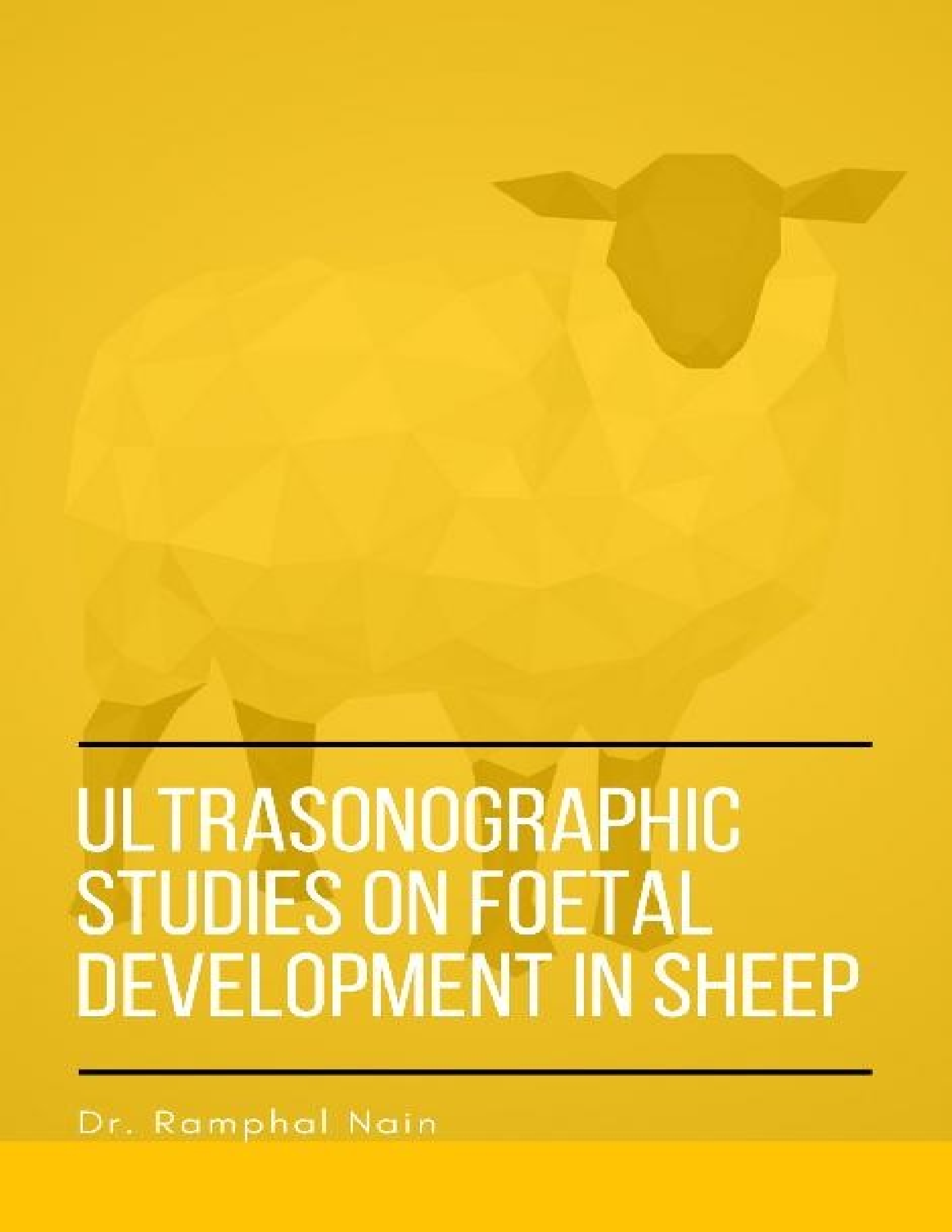

ULTRASONOGRAPHIC STUDIES ON FOETAL DEVELOPMENT IN SHEEP

Dr. Ramphal Nain

ULTRASONOGRAPHIC STUDIES

ON FOETAL DEVELOPMENTAL

IN SHEEP

By

Dr. RAMPHAL NAIN

Dissertation submitted to the Chaudhary Charan Singh

Haryana Agricultural University in partial fulfillment of the

requirements for the degree of:

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in

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COLLEGE OF VETERINARY SCIENCES

Chaudhary Charan Singh

Haryana Agricultural University Hisar

2000

DEDICATED

to my

Reverend parents

Sh. Surat Singh Nain

&

Smt. Mainkur Devi

2

CERTIFICATE I

This is to certify that this dissertation entitled, “ULTRA-SONOGRAPHIC STUDIES ON FOETAL DEVELOPMENT IN SHEEP”, submitted for the degree of Master of Veterinary Science, in the subject of Veterinary Gynaecology and Obstetrics of Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Dr. Ramphal** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

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ACK N O W LE D G M E N T S

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35.

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The umbilicus diameter was measured between Day 39 and Day 90. It shows almost linear pattern of increase. However there was significant change between Day 80 and Day 90.

Ch apt er -I

Introduction

Sheep industry plays an important role with its multipurpose utility of meat, wool, leather products and enriched manure thereby contributing a great deal towards our national economy. The accurate prediction of pregnancy in ewes would greatly increase efficiency of sheep farming.

The profitability of sheep farming could be improved if a simple and a reliable technique is available for the detection of pregnancy. This would enable prompt remating or culling of non-pregnant ewes, more economical use of supplementary feeding in late gestation and more accurate planning of production.

For the last many years, various techniques have been used to diagnose the early pregnancy in sheep such as recto-abdominal rods, vasectomized rams, abdominal ballotment, serum steroid assays and radiography. Most of these techniques have been unsatisfactory due to factors such as expenses, low accuracy rates, impracticability, ewe and human safety considerations and long delay in availability of results.

Introduction

The use of ultrasound system has improved both the reliability and practicality of pregnancy diagnosis in the field. A mode (Amplitude mode) ultrasound applied to the flank region has proven to be reasonably reliable from 50 to 120 days of gestation (Watt *et al.* , 1984).

Doppler systems have been used rectally from 25 days but require animal restraint and operator training (Deas, 1977). Recent improvements in ultrasound technology now enable instantaneous and continuous images of internal anatomy (real time) (Kossoff, 1979; Simpson *et al.* , 1982).

Recently portable real-time B-mode (Brightness mode) ultrasound systems

have been used for pregnancy testing in ewes.

In the non-pregnant ewe or in the pregnant ewe during the first 25 days of pregnancy the uterus is situated within the pelvic cavity. The urinary bladder is taken as a landmark to take the image of uterus with ultrasound.

Pregnancy is diagnosed by imaging fluid in the uterine lumen, evidence of conceptus with heart beat and by the presence of placentomes or by the identification of fetuses.

Lindahl (1971) was the first who reported the use of B-mode real-time ultrasonography with a 5 MHz rectal probe. In view of this reported improvement in accuracy, it was decided to examine further the rectal probe method of early pregnancy diagnosis.

Fowler and Wilkins (1980) used ultrasonic scanning in ewes and found accurate rapid, safe and practicable means of diagnosis of pregnancy.

They reported real-time ultrasound system transabdominal in sheep as reliable to determine pregnancy and fetal numbers from day 50 post-breeding. The rectal use of a 7.5 or 5 MHz probe in the ewe could yield better results, because the early pregnant uterus is in the pelvic canal.

Fredrikson and Swertsson (1986) reported that ultrasonography technique enable to detect the dead fetus or fetal abnormalities in addition to pregnancy diagnosis.

With the advent of B-mode real time ultrasonography it is now possible to obtain images of reproductive organs and developing fetus in small ruminants such as sheep (Buckrell, 1988; Gearhart *et al.* , 1988; Aiumlamai *et al.* , 1992 and Garcia *et al.* , 1993).

It is the noninvasive method and considered superior to non-imaging techniques because it is more accurate and enables to veterinarian to detect viability of conceptus and its dimensions (Logue *et al.* , 1987 and Buckrell, 1988).

An additional advantage of the ultrasound system is the prediction of foetal sex by identifying and locating the genital tubercle (Coubrough and Castell, 1998).

In our country investigation about the use of ultrasonography in general and diagnosis of early pregnancy in sheep in particular are lacking. Keeping in consideration of this fact the present experiment was conducted in Corriedale sheep with the following objectives :-

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Introduction

1.

To obtain images of uterus on day 18th of mating to differentiate pregnant and non-pregnant uterus.

2.

To study fetal development through weekly recording of images of developing fetus.

3.

To assess change in fetal heart beat up to 90 days of gestation.

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Review of Literature

Ch apt er -I I

Review of Literature

In intensive sheep farming, sonographic examination for pregnancy detection and determination of fetal numbers is applied routinely (Fowler and Wilkins, 1984;

White *et al.* , 1984 and Davey, 1986). Generally the findings on non-pregnant uterus as well as the uterus and conceptus during pregnancy are similar in sheep and goats (Tainturier *et al.* , 1983a, b).

Rowson and Moor (1966) conducted the study of development of sheep conceptus during the first fourteen days and found that by Day 13 to 14 the embryonic vesicle lied as a 10 cm long tube in the uterine horn ipsilateral to the corpus luteum of pregnancy. By Day 16 to 18 it extended into the contralateral horn. Eckstein and Kelly (1977) reported that during early pregnancy in ewes, the trophoblast rapidly elongated to occupy both horns and body of uterus by Day 20. King et al (1982) found that on Day 11 trophoblast vesicle began to elongate, by Day 13th vesicle entered the contralateral horn, by Day 18 both horns were occupied in ewes.

Buckrell (1988) reported that real-time p-mode ultrasonography was found to be a reliable method for diagnosis of early pregnancy in sheep.

Buckrell *et al.* (1986) and Gearhart *et al.* (1988) reported that transrectal ultrasonography occasionally allowed the visualization of anechoic sections through embryonic vesicle between Days 14 and 19 of pregnancy in ewes. The expanding vesicle contained inadequate fluid in form of pockets. The embryo in form of series of vesicles could usually found immediately cranial or cranioventrally to the urinary bladder.

Garcia *et al.* (1993) have also reported hypoechoic structures 4 mm in diameter by Day 17 to 19 postbreeding in ewes whereas embryonic fluid and membranes were observed using a 7.5 MHz transducer on Day 15 by Schrick and Inskeep (1993) in same species. Bretzlaff *et al.* (1993) found that transrectal imaging allowed visualization of pregnancy as early as 15 days postbreeding in ewes.

Martinez (1998) reported round or oblong anechoic uterine areas (embryonic vesicle) of more than 3 mm in diameter in the uterine lumen by Day 18 of conception.

Foetal heart beat

Fraser and Robertson (1967, 1968) noted that the foetal heart beats were faster than that of dam (140-200 beats/minute) by ultrasonic foetal pulse detector.

Richardson (1972a,b) reported that there was highly significant correlation of foetal heart beat with the number of days of

gestation. He also observed the mean value ranged from 224 beats per minute at 61 to 70 days of gestation to 182 beats per minute at 91 to 100 days of gestation.

Fukui *et al.* (1984) examined 192 mature ewes mainly Suffolk breed once for a single or multiple pregnancy at 61 to 170 days after mating with probe type fetal pulse detector (Heart tons model UGR-6C; Aloka Co.). Continuous ultrasound was transmitted of 2.25 MHz frequency and 30 mW/cm2 intensity. Pregnancy was diagnosed when the fetal heart was detected. Correct diagnosis for non-pregnant, single, twin pregnancies were 76.9, 74.4 and 88.7 per cent, respectively.

Buckrell (1988) also found that foetal heart could be imaged most by Day 26-28, and by Day 30 should be possible in all ewes. At Day 30 when scanning with 5 MHz head in inguinal region in the standing/ tipped ewe heart of fetus could be imaged immediately in many cases, but required a prolonged scan (1-3 minutes in others).

Shriek and Inskeep (1993) detected the rhythmic pulsation heart beat within

embryonic vesicles on Day 18 or 19 in ewes with transrectal ultrasonography by using 7.5 MHz transducer by keeping ewes in dorsal recumbency in tilting squeeze chute. Garcia *et al.* (1993) confirmed the pregnancy by detection of an embryo proper and by an embryonic heart beat on Days 21 to 34 and later judged against the number of lambs born to each ewe. Botero-Herrea *et al.* (1984) and Bretzlaff *et al.* (1993)

reported the embryo detection in goats at Day 24 to 26 by using a 5 MHz transducer.

Placentomes development

Kahn (1994) observed that placentomes are the cardinal signs of pregnancy. Clotete (1939) found that ewes' placentomes are small nodules on Day 21. At Day 30 the periphery of the nodules begin to form a thin lip around the flat centre. And by approximately Day 90 of gestation the placentome reaches it maximum weight and diameter. The maximum size of placentomes reaches at 74 and 91 day of gestation in sheep and goat, respectively.

Clotete (1939) also reported the largest cotyledons of ewes had a diameter of about 3 cm. However Amoroso (1952) reported that the foetal growth was not limited by the placental development early in pregnancy but gradual increase in weight of placentomes was correlated with a gradual increase in size.

Alexander (1964) found that number and weight of placentomes were quite variable between the two uterine horns in ewes. Riera (1984) noted that sheep usually had 60 and 100 while goats had between 160 and 180 placentomes.

Buckrell et al (1986) used linear array real time ultrasound system rectally to see if the fluid pockets fetus and placentomes could be imaged at Day 20 to25 and

Day 25 to 30, respectively. Pregnant ewes after Day 30 displayed an irregular nonechogenic region anteroventral to the

urinary bladder. Included in this region were button shaped echoic areas assumed to be placentomes and a fetus within the amniotic membrane.

Buckrell *et al.* (1988) also reported that placentomes could be detected by transrectally ultrasonography with a 5 MHz linear transducer at Days 28 to 30 of gestation. Placentomes appeared as small echoic areas on the surface of endometrium.

Haibel (1990) imaged the placentomes in cross view as cup shaped hyperechoic structures, the concave surface directed towards the uterine lumen.

Bretzlaff *et al.* (1993) found that placentomes were readily observable by transrectal ultrasonography through at pregnancy.

Samuel *et al.* (1995) did ultrasonography of abdomen of 5 year old Alpine goat using 5 and 3 MHz sector scanning probes. The accumulation of fluid was determined to be within uterus with placental tissues and placentomes in lumen. They found abnormalities in placenta because placentomes were small and round to oval not of typical-C shaped. They confirmed the case of hydrops uteri on the basis of historical and physical findings.

Doize *et al.* (1997) concluded that placentome size increased rapidly during the first 70 to 90 days of gestation in ewes and does. But they found that in ewes there was a poor correlation of placentome size with gestational age. They used P-mode ultrasonograph with 5 MHz transducer and did transrectal procedure.

Foetal sex determination

The accurate predictability of the sex of fetus can speed up breeding programmes and allows an early selection of pregnant ewes for certain purposes according to the sex of their fetuses. There are several methods of both embryonic and fetal sex determination including Karyotyping, H-Y antigens, and DNA probes for embryos or cells obtained by aminocentesis. Fetal sex can also be determined by ultrasonic examination of fetal structures.

Curran and Ginther (1991) found that ultrasonography could provide not only the gender diagnosis but also the expected accuracy level associated with the diagnosis. However, Noden and Lahunta (1985) reported that ultrasonographic fetal sex determination by identification of the genital tubercle could be done in ewes with a single transrectal examination. The genital tubercle was the embryonic structure that differentiated into the penis in males and clitoris in females. During differentiation the tubercle moved from the initial position between the rear legs towards the umbilical cord in males and towards the tail in females. The fetus was recorded as a male when the genital tubercle located immediately caudal to the umbilical cord and as a female when it was located near the tail.

Although ultrasonography is an accurate technique for fetal sexing, it should be stressed that it requires high resolution ultrasound equipment and considerable practice. There are several anatomical structures which may be confused with the genital tubercle such as cross sectional view

of the umbilical cord, tail and spinal cord. The recognition of fetal landmark is critical to identification and location of the genital tubercle.

Curran also identified the landmarks such as the head, a beating heart, a pulsating umbilical cord, rear legs and the tail. He reported that generally the cross-sectional views were most helpful in identification of the genital tubercle and landmarks described. The frontal view aided in determining the relative location of the genital tubercle to surrounding structures, although this plane of view was more difficult to obtain than the cross sectional view.

