

**Ultrasound Scanning as a Technique in Pregnancy Diagnosis of Saanen Breed at
UniSZA Dairy Goat Farm**

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ABSTRACT

Ultrasonography is not only commonly used in human but also is a useful tool for diagnosis of pregnancy in livestock species, including goats. It was improved with the advent of real-time B-mode (brightness mode) imaging. This tool was used more frequently in sheep compared with goats. The present study was conducted to use B-mode transabdominal probes development of goat pregnancy scanning protocol in UniSZA Dairy Goat Farm (UDGF). Ultra-structure images at different stages of pregnancy and the earliest possible diagnosis of pregnant does were determined. The results of this study showed the following structures: embryonic sacs (days 28-48), foetal (days 28-52), foetal head (days 56-144) and placentome (days 40-146). Single and twin foetuses were differentiated after 56 days of estimated pregnancy. The efficacy of this project was determined based on the frequency of occurrence of the ultra-structures that have been observed. Ultra-structures detected by ultrasound scanner have been identified as indicators for foetal twinning and as a predictor to differentiate stages of pregnancy. In a nutshell, the data of ultra-structures were analysed by percentages of whole stages of pregnancy to detect early pregnancy, twinning of foetus as well as detection of pregnancy. The outcomes of this research will be the fundamental scientific information that could be applied practically as an integrated component of goat management practices in the dairy goat industry for income generation and small farmers in the rural areas for their wellbeing and livelihood as well as for food security and safety of this country.

Keywords: Pregnancy diagnosis, Saanen goats, Ultrasound scanning, Transabdominal probe, UniSZA Dairy Goat Farm

ABSTRAK

Ultrasonografi bukan sahaja digunakan di kalangan manusia tetapi juga sangat berguna sebagai alat untuk diagnosis kebuntingan dalam spesies haiwan ternakan termasuklah kambing. Ianya telah ditambah baik dengan kemajuan pengimejan real-time B-mode (brightness mode). Alat ini kebanyakannya digunakan ke atas biri-biri berbanding kambing. Kajian ini dijalankan menggunakan transabdominal B-mode prob bagi mempelajari protokol imbasan kebuntingan di Ladang Kambing Tenusu UniSZA (UDGF). Pengimejan ultrastruktur ditentukan bagi setiap peringkat kebuntingan dan diagnosis kebuntingan yang terawal. Keputusan kajian ini menunjukkan struktur: kantung embrio (hari ke 28-52), fetus (hari ke 56-144), kepala fetus (hari ke 56-144), placenta (hari ke 40-146). Fetus tunggal dan kembar dibezakan selepas 56 hari anggaran kebuntingan. Keberkesanan projek ini ditentukan berdasarkan kekerapan ultrastruktur yang diperolehi. Ultrastruktur yang dikesan menggunakan imbasan ultrasound akan dikenalpasti sebagai penunjuk kepada fetus kembar dan rujukan untuk membezakan peringkat kebuntingan. Intipatinya, data ultrastruktur dianalisis berdasarkan peratusan sepanjang peringkat kebuntingan untuk mengesan bunting awal, mengjangka umur kebuntingan, fetus kembar termasuklah mengenalpasti kebuntingan. Hasil daripada penyelidikan ini akan memberi kepentingan dari segi maklumat saintifik asas yang mana dapat diaplikasikan secara praktikal sebagai komponen berintegrasi dalam amalan pengurusan kambing dalam industri kambing tenusu bagi menjana pendapatan dan secara tidak langsung membantu penternak kecil-kecilan di kawasan pedalaman untuk kesejahteraan mereka termasuklah menjamin keselamatan makanan di negara ini.

Kata Kunci: Diagnosis kebuntingan, Kambing Saanen, Pemeriksaan ultrasound, transabdominal prob, Ladang Kambing Tenusu UniSZA

INTRODUCTION

Ultrasonography is one of the principal imaging techniques that is established for use in veterinary practices (Abdelghafar et al., 2007). In the 1980s, it was introduced for pregnancy diagnosis at the earliest gestational stage of livestock animals. There are several types of ultrasonography that have been used in livestock fields such as A-mode, B-mode, and Doppler.

