Chapter 1

1.0 INTRODUCTION

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From a total of 861.9 million goats (*Capra hircus*) in the world, 93.5% of them are in Asian (59.7%) and African (33.8%) countries (FAOSTAT, 2008). This statistics is a strong indication of the importance of goats in the livelihoods of people in developing and under-developed countries, especially in Asia. For millions of goat breeders particularly in Asia, undoubtedly, their goat production is highly valued as an asset and main source of their incomes.

According to Malaysia Department of Veterinary Services (2008), consumption of meat from small ruminants (goat and sheep) in Malaysia was approximately 21,000 metric tonnes. However, approximately 2,000 metric tonnes were supplied from local goats, meanwhile, approximately 19,000 metric tonnes of goat meat were imported as either live animals from countries such as Australia and South Africa which constituted more than 90% of small ruminant annual meat requirement. Most of the mutton was imported from Australia and New Zealand. Therefore, continuous dependence of live goat and frozen-mutton importation gives negative impact on the economy and social well being of our country Malaysia.

In view of this, there is a big gap between supply and demand for goat meat in Malaysia that prompts the government to give a very high priority to make agriculture including goat industry as the third engine of economic growth after manufacturing and services sectors. As a result, there is an upsurge of interest in goat business with many breeders and farmers venture into goat production and commercialisation. However, there is a low goat population in Malaysia currently which is approximately 460,000 goats of low quality for commercialisation (Malaysia Department of Veterinary Services, 2008).

Currently, there are progressive research efforts on goats breeding and production have been conducted by the local universities, research institutions, government agencies and private companies to improve the local goat breeds and to multiply the goat population at a very rapid rate to meet the current demand of goat industry in Malaysia.

There are many technologies have been developed to improve goat production in Malaysia. One main problem facing the researchers, entrepreneurs and farmers is to determine pregnancy in goats. Various methods have been suggested to diagnose pregnancy in goat including clinical methods, immunologic tests and oestrus response. This project was undertaken to elucidate the scientific principles of ultrasound scanning to relate structures during pregnancy in goats so that this information could be applied routinely to diagnose pregnancy in goats.

1.2 BACKGROUND

Pregnancy diagnosis is a useful tool in modern goat management, especially when technologies such as artificial insemination and embryo transfer are employed in goat production. Even though there are different pregnancy diagnosis methods available, Malaysian breeders are yet to be convinced on the most efficient and reliable method to be used. Most of goat breeders in Malaysia are still using returning to oestrus response as the main pregnancy diagnosis method, despite of its low accuracy. This is due to lack of reliability of the pregnancy diagnosis methods to be integrated in assisted reproductive technologies in goats. Hormonal method maybe suggested as a choice, however, the information obtained only describes whether the goat is pregnant or not. It could not give information on the foetal development. Besides that, most of hormonal assay takes longer time than ultrasound scanning to confirm the pregnancy status. Owing to that fact, research on ultrasound scanning for pregnancy diagnosis is of highly needed, especially to transform traditional farming into commercial farming.

When scanning is performed within the recommended time frame, accuracy of identifying animals that are not pregnant and counting number of foetuses is very high. Foetal counting and its age at the respective time will enable improvement of feeding management and subsequently improve newborn survival. This will later lead to the rapid enhancement of breeding programme and goat production.

Real-time ultrasonography has revolutionised the technology of reproductive biology in domestic animals. Since 1980s, it has been applied to the reproductive system of the females, particularly to clarify the ovarian follicular dynamics, corpus lutem function and foetal development.

Ultrasound scanning has been applied for pregnancy detection in domestic animals, including goats. In prior to ultrasound scanning, the conventional technique used to study the foetal development involved the measurement, counting and histological evaluation of foetal structures of animals killed at various stages of pregnancy. In contrast, the development of transrectal and transabdominal ultrasonic probes that can be used to visualise foetus as well as uterine structures has opened up new possibilities for examining the dynamics of foetal growth and provided a means for repeated, direct, non-invasive monitoring and measuring of foetus within the uterus. Early utilisation of ultrasound technology in the goat industry has been applied complementarily with embryo transfer procedures, particularly for pregnancy diagnosis and monitoring of foetal viability. Understanding the use of non-invasive ultrasound technology is critical in contemporary livestock production for further improvement in reproductive techniques to provide answers to industrial needs (Medan and Abd El-Aty, 2010).

The introduction of computer systems to ultrasound machines has enabled the storage, processing and presenting of large amounts of data, allowing the production of static two-dimensional grey scale images and real-time imaging (Griffith and Henry, 1973). This real-time B-mode imaging is currently the form of ultrasound most commonly used. Even though there are several other types of ultrasound machine available; M-mode (imaging based on motion), A-mode (imaging based on amplitude reading) and Doppler ultrasound (imaging based on change in frequency), real-time B-mode imaging is of better preference since it provides the actual image at that particular time. These real-time images greatly reduce chance for error and easier to be interpreted by operator.

In reference to Jainudeen and Hafez (2000), real-time B-mode ultrasound machine is differentiated by the display pattern they produce which depends on the type of transducer used. A linear transducer produces a rectangular image, whereas the sector transducer produces a sector image similar to that of a "slice of a pie". The linear array transducer commonly used for transrectal route, meanwhile sector array transducer is used for transabdominal approach.

Currently, research findings have been made on the application of both transrectal and transabdominal ultrasound scanning in small ruminants. Romano and Christians (2008) reported that the earliest pregnancy diagnosis using transrectal ultrasonography using a 7.5 MHz transducer based on the presence of positive signs of pregnancy could be made on day 16 and maximum sensitivity and negative predictive value reached at day 20 following mating. Meanwhile, Santos *et al.* (2007) reported accuracy of 92.6% for foetal sex determination in goats with identification of genital tubercle starting from day 45 of gestation onwards, which is in agreement with Hussein (2008).

As for comparison between reliability of transrectal and transabdominal ultrasound scanning in goat, Karen *et al.* (2009) reported that the days of goat pregnancy which the embryonic vesicle first determined by transrectal and transabdominal ultrasonography were day 17 and day 28, respectively. The foetuses with a beating heart were first determined by transrectal and transabdominal ultrasonography on day 22 and day 30, respectively. This finding suggests that ultrasound scanning could be a reliable tool to diagnose early stage pregnancy in goat.

An early determination of pregnancy can be a useful management tool under more intensive production conditions, or when AI and embryo transfer is employed. Pregnancy diagnosis will identify the females requiring repeat breeding or insemination and/or will allow the separation of pregnant and open females for differential management (Wildeus, 2009). When foetal numbers can be determined, different feeding regimes and vaccination can be applied to single and multiple litter bearing females.

The most promising technique currently available for pregnancy diagnosis in the goat is the use of real-time ultrasound scanning (ultrasonography). The arrival of lower cost, portable veterinary scanners, combined with the advantages of their use (foetal number determination, minimal animal restraint, high throughput), has made the application of this technology economically feasible on the farm level (Wildeus, 2009). Ultrasound examination can be expanded through the application of foetometry, allowing the aging of the foetus. Unlike other pregnancy detection methods, which have inconsistent accuracy, ultrasound scanning gives the exact image of foetus, confirming the pregnancy status.

From the earlier finding by other researchers, non-echogenic vesicle fluid in goat has been detected on days 18 and 21 of gestation using transrectal ultrasonography (Martinez *et al.,* 1998; Padilla-Rivas *et al*., 2005), respectively. However, by using the transabdominal method the vesicle could be detected between days 22 and 26 (Baronet and Vailancourt, 1989; Padilla-Rivas *et al*., 2005). The difference in detection time between those two methods is due to different ultrasound frequency used; transrectal ultrasonography used higher frequency which results in clearer image but shorter penetration distance. On the other hand, transabdominal ultrasonography can be used with precise details at the later stage of pregnancy as compared to transrectal ultrasonography.

Ultrasonic imaging of the heart beat is generally used to detect the foetus and to evaluate the foetal viability and confirm pregnancy. Besides that, it is also very useful to detect abnormality during pregnancy, especially foetal mummification. Heartbeat has been detected on days 21 to 24 of pregnancy (Doize *et al*., 1997; Martinez *et al.,* 1998; Medan *et al*., 2004; Padilla-Rivas *et al.,* 2005) by transrectal sonography and using the transabdominal approach by days 27 to 35 in goats (Hesselink and Taverne, 1994; Dawson *et al*., 1994; Padilla-Rivas *et al*., 2005).

Haibel (1988) reported variation in foetal biparital diameter (BPD) among goat breeds. This finding suggests that it is advisable to make a breed-specific predicting chart for estimation of pregnancy age of goat. The variation most probably caused by different sizes of breed and farm management system, including feeding regimes. The number of foetus also most likely can affect the accuracy of pregnancy age estimation in goat.

1.3 STATEMENT OF PROBLEMS

Major questions that are yet to be answered with regards to ultrasound scanning in goat pregnancy detection include:

a) What is the earliest day of pregnancy to gain significant accuracy in detecting pregnancy using ultrasound scanning in goats?

b) Are there any differences in sensitivity, specificity and accuracy in detecting pregnancy between transrectal and transabdominal probes?

c) How efficient and reliable is ultrasound scanning in detecting pregnancy in does?

d) What is the specific range of suitable gestation stage diagnosis for transrectal and transabdominal probes?

e) Can foetal development in doe be determined using observation from ultrasound scanning with transrectal and transabdominal probes?

f) Is it possible to differentiate structures, shapes and sizes obtained from images using transrectal and transabdominal probes?

g) Would it be possible to estimate the age of foetus using ultrasound scanning, with observation on structures and combination of structures?

h) Is it possible to study the development of foetal organ. e.g. heart using ultrasound in goats?

i) What is the best parameter to be measured for gestational age estimation in pregnant does?

j) Is it possible to determine foetal number using ultrasound in goats?

k) Would it be possible to determine foetal sex using ultrasound in goats?

l) Does different breed of does affect the outcome of ultrasound examination?

m) How can ultrasound detection complement other pregnancy detection methods to achieve a higher efficiency?

n) Is ultrasound examination practical to be applied in large farm operation?

o) Does farm management affect the foetus and foetal structures during pregnancy period as detected using ultrasound scanning?

1.4 JUSTIFICATION

Pregnancy diagnosis is a highly important part of assisted reproductive technologies and it is an integral component of successful livestock management, including goat industry. Nevertheless, information on pregnancy detection via ultrasound scanning in goats is limited and its application is still at infant stage. Hence, research on pregnancy detection via ultrasound scanning is timely before it can be applied routinely and efficiently in goat production. Information on practicality of ultrasound scanning would provide more accurate results and improvise breeding programme. In addition to distinguishing status of pregnancy for each doe, ultrasound scanning provides the advantage to identify stage of gestation (specifically days of pregnancy) which will indeed contribute in confirming pregnancy as well as predicting and estimating the date of kidding. This use of imaging modality will lead to the development of new or modify the established conventional pregnancy detection in goats.

In this research, using ultrasound scanner with transrectal and transabdominal probes, detection, characterisation and measurement will be made on the following:

1. Embryonic vesicle.
2. Heartbeat.
3. Heart size.

4. Foetal number.
5. Placentome; size, time-frame of observation, time of collapse.
6. Chest depth.

The above parameters measured are the common, easily detected and associated significant structures with specific age of foetus during goat pregnancy. The important factors affecting the foetus and foetal-related structures such as the breed, multiple pregnancy, farm management and localities were considered in this project.

1.5 OBJECTIVES

Ultrasound scanning promises a significant future improvement future livestock farm management and scientific studies. In accordance to that, we need to go parallel and get abreast with the technology. Thus, this research is crucial and shall promise a better future in our goat industry.

a) To determine accuracy of pregnancy prediction by using ultrasound scanner.
b) To determine the criteria for twin pregnancy using ultrasound scanner.
c) To predict the gestation age based on ultrasound scanning.
d) To compare the efficacy of two different probes on pregnancy diagnosis.
e) To determine the effect of breeds and unknown date of mating on pregnancy diagnosis using ultrasound scanning.

Chapter 2

2.0 LITERATURE REVIEW

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2.1 TIMELINE OF SIGNIFICANT FINDINGS IN SMALL RUMINANTS ULTRASOUND SCANNING

Below is the summary of significant timeline of findings obtained from various researchers working in the area of ultrasound scanning technologies in small ruminants (Table 2.1).

Table 2.1: Timeline of significant findings by various authors of ultrasound scanning in small ruminant reproduction

Year	Author	Species	Significant finding
1969	Lindahl	Goat	Accurate diagnosis of pregnancy in does from one to 104 days before kidding was made by using an ultrasonic Doppler technique. Detection of pregnancy on day 106 was not possible with external transducers. Prediction of multiple births has not been successful. Instruments with a frequency of 5 MHz seemed to be superior for early detection of pregnancy, whereas those with a frequency of 2.25 MHz might be superior for the detection of foetal heart movement in foetuses near term.
1985	Scheerboom and Taverne	Goat	Visualisation of uterine wall, placentomes, foetal skeleton, kidneys and vascular system during last 3 weeks of gestation.
1986	Pieterse and Taverne	Goat	Detection of hydrometra in goats with real-time ultrasound. Hydrometra caused false positive pregnancy diagnosis via immunologic test because the level of hormone mimics those of pregnant goats.

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Year	Author	Species	Significant finding
1989	Haibel *et al.*	Goat	Real-time ultrasonic foetal head measurement and gestational age in dairy goats via transabdominal approach. Biparietal diameter (BPD) could be accurately measured as early as day 40, but measurement became difficult after day 105 of gestation due to the variability of foetal location and posture. Linear increase in BPD was obtained in the second trimester of pregnancy.
1994	Dawson *et al.*	Goat	Accuracy for determining singles, twins, and triplets at 7 weeks of gestation was 82, 89, and 100%, respectively, with 5.0 MHz transabdominal probe. Two circumscribed fluid filled dark areas seen in the uterine horn indicating a twin was detected at 7 weeks of gestation. The uterus had an intrapelvic location in the first month of pregnancy, thus preventing good visualisation by the transabdominal probe. Foetal heartbeat could be recognised as early as 4 weeks into gestation and the placentomes can be identified at 7 weeks of gestation. The ability to differentiate pregnant from open does was highly accurate when using the transabdominal approach at 5 weeks after breeding. In the second half of gestation, the marked increase in foetal size and normal reduction in the amount of amnio-allantoic fluid often prevented reliable differentiation of foetal structures and an accurate assessment of foetal numbers.

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Year	Author	Species	Significant finding
1997	Doize *et al.*	Goat	Age of foetus was determined by placentomes with equation from days 30 to 90 of gestation. Transrectal probe (5.0 MHz) detected small nodule at 35 days and C-shaped placentomes at 42 days of gestation. Placentome measurement was limited on day 90 onwards. Placentomes were saucer like-shape and the central cavity of the placentomes increased in size as the foetus grows, yet the borders remained thin. Age of foetus was proved 66% correct with correlated equation.
1998	Martinez *et al.*	Goat	Early detection and embryonic growth with 5.0 MHz transrectal probe. On day 20 of gestation, embryos with heartbeat were detected. Non-echogenic density proved as significant indicator in detecting early pregnancy.
1998	Gonzalez de Bulnes *et al.*	Sheep	Embryonic vesicles diameter that were detected with 7.5 MHz linear array transrectal probe were highly correlated with gestational age from days 12 to 19. From day 19 onwards, crown-rump measurements, occipito-snout lengths, thoracic, biparietal and orbit diameters gave highest correlation in determining ages of foetus. Detection of the crown-rump length in an ovine embryo on day 26. Detection of the biparietal diameter left and the occipito-snout length right in a foetus on day 43.

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Year	Author	Species	Significant finding
			Detection of thoracic diameter in a foetus of day 67. The largest anechoic zone in the foetal trunk is the stomach. Transrectal ultrasound device was efficient to observe pregnancy in the first 3 months of gestation only until day 90 because the foetus was not easily accessible later. Single and multiple pregnancies could be detected from day 19, when it was possible to detect the embryo.
1998	Coubrough and Castell	Sheep	Foetal sex determination with 5.0 MHz transrectal probe by identifying and locating genital tubercle between days 60 to 69 post-breeding in ewes. Accuracy of sex determination was 89%.
2004	Gonzalez *et al.*	Goat	Comparison between 7.5 MHz transrectal probe, progesterone assay and pregnancy-associated glycoprotein (PAG) in detecting early pregnancy. Detection on day 26 of gestation using transrectal probe gave 99.4% accuracy.
2004	Medan *et al.*	Goat	Early detection in pregnancy using 7.5 MHz transrectal, 7.5 and 5.0 MHz transabdominal probes. Pregnancy detection gave positive result as early as day 21 of gestation with image of sacs. Pregnancy detection accuracy (100%) on day 60 of gestation. Images of sacs, heartbeats, placentomes and skeletal structures were obtained.
2005	Padilla-Rivas *et al.*	Goat	Pregnancy detection in both 7.5 MHz transrectal and 3.5 MHz transabdominal probes in Boer goats. Positive diagnosis was detected as early as days 22 and 26 of gestation with images of sac and heartbeat with transrectal probe.

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Year	Author	Species	Significant finding
2005	Padilla-Rivas *et al.*	Goat	With transabdominal probe, sac was detected on day 26 and heartbeat on day 33 of gestation. Pregnancy diagnosis on day 26 of gestation gave the best result using transrectal probe with the detection of foetal heartbeat.
2006	Karen *et al.*	Sheep	Determination of foetal number using 3.5 MHz transabdominal probe, progesterone assay and pregnancy-associated glycoprotein (PAG). Positive diagnoses were detected as early as days 50 to 100 of gestation in sheep. Percent of pregnancy diagnosis accuracy on days 76 to 87 of gestation was 71.6% compared to progesterone and PAG; 65.4 and 72.8% respectively.
2007	Santos *et al.*	Goat	Sex determination of Boer goat foetuses using 6.0 and 8.0 MHz transrectal probes by locating the position of genital tubercle. The foetus were diagnosed as male when the genital tubercle was located immediately caudal to the umbilical cord or as female when the genital tubercle was positioned directly below the tail. Daily examination between days 40 to 60 of gestation gave accuracy of 100% for single pregnancies, 96.9% for twin pregnancies and 100% for triplet pregnancies. One time examination between days 45 and 60 of gestation gave accuracy of 94.4% for single pregnancy, 80.0% for twin pregnancy and 100% for triplet pregnancy.
2007	Maico *et al.*	Goat	Sex determination with genital tubercle using 5.0 and 7.5 MHz transabdominal plus 6.0 and 8.0 MHz transrectal probes. Highest pregnancy detection accuracy was during days 40 to 60 of gestation.

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Year	Author	Species	Significant finding
2007	Maico *et al.*	Goat	Detection on scrotal bag for male, and vulva for female was possible on day 45 onwards.
2007	Abdelghafar *et al.*	Goat	Ultrasonic measurement of crown-rump length (CRL) and biprietal diameter to predict gestational age in Saanen goats. Gestational equations were established as follows: CRL = 0.464x – 17.767 BPD = 0.055x – 1.431 Where x = estimated gestational age in days Measuring of CRL and BPD became increasingly difficult as pregnancy approach the third trimester.
2008	Suguna *et al.*	Goat	The embryonic vesicle was detected on days 21 and 28, proper embryo on days 28 and 35 of gestation using the 6.0 MHz transrectal and 5.0 MHz transabdominal probes, respectively. Heartbeat was observed as on days 21 and 35 of gestation with transrectal and transabdominal probes, respectively. Placentomes as a circular 'C' shape structure was detectable on day 42 with an average diameter of 0.97 cm, using the transrectal probe. Using the transabdominal probe, placentomes were detectable on day 50 of gestation. Skeletal structures such as the skull, rib cage and vertebral column were first viewed on day 56 of gestation in both approaches. Singles and twins were differentiated on days 35 and 42 of gestation by the transrectal and transabdominal probes, respectively.
2008	Romano and Christians	Sheep	Studies to determine the earliest date to execute pregnancy diagnosis using 7.5 MHz transrectal probe on ewes.

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Year	Author	Species	Significant finding
2008	Romano and Christians	Sheep	Pregnancy was confirmed by presence of an embryo or extra-embryonic membranes. Accuracy pregnancy diagnosis were 0, 31.3, 40, 70 and 100% on days 15, 16, 17, 18 and 19 of gestation, respectively.
2009	Karen *et al.*	Goat	Estimation on gestational age of Egyptian goats by ultrasonographic foetometry. Age of foetus could be estimated by measuring the crown-rump length, biparietal diameter, trunk diameter, placentomes size, umbilical cord diameter and femur length. Embryonic vesicle and embryo proper with a beating heart were first determined by 7.0 MHz transrectal and 3.5-5.0 MHz transabdominal ultrasonography were days 17, 28, 22 and 31 of gestation, respectively ($P<0.0001$).
2009	Raja Ili Airina	Goat	The highest predictive value (100%) for specific and combination of structures during early stage of gestation were detection of sac, non-echogenic area and foetus-heartbeat-uterine wall-amniotic fluid. Detection of combination of foetal heartbeat, placentome-foetus-heartbeat and foetus-heartbeat-spinal cord-ribs gave 100% predictive value suring middle stage of pregnancy. Predictive accuracy for combinations of structures in late stage, placentome-foetus-heartbeat-spinal cord-ribs, placentome-foetus-heartbeat-spinal cord-ribs-foetal organ and fotus-heartbeat-spinal cord-ribs-foetal organ were 100, 80, and 100%, respectively.

Table 2.1 describes the timeline of significant findings of ultrasound scanning in small ruminants, focusing on goats and sheep. Real time B-mode ultrasonography using transrectal or transabdominal probes appears to be the most preferable method for ultrasonographic pregnancy diagnosis, even though some researchers used other types of ultrasonography techniques such as Doppler method (Lindahl, 1969), A-mode and M-mode. Before late 1980s, ultrasonographic pregnancy diagnosis focuses more on pregnancy status (pregnant or non-pregnant) instead of gestational age estimation (Lindahl, 1969; Scheerboom and Taverne, 1985).

Starting from late 1980s, advantages especially on foetal imaging via real time B-mode ultrasonography leads to another level of pregnancy diagnosis in small ruminants; uterine evaluation and gestational age estimation. B-mode ultrasonography enables more precise data collection on pregnancy such as false pregnancy (Pieterse and Taverne, 1986), foetal number (Dawson *et al.,* 1994; Karen *et al.,* 2006), sex determination (Coubrough and Castell , 1998; Santos *et al.,* 2006; Maico *et al.,* 2007) and foetal survival (Martinez *et al.,* 1998). This foetal imaging helps researchers to measure the development of pregnancy-related structures and correlate them with gestational age.

Early research to correlate pregnancy-related structures with gestational age focuses on diagnosis of early pregnancy. Combination of embryonic vesicles, foetus and foetal heartbeat is considered as the earliest confirmatory evidence of pregnancy. Using transrectal ultrasonography, several researchers managed to correlate the development of crown-rump length (CRL), biparital diameter (BPD) with gestational age (Haibel*,* 1988; Dawson *et al.,* 1994; Martinez *et al.,* 1998; Gonzales de Bulnes *et al.,* 1998) during the first 2 trimesters of pregnancy. However, measuring CRL and BPD becomes increasingly difficult approaching the third trimester of pregnancy even with transabdominal ultrasonography. This is because the increase in foetal size makes them almost impossible to be viewed on the screen as a whole.

In relation to the limitation of measurability of pregnancy-related structures via transrectal probes, some researchers used transabdominal probes complementary with transrectal probes. Despite of the later detection of pregnancy confirmatory structure, which is almost 1 week later as compared to transrectal probes, transabdominal probes offer a longer time-frame for pregnancy-related structures detection throughout pregnancy. Besides, foetal structures such as placentome and heart can be detected and measured throughout the 3 trimesters of pregnancy (Medan *et al.,* 2004; Padilla-Rivas, 2005; Karen *et al.,* 2006; Suguna *et al.,* 2008; Raja Ili Airina, 2009). Foetal sex determination is possible with satisfactorily accuracy using either one of the probes (Coubrough and Castel, 1998; Santos *et al.,* 2006; Maico *et al.,* 2007).

Placentome so far appears to be the easiest to assess pregnancy-related structure via both transrectal and transabdominal ultrasonography. Placentomes are detectable between days 42 and 98 of gestation via transrectal approach. As for transabdominal approach, they were detected between days 50 and 130 of gestation (Suguna *et al.,* 2008). Previous research findings have shown the correlation between placentomes and gestational age (Doize, 1997; Suguna *et al.,* 2008). Unfortunately, one downside of this parameter is inconsistency in measuring the exact same placentome which negatively affects its accuracy. There are about 75 to125 placentomes within uterus of pregnant doe. Hence, the measurement made was based on random selection of placentome instead of the specific one.

Future challenge in ultrasonographic pregnancy diagnosis is to identify a pregnancy-related structure which can be specifically and easily detected during the 3 trimesters of pregnancy. Correlation of this structure with gestational age will give more reliable reference for livestock production management. Besides that, specific gestational age predicting chart for each breeds needs to be established since there is variation reported in pregnancy-related structures detection and measurement in different breeds.

2.2 PREGNANCY DIAGNOSIS IN FARM ANIMALS

Pregnancy diagnosis in farm animals is of considerable economic value, especially in farm production management. According to Jainudeen and Hafez (2000) an early diagnosis of pregnancy is required for the following purposes:

a) To identify non-pregnant animals soon after breeding programme so that production time lost from infertility may be reduced by appropriate treatment or culling.
b) To certify animals for sale or insurance purposes.
c) To reduce waste in breeding programmes using expensive hormonal techniques.
d) To assist in the economic management of livestock.

In general, techniques of pregnancy diagnosis can divided into 3 groups, which are clinical methods, immunologic tests, and return to oestrus observation, as described in Figure 2.1.

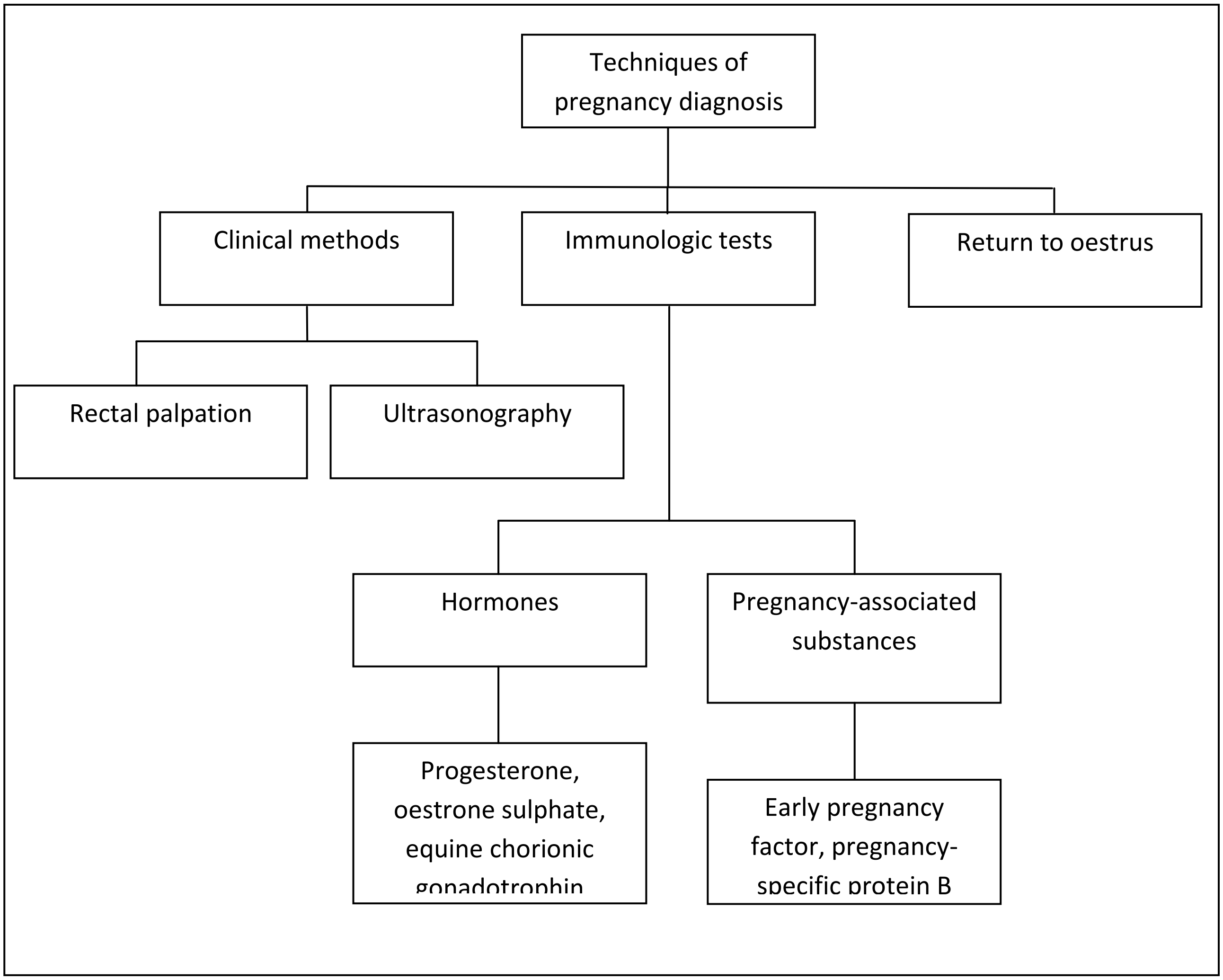

Figure 2.1: Pregnancy diagnosis techniques in farm animal (Adapted from: Jainudeen and Hafez, 2000).

2.2.1 Pregnancy Diagnosis: Clinical Methods

Clinical methods depend on the detection of foetus, foetal membrane and foetal fluids. Two most well known methods that fall into this group are rectal or abdominal palpation and ultrasonography. Even though radiations have been used to diagnose pregnancy in small ruminants, it is now abandoned due to its hazardous effect to the operator.

Rectal palpation is the accepted method of pregnancy diagnosis in big ruminants such as mare, buffalo, and cow. This procedure is performed by palpating the uterus through the rectal wall to detect the uterine enlargement occurring during pregnancy and the foetus or foetal membranes (Jainudeen and Hafez, 2000). One advantage of this technique is it can be performed at an early stage of gestation with high accuracy. However, one downside of this technique is that it requires big pelvic cavity, making it not possible to be practised on small ruminant like goat. Although some farmers practised abdominal palpation, of which performed by palpating the abdominal part of doe, it is not as accurate as rectal palpation and require highly experienced operator. Besides that, the chance of error is even higher.

Ultrasonography on the other hand offers higher accuracy for pregnancy diagnosis. Unlike palpation which requires experience-based diagnosis from unviable structure, ultrasonography provides details of pregnancy status. In addition to that, real-time ultrasonography is considered as the best method of differentiating hydrometra (a fluid-filled uterus which lead to false pregnancy confirmation) from pregnancy; progesterone concentration will remain elevated as in pregnancy (Taverne and Willemse, 1989).

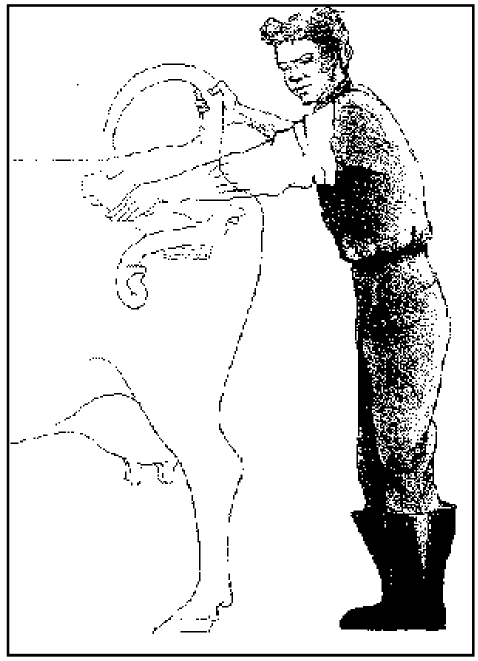

Figure 2.2: Rectal palpation is being performed (Adapted from: http://cal.vet.upenn.edu).

B-mode produces an accurate two-dimensional image of soft tissue cross section. The brightness of the dots on the oscilloscope is projected in various shades of gray, comparable to a black and white succession; they will reveal any motion in the tissue being imaged. Based on this principle, any specific structure and movement will be able to be measured and recorded. B-mode ultrasonography is a non-invasive, accurate and rapid alternative for diagnosing pregnancy and studying the development of the conceptus in livestock (Suguna *et al*., 2008).

Real time B-mode ultrasound machine is differentiated by the display pattern they produce which depends on the type of transducer used (Jainudeen and Hafez, 2000). A linear transducer produces a rectangle image (Figure 2.3), whereas the sector transducer produces a sector image similar to that of a "slice of a pie" (Figure 2.4). The linear array transducer commonly used for transrectal route, meanwhile sector array transducer is used for transabdominal approach. Each probe exhibit different efficiency based on the time they are to be used.

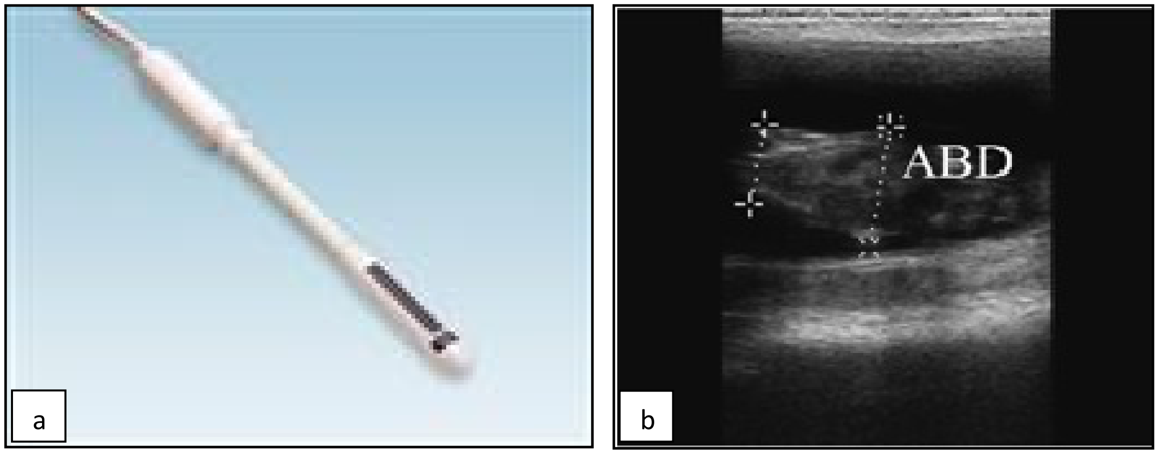

Figure 2.3: Transrectal probe and rectangular image generated. a) Transrectal probe (Adapted from: www.aloka-europe.com) and b) Rectangle image generated from transrectal ultrasound scanning (Adapted from: Ali and Hayder 2007).

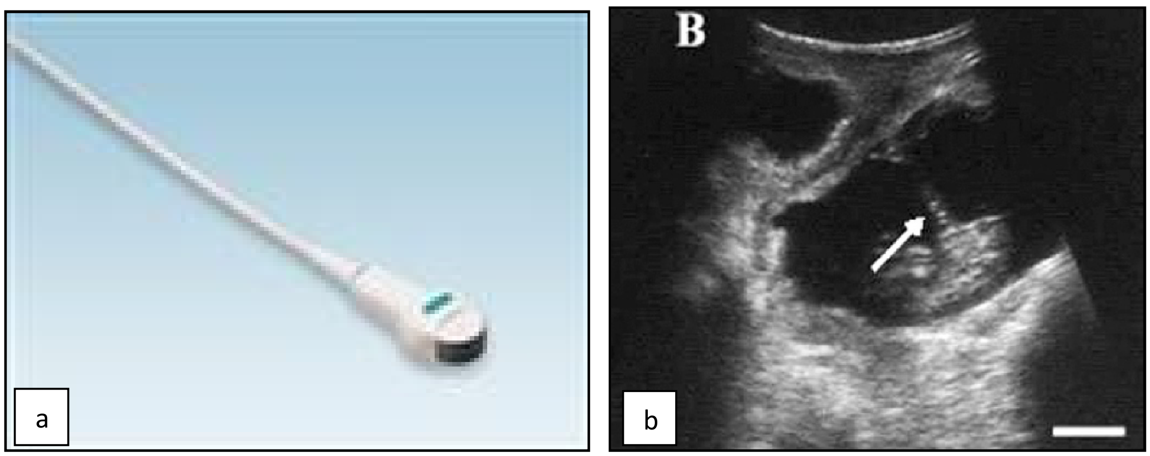

Figure 2.4: Transabdominal probe and sector image generated. a) Transabdominal probe (Adapted from: www.aloka-europe.com) and b) Sector image generated from transabdominal ultrasound scanning (Adapted from: Medan and Abd El-Aty, 2010).

As reported by Martinez *et al.* (1998) and Padilla-Rivas *et al.* (2005) respectively, non-echogenic vesicle fluid in goat has been detected on days 18 and 21 of gestation using transrectal ultrasonography. On the other hand, by using the transabdominal method, the vesicle could be detected between days 22 and 26 (Baronet and Vailancourt, 1989; Padilla-Rivas *et al*., 2005). Between days 35 and 70, both methods appear to be equally accurate.

However, the transabdominal approach is preferred during the second half of pregnancy since it allows larger portion of the pregnant uterus to be visualised.

The arrival of lower cost, portable veterinary scanners, combined with the advantages of their use has made the application of this technology economically feasible on the farm level (Wildeus, 2009). Ultrasound examination can be expanded through the application of foetometry, allowing the aging of the foetus. Using ultrasonography, it becomes possible to study development and growth of the ovine foetal organs and parts (head, eye, stomach, bones, heart, and urinary bladder) and even foetal number determination (Figure 2.5) (Buckrell *et al.,* 1986; Suguna *et al.,* 2008) without endangering pregnancy (Sinclair *et. al.,* 1998; Kaulfuss *et al.,* 1999; Noia *et al.,* 2002).

In other livestock species, such as cattle and sheep, ultrasonography has been used to diagnose pregnancy throughout the gestation. Unfortunately, not much research has been done in goat. Even though there are many reports regarding transrectal and transabdominal ultrasonography in sheep, there is a paucity of information on the suitability of this technique for use in goats (Padilla-Rivas *et al.,* 2005). Moreover, techniques developed for pregnancy diagnosis in ewes have been unsatisfactory in does because of factors such as expense, accuracy, practicality and delayed results (Dawson *et al.,* 1994). This signifies the urgency for more progressive research on ultrasound scanning for pregnancy detection in goats to be conducted.

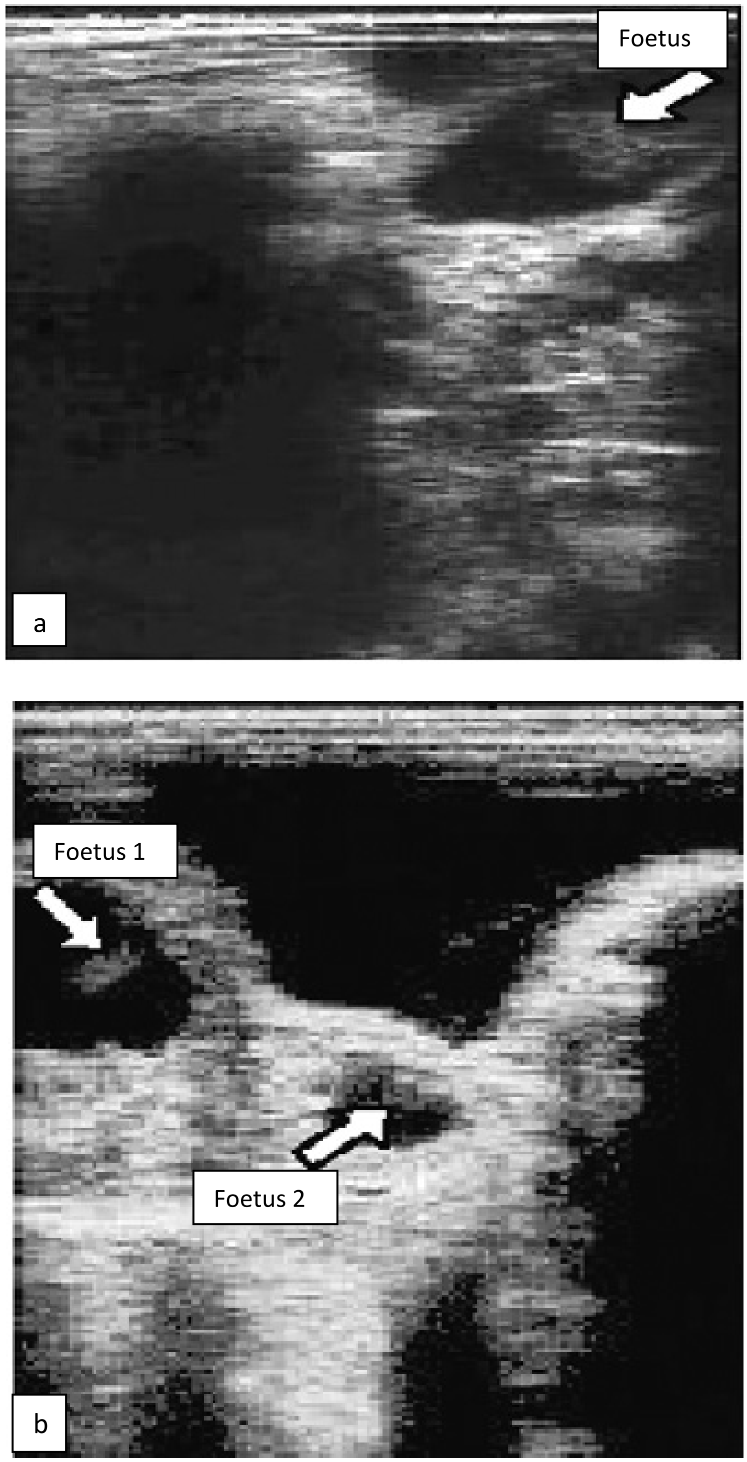

Figure 2.5: Images of single and twin foetuses using transrectal ultrasonography. a) Single foetus (arrow) detected as a distinct echodense structure on day-35 of gestation. b) Twin foetuses (arrows) are apparent as echodense structures within the uterine lumen of a 28-day pregnant doe (Adapter from: Suguna *et al.,* 2008).

2.2.2 Pregnancy Diagnosis: Immunologic Diagnosis

Immunologic diagnosis is defined as technique that rely on detection or measurement of the level of substances originating in the conceptus, the uterus, or ovaries that enter the maternal blood, urine, or milk (Jainudeen and Hafez, 2000). In general, immunologic tests measure two types of substances: pregnancy specifics and pregnancy not specific. Type of immunologic test to be used depends largely on the target substances to be detected and measured.

Pregnancy specific substances refer to the substance that will only appear during pregnancy in maternal blood. Some of these substances originate from the conceptus, meanwhile others may be produced at higher levels of gestation. Its detection is regarded as the solid evidence of pregnancy. Radioimmunoassay is one of the common methods used to detect pregnancy in farm animals.

Serum or milk progesterone (P_4) levels have been widely used to detect pregnancy in goats on days 21 to 22 post-breeding (Corteel *et al.,* 1982). Progesterone concentrations in the blood or in the milk decrease sharply following regression of the corpus luteum (CL) in the non-pregnant goats. On the contrary, the pregnant goats have high P_4 concentrations over the same period since the CL does not regress (De Montigny *et al.,* 1982; Fleming *et al.,* 1990).

Table 2.2: Interpretation of the “On-farm milk progesterone” test for pregnancy diagnosis in cattle (Adapted from: Jainudeen and Hafez, 2000)

Progesterone concentration	Interpretation	Accuracy	Errors
High	Pregnant	75%	1. A cow incorrectly inseminated during the luteal phase (dioestrus) will be in the luteal phase 3 weeks with elevated milk progesterone. 2. A cow with a longer normal cycle length (e.g., 28 days). 3. A cow with a short interoestrus interval (e.g., 17 days). 4. Embryonic mortality between sampling and confirmation of pregnancy by rectal palpation.
Low	Not pregnant	100%	1. Error in animal identification. 2. Human error in differentiating the colour reaction in the ELISA test.

Table 2.2 describes the “On-farm milk progesterone” test for pregnancy diagnosis in cattle, together with its accuracy and justification for error. Despite of the progesterone level based interpretation, a cow with high milk progesterone does not necessarily signify pregnancy, and a cow with low progesterone will not be pregnant. The accuracy of predicting pregnancy has ranged from 75 to 90%. Meanwhile, the accuracy for non-pregnancy is 100%. The explanations on false positive pregnancy diagnosis are listed in Table 2.2. In goat, this test can be used for early diagnosis of pregnancy in milk samples collected 20 days after breeding. Unfortunately, it fails to differentiate pregnancy from hydrometra (a fluid-filled uterus); progesterone concentration will remain elevated as in pregnancy (Jainudeen and Hafez, 2000).

2.2.3: Pregnancy Diagnosis: Return of Oestrus

The conceptus will inhibit regression of CL during pregnancy. Therefore, this will prevent the animal from returning to oestrus. Owing to that pregnancy physiology, livestock that not returning to oestrus after breeding programme is considered to be pregnant. This method is considered to be the simplest and cheapest economically since generally the oestrus sign can be evaluated at approximately day 21 post-breeding. Does may return to oestrus if there is a failure to fertilise, and the non-return to oestrus may indicate pregnancy.

According to Jainudeen and Hafez (2000), a doe in oestrus is restless, bleats frequently, and wags her tail constantly and rapidly. The doe in oestrus may as well have a reduced appetite and a decreased milk production. The vulva may be oedematous and a mucous discharge from the vagina may be observed. Besides that, the doe may occasionally mount on other female, exhibiting homosexual behaviour. The doe usually displays a strong male-seeking behaviour and remain very close to the male. However, without the presence of the male, oestrus is found to be difficult to detect.

Table 2.3: Oestrous cycle, oestrous, and ovulation in farm animals (Adapted from: Jainudeen and Hafez, 2000)

Animal	Length of oestrous cycle (days)	Duration of oestrus (hours)
Ewe	17	24-36
		32-40
Doe	21	20-35
Cow	21	18-19

Table 2.3 describes the length of oestrous cycle and oestrus duration in 3 ruminants; ewe, doe and cow. From the information provided, there are individual variations in term of both oestrous cycle lengths. This variation may negatively affect the

reliability of this method for pregnancy detection. In addition, the indication of non-return to oestrus in pregnant does is the same as given characteristics in goats that either have long cycle, or are pseudopregnant or a physically anoestrus when the goats are synchronised and bred during the non-breeding season (Gonzalez *et. al.,* 2004). Therefore, a diagnosis of pregnancy based on non-return to oestrus is unreliable in goats (Goel and Agrawal*,* 1992; Ishwar, 1995).

2.2.4: Overall Comparison of Efficiency between Currently Available Pregnancy Diagnosis

Table 2.4: Overall comparison between ultrasonography, hormonal assay and return to oestrus responds for pregnancy diagnosis (Modified from Jainudeen and Hafez, 2000)

Pregnancy diagnosis technique	Advantage	Limitation
Clinical methods (Ultrasonography)	1. Real time imaging. 2. Foetal counting. 3. The only method to evaluate embryo viability. 4. Very accurate for pregnancy diagnosis. 5. The only available technique to detect hydrometra (false pregnancy). 6. Non-invasive.	1. Maintenance cost, however, the arrival of many new and cheaper model make it possible for usage at farm level.
Immunologic tests (Hormonal assay)	1. Will give high accuracy if the right procedure is performed (RIA, ELISA). 2. “On-farm” test can be performed at farm level with fast result.	1. Time consuming if RIA is to be performed. 2. Rigid safety standards if RIA is to be performed. 3. Correct interpretation of test results for “On-farm” test depend upon accurate oestrus detection and good recording. 4. Not possible to evaluate embryo viability. 5. Not possible to determine foetal number.
Return to oestrus response	1. The cheapest method available.	1. Low accuracy. 2. Anoestrus affect its reliability. 3. Not possible to evaluate embryo viability. 4. Not possible to determine foetal number.

Table 2.4 describes the overall comparison between the currently available pregnancy diagnosis methods; namely ultrasonography, hormonal assay and return to oestrus response. From the information provided, ultrasonography is found to be the most promising and reliable methods. This is because it offers many other pregnancy related information such as foetal number (single, twin or triplet) as well as embryo viability. Hormonal assay gives higher accuracy as compared to return to oestrus response, but not as accurate as ultrasonography. Despite of its advantage as the cheapest method, return to oestrus observation turned out to be the least reliable method for pregnancy diagnosis.

2.3 DIFFERENT TYPES OF ULTRASOUND SCANNERS

Ultrasound techniques plays major role in industrial application. Ultrasound was proposed as a diagnostic aid back in 1942 (Dussik *et al.,* 1947). Brown (1960) developed the first contact ultrasound scanner allowing the transducers containing crystals to be directly applied to the patient. The application of different type of ultrasound such as A-mode, M-mode, B-mode and Doppler differ with the prediction of the examiner. The real-time brightness or B-Mode imaging is currently the form of ultrasound that is commonly used. The A-mode is used in basic fluid detection equipment such as that used to detect pregnancy in pigs (Holtz, 1982). Meanwhile, M-mode was used to assess movement of heart valves and walls (Edler and Hertz, 1954).

Amplitude depth ultrasound or A-mode ultrasonography is for pregnancy diagnosis in detection of the fluid-filled uterus and is thus no pregnancy-specific. A-mode units emit ultrasonic waves from a transducer placed externally against the skin of the abdomen and directed towards the uterus. Ultrasound waves are converted to electrical energy in the form of audible or visual signal. These units detect fluid filled organs at a depth of 10 to 20 cm (Ishwar, 1995). When a fluid filled structure is detected, some units emit a light or audible signal. Units with an oscilloscope display reflections as peak or blips on the screen. Non-pregnancy is suggested when the peaks are present only in the left half of the screen. Hence, when a fluid filled structure is detected; peaks will also appear on the right half of the screen. Accuracy obtained is 80 to 85% if performed between days 60 to 120 of gestation. Wani (1981) and Watt *et al.* (1984) believed A-mode to be reliable tool from days 50 to 120 of gestation in goats.

An extended urinary bladder, hydrometra or pyometra may give false results that may occur in early gestation or in late gestation because of the decrease in the ratio of uterine fluid to foetal tissue. Neither foetal viability nor foetal numbers can be detected by this method. Earlier work with externally applied A-scan indicated that an accuracy of at least 95% is possible between days 60 to 80 in ewes (Haibel, 1990). However, Meredith and Madani (1980) reported that a positive diagnosis of pregnancy in ewes made on the evidence of ultrasound reflections with an accuracy of 83% on days 61 to 151 after mating. Lindahl (1969) reported that the earliest time at which pregnancy could be detected by using A-scan is between days 40 and 50 after mating.

