"PREGNANCY DIAGNOSIS IN SHEEP BY TRANSABDOMINAL METHOD"

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JUNE, 2011

"PREGNANCY DIAGNOSIS IN SHEEP BY TRANSABDOMINAL METHOD"

Thesis submitted to the

Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar

in partial fulfilment of the requirements
for the award of the degree of

Master of Veterinary Science

in

VETERINARY GYNAECOLOGY & OBSTETRICS

By

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CERTIFICATE

This is to certify that the thesis entitled ***"Pregnancy Diagnosis in Sheep by Transabdominal Method"*** submitted by **Mr. Stanzin Thakchos. MVHK-927** in partial fulfilment of the requirements for the award of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY & OBSTETRICS** of the Karnataka Veterinary, Animal & Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Dedicated to my beloved Mother.....

ACKNOWLEDGEMENT

I take this opportunity to express my deep sense of gratitude, indebtedness and warm regards to ***Dr. V. Chandrashekara Murthy*** *Associate Professor, Department of Gynaecology & obstetrics, and Chairman of my Advisory Committee, who was very helpful to me throughout the course of my study. His guidance, inspiration, immense interest, advices, valuable suggestions, whole hearted encouragement and blessings helped me to grow in my professional and academic competence. I will always remember him with gratitude for the innumerable ways he has influenced my life during my course of time.*

It's my pleasure to acknowledge sincere thanks to ***Dr. T.G. Honnappa*** *Associate Professor, and member of my Advisory Committee, for his valuable help, suggestion, inspiring guidance and support during my research period and preparation of manuscript.*

I wish to express heartfelt thanks to ***Dr. A. Krishnaswamy****, Professor & head of Veterinary Gynaecology & Obstetrics and member of my Advisory Committee, for his moral support, constant encouragement and valuable suggestions that has helped me in completing the research.*

I wish to express my deep and heartfelt gratitude to ***Dr. R.Bhaskaran*** *Professor and Head, Department of instructional livestock farm, who is member of my Advisory Committee, for his valuable help in providing the animals for research work and inspiring guidance rendered during my research work.*

It's my pleasure to express my sincere thanks to ***Dr. Girish Kumar****, Professor, Department of veterinary biochemistry, another member of my Advisory Committee, for his moral support and valuable suggestions that helped me in completing the research.*

I take this opportunity to sincerely thank ***Dr. Sudha,*** *Assistant Professor of the Department for her valuable guidance regarding ultrasound scanning which helped me in completing the research.*

I am highly obliged to my teachers ***Dr. Narsimamurthy*** *(Contract Teacher) of the department who have helped me open heartedly in all means during my post graduate study.*

I owe special thanks to my colleagues ***Drs Mahesh, Mudasir, Arshad, Pradeep, Tahir, Babu, Murthy, Vinod, Adity , Kavya, Anand, Swetha, Kavya, Navya, Vibha, Dost, Hakim & Ashwath*** *for their timely help and advice.*

I may fail in my duty if I do not thank my mother, Sisters and friends for their moral support in my hard days.

There are many others who in various ways, have contributed to and assisted in this work. I express my sincere thanks to them as well.

Bangalore
June, 2011 *(Stanzin Thakchos)*

CONTENTS

Chapter	Title	Page No.
I	INTRODUCTION	1-3
II	REVIEW OF LITERATURE	4-22
III	MATERIALS AND METHODS	23-26
IV	RESULTS	27-43
V	DISCUSSION	44-48
VI	SUMMARY	49-50
VII	BIBLIOGRAPHY	51-65
VIII	ABSTRACT	66

LIST OF TABLES

Table No.	Title	Page No.
1	Percentage of pregnant and non pregnant ewes on the basis of gestational sac	29
2	Percentage of pregnant and non pregnant ewes on the basis of heart beat	29
3	Percentage of pregnant and non pregnant ewes on the basis of placentomes.	29
4	Percentage of pregnant and non pregnant ewes on the basis of leg buds	33
5	Percentage of pregnant and non pregnant ewes on the basis of vertebral column.	33

LIST OF FIGURES

Figure No.	Title	Page No.
01	Percentage of pregnant and non pregnant ewes on the basis of gestational sac.	30
02	Percentage of pregnant and non pregnant ewes on the basis of heart beats.	30
03	Percentage of pregnant and non pregnant ewes on the basis of placentomes.	31
04	Percentage of pregnant and non pregnant ewes on the basis of leg buds.	34
05	Percentage of pregnant and non pregnant ewes on the basis of vertebral column.	34

LIST OF PLATES

Plate No.	Title	Page No.
01	Scanning of the ewes	27
02	Gestational sac at different stages of gestation	35-36
03	Placentomes at different stages of gestation	37-38
04	Leg buds at different stages of gestation	39-40
05	Vertebral column at different stages of gestation	41-43

LIST OF ABBREVIATIONS

A-mode	Amplitude mode
B-mode	Bright mode
%	Per cent
Cm	Centimetre
ng/ml	Nano gram/millilitre
MHz	Mega Hertz
mg/dL	Milligram per decilitre
mg/ml	Milligrams/millilitre
v*iz.*	Namely
RIA	Radio immune assay
ELISA	Enzyme linked immune sorbent assay
KVAFSU	Karnataka Veterinary, Animal & Fisheries Sciences University
bpm	Beats per minute
IU	International unit
oPAG	Ovine pregnancy-associated glycoprotein
boPAG	Bovine pregnancy-associated glycoprotein
PSPB	Pregnancy specific protein B
KDa	Kilo Dalton
oPL	ovine placental lactogen
oCS	Ovine chorionic somatommamotrophin

Introduction

I. INTRODUCTION

The intensive sheep production and the wide spread application of the controlled breeding techniques, such as artificial insemination and out-of season breeding increased the need for an accurate early pregnancy diagnosis.

In India, most of the sheep are bred by natural service and are generally unobserved or unrecorded making fertile breeding impossible to determine early detection of pregnancy. Accurate pregnancy diagnosis is essential for effective flock managemental practices such as the culling of non pregnant females and determination of the number of foetuses, the latter being especially important in sheep herds. Such information would allow producers to group animals based on their nutritional needs, so that they are fed required rations during the later stages of pregnancy (Ishwar, 1995). Furthermore, predictions of the number of foetuses would allow appropriate nutritional management of the ewes in late gestation which will prevent pregnancy toxaemia (Ford, 1983 and Doize *et al*., 1997).

Accurate information on the stage of gestation would be useful to dry off lactating females at an appropriate time and to monitor the ewes near term (Gearhart *et al*., 1988). Thus, segregation of the sheep flocks into pregnant and non-pregnant ewes which might reduce reproductive and production losses that occur in form of abortions, stillbirths and production of weak lambs (Wani *et al*., 1998). Accurate estimation of gestational age is also important to avoid size-related problems during pregnancy (Aiumlamai *et al*., 1992; Tapol *et al*., 1995; Karen *et al*., 2001 and Noia *et al*., 2002).

The traditional methods such as non-return to oestrus and abdominal ballottement are not satisfactory, whereas rosette inhibition test and vaginal biopsy are although accurate. Nevertheless, these methods are impractical under farm conditions (Goel and Agrawal, 1992 and Gordon, 1999). Therefore, pregnancy diagnosis based on visualization of the conceptus by ultrasound in the recent past has been used to detect pregnancy status as well as the litter size (White and Russel, 1987), besides it has also been used to measure placental growth (Kelly *et al*., 1987), fetal growth and thoracic girth (Martinez *et al*., 1998). More recently, foetal growth retardation has been detected using ultrasound (Greenwood *et al*., 2002).

B-mode ultrasonography is an accurate, rapid and safe method for diagnosing pregnancy in sheep (Buckrell *et al*., 1986). Transrectal or transabdominal approaches were 100 per cent accurate for pregnancy diagnosis in sheep (Baronet and Vaillancourt, 1989; Bretzlaff *et al*., 1993; Garcia *et al*., 1993 and Haibel, 1990). Transabdominal ultrasonography has been used with great accuracy for pregnancy diagnosis and estimation of fetal number in Sheep (Buckrell, 1988; Garcia *et al*., 1993 and Anwar *et al*., 2008), Goat (Martínez *et al*., 1998 and Gonalez *et al*., 2004), Deer (Revol and Wilson, 1991) and Reindeer (Vahtiala *et al*., 2004).

Generally, scanners with sector, linear and convex probes at frequencies of 3.5 to 7.5 MHz are used for transrectal and transabdominal examinations. Linear probes are better suited for transrectal examinations, while sector probe is preferred for transabdominal examination of pregnancy (Kuplulu *et al*., 2002 and Santiago-Moreno *et al*., 2005).

In India, The informations regarding the pregnancy diagnosis in ewes using transabdominal ultrasonography are few (Mayura *et al*., 2009).Therefore, the present study is an attempt to evaluate pregnancy in ewes using transabdominal ultrasonography with the following objectives**:**

(i) To determine the earliest stage of gestation at which pregnancy could be detected using transabdominal ultrasonography.

(ii) To describe developmental characteristics of foetus up to day 60 of gestation, and

(iii) To assess the chronological changes of the foetal measurements in the pregnant ewe to assess the stage of gestation and feasibility of using ultrasound for determining the foetal number.

Review of Literature

II. REVIEW OF LITERATURE

Traditional methods of pregnancy diagnosis like visual observation, abdominal palpation, service records and non-return to oestrus are not reliable means of diagnosing early pregnancy. Non-return to oestrus following breeding may often suggest pregnancy, but pathological conditions of the uterus or ovaries, physiological anoestrus late in the breeding season and out of breeding season may cause anoestrus in non-pregnant ewes and does. Also non-return to oestrus is an unreliable method when ewes or does are synchronized and bred during the non-breeding season (Ishwar, 1995).