In the 1950s, ultrasonography began to use in human obstetrics that has been pioneered by Ian Donald (D' Cunha, 2014). During that year, only application of A-mode (amplitude mode) ultrasound which gave a one-dimensional image of uterus started to be applied for human. However, application of ultrasound in livestock for reproduction management until the real-time B-mode (brightness mode) imaging was introduced in the 1980s. Henceforth, real-time B-mode was practised for horse (Ginther, 2014) reproductive management and along with cow (Quintela *et al.*, 2012). For sheep and goat, ultrasound was first applied for gestation diagnosis in the early 1980s (Karen, 2003; Karadev, 2015; Jones and Reed, 2017).

Real-time ultrasonography has been mostly used as a technique to diagnose early pregnancy in small ruminants (Wani *et al.*, 1998). It is a non-invasive modern method which gives rapid and accurate detection of early pregnancy. Moreover, only small false positive occur in diagnosis via ultrasonography. The accuracy of ultrasound scanning can be achieved about 91-100% if it is performed by the experienced operator (Karadev, 2015). B-mode real-time ultrasonography showed two-dimensional images on a screen and direct visualized foetal structures, such as foetal fluids, foetus, foetal heartbeat and placentome. This ultrasound can be performed in two different ways either transrectally (7.5 MHz) or transabdominally (5 MHz). For transabdominal probes approach, it is generally used more than 35 days after breeding while less than 30 to 35 days is best-done transrectally (Matsas, 2007). Each probe has different frequencies, with the range of probe frequencies from 1 to 18 MHz but majority of researchers used between 3.5 and 12 MHz (Jones & Reed, 2017).

Pregnancy diagnosis is a useful technique for the producer to measure the foetal age based on foetal structure detected either via transrectal or transabdominal probes. Foetal age plays an important role in parturition date of does. This information can be a guideline for producers to get ready for kidding preparation. Apart from that, number of foetuses and determination of litter size also could be attained based on detected structure (Jones & Reed, 2017).

Besides, diagnosis of early pregnancy can help the producer decide either the does require artificial insemination or repeat breeding. It is also functioning as a detector in a flock so that it can help the producer to make efficient culling decision to the problem does. When a doe gets detected for early pregnancy, scanning during mid- to late- gestation can be easier for monitoring the changes of foetal ultrastructure development pattern which will be decided soon whether the does need subsequent treatment or management intervention (Jones & Reed, 2017).

For researchers, the information that can be gained from this pregnancy diagnosis technique is ultrasound scanning can be a monitor for foetal development during gestation (Karadev, 2015). In addition, ultrasonography can be safely used for frequent times because Jones and Reed (2017) reported that there was no negative effect occurred from repeated exposure to ultrasound. Thus, this procedure may ease researchers work from getting necropsy to obtain the foetal structure during different stages. However, detection of the embryo is quite difficult during early pregnancy. Therefore, this ultrasound scanning required a highly skilled technician to perform it (Raja Illi Airina *et al.*, 2011).

Abdelghafar *et al.* (2007) reported that only one study has been performed in diagnosis of pregnancy in Saanen goat. In 2017, there were a lot of studies regarding to the pregnancy diagnosis technique using ultrasound scanner but they were using different types of breed. Only some studies that related to Saanen goat in Malaysia. Thus, more information about pregnancy diagnosis on Saanen goats in similar finding is required, especially in Malaysia to obtain consistent and reliable results. The aims of this study were to perform scanning protocol for pregnancy diagnosis in UDFG, to detect early pregnancy and to evaluate the efficiency of the transabdominal probe in determining the criteria of twin pregnancy, including to estimate the foetal age during different pregnancy stages. The findings from this study could be effective way in making rebreed and culling decision for UDFG. Thus, the data could help this farm to increase their reproductive management as well as in productivity such as milk yield in future.

