COMPARISON OF DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN BITCHES

ASHA MERINA KURIAKOSE

Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

Department of Animal Reproduction
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **"COMPARISON OF DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN BITCHES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy
22-8-2005

ASHA MERINA KURIAKOSE

CERTIFICATE

Certified that the thesis entitled **"COMPARISON OF DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN BITCHES"** is a record of research work done independently by **Dr. Asha Merina Kuriakose**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

Dr. Joseph Mathew
(Chairman, Advisory Committee)
Associate Professor and Head
Veterinary College Hospital
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy
22.5.2006

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Asha Merina Kuriakose,** a candidate for the degree of Master of Veterinary Science in Animal Reproduction, agree that the thesis entitled **"COMPARISON OF DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN BITCHES"** may be submitted by Asha Merina Kuriakose, in partial fulfilment of the requirement for the degree.

Dr. Joseph Mathew
(Chairman, Advisory Committee)
Associate Professor and Head
Veterinary College Hospital
College of Veterinary and Animal Sciences, Mannuthy

Dr. T. Sreekumaran
Associate Professor and Head
Department of Animal Reproduction
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. K.N. Aravinda Ghosh
Associate Professor
Department of Animal Reproduction
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

Dr. K.M. Jayakumar
Associate Professor
Department of Clinical Medicine
College of Veterinary and
Animal Sciences, Mannuthy
(Member)

27/9/05

External Examiner

Dr. C. CHANDRAHASAN. M.V.Sc Ph.D.
PROFESSOR AND HEAD
Department of Animal Reproduction, Gynaecology and Obstetrics
Veterinary College & Research Institute

ACKNOWLEDGEMENT

With profound gratitude and indebtedness, I would like to express my deep sense of obligation and gratefulness to the Chairman of the Advisory Committee, ***Dr. Joseph Mathew****, Associate Professor and Head, Veterinary College Hospital, College of Veterinary and Animal Sciences, Mannuthy for his meticulous guidance, sumptuous suggestions, tremendous patience, kind co-operation, parental affection and personal attention to the research work offered to me during the course of my study.*

I deem it my privilege in expressing my sincere thanks and gratitude to ***Dr. T. Sreekumaran****, Associate Professor and Head, Department of Animal Reproduction and member of the advisory committee for his generous encouragement, immense support and valuable suggestions.*

I am deeply indebted to ***Dr. K.N. Aravinda Ghosh****, Associate Professor, Department of Animal Reproduction and member of the advisory committee for his never ending help, meticulous guidance, constant encouragement and untiring efforts in framing up the thesis.*

I acknowledge the support and suggestions given to me by ***Dr. K.M. Jayakumar****, member of advisory committee, Associate Professor, Department of Clinical Medicine for the successful completion of my work.*

I am extremely thankful to ***Dr. G. Ajitkumar****, Assistant Professor, Department of Animal Reproduction, for his valuable help, constructive criticism, suggestions and keen interest shown at every stage of this research work.*

I am extremely thankful to ***Dr. Premni Elias****, Assistant Professor, Department of Clinical Medicine for teaching me the basics of ultrasound examination and also for her valuable advices. I am grateful to her for the support and timely help rendered to me.*

My heart felt thanks are indebted to ***Dr. P. T. Philomina****, Associate Professor and Head, Department of Physiology,* ***Dr. V. Ramnath****, Assistant Professor, Department of Physiology and* ***Dr. Rekha****, Research Assistant, Department of Physiology, for their help and guidance for doing fibrinogen assay.*

I am also grateful to other teachers in the Department of Animal Reproduction, ***Dr. V. Vijayakumaran, Dr. K.V. Athman, Dr. Metilda Joseph*** *and* ***Dr. C. Ibraheemkutty*** *for their help rendered during this period of study.*

I am grateful to ***Dr. E. Nanu****, Dean, College of Veterinary and Animal Sciences, Mannuthy, Kerala Agricultural University for the facilities provided for research work.*

I am indebted to Kerala Agricultural University for awarding me the fellowship for the postgraduate study.

I am very much obliged to ***Dr. K.S. Sujatha*** *the Assistant Professor, Department of Statistics for her help and whole hearted suggestions offered in the statistical analysis of the data.*

I express my sincere thanks to ***Sri. Chandrasekharan****, Assistant Professor, Instrumentation Lab and* ***Mrs. Dhannia*** *for the help and co-operation during the course of my work.*

The help given by ***Sri. Paul, Miss Annie*** *and* ***Miss Sandya*** *in the ARIS Cell and* ***Sri. Madhu****, Office Secretary in students council are warmly remembered.*

My special thanks is due to my colleagues ***Dr. G. Jeba Sujana Dhas, Dr. Ratheesh Babu, Dr. Magnus Paul*** *and* ***Dr. Deepthi.L*** *for the help and support rendered to me at all times.*

With exquisite pleasure and gratitude I acknowledge the help and co-operation rendered to me by my friends ***Vandana, Aparna, Sheena, Deepa, Renju, Prasanna, Jagaveerapandian, Julie, Dhannia, Sheena Chechi*** *and* ***Jaibi****.*

The service of all the labourers of AI Centre, Mannuthy and Kokkalai are greatly acknowledged.

