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PREGNANCY DIAGNOSIS IN GOATS BY USING TWO DIFFERENT ULTRASOUND PROBES

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ABSTRACT

Information on pregnancy detection in goats using real-time ultrasonography is still lacking compared with that in sheep. Ultrasound scanner with transrectal and transabdominal probes was used to observe and identify foetal related structures and images during pregnancy of does. Does were synchronized for estrus and naturally served or artificially inseminated to obtain pregnancy and subsequently scanned using both probes. Observation and identification of sac, non-echogenic area and foetus-foetal heart-uterine wall-amniotic fluid were detected from days 21 to 49 of gestation. Other structures such as placentomes-foetus-foetal heart-spinal cord-ribs-foetal organ (absence and presence) were easily detected during middle stage of gestation (days 51 to 119 of gestation). During third trimester (days 120 to 143 of gestation), only detection of placentomes was observed. Overall accuracy on prediction of each stage of gestation for first, second and third stages of gestation using transrectal and transabdominal probes were 96, 99 and 100%, respectively. In conclusion, early detection of pregnancy is promising with transrectal probe from days 21 to 50 of gestation. Transabdominal probe is practical from days 51 to 145 of gestation. Both probes are needed to detect pregnancy in does since their effectiveness is mutually exclusive for the different stages of gestation.

Key words: Goats, Ultrasonography, Transrectal, Transabdominal, Foetal structures.

INTRODUCTION

A variety of approaches has been explored for the early detection of pregnancy in mammalian species. In recent years, real-time ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants (Romano and Christians, 2008). Methods of choice depend on the availability of equipment, post-breeding period, desired accuracy and experience of the examiner (Ishwar, 1995). With advancement in reproductive biotechnology, there has been an increase in awareness on importance of early pregnancy diagnosis in goats as an integral component of advancement goat management program. Nevertheless, a practical and reliable means of early pregnancy detection in goats is still lacking.

Real-time ultrasonography for early pregnancy detection in goats has been performed either transrectally or transabominally (Padilla-Rivas *et al*., 2005). Ultrasonography pregnancy check is quicker and less stressful for the animals than either laparotomy or laparoscopy (Medan *et al.*, 2004). This technology requires on average 2.5 and 1.5 minutes for transrectal and transabdominal transducers, respectively (Padilla-Rivas *et al.*, 2005). Haibel (1990) reported repeated exposures of the sheep foetuses (probably similar to goats) to ultrasonic waves of 2 different wavelengths (3.5 and 7.5 MHz) were harmless. No foetal deaths or abortions occurred and all of the kids born were morphologically normal and viable (Martinez *et al.*, 1998; Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005).

Ultrasound machines are low cost to run, readily available and the information is obtained instantaneously (Green, 1996; Nyland and Mattoon, 2002). The ability of ultrasound to distinguish fluid from soft tissue and differentiate between soft tissues based on their composition makes it better suited than radiography for examining soft-tissue structures (Nyland and Mattoon, 2002). Therefore, ultrasound appears as a non-invasive alternative compared to many radiographic contrast procedures. Ultrasound may also provide information that previously was only available through exploratory laparotomy (King, 2006).

Medan *et al.* (2004) suggested that early and accurate diagnosis of pregnancy in addition to determination of foetal number is an added advantage for the maintenance of high levels of reproductive efficiency in herd management practices. In other words, pregnancy diagnosis would identify whether the does require repeat breeding or insemination and also would allow the separation of pregnant and open females. Ability to distinguish pregnant from non-pregnant does in an effort to separate the non-pregnant ones from the flock should reduce cost in feeding, labour and medications and therefore, resulted in more profitable goat farming.

To our knowledge, not much concrete report on fetal development during various stages of pregnancy in does is available in the literature. However, ultrasonographic technique using transrectal and transabdominal probes has been used for goat pregnancy diagnosis as reported by Medan *et al.* (2004) and Padilla-Rivas *et al.* (2005). Their findings were variable so that further research is needed to produce consistent and reliable results. The aim of the study was to identify the foetal related structures during pregnancy using transrectal and transabdominal probes.

MATERIALS AND METHODS

Experimental animals: This study was performed at research farm, University of Malaya, Kuala Lumpur and commercial farm in Penang, Malaysia. Jermasia and crossbred Boer does aged between 1 and 7 years old were detected for pregnancy using ultrasound scanner. The ultrasonographic examinations were conducted with the goats in a standing position.