Coubrough and Castell (1998) determined the fetal sex by identifying and locating the genital tubercle in 29 ewes with single fetus at 60 to 69 day post breeding. They conducted the examination once under farm conditions utilizing a real time diagnostic scanner equipped with a linear array 5 MHz transducer. They made the definite diagnosis after lambing and obtained total accuracy 89 and 100 per cent (14/14) of the male fetuses and 76 per cent (10/13) of the female fetuses were correctly diagnosed. In 2 (7%) ewes fetal sex was not determined by them.

Pathological abnormalities

One of the most common pathological condition of the reproductive tract identified in small ruminant by ultrasonography is the hydrometra that is especially common in diary goats but can occur in ewes also. Roberts (1971) found that the normal rate of early embryonic loss in ewes were reported to be 20 to 30 per cent from three weeks

after conception until parturition. Chapman (1980) reported that lambing performance might be slightly lower that predicted by ultrasonography due to prenatal mortality of <me or more embryo of fetuses. Some fetal resorption/abortion unrelated to ultrasonic scanning was normal in most species. Prior to the development of

diagnostic ultrasonography for the early detection of pregnancy fetal attrition had not been easily documented in ewes in most cases and gone unrecorded. Wilkins *et al.* (1982, 1984) found that most reproductive wastages in sheep were between ovulation and Day 30 after breeding as evidence by differences in ovulation rates and embryo counts.

Fredrikson and Siwertsson (1986) found an additional advantage of scanning was the detection of fetal death or abnormalities. Pieterse and Taverne (1986) stated that pyometra, hydrometra, metritis were the potential differential diagnosis in very early pregnancy. Buckrell (1988) found that ultrasound system could help to diagnose the reproductive tract diseases in male arid female animals. He conducted the routine diagnosis for visualization of ovaries by ultrasonography which was not possible at all stages of cycles in sheep and goats.

Buckrell and Gearhart *et al.* (1988) found that ultrasonography was the good tool to predict the pathological conditions of the uterus such as pyometra, hydrometra, mucometra, embryonic deaths, fetal mummifications, hydroallantois and hydroamnion.

They reported that pyometra in ewes could be characterized by an intrauterine fluid accumulation with obvious reflections. The echogenicity

of the fluid was depended upon the relative amount of cellular material in the secretion. A snow storm like image was typically reported. However Gearhart *et al.* (1988) also found fetal attrition at Day 49 and hydrops allantois at Day 110 of gestation in ewes by transabdominal ultrasonography.

Dorn *et al.* (1989) found that ovaries with their developing fetus could only

be identified in some goats by ultrascanning during the first few days of superovulatory treatment. Bretzlaff (1993) reported that hydrometra in ewes might be a condition that gone largely undetected because it might spontaneously resolved. He used ultrasonography to confirm hydrometra but found it unable to reveal whether there was pregnancy and embryonic resorption. He made the designation for hydrometra that if the uterus contained enough fluid and there were no cardinal signs of pregnancy i.e. no visualisation of an embryo, fetus or placentomes. He documented that ultrasonography was the optimal diagnostic technique for hydrometra because it was rapid, non-invasive and provided a visual image of uterine contents. He also reported that hydrometra was less common in sheep than goats.

Bretzlaff *et al.* (1993) found that hydrometra could be recognized when the uterus had fluid without other cardinal signs of pregnancy such as placentomes or a fetus. They reported that early cases of hydrometra with small accumulations of fluid in uterus appeared similar to early pregnancies. That’s why they advised to confirm the positive pregnancy based on uterine fluid without other cardinal signs of pregnancy should always be rechecked after about two weeks.

They reported fetal loss by presence of placentomes that were too small for the expected gestational age or that were not well outlined by the fluid. They found dead/mummified fetuses as fetal parts that had what appeared to be excessive folds of membranes around them or that were barely outlined with very little fluid. They also observed that the loss of one or multiple fetuses in ewe that remained pregnant was not uncommon and could result in confusing artifacts such as large fluid filled pockets with placentomes but no fetus or fetal remanents that were believed to be a

normal fetus that was difficult to be seen.

Schrick and Inskeep (1993) reported the death of sheep embryo by absence of heart beat at Day 25 in one ewe and by Day 40 in another ewe by transrectal ultrasonography.

Samuel and Giller (1995) reported hydrops uteri by observing placentome structure i.e. small and round to oval but were not of typical C-shaped in cross section image. They also reported that there were few placentomes relative to amount of placental tissue, no evidence of fetus and found large volume of fluid in the uterus.

Head, Trunk (Thoracic Depth), CRL, Biparietal Diameter, Fetal Movements

Evans and Sack (1973) reported that by Day 60 the crown rump length of ovine and caprine fetuses surpassed a length of 10 cm with the limits of 5 MHz transducer. Fowler and Wilkins (1984) and Haibel (1988) observed the use of biparietal parameter of the skull for estimation of gestational age in sheep and goats.

Haibel and Perkins (1989) observed that biparietal diameter of ovine and caprine fetuses increased nearly linearly during the course of pregnancy. Sergeev *et al.* (1990) reported that curvilinear relationship ($P<0.05$) for fetal age and thoracic depth of twin fetuses also curvilinear relationship ($P<0.05$) for fetal age and head width. Aiumlamai *et al.* (1992) correlated biparietal diameter of skull, diameter of body trunk with fetal age and it was concluded that these measurements could be used to estimate the age of fetus.

Schrick and Inskeep (1993) determined the pattern of embryonic growth by crown rump length on Day 25, 30, 35 and 40 not varying with the numbers of embryos carried (n=l to 4). Sergeev *et al.* (1990) reported that the fetal dimensions (Head/tho-

rax) were accurate predictors of gestational age due to precise measurements by trans-abdominal approach with smaller variations at 50 to 100 days of gestation in both goats and sheep.

Schrick and Inskeep (1993) measured the length of embryo using the transrectal method correlated with gestation age only during the first 40 days of pregnancy in ewes.

Scheerboom and Taverne (1985) reported that fetuses shown intensive mobility at second and third trimester resulting in frequent positional changes.

Ch apt er -I I I

Material and Methods

The present study was carried out at Sheep Breeding Farm of CCS Haryana Agricultural University, Hisar. Eight Corriedale sheep with known mating date were selected. All the ewes were in good health conditions and were inseminated naturally. The ewes were kept in an open padlock and were provided with adequate ration and water as well as grazed on cotton crop residues for six hours daily. The selected ewes were marked by red dye for easily identification from the flock.

A high quality real-time. B-mode diagnostic ultrasound machine [Pi Medical Vet Scanner-200] equipped with a linear array of switchable frequencies in between 7.5 MHz and 5 MHz transducer designed for intrarectal placement was used. The cord of tranducer was stiffined and lengthened by wrapping a 40 cm PVC pipe around it. For this, the PVC pipe was cut in the mid along its long axis so that cord of the probe can be inserted and removed conveniently. An adhesive tape was wrapped around the distal end of the probe head and its nearest end of pipe, at the middle portion

as well as at another end of the pipe. All the essential connections were made from the main line of electricity through the voltage stabilizer with the ultrasound machine, videocassette recorder and printer.

The machine was set up to obtain good quality images. The experimental eight ewes were removed from the flock for first scanning at Day 18 of mating and subsequent uterine ultrasonography was performed at weekly interval from Day 18 to 90 of gestation.

The ewes were carried in a pen and lightly restrained in standing position by an attendant. The rectum of ewe was made empty by removing the fecal pellets with the help of two fingers, i.e. index and middle finger. The water soluble ultrasonic gel as coupling medium was inserted into the empty rectum to each ewe to displace the rectal gas and to facilitate introduction of the transducer into the rectum. The probe of transducer was also lubricated with the same ultrasonic gel before inserting into rectum to establish a good contact in between the transducer surface and rectal mucosa. The well lubricated transducer head was inserted full length into the rectum 6 inches and gently passed along the rectal floor and rotated 45° to the either side of midline. The anechoic (black) fluid filled bladder was identified immediately as a landmark.

The transducer was positioned dorsal to uterus and the entire uterus was examined systemically. On Day 18 of conception, ewes were diagnosed pregnant with a low/medium of certainty by identifying the echoic (white) streak surrounded by anechoic (black) image of accumulated uterine fluid.

On Day 25 the presence of embryo was confirmed as comma shaped white

structure attached to one side of endometrium with rhythmic pulsations (heart beats). So at this stage of conception ewes were diagnosed with a high degree of certainty. Failure to detect an apparent conceptus without any heart beats on Day 25 resulted in a diagnosis of non-pregnant ewe. The first detection of foetal membranes, fetal parts were noted. Rhythmic pulsations (heart beats) were counted for 5-15 seconds in some embryos and was assumed to be a indicatory embryo viability. It was difficult to count the heart beat due to minor movements of the animal.

At Day 39 head, foetal limbs (fore limbs and hind limbs) with mobility were recorded. Up to Day 32 transducer with 7.5 MHz frequency was used but from Day 39 to Day 90 transducer was set up at 5 MHz for subsequent scanning. In this way the differentiation and organogenesis of various fetal parts such as head, mouth, neck, spinal vertebrae, bony cage, skull bone, forelimbs, hind limbs, hooves, genital tubercle, optic vesicles, urinary bladder, chewing movements of fetus and big picture of scrotum were recorded. With the help of monitor, the dynamics of fetal shape and size, foetal mobility, diameter of internal uterus, conceptus length, crown rump length, placentomes, foetal head, umbilicus, trunk and cranial cavity were documented.

The selected photographic images were printed on paper as well as recorded on TDK and Panasonic Cassettes for subsequent analysis and revaluation.

Ch apt er -I V

Results

1.

Non pregnant uterus

Two nonpregnant animals were scanned. The nonpregnant uterus (Ut) of ewe was found inside the pelvis in the vicinity of the apex of the urinary bladder (UB) (as

shown in Fig. 1). During a transrectal examination a sagittal section through the uterus was produced by holding the probe above the uterus. The cranial border of the uterus was seen as convex structure. No fluid accumulation was seen inside the uterus (Fig.l).

2.

At Day 18 of conception

There was high density echoic tissue producing a white image i.e. conceptus (C) was seen. The conceptus was in a form of echoic streak surrounded by anechoic fluid (F) producing dark image (Fig.2). So at this stage earliest indication of the pregnancy was the embryonic fluid and echoic streak inside the uterus close to cranial border of urinary bladder. The embryonic vesicle was found extended from the one end

to another end of the image obtained. In some animals only accumulated fluid inside the uterus was seen (Fig.2).

3.

At Day 25 of conception

There was increase in the size of the conceptus at Day 25 of conception. It was more wider and longer than at Day 18. There was more accumulation of fluid (F) inside the uterus i.e. volume of the uterine fluid was increased, (shown by arrow in Fig.3 and 4). A clear heart beat inside the fetus was observed and heart beats were counted which were found in the range of 172-165. The shape of fetus was somewhat comma shaped having anechoic line demarkating the fetus in two parts shown by arrow (Fig.5). The embryonic vesicle (EV) was attached to the one side of the endometrium (Ed) (shown by arrow in Fig.3).

4.

At Day 32 of conception

The embryo (E) was seen with increased size. The quantity of uterine fluid (F) also increased. Budding (Bu) took place (as shown in Fig.6 by arrow). There was starting of organogenesis and differentiation at this stage. Fetus with clear heart beat was visible (observed in video) (Fig.6).

5.

At Day 39 of conception

There was again increase in size of fetus (Ft) with increase in more accumulation of uterine fluid (F). There were five extremities seen i.e. head (H), two fore limbs (FL), two hind limbs (HL). Fetus (Ft) was found to be surrounded by hyperechoic fetal membranes i.e. amniotic

membrane AM (shown in Fig.7 by arrow) fetus was visible with clear heart beats (observed in video) (Fig.7).

6.

At 46 Day of conception

The movements of fetal parts were observed along with clear cut heart beats in cardiac cavity (CC) area (shown in Fig.8). Fetus took the ventral position inside the uterus. Size of fetal head (H) was larger than the size at Day 39 of conception (shown in Fig.8, 9 and 10). In one case fetus took a turn with the movements of its trunk (observed in video). The movements of fetus seemed as fetus was floating in the uterine fluid (F). The neck (N) portion of fetus was clearly observed in between head'(H) and trunk (Tr) (shown in Fig.8, 9 and 10). The mouth parts upper jaw (UJ) and lower jaw LJ were seen (Fig.8). The vertebrae of the spinal cord (SV) were developed (Fig.8). In some animals

fetal jerking movements towards the upper side were seen. The aminotic membrane (AM) as a dotted hyperechoic line encircling the fetus was observed (Fig. 10). The bony cage i.e. Ribs (R) were developed. Nodule shaped placentomes were seen (Fig.9).

7.

At Day 53 of conception

The placentomes (P) were seen as typical half moon shaped (Fig. 11). There was a good pulsation observed in the umbilicus (Um) (Fig. 12). The following four nonechogenic area surrounded by echoic lining were seen inside the cross view of the umbilicus (observed in video). These were probably the vessels viz.,

(i)

Right umbilical artery

(ii)

Right umbilical vein

(iii)

Left umbilical artery

(iv)

Right umbilical vein .

It was interesting that the sex of fetus could be predicted at this stage by observing the location of hyperechoic genital tubercle (GT). The genital tubercle was seen in between the umbilicus (Urn) and hind limbs (HL) indicated the male fetus (Fig.12), which was confirmed later on with scrotum and finally on lambing. It seemed that fetus was bringing its forefeet close to its open mouth and appeared that fetus was trying to lick the feet. Bifurcation in hooves were clearly seen shown by arrow (Fig. 13).

8.

At Day 60 of conception

The fetal pathological abnormalities was observed at this stage. Very clear cut picture of the dead fetus (DFt) was seen (Fig. 14). No cardinal signs of the pregnancy was observed. There was absence of the normal reflection from the accumulated fluid because of echoic spots of dead fetus in the anechoic area (Fig. 14). No skeleton, placentomes were observed. So the turbidity of the amniotic fluid (F) confirmed the dead fetus. Actually the same animal had another fetus also which was observed normal. In this way here ultrasonographic techniques also helped to detect the twin pregnancy. The coccygeal vertebral (CV) of tail (T), vertebrae of spinal cord (SV) and bones development of hind limbs (HL) were observed (Fig. 15). A cavity at the point of junction of umbilicus

also seen, which will be developed into the urinary bladder (UB) of the fetus (Fig. 15). Heart beat as well as pulsations in the umbilicus were obtained simultaneously in some animals (observed in video).

Now the size of heart was larger than the previous stages. Pericardial cavity was also clearly seen (observed in video). The size of the fetus was increased showing the lateral view of head (H). The skull bone (SB) encircling the brain lobes shown by arrow (Fig. 18), optic vesicle (OV), nasal bone (nb), nasal passage (np), buccal cavity (be), muzzle (mz), bones of upper jaw (UJ) and lower jaw (LJ) were clearly observed. The size of the spinal vertebrae (SV) was increased and fetus was seemed in sitting position (Fig. 17). Fetus was surrounded by the larger size of placentomes (Fig. 16).

9.

At Day 66 of conception

The size of the placentomes (P) was larger than the previous stages of conception. Now placentomes occupied most of space of uterine lumen. Hypoechoic central cavity of placentome surrounded by echoic periphery zone was observed (Fig. 19). A brightest bilobed hyperechoigenic structure i.e. Genital tubercle (G.T.) was observed (Fig.20).

Fully developed umbilicus (Um) was seen attached to the fetus (Ft) with the good pulsations observed in video (Fig.21).

10.

At Day 73 of conception

Anatomy of the head (H) was observed. The cranial cavities (CRC) of brains covered by skull bone (SB) were seen (Fig.22) fetus was seemed

in sitting position with its head (H) resting on the trunk (Tr). A clear cut neck (N) with a good turn was observed (Fig.22). At this stage again pathological abnormalities of the fetus were detected i.e. dead fetus (Dft) (Fig.23). The turbidity of the uterine fluid (tf) and no cardinal signs of pregnancy (absence of heart beat) confirmed the mortality of fetus (Fig.23).

11.