Confirmation of pregnancy at early stage enables the farmers for better nutritional management and marketing as well as timely culling of barren ewes (Bretzlaff *et al*., 1993). The labaroratory method of pregnancy diagnosis includes assay of estrone sulfate (Tsang, 1978 and Refstal *et al.,* 1991); progesterone (Murray and Newstead, 1988); radiography (Barker and Cawley, 1967); vaginal biopsy (Richardson, 1972a) and pregnancy specific antigen (Ruder *et al*., 1988). However, some of the methods are generally not useful and suitable under the field condition, therefore the method of choice depends upon the cost and availability of equipment, number of days post-breeding, desired accuracy and experience of the examiner.

Under field condition, the techniques commonly used includes rectal abdominal palpation (Ott *et al*., 1981); abdominal palpation and ballotment (Pratt and Hopkins, 1975); palpation of the uterus via laparotomy (Smith, 1980); A-scan ultrasonography (Wani, 1981 and Watt *et al*., 1984) and Doppler ultrasonic (Trapp and Slyter, 1983). The real time B-mode ultrasonography is the common and popular method of pregnancy

diagnosis in small ruminants (Davey, 1986; Haibel, 1990; Bretzlaff *et al.,* 1993; Garcia *et al*., 1993; Karen *et al*., 2001; Anwar *et al*., 2008 and Mayura *et al*., 2009).

Methods of diagnosing pregnancy

2.1 Rectal palpation

Rectal abdominal palpation technique for diagnosing pregnancy in the ewe was first described by Hulet (1972). This technique has also been used in the doe (Ott *et al*., 1981). Ewes are off feed overnight prior to the examination and are placed on a laparotomy cradle for examination. An enema with a soap solution is irrigated gently into the rectum. A rounded tip lubricated hollow plastic rod (1.5 *X* 50 cm) is inserted gently into the rectum to a depth of 30 to 35 cm. The free hand is placed on the posterior abdomen while the rod is manipulated with other hand. The rod is moved up and down and from side to side until an obstruction is encountered and palpated against the abdominal wall or a decision is reached that the ewe or doe is not pregnant (Hulet, 1972). The method is about 97 per cent accurate at 60 days post-mating. Accuracy is greater for single than multiple foetuses.

At the early stage of pregnancy, the sensitivity of the technique for diagnosing pregnancy was low but increases with progress of the pregnancy reaching the highest accuracy (100 per cent) at Days 85 to 109 after mating (Hulet, 1972 and Chauhan *et al*., 1991). In contrast, other studies (Tyrrell and Plant, 1979 and Trapp and Slyter, 1983) reported a lower sensitivity and specificity at Days 60 to 96 after mating with this method.

Though rectal abdominal palpation is simple, quick, accurate and inexpensive, it had a low accuracy in diagnosing multiple foetuses and more hazardous with respect to rectal injury and abortion (Hulet, 1972; Turner and Hindson, 1975; Shelton, 1978; Tyrrell and Plant 1979; Memon and Ott, 1980; Ott *et al*., 1981 and Ishwar, 1995). Therefore, this technique is not a suitable method for diagnosing early pregnancy in sheep.

2.2. Radiography

The technique of radiography is used to detect pregnancy and multiple births with an accuracy of 90 per cent or more, provided ewes are examined later than Day 90 of gestation (Ford *et al*., 1963). In the smaller breeds of ewes this technique could provide an accuracy of 100 per cent at 71 days after breeding (Ford *et al*., 1963).

The foetal skeleton is often radio-opaque after 65 days of gestation. Uterine enlargement suggestive of pregnancy may be detected earlier than this but cannot be differentiated from hydrometra or pyometra. To obviate repeated examinations, it appears that Day 70 after breeding would be a suitable time to predict 100 per cent accuracy in pregnancy detection and foetal count by radiographic examination (Barker and Cawley, 1967). Although this technique is fairly accurate for diagnosing pregnancy but is not practical for examining large number of ewes in the field condition and may be useful for an individual animal when ultrasound equipment is not available. The cost of the equipment and the potential health hazard to the operator may limit its use (West, 1986).

2.3. Vaginal biopsy

Histological evaluation of vaginal biopsies has an accuracy of 97 per cent for diagnosing pregnancy in ewes pregnant for more than 40 days (Richardson, 1972a). The pregnant ewe's vaginal mucosal cells and nuclei were half the size of those in non-pregnant animals, which have polygonal and squamous cells in more than 10 layers.

In pregnant ewes, vaginal epithelium has fewer layers of cells that are usually columnar, cuboidal and primordial. The samples for the biopsy must be taken from the anterior vagina. The correct interpretation of vaginal biopsies from non-pregnant ewes is only 81 per cent (Richardson, 1972a). This method gives no indication of multiple pregnancies. Accuracy is high but the procedure is not practical for field use because of the time and expense involved in obtaining, processing and examining biopsy specimen.

2.4. Palpation of uterus via laparotomy

The gravid uterus can be palpated directly through a small incision in the abdominal wall. Accuracy of direct palpation of the uterus for pregnancy diagnosis was 92 per cent in ewes 4-5 weeks of pregnancy (Hulet and Foote, 1968) whereas in does it approaches 100 per cent after 42 days of gestation (Smith, 1980). At 4-5 weeks post-breeding the uterine horns appears distended and after 6 weeks post-breeding, cotyledons become obvious and the horns are 5-10 cm in diameter.

A small ventral paramedian incision is made large enough to permit entrance of 2-3 fingers just cranial to the udder. An enlarged fluid filled, thin-walled uterus is taken as positive evidence of pregnancy. To prevent infection, aseptic technique is necessary but

is not suitable under the field condition to diagnose pregnancy at large scale (Smith, 1980).

2.5. Abdominal palpation and ballotment

Ewes and does in the late stages of pregnancy can be examined by these techniques. It becomes easier and more reliable with the advancement of gestation but is not suitable for early pregnancy diagnosis. It is easier in thin ewes and does than in fat ewes and does. Pratt and Hopkins (1975) reported an accuracy of 80 to 90 per cent in ewes at 90-130 days of pregnancy.

The gravid uterus or foetus is often palpated through the relaxed abdominal wall by placing a palm on either side of the abdomen and squeezing or lifting upwards. Foetus can be balloted low in the right flank during the last month of gestation in some ewes. Withholding feed and water for at least 12 hour before examination increases the ease of the examination (Pratt and Hopkins, 1975).

The technique of bimanual palpation of small ruminants was developed by Kutty and Sudarsanan (1996). This method includes digital palpation per rectum combined with abdominal manipulation. By using this technique, pregnant ewes were accurately diagnosed based on enlarged cervix, prepubic position of the uterus, palpation of placentomes and/or fetal parts, asymmetry and/or marked distension of uterine horns (Kutty, 1999).

2.6. Hormone assay

Measurement of concentrations of steroid hormones such as progesterone and estrone sulfate at specific time of post-breeding is yet another method of pregnancy diagnosis in small ruminants (Tsang, 1978; Tamanini *et al*., 1986; Worsfold *et al*., 1986; Murray and Newstead, 1988 and Refstal *et al*., 1991). Radioimmunoassay (RIA) and Enzyme linked immune sorbent assay (ELISA) is one of the sensitive tests to detect these hormones in the blood, milk and urine of pregnant ewes.

2.6.1. Progesterone test

Measurement of concentration of progesterone in blood and milk is one of the method for diagnosing pregnancy but is expensive and time consuming. Concentration of plasma progesterone is determined 18 days post-breeding in ewes (Thimonier *et al*., 1977; Tsang, 1978; Dobeli and Schwander, 1985 and McPhee and Tiberghien, 1987). Concentration of plasma progesterone measured in ewes by Thimonier *et al*., (1977) on Day 18 post-breeding showed that all females diagnosed non-pregnant did not lamb, while 83.5 per cent of those diagnosed pregnant did lamb. Accuracy of diagnosing pregnancy and non-pregnancy by measuring the plasma progesterone concentration was 85.7 per cent and l00 per cent, respectively (Thibier *et al*., 1982). Serum progesterone concentration was significantly higher in ewes carrying twins (19.2 ng/ml) and triplet (29.9 ng/ml) than those carrying single foetus (9.2 ng/ml) (Chauhan and Waziri, 1991).

Milk progesterone concentration in the ewes reflects plasma concentration. However, concentration of progesterone in milk was higher in pregnant ewes (Holdsworth and Davies, 1979; Thibier *et al*., 1982; Ozsar *et al*., 1984 and Murray and

Newstead, 1988). Milk progesterone concentration above 10 ng/ ml between 22 and 26 days after breeding was considered as positive for pregnancy with an accuracy of 85.9 per cent and 100 per cent for detecting pregnancy and non-pregnancy in ewes (Holdsworth and Davies, 1979). A concentration of 7.25 ng /ml or more is an indication of pregnancy between Days 19 and 27 post-breeding (Jain *et al,.* 1980). However, milk progesterone concentration varied from day to day, type of milk sample obtained and plasma concentrations of progesterone tend to be more accurate than milk (Bretzlaff *et al*,, 1989). In the pregnant ewes, the plasma progesterone concentration was 4.81 while it was 1.41 ng /ml in ewes, which were mated but failed to conceive 15 days after breeding and serum or plasma tends to give more reliable results than milk (MacDonnell, 1976).

Progesterone test in ewe and doe is a sensitive test for non-pregnancy but is only a fair test for pregnancy. Since elevated progesterone levels indicate the presence of a functional corpus luteum, several conditions like hydrometra, pyometra or early embryonic death may increase the luteal life span and may give false positive results (Thimonier *et al*., 1977). Regarding the fetal sex, the plasma progesterone concentrations of ewes giving birth to male and female lambs were not significantly different (Kalkan *et al*., 1996).

