Chapter 1

1.0 INTRODUCTION

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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 advanced goat management programme. Nevertheless, a practicable and reliable means of early pregnancy detection in goats is still lacking. 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. For example, a reliable technique for early detection of pregnancy would allow early culling of non-pregnant females or rebreeding of barren does. In other words, pregnancy diagnosis would identify whether the females 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 remove the non-pregnant ones from the flock should reduce cost in feeding, labour and medications and therefore, resulted in more profitable goat farming.

A range of pregnancy detection methods have evolved over the years. Various methods had been intended in detecting an early pregnancy in goats as means of improving reproductive efficiency. Ultrasonography, hormone assay and radiography have emerged as the most useful methods utilized today. Older technology described methods of laparotomy, cervical palpation, abdominal palpation or ballottement and rectal-abdominal palpation with a rod have limited utility or have been abandoned. Traditional methods of visual observation, abdominal palpation, service records and non-return to oestrus are not reliable means of diagnosing early pregnancy (Ishwar, 1995).

1.2 BACKGROUND

A variety of approaches has been explored for the early detection of pregnancy and possibly foetal numbers. Methods of choice depend on the availability of equipment, number of days post-breeding, desired accuracy and experience of the examiner (Ishwar, 1995). The application of ultrasound for pregnancy detection in domestic animals is becoming very popular. Pregnancy may be detected in does with all three types of ultrasonography available, i.e. amplitude depth (A-scan), Doppler and real-time B-scan ultrasonography. However, it has been suggested that each can be used under field conditions. The accuracy of diagnosis, timing of examination, foetal numbers and age as well as foetal viability vary considerably among these techniques (Ishwar, 1995). Due to lack of ultrasonography research in goats, basic information on other embryonic growth and related to pregnancy in mammals has been proved to be useful for pregnancy diagnosis study in goats (Martinez *et al.*, 1998).

1.2.1 Application of Ultrasonography in Farm Animals

Since early 1980s, real-time (B-mode) ultrasonography was available but initially considered time consuming and expensive for use in farm animals (Lindahl, 1966; Memon and Ott, 1980; Thwaites, 1981); nevertheless it has been proved to be a reliable means of pregnancy detection in a number of domestic species (Kahn, 1992).

Early foetal sex determination by ultrasonography has been successful in cattle and horses, based on the detection and location of genital tubercle and the external genitalia (Curran and Ginther, 1991; Curran, 1992). Foetal sex can be determined by ultrasonography between days 55 and 75 of pregnancy in horses (Santos *et al.*, 2005) and from day 50 onward in cattle (Curran *et al.*, 1989; Stroud, 1996), emphasizing that in cattle foetal sex can be accurately established around day 60 of pregnancy (Barros and Visintin, 2001). The accuracy of sex identification using ultrasound with transrectal probe is 92.6% on day 49 after breeding. Ultrasound imaging technology provides rapid, non-invasive access to the internal reproductive organs. These sound waves are painless and have no harmful after-effects to the foetus (Pierson and Adams, 1999).

Besides providing high quality and affordable ultra scanner for breeders, experienced operators (ultrasonographers) even provide professional scanning for animals. A few conditions need to be followed as the females must also be held off feed for 18 to 24 hours prior to scanning since animals with too much fill are hard or impossible to detect pregnancy using ultrasound because of food material and gas interfering with the sound waves. Ultrasound technology was proven safe, accurate, rapid and harmless to patient, operators and personnel nearby. These advantages positioned ultrasound scanning as the preference on pregnancy detection for breeders and farmers. Medan *et al.* (2004) suggested that foetal number and gestational age could be determined by ultrasonography.

Other than scanning for pregnant animals, this technology is also practically used to detect genetic disorder in muscling, marbling and fat thickness for cows. Ultrasound was applied to study the prevalence of Hydatid Cysts in the right lung and liver of goats in Turkana, Kenya (Maxson *et al.*, 1996). It had been a tremendous change and improvement in both agriculture and veterinary science sectors.

Ultrasonography has been applied to the ovary and uterus of domestic animals from the 1980s, and established in 1990s, as a practical tool for animal production. In highly developed countries, ultrasound technology is extensively applied by farmers in their farm practices. High quality and compact devices of veterinary ultrasound scanning are manufactured and distributed specifically for field condition on small animals. Demands in ultrasound technology devices were tremendous for the past 20 years. Ultrasound scanners have been used for many years for pregnancy detection of animals. Recently, ultrasound scanning is a standard veterinary diagnostic tool for equine tendon, abdomen, and cardiology of both small and large animals.

The ultrasonographic characterization of corpora lutea (CL) during the oestrous cycle and early pregnancy of ruminants was performed for the first time in cows (Kito *et al.*, 1986; Pierson and Ginther, 1987; Omran *et al.*, 1988; Kastelic *et al.*, 1990). In comparison with real-time transrectal ultrasonography, rectal palpation and plasma progesterone concentration were used to assess the bovine luteal status (Sprecher *et al.*, 1989). Ultrasonography is quicker and less stressful for the animals than either laparoscopy or laparotomy (Schrick and Inskeep, 1993).