MATERIALS AND METHODS

Experimental Animal

Dairy goat breed, namely Saanen, was imported from Melbourne, Australia in December 2017. This breed was selected to be the main dairy goat breed reared at UDFG, Besut, Terengganu. They were fed at *ad libitum* comprising of pellets (SOON SOON Meat Goat Feed – 5180) and forage (*Brachiaria humidicola* and *Pennisetum purpureum*). All the UDFG does that were selected in this experiment underwent natural mating. A total of 41 Saanen does aged between 1 to 2 years old were used in this study.

Materials

The contact gel, KY Jelly was used as a coupling agent for ultrasound transmission, was applied to the right side of the abdomen of the goat by using transabdominal probe.

Pregnancy Scanning Equipment

Real-time scanner (DRAMINSKI ANIMAL *profi*) equipped with different linear array transducers referred to the type of probes to be used. But in this study, the 5.0 MHz transabdominal probe was required in order to detect early pregnancy in Saanen goats.

Transabdominal scanning procedure

Before performing the transabdominal technique, preparation of the doe was required prior to scanning proper, such as the doe needs to be restrained first in order to obtain reliable results. For example, inducing urination in doe was one of the steps that managed to allay the possibility of a false positive result. This condition could be wrongly mistaken for pregnancy by virtue of the fluid in the urinary bladder. A high quality image also may be acquired by removing the hair, so that direct contact between probe and skin are capable (Matsas, 2007). Next, the contact gel (KY Jelly) that had been covered the transducer was applied to the test site at the right area above the udder of the goat, 5 cm in front of the rear leg and 2.5 cm above the teat. Besides, it should be ensured that the skin is in a wet state, elimination of air bubble is also essential between probe and skin. Then, the probe was slowly moving and rotating in that inguinal region (Raja Illi Airina, 2014).

Experimental Design

In Experiment 1 (Detection of pregnancy and non-pregnancy does using ultrasound scanner), it was performed to detect pregnancy and non-pregnancy does based on ultrastructures scanned by 5.0 MHz transabdominal probes. About 29 out of 41 does were detected pregnant by showing embryonic sac, embryonic fluid, foetus, placentome, foetal head or umbilical cord. Obtained ultrastructure from this experiment had been used for Experiment 2 (Detection the earliest pregnancy using transabdominal probe), which was to identify the earliest pregnancy among the does. The earliest pregnancy was determined by referring to previous studies due no record of breeding program. For the Experiment 3 (Identification of single and twin pregnancies using ultrasound scanner), the criteria for single and twin pregnancy were detected by the result from Experiment 1. These criteria can be directly observed when performing the ultrasound scanning technique. Lastly, in Experiment 4 (Prediction of pregnancy age using ultrasound scanner), prediction of foetal age was conducted to predict the parturition date of the does.

RESULTS AND DISCUSSION

Experiment 1: Detection of pregnancy and non-pregnancy does using ultrasound scanner

Ultrasound scanning procedure was conducted for 3 months on the does that underwent natural mating and at the same time showed the physical signs of pregnancy. Some of the pregnancy signs were enlargement of abdomen seen on the right side, udder seen larger when approaching near to the end of pregnancy, non-oestrus and rarely case for swollen legs like women during pregnancy (Herrick, 2018).

Fig. 1 shows the accuracy of pregnant Saanen does of 63% (26 does out of 41) were detected based on the visualization of pregnancy-related structures like embryonic sacs (Fig. 2), embryonic fluid (Fig. 3), foetus (Fig. 3), placentome (Fig. 4), umbilical cord (Fig. 10), and foetal head (Fig. 10). Generally, ultrastructure or combination ultrastructures were detected in agreement with other researchers (Raja Illi Airina et al., 2014; Nizam, 2011; Eman et al., 2016). Nonetheless, another 30% (12 does out of 41) were recognized non-pregnant by showing a blank image (Fig. 5). Then, data of the pregnant does would be used to determine the pregnancy related structure for earliest pregnancy (Experiment 2), single and twin foetuses (Experiment 3) and lastly to obtain foetal age based on the parturition date (Experiment 4). The frequency of ultrastructures detected in pregnant does also recorded in Table 1.

