

# **EARLY PREGNANCY DIAGNOSIS USING ULTRASONOGRAPHY IN GOATS**

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**Thesis submitted in partial fulfillment of the  
requirement for the degree of**

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**Department of Animal Reproduction, Gynaecology and Obstetrics  
COLLEGE OF VETERINARY AND ANIMAL SCIENCES  
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I hereby declare that the thesis entitled "EARLY PREGNANCY DIAGNOSIS USING ULTRASONOGRAPHY IN GOATS " is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

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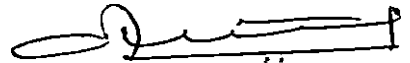


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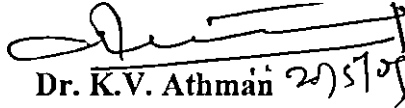
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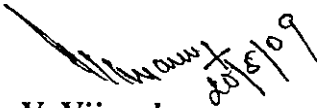
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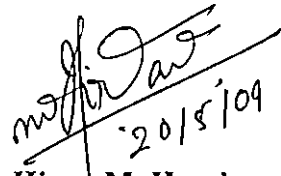
We, the undersigned members of the Advisory Committee of Sreejith, J.R., a candidate for the degree of Master of Veterinary Science in Animal Reproduction, agree that the thesis entitled “ EARLY PREGNANCY DIAGNOSIS USING ULTRASONOGRAPHY IN GOATS .” may be submitted by Sreejith. J.R., in partial fulfillment of the requirement for the degree.



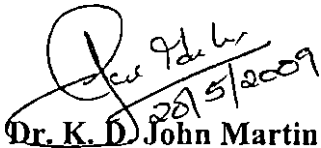
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## *Introduction*

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## 1. INTRODUCTION

Goats are considered as one of the earliest domesticated animals and have played a significant role in the life of man since then. The characteristic adaptability of goats to almost any climate and the capacity to thrive well on any variety of fodder has made them popular among the people, particularly of the rural area. According to the FAOSTAT (2005), world goat population is almost 800 million. India ranks second in the world with regard to goat population, contributing 17 per cent to the total goat population. As per 2003 livestock census, Kerala's share in all India goat population is 1.01 per cent.

In India, goats are predominantly reared by the small and marginal farmers, including landless agricultural labourers (Rekib, 1998) and women. Being small in size, goats are easier to manage, require less space for housing. The early sexual maturity, low age at first kidding, high prolificacy, short generation interval, feeding habits and multiple births are the factors that contribute to rise in goat population. Moreover, goat is known as the "poor man's cow" since it gives wholesome nutritious milk at a lesser cost.

An early and accurate diagnosis of pregnancy is essential for the maintenance of high levels of reproductive efficiency. Accurate pregnancy diagnosis in goats would provide essential information for effective herd management practices like culling or rebreeding of non-pregnant animals and providing adequate nutrition to the pregnant goats. Several methods are employed to diagnose pregnancy in goats. The abdominal palpation technique for diagnosing pregnancy in goats is applicable only during late gestation. The recto-abdominal palpation technique (Hulet, 1972) caused severe rectal damage and abortion (Trapp and Slyter, 1983). Radiography (Richardson, 1972) can be used with high accuracy but is restricted to the later stages of pregnancy and have constraints in the practicability of the equipment for large-scale field application. The health hazards to the operators and animals while using radiographic techniques are also a cause of concern. Hormonal assays can be used for diagnosing pregnancy which include measurement of the level of progesterone

(Pennington *et al.* 1982), oestrone sulphate (Chaplin, 1982) and pregnancy-specific protein B concentrations (Gonzalez *et al.*, 1999) in the milk and plasma. However, many of these methods yield high false positives and negatives especially during the early stages of pregnancy (Karen *et al.*, 2003). External Doppler ultrasonography devices can also be used to determine pregnancy in goats but only after two months of gestation (Trapp and Slyter, 1983). Most of these techniques of pregnancy diagnosis have been unsatisfactory for does because of the expense, accuracy, practicability and delayed results (Dawson *et al.*, 1994).

In recent years ultrasonography has emerged as a promising tool for the early and efficient pregnancy diagnosis in domestic animals. Real-time B-mode ultrasonography is a non-invasive, accurate and rapid technique for early diagnosis of pregnancy, determination of foetal numbers and their viability. The B-mode ultrasound examination can be performed in two different ways: transrectally and transabdominally (Kahn, 1994). There are many reports on transrectal (Schrick and Inskeep, 1993, Garcia *et al.*, 1993) and transabdominal scanning (White *et al.*, 1984, Karen *et al.*, 2006) for pregnancy diagnosis in sheep and counting foetal numbers. In addition, measuring the embryo or foetal parts provides information on the growth status of the embryo or foetus (Karen *et al.*, 2008). Pseudo-pregnancy is one of the common gestational accidents associated with goat and this condition can be easily differentiated from pregnancy using real time B-mode ultrasonography (Hesselink and Taverne, 1994). The ability to identify multiple foetuses and its viability was a real advantage of real-time B-mode ultrasonography over other techniques (Medan *et al.*, 2004).

Many of these reports are on pregnancy diagnosis in sheep and there is a paucity of information on the suitability of these techniques in goats. Hence this work was undertaken with the following objectives:

1. To evaluate the efficacy of transrectal and transabdominal ultrasonography in early pregnancy diagnosis in goats.
2. To identify the optimum stage of gestation for early pregnancy diagnosis in goats.



*Review of Literature*

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## 2. REVIEW OF LITERATURE

### 2.1. PREGNANCY DIAGNOSIS

Early and accurate diagnosis of pregnancy is found important for effective and economic livestock management. Methods for diagnosis of pregnancy in goats included non-return to oestrus, abdominal palpation and ballottement, recto-abdominal palpation, biologic tests like estimation of oestrone sulfate, progesterone, pregnancy specific proteins, radiography, vaginal biopsy, palpation of uterus via laparotomy, and A-mode, Doppler and real-time B-mode ultrasonography. The method of choice depends on the availability of equipment, number of days post-breeding, desired accuracy and experience of the examiner (Ishwar, 1995). Lack of knowledge of techniques to differentiate pregnant from non pregnant animals might result in heavy production losses in the form of abortions, stillbirths and production of weak kids and uneconomical feeding of non-pregnant animals.

#### 2.1.1. Non-return to Oestrus

Mathew (1983) reported that some seasonal tendencies existed in goats in Kerala, and that regular oestrous cycle of 18 to 24 days duration was of very uncommon occurrence especially during the period from January to May.

Krishnakumar (1992) reported that the length of oestrous cycle in Malabari crossbred goats of Kerala was 18-23 days.

Ishwar (1995) reviewed that the length of oestrous cycle in goat ranges from 19-24 days with an average of 21 days. Non-return to oestrus after breeding was one of the signs of pregnancy. However, it was not a reliable method of pregnancy diagnosis in sheep and goat, because of the seasonality in their oestrous behaviour, pathological conditions of the uterus or ovary and physiological anoestrus late in the breeding season and non-breeding season.

### **2.1.2. Abdominal Palpation / Abdominal Ballottement**

Abdominal palpation in late pregnancy was possible in thin-relaxed goats, but was difficult in big bodied goats as they resisted the palpation by tightening the abdominal muscles (Williams, 1986).

Goel and Agarwal (1990) opined that diagnosis by abdominal palpation was not possible at 51- 60 days of pregnancy but the percentage of accuracy increased to 70 per cent at 61- 70 days, 90.3 per cent at 71- 80 days and 95.4 per cent at 80 days and later.

Arthur *et al.* (1996) suggested that beyond 100 days of gestation the foetus could be palpated through the abdominal wall by the ballottement of the foetus with the ewe standing normally. The abdomen was lifted repeatedly, immediately in front of the udder and the foetus could be felt to drop on to the palpating hand.

### **2.1.3. Bimanual palpation technique**

Kutty (1999) developed the bimanual palpation technique and suggested that it could be used for diagnosing pregnancy in small ruminants. He found that this technique could not distinguish hydrometra from pregnancy between day 28 to 30 of gestation.

### **2.1.4. Recto-Abdominal Palpation**

Hulet (1973) predicted pregnancy in sheep by the aid of a hollow plastic palpation rod, with a bullet shaped tip. Rectal trauma, abortion and death have been reported following the examination.

Tyrrell and Plant (1979) observed rectal perforation in some experimental animals, 10 to 12 cm anterior to the anus in the ventral wall, following recto-abdominal palpation with the rod. Bruising on the rectal wall by moving the palpating rod from side to side during the diagnosis was also observed.

Ott *et al.* (1981) used this technique in ewes and does and reported 97 per cent accuracy at day 60 post-mating.

Trapp and Slyter (1983) reported an accuracy of 62.7 per cent in diagnosing pregnancy using recto-abdominal palpation technique between 60 and 96 days of gestation in ewes. The technique caused rectal damage and subsequent infection and death of some animals.

### **2.1.5. Radiography**

Ford *et al.* (1963) concluded that the number of lambs in the uterus could be determined by radiography in Clun Forest ewes with an accuracy of 90 per cent or more, provided the animals are examined later than day 90 of gestation. A probability of accuracy approaching 100 per cent could be observed with smaller breeds since the foetal bones become radio-opaque earlier in pregnancy.

Barker and Cawley (1967) opined that the earliest time at which a foetal skeleton was found was at day 58 of gestation in goats; however, they concluded that day 65 post mating would be the earliest date for detection of a foetal skeleton. If no skeleton was detected by seventy fifth day, the doe could be considered as non-pregnant.

Richardson (1972) radiographically observed fetuses from day 70 of gestation and concluded that the overall accuracy of the method in detecting pregnancy increased with advancing gestation. The accuracy was 52 per cent between 66 and 96 days of gestation and 100 per cent after day 96 post-mating.

### **2.1.6. Palpation of Uterus *via* Laparotomy**

Gravid uterus can be palpated directly through a small incision in abdominal wall. Smith (1980) reported 100 per cent accuracy in direct palpation of uterus after day 42 of gestation. The uterine horns appeared distended at four to five weeks of gestation and after six weeks, the cotyledons became obvious and the horns were five to ten cm in diameter. A small para-median incision was

made to permit entrance of two or three fingers just cranial to the udder and an enlarged thin-walled uterus containing fluid was taken as positive evidence of pregnancy (Ishwar, 1995).

### **2.1.7. Vaginal Biopsy**

Richardson (1972) observed an accuracy of 81 per cent in the diagnosis of pregnancy in Barren ewes using vaginal biopsy. The accuracy after day 40 was 91 per cent and it increased to 100 per cent after day 80 of gestation. Interpretations became doubtful when ewes were in late dioestrus / late anoestrus since the histological appearance of the sections was similar to that of pregnancy.

### **2.1.8. Progesterone Assay**

According to Medan *et al.* (2004), the accuracy of progesterone concentrations in diagnosing pregnancy on day 21 after mating, was 80 per cent for pregnancy and 100 per cent for non pregnancy in Shiba goats. The progesterone cut off value between pregnancy and non pregnancy was 1 ng/ml.

Holdsworth and Davies (1979) suggested progesterone assay at 19- 23 days post-breeding as technique to diagnose pregnancy with high accuracy.

Pennington *et al.* (1982) carried out radioimmunoassay (RIA) of progesterone in milk and reported that in goats, the progesterone level (ng/ ml) was 1 to 2 at oestrus and 5 to 12 at early dioestrus. It reached a plateau (15 to 25 ng/ ml) in the middle of the oestrous cycle and dropped to low concentrations again 3 to 5 days before the next oestrus.

According to Ishwar (1995) elevated progesterone level indicated only the presence of a functional corpus luteum and several conditions like hydrometra, pyometra and early embryonic death might extend the luteal lifespan giving a false positive result. It was reported that progesterone test in doe and ewe could be a good test for diagnosing non- pregnancy but only a fair test for pregnancy.

Engeland *et al.* (1997) used progesterone assay kits for pregnancy diagnosis in dairy goats and concluded that the accuracy of identifying pregnancy was lower than the accuracy of identifying non-pregnancy. It was opined that the presence of high milk and plasma progesterone levels, 20 days after mating could also be due to factors other than pregnancy such as prolonged luteal phase.

Karen *et al.* (2003) reported that in goats, progesterone levels higher than the threshold of 1ng/ ml indicated a functional corpus luteum associated with pregnancy, natural oestrous cycle or ovarian /uterine pathology.

### **2.1.9. Oestrone Sulfate Test**

Oestrone sulfate is a pregnancy-specific hormone produced in increasing amounts by the foeto-placental unit as pregnancy advances. Chaplin (1982) reported a considerable difference in serum and milk oestrone sulfate concentrations between pregnant and non-pregnant does. Oestrone sulfate could be measured in blood, milk or urine by RIA and that by day 50 of gestation its level became high. The optimal sampling time was considered to be 50 days post-breeding.

Worsfold *et al.* (1986) observed an apparent increase in the level of oestrone sulphate from around 70 days post conception in ewes. As oestrone sulphate production was by embryo-endometrial interaction, its level in plasma would be correlated to the number of live foetuses present.

Murray and Newstead (1988) used an enzyme-immunoassay (ELISA) for measuring concentrations of oestrone sulphate in milk to diagnose pregnancy and obtained an accuracy of 82 per cent by day 50 of gestation.

Refstal *et al.* (1991) reported that oestrone sulfate could be detected in the plasma of does from around 40 to 50 days post breeding.

### 2.1.10. Pregnancy-specific proteins

Pregnancy-associated glycoproteins (PAG) and Pregnancy-specific protein B (PSPB) are produced by the placenta throughout the gestation and their plasma levels can be measured by RIA. PAG is produced by the trophoblastic binucleate cells and a level higher than the threshold ( $\geq 1$  ng per ml) was a strong indication of the viable trophoblastic tissues. In pregnant goats the mean concentration of PAG remained higher than in non-pregnant goats. The ability to distinguish pregnant goats from non-pregnant increased as pregnancy progressed due to the steady increase in the PAG concentration in the pregnant goats. These hormones have been identified in various ruminant species including cattle, sheep and goats (Gonzalez *et al.*, 1999).

Karen *et al.* (2003) concluded that ovine pregnancy could be reliably detected using a heterologous radioimmunoassay of PAG from day 22 after breeding. The discriminatory value for the diagnosis of pregnancy by the PAG-RIA test was  $\geq 1$  ng per ml. PAG-RIA tests at days 22, 36 and 50 had significantly higher specificity for the identification of non-pregnant ewes than the progesterone test.

## 2.2. ULTRASONOGRAPHY

Three types of ultrasonography were employed for pregnancy diagnosis in small ruminants (Karen *et al.*, 2001).

### 2.2.1. A-mode ultrasound (Amplitude mode)

Meredith and Madani (1980) used the reflection of ultrasound at nine cm or greater as a positive sign of pregnancy in ewe and reported 96 per cent sensitivity in the period from 61 to 151 days after mating.

By using echo-pulse detectors, the accuracy for detecting pregnant ewes averaged 91 per cent from days 69 to 112 of gestation (Trapp and Slyter, 1983).

Karen *et al.* (2001) opined that A-mode ultrasound could not predict the foetal number and the viability of the foetus.

### 2.2.2. Doppler ultrasound

When the sound wave strikes a moving object, they are reflected back to the transmitting source at a slightly altered frequency. The transducer (probe) when applied to the animal's abdominal wall or inserted into the rectum emits a narrow beam of high frequency wave (ultrasonic). Movement of foetal heart or blood flow in the foetal (umbilical vessels) or maternal (uterine artery) circulation alters the frequency of these waves which are reflected back to the probe, where they are converted to audible sound and amplified (Jainudeen and Hafez, 2000).

Ultrasonic techniques based on the Doppler principle can diagnose multiple pregnancies with an acceptable accuracy, but cannot readily distinguish between females carrying two, three or more than three fetuses (Ishwar, 1995).

Trapp and Slyter (1983) suggested an accuracy of 72.7 per cent using intrarectal Doppler device in sheep between 60 to 96 days of gestation. Abortions and deaths of ewes following the examinations were reported. According to them these deaths were due to rectal damage and subsequent infections.

Fukui *et al.* (1984) used an abdominal ultrasound using Doppler principle for multiple pregnancy diagnosis in sheep. The correct diagnosis of non-pregnancy, single and twin pregnancies was 76.90, 74.40 and 88.70 per cent respectively.