I appreciate with thank for the care and skill with which the neat typing and completing of thesis work done by ***Mr. O.K. Ravindran****, C/o. Peagles, Mannuthy.*

I have no words to express my gratitude to my beloved Daddy, Mummy, Achacha, Maya Chechi, Muth, Tessy and **Appoose** *for their unfailing patience, support and encouragement. Without their blessings, it would not have been possible for me to complete this study in a successful manner.*

Above all, I bow my head to the Sacred Heart of Jesus for the blessings showered on me…… for all the things …… to reach the shore safely.

Asha Merina Kuriakose

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

The relationship of dog accepting human as their master and pack leader dates back to 15,000 years. This intimate bond has grown stronger with the passage of time and the dog in turn has proved itself not only as a loved member of the family but also in many other areas concerning human protection. Traditionally dogs in India were treated like other work animals, without giving any special attention. With the passage of time individual and institutional attempts came up sporadically to protect the canine heritage. This has resulted in awareness regarding scientific breeding and management of pure bred animals. On account of the great demand for pedigreed pups, a number of breeders have taken up commercial breeding of canines, thus contributing for the growth of pure bred canine population. At present in India, particularly in Kerala dog breeding is taken up as an income generating proposition among unemployed people. The current expectation of breeding management clients include supplemental services such as early and accurate pregnancy detection, estimation of stage of gestation and estimation of litter size. All these requires a working knowledge of biological and physiological progress of normal canine pregnancy and of diagnostic and therapeutic modalities applicable to pregnant bitches.

Reproductive pattern of bitches differ from other domestic species. They are non seasonal, mono oestrus, polytocous spontaneous ovulators (Cupps, 1991). During each oestrus cycle, the bitch has prolonged follicular and luteal phases compared to those of the cycling species of farm animals. The inter oestrus interval is identical in pregnant and non pregnant cycles, therefore pregnancy cannot be anticipated by a failure of return to oestrus (Arthur *et al.*, 1996). The main problem in diagnosing pregnancy in the bitches is that overt pseudo pregnancy is very common which exhibit the behavioural and physical changes simulating pregnancy. Other causes of abdominal distension which must be differentiated from pregnancy are pyometra, spleenic enlargement, neoplasia of liver, uterus etc.

Accurate early pregnancy diagnosis in dogs allows the veterinarian to differentiate conception failure from foetal resorption and to diagnose various pathological conditions mimicking pregnancy. It also helps to undertake proper management during pregnancy, to produce healthy litters, thus ensuring economic gains to dog breeder.

Many new and old technologies are available to identify pregnant and non pregnant animal early post breeding. Behavioural and physical changes, abdominal palpation, and radiography (Arthur *et al.,* 1996) are some of the routine methods. Ultrasound scanning (Allen and Meredith, 1981; Shille and Gontarek, 1985; Toal *et al.,* 1986), acute phase protein estimation, (Concannon *et al.,* 1995) and endocrine tests (Concannon *et al.*, 1996; Bunck *et al.,* 2002) are some of the more recent techniques used for pregnancy diagnosis in bitches. Thou (1999) studied the efficacy and accuracy of transabdominal palpation and haematological studies for the diagnosis of pregnancy in bitches. Documented evidence of comparative studies on different techniques of early pregnancy diagnosis are meagre. Few research workers (Narayanan *et al.*, 1996; Deka *et al.*, 2004) recommended transabdominal ultrasonography and estimation of acute phase proteins as diagnostic tools for early pregnancy detection in bitches.

Success in commercial dog breeding depends on the production of large and healthy litters for which optimum reproductive efficiency of the stock has to be ensured. Currently the dog breeding in Kerala is under the threat of an ever-rising incidence of infertility. For the benefit of the dog breeders, research studies have been taken up in canine reproduction under the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy (Simon, 1997; Kadirvel,1998; Geetha, 2000; Becha, 2000). As a progress in this line the present work was undertaken to compare the accuracy and efficacy of various methods of pregnancy diagnosis in order to evolve a suitable and reliable method.