The factor that could cause this failure in natural mating was perhaps due to the incorrect amount of hormones production or the ovary not released the egg. The ovary's does would not producing the egg when it had become cystic. Female that having shorter cycle might be one of the factors for the breeding program failed. Female with shorter cycle had lower fertility compared to the one that had a long or normal cycle which both of

them were fertile (Cooperative Extension, 2012). In addition, injury to males and females reproductive organ also could impact their reproductivity (Cooperative Extension, 2013). In this experiment, the objective to identify between pregnant and non-pregnant does was achieved by DRAMINSKI Animal^{profi} Ultrasound Scanner (transabdominal probe). This study was crucial for proving that the ultrasound scanner was the easiest technique and also could give the fastest result compared to the other pregnancy diagnosis.

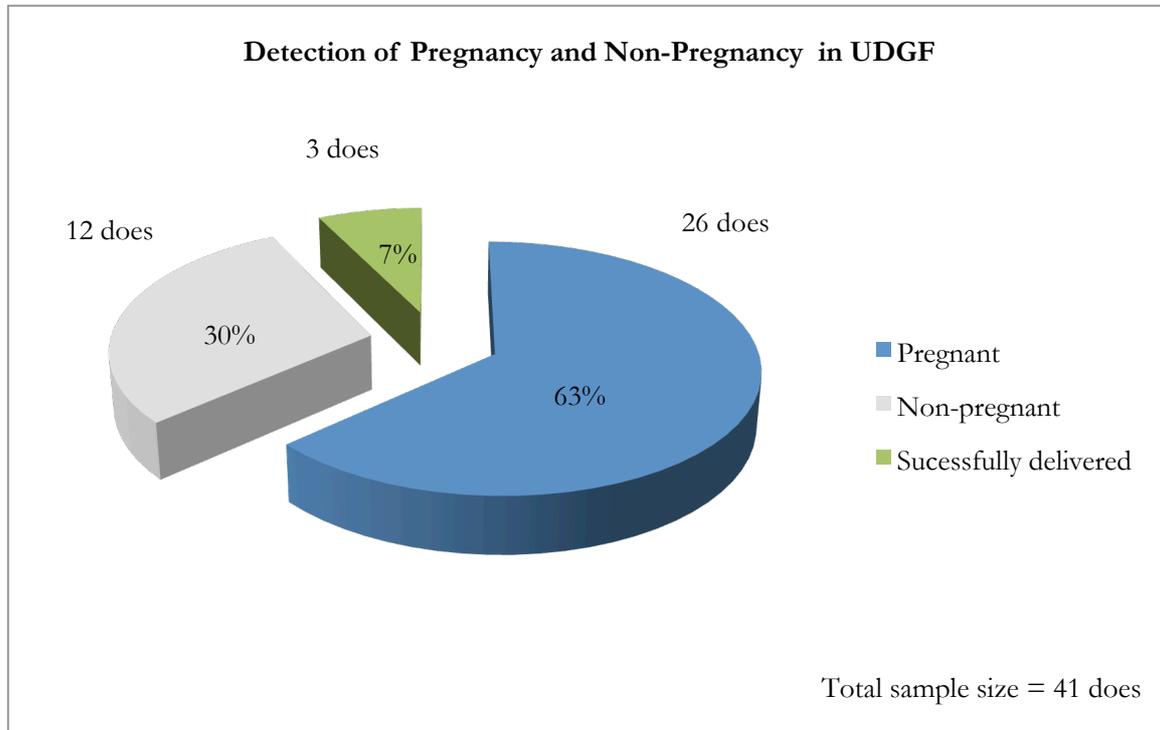


Figure 1 Accuracy of pregnancy and non-pregnancy detected during ultrasound scanning procedure.



Figure 2 Image of embryonic sac (arrow) was presumed days 28-48 of pregnancy.



Figure 3 Image of foetus in embryonic fluid (arrow) was presumed days 28-52 of pregnancy.



Figure 4 Image of placentome (arrow) was presumed days 51-119 of pregnancy.

