Chapter 5

5.0 DISCUSSION

5.1 INTRODUCTION

Even though rapid advancement in the field of caprine reproductive technologies such as embryo transfer, embryo cryopreservation and embryo cloning has been made, pregnancy detection in goat is not given high priority. Currently, pregnancy diagnosis based on physical observation is practically unreliable. Therefore, this experiment was carried out to determine pregnancy in goats using ultrasound scanner. In addition, an attempt was made to estimate age of foetus using transrectal and transabdominal probes throughout the doe's gestation period.

5.2 OVERVIEW OBSERVATION ON FOETAL DEVELOPMENT AND RELATED IMAGES IN REPRODUCTIVE SYSTEM IN DOES WITH TRANSRECTAL PROBE (EXPERIMENT 1)

Images of foetus and related structures such as sac, non-echogenic (NE) area, foetus

with heartbeat, amniotic fluid, uterine wall and placentomes were obtained using transrectal probe and subsequently arranged according to days of pregnancy. Structural images obtained in this experiment were similar with those reported by Martinez *et al. (*1998), Medan *et al. (*2004) and Padilla-Rivas *et al. (*2005). Even though development of embryos theoretically could be identified during preimplantation stage, none of the literature reported to obtain images during this early gestation period. It is apparent that B-mode ultrasound imaging using transrectal probe is unable to identify preimplantation embryos. To date, a sac or embryonic fluid was acknowledged as the first indicator for initial and early stages of pregnancy. Sac was detected with black and white areas (non-

echogenic and echogenic areas). White areas represented the sac's border. Doize *et al.* (1997) suggested that the borders (white line) grew thinner as the embryonic vesicles developed.

Medan *et al.* (2004) and Padilla-Rivas *et al.* (2005) detected a sac on days 22 and 21 of gestation, respectively. Embryonic vesicle was detected on days 17 and 21 by Karen *et al.* (2008) and Suguna *et al.* (2008), respectively. In the present experiment, except for one doe (day 13 of pregnancy) sacs were successfully detected using transrectal probe on day 25 of gestation. Detection of sac was not possible before day 25 and this could be due to small size of the structures, incompetency of operator to position the probe correctly and incapability of probe to detect the structures at this early stage of pregnancy. However, the author believes that with experience, appropriate skills and equipment as well as using a large number of does, some of the structures such as the sac could be detected at initial stage as early as second to third week of pregnancy. Detection of sac needs a specific timing due to limited spectrum of image that could be detected. For example, if it is too early, the structure is too small to be detected and if it is too late, then the sac could have regressed as foetus develops. Martinez *et al.* (1998) detected NE area representing amniotic fluid that facilitated diagnosis of pregnant does as early as day 18 of gestation using 5.0 and 7.5 MHz transrectal probe. In this experiment, non-echogenic (NE) area was detected on day 23 of gestation.

Heartbeat and embryo proper were detected on days 21 (Martinez *et al.,* 1998), 21 (Suguna *et al.,* 2008), 23 (Padilla-Rivas *et al.,* 2005) and 24 of pregnancy (Medan *et al.,* 2004). In the present experiment, heartbeat was detected on day 28 of gestation, which is a few days after sac was observed using transrectal probe. Detection of heartbeat was suggested to give more significant indicators for pregnancy diagnosis (Martinez *et al.,* 1998; Medan *et al.,* 2004). Foetus with detection of heartbeat was

observed together with uterine wall and amniotic fluid. Embryo proper with a beating heart was observed on days 23 and 28 by Karen *et al.* (2008) and Suguna *et al. (*2008), respectively. Suguna *et al.* (2008) detected placentomes (a circular 'C' shape structure) as early as day 42 of pregnancy. In this experiment, placentomes were detected on day 20 of gestation.

On days 51, 66 and 70 onwards, ultrasound scanning using transrectal probe failed to detect any structures or indicators. However, Suguna *et al.* (2008) first viewed skeletal structures such as the skull, rib cage and vertebral column on day 56 of gestation. But there were no reports regarding detection of structural structures using transrectal. This may be due to different breeds, localities and other factors between this research and those of previous related projects.

Based on the results, stages of pregnancy were categorized into two main stages; early and later phase. Transrectal probe proved to be more suitable for early pregnancy (days 13-49 of gestation) compared to later pregnancy (days 50 to 147 of gestation). The early phase can still be divided into 2 stages; initial and early stages of pregnancy. To ease predilection, the first stage (initial stage) was categorized according to oestrous cycle in goat (every approximately 21 days). Indicators of pregnancy were difficult to detect during initial stage but was possible from the early stage of gestation.