Prior to real-time imaging, examination of moving structures such as the heart was possible with Time Motion or M-mode ultrasound (King, 2006). In its current form, the two-dimensional grey scale image allows placement of a cursor line across the desired area and a trace is produced representing the movement of every tissue interface along that line against time. M-mode is now an integral part of echocardiographic examinations and all modern ultrasound machines are equipped with this capability (King, 2006). M-mode permits repeatable, objective measurements to be made at specific stages of the cardiac cycle allowing evaluation of chamber size and wall thickness, assessment of valve motion, calculation of functional criteria including shortening fraction (Curry *et al.,* 1990) and determination of foetal heart rates (Curran and Ginther, 1995; Moreno *et al.,* 1996).

The principle of Doppler ultrasound for pregnancy diagnosis is the detection of movement such as blood flow in the middle uterine artery, umbilical arteries, foetal heart beat and foetal movement. Fraser and Robertson (1967) were the first to apply it on sheep. Shone and Fricker (1969) used the same type of machine on 309 ewes between days 66 and 122 of gestation and recorded 100% accuracy at all stages of pregnancy. Foetal blood from the umbilical artery was the most common diagnostic feature (Ishwar, 1995). Transducer emits ultrasound waves; sound reflected from motionless structures has the same frequency as the transmitted sound whereas sound reflected from moving organ or blood has a different frequency. Differences in frequency are converted to audible sound. Audible signal which could be distinguished by the observer include the foetal heartbeat, arterial blood flow in the middle of uterine artery and umbilical arteries, foetal body movements and maternal intestinal movement. The 'gushing sound' of the middle uterine artery, the 'galloping beat' of the foetal heartbeat and 'swishing sound' of the umbilical blood flow heard were taken as indicators of pregnancy (Wani *et al.,* 1998). The transducer can be applied to the skin of the abdomen or intrarectally using a rectal probe.

A positive diagnosis of pregnancy is made by listening to the rapid, pounding sound of the foetal heartbeat, foetal pulse and foetal movements. Foetal heartbeat and foetal pulse which are faster than the maternal pulse or foetal movement are taken as positive criteria of pregnancy (Lindahl, 1969). Foetal pulse is rapid with swishing sound plus much faster than the maternal pulse that is sharp. External application of the ultrasonic Doppler has been used for detection of pregnancy in does. Pregnancy detection approaches an accuracy of 100% during the last half of gestation (Fraser and Robertson, 1968; Shone and Fricker, 1969) but is not effective on days 50 to 75 or earlier (Lindahl, 1969, 1971). The intrarectal Doppler technique is superior to the external technique when used early in the second trimester for diagnosing pregnancy that may achieve an accuracy of 90% or better (Lindahl 1971; Ott *et al.,* 1981). Results with 2111 ewes indicate that pregnancy can be detected at mid-gestation with an accuracy of higher than 90%. It may also be used earlier which is days 25 to 30 post-breeding but possibility of false results occur when soft faeces around the rectal probe interfere with sound wave transmission. Therefore, it is best to use between days 35 to 40 of gestation (Ishwar, 1995).

False results are unlikely with the Doppler technique when foetal sounds are used as the criteria for pregnancy diagnosis. Hydrometra can cause increased maternal blood flow in the middle uterine arteries but no foetal sounds will be heard. Doppler units with a frequency of 2.25 MHz may be superior in near term pregnancies whereas a 5.0 MHz seems better for detecting earlier pregnancies. Compared to A-scan techniques, the Doppler technique resulted in greater accuracy in ewes, which were at least day 65 of gestation (Lindahl, 1969). The intrarectal Doppler technique also allows detection of pregnancy earlier in gestation than the A-scan technique (Lindahl, 1971). However, Wani *et al.* (1998) reported 72% accuracy in predicting pregnancy in gaddi goats using Doppler technique. The cause might be due to the audition of middle uterine artery sounds in previously kidded does. Accurate detection using Doppler technique is also confounded with the present of multiple foetuses (Ishwar, 1995). However, no explanation was given for this issue.

On the other hand, real-time B-mode ultrasonography offers an accurate, rapid, safe and practical means of diagnosing pregnancy and determining foetal numbers (Ishwar, 1995). B-mode ultrasonic scanning produces a two dimensional images on the screen. For pregnancy examination, it produces a moving image of the uterus, foetal fluid, foetal heartbeat, placentome, head, limbs and other skeletal structures. Examinations should be performed away from direct sunlight under subdued lighting for optimal image visibility. Real-time B-mode ultrasonography is performed using two probes, i.e. transrectal and transabdominal probes. Ideal time for transabdominal scanning is between 40 to 75 days of pregnancy (Ishwar, 1995). Haibel (1990) reported that 25 to 30 days of pregnancy is best done transrectally.

2.4 SUITABILITY OF SCANNING METHOD FOR DIFFERENT STRUCTURES ALONG PREGNANCY PERIOD

Table 2.5: The presence or absence and measurability of foetus and foetal-related structures during different stages of pregnancy

Stages of pregnancy	Structures	Transrectal	Transabdominal	Combination of transrectal and transabdominal
Trimester 1 (days 1-50)	Embryonic vesicles	+ (Gonzalez de Bulnes *et al.* 1998; Medan *et al.* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Romano and Christians, 2008; Karen *et al.,* 2009;	(Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Romano and Christians, 2008; Karen *et al.,* 2009;	+
	Foetus	+ (Gonzalez de Bulnes *et al.,* 1998; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+ (Dawson *et al.,* 1994; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+
	Heart	+ (Martinez *et al.,* 1998; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+ (Dawson *et al.,* 1994; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+
	Head	+ (Gonzalez de Bulnes *et al.,* 1998; Medan *et al.,* 2004; Karen *et al.,* 2009;	+ (Medan *et al.,* 2004; Karen *et al.,* 2009;	+
	Kidney	-	-	-
	Placentome	+ (Doize *et al.,* 1997; Medan *et al.,* 2004; Karen *et al.,* 2009;	+ (Medan *et al.,* 2004; Karen *et al.,* 2009;	+

(continued)

(continued)

Stages of pregnancy	Structures	Transrectal	Transabdominal	Combination of transrectal and transabdominal
Trimester 2 (days 51-100)	Embryonic vesicles	-	-	+
	Foetus	-	+ (Dawson *et al.,* 1994; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009)	+
	Heart	+ (Martinez *et al.,* 1998; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+ (Dawson *et al.,* 1994; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009;	+
	Head	+ (Suguna *et al.,* 2008;	+ (Suguna *et al.,* 2008;	+
	Kidney	+ (Ali and Hayder, 2007)*	+ (Ali and Hayder, 2007)*	+
	Placentome	+	+ (Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009)	+
Trimester 3 (days 101-150)	Embryonic vesicles	-	-	-
	Foetus	-	-	+
	Heart	-	+ (Suguna *et al.,* 2008; Karen *et al.,* 2009)	+
	Head	-	-	-
	Kidney	+ (Ali and Hayder, 2007)*	+ (Ali and Hayder, 2007)*	+
	Placentome	-	+ (Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009)	+

*Studies on ewes that gave significant results equivalent to studies on does.

2.5 PREGNANCY-RELATED STRUCTURES

Pregnancy-related structures refer to the structures that either appear or undergo changes during pregnancy. Some of the commonly identified structures are embryonic vesicles, foetal heart, placentome, foetal head and kidney. Ultrasonography enables the evaluation and measurement of these structures. Recent studies show that such information would allow producers to predict delivery dates and to group animals based on their nutritional needs especially during the last trimester of pregnancy. Hence, further study on correlation between pregnancy related structures and gestational age is of highly important

2.5.1 Embryonic Vesicles

Embryonic vesicles refer to fluid filled vesicles within which the embryo is developed. It appears as a non-echogenic structure (black on screen) on day 21 of gestation using transrectal ultrasonography and day 28 following transabdominal ultrasonography (Suguna *et al.,* 2008). The average diameter of this embryonic vesicle is 1 cm and it can be found in the uterine lumen (Padilla-Rivas *et al.,* 2005). The vesicles can be detected in the direction of 3 to 5 o'clock from bladder via transrectal ultrasonography. Appearance of this embryonic vesicle is considered as an evidence for early pregnancy.

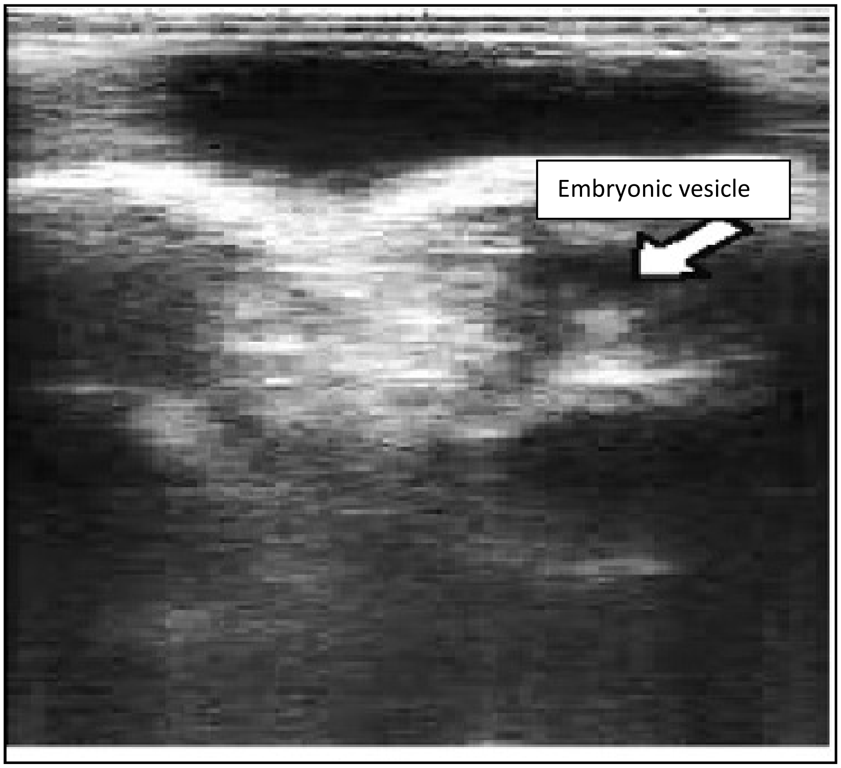

Figure 2.6: Embryonic vesicle (arrow) in the uterine lumen of a doe 21 days pregnant (Adapted from: Suguna *et al.,* 2008).

As pregnancy progresses, embryo will enlarge up to the extent that it is not visible as a whole on the ultrasound machine screen. This is because the foetus will get bigger throughout pregnancy and the vesicles as well need to enlarge to support the foetal development. Owing to this, the application of embryonic vesicle to evaluate gestational age is only limited at the early stage of pregnancy. In a nutshell, appearance of embryonic vesicle can be used to confirm pregnancy; however, it is not suitable for gestational age estimation.

2.5.2 Crown-rump Length

Crown-rump length (CRL) refers to the length of embryo or foetus (Karen *et al.,* 2009). Its measurement is taken as the greatest length of the embryonic or foetal mass before differentiation of foetus into body parts (Figure 2.7). Thereafter, the CRL is measured when the top of the skull and rump could be distinguished (Revol and Wilson, 1991).

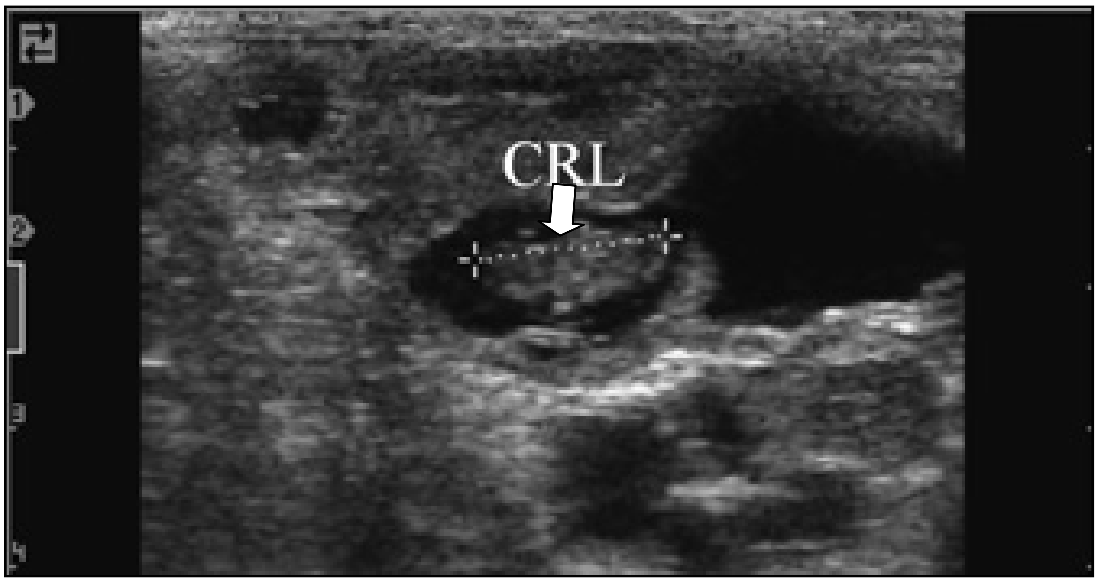

Figure 2.7: Foetus (arrow) at the age of 32 days. Black area enclosed the foetus is the amniotic fluid (Adapted from: Ali and Hayder, 2007).

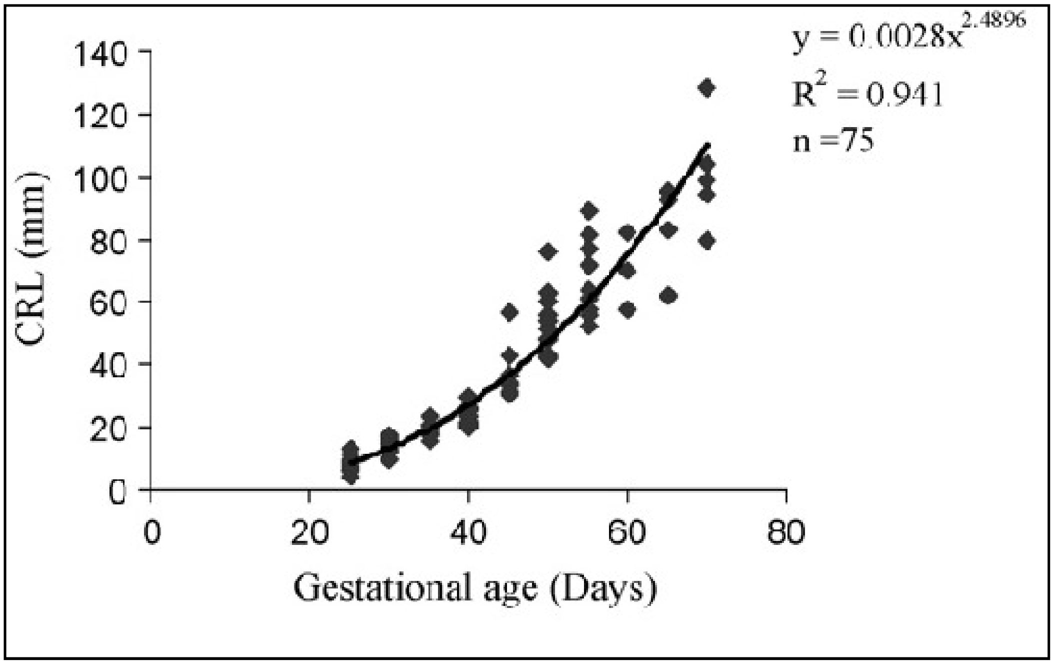

Figure 2.8: Relationship between CRL and gestational age in Egyptian goat (Adapted from: Karen *et al.,* 2009).

Karen *et al.* (2009) reported high correlation between CRL and gestational age between days 25 to 70 of gestation (Figure 2.8). This is in agreement with Martinez *et al.* (1998) who found the correlation between days 19 and 40 of gestation. According to Kahn *et al.* (1989), the imaging of the CRL is limited by the viewing field and the penetration length depth of the ultrasound scanner. After the first half of pregnancy, the length of the foetus usually exceeds the viewing screen of the ultrasound, making it unable to be visualized as a whole. Hence, this limits its reliability during the second half of pregnancy.

2.5.3 Foetal Heart

Foetal heart is considered as the most solid evidence of foetal viability. It is a confirmatory sign that the foetus is alive. Ultrasonic imaging of foetal heartbeat is regularly used to detect embryo and to evaluate embryo viability; this reflects on accurate diagnosis of pregnancy (Martinez *et al.,* 1998). Suguna *et al.* (2008) reported that the earliest heart beat detected by the transrectal method was on day 21, recordable on day 28 and then detectable up to day 98 of gestation. The heartbeat by transabdominal ultrasonography was detected earliest and counted on day 35, up to day 130 of gestation (Suguna *et al.,* 2008). Besides that, the number of foetal heart will as well helps in foetal counting; to confirm whether it is single or multiple pregnancies.

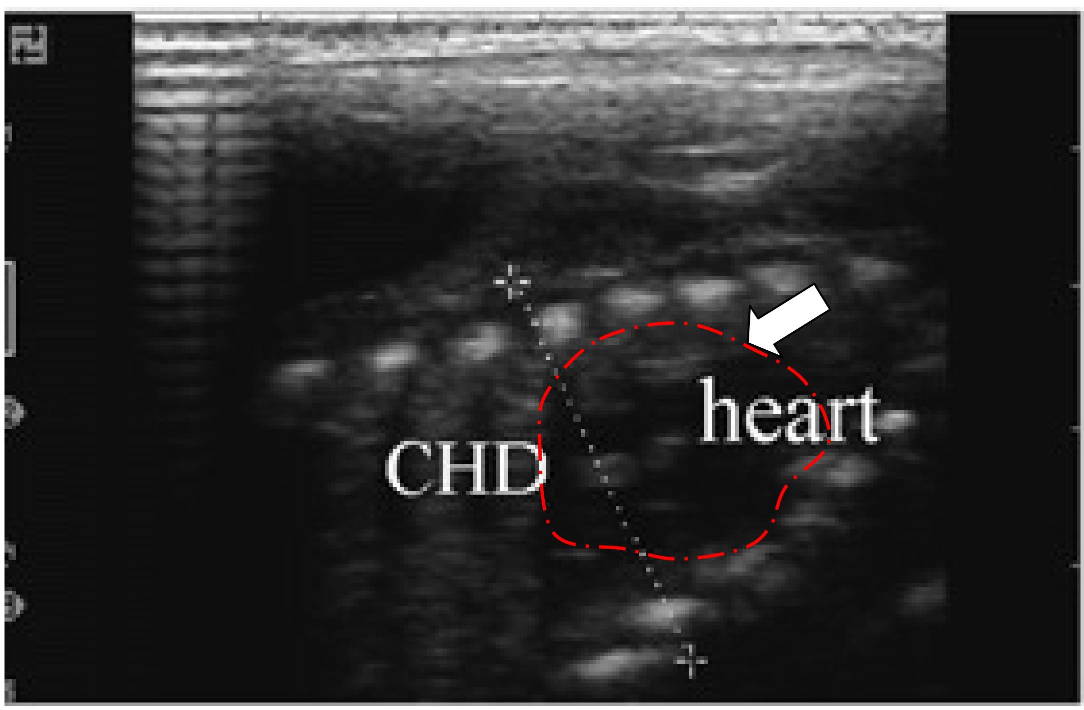

Figure 2.9: Heart (arrow) and chest as observed on day-83 of pregnancy (Adapted from Ali and Hayder, 2007).

Despite of its viability throughout all stage of pregnancy, up to author's knowledge, not much specific research have been done to use data from foetal heart to estimate gestational age. Suguna *et al.* (2008), Karen *et al.* (2009) and Martinez *et al.* (1998) found that there was a significant reduction in heart rate with the advancement of pregnancy. However, the correlation between other measurable parameters especially heart size and anatomical appearance is yet to be reported.

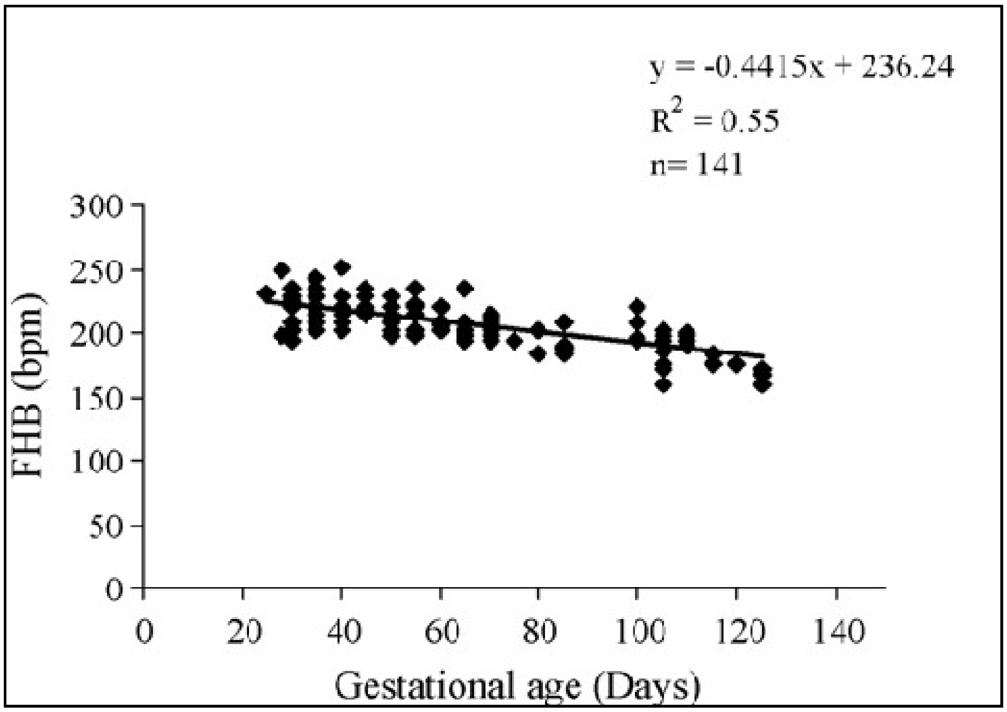

Figure 2.10: Relationship between foetal heartbeat rate (FHB) with gestational age (Adapted from: Karen *et al.,* 2009).

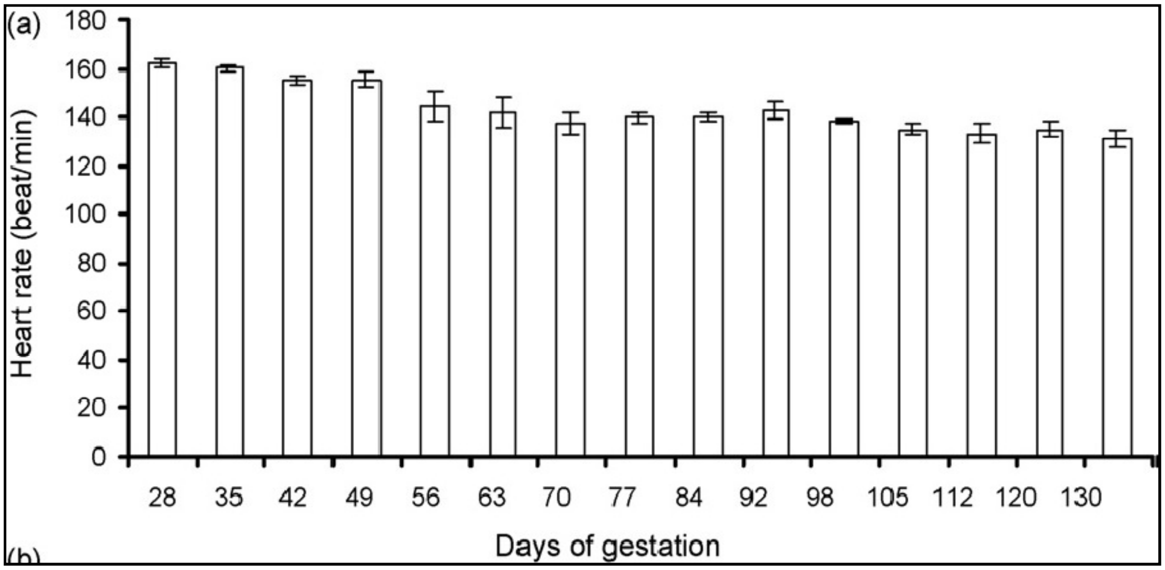

Figure 2.11: Relationship between foetal heartbeat rate (FHB) with gestational age (Adapted from: Suguna *et al.,* 2008).

Figure 2.10 and 2.11 describe the relationship between foetal heartbeat rate (FHB) with gestational age in does. Results obtained in both study showed that foetal heartbeat rate decreased with progressing pregnancy. Similar decreasing trend in FHB were also observed in goats (Martinez *et al.,* 1998), cattle (Kahn, 1989), sheep (Aiumlamai *et al.,* 1992) and buffaloes (Pawshe *et al.,* 1994). However, the absolute value of FHB in both figures is different. This is attributed to the methods used for counting the FHB. Karen *et al.* (2009) used M-mode ultrasound to measure FHB, meanwhile Suguna *et al.* (2008) used stop-watch technique.

2.5.4 Placentome

Ruminants have a cotyledonary placenta instead of a single large area of contact between maternal and foetal vascular systems. The terminology used to describe ruminant placentation is as follows:

- **Cotyledon**: the foetal side of the placenta

- **Caruncle**: the maternal side of the placenta
- **Placentome**: a cotyledon and caruncle together

Caruncles are oval or round thickenings in the uterine mucosa resulting from proliferation of sub-epithelial connective tissue. As shown in the image below, caruncles are readily visible in the non-pregnant uterus. Furthermore, they are the only site in the uterus to form attachments with foetal membranes. Patches of chorioallantoic membrane become cotyledons by developing villi that extend into crypts in the caruncular epithelium.

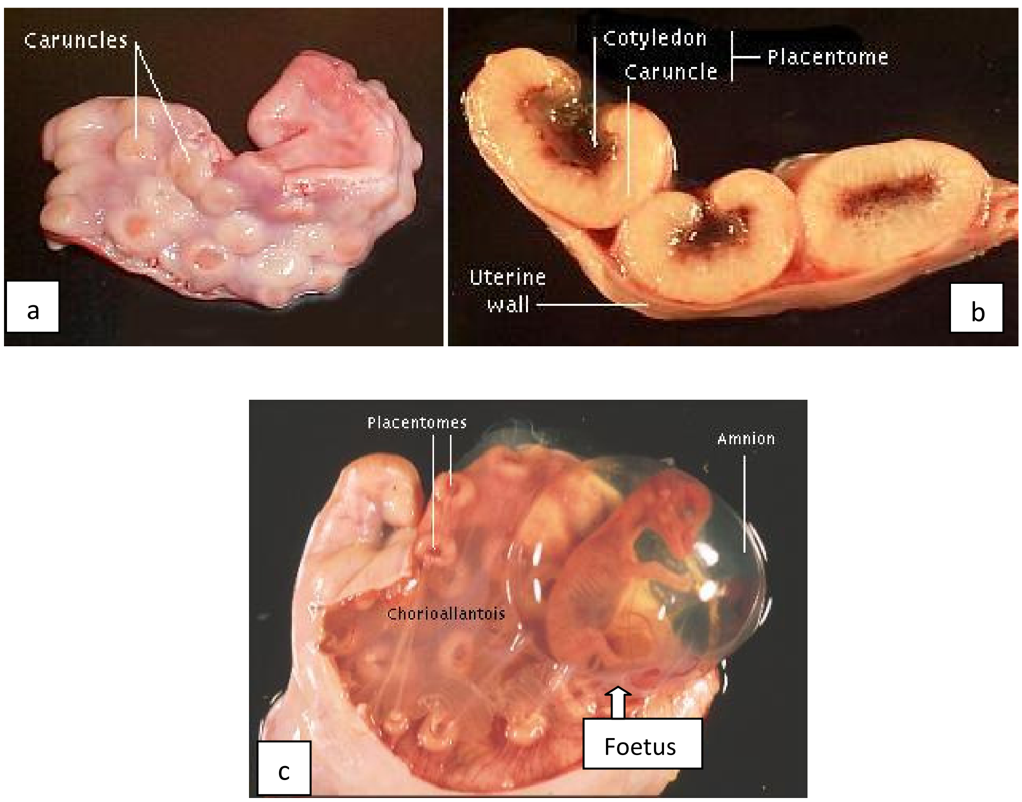

Figure 2.12: Cross-section of caruncles, placentomes and chorioallantois in sheep. a) Caruncles in an incised non-pregnant sheep uterus. b) Cross-section through placentomes from a mid-gestation sheep pregnancy. c) The image to the right shows an incised uterus from a pregnant sheep, roughly day 50 of gestation. The numerous button-shaped structures are placentomes, and the surfaces in view are actually cotyledons - the foetal side of the placentome. The slightly milky-looking membrane covering and between placentomes is the chorioallantois. The foetus is clearly visible inside the amnion (Adapted from: Bowen, 2000).

Pregnant goats, sheep and cattle have between 75 and 125 placentomes in total and they can be easily detected throughout pregnancy (Bowen, 2000). The placentome increases in size and appears as a C- or O-shaped gray image (Lee *et al.,* 2005) (Figures 2.13 and 2.14) throughout pregnancy. The increase in placentome size with advancement of the pregnancy age may be an adaption to provide nourishment to the growing foetus. Previous research studies showed correlation between gestational age and placentome diameter (Doize *et al.,* 1997; Lee *et al.,* 2005; Suguna *et al.,* 2008).

Ali and Hayder (2007) reported degradation of placentome during the last month of pregnancy in sheep which is almost similar in goats (Figure 2.14). Collapsing of the placentome might be an indication for occurrence of a degenerative process (Grunert, 1980; Bjorkman and Dantzer, 1987). On the other hand, these changes in the placentomes may be associated with increased placental perfusion and tissue permeability during late pregnancy (Metcalfe *et al.,* 1988).

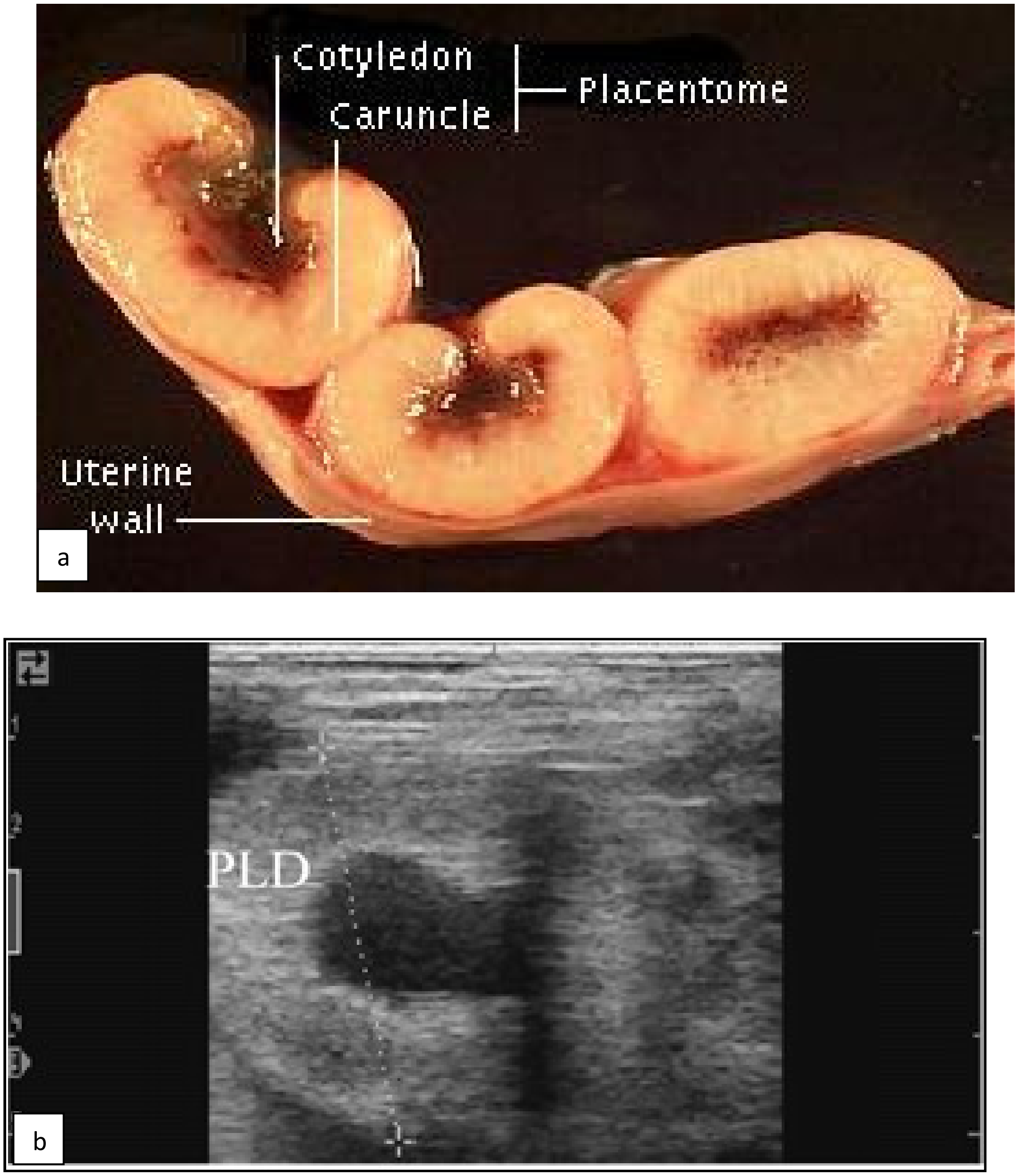

Figure 2.13: Comparison of a) The exact image of C-shaped placentome (Adapted from Bowen, 2000) and b) placentome as observed on day 84 of gestation ultrasonically (Adapted from Ali and Hayder, 2007).

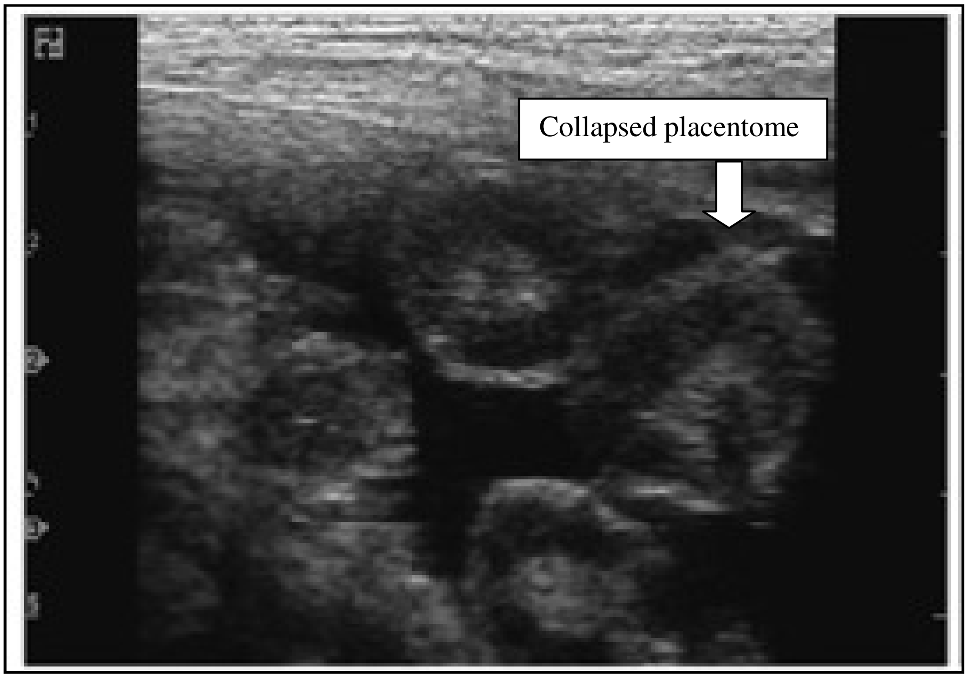

Figure 2.14: Collapsed placentome as observed on day 102 of gestation (Adapted from Ali and Hayder, 2007).

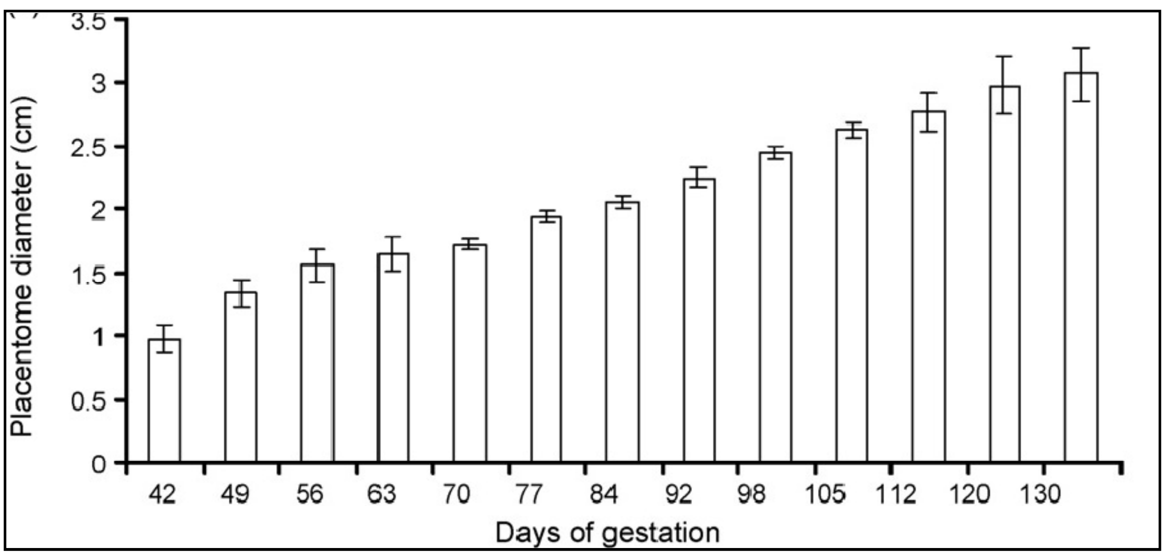

Figure 2.15: Relationship between placentome diameter and gestational age (Adapted from Suguna *et al.,* 2008).

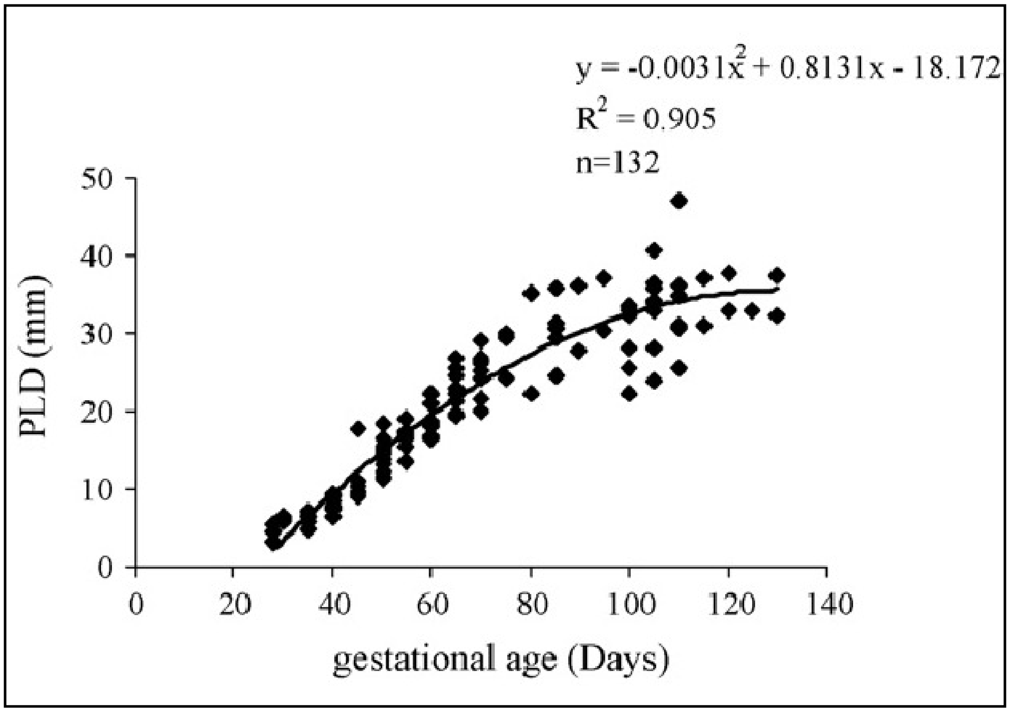

Figure 2.16: Relationship between placentome diameter and gestational age (Adapted from Karen *et al.,* 2009).

Suguna *et al.* (2008) and Karen *et al.* (2009) reported the development pattern of placentome throughout pregnancy in does (Figures 2.15 and 2.16). During the first 2 trimesters of pregnancy, the size of placentome increases steadily. However, the increase rate in placentome size became less during the last trimester of pregnancy, and the size decreases during the last month of pregnancy. In addition to that, the placentome cavity also becomes less echogenic. This indicates that the placentome starts to collapsed during the last trimester of pregnancy. By this time, it starts to collapse with reducing its lumen, while in areas adjacent to the foetus it appears flattened. Collapsing of the placentome might be an indication for occurrence of a degenerative process (Grunert, 1980; Bjorkman and Dantzer, 1987). On the other hand, these changes in the placentomes may be associated with increased placental perfusion and tissue permeability during late pregnancy (Metcalfe *et al.,* 1988).

2.6 TIMEFRAME FOR ULTRASOUND SCANNING PERFORMED FOR PREGNANCY DIAGNOSIS IN SMALL RUMINANT

Table 2.6: Timeframe for ultrasonography as previously conducted in small ruminants

Researcher	Species	Timeframe for ultrasound scanning performed
Doize *et al.,* 1997	Goat	Transrectal ultrasonography was carried out on does every week from breeding to parturition.
Martinez *et al.,* 1998	Goat	The does were scanned once daily from days 13 to 40 after mating.
Karen *et al.,* 2004	Sheep	Ewes were scanned 2 times at 30 to 40 days interval. Gestational ages at the time of scanning of these ewes were calculated retrospectively by subtracting the number of days elapsing between scanning and lambing from the average duration of gestation (150 days).
Suguna *et al.,* 2008	Goat	Gestational age was classified based on 7 days interval (weekly basis) for the first 4 months of pregnancy. During the 5^{th} month of pregnancy, ultrasound scanning only was performed once on each pregnant doe.
Karen *et al.,* 2009	Goat	Transrectal ultrasonography (7 MHz) was carried out on the pregnant does at day 10 post mating on alternate days until day 25 and then once at 3– to 5-day intervals until day 50. Transabdominal ultrasonography (3.5–5 MHz) was carried out on the same animals from days 25 to 130 at 3– to 5-day intervals.

In previously conducted research, different approaches of time-frame for pregnancy diagnosis to be conducted in small ruminants were found. Each approach differs depending on the need of respective experiments. For detailed evaluation of embryo and foetal development during early pregnancy stage, Martinez *et al.* (1998) conducted transrectal ultrasonography on daily basis. Doize *et al.* (1997) and Suguna *et al.* (2008) performed ultrasound scanning on weekly basis to study the development of foetus throughout gestational period. As a result, both researchers managed to record the foetal development up to their targeted stage. On the other hand, Karen *et al.* (2004) conducted ultrasound scanning in pregnant ewes with unknown mating date two times at 30 to 40 days interval. Following that, gestational age was calculated retrospectively by subtracting the number of days elapsing between scanning and lambing date based on the average duration of gestation for ewes which is 150 days.

2.7 EFFECT OF GOAT BREED ON PREGNANCY DIAGNOSIS ACCURACY

Table 2.7: Comparison of diagnosis on selected pregnancy-related structures in different breed of pregnant does

Pregnancy-related structures	Researcher; goat breeds; findings
Allantoic fluid	1. Doize *et al.,* 1997; Alpine and mixed-breed goats; allantoic fluid was first detected on day 21 of gestation. 2. Medan *et al.,* 2004; Shiba goats; allantoic fluid was first detected on approximately day 20 of gestation. 3. Padilla-Rivas *et al.,* 2005; Boer goats; allantoic fluid was first detected on approximately day 20 of gestation. 4. Karen *et al.,* 2009; Egyptian Baladi goats; allantoic fluid was detected on approximately day 17 of gestation.
Placentome	1. Doize *et al.,* 1997; Alpine and mixed-breed goats; placentomes were detected at approximately day 35 of gestation. 2. Medan *et al.,* 2004; Shiba goats; placentomes were detected on approximately day 35 of gestation. 3. Suguna *et al.,* 2008; goat breed was not stated but explained as different from those studied by Medan *et al.,* 2004; the earliest diagnosis of placentome only possible starting from day 42 of gestation. 4. Karen *et al.,* 2009; Egyptian Baladi goats; placentomes were detected on approximately day 28 of gestation.
Foetal BPD	1. Reichle and Haibel, 1991; Pygmy goats; high correlation between foetal BPD and gestational age. 2. Lee *et al.,* 2005; Korean black goats; low correlation of determination (R^2=0.650) between foetal BPD and gestational age. 3. Karen *et al.,* 2009; Egyptian Baladi goats; high correlation of determination (R^2=0.956) between foetal BPD and gestational age.

Table 2.7 describes the comparison between pregnancy-related structures diagnosis in different breed of does. Variation in goat breeds especially on size and fat thickness seems to affect the time for detection and measurement of specific pregnancy-related structures. For instance, placentomes were detectable in pregnant Egyptian Baladi does at

day 28 of gestation (Karen *et al.,* 2009) but only can be detected at day 42 in another breed as studied by Suguna *et al.* (2008). However, placentomes could be detected starting from day 35 of gestation in Shiba (Medan *et al.,* 2004) and Alpine (Doize *et al.,* 1996) does.

Another evident is the detection of allantoic fluid which can be detected at days 20 to 21 in Alpine (Doize *et al.,* 1996), Shiba (Medan *et al.,* 2004) and Boer (Padilla-Rivas *et al.,* 2005) goats, but can be detected as early as day 17 in Egyptian Baladi (Karen *et al.,* 2009) goats. These finding suggests that some breed of goat may show variation with another breed but may show resemblance with the other one. Owing to the findings, it is of highly beneficial for a research on comparison of reliability of equation on gestational age estimation on different breed of does to be conducted.

Chapter 3

3.0 MATERIALS AND METHODS

Chapter 3

3.0 MATERIALS AND METHODS

3.1 GENERAL INTRODUCTION

This study was carried out to diagnose pregnancy in goats using images of ultrasound scanner at three farms. The does underwent AI or/and natural mating after natural oestrus or oestrus synchronisation. The study was performed from October 2008 until November 2010.

3.2 LOCATION OF STUDY

The experiment were carried out at three farms; the Institute of Biological Sciences (ISB) Mini Farm (Livestock), University of Malaya; Kambing Baka Baik Kepala Batas Farm (KBKB), Kepala Batas, Penang and Rumpun Asia Sdn. Bhd. Farm (RASB), Batang Kali, Selangor.

3.3 EXPERIMENTAL ANIMALS

Jermasia, Katjang, Saanen and Boer-crossbred does from ISB Mini Farm (Livestock), University of Malaya, and Rumpun Asia Sdn. Bhd. Farm together with Boer crossbred does from KBKB farm, Kepala Batas, Penang, aged 1 to 7 years old were used in this study. Figures 3.1 to 3.3 show the breed of does used in this experiment. The age of does with no record on birth date were determined by dentition (Figure 3.4). The pregnancy detection was conducted with the does using the ultrasound scanner in a standing position. Goats were fed with Napier grass and commercial pellets twice daily, with access to water *ad libitum*. A total of 673 scanning procedures were carried out in 254 goats. Oestrus synchronisation was carried out on some females before AI or natural mating while the rest were naturally mated.

Figure 3.1: Jermasia breed does.

Figure 3.2: Boer-crossbreed does.

Figure 3.3: Katjang breed does.

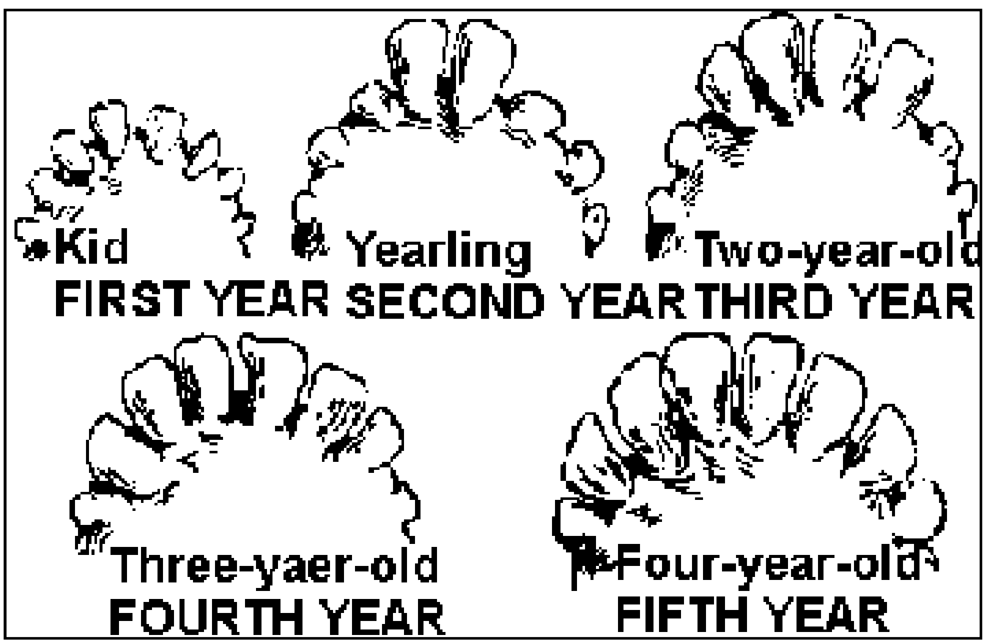

Figure 3.4: Dentition to determine age of goat (Adapted from http://members.psyber.com/macgoats.htm).

3.4 MATERIALS

3.4.1 Equipment and Instruments

Pregnancy detection was conducted using real time B-mode ultrasound scanner, ALOKA SSD500 equipped with a convex 5.0 MHz transabdominal scanner (4.0 cm length) and linear array 7.5 MHz transrectal scanner (shaft length: 35 cm, shaft diameter: 1.4 cm). Desired images were printed by thermic printer Sony UP-860 to obtain hard copies in high density Type II and Type V, paper Sony UPP-110HD.

3.4.2 Disposables/Miscellaneous

Contact gel (carboxymethylcellulose gel) that reacts as a coupling agent for ultrasound transmission was used to the test site of the does in transabdominal approach. For transrectal procedure, the transducer was fastened to a plastic rod lubricated with the gel. Electric shaver was needed to shave pregnancy detection site before ultrasound examination using transabdominal probe took place. Gloves and masks were also used in the experiments.