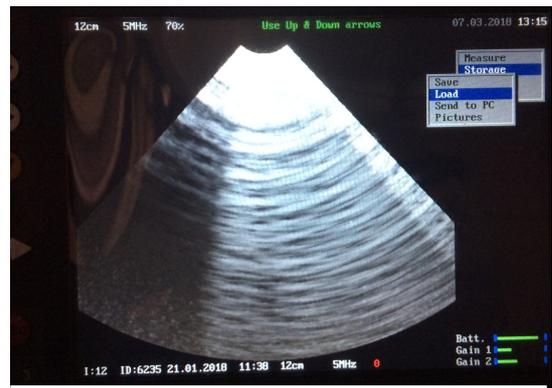


Figure 5 Image of open doe appeared a blank since no pregnancy-related structure was detected.

Table 1: Frequency of pregnancy-related structures detected in pregnant does.

Structure	Frequency detected (times)
Foetus	22
Placentome	20
Embryonic sac	4
Embryonic fluid	2
Twin foetuses	2
Umbilical cord	1

Experiment 2: Detection the earliest pregnancy using transabdominal probe

In order to determine the earliest day of pregnancy, the ultrastructures obtained from this research were referred to previous study by virtue of no record regarding to breeding program. Moreover, only 7% of does that underwent ultrasound scanning procedure were successfully delivered and all of them were verified in the late of gestation when carried out this procedure. Hence, several of the previous studies had been referred to obtain the range of gestation age for the other detected pregnancy-related structure in Experiment 1. Table 2 show the record of ultrastructure or combination ultrastructure detected in gestation period using 5.0 MHz transabdominal probe.

In determining the earliest pregnancy detected, the structure of embryonic sac, foetus and embryonic fluid were detected by Padilla-Rivas et al. (2005), Kailash et al. (2015), and Nizam (2011), respectively. Based on the previous studies, the first-trimester structure that could be detected in this current study was embryonic sacs (days 28 to 48), foetus (days 28 to 52) and embryonic fluid (days 56 to 93) (Suguna, 2008; Nizam, 2011; Raja Illi Airina, 2011; Karadev, 2015).

Table 2: The record of ultrastructure or combination ultrastructure detected in gestation period using 5.0 MHz transabdominal probe.

Pregnancy structure	Range of days
Embryonic sac	28-48
Foetus	28-52
Embryonic fluid	56-93
Umbilical cord	32-116
Foetal head	56-144
Placentome	40-146

Based on the Table 2, it was stated that embryonic sac (Fig. 6) and foetus (Fig. 7) were the earliest ultrastructures that could be seen during early pregnancy.

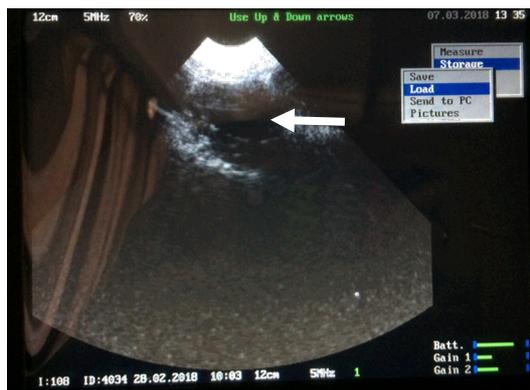


Figure 6 Image of embryonic sac (arrow) was presumed days 28-48 of pregnancy.



Figure 7 Image of foetus in embryonic sac (arrow) was presumed days 28-52 of pregnancy.

Experiment 3: Identification of single and twin pregnancies using ultrasound scanner

About 5% (2 out of 41) of Saanen does were detected having twin pregnancy in this current study and one of them had successfully delivered the kids. The data for parturition date was recorded as required for foetal age prediction in Experiment 4. Based on the result or ultrastructure (Fig. 8 and Fig. 9) obtained, a few criteria for single and twin pregnancies were identified such as quantity of embryonic sac, number of the foetus in uterus, quantity of foetus with cardiac activity or number of placentae (chorionicity).