2.6.2. Estrone sulfate

The presence of a viable foeto-placental unit is accompanied by an increase in estrone sulphate concentrations in the peripheral plasma of ewes. Esterone sulfate was detected in sheep plasma from around 70 days after conception (Tsang, 1978), whereas in does 40-50 days post-breeding (Refstal *et al*., 1991). Enzyme-Linked Immune Sorbent

Assay (ELISA) can be used for measuring concentrations of estrone sulfate in milk as an aid to diagnose pregnancy (Murray and Newstead, 1988). The accuracy of diagnosing pregnancy and non-pregnancy was 82 per cent and 83 per cent, respectively. Estrone sulphate was detectable around Day 70 of gestation with value ranging between 0.1 to 0.7 ng/ml, then its level increased steadily till 2 days before parturition when an upsurge was seen (15-50 ng/ml) (Tsang, 1978). On Day 85 of gestation, there was a significant difference in the level of estrone sulphate between pregnant and non-pregnant ewes. However, due to considerable variation of the hormone levels between individuals, the accuracy for detection of non-pregnancy was only 44 per cent while for detection of pregnancy was 87.9 per cent using the cut-off value of 0.1 ng/ml (Worsfold *et al*., 1986). ELISA test is useful for the measurement of serum estrone sulphate concentrations with optimal accuracy for pregnancy diagnosis between Days 30 to 35 of gestation in ewes (Illera *et al*., 2000).

The concentration of serum estrone sulphate was significantly higher in ewes carrying multiple than those carrying single foetus from Day 80 to 124 of gestation (Illera *et al*., 2000). However, the concentration of estrone sulphate in ovine blood is not reliable for prediction of foetal numbers because of the high variation among individuals (Worsfold *et al*., 1986).

2.6.3. Ovine chorionic somatommamotrophin (oCS) or ovine placental lactogen (oPL)

Ovine placental lactogen (oPL) was studied and purified for pregnancy diagnosis (Chan *et al*., 1978). RIA of oPL achieved 97 per cent and 100 per cent accuracy for

diagnosing pregnant and non-pregnant ewes at Day 64 of gestation, respectively (Robertson et *al*., 1980). Owing to expensive, laborious and time consuming nature of technique, it is also not suitable for field conditions for pregnancy diagnosis.

2.7. Pregnancy proteins

2.7.1. Pregnancy-specific protein B (PSPB)

Pregnancy-specific protein B (PSPB) was first detected in the bovine placenta (Butler *et al*., 1982) and is secreted by binucleate cells of foetal trophoectoderm (Eckblad *et al*., 1985). The physiological role of PSPB during pregnancy is thought to maintain the corpus luteum by stimulating prostaglandin E2 production (Vecchio *et al*., 1995).

Measurements of bovine PSPB by RIA can accurately detect pregnancy (100 per cent) and non-pregnancy (83 per cent) in sheep from Day 26 to 106 of gestation (Butler *et al*., 1982). Willard *et al*., (1987) developed a quantitative RIA test for the measurements of ovine pregnancy specific protein B (oPSPB). oPSPB was detected at 19 (Willard *et al*., 1987; 1995) and 21 days post mating (Wallace *et al*., 1997). Further, it increased steadily until Day 30 and the concentration remained stable up to 20 days prepartum (Willard *et al*., 1995). The concentration dropped rapidly following lambing and it was detected at 12 days (Willard *et al*., 1995) and 3 weeks postpartum (Willard *et al*., 1987).

The accuracy for detecting ewes carrying single and twin lambs was 71 per cent and 81 per cent, respectively, from Day 60 to 120 of gestation (Willard *et al*., 1995). Nevertheless, oPSPB concentrations were not influenced by the sex of the foetus (Wallace *et al*., 1997).

PSPB is one of the useful marker of placental development and function and may provide a reliable indicator of fetal distress and adverse pregnancy outcome. Between Day 50 and100 of gestation, PSPB concentrations were positively correlated with placental weight. In addition, the mass of the foetus in ewes that aborted during late pregnancy was highly correlated with PSPB concentrations up to Day 120 of gestation (Wallace *et al*., 1997).

2.7.2 Ovine pregnancy-associated glycoproteins (oPAGs)

Ovine pregnancy-associated glycoproteins (oPAGs) are synthesized by binucleate cells of trophoblast, and belong to aspartic proteinase family (Xie *et al*., 1991) and most of them are without enzyme activity (Xie *et al*., 1997) with molecular weights between 43 to 67 kDa (Zoli *et al*., 1995 and Xie *et al*. 1997).

The concentration of oPAG in Churra and Merino ewes was detectable in 20 out of 30 ewes at Week 3 and in all ewes by Week 4 after mating (Ranilla *et al*., 1994). The concentration of oPAG increased slowly from 3 to 9 weeks of gestation. Thereafter, plasma profiles of oPAG varied among sheep breeds from week 9 till week17. After lambing, the oPAG levels decreased rapidly reaching the basal value at fourth week postpartum (Ranilla *et al*., 1994, 1997 and Gajewski *et al*., 1999). The concentration of oPAG was influenced by the fetal numbers and the sex of the foetus (Ranilla *et al*., 1994).

Ewes carrying twin foetuses had higher mean oPAG concentrations than those carrying a single foetus from 12 week of gestation to lambing. The variation in the oPAG

concentration was significant at 21 week (Ranilla *et al*., 1997) and ewes carrying male foetuses had higher oPAG concentrations than those carrying female foetuses at Weeks 19, 20 and 21 of gestation (Ranilla *et al*., 1994). Although bPAG and cPAG have been successfully used for detecting pregnancy in cattle (Zoli *et al*., 1992 and Szenci *et al*., 1998) and goats, respectively (Folch *et al.,* 1993 and Gonzalez *et al*., 1999) and there is no data evaluating the accuracy of oPAG assays for diagnosing pregnancy in sheep.

2.8. Ultrasonic techniques

Ultrasonic techniques can be used to examine subsurface structures in living tissues by use of A-scan, B-scan or Doppler techniques. Ultrasound is reflected from moving tissues, such as blood at a slightly changed frequency. One of the most important features of ultrasound, when used for tissue examination, is its safety to the patient and operator (Bishop, 1966). Pregnancy may be detected in ewes and does with all three types of ultrasound techniques.

1. Amplitude depth (A-scan) Ultrasonic techniques,
2. Doppler Ultrasonic techniques,
3. Real time B-scan ultrasonic techniques.

All the above techniques of ultrasound are used under field conditions but the accuracy of diagnosis, timing of examination, foetal numbers and age and foetal viability vary considerably among these techniques. However, real-time ultrasonic techniques are most commonly used in the field conditions.

2.8.1 A – SCAN ULTRASONIC TECHNIQUES

Principles of echo amplitude or amplitude depth versus time ultrasound technique for diagnosis of pregnancy is based on detection of fluid- filled uterus. A-scan units emit ultrasonic waves from a hand-held transducer placed externally against the skin of the abdomen and directed toward the uterus. Ultrasound waves are reflected between different tissues and reflect back to the transducers and converted to electrical energy in the form of audible or visual signals. These units are sensitive at a depth of 10 to 20 cm. The transducer is placed on the lower right flank and in front of the udder of the standing ewe.

Wool of the area should be clipped on the right flank and in front of the udder to facilitate optimal contact. A coupling agent ultrasonic gel or vegetable oil is applied to the transducers to eliminate air spaces between the skin and transducer head. A light or audible signal is emitted by the unit or characteristic blip patterns displayed on an oscilloscope when a fluid filled structure is detected. A-scan ultrasound applied to the flank region has proven to be reasonably reliable from 50 to 120 days of gestation in sheep and goats (Wani, 1981 and Watt *et al*., 1984). An extended urinary bladder, hydrometra or pyometra may often give false positives. False negatives results may occur in early gestation or in late gestation because of decrease in the ratio of uterine fluid to foetal tissue (Watt *et al*., 1984). Neither fetal viability nor the foetal numbers are detected by this method.

Early works with externally applied A-scan ultrasound has indicated an accuracy of at least 95 per cent between 60 and 80 days of gestation in ewes (Haibel, 1990).

However, Meredith and Madani (1980) reported that a positive diagnosis of pregnancy in ewes could be accomplished with an accuracy of 83 per cent 61 to 151 days after mating. Lindahl (1969a,b) reported that the earliest time at which pregnancy could be detected by using A-scan is between 40 and 50 days after mating. However, this A-scan technique is of particular importance in isolated areas where transport or electricity may not be available.

2.8.2. DOPPLER ULTRASONICS

The principles involved in Doppler ultrasonics for diagnosis of pregnancy include detection of fetal heart beat, fetal circulation and fetal movements. Callagan *et al*., (1964) introduced the Doppler technique for diagnosis of pregnancy in humans. Robertson *et al*., (1980b) were the first to apply it to sheep. It detects maternal fetal tissue interfaces (Wani, 1981; Trapp and Slyter, 1983 and Watt *et al*., 1984).

Doppler ultrasonography can be used to assess pregnancy between 140 to 160 days in 5 minutes (Fukui *et al.,* 1984). Shone and Fricker (1969) used the same type of machine on 309 ewes between 66 and 122 days of gestation and recorded 100 per cent accuracy at all stages of pregnancy. Foetal blood from the umbilical artery was the most common diagnostic feature. The foetal heart beat, foetal pulse which is faster than maternal pulse or foetal movement were taken as positive criteria of pregnancy and it could diagnose multiple pregnancies with an acceptable accuracy, but fail to distinguish between ewes carrying twins, triplets, or more than three foetuses (Lindahl, 1969b).

External application of the ultrasonic Doppler has been used for detection of pregnancy in ewes and approaches an accuracy of 100 per cent during the last half of

gestation (Fraser and Robertson, 1968; Lindahl, 1968; Keane, 1969 and Shone and Fricker, 1969). However, it was not effective at 50 days or earlier (Lindahl, 1969a, b, 1971). Due to failure to detect pregnancy before 50 days and cost of the equipments, it is rarely use to diagnose the pregnancy in ewes under field conditions.