Ultrasound imaging examination: Ultrasonographic examinations were conducted using real-time ultrasound scanner (ALOKA SSD500) equipped with a linear array 7.5 MHz transrectal scanner (shit length: 35.0 cm, shaft diameter: 1.4 cm) and a convex 5.0 MHz transabdominal (4.0 cm length). Briefly, with the transrectal approach, faeces were cleared from the rectum. The 7.5 MHz transducer was well-lubricated attached to the tip of a rigid extension rod was introduced. The tip of the transducer was lubricated with carboxymethyl-cellulsoe contact gel. The transducer was inserted gently until the urinary bladder was identifiable. Probe was moved gently forward and backward and rotating it 90 degrees clockwise and counter clockwise. In conducting transabdominal ultrasonography, the contact fluid (carboxymethyl-cellulose gel) was applied to the test side, area of 150 to 200 cm^2 on the right flank above the under after removing the hairs over it. Then, the transducer was placed at the right side of the goat, 5.0 cm in front of the rear leg and 2.5 cm above the teat. Pregnant and non-pregnant goats were determined using real-time monitor by detection of foetal heart, spinal cord, head, limbs, foetal organ and other related structures. Nevertheless, the rapid heartbeat of foetus detected gave a 100% confirmation on positive diagnosis and its viability.

Experimental design: The study was carried out to identify images of foetus and related images in goats throughout pregnancy using both probes and to compare the efficacy of 7.5 MHz transrectal and 5.0 MHz transabdominal probes. Scanning procedures were carried out on 171 does out of which 96 does were having natural oestrus and mated and 75 does were synchronized for oestrus and underwent natural mating or artificial insemination (AI).

Images showing different structures detected were used as indicators to confirm pregnancy and as predictor of different stages of pregnancy in does. The efficacy of both probes was determined by the frequency of occurrence of the observed structures. Three stages of pregnancy based on range of days were designated to determine the percentages of sensitivity, specificity, positive predictive value and negative predictive values during the entire pregnancy period. The sensitivity of diagnosis was defined as the precise assumption made on pregnant does while specificity are the ability to correctly detect non-pregnant does.

RESULTS

Observations on images of foetal and related structures were carried out using both probes. The efficacy of 7.5 MHz transrectal and 5.0 MHz transabdominal probes on percentages of occurrence of foetus and related structures for three stages of pregnancy were observed (Table 1). A total of 141 (82%) goats were detected pregnant from 171 goats scanned in this study. During the first stage of gestation (18 to 50 days), images detected for sac, non-echogenic (NE), foetus, foetal heart, amniotic fluid and uterine wall signifies the percentages of occurrence of the structures detected using transrectal probes. Sac was detected as early as day 18 of gestation. Non-echogenic (NE) areas were observed from days 20 to 24 of pregnancy (Figure 1). Combination of foetus-foetal heart-uterine wall-amniotic fluid was detected as early as 28 days of gestation (Figure 2). No specific indicators were found using transrectal probe at later stages of pregnancy, i.e. on days 51 to 147 of gestation.

During second stage (days 50 to 119) of gestation, percentages on occurrence of placentomes, foetus, foetal heart, ribs, spinal cord and foetal organ shows significant of the structures with transabdominal probes. The first indicator detected was placentomes on day 51 of gestation. In the earlier phase of second stage of gestation, foetus-foetal heart-spinal cord-ribs placentomes were detected from days 84 to 94 of gestation (Figure 3). In figure 4, images with foetus-foetal heart-spinal cord-foetal organs were detected on later phase of second stage of gestation (days 95 to 119). For third stage (days 120 to 147) of gestation, occurrence of placentomes was 100%. Placentomes were detected from days 120 to 146 of gestation (Figure 5). There was no indicator or structure observed in earlier pregnancy using transabdominal probe, i.e. on days 25 until 46 of gestation.

Number of does diagnosed with sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy along gestation period are shown in Table 2. The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy for first stage of gestation were 97, 100, 100, 86 and 96%, respectively. The sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy for second stage of gestation were 99, 100, 100, 95 and 99%, respectively. A 100% value was obtained for specificity, sensitivity, positive predictive value, negative predictive value and overall accuracy for third stage of gestation.

Table 1. Percentages on occurrences of foetus and related structures detected during gestation period using 7.5 MHz transrectal and 5.0 MHz transabdominal probes.