The sac was observed as early as on day 13, non-echogenic (NE) area on day 23, foetus-heartbeat-uterine wall-amniotic fluid on day 28 and placentomes on day 29 of gestation. Thus, it is expected that these structures could be detected in early stage (days 22-49) for the next experiment.

In this experiment, the objective was to develop criteria for pregnancy check in does using transrectal probe. In other words, only foetus and related structures were recorded without paying attention to the details such as different shapes and sizes of

these structures. Therefore, future studies could involve such aspects so that information on the relationship between foetal development and gestation age could further be elucidated.

5.3 OVERVIEW OBSERVATION ON FOETAL DEVELOPMENT AND RELATED IMAGES IN REPRODUCTIVE SYSTEM IN DOES USING TRANSABDOMINAL PROBE (EXPERIMENT 2)

From the results obtained in this experiment using transabdominal probe, images were very much similar with images reported in the literature, except for detection of sac and embryo proper (Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008). These researchers detected caprine foetus with heartbeat as early as days 60, 35 and 33 of pregnancy, respectively. In the present experiment, foetus with heartbeat was detected as early as day 66 of gestation. Besides heartbeat, other structures that were observed were ribs, spinal cord, placentomes and foetal organs. Placentomes were detected as early as day 51 of gestation. Medan *et al.* (2004) and Suguna *et al.* (2008) and reported placentomes detection was on days 50 and 60 of gestation, respectively.

Medan *et al.* (2004) observed spinal cord on day 60 of gestation. Suguna *et al.* (2008) reported detection on skeletal structures such as the skull, rib cage and vertebral column on day 56 of gestation. In this research, ribs were observed from days 66 to 117 of gestation. Spinal cord detection was on days 70 to 117 of gestation. In addition, foetal organs were detected on day 108 of gestation. Foetal organs were represented by uneven non-echogenic area in the foetus detected between spinal cord and ribs.

Transabdominal approach is quicker, more convenient and time saving than transrectal method (Padilla-Rivas *et al.,* 2005). Unlike transrectal approach, images of structures obtained from different stages of pregnancy holds some similarity and were

almost alike from days 66 to 117 of gestation. Placentomes were observed throughout the later stage of pregnancy.

Even though images of foetus and related structures were obtained during the later phase of pregnancy, it is suggested that prediction of more exact gestation age could be determined if more number of pregnant does and detailed identification of pregnancy indicators such as sizes and shapes are taken into consideration. However, this is beyond the scope of this experiment.

Throughout this experiment, transabdominal probe was unable to detect any structures during early stage (13-30 days of gestation). Similar findings were obtained 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.* (2008), 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. In the present study, these structures were not detected. Probably it may be due to the wrong positioning of probe and different wavelengths and equipment used. As mentioned by Karen *et al.* (2008), 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.

Similar to the results obtained in Experiment 1, the information obtained on the images of foetus and related structures during the later phase of pregnancy was used as the basis for the pregnancy indicator criteria in Experiment 3. Consequently, pregnancy was categorized into five stages; initial, early, middle, late and final stages of gestation. This is the first attempt to categorize the caprine pregnancy stages based on images of foetus obtained since most researchers merely reported relationship between structural

images and days of gestation. From the results of the experiment, pregnancy stages (range of days) were chronologically arranged with expected images of structures. Combining findings from Experiments 1 and 2, stages of pregnancy were divided into initial (days 1-21), early (days 22-49), middle (days 50-83), late (days 84-119) and final stage (days 120-147).

5.4 OPTIMIZATION OF PREGNANCY DIAGNOSIS OF DOES USING TRANSRECTAL PROBE (EXPERIMENT 3)

Chronologically arranged structural images obtained from confirmed pregnancy based on specific pregnancy indicators and related structures as suggested in Experiment 1 were used as an attempt to estimate age of foetus using 7.5 MHz transrectal probe. Out of 95 does used in this experiment, 68 does (72%) were confirmed pregnant.

Detection of sac using transrectal probe could be obtained on days 17 to 22 of gestation (Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Karen *et al.,* 2008; Suguna *et al.,* 2008). In this experiment, sac was detected as early as day 21 of gestation. From the observation of this experiment, it is believed that the sac detected as yolk sac formed adjacent to the foetus. Results from present and previous research, it seems that detection of sac using ultrasonography represents the first indicator of pregnancy in goats. Therefore, it is logical to assume that the initiation and formation of sac is in the second to third week of gestation.

Detection of non-echogenic images representing amniotic fluid area, uterine fluid accumulation and allantoic fluid was reported to be on days 17 to 19 of gestation (Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Karen *et al.,* 2008). However, in this experiment, NE area was detected later, that is from days 20 to 25 of gestation. The reason why the detection was later in this research is not known. It could probably due

to different breeds, localities and other factors between this research and those of previous researchers.

