The Foetal Ultrastructural Images Obtained from Pregnant and Non-Pregnant Does Using Transrectal and Transabdominal Probes of Ultrasound Scanner

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ABSTRACT

Real time B-mode ultrasound scanner becomes very effective tools to detect early pregnancy diagnosis in small ruminant such as goat. Ultrasound scanner used in two way which are transrectal and transabdominal probes. Transabdominal probe used as it is less harmful than transrectal probe. The objectives of the study are: 1) to detect the ultrastructural images in non-pregnant does for artificial insemination purposes using ultrasound scanner, 2) to diagnose of difference foetal ultrastructural images obtained during gestation period using ultrasound scanner and 3) to compare the foetal ultrastructural image obtained from transrectal and trans-abdominal probes of ultrasound scanner. There are three pregnant does and 26 non-pregnant does with two successfully delivered out of 29 totals does chosen in this experiment. The ultrastructural images visible are ‘C’ shape placentome, heartbeat, foetus and hydrometra. It is shown that the size of heart determines the age of foetus during gestation period. At the end of this study, the farmers should be exposed to this modern way to diagnose the pregnancy and non-pregnancy in does and another ruminant. The farmer is able to manage their farm management for pregnant does and ready the non-pregnant does with culling or rebreeding for second time. The result of the study would be an alternative reference to increase the production of milk and farm management in goat industry at UDGF and Malaysia especially. The ultrasound technique to diagnose pregnancy in goats.

Keywords: Ultrastructural images, transrectal, transabdominal, placentome, foetus

INTRODUCTION

The pregnancy diagnosis in goats is essential for better efficacy and management of reproduction (Doize et al, 1997), providing information about conception rates after natural mating or artificial insemination (Matsas, 2007) and time for drying-off and parturition date (Doize et al., 1997; Gonzalez et al., 2004). The utilization of an accurate and easily applicable method for pregnancy diagnosis allows the timely repeated insemination, breeding or culling of non-pregnant animals (Amer, 2010). According to Karadaev, (2015), there was no universally accepted classification of methods for pregnancy diagnosis in goats. Therefore, the existing classification in sheep could be applicable also to goats with several modifications. The methods of limited
interest for the practice are the laparoscopy, laparotomy, vaginal biopsy, transabdominal palpation, abdominal inspection, udder evaluation and appearance of milk secretion, live weight increase, rectoabdominal method, while more practical approaches include the non-return to oestrus, hormonal and pregnancy proteins assays, radiography and ultrasonography (Ishwar, 1995). Ultrasonography as a method or monitoring of the reproduction status in small ruminants is becoming increasingly important and popular (Erdogan, 2012). Now, A-2 mode, B-mode and Doppler ultrasound equipment are used for pregnancy diagnosis in goats. Their accuracy and usefulness under field conditions is variable (Ishwar, 1995). In goats, the transabdominal and transrectal approaches are most frequently used.

Real time B-mode ultrasonography is an alternative for diagnosing pregnancy and studying the development of the conceptus in livestock (Sugunaa, 2008). The main criteria for positive diagnosis of pregnancy in goats are the visualization of anechoic cross sections of the uterine lumen (embryonic vesicle), foetus or placentomes in the amniotic fluid. Embryonic vesicles could be detected between the 21st and 28th days, whereas the embryo could be visualized by the 28–35 gestation days using transrectal and transabdominal uterine scans respectively (Hesselink & Taverne, 1994; Martinez et al., 1998; Amer, 2010). During the years, multiple techniques for pregnancy detection in small ruminants have been developed. Each method has advantages and drawbacks. Some of them are invasive or of low accuracy hence of limited practical use (Karadev, 2015). The purposes of the study are: 1) to detect the ultrastructural images in non-pregnant does for artificial insemination purposes using ultrasound scanner, 2) to diagnose of difference foetal ultrastructural images obtained during gestation period using ultrasound scanner and 3) to compare the foetal ultrastructural image obtained from trans-rectal and trans-abdominal probes of the ultrasound scanner.