Russel (1989) suggested that the detection of foetal heartbeat using Doppler principle should provide a means of determining foetal numbers. The difficulty with the method was that the heartbeats or pulses in the major blood vessels of one foetus could be picked up from different positions and angles of the transducer on the abdomen.



Wani *et al.* (1998) employed transrectal ultrasonography based on Doppler principle and succeeded in diagnosing pregnancy in Gaddy goats with 78.10 per cent accuracy.

### **2.2.3. Real time B-mode Ultrasound Scanning of Uterus**

#### **2.2.3.1. Principle**

Ultrasound waves are inaudible to the human ear and operated at frequencies of 1 to 10 MHz. Real time ultrasound systems emits high frequency sound waves from a hand held transducer. The ultrasound is then reflected back from tissue interfaces having varying acoustic impedance and is displayed “live” on a screen. High density echogenic tissue reflects a large proportion of the emitted waves and produces a white image on the screen, whereas non echogenic fluids reflects poorly and produces a dark image (Buckrell *et al.* 1986).

An electric current when applied to the piezoelectric crystals in a transducer produces vibration of the crystals which results in acoustic pressure waves transmitted to the tissue. The sound waves are directed through the tissue by moving transducer or varying the angle of the transducer. Tissues have the ability to either propagate or to reflect the sound wave to varying degrees. The proportion of sound waves that is reflected or echoed is received by the same piezoelectric crystals in the transducer. These are then converted to electrical impulses and displayed on the ultrasound screen as a series of grey dots. The echo-texture of the tissue will determine the proportion of the sound beam reflected. The reflected portion is represented on ultrasound images by shades of grey, extending from black to white. Fluids do not reflect sound wave i.e. they are non-echogenic or anechoic, therefore the image of a liquid containing structure appears black on the screen. At the other extreme, dense tissue (bone) reflects much of the sound waves i.e. hyper-echogenic or hyperechoic. Other tissues are seen in various shades of grey depending on their echogenicity or ability to reflect sound waves. Ultrasound waves travel through the body

(approximately 1540 m/sec) until a tissue reflector is reached. Some of the waves are reflected and return to the crystals. Some of the waves continue to interact with the deeper tissues. The time delay between propagation of the wave and reception of the returning echo is used to calculate the distance from the crystals to the tissue reflector (Pierson *et al.* 1988).

According to Griffin and Ginther (1992), a transducer with high frequency generated a better image resolution, but had a shallower depth of penetration. Therefore, close proximity to the rectal wall during transrectal ultrasonography would improve the visualization and scanning efficiency.

There are linear array transducer and sector transducers. In the linear array transducers, the sound emitting piezo-electric crystals are arranged side-by-side and are stationary, resulting in a rectangular image. In the sector transducers, the crystals are oscillating through angles of 90 degrees, resulting in a pie-slice-shaped image (Bretzlaff *et al.*, 1993).

#### **2.2.3.2. Transrectal scanning**

Fowler and Wilkins (1984) reported an accuracy of 95 per cent in the diagnosis of pregnancy from 40 to 50 days of gestation and over 99 per cent accuracy in the diagnosis of pregnancy above 50 days.

Buckrell *et al.* (1986) observed the gravid uterus in ewes within the pelvic cavity during the first 25 days of pregnancy using transrectal scanning. On day 23 of gestation, a string of small non-echogenic areas ventral to the bladder was observed. At day 25, a foetus was evidenced but no heartbeat was detected. By day 30, button-like echogenic areas assumed to be placentomes and a foetus within the amnionic membrane were detected.

Buckrell (1988) recommended that any ewe diagnosed as pregnant before day 30 of gestation should be re-checked to rule out embryonic death. Any ewe diagnosed as non-pregnant by transrectal scan should be tested high in the groin

region in order to avoid the possibility of false negative diagnosis especially in case of large, mature ewes. It was also opined that does were often less co-operative than ewes to transrectal ultrasonography.

Gearheart *et al.* (1988) detected pregnancy by day 20 post-breeding in ewes with transrectal ultrasonography, but the accuracy was low. As pregnancy advanced, the accuracy in determining the number of embryos increased.

Garcia *et al.* (1993) observed pregnancy in ewes from day 17- 19 of gestation with low or medium degree of certainty. They observed embryo and embryonic heartbeat between day 21 and 23 post-breeding.

Schrick and Inskeep (1993) used transrectal scanning in ewes to detect early pregnancy and suggested that extra-embryonic fluid and membranes could be observed from day 15 post-breeding. Heartbeat within the embryonic vesicles was observed on day 18 or 19. The use of transrectal ultrasonography prior to day 40 of gestation in combination with transabdominal ultrasonography at or beyond day 40 allowed the determination of embryonic and / or foetal loss.

Kaulfuss *et al.* (1997) diagnosed pregnancy by transrectal ultrasound in sheep after day 16 postmating. The fluid-filled trophoblastic vesicles were clearly visible as a non-echogenic small area in the uterine horn. The authors opined that ultrasound diagnosis was a practicable method for field conditions, avoiding negative consequences for pregnancy and the number of embryos. They also suggested that embryonic mortality could also be diagnosed with this technique in sheep.

Gonzalez *et al.* (1998) measured the different embryonic and foetal structures during the first three months of gestation in Manchega dairy ewes employing transrectal ultrasonography and the first structure identified being the embryonic vesicle. It was suggested that the transrectal ultrasonography could be used for the diagnosis of pregnancy until day 90 of gestation as the foetus became inaccessible in advanced pregnancy. The diameter of the embryonic vesicle

increased linearly from day 12 to 26 of pregnancy and the exponential increase of the crown-rump length could only be measured during a limited period, from day 19 to 48 of pregnancy.

Martinez *et al.* (1998) performed transrectal examinations from days 13 to 40 post-mating and observed pregnancy from day 18 of gestation. The mean diameter of the gestational sac was  $4 \pm 0.5$  mm on that day.

According to Karen *et al.* (2001), transrectal B-mode real-time ultrasonography identified the embryonic vesicles as early as 12.8 days after mating in sheep and also opined that the sensitivity of the technique for diagnosing pregnancy earlier than day 25 of gestation was low (12 %). It was concluded that the optimum time for using transabdominal or transrectal scanning in sheep ranged from 25 to 100 days of gestation.

Medan *et al.* (2004) suggested that the first sign of pregnancy in goats using transrectal ultrasonography was the appearance of a gestational sac which was circular or an elongated anechoic structure. The gestational sacs, embryo heartbeat and placentomes were detected on  $20.20 \pm 0.60$ ,  $24.30 \pm 0.70$  and  $35.40 \pm 1.00$  days of gestation respectively. As pregnancy progressed the size of the placentomes increased and appeared as 'C' shaped or 'O' shaped grey image. Skeletal structures were observed by two months of pregnancy.

Singh *et al.* (2004) observed 66 per cent accuracy in diagnosing pregnancy on ultrasonography in doe between day 17 to 19 of gestation and it increased to 100 per cent on day 34 of gestation in Jamunapari goats.

Dhoble *et al.* (2005) observed that the earliest indication of pregnancy in goats using ultrasonography was the demonstration of embryonic fluid inside the uterus. The earliest detection of pregnancy with an accuracy of 100 per cent was possible on day 23 or 24 of gestation and the placentomes became first visible on the days 25 to 35 of gestation as button-like protrusions, clearly smaller than the embryos.

According to Hoque *et al.* (2005) the conceptus was observed on day 20 post mating and observed foetus up to day 60 of gestation by the transrectal ultrasonographic technique. After day 60, the visualization of fetuses became difficult as the gravid uterus descended into the abdominal cavity. It was observed that repeated transrectal ultrasonography was stressful to the animal and caused straining, bleeding and ballooning of the rectum.

Kumar *et al.* (2005b) reported that on day 18 of gestation, multiple fluid pockets were visible inside the lumen of the uterus immediately cranial to the urinary bladder with a 7.5 MHz transducer in Black Bengal goats. The authors observed echoic conceptus on day 23, foetal heartbeat on day 26, placentomes on day 40 and bony rib-cage on day 50 of gestation.

Lee *et al.* (2005) performed transrectal sonography for pregnancy diagnosis in Korean black goats using a 5 MHz linear array transducer between 30 to 45 days post-breeding and opined that when multiple fluid-filled uterine enlargement, embryo proper or foetal heartbeat was detected, the animal could be declared pregnant.

Padilla *et al.* (2005) observed fluid accumulation within the uterine lumen from 19.5 days of gestation onwards in Boer goats. For maximum reliability, the transrectal ultrasonography could be recommended and the observation of the foetal heartbeat from 22.9 days would be considered as the conclusive evidence of the presence of a live foetus.

Aliya and Hayderb (2007) observed embryos and amniotic vesicles on  $25.38 \pm 1.20$  and  $28.67 \pm 1.00$  days of gestation respectively in Ossimi sheep. The ossification of the head, ribs and vertebrae were observed by  $44.80 \pm 2.50$ ,  $50.25 \pm 3.40$  and  $51.50 \pm 2.50$  days respectively. The placentomes increased in size until day  $82.73 \pm 7.70$  days and then began to collapse. Transrectal scanning could be performed efficiently until day 48 of pregnancy and beyond that most of

the foetal parts, except the placentomes became out of the range of the ultrasound beam.

According to Matsas (2007) transrectal ultrasonography could be employed for the diagnosis of non-pregnancy in goats less than 30 days post-breeding. He also reported that diagnosis of non-pregnancy less than 30 days after breeding was probably achieved by the transrectal approach. A positive diagnosis of pregnancy was made when the embryo, foetus or placentomes was seen.

Amer (2008) utilized B-mode transrectal and transabdominal ultrasonography for early pregnancy diagnosis and foetometry in Egyptian Baladi goats. Cent percent accuracy was achieved in detecting pregnancy and suggested that the transrectal probe was more reliable enabling recognition of foetal fluid (5 days earlier) and heartbeat (4 days) than the transabdominal probe. The observation of heartbeat was recommended as a conclusive evidence of the presence of a live foetus. He stated that ultrasonography was a reliable mean to provide early detection of gestation as early as 19 to 27 days after mating. He observed a small non echogenic vesicle, about 1 cm in diameter, in the uterine lumen from day  $19.5 \pm 0.30$  onwards.

Dawane *et al.* (2008) conducted ultrasonographic examination in sheep using 7.5 MHz linear transducer by transrectal route for early pregnancy diagnosis and observed an accuracy of 90 per cent on day 24 of gestation. From day 24 to 30 of gestation, embryo was detectable, while heartbeat was first observed from day 25 to day 30 of gestation and placentomes were detectable from day 30 to 50 of gestation.

Romanoa and Christiansb (2008) concluded that the early pregnancy diagnosis in sheep, using a 7.5 MHz transducer by transrectal route, was at day 16 and maximum sensitivity and negative predictive values was reached at day 20 following breeding.

Suguna *et al.* (2008) used transrectal real-time ultrasound scanning for the early diagnosis of pregnancy in goats and monitored the embryonic and foetal development in goats. They detected the embryonic vesicle as a fluid filled non-echogenic area on day 21 and embryo proper on day 28 using transrectal approach. Heartbeat was observed as early as day 21 of gestation and was recordable by day 28 of gestation. Placentomes were detectable as 'C' shaped structure on day 42 with an average of 0.97 cm using the rectal probe. Skull, ribcage and vertebral column were first viewed on day 56 of gestation. It was difficult to observe the foetus transrectally after day 98 of gestation since the uterus reached a more cranio-ventral abdominal position.

### **2.2.3.3. Transabdominal scanning**

Lindhal (1976) suggested that pregnancy could be determined with an accuracy of 100 per cent in ewes from mid gestation to term by transabdominal ultrasonography.

White *et al.* (1984) stated that pregnancy could be diagnosed with an accuracy of 100 per cent in sheep between days 50 and 100 of gestation. It was opined that pregnancy could be diagnosed as early as 30 days of gestation on the basis of the imaging of the fluid filled uterus and placental material.

According to Buckrell (1988) the ideal time for diagnosis of pregnancy in ewes appeared to be between day 45 and 50 post-breeding. However, uterine fluids, placentomes, foetus and foetal movements could be imaged by placing a 5 MHz transducer in the fleece-less inguinal region in the standing ewe 25-30 days post-breeding.

Russel (1989) stated that pregnancy could be detected in goats by about 30 days post-conception by identifying the fluid-filled uterus by transrectal ultrasonography. Cotyledons were observed from about day 40 of gestation as white circular structures with hollow centres. Individual fetuses could be identified by days 45-50 and foetal bones from about day 75 of gestation. False or

phantom pregnancies could be diagnosed readily as a large fluid-filled area devoid of any structures like cotyledons or membranes and foetus using scanning.

Aiumlamai *et al.* (1992) reported the details of weekly transabdominal ultrasound scanning in seven Swedish peltssheep ewes starting from one and a half to two months of gestation until parturition using a 3.5 and 7.5 MHz transducer for determining pregnancy status, the number of foetus and the foetal viability.

Bretzlaff *et al.* (1993) diagnosed pregnancy reliably from 30 days of gestation to term with transabdominal use of sector scanners in small ruminants. For transabdominal scanning they preferred the use of sector transducers because of the larger abdominal area that could be scanned. The authors suggested that linear-array transducers could be used for transabdominal scanning but could not scan a large area as the sector scanner took more time to scan the desired area. According to the authors, the placentomes became the cardinal sign of pregnancy after day 50 of gestation and as pregnancy progressed, the developing conceptus and placentomes became the major criteria for diagnosing pregnancy.

Dawson *et al.* (1994) recognized non-pregnant does readily and accurately with an accuracy of 100 percent at five and seven weeks of gestation. He diagnosed pregnancy based on the recognition of fluid filled uterus, placentomes, foetal structures such as head, thorax, limbs and foetal body movements.

Hesselink and Taverne (1994) concluded that transabdominal scanning of goats, performed between days 40 and 70 after mating, yields information about pregnancy, foetal viability and single or multiple pregnancy while at the same time making a reliable differential diagnosis of pregnancy and hydrometra. They suggested that sonographic examination should preferably be done from the right side, because from the left side the filled rumen can impede the proper observation of the uterus. In most cases the non-pregnant uterus was not visible



with transabdominal scanning, because the echogenicity of non-pregnant uterine tissues does not contrast with that of corresponding tissues. During the transabdominal scanning, the first sign of pregnancy that they observed was the fluid in the uterine lumen from about day 22 of gestation. They suggested that pregnancy should be diagnosed based upon the presence of fluid together with placentomes or foetal parts or membranes.

Arthur *et al.* (1996) stated that a B- mode ultrasound sector transducer probe, using the transabdominal approach as an accurate mean of differentiating pregnant and non- pregnant ewes and also to determine the foetal numbers. Pregnancy could be detected by day 30 of gestation and foetal numbers by day 45-50 of gestation.

According to Karen *et al.* (2001), transabdominal scanning achieved high accuracy for pregnancy diagnosis (94 to 100 percent) on day 29 to 106 of gestation in sheep.

Hoque *et al.* (2005) detected foetus earliest on day 30 post breeding and was clearly visible up to day 120 of gestation in goat. Sector scanning provided a wider view of the abdomen and allowed visualization of more uterine area and accurate diagnosis of multiple fetuses.

In goats, using 3.5 MHz transducer, Padilla *et al.* (2005) succeeded in detection of fluid accumulation within the uterus and foetal heartbeat earliest by day 24.70 and 27 days post-breeding respectively. They reported that the visualization of foetal heartbeat took three or more days later than the visualization of gestational sac and preferred it as an indicator of pregnancy and conclusive proof of the presence of a live foetus.

Transabdominal ultrasonography was successfully used by Tasal *et al.* (2006) to diagnose pregnancy in sheep with 94.10 per cent accuracy between 46 and 60 days of gestation and 100 per cent accuracy between 61 and 81 days of

gestation based on the visualization of fluid filled uterine horns, embryonic vesicles, foetal body and placentomes.

Abdelghafar *et al.* (2007) performed transabdominal ultrasonography in Saanen goats between day 26 and 110 of gestation and achieved an accuracy of 100 per cent in diagnosing pregnancy. The observation of fluid-filled gestational sacs or cotyledons or foetal parts was taken as positive indication of pregnancy.