Figure 3.5: Ultrasound machine ALOKA SSD500. This ultrasound machine comprises of three major parts; monitor, probes and printer. ALOKA SSD500 can be used for B-mode and M-mode ultrasound diagnosis (Adapted from http://www.medwrench.com).

3.5 METHODS

3.5.1 General Overview

In this study, five experiments were carried out. Females that were synchronised for oestrus or naturally oestrus were restrained in a standing position before pregnancy detection. Ultrasound machine and probes were properly set up. Data such as tag number of the goat and date were keyed in. Ultrasound scanning examinations were executed using two probes; transrectal and transabdominal probes. Transrectal probe was preferable in early stages whereas transabdominal probe gave effective result in later stages of pregnancy.

3.5.2 Transrectal Scanning Procedure

With transrectal approach, faeces were cleared from the rectum. The 7.5 MHz transducer was attached to the tip of a rigid extension rod. The tip of the transducer was lubricated with carboxymethylcellulose contact gel. The transducer was inserted gently until the urinary bladder was identifiable. Probe was moved gently forwards and backwards and rotated 90 degrees clockwise and 180 degrees counter-clockwise. Images of embryonic vesicles, placentomes, heart, heartbeat and foetus were positive indicators of pregnancy in early stages.

3.5.3 Transabdominal Scanning Procedure

Transabdominal ultrasonography was performed at middle, late and final stages of gestation using a 5.0 MHz transducer. First, the test site area of 150 to 200 cm^2 was shaved. The contact fluid, carboxymethylcellulose gel was later applied to the test site and the transducer was placed at the right side of the goat, 5 cm in front of the rear leg and 2.5

cm above the teat. Then, surface probe was covered with gel and probe was orientated perpendicularly towards the ventral abdominal wall. The observed images of placentomes, heart, skeletal structures, heartbeat, spinal cord, head, limbs and foetus which were positive indicators of pregnancy in later stages were printed. The images were analysed and compared for the different stages of pregnancy. Detection of pregnancy on does differ with stages of gestation.

3.6 EXPERIMENTAL DESIGN

A total of 673 scanning procedures were carried out on naturally mated and artificially inseminated does. Figures 3.6 and 3.7 show AI being conducted on the does. A total of 5 experiments using ultrasound scanner with 7.5 MHz transrectal and/or 5.0 MHz transabdominal probes were carried out in this study. In Experiment 1, preliminary study for pregnancy-related structure was conducted on a total of 92 naturally mated Boer-crossbred does. Later, in Experiment 2, a flock test was conducted on 119 does from different breeds with unknown breeding status. Specific evaluation and measurement of pregnancy-related structures using transrectal and transabdominal probes was performed on 11 does in Experiments 3 and 4, respectively. In Experiment 5, equations for gestational age estimation based on data from Experiments 3 and 4 were tested on 43 pregnant does to determine its accuracy. Images showing specific structures were used as indicators to optimise the prediction for different stages of pregnancy. The introduction of bucks to females and date of mating were unknown to the researcher. The does that were naturally mated and/or AI were scanned using both probes. Percentage of correct diagnosis was evaluated based on the predetermined optimal combination of structural images.

Figure 3.6: Artificial insemination (AI) is being performed on oestrus synchronized does.

Figure 3.7: Close up: AI is being performed on oestrus synchronized does.

3.6.1 Observation on Foetal Structures and Related Images in Reproductive System of Does using Transrectal and Transabdominal Probes (Experiment 1)

This experiment is the preliminary study to evaluate the detectable pregnancy related structures in pregnant does. A total of 92 naturally-mated does at KBKB Farm were used in the experiment. The pregnant does were diagnosed using 7.5 MHz transrectal probe throughout the gestation period. The foetal structural images and other related images were recorded. The age of foetus involved in this experiment was determined retrospectively after obtaining date of kidding (approximately 147 days). Images from the non-pregnant does were obtained as control. The foetal structural images and other related images obtained were sequentially arranged and correlated with the specific period of pregnancy. Parameters on early pregnancy such as sac, non-echogenic (NE) area, placentomes, uterine wall, amniotic fluid and foetus with heartbeat were observed and recorded during pregnancy detection. The objective of the experiment was to observe foetal structures and related images in reproductive system using 7.5 MHz transrectal and 5.0 MHz transabdominal probes. Analysis obtained from the experiment was fact finding for Experiment 2.

3.6.2 Gestational Age Estimation based on Analysis of Pregnancy-related Structures in Different Breeds of Goats based on Characteristics Derived from Experiment 1 (Experiment 2)

This experiment aims to validate the criteria of pregnancy-related structures as obtained from Experiment 1 in different breeds of goats. The study was conducted on 119 mature females aged 2 to 6 years from different breeds of goat (50 Boer-crossbred does, 5 Saanen does, 43 Jermasia does and 21 Katjang does). All the does were mated naturally at either unknown dates or the actual mating dates were not informed to the technician before ultrasound scannings were carried out. The does were assigned into 4 different groups according to their breeds before ultrasound scanning was conducted. When the foetus or embryo imaged, the following pregnancy-related structures were identified and recorded:

a) Placentome echogenicity: the echogenicity of two largest placentome were classified into three groups based on colours; white, white-grey, and grey. The observed placentome had a circular shape when viewed in a longitudinal section or a regular C-shaped in cross section.

b) Embryonic sac: the non-echogenic areas (black on the screen) in the uterus were assumed to represent the fluid filled early conceptus which was later confirmed as pregnancy. The visualisation of whole shape embryonic sac is considered as significant indicator for early pregnancy.

c) Foetus: foetus was confirmed from heartbeat detection and its independent movement. Later, it was classified based on its visualization on screen.

d) Heart echogenicity: the echogenicity of heart was classified into four groups based on colours; white, white-grey, grey-black and black. The heart was confirmed by observation of rhythmic pulsation and thoracic area.

e) Heart size: size of heart was classified into 3 groups based on their appearance on screen; small, medium, large.

Chronologically arranged images during pregnancy obtained from Experiment 1 formed the bases as a reference for images acquired from Experiment 2.

3.6.3 Optimisation of Pregnancy Diagnosis of Does with Transrectal Probe (Experiment 3)

A total of 11 naturally mated does at ISB Farm and RASB Farm were used in this experiment. It was designed to optimise pregnancy diagnosis in does using ultrasound scanner with 7.5 MHz transrectal probe. Ultrasonography was conducted on weekly basis, and a total 198 scanning procedure were performed. Briefly, structural and related images were categorised on weekly basis. Specific does of different pregnancy stages were selected and were diagnosed using 7.5 MHz transrectal probe starting from day-28 of gestation. The foetal structural and other related images, such as presence of foetal head, foetal heart, placentomes and foetal heart beat were recorded and measured. The age of foetus involved in this experiment was also confirmed with the mating dates. Ultrasonography was performed until the day of which the pregnancy-related structures hard to be visualised transrectally. Various measurements of pregnancy-related structures were analysed and formed the percent predicted accuracy for different stages of pregnancy. The reliability for the structures with highest predictive value was diagnosed and confirmed in Experiment 5.

3.6.4 Optimisation of Pregnancy Diagnosis of Does with Transabdominal Probe (Experiment 4)

A total of 11 naturally mated does at ISB Farm and RASB Farm were used in this experiment. It was designed to optimise pregnancy diagnosis in does using ultrasound scanner with 5.0 MHz transabdominal probe. Ultrasonography was conducted on weekly basis, and a total of 198 scanning procedure were performed. Briefly, structural and related images were categorised on weekly basis. Specific does of different pregnancy stages were selected and were diagnosed using 5.0 MHz transabdominal probe starting from day 28 of gestation. The foetal structural and other related images, such as presence of foetal head, foetal heart, foetal number, placentomes and foetal heart beat were recorded and measured. The age of foetus involved in this experiment was confirmed with the mating dates. Ultrasonography was performed until the day of which the pregnancy-related structures hard to be visualized transrectally. Various measurements of pregnancy-related structures were analyzed and formed the percent predicted accuracy for different stages of pregnancy. The reliability for the structures with highest predictive value was diagnosed and confirmed in Experiment 5.

3.6.5 Prediction of Gestational Age based on Equations on Placentome Diameter and Heart Size Derived from Experiments 3 and 4 Using Transrectal and Transabdominal Probes (Experiment 5)

The objective of this experiment was to determine the efficacy of ultrasound scanning in detecting different stages of pregnancy in goats using the optimal combination of structural image obtained from Experiments 3 and 4 using transrectal and/or transabdominal probes. Ultrasound scanning was performed one time on 43 pregnant does of which their mating dates were unknown to the author. Equations and data derived from Experiments 1 to 4 were used to determine gestational age. Confirmations were made based on the actual delivery date.

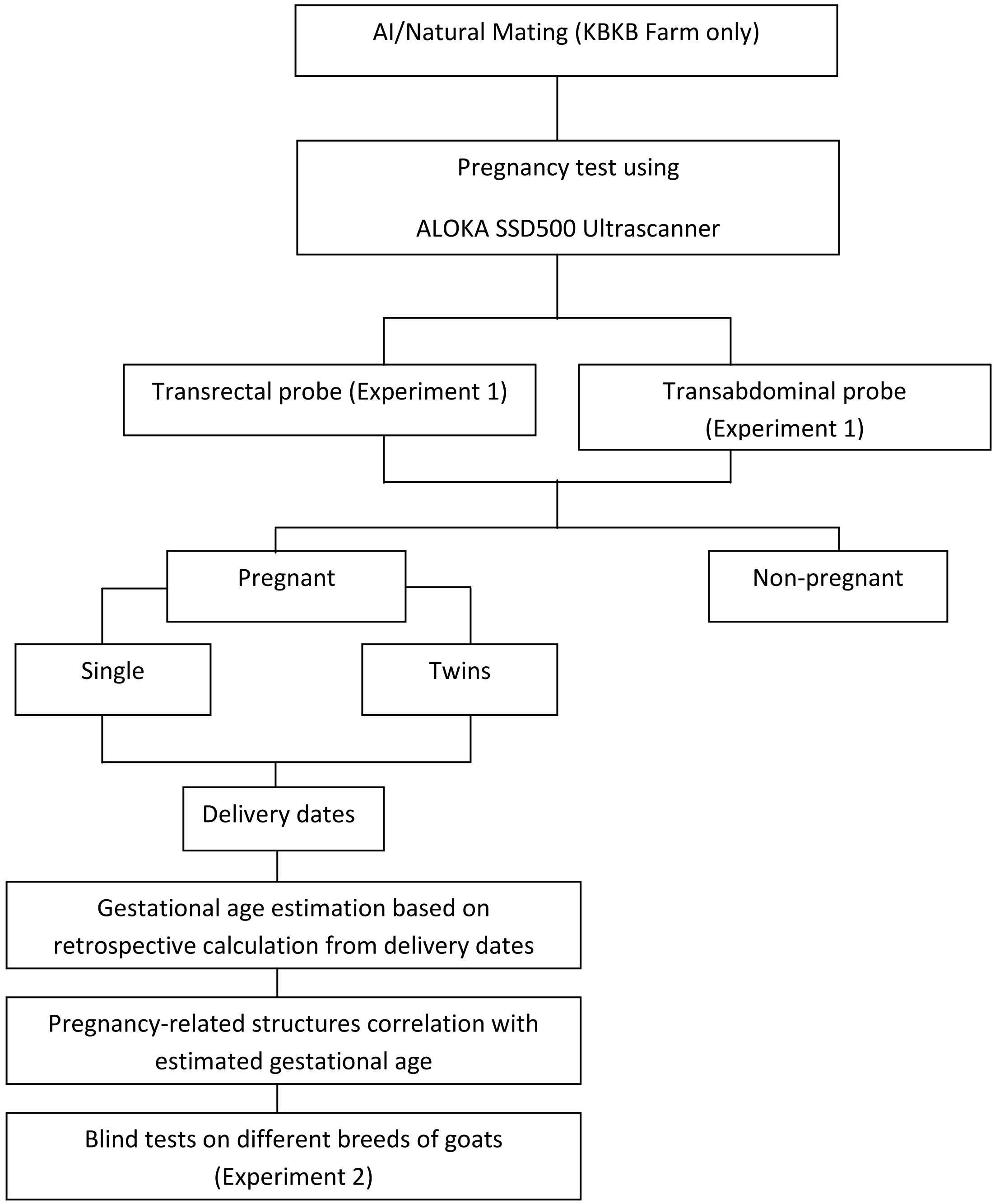

Figure 3.8: A schematic overview of the experimental design for diagnosis of pregnant does using transrectal and transabdominal probes (Experiment 1) and flock test (Experiment 2).

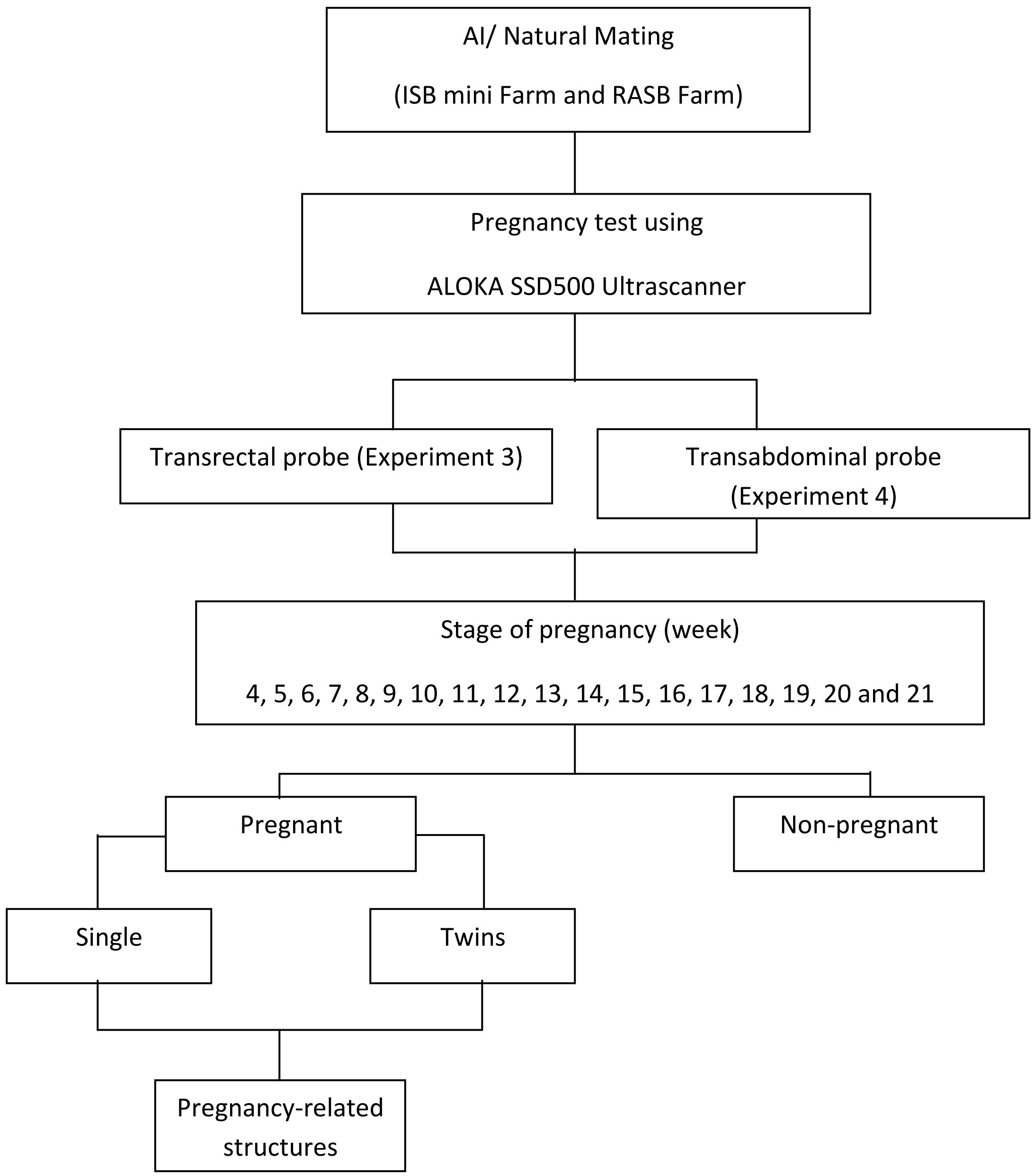

Figure 3.9: A schematic overview of the experimental design for optimisation of pregnancy diagnosis in does using transrectal (Experiment 3) and transabdominal (Experiment 4) probes.

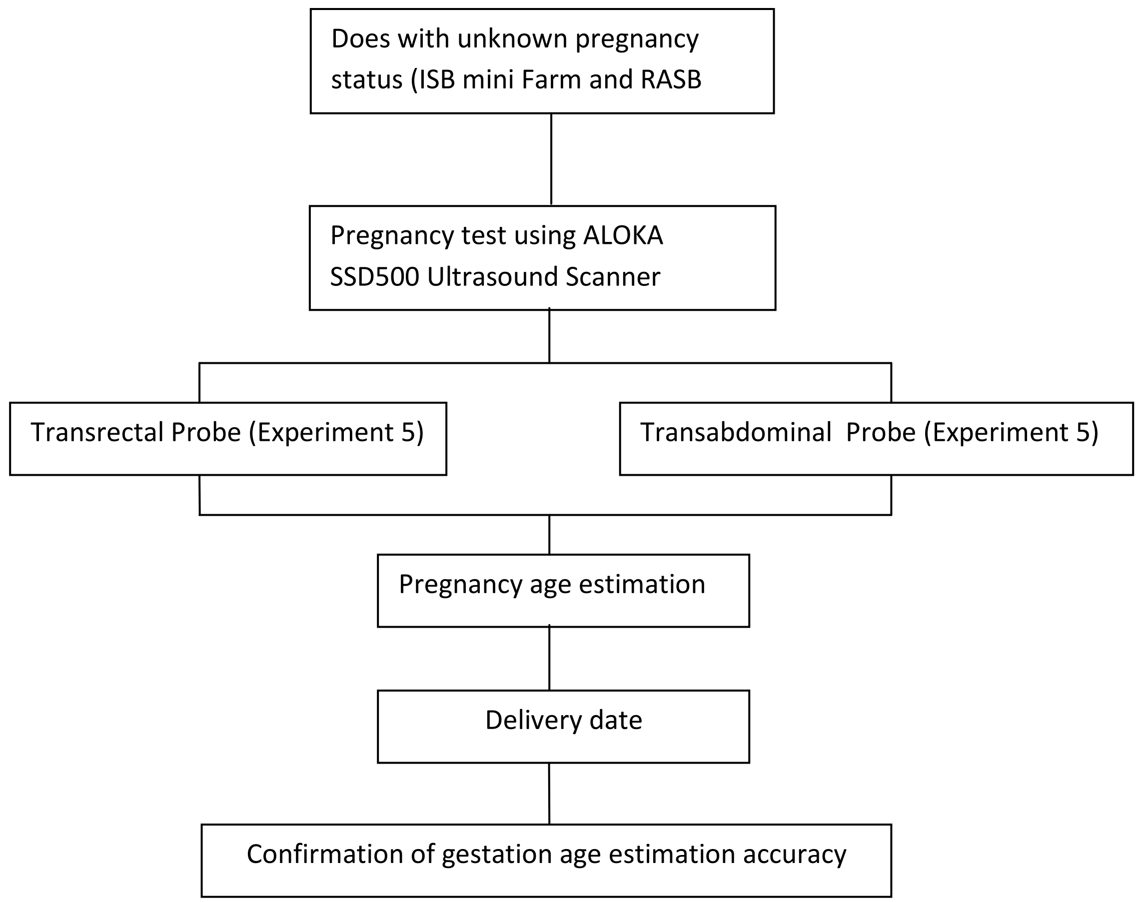

Figure 3.10: A schematic overview of gestational age estimation based on analysis of pregnancy-related structures in does (Experiment 5).

Chapter 4

4.0 RESULTS

Chapter 4

4.0 RESULTS

4.1 OBSERVATION ON FOETAL STRUCTURES AND RELATED IMAGES IN REPRODUCTIVE SYSTEM OF DOES USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 1)

Table 4.1 describes the characteristic of ultrasonographic images of pregnancy diagnosis in pregnant does via both transrectal and transabdominal probes based on gestation age classification. Gestational age was classified according to the 5 months of pregnancy period; 0-30 days (month 1), 31-60 (month 2), 61-90 (month 3), 91-120 (month 4) and 121-147 (month 5). During the first month of pregnancy, embryonic sacs and foetal heart images were detected using both transrectal and transabdominal probes. On the second month of pregnancy (days 31-60 of gestation), both probes showed equal reliability in pregnancy-related structures detection. Meanwhile, on the third month of pregnancy (days 61-90 of gestation), visualisation of pregnancy-related structures images by transrectal probe was limited due to the increasing in foetal size. Placentome was the only structures could be viewed at this stage. However, the pregnancy-related structures were easily accessed via transabdominal probe.

During the subsequent month (days 91-120 of gestation), only a few pregnancy-related structures such as foetal heart and placentomes could be visualised by transabdominal probe in which foetal heart appeared to be less echogenic and beat slowly. Approaching day 120 of gestation, placentomes started to degrade. During days 121-147, foetus would be extremely difficult to be visualised transrectally, and even for transabdominal ultrasonography, only some part of foetus could be observed. Foetal heart, when detected, appeared as black structure with slow rhythmic pulsation.

Figures 4.1 to 4.4 and Figures 4.5 to 4.10 show the images of transrectal and transabdominal probes, respectively. Pregnancy diagnosis using transrectal probe detected ovaries with developing follicles in non-pregnant does (Figure 4.1), but not in pregnant does. For transabdominal probe, images recorded from pregnant does appeared as blank since no pregnancy related structures was detected (Figure 4.5). From the results, for early pregnancy, the pregnancy could be confirmed by the detection of embryonic sac via transrectal probe (Figure 4.2) and transabdominal probe. Foetus with very rapid heartbeat could also be observed using both probes (Figures 4.3 and 4.6). Foetal heart, if observed, would be visible as rapid-beating white structure. Meanwhile, at pregnancy ages of 31-60 days, the heart of the foetus started to change its echogenicity which could be observed through the change in colour, from white to grey. At day 30, foetal counting was possible to be done via transrectal probe (Figures 4.3 and 4.4). Pregnancy-related structure, placentome (Figure 4.7), was first detected at day 44 of pregnancy and grew bigger throughout the pregnancy. Foetal structures like spine, leg and sometimes scrotum would be viable.

At the ages between days 61-90 of gestation, the foetus grew bigger and difficult to be visualised via both probes (Figure 4.8). However, ossification process was observed to be continued as bone structures like head, spine, and leg could be observed easily. Heart and thorax could be detected easily via transabdominal probe and the change in echogenicity of the heart continued as the colour turn from grey to black (Figure 4.8). At the ages between 91-120 days, the placentome started to change its echogenicity and its number increased. Heart was observed as more non-echogenic (Figure 4.9). The heartbeat was visible as slower compared to the earlier stage. Foetus was hard to visualise transrectally, and even for transabdominal ultrasonography, only some part of foetus could be observed. At the late age of pregnancy, days 121-147, foetus was extremely hard to visualise transrectally, and even for transabdominal ultrasonography, only some part of foetus could be observed such as placentomes (Figure 4.10) and foetal heart. Placentomes were observed to be degraded and grey in colour.

Table 4.1: Characteristic of ultrasonographic images of pregnancy diagnosis in pregnant does via both transrectal and transabdominal probes based on gestation age classification

Classification of gestation age	Characteristic of images recorded
Below 30 days	1. A clump of sac with diameter of more than 1 cm could be observed in the direction of 3-5 o'clock from bladder (transrectal probe) 2. A clump of sacs near bladder. Foetus could be observed in either one or two (twin) of those sacs (transabdominal probe) 3. Usually, if foetus detected, heart would be observed as a rapid beating round shaped white coloured structure (transrectal probe)
31 to 60 days	1. Rapid echo-dense heartbeat and whole foetus could be detected easily (transabdominal probe) 2. Foetus would be quite big to be observed. However, structures like spine, leg and sometimes scrotum would be viable 3. Echo-dense placentome could be observed (transrectal and transabdominal probes) 4. At around day 60, the heart starts to change its echogenecity (white–grey in colour) (transrectal and transabdominal probes)
61 to 90 days	1. Foetus would be too big to be observed, however, echo-dense structures like spine and bone could be detected easily (transrectal probe) 2. Quite big echo-dense placentome could be observed and measured (transrectal and transabdominal probes) 3. Big echo-dense placentome could be observed around amniotic fluid, however, they are scattered (transabdominal probe) 4. Heart and thorax could be detected easily. Heart starting to turn from echo-dense to non-echo (grey to black in colour) (transabdominal probe)

(continued)

(continued)

Days of pregnancy	Characteristic of images recorded
91 to 120 days	1. At around day-120, placentome starts to change its echogenicity and their number increase (transabdominal probe) 2. Heart would be observed as more non-echogenic. The heartbeat visible as slower compared to the earlier stage (transabdominal probe) 3. Foetus would be hard to visualise transrectally, and even for transabdominal ultrasonography, only some part of foetus could be observed (transrectal and transabdominal probes)
120 days to delivery days	1. Foetus would be hard to visualise transrectally, and even for transabdominal ultrasonography, only some part of foetus could be observed (transrectal and transabdominal probes) 2. Non-echodense and very big heart could be observed (transabdominal probe) 3. Placentome would appear as less-echogenic. Usually it will be in grey in colour (transabdominal probe) 4. Placentomes are highly packed around the amniotic fluid (transabdominal probe) 5. Heart, if detected, would be non-echodense and beats slowly (transabdominal probe)

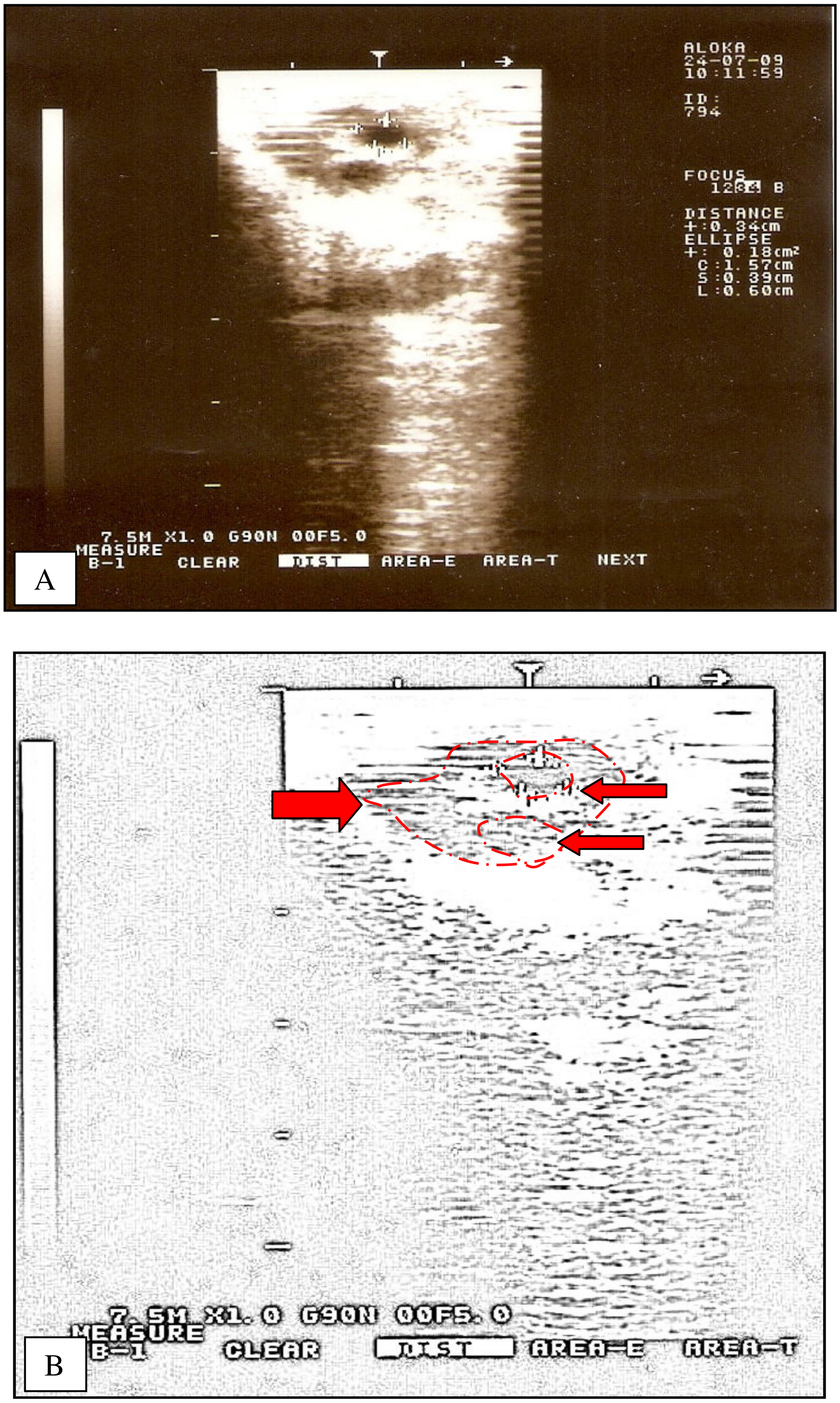

Figure 4.1: A) Original image of an open doe via transrectal ultrasonography. No pregnancy related structure detected. Ovary with follicle could be detected easily. B) Labeled image of ovary (arrow: →) and follicles (arrows: ←) as detected in non-pregnant does.

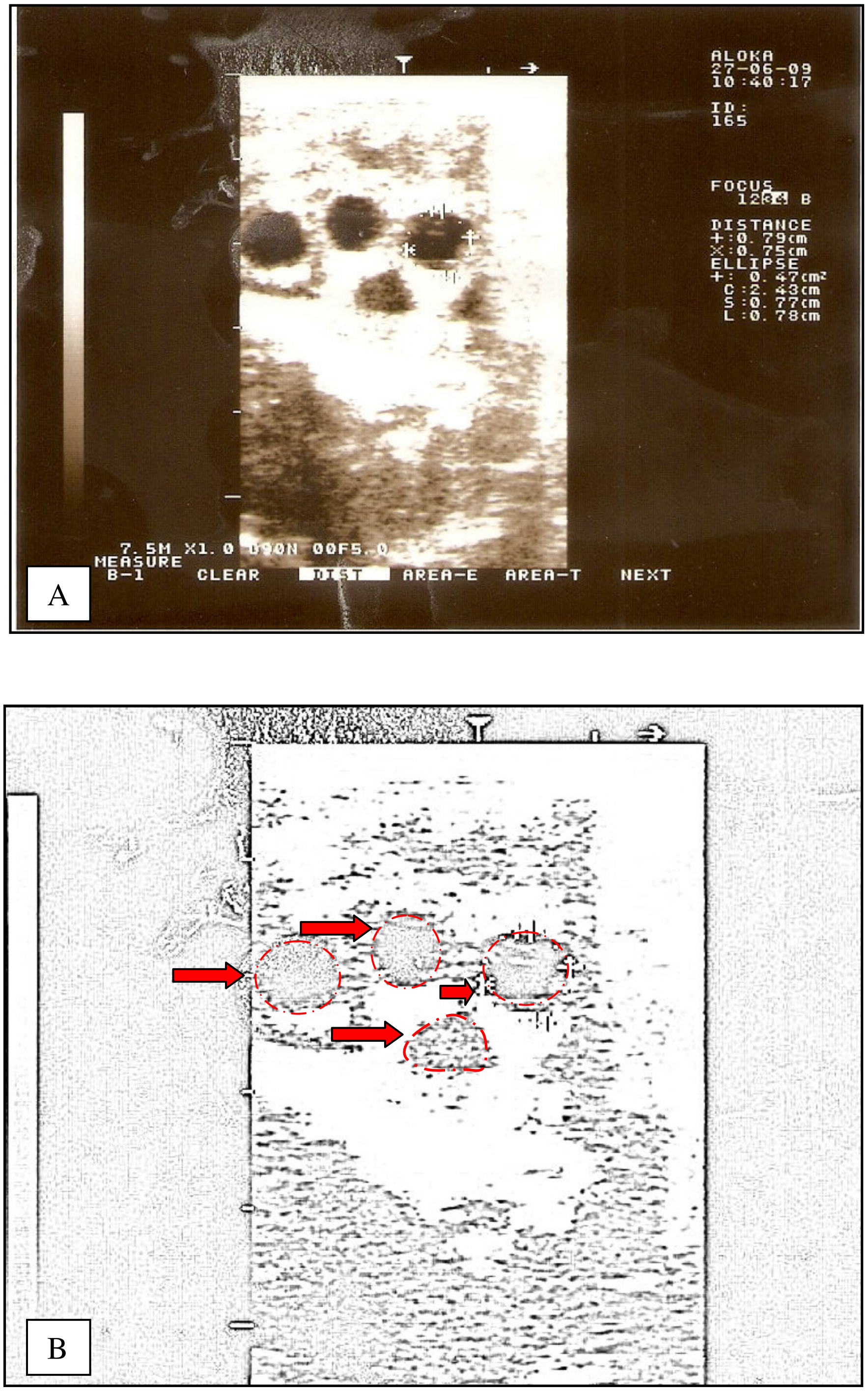

Figure 4.2: A) Original image of pregnant doe as visualized by via transrectal ultrasonography (presumed day 21 of pregnancy). Embryonic sacs were detected at 3-5 o'clock direction to the urinary bladder. B) Labeled image of detected embryonic sacs (arrows).

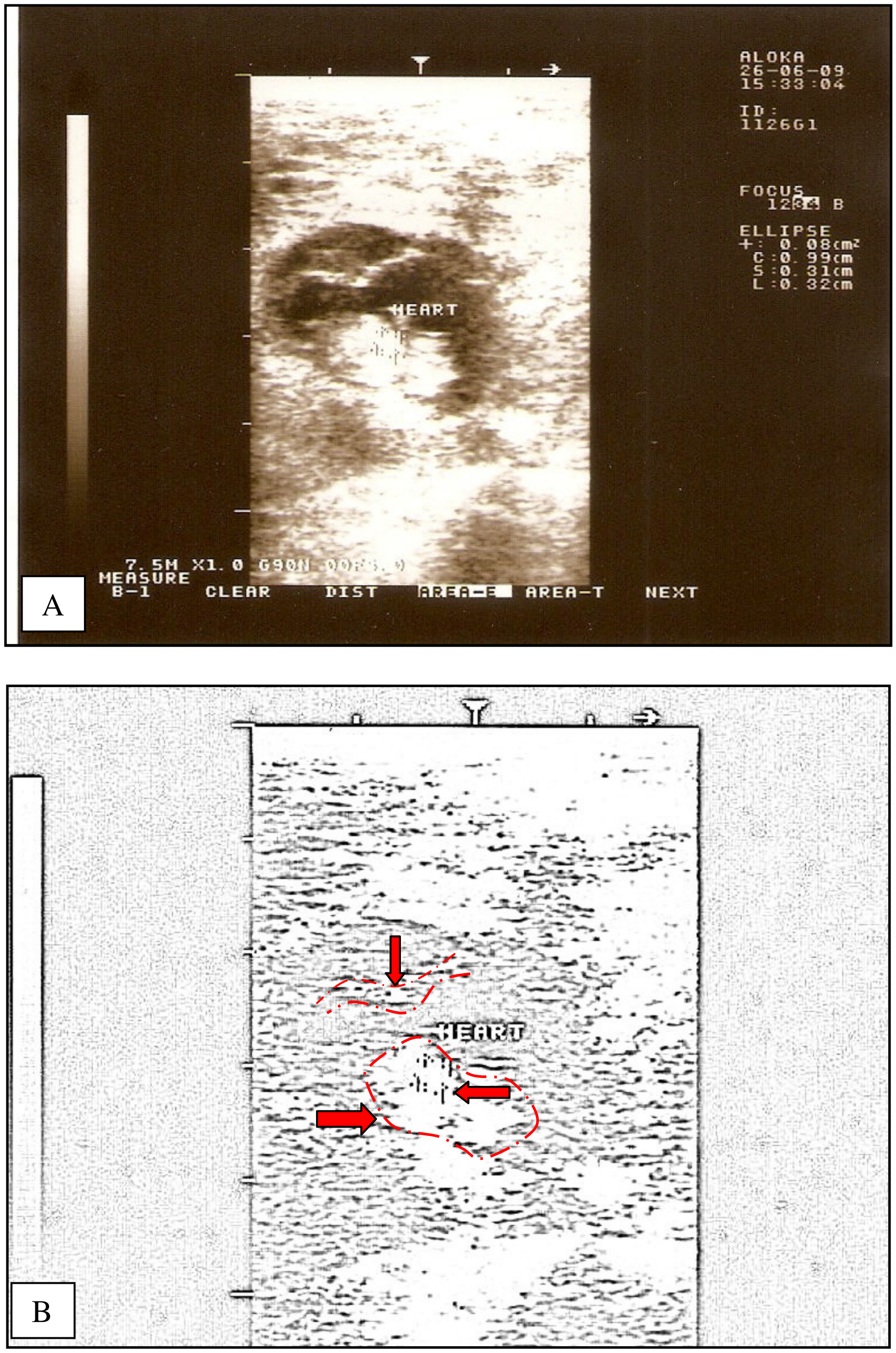

Figure 4.3: A) Original image of pregnant doe as visualized by via transrectal ultrasonography (presumed day 30 of pregnancy). B) Labeled image of detected foetus (arrow: →), foetal heart (arrow: ←) and umbilical cord (arrow: ↓).

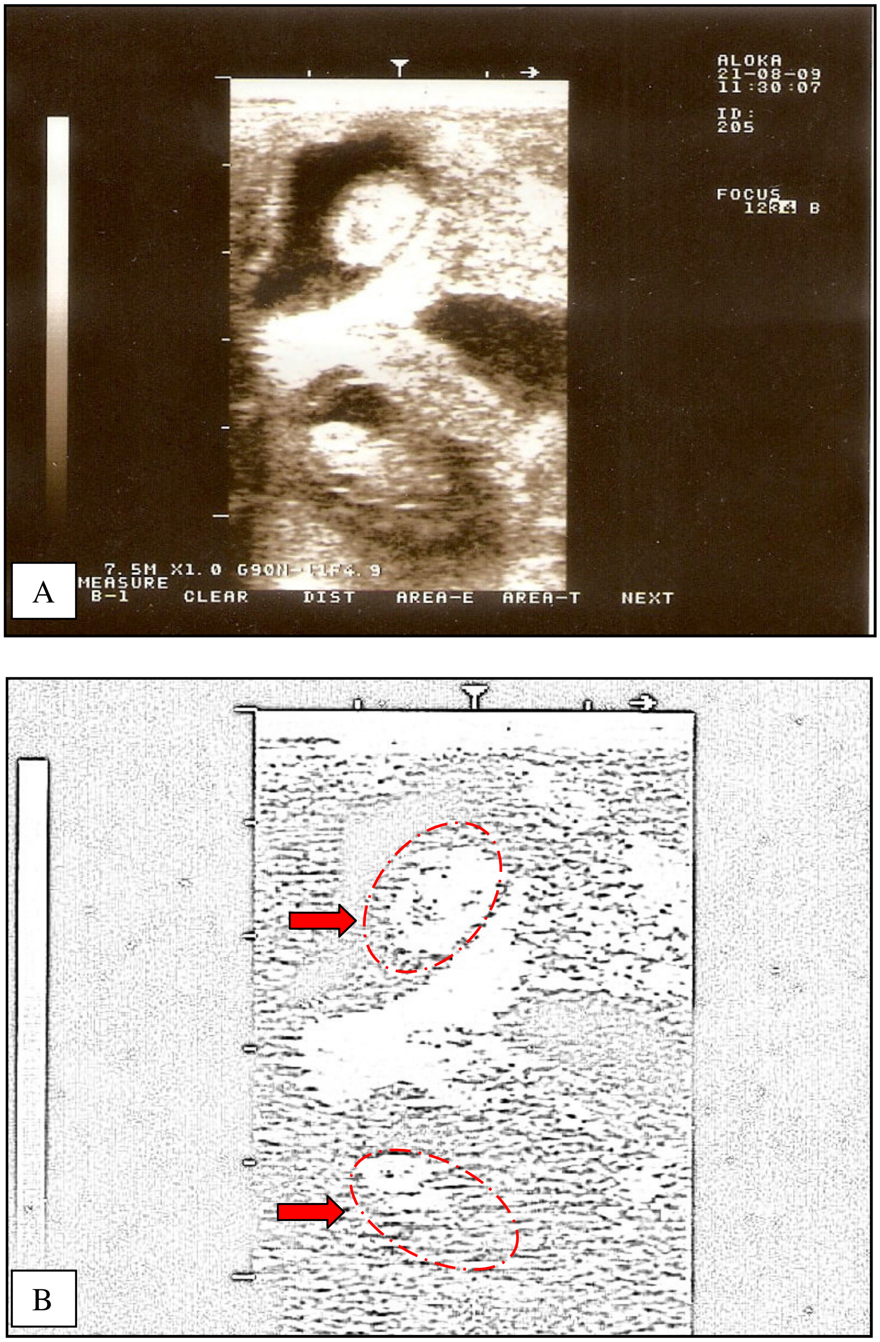

Figure 4.4: A) Original image twin foetuses as visualised by via transrectal ultrasonography (presumed day 30 of pregnancy). B) Labeled image of detected foetuses (arrows).

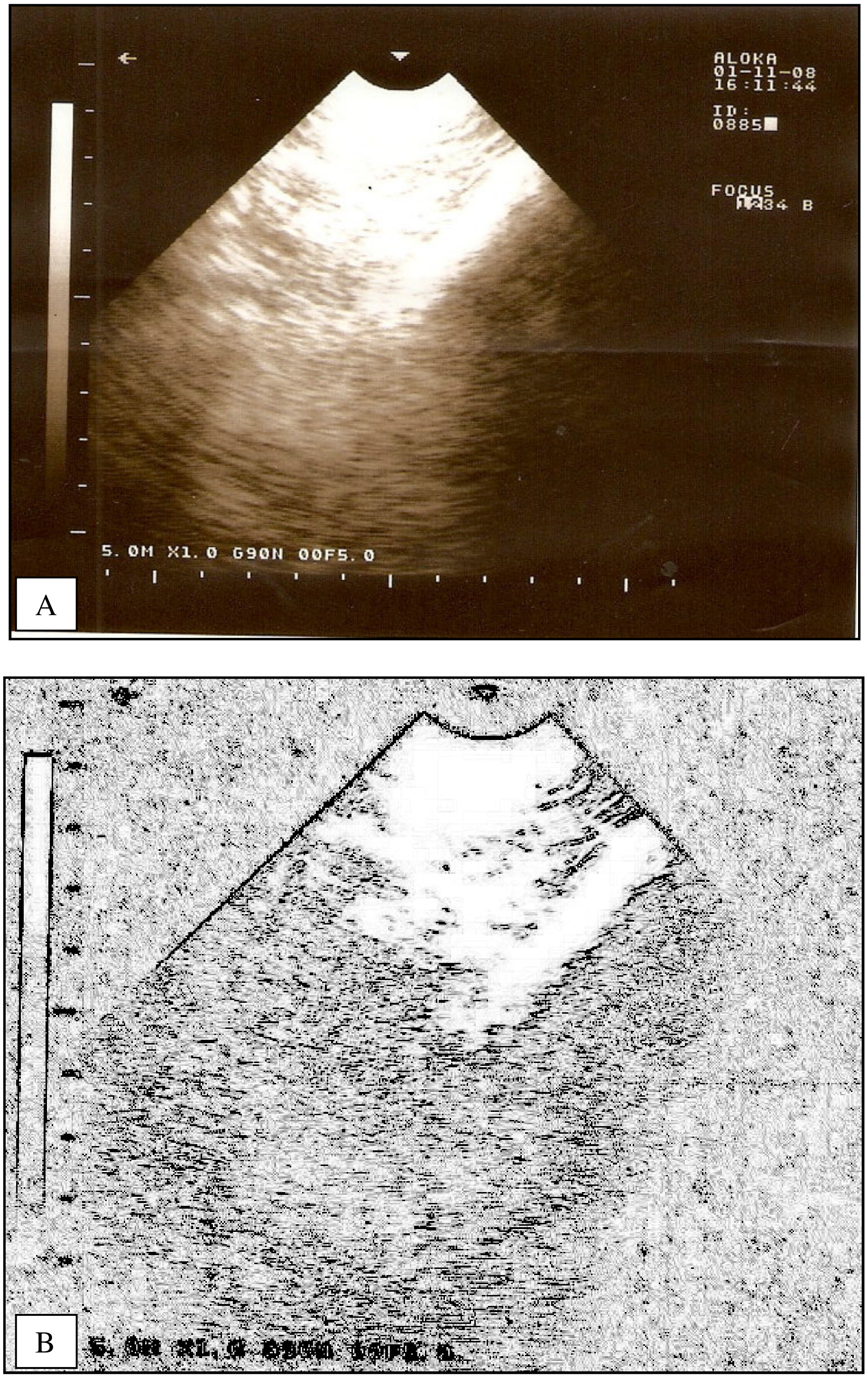

Figure 4.5: A) Image of an open doe via transabdominal probe. No pregnancy-related structure detected. B) Labeled image. Note: the image appeared as blank since no pregnancy-related structure was detected.

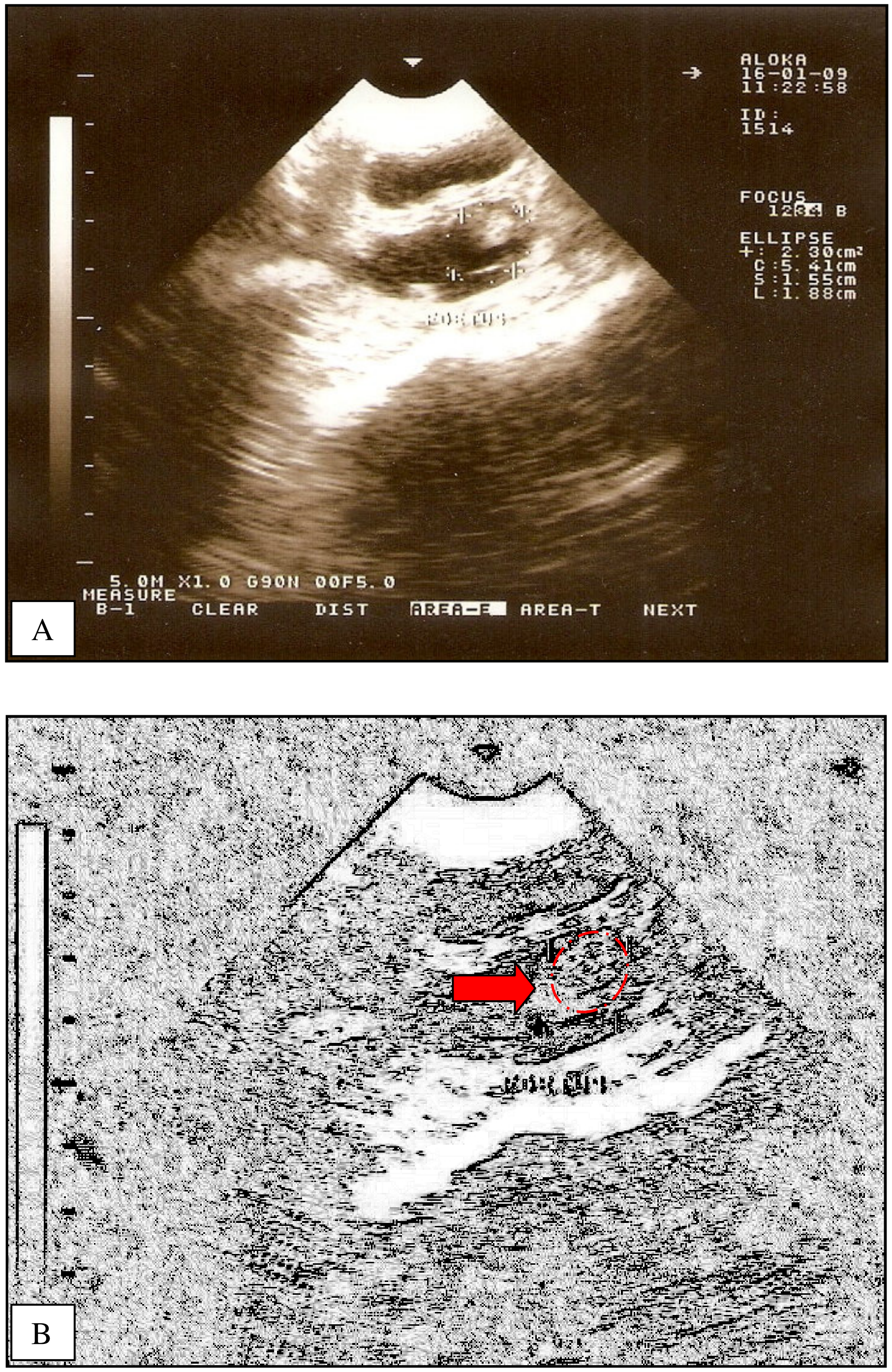

Figure 4.6: A) Original image of foetus as detected using transabdominal probe (presumed day 29 of gestation). B) Labeled image of foetus detected (arrow).