Review of Literature

2. REVIEW OF LITERATURE

2.1 OPTIMAL TIME FOR BREEDING

Canines are unique in their reproductive pattern. They have prolonged follicular and luteal phases. There is considerable variation in the time of ovulation, in relation to the onset of vulval swelling and serosanguineous discharge of early proestrus in bitches. Therefore, breeding at a predetermined days after the onset of proestral bleeding in bitches resulted in apparent infertility. There are several investigative methods for identifying the optimal mating time which include measurement of plasma hormone, examination of exfoliated vaginal cells, and vaginal endoscopy. England and Concannon (2002) opined that onset of behavioural oestrus, timing of vulval softening and exfoliative vaginal cytology are the cheapest and simple methods used to determine the optimum time for breeding.

2.1.1 Clinicogynaecological Examination

Hewitt and England (2000) recorded relationship between the onset of proestrus and the day of ovulation in 292 bitches and found that some bitches ovulated as early as five days or as late as 30 days after signs of proestrus had been observed. The vulva and perineal tissue became enlarged and odematous, with a distinct vulval softening and decrease in turgidity occurring at the time of LH surge. According to them, behaviour of the bitch in response to the dog (standing oestrum) and the occurrence of vulval softening could help to estimate the time of ovulation since both of these events occur approximately two days before ovulation.

England and Concannon (2002) opined that for the determination of the optimum time for breeding, a combination of the onset of standing oestrus behaviour and the timing of vulval softening could be used. Each of these events occured on an average one or two days before ovulation.

2.1.2 Vaginal Cytology

Sokolowski (1980) reported that the vaginal smear could be used to approximate the time of ovulation in bitch and thus help maximum fertility.

Johnson (1986) stated that as oestrus approaches there was a gradual increase in the maturity of the epithelial cells and a decrease in white blood cells. During oestrus, superficial cells were the predominant cell type eventually accounting for more than 90 per cent of the exfoliated epithelial cells.

Olson *et al.* (1987) reported that the vaginal smears obtained during oestrus did not contain neutrophils. Erythrocytes diminish in number but could be observed through out oestrus and in early diestrus in many bitches. According to them 90 per cent or more of the epithelial cells in a smear from an oestrus bitch were superficial cells.

According to England and Allen (1989) bitches exhibited a characteristic ferning pattern after the peak in plasma oestrogen concentration and they opined that a ferning index when combined with conventional vaginal cytology could be used in determination of the optimum mating time in bitch.

Wright (1991) estimated the ovulation time in eleven Labrador bitches by different methods. It was found to occur nine to twenty days after the start of the cycle, zero to four days after the onset of positive postural reflexes, four days before and seven days after reaching a vaginal cytological eosinophilic index (EI) of 100 per cent and over a range of 2.5 to 5.5 days based on plasma progesterone concentration of 4 to 10 ng/ml.

According to Arthur *et al.* (1996) there were large numbers of erythrocytes at the onset of proestrus and at the time of oestrus the number of erythrocytes reduced, and the smear consisted of superficial cell types from the stratified squamous epithelium such as anuclearcells, cells with pyknotic nuclei and large intermediate cells. Towards the end of oestrus, leucocytes appeared in the smear, and became dominant cell type during metestrus. In anoestrus, nucleated basal and intermediate cells of the stratified squamous epithelium together with a few neutrophils formed the characteristic smear.

Simon and Athman (1998) stated that during proestrus, the parabasal, intermediate and some superficial cells were exfoliated. Red blood cells, white blood cells and bacteria were also present. During proestrus the percentage of superficial cells increased until it was nearly 100 per cent by the beginning of oestrus. Background debris were usually absent during oestrus. An abrupt clearing of the background usually indicated the occurrence of LH surge.

Hewitt and England (2000) mentioned about the cornification Index calculated from the formula, Number of cornified cells (Anuclear cells) / Total number of epithelial cells X100 and opined that the optimal time for breeding was when the cornification index was 80 per cent or over.

England and Concannon (2002) advised breeding of bitches throughout the period when 80 to100 per cent of epithelial cells were superficial cells as it was typically coincident with the fertile period.

2.2 TRANSABDOMINAL PALPATION

England (1998) reported abdominal palpation as a reliable method of pregnancy diagnosis in bitches. According to him the ease with which it may be carried out depends upon several factors 1.Temperament of the bitch, a nervous bitch will tense her abdominal muscles and make diagnosis extremely difficult. 2.The size of the bitch, it is much easier to make a diagnosis in a small bitch where only one hand is required for palpation. 3. The period of gestation at which examination is made. 4. Whether the bitch is normal or grossly fat, extremely fat bitches make pregnancy diagnosis by palpation difficult. 5. The number of foetuses in utero, if only one or two foetuses are present they may carried well forward and will be difficult to detect.