Aliya and Hayderb (2007) assessed embryonic and foetal development in Ossimi sheep using B-mode ultrasonography and suggested that transabdominal scanning could be done as early as 24 days of breeding. However, the scanning view was much inferior to that of the transrectal one and it became better as the pregnancy advanced.

According to Matsas (2007) transabdominal approach was generally used for examinations made more than 35 days after breeding and the ideal time for it was roughly from 40 to 75 days of gestation. It was also reported that the placentomes could be seen by 30-35 days of gestation as echogenic densities in the uterine wall. By 45- 50 days, they were more easily seen as C-shaped densities with the concave surface directed towards the uterine lumen.

Amer (2008) opined that fluid- filled vesicles could be reliably located from  $24.70 \pm 0.40$  days of gestation and foetal heartbeat from  $27 \pm 0.60$  days onwards in Egyptian Baladi goats using a transabdominal probe.

Anwar *et al.* (2008) detected pregnancy with 100 per cent accuracy by appreciating anechoic elongated structure within the uterine fluid in ewes using transabdominal ultrasonography by day 42 of gestation.

Suguna *et al.* (2008) detected embryonic vesicle on day 28 and embryo proper on day 35 employing transabdominal scanning in goats. The heartbeat was detectable on day 35, placentomes on day 50 and skeletal structures on day 56 of gestation. It was opined that the best approach for measuring the diameter of

placentome, head and thorax during later stages of gestation was the transabdominal scanning

#### **2.2.4. Foetal number**

Lindhal (1976) reported an accuracy of 84 per cent in detecting single and multiple fetuses in ewes using transabdominal scanning 28 to 52 days before lambing. No attempts were made to distinguish twins and triplets and both were identified as multiples.

White *et al.* (1984) stated that high levels of accuracy in determination of foetal numbers in sheep were achieved only from 45 to 50 days of gestation by transabdominal scanning. It was concluded that beyond about 100 days of gestation, the size of the fetuses in relation to the area imaged was such that it became increasingly difficult to determine numbers rapidly.

Fukui *et al.* (1986) reported an accuracy of 68.20 per cent in predicting single pregnancies and 66.70 per cent in predicting multiple pregnancies in ewes using real-time ultrasonography. It was suggested that prediction of the number of foetuses could help in initiating earlier treatment options for prevention of lambing difficulties. Further, identification of ewes carrying single or multiple foetuses was desirable for correct feeding management during late gestation.

Russel (1989) identified individual fetuses by day 45 to 50 and counted the foetuses accurately between day 50 and 100 of gestation in sheep. At later than days 100 of gestation it was difficult to accurately determine the number of foetuses because of the size of the foetuses and the position of the uterus within the abdomen. It was opined that feeding in relation to foetal numbers offered a means of manipulating birth weight. It could lead to substantial reduction in the mortality of both offspring and dam. Lamb mortality was very closely related to birth weight making both underweight and overweight lambs at risk.

Bretzlaff *et al.* (1993) opined that identification of single and multiple foetuses by transabdominal scanning was most accurate from 45 to 90 days of gestation.

Schrack and Inskip (1993) suggested that determination of the number of foetuses *in utero* would help in the allotment of ewes to appropriate nutritional regimens during gestation.

Dawson *et al.* (1994) suggested that transabdominal scanning at seven weeks of gestation was more accurate than at five weeks for predicting foetal numbers in does. It was opined that accuracy of predicting foetal numbers was low prior to sixth week of gestation as the individual foetal or placental structures were not readily visible.

Hesselink and Taverne (1994) reported that the accuracy in differentiating a singleton from a twin pregnancy, using transabdominal scanning was higher than that of twins from triplets or quadruplets and also opined that the accuracy would depend on the equipment, operator experience and stage of pregnancy.

Medan *et al.* (2004) obtained an accuracy of 91.70 per cent on day 60 of gestation by employing real time B-mode transrectal ultrasonography and suggested that determination of foetal number would help in separating does carrying single, twins or triplets for differential management.

Padilla *et al.* (2005) proposed that the best time to distinguish between single and twin foetuses in goats with a 7.5 MHz rectal probe was between 28 and 40 days of gestation and difficulty in identifying the foetal numbers increased with multiple pregnancies.

Abdelghafar *et al.* (2007) obtained an overall accuracy of 79.30 per cent in the determination of foetal numbers and the corresponding values with respect to singleton, twins and triplets were 88.2, 77.7 and 50 per cent respectively.

According to Matsas (2007), the optimal time for counting the foetal numbers was between 40 and 70 days for linear array transducer and 45-90 days post-breeding for sector scanners. It was reported that the diagnosis of triplets was more difficult than twins and the foetal age could be estimated by measuring the width of the foetal head between 40 and 100 days of gestation.

Suguna *et al.* (2008) differentiated singleton from twins on day 35 and 42 of gestation in goats by transrectal and transabdominal scanning respectively. The proposed best period for differentiation of singleton from twins was between fifth to seventh week of gestation.

### 2.2.5. Foetal growth

Aiumlamai *et al.* (1992) observed that the biparietal diameter of the skull and the diameter of the body trunk were correlated with the foetal age and it was concluded that these measurements could be used to estimate the age of the foetus. High correlation was also observed with the biparietal diameter of the skull as well as the diameter of the body trunk with the gestational age.

Schrick and Inskeep (1993) observed that in ewes the crown-rump length of embryos on days 20, 25, 30, 35 and 40 averaged  $6 \pm 1$ ,  $10 \pm 2$ ,  $17 \pm 2$ ,  $25 \pm 2$  and  $36 \pm 4$  mm, respectively and that the pattern of embryonic growth, did not differ with the number of embryos carried.

Doize *et al.* (1997) reported that placentome diameter in dairy goat breeds were correlated with the gestational age upto day 90. The transrectal measurements of the placentomes in the vicinity of the uterus were made. The relationship between gestational age (GA) in days and placentome diameter (PD) in millimeters was  $GA=28.74+1.80(PD)$ . He also reported that this technique was accurate within 14 days in most of the goats studied. He suggested that measurement of placentomes by transrectal ultrasonography could be used as a technique to ascertain the gestational age in goats. It was determined accurately with a range of  $\pm 7$  days and  $\pm 14$  days in 66 and 96 per cent of pregnant does

respectively. Further it was concluded that there was a poor correlation of placentome size with gestational age in ewes.

According to Martinez *et al.* (1998) crown-rump length can also be used to estimate gestational age upto day 40 of pregnancy.

Lee *et al.* (2005) identified ultrasonography as a technique for the estimation of gestational age in Korean black goats. It was observed that the trunk diameter, length of long axis and short axis of the heart were useful parameters in the estimation of the foetal age when a transabdominal ultrasonography was conducted. As pregnancy advanced, the concave circular shape of the ruminant placentome changed to a 'C' shaped or 'O' shaped grey image. It was also observed that the diameter of the placentome was a poor parameter in the estimation of gestational age in goats.

Gaikwad *et al.* (2007) confirmed pregnancy on day 24 using transrectal scanning and measurement of the foetal CRL was taken on days 35, 42, 49, 56, 63 and 70 of gestation using transrectal and transabdominal probe in Osmanabadi goats. The corresponding mean CRL in millimeters were  $27.82 \pm 0.73$ ,  $46.35 \pm 1.32$ ,  $65.32 \pm 1.18$ ,  $82.85 \pm 0.88$ ,  $99.66 \pm 0.94$  and  $117.27 \pm 1.12$ .

Amer (2008) successfully employed transabdominal convex probe from day 40 to 89 of gestation to measure CRL and from day 40 to 109 to measure bi-parietal diameter in goats.

Suguna *et al.* (2008) observed that the diameter of the head increased significantly during gestation from  $1.97 \pm 0.00$  cm to  $6.57 \pm 0.40$  cm. It was reported that the placentome diameter from day 42 increased significantly and attained a maximum diameter on day 130 of gestation. Analysis of data showed that the placentome diameter had a significantly high correlation ( $r=0.99$ ) with gestational age.

### 2.2.6. Foetal sex

According to Abdelghafar *et al.* (2007) ultrasonography was the only method to detect foetal sex. The accuracy of determining the foetal sex depended on the position of the foetus and the skill of the sonographer.

Santos *et al.* (2007) suggested that foetal sexing in goats highly depended on the time of genital tubercle migration. The migration of the genital tubercle occurred around day 50 of pregnancy and it was concluded that the examinations should be performed between days 55 and 70 of pregnancy in order to avoid mistakes resulting from individual or breed specific variations. Foetal sex was determined by visualizing the external genitalia and the location of the genital tubercle. The foetus was recorded as a male when the genital tubercle was located immediately caudal to the umbilical cord and as a female when it was located near the tail.

Amer (2008) stated that the genital tubercle in male fetuses could be identified from day 40 onwards. The accuracy of sex diagnosis did not differ between single and multiple pregnancies. An accuracy of 83.3 and 70.2 per cent were obtained in diagnosing the sex of single foetuses and multiple foetuses respectively. The migration of the genital tubercle occurred around day 50 of pregnancy and he suggested that examinations should be performed between day 55 and 70 of pregnancy to avoid mistakes resulting from individual and breed specific variations.

### 2.3. GESTATIONAL ACCIDENTS

According to Roberts (1971) the normal rates of each embryonic loss in the ewe are reported to be 20 to 30 per cent from weeks after conception until parturition.

Gearheart *et al.* (1988) and Schrick and Inskeep (1993) detected embryonic death in ewes by the absence of heartbeat, smaller size of the embryo and less fluid surrounding the particular embryo.

Garcia *et al.* (1993) reported a low rate of embryonic loss of seven per cent. Hesselink and Taverne (1994) diagnosed hydrometra as non-echogenic fluid compartments separated by double-layered thin tissue walls. There was fluid in the uterus in the absence of fetuses and placentomes. They also opined that the absence of foetal movements and heartbeats could be considered as the first ultrasonographic signs of foetal death.

According to Padilla *et al.* (2005) foetal death at an advanced stage of pregnancy was characterized by the lack of foetal movement or heartbeat and there was often an associated lack of defined structures or surrounding foetal fluid.

Kumar *et al.* (2005a) detected foetal mortality in a goat by using ultrasound technique. He opined that heartbeat should be taken as a criterion to know the viability of the foetus. He observed absence of heartbeat, an increase in cloudiness with decrease in amount of foetal fluid and size of foetus.

#### 2.4. GESTATION LENGTH

According to Roberts (1971) the normal length of gestation in goats ranged from 148 to 156 days. Sudarsanan and Raja (1973) observed that the length of gestation ranged from 142 to 152 days with a mean of 146.20 days in Malabari goats.

Kuriakose (1981) reported that the average gestation length in Malabari, Alpine X Malabari and Saanen X Malabari does was found to be  $146.66 \pm 0.53$  days. Biswas and Kaul (1994) reported that the average gestation length in Himalayan Chegu goats was  $150.60 \pm 0.30$  days. They suggested that weight of



the doe at kidding, sex of the kid, season of kidding, interaction of any of these traits and kid weight did not affect the gestation length significantly.

Prasanth (1995) concluded that the average gestation in Alpine X Malabari does was  $149.85 \pm 4.45$  days. Jainudheen and Hafez (2000) opined that the average gestation length in goat was 150 days.

According to Smith (2007) the normal length of gestation was about 150 days varying from 147 to 155 days. He observed that there was little effect of litter size on gestation length and twins and triplets were more common than singletons, except in primiparous animals.

Juliet (2008) observed that the average gestation length in crossbred Malabari goats was  $146.03 \pm 0.76$  days.

## 2.5. LITTER SIZE

Prasanth (1995) reported that the incidence of single, twin, and triplet birth was 40, 48 and 12 per cent respectively in Malabar X Alpine crossbred goats with an average of 1.7 kids per kidding.

James *et al.* (2002) observed a higher twinning percentage in goats maintained in Kerala.

According to Afsal (2003), the incidence of single, twin and triplet birth was 32.26, 58.07 and 9.68 per cent respectively in Malabari crossbred does.

## *Materials and Methods*

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### **3. MATERIALS AND METHODS**

#### **3.1. EXPERIMENTAL ANIMALS**

The material for the present study consisted of crossbred female goats maintained at the University Goat and Sheep Farm and those presented for Artificial Inseminations (A.I) at the Artificial Insemination Centres of the Department of Animal Reproduction and Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy and University Veterinary Hospital, Kozhikode, Thrissur.

From among them thirty apparently healthy does with the history of breeding were selected for the study and the selected goats were randomly grouped into three with ten animals each. They were subjected to B-mode real-time ultrasound scanning transrectally (7.5 MHz probe) and transabdominally (3.5 MHz probe). The experiment was conducted during the period from June 2008 to April 2009.

##### **3.1.1. Group I**

This group consisted of ten goats, which were subjected to ultrasound scanning between third and fourth week (15- 28 days) post-breeding.

##### **3.1.2. Group II**

This group consisted of ten goats, which were subjected to ultrasound scanning between fifth and sixth week (29- 42 days) post breeding.

##### **3.1.3. Group III**

This group consisted of ten goats, which were subjected to ultrasound scanning between seventh and eighth week (43- 56 days) post breeding.

Typical ultrasonographic imaging of the conceptus and associated structures were used for the diagnosis of pregnancy. Does were diagnosed pregnant when there was the detection of gestational sac, embryo, embryonic heartbeat, placentomes or foetal skeleton. Viability of the foetus/ foetuses and foetal number were also observed. Ultrasonographic measurements of the gestational sac diameter (GSD), crown-rump length (CRL) of the foetus and diameter of the placentomes (PD) at various days of gestation were recorded. All the goats were followed up till kidding. The accuracy in diagnosing pregnant and non-pregnant status by the transrectal and transabdominal ultrasound scanning techniques was compared using the actual kidding data.

## 3.2. ULTRASOUND SCANNING

### 3.2.1. Equipment

Ultrasound equipment (DC-6 VET DIAGNOSTIC ULTRASOUND SYSTEM MINDRAY, BIO-MEDICAL ELECTRONICS Co, Ltd and and HS 2000, HONDA ELECTRONICS Co, Ltd), which produces two dimensional gray scale real time images, was used for the ultrasound scanning. It produces a moving image of the uterus, foetal fluid, embryo/foetus, foetal heart flickering and placentome. A linear array transducer (5 to 8 MHz) and a sector transducer (3 to 5 MHz) were used (Plate 1 and 2).

### 3.2.2. Transrectal scanning

Ultrasonographic examination was performed using a real time B-mode scanner equipped with linear array transducer. The does were restrained in standing position. Ultrasound gel-couplant was applied to the transducer to assure good acoustic transmission. After clearing the faeces from rectum the transducer was introduced gently into the rectum in a downward fashion until the bladder appeared on the screen. The uterine horns were observed cranial to the bladder and then the probe was rotated laterally at 90 clockwise and 180 anticlockwise to

visualize typical echoic/anechoic structures viz. foetal fluid, embryonic vesicles, embryo/foetus, foetal heart flickering and placentome (Martinez, *et al.*, 1998). Does were diagnosed pregnant from the observation of gestational sac, embryo/foetus, embryonic/foetal heartbeat, placentomes or foetal skeleton.

### **3.2.3. Transabdominal scanning**

To perform transabdominal ultrasonography, the hair on the right inguinal region and abdomen cranial to the pelvic brim was removed. Ultrasound gel-couplant was applied to the hairless area and to the transducer to assure good acoustic transmission. Goats were placed in left lateral recumbancy and ultrasonographic examination was done (Karen *et al.*, 2008). Ultrasonography was done from the right side, as on left side the filled rumen could impede proper observation of the uterus (Hesselink and Taverne, 1994). Embryonic vesicles, embryo, foetus, foetal heartbeat and placentomes were observed. Does were considered positive when there was the detection of gestational sac, embryo/foetus, embryonic/foetal heartbeat, placentomes or foetal skeleton (Plate 3).

## **3.3. ULTRASONOGRAPHIC IMAGING OF CONCEPTUS AND ASSOCIATED STRUCTURES**

### **3.3.1. Gestational sac**

The presence of a discrete, intrauterine, sharply demarcated, anechoic, round or oval structure was recorded as gestational sac (Singh *et al.*, 2004).

### **3.3.2. Embryo/ foetus**

According to Martinez *et al.* (1998), the confirmatory sign of pregnancy in goat was the detection of proper embryo. The presence of the embryo was recorded. The embryo proper appeared as an echogenic structure within the anechoic fluid.