Probes	Structures	Range of days		
		18-50 % (n)	**51-119 % (n)**	**120-147 % (n)**
Transrectal	Sac	47 (17/36)	0	0
	Non-echogenic area	37 (13/35)	0	0
	Foetus	47 (17/36)	0	0
	Foetal heart	47 (17/36)	0	0
	Amniotic fluid	47 (17/36)	0	0
	Uterine wall	47 (17/36)	0	0
Transabdominal	Placentome	0	40 (34/85)	100 (19/19)
	Foetus	0	69 (59/85)	0
	Foetal heart	0	69 (59/85)	0
	Ribs	0	69 (59/85)	0
	Spinal cord	0	69 (59/85)	0
	Foetal organ	0	44 (37/85)	0

n = number of structures detected/total number of structures.

Table 2. Percentages of does that were diagnosed positive and negative during initial and early stages of gestation

Diagnosis*	Range of days		
	18-50 % (n)	**51-119 % (n)**	**120-147 % (n)**
a: Correct positive	100 (36/36)	**100 (85/85)	100 (20/20)
b: Incorrect positive	0 (0/36)	0 (0/85)	0 (20/20)
c: Correct negative	86 (6/7)	95 (19/20)	100 (3/3)
d: Incorrect negative	14 (1/7)	5 (1/20)	0 (0/3)
e: Sensitivity (%)	97% (36/37)	***99% (85/86)	100% (20/20)
f: Specificity (%)	100% (6/6)	100% (19/19)	100% (3/3)
g: Positive predictive value (%)	100% (36/36)	100% (86/86)	100% (20/20)
h: Negative predictive value (%)	86% (6/7)	95% (19/20)	100% (3/3)
Overall accuracy (%)	96%	99%	100%

a: Pregnant does that were correctly diagnosed; b: Pregnant does that were incorrectly diagnosed; c: Non-pregnant does that were correctly diagnosed; d: Non-pregnant does that were correctly diagnosed; e: Sensitivity [a/(a+d)]; f: Specificity [c/(b+c)]; g: Positive predictive value [a/(a+b)]; h: Negative predictive value [c/(c+d)]; i: Overall accuracy [(e+f+g+h)/4]; * refers to the confirmation of does whether kidding or not kidding; ** refers to percentage (no. of does correctly or incorrectly per total does either positively or negatively diagnosed; *** refers to percentage (actual no. of does/total no. of does for respective defined diagnose).

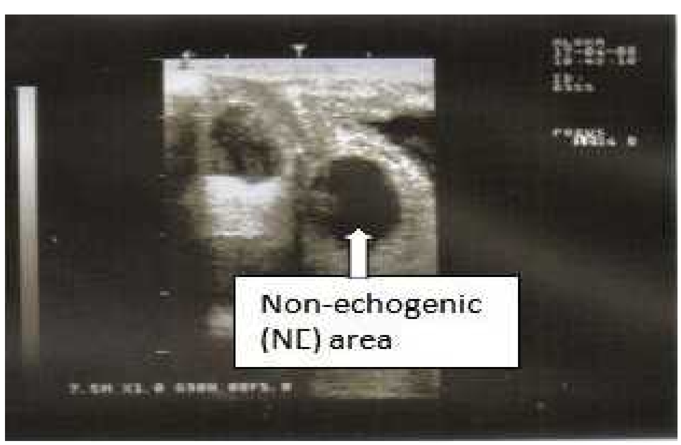

Figure 1: Image of Non-echogenic (NE) area (arrows) was detected on days 20 to 24 of gestation (early stage)

using transrectal probe

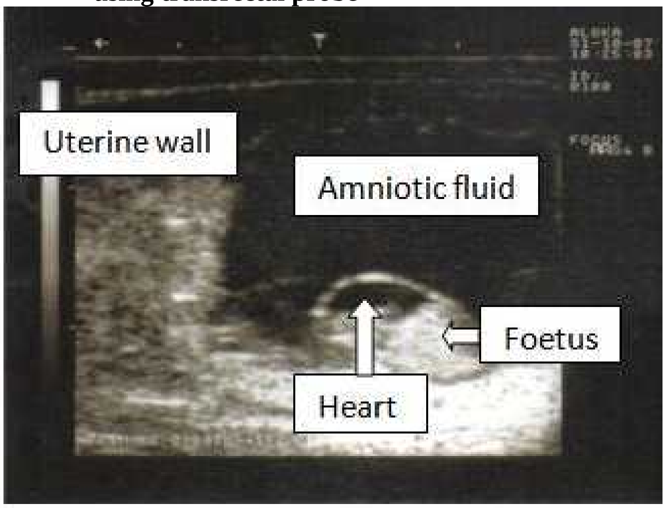

Figure 2: Image of foetus-heartbeat- uterine wall-amniotic fluid (arrows) was detected on day 28 of gestation (early stage) using transrectal probe

