Chapter 2

2.0 REVIEW OF LITERATURE

2.1 TIMELINE OF SIGNIFICANT FINDINGS IN GOAT ULTRASOUND IMAGING

Table 2.1 shows timeline of significant findings by various authors of ultrasound imaging in goat reproduction. This timeline covers from 1985 until 2008.

Table 2.1: Timeline of significant findings by various authors of ultrasound imaging in goat reproduction

Year	Author	Significant finding
1985	Scheerboom and Taverne	Visualization of uterine wall, placentomes, foetal skeleton, kidneys and vascular system during last 3 weeks of gestation.
1994	Dawson *et al.*	Accuracy for determining singles, twins and triplets at 7 weeks of gestation was 82%, 89% and 100%, respectively, with 5.0 MHz transabdominal probe.
		Two circumscribed fluid filled dark areas seen in the uterine horn indicating a twin was detected at 7 weeks of gestation.
		The uterus has an intrapelvic location in the first month of pregnancy, thus preventing good visualization by the transabdominal approach.
		Foetal heartbeat can be recognized as early as 4 weeks into gestation and the placentomes can be identified at 7 weeks of gestation.
		The ability to differentiate pregnant from open does was highly accurate when using the transabdominal approach at 5 weeks after breeding.
		In the second half of gestation, the marked increase in foetal size and normal reduction in the amount of amnio-allantoic fluid often prevented reliable differentiation of foetal structures and an accurate assessment of foetal numbers.

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Year	Author	Significant finding
1997	Doize *et al.*	Age of foetus was determined by placentomes with equation from day 30 to 90 of gestation. Transrectal probe (5.0 MHz) detected small nodule at 35 days and C-cup placentomes on 42 days of gestation. Placentomes measurement was limited on day 90 onwards. Placentomes growth was rapid during days 70 to 90 of gestation. Placentomes are saucer like-shape and the central cavity of the placentomes increased in size as the foetus grow, yet the borders remains thin. Age of foetus was proved 66% correct with correlated equation.
1998	Martinez *et al.*	Early detection and embryonic growth with 5.0 MHz transrectal probe On day 20 of gestation, embryos with heartbeat were detected. Non-echogenic density proved as significant indicator in detecting early pregnancy.
1998	Gonzalez de Bulnes *et al.* (sheep)*	Embryonic vesicles diameter that were detected with 7.5 MHz linear array transrectal probe were highly correlated with gestational age from day 12 to 19. From day 19 onwards, crown–rump measurements, occipito-snout lengths, thoracic, bipariental and orbit diameters gave highest correlation in determining ages of ewes. Detection of the crown-rump length in an ovine embryo on day 26.

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Year	Author	Significant finding
		Detection of the biparietal diameter left and the occipito-snout length right in a foetus on day 43.
		Detection of thoracic diameter in a foetus of 67 days. The largest anechoic zone in the foetal trunk is the stomach.
		Transrectal ultrasound device is efficient to observe pregnancy in the first three months of gestation only until days 90 to 91 because the foetus is not easily accessible later.
		Single and multiple pregnancies could be detected from day 19, when it is possible to detect the embryo.
1998	Coubrough and Castell (sheep)*	Foetal sex determination with 5.0 MHz transrectal probe by identifying and locating genital tubercle between 60 to 69 days post-breeding in ewes.
		Accuracy on sex determination was 89%.
2004	Gonzalez *et al.*	Comparison between 7.5 MHz transrectal probe, progesterone assay and pregnancy-associated glycoprotein (PAG) in detecting early pregnancy.
		Detection on day 26 of gestation using transrectal probe gave 99.4% accuracy.
2004	Medan *et al.*	Early detection in pregnancy using 7.5 MHz transrectal, 7.5 and 5.0 MHz transabdominal probes.
		Pregnancy detection gave positive result as early as 21 days of gestation with image of sacs.
		Accuracy (100%) on day 60 of gestation.
		Images on sacs, heartbeats, placentomes and skeletal structures were obtained.

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Year	Author	Significant finding

2005	Padilla-Rivas *et al.*	Pregnancy detection in both 7.5 MHz transrectal and 3.5 MHz transabdominal probes in Boer goats. Positive diagnosis was detected as early as 22 and 26 days of gestation with images of sac and heartbeat with transrectal probe With transabdominal probe, sac was detected on day 26 and heartbeat was visible on day 33 of gestation. Pregnancy diagnosis on day 26 of gestation gave the best result.
2006	Karen *et al.* (sheep)*	Determination of foetal number using 3.5 MHz transabdominal probe, progesterone assay and pregnancy-associated glycoprotein (PAG). Positive diagnoses were detected as early as 50 to 100 days of gestation in sheep. Percent of accuracy on 76 to 87 days of gestation was 71.6% compared to progesterone and PAG; 65.4% 72.8%, respectively.
2007	Maico *et al.*	Sex determination with genital tubercle using 5.0 and 7.5 MHz transabdominal plus 6.0 and 8.0 MHz transrectal probes. Highest accuracy was during 40 to 60 days of gestation. Detection on scrotal bag for male, and vulva for female was possible on day 45 onwards.
2008	Karen *et al.*	Estimation on gestational age of Egyptian goats by ultrasonographic fetometry. Age of embryo of foetus can be estimated by measuring the crown-rump length, bipariental diameter, trunk diameter, placentomes size, umbilical cord diameter and femur length.