### 3.3.3. Foetal heartbeat and viability

Foetal viability was assessed based on the observation of foetal heartbeat. The observation of heartbeat was a conclusive evidence of the presence of a live foetus (Padilla *et al.*, 2005).

### 3.3.4. Placentomes

The presence of placentomes was recorded. Placentomes appeared as a hyper-echogenic, 'C' or 'O' shaped structure as pregnancy advanced (Suguna *et al.*, 2008).

### 3.3.5. Foetal skeleton

The day of appearance of foetal skeleton and vertebral column was observed. The foetal skeleton was an echogenic structure and appeared as white in colour in the screen.

### 3.3.6. Foetal number

The number of foetus was counted in all the three groups and compared with the actual kidding data (Dawson *et al.*, 1994).

## 3.4. MEASUREMENT OF GSD, CRL AND PD

The measurements of the gestational sac diameter (GSD), crown-rump length (CRL) of the foetus and diameter of the placentomes (PD) in millimeters (mm) at various days post-breeding were taken by both transrectal and transabdominal ultrasound scanning. The cross-sectional diameter of the gestational sac in millimeter was measured at their maximal diameter (Martinez *et al.*, 1998). The measurement from the crown to the rump of the embryo/ foetus in millimeter was recorded (Amer, 2008). The diameter of a representative placentome in a group was measured (Doize *et al.*, 1997).

### 3.5. GESTATIONAL ACCIDENTS

Gestational accidents like embryonic death, abortion, stillbirth, hydrometra if any till kidding were observed.

### 3.6. STATISTICAL ANALYSIS

Data obtained were statistically compiled and analysed using standard statistical procedure (Snedecor and Cochran, 1989).



Plate 1. MINDRAY ultrasound equipment



Plate 2. Transabdominal (sector) and Transrectal (linear) ultrasound probes



Plate 3. Transabdominal ultrasound scanning



## *Results*

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## 4. RESULTS

The study was carried out to evaluate the efficacy of transrectal and transabdominal ultrasonography in early pregnancy diagnosis in goats and to identify the optimum stage of gestation for early pregnancy diagnosis using B-mode ultrasonography in goats.

Pregnancy diagnosis was carried out using transrectal and transabdominal ultrasonography. The accuracy for the diagnosis of pregnancy, ultrasonographic imaging of the conceptus and associated structures and its measurement, foetal viability and foetal number were assessed by both the techniques of scanning.

### 4.1. ULTRASOUND SCANNING FOR PREGNANCY DIAGNOSIS

Accuracy of transrectal and transabdominal scanning in pregnancy diagnosis in animals of group I, II and III are presented in Table 1 and Fig. 1.

#### 4.1.1. Pregnancy Diagnosis by Transrectal Scanning

##### 4.1.1.1. *Group I*

Among the ten animals of this group, scanned between third and fourth week post-breeding, seven animals were confirmed as pregnant and three as non-pregnant based on the kidding data (Table 1).

Six animals were diagnosed as pregnant and three as non-pregnant by transrectal scanning. There was one doubtful case in which pregnancy could not be ascertained. Thus the accuracy of transrectal scanning in this group was 90 per cent. The data is presented in Table 1 and Fig. 1.

#### **4.1.1.2. Group II**

Ten animals were scanned between fifth and sixth week post-breeding, and six animals were confirmed as pregnant and four as non-pregnant based on the kidding data.

All the pregnant animals of group II were diagnosed correctly by transrectal scanning. There were no doubtful cases in this group. The accuracy for the diagnosis of pregnancy in this group using transrectal scanning was 100 per cent (Table 1 and Fig. 1).

#### **4.1.1.3. Group III**

Of the ten animals of group III, which were scanned between seventh and eighth week post-breeding, seven were confirmed as pregnant and three as non-pregnant based on the kidding data.

These animals were accurately diagnosed by transrectal scanning as pregnant and non-pregnant. There were no doubtful cases in this group also. The accuracy for the diagnosis of pregnancy using this technique was 100 per cent in this group (Table 1 and Fig. 1).

### **4.1.2. Pregnancy Diagnosis by Transabdominal Scanning**

#### **4.1.2.1. Group I**

Of the seven pregnant animals of this group, only two could be correctly identified as pregnant by transabdominal scanning. The pregnancy status of four animals was doubtful as pregnancy could not be ascertained and one animal wrongly identified as non-pregnant (false negative). There were three true negatives. The accuracy was 50 per cent in the diagnosis of pregnancy in this group by transabdominal scanning (Table 1 and Fig. 1)

#### **4.1.2.2. Group II**

All animals in this group were diagnosed correctly as pregnant and non-pregnant by transabdominal scanning. There were six pregnant and four non-pregnant animals. In this group, there were no doubtful cases. Thus the accuracy for detection of pregnancy status by transabdominal scanning was 100 per cent in this group by this technique (Table 1 and Fig. 1).

#### **4.1.2.3. Group III**

Among the ten animals scanned between seventh and eighth week post-breeding, seven were correctly diagnosed as pregnant and three as non-pregnant by this method of scanning. In this group also there were no doubtful cases. The accuracy of transabdominal scanning for detecting status of pregnancy in this group was also 100 per cent (Table 1 and Fig. 1).

### **4.2. ULTRASONOGRAPHIC IMAGING OF CONCEPTUS AND ASSOCIATED STRUCTURES**

#### **4.2.1. Imaging by Transrectal Scanning**

The data of the detection of conceptus and associated structures of group I, II and III by transrectal scanning are given in Tables 2, 3, 4 and 5.

##### **4.2.1.1. Embryonic vesicle**

The embryonic vesicle, which appeared as a discrete, intra-uterine, sharply demarcated anechoic, round or oval structure was detected earliest on day 19 of gestation by transrectal scanning (Table 3 and Plate 7). It was evident in six of the seven pregnant animals of group I and all the pregnant animals of group II and III (Table 2 and Plates 4 to 8).

#### **4.2.1.2. Embryo/foetus**

The presence of the embryo, which appeared as an echogenic structure within the anechoic fluid, was first observed on day 22 post-breeding by transrectal ultrasonography (Table 3 and Plate 5). It was evident in five of the seven pregnant animals of group I and all the animals of group II and III by transrectal scanning (Table 2 and Plates 5 to 9).

#### **4.2.1.3. Foetal heartbeat**

The foetal heartbeat was first detected as a flickering on day 24 post-breeding by transrectal scanning in group I (Table 3). It was observed in four of the seven pregnant animals of group I and all the pregnant animals of group II and III (Table 2).

#### **4.2.1.4. Foetal viability**

Foetal viability was assessed based on the observation of foetal heartbeat. The foetal heartbeat was first observed on day 24 of gestation in the pregnant animals of group I by transrectal scanning (Table 3).

#### **4.2.1.5. Placentomes**

Placentomes were not observed by transrectal ultrasound technique, in the pregnant animals scanned between third and fourth week of gestation (group I). By transrectal scanning, placentomes were first observed as a concave-shaped echogenic structure on day 42 of gestation (Table 4 and Plate 9). This was detected in two of the six pregnant animals of group II and all pregnant animals of group III (Table 2). It changed from the concave-shaped structure to a 'C' or 'O' shaped structure as pregnancy advanced (Plates 10 and 11).

#### **4.2.1.6. Foetal skeleton**

Foetal skeleton was not observed in the pregnant animals scanned between third and fourth week and fifth and sixth week of gestation (Table 2). Foetal skeleton was first observed on day 54 of gestation in the pregnant animals scanned between seventh and eighth week of gestation by employing transrectal scanning (Table 5). The foetal skeleton was detected in three of the seven pregnant animals of group III (Table 2 and Plate 10).

#### **4.2.2. Imaging by Transabdominal Scanning**

The data of the detection of conceptus and associated structures of group I, II and III by transabdominal scanning are given in Tables 2, 3, 4 and 5.

##### **4.2.2.1. Embryonic vesicle**

The embryonic vesicle was first detected by transabdominal scanning on day 26 of gestation (Table 3). It was observed only in two of the seven pregnant animals of the group I. However, it was detected in all the pregnant animals of group II and III (Table 2 and Plates 12 to 14).

##### **4.2.2.2. Embryo/foetus**

The embryo was first detected on day 28 of gestation by transabdominal scanning (Table 3 and Plate 12). This could be observed in only one of the seven pregnant animals of group I. But it was detected in all the animals of group II and group III (Table 2 and Plates 12 to 14).

##### **4.2.2.3. Foetal heartbeat**

Foetal heartbeat was not observed in pregnant animals of group I by transabdominal scanning (Table 3). By transabdominal scanning, foetal heartbeat

was detected earliest on day 34 of gestation. Foetal heartbeat was observed in five of the six pregnant animals of group II and all the animals of group III.

#### **4.2.2.4. Foetal viability**

By transabdominal scanning, the foetal heartbeat was detected earliest on day 34 of gestation and foetal viability could be assessed from that day of gestation employing this approach of scanning (Table 4).

#### **4.2.2.5. Placentomes**

Placentomes were not observed by transabdominal ultrasound technique, in the pregnant animals scanned between third and fourth week of gestation (group I). Placentomes were first observed as a concave-shaped echogenic structure on day 42 of gestation by transabdominal scanning (Table 4). This was detected in two pregnant animals of group II and all pregnant animals of group III (Table 2). As pregnancy advanced, the concave-shaped structure changed to a 'C' or 'O' shaped structure (Plates 15 to 17).

#### **4.2.2.6. Foetal skeleton**

Foetal skeleton was not observed in the pregnant animals scanned between third and fourth week and fifth and sixth week of gestation (Table 2). Foetal skeleton was first observed on day 54 of gestation in the pregnant animals scanned between seventh and eighth week of gestation by employing this approach of scanning (Table 5). The foetal skeleton was detected in three of the seven pregnant animals of group III by employing transabdominal scanning (Table 2 and Plates 15 to 17).

### 4.3. ULTRASONOGRAPHIC MEASUREMENT OF GSD, CRL AND PD

#### 4.3.1. Measurement by Transrectal Scanning

Measurements of GSD, CRL and PD were recorded and the details observed by transrectal scanning are given in Table 6 and Fig. 3, 4 and 5. The earliest day of detection of the gestational sac was on day 19 of gestation using transrectal scanning. The mean diameter recorded was 5.1 mm on day 19 and 27 mm on day 36 of gestation by transrectal scanning. The GSD recorded by transrectal scanning had high correlation ( $r = 0.974$ ) with gestational age.

The embryo was first detected on day 22 of gestation by transrectal scanning and the mean crown-rump length ranged from 7.2 mm on day 22 to 34.4 mm on day 43 of gestation using transrectal scanning. The CRL recorded by transrectal scanning had a high correlation ( $r = 0.969$ ) with the gestational age.

Placentomes were first detected on day 42 of gestation as concave-shaped structures by using transrectal scanning. The diameter of the placentomes recorded by transrectal on day 42 of gestation was 8.4 mm and it was 19.6 mm on day 56 of gestation by transrectal scanning. The placentome diameter recorded by transrectal scanning also had a high correlation ( $r = 0.973$ ) with the gestational age.

#### 4.3.2. Measurement by Transabdominal Scanning

Ultrasonographic measurements of the GSD, CRL and PD were recorded. The details of the measurement by transabdominal scanning are given in Table 6 and Fig. 3, 4 and 5. The gestational sac was first detected on day 26 of gestation by transabdominal scanning. The mean diameter recorded was 15.7 mm on day 26 and 34.4 mm on day 36 of gestation by transabdominal scanning. The GSD recorded by transabdominal scanning had a high correlation ( $r = 0.985$ ) with the gestational age.



The embryo was first detected on day 28 of gestation by transabdominal scanning and the mean crown-rump length recorded was 16.7 mm on that day and as 32.7 mm on day 43 of gestation by transabdominal scanning. The CRL recorded had a high correlation ( $r = 0.943$ ) with the gestational age.

Placentomes were first detected on day 42 of gestation as concave-shaped structures by transabdominal scanning. The diameter of the placentomes recorded on day 42 of gestation was 8.5 mm and was 22.1 mm on day 56 of gestation by transabdominal scanning. The placentome diameter also had a high correlation ( $r = 0.992$ ) with the gestational age.

#### 4.4. FOETAL NUMBER

##### 4.4.1. Foetal Number Estimation by Transrectal Scanning

The number of foetuses were counted in all the three groups and compared with the actual kidding data. The number of foetuses estimated by transrectal scanning and actual kidding is presented in Table 7. According to the actual kidding data, there were four singletons, twelve twins and four triplets.

Out of the seven predictions made by transrectal scanning in group I, five were correct. All the foetal number predictions made in group II were accurate and in group III, five predictions of the seven were correct. Thus the accuracy for prediction of foetal number using transrectal scanning in pregnant animals of group I and III was 71.43 per cent (Fig. 2). The accuracy for the prediction was 100 per cent in pregnant animals of group II (Table 8). There were four singletons which were accurately predicted by transrectal scanning. Of the four triplet births, foetal number was accurately diagnosed in two animals by transrectal scanning. Out of the twelve twin births, ten were diagnosed accurately. Thus the accuracy for the prediction of singletons, twins and triplets by transrectal scanning was 100, 83.33 and 50 per cent respectively. The overall accuracy for the prediction of foetal numbers by transrectal scanning was 80 per cent (Table 9).

#### 4.4.2. Foetal Number Estimation by Transabdominal Scanning

By transabdominal ultrasonography, it was not possible to predict foetal number accurately in pregnant animals of group I. Of the six predictions in group II, four were accurate and in group III, six of the seven predictions were correct. Thus the accuracy for the prediction of foetal number in group II by transabdominal scanning was 66.66 per cent while the accuracy was 85.71 per cent in group III (Table 8 and Fig 2). By transabdominal scanning, two of the four singletons were accurately predicted. Seven of the twelve twins and one of the four triplets were correctly determined by transabdominal scanning. The accuracy for the prediction of singletons, twins and triplets was 50, 58.33 and 25 per cent respectively. The overall accuracy was 50 per cent for the prediction of foetal numbers by transabdominal scanning (Table 9).

#### 4.5. GESTATIONAL ACCIDENTS

Gestational accidents like embryonic death, abortion, still-birth, hydrometra, if any, until kidding were observed. There was one still-birth and one abortion in two animals during the period. The animal in which still-birth was observed was scanned on day 34 of gestation. The animal which aborted in the third month of gestation was scanned on day 49 of gestation (Table 7).

#### 4.6. LITTER SIZE

Among the twenty pregnant animal of the study, there were four singletons, twelve twins and four triplets. There was one still-birth and one abortion. There were eighteen male kids and seventeen female kids. The average birth weight of the kids was 1.95 kg. The average birth weight of male kids was 1.98 kg while the average birth weight of female kids was 1.92 kg. The average weight of singletons, twins and triplets was 2.45 kg, 1.66 kg and 1.98 kg respectively (Table 10).

#### 4.7. GESTATION LENGTH

The average gestation length in the does was 146.47 days. The average gestation length in singletons, twins and triplets was 149.25, 146.73 and 143 days respectively (Table 10).