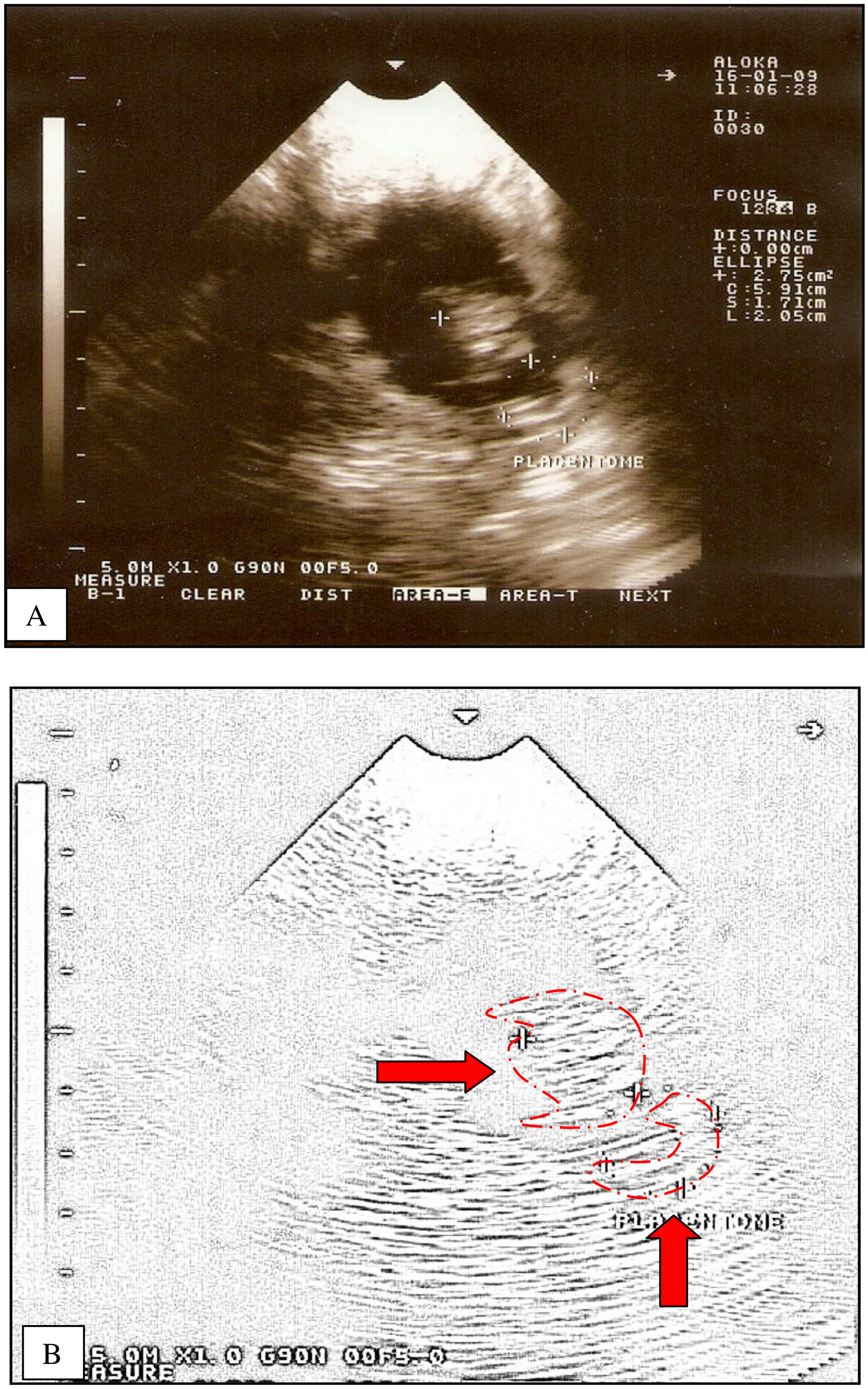

Figure 4.7: A) Original image of foetus as detected using transabdominal transducer (presumed day 52 of gestation). Placentomes were detected around the embryonic fluid enclosing foetus. B) Labeled image of foetus (arrow: →) and placentomes (arrow: ↑).

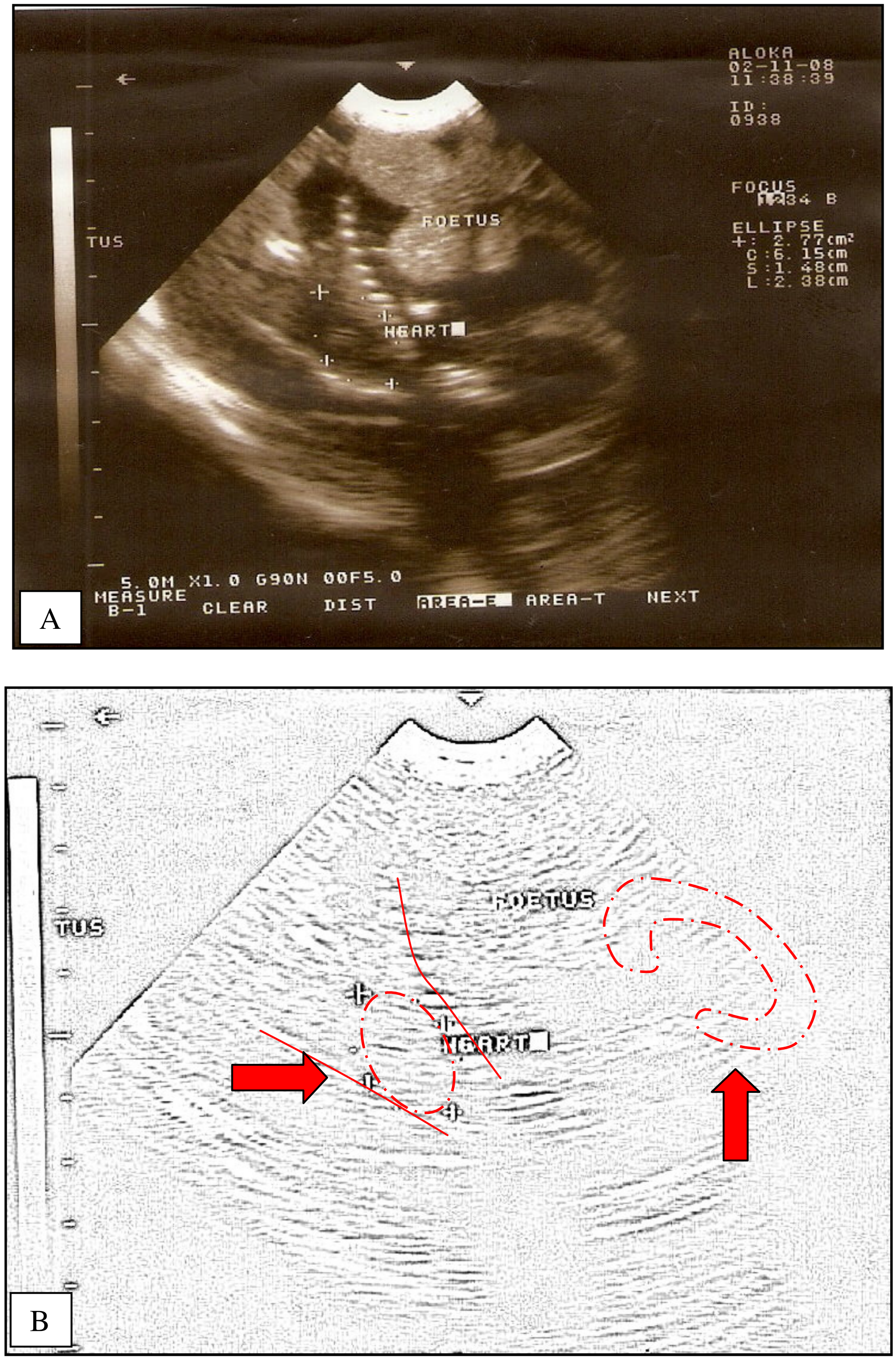

Figure 4.8: A) Original image of foetus as detected using transabdominal probe (presumed day 75 of gestation). Placentomes were detected around the embryonic fluid enclosing foetus and heart detected as grey coloured structure with fast rhythmic beating enclosed within thoracic area. B) Labeled image of foetus (arrow: →) and placentomes (arrow: ↑).

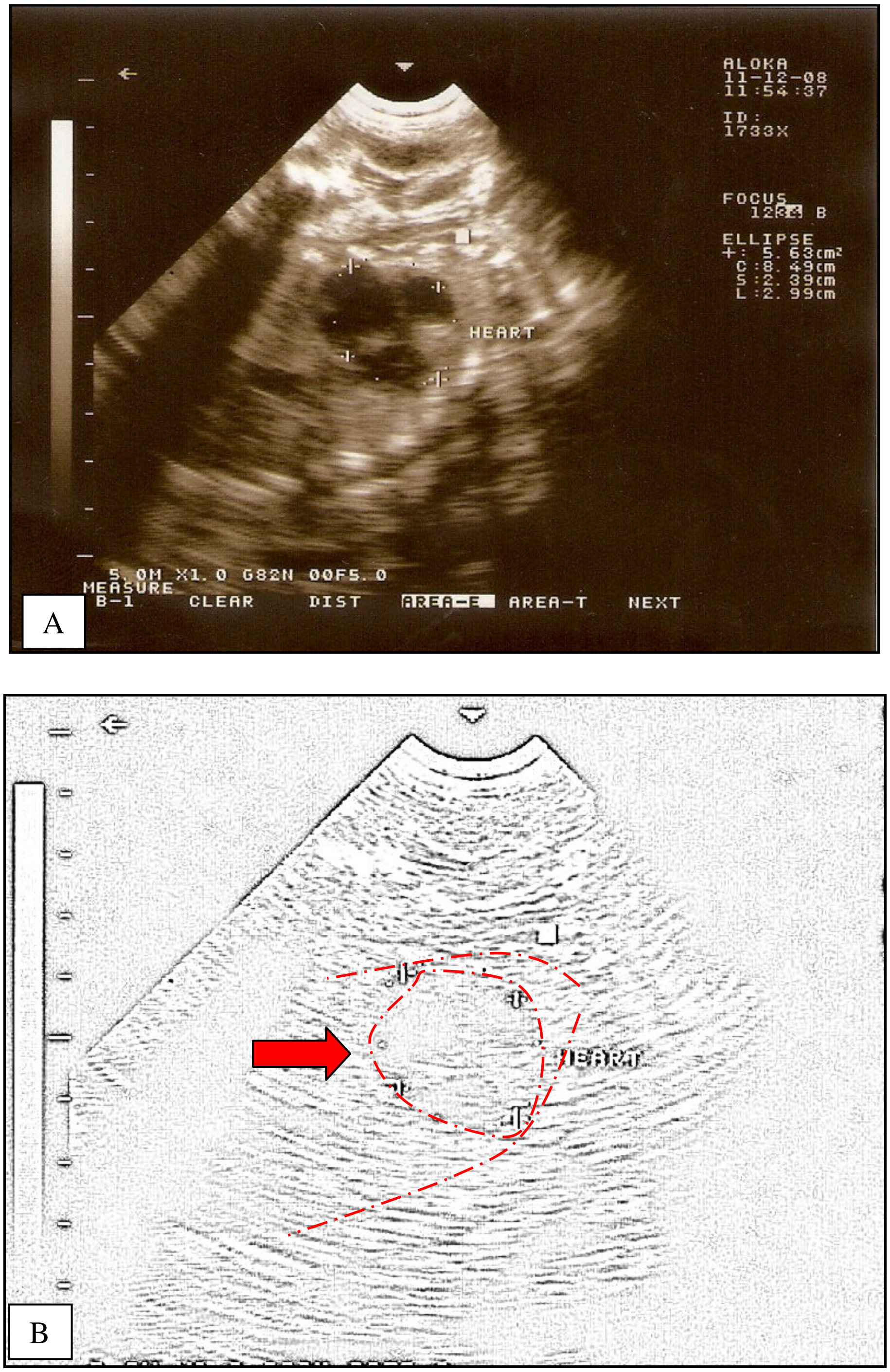

Figure 4.9: Image of foetus as detected using transabdominal probe (presumed day 119 of pregnancy). Heart detected (arrow) as grey-black coloured structure with slower rhythmic beating as compared to earlier stage of pregnancy. A) Original image. B) Labeled image.

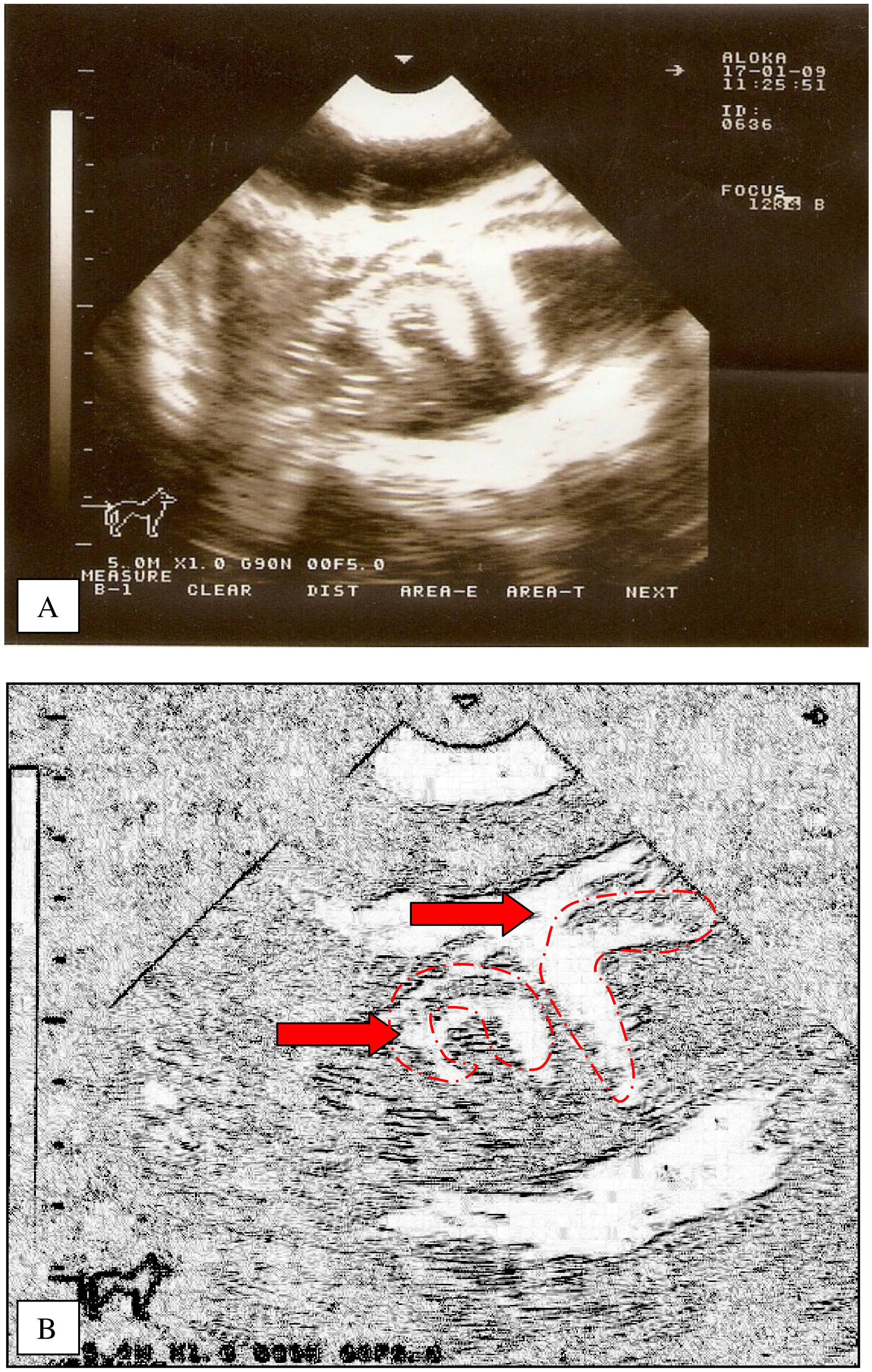

Figure 4.10: A) Original image of placentomes as detected using transabdominal probe (presumed day 147 of pregnancy). B) Labeled image of C-shaped placentomes (arrows).

Table 4.2: Comparison of detectability for transrectal and transabdominal probes throughout gestation

Gestation age classification	Structures	Transrectal probe	Transabdominal probe
Below 30 days	Embryonic sac	+ (day 29)	+ (day 29)
	Foetus	+ (day 29)	+ (day 29)
	Foetal heart	+ (day 29)	+ (day 29)
31-60 days	Embryonic sac	+ (day 45)	+ (day 48)
	Foetus	+ (day 44)	+ (day 52)
	Foetal heart	+ (day 44)	+ (day 52)
	Foetal head	+ (day 45)	+ (day 60)
	Foetal legs	-	+ (day 44)
	Umbilical cord	+ (day 32)	+(day 32)
	Placentome	+ (day 44)	+ (day 44)
61-90 days	Foetus	-	-
	Foetal heart	-	+ (day 75)
	Foetal head	-	+ (day 66)
	Foetal legs	-	-
	Foetal organs	-	+ (day 76)
	Umbilical cord	-	+ (day 76)
	Placentome	+ (day 75)	+ (day 76)
91-120 days	Foetus	-	-
	Foetal heart	-	+ (day 116)
	Foetal head	-	-
	Foetal legs	-	-
	Foetal organs	-	+ (day 101)
	Placentome	+ (day 110)	+ (day 110)
121-147 days	Foetal heart	-	+ (day 144)
	Foetal organs	-	+ (day 144)
	Placentome	-	+ (day 144)

Tables 4.2 summarise and compare the detectability for transrectal and transabdominal probes throughout pregnancy period. This table is the summarised version for Table 4.1; it classifies the detectability of specific pregnancy-related structures throughout gestational period with regards to different kind of probes. For gestational age below 30 days, embryonic sacs, foetus and foetal heart were observed as early as day 29 of pregnancy via both probes. The structures appeared to be bigger on screen when scanning done using transrectal probe as compared to transabdominal probes. However, using

electronic calipers, the measurement of images from both probes appeared to be not significantly different. Embryonic sacs appeared as black non-echogenic area. Meanwhile, foetus and foetal heart appeared to be echodense (white in colour). Active foetal movement was not observed.

In the following gestational stage, days 31-60 of gestation, both probes were recorded to be able to observe 6 pregnancy-related structures; embryonic sacs (transrectal:day 45; transabdominal: day 48), foetus (transrectal:day 44; transabdominal: day 52), foetal heart (transrectal:day 44; transabdominal: day 52), foetal head (transrectal:day 45; transabdominal: day 60), umbilical cord (transrectal:day 32; transabdominal: day 32) and placentomes (transrectal:day 44; transabdominal: day 44). Transrectal probe was not able to detect foetal leg but transabdominal probe managed to detect it on day 44 of gestation. At this stage of pregnancy, both probes proved to be able to provide approximately equal detection ability.

Foetus was observed to undergo rapid increase in size during the third month of gestation (days 61-90). Foetus was too big to be visualised on the screen for both probes. Besides that, transrectal probe only can be used to detect placentomes (day 75) using conventional procedure. On the other hand, transabdominal probe seems to be the more reliable method since it can visualise almost all pregnancy-related structure except foetal body as a whole and foetal legs. Foetal organs such as stomach were observed on day 76 of gestation. In addition to that, pregnancy-related structures could be detected easily via transabdominal probe at this stage of pregnancy.

Foetal structure underwent more rapid increase during days 91-120 of gestation. Bony structures ossified and increase in size. Owing to that, only a few pregnancy-related structures could be observed at this stage. Placentome was found to be difficult to be detected with transrectal probe, but it was detected when the abdominal part of the doe was lifted a bit when ultrasound scanning was conducted. Transabdominal probe was observed to detect foetal heart (day 116), foetal organs (day 101) and placentome (day 110). Other pregnancy-related structures were found to be difficult to be recorded at this stage.

At the final stage of pregnancy, days 121-147 of gestation, transrectal probe was found not to be able to detect any pregnancy-related structures. Meanwhile, transabdominal probe was able to detect foetal heart (day 144), foetal organs (day 144) and placentome (day 144) at this stage. All structures (foetal heart, foetal organs and placentomes) appeared to be less echodense at this final stage of pregnancy.

Table 4.3: Range of pregnancy-related structures detection throughout pregnancy using both transrectal and transabdominal probes

Structure	Transrectal probe	Transabdominal probe
	Range detected	Range detected
Embryonic sacs	Days 29 - 45	Days 29 - 48
Whole foetal body	Days 29 - 44	Days 29 - 52
Foetal heart	Days 29 - 44	Days 29 - 144
Foetal head	Days 45 - 60	Days 60 - 66
Foetal organs	-	Days 76 - 144
Placentome	Days 44 - 110	Days 44 - 144

In this experiment, one of the main objectives was to identify pregnancy-related structures that could be measured the most throughout pregnancy and their development. The finding will be used in Experiments 3 and 4 for more specific observation and measurements. The results from this experiment suggested that there were six pregnancy-related structures which fulfill the criteria. The structures are embryonic sacs, foetal body, foetal heart, foetal head, foetal organs and placentomes.

Table 4.3 describes the range of pregnancy-related structures detection throughout pregnancy using both transrectal and transabdominal probes. Embryonic sacs could be detected on days 29-45 and days 29-48 using transrectal and transabdominal probes respectively. Meanwhile, whole foetal body was detected on days 29-44 and days 29-52 using transrectal and transabdominal probes. Foetal heart appeared to be the easiest pregnancy-related structure to be detected during pregnancy. Transrectal and transabdominal probes were able to detect it on days 29-44 and days 29-144 of gestation, respectively. From this finding, foetal heart appeared to be a potential structure to be specifically studied in Experiments 3 and 4.

Transrectal and transabdominal probes were able to detect it on days 29-44 and days 29-144 of gestation, respectively. Foetal head was detected on days 45-60 of gestation using transrectal probe and days 60-66 of gestation via transabdominal probe. Foetal organs only were detected using transabdominal probe on days 76-144 of gestation. Transrectal and transabdominal probes detected placentomes on days 44-110 of gestation and days 44-144 of gestation, respectively. With relation to their detection, placentomes also showed changes in their echogenicity.

4.2 GESTATIONAL AGE ESTIMATION BASED ON ANALYSIS OF PREGNANCY-RELATED STRUCTURES IN DIFFERENT BREEDS OF GOATS BASED ON CHARACTERISTICS DERIVED FROM EXPERIMENT 1 (EXPERIMENT 2)

Table 4.4: Analysis of images obtained from confirmed pregnant does

Breed	Tag	Date of scanning	Structures observed	Estimated age	Single/ Multiple pregnancy*	Results**
Boer cossbred	B0043	12/9/2009	• Degraded placentome • Black-grey colour heart	4 months	Single	+
	B0045	12/9/2009	• Degraded placentome • Black-grey colour heart	4 months	Single	+
	B0046	12/9/2009	• Degraded placentome • Black-grey colour heart	4 months	Single	+
	B0051	12/9/2009	• Very big black colour heart • Degraded placentome	4 ½ months	Twin	-
	B0054	12/9/2009	• Degraded placentome • Black-grey colour heart	4 months	Single	+
	B0061	12/9/2009	• Grey colour heart • Early degraded placentome	3 months	Triplet	-
	B0067	12/9/2009	• Degraded placentome • Black-grey colour heart.	4 months	Twin	+

(continued)

(continued)

Breed	Tag	Date of scanning	Structures observed	Estimated age	Single/ Multiple pregnancy*	Results**
Saanen	S8	4/9/2009	• Non-echogenic area	1 month	-	-
	S26	4/9/2009	• Degraded placentome • Foetus was hard to be visualized on the ultrasound screen.	4 ½ months	Single	+
	S29	4/9/2009	• Degraded placentome • Black-grey colour heart	4 months	Single	+
	S30	4/9/2009	• Degraded placentome • Black-grey colour heart.	4 ½ months	Single	+
	S36	4/9/2009	• Placentome was hard to be visualized but the size was not big	4 months	Single	+
Jermasia	J0076	19/11/2009	• Early phase of degraded placentome • Grey colour heart with medium size.	3 months	Single	+
	J0096	22/10/2009	• White-grey colour heart with small size • Abdominal part of foetus still visible on ultrasound screen • Echo-dense (white colour)	2 months	Single (Abortion)	+

(continued)

(continued)

Breed	Tag	Date of scanning	Structures observed	Estimated age	Single/ Multiple pregnancy*	Results**
Jermasia			placentome			
	HG3	22/10/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Twin	+
	HG384	19/11/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Twin	+
	HF284	19/11/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Single	+
	HE605	18/7/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Twin	+
	J0046	18/7/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Twin	+
	J0113	1/10/2009	• No non-echogenic other than bladder structure detected.	Not pregnant	Single (Abortion)	-

(continued)

(continued)

Breed	Tag	Date of scanning	Structures observed	Estimated age	Single/ Multiple pregnancy*	Results**
Katjang	K0035	9/3/2009	• White-grey colour heart with small size • Abdominal part of foetus still visible on ultrasound screen • Echo-dense (white coloured) placentome.	2 ½ months	Single	+
	K0039	25/1/2009	• Early phase of degraded placentome • Grey colour heart with medium size	3 months	Single	+
	K0019	14/2/2009	• White-grey colour heart with small size • Abdominal part of foetus still visible on ultrasound screen • Echo-dense placentome	2 ½ months	Single	+

(continued)

(continued)

Breed	Tag	Date of scanning	Structures observed	Estimated age	Single/ Multiple pregnancy*	Results**
Katjang	K0047	8/3/2009	• White-grey colour heart with with small size • Foetal organ still visible on ultrasound screen	2 months	Single	+
	K0063	9/3/2009	• Small foetus • Clump of sac	1 month	Twin	-
	K0064	18/12/2008	• Early phase of degraded placentome • Grey colour heart with medium size.	3 ½ months	Single	-

*Single or twins was determined after delivery.
**Results were considered as positive (+) if the doe delivered within less than one month difference from estimated time and negative (-) if the doe delivered within more than one month difference from the estimated time.

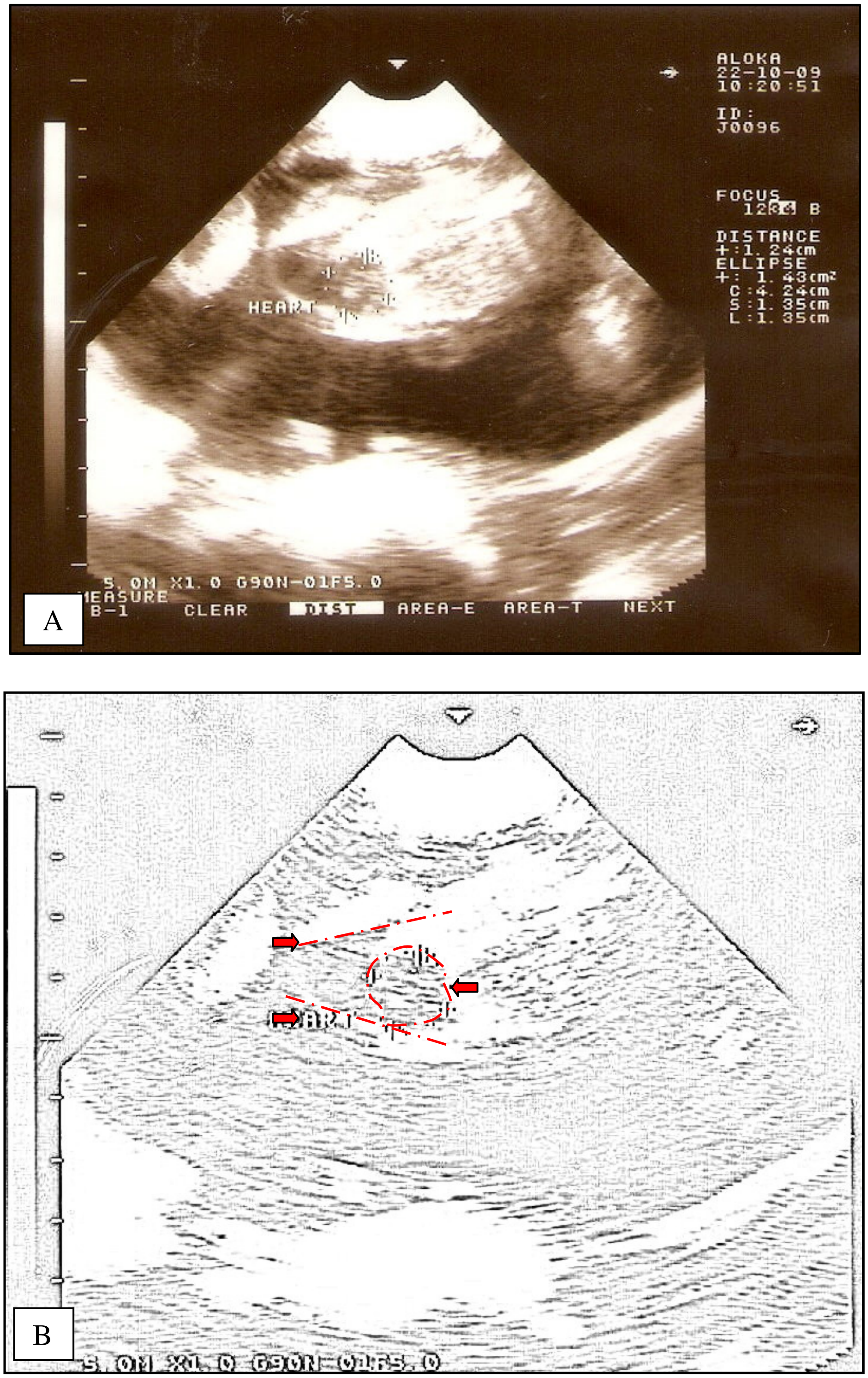

Figure 4.11: Foetal heart (arrow: ←) and V-shaped thoracic area (arrows: →) at gestational age of approximately 60 days. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the actual mating date.

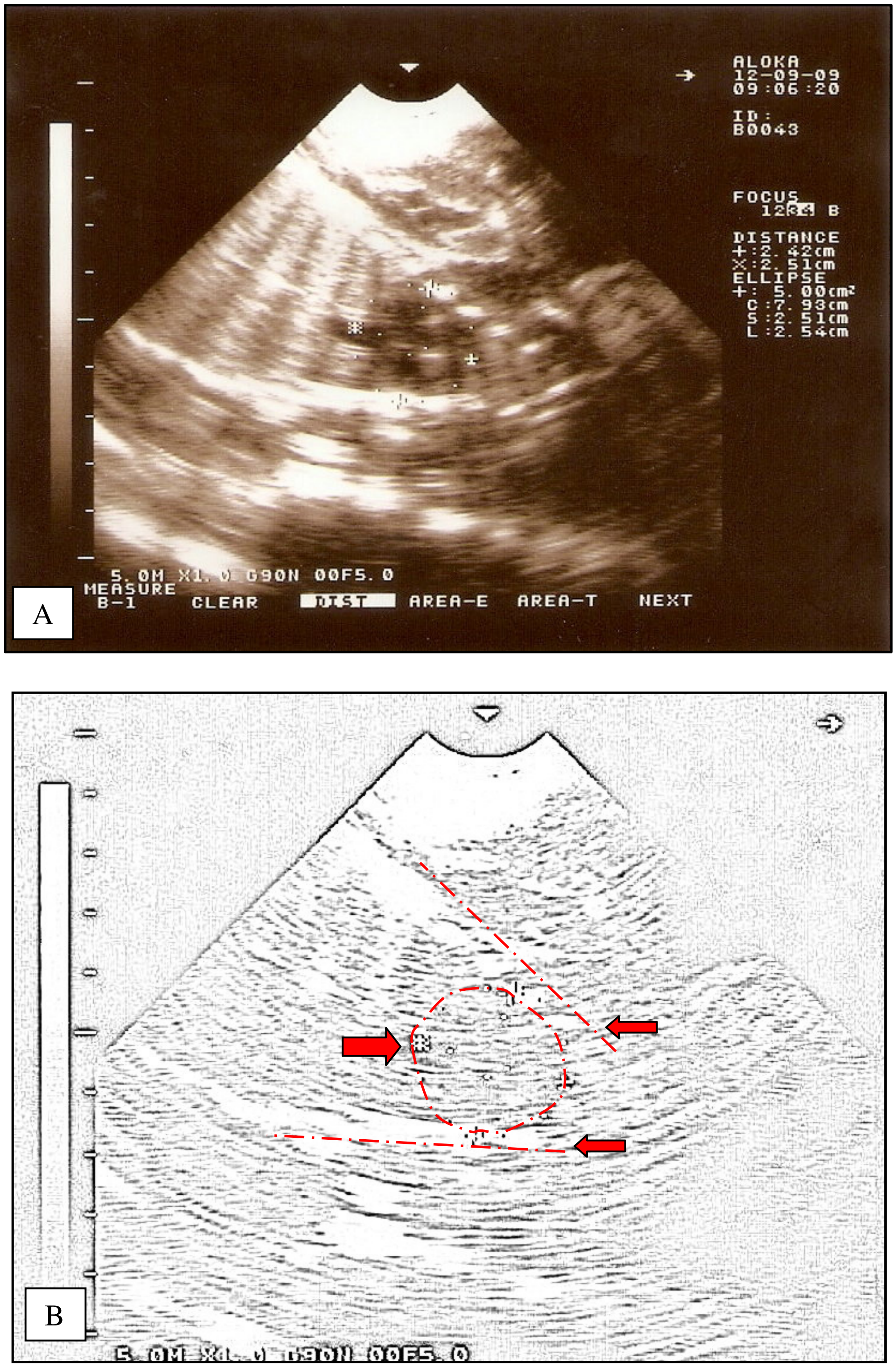

Figure 4.12: Foetal heart (arrow: →) V-shaped thoracic area (arrows: ←) at gestational age of approximately 120 days. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the delivery date.

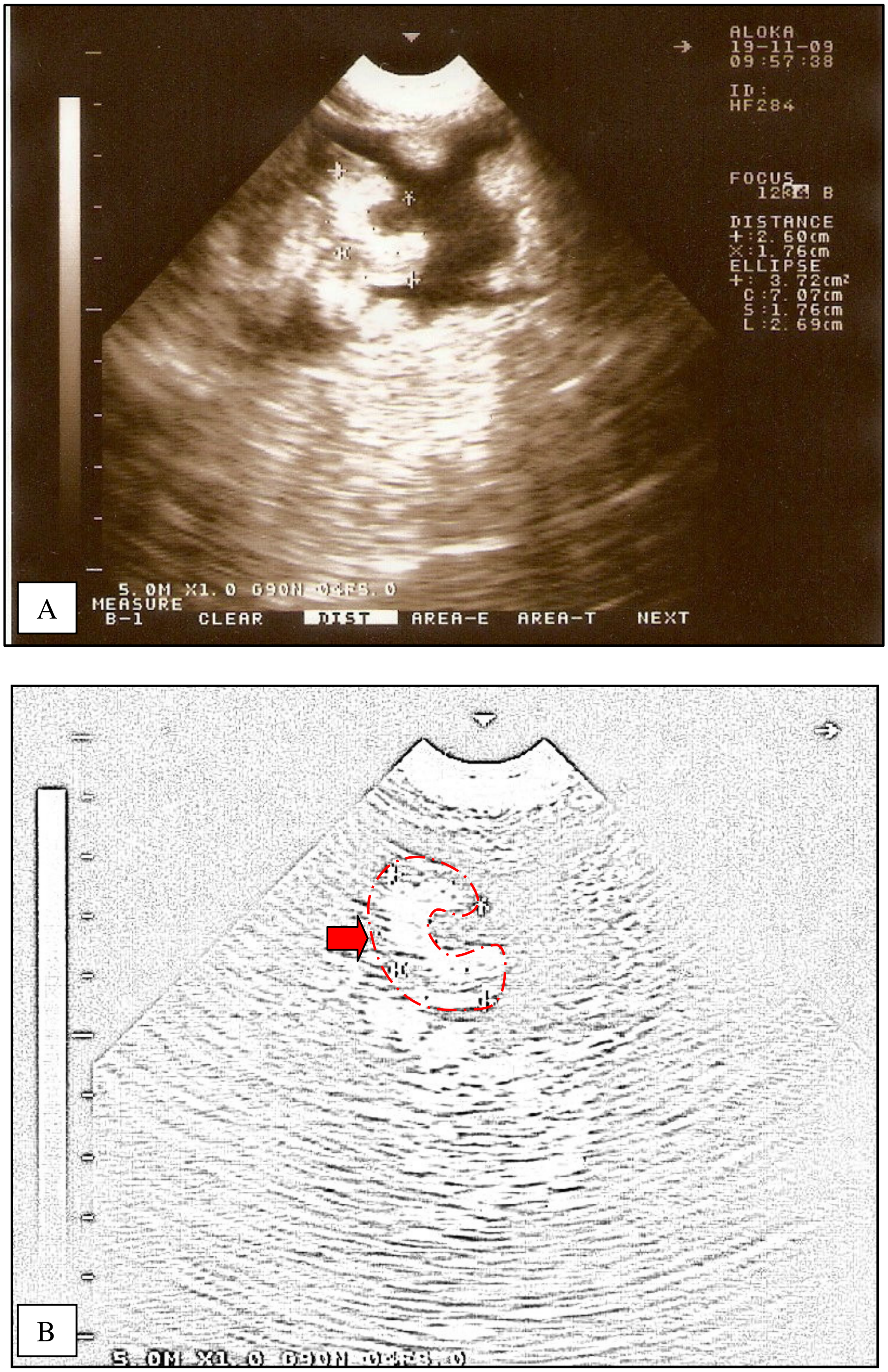

Figure 4.13: Placentome (arrow) at gestational age of approximately 60 days appear as echogenic structure. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the actual mating date.

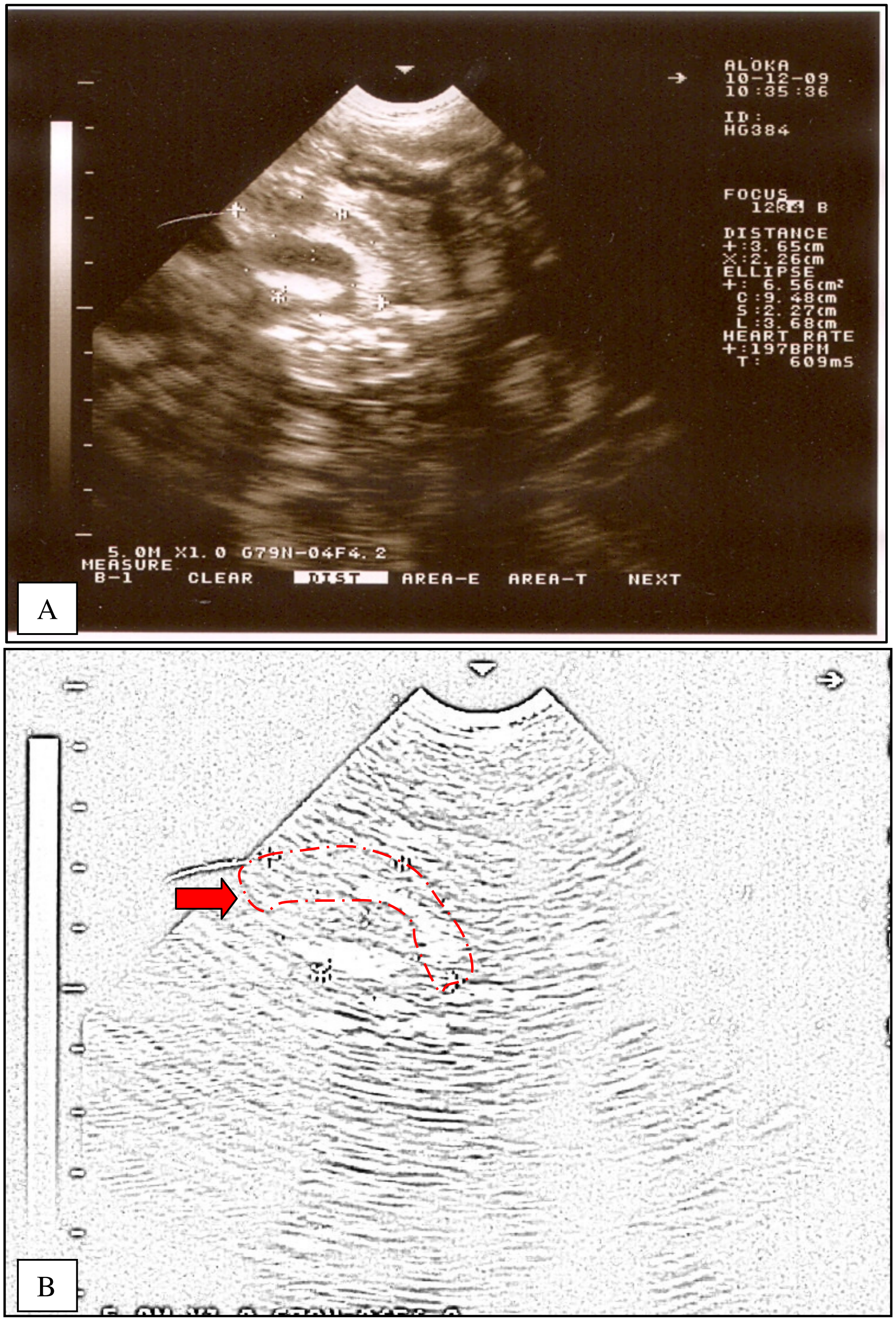

Figure 4.14: Placentome (arrow) at gestational age of approximately 120 days appears as less echogenic structure (grey colour instead of white). A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the delivery date.

Table 4.5: Summary of pregnancy detection and gestational age accuracy estimation based on criteria derived from Experiment 1

Goat breed	No. of correctly diagnosed positive for pregnancy (%)	No. of correctly diagnosed negative for pregnancy (%)	Correct kidding time estimation for single pregnancy (%)	Correct kidding time estimation for multiple pregnancies (%)	Overall correct kidding time estimation (%)
Boer-crossbred	7/7 (100%)	43/43 (100%)	4/4 (100%)	1/3 (33.33 %)	5/7 (71.43 %)
Saanen	4/5 (80%)	-	4/4 (100%)	-	4/4 (100%)
Jermasia	7/7 (100%)	35/36 (97.22%)	3/3 (100%)	4/4 (100%)	7/7 (100%)
Katjang	7/7 (100%)	14/14 (100%)	4/6 (66.67%)	1/1 (100%)	5/7 (71.43%)

From the results obtained (Tables 4.4 and 4.5), in Boer-crossbred does, the accuracy of positive pregnancy diagnosis was 100%, negative pregnancy diagnosis was 100%, while overall accurate gestation age determination was 71.43%, with 100% correct gestation age estimation for single pregnancy and 33.33% for multiple pregnancies (twin and triplet). Meanwhile, in Jermasia does, the accuracies were 100, 97.22, 100, 100, and 100%, respectively. In Katjang does, the accuracies recorded were 100, 100, 71.43, 66.6 and 100%, respectively. In Saanen does, the accuracy of positive pregnancy diagnosis was 80%, overall accurate gestation age was 80%, and no multiple pregnancies was recorded. The size of foetal parts was observed as variable, depending on foetal number. However, the pattern of echogenicity changes in pregnancy-related structures was almost similar among the breeds studied (Table 4.4).

4.3 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSRECTAL PROBE (EXPERIMENT 3)

Ultrasound scanning was conducted on the does underwent breeding programme starting from day 28 (week 4) post-mating. Following that, the scanning was conducted on weekly basis until week 21 of gestation. Table 4.6 describes the details of pregnancy-related structures detected throughout pregnancy using transrectal probe. Figures 4.16 - 4.21 show the selected images recorded using transrectal probe at certain age of pregnancy. The earliest pregnancy-related structures were embryonic fluids, foetus (Figures 4.15 and 4.16) and foetal heart on week 4 of gestation. Embryonic vesicles visualised as a group of non-echogenic round or oval shape structure detected at the direction of 12 - 3 o'clock from urinary bladder and measurable. Meanwhile, no active movement of foetus was detected at this age, but the crown-rump length could be measured. On the other hand, foetal heart appeared as small-strong beating white structure with undefined shape. Hence, foetal heart area could not be measured at this age.

On the following week (week 5 of gestation), all three structures earlier detected plus umbilical cord were visualised. Umbilical cord appeared as echogenic thread-like structures which connect foetus and the border of embryonic sac. Embryonic vesicles were detected at the same position as in earlier week. One interesting observation was that the first active movement of foetus was detected at this gestational age. The shape of foetal heart was still undefined and the measurement of foetal heart area could not be done. At the age of week 6 of gestation, first body differentiation was observed; foetal head, trunk and legs were clearly distinguished from each other (Figure 4.17). The size of embryonic sac increased but still was visible as a whole on screen. Another additional pregnancy-related structure first detected at week 6 of gestation was placentome which appeared as small C-shaped echodense structure.

During the subsequent week (week 7 of gestation), the size of embryonic sacs and foetus was greatly increased. Embryonic sacs could not be visualised on the viewing screen, and it became very difficult to get full view of foetus on the screen. On the other hand, the size and number of placentomes increased and the placentomes were detected as C- or O-shaped echodence structures. The differentiated body parts increased in size (Figure 4.18). Foetal heart, unfortunately, still did not show defined shape. Starting from week 8 of gestation, foetus could not be visualised as a whole on the viewing screen. Only certain part of foetal body could be recorded at certain scanning session. Placentomes increased in size and numbers during week 8 of gestation. At this gestational age, foetal heart appeared as a small strong beating white-grey structure with defined shape, thereby facilitating foetal heart area measurement (Figure 4.19).

During weeks 9-10 of gestation, foetus underwent more development especially increase in pregnancy-related structures size (Figure 4.20). Foetal heart could be detected easily on week 9, but difficult at week 10 of gestation. Only 1-2 foetal legs could be viewed on the screen for both foetal ages. Starting from week 11 of gestation, it was extremely difficult to detect pregnancy-related structures, except for placentomes (Figure 4.21). As pregnancy progressed, placentomes changed their echogenicity from echodense (detected as white in colour) to less echodense (detected as grey in colour) (Figure 4.21).

Table 4.6: Details of pregnancy-related structures detected throughout pregnancy using transrectal probe

Gestational age (weeks)	Structures detected	Description	Measurability
4	Embryonic fluid	Embryonic vesicles visualised as a group of non-echogenic round or oval shape structure detected at the direction of 12-3 o'clock from urinary bladder (Figure 4.16)	Yes
	Foetus	Foetuses were detected and measureable. No active movement of foetus was observed. However, foetal counting could be done (Figure 4.16)	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined	No
5	Embryonic fluid	Embryonic vesicles visualised as a group of round or oval shape structure detected at the direction of 12-3 o'clock from urinary bladder	Yes
	Foetus	First active movement of foetus was observed. Foetal counting could be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined	No
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structures which connect foetus and the border of embryonic sac	No
6	Embryonic fluid	Size of embryonic sac increased. However, it still is visible as a whole on screen	Yes
	Foetus	Foetus moves actively. Foetal counting could be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined (Figure 4.17)	Yes
	Placentome	Appeared as small C-shaped structure	Yes

(continued)

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Gestational age (weeks)	Structures detected	Description	Measurability
6	Umbilical cord	Appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac (Figure 4.17)	No
	Foetal head	Started to be differentiated from the foetal trunk. Shape of foetal head was definable at this gestational stage (Figure 4.17)	Yes
	Foetal leg	Both rear and hind leg of foetus could be detected and measured	Yes
7	Embryonic fluid	Size of embryonic sac increased. Embryonic sac cannot be visualised as a whole on screen	No
	Foetus	Moved actively and increased in size. It becomes more difficult to visualize foetus as a whole on the screen (Figure 4.18)	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shape structure	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac	No
	Foetal head	Size of foetal head increased (Figure 4.18)	Yes
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively (kicking) (Figure 4.18)	Yes
8	Embryonic fluid	Size of embryonic sac increased. Embryonic sac cannot be visualised as a whole on screen	No
	Foetus	Foetus moves actively and increased in size. It cannot be visualised as a whole on the screen. Foetal counting was difficult to be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white-grey structure. The shape of the heart was definable (Figure 4.19)	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shape structure	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac	No

(continued)

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Gestational age (weeks)	Structures detected	Description	Measurability
8	Foetal head	Size of foetal head increased. One foetal head will fit the whole viewing screen	Yes
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively	Yes
9	Embryonic fluid	Embryonic fluid became too big to be viewed on the screen. Only some part of it can be visualised	No
	Foetus	Foetus increased in size and foetal organ can be viewed. Foetal counting was very difficult to be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white-grey structure. The shape of the heart was definable and measurable	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shape structure	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac	No
	Foetal head	Size of foetal head increased. One foetal head will fit the whole viewing screen. BPD measurement was almost impossible	No
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively. Only 1-2 legs can be viewed at one time	No
10	Foetus	Foetus was very difficult to be viewed. Foetal counting was extremely difficult to be done	No
	Foetal heart	Foetal heart appeared as medium-sized strong beating white-grey structure. The shape of the heart was definable and measurable. However, it was difficult to detect the heart at this stage	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shape structure. Placentome can be detected easily (Figure 4.20)	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac. However, umbilical cord became more difficult to be viewed	No

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Gestational age (weeks)	Structures detected	Description	Measurability
10	Foetal head	Size of foetal head increased. One foetal head will fit the whole viewing screen. BPD measurement was almost impossible	No
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively. Only 1-2 legs can be viewed at one time	No
11	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shape structure (Figure 4.21). Placentome can be detected easily	Yes
12	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome can be detected easily	Yes
13	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
14	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
15	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
16	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
17	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
18	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
19	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes

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Gestational age (weeks)	Structures detected	Description	Measurability
20	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes
21	Placentome	Size and number of placentome increased. Placentome appeared as less echogenic (grey-coloured) C-shape structure. Placentome was difficult to be detected	Yes

Table 4.7: Measurability of pregnancy-related structures throughout pregnancy via transrectal procedure

Pregnancy age (days)	Pregnancy-related structures													
	Embryonic sacs		Amniotic fluid		Crown rump length		Heart size		Heartbeat		Foetal-head diameter		Placentome diameter	
	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA
21-28	+	+	+	+	+	+	-	-	-	-	-	-	-	-
29-35	+	+	+	+	+	+	+	-	+	-	-	-	-	-
36-42	+	+	+	+	+	+	+	-	+	-	+	+	+	+
43-49	-	-	-	-	-	-	+	-	+	-	+	+	+	+
50-56	-	-	-	-	-	-	+	+	+	+	+	+	+	+
57-63	-	-	-	-	-	-	+	+	+	+	+	+	+	+
64-70	-	-	-	-	-	-	+	+	+	+	+	+	+	+
71-77	-	-	-	-	-	-	-	-	-	-	-	-	+	+
78-84	-	-	-	-	-	-	-	-	-	-	-	-	+	+
85-91	-	-	-	-	-	-	-	-	-	-	-	-	+	+
92-98	-	-	-	-	-	-	-	-	-	-	-	-	+	+
99-105	-	-	-	-	-	-	-	-	-	-	-	-	+	+
106-112	-	-	-	-	-	-	-	-	-	-	-	-	+	+
113-119	-	-	-	-	-	-	-	-	-	-	-	-	+	+
120-126	-	-	-	-	-	-	-	-	-	-	-	-	+	+
127-133	-	-	-	-	-	-	-	-	-	-	-	-	+	+
134-140	-	-	-	-	-	-	-	-	-	-	-	-	+	+
141-147	-	-	-	-	-	-	-	-	-	-	-	-	+	+

DA: Detectable

MA: Measurable

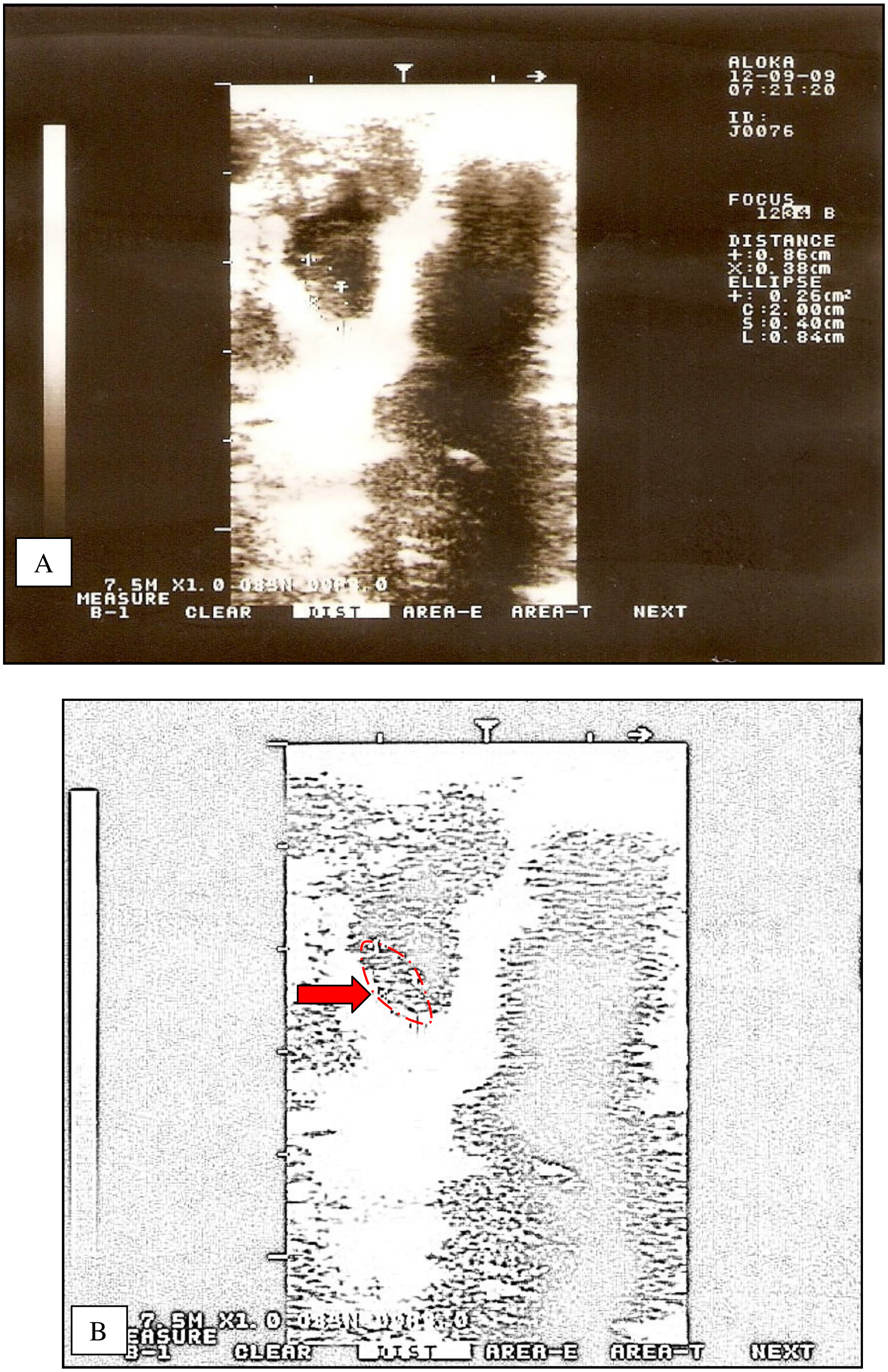

Figure 4.15: Single foetus at day 23 (week 4) of pregnancy. A) Original image detected foetus (arrow). B) Labeled image of the detected foetus (arrow).

