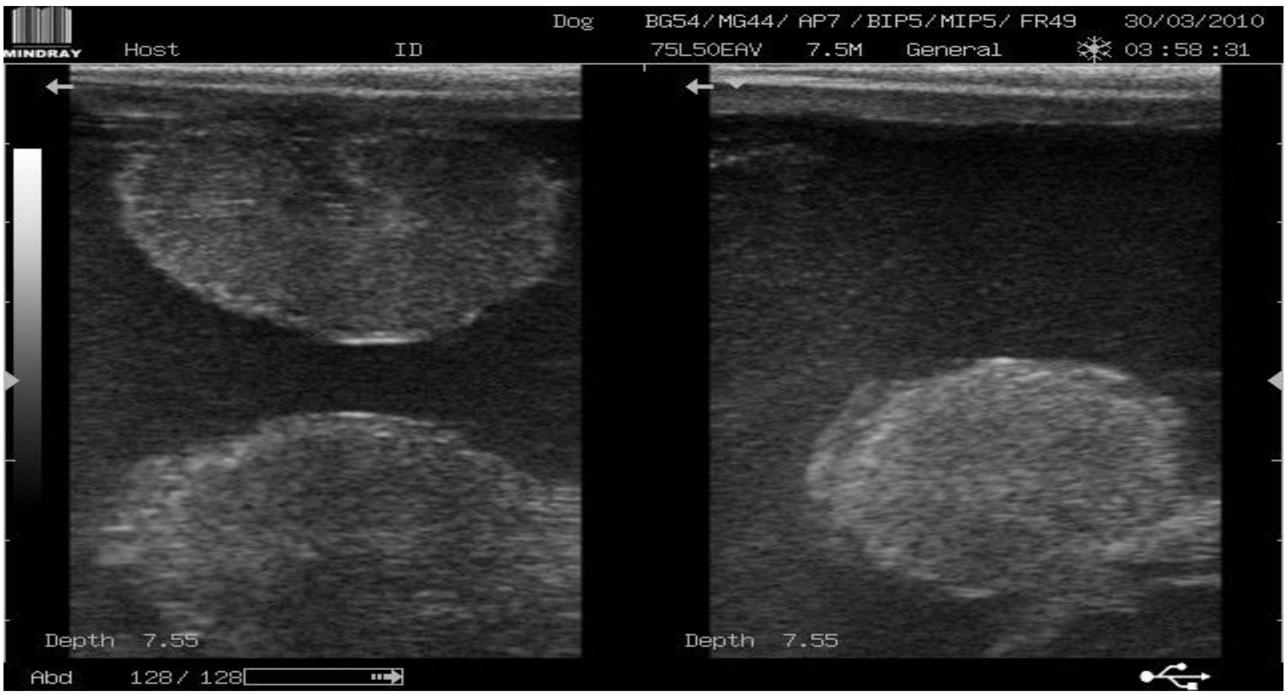
Determination of gestational age in dairy cattle using transrectal ultrasound measurements of placentome size



Gerben Blankenvoorde
3050270

Supervisors:
Peter Vos (University Utrecht, The Netherlands)
Richard Laven (Massey University, New Zealand)

Massey University Universiteit Utrecht

Part I: General introduction
1. Introduction

The dairy industry in New Zealand is seasonal in terms of calving. Most of the New Zealand herds have their calving season in the late winter or early spring. Therefore it is important for the New Zealand farmer to achieve an inter-calving interval of 365 days in order to coincide with the pasture based management of the dairy herd. When a cow fails to conceive within the breeding season the farmer has to carry her over to the next breeding season or remove her from the farm. Both options have an economic disadvantage. To achieve an inter-calving interval of 365 days the cows have to conceive within the breeding season of 12 -14 weeks since the gestation length is on average 280 days. Adequate pregnancy control is therefore necessary for the farmer and this helps to improve the reproductive efficiency of the farm. Good farm management requires reliable pregnancy control techniques including an adequate estimation of the cow's gestation age. At the conclusion of the pregnancy control the farmer can make decisions such as: which cows need to be removed from the farm, what is the optimal dry-off period should be and whether a cow needs food supplementation.

This report is structured into two sections: a general section and the more specific section on placentome research. The general section includes the physiology of the pregnancy, the pregnancy control options and a placentome literature study.

1.1 Pregnancy
1.1.1Early pregnancy

After a successful fertilization and fusion of the male and female pro-nuclei a zygote is formed. A zygote is the single celled embryo. The embryonic period is traditionally defined as the period between fertilization and the end of the organogenesis on the 42nd day of gestation[1]. The zygote undergoes a series of mitotic divisions called cleavage divisions. After the first division the embryonic cells are called blastomeres and during these first stages the embryo is in the oviduct[2]. After 2 – 5 days the embryo arrives in the uterus whereafter 4 – 7 days and a couple of cleavage divisions the embryo becomes a morula. During the morula stage cells begin to separate into two distinct populations and water diffuses through the zona pellucida into the embryo and begins to form a fluid filled cavity and the embryo is called a blastocyst. Around day 9 – 11 after fertilization the blastocyst hatches from the zona pellucida and forms a hatched blastocyst. The hatched blastocyst undergoes massive growth. By day 18 the blastocyst occupies space in both uterine horns and is about 40 cm in length with a diameter of 2 mm[1.]. At this stage the blastocyst prevents luteolysis from the corpus luteum by producing specific proteins called interferons[3]. The interferons are produced by the trophoblastic cells of the blastocyst and act on the endometrial cells of the uterus to inhibit the oxytocin receptor production. By inhibiting the production of the oxytoxin receptor, the producing of PGF2α through oxytocin will be prevented. This will then cause the low concentration of PGF2α, and luteolysis will therefore be prevented and pregnancy will continue. The blastocyst produces maximum amounts of interferons between day 16 and 19 of gestation and continues secretion until day 38 of gestation[4]. The hatched blastocyst consist of the inner cell mass, the trophoblast and the blastocoele. At this stage the embryo begins to form extra embryonic membranes. Figure 1 illustrates the development of the young embryo membranes.

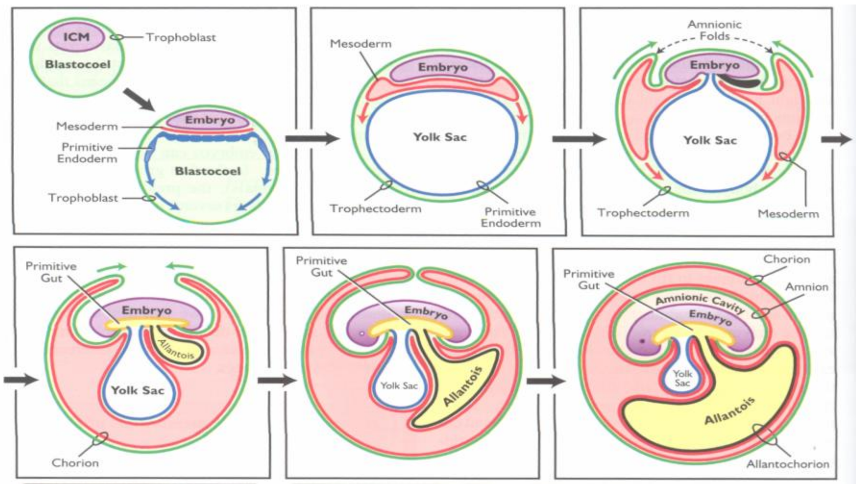


Figure 1*[2]*: *Schematic diagram illustrating the typical development of extraembryonic membranes in mammals*

The mesoderm begins to develop between the primitive endoderm and the embryo. The mesoderm continues to grow, pushes against the trophectoderm and fuse with it to form the chorion. The chorion folds upward forming structures called amnionic folds. These folds continue to grow upward around the embryo and form the amnion. The amnion compartment is filled with fluid to protect the embryo by mechanical shocks. The amnion cavity is rectally palpable between day 30 and 45 as a turgid balloon in the uterus. The allantois is a diverticulum from the primitive gut which contain liquid waste from the embryo[2]. During pregnancy the allantois becomes filled with fluid and presses against the chorion this newly formed membrane is called the allantochorion. The allantochorion will provide the surface for attachment to the endometrium. At day 24 the allantois is about 1 meter long and 3 mm in diameter. Up untill day 35 of gestation the ends of the allantochorion membrane degenerate leading to shorting of the allantois[1].

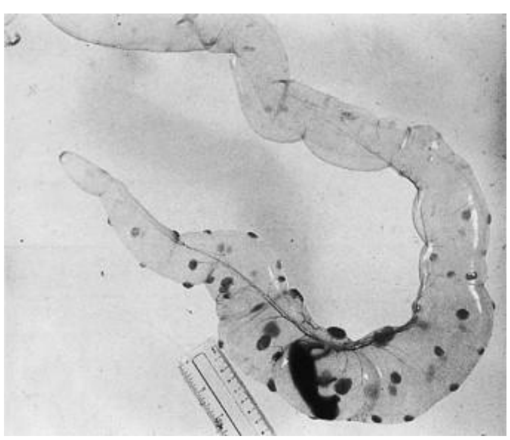


Figure 2*[5]*: *Bovine fetus at 60 days. The amnion (around the fetus) and the allantochorion with the formation of cotyledons are visible.*

The placenta consists of a fetal component derived from the chorion and a maternal component from the uterine endometrium. Attachments of the embryo to the uterine endometrium provide the embryo adequate nutrition and protection during development. Ruminants have a cotyledonary placenta. A placentome is a placental unit of trophoblastic origin consisting of blood vessels and connective tissue[2]. The placentome consist of a fetal cotyledon and a maternal caruncle originating from the caruncular region of the uterus. The placentomes form specific zones for metabolic exchange. At about 25 days of pregnancy in cattle the chorion initiates attachment to the caruncles of the uterus. During this formation villi from the chorion protrudes into crypts in the caruncle. Attachment is finished around day 40 in cows. The border between the chorion and the endometrium consist of the chorion and the endometrial epithelium with partial erosions filled with binucleate giant cells. The binucleate giant cells are formed around day 18-20 and originate from trophoblast cells. The cells migrate from the chorion epithelium to the endometrial epithelium. Around 20 % of the placenta consists of the binucleate cells[2]. The cells help the conceptus to exchange molecules from the fetal to the maternal placenta (*see fig. 3*). The binucleate giant cells secrete pregnancy specific protein B and are important in producing progesterone and estrogens[2].

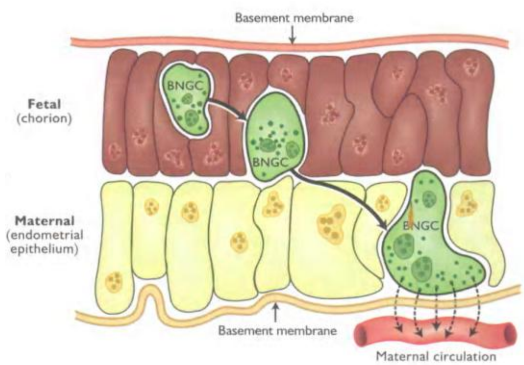


Figure 3*[2]*: Binucleate giant cells migrate from the chorion to the endometrial epithelium. These cells secrete placental lactogen and pregnancy specific protein B.

1.1.2 Embryonic/Fetal development

The first somites from the embryo are formed around day 19 of gestation. At this time the embryo is 3-4 mm long, the neural tube close and the first heartbeats can be detected. On day 22 the optic vesicles, hepatic primordium and the mesonephros are formed[1]. The limbs originate at day 25 – 26 of gestation, at day 26 the embryo curves and is 7 – 8 mm long. Up to day 50 it grows by an average of 1,1 mm/day[6]. Around day 35 folds around the eyes are visible, the neck is clearly developed and digits are recognizable on all four limbs. On day 40 of gestation the genital tubercle is located on the medium line between the posterior limbs. At day 45 the fetus loses its rudimentary embryonic shape and the face, neck, limbs and tail get length, rudimentary eyelids and ear pinnae are seen *(see fig. 5).* At day 40 of gestation the jejunum and ileum can be differentiated but the differentiation of the large intestines comes later staring at day 130 of gestation. The mesonephros and the liver fill almost the entirely abdomen during the first few months of gestation. Around day 60 of gestation the stomach divided in the 4 compartments. At this

stage of gestation the omasum is the largest part of the stomach, after 6 months the abomasum starts growing faster and will be the largest part at birth *(see fig. 4).* The mesonephros atrophies around day 70 of gestation and approximately at day 90 the kidneys have the 'normal' posses and lobular shape[1]. The bones start to ossify somewhere between day 50 and 100. Further on in this report will be mentioned on which day of gestation the different bones ossified.
In the third month of gestation hair follicles near the eyes and lips are found. In the fourth month the claws are cornified and there is dental development. In the fifth month testicles descent and in the sixth month hair is formed at the eyelashes, ears and the end of the tail. In the seventh month hair is formed all over the body and the eyelids are open. In the eight month a full coat of short hair is formed and in the ninth month a hair tuft arises at the end of the tail[1].