Table 3. Detection of pregnancy associated structures among the pregnant animals of group I using transrectal (TR) and transabdominal (TA) scanning

Animal no:	Day of gestation	Type of scanning	Gestational sac	Foetus	Foetal heartbeat	Placentomes	Foetal skeleton
1	19	TR	-	-	-	-	-
		TA	-	-	-	-	-
2	19	TR	+	-	-	-	-
		TA	-	-	-	-	-
3	22	TR	+	+	-	-	-
		TA	-	-	-	-	-
4	24	TR	+	+	+	-	-
		TA	-	-	-	-	-
5	24	TR	+	+	+	-	-
		TA	-	-	-	-	-
6	26	TR	+	+	+	-	-
		TA	+	-	-	-	-
7	28	TR	+	+	+	-	-
		TA	+	+	-	-	-

+ Detected

- Not detected

Table 4. Detection of the pregnancy associated structures among the pregnant animals of group II using transrectal (TR) and transabdominal (TA) scanning

Animal no:	Day of gestation	Type of scanning	Gestational sac	Foetus	Foetal heartbeat	Placentomes	Foetal skeleton
1	31	TR	+	+	+	-	-
		TA	+	+	-	-	-
2	34	TR	+	+	+	-	-
		TA	+	+	+	-	-
3	34	TR	+	+	+	-	-
		TA	+	+	+	-	-
4	36	TR	+	+	+	-	-
		TA	+	+	+	-	-
5	42	TR	+	+	+	+	-
		TA	+	+	+	+	-
6	42	TR	+	+	+	+	-
		TA	+	+	+	+	-

+ Detected

- Not detected

Table 5. Detection of the pregnancy associated structures among the pregnant animals of group III using transrectal (TR) and transabdominal (TA) scanning

Animal no:	Day of gestation	Type of scanning	Gestational sac	Foetus	Foetal heartbeat	Placentomes	Foetal skeleton
1	43	TR	+	+	+	+	-
		TA	+	+	+	+	-
2	43	TR	+	+	+	+	-
		TA	+	+	+	+	-
3	49	TR	+	+	+	+	-
		TA	+	+	+	+	-
4	50	TR	+	+	+	+	-
		TA	+	+	+	+	-
5	54	TR	+	+	+	+	+
		TA	+	+	+	+	+
6	55	TR	+	+	+	+	+
		TA	+	+	+	+	+
7	56	TR	+	+	+	+	+
		TA	+	+	+	+	+

+ Detected

- Not detected

Table 6. Gestational sac diameter, crown-rump length and placentome diameter at various day of gestation by transrectal and transabdominal scanning

Day of gestation	GSD mean (mm)		CRL mean (mm)		PD mean (mm)	
	TR	TA	TR	TA	TR	TA
19	5.1	-	-	-	-	-
22	8.3	-	7.2	-	-	-
24	15.5	-	9.4	-	-	-
26	16.8	15.7	15.8	-	-	-
28	18.6	17.5	17.2	16.7	-	-
31	20.2	21.9	16.6	16.6	-	-
34	24.0	28.9	20.2	19.9	-	-
36	27.0	34.4	22.7	21.1	-	-
42	-	-	32.3	29.2	8.4	8.5
43	-	-	34.4	32.7	8.6	8.6
49	-	-	-	-	16.4	16.0
50	-	-	-	-	16.8	17.0
54	-	-	-	-	18.6	19.4
55	-	-	-	-	19.0	22.3
56	-	-	-	-	19.6	22.1
'r' value	0.974	0.985	0.969	0.943	0.973	0.992

GSD - Gestational sac diameter

CRL - Crown-rump length

PD - Placentome diameter

r - Coefficient of correlation with gestational age

TR - Transrectal scanning

TA - Transabdominal scanning

Table 7. Foetal number predicted at various days of gestation by transrectal (TR) and transabdominal (TA) scanning and actual kidding in pregnant animals of all groups

Day of gestation	Foetal number predicted		Actual Kidding
	TR	TA	
19	2/3	-	2
19	-	-	2
22	1	-	1
24	2	-	2
24	1	-	1
26	3	-	3
28	2	-	2
31	2	2/3	2
34	3	2/3	3
34	2	2	2 (still-birth)
36	1	1	1
42	2	2	2
42	2	2	2
43	1	1	1
43	2	2	2
49	2	3	3 (abortion)
50	2	2	2
54	2	2	2
55	2	2	2
56	2/3	2/3	3



Table 8. Accuracy of transrectal (TR) and transabdominal (TA) scanning in foetal number prediction in group I, II and III

Group	Weeks of gestation	No: of predictions		No: of correct predictions		Accuracy (%)	
		TR	TA	TR	TA	TR	TA
I	3- 4	7	0	5	*	71.43	*
II	5- 6	6	6	6	4	100	66.66
III	7- 8	7	7	5	6	71.43	85.71

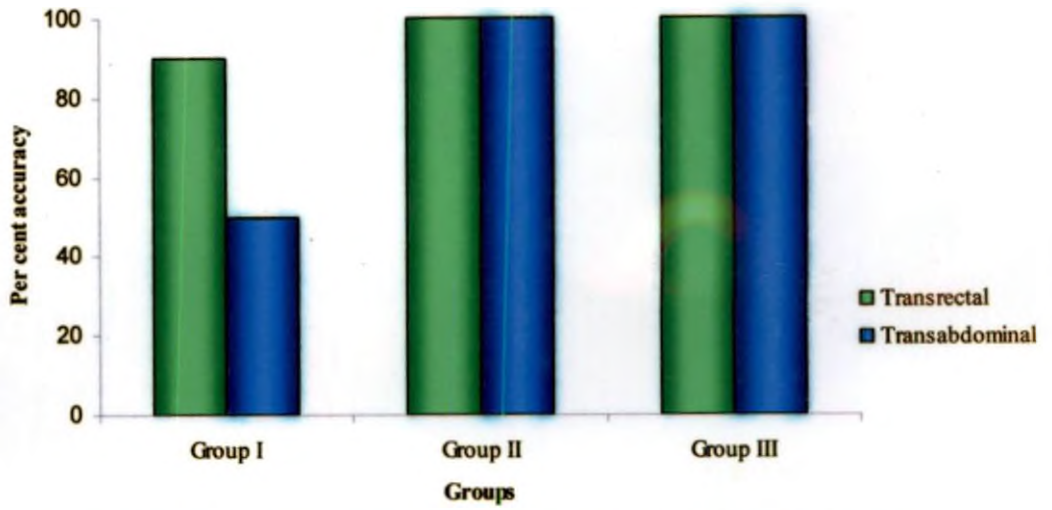
\*: accuracy could not be predicted

Table 9. Accuracy of litter size prediction by transrectal (TR) and transabdominal (TA) scanning

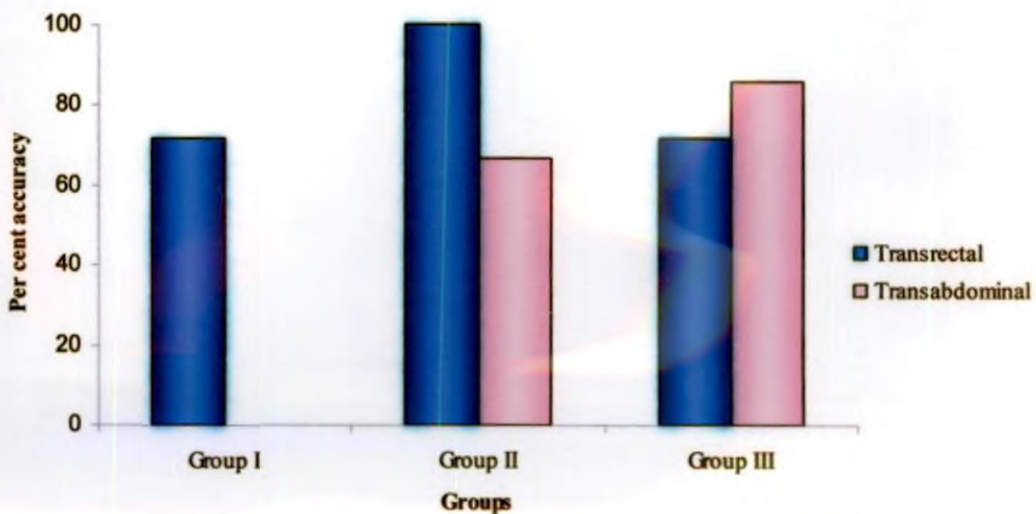
Litter size	Number on actual kidding	No: of correct predictions		Accuracy (%)	
		TR	TA	TR	TA
Singletons	4	4	2	100	50
Twins	12	10	7	83.33	58.33
Triplets	4	2	1	50	25
Overall	20	16	10	80	50

Table 10. Average Birth weight and gestation length of singletons, twins and triplets

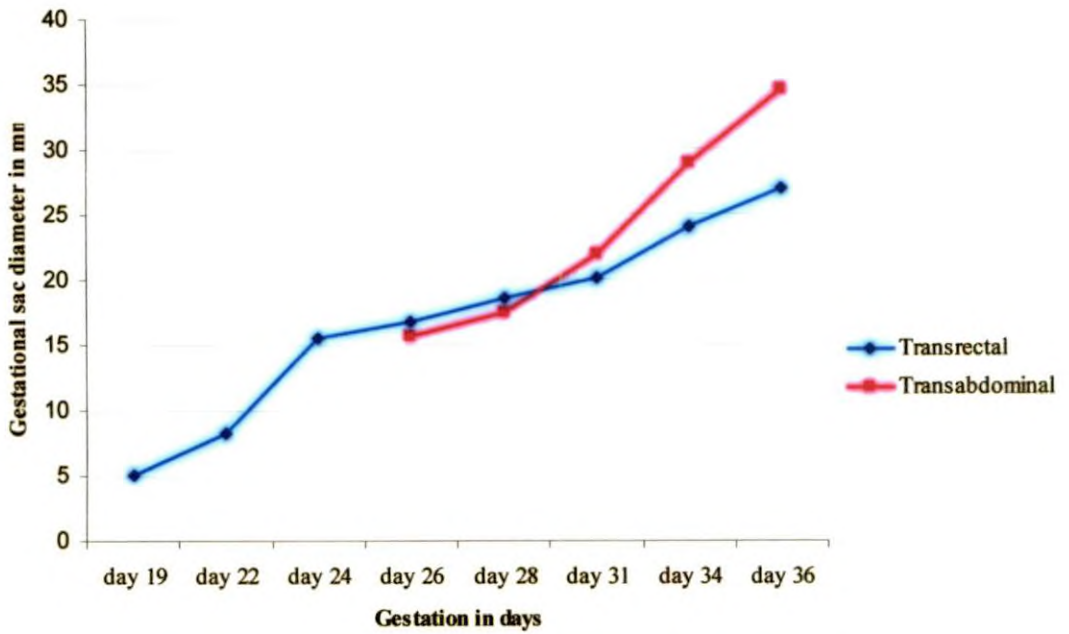
Birth weight (kg)	Average	1.95
	Male	1.98
	Female	1.92
	Singletons	2.45
	Twins	1.66
	triplets	1.98
Gestation length (days)	Average	146.47
	Singletons	149.25
	Twins	146.73
	Triplets	143



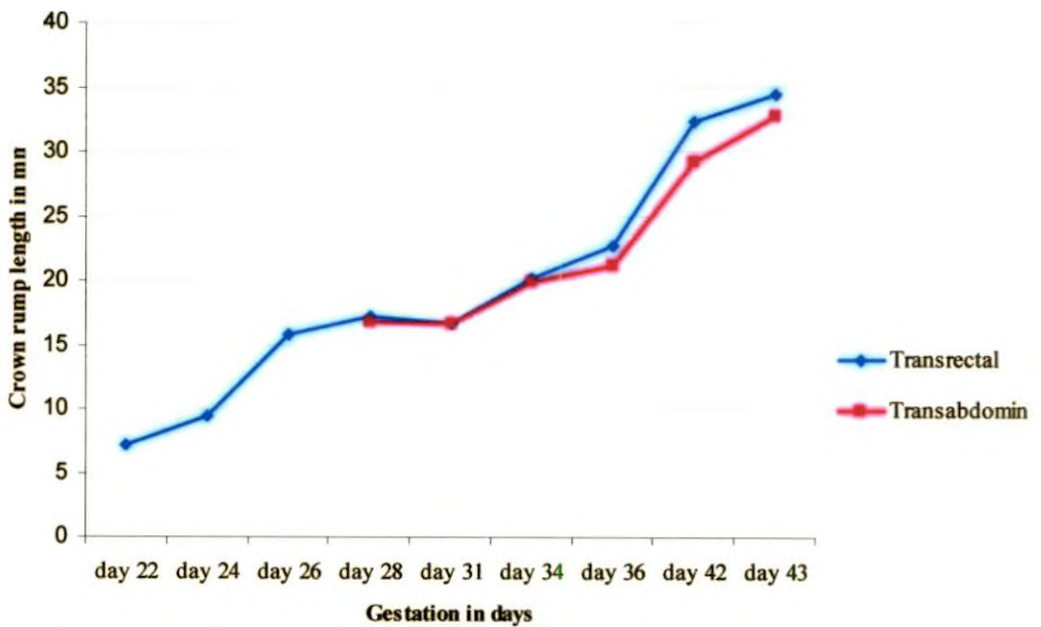
**Fig. 1. Accuracy of transrectal and transabdominal ultrasound scanning for the detection of pregnancy in goats**



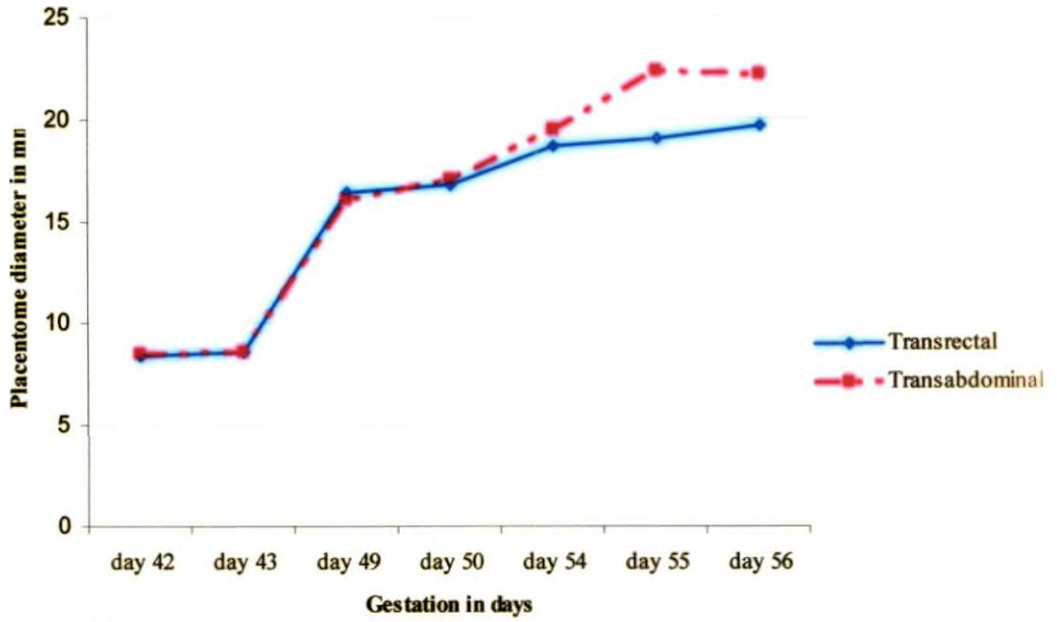
**Fig. 2. Accuracy of transrectal and transabdominal scanning for prediction of foetal number in goats**



**Fig. 3. Gestational sac diameter estimated at various days of gestation by transrectal and transabdominal scanning**



**Fig. 4. Crown Rump length estimated at various days of gestation by transrectal and transabdominal scanning**



**Fig. 5. Placentome diameter estimated at various days of gestation by transrectal and transabdominal scanning**

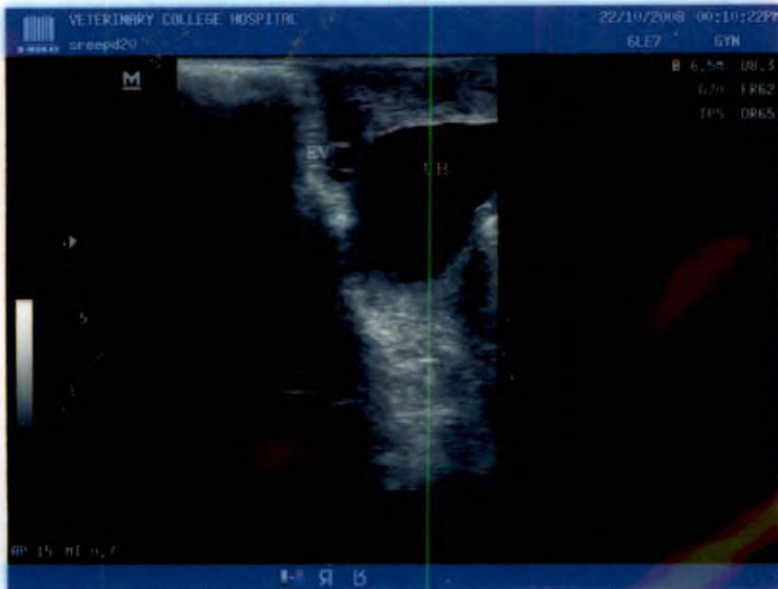


Plate 4. Transrectal ultrasonogram of uterus of a doe on Day 19 of pregnancy. The anechoic fluid of an embryonic vesicle (EV) inside the uterus can be seen. The urinary bladder (UB) is observed as fluid filled dark anechoic structure

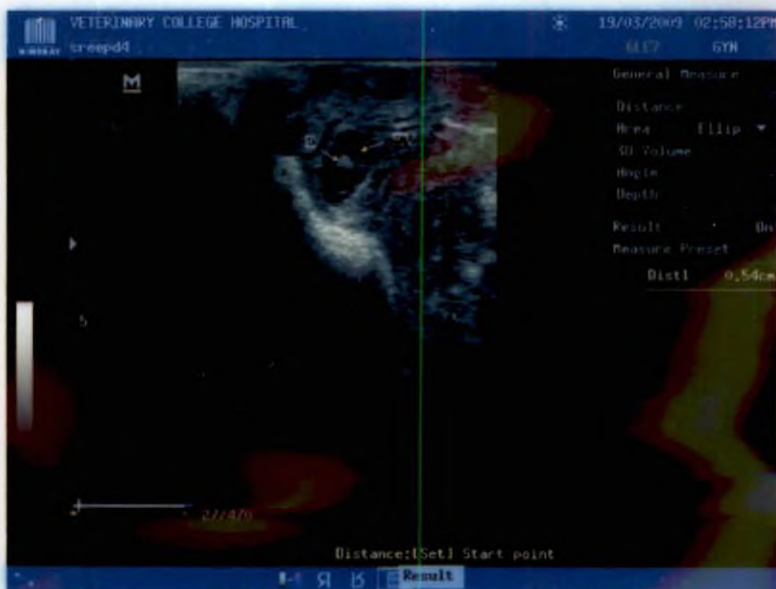


Plate 5. Transrectal ultrasonogram of uterus of a doe on Day 22 of pregnancy. The anechoic fluid of an embryonic vesicle (EV) inside the uterus can be seen. The embryo (E) can be visualized as an echogenic structure within the embryonic vesicle





Plate 6. Transrectal ultrasonogram of uterus of a doe on Day 26 of pregnancy. Three anechoic areas representing embryonic vesicle (EV) and the image of embryo within them can be seen.