2.8.2.1. DOPPLER ULTRASONIC TECHNIQUES – RECTAL

The intra-rectal Doppler technique is superior to the external technique when used early in the second trimester for diagnosing pregnancy with an accuracy of greater than 90 per cent. It may also be used 25 to 30 days post-breeding but false negatives occur. Therefore, it is best to use between 35 to 40 days of gestation (Lindahl, 1971 and Ott *et al*., 1981). With Doppler technique, fetal viability can be detected but accuracy of detection of multiple foetuses is difficult. Compared to A-scan technique, the Doppler technique resulted in greater accuracy in ewes, which were at least 65 days pregnant (Lindahl, 1969b). The intra-rectal Doppler technique also allowed detection of pregnancy earlier in gestation than the A-scan technique (Lindahl, 1971).

2.8.3 B- SCAN ULTRASONIC TECHNIQUES

Real-time B-mode ultrasonography was first introduced to veterinary practice for early pregnancy diagnosis (Taveme, 1984). B-mode real time ultrasonography is one of the reliable method for diagnosing pregnancy and to estimate fetal numbers in small ruminants (Dawson *et al*., 1994; De Bulnes *et al*., 1998; Anwar *et al*., 2008 and Karen *et al*., 2009). Transrectal or transabdominal B-mode real time ultrasonography has been used to predict gestational age by means of fetal measurements in small ruminants by numerous researchers and foetal measurements used in those studies were biparietal

diameter (Aiumlamai *et al*., 1992; De Bulnes *et al*., 1998; Greenwood *et al*., 2002 and Karen *et al*., 2009), trunk diameter (De Bulnes *et al*., 1998; and Karen *et al*., 2009), crown-rump length (De Bulnes *et al*., 1998), fetal heart diameter (Parraguez *et al*., 2000 and Karen *et al*., 2009) and metacarpus length (Greenwood *et al*., 2002). The relationship between placentome size and gestational stage was investigated by Doize *et al*., (1997) and Karen *et al*., (2009). According to results of these studies, gestation age of ewes or does was accurately estimated by foetal ultrasound measurements. Multiple regression equations developed from measurement of biparietal diameter and metacarpal bone length could provide further improvement in prediction of gestational age (Greenwood *et al*., 2002).

Until recently there were no satisfactory techniques for determining foetal numbers in ewes and does. The measurement of blood metabolites and hormones concentrations was not sufficiently accurate, and the need for laboratory analysis precludes immediate results. Real-time, B-scope ultrasonic scanning offers an accurate, rapid, safe and practical means of diagnosing pregnancy and determining fetal numbers. It produces a moving image of the uterus, foetal fluids, foetus, foetal heart beat and placentomes (Karen *et al*., 2001).

2.8.3.1. BY TRANSRECTAL METHOD

By using transrectal ultrasonography (7.5 MHz), embryonic vesicle of the pregnant ewe was identified at Day 12 after mating, while the first visualization of the embryo was accomplished at Day 19 (Gonzalez *et al*., 1998) or Day 20 (Schrick and Inskeep, 1993). By using 5 MHz transrectal probe, the first signs of pregnancy in form of

circular and elongated anechoic images located in utero cranial to bladder were observed in ewe at Days 17 to 19 (Garcia *et al*., 1993 and Doize *et al*., 1997), while embryo could be detected at Day 25 after mating (Buckrell *et al*., 1986).

The specificity of 7.5 MHz transrectal ultrasonography to detect non-pregnancy was low during the first two months of gestation (Schrick and Inskeep, 1993). The false positive diagnoses were attributed to embryonic or fetal death and the sensitivity of 5 MHz transrectal ultrasonography for detecting pregnant ewes was greatly variable (12 per cent to 98.7 per cent) at less than Day 25 of gestation (Gearhart *et al*., 1988). Thereafter, the sensitivity increased with the progression of pregnancy and the accuracy was ranged between 65 per cent and 87 per cent at Days 25 to 50, depending on the breed, age and parity of the ewes, experience of the operator and the technique of the examination (Buckrell *et al*., 1986; Gearhart *et al*., 1988 and Garcia *et al*., 1993).

Trans-rectal techniques have relatively poor applicability owing to care and labour connected to faecal repletion and rectal brittleness (Kaulfuss *et al*., 1996; Gonzalez *et al*., 1998; Karen *et al*., 2001; Padilla-Rivas *et al*., 2004 and Viñoles *et al*., 2004).

2.8.3.2. BY TRANSABDOMINAL METHOD

By using transabdominal approach, pregnancy was first verified at Day 25 (Gearhart *et al.,* 1988) or Day 30 after breeding (Bretzlaff *et al*., 1993a). The sensitivity and specificity of the technique were high after Day 29 (Taverne *et al*., 1985) reaching nearly 100 per cent from Days 46 to 106 of gestation (White *et al*., 1984; Fowler and Wilkins 1984; Davey 1986; Gearhart *et al*., 1988; Anwar *et al*.,2008 and Mayura *et*

al.,2009). However, the lower specificity was lower at Days less than 40 to 100 after mating (Logue *et al*., 1987 and Anwar *et al*., 2008). Transabdominal scanning is similar in ewe or doe. The scanning is performed on the standing or dorsal recumbent ewe and doe. The lubricated transducer head is placed against the clipped skin in the inguinal region across the abdomen cranial to the pelvic brim, on the sides of udder.

The ideal time for transabdominal scanning is between 40 to 75 days of gestation, when the pregnant uterus is lying against the right body wall. Real-time ultrasound system used transabdominally in ewes was reliable to determine pregnancy and fetal numbers from 50 days after breeding (Fowler and Wilkins, 1984; White *et al*., 1984; Davey, 1986 and Anwar *et al*., 2008). In case of negative transabdominal scan at 35 days or less, a transrectal scan may be performed to confirm. The lubricated transducer is inserted into the rectum and slowly rotated from side to side. Faeces should be removed, wiped or cleaned from the transducer, otherwise it may give a poor-quality image or no image.

Pregnancy is confirmed by imaging fluid in the uterine lumen, by finding evidence of placentomes or by identification of one or more foetuses (White and Russel, 1984 and Buckrell *et al*., 1986). The foetus and foetal heart beat were frequently visible after Day 25. Fetal viability can be assessed by visualizing fetal movement or the fetal heart beat during real time imaging. Placentomes were routinely found by Day 26-28 of post-breeding and appear like echogenic densities in the uterine wall (Buckrell *et al*., 1986). The optimal time for counting fetal numbers accomplished between 45 and 90 days of gestation (White and Russel, 1984; Wilkins and Fowler, 1984 and Haibel, 1990).

After 90-100 days of gestation, foetuses become too large to be consistently differentiated from each other and twins can be more accurately diagnosed than triplets.

Another advantage of real-time ultrasonics is that it can distinguish a pregnancy from hydrometra, pyometra and foetal mummification (Haibel, 1990). Foetal age in ewe and doe was determined by the use of real-time ultrasonics at 40-100 days of gestation by measuring width of the foetal skull (Haibel, 1988; Reichle and Haibel, 1999 and Anwar *et al*., 2008). This technique was helpful in predicting parturition date when the actual date of breeding is not known. Trans-abdominal echotomography actually represents the best available method for a sure, easy and reliable pregnancy diagnosis in the sheep (Haibel, 1988; Doizè *et al*., 1997 and Garcia *et al*., 1993).

Real-time ultrasonography for diagnosing pregnancy in ewes and does can be rapidly learned and experienced examiner can detect pregnancy with of 91-100 per cent accuracy (White *et al*., 1984; Logue *et al*., 1987 and Buckrell, 1988). Rarely, false positives results were obtained which may be due to early embryonic death or unobserved abortion or sometimes misinterpreting the urinary bladder as the uterus (White *et al*., 1984; Fowler and Wilkins, 1984 and Haibel, 1990). False negatives may often meet with failure to image the tract early in gestation or operator inexperience (White *et al*., 1984 and Haibel, 1990). In addition to assess fetal viability, numbers and age could be assessed by real-time ultrasonography besides it is helpful in diagnosing diseases of the reproductive tract (Buckrell, 1988).

2.9. Recommendations for the diagnosis of pregnancy by ultrasonography

Following are the recommendations for pregnancy diagnosis using ultrasound (Buckrell, 1988).

1. Feed and water should be withheld for 12 hour prior to diagnosis;
2. Rectal scanning should be avoided unless early diagnosis is essential, in which it is better to use 5 MHz head from Day 25;
3. For trans-abdominal scanning, the 5 MHz head should be placed high in the fleece-less groin, close to the udder;
4. Any ewe and doe diagnosed as non-pregnant by rectal scan should also be tested high in the groin to avoid false negatives;
5. Does versus ewe's early diagnosis**:** does are often less co-operative than ewes when rectal probes are inserted. Transabdominal diagnosis of Days 25-30 is easy and accurate when using a 5 MHz head in the standing doe;
6. Fetal counts are made on standing ewes or does, 20 cm above the udder by the use of a 5 MHz head from Day 40-50 and a 3 MHz head from Day 50-100;
7. Late term diagnosis of pregnancy and fetal monitoring is recommended with a 3 MHz head on the clipped ventral abdomen.

Materials and Methods

II. MATERIALS AND METHODS

3.1 Experimental animals

The present study was conducted on twenty ewes of Bannur breed which were diagnosed as pregnant and being maintained at the Department of Instructional Livestock Farm Complex, KVAFSU, Veterinary College, Bangalore and ten ewes which were found pregnant when presented for pregnancy diagnosis at the Department of Gynaecology & Obstetrics, Veterinary College, Bangalore. The animals being maintained at the Department of Instructional Livestock Farm Complex were maintained in semi intensive rearing system with proper breeding records and mating being carried out under strict veterinary supervision. The ewes in the farm were left with breeding rams which were marked with colour on the brisket to identify the ewes in oestrus and breeding during the night time. Breeding was carried out with two fertile rams starting from the last week of November 2010 till second week of January 2011. The ewes mated by the rams were identified by their marking on the rump region. The marked ewes were subjected for pregnancy diagnosis by ultrasound scanning after 25 days of mating. All the ewes found pregnant on day 25 of scanning were repeatedly examined once in five days till 60 days of gestation and then once a week till lambing. The ewes diagnosed as pregnant at the Department of Gynaecology & Obstetrics, Veterinary College, Bangalore were included only if the proper breeding dates were available with the owner and the owners willing to present their ewes for further follow up.