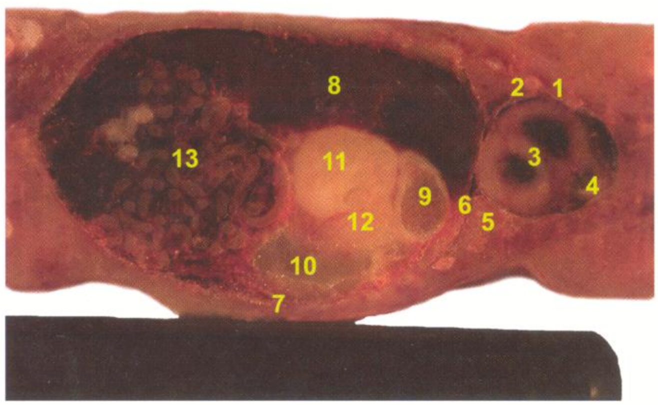


Figure 4[1]: *Frontal view of the thoraric and abdominal cavities of a bovine fetus at 4 months of gestation. 1: Thoraric cavity; 2: ribs; 3: heart; 4: pulmonary trunk; 5: lungs; 6: diaphragm; 7: abdominal cavity; 8: liver; 9:reticulum; 10 rumen; 11: omasum; 12 abomasum; 13 intestines.*

1.1.3 Changes in genital organs during pregnancy

1.1.3.1 Ovaries[5]

Throughout the whole period of gestation the corpus luteum remains at the maximum size. The corpus luteum in the ovary is mostly at the side of the pregnant horn. As pregnancy advances the position of the ovaries changes. In heifers and young cows they lie in the pelvic cavity. The ovaries in non - pregnant multiparous cows are often found in the abdominal cavity 5 – 8 cm in front of the pelvis. With increase in weight of the uterus the ovaries in the pregnant cow also sink into the abdomen and is even beyond reach for rectal examination after day 100, although in some cases the ovaries will be detected up untill 150 days of pregnancy.

1.1.3.2 Uterus[5]

The uterus starts to increase in size around day 28 of gestation and the amnion compartment becomes palpable as a kind of balloon from 2 cm in diameter. The amniotic compartment grows up to 3 and 5 cm on days 35 and 60 of gestation respectively. The allontoic compartment is much longer but not palpable around day 28. The diameter from the pregnant horn is 5.0 -6.5 cm at 45 days of gestation, 6.5-7.0 cm at 60 days, 8.0 -10.0 cm at 90 days, 12 cm at 120 days and 18 cm at 150 days of gestation. (See table 2). Around day 90 of gestation the uterus in some animals is still situated high up at the pelvic brim but in some multiparous cows the uterus lies in the abdomen. Around the fourth month of gestation in all cows the uterus sinks below the pelvic brim. It is generally believed that the majority of the cows (60%) are right-side pregnant.

1.1.3.3 Uterine arteries[5]

During the pregnancy the uterine artery growth and a characteristic change in the pulse wave is noticed. The change of pulse is called fremitus or ‘gefässchwirren’. Fremitis can be noticed around day100 of gestation in the uterine artery at the side of the pregnant horn and around day 150 at the side of the non –pregnant horn.

1.2 Pregnancy control

Adequate pregnancy control is possible at different stages of gestation. In an early stage of gestation pregnancy can be determined by multiple characteristics. Table 1 shows a list of methods of pregnancy diagnosis and the earliest time they can be used.

Table 1[5] *Methods of pregnancy diagnosis*

Method	Earliest time
Early pregnancy factor (EPF)/early conception factor (ECF)	3 days
Failure to return to oestrus and persistence of corpus luteum	21 days
Progesterone concentration in plasma and milk	21–24 days
Assay of pregnancy-specific protein B (PSPB)	24 days
Palpation of the allantochorion (membrane slip)	33 days
Unilateral cornual enlargement and disparity in size, thinning of the uterine wall, fluid-filled fluctuation of enlarged horns	35 days
Palpation of the early fetus when the amnion loses its turgidity	45–60 days
Palpation of the carnucles/cotyledons	80 days
Hypertrophy of the middle uterine artery until presence of fremitus	85 days
Oestrone sulphate in blood or milk	105 days
Palpation of the fetus	120 days

1.2.1 Early pregnancy factor

Early pregnancy factor is an immunosuppressive glycoprotein produced by the cow associated with pregnancy. The early pregnancy factor can be detected with test kits in serum and milk as early as 3 days after artificial insemination. Studies for test sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 86%, 4%, 49%, 23%, and 46%, respectively[7]. Therefore the early pregnancy factor test is not a very reliable manner of pregnancy control.

1.2.2 Pregnancy – specific protein B

The pregnant –specific protein B is detected in maternal serum after 24 days of gestation. The protein is secreted by the binucleate cells of the trophoblastic ectoderm[2]. Since it has a long half- live time it can also be detected in serum for many weeks post – partum. The test should not be used before 70 days post-calving[33]. After embryonic death or fetal death PSP can be detected in serum in the same concentration. Using a ELISA - PSP to detect the pregnant specific protein 94 % sensitivity at 30 days of gestation has reported[33.]

1.2.3 Milk progesterone tests

For using the milk progesterone test, milk samples have to be collected from day 21 untill day 24 after insemination/mating, with an optimum for 24 days after insemination[5]. Progesterone is the hormone produced by the corpus luteum, low progesterone indicates that corpus luteum has regressed and the cow is not pregnant. High progesterone indicates that the cow has a functional corpus luteum and the cow may be pregnant. The tests are ELISA tests. This test gives a 97.2 % sensitivity of early non – pregnancy diagnosis (repeated milk samples collected between day 18 and 24 post – insemination). Therefore it is the best test for an early non – pregnancy diagnosis[8]. For early pregnancy diagnosis the specificity is around 80% [7]. Reasons for false positive tests are estrus detection errors, pyometra, luteal or follicular cysts, variation in estrus length between cows and early embryonic mortality.

1.2.4 Rectal examination

Corpus Luteum;
A corpus luteum is formed in the ovary after ovulating of the preovulatoir follicle, and secretes progesterone to support the establishment of pregnancy. In non-pregnant cows the corpus luteum starts to regress 17-18 days after ovulation but remains functional for up to 200 days during pregnancy[9]. A CL is the main source of progesterone for the maintenance of pregnancy, the placenta produces only a small amount progesterone. Removal of the ovary prior to 200 days usually results in abortion. 24 days after mating/insemination the corpus luteum in a pregnant cow is 30.2 mm on average and 23,.8 mm on average in a non-pregnant cow[10].

Some characteristics of rectal pregnancy examination used for pregnancy diagnosis are shown in table 2.

***Table 2**[7] Uterine position/diameter and structures during pregnancy*

Days of gestation	Uterine position	Pregnant horn diameter	Palpable Structures
35-40	Pelvic floor	Slightly enlarged	Uterine asymmetry/fetal slip
45-50	Pelvic floor	5.0 - 6.5 cm	Uterine asymmetry/fetal slip
60	Pelvis/abdomen	6.5 – 7.0 cm	Membrame slip
90	Abdomen	8.0 – 10.0 cm	Small placentomes/fetus(10 – 15 cm long)
120	Abdomen	12 cm	Placentomes/fetus (25 -30 cm long)
150	Abdomen	18 cm	Placentomes/fetus (35 – 40 cm long) fremitis

1.2.5 Ultrasound

Ultrasound scanning is another diagnostic tool for pregnancy diagnosis. Real – time B mode scanning is the method of choice for early pregnancy diagnosis in the cow. For early pregnancy a 5 – 8 MHz linear transducer is required, whereas a 3,5 MHz transducer may be used for late pregnancies. Using Doppler, it is possible to identify the fetal heart from 6 – 7 weeks with a rectal probe.

The advantage of using ultrasound scanning after insemination is that it enables relative earlier pregnancy diagnosis as well as offering high accuracy pregnancy diagnosis. Using real – time B mode a 95% sensitivity on day 26 of gestation until an almost 100% sensitivity on day 29 is found[1]. In the literature different dates are noticed of accurate pregnancy scanning have been observed. Boyd et al. (1988) and Pierson et al. (1984) reported pregnancy in cattle as early as 9 and 12 days respectively. Kastelic et al[6], observed that ultrasound pregnancy diagnosis between day 10 and 16 has an accuracy of < 50 % and which improved by day 18 until day 22 (100%). Most of the embryonic deaths occur before day 25 but will continues thereafter.10 – 15 % between 25 and 42 days, 6.3% between 42 and 56 % and 3.4% between 56 and 98 days[1]. If early embryonic loss stays unnoticed, early pregnancy scanning could be a disadvantage and will reduce reproductive efficiency through extending the calving interval[12].

The embryo can first be seen by ultrasound scanning through the detection of the heartbeat, by some embryos heartbeats can be seen at day 19 but the heartbeats are generally visible at day 25. Although embryos can be seen by day 19 the most practical method is to scan pregnant animals expected to have embryos > 25 days. Fluid accumulation increases considerably at approximately day 25, thereafter the embryo detaches from the uterine wall and is easier to detect by ultrasound scanning. The uterine lumen contains a variable quantity of anechogenic fluid produced by the conceptus. After 27 days of pregnancy it is possible to confirm the pregnancy diagnosis by fluid accumulation in the uterine lumen. Fluid in the uterus during oestrus may be confused with pregnancy and this may cause a potential diagnostic error. Before day 30 of gestation it is more difficult to observe the embryo because the young embryo is often located near the uterine wall in a small amount of fluid. Careful examination of the anechogenic area mostly reveals the presence of the embryo[27].

There are some landmarks for a veterinarian to detect normal development of the conceptus[1]. After day 30 of gestation it is possible to visualize the amniotic membrane. The amniotic vesicle is clearly visible through the reflections due to the round shape *(see fig. 5).* After day 35 the first placentomes can be viewed. The attachment of the umbilical cord from the embryo to the uterus starts at day 40 of gestation. Ossified ribs in the fetus can be viewed from day 50 until day 60 of gestation *(see fig. 6).* The sternum starts to ossify between day 81 and 85.

The first centres of ossification in the skull, which are very echogenic, appear at the end of the 2nd month of gestation. The ossification of the skull completes by day 100[13]. The head of the fetus is visible by ultrasound scanning during the entire gestation. The eyes can be seen and are an indicator of the gestation length and increase rapidly during the first 6 months of gestation (see table 3)

The ossification of the cervical, thoracic, lumbar and sacral vertebrae starts at day 61 to 65 whereas the ossification of the coccygea vertebrae starts at day 86[13]. The scapula, ilium and ischium ossified approximately at 70 days of gestation. The long bones of the limbs start to ossify around day 61 – 65 and the digits begin to ossify between day 81 and 85 of gestation. Kahn[14] (1989) found a relationship between multiple fetal structures and the gestation age which can be used to estimate the gestation age.
The heartbeat is easy to visualize with the ultrasound. The frequency of the heartbeat varies with the gestation age. Moreover, within one fetus the heartbeat varies at one day due to individual fetal activity[1] *(See table 3).*

During the first stage of gestation the abdomen is almost entirely filled with the liver and the mesonephros, during the last stage the liver growth slows down. Around 60 days the stomach is divided into the four compartments whereas the abomasum and the omasum appear as hyperechogenic spots and the rumen as a hypoechogenic spot. Other organs in the abdomen are not that easy to identity. In the first part of gestation the kidneys are occupy a large volume in the abdomen, around day 90 of gestation the kidneys get their 'normal' location and shape.
Examination of the cow for ultrasound fetal sexing can be done between 54 and 100 days of pregnancy, but the ideal moment is between 60 and 70 days of gestation[1]. This report, however, will not discuss ultrasound fetal sexing.