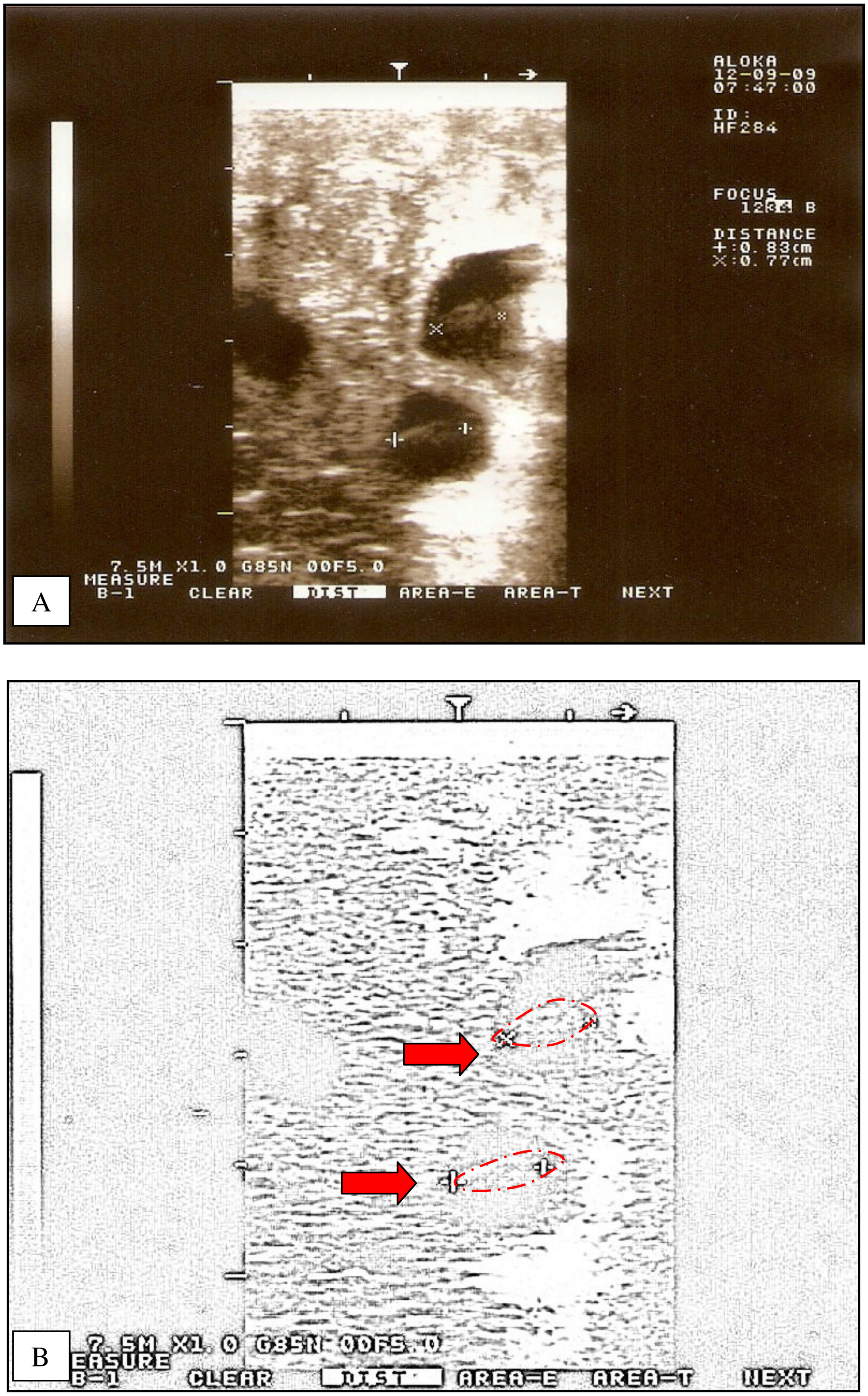

Figure 4.16: Twin foetuses as observed on day-23 (week 4) of pregnancy. A) Original image of the detected foetuses. B) Labeled image of the detected foetuses (arrows).

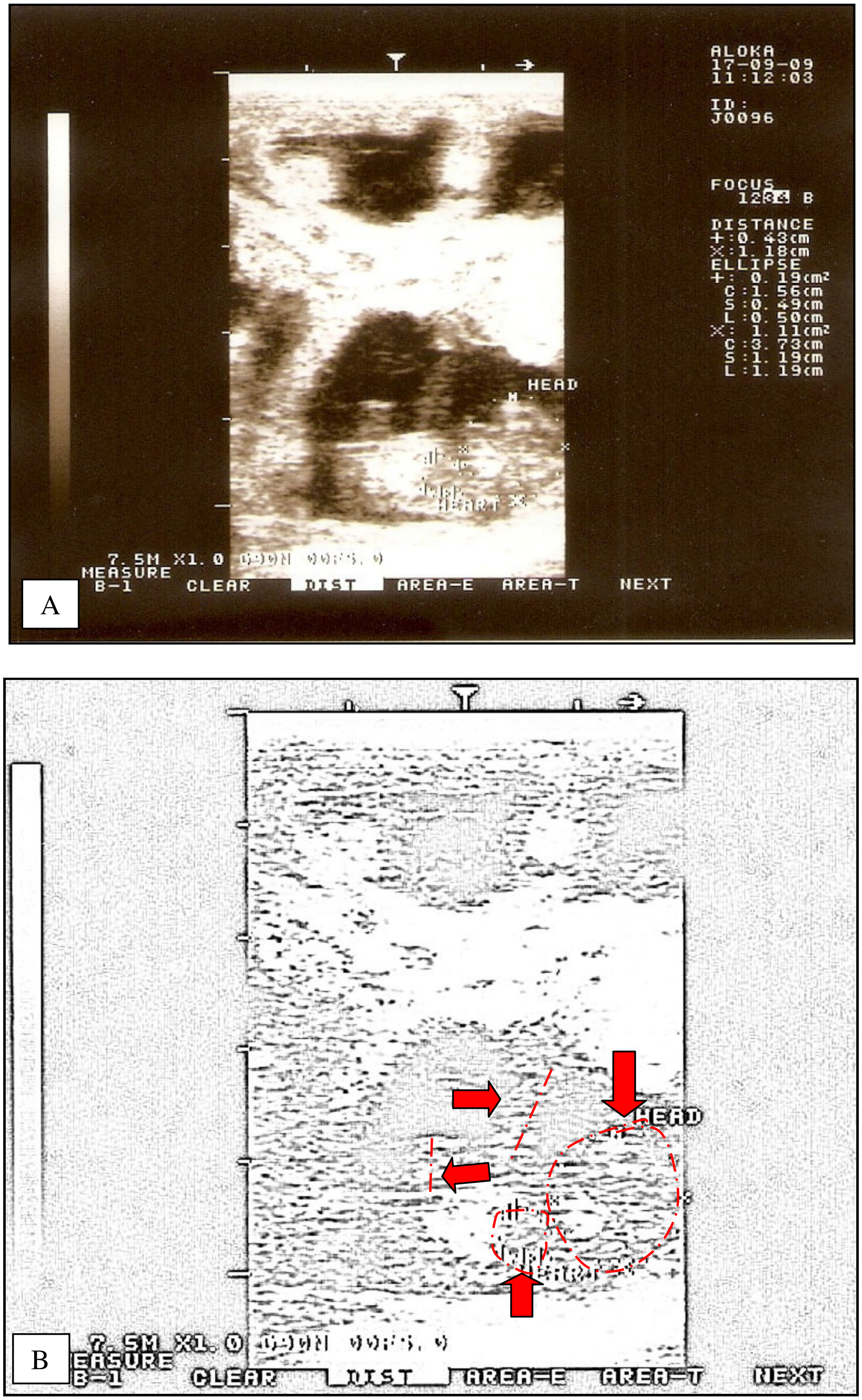

Figure 4.17: Foetus as observed on day-38 (week 6) of pregnancy. A) Original image. B) Labeled image: foetal head (arrow: ↓), umbilical cord (arrow: →), leg (arrow: ←) and heart (arrow: ↑) can be detected.

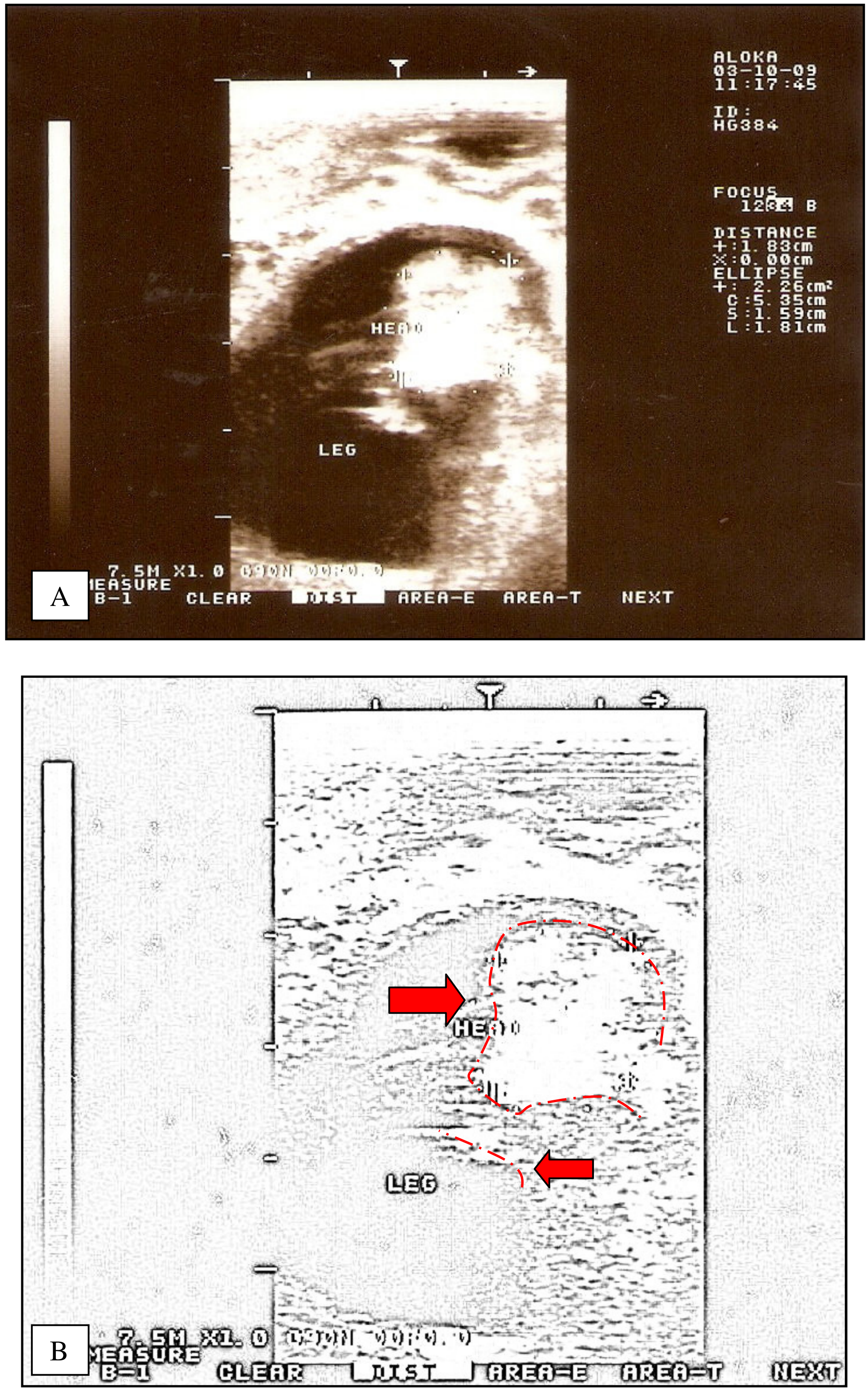

Figure 4.18: Foetus as observed on day 46 (week 7) of gestation. Foetal head, umbilical cord, leg and heart can be detected easily. Foetal head was well differentiated from foetal trunk at this age. A) Original image. B) Labeled image: head (arrow: →) and leg (arrow: ←).

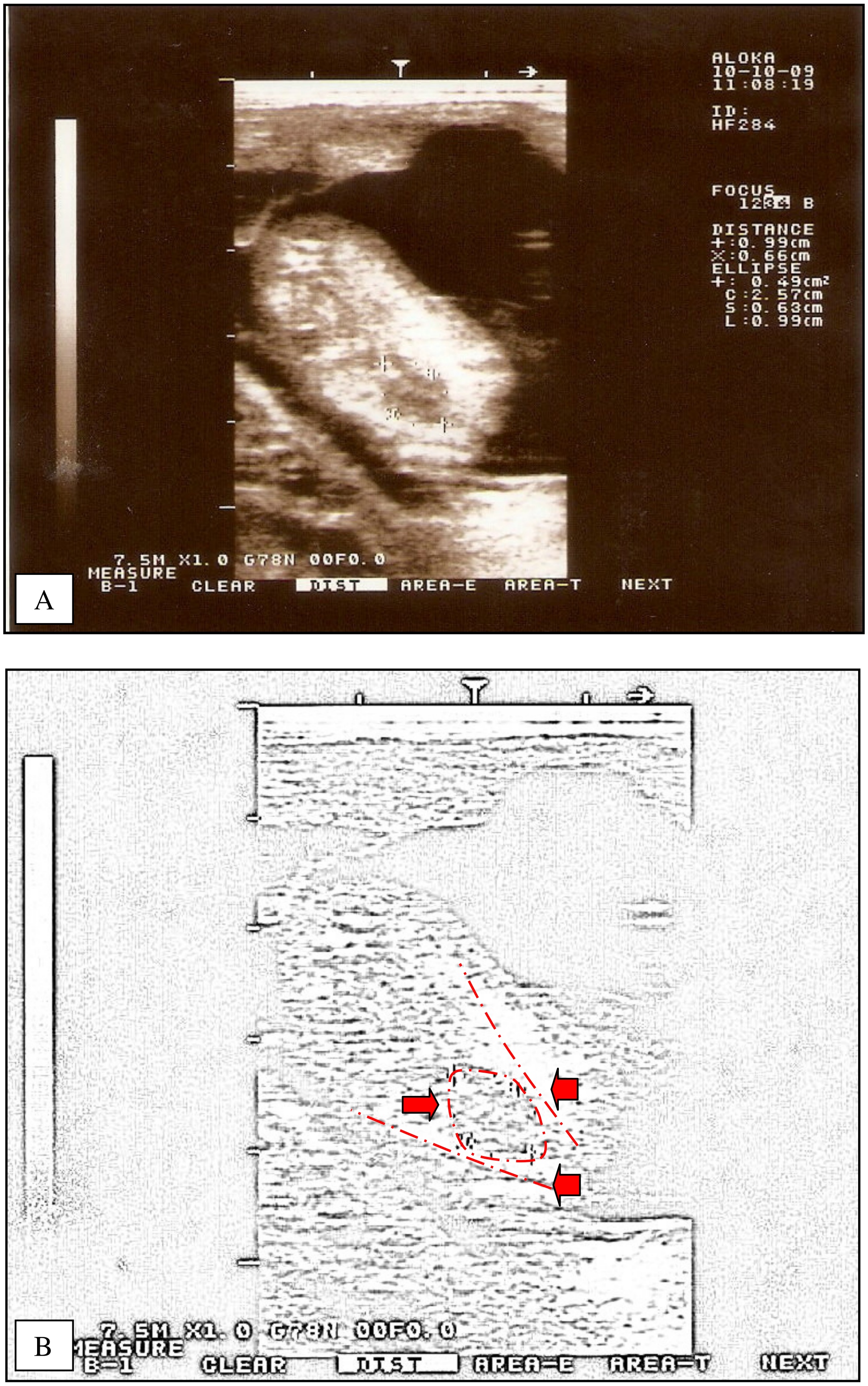

Figure 4.19: Foetus as observed on day 53 (week 8) of gestation. Foetal heart shape, thoracic area and foetal trunk can be accessed. Foetal heart colour changed from white to grey, results in definable shape. A) Original image. B) Labeled image: heart (arrow: →) and ribs (arrow: ←).

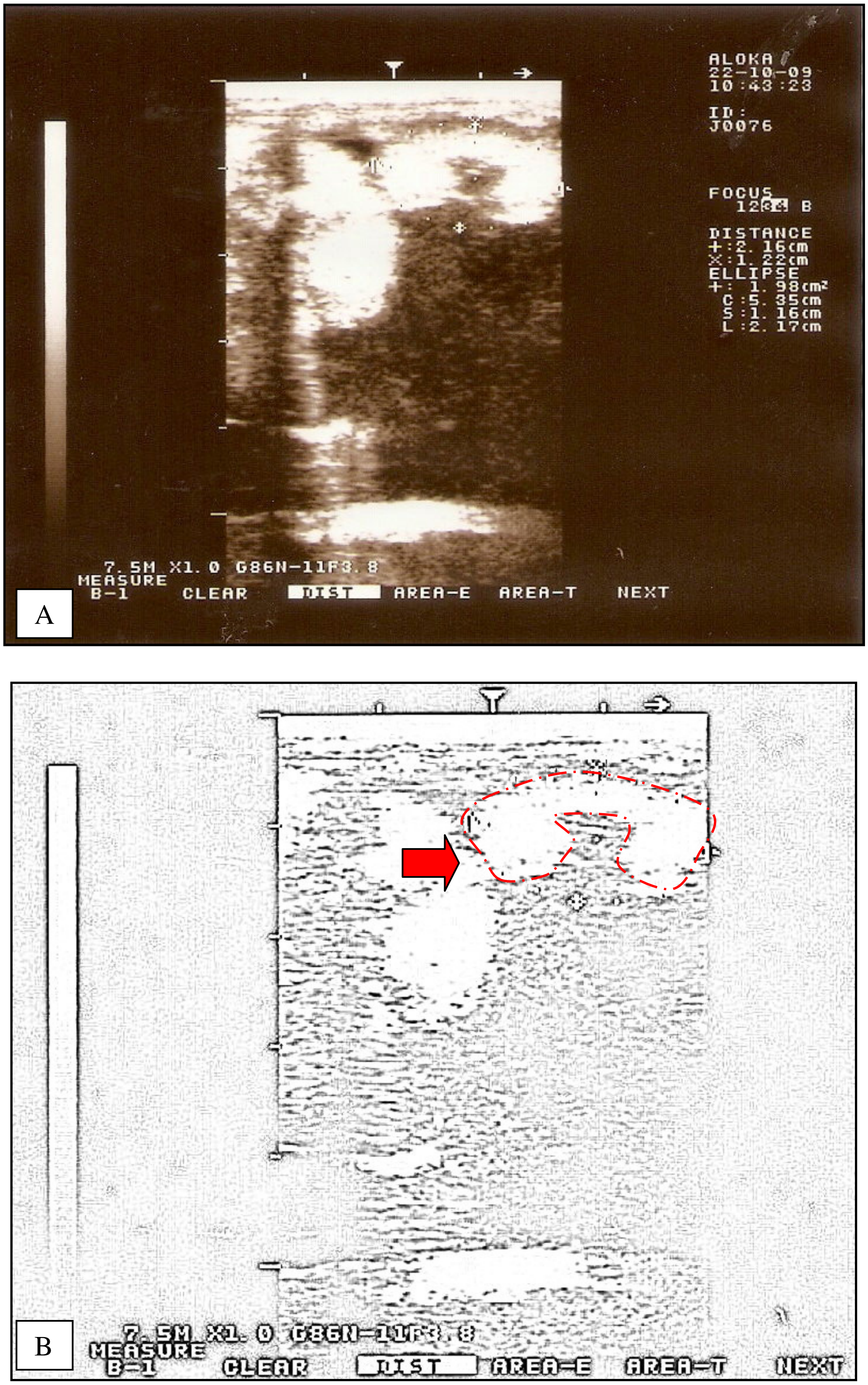

Figure 4.20: Image recorded on day 65 (week 10) of gestation. Foetus and foetal head appeared to be too big visualised as a whole on screen. Only placentome can be visualised as a whole at this age. A) Original image. B) Labeled image: placentome (arrow).

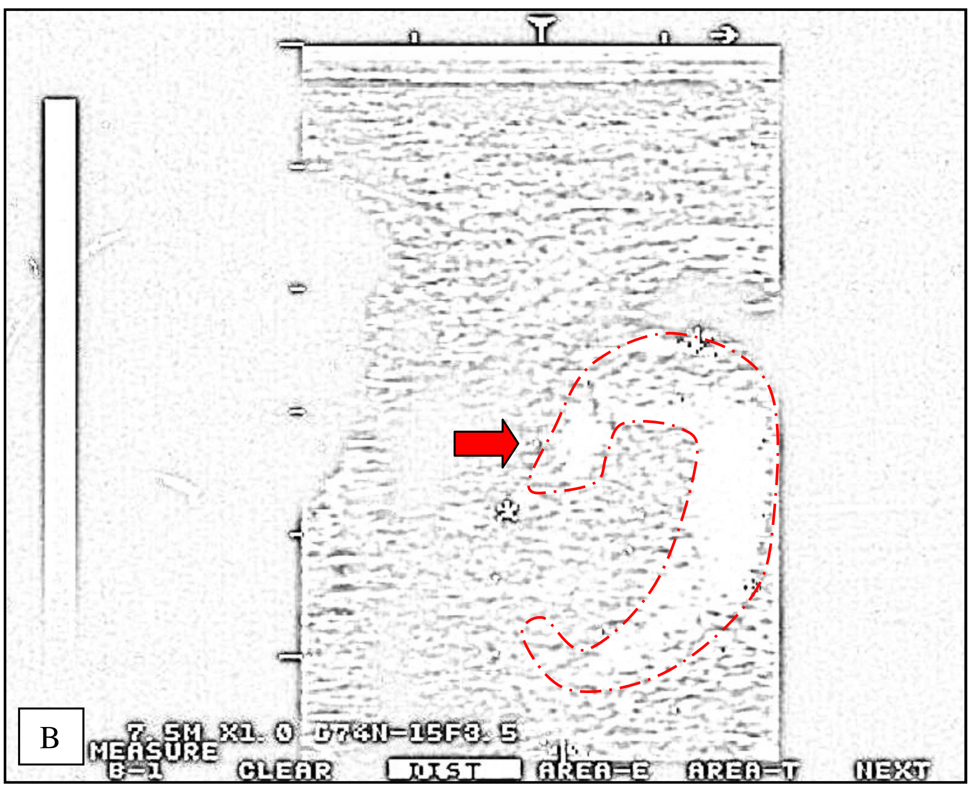

Figure 4.21: Image recorded on day 72 (week 11) of gestation. Foetus and other pregnancy-related structures were difficult to be visualised on screen. Only placentome can be visualised as a whole at this age. A) Original image. B) Labeled image: placentome (arrow: →).

4.4 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSABDOMINAL PROBE (EXPERIMENT 4)

Ultrasound scanning was conducted on the does underwent breeding programme starting from day 28 (week 4) post-mating. Following that, the scanning was conducted on weekly basis until week 21 of gestation. Table 4.7 describes the details of pregnancy-related structures detected throughout pregnancy using transrectal probe. Figures 4.22 - 4.39 show the selected images recorded using transabdominal probe at certain age of gestation. The earliest pregnancy-related structure detected was embryonic fluids which appeared as a group of non-echogenic round- or oval-shape structures, and measurable at week 4 of gestation.

On the following week (week 5 of gestation), embryonic vesicles, foetus and foetal heart were visualised. Embryonic vesicles were detected at the same position as in earlier week and foetus was observed in one of the vesicles (Figure 4.22). No active movement of foetus was detected at this gestational age. The shape of foetal heart was still undefined and the foetal heart area measurement could not be done. At the age of 6 week of gestation, two additional pregnancy-related structures were first detected: umbilical cord and placentome. Umbilical cord appeared as echogenic thread-like structures which connect foetus and the border of embryonic sac and placentome as small C-shaped echodense structure (Figure 4.24). Embryonic vesicles increased in size, but still could be visualised individually as a whole round or oblong shaped non-echogenic sac (Figure 4.23). Foetal heart did not show much development and still could not be measured (Figure 4.23). First active foetal movement was observed.

During the subsequent week (week 7 of gestation), the size of embryonic sacs greatly increased and it was difficult to get its full view on the screen. On the other hand, the size and number of placentomes increased and they were detected as C- or O-shaped echodence structures (Figure 4.25). Foetal head appeared as an echodense structure which was segregated from foetal body (Fig. 4.25) and foetal legs were observed to move with kicking motion. Foetal heart, unfortunately, still did not show defined shape. Starting from week 8 of gestation, embryonic sacs and foetus could not be visualised as a whole on the viewing screen. Only certain part of foetal body could be recorded at certain time (Figure 4.26). Placentomes increased in size and numbers (Figure 4.27). At this gestational age foetal heart appeared as small strong beating white-grey structure with defined shape. Hence, measurement of foetal heart area could be made at this stage.

During weeks 9-10 of gestation, foetus underwent more development especially increased in pregnancy-related structures size. Bony structures especially foetal head increased in size and became more ossified (Figure 4.28). Foetal heart could be detected easily on both weeks. Only 1-2 legs could be viewed on the screen for both ages (Figure 4.29). Scrotum was observed on week 10 of gestation as a hemisphere echodense structure positioned between the two hind legs of foetus (Figure 4.29). Starting from week 11 of gestation, it was extremely difficult to detect embryonic fluids and foetal trunk. Foetal heart was observed to change its colour from white-grey to grey (Figure 4.31). Bony structures became more ossified and increased in size (Figure 4.30). Scrotum still could be observed between the two hind legs of the foetus.

Five pregnancy-related structures were recorded during weeks 12-13 of gestation: placentome, foetal heart, foetal head, foetal leg and scrotum. At week 12 of gestation, foetal heart appeared as strong beating small grey structure (Figure 4.32). Its size increased at week 13 of gestation and the heartbeat appeared to be less rapid. Placentomes increased in size and started to become less echodense. Scrotum still could be observed as echodense hemisphere structure positioned between the two hind legs of foetus (Figure 4.33).

Scrotum was found to be accessible up to the week 14 of gestation together with foetal heart (Figure 4.34) and placentomes. Some foetal organs like stomach was detected as well, but they were not measured. Starting from week 15 of gestation until delivery dates, only foetal heart and placentomes could be viewed as a whole and measured easily (Figures 4.35 - 4.39). Both structures underwent changes in size and became less echodense as pregnancy progress.

Table 4.8: Details of pregnancy-related structures detected throughout pregnancy using transrabdominal probe

Gestational age (weeks)	Structures detected	Description	Measurability
4	Embryonic vesicles	Visualised as a group of non-echogenic round or oval shape structure	Yes
5	Embryonic vesicles	Visualised as a group of non-echogenic round or oval shape structure (Figure 4.22)	Yes
	Foetus	Foetuses were detected and measureable (Figure 4.22). No active movement of foetus was observed. However, foetal counting could be done	Yes
	Heartbeat	Foetal heart appeared as very small strong beating white structure. The shape of the heart was not defined	No
6	Embryonic vesicles	Embryonic vesicles increased in size, but still can be visualised individually as a whole round or oblong shaped non-echogenic sac (Figure 4.23)	Yes
	Foetus	Movement of foetus was observed. Foetal counting could be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined (Figure 4.23)	No
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structures which connect foetus and the border of embryonic sac	No
	Placentome	Appeared as small nodule c-shaped echodense structure (Figure 4.24)	Yes
7	Embryonic vesicles	Embryonic vesicles increased in size, but still can be visualised individually as a whole round or oblong shaped non-echogenic sac (Figure 4.25)	Yes
	Foetus	Movement of foetus was observed. Foetal counting could be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white structure. The shape of the heart was not defined	No
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structures which connect foetus and the border of embryonic sac	No

(continued)

(continued)

Gestational age (weeks)	Structures detected	Description	Measurability
7	Placentome	Appeared as small nodule c-shaped echodense structure (Fig. 4.25)	Yes
	Foetal head	Foetal head appeared as an echodense structure which is segregated from foetal body (Fig. 4.25)	Yes
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively (kicking)	Yes
8	Embryonic fluid	Size of embryonic sac increased. Embryonic sac cannot be visualised as a whole on screen	No
	Foetus	Foetus moves actively and increased in size. It cannot be visualised as a whole on the screen. However, foetal counting still possible to be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white-grey structure. The shape of the heart was definable	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure (Figure 4.27)	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac	No
	Foetal head	Size of foetal head increased and foetal head is very well differentiated from foetal body (Figure 4.26). One foetal head will fit the whole viewing screen	Yes
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively	Yes
9	Embryonic fluid	Size of embryonic sac increased. Embryonic sac cannot be visualised as a whole on screen	No
	Foetus	Foetus moves actively and increased in size. It cannot be visualised as a whole on the screen. However, foetal counting still possible to be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white-grey structure. The shape of the heart was definable	Yes

(continued)

(continued)

Gestational age (weeks)	Structures detected	Description	Measurability
9	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped	Yes
	Umbilical cord	Umbilical cord appeared as echogenic thread-like structure which connects foetus and the border of embryonic sac	No
	Foetal head	Size of foetal head increased and the skull become more ossified (Figure 4.28)	Yes
	Foetal leg	Foetal legs were well differentiated from other body parts and moves actively (kicking)	Yes
10	Embryonic fluid	Size of embryonic sac increased. Embryonic sac cannot be visualised as a whole on screen	Yes
	Foetus	Foetus moves actively and increased in size. It cannot be visualised as a whole on the screen. However, foetal counting still possible to be done	Yes
	Foetal heart	Foetal heart appeared as small strong beating white-grey structure. The shape of the heart was definable	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure	Yes
	Umbilical cord	Umbilical cord increased in size	No
	Foetal head	Size of foetal head increased and the skull become more ossified	Yes
	Foetal leg	Foetal legs became more ossified and moved actively (Figure 4.29)	Yes
	Scrotum	Scrotum could be observed as echodense sac-like structure which is positioned between the two hind legs of foetus (Figure 4.29)	No
11	Foetal heart	Foetal heart appeared as small strong beating grey structure. The shape of the heart was definable (Figure 4.31)	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure	Yes
	Foetal head	Size of foetal head increased and the skull become more ossified (Figure 4.30)	Yes
	Foetal leg	Foetal legs became more ossified and moved actively	Yes

(continued)

(continued)

Gestational age (weeks)	Structures detected	Description	Measurability
11	Scrotum	Scrotum could be observed as echodense sac-like structure which is positioned between the two hind legs of foetus	No
12	Foetal heart	Foetal heart appeared as small strong beating grey structure. The shape of the heart was definable (Fig. 4.32)	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure	Yes
	Foetal head	Size of foetal head increased and the skull become more ossified	Yes
	Foetal leg	Foetal legs became more ossified and moved actively. Foetal leg was difficult to be visualized as a whole	No
	Scrotum	Scrotum could be observed as echodense sac-like structure which is positioned between the two hind legs of foetus	No
13	Foetal heart	Foetal heart appeared as medium sized grey structure with rhythmic pulsation. The shape of the heart was definable. Foetal heartbeat appeared to be less rapid	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure. Some placentomes, especially those with bigger sizes started to become less echodense	Yes
	Foetal head	Size of foetal head increased and the skull become more ossified. It was difficult to visualised foetal head as a whole on viewing screen	No
	Scrotum	Scrotum could be observed as echodense sac-like structure which is positioned between the two hind legs of foetus (Figure 4.33)	No
14	Foetal heart	Foetal heart appeared as medium sized grey structure with rhythmic pulsation (Figure 4.34). The shape of the heart was definable. Foetal heartbeat appeared to be less rapid	Yes

(continued)

(continued)

Gestational age (weeks)	Structures detected	Description	Measurability
14	Placentome	Size and number of placentome increased. Placentome appeared as echogenic (white-coloured) C-shaped or O-shaped structure. Some placentomes, especially those with bigger sizes started to become less echodense	Yes
	Scrotum	Scrotum could be observed as echodense sac-like structure which is positioned between the two hind legs of foetus	No
15	Foetal heart	Foetal heart appeared as medium sized grey-black structure with rhythmic pulsation (Figure 4.35). The shape of the heart was definable. Foetal heartbeat appeared to be less rapid	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes
16	Foetal heart	Foetal heart appeared as medium sized grey-black structure with rhythmic pulsation (Figure 4.36). The size of heart increased	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes
17	Foetal heart	Foetal heart appeared as medium sized black structure with rhythmic pulsation (Figure 4.37). The size of heart increased	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes
18	Foetal heart	Foetal heart appeared as medium sized black structure with rhythmic pulsation. The size of heart increased	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes
19	Foetal heart	Foetal heart appeared as medium sized black structure with rhythmic pulsation. The size of heart increased	Yes

(continued)

(continued)

Gestational age (weeks)	Structures detected	Description	Measurability
19	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes
20	Foetal heart	Foetal heart appeared as medium sized black structure with rhythmic pulsation. The size of heart increased	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure (Figure 4.38)	Yes
21	Foetal heart	Foetal heart appeared as medium sized black structure with rhythmic pulsation. The size of heart increased (Figure 4.39)	Yes
	Placentome	Size and number of placentome increased. Placentome appeared as less echodense (grey-coloured) C-shaped or O-shaped structure	Yes

Table 4.9: Measurability of pregnancy-related structures throughout pregnancy via transabdominal procedure

Pregnancy age (days)	Pregnancy related structures													
	Embryonic sacs		Amniotic fluid		Crown rump length		Heart size		Heartbeat		Foetal-head diameter		Placentome diameter	
	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA	DA	MA
21-28	-	-	-	-	+	-	-	-	-	-	-	-	-	-
29-35	+	+	+	+	+	+	-	-	+	-	-	-	-	-
36-42	+	+	+	+	+	+	-	-	+	-	-	-	+	+
43-49	+	+	+	+	+	+	-	-	+	-	+	+	+	+
50-56	+	-	+	-	-	-	+	+	+	-	+	+	+	+
57-63	+	-	+	-	-	-	+	+	+	-	+	+	+	+
64-70	+	-	+	-	-	-	+	+	+	-	+	+	+	+
71-77	+	-	+	-	-	-	+	+	+	-	+	+	+	+
78-84	+	-	+	-	-	-	+	+	+	-	+	+	+	+
85-91	+	-	+	-	-	-	+	+	+	-	+	+	+	+
92-98	+	-	+	-	-	-	+	+	+	-	+	-	+	+
99-105	+	-	+	-	-	-	+	+	+	-	+	-	+	+
106-112	+	-	+	-	-	-	+	+	+	-	+	-	+	+
113-119	+	-	+	-	-	-	+	+	+	-	+	-	+	+
120-126	+	-	+	-	-	-	+	+	+	-	+	-	+	+
127-133	+	-	+	-	-	-	+	+	+	-	+	-	+	+
134-140	+	-	+	-	-	-	+	+	+	-	+	-	+	+
141-147	+	-	+	-	-	-	+	+	+	-	+	-	+	+

DA: Detectable

MA: Measurable

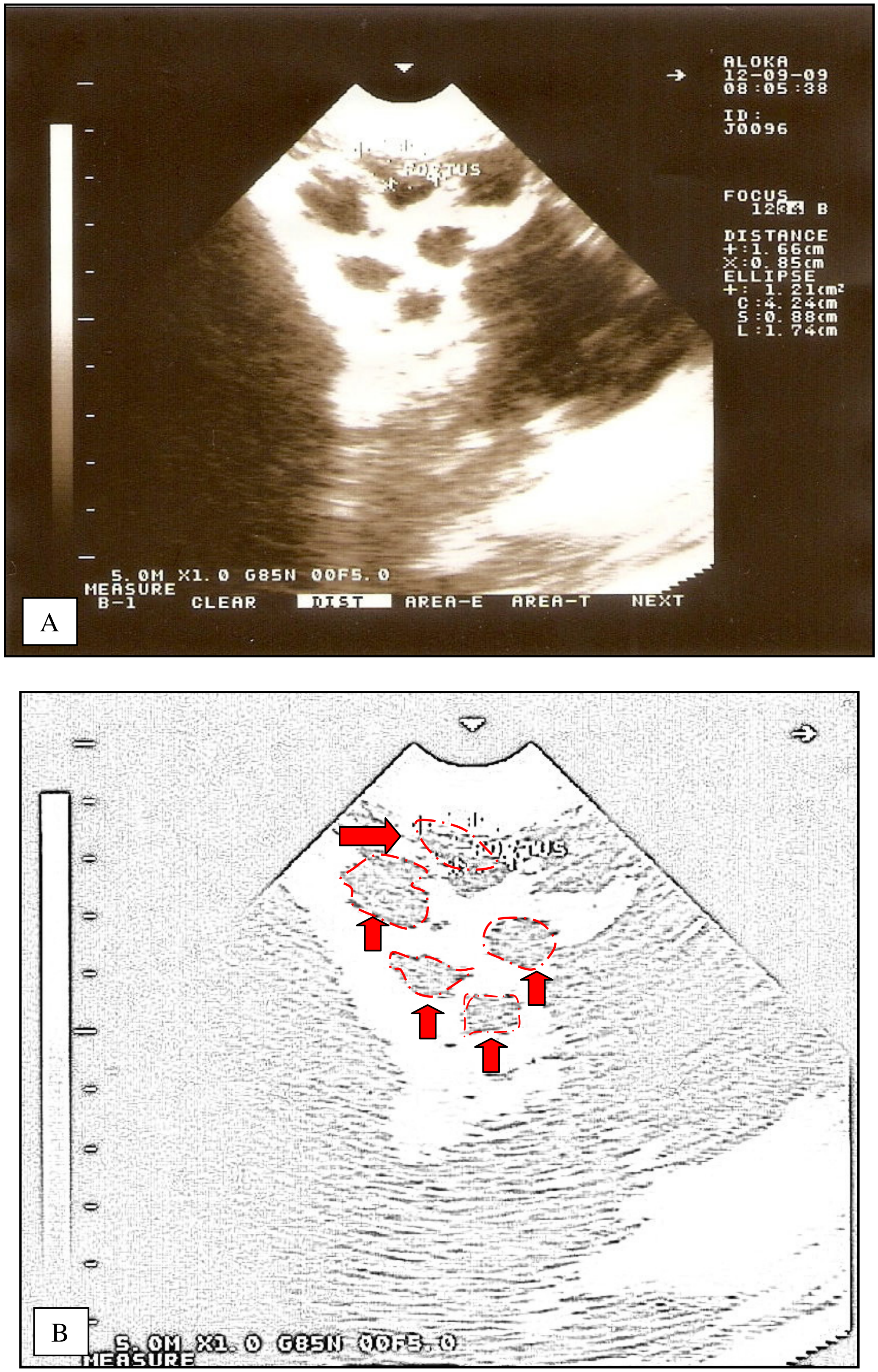

Figure 4.22: Image of pregnant doe at day 33 (week 5) of gestation. A) Original image. B) Labeled image: foetus (arrow: →), allantoic fluid (arrows: ↑).

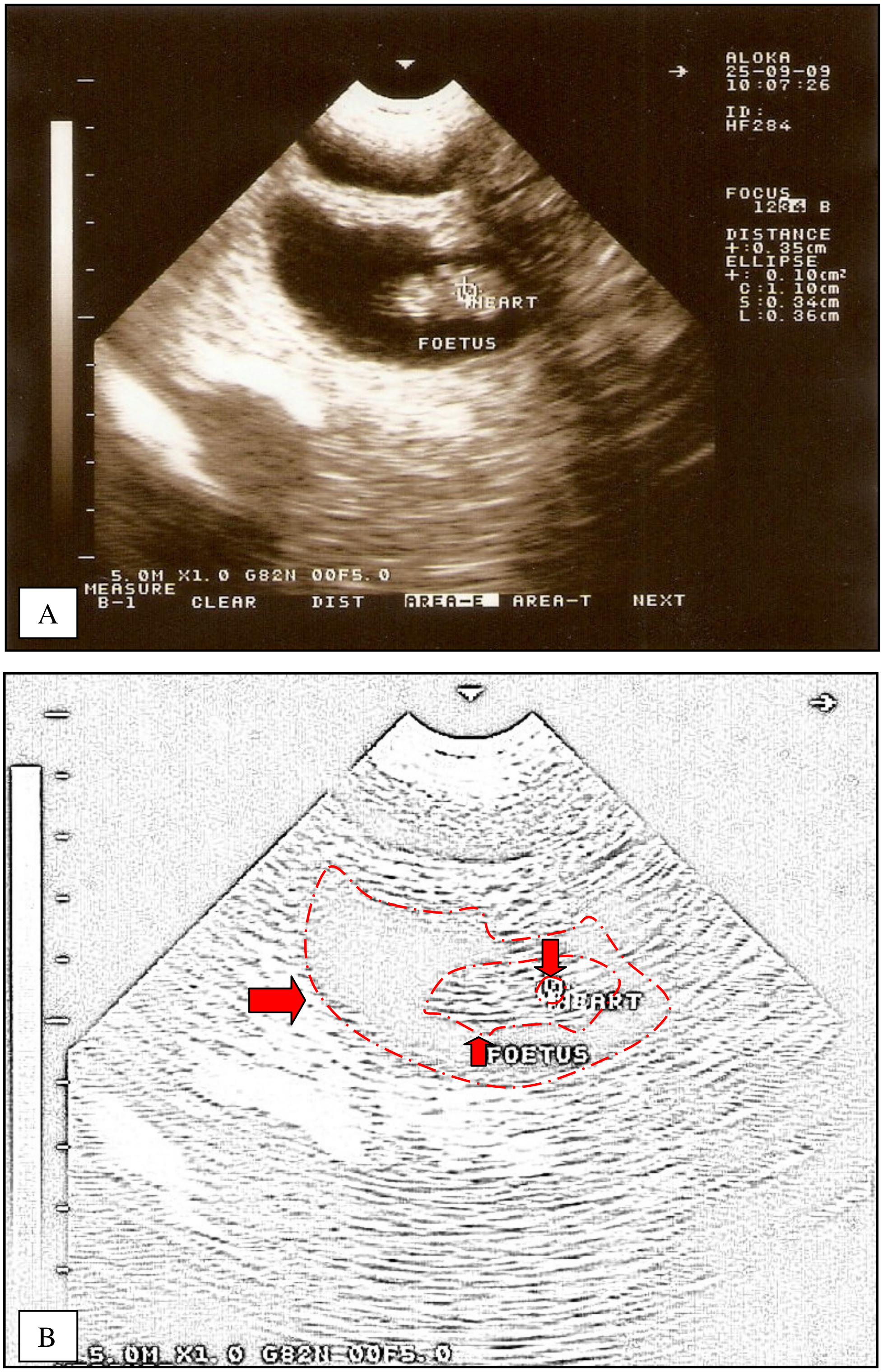

Figure 4.23: Image of pregnant doe at day 36 (week 6) of gestation. Foetus and heart were detected. A) Original image. B) Labeled image: embryonic sac (arrow: →), foetus (arrow: ↑) and foetal heart (arrow: ↓).

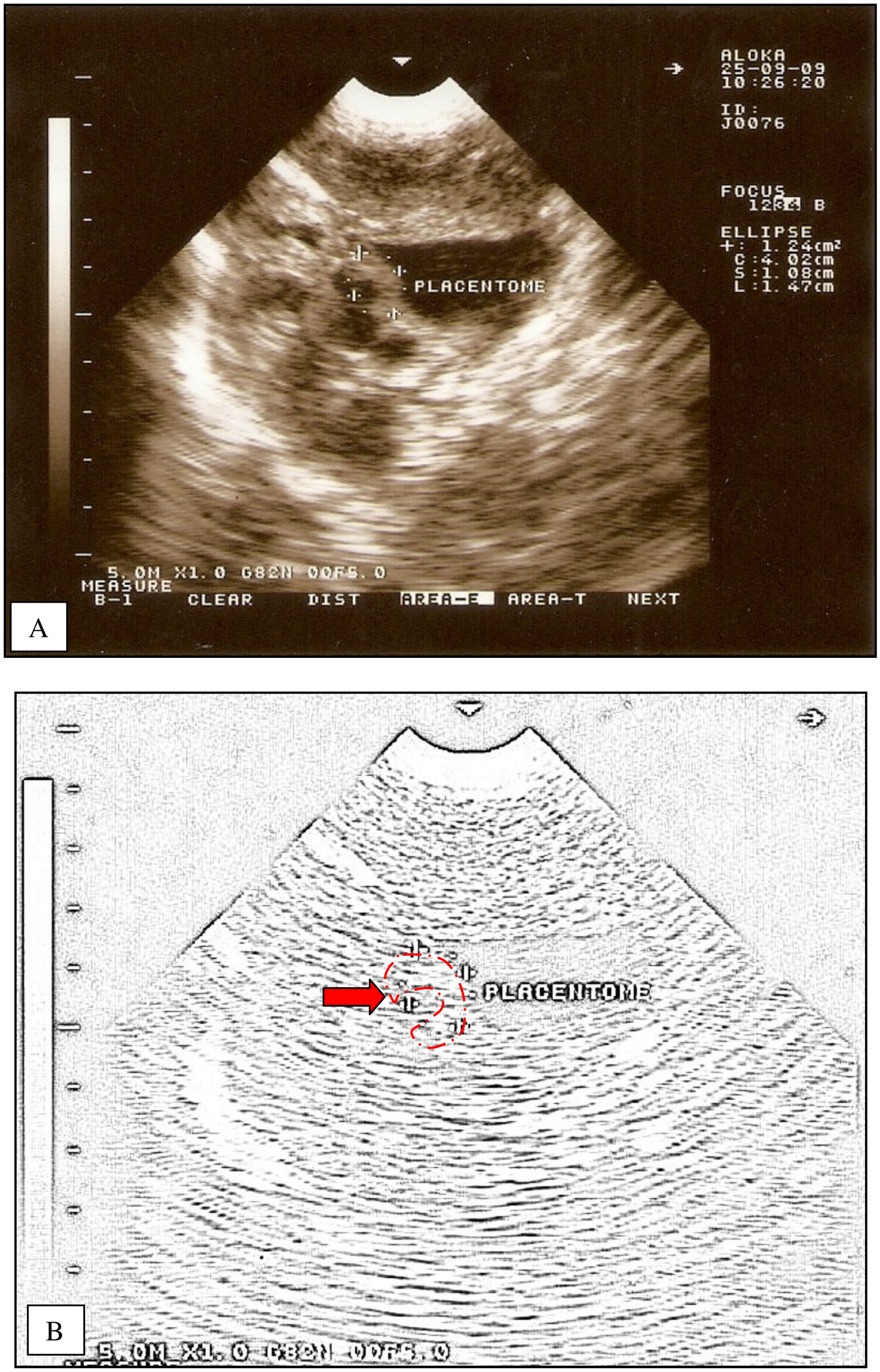

Figure 4.24: Image of pregnant doe at day 36 (week 6) of gestation. C-shaped placentome was detected. A) Original image, B) Labeled image: placentome (arrow: →).