MATERIALS AND METHODS

Experimental animals

The total of 29 pregnant and non-pregnant does from Pasir Akar Farm, Universiti Sultan Zainal Abidin have been used in this study. The ultrasound scanning done by standing position. Imago-S ultrasound scanner used in this study equipped with different linear array transducer referred to the type of probes to be used. The trans-abdominal probes equipped with 3.5 MHz and 7 MHz linear array transducer while trans-rectal probes equipped with 10 MHz linear array transducer. The linear array can be changed according to the needs because the ultrasound scanner was digital touch screen.

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Detection 7 (21/02/2020)
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**Ultrasound procedure**

Vaseline act as the lubricant or coupling agent for ultrasound probes. Vaseline used between the transducer and the skin on right abdomen of the goat to allow for the best sound conduction, smooth movement of the transducer and give the clear image of foetus. The shaver used to shave the fur at the right abdomen of the does to get the clear ultrastructural images before the Vaseline applied. It affected the result to get the clear image if it is not shaved before ultrasound scanning procedure. The present of hair and wools on abdominal area may
disrupt the result of images during pregnancy scanning. The hair needs to be shaved first by goat hair shaver before the diagnosis started. The shaving of abdominal wool may be considered to facilitate trans-abdominal scanning during mid to late gestation because the probe is placed on the abdomen.

**Trans-abdominal probes procedure**

The trans-abdominal probe had no issues of animal welfare that could affect embryonic death. The animals were less stressful and less invasive and could be done faster as minimal restrain required by trans-abdominal probe. The observation could be performed in standing position for both trans-rectal and trans-abdominal without used of sedation on the animal. The present of hair and wools on abdominal area may disrupted the result of images during pregnancy scanning. The hair needed to be shaved first by goat hair shaver before the diagnosis started. The shaving of abdominal wool may be considered to facilitate trans-abdominal scanning during mid to late gestation because the probe was placed on the abdomen. The Vaseline applied to the probe surface and the right abdominal wall above the udder of does, 5cm from the rear leg and 2.5 cm above the udders. The function of lubricant also important as it enable the tight bond between the goat skin and transducer make the result more accurate and clearly visible. The probe was slowly moving and rotating in the inguinal area. The probes moved until the ultrastructural images visible. It may take time for the beginners to have the clear images. The pregnant does were decided by detection of amniotic fluids, heartbeat and placentome.

**Transrectal probe procedure**

Other than trans-abdominal probes, trans-rectal probes method also qualified for ultrastructural scanning. Trans-rectal procedure does not follow the same step as the trans-abdominal procedure because it includes the internal diagnosis. The Vaseline applied to the probe’s surface to give clear image during the diagnosis. Faeces should be removed, wiped or cleaned from the transducer, otherwise it may give a poor-quality image or no image. The lubricated transducer is inserted inside the rectum and slowly rotated from side to side. Pregnancy is confirmed by imaging fluid in the uterine lumen, by finding evidence of placentomes or by identification of one or more foetuses.

**Experimental design**

The does underwent natural mating or artificial insemination were observed physically. Experiment 1: Diagnose the ultrastructural images of pregnant and non-pregnant does for artificial insemination purposes. Non-pregnant does will undergo artificial insemination for another student study while the pregnant does undergo Experiment 2: Detection of different foetal ultrastructural images obtained with prediction of foetal age using ultrasound scanner. The ultrasound scanning of detected pregnant does in experiment 1 will continued every week until the parturition period. The different ultrastructural images are heartbeat, placentome and another organ. At the same time, Experiment 3: Comparison the foetal ultrastructural image obtained from trans-rectal versus trans-abdominal, proceed. Basically, the same doe will undergo two different probes for ultrastructural scanning. Trans-abdominal probes procedure went first before trans-rectal probes procedure to reduce the uncomfortable condition in doe. The comparation between the probes are to observed the clear images obtained and recommend the best probes for next study.