Presence of foetus with heartbeat is a definitive indicator of pregnancy. Detection of heartbeat and embryo proper were detected from days 21 to 24 of gestation by previous author (Martinez *et al.,* 1998; Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005; Suguna *et al.,* 2008). The foetal heartbeat can be detected slightly a few days after detection sac. Different range of days may be due to different breed, equipment used and operator skills. Padilla-Rivas *et al.* (2005) reported that foetal heartbeat as an indicator of pregnancy is preferred because it provides conclusive proof of the presence of a live foetus. In this study, foetus with heartbeat was detected as early as day 26 of gestation. In addition, ultrasound scanning with positive presence of foetus was proved to be reliable in determining embryonic mortality based on presence and absence of heartbeat from day 21 of pregnancy (Martinez *et al.,* 1998). Hence, it is important to detect open females after abortion for immediate separation and rebreeding or culling of females.

Pregnancy detection with heartbeat is very accurate; however, we encountered a few problems in this experiment. For example, we faced difficulty to detect foetus as well as the heartbeat especially at early pregnancy stage (approximately before day 26 of gestation) whereby the size of the foetus was thought to be relatively small and or the heartbeat had not been initiated yet. In this experiment, detection of heartbeat and foetus were categorized as specific structures separately. In other words, foetus detected means that presence of foetus with or without heartbeat and heartbeat detected means that presence of both heartbeat and foetus.

Other than that, there was an insignificant indicator (an echogenic structure near the bladder) during Experiment 1. The echogenic area was first predicted as chained

placentomes but it turns out to be irrelevant in this study. Negative diagnosed was confirmed when we double-checked the same does and a few turn out non-pregnant on the second scanning. Nevertheless, it was also important that we managed to avoid doing the same mistakes in other does in this experiment. No attempt was made to differentiate single and twins in this research but Medan *et al.* (2004) and Suguna *et al.* (2008) reported differentiation of singles and twins were possible on day 60 and 35 of gestation, respectively.

Table 4.4 shows that percent predictive value for specific and combination of structures detected at early stage of gestation using transrectal probe. It is further illustrated in simpler presentation as shown in Figures 4.26 and 4.27 for specific and each structural image or combination of structures, respectively. Using transrectal probe, specific structures such as sac, NE area, foetus, heartbeat, uterine wall and amniotic fluid were observed during the third to fifth week of gestation. Besides singular specific structures, combination of structures was also detected. These combinations of structures include foetus-heartbeat and foetus-heartbeat-uterine wall-amniotic fluid. For the highest predictive value (31%), it was observed that the sac was detected during earlier stage (days 21-27 of gestation) than that of combination of foetus-heartbeat-uterine wall-amniotic fluid at later stage (days 26-44 of gestation). It is evidence that it is not necessary to detect the combination of structures for a positive diagnosis because even if any one of the specific structures were detected, pregnancy could still be estimated as positive early pregnancy stage diagnosis as shown in the results of this research. It is suggested that foetus-heartbeat detection could be the minimal criterion to detect early stage of gestation. However, it is more desirable if combination of structures of foetus-heartbeat-uterine wall-amniotic fluid were obtained to give more convincing and definitive confirmation for early stage pregnancy using the transrectal probe.

Not much research was conducted to determine placentomes during early stage of pregnancy. Detection of placentomes on days 28 to 30 during early stage of gestation was reported by Doize e*t al.* (1997) and Karen *et al.* (2008). In this experiment, using transrectal probe, placentomes was detected only once that is on day 30 of gestation. Probably during this early stage, placentomes are not fully developed yet to enable to be detected using ultrasound probes. Hence, placentomes were not a preferred indicator for early pregnancy.

It could be generalized that expected structures to be detected from days 16 to 25 are sac and NE area, days 26 to 44 of gestation, foetus, heartbeat, uterine wall and amniotic fluid and beyond days 50 none of the structures are able to be detected using the transrectal probe. As explain earlier, period or length of pregnancy was assumed 147 days. Thus, we concluded detection of sac was around 2 to 4 weeks easier indication in term of cycle of oestrus (after 1st oestrus cycle). Nevertheless, if embryonic vesicles were encountered later than the 4th week, does might be having a non-uniform cycle or kidding date was miscalculated due to management problems. Detections on pregnancy were carried out but determination on stage of foetal during gestation period was our priority as well.