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Year	Author	Significant finding

		Embryonic vesicle and embryo proper with a beating heart were first determined by 7.0 MHz transrectal and 3.5-5.0 MHz transabdominal ultrasonography were days 17, 28, 22 and 31 of gestation, respectively ($P < 0.0001$).
2008	Suguna *et al.*	The embryonic vesicle was detected on days 21 and 28 and the embryo proper on days 28 and 35 of gestation using the 6.0 MHz transrectal and 5.0 MHz transabdominal methods, respectively. Heartbeat was observed as on days 21 and 35 of gestation with transrectal and transabdominal approaches, respectively. Placentomes as a circular 'C' shape structure was detectable on day 42 with an average diameter of 0.97 cm, using the transrectal probe. Using the abdominal probe, placentomes were detectable on day 50 of gestation. Skeletal structures such as the skull, rib cage and vertebral column were first viewed on day 56 of gestation in both approaches. Singles and twins were differentiated on days 35 and 42 of gestation by the transrectal and transabdominal approaches, respectively.
2008	Romano and Christians (sheep)*	Studies to determine the earliest date to execute pregnancy diagnosis using 7.5 MHz transrectal probe on ewes. Pregnancy was confirmed by presence of an embryo or extra-embryonic membranes. Accuracy rates were 0%, 31.3%, 40%, 70% and 100% on 15, 16, 17, 18 and 19 days of pregnancy, respectively.

*Studies on ewes that gave significant results equivalent to studies on does.

2.2 HISTORICAL BACKGROUND OF ULTRASOUND SCANNING IN PREGNANCY DIAGNOSIS

Ultrasound has many industrial applications but its initial veterinary application, for example the detection of ovine pregnancy was reported in 1966 (cited by King, 2006). Ultrasound was first used in animals as a means of determining back fat thickness in relation to carcass quality (Temple *et al.,* 1956) and a review of the current veterinary literature indicated that this remains its most common application. Ultrasonography was introduced in animal reproduction for depicting pregnancies in horses (Palmer and Driancourt, 1980) and cats (Mailhac *et al.,* 1980). Kahn (1992) reported that ultrasonography scanning is practically used to detect pregnancy in numerous animals. Some are considered important in research and some are for conservation of wild life as well as exotic species. Importance of ultrasonography also revolves in detecting pregnancy for exotic animals such as gorillas and rhesus monkeys (Sabbagha *et al*., 1975; Nyland *et al.*, 1984). In recent years, real-time ultrasonography has been used more frequently for pregnancy diagnosis in small ruminants (Romano and Christians, 2008).

The benefits of ultrasound as a veterinary diagnostic imaging procedure are numerous. Routine examinations have been shown that the procedure is safe. No report on harmful biological effects was raised although constant review is undertaken to ensure this remains the case. Ultrasound is considered a safe procedure for the patient, the operator and nearby personnel, allowing it to be performed in any location without the need for specific safety precautions (Preston and Shaw, 2001). It is also less invasive, therefore, well tolerated in unsedated animals, making serial examinations to monitor progression of the condition, response to treatment or to practice scanning techniques possible and practicable (Nyland and Mattoon, 2002). Although it takes practice and experience to achieve competency and confidence with the technique, specific applications can be easily and rapidly learned even by inexperienced operators (Dickie *et al.,* 2003).

Ultrasound machines are 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).

Teixeira *et al.* (2008) reported that ultrasound was also reliable to select embryo donors with a high probability of having produced zygotes. Selected does later undergo further processing by DNA microinjection to participate in a transgenesis goat programme. Transgenic animals depicts as an advanced technology and may provide a frog-leap in animal biotechnology, therefore, complemented technology such as ultrasound scanning was believed to have a promising prospect. Ultrasound scanning straight forward method was able to simplified procedure in determining donors and may probably a preferred technique to surgical procedure such as laparotomy. It is proven less stressful to the animal. The sensitivity, specificity and efficiency of ultrasonographic observation to determine the occurrence of ovulations in goat was 100%. There are a few drawbacks, especially in determining presence of multiple ovulations but ultrasound proves to be a potential tool for the embryo biotechnology. In goats, several evaluations have been done using rectal scanning to verify and monitor the ovarian follicular dynamics (Ginther and Kot, 1994; Cruz *et al.,* 2005), the time of ovulation (Gonzalez-Bulnes *et al.,* 2004) and to determine with accuracy the number of CL present 6 to 7 days following both hormonally induced oestrus and superovulation (Gonzalez de Bulnes *et al.,* 1999; Riesenberg *et al.,* 2001).

2.3 DIFFERENT TYPES OF ULTRASOUND SCANNERS

Ultrasound techniques plays major role in industrial application. Ultrasound was proposed as a diagnostic aid back in 1942 (Dussik *et al.,* 1947). Brown (1960) developed the first contact ultrasound scanner allowing the transducers containing crystals to be directly applied to the patient. The application of different type of ultrasound such as A-mode, M-mode, B-mode and Doppler differ with the predilection of the examiner. The real-time brightness or B-mode imaging is currently the form of ultrasound that is commonly used. The A-mode is use in basic fluid detection equipment such as that used to detect pregnancy in pigs (Holtz, 1982). Meanwhile, M-mode was used to assess movement of heart valves and walls (Edler and Hertz, 1954).

Amplitude depth ultrasound or A-mode ultrasonography is for pregnancy diagnosis in detection of the fluid-filled uterus and is thus no pregnancy-specific. A-mode units emit ultrasonic waves from a transducer placed externally against the skin of the abdomen and directed towards the uterus. Ultrasound waves are converted to electrical energy in the form of audible or visual signal. These units detect fluid filled organs at a depth of 10 to 20 cm (Ishwar, 1995). When a fluid filled structure is detected some units emit a light or audible signal. Units with an oscilloscope display reflections a peak or blips on the screen. Non-pregnancy is suggested when the peaks are present only in the left half of the screen. Hence, a fluid filled structure is detected; peaks will also appear on the right half of the screen. Accuracy obtained is 80 to 85% if performed between 60 to 120 days of gestation. Wani (1981) and Watt *et al.* (1984) believed A-mode to be reliable tool from 50 to 120 days of gestation in goats.