Plate 7. Transrectal ultrasonogram of uterus of a doe on Day 34 of pregnancy. Two anechoic areas representing embryonic vesicle (EV) and the image of embryo within them (E) can be seen.



Plate 8. Transrectal ultrasonogram of uterus of a doe on Day 34 of pregnancy. Three anechoic areas representing embryonic vesicle (EV) and the image of embryo within them (E) can be seen.

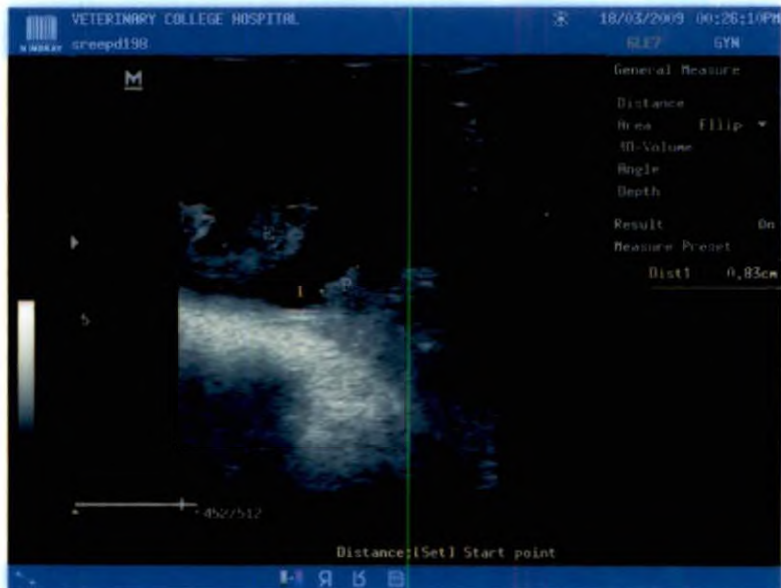


Plate 9. Transrectal ultrasonogram of uterus of a doe on Day 42 of pregnancy. The image of foetus (E) and placentome (P) can be seen.



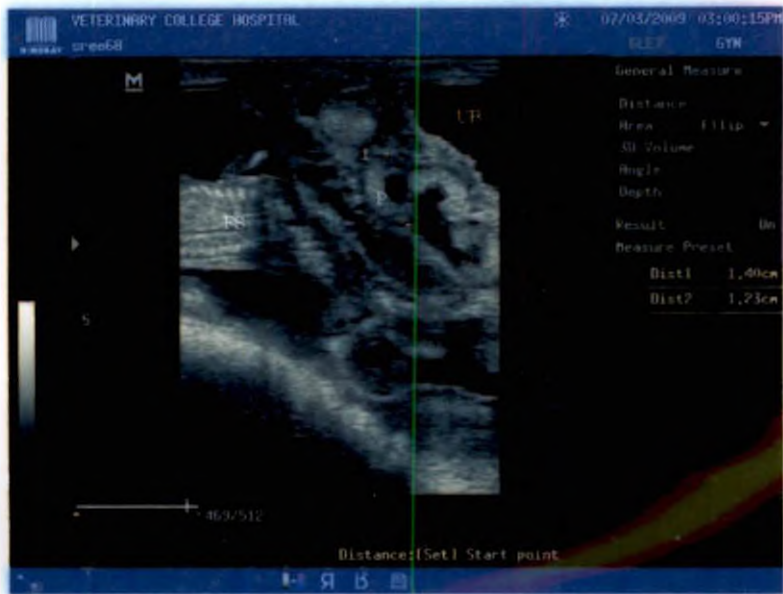


Plate 10. Transrectal ultrasonogram of uterus of a doe on Day 55 of pregnancy. Image of Foetal skeleton (FS) and the image of placentomes (P) can be seen.

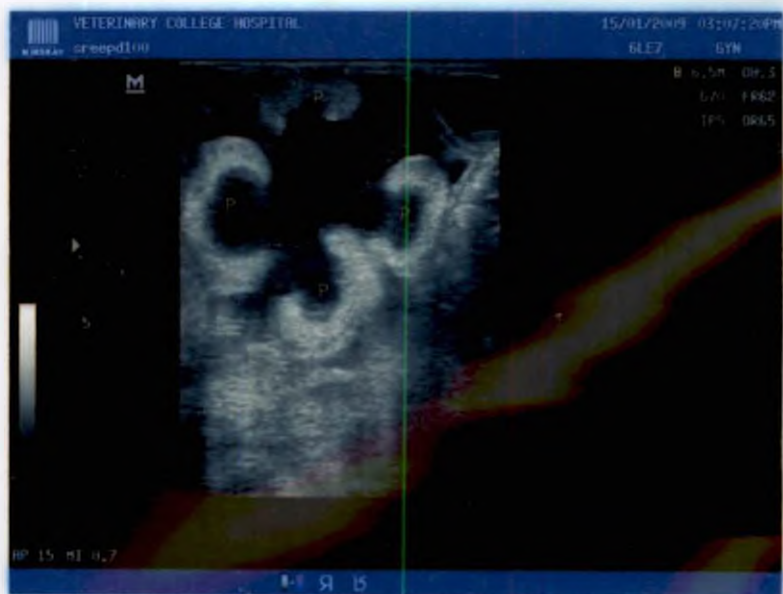


Plate 11. Transrectal ultrasonogram of uterus of a doe showing echogenic 'C' shaped placentomes



Plate 12. Transabdominal ultrasonogram of uterus of a doe on day 28 of pregnancy. Anechoic areas representing embryonic vesicle (EV) and the image of embryo (E) within them can be seen.



Plate 13. Transabdominal ultrasonogram of uterus of a doe on Day 34 of pregnancy. Anechoic areas representing embryonic vesicle and the image of embryo within them (E) can be seen.



Plate 14. Transabdominal ultrasonogram of uterus of a doe on day 42 of pregnancy. Echogenic foetus (E) within the embryonic vesicle can be seen.



Plate 15. Transabdominal ultrasonogram of uterus of a doe on Day 54 of pregnancy. Echogenic foetal skeleton (FS) and placental structures (P) can be seen.





Plate 16. Transabdominal ultrasonogram of uterus of a doe on day 55 of pregnancy. Echogenic foetal skeleton (FS) and placentomes (P) can be seen.



Plate 17. Transabdominal ultrasonogram of uterus of a doe on day 56 of pregnancy. Echogenic foetal skull (F) and placentomes (P) can be seen.

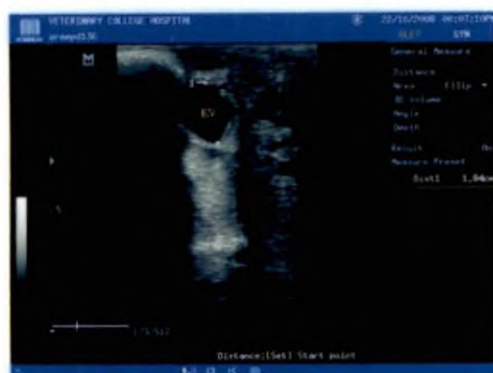


Plate 18. Measurement of gestational sac diameter by electronic calipers, as indicated by two crosses on the image

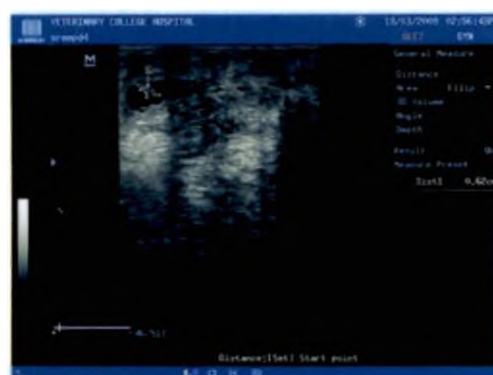


Plate 19. Measurement of crown rump length using electronic calipers

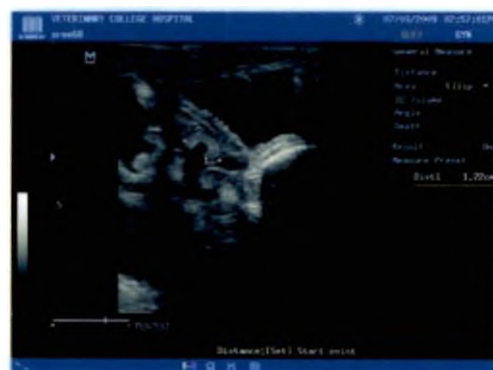


Plate 20. Measurement of placental diameter using electronic calipers.

## *Discussion*

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## 5. DISCUSSION

The study was carried out to evaluate the efficacy of transrectal and transabdominal ultrasonography in early pregnancy diagnosis in goats and to identify the optimum stage of gestation for early pregnancy diagnosis using B-mode ultrasonography in goats. Both techniques were used to determine early pregnancy diagnosis in goats and their efficiency was compared with the actual kidding data. The optimum stage of gestation for early pregnancy diagnosis by both techniques was identified by the present study.

### 5.1. ULTRASOUND SCANNING FOR PREGNANCY DIAGNOSIS

Accuracy of transrectal and transabdominal scanning in pregnancy diagnosis in animals of group I, II and III are presented in Table 1.

#### 5.1.1. Pregnancy Diagnosis by Transrectal Scanning

##### 5.1.1.1. Group I

The accuracy of transrectal ultrasonography in pregnancy diagnosis of the animals scanned between third and fourth week post-breeding (group I) is presented in Table 1. The accuracy for the detection of pregnancy status was 90 per cent in this group. In the present study, one animal scanned on day 19 post-breeding could not be confirmed as pregnant by transrectal scanning. However, from day 22 post-breeding (fourth week) all animals were accurately identified as pregnant and non-pregnant by transrectal scanning. Buckrell (1988) reported that the accuracy could exceed 95 per cent as early as day 25 post-breeding by using transrectal scanning in goats and sheep. Bretzlaff *et al.* (1993) observed pregnancy by 19 to 22 days of gestation in goats by transrectal ultrasonography which was similar to the present study. Garcia *et al.* (1993) reported an accuracy of 87 per cent by day 24 to 26 of gestation in ewes. Martinez *et al.* (1998) observed pregnancy from day 18 onwards which was one day earlier than the

present study. Singh *et al.* (2004) obtained an accuracy of 83 and 100 per cent on day 21 to 23 and on day 24 to 26 respectively in Jamunapari goats. Gonzalez *et al.* (2004) observed an accuracy of 99.4 per cent on day 26 of gestation in goats. Deokate *et al.* (2005) and Dhoble *et al.* (2005) reported an accuracy of 100 per cent on day 24 of gestation in goats which was in agreement with the present study. The reason for lower accuracy in the third week post-breeding in the present study might be due to breed difference, operator experience or due to the resolution of the probe.

#### **5.1.1.2. Group II**

The accuracy for the diagnosis of pregnancy using transrectal scanning was 100 per cent in all the animals scanned between fifth and sixth week (Table 1). Hesselink and Taverne (1994) detected pregnancy in all cases by day 30 of gestation. Garcia *et al.*, (1993) found that the accuracy was 95 per cent in ewes between day 32 and 34 of gestation. Kaulfuss *et al.* (1997) observed that the accuracy of transrectal scanning was 100 per cent on day 35 post-breeding in sheep. These reports were similar to the observations of the present study.

#### **5.1.1.3. Group III**

The accuracy of transrectal scanning in the diagnosis of pregnancy was 100 per cent in all the animals scanned between seventh and eighth week post-breeding. Karen *et al.* (2004) reported an accuracy of 96.8 per cent while Romanoa and Christiansb (2008) observed accuracy of 100 per cent in diagnosing pregnancy in sheep between 41- 50 days post-breeding.

### **5.1.2. Pregnancy Diagnosis by Transabdominal Scanning**

#### **5.1.2.1. Group I**

Ten animals were scanned between third and fourth week post-breeding and the accuracy of transabdominal scanning was 50 per cent in the diagnosis of



pregnancy (Table 1). Pregnancy could be identified from day 26 post-breeding employing transabdominal scanning (Table 3). Abdelghafar *et al.* (2007) observed an accuracy of 100 per cent in diagnosing pregnancy in Saanen goats between day 26 and 110 of gestation. Anwar *et al.* (2008) obtained an accuracy of 30 per cent at 26-30 days of gestation by transabdominal scanning. Aliya and Hayderb (2007) suggested that transabdominal scanning could be done as early as 24 days of breeding in sheep.

### **5.1.2.2. Group II**

Among the ten animals scanned between fifth and sixth week post-breeding in this group, six animals were confirmed as pregnant and four animals as non-pregnant by transabdominal scanning. The accuracy of transabdominal scanning in diagnosing pregnancy reached 100 per cent in this group (Table 1). According to Bretzlaff *et al.* (1993) and Arthur *et al.* (1996) the pregnancy diagnosis was quite reliable from day 30 of gestation with transabdominal scanning in goats. Dawson *et al.* (1994) observed 100 per cent accuracy for the detection of pregnancy from fifth week post-breeding in Alpine goats. Anwar *et al.* (2008) obtained an accuracy of 100 per cent in pregnancy diagnosis in sheep by day 42 of gestation. All these reports were in accordance with the present study.

### **5.1.2.3. Group III**

Seven animals were diagnosed as pregnant and three as non-pregnant and the accuracy of transabdominal scanning for diagnosing pregnancy in this group was 100 per cent (Table 1). Fowler and Wilkins (1984) reported an accuracy of 95 per cent in the diagnosis of pregnancy from 40 to 50 days of gestation and over 99 per cent accuracy in the diagnosis of pregnancy above 50 days in ewes. White *et al.* (1984) observed an accuracy of 100 per cent in diagnosing pregnancy from day 50 of gestation in sheep using transabdominal scanning.

Aiumlamai *et al.* (1992) observed an accuracy of 100 per cent in sheep from 45 days of gestation. Karen *et al.* (2006) observed an accuracy of 100 per cent for transabdominal scanning for pregnancy diagnosis in ewes from 43- 56 days of gestation. These reports were in agreement with the present study. Tasal *et al.* (2006) obtained a lesser accuracy of 94.10 per cent to diagnose pregnancy in sheep between 46 and 60 days of gestation.