Other than detecting pregnant and non-pregnant does, the need to estimate age of gestation is vital. In this experiment, using transrectal probe, the pregnancy indicators such as sac, NE area, foetus, heartbeat, uterine wall and amniotic fluid were positively detected from days 15 to 44 of pregnancy. Distribution of structures in early stage of gestation was recorded to show the development and to estimate age of foetus using structures or combination of structures. The structures with highest occurrences or distribution can be assumed as indicator for that specific stage, in this case early stage of gestation. Distribution on detection of structures was later focused to specific range of days during the early pregnancy. Consequently, early stage of gestation was divided

into certain range of days to relate it to the frequency of different structures observed. It is evidence that occurrence of sac and NE area were obviously high during days 16 to 25 of gestation while combination of foetus-heartbeat-uterine wall-amniotic fluid were mostly detected during days 26 to 44 of gestation. In summary, when using the transrectal probe, it is suggested that structures of sac or NE area could be used as a criterion to detect earlier foetal age (days 16-26 of gestation) whilst combination of foetus-heartbeat-uterine wall-amniotic fluid to detect later pregnancy (days 27-44 of gestation).

A total of one out of 67 pregnant does and one from 28 non-pregnant does was negatively diagnosed during initial and early stage, respectively. For initial stage (days 1-20) on either does or ewes, there were very few reports on percentage of accuracy, sensitivity or specificity. Romano and Christians (2008) reported overall accuracy on ewes in detecting pregnancy was 100% as early as day 19 using transrectal probe. Scanning examinations was done on dorsal recumbency of ewes. In this study, the percentage for sensitivity and negative predictive value was 100% but percentage for specificity and positive predictive value was low. This was due to misdiagnosed of pregnant does (3/4) that was predicted open female. During this stage, a common indicator, the NE area was not detected. This may due to the positioning of probe, different equipment used and the positioning of patient e.g. does or ewes. Furthermore, this is the initial fact finding experiment to form the basis of detailed subsequent experiments.

Gonzalez *et al.* (2004) reported 99.4% overall accuracy on day 26 of gestation. In this experiment, early stage of gestation gave more than 80% of sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy. The value obtained during this stage was high and shows consistency in distribution of structural images. Percentage on negative predictive value was lower and resulted from

misdiagnosed of a doe that was non-pregnant but detected as pregnant. A structure suspected as sac was observed but the structure may be irrelevant to this experiment. No structures were detected after day 50 of gestation, so sensitivity, specificity, positive predictive values, negative predictive values and overall accuracy for middle, late and final stage of gestation were not analyzed for the transrectal probe. The overall accuracy for initial and early of gestation in this experiment was 66% and 94%, respectively. For initial scanning, it would be desirable to repeat scanning a week later to confirm the accuracy of the preceding results. From the results of this research, it seems that detection of pregnancy gave the best accuracy from third week of pregnancy using transrectal probe. The percentage of accuracy using transrectal probe was quite high and proved reliable in pregnancy diagnosis. No specific reasons for variations in accuracy results were reported in the literature. Therefore, in future more research is needed to elucidate this issue.

In summary, transrectal probe gave best result in detecting earlier stage (days 20 to 49). The best criterion for specific and combination of structures during early stage of pregnancy are sac and non-echogenic (NE) area between days 16 to 25 of gestation together combination of foetus-heartbeat-uterine wall-amniotic fluid between days 26 to 44 of gestation. From the result, it was concluded that from middle stage onwards, transrectal approach was not practical.

5.5 OPTIMIZATION OF PREGNANCY DIAGNOSIS OF DOES USING 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 estimate age of foetus. Seventy nine

percent (118/150) does were confirmed pregnant using the pregnancy indicators and combination of related structures decided.

Detection of placentomes was possible on days 50 to 60 of pregnancy (Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008). The findings of the present study were similar as previous researchers, which is detection of placentomes could be as early as day 50 of gestation (middle stage of gestation). The structures vary in sizes. Placentomes increases in size as the foetus grew to enable observation of placentomes with no difficulty. Doize *et al.* (1997) reported gestation age estimation based on placentomes size using callipers. In the present study, however, it was difficult to correlate gestation age with the size of placentomes due to variation and inconsistent in size of placentomes along the gestation period. Therefore, placentomes probably can be correlated with age of foetus if proper equipment is being used. However, further studies are needed to validate this proposal. Nevertheless, placentomes could be a good pregnancy indicator.

The detection of placentomes was equally important as heartbeat detection in detecting pregnancy in goats using transrectal probe. Heartbeat was detected from days 33 to 60 of pregnancy (Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008). In this experiment, heartbeat was detected as early as day 56 of gestation.

Medan *et al.* (2004) and Suguna *et al.* (2008) detected spinal cord on day 60 of gestation. Suguna *et al.* (2008) reported detection on other skeletal structures such as the skull, rib cage and vertebral column on day 56 of gestation. In this present research, similarly spinal cord and ribs were detected as early as days 51 and 65 of gestation, respectively. Observation on head and radius was detected by Medan *et al.* (2004) at day 70 of gestation. However, we were unable to confirm these structures using transabdominal probe since they were not used as pregnancy indicators in this study. As

mention earlier (Section 2.4), time and positioning of probe were major factors affecting the success of obtaining the expected structural images. For example, positioning the transabdominal probe at the right flank of abdomen is preferred for it is easier to detect images that would give higher positive results since this side of abdomen is free from rumen interference.