At Day 80 of conception

At this stage the excellent feature of fully developed fetus was observed i.e. the chewing movements of the fetal mouth were seen (observed in video). It seemed that fetus was taking some thing inside the mouth. Full view of the head (H) and turning of head towards trunk was seen (observed in video). The nasal bones (nb), eye ball (eb),

lower jaw (LJ), upper jaw (UJ) and tongue (T) in mouth was clearly seen (Fig.24). The movements of the fetal forelimbs (FL) were also seen. The fetus surrounded by placentomes (P) was seen.

12.

At Day 90 of conception

A good picture of the scrotum (Sc) (Fig.25) was seen. It was located in between tail and hind limbs (HL). Very large clear-cut image was observed. It was the same fetus in which sex of fetus (Fig. 12) had been already predicted i.e. male by observing the location of genital tubercle. In this way the presence of scrotum (Sc) and lambing the male kid confirmed the prediction (Fig.25).

Discussion

Ch apt er -V

Discussion

From present study, ultrasonography appeared a good tool to study the fetal development in ewes. Now-a-days with the help of B-mode real time ultrasound system it has become possible to study the live images of reproductive organs and developing fetus within the uterus of small ruminants such as sheep and goats (Buckrell, 1988; Gearhart *et al.,* 1988; Aiumlamai *et al.,* 1992 and Garcia *et al.,* 1993).

The ultrasonography is a recent technique to diagnose the early pregnancy, determine the fetal numbers, their pattern of growth, viability, dimensions, mortality and gender of the growing fetus (Fredrikson and Swertsson, 1986; Logue *et al.,* 1987; Buckrell, 1988; Coubrough and Castell, 1998).

According to present study, the non-pregnant uterus of two ewes showed the homogenous image of genital tract with a coarsely granular echo. Similar results

were obtained by Kahn (1994) who observed the non-pregnant genitalia in sheep and goats using a 7.5 MHz probe.

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Discussion

To differentiate pregnant and nonpregnant uterus, the fluid accumulation was the main criteria. In non-pregnant reproductive tract there was no finding of fluid accumulations. The nonpregnant uterus was found inside the pelvis in the vicinity of the apex of the urinary bladder. Similar observations have also been made by (Buckrell, 1986) who reported that uterus lies in pelvic cavity in nonpregnant or pregnant ewes during the first 25 days of conception. In present study the transducer of 7.5 MHz provided the optimum details in comparison to probe of 5 MHz frequency. Because more powerful transducer with high frequency has a stronger signal. It also supports the reports made by Kahn (1994).

At Day 18 of conception scanning with a 7.5 MHz probe showed a white image i.e. conceptus in form of echoic streak surrounded by dark image of anechoic fluid.

Buckrell (1988) also reported uterine fluid in ewes at Day 16 to 17 in some cases and at Day 20 to 23 with regularity.

Generally the findings on nonpregnant uterus as well as the uterus and conceptus during pregnancy are same in sheep and goats (Tainturier *et al.*, 1983a,b). The study of the development of the sheep conceptus during the first 14 days revealed that by Day 13 to 14 the embryonic vesicle lies as 10 cm long tube in the uterine horn ipsilateral to the Corpus luteum of pregnancy. By Day 16 to 18 it extends into the contralateral horn (Rowson and Moor, 1966).

38

Discussion

Eckstein and Kelly (1977) found that during early pregnancy in ewes the trophoblast rapidly elongated to occupy both horns and body of uterus by Day 20. King et ql. (1982) reported that trophoblast vesicle begins to elongate at Day 11, it enters the contralateral horn at Day 13 and by Day 18 the both horns are occupied in ewes.

Buckrell *et al.* (1986) and Gearhart *et al.* (1988) reported occasional visualization of anechoic sections through embryonic vesicle between Day 18 and 19 of pregnancy in ewes. They found the expanding vesicles containing inadequate fluid immediately cranial or cranioventrally to the urinary bladder.

Garcia *et al.* (1993) have also reported hypoechoic structure 4 mm in diameter by Day 17 to 19 post-breeding in ewes whereas embryonic fluid and membranes were observed using 7.5 MHz transducer on Day 15 by Schrick and Inskeep (1993) in same species.

However, Bretzlaff *et al.* (1993) found that transrectal imaging allowed visualization of pregnancy as early as 15 days post breeding in ewes. But Martinez (1998) reported round or oblong nonechogenic uterine areas i.e. embryonic vesicle of more than 3 mm in diameter in the uterine lumen by Day 18.

In the present study embryonic fluid was the earliest indication of pregnancy.

The echoic streak was visible less than 0.8 cm in length and 0.25 cm in width, situated close to the urinary bladder. Similar observations were also reported by Schrick and Inskeep (1993). They observed the fetus at Day 20.

Discussion

In current study the embryonic vesicle was found extended from one end to another end of the image while in some animals only accumulated fluid inside the uterus

was seen. Gearhart *et al.* (1988) detected positive pregnancy at Day 20 with 5 MHz probe. In present study the probe of same frequency was tried at Day 18 but, it could not provide optimum details.

In the current study the scanning of ewes on Day 25 of conception with 7.5 MHz probe showed a comma shaped embryo with clear heart beats (172-165 per minute) attached to one end of the endometrium. From the detection of heart beat it is believed that pregnancy can be confirmed on this day. In other studies the researchers observed beating heart on various days.

Buckrell (1988) also found that fetal heart beat could be imaged in most fetuses by Day 26-28 and by Day 30 should be possible in all. Schrick and Inskeep (1993) detected the rhythmic pulsation i.e. heart beat within embryonic vesicle on Day 18 or 19 by using 7.5 MHz probe in ewes. Garcia *et al.* (1993) observed the live embryo on Day 21 to 34 by detecting the heart beat. Botero Herra *et al.* (1984) and Bretzlaff *et al.* (1993) reported the embryo detection in goats at Day 24 to 26 using 5 MHz transducer.

Gearhart *et al.* (1988) detected the fetal heart beat in sheep at Day 25 via transrectal scanning while at Day 29 via transabdominal scanning.

Discussion

In the present study heart beat was considered as one of most important criteria to detect viability of conceptus which is in agreement with Pierson and Ginther (1984) who also considered heart beat as an important criteria to detect the conceptus viability in other live stocks.

In current study the conceptus with heart beats (172-165) at Day 25 using MHz transducer was seen with clarity. Although it was very difficult to count the heart beats due

to minor movements of the animal. But still beats were calculated in 5 seconds and multiplied by 12 to obtain the beats per minute. In present study the beats were found in the range of 172-155 per minute from Days 25 to 90 of gestation. In this way these were not significant different ($P>0.05$) except the values were higher around Day 25 and 32. While Richardson (1972a) found a significant negative correlation of fetal heart beats with days of gestation. He observed the mean values (224 beats per minute) at 61 to 70 days of gestation. The findings of current study are not in consistent to findings of above mentioned researchers. The possible reason may be differences in the equipments used to count the heart beats and individual subjectivity.

But the results of current study i.e. mean value in the range of 168 at Day 90 are similar to reports of Richardson (1972a) observed 182 beats/minute at Day 91 to 100 of gestation.

The internal uterus diameter (embryonic vesicle diameter) measured from Day 18 to 39. It was 15 mm at Day 18, 17.5 mm at Day 25 and 22.5 mm at Day 39. Gonzalez de Bulnes (1998) who also measured the

Discussion

embryonic vesicle diameter in Manchega diary ewes at Day 26 which was 25.3±4.2 mm. But the results of the present study are not in consistent with the findings of Gonzalez de Bulnes (1998). The possible reason may be species variations.

At Day 39 hyperechoic amniotic membrane emerging from the conceptus was observed. However Schrick and Inskeep (1993) observed the extraembryonic membrane and fluid in the uterine horn by Day 15 in ewes subsequently confirmed the pregnancy using Aloka 500 ultrasound system. Kahn (1994) also observed the similar amnion in between Day 25 and 30. In the present study the reason for late observation of

extraembryonic membrane might be variation in kind of ultrasound system.

In the present study the placentomes were visible nodule shaped at Day 46, typical half moon or cup shaped at Day 53 and button shaped at Day 66 of gestation. As the cardinal sign of pregnoncy in all experimental animals. Doize *et al.* (1997) also recorded the various shapes of placentomes in sheep and goats. They found small nodule shaped placentome at Day 32 in ewes and at Day 35 in goats. But at Day 42 the placentomes of all goats were become cup-shaped whereas 9 out of 12 ewes presented cup shaped placenta at Day 39 of gestation.

They also concluded that development of the placental tissue was rapid and maximum size was reported at Day 74 in ewes. But in contrast placentomes in does exhibited a slower growth with the largest diameter at Day 91 of gestation.

Discussion

Buckrell *et al.* (1986) used linear array real time ultrasound system rectally to image the placentome at Day 20 to -25 and 25 to 30 and found that pregnant ewes displayed an irregular echoic region anterioventral to urinary bladder after Day 30. They observed button shaped echoic structure in cross section view. They also detected the placentomes with 5 MHz probe transrectally at Day 28 to 30 and placentomes appeared as small echogenic areas on the endometrial surface. Haibel (1990) also found cup-shaped hyperechogenic structure, the concave surface was directed towards the uterine lumen.

Bretzlaff *et al.* (1993) found that placentomes were readily observable with the help of transrectaql ultrasonography through at pregnancy.

The placentome diameter was found near 1 cm at 39 while it reached over 2.5 cm by Day 73 and 2.75 cm by Day 90. There was no significant change in diameter

in between Day 73 and 90. This finding is in consistent with Doize *et al.* (1997) who concluded that placentome size increased rapidly during the first 70 to 90 days of gestation in ewes and does. But they found a poor correlation of placentome size with gestational age. The observation of current study regarding the diameter of placentome also matches with the reports of Clotete (1993) who concluded that largest placentome of ewes had a diameter of about 3 cm.

However Amoroso (1952) reported that foetal growth is not limited by the placental development early in pregnancy but gradual increase in weight of placentomes was correlated with a gradual increase in size.

Discussion

Gonzalez de Bulnes *et al.* (1998) observed great variation in size of placentomes of ewes. Kelly *et al.* (1987) pointed out the potential value of the measurements of placentomes to estimate the placental growth in ewes.

Riera (1984) found 60-100 placentomes in sheep while 160-180 placentomes in goats.

Alexander (1984) found variation in number of placentomes in both uterine horns of the ewes.

In this way the results of current study are coinciding with the work of researchers, mentioned above i.e. there was no significant change in dimensions of placentomes in between Day 73 and 90. Placentome number increased and occupied most of the space of uterine lumen at Day 66 which is inconsistent with the reports of Kahn (1994).

In present study the various shapes of developing conceptus were recorded starting from streak shaped at Day 18, almost comma shaped at Day 25 in some animals

while oval or oblong shaped in other animals and C-shaped at Day 32. The anechoic shadow (demarkation line) in the embryo making its two parts i.e. head and trunk was seen at Day 25 and budding of extremities was observed at Day 32 while Garcia *et al.* (1993) reported the budding of forelimbs in ewes at Day 35 of gestation. So current study has revealed the budding earlier than the other researchers in ewes.

Discussion

In present study, five extremities i.e. Head, two forelimbs, two hind limbs at Day 39 of gestation were seen very cleanly. But the proper shape of the developing organs of fetus i.e. head, neck, trunk, bony parts of upper jaw, lower jaw, vertebrae of spinal cord and ribs were visible with the nice pictures at Day 46. The results are inconsistent with Aiumlamai *et al.* (1992) who observed the bony parts of skull and thorax i.e. ribs at Day 44 to 63. At the same time a dotted hyperechoic line i.e. amniotic membrane encircling the whole fetus was also seen. The fetal movements were observed at Day 46. In one case fetus took the ventral position in side the uterus with the movements of its trunk. These movements of fetus seemed as the fetus was floating in the uterine fluid. At the same time jerking movements of fetuses were also observed in some other scanning. The similar findings were also reported by Scheerboom and Taverne (1985). They observed the intensive mobility of fetus at second and third trimester resulting in frequent positional changes.

In the present study fetal head diameter i.e. biparietal diameter was measured from Day 39 to Day 90 of gestation and there was typical, linear pattern of growth i.e. Linear increase in biparietal diameter. Several other workers also measured the biparietal diameter of ovine and caprine fetuses. Fowler and Wilkins (1984) and Haibel (1988) observed the biparietal diameter to estimate the gestation age in sheep and goats.

Haibel and Perkins (1989) observed that biparietal diameter of ovine and caprine fetuses increased linearly during the course of pregnancy. Haibel (1988)

readily identified the fetal head and measured the width at Day 40 of pregnancy in diary goats.

Sergeev *et al.* (1990) concluded that there was a very good relationship between fetal age and linear measurement of fetal head width. The high correlation observed indicated that head width was useful parameter of for predicting the fetal age in sheep. They also reported that measurement of head were the accurate predictors of gestational with smaller variations at Day 50 to 100 in both sheep and goats.

Reichle and Haibel (1991) also reported that fetal head measurement is not very precise earlier than Day 36. In the present study fetal head was routinely seen from Day 39-to 90 of gestation. But in later stages of the pregnancy it was become very difficult to scan the whole size of head because of large size of fetus i.e. big fetus could not be scanned in single image. The similar problems were also faced by Reichle and Haibel (1991) who found difficulties to obtain a symmetrical fetal head image at the end of second trimester due to large size of fetus and its location within the abdomen. This range is similar to that of goats from Day 39 to 100 (Haibel, 1988).

Kelly and Newnham (1989) found similar fetal head measurement for single and twin fetuses up to about Day 80. In the current study biparietal diameter was near to 1 cm at Day 39, 3 cm at Day 66 and near 5 cm at Day 90, which are almost in consistent with the findings of Haibel and Perkins (1989) and Haibel *et al.*, 1989) who found the

biparietal diameter grow from 0.75 to 1 cm at Day 40, 2.3 to 2.6 around Day 70 and 3.0 to 4.5 cm at about Day 100.

Gonzalez de Bulnes *et al.* (1998) found that with more advance in pregnancy when it was not possible to distinguish the fetus entirely the most suitable measurements were the biparietal diameter but it was difficult to image them in appropriate plane. They recorded the biparietal diameter 0.6 cm at Day 32 and 3.9 cm at Day 90. In present study the black spot in lateral view of the head was detected as eye bud at Day 46. The vertical and horizontal diameters of eye ball were found 0.5 cm and 0.7 cm at Day 46, 0.5 cm and 0.75 cm at Day 60, 0.75 and 0.75 cm at Day 66, 1.0 and 1.0 at Day 80, 1.75 and 2 cm at Day 90 of gestation respectively. So these findings are similar to Gonzalez de Bulness who reported that the orbit diameter increased from 0.2 cm at Day 36 to 1.7 cm at Day 90 with the potential curve.

In present study bifurcation in hooves were clearly seen at Day 53 in Corriedale ewes but comparable data is not available in other breeds of sheep and goats. Therefore it was the new finding in current study.

It was also possible to obtain the clear picture of vertebral column and it was amazing to find the individual vertebrae in different regions of the vertebrae column. However, in present study the coccygeal vertebrae were seen at Day 60. These finding are in agreement with the findings of Gonzalez de Bulnes *et al.* (1998) who reported the measurements of cervical, thoracic and lumbar vertebrae width from Day 50 where the

Discussion

measurements of coccygeal vertebrae were not possible until Day 57, and these values increased linearly with time.

At Day 53 of conception rhythmic pulsations in umbilicus was clearly seen

along with four anechoic spots encircled by echoic lining within the umbilicus. Probably these are vessels of the umbilicus viz., (i) Right umbilical artery (ii) Right umbilical vein (iii) Left umbilical artery (iv) left umbilical vein, to our knowledge there are no reports regarding the ultrasonographic studies of the umbilical vessels.

In present study fetus appeared licking its feet as was trying to bring the forefeet near to mouth on Day 53. The measurements of fetal limbs revealed that these were increased in length at Day 53 in comparison to previous scanning.