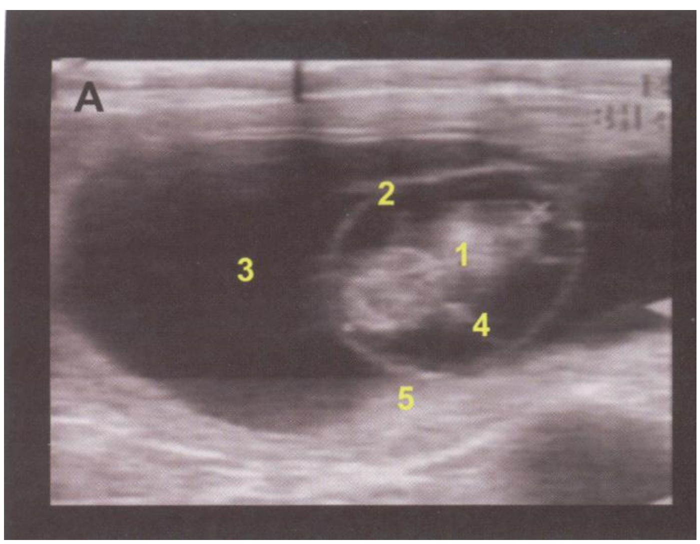


Figure 5: *40 day old embryo (1) measuring 21 mm. Probe 7,5 MHz. Starting on day 30 of gestation, there is generally enough amniotic (2) and allantoic (3) fluid in the uterus to be able to position and view the bovine embryo in the centre of the zone of accumulated fluid. On these ultra sonograms, the head, limbs (4) and placentome (5) are visible.*

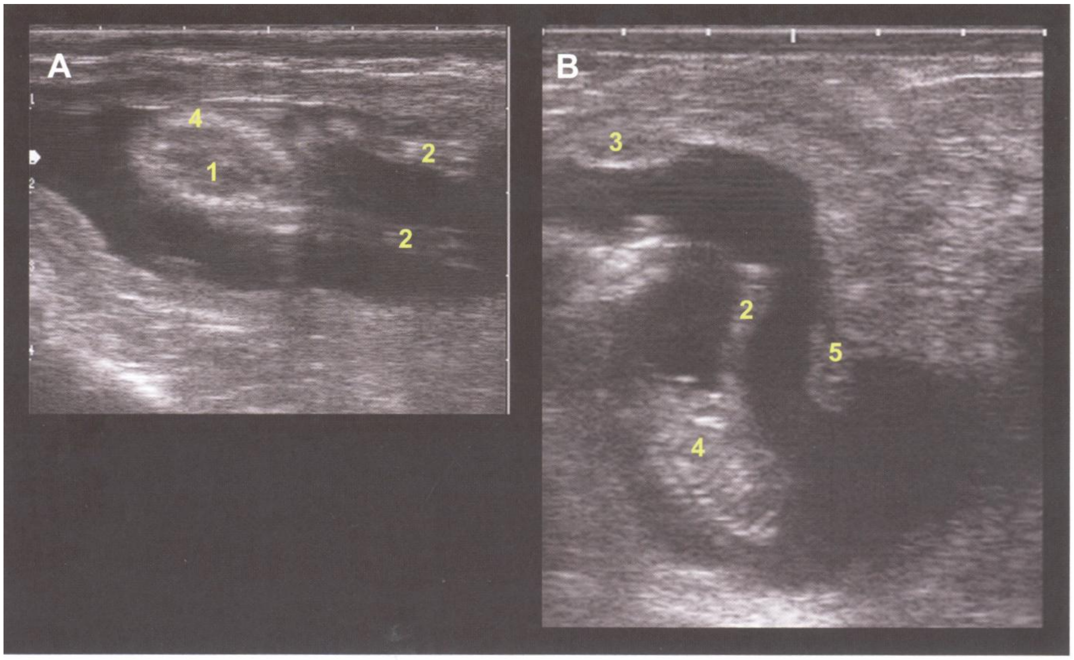


Figure 6*[1]*: *Fetus on day 59 with the first view of the ribs. A: transverse section of the fetus at the level of the anterior portion of the thorax. B: longitudinal section. 1: fetus 2: anterior limb 3: placentome 4: ribs 5: umbilical cord*

According to DesCôteaux[1] the crown rump length is the easiest and most precise parameter in estimating gestational age till day 55 of gestation. After day 55 the diameter of the trunk and the head are more practical because it is not possible to provide a view of the entire crown rump. In ***table 3*** and ***4*** are some embryonic and fetal measurements summarized which can be used to predict the gestation age.
In our study we will measure the placentome length at different time points during gestation to investigate whether the placentome length as measured by transrectal ultrasound scanning is a quick and reliable benchmark to predict the gestational age of a pregnant cow.

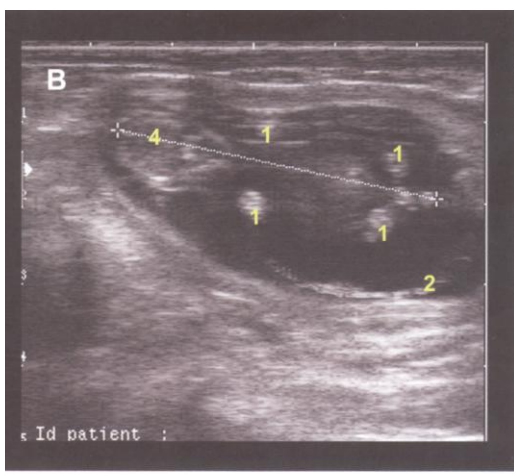


Figure 7*[1]*: *Evaluation of gestational stage by measuring the crown-rump length of a 53 day old fetus. 1: limbs 2: amnion 4: head*

Table 3*[1] *Embryonic and fetal measurements and their moment of apparition in ultrasound scanning between 25 and 55 days of gestation

	Day 25	Day 30	Day 35	Day 40	Day 45	Day 50	Day 55
Crown-rump length	0.5-0.7	0.8-1.2	1.3-1.7	1.7-2.4	2.3-2.6	3.5-4.5	4.5-6.0
Cardic freq.(b/min)	140-150	160-180	170-190	170-290	170-190	180-200	180-200
Allantois	+	+	+	+	+	+	+
Amnion		+	+	+	+	+	+
Spinal column		+	+	+	+	+	+
Anterior limbs		+	+	+	+	+	+
Posterior limbs			+	+	+	+	+
Trunk diameter			0.6	0.9	1.2	1.5	1.7
Placentome (cm)			0.3	0.5	0.6	0.8	1.0
Claws					+	+	+
Eye diameter						0.3	0.4
Ribs						+	+

Table 4*[10] *Fetal measurement in ultrasound scanning between 60 and 270 days of gestaion.

	Day 60	Day 70	Day 80	Day 90	Day 100	Day 120	Day 140	Day 150	Day 180	Day 210	Day 240	Day 270
Crown-rump length	6-7	9-13	12-13	13-17	19	22-32	33	37-40	46-54	60-70	60-82	65-88
Eye diameter (mm)	4	6	8	10	12	16	19	20	24	26	27	27
Trunk diameter (cm)	1.7-2.2	2.3-2.9	3.0-3.7	3.9-4.5	5.1-5.3	7.0-8.7	8.9-14.8					
External skull diameter (cm)	1.6-1.8	2.0-2.3	2.5-2.8	3.2-3.3	3.8-4.0	4.9-6.2	5.9-9.6					
Heart rate (b/min)	160-195			150-180		145-165		140-155	135-155	135-145	125-135	120-125

2. Literature study

2.1 Determination of gestation age

In the dairy industry determination of the gestation age is an essential part of the reproductive efficiency. Multiple characteristic can be used for this purpose. In cattle fetal measurements are commonly used. White et al[15] (1985) found that gestational age (between 20 and 140 days) could be estimated by measurements of certain uterine and fetal dimensions. Crown-rump length provided the most precise estimate of gestational age (sd 4.5 days). The head length and the diameters of the trunk, head and nose being intermediate and the uterine diameter the least (sd 12,6 days). Harris et al[16] (1983) found a crown-rump length from 1 cm on day 57 until a length of 59.1 cm at day 200. This results in the regression equation Y(fetal age in days) = 54.6 plus or minus 2.46X(crown-rump length). Thomsen[17] showed a linear correlation between log weight and log length, log weight and log head circumference, and log body length and log head circumference. From these relationships he calculated the fetal age. These findings are derived from fetuses collected from slaughtered animals. Richardson has created the following formula for calculating the age of the fetus from its crown – anus length[5] X (day of gestation)= 2.5 (Y (crown – anus length) + 21).All of these fetal measurements are, however, under practical conditions time consuming and visualization of the fetus is difficult especially later in gestation.

From day 60 of gestation until the end of gestation placentomes are relative easy to visualize and simple to measure by using ultrasound scanning. Placentome measurement may be an alternative for the prediction of the gestation age. Although veterinarians use placentomes to predict the gestational age no scientific evidence is published to evaluated their use in predicting the gestation age in cattle.

Placentomes can be ultrasonically identified starting on day 35, 2 (+/- 1.0) of gestation and are visible close to the embryo according to Curran et al [18] (1986). The book Ruminant and Camelid Reproductive Ultrasonography 2010 mentioned the following placentome sizes on day 35 (0.3 cm), day 40 (0.5cm), day 45 (0.6cm), day 50 (0.8cm) and day 55 (1.0cm). This book does not, however, provide a reference to any report which published these placentome sizes.

2.2 Placentome shape

There are two shapes of placentomes identified by Laven and Peters, normal and flat[19.] The normal cow placentome is a concave one with chorion tissue at the outside and caruncular tissue at the inner side. The bulk of the caruncular stalk consists mostly of large, vigorously spiraled arteries[20]. Sheep and goats have a cup- like shape of the placentomes[20]. No significant (P= 0.10) effect of gestation age was found on the shape of the placentomes during pregnancy in cows, whereas ovine placentomes change from type in late gestation[20]. There was no evidence that the proportion of uteri with flat placentomes change during the pregnancy[19]. According to Zhu et al. (2007) the cow

placentome does not exhibit distinct changes in type with advancing gestation, although it gradually increases in size and vascularity[20]
The pregnant horn had a significantly higher weight percentage of flat placentomes compared to the non – pregnant horn[19].

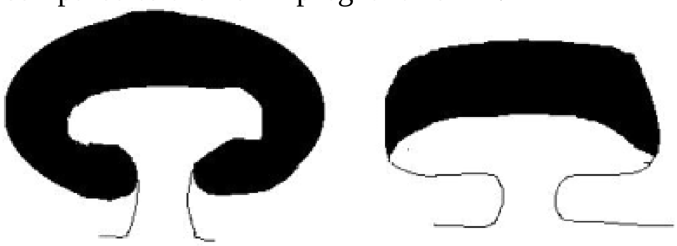


Figure 8[19] *Two different shapes of the bovine placentome*

2.3 Placentome number

Laven and Peters[19] (2001) did research to placentomes in uteri from 47 slaughtered animals. They found fewer ($P< 0.05$) number of placentomes in the non-pregnant horn as compared to the pregnant horn. From the 47 uteri 10 uteri had no placentomal development in the non –pregnant horn, this has not been previously reported. No significant change in the total number of placentomes during gestation was found (see table 5). Moreover, no correlation was found between the number of placentomes in the pregnant horn and the corresponding number in the non-pregnant horn. Moreover no correlation was found between the placentome number and sire, sex of fetus and fetal age[21]. According to Senger[2] there were on average between 70 and 120 cotyledons distributed across the surface of the chorion.