2.2.1 Transabdominal Palpation at 10 to 20 Days Post Breeding

Sokolowski (1980) stated that at about 19th day after breeding ovoid enlargements occur in the uterine horns in which the developing embryo was situated. About day 20 the developing uterus had spherical swellings approximately 10 to 15 mm in diameter.

According to Arthur *et al.* (1996) at day 18 to 21 the embryos represented a series of tense, oval distensions in the cornua about twelve millimetre long by nine millimetre broad. They found it difficult to detect embryos in large and fat bitches at this stage by palpation.

2.2.2 Transabdominal Palpation at 20 to 30 Days Post Breeding

Harrop (1960) described in detail about the pregnancy diagnosis by palpation at different stages of gestation. According to him 18 to 21 days was the first stage at which the pregnancy diagnosis by transabdominal palpation could be carried out. The embryo represented a series of tense oval distensions of the cornua, about half an inch long. In small easily manipulated bitches it was possible to count the embryos. In large fatty bitches it was impossible to detect the embryos at this stage.

According to him 24 to 30 days was the optimum period for the early diagnosis of pregnancy in the bitch. The embryos were spherical in shape and about one inch in diameter to the touch.

Sokolowski (1980) stated that palpation of the abdomen between day 20 and 28 after breeding was helpful to diagnose pregnancy. After day 28 it was extremely difficult to palpate the pregnant uterus, because the spherical shape of the uterine enlargements changed to an ovoid shape and increase in size to 15 to 30 mm in diameter depending on the size of the bitch.

Jones and Joshua (1982) opined that at 21 days each foetal unit was in the size of a pea, about 0.75 to 1.0 cm in diameter and was very successful to undertake pregnancy diagnosis by palpation at this stage in small relaxed bitches.

Burke and Badertscher (1986) reported that at 21 days of gestation the swellings measure approximately one centimetre in diameter by abdominal palpation, by day 24 to 26 they were nearly two centimetre in diameter and by day 32 to 35 it averaged to about 3 to 3.5cm in small breeds, about 4 cm in medium breeds and about 6 to 7 cm in large breeds.

According to Barr (1988) and Ferguson (1990) detection of pregnancy by palpation was usually optimal between 24 and 30 days of gestation in the dog. Similarly Allen *et al.* (1991) opined that, an experienced person could correctly diagnose pregnancy by palpation between 25 and 30 days of gestation. But there were certain factors such as the size of the animal, its temperament, period of gestation, obesity etc which affected the accuracy of abdominal palpation method.

According to Gangadhar (1995) the optimum time for pregnancy diagnosis by abdominal palpation was 28 days after breeding and found the size of each foetal swelling about two centimetre in diameter.

England (1998) stated that the optimum time for diagnosis by abdominal palpation in bitches was one month after mating and at this stage the conceptuses were spherical in outline and vary between 15 and 30 mm in diameter

Thou (1999) recorded earliest results of pregnancy diagnosis in dogs by transabdominal palpation at 21 days after breeding in a Pomeranian bitch. At 30 to 35 days post breeding, the accuracy of palpation in Alsatian and Pomeranian was 88.9 and 100 per cent respectively.

Gradil *et al.* (2000) palpated pregnancy at 26 to 28 days post breeding and opined that by day 28 uterine swelling were of three to five centimeter in diameter for a middle sized dog. After day 30 of gestation, the uterus enlarged rapidly and occupied a more cranioventral position and it became more difficult to palpate as discrete swellings.

Purswell *et al.* (2000) reported that abdominal palpation was best accomplished at 25 to 28 days when the 'string of pearls' effect of the uterus was most obvious. After 30 to 35 days post breeding, the uterine swellings were elongated, became more oval and more fluctuant. At this time it was difficult to differentiate a gravid uterus from other abdominal contents.

2.2.3 Transabdominal Palpation at 30 to 40 Days Post Breeding

Harrop (1960) opined that positive diagnosis of pregnancy after 35 days of gestation were difficult as the constricted portion of the cornua appeared as tubes of uniform diameter.

According to Arthur *et al.* (1996) foetal growth was rapid at 40 to 50 days of gestation. At day 45 it was possible to palpate the posteriorly situated foetuses.

Gradil *et al.* (2000) reported that the uterine swellings became almost confluent and were more pliable and more difficult to palpate as distinct entities between 35 to 40 days of gestation.

2.2.4 Transabdominal Palpation at 40 to 65 Days Post Breeding

Harrop (1960) detected the most posteriorly situated foetuses between 45 to 55 days by transabdominal palpation and individual foetus was palpable at 55 to 63 days.