It was very amazing and excellent finding at the Day 53 that in current study the gender of the fetus as male was predicted in one case. The prediction was made by identifying and locating the genital tubercle i.e. bilobed brightest hyperechoic structure found in between the umbilicus and hind limbs. The prediction was confirmed later on with the help of the very clear and big picture of scrotum in same animal at Day 90 and lambing of the male kid again confirmed the prediction. Muller and Wittkowski (1986) reported that ultrasonography is easy to apply, harmless, quite reliable for sexing in farm animals but these workers suggested second ultrascanning to enhance the accuracy of six diagnosis. The genital tubercle and development or absence of scrotum are two major criteria that have

been used by ultrasonographers to diagnose the gender of fetus between Day 48 to 113 with a success of 92 to 100 per cent in cattle (Perry, 1990).

Curran and Ginther (1991) reported that ultrasonography could not only the diagnose the sex of fetus but the expected accuracy level of diagnosis also. However Noden and Lahunta (1985) identified the genital tubercle which moved from initial position towards the site in between hind limbs and umbilicus in males while in females

it was found below the tail. In current study fetus was predicted as male because the genital tubercle was situated immediately caudal to the umbilicus. These findings of present study are similar to the Coubrough and Castell (1998) who observed the fetal sex by identifying and locating the genital tubercle in ewes with single fetus at Day 60 to 69 with 5 MHz probe, with a total accuracy of 89 and 100 per cent of male and 76 per cent of female fetuses. At Day 66 of conception again a bilobed brightest structure hyperechogenic i.e. genital tubercle was seen.

Although ultrasonography is an accurate technique for fetal sexing still it requires skill and high resolution ultrasonic equipment. The recognition of fetal land mark such as head, heart, pulsating umbilical cord, rear legs and tail is essential because these anatomical structures may confuse the diagnosis of genital tubercle (Curran, 1992).

At Day 60 of conception a fluid filled anechoic cavity was clearly visible inside the abdominal region of developing fetus with 5 MHz probe.

Discussion

It was observed at the point of junction of umbilicus. Probably it was the developing urinary bladder of the fetus. It was again great achievement in the current study to document the urinary bladder at earliest period i.e. Day 66. Since Aiumlamai *et al.* (1992) detected the urinary bladder at Day 97 to 103 of gestation in ewes. Gonzalez de Bulnes *et al.* (1998) also easily monitored anechoic cavity of the stomach in the abdomen from Day 50 of gestation. The pericardial cavity and details of anatomical structures of heart i.e. septum and values, were observed at Day 60. While Aiumlamai *et al.* (1992) reported the heart chambers at Day 97 to 103. The lateral view of head of the fetus showed the developing bony parts on the same day i.e. skull bone, nasal bone,

nasal passage, buccal cavity, upper jaws, lower jaw and vertebral of spinal cord. Similar results were obtained by Kahn (1994).

At Day 73 of conception anatomy of the full size head was seen. It was showing the cranial cavities containing brain-lobes. The measurements of cranial cavities i.e. length and width were obtained between Day 46 to 73. These measurements showed the almost linear pattern of growth.

The measurements could not be taken before Day 46 because the proper shape of head was not fully formed. The measurements regarding the cranial cavities were not possible beyond the Day 73 due to large size of the fetus. Because it was difficult to scan the full size fetal head. No parallel reports are available.

Discussion

From the clear picture of brain hemisphere it is believed that further study can be conducted on brain with the help of ultrasonography in small ruminants.

At Day 80 of conception the great and excellent feature of present study was to observed the opening and closing activities of fetal mouth. It seemed that fetus was chewing something in the mouth. Although very least literature is available about the ultrasonographic studies of fetal movements in ewes. But there are no reports regarding the typical chewing movements of fetus. Therefore it was the greatest achievement of the current study to reveal hidden discovery i.e. fetus learns the skills of chewing within uterus. The paddling movement of forelimbs were also recorded. Probably it helped the fetus to stir the amniotic fluid.

The big good picture of scrotum was observed at Day 90 of conception in same fetus which was earlier predicted male. So this anatomical structure of scrotum confirmed the prediction. No parallel reports are available.

In present study the crown rump length was also measured from Day 39 to 53. Beyond Day 53 it was not possible to measure the dimensions of crown rump length due to large size of fetus. At Day 39 it was 4 cm while at Day 53 it was 9 cm. These results are similar to the findings of Evans and Sack (1973) who reported that crown rump length of ovine and caprine fetuses 4 cm at Day 40, 7 cm at Day 50 and it surpasses a length of 10 cm at Day 60 which is limits of 5 MHz transducer.

Discussion

In current study it was difficult to record the crown rump length after Day 53 because full image of the large sized fetus could not be taken after Day 53. However Gonzalez de Bulnes *et al.*, (1998) claimed to measure the crown rump length and trunk diameter of embryo at Day 19 to onwards. Schrick and Inskeep (1993) measured the crown rump length of embryo at Day 20, 20, 30, 35 and 40 averaged 6±1, 10±2, 25±2, 36±4 mm, respectively.

In one case the twin pregnancy was also observed at'Day 60 using 5 MHz transducer. There were two fetuses, one was dead fetus while other was normal live fetus. So the results are in consistent with the findings of Bretzlaff *et al.* (1993). They also reported that loss of one/ multiple fetus in ewe that remained pregnant was not uncommon. Schrick and Inskeep (1993) also noted the two ewes having each had an embryo died by Day 25 or Day 40 respectively (diagnosed by absence of previously observed heart beat) but each ewes maintained the remaining embryo to term.

At Day 60 in one animal and at Day 73 in another animal pathological abnormalities of the fetus were observed in present study. The fetal mortalities were diagnosed by absence of the cardinal signs of pregnancy. Because there was no heartbeat which is the major criteria for the viability of fetus. There were echoic spots (white

spots) in the anechoic area indicating the absence of normal reflection from the uterine fluid. There were no

normal skeleton. There were no presence of placentomes which are also the major cardinal symptoms to confirm the live fetus. Finally the turbidity of the uteirne fluid as well no cardinal signs of pregnancy confirmed the dead fetus.

Roberts (1971) observed the fetal mortality 20-30 per cent from Day 20 to parturition. Chapman (1980) reported fetal mortality was the responsible for reducing the lambing performance as predicted by ultrasonography because fetal resorption/abortion is normal in most of the species. Fetal attrition is not easily documented prior to the diagnosis by ultrasonography in early stages and goes unrecorded in ewes.

Buckrell and Geart *et al.* (1988) observed pyometra ewes by obvious reflections from intrauterine fluid. They reported snow like images as the typical indication of pathological abnormalities.

Gearhart *et al.* (1988) observed fetal attrition at Day 49 and hydrops allantois at Day 110 in ewes. However Bretzlaff (1993) could not differentiate in between pregnancy and embryonic resorption that’s why hydrometra goes undetected. He also concluded that hydrometra is less common in sheep than goats. Bretzlaff *et al.*, 1993 also advised to confirm the positive pregnancy based on uterine fluid without other cardinal signs of pregnancy should always be rechecked after about 2 weeks.

Schrick and Inskeep (1993) reported the death of sheep embryo by absence of heart beat at Day 25 and Day 40. But Samuel and Giller

(1995) confirmed the hydrops uteri by observing few numbers of placentomes without any typical C-shaped and no evidences of fetus. Kahn (1994) reported that one reason a small number of erroneous diagnosis can not be prevented was that an animal found pregnant in very early pregnancy can suffer embryonic loss and latter be counted as a false positive diagnosis may be made when pathological abnormalities are wrongly interpreted as signs of pregnancy. Still Buckrell et al (1986) and Buckrell (1988) found that the accuracy of diagnosis as pregnant can exceed 95 per cent as early as Day 25 by using the transrectal method of ultrasonography.

So B-mode ultrasonography is the best noninvasive techniques to study the developing fetus.

Ch apt er -VI

Summary and Conclusions

The present study was carried out on eight Corriedale sheep of known mating date to differentiate the pregnant and nonpregnant as well as to observe the sequential events and heart beats of the developing fetus, with the help of ultrasonography. To study the genitalia of nonpregnant uterus, two ewes were also scanned.

At Day 18 : A black anechoic area i.e. uterine fluid containing a hyperechoic structure in form of long white streak i.e. conceptus was identified in six ewes while two ewes were diagnosed as nonpregnant.

At Day 25: The comma shaped conceptus with heart beat and a demarcation line making its two parts, as well as attached to endometrium was visible.

At Day 32: The C-shaped conceptus with budding of fetal extremities was observed.

At Day 39 : The amniotic membrane encircling the developing fetus with budding of

head, limbs formation was visible.

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Summary and Conclusions

At Day 46 : The fully fetus with development of the head, neck, trunk having - cardiac cavity, mouth parts, lower jaw, upper jaw, spinal vertebrae, bony cage was visible Amniotic membrane as dotted echoic line was visible. i The ventrally lied fetus was seen with vigerous movements. Nodule shaped placentomes were observed.

At Day 53: The typical halfmoon shaped placentome, genital tubercle, pulsating umbilicus, bifuracation in hooves and licking of the forefeet was identified.

At Day 60: A dead fetus was visible. The urinary bladder, genital tubercle, Coccygeal vertebrae, beating heart in pericardial cavity and pulsating umbilicus was identified.

At Day 66: Full view of button shaped placentomes was visible. The genital tubercle and fully developed umbilicus was seen. At Day 73: Fetal head showing cranial cavities having brain lobes, and head resting on trunk was identified. Again a dead fetus was also seen. At Day 80: Full anatomy of the head and mouth along with typical chewing fetal movements were seen.

At Day 90: Full anatomy of fetal parts and good picture of scrotum was visible.

The following conclusions were drawn from the present study:-

1.

Accuracy of pregnancy diagnosis with ultrasdonography is 100 per cent from Day 25 onwards as heart beat is the cardinal sign of viable fetus can be visualized.

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Summary and Conclusions

2.

Conceptus changes its shape i.e. from a long streak at Day 18, comma shape at Day 25, C-shaped at Day 32, with extremities at Day 39 and fully developed fetus identified at Day 46.

3.

There is no major change in heart beats. Mean value in range of 172 to 165 beats/minute were observed.

4.

There is no significant change in diameter of the placentome in between Day 73 and 90 of gestation.

5.

Fetal sex can be diagnosed easily at Day 53 in male but not easy to identify the female fetus.

6.

Fetal mortalities and any other pathological abnormalities can be diagnosed at Day 60 and onwards.

7.

The viable fetus along with the dead fetus in same animal i.e. twin pregnancy can be diagnosed at Day 60.

8.

Fetus learns the typical chewing movements of mouth and paddling movements of limbs during the gestation can be diagnosed at Day 80.

9.

The full sized scrotum can be scanned at Day 90. Ultrasonography is noninvasive method and superior to all other method for early, pregnancy diagnosis, the study of developing fetus, genitalia and prediction of the fetal

sex.

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ABSTRACT

a)

Title of thesis:

ULTRA-SONOGRAPHIC STUDIES

ON FOETAL DEVELOPMENT IN

SHEEP

b)

Full name of degree holder

:

DR. RAMPHAL NAIN

c)

Title of degree

:

Master of Veterinary Science in

Veterinary Gynaecology &

Obstetrics

d)

Name and address of

:

Dr. S.K. Verma

Major Advisor

Professor**,** Department of Animal

Reproduction Gynaecology &

Obstetrics

CCS Haryana Agricultural

University, Hisar- 125 004.

e)

Degree awarding

:

CCS Haryana Agricultural

University/Institute

University, Hisar- 125 004.

f)

Year of award of degree

:

2000

g)

Major subject

:

Veterinary Gynaecology and

Obstetrics

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Total number of pages in thesis

:

i)

Number of words in the abstract

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Approximately 300

An experiment was conducted at Sheep Breeding Farm of CCS Haryana Agricultural University Hisar to study the foetal development in eight Corriedale ewes. The ewes were scanned transrectally by B-mode real time ultrasound machine with a linear array of 7.5 MHz and 5 MHz frequencies from Day 18 to 32 and Day 39 to 90 of gestation at weekly intervals, respectively.

Ultrasonic gel was used as coupling medium. The transducer head was inserted into the rectum searching urinary bladder as a land mark, uterus was identified. The six ewes were diagnosed pregnant at Day 18 by observing the accumulated uterine fluid containing a white streak i.e. conceptus while the uterus of remaining two ewes showed homogenous coarsely granular echo i.e. nonpregnant. But the pregnancy was confirmed at Day 25 by pulsating heart within the embryo. The Amnion and typical half-moon shaped placentomes were seen at Day 39. No significant change in placentomal size was observed from Day 73 to 90. The streak, comma and C-shaped conceptus was visible at Day 18, 25 and 32 respectively.

The fetus with developing extremities was seen at Day 39. But fully developed fetus having head, eye ball, neck, trunk, jaw bones, spinal vertebrae and bony cage, with jerking fetal movements were observed at Day 46. A typical increase in biparietal diameter was measured from Day 39 to 90. It was difficult to count the

heart beats due to movements of animal. Still the heart beats were calculated and found in range of 172-155 per minutes. Bifurcation of hooves was visible at Day 53. Tail was seen at Day 60. The pulsating umbilicus was observed at Day 53. A fetus was predicted male by location of genital tubercle in between umbilicus and hind limbs, the presence of scrotum at Day 90 and lambing confirmed the prediction. The urinary bladder of fetus was seen at Day 66. The fetus was seen with the excellent and new finding i.e. chewing the fluid and paddling movements of forelegs at Day 80. The crown rump length was found 4 and 9 cm at Day 39 and 53, respectively. The twin pregnancy was also observed at Day 60. The presence of turbid uterine fluid having no cardinal signs of pregnancy confirmed the fetal mortality at Day 60 and 73. A nice picture of scrotum was seen at Day 90 of the gestation. Therefore, B-mode real-time ultrasonography is the accurate, rapid, safe, noninvasive and superior practical means to diagnose the earliest pregnancy as well as to study the developing fetus.

MAJOR ADVISOR

(SIGNATURE OF THE STUDENT)

HEAD OF THE DEPARTMENT

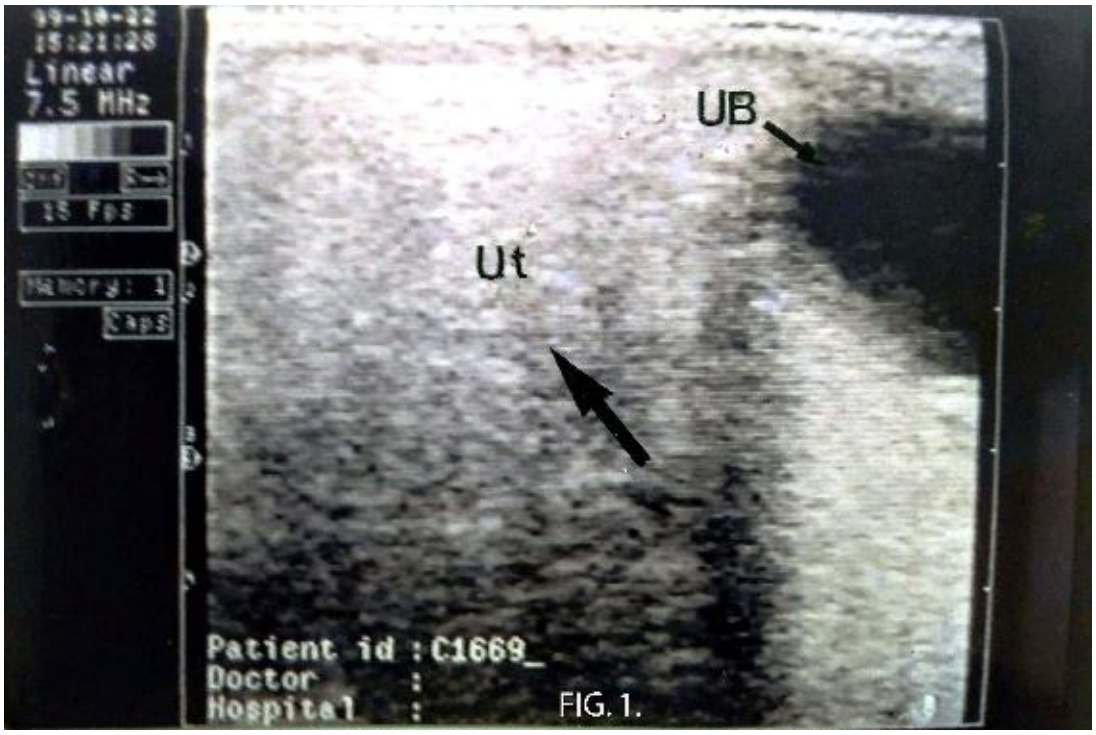

Fig. 1.