Medan *et al.* (2004) reported the ability to identify multiple foetuses as a clear advantage of ultrasonography. Padilla-Rivas *et al.* (2005) using 7.5 MHz transrectal probe, was able to distinguish single and twins is between days 28^{th} and 40^{th} of pregnancy. No attempt was made to determine foetal numbers in this study; however, it has been observed that single foetus was detected mainly under the udder area while twin foetuses were often observed at the belly or abdomen area. Further studies are needed to confirm this observation.

Reviewing the literature, it is noted that no reports were available on these detection on combinations of structural images during goat pregnancy (Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008). As far as we know, this is the first attempt to relate combinations of structures age of gestation in does. In this study, the demarcation of second trimester onwards (middle, late and final stages of gestation), was developed based on combinations of structural clusters along the later periods of pregnancy. In other words, the stages were proposed when majority of combination of structures were detected within certain range of days during later period of pregnancy.

The highest predictive values during middle stage were foetus-heartbeat (30%), followed by placentomes-foetus-heartbeat (20%) and foetus-heartbeat-spinal cord-ribs (20%). Placentomes alone were observed during early middle stage (i.e. days 50 to 66 of gestation). Other combinations involving along with other structures such as spinal cord and ribs were also observed (days 56 to 83 of gestation). Therefore, from the

results of this experiment, it is suggested that the above combinations of structures would confirmed the middle stage of pregnancy.

During late stage, the structures detected were almost the same as middle stage of gestation. The highest predictive values during late stage were placentomes-foetus-heartbeat-spinal cord-ribs-foetal organ (29%), followed by foetus-heartbeat-spinal cord-ribs-foetal organ (25%), placentomes-foetus-heartbeat-spinal cord-ribs (19%), foetus-heartbeat-spinal cord-ribs (10%), foetus-heartbeat (10%) and placentomes (7%). The structures in late stage showed development in foetal skeletal structures. Age of embryo of foetus can be estimated by measuring the crown rump length, biparietal diameter, trunk diameter and femur length were reported to be reliable in determination of age of gestation (Karen *et al.,* 2008). In this research, detection on skeletal structures and foetal organs was proved to be main indicators for late stage of gestation. In addition, foetal organ showed foetus undergoes internal development. Non-echogenic area (e.g. kidney) in various shapes with very thin borders was detected between ribs and spinal cord. During final stage, no structures except placentomes were detected due to limitation of the ultrasound probe. This is similar as reported by other researchers (Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005; Suguna *et al.,* 2008).

All animals were positively diagnosed and obtained 100% overall accuracy during late and final stages of gestation. During middle stage of gestation, two does were negatively diagnosed as pregnant and non-pregnant, respectively. This may due to misinterpretation on certain structures that were mistaken as pregnancy indicator. As for the negative diagnosed on non-pregnant does, the different approach on positioning of probe might lead to fail in detecting any structures. Percentages of sensitivity and specificity for middle stage were 96% and 92%, respectively. The overall accuracy for middle, late and final stages of gestation in this experiment were 94%, 100% and 100%, respectively. No structures were detected before day 50 of gestation, so sensitivity,

specificity, positive predictive values, negative predictive values and overall accuracy for initial and early stage of gestation were not analyzed for the transabdominal probe.

From the results, no indicator were observed or detected from days 13 to 48 in early phase of gestation. This proves that transabdominal probe was most practical at later phase (second and third trimesters). However, the limitation of transabdominal probe is unable to detect the foetal structures except for placentomes starting days 120 until birth. In summary, the best combinations of structures for middle stage are foetus-heartbeat, placentomes-foetus-heartbeat and foetus-heartbeat-spinal cord-ribs; while the best combinations of structures for late stage of gestation were placentomes-foetus-heartbeat-spinal cord-ribs-foetal organ, foetus-heartbeat-spinal cord-ribs-foetal organ and placentomes-foetus-heartbeat-spinal cord-ribs. Placentomes was the best criterion for final stage of gestation.

5.6 PREGNANCY DIAGNOSIS IN DOES UNDERGO NATURAL MATING AND AI WITH TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 5)

This experiment confirmed the accuracy and reliability of combinations of foetal structures as proposed from Experiments 3 and 4. The experiments were carried out with both probes since the efficacy were proven on different stages of goat pregnancy (Sections 5.4 and 5.5). Medan *et al.* (2004) also carried out goat pregnancy detection research with both probes.