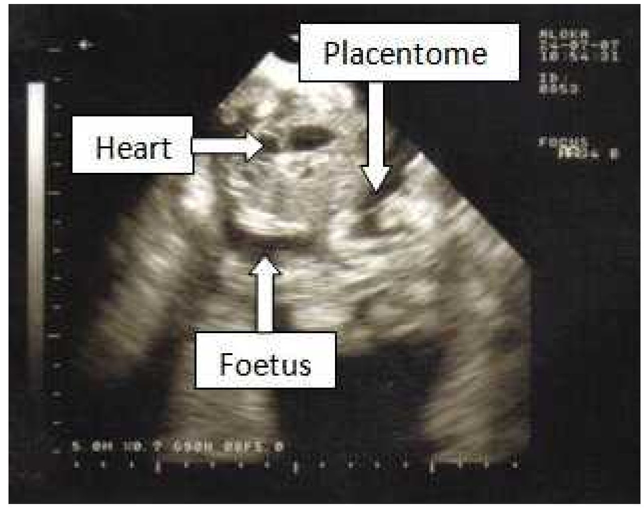

Figure 3: Image of placentomes-foetus-heartbeat (arrows) was detected on day 72 of gestation (middle stage) using transabdominal probe

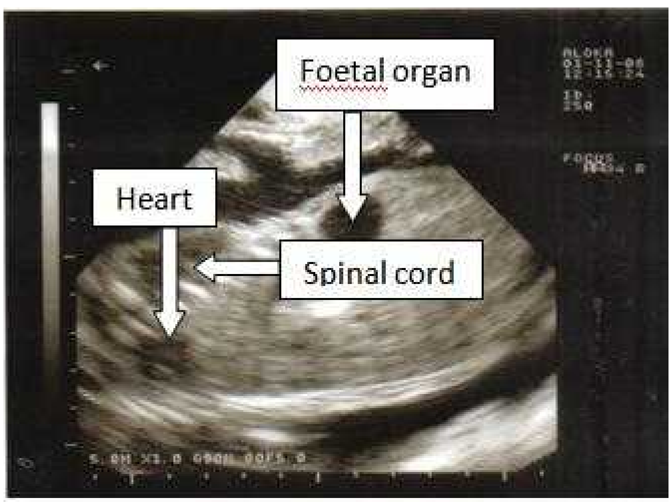

Figure 4: Image of foetus-heartbeat-spinal cord-ribs-foetal organ (arrows) was detected on day 103 of gestation (late stage) using transabdominal probe

Figure 5: Image of placentomes (arrows) was detected on day 143 of gestation (late stage) using transabdominal probe

DISCUSSION

The significant finding obtained from this study was the identification of foetus and foetal-related structures throughout the gestation period using transrectal and transabdominal probes. Structural images obtained in this experiment were similar with those images reported by Martinez *et al.* (1998); Medan *et al.* (2004) and Padilla-Rivas *et al.* (2005). Even though embryo development of embryos theoretically could be identified during pre-implantation stage, none of the literature reported to obtain images during this period of time. B-mode ultrasound imaging using transrectal probe was unable to identify pre-implantation embryos. To date, a sac or embryonic fluid was acknowledged as the first indicator for pregnancy detection. Sac was detected with black and white areas (non-ehogenic and echogenic areas). White areas represented the sac's border.

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. This corroborates with the finding of the present experiment where by sacs were successfully detected on day 18 of gestation using transrectal probe. The difficulty of detection on sac at this early stage may due to size of the sac, positioning of the probe, incapability of probe to detect or incompetency of operator in detecting the sac at this early stage of pregnancy. It is suggested that formation of sacs in goats could be initiated and formed from second to fourth week of gestation. The author assumed that sac was yolk sac that had regression simultaneously as foetus develop. Non-echogenic (NE) area was detected on days 20 to 24 of gestation using transrectal probe. 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 probes.

Foetal heart and embryo proper were detected on days 21 (Martinez *et al.*, 1998; Suguna *et al.*, 2008), 23 (Padilla-Rivas *et al.*, 2005), 24 of pregnancy (Medan *et al.*, 2004). In the present experiment, foetal heart was detected on day 28 of gestation, which is a few days after sac was observed using transrectal probe. Detection of foetal heart was suggested to give more significant indicator 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 22 and 28 by Karen *et al.* (2008) and Suguna *et al.* (2008), respectively. On days 51, 66 and 70 onwards, ultrasound scanning using transrectal probe failed to detect any structures or indicators. In a nutshell, using transrectal probe, we observed sac on day 18, NE area on days 20 to 24 and foetus-foetal heart-uterine wall-amniotic fluid on day 28.