Transrectal ultrasonogram of nonpregnant uterus of an ewe with a 7.5 MHz transducer showing the uterus (Ut) and the urinary bladder (UB) in a sagittal section with dorsoventrally directed beam. The uterus appeared homogenous coarsely granular close to the anterior border of bladder.

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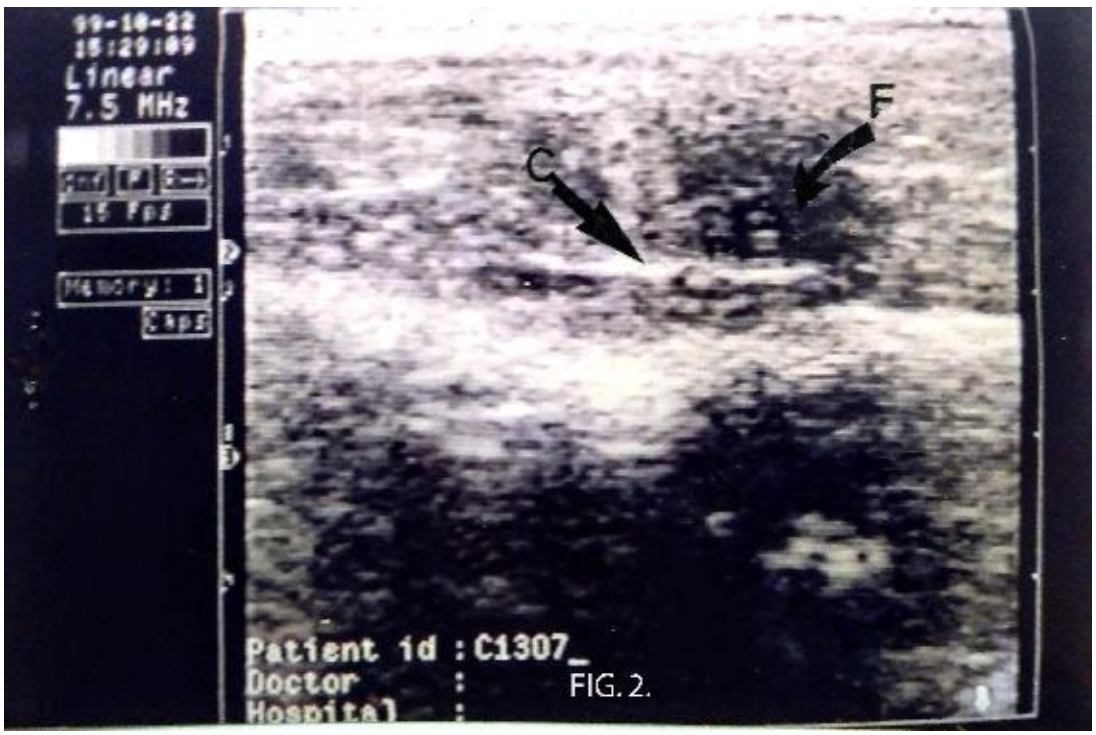

Fig. 2.

Transrectal ultrasonogram of uterug of an ewe at Day 18 of pregnancy with a 7.5 MHz transducer. The scan is showing echoic streak of conceptus (C) in anechoic fluid (F)

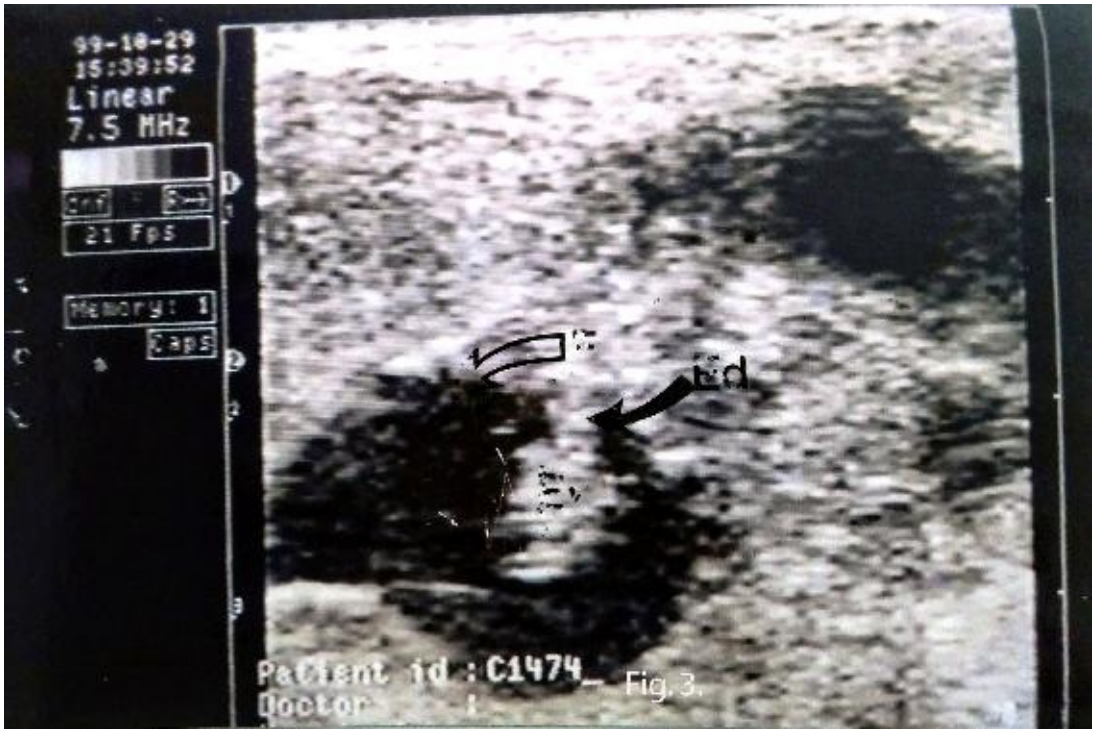

Fig. 3.

Transrectal ultrasonogram of uterus of an ewe at Day 25 of pregnancy with a 7.5 MHz transducer. The scan is showing embryonic vesicle (EV) attached to one side of endometrium (Ed).

Fig. 4.

Transrectal ultrasonogram of uterus of an ewe at Day 25 of pregnancy with a 7.5 MHz transducer. The scan is showing dark image i.e. accumulated uterine fluid (shown by arrow)

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Fig. 5.

Transrectal ultrasonogram of uterus of an ewe at Day 25 of pregnancy with a

7.5 MHz transducer. The scan is showing anechoic line shown by arrow demarkating the fetus in two parts

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Fig. 6.

Transrectal ultrasonogram of uterus of an ewe at Day 32 of pregnancy with a 7.5 MHz transducer. The scan is showing echoic embryo (E) surrounded by anechoic embryonic fluid (F). Embryo with budding (Bu) of limbs (shown by arrows) is visible

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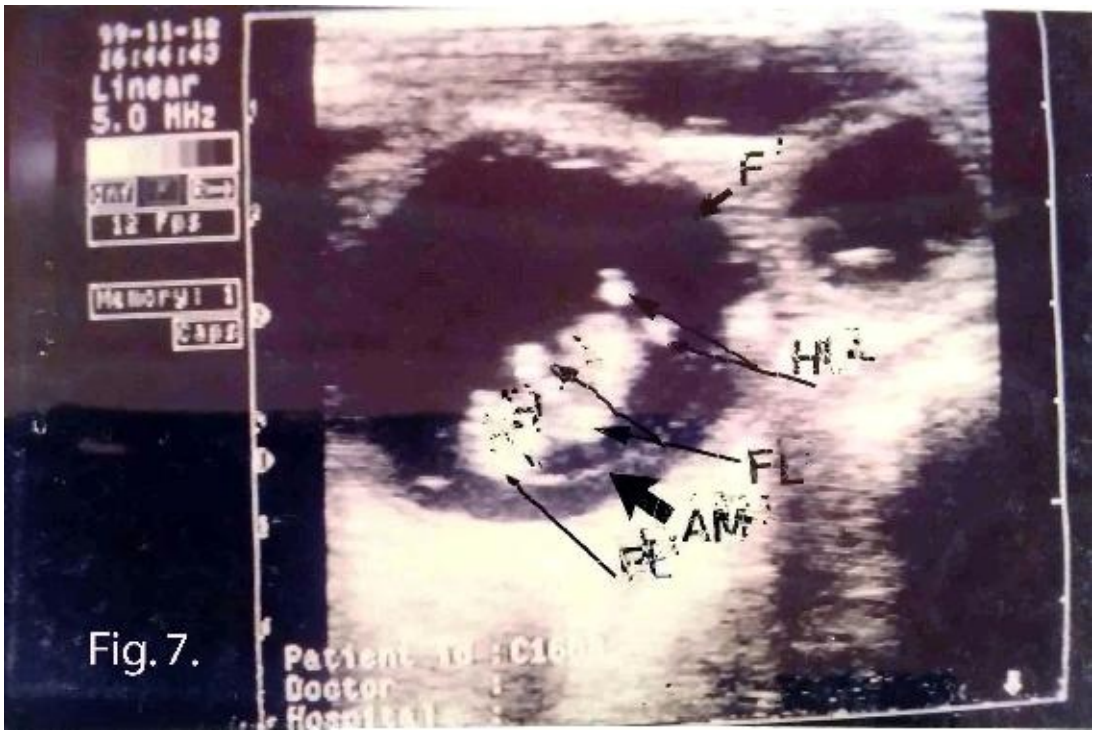

Fig. 7.

Transrectal ultrasonogram of uterus of an ewe at Day 39 of pregnancy with a 5 MHz transducer. The scan is showing echoic fetus (Ft) surrounded by anechoic embryonic fluid (F). embryo with budding of head (H), forelimbs (FL) and hind limbs (HL) is visible (shown by arrows). The hyperechoic amniotic membrane (A.M.) encircling the embryo is also clear visible

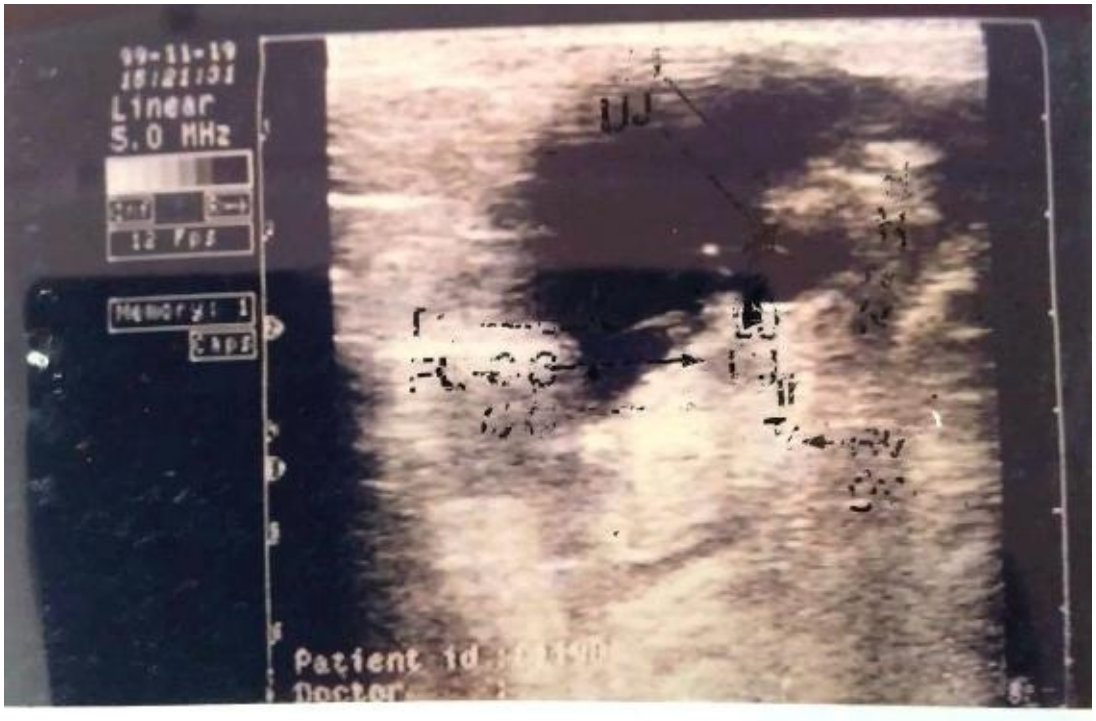

Fig. 8.

Transre ctal ultrasonogr am of uterus of an e we at Day 46 of pregnancy with a 5 MHz transducer. The scan is showing cardiac cavity (CC) area, head (H), neck (N), trunk (Tr), spinal vertebrae (SV), Forelimbs (FL) and mouth parts i.e. Upper jaw (UJ) and lower jaw (LJ) bones of fully developed fetus

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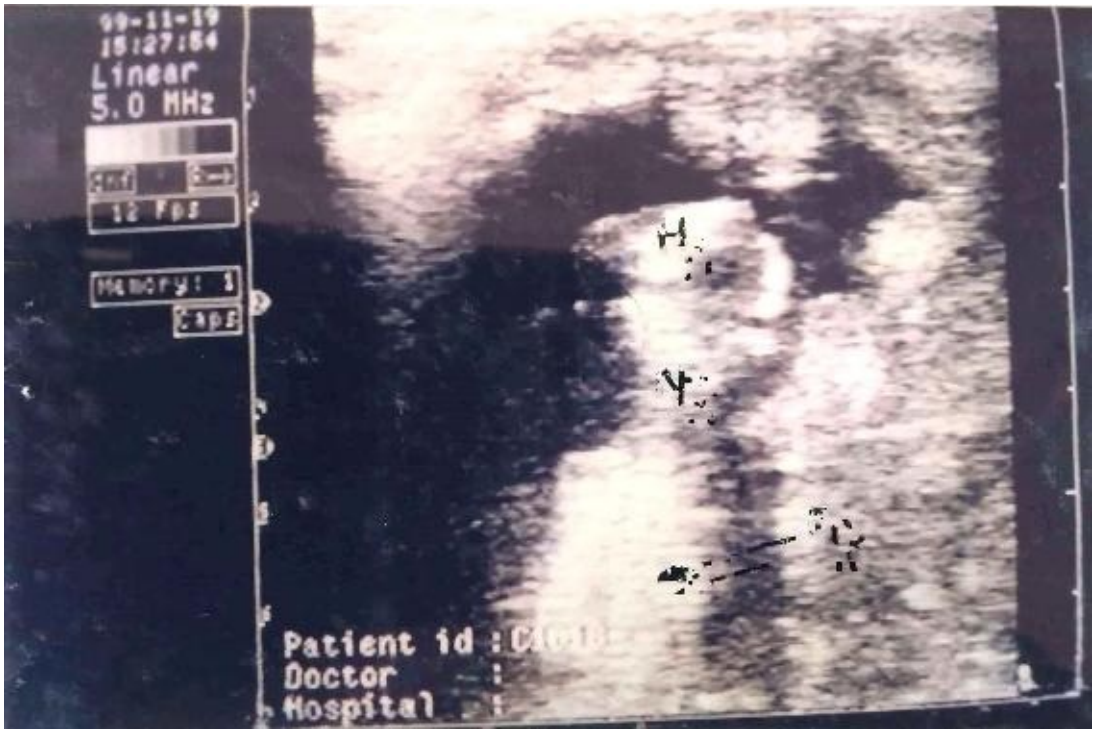

Fig. 9.