Like the other ultrastructure, twin foetuses could be recognized in a certain age of gestation period. According to Amer (2010), the transabdominal probe could be detected the single and twin foetuses on days 90 to 109. Instead, Suguna et al. (2008) reported that on the days 40 to 70 of gestation was the best period for counting the foetal number. Moreover, it was also the correct time to ensure foetus does not conceal each other and the visualization of the whole uterine could be obtained. Therefore, the twin pregnancy (Fig. 9) that visualized in this study was predicted on the days 40 to 109 of a gestation period.

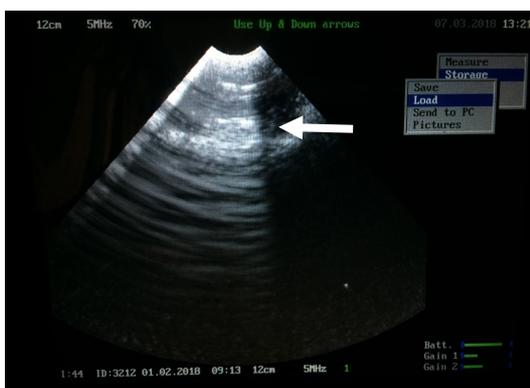


Figure 8 Image of single foetus (arrow) was presumed days 28-52 of pregnancy.

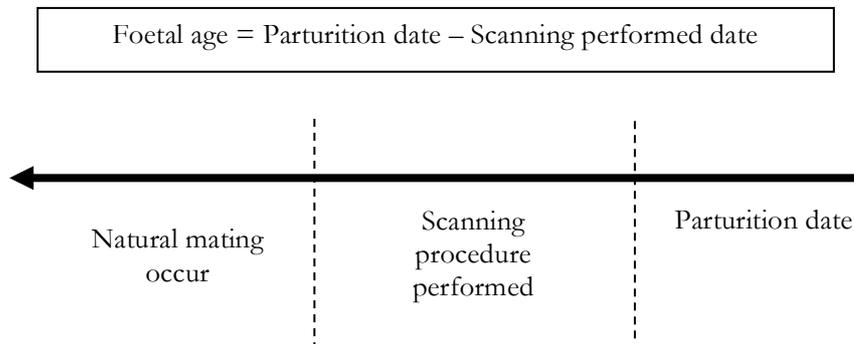


Figure 9 Image of twin fetuses in amniotic fluid (arrow) was presumed days 40-109 of pregnancy.

Experiment 4: Prediction of pregnancy age using ultrasound scanner

Mostly prediction of foetal age was according to the record of breeding program in a farm. However, parturition date could be used to estimate the gestation age despite of using breeding record. About 5% of does had used this calculation, whereas the other samples were referred to record of previous study due to breeding program had not started yet in UDFG.

The calculation to estimate the foetal age is given as suggested below.



*Assumption 147 days of gestation length

The proposed prediction of age of foetus based on parturition date is given in Fig. 10 and 11.