According to England (1998) after day 45, the uterine horns tend to fold upon themselves resulting in the caudal portion of each horn being positioned against the ventral abdominal wall and the cranial portion of the same horn being positioned dorsally. After day 55, the foetuses could be identified especially if the forequarters of the bitch were elevated and the uterus is manipulated caudally towards the pelvis.

Gradil *et al.* (2000) stated that after day 45 to 50 of gestation the individual foetuses were palpable and easily identified.

2.2.5 Accuracy of Diagnosis by Abdominal Palpation

According to Allen and Meredith (1981) palpation of the abdomen in the period from 26 to 35 days after mating was found to be 87 per cent accurate in diagnosis of pregnancy. Earliest pregnancy detection were obtained at 21 days after first mating, but correct positive results were obtained in only 52 per cent from day 21 to day 25 post breeding by abdominal palpation. During period from day 25 to 35, 75 per cent of the test gave correct positive results.

Shille and Gontarek (1985) found 75 per cent accuracy in diagnosing pregnancy at 40 days of gestation in bitches by palpation whereas, Taverne *et al.* (1985) reported 93 per cent specificity between days 25 and 35 of pregnancy by transabdominal palpation.

Out of fifty five bitches examined for pregnancy by Toal *et al.* (1986), the accuracy of pregnancy detection and foetal counting by abdominal palpation, was 88 per cent and 12 per cent respectively. He reported that palpation was less reliable than ultrasound for determining pregnancy and litter size.

According to Allen *et al.* (1991) Abdominal palpation was more accurate from 24 to 35 days post breeding.

Deka *et al.* (2004) reported that earliest pregnancy could be diagnosed by abdominal palpation on 23rd day post service with 65 to 71 per cent accuracy which improved to 100 per cent from 28th day to term.

2.3 ULTRASOUND SCANNING

In recent years there has been considerable interest in the use of ultrasonic method for the diagnosis of pregnancy in bitches. Diagnostic ultrasound usually employs sound waves of frequencies between one and 10 MHz (Barr, 1988; Mattoon and Nyland, 1995). Nyland *et al.* (1995) and Vyas *et al.* (2003) opined that small dogs below10kg could be examined with 7.5 or 10 MHz transducers. Medium sized dogs required frequencies of 5.0 MHz whereas, large breed dogs sometimes required 3 MHz or lower frequencies.

2.3.1 Ultrasound Scanning at 10 to 20 Days Post Breeding

Bondestam *et al.* (1983) reported difficulty in detecting foetuses at 21 days of gestation and suggested ultrasound scanning during sixth week of gestation.

Cartee and Rowles (1984) were able to discern fluid density enlargement of the uterus and presence of tissue echoes suggestive of fetus 14 days after the last breeding in four bitches. They observed small hyper echoic area within the lumen on post breeding day 10 and hyperechoic intraluminal embryo averaging 10 millimetre in length at 17 to 23 days post breeding.

Concannon (1986) reported that uterine swelling at implantation sites was about one centimetre in diameter by day 20 and represent localized uterine oedema, expansion of the embryonic membranes and early placental development.

Yeager *et al.* (1992) detected the gestational sacs at 17 days to 20 days after the LH surge, and found to be one to two millimetre in diameter and one to four millimetre in length.

Kahn (1994) opined that hypoechoic areas of a few millimetre in diameter could be found in the regions of the developing conceptuses as early as second week of pregnancy.

Zambelli *et al.* (2002) reported the earliest ultrasonographic observation of the gestational sac on day 10 after mating, while the embryo could be measured only at day 18 by ultrasound scanning.

England *et al.* (2003) reported that the diameter of gestational sac was two millimetre at 20 days post breeding.

2.3.2 Ultrasound Scanning at 20 to 30 Days Post Breeding

According to Bondestam (1983) all foetuses detected before 28th day of gestation were small and details of the shape of the foetus could not be demonstrated except for beating hearts.

Cartee and Rowles (1984) found a hyperechoic intraluminal embryo averaging 10 millimetre length in all bitches examined between 17 to 23 days of gestation and reported that foetal and cardiac activities appeared at 28 days of gestation.

Shille and Gontarek (1985) reported that the foetal movements and heart beat could not be identified until days 28 and 35 respectively. During the period of 27 to 30 days uterus had an anechoic lumen containing a hyperechoic embryo. The embryo was semicircular in shape.

Taverne *et al.* (1985) opined that a reliable pregnancy diagnosis using 5MHz ultrasound was possible in most cases from day 25 of gestation.

Toal *et al.* (1986) examined six dogs on 21st and 22nd day following initial breeding and diagnosed four out of six as pregnant. Ultrasound failed to estimate correctly the littersize in each instance and there were no false positives.