3.2 Ultrasonography

A real time B-mode ultrasound scanner equipped with a 3.5 and 5 MHz sector probe (Honda -Co., Ltd., Japan) was used for scanning. Scanning was performed after shaving the inguinal region, in front and on either side of udder of the animal. The animal was lightly restrained by one person in standing position and also in dorsal recumbent position (Plate 1A). One of the hind legs of the ewe was folded up at the time of scanning for proper placement of the probe (Plate 1B). An ultrasound coupling gel was applied each time to the probe to develop good contact and to remove air between probe and animal skin. The veterinary ultrasound machine used in the present study has been provided with software for determining the stage of pregnancy in ewes on the basis of biparietal head diameter of foetus.

Each animal was subjected for scanning for at least five minutes & following observations were recorded for pregnancy diagnosis.

3.2.1 Determination of pregnancy

3.2.1A-Gestational sac

Gestational sac appears as circular and elongated anechoic areas located cranial to the bladder at Day 25 (Gearhart *et al.,* 1988) or Day 30 after breeding (Bretzlaff *et al*., 1993).

3.2.2 B-Foetal heart rate

By using Transabdominal ultrasonography, the rhythmic pulsations within the ovine foetal heart was measured (Aiumlamai *et al*., 1992 and Hasan, *et al*., 2007)

3.2.3 C-Placentomes

Placentomes were detected by transabdominal ultrasonography for the diagnosis of pregnancy in sheep (Doize *et al*., 1997 and Gonzalez *et al*., 1998).

3.2.4 D- Leg buds

Leg buds were detected by using the transabdominal ultrasonography method (Anwar *et al.*, 2008).

3.2.1 E- Vertebral Column

Pregnancy was also detected by locating the vertebral column of the ovine foetus (Anwar *et al.*, 2008).

3.2.2 Estimation of gestational age

When the date of mating is unknown, monitoring fetal development allows estimation of gestational age. An attempt was made to predict the stage of gestation on the basis of head diameter.

3.2.2 A-Foetal head diameters

For the purpose of estimation of the gestational age, biparietal diameter of foetal head was obtained and the ultrasound machine used in the present study would provide the gestational age in days on the basis of foetal head diameter. In every animal, atleast three readings were obtained and the average gestational age was determined.

3.3 Determination of the foetal number

An attempt was made to determine the foetal number on the basis of appearance of foetal head by using transabdominal ultrasonography (3.5 MHz).

PLATE 1

Plate 1A: Scanning of the ewe in dorsal recumbency

Plate 1B: Scanning of the ewe in standing position

Results

IV. RESULTS

The present study was conducted to diagnose pregnancy in ewes using the real time B- mode ultrasonography by taking the following criteria or chronological changes, which are as follows;

1. Gestational sac

In the present study, gestational sac was detected between 25-30 days in 5 out of 30 ewes with the accuracy of 16.6 per cent. The presence of anechoic areas cranial to the bladder was considered as positive for pregnancy (Table 1, Plate 2, and Fig. 1).

2. Heart beat

In the present investigation the heart beat could be detected for the first time at 31-35 days with accuracy of 36.6 per cent. The accuracy of pregnancy detection by heart beat was 60, 86.6 and 100 per cent respectively at 36-40, 41-45 and 46-50 days after mating (Table 2 and Fig. 2).

3. Placentomes

No placentomes were detected at 25-30 days after the mating. But, it was detected in eleven out of thirty ewes (36.6 percent) as small circular, concave structures between 31-35 days. The frequency of detection of placentomes increased with the increase in gestation age and the percentage accuracy of detecting pregnancy was 53.3, 80.00, and 100 per cent between 36-40 days and 41-45 46-50 days after mating respectively (Table 3, Plate 3, and Fig. 3).

Table 1. Percentage of pregnant and non pregnant ewes based on observation of gestational sac

25-30Days after mating	No. Of observations	Percentage
Gestational sac observed	5	16.6%
Gestational sac not observed	25	84.4%
Total	30	100%

Table 2. Percentage of pregnant and non pregnant ewes based on foetal heartbeat

Days after mating	Heart beat observed	Heartbeat not observed	Total
25-30	0%(0)	100% (30)	30
31-35	36.6% (11)	63.3%(19)	30
36-40	60% (18)	40% (12)	30
41-45	86.6% (26)	13.3% (04)	30
46-50	100%(30)	0% (0)	30
51-55	-	-	-
55-60	-	-	-

Table 3. Percentage of pregnant and non pregnant ewes based on placentomes

Days after mating	Placentomes observed	Placentomes not observed	Total
25-30	0%(0)	100%(30)	30
31-35	36.6% (11)	63.3% (19)	30
36-40	53.3%(16)	46.4% (14)	30
41-45	80.0% (24)	20.0% (06)	30
46-50	100.0% (30)	0% (0)	30
51-55	-	-	-
55-60	-	-	-

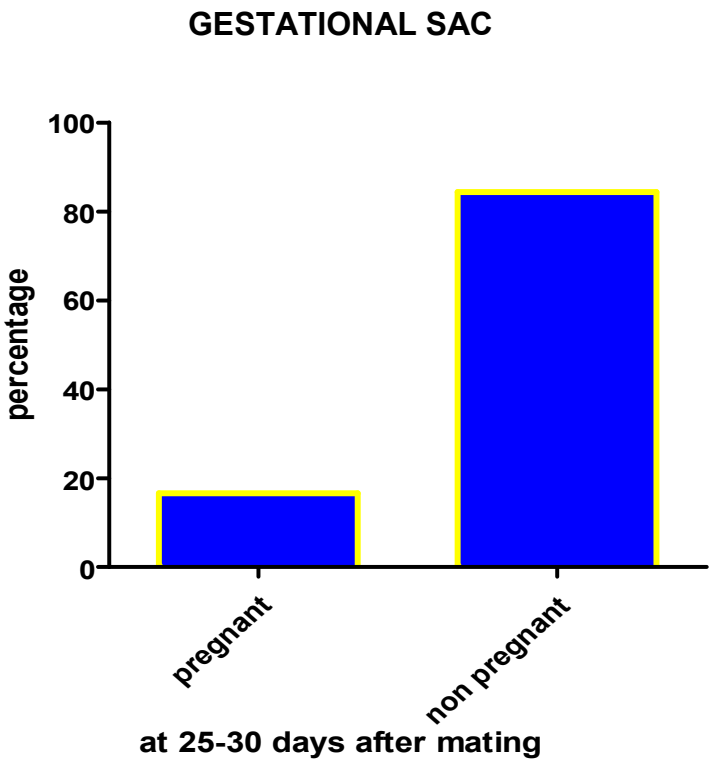

Fig. 1: Percentage of pregnant and non pregnant ewes on the basis of gestational sac

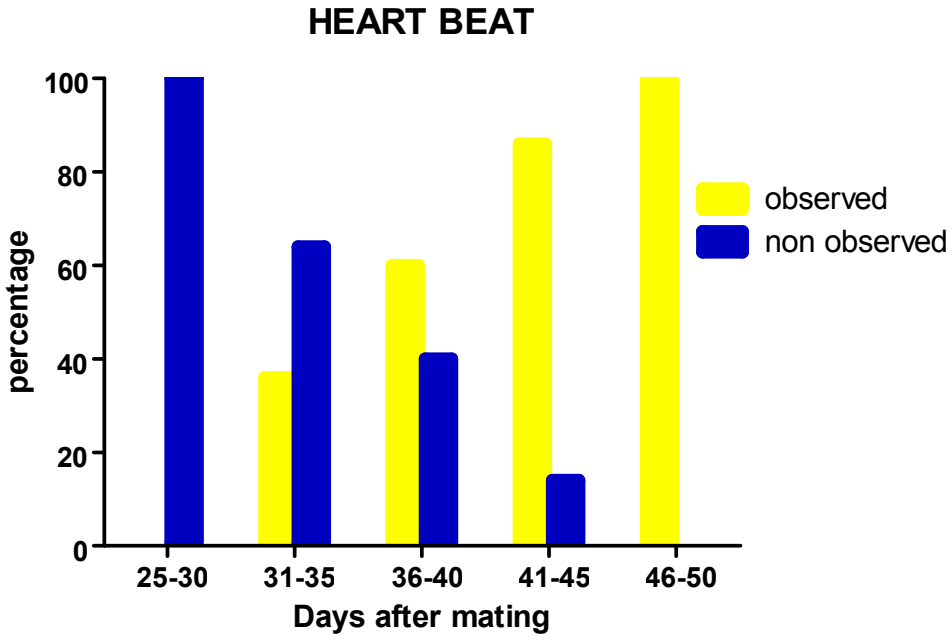

Fig. 2: Percentage of pregnant and non pregnant ewes on the basis of foetal heart beats

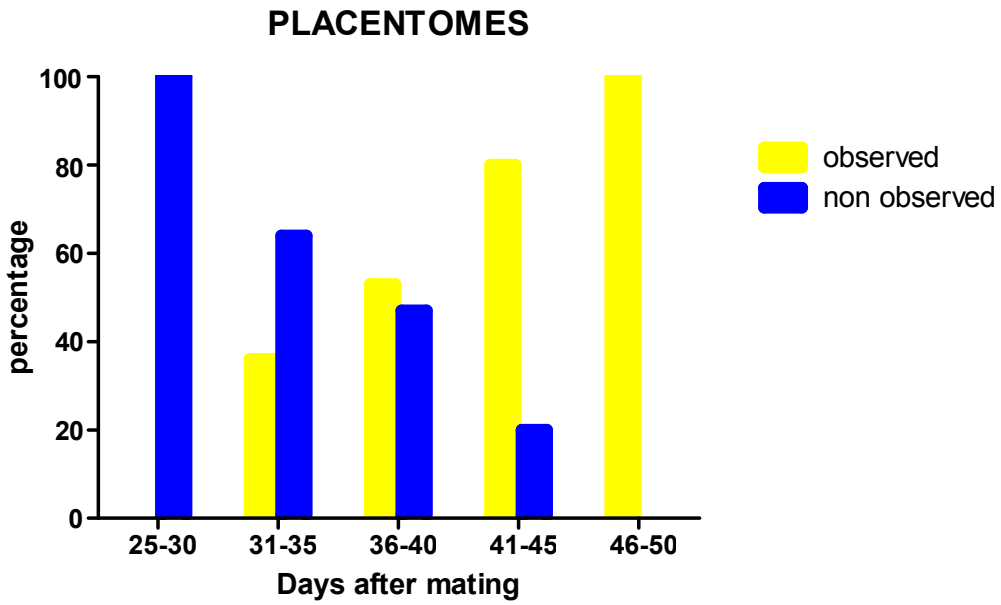

Fig. 3: Percentage of pregnant and non pregnant ewes on the basis of placentomes

4. Leg buds

Leg buds could not be detected till 36-40 days after the mating. However, the detection of leg buds was first observed at 36-40 days in seven ewes (23.3 per cent). The accuracy of pregnancy by detection of leg buds was 56.6, 76.6 and 100 per cent between 41-45, 46-50 and 51-55 days respectively (Table 4, Plate 4 and Fig. 4).