Table 5[19]. *Number of placentomes in each horn in relationship to the estimated gestational age*

Days of gestation	Pregnant horn			Non-pregnant horn		
	Mean	SEM	Range	Mean	SEM	Range
< 71	43	3.9	31–55	9.4	4.7	0–16
71–100	44.4	3.9	31–65	17.8	5.6	0–32
101–130	43.3	3.3	31–56	16.1	5.6	0–42
131–160	57.2	3.3	42–69	21.1	5.6	0–38
161–190	43.9	3.3	35–55	18.9	5.3	20–35
191–220	48.3	1.7	45–54	26.9	6.67	0–35
221–250	53.1	10.3	26–88	22.2	1.7	22–36
251 +	48.9	3.3	40–58	22.8	8.9	0–51

2.4 Placentome weight

Laven and Peters[19] also examined the placentome weights. They found an overall significantly lower placentome weight in the non – pregnant horn (P < 0.01) compared to the pregnant horn for the individual average placentome weight as well the total placentome weight. No correlation was found between the total weight of the placentomes and the total number of placentomes. There was no correlation between the number of placentomes and the weight of individual placentomes (p=0.09). Therefore no compensatory increase in placentome weight was noticed when fewer sites were filled. The increase of weight in the pregnant horn decrease with time, between 60 and 190 days there is a significant (P <0.05) increase in the total weight but there is no significant (P > 0.1) increase after this time. However, Anthony et al[21] found an increase of individual placentome weight as well for total placentome weight in beef cattle between 200 en 260 days. On day 200 the total placentome weight was 2600 gram and the average placentome weight was 31 gram, on day 260 was the placentome weight was 4873 and 52.5 gram respectively. According to Reynolds et al[22](1990), placentome weight increase exponentially between 100 and 250 days of gestation, with a relative decreased rate onwards the end of the gestationperiod. *(see table6).*

Table 6[22]*: weight (means +/- s.e.m.) of gravid uterine tissues of cows*

Tissue wt (kg)	Day of gestation			
	100	150	200	250
Gravid uterus	3·61 ± 0·16	12·73 ± 0·56	28·79 ± 1·59	47·34 ± 3·64
Uterus	—	1·99 ± 0·13	3·75 ± 0·20	4·28 ± 0·32
Fetus	0·38 ± 0·01	3·10 ± 0·11	12·63 ± 0·38	27·75 ± 2·13
Fetal membranes	0·25 ± 0·02	0·46 ± 0·03	1·15 ± 0·77	1·45 ± 0·92
Fetal fluids	—	6·01 ± 0·45	7·86 ± 1·07	9·05 ± 0·85
Caruncles	0·13 ± 0·001	0·71 ± 0·05	2·12 ± 0·14	3·11 ± 0·29
Cotyledons	0·16 ± 0·01	0·46 ± 0·03	1·28 ± 0·11	1·69 ± 0·28
Curved crown–rump length (cm)	23·0 ± 1·0	48·5 ± 0·8	75·6 ± 1·3	90·1 ± 2·4

Numbers of observations were 4 on Day 100, 5 on Day 150, 6 on Day 200 and 6 on Day 250 of gestation for all tissue weights and also for curved crown–rump length.

2.5 Placentome length

Senger[2] mentioned a placentome length of 5 to 6 cm in diameter at the end of gestation with no reference to a report.Laven and Peters[19] show that there was a significant (P < 0.01) increase in the average length of placentomes with increased gestational age. There was no correlation (P >0.05) between mean placentome length in the pregnant horn and the total number of placentomes[19]. The largest placentomes are found near the fetus in the pregnant horn whereas the placentomes at the tip are the smallest. The largest placentomes in the non – pregnant horn are those in het mid – horn region. Placentome length increased linearly during gestation. After 190 days of gestation the rate of increase slowed considerably in spite of the fact that the growth in fetal weight is the greatest during this period. Change in vasculature that increase the fetus/maternal exchange could be an explanation for the high fetus/ placentome ratio in the last stage of gestation[23]. No significant increase of the placentome length showed between 190 and 280 days of gestation[19]. This is the reason that ultrasound scanning of placentomes in the pregnant cow is unlikely to be useful for diagnosing the stage of gestation after 190 days.

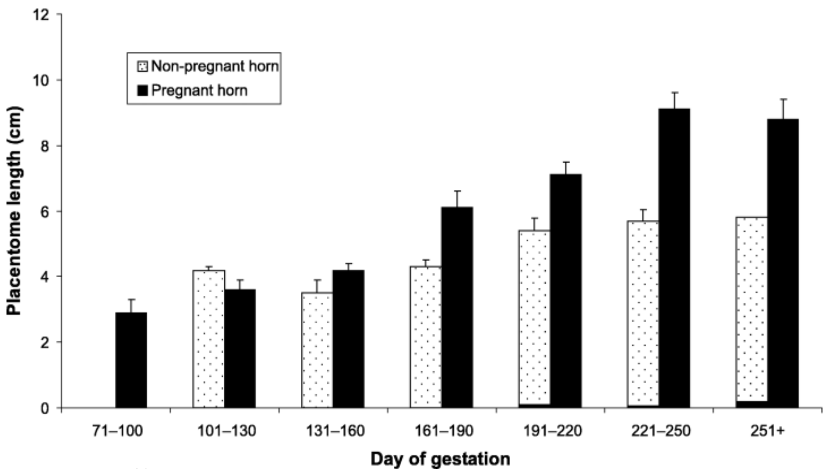


Figure 9 [19]***:*** *growth in placentome length in the pregnant and the non- pregnant horn.*

Some research of placentome size has been done by cloned and IVF pregnancies. Heyman et al[28] (2002) found no reference values for placentome size in cattle which is why they made a control group of 13 animals. They conducted a transabdominal scan of cows from 120 days of gestation every 14 days and found that the mean size of placentomes between 4 and 6 months of pregnancy were significantly higher in cows which carried somatic clones than in the control group. A possible explanation for this placental pathology is the high level of maternal serum pregnancy protein PSP60. The placentome size is recorded by transabdominal scanning and is shown in surface area (cm^2) and therefore not very useful for the placentome study.

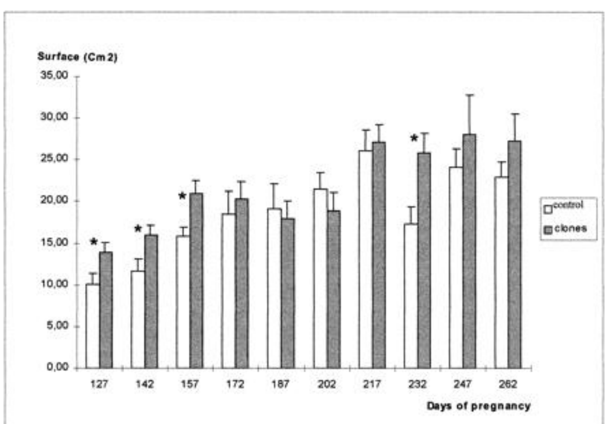


Figure 10 [28]***:*** *Evolution of placentome size during pregnancy after somatic cloning X-axis, stage of pregnancy (days); Y-axis, mean size of placentomes (cm^{2} +/- SEM) * Significantly differtent from controls. (P < 0.005)*

2.6 Placentomes size in Goat and Sheep

However there is no report published which uses placentome size in cattle to predict the gestation age, Doizé et al[24](1997) published a report which determine the gestation age in goat and sheep by using transrectal ultrasonographic measurements of placentomes. In ewes placentomes can be detected as small nodules by day 21 of gestation. At day 30 the periphery of the placentome begins to rise and form a thin lip around a flat centre. Placentomes can be detected using ultrasonographic at day 32 of gestation in ewes and at day 35 of gestation by does. By 90 days the placentomes reach the maximum weight and diameter[25]. Adyn et al[26] found a maximum diameter of the placentomes in ewes at week 11 (3.41 + or – 0.04 cm) in the second half of gestation placentome decrease in length till 1.73 + or – 0.18 cm before birth. This is in agreement with Doizé et al[24], they found that placentomes reach a maximum diameter at day 74 in ewes and at day 91 in does. The largest placentomes had a diameter of about 3 cm in ewes and about 2,5 cm in does.

After transrectal ultrasound scanning of 169 does with estimate gestation age *Doizé et al* calculate a linear regression relationship between fetal age in days and placentome size in mm. Gestational age = 28.74 + 1.80 * placentome length + e with a correlation coefficient from 0.7034. In the following experiment with 63 does they determine the gestation age after placentome measurement and found a correctly predicted gestation age in 66% of the measurements within a margin of +/- 7 days and in 96 % within a margin of +/- 14 days. In conclusion transrectal ultrasound measurement of placentome size during the first 90 days of gestation could be used as an indication of gestation age.

Doizé et al. used 132 ewes which were scanned using transrectal ultrasound in order to calculate a linear regression equation. The linear regression equation was less accurate but still significant. The linear relationship in ewes is Gestational age = 47,.98 + 0.62 * Placentome length + e with a correlation coefficient from 0.1559. In conclusion transrectal ultrasound measurement of placentome size is not useful for predicting the gestation age in sheep. This is in agreement with Aydn et al.. *Doizé et al* offer some possible explanations for the poor relationship between the size of the placentomes and the gestation age. It may, for example, be due to the number and size of placentomes within the uterus, given that only one placentome was scanned at each time point. The smallest placentomes were found near the tip of the horns and the bigger ones in the middle of the horn. An other possibility for the poor relation could be the variation in number and size between females and breeds of ewes[24].

Part II: Research project

Determination of gestation age in dairy cattle between 60 and 170 days of gestation using transrectal ultrasound measurements of placentome size

1. Introduction

Determination of gestational age during pregnancy diagnosis is an essential part of assessing reproductive efficiency. In cattle, fetal measurements are commonly used for this purpose. However, obtaining these measurements can be time consuming and visualization of the fetus is difficult to achieve, particularly in the latter stages of gestation. A possible alternative could be the measurement of placentome size. This might be viable because, from 60 days of gestation onwards, placentomes are relative easy to visualize and simple to measure using ultrasound and they grow linear in size until day 190 during gestation. Although there is anecdotal evidence of the use placentomes, as yet there have been no published studies evaluating their use in predicting gestational age.

In the cow the allantochorion membrane and endometrium become modified for exchange at specialized points of contact, namely the placentomes. The structure composed of maternal caruncle and fetal cotyledon, is the organ through which respiratory gases, nutrients and wastes are transported[19]. Placentomes can be ultrasonically first seen around day 35[29]. Placentome length increased linearly during gestation. However after 190 days of gestation the rate of increase of the placentome length slowed considerably. Between 190 and 280 days of gestation no significant difference in placentome length is showed[19].

1.1 Objective and hypothesis

The main objective of this study is to investigate the possible relationship between placentome characteristic and gestational age in dairy cattle from day 60 until day 170 of gestation.

This objective has been structured in such a way that it will address the following three research questions:

-What is the relationship between the length, the perpendicular length and area of the placentomes measured through ultrasound scanning and the gestation age?

-Which independent factors (breed, age, farm or horn) have a significant effect on the length and perpendicular length of the placentomes measured through ultrasound scanning?

-To what extend are placentome characteristics measured by ultrasound scanning useful for predicting the gestation age.

Hypothesis: *Transrectal ultrasound measurements of placentome size are useful (sd <= 5 days) for predicting the gestational age in dairy cattle. Breed, age, farm and uterine horn have no significant effect on the placentome size.*

2Material & methods

2.1 Cows

For this trail 62 cows were initially selected. All of the cows were seasonal breeders from a spring herd, and throughout the whole trial all of them were pasture-based managed. The animals were housed on two different farms. 32 cows were held on a farm from Diary NZ in Taranaki, the other 30 cows were from a Massey University farm, the Massey no.4 farm. The animals were selected after artificial insemination. Cows on the Taranaki farm were inseminated after showing signs of estrous behavior in their nature cycle. On the Massey farm all of the cows were synchronized before insemination. All the insemination data was taken between the 11th of October 2009 and the 10th of November 2009. Two cows from the Massey farm were removed from the research proceedings for the following reasons: One cow did not conceive and the other had an abortion. The selected cows were further divided into the following statistical groups. *(See table 1)*

Table 1

Farm	Taranaki (N=32)	Massey (N=28)	
Breed	Jersey (N=15) (14 Taranaki & 1 Massey)	Crossbred (Friesian * Jersey N(=17) (2 Taranaki & 15 Massey)	Friesian (N=28) (16 Taranaki & 12 Massey)
Age	Primiparous (N=31)	Multiparous (N=29)	

2.2 Scanning

Cows were scanned transrectal with a 7, 5 MHz linear probe (Mindray DP6600). In this study the cervix was used as landmark for the placentome scanning. Because the placentomes at different places on the uterus have different sizes[19], with the largest placentomes at the corpus of the uterus, it is important to use placentomes at the same place on the uterus to standardize the study. Placentomes were chosen one probe length (6 á 7 cm) cranial from the cervix because of these were the best to find after multiple scanning over time in the same animal. This because of the placentomes in the middle of the uterus horns sinks in the abdomen after 4 or 5 months of gestation and it is not possible anymore to reach the corpus of the uterus.
Around 4 different placentomes on the left uterus horn and 4 placentomes on the right uterus horn were scanned. Placentomes were scanned on their maximum size and pictures were saved on a USB storage. Scanning started approximately 60 days after insemination. Until day 170 of gestation each cow were scanned once every 10 days on the Massey farm. *(see fig. 1).* On the Taranaki farm each cow was scanned once every 10 days until day 130 and thereafter once every 30 days until day 160 of gestation. Determination of the pregnant and the non-pregnant horn took place on day 100 and day 110 of gestation.