### **5.1.3. Comparison of transrectal and transabdominal scanning in pregnancy diagnosis**

In the animals scanned between third and fourth week post-breeding (group I), the accuracy for pregnancy diagnosis by transrectal and transabdominal scanning was 90 and 50 respectively. The accuracy reached 100 per cent in group II and III by both the methods of scanning. Bretzlaff *et al.* (1993) opined that after day 50 of gestation, it became easier to visualize foetuses by the transabdominal approach because of the anterior-ventral displacement of the uterus and its contents. The uterus had an intra-pelvic location in the first month of pregnancy, thus preventing good visualization by transabdominal scanning (Dawson *et al.*, 1994). Kahn (1994) opined that the transrectal scanning was more accurate than transabdominal method until day 35 of gestation in sheep and goats and that between days 35 and 70 both methods appeared to be equally accurate. Karen *et al.* (2001) opined that the optimum time for using transabdominal or transrectal scanning in sheep ranged from 25 to 100 days of gestation. Aliya and Hayderb (2007) opined that the imaging of transabdominal scanning was much inferior to that of the transrectal one and it became better as the pregnancy advanced. They also observed that transrectal scanning could be performed efficiently until day 48 of pregnancy and beyond that most of the foetal parts, except the placentomes became out of the range of the ultrasound beam. All these reports are in accordance with the present study.

## 5.2. ULTRASONOGRAPHIC IMAGING OF CONCEPTUS AND ASSOCIATED STRUCTURES

### 5.2.1. Imaging by Transrectal Scanning

#### 5.2.1.1. Embryonic vesicle

The first structure identified in diagnosing pregnancy was the embryonic vesicle. It appeared as a discrete, intrauterine, sharply demarcated anechoic, round or oval structure and was detected earliest on day 19 by transrectal scanning in one animal of group I (Table 3). It was evident in six of the seven pregnant animals of group I. By transrectal scanning, embryonic vesicle was observed in all the pregnant animals of other groups (Table 2).

The first ultrasonographic sign of a pregnant uterus in sheep and goat was the visualization of fluid-filled vesicles (Buckrell 1988, Gonzalez *et al.* 1998, Medan *et al.* 2004). Padilla *et al.* (2005) and Amer (2008) observed the embryonic vesicle by transrectal scanning in goats on 19.5 and  $19.5 \pm 0.30$  mean days of gestation respectively which was similar to the observation made in this study. Medan *et al.* (2004) observed the embryonic vesicle in goats on  $20.2 \pm 0.60$  days after mating employing transrectal scanning. Singh *et al.* (2004) detected circular and elongated embryonic vesicle during 17-19 days of gestation in goats with less accuracy while Dhoble *et al.* (2005) detected it on day 23 of gestation by transrectal scanning. Martinez *et al.* (1998) and Kumar *et al.* (2005b) visualized multiple non-echogenic areas on day 18 in pregnant goats, which was one day earlier than the present study. Suguna *et al.* (2008) detected embryonic vesicle on day 21 post-breeding in goats by transrectal scanning.

#### 5.2.1.2. Embryo/foetus

The presence of embryo proper which appeared as an echogenic structure within the anechoic fluid was first observed on day 22 of gestation by transrectal scanning (Table 3). Hesselink and Taverne (1994) opined that the mere presence of fluid in the uterus was not the only indication of pregnancy, because of the

possibility of hydrometra in goats and suggested that detection of the foetus was required for the confirmatory diagnosis of pregnancy. According to Martinez *et al.*, (1998), the confirmatory sign of pregnancy in goat was the detection of proper embryo. Kumar *et al.* (2005b) observed echoic conceptus in goats on day 23 and Dawane *et al.* (2008) observed it between days 24 and 30 of gestation in sheep. These reports were in agreement with the present study. Bretzlaff *et al.* (1993) detected embryo in goats by 24 days of gestation. Singh *et al.* (2004) observed embryo on day 25 post-breeding. Buckrell *et al.* (1986) and Aliya and Hayderb (2007) observed a foetus on day 25 and  $25.38 \pm 1.20$  days of gestation in sheep by transrectal ultrasonography. Garcia *et al.* (1993) visualized the embryo proper from 21 days of gestation in sheep. Embryo proper was frequently observed on day 25 in ewes (Schrick and Inskeep, 1993) but it was observed by 19 days of gestation in goats (Gonzalez *et al.*, 1998 and Martinez *et al.*, 1998).

#### **5.2.1.3. Foetal heartbeat**

Foetal heartbeat was first detected on day 24 of gestation as a flickering movement by transrectal scanning (Table 3). Medan *et al.* (2004) observed the foetal heartbeat on  $24.30 \pm 0.70$  days post-breeding while Kumar *et al.* (2005b) detected it on day 26 of gestation in goats using transrectal scanning. Padilla *et al.* (2005) observed the foetal heartbeat from 22.9 days of gestation in Boer goats. Garcia *et al.* (1993) detected the embryonic heartbeat from day 21 to 23, Buckrell (1988) from day 26 and Dawane *et al.* (2008) from day 25 to 30 of gestation in pregnant ewes. Kumar *et al.* (2005b) could find conceptus heartbeat on day 30 post-breeding in black Bengal goats.

#### **5.2.1.4. Foetal viability**

Foetal viability was assessed based on the observation of foetal heartbeat. The heartbeat of the foetus was detected from day 24 of gestation by transrectal scanning in pregnant animals of all groups (Table 3, 4, 5). Heartbeat was considered as a criterion to know the viability of the foetus (Kumar *et al.*, 2005a).

Padilla *et al.* (2005) and Amer (2008) opined that the observation of heartbeat was a conclusive evidence of the presence of a live foetus.

#### **5.2.1.5. Placentomes**

Placentomes were not observed in the pregnant animals scanned between third and fourth week post-breeding (group I). Placentomes were first observed as a concave-shaped echogenic structure directed towards the uterine lumen by transrectal scanning from day 42 in the pregnant animals of group II (Table 4). In group III animals, placentomes changed from the concave-shaped structure to a 'C' or 'O' shaped structure. Suguna *et al.* (2008) observed placentomes on day 42 of gestation in goats by transrectal scanning which was similar to the present study. Buckrell (1988) observed placentomes from day 26 to 28 of gestation. Medan *et al.* (2004) observed placentomes on  $35.40 \pm 1.00$  days of gestation by transrectal scanning and opined that the size of the placentomes increased and appeared as 'C' shaped or 'O' shaped grey image as pregnancy progressed. Dhoble *et al.* (2005) observed placentomes on the days 25 to 35 of gestation as button-like protrusions in goats by transrectal scanning. Dawane *et al.* (2008) observed placentomes by transrectal scanning from day 30 to 50 of gestation in sheep. The early detection of placentomes might be attributed to the breed difference, resolution of the probe and operator expertise.

#### **5.2.1.6. Foetal skeleton**

Foetal skeleton was not observed in the pregnant animals scanned between third and fourth week post-breeding and fifth and sixth week post-breeding (group I and II). Foetal skeleton was first observed from day 54 of gestation by transrectal scanning in the pregnant animals scanned between seventh and eighth week post-breeding (table 5). Medan *et al.* (2004) detected skeletal structures at two months of pregnancy in goats by transrectal scanning. Kumar *et al.* (2005b) observed rib-cage on day 50 of gestation in goats by the

same technique. Suguna *et al.* (2008) detected skeletal structures from day 56 of gestation by this method of scanning.

## **5.2.2. Imaging by Transabdominal Scanning**

### **5.2.2.1. Embryonic vesicle**

The earliest day for detection of embryonic vesicle as fluid-filled cavities, employing transabdominal scanning was on day 26 post-breeding in the present study (Table 3). It was evident in only two of the seven pregnant animals of group I and all the pregnant animals of group II and III.

White *et al.* (1984) observed the fluid filled uterus as early as 30 days of gestation in sheep by transrectal scanning. Russel (1989) stated that pregnancy could be detected in goat by about 30 days post-conception by identifying the fluid-filled uterus. Padilla *et al.* (2005) succeeded in detection of fluid accumulation within the uterus by mean day 26.4 of gestation on scanning at 3.5 day interval and it improved to 24.7 days on scanning daily. Amer (2008) opined that fluid-filled vesicles could be reliably located from  $24.70 \pm 0.40$  days. Suguna *et al.* (2008) detected embryonic vesicle from day 28 of gestation in goat. Hesselink and Taverne (1994) observed pregnancy from about day 22 of gestation in goat in standing position. Late day of detection of embryonic vesicle by transabdominal scanning in our study may be attributed to the lateral recumbency position, breed difference and resolution of the transabdominal probe used.

### **5.2.2.2. Embryo/foetus**

The foetus/ embryo were first detected on day 28 post-breeding by transabdominal scanning in the present study. Hoque *et al.* (2005) detected foetus earliest on day 30 post breeding. Suguna *et al.* (2008) detected embryo proper on day 35 by employing transabdominal scanning in goats. Karen *et al.* (2008) detected embryo on  $30.36 \pm 4.75$  days in goats by transabdominal scanning. All these reports were in agreement with the present study.

### **5.2.2.3. Foetal heartbeat**

Foetal heartbeat was first observed as flickering movement on day 34 post-breeding employing transabdominal scanning technique (Table 4). Hesselink and Taverne (1994) reported that action of heart was perceptible in all cases from day 35 post-breeding by transabdominal scanning. Padilla *et al.* (2005) observed foetal heartbeat on day 33 post-breeding by scanning at 3.5 day interval and it improved to day 27 on daily basis scanning. Suguna *et al.* (2008) observed foetal heartbeat on day 35 post-breeding in crossbred goats. These reports are similar to the present study. Amer (2008) observed foetal heartbeat from  $27 \pm 0.60$  days onwards using a transabdominal probe. The difference in the day of detection may be due to the breed difference and operator expertise.

### **5.2.2.4. Foetal viability**

Foetal viability was assessed based on the observation of foetal heartbeat. The heartbeat of the foetus was detected from day 34 of gestation by transabdominal scanning in pregnant animals. (Table 3, 4, 5). No foetal heartbeat could be observed by transabdominal scanning in the pregnant animals scanned between third and fourth week of gestation (group I). The detection of heartbeat is the confirmatory sign of viability of the foetus (Suguna *et al.*, 2008).

### **5.2.2.5. Placentomes**

Placentomes were not observed in the pregnant animals scanned between third and fourth week post-breeding (group I). Placentomes were first observed as a concave-shaped echogenic structure directed towards the uterine lumen by transabdominal scanning from day 42 in the pregnant animals of group II (Table 4). In group III animals, placentomes changed from the concave-shaped structure to a 'C' or 'O' shaped structure. Aiumlamai *et al.* (1992) detected placentomes in pregnant sheep from day 44 of gestation by transabdominal scanning. By transabdominal scanning, Dawson *et al.* (1994) identified placentomes on the seventh week post-breeding in goats. Bretzlaff *et al.* (1993) reported that

placentomes become the cardinal sign of pregnancy after day 50 of gestation. Matsas (2007) observed that by 45- 50 days, placentomes were more easily seen as C-shaped densities with the concave surface directed towards the uterine lumen. Anwar *et al.* (2008) observed placentomes in all the pregnant ewes from 45-50 days post-breeding using transabdominal scanning. Suguna *et al.* (2008) could observe placentomes on day 50 of gestation in crossbred goats by transabdominal scanning. All these reports were similar to the present study.

#### **5.2.2.6. Foetal skeleton**

Foetal skeleton was not observed in the pregnant animals scanned between third and fourth week post-breeding and fifth and sixth week post-breeding (group I and II). Foetal skeleton was first observed from day 54 of gestation by transabdominal scanning in the pregnant animals scanned between seventh and eighth week post-breeding (table 5).

Anwar *et al.* (2008) observed vertebral columns of the foetuses in pregnant ewes with 100 per cent accuracy from 51-55 days post-breeding by transabdominal scanning which was a similar observation to the present study. Suguna *et al.* (2008) detected skeletal structures from day 56 of gestation by transabdominal scanning.

### **5.3. MEASUREMENT OF GSD, CRL AND PD**

#### **5.3.1. Measurement by Transrectal Scanning**

The details of Gestational sac diameter (GSD), crown-rump length (CRL) and placentome diameter (PD) at various day of gestation by transrectal and transabdominal scanning are presented in Table 6.

The earliest day of detection of the gestational sac was on day 19 of gestation using transrectal scanning and the mean diameter recorded was 5.1 mm on that day. Martinez *et al.* (1998) detected non-echogenic areas in the uterus about  $4.0 \pm 0.3$  mm by day 18 post-breeding in goats by transrectal scanning.



Singh *et al.* (2004) and Garcia *et al.* (1993) observed circular and elongated embryonic vesicle, in goat and sheep respectively, with a mean diameter of 4 mm during 17-19 days of gestation by transrectal scanning. After day 42 of gestation, the gestational sac began to elongate and the measurement of GSD was not possible. The GSD recorded by transrectal scanning had high correlation ( $r = 0.974$ ) with gestational age.

In the present study the embryo was detected earliest on day 22 of gestation by transrectal scanning and its mean crown-rump length was 7.2 mm using transrectal scanning. Schrick and Inskeep (1993) observed that in ewes the crown-rump length of embryos on days 20, 25, 30, 35 and 40 averaged  $6 \pm 1$ ,  $10 \pm 2$ ,  $17 \pm 2$ ,  $25 \pm 2$  and  $36 \pm 4$  mm, respectively and reported that beyond day 40 of gestation it was difficult to determine the size and number of foetuses due to the inability to image an entire foetus in a single frame on the screen. Martinez *et al.* (1998) reported that the mean CRL observed by transrectal scanning was  $5.3 \pm 0.3$  mm on  $20.7 \pm 0.5$  days, reaching  $34.2 \pm 0.6$  on day 40 of gestation. The CRL recorded by transrectal scanning had a high correlation ( $r = 0.969$ ) with the gestational age which was similar to the study of Martinez *et al.* (1998) who also found high correlation ( $r = 0.94$ ) with gestational age.

After day 43 of gestation it was difficult to measure the crown-rump length due to the difficulty to image the entire foetus in the screen. Similar observations were made by Gonzalez *et al.* (1998) and Aliya and Hayderb (2007). They opined that transrectal scanning could be performed efficiently until day 48 of pregnancy and beyond that most of the foetal parts became out of the range of the ultrasound beam. Kumar *et al.* (2005b) opined that after day 50 it became difficult to get full length of the foetus.

Placentomes were first detected on day 42 of gestation as concave-shaped structures by transrectal scanning. The mean diameter of the placentomes recorded by transrectal scanning on day 42 post-breeding was 8.4 mm. Suguna *et al.* (2008) observed mean diameter 9.7 mm for placentomes on day 42 of

gestation by transrectal scanning. Significant increase in placentomes diameter with advancement of gestation and high correlation ( $r = 0.99$ ) obtained by Doize *et al.* (1997) was in agreement with the present study.

### 5.3.2. Measurement by Transabdominal Scanning

The earliest day of detection of the gestational sac was on day 26 of gestation using transabdominal scanning. The gestational sac was first detected on day 26 of gestation by transabdominal scanning. The mean diameter recorded was 15.7 mm on day 26 and 34.4 mm on day 36 of gestation by transabdominal scanning. The GSD recorded by transabdominal scanning had a high correlation ( $r = 0.985$ ) with the gestational age.

The embryo was detected earliest on day 28 of gestation by transabdominal scanning and the mean crown-rump length recorded was 16.7 mm on that day. After day 43 of gestation it was difficult to measure the crown-rump length due to the difficulty to image the entire foetus in the screen. The CRL recorded had a high ( $r = 0.943$ ) correlation with the gestational age. Gaikwad *et al.* (2007) observed mean CRL in millimeters were  $27.82 \pm 0.73$ ,  $46.35 \pm 1.32$  on days 35, 42 of gestation using transrectal and transabdominal probe in Osmanabadi goats. These values were higher than that obtained in the present study. This might be due to the difference in the breed. The size of the embryo/ foetus increased and became too large to be completely visualized on the screen after 50 days of gestation (Bretzlaff *et al.*, 1993).

Placentomes were first detected on day 42 of gestation as concave-shaped structures by using transabdominal scanning. The mean diameter of the placentomes recorded by transabdominal scanning on day 42 post-breeding was 8.5 mm. The placentome diameter also had a high correlation ( $r = 0.992$ ) with the gestational age. Suguna *et al.* (2008) also observed a significantly high correlation ( $r=0.99$ ) for placentome diameter with gestational age. Karen *et al.* (2008) also reported a high correlation of placentome diameter with gestational

age. As pregnancy advanced the placentomes increased in size and appeared as 'C' or 'O' shaped grey images (Lee *et al.*, 2005) which was in accordance with the present study.