As suggested from preceding experiment, transrectal probe was more suitable in early stage of pregnancy detection. Detection of specific and combinations of structures gave 100% predictive value confirming early stage of gestation. Detection of sac and NE area shows a higher frequency during days 23 to 26 of gestation compared to 29 to

36 of gestation. This may be due to the sac regression phase, thus less visibility with the transducer. Combination foetus-heartbeat-uterine wall-amniotic fluid was high on days 29-36 of gestation. This may be due to the visibility of foetus size and foetus development. Therefore, we may conclude that foetal development with heartbeat on does started on day 29 of gestation onwards. Initiation of sac commenced on day 23 and development of foetus on 29 days onwards. This information may be useful to relate to gestation age and early foetal development.

Detection using transabdominal probe especially for combinations of structures was more complex than transrectal probe due to different combinations of multiple structures detected along the later gestation period (i.e. second and third trimesters). Combination of placentomes-foetus-heartbeat showed the highest occurrence in middle stage of gestation. Placentomes was easily detected and it was a definite clear indicator for pregnancy, but detecting placentomes on its own gave difficulty in predicting the age of foetus. Combination of foetus-heartbeat-spinal cord-ribs also predicted gestation age during the middle stage. Spinal cord and ribs can be easily differentiated when heartbeat was detected. However, these structures might not be found in all does. Absence of certain structures could be resulted from the incomplete development of foetus or the size of foetus was too small to be noticed. This was due to structures that were present in clusters. Combination of foetus-heartbeat was detected in early phase of middle stage of gestation. This may be related to the size of foetus, however, combination placentomes-foetus-heartbeat was also detected on the same day (e.g. day 60 of gestation). This may due to positioning of the probe. Nevertheless, as long as the foetus was detected with heartbeat using the transabdominal probe, if part of the foetus was not clearly detected, it may be sufficient to indicate middle stage of gestation. More complex structures, such as foetus-heartbeat-spinal cord-ribs may indicated middle stage, but late phase of middle stage (days 72-83 of gestation).

As for the late stage of gestation, combinations of placentomes-foetus-heartbeat-spinal cord-ribs, placentomes-foetus-heartbeat-spinal cord-ribs-foetal organs and foetus-heartbeat-spinal cord-ribs-foetal organs gave high occurrences and predictive values. Foetus-heartbeat-spinal cord-ribs were also detected on late stage of gestation, but on the early late phase (days 84-91 of gestation); placentomes-foetus-heartbeat-spinal cord-ribs-foetal organs gave the highest occurrence as the foetal development was clearer and more discernible. The black area indicating fluid was marked as foetal organ as reported by Scheerboom and Taverne (1985). The foetal organ was visible and a clear indicator for late stage. Combination of foetus-heartbeat-spinal cord-ribs-foetal organs was the same combination of placentomes-foetus-heartbeat-spinal cord-ribs-foetal organs, in absence of placentomes. Even though placentomes was detected throughout the later phase, sometimes the size of foetus gave difficulty in detecting the placentomes. Nevertheless, the presence of foetal organ was good enough to indicate late stage of gestation. The main problem is in identifying the structures of foetal structures. Some structures were clear (elongated or circular black area), but some of the structures were only visible after movement of foetus. Experience and skills were required to make this prediction a success. During final stage of gestation, presence of placentomes alone gave highest prediction. The foetus was too big to be detected unless the position of foetus was different. It may vary in does, but we can also differentiate the stage by looking at the echo density of placentomes. From the results, none of the pregnant does where negatively diagnosed. Percent on accuracy for each stage was 100% with either transrectal or transabdominal probes for early, middle and final stage of gestation. Different from late stage of gestation, percentage of accuracy were 97% using transabdominal probe. This may be due to similarity of indicators on combinations of structures detected.

Haibel (1990) reported exposures to 3.5 and 7.5 MHz resulted in no foetal death or abortions occurred with kids were born morphologically normal and viable. Exposures to two different wavelengths seemed to be harmless. If there were any abortions cases reported, this may be due to nutritional regimes to mothers and hygiene in farm. In this study, scanning procedures were carried out on does by more than once and by two different wavelengths of transducer; 7.5 MHz transrectal and 5.0 MHz transabdominal probes. Most researchers carried out their experiments using both transabdominal and transrectal approaches in goats (Medan *et al.,* 2004; Padilla-Rivas *et al.*, 2005; Maico *et al.,* 2007; Karen *et al.,* 2008*;* Suguna *et al.,* 2008). Only Martinez *et al.* (1998) applied only transrectal approach in his studies. Nevertheless, practicality and efficacy of probes depend on objectives of the experiment, individual skills, availability and capital.