However, an extended urinary bladder, hydrometra or pyometra may give false results that may occur in early gestation or in late gestation because of decrease in the ratio of uterine fluid to foetal tissue. Neither foetal viability nor foetal numbers can be detected by this method. Early work with externally applied A-scan indicated that an accuracy of at least 95% is possible between 60 to 80 days of gestation in ewes (Haibel, 1990). However, Meredith and Madani (1980) reported that a positive diagnosis of pregnancy in ewes is made on the evidence of ultrasound reflections with an accuracy of 83% on days 61 to 151 after mating. Lindahl (1969) reported that the earliest time at which pregnancy could be detected by using A-scan is between 40 and 50 days after mating.

Prior to real-time imaging, examination of moving structures such as the heart was possible with Time Motion or M-mode ultrasound (King, 2006). In its current form, the two-dimensional grey scale image allows placement of a cursor line across the desired area and a trace is produced representing the movement of every tissue interface along that line against time. M-Mode is now an integral part of echocardiographic examinations and all modern ultrasound machines are equipped with this capability (King, 2006). M-mode permits repeatable, objective measurements to be made at specific stages of the cardiac cycle allowing evaluation of chamber size and wall thickness, assessment of valve motion, calculation of functional criteria including shortening fraction (Curry *et al.,* 1990) and determination of foetal heart rates (Curran and Ginther, 1995; Moreno *et al.,* 1996).

The principle of Doppler ultrasound for pregnancy diagnosis is the detection of movements such as blood flow in the middle uterine artery, umbilical arteries, foetal heart beat and foetal movements. Fraser and Robertson (1967) were the first to apply it on sheep. Shone and Fricker (1969) used the same type of machine on 309 ewes between days 66 and 122 of gestation and recorded 100% accuracy at all stages of pregnancy. Foetal blood from the umbilical artery was the most common diagnostic feature (Ishwar, 1995). Transducer emits ultrasound waves; sound reflected from motionless structures has the same frequency as the transmitted sound whereas sound reflected from moving organ or blood has a different frequency. Differences in frequency are converted to audible sound. Audible signals which could be distinguished by the observer include the foetal heartbeat, arterial blood flow in the middle of uterine artery and umbilical arteries, foetal body movements and maternal intestinal movement. The ‘gushing sounds’ of the middle uterine artery, the ‘galloping beat’ of the foetal heartbeat and ‘swishing sound’ of the umbilical blood flow heard were taken as indicators of pregnancy (Wani *et al.,* 1998). The transducer can be applied to the skin of the abdomen or intrarectally using a rectal probe.

A positive diagnosis of pregnancy is made by listening to the rapid, pounding sound of the foetal heartbeat, foetal pulse and foetal movements. Foetal heartbeat and foetal pulse which are faster than the maternal pulse or foetal movement are taken as positive criteria of pregnancy (Lindahl, 1969). Foetal pulse is rapid with swishing sound plus much faster than the maternal pulse that is sharp. External application of the ultrasonic Doppler has been used for detection of pregnancy in does. Pregnancy detection approaches an accuracy of 100% during the last half of gestation (Fraser and Robertson, 1968; Lindahl, 1968; Shone and Fricker, 1969) but is not effective on day 50 to 75 or earlier (Lindahl, 1969, 1971). The intrarectal Doppler technique is superior to the external technique when used early in the second trimester for diagnosing pregnancy that may achieve an accuracy of 90% or better (Lindahl 1971; Ott *et al.,* 1981). Results with 2111 ewes indicate that pregnancy can be detected at mid-gestation with an accuracy of higher than 90%. It may also be used earlier which is 25 to 30 days post-breeding but possibility of false results occur when soft faeces around the rectal probe interfere with sound wave transmission. Therefore, it is best to use between 35 to 40 days of gestation (Ishwar, 1995).

False results are unlikely with the Doppler technique when foetal sounds are used as the criteria for pregnancy diagnosis. Hydrometra can cause increased maternal blood flow in the middle uterine arteries but no foetal sounds will be heard. Doppler units with a frequency of 2.25 MHz may be superior in near term pregnancies whereas a 5.0 MHz seems better for detecting earlier pregnancies. Compared to A-scan technique, the Doppler technique resulted in greater accuracy in ewes, which were at least 65 days pregnancy

(Lindahl, 1969). The intrarectal Doppler technique also allows detection of pregnancy earlier in gestation than the A-scan technique (Lindahl, 1971). However, Wani *et al.* (1998) reported 72% accuracy in predicting pregnancy in gaddi goats using Doppler technique. The cause might due to the audition of middle uterine artery sounds in previously kidded does. Accurate detection using Doppler technique is also confounded with the present of multiple foetuses (Ishwar, 1995). However, no explanation was given for this issue.

On the other hand, real-time B-mode ultrasonography offers an accurate, rapid, safe and practical means of diagnosing pregnancy and determining foetal numbers (Ishwar, 1995). B-mode ultrasonic scanning produces a two dimensional images on the screen. For pregnancy examination, it produces a moving image of the uterus, foetal fluids, foetal heartbeat, placentomes, head, limbs and other skeletal structures. Examinations should be performed away from direct sunlight under subdued lighting for optimal image visibility. Real-time B-mode ultrasonography is performed using two probes, i.e. transrectal and transabdominal probes. Ideal time for transabdominal scanning is between 40 to 75 days (Ishwar, 1995). Haibel (1990) reported that 25 to 30 days is best done transrectally.