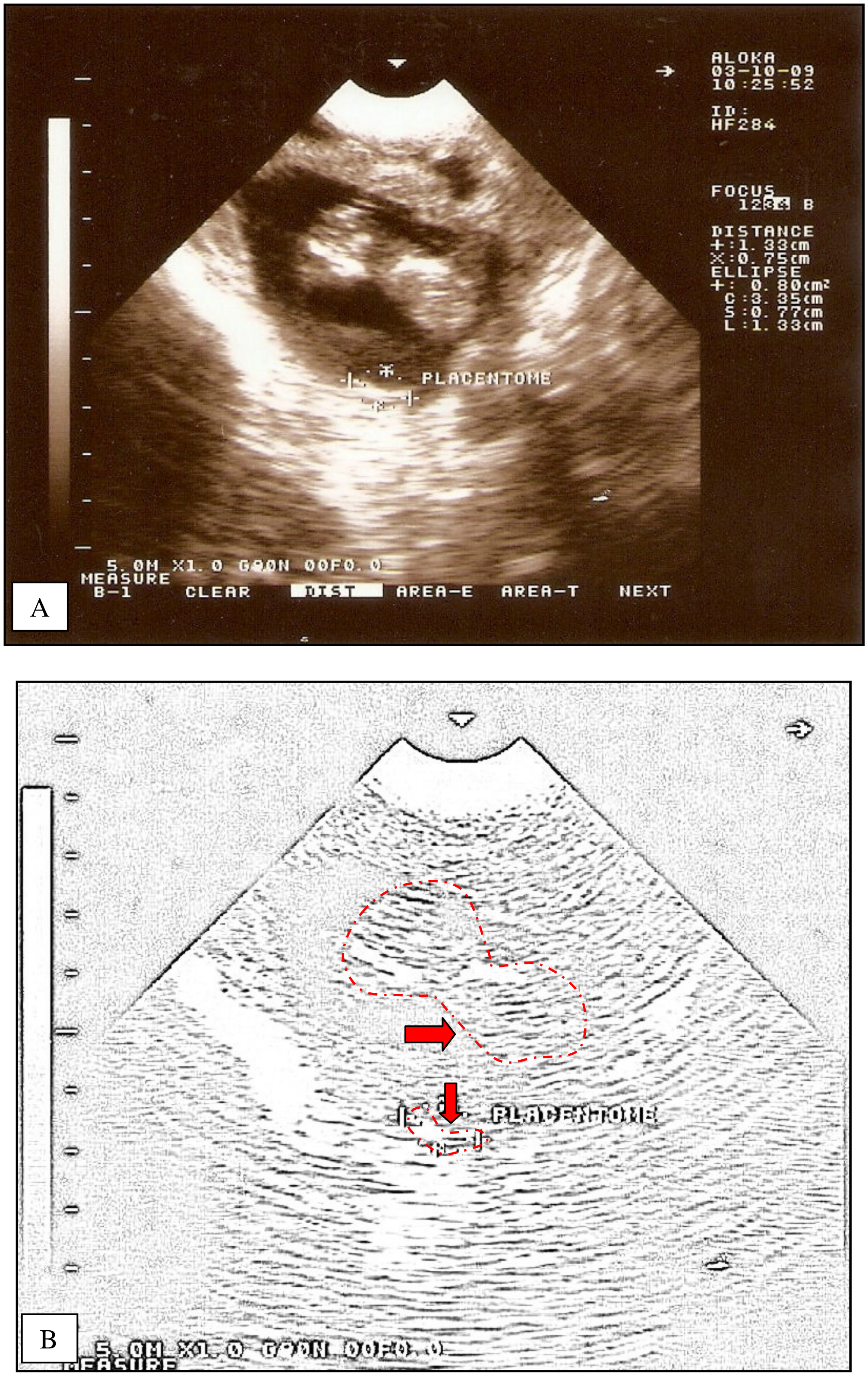

Figure 4.25: Image of pregnant doe at day 46 (week 7) of gestation. Foetal head, body (arrow: →) and placentome (arrow: ↓) were detected. A) Original image. B) Labeled image.

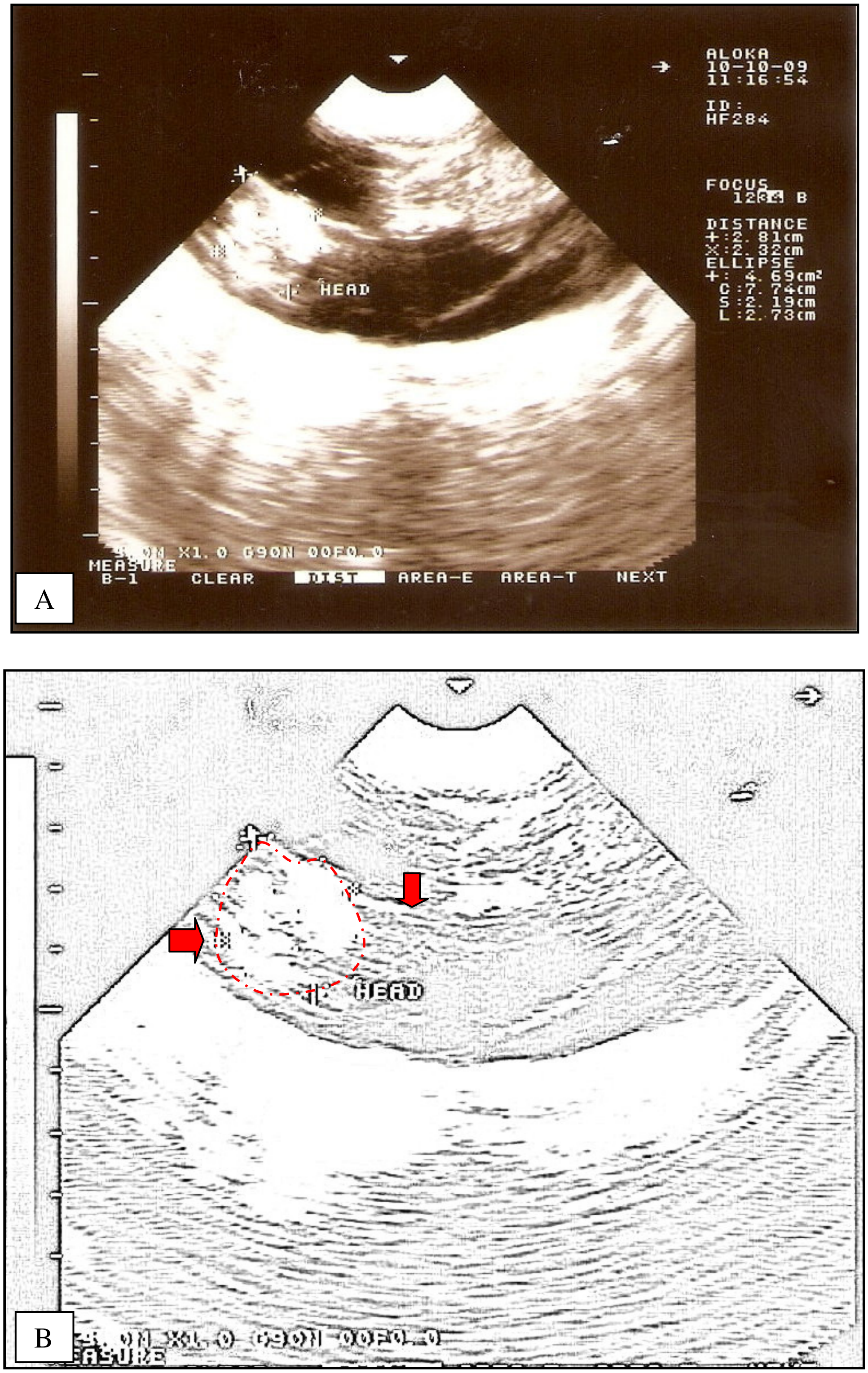

Figure 4.26: Image recorded at day 53 (week 8) of gestation. Foetal head (arrow: →) increased in size and foetal neck (arrow↓) can be detected easily. Foetal heart and thoracic area can be detected as well. A) Original image. B) Labeled image.

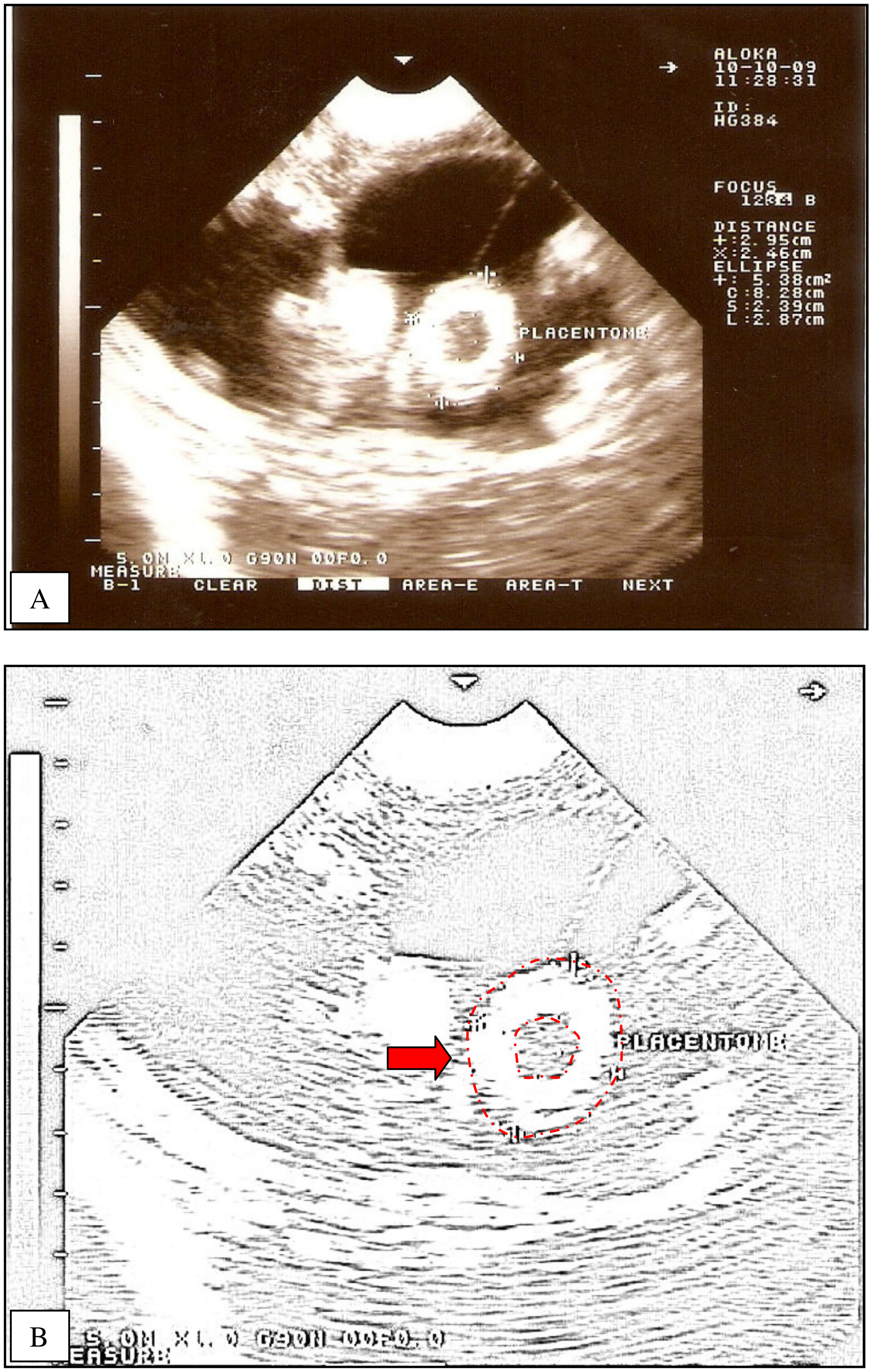

Figure 4.27: Image recorded at day 53 (week 8) of gestation. Placentome (arrow) can be detected and measured easily. A) Original image. B) Labeled image.

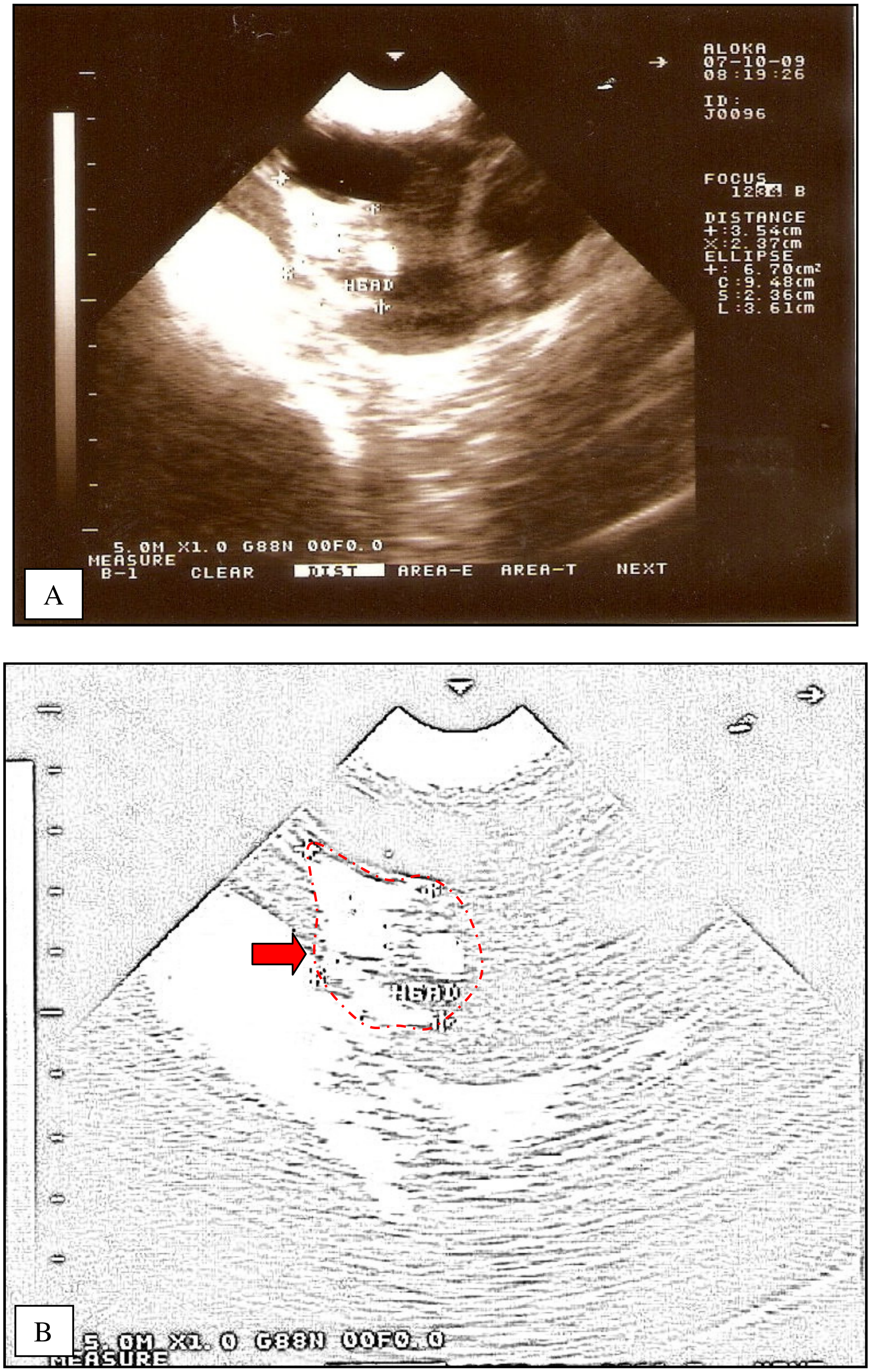

Figure 4.28: Image recorded at day 60 (week 9) of gestation. Bony structure becomes more echodense. Foetal head (arrow: →) can be detected easily. A) Original image. B) Labeled image.

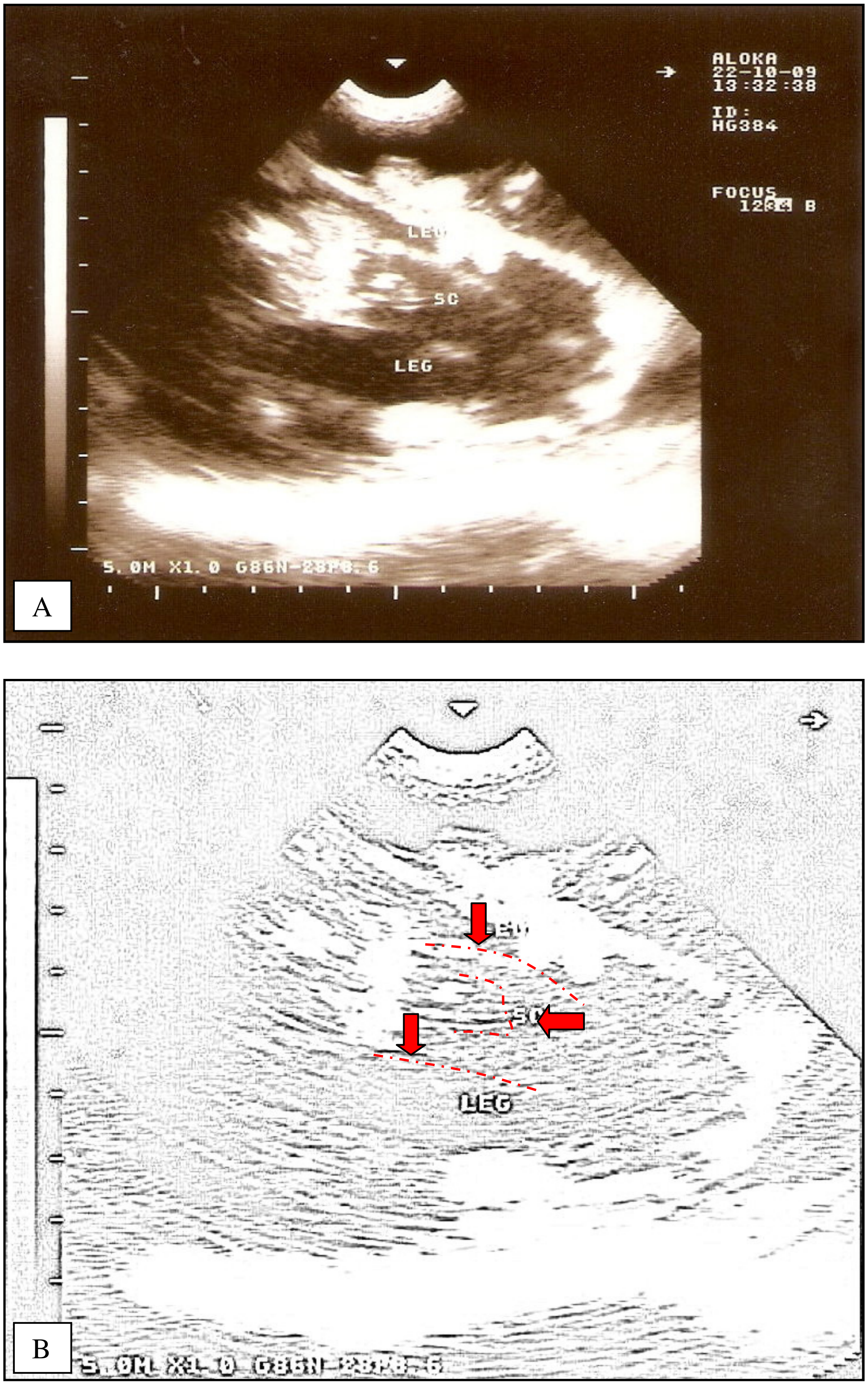

Figure 4.29: Image recorded at day 65 (week 10) of gestation. Legs (arrows: ↓) and scrotum (arrow: ⟵) can be observed. A) Original image. B) Labeled image.

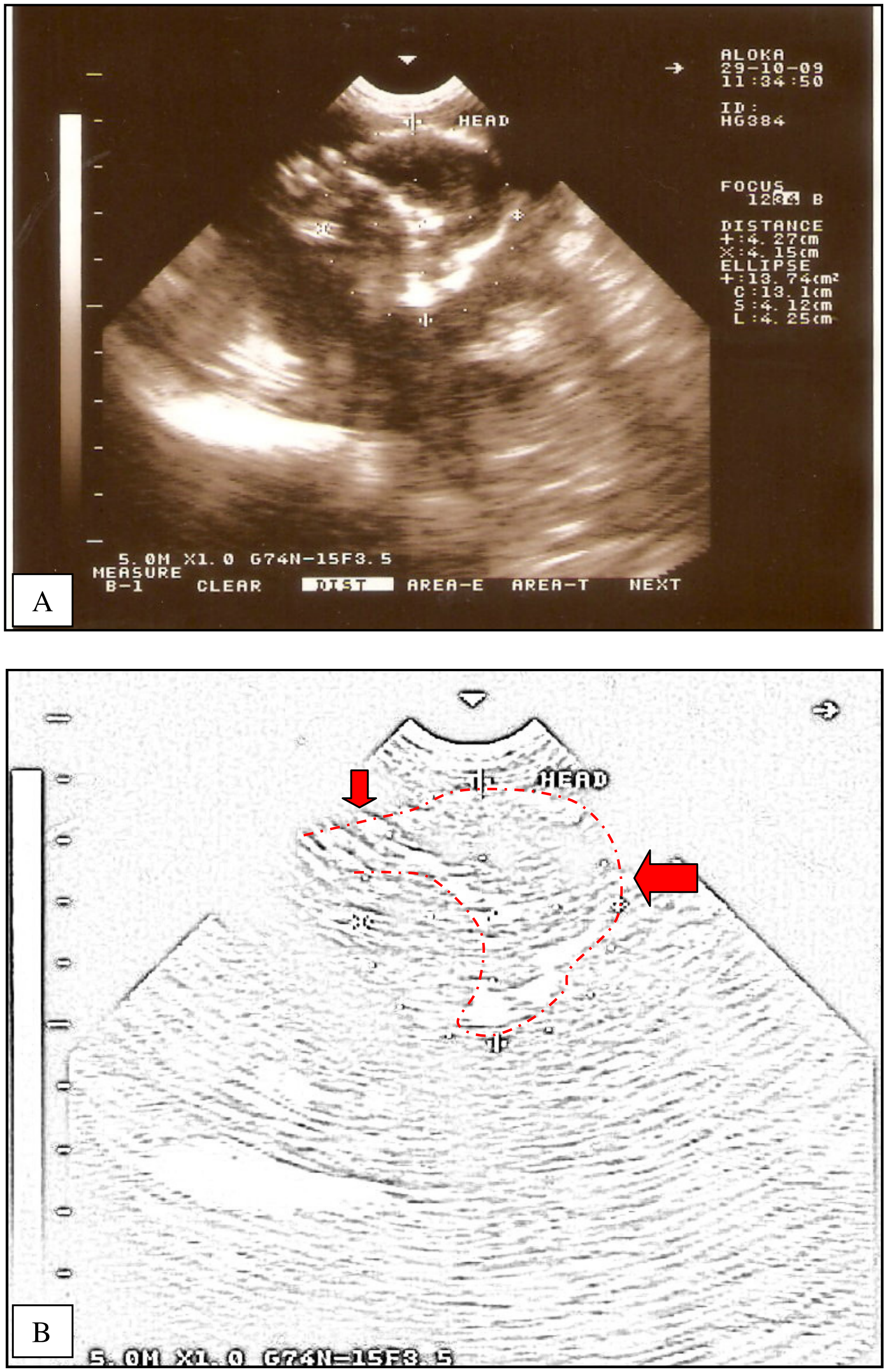

Figure 4.30: Image recorded at day 72 (week 11) of gestation. Foetal head (arrow: ←) and neck (arrow: ↓) became more ossified. A) Original image. B) Labeled image.

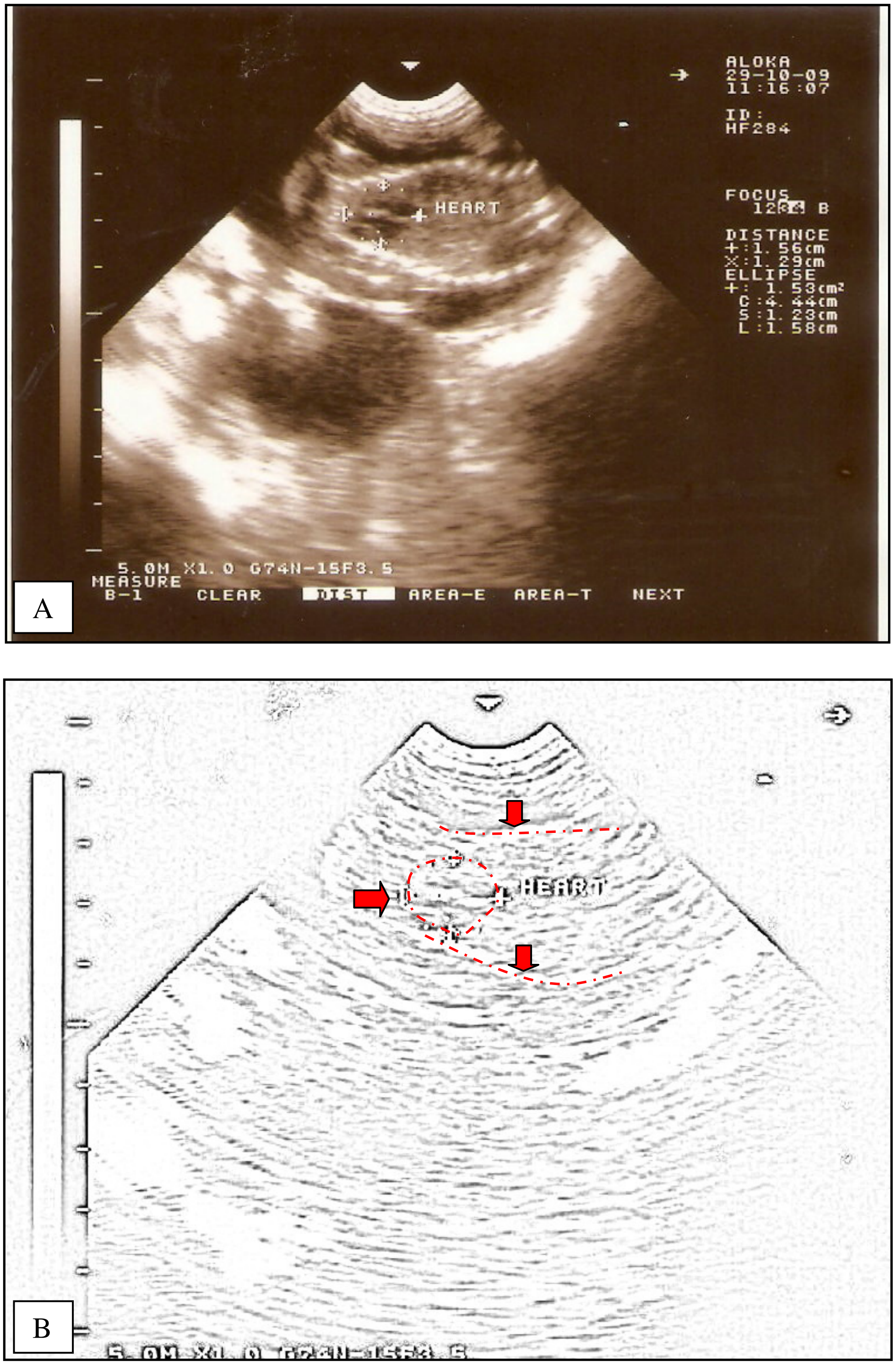

Figure 4.31: Image recorded at day 72 (week 11) of gestation. Foetal heart (arrow: →) and ribs (arrows: ↓) could be detected easily. A) Original image. B) Labeled image.

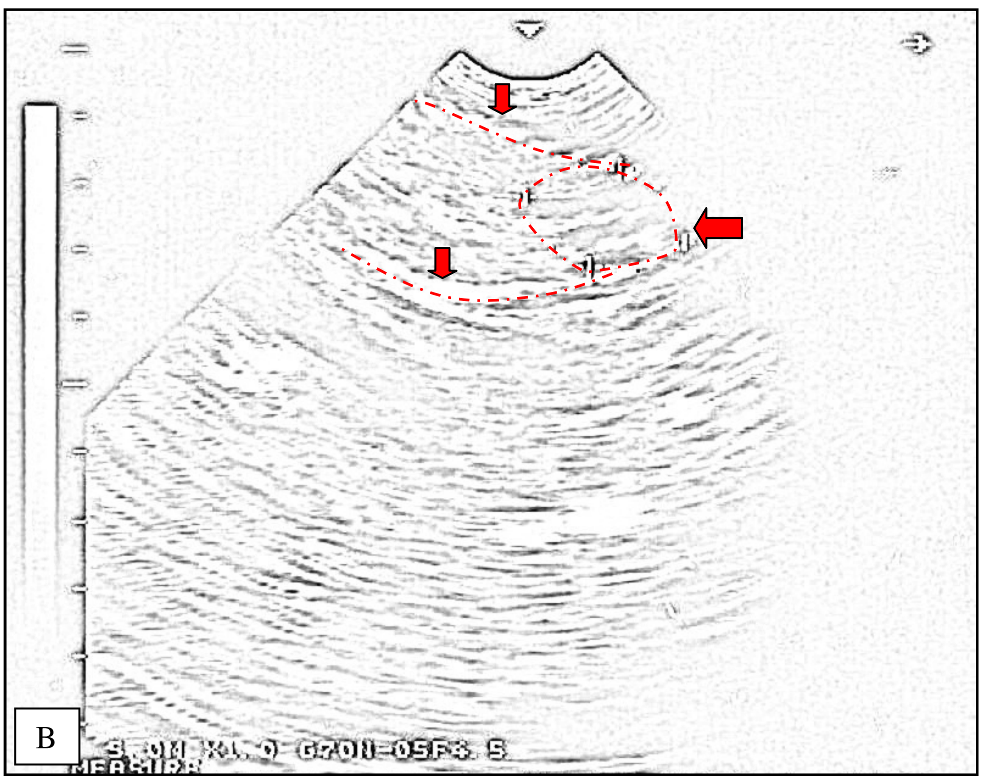

Figure 4.32: Image recorded at day 84 (week 12) of gestation. Foetal heart (arrow: ⟵) and ribs (arrows: ↓) could be detected easily. A) Original image. B) Labeled image.

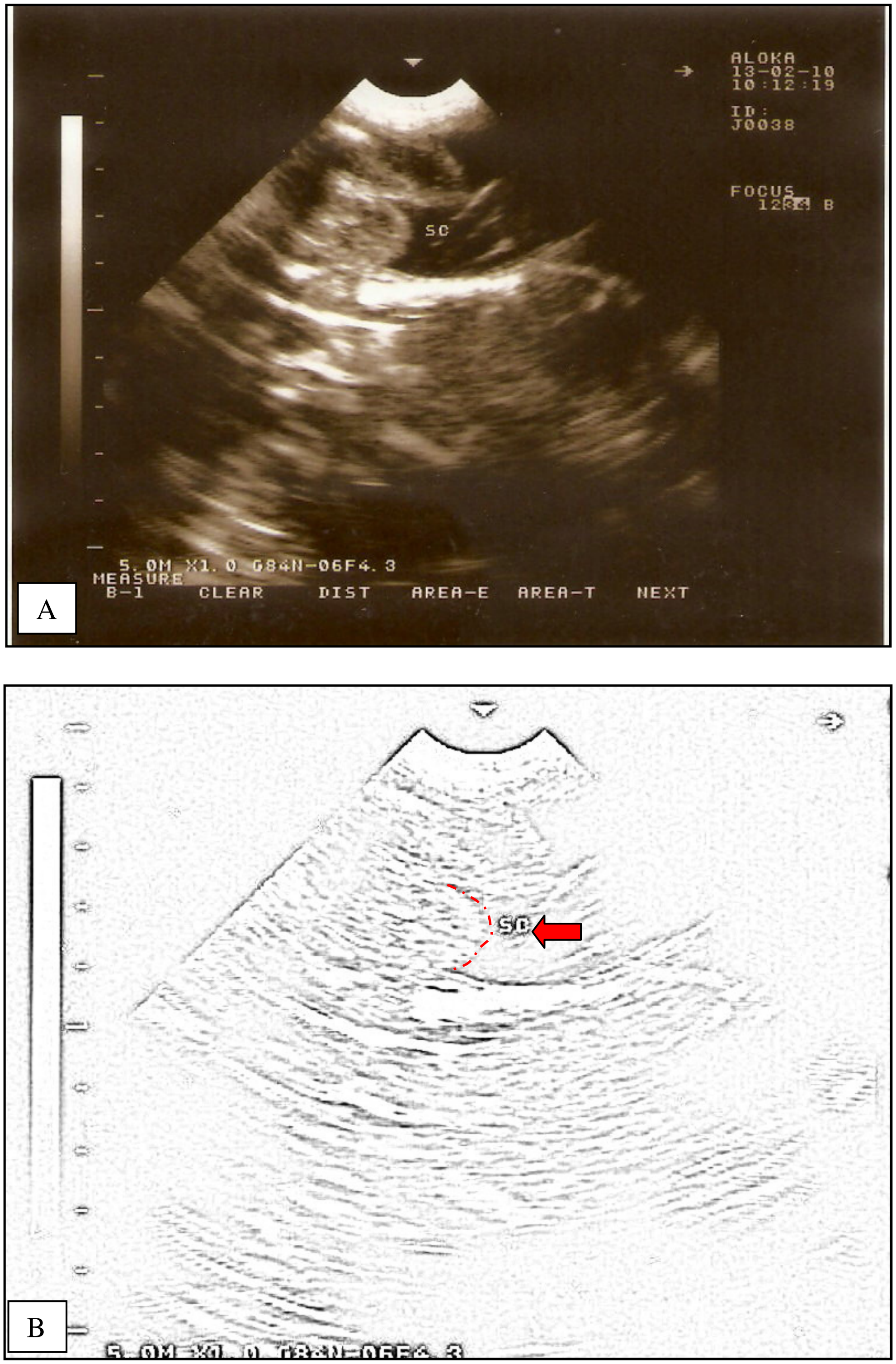

Figure 4.33: Image recorded at day 91 (week 13) of gestation. Scrotum could be detected (arrow). A) Original image. B) Labeled image.

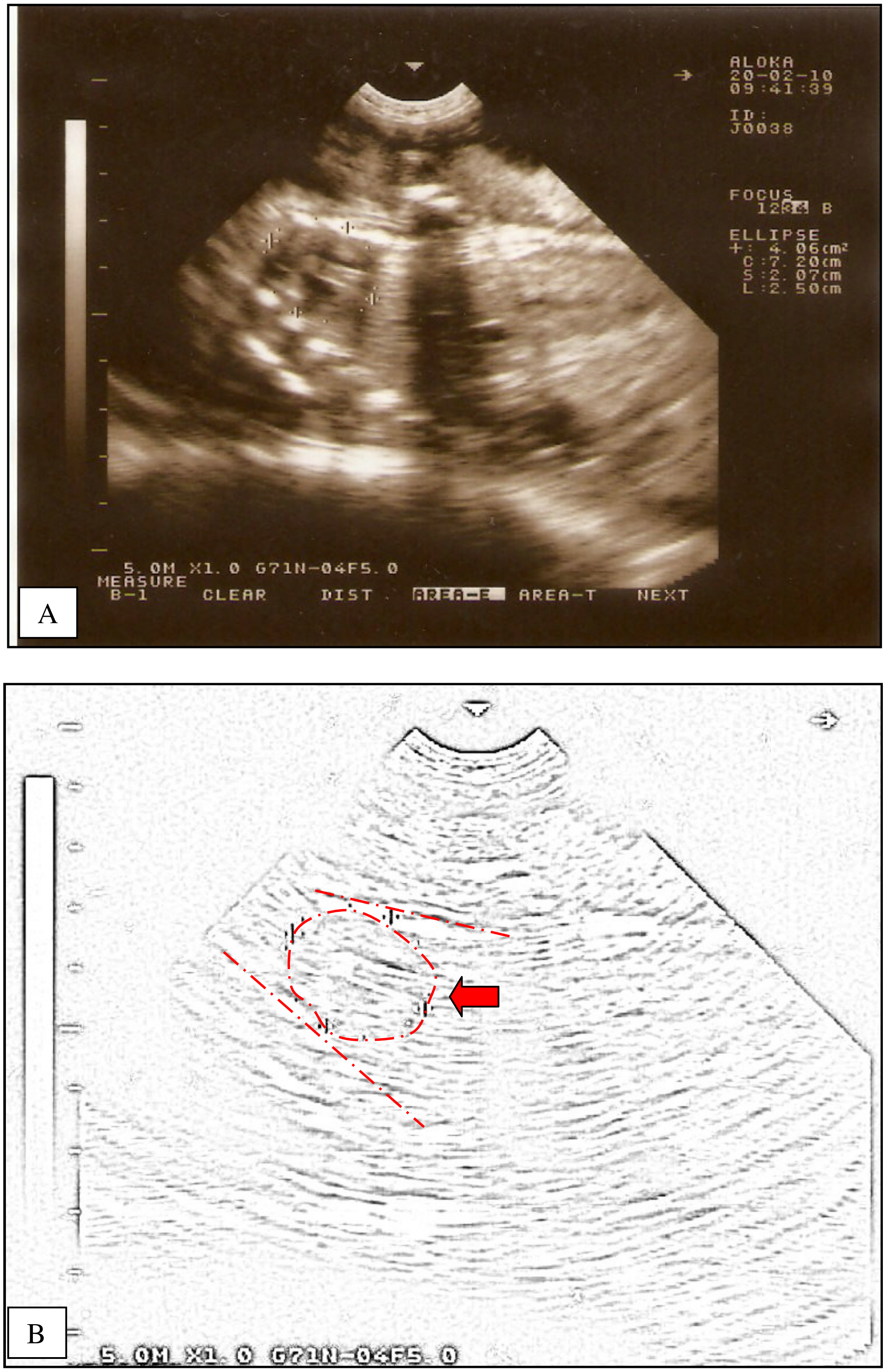

Figure 4.34: Image recorded at day 98 (week 14) of gestation. High increase in foetal heart size (arrow) as compared to two weeks earlier could be observed. A) Original image. B) Labeled image.

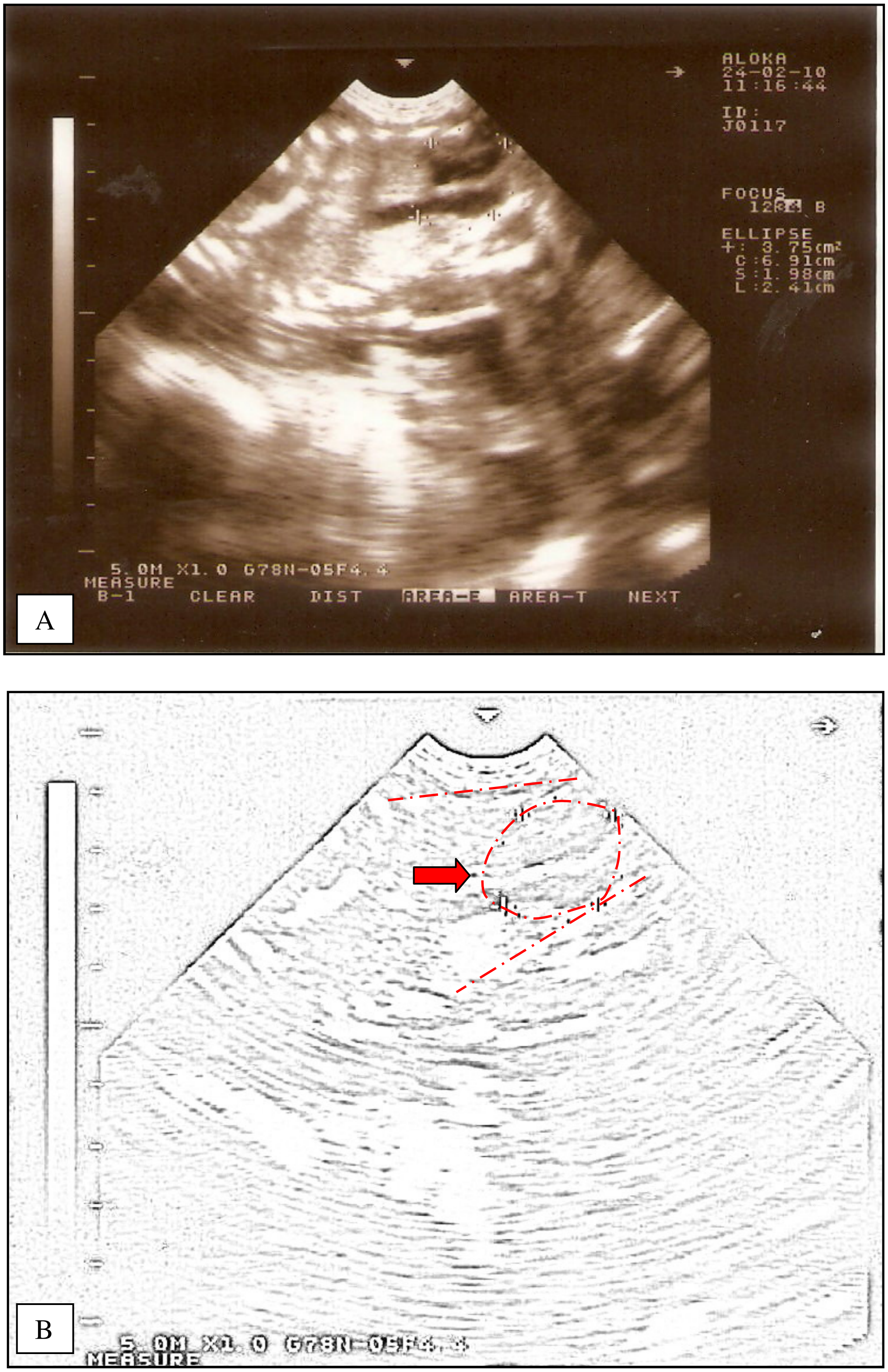

Figure 4.35: Image recorded at day 105 (week 15) of gestation. Foetal heart (arrow) started to change its echogenicity and became black in colour. A) Original image. B) Labeled image.

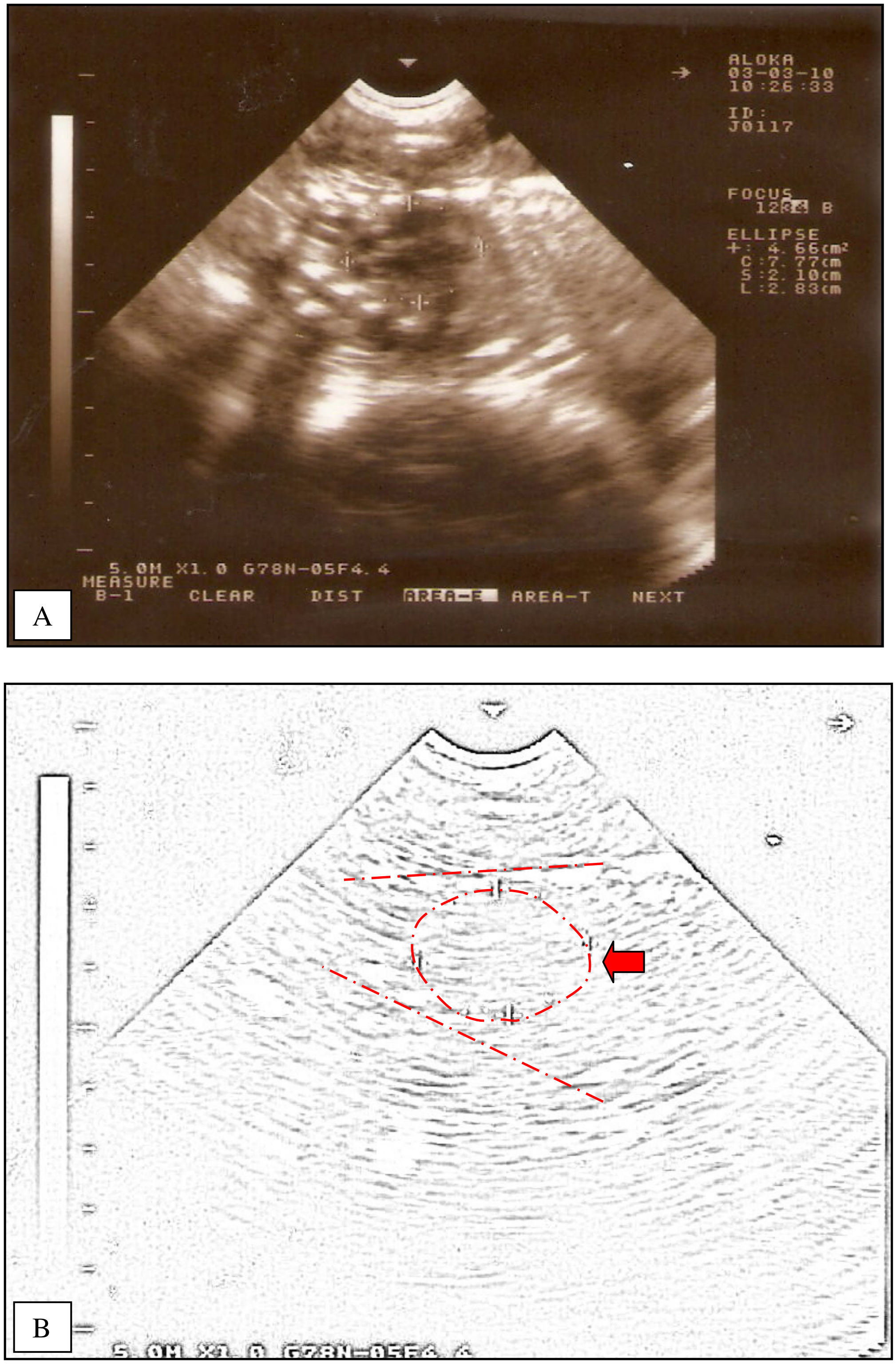

Figure 4.36: Image recorded at day 112 (week 16) of gestation. Foetal heart (arrow) increased in size (4.66 cm^2) as compared to one week earlier (Figure 4.35: 3.75 cm^2).

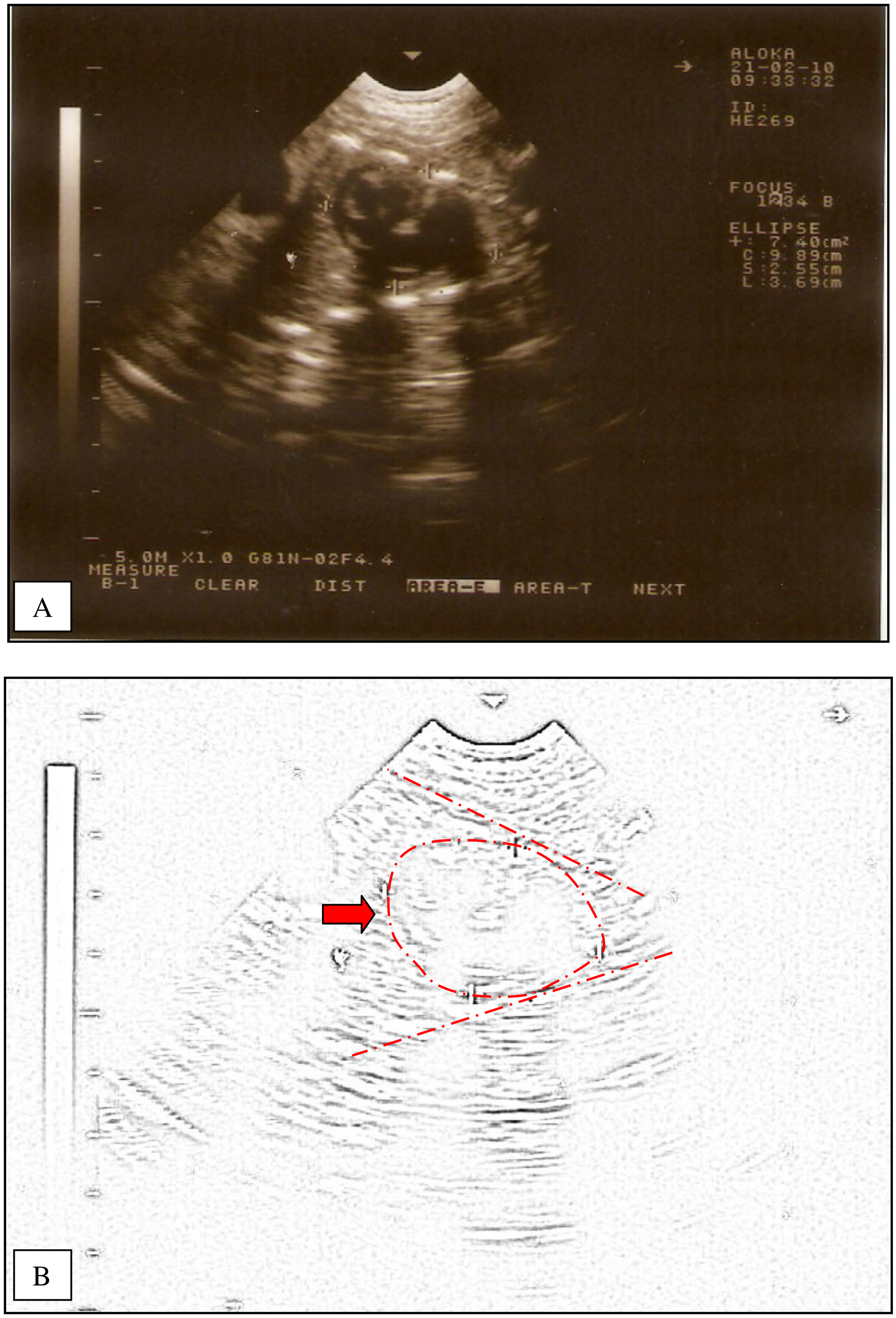

Figure 4.37: Image recorded at day 119 (week 17) of gestation. Foetal heart (arrow) increased in size and appeared as less echogenic structure (black in colour).

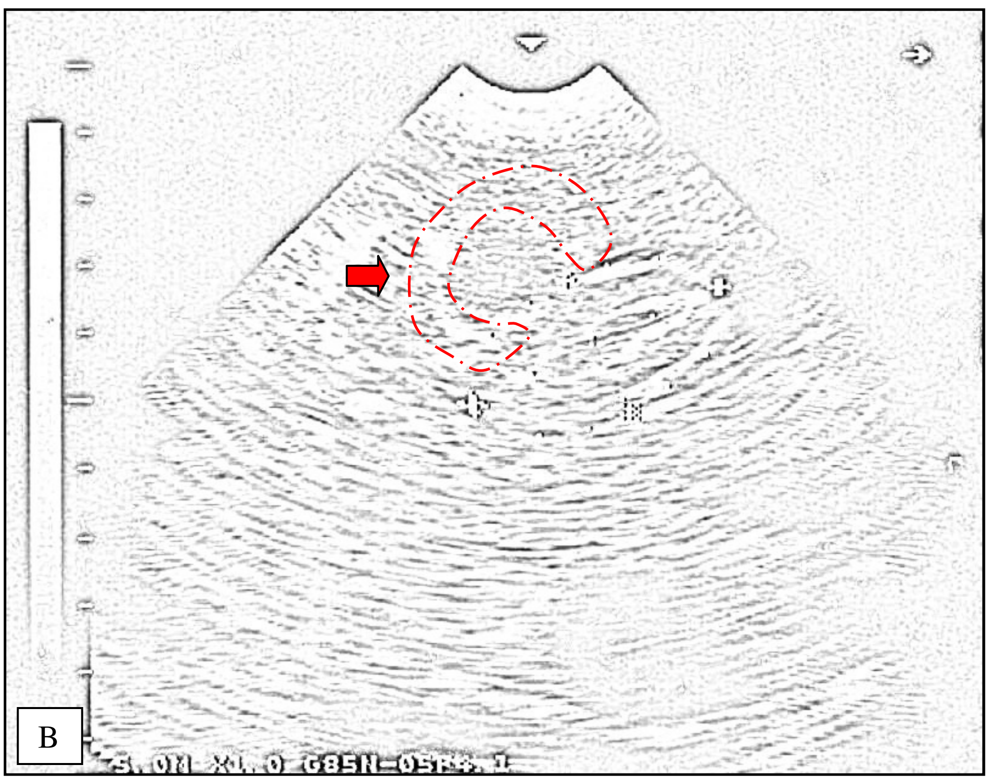

Figure 4.38: Image recorded at day 135 (week 20) of gestation. Placentome (arrow) have degraded and change in colour. A) Original image. B) Labeled image.