**RESULTS**

The detection of non-pregnant does ultrastructural images based on the blank images captured by ultrasound scanner. There was no indicator detected on nonpregnant does or does in the first trimester because the embryonic fluid does not fill the uterine wall (Fig. 1). The black images detected by ultrasound represent the embryonic fluid while the white images captured represent the foetus structure such as bone. The ultrastructural images could be seen clearly as it produced two dimensional images on screen which can be photographed by a polaroid camera and produced a moving image of the uterus, foetal heart beat and placentomes. The percentages
of pregnant does were very low along the experiment conducted in Pasir Akar farm. The experiment on 29 does detected that only 7% of the does were pregnant (two does) while the detection of non-pregnant does were 93% (27 does out of 29). The detection of pregnant does were based on the structural images visible during the experiment. The ultrastructural images for examples amniotic sac, embryonic fluid, foetus, umbilical cord, foetus head, placentome and heart (heartbeats).

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4  3447   -  Blank image
5  6015   +  Foetus movement and placentome

Detection 6 (19/02/2020)
No. Tag No. Pregnancy  Ultrastructure
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Detection 7 (21/02/2020)
No. Tag No. Pregnancy  Ultrastructure
1  6015   +  Heartbeat and placentome

Detection 8 (26/02/2020)
No. Tag No. Pregnancy  Ultrastructure
1  6015   +  Heartbeat

**Fig. 1.** The figure shows no significant structure was detected on non-pregnant (tag no. 3447).

The possible measurable ultrastructure was fluid filled uterus or embryonic fluid. Embryonic fluid is one part of embryonic sac which cannot be fully seen on ultrastructural images. The fluid filled uterus measurements was limited since the uterus rapidly become large. The reliable pregnancy diagnosis must not base upon the presence of fluid in the uterus because the possibility of hydrometra (Hesselink & Taverne, 1994). The example of hydrometra had been detected in a doe at Pasir Akar farm (Fig. 8). The placentome detected at Day 131 of gestation have been observed in Fig. 2. The ‘C’ shape of placentome appeared in the ultrasound screen at late of pregnancy due to foetal enlargement. The shape observed depend to the section of the ultrasound scanning probes. The heart beat detected at Day 140 of gestation shown in Fig. 3. The ultrasound image of heartbeat was not easy to capture as the foetal movement make it presence and disappeared at a moment. The heart valve also can be seen as well as the heart ultrastructural image (Fig 4). The heart valve moves in and out when the foetus heart beating during the ultrasound procedure. The structure of foetus clearly shown the head, leg, mouth and part of the body of the foetus at late of gestation period captured by transabdominal probes in Fig. 5. It is important to have accurate record of goat age for breeding by knowing the mature age but the foetal age rarely recorded. The foetal age can be assumed using the parturition date.
**Fig. 2.** Detection of placentome ultrastructural image on Day 131 of gestation (tag no. 6015).

**Fig. 3.** The foetal heartbeat detected (tag no. 6015)

**Fig. 4.** The foetal heartbeat and valve detected (tag no. 4158).
The image captured from transabdominal probes gave the blur placentome ultrastructural image seen on the screen. The placentome is the combination of caruncle and cotyledon. Figure of transabdominal probes only shows the placentome without clear image of cotyledon while figure of transrectal probes shows the clear placentome ultrastructural image with cotyledon in the middle of the structure. The structural image of placentome in Fig. 6 captured more than one placentome while in Fig. 7, only one placentome detected because the screen cannot fit all the uterus structure at late pregnancy.