However, most modern units combine two-dimensional B-Mode and Doppler imaging, known as Duplex imaging (Curry *et al.,* 1990) allowing direction of the Doppler beam by means of a movable cursor line on the B-Mode image. This improves the accuracy with which flow can be identified, localised and interrogated. Two forms of Doppler have emerged, i.e. pulsed wave (PW) (Wells, 1969) and continuous wave (CW) (Light, 1969). Pulsed wave selectively interrogates flow within a small segment selected by the presence of a gate on the cursor line, therefore, allowing the location and uniformity of flow to be determined although the maximum velocity of flow that can be measured is limited. Continuous wave Doppler lacks depth discrimination, thus combines all the flows encountered along the length of the cursor into one trace although it does allow accurate measurement of high velocity flow (King, 2006).

2.4 FACTORS AFFECTING ACCURACY RATE OF PREGNANCY DIAGNOSIS USING ULTRASOUND SCANNING

To our knowledge, the factors affecting accuracy rate using either transrectal or transabdominal ultrasonographies for the detection of early pregnancy have not been investigated in goats. At present, the main problem affecting accuracy of pregnancy detection using ultrasonography in farm animals has been the lack of a simultaneous, non-invasive and accurate standard method for different groups of animals. Accuracy rate using ultrasound scanners also varies with different materials and method used. The age and breed of the animals and the experience of the operators are among the main factors responsible for these variable results (Buckrell, 1988; Bretzlaff and Romano, 2001). Succinctly, four main aspects need to be considered to increase accuracy, which are the operator's skills, availability of equipment, the patient (e.g., doe) and the foetus to be observed.

In order to acquire the highest accuracy rate, operator's competency in interpretation of desired images is highly important. Meaningful information on image indicators during pregnancy may be difficult to obtain and scarcely reported in the literature. Thus, predicted results on images during specific time of pregnancy solely depend on operator's experiences and skills. Although specific skills and information may be rapidly acquired, it requires a constant and regular practice in order to achieve a broad-base competency with the technique and confidence in the results (King, 2006). Other than that, thorough knowledge of the relevant internal reproductive anatomy of the female is also a necessity. Operators are required to have competency in differentiating the normal and abnormal anatomical structures associated with pregnancy in order to avoid any misdiagnosis.

Equipment's availability holds an important role as well. Transrectal ultrasonography offers several advantages over other methods used for pregnancy diagnosis. These include an earlier time of pregnancy diagnosis, earlier determination of conceptus number, embryo or foetus viability, the estimation of embryo or foetus age, reduction of misdiagnosis, sexing and the diagnoses of abnormal pregnancies (Bretzlaff *et al.,* 1993; Coubrough and Castell, 1998; Romano*,* 1998). This explains researchers prefer transrectal to transabdominal approach.

Transrectal transducers was proved to be a better tool in detecting early pregnancies or foetal numbers, but the level of accuracy was predominantly depending on the probe frequency. Usually, a higher frequency was applied with the transrectal transducer. Higher frequency probes are designed for view nearby objects in which they deliver higher resolution and clearer image at a close range. Definite images can be observed by experienced operators thus determining early pregnancy, foetal number and sex foetal differential. However, with a lower frequency such as 3.5 MHz, deeper penetration is achieved (Padilla-Rivas *et al.*, 2005). Thus, a lower frequency transducer is a better preference to detect pregnancy using certain indicators such as embryonic vesicles in early stages of gestation.

Transabdominal probe was reliable as has been shown that sac was detected as early as 26 days of gestation (Gonzalez *et al.,* 2004). Ultrasound penetration ability with transabdominal probe also plays an important role. For this reason, some researchers shaved test site while others clipping away the hair overlying the region of interest. Therefore, precaution must be taken to avoid misdiagnosis as the beam cannot penetrate the air trapped between the hairs as suggested by Herring and Bjornton (1985), Green (1996) and Nyland and Mattoon (2002). Other than that, judicious positioning of the does with transabdominal probe is also important. Ultrasound is unable to penetrate gas filled or bony structures, therefore acoustic windows must be found which avoid the interposition of bone or gas between the transducer and the region of interest.

Kahn (1992) reported there are three periods or lengths of time for sonographic diagnosis on early pregnancies (the first 2 months of gestation). First, detection can be carried out the first 14 days of pregnancy whereby, early detection of pregnancy may be visible but diagnostic accuracy was low. This diagnosis is normally used for research purposes. Second, the succeeding period (days 14-28 of gestation) gives reliable diagnosis but needs favourable conditions. Normally, accuracy of diagnosis is low under field condition. Third, days 29-56 of pregnancy gives reliable accurate results of diagnosis even under practical field conditions and on large-scale basis.

The earliest detection may not give the highest accuracy but gives advantage over time. Signs of pregnancy can be described in some cases and used for research purposes, but accuracy of diagnosis is low under field conditions. Romano and Christians (2008) prioritize on obtaining the earliest period to detect pregnant ewe and reported day 20 of gestation gave 100% accuracy. The succeeding period which is the second length of time, promises reliable diagnosis but results are often difficult to achieve and needs favourable conditions plus scanning can be time consuming. Even though the third period may take a while, sonography provides accurate results under practical circumstances and on a large-scale basis. All factors mentioned earlier may impact in diverse results.