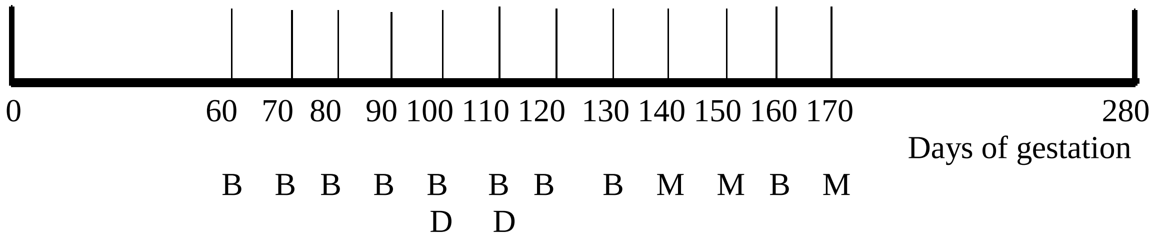


Figure 1: *Scanning was performed during different stages of gestation. B= Massey + Taranaki M = only Massey, D = determination of the pregnant horn.*

2.3 Pictures processing and analyzing

Image J was used for measuring the placentome size. Image J is a program for picture processing and analyzing. The scale from the scanner was saved in the same screen as the placentome pictures. The length, the perpendicular length and the area of the placentomes were measured in mm. The longest axis of the placentome is the length of the placentome. The axis right – angle on the longest axis is the perpendicular length of the placentome. The area of the placentome was measured by drawing a line around the whole placentome afterwards Image J calculated the area in mm^2.

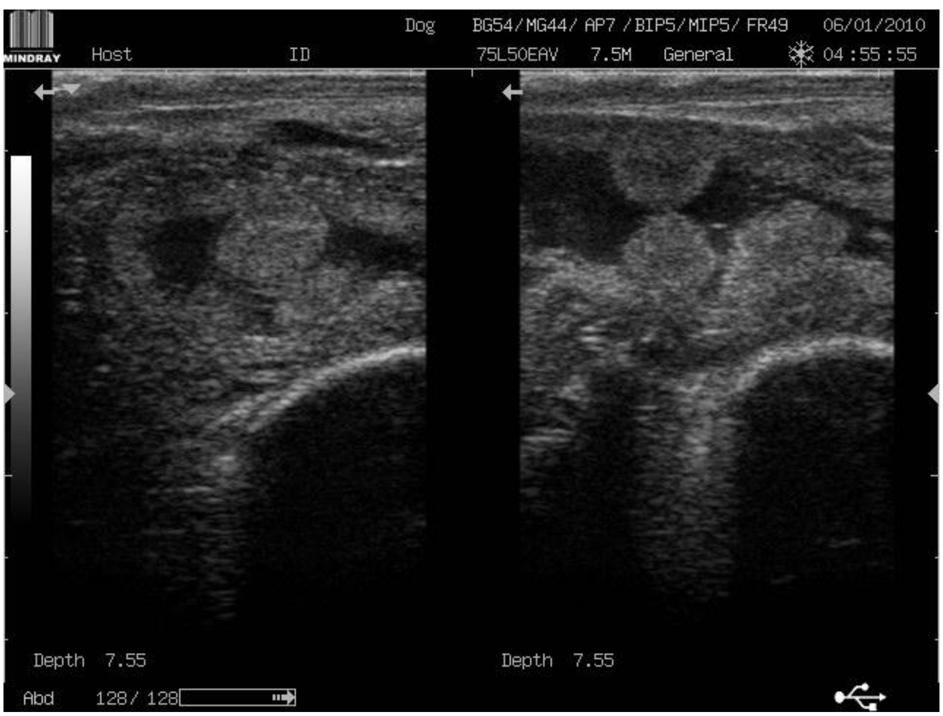


Figure 2 *Scanning picture. Three placentomes in the pregnant horn on day 74 of gestation are shown*

Figure 3 *Scanning picture. Four placentomes in the non - pregnant horn on day 96 of gestation are shown*

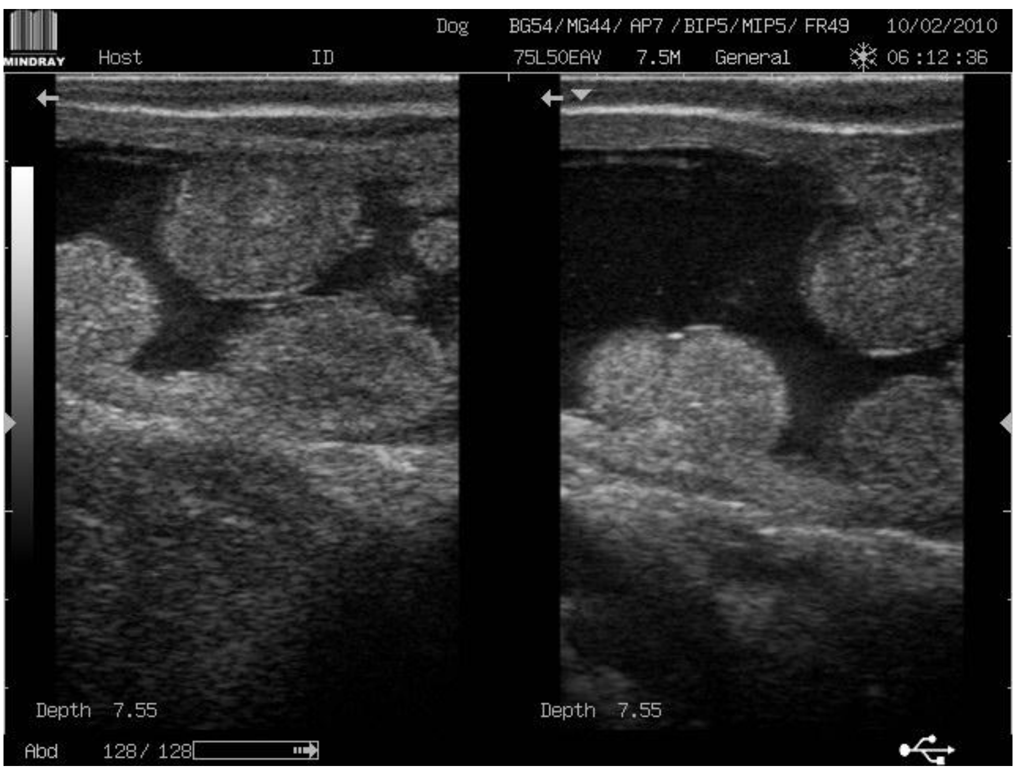


Figure 4: *Scanning picture. Three placentomes in the pregnant horn on day 110 of gestation are shown.*

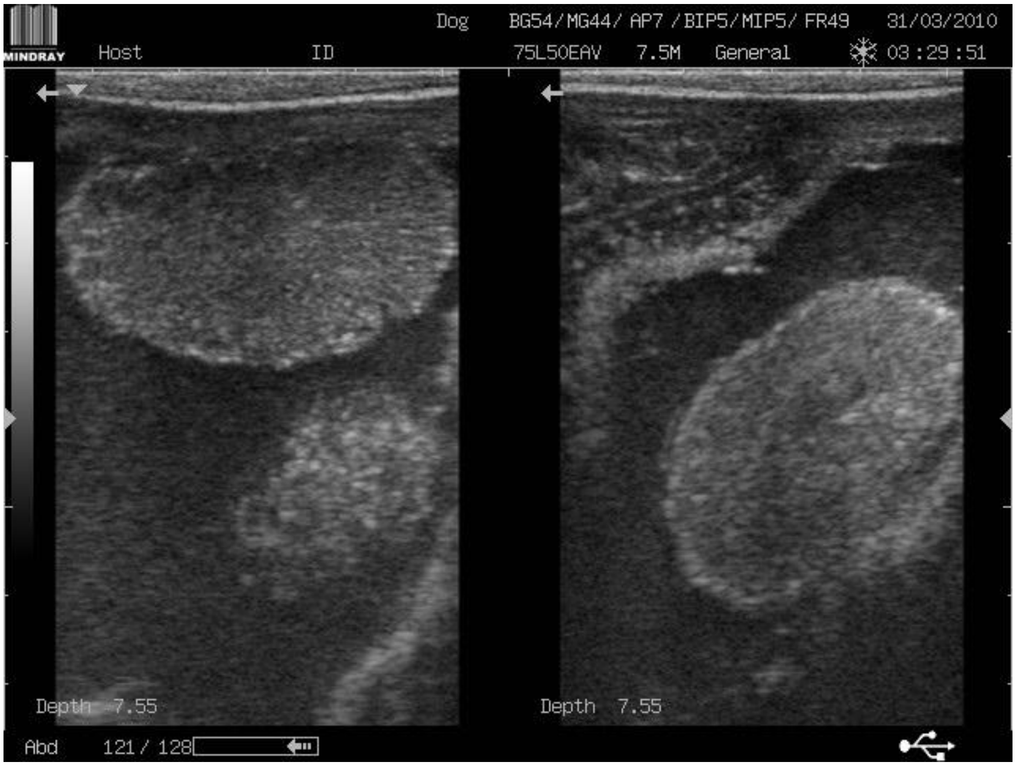


Fig. 5. *Scanning picture. Two placentomes in the pregnant horn on day 159 of gestation are shown*

2.4 Dataset:

The pictures were processed and analyzed, such that the resulting dataset contained data from 3529 placentomes from 60 cows. From each placentome the following information was recorded: the length, the perpendicular length, the area and the origin of the placentome (pregnant horn or the non – pregnant horn.). Scanning took place between 15 December 2009 and 31 March 2010. The number of days of gestation (artificial insemination date minus the scan date) vary between 52 and 171 days.

2.5 Statistical analysis

A repeated measured mixed model was used to analyze the data. The statistical work has been done by Moniek van den Bosch[30]. She has been working with me on the placentome study. She used mixed models (PROC Mixed, SAS 9.2, 2008). Whereas I used mixed model with SPSS 16 for the first part. Repeated measurements were added over time for the cows. Placentome length, perpendicular placentome length and the placentome area were added as dependent variable. Independent variables were farm (Massey or Taranaki), breed (Holstein Friesian, Crossbred and Jersey), age (multiparous or primiparous) and days of pregnancy. The mixed models were also used for regress the placentome data for farms against the gestational age.

3. Results

3.1 Placentome size

Before we start analyzing the dataset, the cows were classified, based on the number of days of pregnancy (based on artificial insemination data), in classes of 10 days. Classification of the cows and the number of observations per class and per uterine horns are shown in table 2.

Table 2: *Classification of the cows per class and the number of observations per uterine horns (pregnant horn or non-pregnant horn)*

Actual gestation age (Days)	**Number of cows per class (N)**	**Classified gestation age (Days)**	**Number of observations in the pregnant horn**	**Number of observations in the non-pregnant horn**
52-55	6	50	16	0
55-65	17	60	67	0
65-75	8	70	14	28
75-85	36	80	126	141
85-95	56	90	184	218
95-105	58	100	219	214
105-115	53	110	213	222
115-125	55	120	241	237
125-135	56	130	278	271
135-145	22	140	68	74
145-155	25	150	105	106
155-165	44	160	190	195
165-171	17	170	52	50

Figures 5, 6 and 7 show the mean length, mean perpendicular length and mean area of the placentomes including the standard error bars at the different classes of gestational age.