Transrectal probe was reported in detecting pregnancy much earlier compared to transabdominal probe (Martinez *et al.,* 1998). Transrectal approach also effortlessly acquired interpretable images available during early stage of gestation. Despite the fact that the insertion of a transrectal probe caused slightly discomfort to patient, no injuries or damages inflicted if it were handled by a helper and probe was manipulated gently. In this study, the author was able to observe development of foetus during early phase. With advanced technology, structural development of foetus from implantation to foetus development may be possible using ultrasonography. Transabdominal probe on the other hand was more practical in later stage and effective in detecting foetal number or even sex differential (Dawson *et al.,* 1994; Maico *et al.,* 2007). In addition, transabdominal approach was believed to be an easier and safer method compared to transrectal approach. Development of foetus during second and third trisemesters was also possible using transabdominal probe. Even though, shapes and sizes were almost the same during middle to late stage of gestation, but the presence of foetal organ gave

an idea in organogenesis of foetus. In this study, having both probes was vital. Confirmation of pregnancy status was by detection of heartbeat by either transrectal or transabdominal probes. If necessary, pregnancy diagnosis in certain does was scanned more than once by both probes to ensure high efficiency of pregnancy diagnosis.

The importance to differentiate pregnant does from open females is described in Review of Literature section (Chapter 2). Using ultrasonography, it may help to plan early for the non-pregnant does to undergo rebreeding programme. This results in increase in does reproductive efficiency. As for pregnant does, appropriate management during pregnancy, for example feeding regimes could be design to ensure the health and survival of kids. Other than that, before any medication is given to does, confirmation on pregnancy status prevents the possibility of abortion. Some medications are not suitable to be given to pregnant does as they may be harmful to foetus that may lead to abortion.

From the results obtained in this study, the accuracy in detecting pregnancy status was 100% using both probes. This is similar to the previous experimental results and comparable with those reported by previous researcher (Medan *et al.,* 2004; Karen *et al.,* 2008*;* Suguna *et al.,* 2008). The need to differentiate pregnant and non-pregnant does is undeniably important as well as the necessity to predict the time of parturition should not be overlooked. In this experiment, does were scanned randomly and age of foetus was determined prior to date of kidding. We found out that detection of combinations of structures were reliable particularly detection of foetus-heartbeat-uterine wall-amniotic fluid (days 29-45 of gestation) and foetus-heartbeat-spinal cord-ribs-foetal organ (days 105-119 of gestation) using transrectal and transabdominal probes, respectively. Estimation on age of foetus makes it possible to discern date of mating. In a given practical situation, for example in a synchronized AI programme with subsequent natural mating, this information could be useful to determine whether

the does were pregnant due to AI or natural mating. If this is possible, then AI efficiency can be compared with natural mating as well as estimation and management for the respective parturition dates could be arranged accordingly. Those does that were confirmed not pregnant could be rebred or culled from the herd as well as possibly used as donors and recipients for embryo transfer programme.

Knowing the date of parturition resulted from ultrasound scanning would value add in farm management. For example, specific numbers of kids would be expected on specific period of time resulted from synchronization-AI/natural mating programme. Therefore, pre-nutritional arrangement could be designed for the expecting does with the anticipated kids. Another benefit of knowing the age of gestation is helping in decision-making in terms of transporting pregnant does in which when age of foetus were estimated late or final stages of gestation, it is best not to transport them as it might affect the survival of prospective kids. Thus, this technology is practical and can be applied for the goat farming enhancement.

Summarizing the results, the author suggested possible different range of days for each stage of gestation. This was based on different combinations of structures that were observed related to specific range of days. The middle and late stages of gestation were consistently shown to have wider range of days compared to other stages of pregnancy. The detailed delineation of this issue was beyond the scope of the present study. Nevertheless, it is hoped that having 5 stages of gestation as suggested in this study would ease the prediction on date of kidding.

5.7 GENERAL DISSCUSSION

Knowledge on growth and development of specific foetal structures at specific gestation stage could be exploited both for scientific information and practical application.

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 gestation. Practically, the exact age of pregnancy could be confirmed as Day 0 resulted from AI or natural mating by knowing the specific foetal structures during gestation age. Additionally, gestational age of transferred embryo could also be estimated. However, such information in goat is scarce in the literature. In this study, an attempt was made to determine the specific foetal structures or in combinations of structures during goat pregnancy using transrectal and transabdominal probes. Even though specific foetal structures were detected during pregnancy, gestation age was deliberately estimated based on kidding date retrospectively. In the present study, the exact date of mating was unable to be determined due to the geographical location (300 km away from University of Malaya) as well as logistic management (incomplete farm records) of the farm making it difficult to conduct continuous and intense scanning in short time intervals. Therefore, it is suggested that in future, more focused research on the day of fertilization related to natural mating and AI as well as embryo transfer is needed. Concurrently, appropriate management practices such as reliable farm records and related technologies such as oestrus detection should be employed.