Using transabdominal probe, from the results obtained in this experiment, images were very much similar with images those reported in the literature. Padilla-Rivas *et al.* (2005), Suguna *et al.* (2008) and Medan *et al.* (2004) reported detection of goat foetus with heartbeat as early as days 33, 35 and 60 of pregnancy; respectively. In the present experiment, foetus with foetal heart was detected as early as day 66 of gestation. Besides foetal heart, other structures that were observed were ribs, spinal cord, placentomes and foetal organs. From the results, placentomes was detected as early as day 51 of gestation. Suguna *et al.* (2008) and Medan *et al.* (2004) 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 as early as 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 NE area in the foetus, theoretically 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 119 of gestation. Placentomes were also observed throughout the later stage of pregnancy. This may be due to the growth curve of foetus in does. However, the limitation of transabdominal probe is unable to detect the foetal structures, except for placentomes starting day 120 until birth. Only placentomes were detected for third stage of gestation. During second stage of gestation, detection of placentomes-foetus-foetal heart-spinal cord-ribs was observed until day 119.

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 stage of gestation). Combination of structures such as placentomes-foetus-foetal heart-spinal cord-ribs showed the highest percentage of occurrence in early second stage of gestation. Placentomes were easily detected and it was a definite clear indicator for pregnancy. Spinal cord and ribs can be easily differentiated when foetal heart was detected. However, these structures might not be easily detected 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. Other than that, this may be related to the size of foetus and the positioning of the probe. As for the later phase of second stage of gestation, placentomes-foetus-foetal heart-spinal cord-ribs-foetal organs gave a high percentage of occurrences. The black area indicating fluid was marked as foetal organ as reported by Scheerboom and Taverne (1985). The main problem is in identifying some of the structures of foetal structures which were clear (elongated or circular black area), however some of the structures were only visible after movement of foetus. Experience and skills are required to make this identification a success. During third stage of gestation, presence of placentomes alone was detected. The fetus was too big to be detected unless the position of fetus was different.

From the results of the present study, percentage of accuracy for first, second and third stages of gestation were 96, 99 and 100%, respectively, regardless using transrectal or transabdominal probes. The first stage of gestation gave slightly a lower percentage of positive diagnosis using transrectal probe that could be due to the difficulty of identifying structures from days 18 to 22 of gestation.

The information obtained from this study alongside with future refined studies not only elucidates the principles of ultrasonography during goat foetal development during pregnancy but also extend to other related reproductive processes such as detection of folliculogenesis and ovulation mechanism as well as detection of reproductive organ abnormality. Ultrasound scanning technology if perfected could be coupled with other biotechnologies to be used routinely and efficiently in the goat farm management.

In summary, images of foetus and foetal-related structures were identified throughout the pregnancy stages in goats using two probes which are transrectal probe for first stage of gestation (days 18-50 of gestation) and transabdominal probe for second and third stages of gestation (days 51-143 of gestation) of pregnancy. We conclude that from second stage of gestation onwards, transrectal approach is not practical due to the difficulties in obtaining images. This probes that transabdominal probe is more practical at later phases (second and third stages of gestation). Observation and identification of sac, NE area and foetus-foetal heart-uterine wall-amniotic fluid were detected from days 21-49 of gestation. Combinations of placentomes-foetus-foetal heart-spinal cord-ribs and placentomes-foetus-foetal heart-spinal cord-ribs-foetal organ were easily detected during second stage of gestation. During third stage of gestation, only detection of placentomes was observed. Overall accuracy on pregnancy detection for each stage of gestation, i.e. first, second and third stages of gestation using transrectal and transabdominal probes were 96, 99 and 100%, respectively. The findings obtained from this study gives significant information on goat foetal structures and pregnancy detection using ultrasound. With refinement and detailed research on the basic structural images during pregnancy of does would give new impetus to revolutionize future pregnancy diagnosis in goats both for academic, research and industry. In addition, ultrasound technology along with progesterone assays and pregnancy-associated protein for pregnancy diagnosis in does is a useful tool in reproductive biotechnology that is an integral component with other reproductive technologies, such as AI, embryo transfer, cryopreservation, *in vitro* fertilisation, cloning, gene transfer and stem cell research.

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