1.2.2 Ultrasound Scanning in Goats

Real-time ultrasonography for early pregnancy detection in goats has been performed either transrectally or transabdominally (Padilla-Rivas *et al.*, 2005). Although transrectal or transabdominal real-time ultrasonography has been shown to provide a reliable means of pregnancy diagnosis in sheep (Buckrell, 1988; Haibel and Perkins, 1989; Garcia *et al.*, 1993; Schrick and Inskeep, 1993; Kaulfuss *et al.*, 1996), there have been only a few reports in the goat (Martinez *et al.*, 1998; Medan *et al.*, 2004; Padilla-Rivas *et al.*, 2005).

Real-time scanning of the uterus, in which an image is displayed on a video monitor, allows the operator to make a precise and accurate decision as to the state of pregnancy. Haibel (1990) reported repeated exposures of the sheep foetuses (probably similar to goats) to ultrasonic waves of two 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).

This technology took only measurements on average 2.5 and 1.5 minutes for transrectal and transabdominal transducers, respectively (Padilla-Rivas *et al.*, 2005). Successful rate depends on instrument, operator's experience and laboratory facilities. Real-time ultrasonography for diagnosing pregnancy in does can be rapidly learned. The experienced examiner can expect an accurate diagnosis of 91-100% (Ishwar, 1995).

Real-time ultrasonography is recommended as a reliable means of pregnancy detection as early as 26 days after conception (Medan *et al.*, 2004). Foetal heartbeat would be detected from day 23 onwards in Boer goats (Padilla-Rivas *et al.*, 2005). Ultrasonic imaging of the heartbeat is regularly used to detect the embryo and to evaluate embryo viability (Ishwar, 1995). Pregnancy diagnosis may be based on the images of uterine fluid and the amniotic vesicle or the embryo with its heartbeat (Medan *et al.*, 2004).

Padilla-Rivas *et al.* (2005) addressed the issue of pregnancy diagnosis using real-time ultrasonography in goats, while comparing the transrectal and transabdominal approaches. Visualization by transrectal transducer was a small non-echogenic (i.e. fluid filled) vesicle (1 cm in diameter), in the uterine lumen reliably detected from day 22 onwards. Transrectal transducers with different wavelengths, responded as early as 20 days after service if relying on the presence of a fluid-filled vesicle and from day 23 onwards when emphasizing on the identification of foetal heartbeat (Hesselink and Taverne, 1994; Martinez *et al.*, 1998; and Padilla-Rivas *et al.*, 2005).

Ultrasonography may serve as a reliable means to detect pregnancy in goats early in the fourth week after mating when using a 3.5 MHz transabdominal transducer (Padilla-Rivas *et al.*, 2005). Transabdominal transducer visualized fluid-filled vesicles were reliably located from day 26 onwards, whereas the foetal heartbeat was first seen as early as day 33 (Padilla-Rivas *et al.*, 2005). With a transabdominal 3.5 or 5 MHz transducer, results are obtained when diagnosed approximately 28 days of gestation (Hesselink and Taverne, 1994; Martinez *et al.*, 1998; Padilla-Rivas *et al.*, 2005). With B-mode ultrasonography, gestational age, foetal number and viability of the foetuses may be identified, depending on suitable equipment and operator's experience (Bretzlaff and Romano, 2001).

Both transducers proved to be a reliable tool in pregnancy detection in goats as ultrasound imaging gave very high percentage of accuracy in pregnancy diagnosis in goats (97.3%) approximately 55 to 60 days of gestation. However, the ability of these transducers for foetal numbers or twin detection was poor. This may be worsened due to inexperienced operators as twin detection accuracy was highly correlated with experience of the operators (Fowler and Wilkins, 1984).

Ultrasound was also proved to be capable to detect genital tubercles located in small ruminants, thus enabling to determine and differentiate the gender of foetus (Coubrough and Castell, 1998; Burstel *et al.*, 2001, 2002; Oliveira *et al.*, 2005; Santos *et al.*, 2005). It has been suggested that the optimal period of pregnancy is crucial to obtain the highest efficiency in gender determination (Coubrough and Castell, 1998). Accuracy of foetal sexing under field conditions is high in goats when ultrasound imaging is properly timed during pregnancy and when it is performed with proper equipment by experienced operators. Transrectal ultrasonography using the linear transducer is more effective for sexing goat foetuses between days 40 and 60 of pregnancy in both single and twin pregnancies (Maico *et al.*, 2007).