**DISCUSSION**

In this study, the transabdominal probes used to detect the non-pregnant does for artificial insemination procedure. The number of 27 does detected non-pregnant by using transabdominal probes of ultrasound scanner underwent natural mating at UDGF. It is important to determine the does were not pregnant before artificial insemination procedure to avoid abortion of foetus. Tissues, such as ovary or uterine stroma, that
reflect intermediate proportions of ultrasound waves are called echoic or echogenic and their images appear in different shades of grey on the screen (Gonzalez-Bulnes et al., 2010). In most study, the uterus of non-pregnant does is not visible using transabdominal probes scanning by virtue of echogenicity of non-pregnant uterine tissues does not contrast enough with surrounding tissues (Hesselink & Taverne, 1994). The expected non-pregnant does can be distinguished between the pregnant does by the sign of enlargement of the right side of the does’ abdomen in the view of fact that the rumen present on the left side.

However, sometimes the large abdomen owing to the access volume of feed intake. Does with small udder size expected not to be pregnant. The increase of udder size only can be seen in late pregnancy. Other sign of non-pregnant does are tail wagging, mucus discharge from the vagina, red vulva and mount each other. This assumption cleared by using ultrasound scanning technique as it gives more accurate information to estimate pregnancy. Although ultrasound scanner gave accurate data, it is not affordable price to the small farmers. The ultrasound scanning on those goats gave the result of majority non-pregnant does. The screen of ultrasound scanner captured the image of blank uterus as the wall of the uterine does not contain any fluid filled. The black ultrasound image represents the fluid in the uterus while the white image possibly the foetus structure of head, bone or any organs.

One of the goats with pseudopregnancy detected. Initially, the goat was expected to be pregnant due to the bigger abdomen observed. The ultrasound scanning shows the image of hydrometra, false pregnancy. Hydrometra are the terms used as synonymous with pseudopregnancy in goats. Pseudopregnancy occur when fluid accumulates inside the uterus with the persistence of corpus luteum and absence of foetus. It is important to know the pathological condition as it cause the temporary infertility in dairy goats (Almubarak, 2018). It is clearly shown that ultrasound technique is the best method to diagnosis the pregnant and non-pregnant does. The first objective to detect the non-pregnant does for artificial insemination achieved by IMAGO touch screen ultrasound scanner.

![Fig. 8. Hydrometra detected at Pasir Akar farm by transabdominal probes (tag no. 6016).](image)

The first detected pregnant doe on 21st January in the late pregnancy. The doe expected to be pregnant as the abnormal size of udder and the abdomen observed. Transabdominal probes of ultrasound scanning used to confirm the pregnancy. During the diagnose procedure, the foetus movement in doe felt. The scanning procedure takes less than 10 minutes as the doe do not cooperate well and heartbeat of the foetus recorded. According to Padilla et al. (2005), foetal heart can be detected on Day 23 of pregnancy. The foetus with the heartbeat was observed on Day 28 by Suguna et al. (2008). Detection of foetal heart was the most accessible and reliable for pregnancy confirmation due to the detection of foetal give the accuracy of 100% were correctly diagnose (Airina et al., 2011). That clearly visible heartbeat showed movement of heart valve in and out divided into two chambers with grey and black colour ultrastructural images. Fig. 4 shows the size heart of doe 4158 bigger than the size of heart in Fig. 3, doe 6015 due to the age of the foetus for doe 4158 older than doe 6015. The heartbeat taken in Fig. 4 was two days before the doe underwent parturition but the foetus died due to long delivery time.
Goats have a cotyledonary placenta which is numerous smaller placentae instead of having a large contact area between the mother and foetal vascular system (Bowen, 2000). The gross structure of placenta is cotyledon as the foetal side of the placenta, caruncle as the maternal side of placenta and placentome as combination of cotyledon and caruncle. Caruncle have oval thickening and visible in non-pregnant uterus (Bowen, 2000). Placentomes easily detected throughout the pregnancy (Airina et al., 2011). Pregnant does have around 75 to 125 total placentomes (Bowen, 2000). In this study, the pregnancy diagnosis and the ultrastructural image obtained in the middle and late gestation. As mention above, the researcher has their own result in the experiment. The accuracy of ultrasound image detected due to some factor that affecting them such as different frequencies used for each researcher.