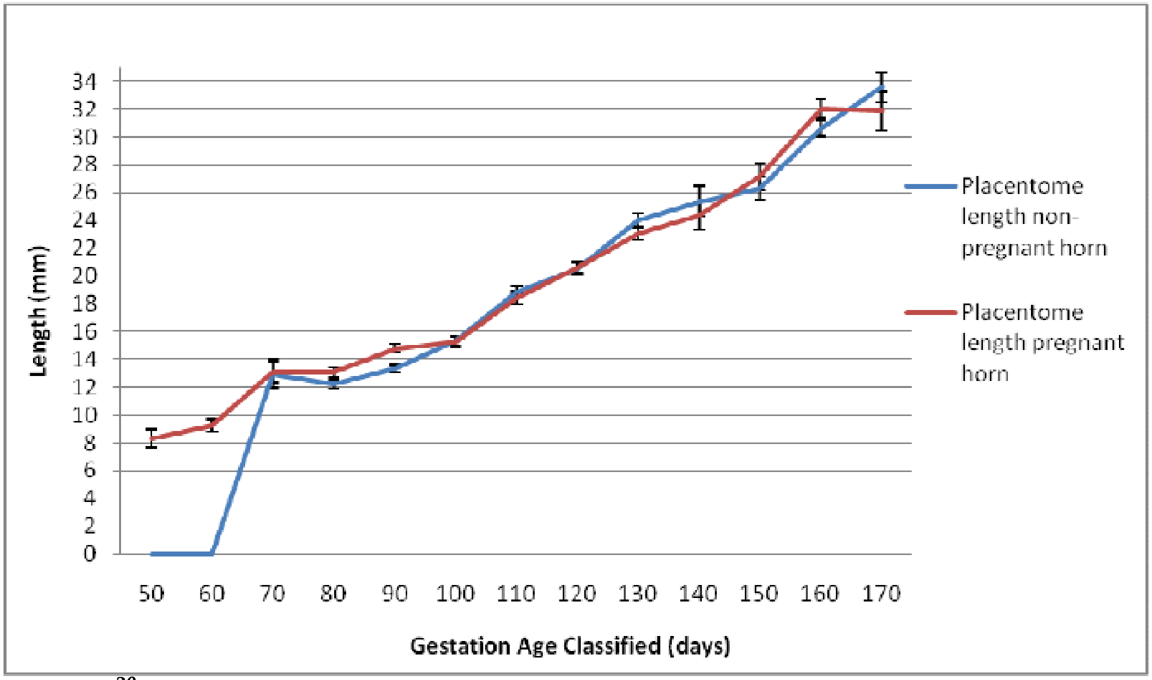


Figure 5[30]: Mean length (mm) of the placentomes found by transrectal ultrasound scanning for the pregnant (red line) and non-pregnant (blue line) horn. On the X-axis the classified days of gestation are shown.

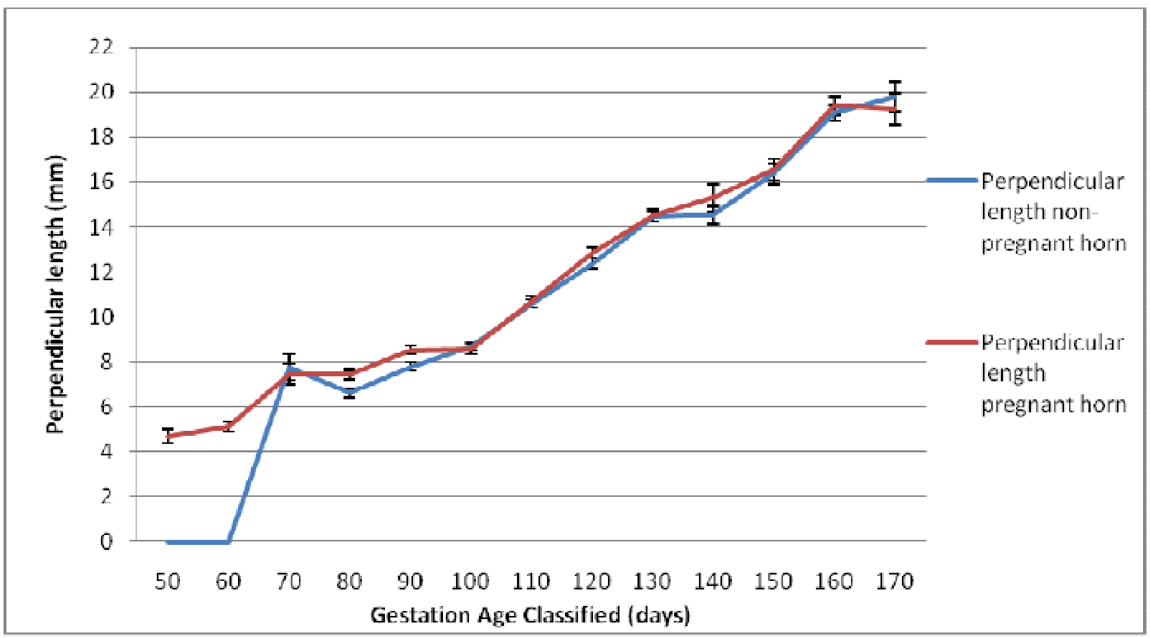


Figure 6[30]: Mean perpendicular length (mm) of the placentomes found by transrectal ultrasound scanning for the pregnant (red line) and non-pregnant (blue line) horn. On the X-axis the classified days of gestation are shown.

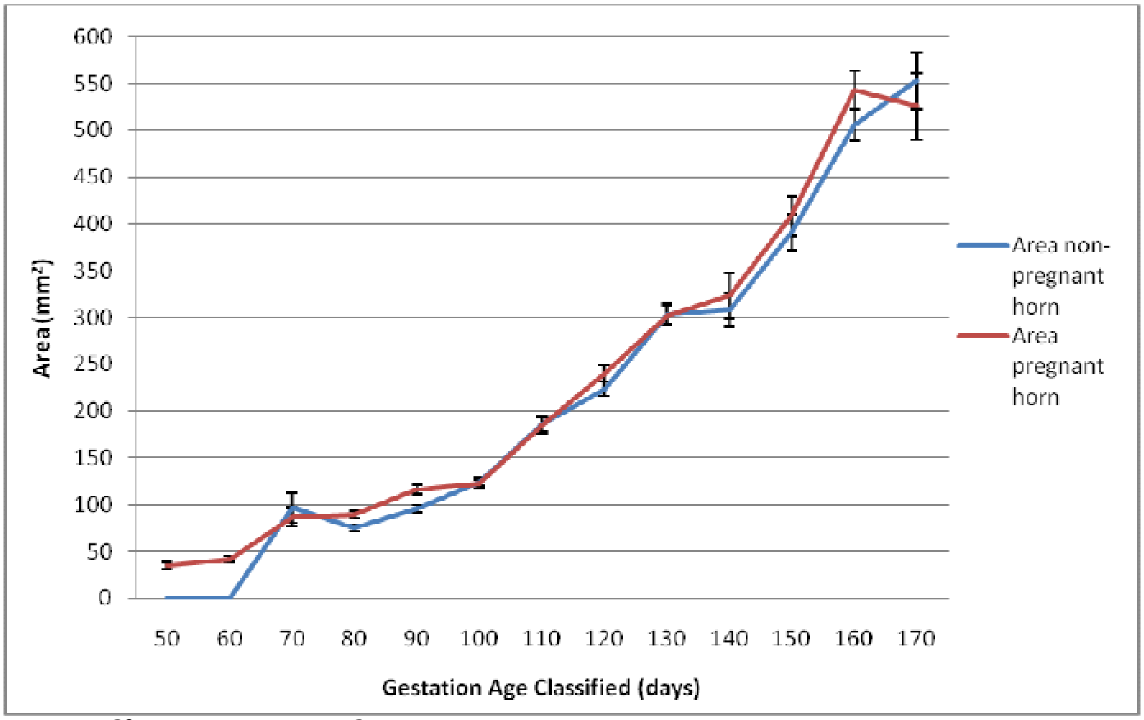


Figure 7[30]: Mean area (mm^2) of the placentomes found by transrectal ultrasound scanning for the pregnant (red line) and non-pregnant (blue line) horn. On the X-axis the classified days of gestation are shown.

Placentome length, perpendicular placentome length and placentome area increase significantly ($P < 0.05$) every 10 days from day 100 of gestation till day 130 of gestation and from day 140 until day 150 of gestation.
The correlations between the placentome length, the perpendicular placentome length and the placentome area are highly significant (see table 3).

Table 3: *Correlation between the placentome length, perpendicular placentome length and the placentome area*

	Length		**Perpendicular**		**Area**	
	r	**P-value**	**r**	**P-value**	**r**	**P-value**
Length	-	-	0.81	<0.001	0.92	<0.001
Perpendicular	0.81	<0.001	-	-	0.89	<0.001
Area	0.92	<0.001	0.89	<0.001	-	-

3.2 Linear regression:

Analysis of the dataset showed that there was no significant effect of the independent variables breed, age and uterine horn on placentome size ($P > 0.05$). A significant effect of the independent variables farm and days of pregnancy were found on the placentome size ($P< 0.001$). Due to a significant farm effect on the placentome size two different linear regression equations were calculated *(see table 4).* The regression model used was:

$\mathbf{Y = \beta_0 + \beta_{1} * X + e}$

Where Y is the gestation age of the cow in days, β_0 is the intercept and β_1 is the slope of the regression coefficient, X is the length (mm or mm^2) of the placentome and e is the residual error corresponding to observation Y. The residual error is an observable estimate of the unobservable statistical error.

Table 4: *Linear regression equations of the gestation age and the placentome measurements per farm.*

	Farm	**Intercept(β_0)**	**P-value**	**Slope (β_1)**	**P value**	**R^2**
Length	Massey	68.36±2.34	<0.0001	2.38±0.10	<0.0001	0.59
	Taranaki	53.43±2.09		3.09±0.10		0.68
Perpendicular length	Massey	66.56±2.03	<0.0001	4.13±0.15	<0.0001	0.65
	Taranaki	52.76±1.77		5.31±0.14		0.77
Area	Massey	91.84±1.40	<0.0001	0.10±0.004	<0.0001	0.61
	Taranaki	82.63±1.24		0.14±0.005		0.68

3.3 Limits of agreement

The linear regression equations found for the Taranaki farm and the Massey farm were used to predict the gestation age of the cows per farm. The actual and predicted gestational age were plotted against each other for placentome length, perpendicular placentome length and placentome area. *Figure 8* shows a significant linear relation between the predicted and the actual gestation age for the placentome length for both farms. Because of the high correlation between the placentome length and the perpendicular length and the placentome area only the placentome length is shown in this report.