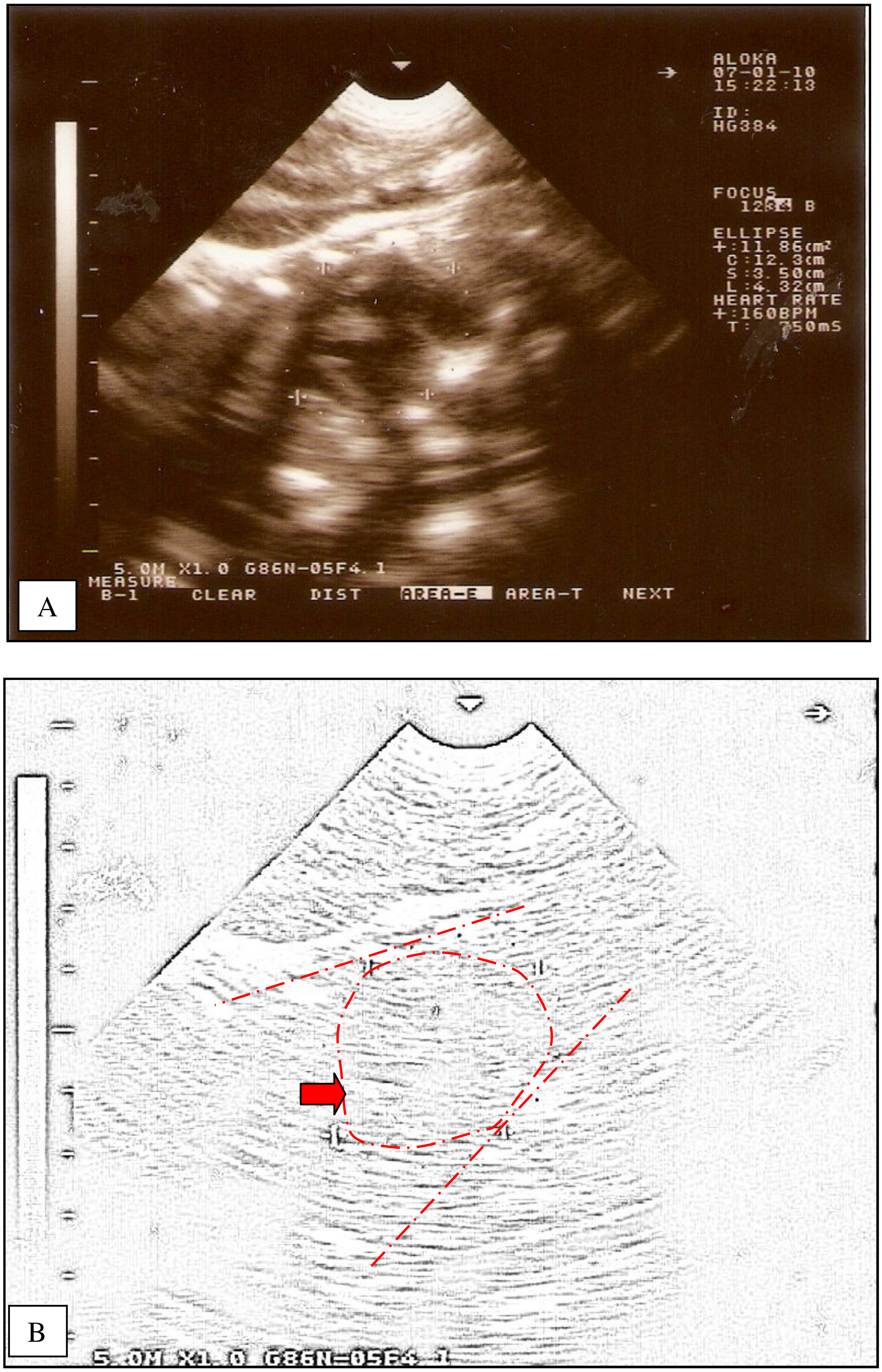

Figure 4.39: Image recorded at day 142 (week 21) of gestation. Heart achieved maximum size and appeared as slow beating non-echogenic structure.

4.5 PREDICTION OF GESTATIONAL AGE BASED ON EQUATIONS ON PLACENTOME DIAMETER AND HEART SIZE DERIVED FROM EXPERIMENTS 3 AND 4 USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 5)

4.5.1 Correlation between foetal heart development and gestational age

Out of 11 does underwent natural matings, all were diagnosed to be pregnant with 6 single and 5 twin pregnancies. Confirming the results as described in previous sections, for gestational age of less than 4 weeks, in summary, the heart appeared as an undefined shape with white-coloured structure with rapid heartbeat. In both single and twin pregnancies, the hearts grew slightly bigger and change its colour to white-grey at the age of 8 weeks. Later, at the age of weeks 9 to 12, the heart colour observed was grey and the heart size increased steadily. At the gestational age of 13 to 16 weeks, the heart started to change its colour to black, and the size increase dramatically for both single and twin pregnancy. During the 5th month of pregnancy (weeks 17 to 21 of gestation), the heart was observed as black slow-beating structures.

On the 8th week of pregnancy, the heart showed definable shape (white-grey in colour). Hence, measurements of heart size were taken on weekly basis starting from week 8 of gestation up to the delivery date. However, 2 does bearing single pregnancy aborted during the gestation period. Foetal mummification was detected in one doe, of which foetal heart and thoracic area were detected but heartbeat was absent. For both cases, measurements were only taken up to the week before abortion. To minimise technical error, heart area only was measured at a fixed angle and foetal position (Figure 4.40).

Table 4.10: Changes in foetal heart echogenicity and area throughout both single and twin pregnancy

Gestational age,x (weeks)	Heart colour	Heart size for single pregnancy (cm^2)	Heart size for twin pregnancy (cm^2)
<4	White	-	-
4<x<8	White-grey	$0.36 \pm 0.07^{a,x}$	$0.41 \pm 0.01^{a,x}$
8<x<12	Grey	$2.33 \pm 0.43^{a,x}$	$2.30 \pm 0.32^{a,x}$
12<x<16	Grey-black	$5.86 \pm 1.30^{a,x}$	$7.66 \pm 0.59^{a,x}$
16<x<21	Black	$7.90 \pm 1.22^{a,x}$	$14.09 \pm 1.24^{b,y}$

^{a,b} means with different superscripts in a row were significantly different (P<0.05).
^{x,y} means with different superscripts in a column were significantly different (P<0.05).

Figure 4.40 shows the fixed position for foetal heart measurement to be taken. Foetal heart was visualised as oval-shaped non-echogenic structure within the thoracic area. Figures 4.41 to 4.48 describe the relationship between gestational ages and measured pregnancy-related structures, namely heart area and placentomes. Correlation coefficient, R, for each relationships are 0.749 (gestational age and placentome diameter; Figures 4.41 and 4.42), 0.954 (gestational age and heart area for single pregnancy in Jermasia does; Figure 4.43 and 4.44), 0.929 (gestational age and heart area for single pregnancy in Jermasia and Boer-crossbred does; Figures 4.45 and 4.46) and 0.872 (gestational age and heart area for twin pregnancy in Jermasia does; Figures 4.47 and 4.48).

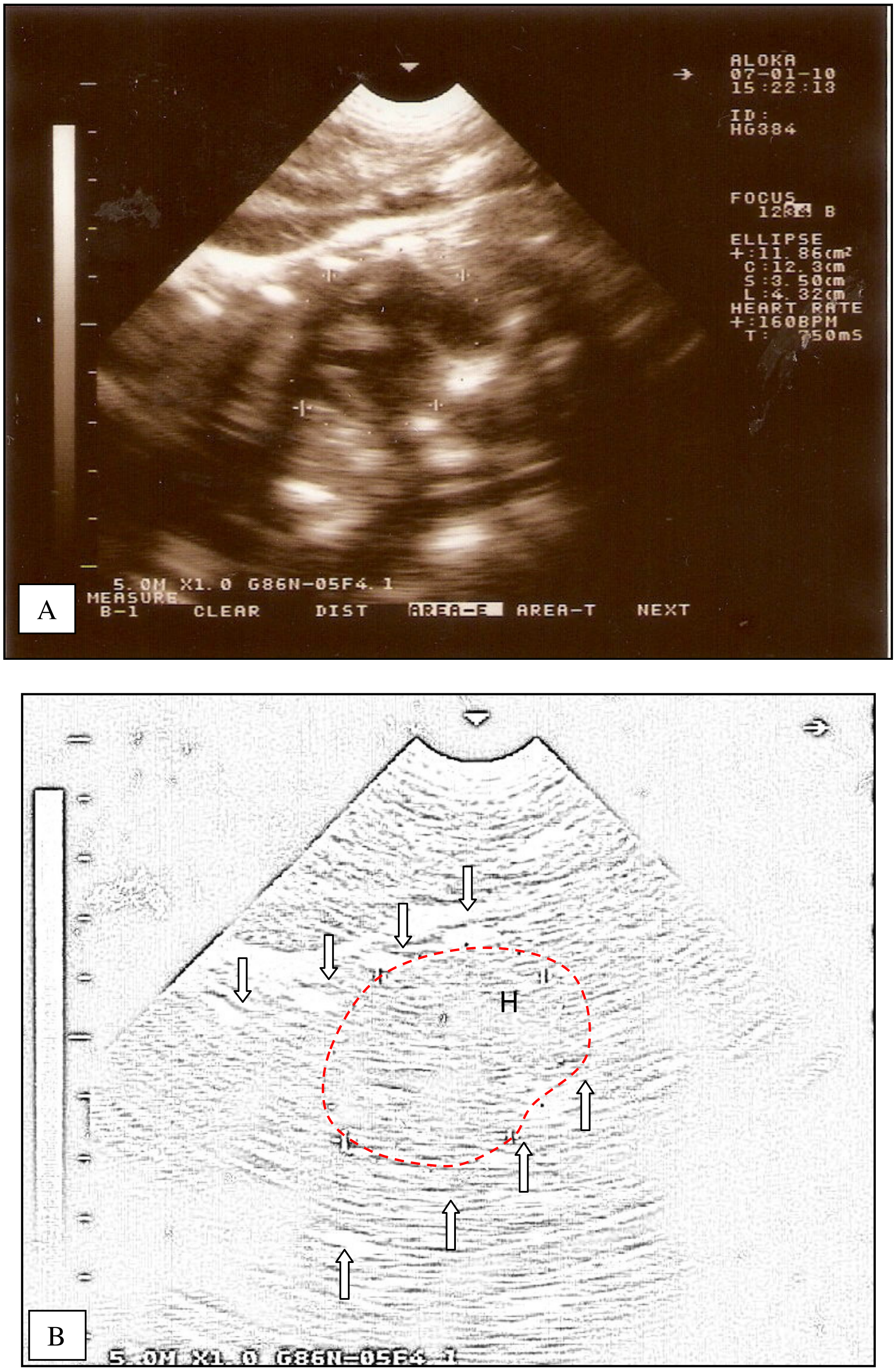

Figure 4.40: Fixed foetal position for heart measurement to be made. Foetal heart (H) appeared as non-echogenic structure between the white dots (arrows) which represent ribs. A) Original image. B) Labeled image.

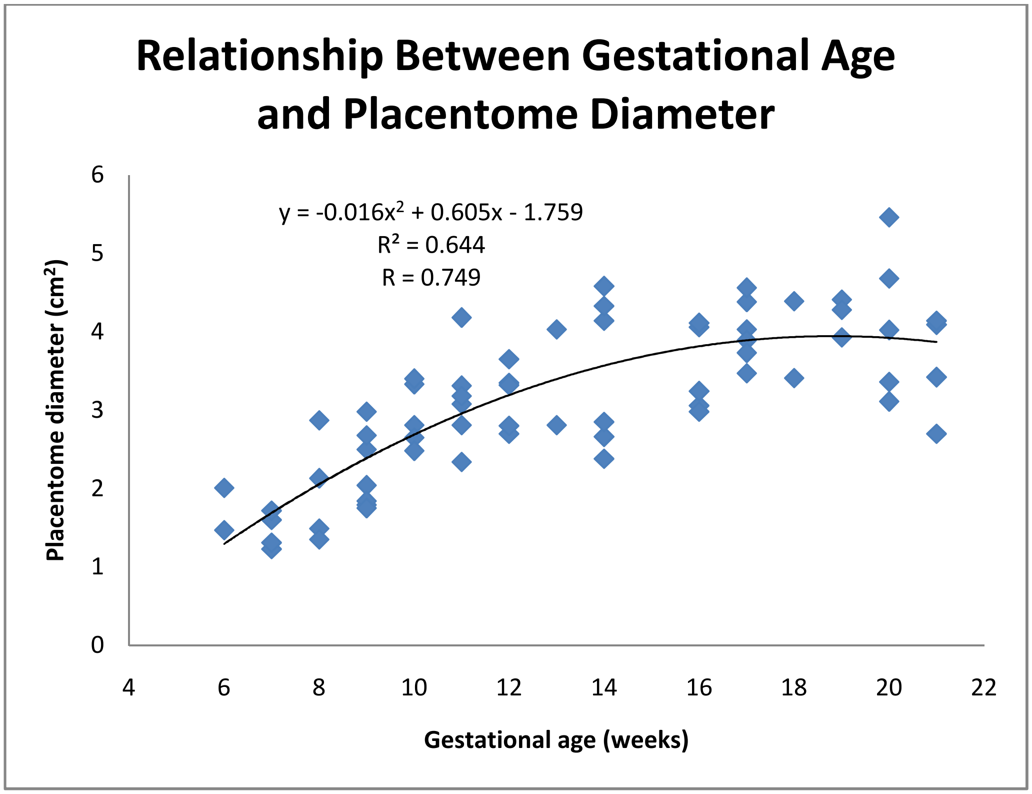

Figure 4.41: Polynomial regression between placentome diameter and gestational age in Jermasia and Boer-crossbred does.

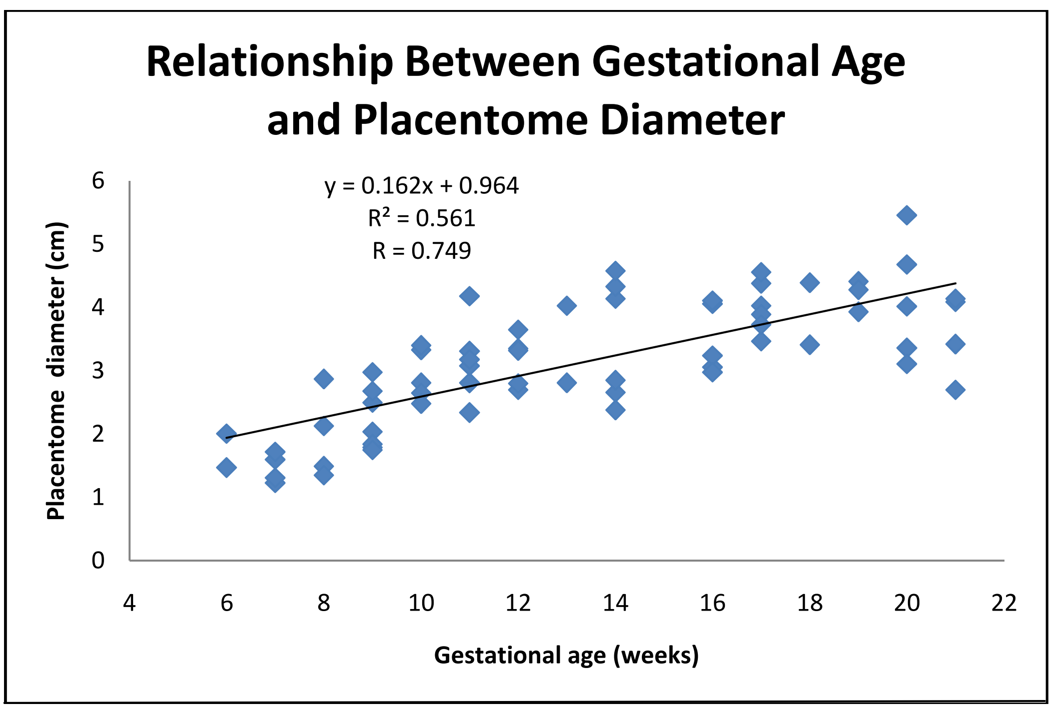

Figure 4.42: Linear regression between placentome diameter and gestational age in Jermasia and Boer-crossbred does.

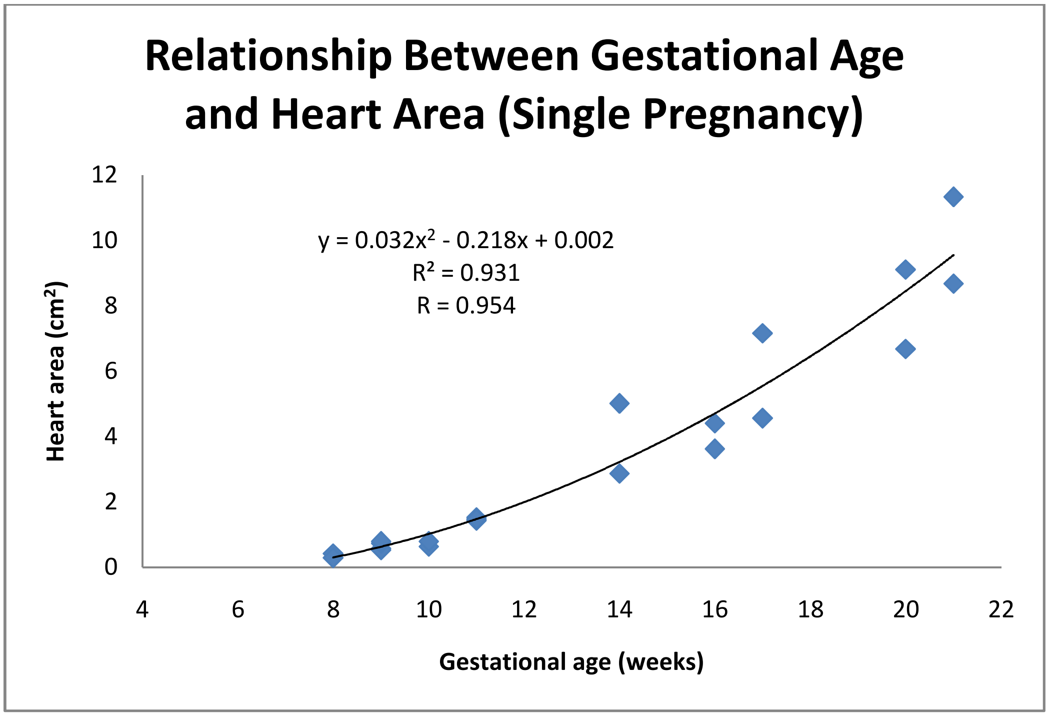

Figure 4.43: Polynomial regression between gestational age and heart area in Jermasia does (single pregnancy).

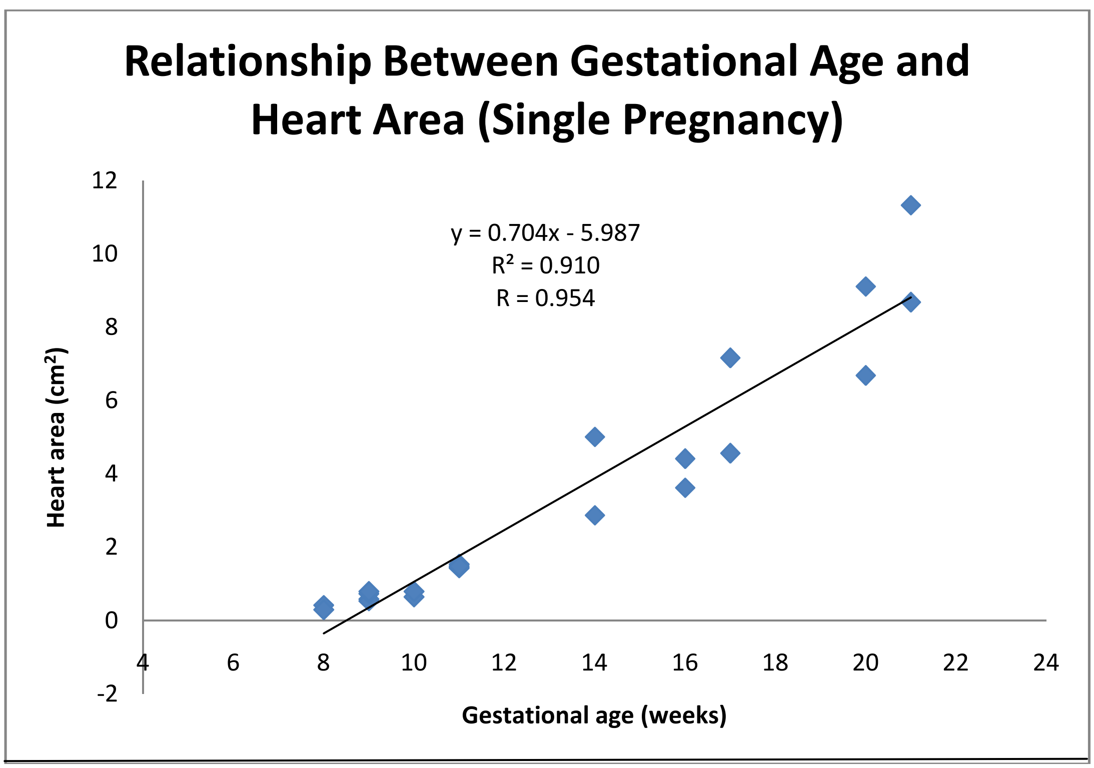

Figure 4.44: Linear regression between gestational age and heart area in Jermasia does (single pregnancy).

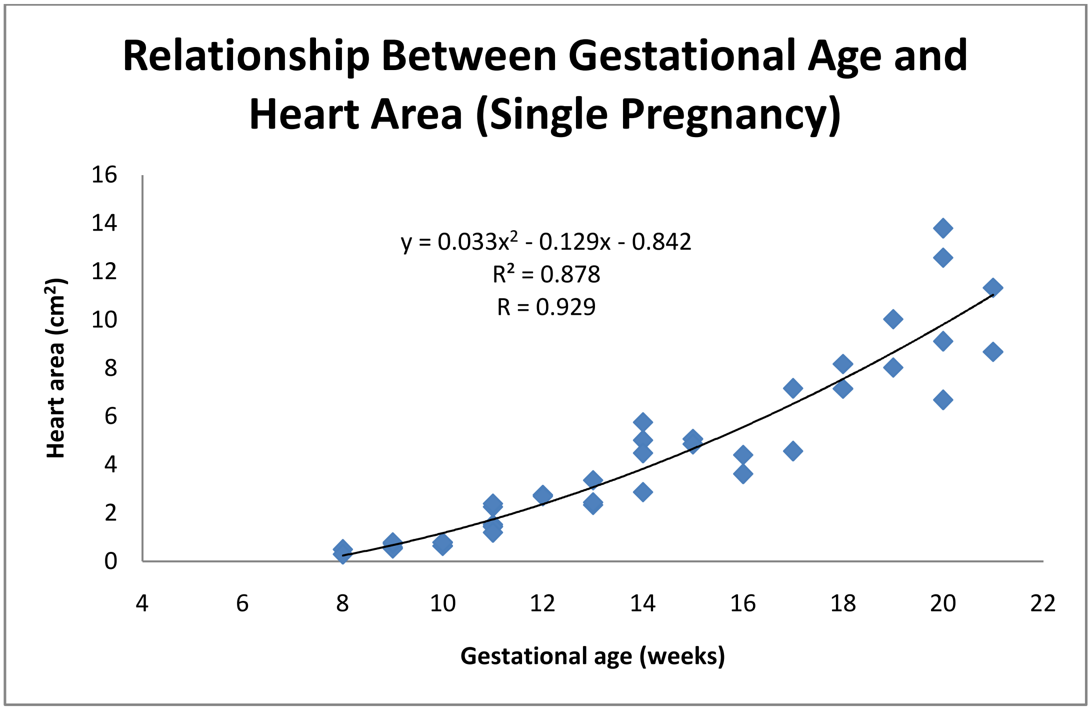

Figure 4.45: Polynomial regression between heart area and gestational age in Jermasia and Boer-crossbred does (single pregnancy).

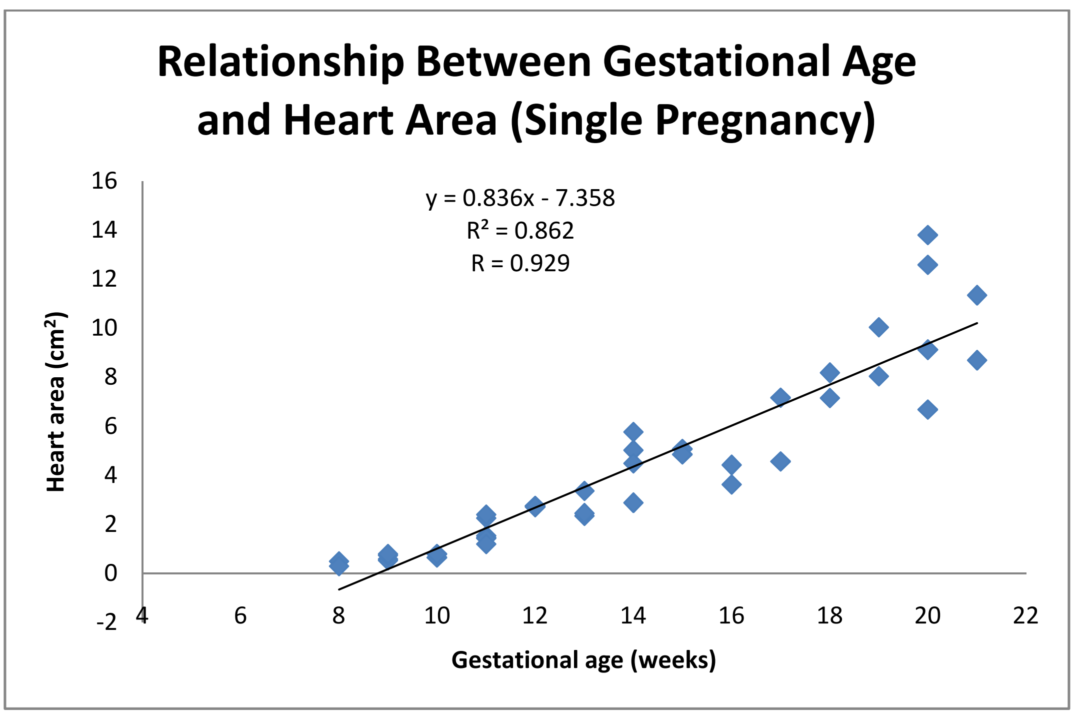

Figure 4.46: Linear regression between heart area and gestational age in Jermasia and Boer-crossbred does (single pregnancy).

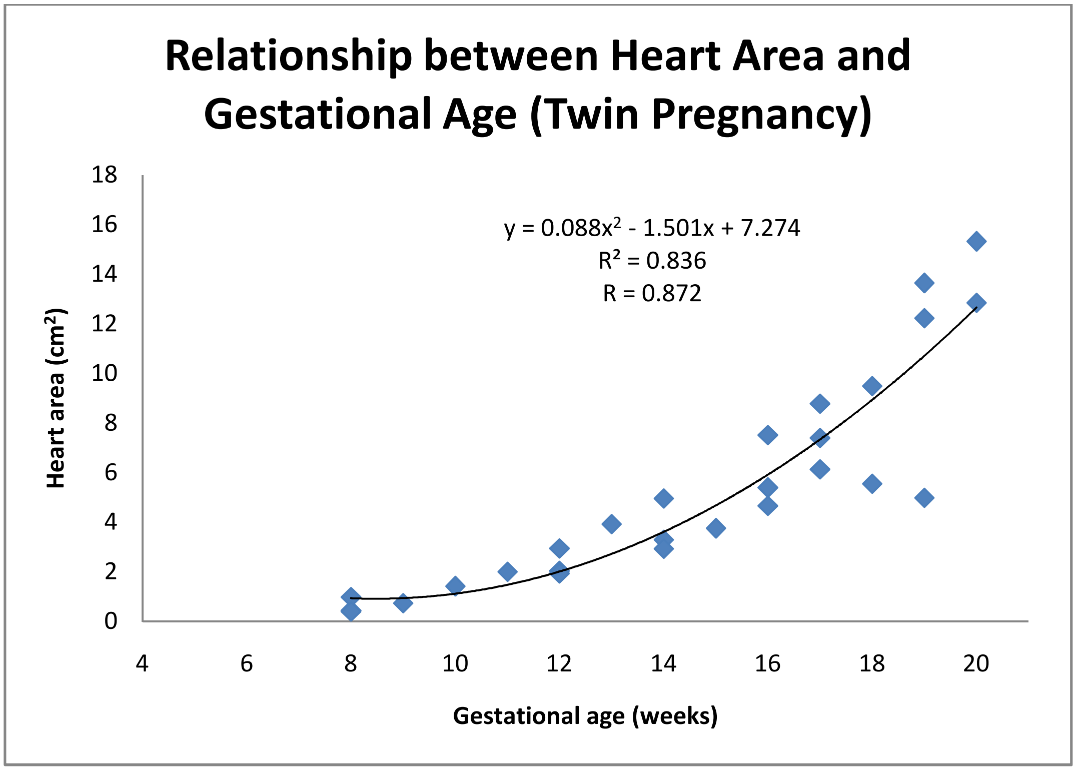

Figure 4.47: Polynomial regression between heart area and gestational age in Jermasia does (twin pregnancy).

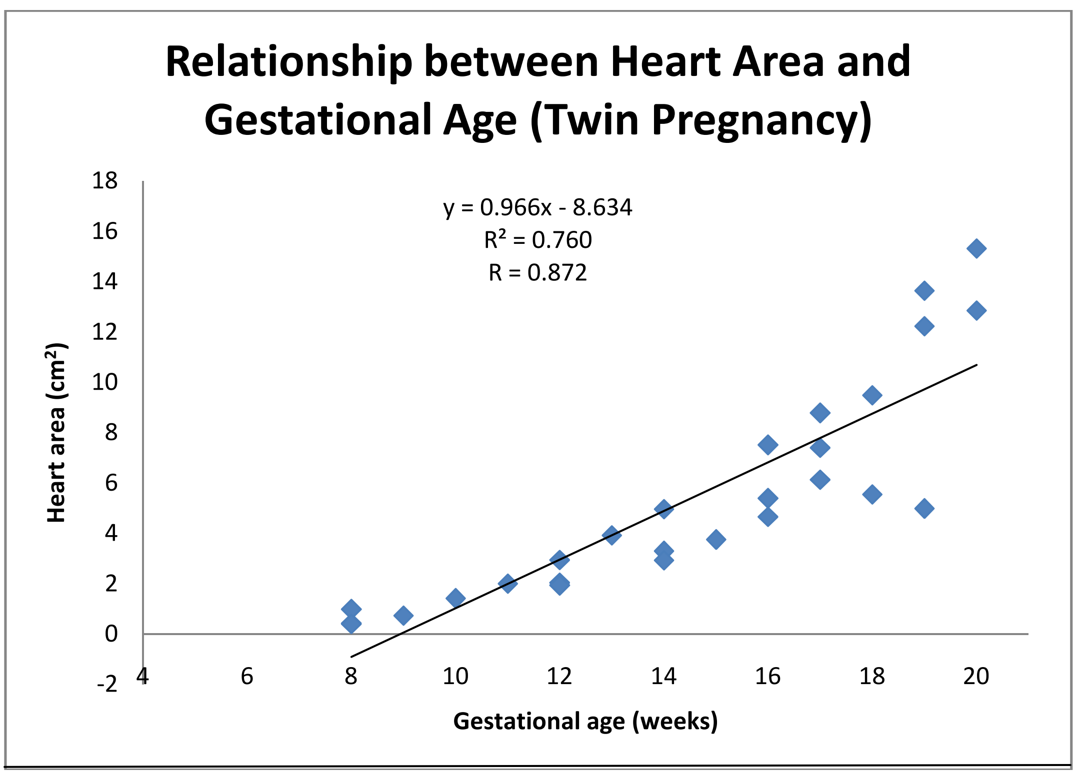

Figure 4.48: Linear regression between heart area and gestational age in Jermasia does (twin pregnancy).

4.5.2 Flock test for gestational age estimation from placentome diameter

Tables 4.11 to 4.14 describe the accuracy of gestational age estimation based on equations obtained from previous and current researches using placentome diameter and gestational age relationship; Karen *et al.* (2009) (Table 4.11) and Suguna *et al.* (2008) (Table 4.12), equations obtained from this research (Tables 4.13 and 4.14). Application of equation from Karen *et al.* (2009) gave 38% (5/13) accuracy for does kidded within 1 week time from estimated date, 38% (5/13) for 2 weeks difference and 46% (6/13) for 3 weeks different. Meanwhile, equation from Suguna *et al.* (2008) gave 31% (4/13), 38% (5/13) and 54% (7/13) accuracy respectively. Equation from current study, which based on polynomial regression, was found to be 8% (1/13), 31% (4/13) and 38% (5/13) accurate for each time-frame. Another equation, which based on linear relationship between placentome diameter and gestational age gave 8% (1/13), 23% (3/13) and 31% (4/13) accuracy for each timeframe.

Table 4.11: Accuracy of gestational age estimation based on Karen *et al.* (2009) equation using placentome diameter and gestational age relationship

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	5	5/5 (100%)	1/5 (20%)	1/5 (20%)	1/5 (20%)
Katjang	5	5/5 (100%)	3/5 (60%)	4/5 (80%)	4/5 (80%)
Saanen	3	3/3 (100%)	1/3 (33%)	1/3 (33%)	1/3 (33%)
Overall accuracy		13/13 (100%)	5/13 (38%)	6/13 (46%)	6/13 (46%)

Table 4.12: Accuracy of gestational age estimation based on Suguna *et al.* (2008) equation using placentome diameter and gestational age relationship

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	5	5/5 (100%)	2/5 (40%)	2/5 (40%)	2/5 (40%)
Katjang	5	5/5 (100%)	2/5 (40%)	2/5 (40%)	3/5 (60%)
Saanen	3	3/3 (100%)	0/3 (0%)	1/3 (33%)	2/3 (67%)
Overall accuracy		13/13 (100%)	4/13 (31%)	5/13 (38%)	7/13 (54%)

Table 4.13: Accuracy of gestational age estimation based on current research equation (polynomial regression) using placentome diameter and gestational age relationship

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	5	5/5 (100%)	0/5 (0%)	0/5 (0%)	1/5 (20%)
Katjang	5	5/5 (100%)	1/5 (20%)	4/5 (80%)	4/5 (80%)
Saanen	3	3/3 (100%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
Overall accuracy		13/13 (100%)	1/13 (8%)	4/13 (31%)	5/13 (38%)

Table 4.14: Accuracy of gestational age estimation based on current research equation (linear regression) using placentome diameter and gestational age relationship

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	5	5/5 (100%)	0/5 (0%)	1/5 (20%)	1/5 (20%)
Katjang	5	5/5 (100%)	1/5 (20%)	2/5 (40%)	2/5 (40%)
Saanen	3	3/3 (100%)	0/3 (0%)	0/3 (0%)	1/3 (33%)
Overall accuracy		13/13 (100%)	1/13 (8%)	3/13 (23%)	4/13 (31%)

4.5.3 Flock test for gestational age estimation from foetal heartbeat rate

Table 4.15: Accuracy of gestational age estimation based on Karen *et al.* (2009) equation using foetal heartbeat rate and gestational age relationship

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	3	3/3 (100%)	1/3 (33%)	1/3 (33%)	1/3 (33%)
Jermasia	7	7/7 (100%)	2/7 (29%)	3/7 (43%)	4/7 (57%)
Overall accuracy		10/10 (100%)	3/10 (30%)	4/10 (40%)	5/10 (50%)

Table 4.15 shows the accuracy of gestational age from foetal heartbeat rate using equation obtained from Karen *et al.* (2009). For delivery within 1 week from the estimated date, this equation was found to be 30% (3/10) accurate. Meanwhile, 4 out of 10 (40%) does delivered within 2 weeks difference from the estimated time. This equation was found to reach its maximum accuracy at 50% (5/10) at 3 weeks difference in delivery time.

4.5.4 Flock test for gestational age estimation from heart area

Table 4.16: Accuracy of gestational age estimation based on current research equation (polynomial regression) using heart area and gestational age relationship from Jermasia does

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	17	100%	12/17 (71%)	13/17 (76%)	16/17 (94%)
Katjang	8	100%	6/8 (75%)	6/8 (75%)	7/8 (88%)
Jermasia	4	100%	1/4 (25%)	2/4 (25%)	3/4 (75%)
Saanen	4	100%	1/4 (25%)	1/4 (25%)	2/4 (50%)
Overall accuracy		100%	20/33 (61%)	22/33 (67%)	28/33 (85%)

Table 4.17: Accuracy of gestational age estimation based on current research equation (linear regression) using heart area and gestational age relationship from Jermasia does

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	17	100%	9/17(53%)	14/17 (82%)	15/17 (88%)
Katjang	8	100%	6/8 (75%)	6/8 (75%)	7/8 (88%)
Jermasia	4	100%	1/4 (25%)	1/4 (25%)	1/4 (25%)
Saanen	4	100%	1/4 (25%)	1/4 (25%)	2/4 (50%)
Overall accuracy		100%	17/33 (52%)	22/33 (67%)	25/33 (76%)

Table 4.18: Accuracy of gestational age estimation based on current research equation (polynomial regression) using heart area and gestational age relationship from Jermasia and Boer-crossbred does

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	17	100%	10/17 (59%)	16/17(94%)	17/17 (100%)
Katjang	8	100%	6/8 (75%)	6/8 (75%)	7/8 (88%)
Jermasia	4	100%	2/4 (50%)	3/4 (75%)	4/4 (100%)
Saanen	4	100%	1/4 (25%)	2/4 (50%)	3/4 (75%)
Overall accuracy		100%	19/33 (58%)	27/33 (82%)	31/33 (94%)

Table 4.19: Accuracy of gestational age estimation based on current research equation (linear regression) using heart area and gestational age relationship from Jermasia and Boer-crossbred does

Breed	Total number scanned	Positive pregnancy accuracy (%)	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 weeks difference (Accuracy %)	Kidded within ±3 weeks difference (Accuracy %)
Boer-crossbred	17	100%	10/17 (59%)	16/17 (94%)	16/17 (94%)
Katjang	8	100%	5/8 (63%)	6/8 (75%)	6/8 (75%)
Jermasia	4	100%	1/4 (25%)	2/4 (50%)	4/4 (100%)
Saanen	4	100%	1/4 (25%)	1/4 (25%)	1/4 (25%)
Overall accuracy		100%	17/33 (52%)	25/33 (76%)	27/33 (82%)

Tables 4.16-4.19 describe the results of blind test conducted on 33 does from different breeds; 17 Boer-crossbred, 8 Katjang, 4 Jermasia and 4 Saanen does. For polynomial relationship based on data obtained from Jermasia does only (Table 4.16), a total of 20 out of 33 does (61%) delivered within 1 week from or before the estimated delivery time. Meanwhile, 67% (22/33) delivered within 2 weeks time and 85% (28/33) within 3 weeks time. Table 4.17 shows the accuracy of gestational age estimation for linear relationship based on data from Jermasia does. The equation derived gave 52% (17/33)

accuracy for delivery within 1 week difference, 67% (22/33) for 2 weeks difference and 76% (25/33) accuracy for 3 weeks difference. On the other hand, polynomial relationship between gestational age and foetal heart area based on data obtained from Jermasia and Boer-crossbred does (Table 4.18) was found to be 58% (19/33) accurate for 1 week difference, 82% (27/33) for 2 weeks difference and 94% (31/33) for 3 weeks difference. Linear relationship (Table 4.19) gave 52 (17/33), 76 (25/33) and 82% (27/33) accuracy, respectively.

Table 4.20: Accuracy of gestational age estimation based on current research equation (polynomial) using heart area and gestational age relationship from Jermasia does, when conducted during different gestational age

Actual gestational age	No. of does	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 week difference (Accuracy %)	Kidded within ±3 week difference (Accuracy %)
0-4 weeks	-	-	-	-
5-8 weeks	1	1/1 (100%)	1/1 (100%)	1/1 (100%)
9-12 weeks	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
13-16 weeks	7	5/7 (71%)	6/7 (86%)	7/7 (100%)
17-21 weeks	20	9/20 (45%)	10/20 (50%)	15/20 (75%)
Overall accuracy		20/33 (61%)	22/33 (67%)	28/33 (85%)

Table 4.21: Accuracy of gestational age estimation based on current research equation (linear) using heart area and gestational age relationship from Jermasia does, when conducted during different gestational age

Actual gestational age	No. of does	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 week difference (Accuracy %)	Kidded within ±3 week difference (Accuracy %)
0-4 weeks	-	-	-	-
5-8 weeks	1	1/1 (100%)	1/1 (100%)	1/1 (100%)
9-12 weeks	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
13-16 weeks	7	4/7 (57%)	7/7 (100%)	7/7 (100%)
17-21 weeks	20	7/20 (35%)	9/20 (45%)	12/20 (60%)
Overall accuracy		17/33 (52%)	22/33 (61%)	25/33 (76%)

Table 4.22: Accuracy of gestational age estimation based on current research equation (polynomial) using heart area and gestational age relationship from Jermasia and Boer-crossbred does, when conducted during different gestational age

Actual gestational age	No. of does	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 week difference (Accuracy %)	Kidded within ±3 week difference (Accuracy %)
0-4 weeks				
5-8 weeks	1	1/1 (100%)	1/1 (100%)	1/1 (100%)
9-12 weeks	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
13-16 weeks	7	6/7 (86%)	7/7 (100%)	7/7 (100%)
17-21 weeks	20	7/20 (35%)	14/20 (70%)	18/20 (90%)
Overall accuracy		19/33 (58%)	27/33 (82%)	31/33 (94%)

Table 4.23: Accuracy of gestational age estimation based on current research equation (linear) using heart area and gestational age relationship from Jermasia and Boer-crossbred does, when conducted during different gestational age

Actual gestational age	No. of does	Kidded within ±1 week difference (Accuracy %)	Kidded within ±2 week difference (Accuracy %)	Kidded within ±3 week difference (Accuracy %)
0-4 weeks				
5-8 weeks	1	1/1 (100%)	1/1 (100%)	1/1 (100%)
9-12 weeks	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
13-16 weeks	7	5/7 (71%)	7/7 (100%)	7/7 (100%)
17-21 weeks	20	6/20 (30%)	12/20 (60%)	14/20 (70%)
Overall accuracy		17/33 (52%)	25/33 (76%)	27/33 (82%)

Tables 4.20-4.23 provide information on accuracy of gestational age estimation in tested does at different actual gestational age. Gestational age estimation at weeks 5 to 12 gave 100% accuracy (6/6) for only 1 week difference between the estimated gestational age and the actual one. For weeks 13 to 16, polynomial equation derived from data on Jermasia does (Table 4.20) only gave 71% (5/7) accuracy for 1 week gestational age difference, 86% (6/7) accuracy for 2 weeks difference and 100% (7/7) accuracy for 3 weeks difference. Linear equation from the same data (Table 4.21) was found to be 57 (4/7), 100 (7/7) and 100% (7/7) accurate, respectively, for weeks 13 to 16 of gestation. Polynomial relationship based on data obtained from Jermasia and Boer-crossbred does

(Table 4.22) was found to be 86 (6/7) and 100% (7/7) accurate for 1 week and 2 weeks difference, respectively, at weeks 13 to 16 of gestation. Linear relationship (Table 4.23), on the other hand, gave 71 (5/7) and 100% (7/7) accuracy for respective gestational age. Reduction in accuracy was observed in all results obtained from the four equations on weeks 17 to 21 of gestation.

4.5.5 Summary of accuracy of gestational age estimation using different pregnancy-related structures

Table 4.24: Summary of accuracy for gestational age estimation using equations from previous researchers and current findings

Pregnancy-related structures	Equation from previous research		Equation from current research	
	Equation	Accuracy (%)	Equation	Accuracy (%)
Placentome	$y = -0.0031x^2 + 0.8131x - 18.172$ Where; y : Placentome diameter (mm) x : gestational age (days) (Karen *et al.,* 2009)	46% (n=13)	$y = -0.016x^2 + 0.605x - 1.759$ Where; y : Placentome diameter (cm) x : gestational age (weeks)	38% (n=13)
	$y = 42.5x – 5.2$ Where; y: gestational age (days) x: placentome diameter (cm) (Suguna *et al.,* 2008)	54% (n=13)	$y = 0.162x + 0.964$ Where; y : Placentome diameter (cm) x : gestational age (weeks)	31% (n=13)
Heart area size	-	-	Polynomial regression (Equations derived from data obtained from Jermasia does only): For single pregnancy, $y = 0.032x^2 - 0.218x - 0.002$ y : Heart area (cm^2) x : gestational age (weeks) For twin pregnancy $y = 0.088x^2 - 1.501x + 7.274$ y : Heart area (cm^2) x : gestational age (weeks)	85% (n=33)

(continued)

(continued)

Pregnancy-related structures	Equation from previous research		Equation from current research	
	Equation	Accuracy (%)	Equation	Accuracy (%)
Heart area size	-	-	Linear Regression (Equations derived from data obtained from Jermasia does only): For single pregnancy, $y = 0.704x - 5.987$ y : Heart area (cm^2) x : gestational age (weeks) For twin pregnancy $y = 0.966x - 8.634$ y : Heart area (cm^2) x : gestational age (weeks)	76% (n=33)
			Polynomial regression (Equations derived from data obtained from Jermasia and Boer-crossbred does): For single pregnancy, $y = 0.033x^2 - 0.129x - 0.842$ y : Heart area (cm^2) x : gestational age (weeks)	94% (n=33)

(continued)

(continued)

Pregnancy-related structures	Equation from previous research		Equation from current research	
	Equation	Accuracy (%)	Equation	Accuracy (%)
Heart area	-	-	Linear Regression (Equations derived from data obtained from Jermasia and Boer-crossbred does): For single pregnancy, $y = 0.836x - 7.358$ y : Heart area (cm^2) x : gestational age (weeks)	82% (n=33)
Heartbeat rate	$Y = -0.4415x + 236.24$ Where; Y : FHB (bpm) X : gestational age (days) (Karen *et al.,* 2009)	50% (n=10)	-	-

Table 4.24 summarises all the blind tests conducted in current research according to pregnancy-related structures (Tables 4.9 - 4.17). Estimated gestational age is considered as accurate if the doe delivers within 3 weeks difference from the estimated time, which is less than one month difference of the actual deliver date, an acceptable time period for farm level management. Gestational age estimated from placentome diameter using equation derived by Suguna *et al.* (2008) was found to be 54% accurate and 46% accurate using equation derived by Karen *et al.* (2009). Two equations were derived from this experiment based on linear and polynomial relationship of gestational age and foetal heart area. Both linear and polynomial equations gave 31 and 38% accuracy, respectively. Equation which relates foetal heartbeat with gestational age (Karen *et al.,* 2009) was tested to be 50% accurate. On the other hand, for data obtained from Jermasia does alone, equation obtained from linear and polynomial regression of foetal heart size for gestational

age estimation gave 85 and 88% accuracy, respectively. Meanwhile, for data obtained from Jermasia and Boer-crossbred does, linear and polynomial equation gave 81 and 92% accuracy, respectively.

Chapter 5

5.0 DISCUSSION

Chapter 5

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5.1 INTRODUCTION

Pregnancy detection in goat is crucial for farm production management. Ruminant breeders in developed countries have applied ultrasound scanning to assist their breeding programme owing to its reliability. Unfortunately, majority of goat farm in Malaysia applied pregnancy diagnosis which based on physical evaluation and oestrus observation which is practically unreliable. This is due to lack of information on its application at farm level in Malaysia. Besides that, there is also paucity on research of ultrasound scanning in small ruminant pregnancy diagnosis. Therefore, this experiment was carried out with aims to determine the most reliable and easy to access indicator for pregnancy in Malaysian local goats, as well as to establish equation for gestational age estimation using transrectal and transabdominal probes. Ultimately, a flock test was conducted on groups of goat from different breeds, ages, and management system to validate the reliability of equation obtained.

5.2 OBSERVATION ON FOETAL STRUCTURES AND RELATED IMAGES IN REPRODUCTIVE SYSTEM OF DOES USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 1)

Images of foetus and related structures such as sac, non-echogenic area, foetal heart, foetal head, amniotic fluid, ribs, and placentomes were obtained using both transrectal and transabdominal probes. Following that, the images were arranged according to days of pregnancy. The aim of this study was to identify the pregnancy-related structures which can be detected throughout pregnancy period; specificity and time-frame for detection. The data obtained from this experiment will be used to determine which pregnancy-related structures to be focused on its development evaluation in Experiments 3 - 4. Generally, pregnancy-related structures detected were in agreement with other researchers (Martinez *et al.,* 1998; Medan *et al.,* 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009; Raja Ili Airina, 2009).

Allantoic fluids or embryonic fluid which appeared in form of circular and elongated non-echogenic sacs located in utero cranial of the bladder was acknowledged as the first indicator for initial and early stage of pregnancy (Doize *et al.,* 1997; Martinez *et al.,* 1998; Suguna *et al.,* 2008; Karen *et al.,* 2009). Doize *et al.* (1997) suggested that the borders (white line) grew thinner as the embryonic vesicles developed. This structure was very easy to access and visualise at the early stage of pregnancy.

In current experiment, embryonic vesicles were detected from days 29 - 45 and days 29 - 48 of gestation, respectively, using transrectal and transabdominal probes. Suguna *et al.* (2008) reported that vesicles were detected on day 21 and proper embryo on day 28 of gestation via transrectal approach. Meanwhile, embryonic vesicles were detected on day 28 of gestation via transabdominal approach. In another research, Karen *et al.* (2009) found that embryonic vesicles was first detected using transrectal and transabdominal probes on approximately days 17 and 28 of gestation, respectively. The delay of embryonic sacs detection between current experiment and previous findings could be attributed to the method used for image collection. In the present experiment, images were taken randomly and gestational age was determined retrospectively after obtaining delivery dates (approximately 147 days). On the other hand, earlier research studies involved recording the images at fixed time duration after mating. The latter approach will be applied in Experiments 3 and 4 for more accurate evaluation.