5. Vertebral column

The vertebral column was detected in six ewes out of thirty with accuracy of 20 per cent at 36-40 days after the mating. The percentage of pregnancy was increased with the advancement of gestation and was 46.6, 66.6, 80 and 100 per cent at 41-45, 46-50, 51-55 and 56-60 days of pregnancy respectively (Table 5, Plate 5 and Fig. 5).

6. Foetal number

In the present study, in all the animals subjected to the ultrasound scanning for the determination of the foetal number, a single gestational sac or single foetal head was visualised suggesting the absence of twining or triplets. In subsequent scanning and also on lambing, the presence of single foetus was confirmed.

7. Determination of Gestational age

The gestational age was determined on the basis of foetal head diameter and the approximate lambing date was determined as 150 days for Bannur breed. Gestational age calculated on the basis of foetal head diameter measurements. Further, every animal was closely followed to determine if the lambing occurred around the predicted date of lambing. In the present study, however, none of the ewes delivered around the predicted day of lambing and all the animals delivered between 13 to 17 days beyond the expected day of lambing.

Table 4. Percentage of pregnant and non pregnant ewes based on observation of leg buds

Days after mating	Leg bud observed	Leg bud not observed	Total
25-30	0%(0)	100%(30)	30
31-35	0%(0)	100%(30)	30
36-40	23.3%(7)	76.6%(23)	30
41-45	56.6%(17)	43.3%(13)	30
46- 50	76.6%(23)	23.3%(07)	30
51-55	100%(30)	0%(0)	30
55-60	-	-	-

Table 5. Percentage of pregnant and non pregnant ewes based on observation of vertebral column

Days after mating	Vertebral column observed	Vertebral column not observed	Total
25-30	0%(0)	100%(30)	30
31-35	0%(0)	100%(30)	30
36-40	20%(06)	80%(24)	30
41-45	46.6%(14)	53.3%(16)	30
46-50	66.6%(20)	33.3%(10)	30
51-55	80%(24)	20%(06)	30
56-60	100%(30)	0%(0)	30

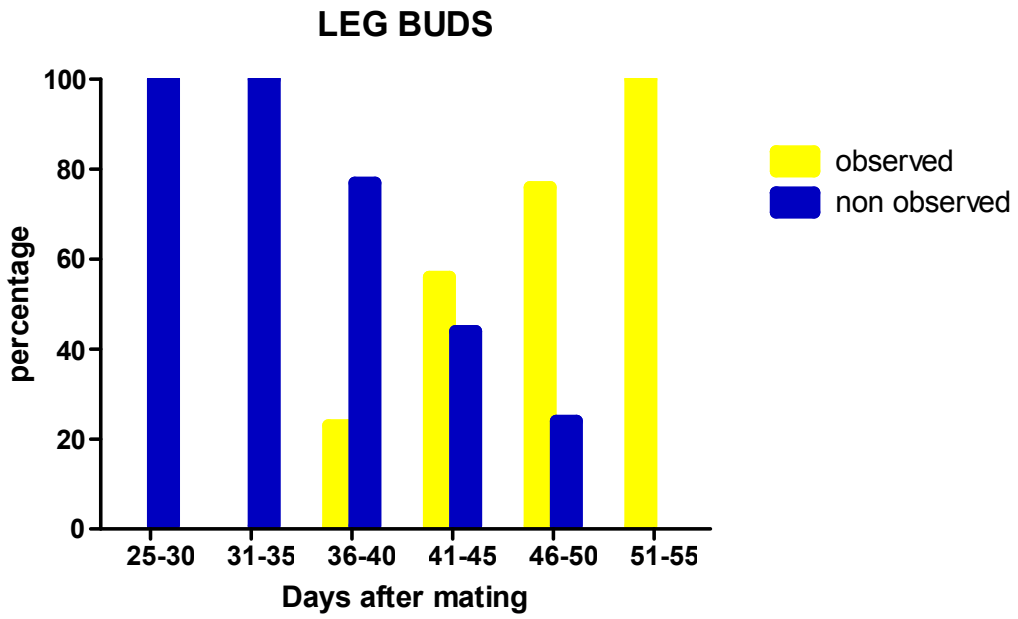

Fig. 4: Percentage of pregnant and non pregnant ewes on the basis of leg buds

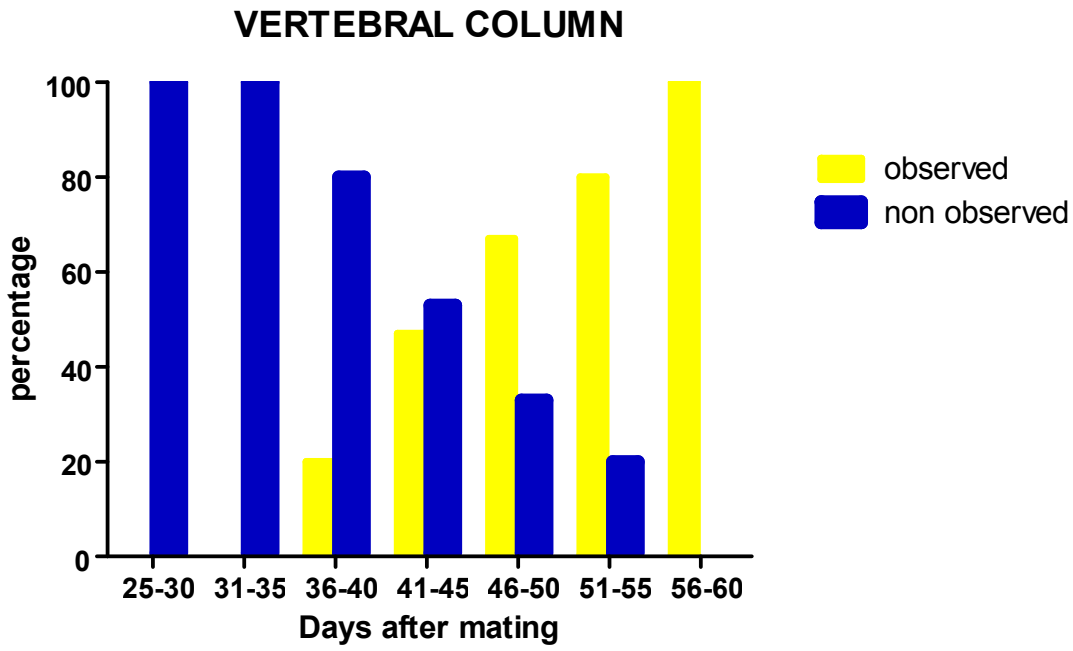

Fig. 5: Percentage of pregnant and non pregnant ewes on the basis of vertebral column

PLATE 2: Gestational sac at 25-30 days of gestation

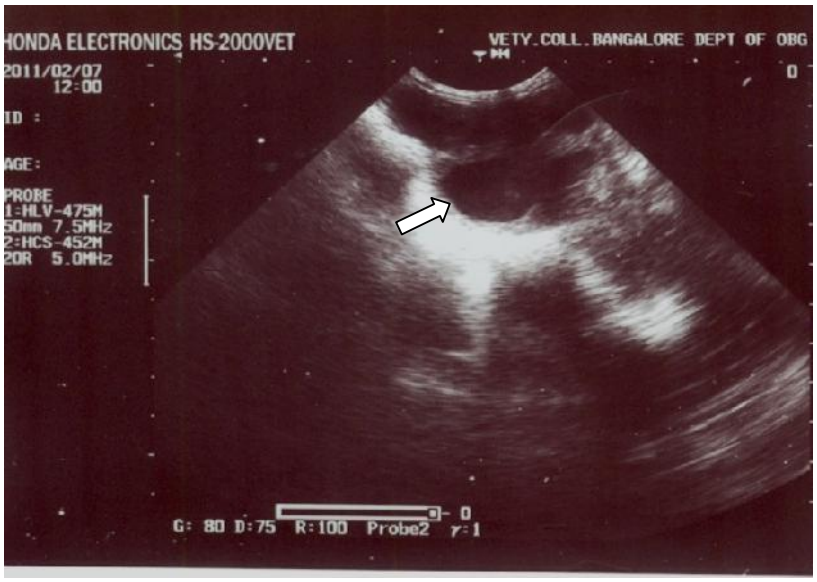

Plate 2A: Anechoic area (gestational sac) below the bladder at 25 days after mating

Plate 2B: Gestational sac with embryonic vesicles at 26 days after mating.