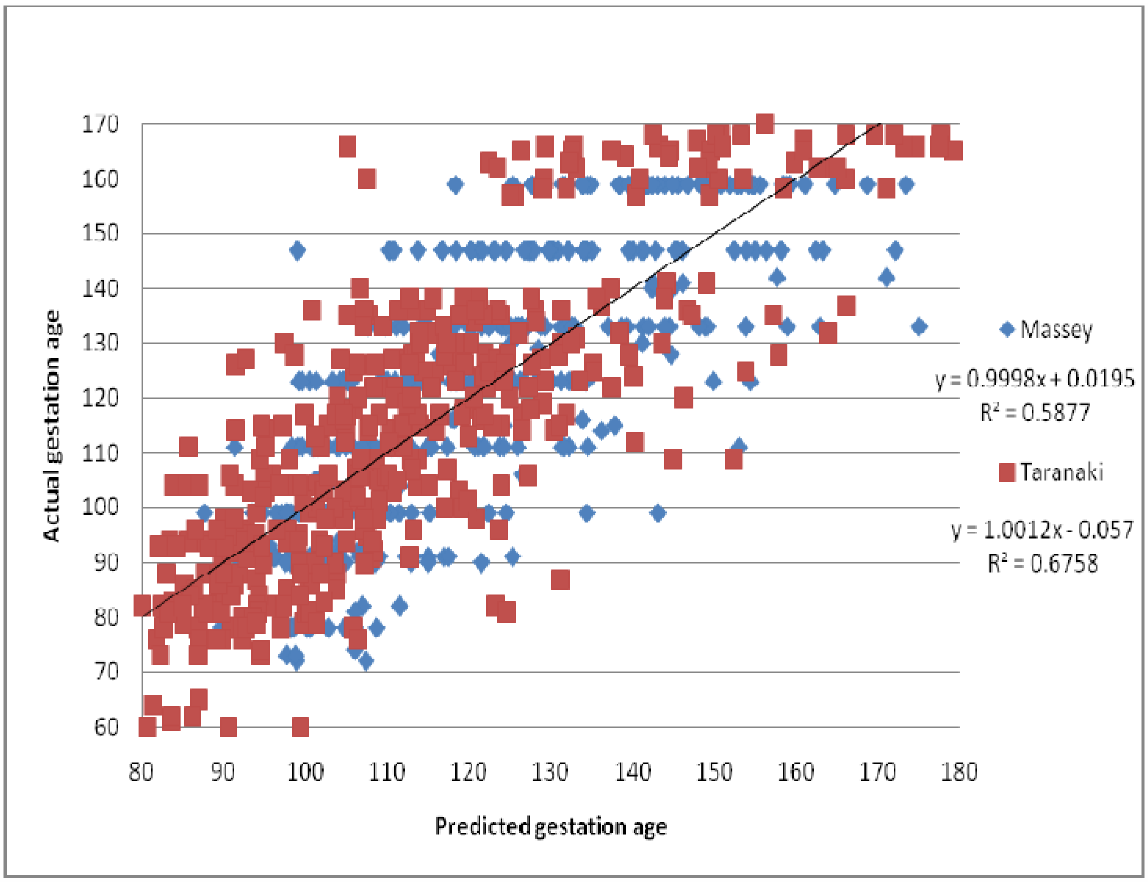


Figure 8[30]*: the relationship between the predicted and the actual gestation length for the Massey and the Taranaki farm.*

Since we were interested in the similarity between the predicted and the gestational length we determined the limits of agreement. As such we determine the limits within which 95% of the difference lies. The standard limits of agreement plots for both farms are shown in *figure 9* and *10*

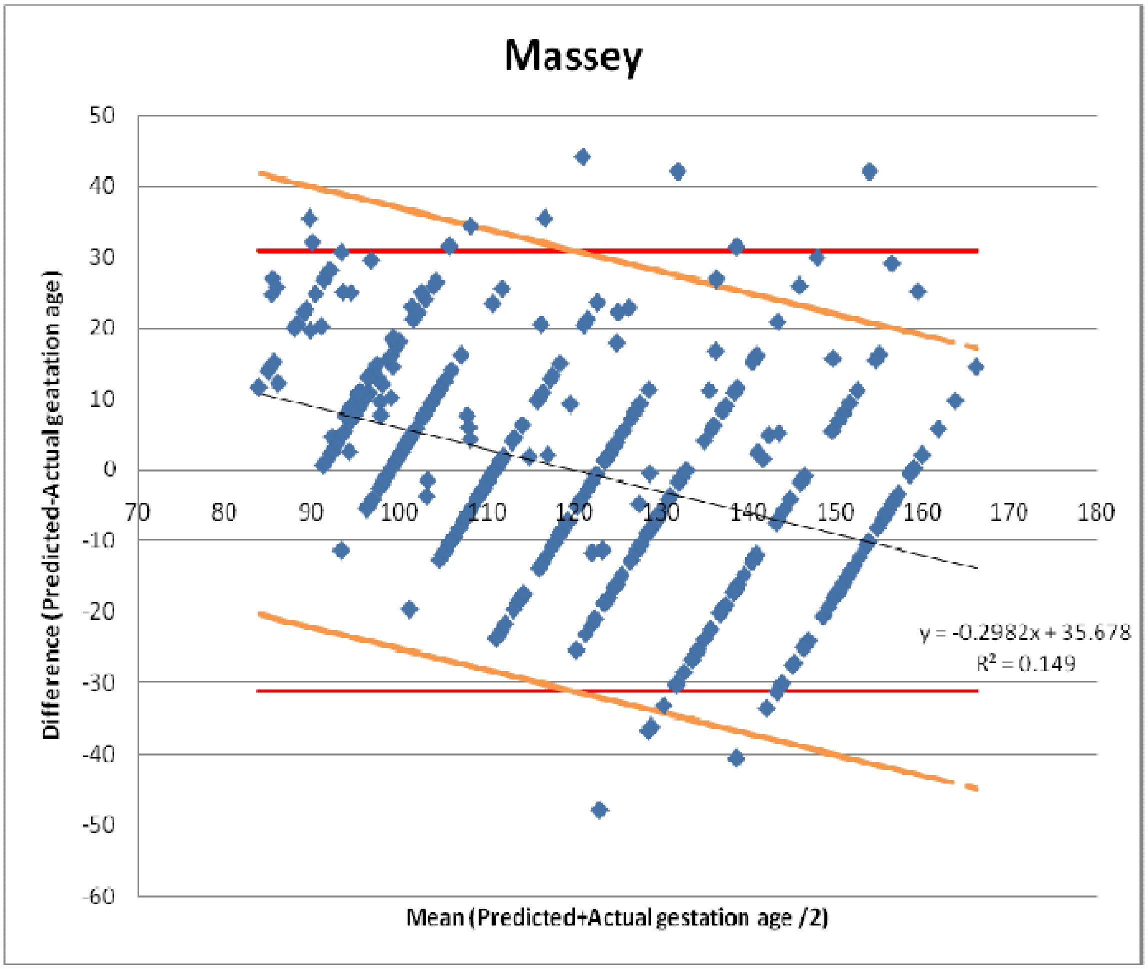


Figure 9[30]: Plots for limits of agreement on the Massey farm. The red line is the 95% limit of agreement of the mean difference. The orange line is the 95% limit of agreement from the regression line.

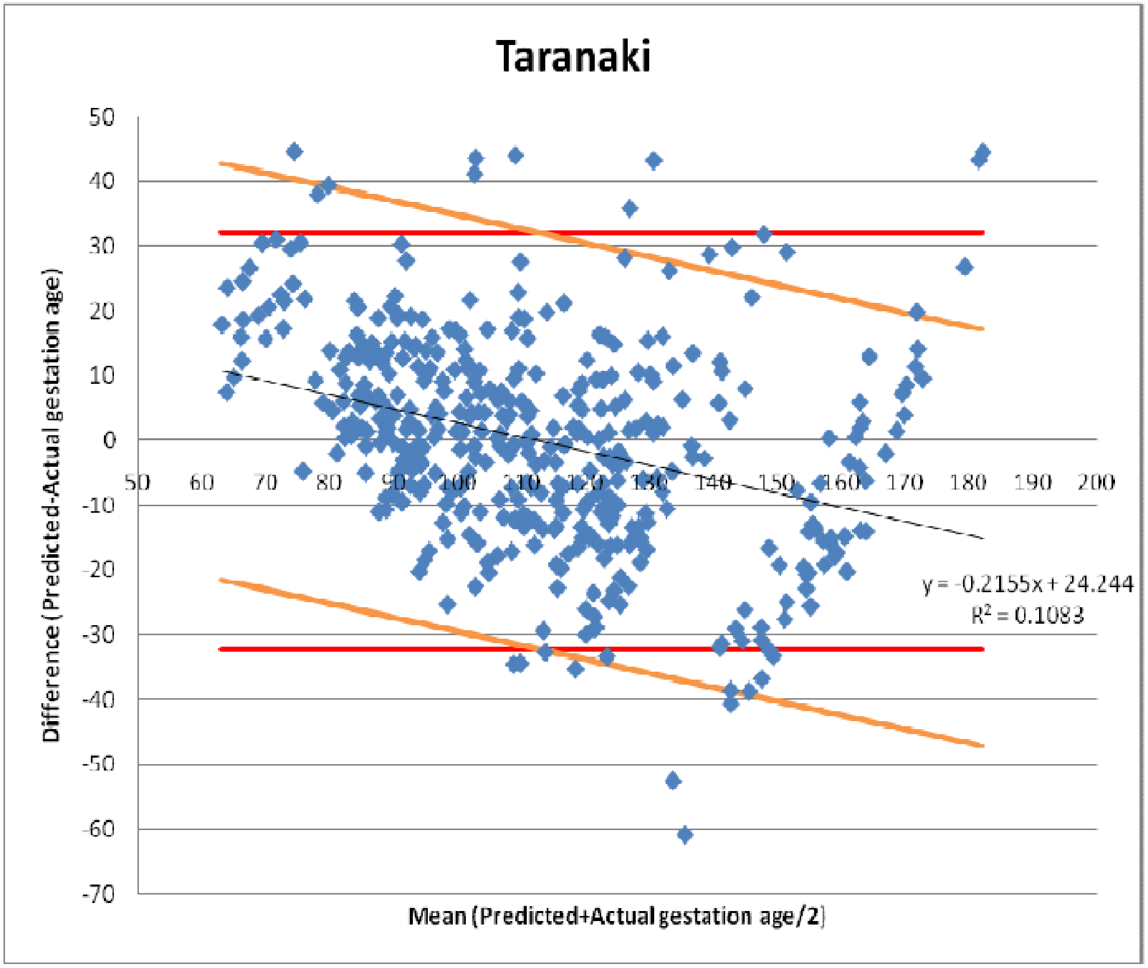


Figure 10[30]: *Plots for limits of agreement on the Taranaki farm. The red line is the 95% limit of agreement of the mean difference. The orange line is the 95% limit of agreement from the regression line.*

The linear relation found for the Taranaki and Massey farm shows that the gestation age is overestimated until 120 days of gestation for the Massey farm and until 110 days of gestation for the Taranaki farm. After 120 and 110 days of gestation the gestional age is underestimated. The mean difference for all measurements on the Massey farm was 0.009 with a standard deviation of 15.84 days. The 95% limits of agreement are shown within the red line. The upper limit is 31.04 days and the lower limit is -31.04 days. The orange line shows the 95% limit of agreement of the regression equation. For the Taranaki farm is the mean difference of all measurements was 0.082 with a standard deviation of 16.41 days. The upper limit is 32.16 days and the lower limit is -32.16

4. Discussion

A linear relation was found between placentome length, perpendicular placentome length, placentome area and the gestational age. In table 4 are the linear regression equations are shown. Due to the farm effect on the placentome size there are different linear regressions equations calculated for the Massey farm and the Taranaki farm.

In this study placentome size increased significantly every 10 days from day 100 until day 130 days of gestation and from day 140 until 150 days of pregnancy. This would therefore indicate that the placentome increases most rapidly during the stages of pregnancy that falls between 100 and 130 and 140 until 150 days of pregnancy. This is in contrast with Laven and Peters[19](2001) which found a linear increase of the placentomes throughout gestation. With a significant linear increase from day 70 until day 190 and no significant difference in the length of the placentomes between 190 and 280 days of gestation. Laven and Peters measured the placentome size in uteri collected from an abattoir so in their case it was possible to take a more accurate measurement. It could be possible that the difference in results we found between these two studies is a consequence of the different measurement techniques.

As independent variables we used: horn, breed, age, farm and days of pregnancy. From these independent variables had the farm and the days of pregnancy had a significant effect on the placentome size. Whereas the horn, the breed and the age of the cows had no significant effect on the placentome size.

4.1Pregnant horn versus non-pregnant horn

There was no difference found between the placentome length, perpendicular placentome length or the placentome area between the pregnant and the non-pregnant horn. Laven and Peters[19] showed that at all stage the average placentomes length in the pregnant horn was significantly greater than that in the non-pregnant horn except between 101 and 130 days of pregnancy. A possible explanation for this lack of difference between the pregnant and the non-pregnant horn is the location we used for scanning. Placentomes near the cervix are not as big as placentomes in the corpus region. Another explanation is misdetection of the pregnant horn. During the first three scan moments we decide which of the horn the pregnant one was. This meant that we collected three independent observations per horn. In only 10 of the cows were the three observations the same that is, three times left or three times right. It would therefore have been better if we conducted more observations such as detection of fremitus between day 100 and 130 in the uterine artery.

4.2 Age, breed and farm

There has not been a comparative study of the influence of breed, age and farm on the placentome size.

This study found no significant influence of the age (primiparous or multiparous) on the placentome size. By predicting the gestation age of a cow it is not necessary to distinguish between a primiparous and a multiparous cow. This study did not use nuliparous (heifers) for placentome size measurements. Further research is required to determine whether there is a significant influence of the age (nuliparous versus primi– or multiparous) on the placentome size.