According to Barr (1988) the pregnancy diagnosis could be established with consistency between 24 and 28 days post breeding by ultrasound scanning.

Allen and England (1990) described in detail about the diagnosis of early pregnancy and the number of conceptuses using B-mode ultrasound.

Yeager and Concannon (1990) reported that the embryonic mass and heart beat were first detected at 23 to 25 days after the LH surge.

Yeager *et al.*(1992) detected placental layers in the uterine wall at day 22 to 24, embryo and heart beat at day 23 to 25, yolk sac membrane at day 25 to 28, and allantoic membrane at day 27 to31 by ultrasound scanning.

Kahn (1994) reported that at 20 to 30 days of gestation the uterine enlargement of the bitch were ovoid in shape and easy to count the foetal numbers at this stage by ultrasound scanning.

According to Gradil *et al.* (2000) the embryo proper could be imaged by day 24 of gestation by sonography.

Bhadwal and Mirakhur (2000) reported that the earliest gestation age of foetus was 26 days when it could easily be diagnosed as almost a round anechoic cavity containing hypo to hyper echoic mass. By day 30 conceptual swelling was seen as round to oval and contained elongated hyperechoic structure surrounded by anechoic amniotic fluid.

England *et al.* (2003) found the body of the embryo within the uterine fluid at 23 days of gestation as one millimetre mass. It increased in length up to one centimetre at 28th day of gestation. The diameter of the gestational sac was two millimeter and no embryonic mass was visible at 20th day of gestation.

2.3.4 Ultrasound Scanning at 30 to 40 Days Post Breeding

Allen and Meredith (1981) reported that from day 32 to day 62 there were 90 per cent correct positive result by A mode –ultrasound scanning. They found 100 percent accuracy in diagnosing pregnancy using ultrasound scanning from 40 days of gestation.

Bondestam *et al.* (1983) reported that after 40th day details of stomach, urinary bladder and umbilical vein of foetus could be demonstrated by ultrasound scanning.

Toal *et al.* (1986) examined 10 bitches between 24 to 32 days following breeding and identified five bitches as being pregnant and there were no false positives.

Yeager *et al.* (1992) reported altered relationships between foetal size and extra foetal structures after day 38 by ultrasound scanning. Body diameter became greater than half of the diameter of the chorionic cavity on day 38 to 41.

Kahn (1994) reported that the ampullary shape of the uterus starts to diminish from day 35 to 40 and changes to a more tubular shape. It was difficult to follow a string of neighbouring conceptuses on a longitudinal section of single uterine horn by ultrasound scanning at this stage.

2.3.5 Ultrasound Scanning at 40 to 60 Days Post Breeding

Twenty two dogs were examined by Toal *et al.* (1986) between 43 and 54 days following breeding. Ultrasound correctly identified sixteen bitches as being pregnant with no false positives.

Khan (1994) opined that at 51 to 65 days of gestation the foetuses will not float inside the uterus and it became difficult to distinguish foetus from maternal tissues. Foetal heart beat and mineralised foetal skeleton could be monitored clearly during this stage.

2.3.6 Foetal viability

Helper (1970) detected foetal heart beats in all bitches from 32 days of gestation using ultrasound scanning. Bondestam (1983) reported that foetal viability could be assessed by observing heart beats at 28^{th} day of gestation.

Cartee and Rowles (1984) observed foetal heart beating at 120 to 140 beats/minute from day 28. Hence foetal viability could be readily assessed from this stage onwards by both cardiac and generalized activity.

Poffenbarger and Feeney (1986) reported that a diminution of foetal movement and detection of fetal heart beat less than twice maternal heart rate signal foetal distress, which warrants intervention of some kind.

According to Barr (1988) the foetal viability could be assessed by heart rate and foetal motion monitored from day 28 onwards by sonography

Yeager and Concannon (1990) detected embryonic mass and foetal heart beat at 23 to 25 days after the LH surge and Yeager *et al.* (1992) found foetal movement at day 34 to 36 of gestation. They opined that the foetal viability could be diagnosed as early as 25 days of pregnancy through sonography as indicated by the pulsating cardiac movements with 97.62 per cent accuracy.

Kahn (1994) opined that the location of the pulsating heart could aid in the differentiation between yolksac and embryo.

According to Purswell *et al.* (2000) assessment of foetal viability by noting foetal movements and heart beats could be done at 28 days post breeding and foetal heart beats below 200 beats /minute during pregnancy indicated foetal stress.

Bhadwal and Mirakhur (2000) found flickering of heart beat as a sign of foetal viability at day 35 and at this stage foetus was observed as a large elongated and curved mass.