Plate 2C: Gestational sac at 28 days of gestation

Plate 2D: Gestational sac with embryonic vesicle at 30 days after the mating

PLATE 3: Placentomes at different stages of gestation

Plate 3A: Placentomes at 31-35 days of gestation

Plate 3B: Placentomes at 36-40days of gestation

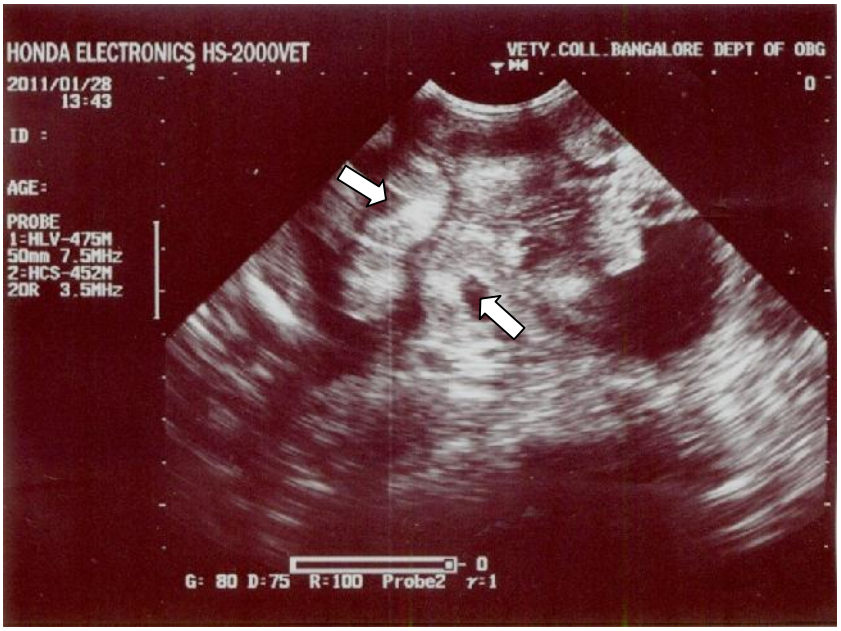

Plate 3C: Placentomes at 41-45 days of gestation

Plate 3D: Placentomes at 46-50 days of gestation

PLATE 4: Leg buds at different stages of gestation

Plate 4A: Foetal leg buds at 31-35 days of gestation

Plate 4B: Foetal leg buds at 36-40 days of gestation

Plate 4C: Foetal leg buds at 41-45 days after mating

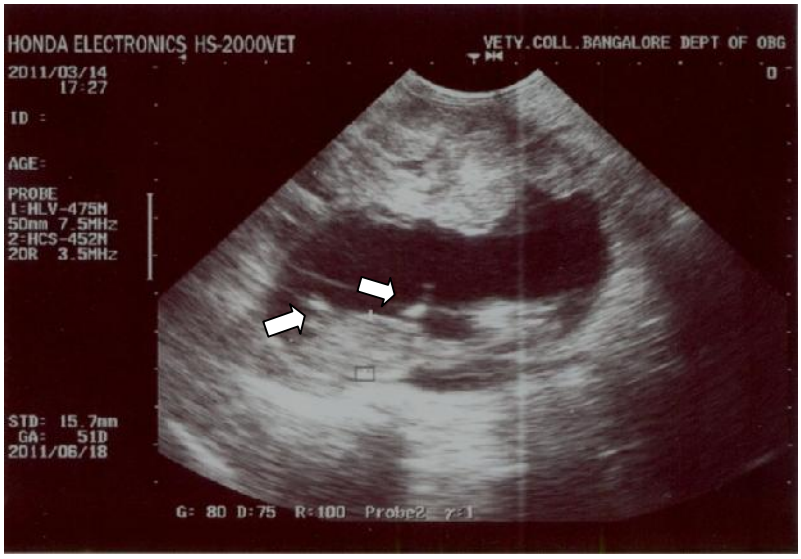

Plate 4D: Foetal leg buds at 46-50 days of gestation

PLATE 5: Vertebral column at different stages of gestation

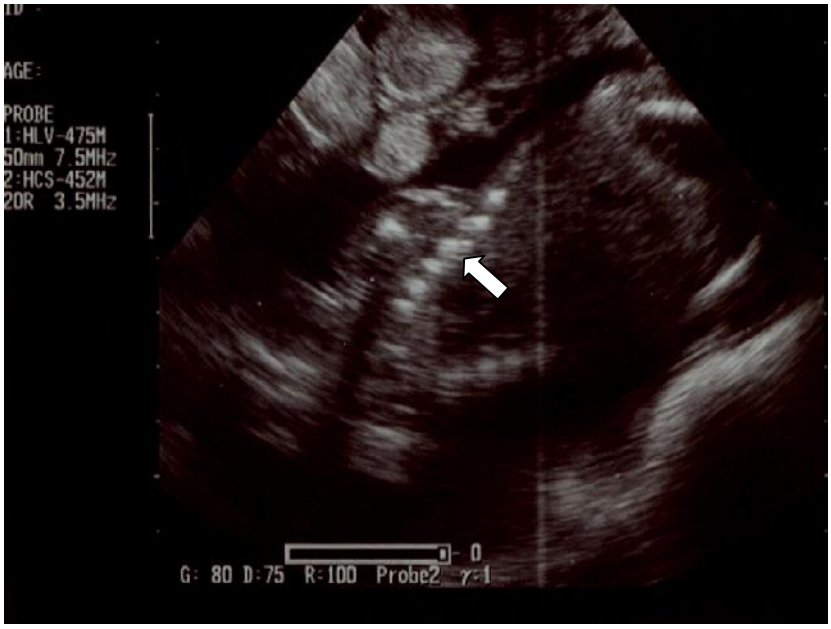

Plate 5A: Vertebral Column of foetus at 36-40 days of gestation

Plate 5B: Vertebral Column and head of foetus at 41-45 days of gestation

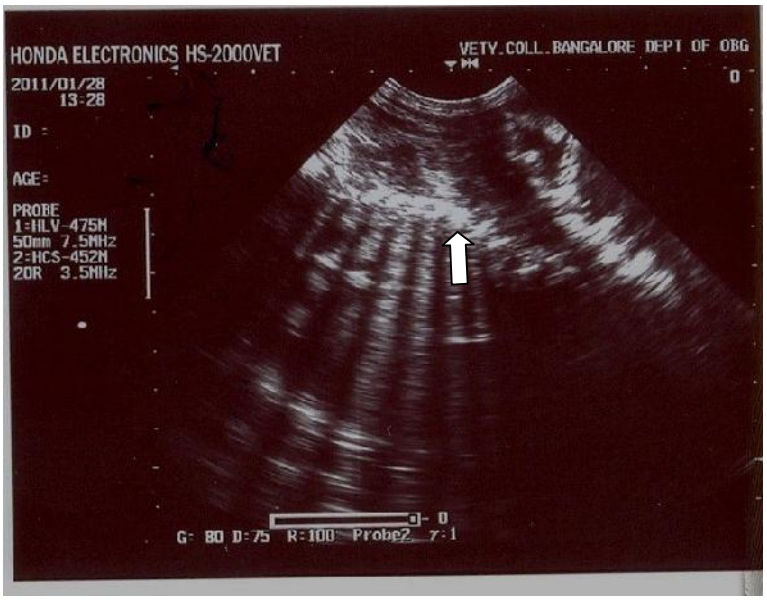

Plate 5C: Vertebral Column of foetus at 46-50 days after the mating

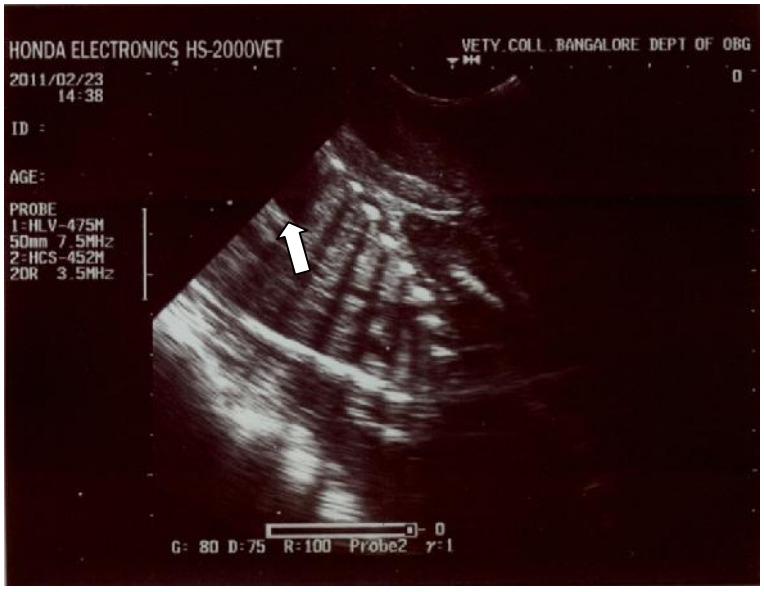

Plate 5D: Vertebral Column and heart of foetus at 51-55 days of gestation.

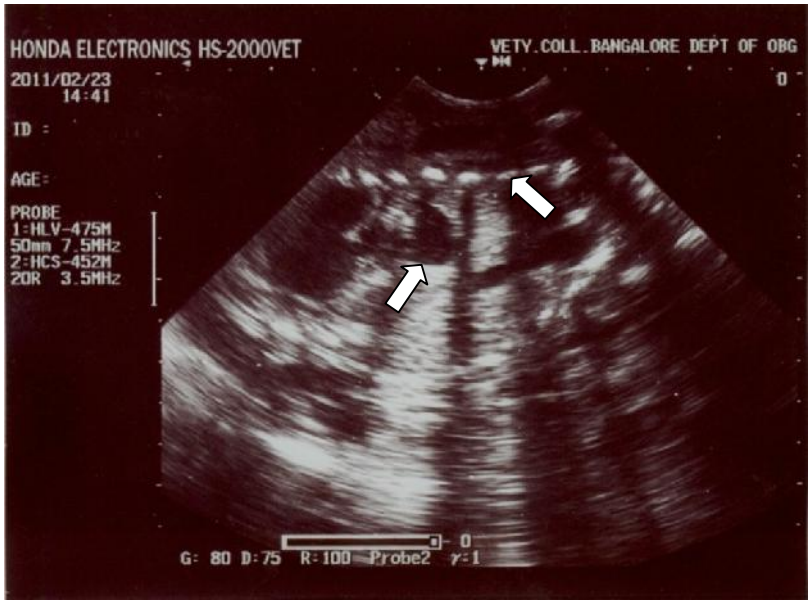

Plate 5E: Vertebral Column and heart of foetus at 56-60 days of gestation

Discussion

V. DISCUSSION

Previous studies have amply demonstrated that transabdominal ultrasonography using probes 3.5 & 5 MHz of frequency was found most suitable for pregnancy diagnosis in sheep (Wilkins and Fowler, 1984; Anwar *et al*., 2008 and Mayura *et al*., 2009). An accuracy of 95% has been reported for diagnosis of pregnancy from 40 to 50 days using a 3 MHz probe (Fowler and Wilkins, 1984).In the present study, 100 percent detection of pregnancy at 40 to 50 days of post mating was made using similar probes.