Accuracy rate also proved to be higher when study was performed during optimal time in detecting the earliest indicator in pregnancy due to the rapid increase in the volume of the embryonic fluid making it more discernible. The optimal time for counting foetal numbers is between 45 to 90 days of gestation (White *et al.,* 1984; Wilkins and Fowler, 1984; Haibel, 1990). After 90 to 100 days of gestation, foetuses become too large to be consistently differentiated from each other. Twins can be more accurately diagnosed than triplets.

Different position in animals plays important role in acquiring high accuracy. Karen *et al.* (2004) performed ultrasonography examinations in Awassi x Merino ewes, gave a significant different in accuracy rate since the pregnant uterus tops over the pelvic brim and descends into the abdominal cavity at an early stage of pregnancy especially by pluriparous ewes (Buckrell, 1988; Bretzlaff *et al.*, 1993). Thus, she took the initiative to lift the abdomen wall of the ewe. However, most literature in goats believed standing position to be the easiest way to conduct the experiment.

Age, breed and status of does may be a slight factor, as nulliparous gave higher accuracy than pluriparous ewes (Karen *et al.,* 2004). Some does were deprived from feed or water before the examination. Fasting minimizes the presence of intestinal gases, which might obscure images of reproductive tract and consequently, increases in correct diagnosis.

A positive diagnosis of pregnancy is assured by imaging the embryo or foetus or placentomes surrounded by fluid. The condition occurs commonly enough in goats to advise caution against making a positive diagnosis of pregnancy until the embryo or foetus can be seen. The urinary bladder should not be confused with a fluid filled uterus. Visualizing foetal movement or beating of the foetal heart during real-time imaging can assess foetal viability. Later, open or pseudo-pregnant or often culled or given prostaglandin to make them come in oestrus. Thus, there is great emphasis plays on obtaining highly accurate pregnancy test. False positive diagnosis may due to early embryonic death, unobserved abortion or sometimes misinterpreting the urinary bladder as the uterus but reported rare (Fowler and Wilkins, 1984; White *et al.,* 1984; Ley, 1985; Haibel, 1990). False negative may result from failure to image the tract early in gestation or inexperienced operator (White *et al.,* 1984; Haibel, 1990).

2.5 **RELATIONSHIP BETWEEN STRUCTURES DETECTED BY ULTRASOUND SCANNER DURING PREGNANCY**

Pregnancy is confirmed by imaging fluid in the uterine lumen, by finding evidence of placentomes or by identification of one or more foetuses (White and Russel, 1984; Buckrell *et al.,* 1986). Embryology is important that complements the early pregnancy detection in goats. Pregnancy signal was initiated given out on day 12 to the uterus to stop releasing prostaglandin and consequently the corpus luteum persists in order to maintain pregnancy. If the embryo dies before this, the cycle length will be normal and oestrous cycle will continue as usual. Embryo is growing on day 10 but it is not visible to the naked eye. After fertilization, zygote developed the morula, a 16 or 32 cell structure formed by the division and redivision of the zygote. The blastocyst is a structure with a fluid filed cavity (blastocoele) surrounded by the cell formed into a layer. The inner cell mass is the mound of cells that will form the embryo. The cells differentiate to form the specific organ in the embryo. The first stage of differentiation is three layers of primitive cell; ecdoterm, mesoderm and endoderm. Figure 2.1 shows the stages of development of a fertilized egg.

At the moment, no research has been reported to successfully obtain images of these stages of embryo *in vivo*. The first sign of pregnancy was the appearance of gestational sac, a circular or elongated non-echogenic structure located in the uterus cranial to the bladder. The gestational sac could be detected with ultrasound on day 21 of gestation (Medan *et al.*, 2004). A placenta consists of foetal and parental tissue for purpose of physiological

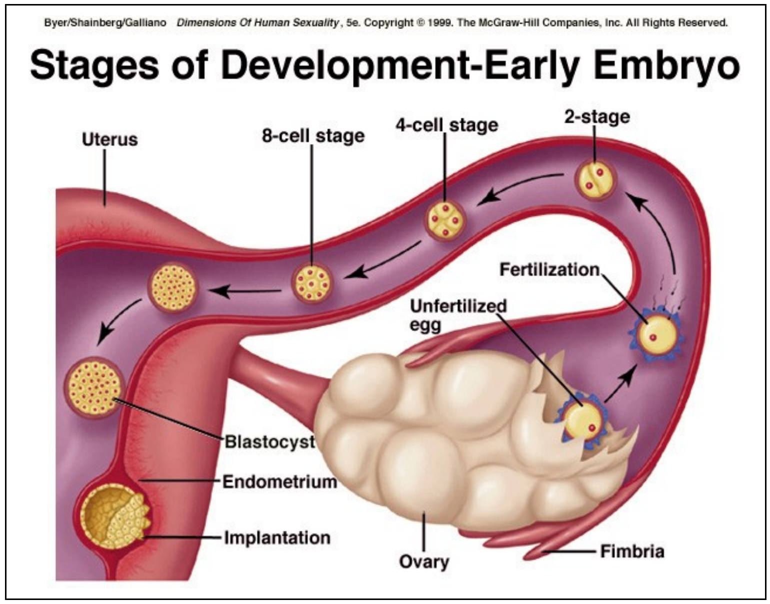

Figure 2.1: The stages of development of a fertilized egg. (Adapted from Dimensions of Human Sexuality. Byer/Shanberg/ Galliano, McGraw-Hill Companies, 1999).