Chandolia *et al.* (2003) successfully used ultrasound scanning for diagnosis of obstetrical cases in bitches to assess the foetal status.

2.3.7 Foetal Measurements

Cartee and Rowles (1984) measured the diameter of uterus and length of embryo at various stages of gestation. The length of the embryo at 17 to 23 days and at 27 to 30 days were averaging 10mm and 18.5 mm, respectively and average crown rump length (CRL) at 34 to37 days, 38 to 45 days, and 46 to 49 days were 25.5mm, 71.0mm and 89mm respectively in German Shepherd and Irish Setters.

Shille and Gontarek (1985) found gestational sac diameter on days 27 to 34, 35 to 44 and 47 to 56 after ovulation ranged from 23 to 30, 25 to 49 and 46 to 89mm respectively in Grey hound bitches.

England *et al.* (1990) and Yeager and Concannon (1990) suggested that measurement of foetal sacs and other diameters were useful indicators for early diagnosis of pregnancy.

Yeager and Concannon (1990) measured the diameter of gestational sac at 20 days and 25 days of gestation and found to be one to two millimeter in diameter and one to four millimeter in length and 8.2± 0.3 mm in diameter and 20.3±1.1mm length respectively in Beagle bitches.

According to Yeager *et al.* (1992) the CRL increased from 0.3±0.05 cm on day 24 to 9.2±0.2cm on day 48. Body diameter increased from.0.2±0.03cm on day 24 to 4.6±0.15cm on day 60. Head diameter increased from 1.8±0.05cm on day 34 to 2.7±0.04cm on day 60. Of the foetal structures, head diameter was the most accurate for estimation of gestational age.

Mattoon and Nyland (1995) opined that the first sign confirming pregnancy was detection of gestational sac by sonography as early as 20 days post breeding.

Zambelli *et al.* (2002) found highly positive correlation between the anatomic and the ultrasonographic measurement of the external diameter of the gestational sacs (r =0.9967) and the length of the embryos/foetuses(r =0.9964). They also found a linear correlation between the external diameter of the gestational sac and the gestational age.

England *et al.* (2003) reported the diameter of gestational sacs at 20 days, 22 days and 23 days as two millimeter, four millimeter, six millimeter respectively and the length of embryo at 24 and 28 days of gestation as one millimetre and 2 millimetre respectively.

Kutzler *et al.* (2003) estimated gestational age using two published tables correlating embryonic vesicle diameter (EVD), crown rump length (CRL), Body diameter (BD) and biparietal diameter (HD) to the LH surge in mid gestation Beagle bitches and Body diameter and biparietal diameter to the LH surge in late gestation Retrievers.

Oral and Alacam (2004) examined ten pregnant Siberian husky bitches by Ultrasound scanning and foetal heart diameter were taken at various stages of gestation. They found a linear increase of foetal heart diameter between 35 and 65 days and calculated the gestational age by using the formula gestational age(day)=foetal heart diameter(cm)x19.0599+24.7685 and found only 0.6805 days of variation between the estimated and actual parturition days

2.3.8 Foetal Number

Bondestam *et al.* (1983) opined that the period between the 28th and 35th day of gestation found to be the most suitable time for counting the foetuses by ultrasound scanning.

Shille and Gontarek (1985) and Poffenbarger and Feeney (1986) observed low accuracy of predicting actual foetal number was associated with overlapping foetuses or mistaking them as already counted due to the acoustic artifacts.

Toal *et al.* (1986) reported that foetal counting by ultrasound was inaccurate for litter size determination. The accuracy of correct litter size estimation using ultrasound increased as pregnancy progressed, ranging zero per cent for the first trimester, 20 per cent for the first part of second trimester, 38 per cent for the second part of the 2nd trimester, and 50 per cent for the 3rd trimester. The larger size of the foetus in small litters permitted easier recognition and more accurate counting.

According to Barr (1988) estimation of litter size by ultrasound scanning was less easy, but the period between the 28th and 35th day of gestation was the best time for counting the foetuses.

England (1998) found the efficacy of prediction of litter size to be 97 percent in early stages of gestation, which dropped to 20 percent during later stages of pregnancy.

Deka *et al.* (2004) examined 66 pregnant bitches at various stages of gestation using radiography and ultrasonography and found an overall accuracy in detection of littersize through sonography as 96.78 per cent.

2.3.9 Gestational Accidents

Allen *et al.* (1989) reported a case of hydrops foetalis diagnosed in a near term bitch using real time ultrasonography. The effected foetus was identified by the presence of intra thoracic and subcutaneous fluid.