Transrectal and transabdominal probes were more practical in early and late phases of pregnancy, respectively. Factors such as different equipment used, breed of does and the operator's competency could contribute to variable findings as mentioned in Section 2.4. Results obtained from this study gave significant information on goat foetal structures and pregnancy detection using ultrasound; however, there were constraints and weaknesses that could be overcome and improved in future research.

Romano and Christians (2008) detected pregnancy as early as 20 days of gestation at dorsal recumbence in ewes. However, there is no report in the literature of using dorsal recumbence scanning in does. In our study, standing position of the does

was carried out and detection of pregnancy was obtained as early as day 22 of gestation. It is believed that no effect of pregnancy detection efficiency between the two positions.

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 when twin foetuses were present, the detection would likely be at the belly whereas for single foetus, the detection would normally be at the udder. The main focus of the study is to detect different structures during gestation period. Detailed studies on shapes and sizes of the foetal structures were not given much attention. This limitation is partly due to inability of machine to measure the parameter structures precisely as reported by Karen *et al.* (2008) and Suguna *et al.* (2008).

As technology advances, detection was possible with 3-dimension and colour machine but there are limitations. Machine evolved with great advance but the high technology machine is expensive and needs new skills. Thus, the basic B-mode ultrasonography as used in this study is sufficient to detect does pregnancy which is convenient and simple to use.

In human pregnancy detection using ultrasound, scientific calculations produced indexes and matrix formula that instantly estimate the age of foetus. Therefore, this approach could be designed for goat. In future studies, therefore, with proper machine, skills as well as proper experimental design based on human experience, goat foetal age could be determine instantly using the ultrasound scanning. 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.

In summary, this study achieves the objectives of developing ultrasound goat pregnancy diagnosis protocol using two probes which is transrectal probe for first trimester and transabdominal probe for second and third trimesters of pregnancy. However, for future studies it is recommended that further ultrasound scanning improvements need to be carried out. These include ultrasound scanning as early as on day 22 of gestation, determining the exact date of mating or AI and relating the age of foetus with specific or combination of structures. Based on the present experience, preferably both probes are to be used for scanning session for individual does to complement, confirm and increase efficiency of diagnosis. It is noteworthy to mention that Padilla-Rivas *et al.* (2005), Karen *et al.* (2008) and Suguna *et al.* (2008) also used both probes in their research. Therefore, ultrasound technology along with progesterone assays and pregnancy-associated protein (PAG) for pregnancy diagnosis in does is a useful tool in reproductive biotechnology that is integral component with other reproductive technologies such as AI, embryo transfer, cryopreservation, IVF, ICSI, cloning, gene transfer and stem cell research.

Chapter 6

6.0 CONCLUSIONS

a) Ultrasound pregnancy detection protocol in goats using transrectal and transabdominal probes was developed.

b) The first trimester (days 21-49 of gestation) could be detected using transrectal probe.

c) The second and third trimesters (days 50-143 of gestation) could be detected using transabdominal probe.

d) The best specific and combination of structures for early stage of gestation (days 21-49 of gestation) were sac, non-echogenic (NE) area and foetus-heartbeat-uterine wall-amniotic fluid.

e) Combinations of foetus-heartbeat, placentomes-foetus-heartbeat and foetus-heartbeat-spinal cord-ribs were proven reliable during middle stage of gestation (days 50-83 of gestation).

f) During late stage of gestation (days 84-119 of gestation), combinations of placentomes-foetus-heartbeat-spinal cord-ribs, placentomes-foetus-heartbeat-spinal cord-ribs-foetal organ and foetus-heartbeat-spinal cord-ribs-foetal organ were dependable.

g) During final stage of gestation (days 120-143 of gestation), only detection of placentomes gave the best criterion.

h) Efficacy of ultrasonography in goat pregnancy detection was 100% accuracy in detecting pregnancy status.

i) Accuracy on age estimation for each stage of gestation, i.e. early, middle, late, and final stages using transrectal and transabdominal probes were 100%, 100%, 97% and 100%, respectively.

j) Foetal development using transrectal and transabdominal probes were concluded from specific and combinations of structures with predictive values for each stage of gestation.

k) Ultrasonography is proven reliable in detecting pregnancy diagnosis in goats.

l) In conclusion, it is suggested that both probes are needed to detect pregnancy in does since their effectiveness is mutually exclusive for the different trimesters of pregnancy. In other words, early detection of pregnancy is promising with transrectal probe from days 21-49 of gestation. Transabdominal probe was practical from days 50-147 of gestation.