Where the necessary equipment and skills are available, real-time ultrasonography is a convenient and reliable means of early pregnancy detection in goats, particularly if a rectal transducer with a high resolution is available and the foetal heartbeat is taken as proof of the presence of a live foetus. Under these circumstances, on the average, pregnancy may be diagnosed in goats from day 23 onwards (Padilla-Rivas *et al.*, 2005).

Ultrasonography is useful to access the function and topography of corpus luteum using transrectal probe. Ultrasonography helps characterize the evolution pattern of the CL and to compare luteal function which is a valuable tool to differentiate between phases of normal oestrous cycles (Simoes *et al.*, 2005). Recently, with the development of real-time ultrasound, the detection and measurement of CL in goats were also studied (De Castro *et al.*, 1999; Simoes *et al.*, 2005), including their relationship with the progesterone levels (Orita *et al.*, 2000; Medan *et al.*, 2003).

1.2.3 The Principle of Ultrasound Scanning

The principle for ultrasonography can be explained as for underwater sonar or echo sounding. Ultrasounds are sound waves with a higher frequency that can be heard by the human ear. Ultrasonic sound wave of about 2 million Hz (cycles) per second can penetrate through living tissues and will be reflected from tissue interfaces. In the body, an apparatus sends an ultrasonic wave at a speed of about 1,500 metres per second. The wave will be refracted or 'broken-up' at the interface between two type of tissues and part of the wave will be reflected back and detected by the apparatus. The rest of the ultrasonic wave continues deeper into the body, and is reflected as an echo from the surface of tissues lying further inside the body. The densities of the respective tissues determine how much echo is reflected and the speed of the sound wave as it passes through them. The time taken for the reflected wave to return to the ultrasound <u>probe</u> indicates how deep the tissue lies within the body. Ultrasound probe is the part of an <u>ultrasound</u> scanner that contains the crystals that send out and receive the actual sound waves.

Attenuation is another theory that helps in detecting pregnancy in animals. Attenuation is reduction of the intensity of the sound beam as a result of refraction, reflection, scattering and absorption. This phenomenon happens when ultra-waves were unable to catch object images. This may be due to fluid-filled cavity such as bladder, amniotic fluid and fluid in sac. The image on the monitor is 'real-time' because the transmission and reception of ultrasound waves is constant and the display is updated rapidly at many frames per second. Real-time, B-mode imaging refers to the use of pulsed ultrasonography to obtain moving images of structures in grey scale. Images are constructed from returning echoes and continuously updated on the computer monitor by digital computer reconstruction. The brightness of the imaged structure is proportional to the strength of the returning echoe.

1.3 JUSTIFICATION

Studies on pregnancy in does are vital; however, information on pregnancy detection in goats is limited. Thus, it is important to know the efficacy of ultrasound scanning using two different probes, namely transrectal and transabdominal probes. Information on practicality of different probes would provide a more accurate result and save ample time. Other than distinguishing status of pregnancy for each does, the ability to identify stage of gestation (days of pregnancy) would help in predicting and estimating the date of kidding. This will increase the efficiency in herd management programme.

1.4 STATEMENT OF PROBLEMS

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

- a) What is the earliest day of pregnancy to gain significant accuracy in detecting pregnancy using ultrasound in goats?
- b) Are there any differences in sensitivity, specificity and accuracy in detecting either transrectal or transabdominal probe?
- c) Is it possible to determine the specific range of gestation age for transrectal and transabdominal probes?
- d) Is it possible to differentiate structures, shapes and sizes obtained from images using transrectal and transabdominal probes?
- e) Would it be possible to estimate the age of foetus using ultrasound scanning?
- f) Is it possible to determine age of foetus with observation on structures and combination of structures?
- g) How efficient and reliable is ultrasound scanning in detecting pregnancy in does?
- h) Can foetal development in doe be determined using observation from ultrasound scanning with transrectal and transabdominal probes?
- i) Is it possible to determine single, twin and triplet kids using ultrasound in goats?
- j) Is sex determination possible using ultrasound in goats?
- k) Is it possible to know the development of foetal organ, e.g. kidney using ultrasound in goats?
- 1) Is ultrasound examination practical in a large farm operation?
- m) How can ultrasound detection complement other pregnancy detection methods to achieve a higher efficiency?
- n) Does different breed of does affect the outcome of ultrasound examination?

1.5 OBJECTIVES OF THE STUDY

Ultrasound technology obviously promises a better future in animal science and managements. Therefore, we need to go parallel and get abreast with the technology. Thus, this research is vital and shall promise a better future in our goat industry.

Objectives of this study were:

- a) To determine the efficacy of ultrasonography in goat pregnancy detection.
- b) To monitor development of foetus by means of two different probes :
 - o transabdominal probe
 - o transrectal probe.
- c) To predict the best combination of structures observed for each stage of pregnancy.
- d) To estimate age of foetus with predicted combination of structures observed.
- e) To confirm the accuracy of predicted structural image combinations in estimation age of foetus.