exchange. Although nutrients and waste materials are exchange between the two circulation, blood does not pass directly from the foetus to the maternal blood supply and *vice versa*. Although the two are close, there is always tissue between them. In goats, there are specialized areas of contact, the cotyledons. Caruncles or placentomes provide nutrients for the kid, as placenta is for babies in human. Detection of placentomes was an earlier indicator of pregnancy before detection of heartbeat. Placentomes appear as echogenic densities in the uterine wall and visible as small nodules. As pregnancy progressed, placentomes increased in size and appeared as C-shaped by 45 to 50 days when viewed in cross-section are routinely found on days 26 to 28 using transrectal probe and 40 days of post-breeding using transabdominal probe (Buckrell *et al.,* 1986) or O-shaped grey image that is possible with ultrasound transabdominal transducer on day 60 of gestation (Medan *et al.*, 2004). They are seen as small discs rich in blood vessels. They begin to lie against the uterine caruncles at about day 17 of pregnancy. On day 30, attachments have been formed and the embryo no longer depends on the uterine milk for its nutrients. Caruncles or cotyledons are the points of attachments of the placenta to the uterine wall.

The amnion and the allantochorion are membranes around the outside of the embryo. The amnion contains fluids in which the embryo is suspended. While, the allontochorion attaches to the lining of the uterus to form the placenta. Medan *et al.* (2004) reported that the image of the embryo with umbilical cord was discovered as early as 30 and 33 days of gestation. The amniotic fluid and umbilical cord was distinctively portrayed in still images. The principle of ultrasound enables the sonographers to differentiate the foetuses that are high echogenic density surrounded by non-echogenic area that represent the amnion fluid (Martinez *et al.,* 1998).

After differentiation of the tissue and organs, the embryo becomes known as a foetus. When the circulatory system develops rapidly and by the third week heartbeat is detectable. The detection on heartbeat plays major roles in confirming the presence of embryo. Rhythmic pulsation is an indicator of embryo viability. Heartbeat was detected as early as 20 days of post-breeding (Martinez *et al*., 1998). The embryos of pregnant animals were detected and confirmed by detection of heartbeat on 24 days of gestation (Medan *et al.*, 2004). Using transrectal probe, bladder lies as a reference for the reproductive site. The bladder can be identified by viewing the characteristics triangular-shape neck as the transducer is directly in the rectum. The bladder wall can usually be seen as echogenic white line separating the anechoic lumen of the bladder from the anechoic luminal sections. The foetus appears as an echogenic mass within the uterine lumen. The foetus and the foetus heartbeat are visible after days 25 and 35 with transrectal and transabdominal probes, respectively. Foetal viability can be assessed by visualizing foetal movement or beating of the foetal heart during real-time imaging. On day 60 of gestation onwards, other structures such as ribs, long bones and head were detected using transbdominal probe. Medan *et al.* (2004) obtained image of the thorax in a goat foetus using 5.0 MHz transabdominal transducer. The heart appears as an anechoic structure between the white dots that represent the ribs. On day 70 of gestation, images of long bone (radius) and head were detected. Certain structures provide the ability to estimate gestational age such as determination of crown-rump length of the embryo, size of placentomes, biparietal diameter of skull and length of long bones. Figure 2.2 shows pictures of foetus taking form.

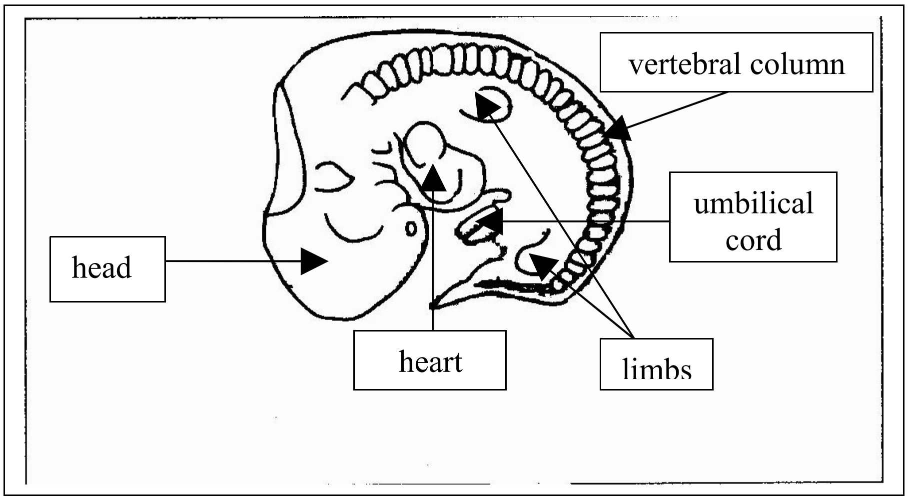

Figure 2.2: A foetus taking form. (Adapted from Anatomy and physiology of the goat. Robert North, Agfact A7.9.5, second edition 2004).

It is important to know basic knowledge of goat anatomy and reproductive site of female does. This is avoiding any misinterpretation of images. The uterus is often called the womb. When the does is not pregnant, the uterus is 2 to 3 cm long. It has two horns extending from it. The lining of the uterus is dark pink because it is covered with tiny blood vessels that are there to provide nutrients for the growing embryo. The caruncles, mention earlier as small raised areas on the uterine wall, are the points of attachment for the placenta. Cranial to the uterus is the rectum. The rectum is where the transrectal probe is being inserted. Probe will be carefully inserted until an image of bladder, structure in black, due to non-echogenic density showing it consists of fluid was detected. The probe is then rotated 90 degrees clockwise and non-echogenic density showing it consists of fluid. Figure 2.3 shows the normal position of the foetus in doe's abdomen (cross-section).