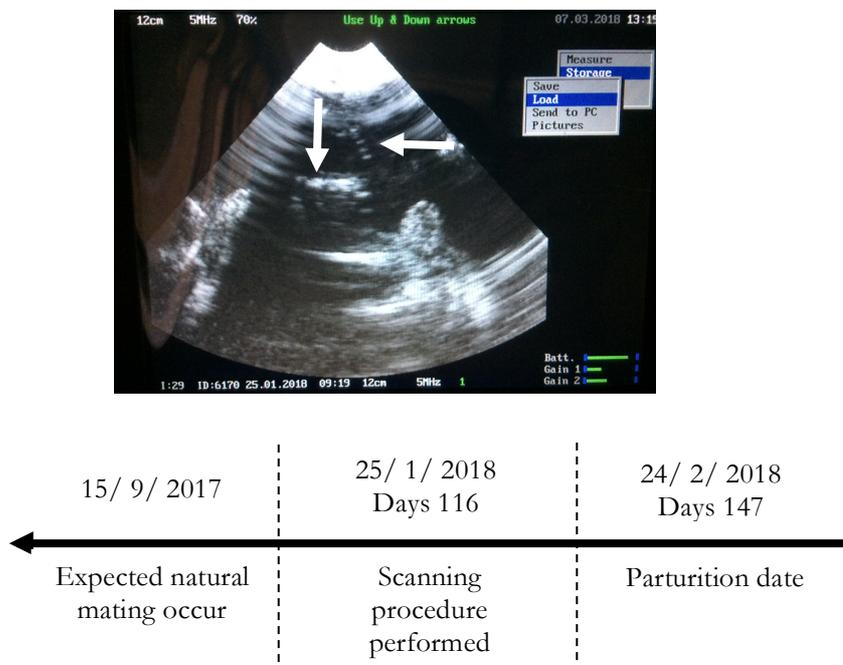


Figure 10 Image of pregnant doe shows foetal head and umbilical cord detected at day 116. Gestation age was presumed from parturition date.



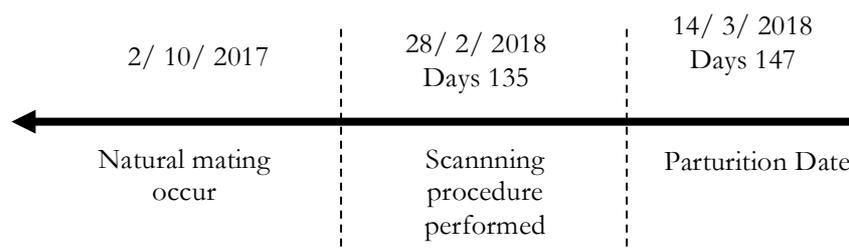


Fig. 11: Image of pregnant doe shows C-shaped placentome detected at day 132. Gestation age was presumed from parturition date.

Suguna (2008) reported that foetal head was detected on day 56 of gestation. Even so, a foetal head was observed on days 60 to 66 of gestation by Nizam (2011). In current finding, the does that was detected with foetal head structure (Fig. 10) was at day 116 of gestation by using the formula that stated in the result of Experiment 4 and this data was in agreement with the previous study that mentioned above. Then, it could be concluded foetal head visualized starting from days 56 to 144 of a gestation period.

On the day 116 of pregnancy, umbilical cord (Fig. 10) also could be detected in this study. However, Nizam (2011) reported that the structure of umbilical cord only could be seen on days 32 to 76 of gestation. The late detection of umbilical cord between current experiment and the previous finding could be attributed due to the type of ultrasound scanner used for pregnancy diagnosis or perhaps could be due to the image collection. Thus, it can be concluded that the early detection umbilical cord perhaps on day 32 while for late detection on day 116 of gestation.

In previous research, the first detection of placentome by Raja Illi Airina (2014) was on day 51 and also in the third stages of pregnancy (on days 120 to 146). In the middle of pregnancy, placentome was recognized as a small nodule C-shaped echodense structure while it getting increase in size and appeared in white-coloured C-shaped in the third stages of pregnancy. In another research, Dawson (1999) reported that the C-shape structure could be observed more than 40 days of pregnancy. This finding had shown that Dawson (1999) detected the structure earlier than Raja Illi Airina (2014). However, the earlier detection of placentome might be happening because of different type of breed using in their research. Placentome (Fig.11) was detected on day 135 in this experiment was in an agreement with the previous studies mentioned above where the result was still within the range of their data collection. Thus, the placentome could be detected on 40 days onwards.

CONCLUSION

In conclusion, ultrasound scanner machine is useful in farm management to detect pregnancy and non-pregnancy in does. Moreover, it is also reliable to be applied not only in small scale management but also in large farm operation. Most structures detected in this study were foetus and placentome while the minority structures were umbilical cord and foetal head. This research also found that single and twin pregnancies could be detected using transabdominal probe of the ultrasound scanner. The structures detected were foetuses, embryonic fluid and placentae. The earliest structures that detected by 5.0 MHz transabdominal probe were embryonic sac and foetus, respectively on days 28 to 48 and on days 28 to 52 days of gestation. Besides, foetal age also could be determined based on parturition date even though the experiment was carried out with unknown breeding program. For instance, the umbilical cord visualized in this study was estimate at the age of 116 days of gestation based on the calculation. However, the record of breeding program is highly required to ensure the study can be performed easily.

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