No significant influence of breed was found on the placentome size. This study used Holstein Friesian, Jersey and crossbred (Jersey*Friesian) cows. A Jersey is a small cow and the Friesian is a relative big cow so it is not plausible that there is a significant influence between a breed and the placentome size. A Jersey and a Holstein Friesian are either dairy cows, further research is therefore required to determine the influence of beef cattle or dairy cows on the placentome size.

A significant farm effect was found on the placentome size. This is contrary to the expectations stated in our hypothesis that there would be no effect of the farm on the placentome size. Vonnahme et al.[31] (2003) found no difference in placentomal measurements in a group ewes fed on 50% of total digestible nutrients. The fetuses were markedly smaller in the restricted group, however, the placentomes were not significant smaller. In a different study, Long et al.[32] (2009)found a reduced placentome area and reduced cotyledonary weights in nutrion restricted cows. Both dairy herds on the Massey farm and on the Taranaki farm were pasture based managed. For an explanation for the significant difference in placentome size between the farms further research is required to establish the factors which influence placentome development in cows.

4.3 Comparison between sheep and goats versus cows

In contrast to sheep and goat the bovine placentomes do growth considerably throughout gestation. In ewes and does the placentomes reach a maximum diameter around day 74 in ewes and around day 91 in does and decreases in size further on in gestation. *Doizé et al* [24] calculate a linear regression relationship between fetal age in days and placentome size in mm for does : (28.74 + 1, 80 * placentome length + e) and for ewes (47.98 + 0.62 * Placentome length + e). The linear regression relationship between fetal age in days and placentome length in mm for the Taranaki and the Massey farm were respectively (53.43 + 3.09 * placentome length + e and 68.36 + 2.38 * placentome length + e). This means that the placentomes in cows grow faster than in ewes and does. Likely this observation may be explained by difference in sizes of the animals or by the different type of placentome shapes. In comparison to the limits of agreements plot for cattle (fig 9 and 10) which show a 95% prediction intervals from ±32,82 and ±31.68 days in goats the gestational age can be predicted within a marge from 14 days for 96% correctly[24]. This is more accurate than in cows. It may be explained by the difference in gestational length between cows and small ruminants. Moreover they used only one placentome per time point as we used around 8 placentomes per time point so the results are more accurate.

5. Conclusion

The objective of this study was to investigate whether transrectal ultrasound scanning of the placentomes between 60 and 170 days of pregnancy is a useful tool to predict the gestational age.

Linear relations were found between the placentome characteristics (length, perpendicular length and area) and the gestation age. The correlation coefficient from the linear regression equations are between 0.59 R^2 and 0.77 R^2 *see table 4*. So the gestation age can be estimate by using transrectal scanning of the placentomes but the large standard deviation (15.84 and 16.41 for the Massey and Taranaki farm respectively) by the limits of agreement make the use of placentome scanning not an useful tool for predicting the gestational age.

The independent factors breed, age and uterine horn had no influence on the placentome size. Although there is a significant farm effect on the placentome size. The high prediction intervals together with the significant effect of the farm on the placentome size make that placentome scanning is not useful to predict the gestational age of cattle.

There are fetal dimensions which are more accurate predictors of gestational age. The crown – rump length provided the most precise estimate of gestational age (sd 4,5 days)[15]. Before 55 days it is possible to provide a view of the entire crown-rump length. After day 55 the diameter of the trunk or the head can be used to predict the gestation age[1]. Although time consuming these fetal measurements can better be used to predict the gestational age.

The hypothesis that transrectal ultrasound measurements of placentome size are useful (sd <= 5 days) for predicting the gestational age in dairy cattle and breed, age, farm and uterine horn have no significant effect on the placentome size will be rejected since the standard deviation on the Massey and Taranaki farm are 15.84 and 16.41 days respectively and there is a significant effect of the farm on the placentome size.

6. Remarks

This report is the result of my research project in New Zealand at the Massey University in Palmerston North.
First I would like to thank Richard Laven and Folusho Doris Adeyinka from the Massey University for their excellent help and support during this research in New Zealand. Also I like to thank Moniek van den Bosch for the good cooperation on the research and the statistical work she did for me.
Finally I thank Peter Vos from the University of Utrecht for his help by writing this report.

Gerben

References:

1 Luc DesCôteaux, Jill Colloton, Giovanni Gnemmi, 2010 Practical atlas of ruminant and camelid reproductive ultrasonography. 81 -124.

2 Senger, P. L. 2003. Pathways to pregnancy and paturition. 306-323.

3 D. L. Northey and L. R. French, 1980 Effect of Embryo Removal and Intrauterine Infusion of Embryonic Homogenates on the Lifespan of the Bovine Corpus Luteum. J ANIM SCI, 50:298-302.

4 Godkin JD Lifsey GJ Gillepsie, BE, 1980, Characterization of bovine conceptus proteins produced during the peri- and postattachment periods of early pregnancy Biol. Reprod 38, 703.

5 Arthur's Veterinary Reproduction and Obstetrics 2001 (Eighth Edition), 57-97.

6 Pierson RA, Ginther OJ, (1984) Ultrasonography for detection of pregnancy and study of embryonic development in heifers. Theriogenology 22: 225 – 233.

7 Broaddus B, de Vries A, 2005 A Comparison of Methods for Early Pregnancy Diagnosis.

8 Pennington, J.A., L.H. Schultz, and W.F. Hoffman. 1985. Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding: field study in dairy cattle. J. Dairy Sci. 68:2740–2745.

9 Beindorf N, Nagai R, Shirasuna K, Herzog K, Hoefmann K, Sasaki M, Bollwein H, Miyamoto A, (2010) Vascular changes in the corpus luteum during early pregnancy in the cow. J Reprod Dev. 56(2):263-270

10 Taylor C, Rajamahendran R. (1991) Follicular dynamics and corpus luteum growth and function in pregnant versus nonpregnant cows. J Dairy Sci.;74(1):115-23.

11 Kastelic, J.P., S. Curran, and O.J. Ginther. (1989). Accuracy of ultrasonography for pregnancy diagnosis on days 10 to 22 in heifers. Theriogenology 31:813.

12 Fricke, P. M. (2002). Scanning the future-ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918.

13 Gjesdal F, (1969) Age determination of bovine foetuses Acta Vet Scand. 10(3): 197-218.

14 Kahn W (1989) Sonographic fetometry in the bovine Theriogenology 31: 1105-1121.

15 White I, Russel A, Wright I, Whyte T,. (1985). Real-time ultrasonic scanning in the diagnosis of pregnancy and the estimation of gestational age in cattle. The Veterinary Record 117:5.

16 Harris RM, Snyder BG, Meyer R, (1983) The relationship of bovine crown-rump measurement to fetal age. Agri-pracitce. 1983. 4:6,16-22. 7 ref.

17 Thomsen JL. Body Length, (1974) Head Circumference, and Weight of Bovine Fetuses: Prediction of Gestational Age. Dept. of Anthropology. University of Copenhagen.

18 Curran S, Pierson RA, Ginther OJ. (1986) Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J. Amer. Vet. Med. Assoc.189:1295.

19 Laven, RA, Peters AR, (2001) Gross morphometry of the bovine placentomes during gestation. Reprod Dom Anim 36, 289-296.

20 Zhu M J, Du M, Hess BW, Means WJ, Nathanielsz PW, Ford SP (2007) Maternal nutrient restriction upregulates growth signaling pathways in the cotyledonary artery of cow placentomes. Placenta 28: 361-368.

21 Anthony RV, Bellows RA, Short RE, Staigmiller RB, Kaltenbach, CC, Dunn TG, (1986) Fetal growth of Beef calves. II. Effect of sire on prenatal development of the calf and related placental characteristics. J. Anim. Sci. 62:1375-1387.

22 Reynolds LP, Millaway DS, Kirsch JD, Infeld JE, Redmer DA, (1990) Growth and in-vitro metabolism of placental tissues of cows from day 100 to day 250 of gestation; J. Reprod. Fert. 89, 213-222.

23 Leiser R, Krebs C, Ebert B, Dantzer V, (1997): Placental vascular corrosion cast studies: a comparison between ruminants and humans. *Microsc Res Techniques* 38 **,** 76–87.

24 Doize F, Vaillancourt D, Carabin H, Belanger D, (1997) Determination of gestational age in sheep and goats using transrectal ultrasonographic measurement of placentomes. Theriogenology 48:449-460.

25 Alexander G. (1964). Studies on the placenta of sheep. J. Reprod Fertil 7: 289-305.

26 Aydn, I, Celik HA, Sendag S, Dinc DA, (2008) Determination of placentome development and gestational age by ultrasonographic measurements. Vet Bilimleri Dergisi 24: 1, 29-34. 29 ref.

27 Stroud BK, (1994) Clinical applications of bovine reproductive ultrasonography. Comp Cont Educ 16(8):1085-1097.

28 Heyman Y, Chavatte-Palmer P, (2002) Frequency and occurrence of late gesational losses from cattle cloned embryos. Bio of Reprod 66(1):6-13.

29 Kolour AK, Batavani RA, Ardabili FF, (2005) Preliminary observations on the effect of parity on first day ultra-sonic detection of embryo and its organs in bovine. J Vet Med A 52: 74-77.

30 Bosch M vd, (2010) Internship Report ADP-70424

31 Vonnahme KA, Hess BW, Hansen TR, McCormick RJ, Rule C, Moss GE, Murdoch WJ, Nijland MJ, Skinne DC, Nathanielsz PW, Ford SP (2003) Maternal Under nutrition from Early- to Mid-Gestation Leads to Growth Retardation, Cardiac Ventricular Hypertrophy, and Increased Liver Weight in the Fetal Sheep Bio of Rep vol. 69 no. 1 133-140

32 Long NM, Vonnahme KA, Hess BW, Nathanielsz PW, Ford SP, (2009) Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine, J ANIM SCI .jas.2008-1672

33 Romano JE, Larson JE. Accuracy of pregnancy specific protein-B test for early pregnancy diagnosis in dairy cattle. Theriogenology 2010 Oct 1;74(6):932-9.

Attachment: I

DETERMINATION OF GESTATIONAL AGE IN DAIRY CATTLE USING TRANSRECTAL ULTRASOUND MEASUREMENTS OF PLACENTOME SIZE

Richard Laven[1], Folusho Doris Adeyinka[1], Moniek van Den Bosch[2], Gerben Blankenvoorde[3]

1 Massey University, Palmerston North, 2 Wageningen University, Wageningen, 3University of Utrecht, Utrecht, New Zealand

Determination of gestational age during pregnancy diagnosis is an essential part of assessing reproductive efficiency. In cattle, fetal measurements are commonly used for this purpose. However, obtaining these measurements can be time consuming and visualization of the fetus difficult to achieve, particularly later in gestation. Measurement of placentome size could be an alternative, as from 60 days of gestation onwards placentomes are easy to visualize and simple to measure using ultrasound and they grow in size during gestation. However, although there is significant anecdotal evidence of their use there have been no published studies evaluating their use in predicting gestational age. 58 dairy cattle from two farms were examined using transrectal ultrasound every 10 days from day 60 until drying off (approximately 180 days). Scanning sites were determined by first locating the cervix of the animal followed by locating the placentomes that were approximately one probe length (6 cm) distal from the cervix. Three to four placentomes were measured in each horn at each time point, images of each placentome were captured for subsequent measurement using Image J software. Two measures were recorded per placentome - longest length and width perpendicular to that measurement. A repeated measures mixed model was used to analyze the data, with placentome measurement (either length or perpendicular length) as the dependent variable and farm, breed, age (first lactation or older), horn (pregnant or non-pregnant) and the number of days pregnant as independent variables. The analysis showed that there was no significant effect of horn, age or breed on placentome size ($P>0.05$) but there was a significant association between gestational age over the period of the study ($P< 0.001$) and a significant farm effect ($P< 0.001$). A regression equation was thus calculated for each farm. For each farm this showed that placentome size tended to overestimate gestation length below 130 days, and underestimate it after that. Again for both farms, the 95% and 75% prediction intervals were ± 31 and 20 days respectively. These high prediction intervals combined with the presence of a farm effect suggest that placentome size is not a useful measure of gestation age. Further research is required to establish the factors which influence placentome development in normal cows, particularly those responsible for the farm effect seen in this study.