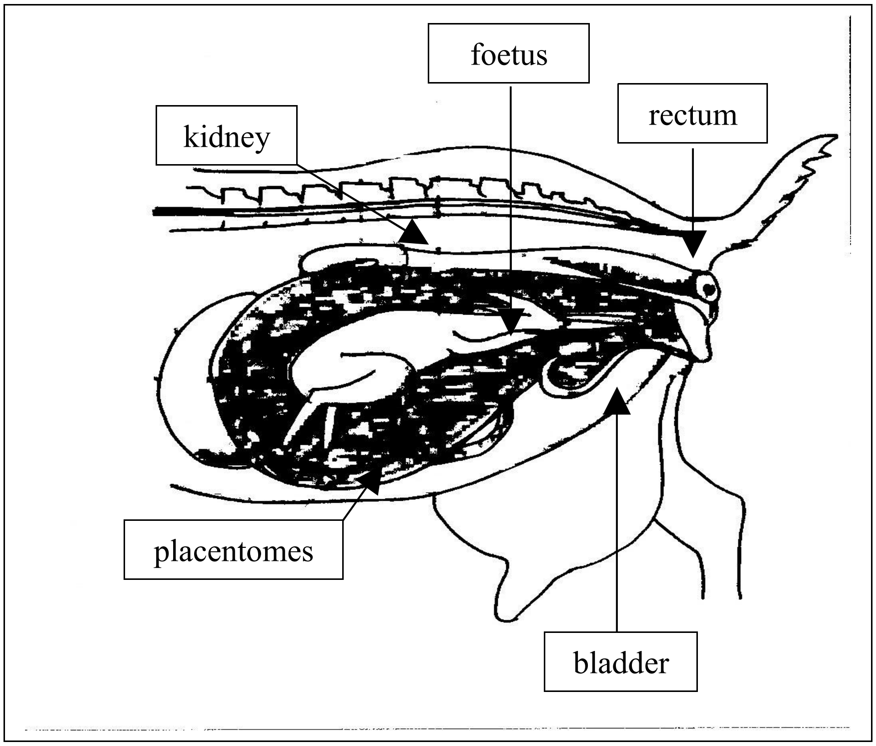

Figure 2.3: The normal-position of the foetus in the doe's abdomen (cross-section) (Adapted from Anatomy and physiology of the goat. Robert North, Agfact 7.9.5, second edition 2004).

2.6 SUITABILITY OF SCANNERS FOR DIFFERENT STRUCTURES ALONG THE PREGNANCY PERIOD

Different scanners gave different results along the pregnancy period. Table 2.2 shows development of foetal structures such as non-echogenic area and sac during early stage plus ribs and genital tubercles at later stages of pregnancy that were detected using transrectal and transabdominal probes with accuracy rate. Images of ultrasound scanning by different authors were rearranged by period of gestation to show development of foetal structures using different probes (Appendix Figures 1.1- 1.27).

Table 2.2: Development of foetal structures detected in goats using transrectal and transabdominal probes with accuracy rate along gestation period

Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
15-19 (14)	Accuracy rate was 0%, 31.3%, 40%, 70% and 100% on days 15, 16, 17, 18 and 19 of pregnancy; respectively (Romano and Christians, 2008).	Presence of an embryo or extra-embryonic membranes using 7.5 MHz transrectal probe on ewes (Romano and Christians, 2008).	NA
	NA	Embryonic vesicle was first detected on day 16.98±1.97 of gestation with 7.0 MHz transrectal probe (Karen *et al.,* 2008).	NA
20-29 (9)	NA	Non-echogenic density proved as significant indicator in detecting early pregnancy at day 18 of gestation (Martinez *et al.,* 1998).	NA
	NA	Image on non-pregnant does uterus as reference for open female (Medan *et al.,* 2004; Padilla-Rivas *et al.,* 2005).	NA
	NA	Embryos with heartbeat were detected using 5.0 MHz transrectal probe on day 20 of gestation (Martinez *et al.,* 1998).	NA
	NA	Image of sacs detected with 7.5 MHz transrectal probe on day 21 of gestation	NA

(Medan *et al.,* 2004*).*

(continued)

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Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
20-29 (9)	NA	The embryonic vesicle was detected on day 21 using 6.0 MHz transrectal probe (Suguna *et al.* 2008).	The embryonic vesicle was detected on day 28 using 5.0 MHz transabdominal ultrasonography (Suguna *et al.* 2008).
	NA	Heartbeat was observed on day 21 using 6.0 MHz transrectal probe (Suguna *et al.* 2008).	NA
	NA	NA	Embryonic vesicle was detected on day 23 using (3.5-5.0 MHz) transabdominal ultrasonography of gestation (Karen *et al.,* 2008).
	Pregnancy diagnosis on day 26 of gestation gave the best result using transrectal approach (Padilla-Rivas *et al.,* 2005*).*	Images of sac and heartbeat were detected with 7.5 MHz transrectal probe on 22 and 26 days of gestation (Padilla-Rivas *et al.,* 2005*).*	NA
	Detection on day 26 of gestation using 7.5 MHz transrectal probe gave 99.4% accuracy (Gonzalez *et al.,* 2004).	NA	NA
	NA	NA	Sac was detected using 3.5 MHz transabdominal probe on day 26.4 of gestation (Padilla-Rivas *et al.,* 2005*).*