Transrectal ultrasonogram of uterus of an ewe at Day 46 of pregnancy with a 5 MHz transducer. The scan is showing head (H), bony cage of ribs (R) and spinal vertebrae of fetus (SV). Nodule shaped placetomes (P) were seen

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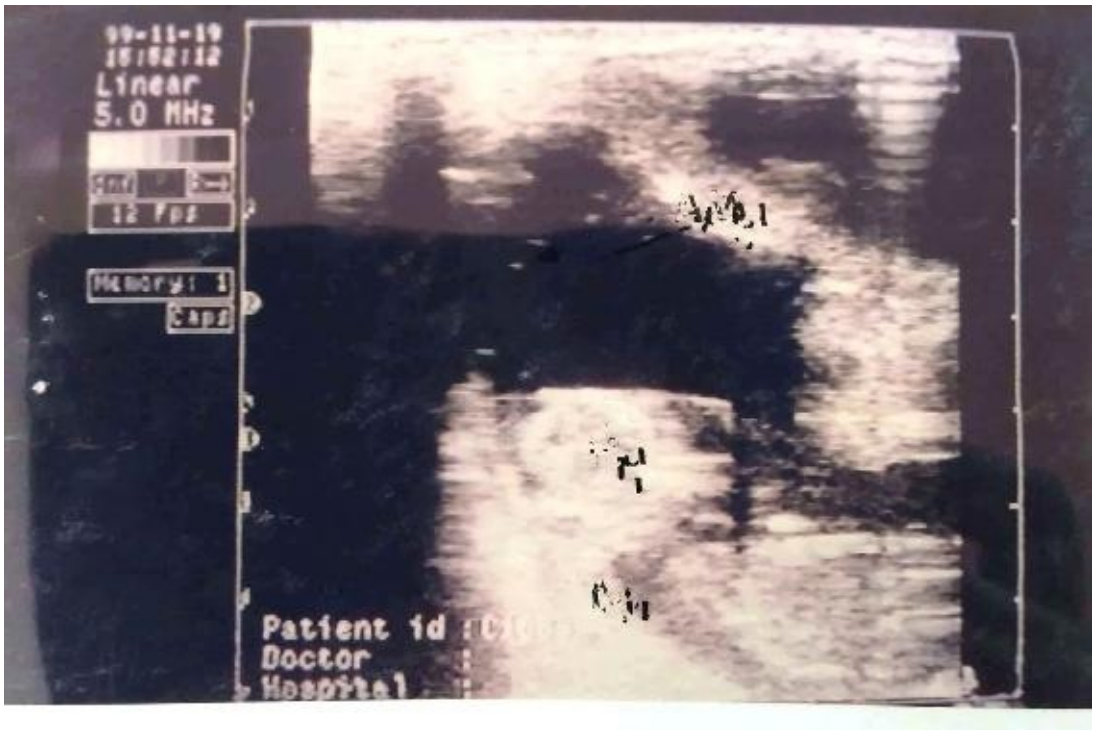

Fig. 10.

Transrectal ultrasonogram of uterus of an ewe at Day 46 of pregnancy with a 5 MHz transducer. The scan is showing fetus lies ventrally. Head (H) is clear visible. Fetus is surrounded by dotted hyperechoic amniotic membrane (A.M.)

Fig. 11.

Transrectal ultrasonogram of uterus of an ewe at Day 53 of pregnancy with a 5

MHz transducer. The scan is showing placentome (P) i.e. typical half-moon shaped structure.

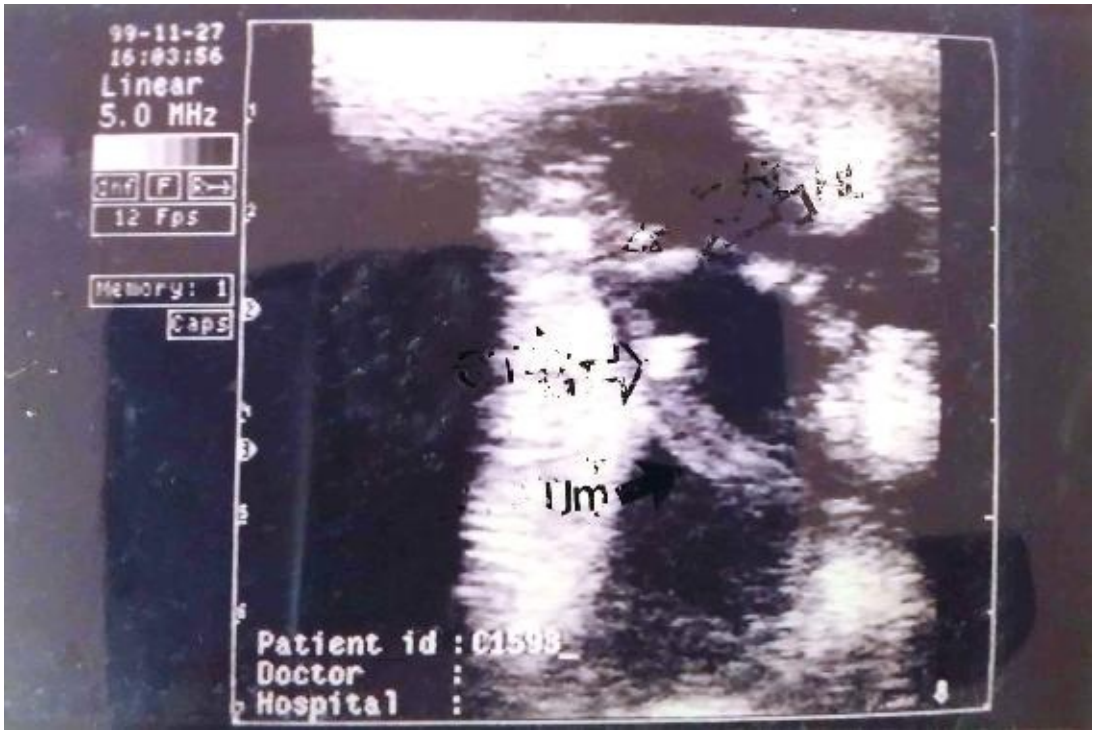

Fig. 12.

Transrectal ultrasonogram of uterus of an ewe at Day 53 of pregnancy with a 5 MHz transducer. The scan is showing hyperechoic bilobed genital tubercle (GT) lies in between the umbilicus (Urn) and hindlimbs (HL). Fetus was predicted as male on the basis of this structure. Bones of hind limbs are also visible

Fig. 13.

Transrectal ultrasonogram of uterus of an ewe at Day 53 of pregnancy with a 5 MHz transducer. The scan is showing bifurcation of hooves (shown by arrow)

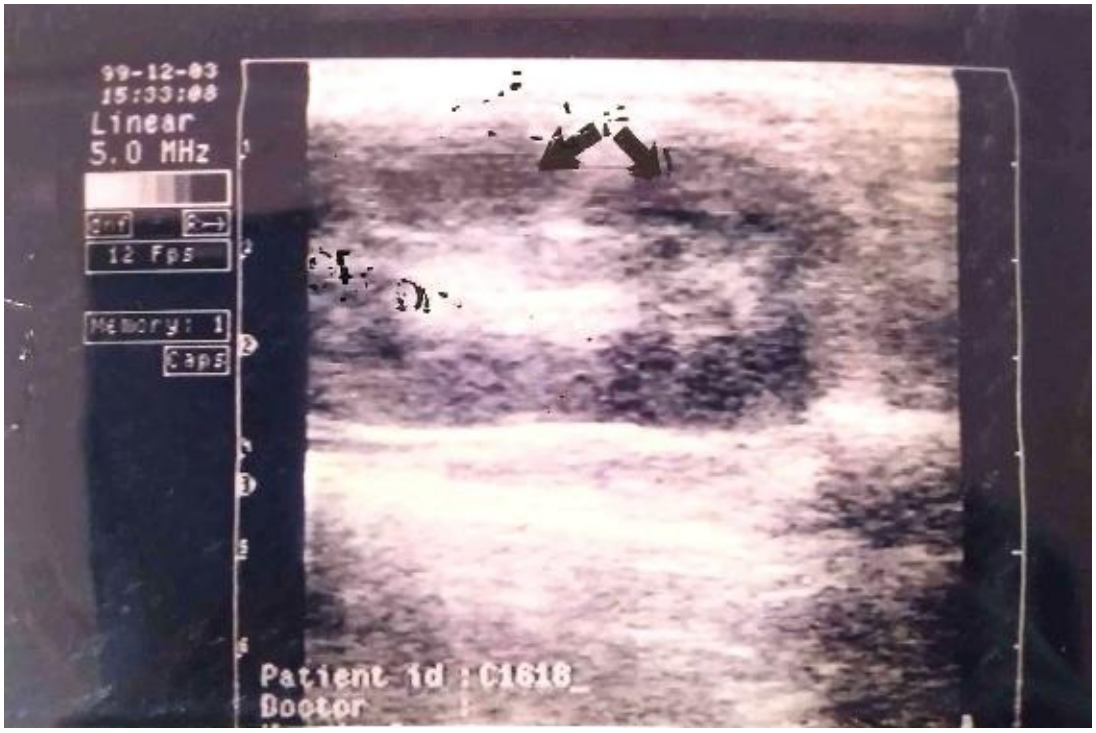

Fig. 14.

Transrectal ultrasonogram of uterus of an ewe at Day 60 of pregnancy with a 5 MHz transducer. The scan is showing pathological abnormalities i.e. dead fe-

tus (DFt). No normal skeleton of the fetus, but flickering echoic area in fetal fluid was seen

Fig. 15.

Transrectal ultrasonogram of uterus of an ewe at Day 60 of pregnancy with a 5 MHz transducer. The scan is showing coccygeal vertebrae (CV) of tail (T), hindlimbs (HL) bones. The spinal vertebrae (SV) are also visible with clarity

Fig. 16.

Transrectal ultrasonogram of uterus of an ewe at Day 60 of pregnancy with a 5 MHz transducer. The scan is showing lateral view of head (H), skull bone (SB), spinal vertebral (SV), optic vesicle (OV), nasal bone (nb), nasal passage (np), buccal cavity (be), muzzle (mz), lower jaw (LJ) and upper jaw bones (UJ). Fetus is surrounded by placentomes (P)

Fig. 17.

Transrectal ultrasonogram of uterus of an ewe at Day 60 of pregnancy with a 5 MHz transducer. The scan is showing large sized cervical vertebrae of spinal cord (shown by arrow) and fetus is in sitting position

85

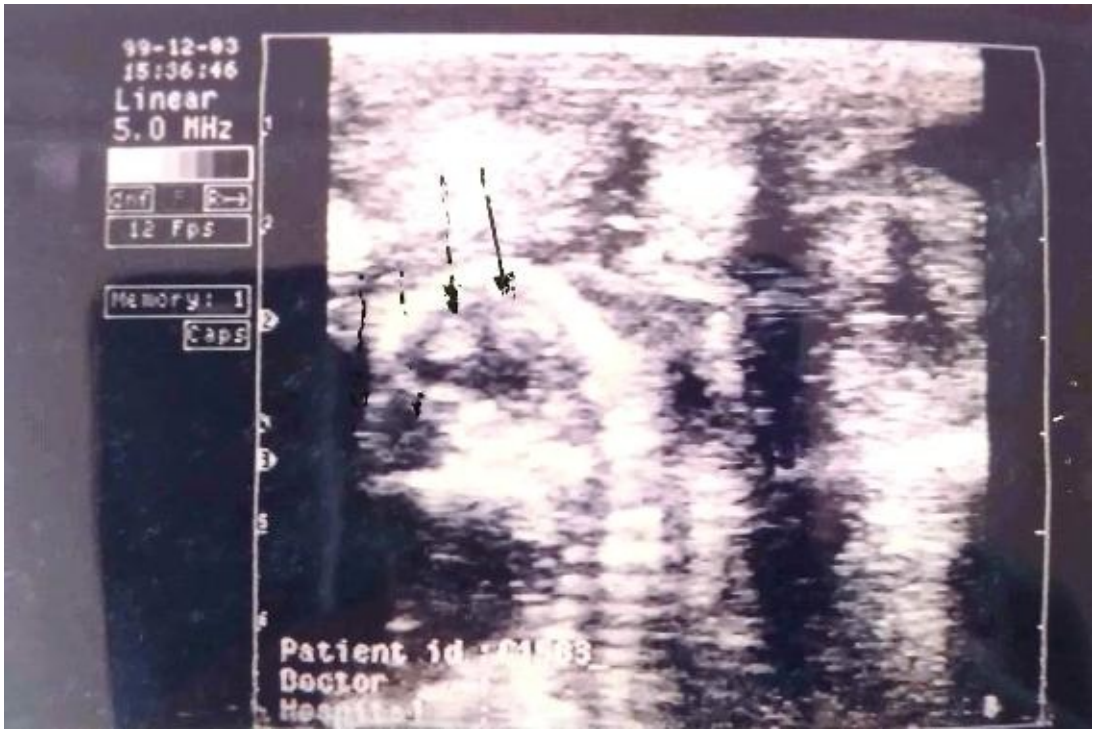

Fig. 18.

Transrectal ultrasonogram of uterus of an ewe at Day 60 of pregnancy with a 5 MHz transducer. The scan is showing brainlobes (shown by arrows)

86

Fig. 19.

Transrectal ultrasonogram of uterus of an ewe at Day 66 of pregnancy with a 5 MHz transducer. The scan is showing full size placentome (P) occupying most of the space of uterine lumen. The hypoechoic central cavity of the placentome (P) is shown by arrow

Fig. 20.

Transrectal ultrasonogram of uterus of an ewe at Day 66 of pregnancy with a 5 MHz transducer. The scan is showing genital tubercle (GT) as brightest bilobed hyperechoic structure

88

Fig. 21.

Transrectal ultrasonogram of uterus of an ewe at Day 66 of pregnancy with a 5 MHz transducer. The scan is showing umbilicus (Um) attached with fetus (Ft)

89

Fig. 22.

Transrectal ultrasonogram of uterus of an ewe at Day 73 of pregnancy with a 5 MHz transducer. The scan is showing cranial cavity (crc), covered by skull bone (SB), containing brane lobes. The head (H) of fetus with turning at neck (N), resting on trunk (Tr) and fetus seems to be in sitting position

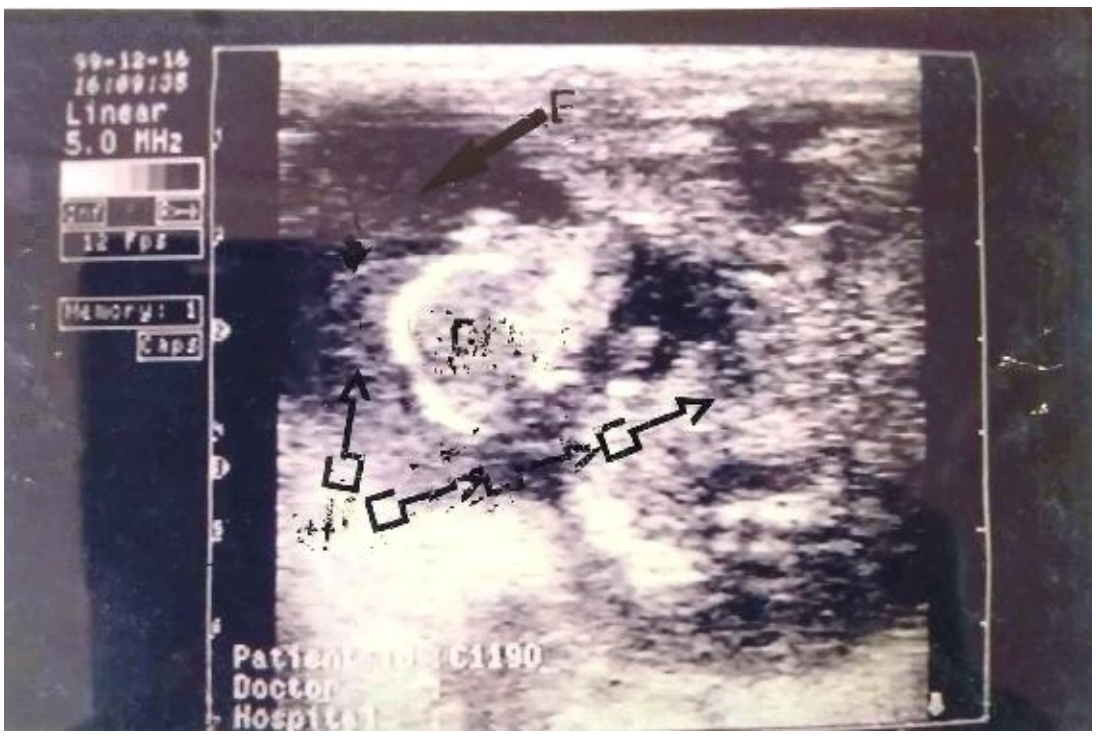

Fig. 23.