#### 5.4. FOETAL NUMBER

##### 5.4.1. Foetal Number Estimation by Transrectal Scanning

The overall accuracy for the prediction of foetal numbers by transrectal scanning was 80 per cent (Table 9). The accuracy for prediction of foetal number using transrectal scanning in pregnant animals of group I and III was 71.43 per cent. The accuracy for the prediction was 100 per cent in pregnant animals of group II (Table 8).

The accuracy for the prediction of singletons, twins and triplets by transrectal scanning was 100, 83.33 and 50 per cent respectively. Fowler and Wilkins (1984) and Padilla *et al.* (2005) reported that the best time to distinguish between twins and singletons with a rectal probe was between day 28 and 40 of gestation. This was in accordance with the present study. Padilla *et al.* (2005) also observed that difficulty in identifying the foetal numbers increased with multiple pregnancies which was similar to the present study. Matsas (2007) opined that the optimal time for counting the foetal numbers by transrectal scanning was between 40 and 70 days of gestation. Suguna *et al.* (2008) differentiated singleton from twins on day 35 of gestation in goats by transrectal scanning.

##### 5.4.2. Foetal Number Estimation by Transabdominal Scanning

The overall accuracy for the prediction of foetal numbers by transabdominal scanning was 50 per cent (Table 9). By transabdominal ultrasonography, it was not possible to predict foetal number accurately in pregnant animals group I. The accuracy for the prediction of foetal number in

group II by transabdominal scanning was 66.66 per cent while the accuracy was 85.71 per cent in group III (Table 8).

The accuracy for the prediction of singletons, twins and triplets was 50, 58.33 and 25 per cent by transabdominal scanning respectively. Fowler and Wilkins (1984) could detect singles but could not differentiate multiples by transabdominal scanning. This was in accordance with the present study. White *et al.* (1984) reported that it was difficult to distinguish twins from triplets and quads by transabdominal scanning. Gearheart *et al.* (1988) observed an accuracy of 100 per cent in determining singletons and 97.3 per cent in determining twins on day 51 to 75 days of gestation by transabdominal ultrasound scanning. Hesselink and Taverne (1994) reported that the accuracy in differentiating a singleton from a twin pregnancy using transabdominal scanning was higher than that of a twin from a triplet and a quadruplet. Karen *et al.* (2006) obtained a sensitivity of 53.8 per cent in the diagnosis of multiples and 78.6 per cent in ewes in the determination of singles using transabdominal scanning between days 43 and 56 of gestation. Matsas (2007) reported that the diagnosis of triplets was more difficult than twins which were similar to the observation made by the present study. Suguna *et al.* (2008) differentiated singleton from twins on day 42 of gestation in goats by transabdominal scanning.

Bretzlaff *et al.* (1993) opined that the identification of triplets or more foetuses was about 50 per cent by ultrasonography and that identification of single and multiple foetuses by transabdominal scanning was most accurate from 45 to 90 days of gestation. Hesselink and Taverne (1994) opined that the accuracy would depend on the equipment, operator experience and stage of pregnancy. According to Matsas (2007), the optimal time for counting the foetal numbers was between 40 and 70 days for transrectal scanning and 45-90 days post-breeding for transabdominal scanning. Suguna *et al.* (2008) proposed that the best period for differentiation of singleton from twins was between fifth to seventh weeks of gestation. More than two kids were difficult to identify (Padilla *et al.* 2005). Arthur *et al.* (1996) opined that foetal numbers could be counted

accurately by day 45-50 of gestation using transabdominal approach. Dawson *et al.* (1994) opined that transabdominal scanning at seventh week of gestation was more accurate than fifth week for prediction of foetal numbers.

#### 5.6. GESTATIONAL ACCIDENTS

There was one still-birth and one abortion in two animals during the period. The animal in which still-birth was observed was scanned on day 34 of gestation. The animal which aborted in the third month of gestation was scanned on day 49 of gestation (Table 7). Wurst *et al.* (2007) opined that the combination of transrectal and transabdominal ultrasonography was safe for pregnancy diagnosis in ewes. Real-time ultrasonography could rule out uterine pathology as foetuses were demonstrated from fifth week of gestation (Dawson *et al.* 1994). According to Roberts (1971) the normal rates of each embryonic loss in the ewe are reported to be 20 to 30 per cent from weeks after conception until parturition. Garcia *et al.* (1993) reported a low rate of embryonic loss of seven per cent. The reason of abortion might not be due to scanning but due to some managerial or infectious causes.

#### 5.7. LITTER SIZE

Among the twenty pregnant animals of the study, there were four singletons, twelve twins and four triplets (Table 9). There was a still-birth and an abortion. There were eighteen male kids and seventeen female kids. The average birth weight of the kids was 1.95 kg. The average birth weight of male kids was 1.98 kg while the average birth weight of female kids was 1.92 kg. The average weight of singletons, twins and triplets was 2.45 kg, 1.66 kg and 1.98 kg respectively (Table 10). Krishnakumar (1992) reported that the mean birth weight was 1.92 kg which was similar to the observation made in the study. James *et al.* (2002) observed a higher twinning percentage in goats maintained in Kerala which was in agreement with the present study. Afsal (2003) also reported that the incidence of twins was more in Malabari crossbred does.

## 5.8. GESTATION LENGTH

The average gestation length in the does was 146.47 days. The average gestation length in singletons, twins and triplets was 149.25, 146.73 and 143 days respectively (Table 10). This was similar to the observation made by Sudarsanan and Raja (1973). They observed that the length of gestation ranged from 142 to 152 days within a mean of 146.20 days in Malabari goats. Similar observations were made by Kuriakose (1981) and Juliet (2008). Biswas and Kaul (1994) reported that the average gestation length in Himalayan Chegu goats was  $150.60 \pm 0.30$  days. Prasanth (1995) found that the average gestation length in Alpine X Malabari does was  $149.85 \pm 4.45$  days. Singh *et al.* (2004) reported an average gestation length of  $150.2 \pm 2.68$  days in Boer goats.

## CONCLUSION

Real-time B-mode ultrasound scanning offered a safe, non-invasive and accurate technique for early pregnancy diagnosis, imaging of the pregnancy associated structures and its measurement, estimation of foetal number and assessment of foetal viability in goats. The pregnancy could be diagnosed on day 19 of gestation by detection of embryonic vesicle but reached 100 per cent accuracy from fourth week of gestation by transrectal scanning. By transabdominal scanning, the earliest day of detection of embryonic vesicle was on day 26 of gestation and reached 100 per cent accuracy by fifth week of gestation. Real-time ultrasound scanning by both transrectal and transabdominal scanning was found to be reliable, safe and accurate for the diagnosis of pregnancy in goats from fifth week of gestation.

By transrectal scanning the optimum time for foetal number estimation was fifth and sixth week post-breeding while that for transabdominal scanning was seventh and eighth week post-breeding. The disadvantage was the difficulty in differentiation of triplets and expertise required and also the initial cost involved.

## *Summary*

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## 6. SUMMARY

The present study was carried out to evaluate the efficacy of transrectal and transabdominal ultrasonography in early pregnancy diagnosis in goats and to identify the optimum stage of gestation for early pregnancy diagnosis using B-mode ultrasonography in goats. The animals for the present study consisted of crossbred female goats maintained at the University Goat and Sheep Farm and those presented for Artificial Inseminations (A.I) at the Artificial Insemination Centre ancillary to Department of Animal Reproduction and Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy and University Veterinary Hospital, Kokkalai.

From among them thirty apparently healthy does with the history of breeding were selected for the study and these goats were randomly grouped into three consisting of ten animals each. Group I consisted of ten goats which were scanned between third and fourth week (15- 28 days) post-breeding. Ten goats scanned between fifth and sixth week (29- 42 days) post-breeding, were included in group II and group III consisted of ten goats which were scanned between seventh and eighth week (43- 56 days) post-breeding. These animals were subjected to B-mode real-time ultrasound scanning transrectally (7.5 MHz probe) and transabdominally (3.5 MHz probe).

In group I, six animals were diagnosed as pregnant and three as non-pregnant by transrectal scanning. There was one doubtful case in which pregnancy could not be ascertained. All the pregnant animals of group II and III were diagnosed correctly by transrectal scanning. There were no doubtful cases in these groups. The accuracy of transrectal scanning in group I, II and III was 90, 100 and 100 per cent respectively. Of the seven pregnant animals of group I, only two could be correctly identified as pregnant by transabdominal scanning. The pregnancy status of four animals was doubtful as pregnancy could not be ascertained and one animal wrongly identified as non-pregnant. All the pregnant animals of group II and III were diagnosed correctly by transabdominal scanning. There were no doubtful cases in these groups for transabdominal scanning. The accuracy for corresponding weeks was 50, 100 and 100 per cent respectively. By



transrectal scanning, pregnancy was first detected on day 19 and by transabdominal scanning pregnancy could be confirmed on day 26 of gestation.

The embryonic vesicle, which appeared as a discrete, intrauterine, sharply demarcated anechoic, round or oval structure was detected earliest on day 19 by transrectal scanning and on day 26 of gestation by transabdominal scanning. It was evident in all the pregnant animals from the fourth week (day 22) of gestation by transrectal scanning. The embryo was first observed on day 22 and on day 28 by transrectal and transabdominal scanning respectively. The foetal heartbeat which was an indication of foetal viability was detected earliest on day 24 of gestation. But by transabdominal ultrasonography, it could be detected only on day 34 of gestation. Placentomes and foetal skeleton were observed on day 42 and 54 of gestation respectively using both methods of scanning. Placentomes changed from the concave-shaped structure to a 'C' or 'O' shaped structure as pregnancy advanced.

The mean diameter of embryonic vesicle recorded was 5.1 mm on day 19 and 27 mm on day 36 of gestation by transrectal scanning while, the mean diameter recorded was 15.7 mm on day 26 and 34.4 mm on day 36 of gestation by transabdominal scanning. The mean crown-rump length ranged from 7.2 mm on day 22 to 34.4 mm on day 43 of gestation using transrectal scanning. By transabdominal scanning, the mean crown-rump length recorded was 16.7 mm on day 28 and 32.7 mm on day 43 of gestation. The diameter of the placentomes recorded by transrectal and transabdominal scanning on day 42 of gestation was 8.4 mm and 8.5 mm respectively.

The overall accuracy for the prediction of foetal numbers by transrectal and transabdominal scanning was 80 and 50 per cent respectively. The accuracy for prediction of foetal number using transrectal scanning in pregnant animals of group I and III was 71.43 per cent. The accuracy for the prediction was 100 per cent in pregnant animals of group II. There were four singletons which were accurately predicted by transrectal scanning. Of the four triplet births, foetal number was accurately diagnosed in two animals by transrectal scanning. Out of the twelve twin births, ten were diagnosed accurately. Thus the accuracy for the

prediction of singletons, twins and triplets by transrectal scanning was 100, 83.33 and 50 per cent respectively. By transabdominal ultrasonography, it was not possible to predict foetal number accurately in pregnant animals group I. The accuracy for the prediction of foetal number in group II by transabdominal scanning was 66.66 per cent while the accuracy was 85.71 per cent in group III. By transabdominal scanning, two of the four singletons were accurately predicted. Seven of the twelve twins and one of the four triplets were correctly determined by transabdominal scanning. The accuracy for the prediction of singletons, twins and triplets was 50, 58.33 and 25 per cent respectively. All the foetal measures by transrectal and transabdominal scanning were highly correlated ( $r > 0.9$ ) with gestational age.

Gestational accidents during the present study include one abortion and one still-birth which might not be due to scanning but other reasons. Based on kidding data, there were eighteen male kids and seventeen female kids. The average birth weight of the kids was 1.95 kg. The average birth weight of male kids was 1.98 kg while the average birth weight of female kids was 1.92 kg. The average weight of singletons, twins and triplets was 2.45 kg, 1.66 kg and 1.98 kg respectively. The average gestation length in the does was 146.47 days. The average gestation length in singletons, twins and triplets was 149.25, 146.73 and 143 days respectively.

The present study revealed that by real-time B-mode ultrasound transrectal scanning, pregnancy diagnosis could be made as early as on day 19 of gestation by detection of embryonic vesicle but the accuracy level reached 100 per cent, only from fourth week of gestation. By transabdominal scanning, pregnancy diagnosis could be made from day 26 of gestation and accuracy level reached 100 per cent only by fifth week of gestation. Real-time ultrasound scanning by both transrectal and transabdominal scanning were found to be reliable, safe and accurate for the diagnosis of pregnancy in goats from fifth week of gestation. By transrectal scanning the optimum stage for foetal number estimation was fifth and sixth week of gestation while that for transabdominal scanning was seventh and eighth week of gestation. Singletons could be predicted accurately, but there was difficulty in differentiation of triplets.

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# **EARLY PREGNANCY DIAGNOSIS USING ULTRASONOGRAPHY IN GOATS**

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**Abstract of the thesis submitted in partial fulfillment of the  
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## ABSTRACT

The objective of the study was to evaluate the efficacy of B-mode ultrasonography in early pregnancy diagnosis in goats and to identify the optimum stage of gestation for early pregnancy diagnosis using of transrectal and transabdominal ultrasonography.

Thirty apparently healthy does with the history of breeding were selected for the study and these goats were randomly divided into three groups consisting of ten animals each. Group I consisted of ten goats which were scanned between third and fourth week (15- 28 days) post-breeding. Ten goats scanned between fifth and sixth week (29- 42 days) post-breeding, were included in group II and group III consisted of ten goats which were scanned between seventh and eighth week (43- 56 days) post-breeding. These animals were subjected to B-mode real-time ultrasound scanning transrectally (7.5 MHz probe) and transabdominally (3.5 MHz probe).

The accuracy of transrectal scanning in group I, II and III was 90, 100 and 100 per cent respectively and the accuracy for corresponding weeks was 50, 100 and 100 per cent respectively for transabdominal scanning. The embryonic vesicle was detected earliest on day 19 of gestation by transrectal scanning and on day 26 by transabdominal scanning. The embryo was first observed on day 22 and day 28 by transrectal and transabdominal scanning respectively. The foetal heartbeat which was an indication of foetal viability was detected earliest by transrectal scanning on day 24 of gestation. But by transabdominal ultrasonography, it could be detected only on day 34 of gestation. Placentomes and foetal skeleton were observed on day 42 and 54 of gestation respectively using both methods of scanning.

The mean diameter of gestational sac recorded was 5.1 mm on day 19 and 27 mm on day 36 of gestation by transrectal scanning. The mean diameter of gestational sac recorded was 15.7 mm on day 26 and 34.4 mm on day 36 of



gestation by transabdominal scanning. The mean crown-rump length ranged from 7.2 mm on day 22 to 34.4 mm on day 43 of gestation using transrectal scanning. By transabdominal scanning, the mean crown-rump length recorded was 16.7 mm on day 28 and 32.7 mm on day 43 of gestation. The diameter of the placentomes recorded by transrectal and transabdominal scanning on day 42 of gestation was 8.4 mm and 8.5 mm respectively. All the foetal measures by transrectal and transabdominal scanning were highly correlated ( $r > 0.9$ ) with gestational age.

The overall accuracy for the prediction of foetal numbers by transrectal and transabdominal scanning was 80 and 50 per cent respectively. The accuracy for prediction of foetal number using transrectal scanning in pregnant animals of group I and III was 71.43 per cent. The accuracy for the prediction was 100 per cent in pregnant animals of group II. By transrectal scanning, the accuracy for the prediction of singletons, twins and triplets by transrectal scanning was 100, 83.33 and 50 per cent respectively.

By transabdominal ultrasonography, it was not possible to predict foetal number accurately in pregnant animals group I. The accuracy for the prediction of foetal number in group II by transabdominal scanning was 66.66 per cent while it was 85.71 per cent in group III. . The accuracy for the prediction of singletons, twins and triplets by ultrasonography was 50, 58.33 and 25 per cent respectively.

In conclusion, transrectal scanning was accurate for pregnancy diagnosis from fourth week of gestation and transabdominal from fifth week of gestation and that real-time ultrasound scanning by both transrectal and transabdominal approaches was found to be reliable, safe and accurate for the diagnosis of pregnancy in goats from fifth week of gestation.