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Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
20-29 (9)	NA	Embryo proper with a beating heart was detected on day 23 of gestation using 7.0 MHz transrectal (Karen *et al.,* 2008).	Embryo proper with a beating heart was detected on day 31 of gestation using (3.5-5.0 MHz) transabdominal ultrasonography (Karen *et al.,* 2008).
	NA	Embryo proper was detected on day 28 of gestation (Suguna *et al.,* 2008).	NA
30-44 (14)	NA	NA	Heartbeat was visible on day 33 of gestation using 3.5 MHz transabdominal probe (Padilla-Rivas *et al.,* 2005).
	NA	Placentomes were detected on day 35 of gestation using 7.5 MHz transrectal probe (Medan *et al.,* 2004).	NA
	NA	Embryo with umbilical cord was detected on day 30 and 35 of gestation using 7.5 MHz transrectal probe (Medan *et al.,* 2004).	NA
	NA	Heartbeat was detected on day 35 of gestation (Suguna *et al.* 2008).	NA

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Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
	NA	Embryo was detected on day 40 of gestation using 7.5 MHz transrectal probe (Martinez *et al.,* 1998).	NA
	Age of foetus was proved 66% correct with correlated equation (Doize *et al.,* 1997).	Transrectal probe (5.0 MHz) detected small nodule at 35 days and C-cup placentomes on 42 days of gestation (Doize *et al.,* 1997).	NA
30-44 (14)	NA	NA	Embryo proper was detected on day 35 of gestation (Suguna *et al.,* 2008).
	NA	NA	Twin was detected with 5.0 MHz transabdominal probe at day 40 of pregnancy (Medan *et al.,* 2004).
	NA	Singles and twins were differentiated on day 42 of gestation (Suguna *et al.,* 2008).	Singles and twins were differentiated on day 42 of gestation (Suguna *et al.,* 2008).
	NA	Placentomes was detected on days 35-42 (Suguna *et al.,* 2008).	NA
45-75 (30)	NA	NA	Placentomes was detected on day 50 (Suguna *et al.* 2008).

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Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
45-75 (30)	Highest accuracy was during 40 to 60 days of gestation (Maico *et al.,* 2007).	6.0 and 8.0 MHz transrectal probes to detect male and female fetuses (Maico *et al.,* 2007).	Detection on scrotal bag for male foetuses was possible day 45 onwards 5.0 and 7.5 MHz transabdominal (Maico *et al.,* 2007).
	Accuracy on sex determination was 89% using 5.0 MHz transrectal probe (Coubrough and Castell, 1998).	Foetal sex determination with 5.0 MHz transrectal probe by identifying and locating genital tubercle betweeen 60 to 69 days post-breeding in ewes (Coubrough and Castell, 1998).	NA
	NA	NA	Images on sacs, heartbeats, placentomes and skeletal structures were obtained with 7.5 and 5.0 MHz transabdominal probes on day 60 of gestation (Medan *et al.,* 2004).
	NA	NA	Images on radius and head were obtained with 7.5 and 5.0 MHz transabdominal probes on day 70 of gestation (Medan *et al.,* 2004).

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Day of gestation (interval days)	Accuracy rate	Transrectal probe	Transabdominal probe
45-75 (30)	NA	Skeletal structures such as the skull, rib cage and vertebral column were first viewed on day 56 of gestation (Suguna *et al.,* 2008).	Skeletal structures such as the skull, rib cage and vertebral column were first viewed on day 56 of gestation (Suguna *et al.,* 2008).
76-106 (30)	Percent of accuracy on 76 to 87 days of gestation was 71.6% compared to progestrone and PAG; 65.4% 72.8%, respectively, with 3.5 MHz transabdominal probe (Karen *et al.,* 2008).	NA	NA
107-126 (19)	NA	NA	NA
127-147 (20)	NA	NA	Uterine wall, placentomes, foetal skeleton, kidneys and vascular system during last 3 weeks of gestation (Scheerboom and Taverne, 1985).

*NA=not applicable

2.7 IMPROVEMENT OF ULTRASOUND SCANNING

King (2006) reported the development and advances of diagnostic ultrasound in animals. Recent advances in computer technology have significantly influenced equipment design and the miniaturisation of transducers that simultaneously allows intraoperative and intravascular applications. Software advances have improved the ability to manipulate and process data, leading to an increased amount of information being obtained from each examination and the advent of remote diagnosis. Diagnostic or therapeutic purposes can be executed with the technology obtained from ultrasound guided interventional techniques. Many of these developments have been introduced by equipment manufacturers but have found specific applications in the veterinary field. Currently, ultrasound is engaged in miscellaneous situation, not just as a diagnostic tool in the routine clinical workup of a range of species, but ultrasound expands its application to disease screening, conservation projects, commercial services, herd management and clinical research (King, 2006).

Professional courses of ultrasound were offered at several academic institutes to generate proficient and skilled sonographers and accordingly significant increased the number of competent sonographers in general veterinary practice. The improved quality of portable machines emerged as the recent introduction of mobile small animal ultrasound services. This new and handy gadget creates a trend and is believed likely to continue. A non-invasive characteristic in ultrasound helped in health screen for small animals. Ultrasound led to the introduction of congenital disease screening schemes including polycystic kidney disease (PKD) in cats (Beck and Lavelle, 2001; Cannon *et al.,* 2001) and cardiac conditions in dogs (Dukes-McEwan, 2003). Farm animal reproductive applications dominate the literature with ultrasound having found widespread use as a herd management tool (Ribadu and Nakao, 1999). Commercial transcutaneous sheep scanning services to identify pregnancy and determine foetal numbers are obtainable by non-veterinary sonographers (White and Russel, 1984). However, current legislation is under review to allow non-veterinary personnel to perform the same service in cattle using transrectal probe. Ultrasound also permits foetal sexing given the right time of gestation, machine availability and operator (Coubrough and Castell, 1998; Ribadu and Nakao, 1999).