Transrectal ultrasonogram of uterus of an ewe at Day 73 of pregnancy with a 5 MHz transducer. The scan is showing pathological abnormalities i.e. dead fetus (DFt). No normal reflection from the accumulated fluid because of echoic spots (shown by arrows) in the dark image. The skeleton of fetus is also smaller than the normal size. The turbidity of the fluid confirms the dead fetus. No cardinal signs of pregnancy seen

Fig. 24.

Transrectal ultrasonogram of uterus of an ewe at Day 80 of pregnancy with a 5 MHz transducer. The scan is showing horizontal sagittal section of mouth. The nasal bone (nb), bones of lowe jaw (LJ), upper jaw (UJ) and tongue (T) in mouth, eye ball (eb), fore limbs (FL) are clear visible. The placentome (P) lies just infront of the mouth

Fig. 25.

Transrectal ultrasonogram of uterus of an ewe at Day.90 of pregnancy with a 5 MHz transducer. The scan is showing the picture of scrotum (Sc) which is situated in between hind limbs (HL) and below the tail (T) (shown by arrow)

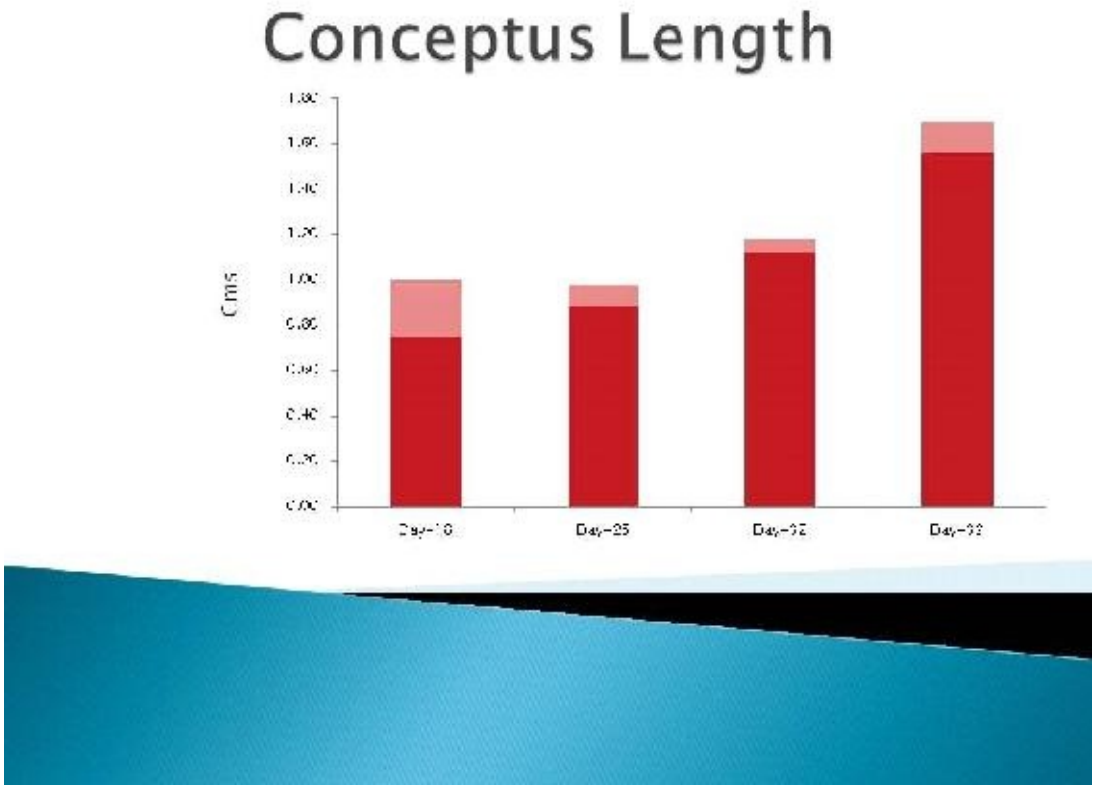

Fig. 26.

Conceptus Length

Since the fetal parts were not differentiated because the observation from Day 18 to Day 39 are referred as the value of conceptus. The conceptus length is increased from Day 18 to Day 39 or almost linearly. The values at Day 18 were less than 0.8 cm while it reached up to close to 1.6 cm by Day 39

94

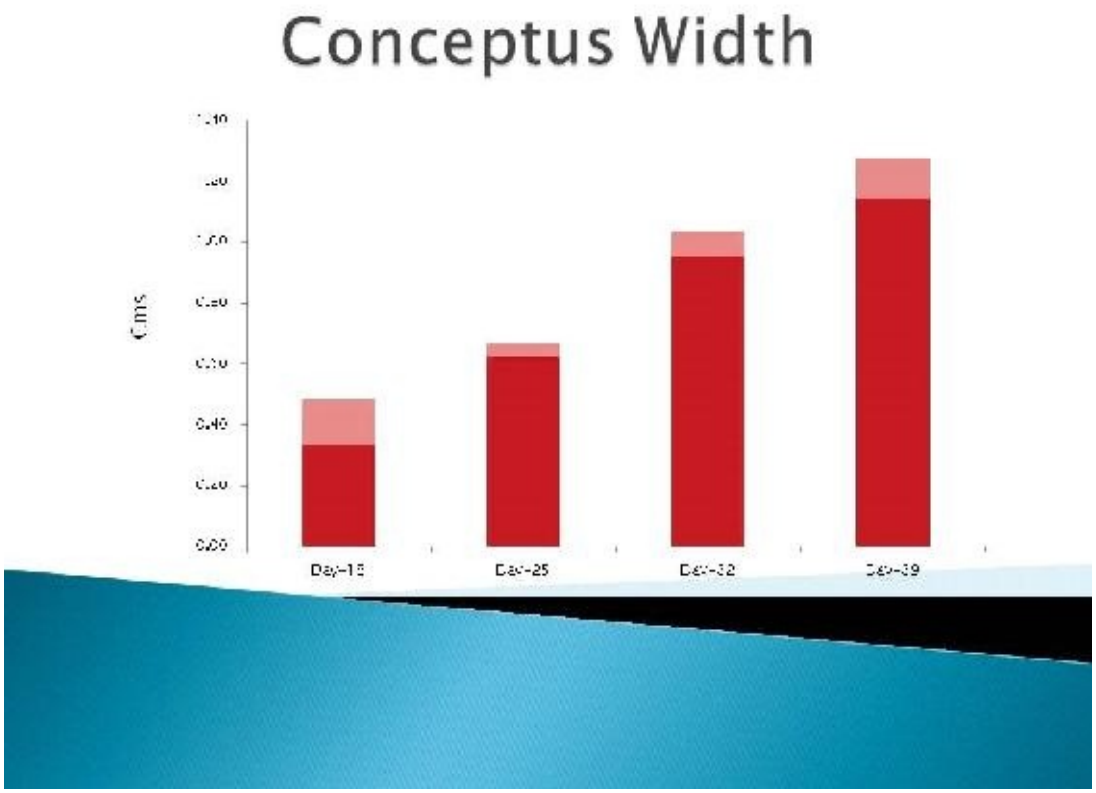

Fig. 27.

Conceptus Width

The conceptus width also shows linear pattern of growth, the values reached close to 1.2 cm by Day 39.

95

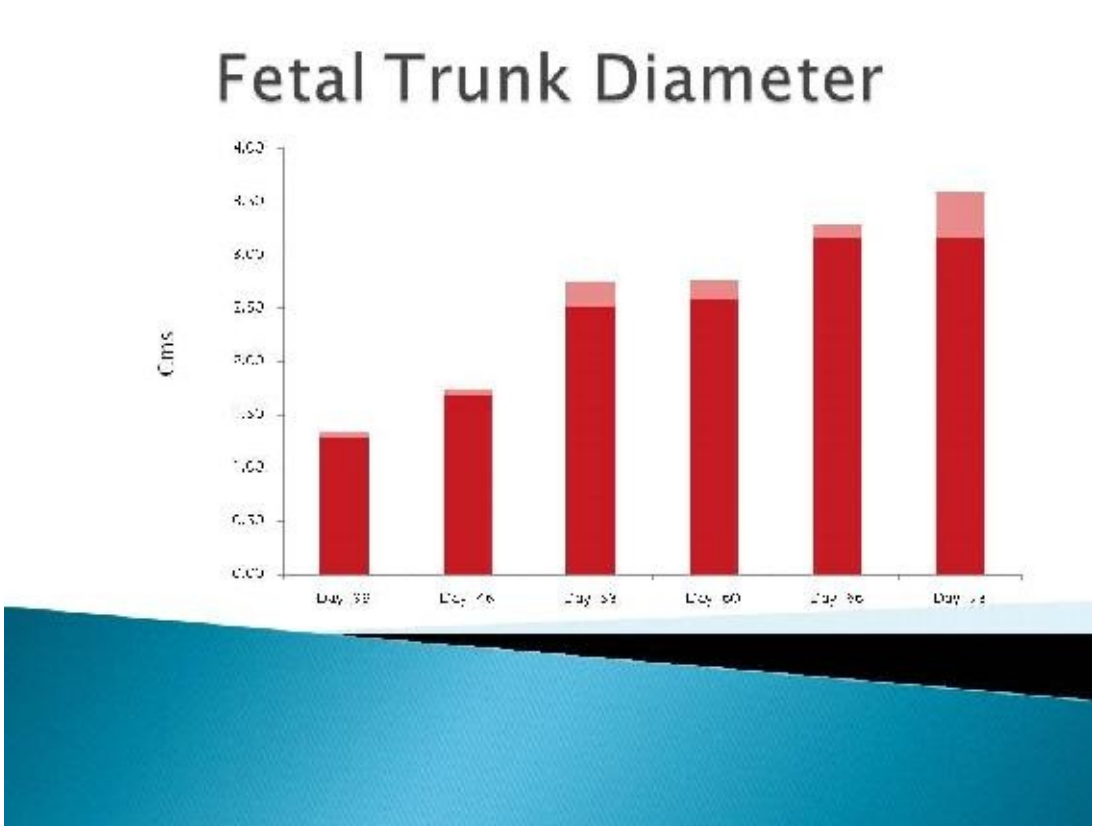

Fig. 28.

Fetal Trunk Diameter

The trunk diameter shows significant increase between Day 46 and Day 53, and between Day 60 and Day 66. The trunk diameter could not be measured after Day 73 due to large size of fetus and limitations of transducer

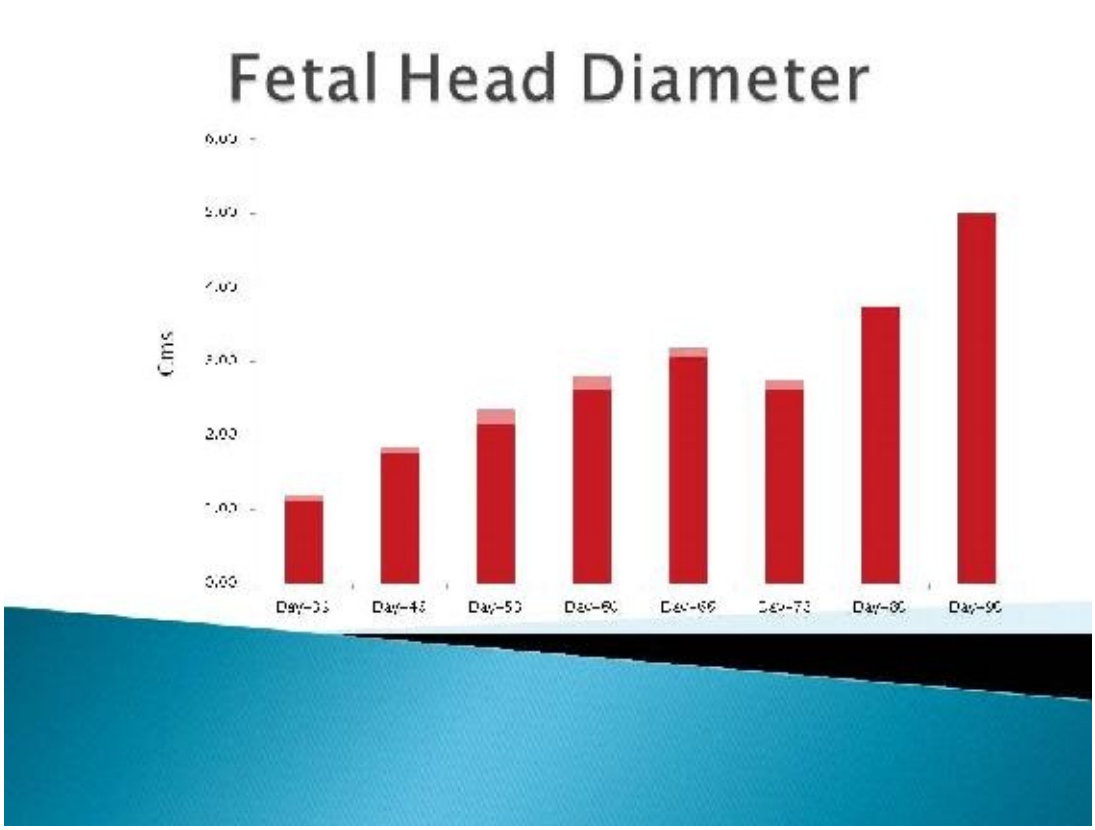

Fig. 29.

Fetal Head Diameter

The head diameter shows a typical linear pattern of growth. The values at Day 39 are close to 1 cm and reached around 5 cm by Day 90

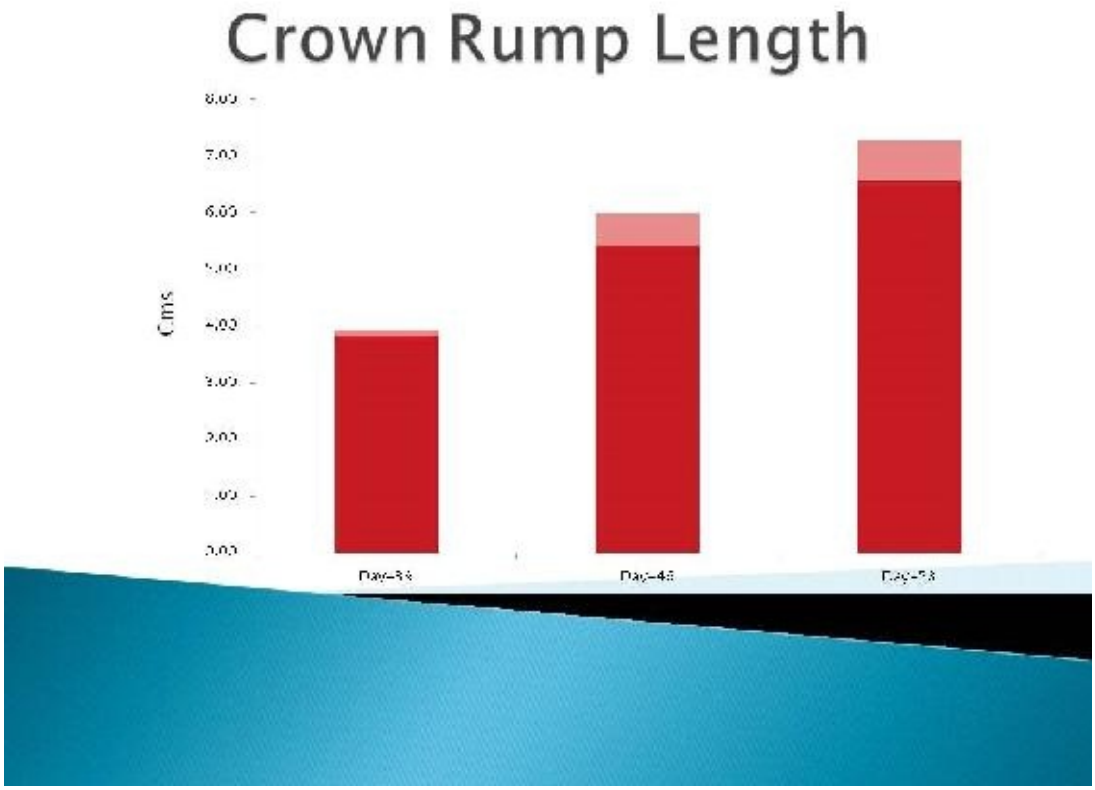

Fig. 30.