Haibel, (1990) and Taveme, (1984) reported that pregnancy could be diagnosed between 4 and 5 weeks after mating. In the present study, pregnancy could be detected by 25 to 30 days with accuracy of 16.6 per cent only. The delay in detecting pregnancy as compared to the previous reports at early stages may be attributed to the factors such as breed, age and parity of the ewes, experience of the operator and the technique of the examination as opined by various workers (Buckrell *et al*., 1986; Gearhart *et al*., 1988 and Garcia *et al*., 1993).

5.1 Gestational sac

In the present investigation, the gestational sac, in the form of circular and elongated fluid filled anechogenic images located cranial to bladder, could be seen in only five ewes with 16.6 per cent accuracy at 25 to 30 days after mating. The findings of current study is in accordance with Gearhart *et al*., (1988), Taverne *et al*., (1985) and Bretzlaff *et al*., (1993) who also reported that pregnancy could be first verified at 25 to 30 days after the breeding. No false positive cases were recorded in the present study. False positives results are rare and are attributed to the early embryonic death, unobserved

abortion and sometimes misinterpreting the urinary bladder as the uterus (White *et al*., 1984; Fowler and Wilkins, 1984 and Haibel, 1990).

5.2 Foetal Heart beat

The fetal heartbeat is one of the preferred indicator of pregnancy in ewes because it provides conclusive evidence of the presence of a live foetus (Aiumlamai, *et al*., 1992 and Amer, 2007). In the present study, heart beat was observed for the first time at 31-35 days of gestation in eleven ewes out of thirty ewes with 36.6 per cent of accuracy. The heart beat became more prominent with the advancement of gestation and the rate of accuracy detected was 60.0, 86.6 and 100 per cent respectively at 36-40, 41-45 and 46-50 days of pregnancy, respectively.

Similar observation have been reported where in fetal heart beats could be determined clearly as early as 25 to 30 day of pregnancy and the foetus could be visualized by transrectal ultrasound (Chavez, *et al*., 1996; and Hasan, *et al*., 2007). However, Aiumlamai, *et al*., (1992) reported that fetal hearts were clearly seen at day 44 to 63 days of gestation and heart chambers and valves are distinguishable from day 97 to 103 of gestation by transabdominal ultrasonography. Further, he also reported that the foetal heart rate reached a plateau by 7 weeks before lambing (167 ± 1.5 beats per minute) and decreased to 139.0 ± 15.7 and 117.0 ± 9.2 bpm at 3 weeks before and at lambing respectively. Hence, the investigation of the present study revealed that pregnancy diagnosis by transabdominal ultrasound can be done at 46 to 50 days with 100 per cent accuracy.

5.3 Placentomes

In the present study, placentomes were detected with accuracy of 36.6, 56.6, 80 and 100 per cent at 31-35, 36-40, 41-45 and 46-50 days, respectively. Studies by Russel (1989) revealed that placentomes could be identified from about day 40 as echoic circular structures in sheep. On Day 42, the ovine placentomes appears to be cup-shaped form and reaching the maximum size by Day 74 (Doize, *et al*., 1997). Placentomes were detected with 100 per cent at 45-50 days after the mating (Anwar *et al*., 2008). However, a poor correlation between placentomes size and ovine gestational age has been reported which was ascribed to great variation in the size of placentomes in different breeds (Doize *et al*., 1997 and Gonzalez *et al*., 1998). Hence, the finding of present study is in agreement with the previous reports that have also employed transabdominal ultrasonography using a 3.5 MHz probe (Russel, 1989 and Anwar *et al*., 2008).

5.4 Foetal leg buds

In the present study, the leg buds were first observed at 36-40 of gestation with accuracy of 23.3 per cent and was seen in all the thirty ewes by 51-55 days after the mating. Similar observations have also been reported previously by Anwar *et al*., (2008). It can be opined that the pregnancy diagnosis by transabdominal ultrasound based on foetal leg buds could be detected with 100 per cent accuracy between 51 to 55 days of gestation.

5.5 Vertebral Column

The vertebral column was first seen at 36-40 days of gestation and the percentage of accuracy of detecting foetal vertebral column increased with the increase in gestation

period. The pregnancy could be detected in all the ewes by 55-60 days of gestation which is similar to the findings of Anwar *et al*., (2008), confirming his finding that pregnancy diagnosis by transabdominal ultrasound based on vertebral column can be done by 55-60 days of pregnancy with 100 per cent accuracy.

5.6 Foetal Number

In the present investigation, no twins could be detected on scanning which were later confirmed at lambing. The pregnancy was diagnosed in all ewes by 40 to 50 days after the mating. This is in agreement with Goel and Agrawal (1992) who opined that differentiation between twins and triplets or quadruplets at any stage of gestation using the transabdominal ultrasound technique is very difficult. In continuation to this, Karen *et al.,* (2001, 2004) reported that the accuracy of ultrasound in detecting ewes carrying twins or more was highly disappointing.

5.7 Head Diameter

In the present study, prediction of stage of gestation on the basis of biparietal head diameter was also recorded. The animals which were scanned positive for the pregnancy were later subjected for measurement of head diameter of the foetus to predict the stage of gestation. Further, the date of lambing was retrospectively compared with the date of scanning and the gestational stage was predicted by use of head diameter. Surprisingly, difference of around 15±2days was found between the date of lambing and the date of ultrasound examination based on the head diameter of foetus. The difference in the length of gestation could be attributed to the calibration of the machine to exotic breeds which have greater head diameter compare to local breeds. In the light of the observations made

in the present study, it is necessary for standardising the imported ultrasound machines before use on indigenous breeds for predicting the gestational age based on the biparietal head diameter of the foetus.

Summary

VI. SUMMARY

The present study was conducted for pregnancy diagnosis in sheep using B-mode transabdominal ultrasonography at Department of Instructional Livestock Farm Complex Unit, KVAFSU, Veterinary College, Bangalore. Thirty ewes of mixed age, Bannur breed were selected for the study and ewes were left with two fertile rams with colour marking on the brisket for identifying oestrus and breeding. The study was carried out during the last week of November 2010 till starting second week of January 2011.

Detection of pregnancy was made using parameters like gestational sacs, heartbeat, placentomes, leg bud and vertebral column by transabdominal ultrasonography starting from 25 days after the mating. The gestational sac was observed in five ewes out of thirty (16.6 per cent) at 25 to 30 days after the mating. The heartbeat of the foetus were first detected at 31 to 35 days of gestation in eleven ewes (36.6 per cent) and the accuracy of detecting heart beat was 100 per cent at 46 to 50 days. The rate of detection of foetal heart beat became more prominent with the progression of gestation and which can be used to access foetal viability. The placentomes were identified as echoic, circular to cup-shaped structures from day 30-35 and the same was observed in eleven ewes (36.6 per cent). Placentomes were visible ultrasonographically with 100 per cent accuracy at 46 to 50 days of gestation and the size of placentomes increased as the gestation period advanced.

In the present study, the foetal leg buds were first visualized at 36 to 40 days of gestation in seven ewes (23.3 per cent). The size of leg buds increased as the gestation period advanced and was detected in all the ewes at 51 to 55 days of gestation (100 per

cent).The foetal vertebral column was first observed at 36 to 40 days of gestation in only six ewes (20 per cent) and in all ewes (100 per cent) by 55-56 days of gestation.

None of the ewes subjected for pregnancy diagnosis carried twins which were later confirmed at lambing.

In conclusion, the transabdominal technique proves to be convenient, reliable and accurate enough; it is also less labour-consuming and less stressful for animals. The real-time transabdominal ultrasonography pregnancy can be easily utilized for pregnancy diagnosis in ewes at field level with accuracy of 100 per cent between 40 to 50 days of gestation taking placentomes and heart beat as criteria whereas 100 per cent pregnancy can be detected at 50 to 55 days taking leg buds and vertebral column as criteria for pregnancy diagnosis.

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Abstract

VIII. ABSTRACT

The present study was conducted to detect the pregnancy by using B-mode real time ultrasound using 3.5 and 5 MHz probe by transabdominal method on twenty Bannur ewes maintained at Department of Instructional Livestock Farm Complex and ten pregnant ewes presented for the pregnancy diagnosis at the Department of Gynaecology and Obstetrics, Veterinary College Bangalore, were used for the present study. The objective of the study was to determine the earliest day at which pregnancy could be detected and describe the chronological characteristics of pregnancy from Day 25 to Day 60 of gestation. The present study revealed that the pregnancy could be detected at 25 days after mating by detection of gestational sac with 23.3 percent accuracy. The foetal heartbeat and placentomes were detected with 100 percent accuracy from 45 to 50 days of gestation. Further, leg buds and vertebral column were seen in all the ewes at 55 to 60 days of gestation. It is concluded that pregnancy diagnosis in sheep with 100 percent accuracy can be accomplished between 40 to 50 days post mating using transabdominal ultrasonography.

Keywords : B-mode, transabdominal ultrasonography, gestational sac, placentomes, foetal heartbeat, leg buds, vertebral column