The mere presence of fluid in the uterus is not the only indication of pregnancy, because of the possibility of hydrometra in goats (Hesselink and Tavern, 1994). Therefore, detection of the foetus is required for the confirmatory diagnosis of pregnancy (Suguna *et al.,* 2008). In this experiment, foetus with heartbeat was detected on day 29 of gestation using both transrectal and transabdominal probes. Other researchers reported detection of foetus days 21 (Martinez *et al.,* 1998; Suguna *et al.,* 2008), 23 (Padilla-Rivas *et al.,* 2005) and 24 of gestation (Medan *et al.,* 2004). Martinez *et al.* (1998) recorded heartbeat rate and detection from days 21 to 40 of gestation using transrectal probe. This is in agreement with current finding which recorded heartbeat from days 29 to 44 of gestation via transrectal route. Meanwhile, heartbeat was detected on days 29 to 144 of gestation via transabdominal probe. Suguna *et al.* (2008) reported detection of heartbeat between days 35 to 130 of gestation. The differences might be attributed to the effect of breed of does and model of ultrasound machine used. In the present study, ultrasound scanning was conducted on Boer-crossbred does using ALOKA SSD 500 ultrasound machine, while Pie Medicals ultrasound machine was used by Suguna *et al.* (2008) on unstated breed of does.

In this experiment, placentomes were detected on days 44 to 110 and days 44 to 144 of gestation with transrectal and transabdominal probes, respectively. Doize *et al.* (1997) reported detection of placentomes between days 30 to 90 of gestation via transrectal probe. After day 90 of gestation, the placentomes were difficult to be viewed due to the increase in foetal size and other pregnancy-related structures. This is in agreement with Suguna *et al.* (2008) who detected placentomes up to day 98 of gestation. Placentomes were detected up to later days of gestation using transrectal probe in this experiment might owe to the lifting of abdomen during scanning which was earlier found to improve the accuracy in ewes (Karen *et al.,* 2004). Karen *et al.* (2009) found that placentomes could be detected at range days 28 to 130 of gestation using transrectal and transabdominal probes alternately.

The first skeletal structure viewed in this experiment is the foetal head on days 45 to 60 of gestation using transrectal probe and days 60 to 66 of gestation using transabdominal probe. Other skeletal structures were observed a few days later but the images were not recorded. Suguna *et al.* (2008) first viewed skeletal structures including foetal head, rib cage and vertebral column on day 56 of gestation. On the other hand, Hussein (2008) identified foetal head of Egyptian Baladi goats at approximately day 40 of gestation and Karen *et al.* (2009) first viewed foetal head on approximately day 30 of gestation using transrectal probe. The measurement of foetal head diameter also known as bi-parietal diameter (BPD) is routinely performed in human prenatal examination and estimation of age, however, not much report have been reported on ruminants especially goats (Lee *et al.,* 2005).

Based on the results, stages of pregnancy were categorised into monthly basis. In the present study, transrectal probe was shown to be more suitable for the first 3 months of pregnancy as compared to the later stage (months 4 to 5 of gestation). Most of the pregnancy-related structures only could be measured up to the third month of gestation (day 90 of gestation). Meanwhile, transabdominal probe was found to be effective starting from the second month of gestation until delivery date. Earlier research studies focused on early pregnancy evaluation which includes pregnancy age and foetal sex (Haibel, 1988; Doize *et al.,* 1997; Martinez *et al.,* 1998; Gonzalez de Bulnes *et al.,* 1998; Gonzalez *et al.,* 2004; Medan *et al.,* 2004; Santos *et al.,* 2006). As shown from current result, the diagnosis using transrectal probe only accurate until up to the third month of gestation.

Only a few researchers (Padilla-Rivas *et al.,* 2005; Maico *et al.,* 2007; Suguna *et al.,* 2008; Karen *et al.,* 2009) applied transabdominal probe in goat pregnancy diagnosis despite of its advantages which are quicker, more convenient and time saving than transrectal method (Padilla-Rivas *et al.,* 2005). This shows paucity in pregnancy diagnosis via transabdominal ultrasonography. Hence, further research is of highly important to optimise the potential of transabdominal probe in goat pregnancy diagnosis. Data obtained from current finding will be a guide for identification and evaluation of pregnancy-related structures in Experiments 3 and 4.

Throughout this experiment, transabdominal probe was able to detect embryonic sacs, foetus and heartbeat during the later part of the first month of gestation (day 29 of gestation). Similar findings were reported by Medan *et al.* (2004). However, Padilla-Rivas (2005) reported that heartbeat was detected on day 27 of gestation using 5.0 MHz transabdominal probe. Suguna *et al.* (2008) and Karen *et al.* (2009), in their studies, reported that embryonic vesicle and embryo proper was detected on days 28, 35, 28 and 31 of gestation using the 5.0 and 3.5 - 5.0 MHz transabdominal probe, respectively. However, Medan *et al.* (2004) reported that no pregnancy-related structure was detected at gestational age earlier than 30 days. The differences of the finding may be due to the different positioning of probe and different wavelengths and equipment used. As mentioned by Karen *et al.* (2009), whether breed differences is account for this variable results, it is open to conjecture. Nevertheless, transabdominal probe proves to be more practical and appropriate to detect later stage of pregnancy, specifically from days 50 to 147 of gestation.

In this experiment, the objective was to develop criteria for pregnancy check in does using transrectal and transabdominal probes. This experiment is the preliminary study for the main aim of this research. In other words, only foetus and related structures were recorded without paying attention to the details such as sizes of these structures. Therefore, future studies (Experiments 3 and 4) would involve such aspects so that information on the relationship between foetal development and gestation age could further be elucidated.

5.3 GESTATIONAL AGE ESTIMATION BASED ON ANALYSIS OF PREGNANCY-RELATED STRUCTURES IN DIFFERENT BREEDS OF GOATS BASED ON CHARACTERISTICS DERIVED FROM EXPERIMENT 1 (EXPERIMENT 2)

Chronologically arranged structural images obtained from confirmed pregnancy based on specific pregnancy-related structures as suggested in Experiment 1 were used as an attempt to estimate age of foetus using 7.5 MHz transrectal and 5.0 MHz transabdominal probes. Other than detecting pregnant and non-pregnant does, the need to estimate age of gestation is vital (Raja Ili Airina, 2009). Since no specific measurement was made on pregnancy-related structures in Experiment 1, gestational age estimation was based on detection of pregnancy-related structures and its rough description especially on size (small, medium and big) and echogenicity. Out of 119 does with unknown breeding status involved in this study, 26 (22%) were detected to be pregnant after being scanned. However, only 25 delivers, while the other one was later confirmed as false pregnancy. Therefore, the data was not included for determination of gestational age estimation accuracy. The diagnosis of the remaining 93 non-pregnant does was found to be 100% accurate from delivery date.

In present study, the ultrasonographic presentation of the foetus at the time of examination is considered as decisive factor. The incorrect position of foetus, obstructing the visualisation of anatomic structures for gestational age estimation, impairs ultrasound scanning especially in multiple pregnancies (Hussein, 2008). Padilla-Rivas *et al.,* (2005) reported that the size of doe would affect the time of detection for pregnancy-related structures of which detection of foetal heartbeat was only possible at day 34 of pregnancy in one doe they examined, which was very big in size (weighed 74 kg). Meanwhile, the size of foetal parts is observed as variable, depending on foetal number. However, the pattern of echogenicity changes in pregnancy-related structures in current research was almost similar among the breeds studied.

As for relationship between gestation age and pregnancy-related structures developed for Boer crossbred (Experiment 1), the criteria could be applied to all the breeds of goats studied. However, in multiple pregnancies, the diagnosis related to age tended to be inconsistent. From the results of this study, it is suggested that changes in echogenicity of pregnancy-related structures and general classification of their respective sizes derived from Boer crossbred does are probably applicable as indicators in determining gestational age in other breeds of goat, especially for single pregnancy. However, for more accurate diagnosis, more in depth study should be done especially on the exact measurement of pregnancy-related structures and different correlation is suggested to explain the difference between single and multiple pregnancies.

Hasselink and Taverne (1994) stressed the potential role of real time B-mode ultrasound for detecting pathological conditions such as pyometra, hydrometra or foetal death. In this experiment, one false positive pregnancy diagnosis was done in one Saanen doe. In that one particular doe, big non-echogenic areas were observed, leading to gestational age estimation of one month. However, from follow up ultrasound scanning conducted on that particular doe, no foetus or any other pregnancy related structures was detected. Besides that, the doe did not deliver on the estimated date. This finding indicates the importance of detecting foetus with proper heartbeat as the prime indication of pregnancy rather than depending on the fluid detection alone. This is in agreement with previous researchers suggestions (Martinez *et al.,* 1998; Suguna *et al.,* 2008).

Foetuses were considered to be at certain stage of gestation based on size and echogenicity of pregnancy-related structures especially placentomes. The increase in placentome diameter with advancement in gestation may be an adaptation to provide nourishment to the growing fetus (Suguna *et al.,* 2008). Several researchers found high correlation between the gestational age and placentome diameter (Doize *et al.,* 1997; Suguna *et al.,* 2008). On the contrary side, Lee *et al.* (2005) reported a lower correlation ($r = 0.574$); which could be ascribed to the variation in placentome size, their location, breed, etc. As pregnancy progresses, the placentome increases in size and appears as a C- or O-shaped gray image (Medan *et al.,* 2004; Lee *et al.,* 2005), which is in accordance with the present findings.

Reviewing the literature, it is noted that no report were available on relationship between heart echogenicity and gestational age in goat. Previous researches which relate heart with gestational age focused more on heartbeat rate (Martinez *et al.,* 1998; Suguna *et al.,* 2008; Karen *et al.,* 2009) of which they observed a declining heartbeat rate with advancement of pregnancy. However, they differ in foetal heartbeat rate counting method; Karen *et al.* (2009) used M-mode ultrasound to estimate the frequency of heart beats, while stopwatch technique was used in study of Martinez *et al.* (1998) and Suguna *et al.* (2008). In present research, foetal heartbeat rate was not measured since the objective is more on qualitative rather than quantitative study. In the meantime, heart echogenicity appeared to change as pregnancy progress; from echodense (white in colour) to non-echogenic (black in colour). From the flock test (Experiment 2), this criterion gave fairly accurate result, suggesting the potential of further research to be done (Experiments 3 and 4).

5.4 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSRECTAL PROBE (EXPERIMENT 3)

This experiment was conducted to obtain specific qualitative evaluation of pregnancy-related structures in pregnant does using 7.5 MHz linear array transrectal probe. Out of 11 (8 Jermasia and 3 Boer-crossbred) does underwent breeding programme, 11 were confirmed to be pregnant and were subjected to transrectal ultrasonography on weekly basis starting from day 21 of gestation until delivery date. Despite the fact that the introduction of a rectal probe may cause slight discomfort to the doe, no injury or damage is inflicted if the animals are steadied by a helper and the probe is handled gently (Padilla-Rivas *et al.,* 2005). In this experiment, the ultrasound scanning session was conducted gently and no injury was observed on the animals.

Previous researchers detected embryonic vesicles using transrectal probe on days 17 to 22 of gestation (Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009). In this experiment, embryonic vesicle was firstly detected in all does at day 23 of gestation (week 4 of gestation). The delay might due to the effect of breed and operator's lack of experty in conducting early stage pregnancy diagnosis. The vesicles with diameter of approximately 1 cm could be observed at 12-3 o'clock direction from the bladder. With reference to the results from present and previous researches, it seems that detection of sac using ultrasonography represents the first indicator of pregnancy in does.

Therefore, it is logical to assume that the initiation and formation of sac is in the second to third week of gestation (Raja Ili Airina, 2009).

Detection of non-echogenic area representing amniotic fluid area, uterine fluid accumulation and allantoic fluid was reported to be on days 17 to 19 of gestation (Martinez *et al.,* 1998; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Karen *et al.,* 2009). Nevertheless, in this experiment, non-echogenic area was detected later together with foetus, that is on day 23 of gestation (week 7 of gestation). The later detection in this research could probably due to different breeds, localities and other factors between this research and those of previous researchers.

Certainly, early diagnosis of gestation is accepted to be highly accurate when the embryo and its heartbeats are detected (Martinez *et al.,* 1998). In this study, presence of foetal heartbeat was detected on week 4 of gestation (days 21-28 of gestation). This is in agreement with that reported by Medan *et al.* (2004) (days 24.3 of gestation), Padilla-Rivas *et al.* (2005) (days 22.9 of gestation) and Karen *et al.* (2009) (days 22.36 of gestation). Even so, Martinez *et al.* (1998) reported earlier detection of foetal heartbeat, which was on days 20.7 of gestation (week 3 of gestation). Thus, it is suggested that weeks 3 to 4 of gestation are the best time period for early stage foetal viability confirmation using transrectal probe.

In current research, foetal heart was detected up until week 10 of gestation using transrectal probe. Measurement of foetal heart was only could be made starting from week 8 of gestation. Current experiment found that the colour of foetal heart changes from white to white-grey starting from week 8 of gestation, giving defined shape of the heart. Before week 8 of gestation, the heart size was found to be too small, which was close to resolution limit of the transducer, making it very difficult to be measured, which is in agreement with

Martinez *et al.* (1998). The maximum day of foetal heart visibility using transrectal probe in current research is less as compared to Suguna *et al.* (2008) who detected foetal heart up to day 98 of gestation (week 14 of gestation). The difference in detection time may due to the difference in probe frequency; Suguna *et al.* 2008 used 6.0 MHz transrectal probe which has farther penetration but less clear visualisation, which is in contrast as compared to the 7.5 MHz transrectal probe used in current research. As pregnancy advances, it was difficult to observe the foetus transrectally, as the uterus reaches a more cranio-ventral abdominal position.

First active movement of foetus was detected on day 35 of gestation (week 5 of gestation). Below that age, foetus was observed to be in static position with rapid heartbeat together with umbilical cord. To the best of author knowledge, this is the first report which discusses the time foetal move actively. This information will help in determining foetal age without any measurement to be made; small size foetus with no active movement would be considered to be in weeks 3 to 4 of gestation. Moreover, detection of foetus will assist in foetal counting at early gestation stage, before the size of foetus increased. Finding from this research suggested weeks 5 to 7 of gestation as the most appropriate time for foetal counting using transrectal probe. This is because at this time duration, individual foetus moves and could be visualised on the viewing screen. Suguna *et al.* (2008) reported same finding with current research. Furthermore, Padilla-Rivas *et al.* (2005) suggested that the best time for foetal counting is from weeks 4-6 of gestation (days 28-40 of gestation).

Placentome was first detected on day 36 of gestation (week 6 of gestation). It appeared as small C-shaped echodense structure. Meanwhile, Suguna *et al.* (2008) firstly detected placentome on day 42 of gestation. However, most researchers have detected placentomes via transrectal and transabdominal ultrasonography by days 25–40 of gestation as a small nodule to white circular structure with a echodense centre (Haibel, 1990; Doize *et al.,* 1997; Medan *et al.,* 2004, Karen *et al.,* 2009) some of which seems to be a little earlier than in the current study (day 36 of gestation). Even though Doize *et al.* (1997) reported detection of placentome in does as small nodules on day 32 of gestation, the authors as well reported that placentome developed as C-shaped structure on day 42 of gestation, which is in agreement with current research finding timeline. From the present author's experience, placentome is the easiest pregnancy-related structure to be detected throughout pregnancy owing to its unique structure and many in number. In ruminants such as cattle, sheep and goat, there are around 75-125 placentomes (Bowen, 2000).

Throughout gestation, the size of placentome increased. The increase in placentome diameter with an advancement of gestation may be an adaptation to provide nourishment to the growing foetus (Suguna *et al.,* 2008). The validity of this observation is confirmed by earlier findings of placentome development throughout gestation (Doize *et al.,* 1997; Lee *et al.,* 2005; Suguna *et al.,* 2008). Those researchers reported correlation between gestational age and placentome diameter. Further analysis of foetal measurement will be discussed in Experiment 5. Unlike earlier research studies, current research managed to detect placentome until the delivery date even though the detection became gradually difficult as pregnancy progress. Earlier researchers reported that placentomes only could be measured up to days 90 to 100 of gestation (Doize *et al.,* 1997; Suguna *et al.,* 2008) which could be due to the variation and difficulty in imaging the distended uterus (Doize *et al.,* 1997). In current experiment, the difficulty was solved by lifting the doe abdominal part when pregnancy scanning was conducted.

As pregnancy progresses, the placentome increases in size and appears as a C- or O-shaped gray image (Lee *et al.,* 2005), which is in accordance with the current findings. During the last month of pregnancy, placentomes were found to be less echogenic, while its attached surface appeared more echointensive. By this time, it started to collapse with reducing its lumen, while in areas adjacent to the foetus it appeared flattened. Collapsing of the placentome might be an indication for occurrence of a degenerative process (Grunert, 1980; Bjorkman and Dantzer, 1987). On the other hand, these changes in the placentomes may be associated with increased placental perfusion and tissue permeability during late pregnancy (Metcalfe *et al.,* 1988).

In current research, bony structures especially foetal were found to start differentiated from week 6 (day 38) of gestation. Figure 4.17 shows the differentiated foetal head, legs and trunk. Earlier than this age, foetus was observed as an elongated echodense structure with rhythmic pulsation of heart in the middle, located within an echogenic sac (Figures 4.15 and 4.16). Foetal head and bony structures were accessible until week 10 of gestation. Beyond this age, it was impossible to view the image as a whole because the images became distorted. This finding is similar to those reported by Ali and Hayder (2007) who detected ossification in foetal head, ribs and vertebrae in sheep at approximately day 45, 50, and 52 of gestation, respectively. Karen *et al.* (2009) firstly identified foetal head at day 30 of gestation, while Suguna *et al.* (2008) at day 56 of gestation. The difference might due to the breed studied as well as frequency of probe used.

5.5 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSABDOMINAL PROBE (EXPERIMENT 4)

This experiment was designed similar to that of Experiment 3, except it was carried out using 5.0 MHz transabdominal probe. Chronologically arranged structural images were obtained along pregnancy period as an attempt to evaluate foetal development. A total of 11 does were confirmed to be pregnant and their foetal development was studied extensively.

Most of the earlier research studies focused on application of transrectal probe (Buckrell *et al.,* 1986; Doize *et al.,* 1997; Martinez *et al.,* 1998; Gonzalez *et al.,* 2004; Karen *et al.,* 2004) instead of transabdominal probe for pregnancy diagnosis. A few researchers studied the comparison between both probes throughout pregnancy period (Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009). The preference on studies of transrectal rather than transabdominal probe is due to their advantage on early stage pregnancy diagnosis. Transrectal probe detected pregnancy-related structures 4 to 7 days earlier compared to transabdominal probe (Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009). On the downside, transrectal probe only reliable for pregnancy-related structures detection up until mid-stage of pregnancy (approximately month 3 of gestation) (Buckrell *et al.,* 1986; Doize *et al.,* 1997; Martinez *et al.,* 1998; Gonzalez *et al.,* 2004; Karen *et al.,* 2004; Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009). This is in preference to the advantages of transabdominal probe; that is longer detection time.

In current research, transabdominal probe detected non-echogenic areas at week 4 of gestation. However, foetus and heartbeat only were observed on one week later (week 5 of gestation) compared to transrectal probe which detected all three structures (non-echogenic area, foetus and foetal heart) at week 4 of gestation. The detection time of non-echogenic areas in this current research is in agreement with those reported by previous researchers (Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009). The detection time for foetus with foetal heartbeat in current experiment was week 5 of gestation, which is is in agreement with Karen et al. (2009) who detected the structures on approximately day 30 of gestation (week 5 of gestation), but a bit later than Padilla-Rivas *et al.* (2005), who detected the structures on approximately at the range of day 23 to 29 of gestation (approximately week 4 of gestation). Even though there is difference between current results and previous research findings in term of non-echogenic area, foetus and heartbeat detection time, the difference was only within 1 week, and most probably subjected to the different breeds of doe studied as well as technical expertise of the operator.

In this experiment, application of transabdominal probe for foetal counting was found to be highly reliable between weeks 5 to 10 of gestation. The animals were designated as bearing twins when two heads and/or two beating hearts and/or two bodies were recognised (Abdelghafar *et al.,* 2007). After week 10 of gestation, foetus still could be detected, but its big size may hinder the detection of the other foetus. Geol and Agrawal (1992) found that it was difficult to differentiate between twins, triplets or quadruplets at any stages of gestation. This point is solidified by Abdelghafar *et al.* (2007) who reported that the accuracy of transabdominal ultrasonography in determining singles, twins and triplets in Saanen goats was 88.2, 77.7 and 50%, respectively, when ultrasonography was conducted at random time throughout gestation. On the other hand, in ewes, Karen *et al.* (2001) reported that the accuracy of ultrasound in detecting two foetuses or more was disappointing. Current research finding suggests that to obtain accurate foetal number using transabdominal probe, ultrasound scanning on pregnant doe should be conducted between weeks 5 to 10 of gestation.

Foetal heartbeat is the best indicator for foetal viability. Foetal death in pregnancy was characterised by the lack of foetal movement or heartbeat, and there was often an associated lack of defined structures or non-echogenic area (Padilla-Rivas *et al.,* 2005). One foetal death was observed in this experiment at day 98 of gestation (week 14 of gestation) which was confirmed by abortion on approximately 4 days later. For this condition, foetal trunk, thoracic area and other pregnancy-related area were detected, but not foetal heartbeat. Earlier research studies (Haibel, 1990; Padilla-Rivas *et al.,* 2005) reported that in spite of the repeated exposure of the foetuses to ultrasonic waves, no foetal death or abortion occurred and all the kids born were morphologically normal and viable. Foetal death as reported in current research most probably due to nutritional condition, of which the particular aborted doe was quite small in size. Despite of the loss due to foetal death, this finding solidified the advantage of ultrasound scanning in evaluating foetal condition throughout pregnancy. It would seem important to study the extent and timing of embryonic and foetal wastage in view of their negative impact on reproductive performance (Martinez *et al.,* 1998).

Foetal heart was detectable from week 5 of gestation until delivery date using transabdominal ultrasound. Nevertheless, measurement of foetal heart only could be made starting from week 8 of gestation, of which the heart became less echodense and gave defined shape. No literature on foetal heart development (area/size) throughout pregnancy period was found by author. As pregnancy advances, the foetal heart increased in size and showed declining heartbeat rate. The declining pattern in heartbeat rate is in agreement to those reported in earlier literature (Martinez *et al.,* 1998; Suguna *et al.,* 2008; Karen *et al.,* 2009). The exact value and measurement of foetal heart size will later be discussed in Experiment 5.

Measurement of foetal heart area was taken at a fixed angle (Figure 4.40) to minimise variation. Different angle would give different image of the pregnancy-related structure. This is supported by finding by Azevedo *et al.* (2009) who reported different accessibility of foetal organ visualisation for foetal sexing from different plane of scanning, suggesting that one specific angle is needed for one specific image visualisation. In addition, placentome also give two different shape when imaged from different angle; O-shaped when viewed in a longitudinal section or a regular C-shaped in cross section (Karen *et al.,* 2009). Hence, the need for certain angle of foetal heart imaging by ultrasound scanning is verified.

Current research firstly detected placentome on day 36 of gestation (week 6 of gestation). This finding is earlier to those reported by Suguna *et al.* (2008), who reported first detection of placentome on day 50 of gestation (week 8 of gestation), using 5 MHz sector array transducer. The frequency of probe used in current experiment is the same with Suguna *et al.* (2008), but the model of ultrasound machine is different. From the present author's experience, placentome is the easiest pregnancy-related structure to be detected throughout pregnancy owing to its unique structure and many in number. In agreement to observation in Experiment 3, placentomes were found to be less echogenic during the later stage of pregnancy.

Previous research studies reported that placentomes were detectable up until day 130 to 140 of gestation (Suguna *et al.,* 2008; Karen *et al.,* 2009). Current research study found that the placentomes could be visualised up until delivery day which is approximately day 147 of gestation. At this age, placentomes were observed to be highly packed on the viewing screen. It was a bit difficult to detect the placentome at the later stage of pregnancy since the foetal body has become very big and may block the placentomes. Nevertheless, current research study overcomes this problem by massaging and adjusting the abdominal part of the doe to align the foetus position with the probe image spectrum. For placentomes measurement which will be further described in Experiment 5, only the largest placentomes on the viewing screen were measured.

On the other interesting aspect, foetal sexing, current research reported detection of scrotum between weeks 10 to 14 of gestation in 4 does. Scrotum could be observed as an echodense semi-hemisphere structure positioned between two hind legs of the foetus (Figure 4.29). All foetuses with scrotum detected were confirmed to be male after delivery of the kids. In one doe bearing twin, one of its kid was male and the other was female. Scrotum was not detected in the remaining 7 does due to the difficulty to get the right angle for visualisation. Out of the 7 does, 4 gave birth to female kids and 3 delivered male kids. Earlier research studies focused on evaluation of the final tubercle for foetal sexing (Santos *et al.,* 2006; Azevedo *et al.,* 2009). With positive male diagnosis, a bilobar hyperechoic structure appeared near or in close proximity to the umbilicus. Whereas, for a positive female diagnosis, this structure was absent on scanning this region (Inomata *et al.,* 1982; Azevedo *et al.,* 2009). Unfortunately, due to lack of this specific technical skill, foetal sexing in this experiment was only based on scrotum.

Applying 5.0 MHz transabdominal probe, current research found that bony structures, especially foetal head (Figure 4.26), were found to start differentiated from week 7 of gestation. These structures were accessible up to week 13 of gestation. This finding is in agreement with Abdelghafar *et al.* (2007), who detected foetal head from days 46 to 95 of gestation (weeks 7 to 14 of gestation). As pregnancy advances, these bony structures became more ossified and increased in size, making it difficult to be viewed as a whole on the screen. This limitation is also caused by compression of the foetal head by other foetal parts (Abdelghafar *et al.,* 2007). Similar difficulties in viewing foetal head during the late stage were found in llama (Haibel and Fung, 1991) and during the later part of pregnancy in ungulate species (Place *et al.,* 2002). These findings are the solid evidence of limitation of foetal head as gestational age indicator throughout the whole gestational age; gestational equations derived from foetal head measurement could be used confidently but only within a limited gestation age.

Evaluating all the accessible pregnancy-related structure, current research suggests that two of them are suitable to be used as gestational age indicator; heart area size and placentome diameter. Even though combination of pregnancy-related structure could be used as gestational age indicator (Raja Ili Airina, 2009), as they were accessible at specific time of pregnancy, quantification through specific measurement is more reliable. Accessibility of foetal heart and placentome throughout pregnancy fulfills the criteria for the suitable gestational age indicator. Earlier research studies discussed on the reliability of placentome (Doize *et al.,* 1997; Martinez *et al.,* 1998; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008; Karen *et al.,* 2009), but this research is the first to relate foetal heart area size with gestational age. Detailed explanation will be described in Experiment 5.

5.6 PREDICTION OF GESTATIONAL AGE BASED ON EQUATIONS ON PLACENTOME DIAMETER AND HEART SIZE DERIVED FROM EXPERIMENTS 3 AND 4 USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 5)

Specific measurements on placentome diameter foetal and heart area size were taken throughout gestation of 11 pregnant does from Jermasia and Boer-crossbred does using transrectal and transabdominal probes starting from first detection time. Placentome measurement was made only on the largest placentomes in the scanning field. Meanwhile, measurement of heart area was made by taking as oval shape of possible of the heart at a specific angle (Figure 4.40). Graphs showing relationship between both pregnancy-related structures and gestational age were plotted to study the growth pattern.

Figures 4.41 and 4.42 describe the polynomial and linear relationships, respectively, between placentome diameter and gestational age. Throughout pregnancy period, placentome diameters were observed to increase steadily until week 18 of gestation. Beyond that age, the increase in size was shown to have stopped. Collapsing of placentomes at late stage of pregnancy as earlier described in Experiments 3 and 4 explains the growth pattern on this pregnancy-related structure. On the other hand, both polynomial and linear relationships give low coefficient of determination; $R^2 = 0.644$ and $R^2 = 0.561$, respectively. The higher value of R^2 for polynomial regression as compared to linear regression may due to the actual development which resemble polynomial pattern. Both coefficient of determination value obtained in this experiment are higher than those reported by Lee *et al.* (2005) ($R^2 = 0.33$) but lower than those reported by Doize *et al.* (1997) ($R^2 = 0.70$) and Karen *et al.* (2009) ($R^2 = 0.86$).

In current research, placentome has shown medium correlation (R = 0.749) with gestational age. Meanwhile, Suguna *et al.* (2008) reported that placentome diameter showed a significant high correlation (R = 0.99) with gestational age. The difference may due the different type of breeds used in respective studies.

The current study is the first that incorporate heart area size as a biometric index predicting gestational age in does. Figures 4.43 to 4.48 describe the development of foetal heart size throughout pregnancy. The graph plotted shows polynomial function as observed on the rapid development of heart size starting from the week 8 of gestation, suggesting that this criterion is a reliable indicator for pregnancy age during that period. Each graph shows polynomial and linear relationship based on data obtained either from Jermasia breed only (Figures 4.43 and 4.44) or combination of Jermasia and Boer-crossbred (Figures 4.45 and 4.46), as well as twin pregnancy (Figures 4.47 and 4.48). The value of coefficient of determination, R^2, in polynomial regression in Jermasia and combination breed of does (R^2 = 0.931, 0.878, respectively) was found to be slightly higher than those in linear regression (R^2 = 0.910, 0.862, respectively). The polynomial growth pattern of the foetal heart during pregnancy may be the cause for this difference. For twin pregnancy (polynomial vs. linear; R^2 = 0.836 and R^2 = 0.760, respectively), the relationship was based on data from Jermasia does since no such pregnancy detected in Boer-crossbred does in this study.

Flock test was conducted to evaluate the reliability and accuracy of regression equations derived from current research. For placentome diameter, equations derived in earlier researchers (Suguna *et al.,* 2008; Karen *et al.,* 2009) were tested as well as control. Equations from both research studies relate placentome diameter with gestational age in days, while current research relates the measurement with gestational age on weekly basis.

Therefore, the estimated gestational age using equations from the previous researchers was converted into week by considering days 1 to 7 of gestation as week 1 of gestation, and so forth. Details of the equations are described in Table 5.1. Gestational age estimation was considered to be highly accurate if the doe delivers within 1 week difference from the estimated delivery date. For academic purposes, time-frame for maximum achievable accuracy was studied.

Table 5.1: Details of equations used for gestational age relationship estimation from placentome diameter

Researcher	Year	Types of regression	Equation	R and/or R^2 value
Suguna *et al.*	2008	Linear	$y = 42.5x - 5.2$ Where; y: gestational age (days) x: placentome diameter (cm) n = 6	*R^2 value was not given R = 0.99
Karen *et al.*	2009	Polynomial	$y = -0.0031x^2 + 0.8131x - 18.172$ Where; y : Placentome diameter (mm) x : gestational age (days) n = 15	$R^2 = 0.86$ *R value was not given
Current author	2011	Linear	$y = 0.162x + 0.964$ Where; y : Placentome diameter (cm) x : gestational age (weeks) n = 11	$R^2 = 0.561$ R = 0.749
Current author	2011	Polynomial	$y = -0.016x^2 + 0.605x - 1.759$ Where; y : Placentome diameter (cm) x : gestational age (weeks) n = 11	$R^2 = 0.644$ R = 0.749

From the flock test, gestational age estimation was found to achieve its maximum accuracy within 3 weeks difference. Application of equation from Suguna *et al.* (2008) gave 31% (4/13) accuracy for does kidded within 1 week time from estimated date, 38% (5/13) for 2 weeks difference and 54% (7/13) for 3 weeks different (Figure 4.12). Meanwhile, equation from Karen *et al.* (2009) gave 38% (5/13), 46% (6/13) and 46% (6/13) accuracy respectively (Figure 4.11). Equation from current study, which was based on polynomial regression, was found to be 8% (1/13), 31% (4/13) and 38% (5/13) accurate for each timeframe (Figure 4.13). Another equation, which was based on linear relationship between placentome diameter and gestational age, gave 8% (1/13), 23% (3/13) and 31% (4/13) accuracy for each time-frame (Figure 4.14).

Current research shows that polynomial relationship based on data obtained from current research between placentome diameter and gestational age gives higher accuracy as compared to linear relationship; maximum accuracy = 38% and 31%, respectively. On the contrary, control equations from earlier research studies indicate that linear relationship (Suguna *et al.,* 2008) gives better accuracy than polynomial relationship (Karen *et al.,* 2009); maximum accuracy = 54% and 46%, respectively. Control equations give higher accuracy than those obtained from current research. From author's opinion, the difference may be due to the method of which placentome measurement was taken as well as breed effect.

In all research studies (Doize *et al.,* 1997; Martinez *et al.,* 1998; Suguna *et al.,* 2008; Karen *et al.,* 2009), measurement was taken on only the largest available placentome on the viewing screen, which was taken on random basis, instead of the specific one throughout pregnancy. The high number and random location of placentome attached on the chorio-allantois membrane (Bowen, 2000) making it impossible to evaluate the development of one specific placentome throughout pregnancy. The same difficulties occurred during flock test when identifying placentome for gestational age estimation; the placentome may be the largest on the viewing screen, but not on the whole chorio-allantois membrane. This limitation caused the low accuracy in gestational age estimation even though current research agreed with previous studies that placentome diameter increased with advancement of pregnancy.

Tables 4.16 - 4.19 describe the results of flock test conducted on 33 pregnant does using heart area size as indicator for gestational age estimation. They were divided into their respective breeds to determine its effect on gestational age estimation accuracy. Estimation was considered to be highly accurate, if the doe delivers within 1 week difference from the estimated delivery date, considering the individual physiology variation. Throughout pregnancy, the equations derived in current research was found to be fairly accurate for 1 week difference in delivery date, and achieve its maximum accuracy within 3 weeks difference in delivery time. Both polynomial relationships obtained from Jermasia does alone (Table 4.16) and combination of Jermasia and Boer-crossbred does (Table 4.18) gave higher maximum accuracy for 3 weeks difference time for actual delivery as compared to those of linear regression (Tables 4.17 and 4.19); 85 and 94% vs. 76 and 82%, respectively. This result is supported by the actual polynomial pattern showed by foetal heart throughout pregnancy (Figures 4.43 - 4.48). As explained earlier, no previous literature has been found on foetal heart development, thus it is believed that current research is the first to evaluate this new aspect of pregnancy-related structure. Combination of breed (Jermasia and Boer-crossbred) was found to slightly improve the accuracy of gestational age estimation equation.

In reference to breed effect, Boer-crossbred does give high maximum accuracy when the polynomial equations obtained from Jermasia and breed combination were applied, 94 (16/17) and 100% (17/17), respectively. In addition, linear regression as well gave high accuracy for both cases, 88 (15/17) and 94% (16/17), respectively. This finding indicates the suitability and reliability of the equation on Boer-crossbred does. Katjang and Jermasia breed were found to give lower maximum accuracy, but still higher compared to those of Saanen breed. The difference number of does tested for each breed might influence the variation. Nevertheless, earlier research studies suggested a specific predicting chart for each goat breed (Haibel,1990; Karen *et al.,* 2009).

The effect of goat breed was supported by the variation of correlation of determination (R^2) in bi-parietal diameter and gestational age reported by different researcher. Karen *et al.* (2009) found that foetal bi-parietal diameter in Egyptian Baladi goats was highly significant in correlation of determination ($R^2 = 0.956$) with the gestational age between days 30 and 105 of gestation. Similar results were reported by Haibel (1988) in dairy goats between days 39 and 100 and by Reichle and Haibel (1991) in pygmy goats (small sized breed) between days 36 and 102 of gestation. On the other hand, Lee *et al.* (2005) reported a lower correlation of determination ($R^2 = 0.65$) in Korean black goats between days 60 and 135 of gestation. As discussed earlier, bi-parietal diameter was not studied in current research due to its limitation, which could only be applied at narrow spectrum of gestational age. Meanwhile, heart area was measured and gave R^2 value of 0.931, 0.910, 0.878 and 0.862 for equation from data obtained from polynomial regression (Jermasia doe), linear regression (Jermasia doe), polynomial regression (Jermasia and Boer-crossbred doe) and linear regression (Jermasia and Boer-crossbred doe), respectively.

Boer-crossbred, Jermasia and Katjang breeds used in flock test of current experiment have almost similar body shape, especially the kids although the size of the adults is different. On the contrary, Saanen breed, which is the dairy goat has longer body (neck to tail) as compared to the other breed used. This may explain the similarities and differences in accuracy between the breeds. Equations derived from current research was shown to give high accuracy in Boer-crossbred, Katjang and Jermasia goats, but fairly accurate in Saanen goats. For future improvement, more samples are needed for flock test in order to solidify current finding.

In order to evaluate the reliability of equations based on heart area size derived from current research at different gestational age, tested does were divided into groups of five gestational stages (Tables 4.20 - 4.23); weeks 1 to 4, 5 to 8, 9 to 12, 13 to 16, and 17 to 21 of gestation. From the results, all equations gave maximum accuracy of 100% (13/13) for gestational age of weeks 5 to 16 of gestation; the does delivered latest within 3 weeks from the estimated time. Polynomial and linear relationships derived based on data from Jermasia does alone gave 85 (11/13) and 77% (10/13) accuracy, respectively, for the delivery within 1 week from the estimated time, for diagnosis between weeks 5 to 16 of gestation. On the other hand, polynomial and linear relationships derived from data combination of goat breeds were found to be 92 (12/13) and 85% (11/13) accurate, respectively, for the delivery within 1 week from the estimated time, for diagnosis between weeks 5 to 16 of gestation. The high accuracy obtained from this flock test validates the reliability of foetal heart area as criterion for gestational age estimation during weeks 5 to 16 of gestation.

In the meantime, gestational age estimation based on foetal heart area size at weeks 17 to 21 of gestation was found to be fairly accurate. Polynomial and linear regression based on data from Jermasia does gave maximum accuracy of 75 (15/20) and 60% (12/20), respectively. Meanwhile, polynomial and linear regression based on combined data from Jermasia and Boer-crossbred does were found to be 90 (18/20) and 70% (14/20) accurate for 3 weeks delivery date difference. As for delivery time within 1 week difference, the equations gave 45 (9/20), 35 (7/20), 35 (7/20) and 30% (6/20) accuracy, respectively. This finding shows inconsistent accuracy for does at the last month of pregnancy. Even so, for application purpose, it is highly advisable to compliment the echogenicity characteristic of foetal heart to confirm the doe is at fifth month of pregnancy, since it appear as slow beating oval-shaped non-echogenic area at this age, rather than depending on the measurement alone.

Among all four equations derived from current research, polynomial regression of data from Jermasia and Boer-crossbred does was found to be the most reliable. Its accuracy for one week delivery time difference, 58% (19/33), is the second highest, but its maximum accuracy, 94% (31/33), is the highest as compared to the others. Polynomial regression of data from Jermasia does alone gives the highest accuracy for 1 week delivery time difference, 61% (20/33), but its maximum accuracy was only 85% (28/30). Addition in sample number in the earlier equation seems to have improved its maximum accuracy. In current research, the number of goat available for breeding was limited. Therefore, in future research, more samples should be included in establishing the relationship to ameliorate its reliability especially at the last month of pregnancy.

Another flock test attempted to test the reliability of foetal heart rate in determining gestational age (Table 4.15). Skills for heartbeat rate using M-mode ultrasound scanning was only obtained by operator after several weeks parameters measurement has started. Therefore, relationship between heartbeat rate and gestational age could not be obtained in this research. Nevertheless, equation obtained from Karen *et al.* (2009) was used in this study to serve this purpose. From the flock test, the equation gave accuracy of 30% (3/10) for 1 week delivery time difference and maximum accuracy of 50% (5/10) for 3 weeks delivery time difference. For prospective research study, it is highly suggested for this parameter to be evaluated and tested.

Comparing the accuracy between placentome diameter and heart area size as criterion for gestational age estimation, heart area size was found to be more accurate. Results based on equation from Suguna *et al.* (2008) which used placentome diameter for gestational age estimation gave 31% (4/13) accuracy for 1 week difference in delivery time, and maximum accuracy of 54% (7/13) for 3 weeks difference in delivery time. Polynomial regression of placentome diameter in current experiment gave 8 (1/13) and 38% (5/13), respectively. On the contrary, as discussed earlier, polynomial regression of heart area size of Jermasia and Boer-crossbred does gave 58 (19/33) and 94% (31/33) accuracy, respectively, which is way higher than those using placentome diameter. The large number of placentome (Bowen, 2000) as compared to the specific one heart for each foetus explains this difference. Nevertheless, as forethought, it is advisable to evaluate the placentome size as well as its echogenicity to corroborate the gestational age estimation together with foetal heart measurement.

5.7 GENERAL DISCUSSION

When the date of mating is unknown, estimation of the gestational age in small ruminants is desirable for reproductive management to dry off the lactating females at appropriate time, observe the female near term (Doize *et al.,* 1997) and to induce kidding (Karen *et al.,* 2009). Knowledge on growth and development of specific foetal structures at specific gestation stage could be exploited both for these purposes. Scientifically, formation of foetal structures such as heart, spinal cord and ribs at specific stage of gestation could increase the information anatomy and physiology of foetus during pregnancy. Practically, the exact age of pregnancy could be confirmed as day 0 resulted from AI or natural mating by knowing the foetal structures during gestation age.

In human pregnancy detection using ultrasound, scientific calculations produced indexes and matrix formula that instantly estimate the age of foetus. A correlation was seen between gestational age, bi-parietal diameter, femoral length and renal length measurement in human foetus (Ansari *et al.,* 1997; Konje *et al.,* 2002; Zalel *et al.,* 2002). Therefore, this approach could be designed for goat. The information obtained from such studies may bring to a better discovery on the anatomy and physiology of goat foetal development as well as practical application of pregnancy detection which will be beneficial to the goat industry.

Present study has successfully identified foetal heart as a reliable indicator for gestational age estimation. It is found that polynomial relationship between foetal heart area size and gestational age give acceptable accuracy when tested on flock from different goat breeds, gestational age and farm management. Moreover, as discussed earlier, foetal heart was found to be less echogenic as pregnancy advances. This visible change in heart colour (white to black) will complement in more accurate estimation. In comparison with placentome diameter, study of foetal heart is more reliable owing to its specificity; only one heart for one foetus, but 75 – 125 placentomes within one pregnancy). Confirmation of foetal number, foetal viability and foetal age are the obvious advantages of foetal heart evaluation as compared to other pregnancy-related structures.

One difficulty faced during the study was the availability for does to undergone breeding programme for weekly data collection (Experiments 3 and 4). Besides that, logistic management (incomplete farm records) of the farm have caused lost of birth records of some of the does tested in Experiments 5; only birth date of 33 out of 43 diagnosed does were recorded. Due to that, the data for the remaining 10 does were discarded in current research. Therefore, in future research, it is highly suggested that more does to be used to study the relationship between the parameters, as well as better farm record to avoid the waste of sample. Besides that, correlation between foetal heart area, foetal heartbeat rate, kids' birth weight and gestational age is believed to breakthrough finding in future research.

Real time B-mode ultrasonography has been used for pregnancy diagnosis in goats (Dawson *et al.,* 1994; Doize *et al.,* 1997; Martinez *et al.,* 1998; Gonzalez *et al.,* 2004; Medan *et al.,* 2004; Suguna *et al.,* 2008; Karen *et al.,* 2009). Unfortunately, information on the best pregnancy-related structure that was as well tested to give high gestational age estimation accuracy in goat is scarce in the literature. Earlier research studies focused on establishment of relationship between pregnancy-related structures and gestational age at early to mid stage of pregnancy; not much information was obtained on its accuracy when tested at farm level. Furthermore, in earlier research studies, gestational age was sometimes deliberately estimated based on kidding date retrospectively (Raja Ili Airina, 2009). In this study, an attempt was made to establish relationship between the most accessible pregnancy-related structures with gestational age. In addition to that, a flock test was conducted on does from different breed and farm management to validate the reliability of the equations at farm level.

In the present study, preliminary study (Experiment 1) was conducted at Kambing Baka Baik Kepala Batas Farm (KBKB) which located 300 km away from the University of Malaya. Upon identification of pregnancy-related structures, flock test was conducted as preliminary test for their accuracy (Experiment 2). Following that, specific evaluation and measurement on pregnancy-related structures were conducted on weekly basis at Institute of Biological Sciences (ISB) Mini Farm (Livestock), University of Malaya, using both transrectal and transabdominal probes (Experiments 3 and 4). Ultimately, equations based on the most accessible pregnancy-related structures (placentome diameter and heart area size) were derived and tested on does with unknown mating status at ISB Mini Farm and Rumpun Asia Sdn. Bhd. Farm (RASB), Batang Kali, Selangor.

Transrectal and transabdominal probes were more practical in months 1 to 3 and late month 1 to 5 of gestation, respectively. The earliest pregnancy-related structure in current research was detected 4 to 7 days earlier using transrectal probe, as compared to transabdominal probe. Difference in probe frequency and route may contribute to the delay in detection using transabdominal probe. Results obtained from this study gave significant information on goat foetal structures, pregnancy detection and gestational age estimation using ultrasound; however, there were constraints and weaknesses that could be overcome and improved in future research. As mentioned earlier, one of the limitation in this study is the limited number of does available for breeding programme (Experiments 3 and 4) as well as incomplete farm record of the farm of which the delivery date of some diagnosed does in Experiment 5 was not found. Due to that, the diagnosed does without delivery date record were discarded from the flock test analysis. For future melioration, a bigger number of does would be helpful to corroborate the finding.

Although in sheep, the time of pregnancy detection with transabdominal probe may be advanced by positioning the animals in dorsal recumbency (Schrick and Inskeep, 1993), in goats, this would lead to a great deal of resistance and struggling and, thus, does not seem practical (Padilla-Rivas *et al.,* 2005). Thereof, in current study, ultrasound scanning using both probes was conducted in standing position of the does.

Attention to small details in positioning the probe on scanning area especially using transabdominal probe should be taken into consideration in order to obtain higher accuracy and shorter scanning duration. This is more important especially during early phase of middle stage of gestation. It has been observed in this study that using transabdominal probe, the foetus could be detected at earlier stage (months 1 to 2 of gestation) by placing the probe just under the udder of the doe. Following that (months 3 to 4 of gestation), the foetus could be detected at the area between the udder and 3-4 cm below it. During the last month of gestation, the foetus could be observed at the abdominal area 6 - 8 cm from the udder, near the belly button of the doe. Meanwhile, foetus only could be detected up to the third month of pregnancy using transrectal probe, which is in agreement with earlier research studies (Martinez *et al.,* 1997; Suguna *et al.,* 2008; Karen *et al.,* 2009). As for the foetal counting, both transrectal and transabdominal probes has shown to be reliable at the range of weeks 5 to 7 and weeks 5 to 10 of gestation, respectively.

Placentome and foetal heart were found to be fulfill the criteria as gestational age indicator; easy to access and develop in size throughout pregnancy. Comparison made from flock test between those two pregnancy-related structures suggested that foetal heart area is a more reliable indicator (maximum accuracy of 94% vs. 38%). This is due to the specificity of the structure, of which there is only one heart to be evaluated for each foetus, but 75 – 125 of placentomes are scattered and attached on chorio-allantois membrane;

measurement of placentome was based on the largest placentome detected on the viewing screen instead of one specific heart as in foetal heart measurement.

In summary, this study achieved the objective of developing ultrasound goat pregnancy diagnosis protocol and equation for gestational age estimation using two probes which are transrectal and transabdominal probes. To the best of author's knowledge, current research is the first that report the relationship between foetal heart area size and gestational age. The age of foetus can be accurately estimated by measuring the size of foetal heart area for pregnancy between weeks 8 to 21 of gestation; for earlier stage of pregnancy, gestational age can be ensured from the morphology (appearance) of foetal heart. Nevertheless, for future studies, it is recommended that further ultrasound scanning improvement need to be carried out. These include to establish specific gestational age estimation equation for different breed of does. Increasing the sample number will be of great improvement for future research. Based on the present experience, for farm with proper planned breeding programme (farm record), either one of the probe could be used at suitable time for pregnancy diagnosis. On the contrary, for does with unknown breeding status, preferably both probes are to be used for scanning session for individual does to complement, confirm and increase the efficiency of diagnosis. It is noteworthy to mention that Padilla-Rivas *et al.* (2005), Suguna *et al.* (2008) and Karen *et al.* (2009) also used both probes in their research. Therefore, ultrasound technology through measurement of specific pregnancy-related structure is reliable for gestational age estimation in pregnant does.

Chapter 6

6.0 CONCLUSIONS

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a) The earliest day to confirm pregnancy using transrectal and transabdominal probes are on days 28 and 35 of gestation, respectively.

b) There are differences in sensitivity, specificity, and accuracy in detecting pregnancy between transrectal and transabdominal probes.

c) Ultrasound scanning is highly reliable and efficient in detecting pregnancy in does.

d) Transrectal probe is reliable and efficient during the first 3 months of pregnancy, and it detect confirmatory signal of pregnancy at approximately 1 week earlier than transabdominal probe. Meanwhile, transabdominal probe is reliable and efficient during the whole 5 months of pregnancy.

e) Foetal development in doe can be determined using observation from ultrasound scanning with transrectal and transabdominal probes.

f) Structures, shapes and sizes obtained from images using transrectal and transabdominal probes can be differentiated and measured.

g) Gestational age of foetus can be estimated using ultrasound scanning.

h) It is possible to study the development of specific foetal organ using ultrasound scanning in does.

i) It is possible to determine the age of foetus with observation on structures and combination of structures especially the changes in its echogenicity and size.

j) Foetal heart area is the best parameter to be measured for gestational age estimation in pregnant does.

k) Foetal number could be determined using transrectal and transabdominal probes within weeks 5 to 7 and weeks 5 to 10 of gestation, respectively.

l) Foetal sex could be determined using ultrasound in pregnant does via observation of scrotum.

m) Different breed of does affect the accuracy of ultrasound examination.

n) Ultrasound detection complements other pregnancy detection methods by providing information on foetal number, viability and gestational age estimation.

o) Ultrasound examination is practical to be applied in large farm operation.

p) Farm management does not significantly affect the foetal structures detected by ultrasound scanning.

q) In a nutshell, transrectal probe is favourable for early stage to mid stage of pregnancy, weeks 4 to 10 of gestation. Although transabdominal probe detected confirmatory sign of pregnancy (foetus with heartbeat) at approximately 1 week later than transrectal probe, it is practical to be used at the remaining days of pregnancy.