Crown Rump Length

The crown rump length could be measured only between Day 39 and 53 because of the full fetus was seen during these days. There was significant increase in crown rump length between Day 39 and 46 and again significant increase between Day 46 and 53.

98

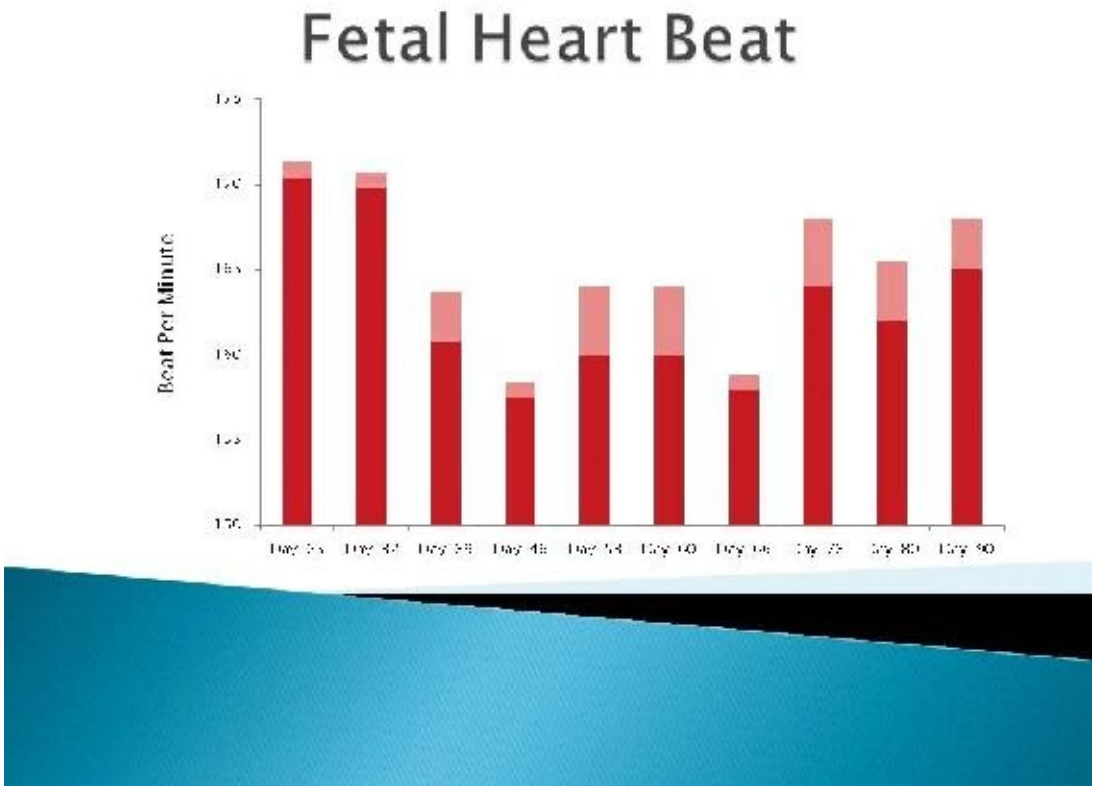

Fig. 31.

Fetal Heart Beat

The heart beat ranged between 172 to 155 beatsd per minute and there were variations among values. However these were not significant different (P>0.05). It appeared the values were higher around Day 25 and Day 32.

99

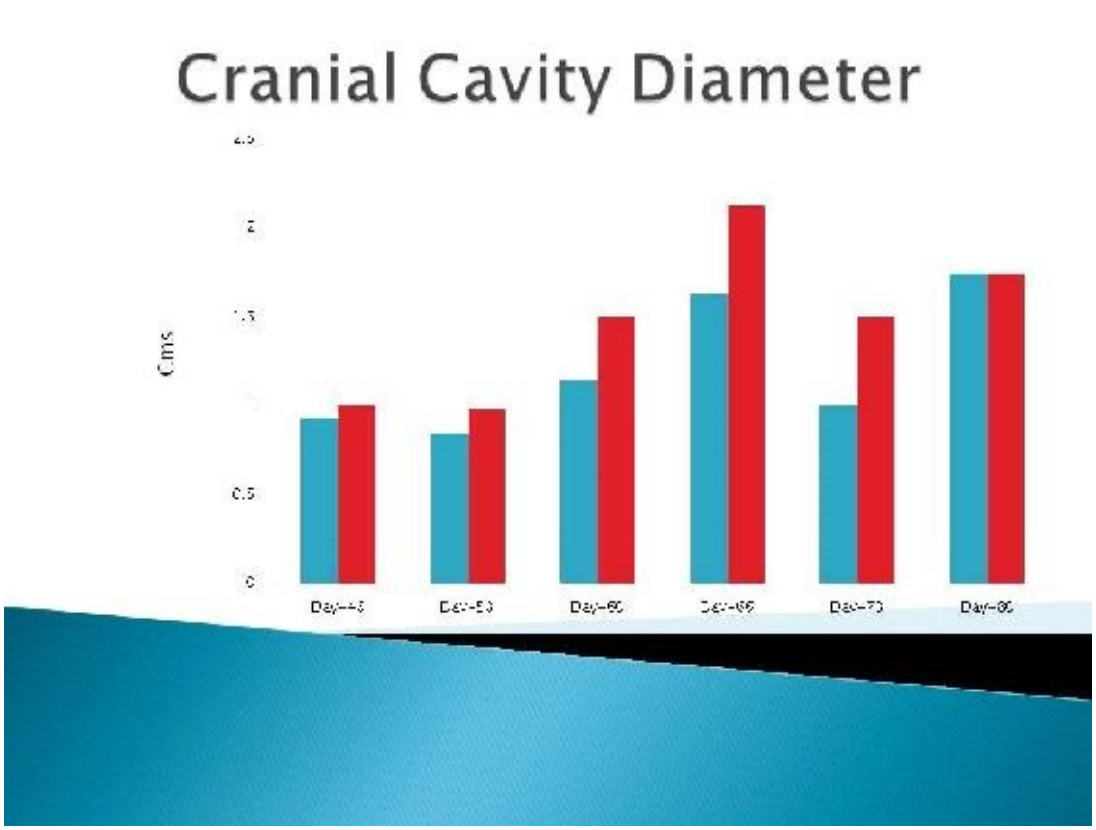

Fig. 32.

Cranial Cavity Diameter

A very good image of cranial cavity containing brain lobes was obtained between Days 46 to 73. The cranial cavity also shows almost linear pattern of growth between Day 46 and Day 73.

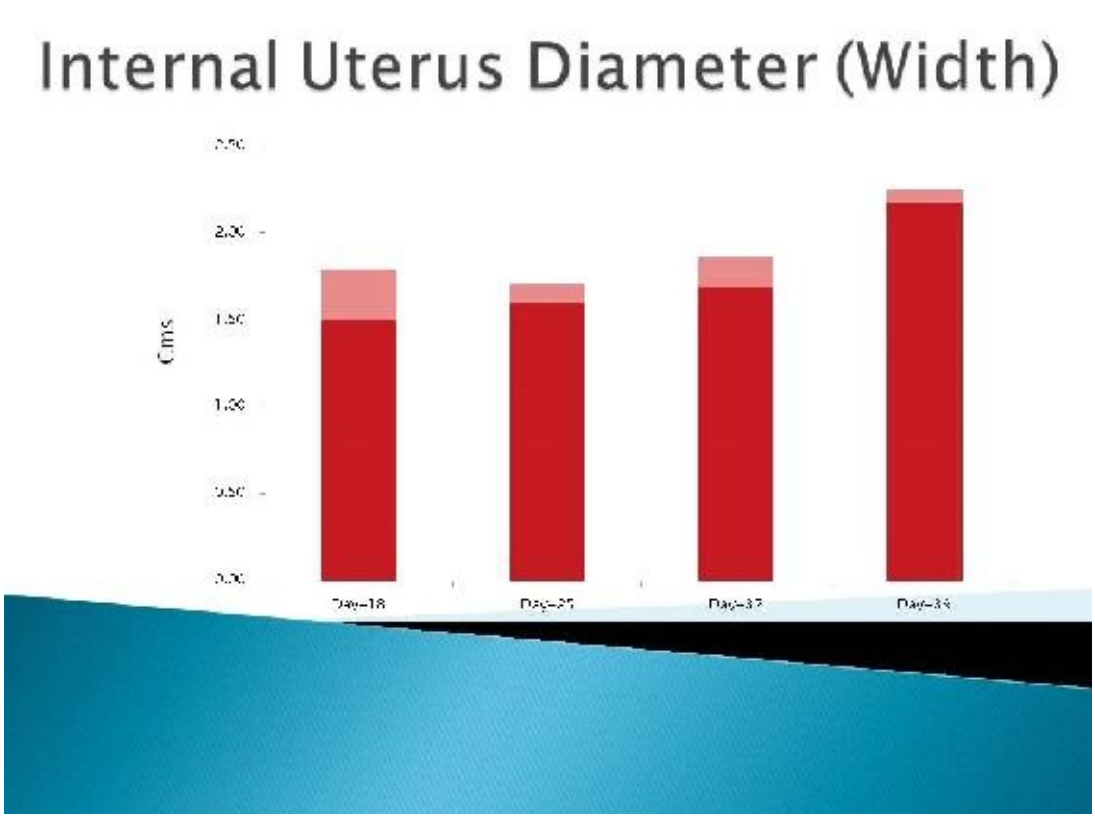

Fig. 33.

Internal Uterus Diameter

The internal uterus diameter was measured between Day 18 to Day 39 and later on it became difficult to obtain the full view of the uterus. The values on Day 18 were close to 1.5 cm that reached up to over 2.25 cm by Day 39

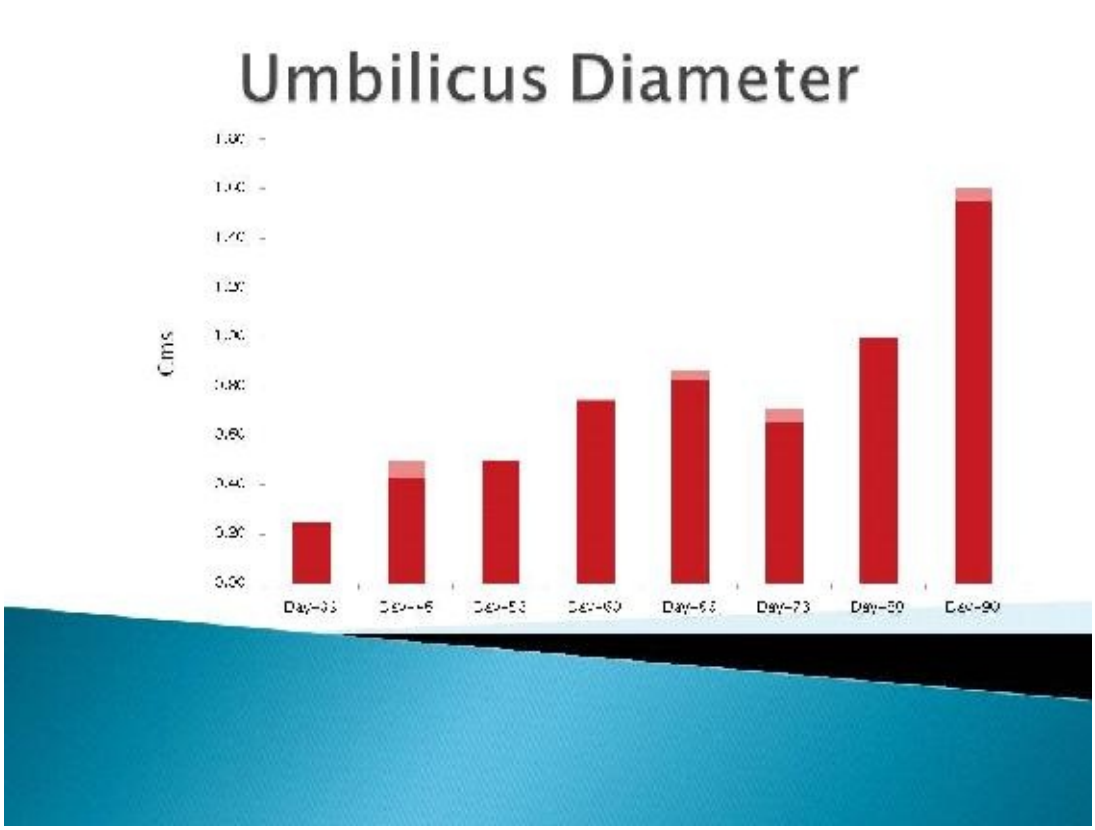

Fig. 34.

Placentomes Diameter

The good pictures of placentomes were obtained and diameter were measured on a cross section view. The values were close to 1 cm on Day 39 and that reached to over 2.5 cm by Day 73 and 2.75 cm by Day 90. There was no significant change between Day 73 and Day 90

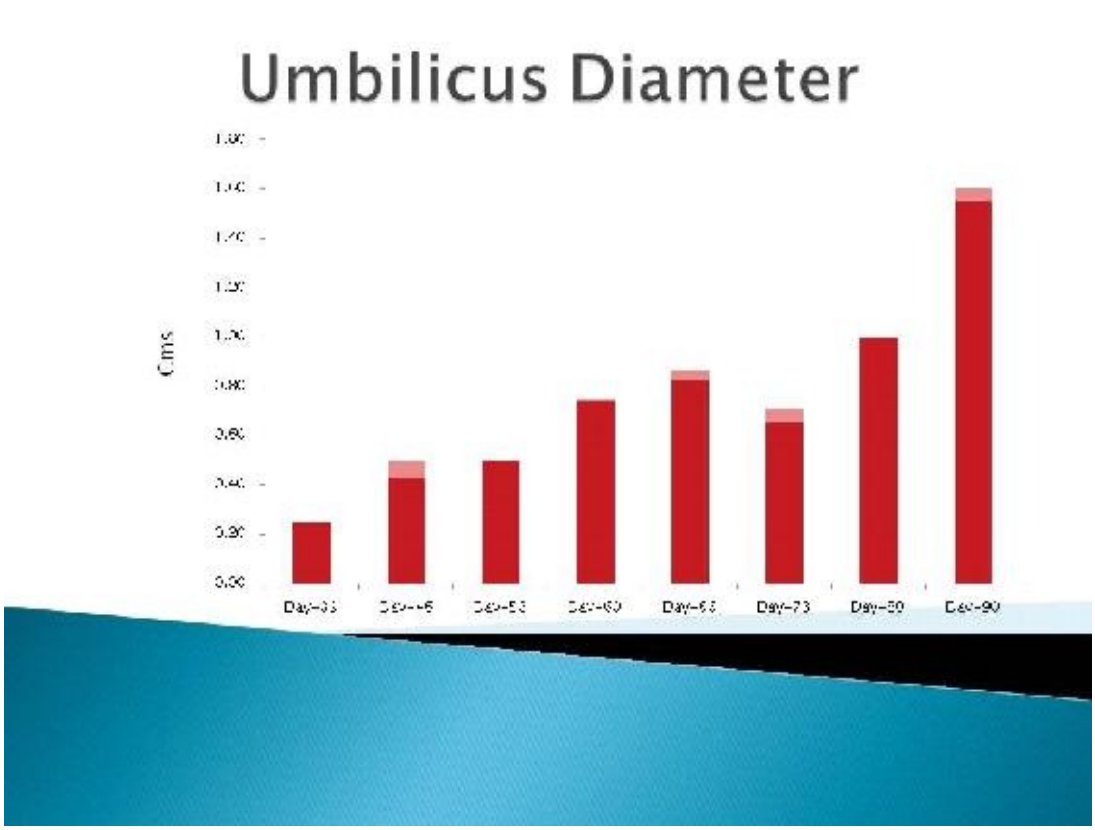

Fig. 35.

Umbilicus Diameter

The umbilicus diameter was measured between Day 39 and Day 90. It shows almost linear pattern of increase. However there was significant change between Day 80 and Day 90.

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