Ultrasound probes classified by frequencies and arrangement of crystals as linear array. Ultrasound scanning procedure were scanned in standing position for both transrectal and transabdominal probes. The ultrastructural image of transabdominal probes (3.5MHz/7.5MHz) are not clearly as image of transrectal probes (10MHz) as shown in Fig. 6 and Fig.7. The higher the frequencies used for diagnose the pregnancy, the smaller correspond wavelength cause the low quality of image enhance (Gonzalez-Bulnes et al., 2010). Tissue-penetration was diminished with attenuation of higher wavelength (Gonzalez-Bulnes et al., 2010).

The accuracy of transabdominal ultrasonography for diagnosing pregnancy from Day 25 to 30 of pregnancy onwards, and Day 60 as 7.5 MHz transrectal probes do not have enough penetration to permit seeing the entire uterus (Gonzalez-Bulnes et al., 2010). Clear images recorded as a result for transrectal probes scanning. Although the blur placentome image by transabdominal probes, it is recommended due to combination of speed and accuracy of pregnancy evaluation. The technique used must considered the safety of doe and foetus. The viability of embryo was important to avoid mistake cause foetus abortion.

According to Medan (2010), the benefits of ultrasound as a diagnostic imaging procedure in animal reproduction are numerous. Importantly, routine examinations have been shown that no harmful biological effects. Ultrasound is considered as a safe procedure for the animal, the operator and nearby personnel, allowing it to be performed in any place without the need for special safety precautions. Ultrasound is non-invasive and well tolerate in animals, treatment and practice scanning techniques. It is a wave form of non-ionising energy and has no relation to X-rays which can damage the tissues because of their ionising effect on living cells. No significant heating produced when the low intensity of pulsed ultrasound used for diagnostic purposes. Ultrasound is considered very save with no harmful bioeffects compared with other diagnostic aids, X-rays.

Trans-rectal procedure can detect early pregnancy in does earlier than trans-abdominal. Trans-abdominal probe is more effective because it does not cause harm to the animals. B-mode real-time ultrasonography is an accurate, rapid and simple imaging technique used for the detection of pregnancy in goats. Transrectal or transabdominal ultrasonography applications can be used with a nearly 100% accuracy rate (Erdogan, 2012). The used of trans-abdominal probe are more accurate and time saving as the result shows trans-abdominal technique need only 1.5 minutes rather than 2.5 minutes in trans-rectal probe (Padilla-Rivas et al., 2005). Trans-abdominal ultrasound scanning was observed on day 40, 50, 60, 70, 80 and 90 after breeding using 5 MHz transducer (M. Medan et al., 2004). The trans-abdominal probe then slowly moved to the inguinal region till urinary bladder appear (Airina et al., 2011). During early pregnancy, the fluid filled the gravid uterus and displaced by the rumen towards the right of the abdomen, making scanning on the right side of the animal are the best option.

CONCLUSION

The structure detected in this study were blank uterus wall for non-pregnant doe, placentome, heartbeat and foetus images and one with hydrometra. The foetal movement and heartbeat easily visible on late pregnancy. The size of foetus heart determines the age of the foetus during gestation period. Placentomes have two size which is ‘C’ shape and ‘O’ shape differently according to the angle of diagnose area. In this study, the ‘C’ shape of placentome observed. Both transrectal and transabdominal probes have its own benefit in pregnancy
diagnosis. An additional advantage is aspects that related to management and improvement of reproduction. On the other hand, the most beneficial factors of ultrasonography are that its efficiency is always dependent on the expertise of the ultrasound, and that the learning-time is slow to get the perfect clear image of ultrastructural. Regarding of these issues, being an image-based technique, can be only obtained by training and reading of specific guides such as journals. It is hope that the used of both ultrasound scanner probes will gave the accurate effect in does at UDGF to improve the breeding programs.

ACKNOWLEDGMENTS

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REFERENCES


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