England (1992) found the components of embryonic resorption as reduced volume and changes in echogenisity of embryonic fluid, loss of embryonic mass and absence of heart beat. Collapse of the conceptus with thickening and increased bulging of the uterine wall and reduced size in comparison with adjacent conceptuses were reported. Following resorption, the uterus was moderately hypoechoic in appearance, similar to after parturition.

Ettinger and Feldman (2000) reported about the incidence of early embryonic death and spontaneous abortion in bitches. According to them the bitch consume the aborted foetus or resorption of the conceptuses may occur until day 45 of pregnancy without noticeable signs.

Bhadwal and Mirakhur (2001) described a case of pregnancy at 35th day of gestation which was going to abort as an anechoic area with hyper echoic mass attached inside. No foetal movement or heart beat were detectable.

According to Hopper *et al.* (2004) out of 161 canine pregnancies diagnosed by ultrasonography incidence of foetal resorption, abortion, stillbirth, neonatal mortality and congenital abnormalities were 7.4, 8.4, 8.15 and 9.6 percent respectively.

2.3.10 Accuracy of Diagnosis by Ultrasonography

Allen and Meredith (1981) found the optimum period for using A –mode ultrasound as 32 to 62 days after mating. In this period the accuracy of detecting pregnancy was 90 per cent as against 83 per cent in nonpregnant bitches.

Shille and Gontarek (1985) done abdominal palpation and ultrasonography for pregnancy diagnosis in bitches on day 19 to 22, 26 to 30, 34 to 38 and 40 to term after ovulation, correct diagnosis were made in 33, 42, 50 and 75 per cent of the bitches by palpation and in 42, 67, 75,and 83 per cent of bitches by ultrasonography.

Toal *et al.* (1986) examined fifty five bitches for pregnancy at different intervals following breeding and found that ultrasound was 94 per cent accurate for detection of pregnancy and 36 per cent accurate for foetal counting. They opined that variable foetal posture, large foetal size with diminished amniotic fluid and the stacked and overlapping distribution of foetuses in large litters hampered counting.

Thuroczy and Balogh (2003) reported that pregnancy diagnosis by ultrasound was possible from day 21 after mating and the number of foetuses and their viability could be shown between day 25 and 35 of pregnancy. From day 50, foetal organs were viewed and disorders of fetal development could be detected.

Deka *et al.* (2004) reported that earliest pregnancy diagnosis with 100 per cent accuracy was by 18th day post service by ultrasonography. Accuracy of estimating foetal viability was 97.62 per cent and litter size 96.78 per cent by 25th day of pregnancy .

2.4 .HAEMATOLOGICAL STUDIES

2.4.1 Haemogram

2.4.1.1 Total Erythrocyte Count

Doxey (1966) reported that normal erythrocyte count ranged between 5.5 to 8.0 million/cmm with its average as 6.44 million /cmm in healthy dogs. He reported a steady fall in the red cell count values as pregnancy progressed and a lowest value were obtained at term and just after parturition. Similar observations were reported by Schalm *et al.* (1975) who noticed the range as 5.5 to 8.5 million /cmm in normal dogs and reported that during gestation the erythrocyte number became gradually reduced from the mean normal value of 8.85 million to 4.53 million / cmm. Hinton and Jones (1978) reported total erythrocyte count in between 2.77 to 7.39×10^{12} /L in nonpregnant dogs. Allard *et al.*(1989) reported that the red cell count, haemoglobin concentration and PCV were decreased during pregnancy. Prabhakaran *et al.* (1996) did not find significant variation in RBC value during pregnancy and lactation in bitches. Thou (1999) found the average erythrocyte count on 21 to 25, 30 to 35,and 45 to 50 for pregnant and non pregnant as 6.81±0.16, 6.29±0.13, 5.49±0.11 and 7.38±0.17, 7.3±0.18, 7.43±0.16 million/cmm respectively and reported a significant variation in the erythrocyte count between pregnant and non pregnant bitches.

2.4.1.2 Haemoglobin (Hb)

Doxey (1966) observed a steady fall in the haemoglobin values as pregnancy progressed and the lowest value at term or just after parturition. Hinton and Jones (1978) reported a range between 6.5±0.4 to 20.4±0.3 g/dl in apparently healthy nonpregnant animals. According to Saror *et al.*(1979) normal value of haemoglobin in dog was 14.2±1.6. gm/dl. Benjamin (1985), Singh *et al.* (1987) and Sastry (1989) reported the value of haemoglobin in the range of 12 to 18 g/dl in non pregnant bitches. According to Prabhakaran *et al.* (1996) haemoglobin content, packed cell volume, erythrocyte and leucocyte counts did not show much variation between pregnancy and non pregnancy. Thou (1999) given mean values of haemoglobin on days 21 to 25, 30 to 35 and 45 to 50 in pregnant as 12.47±0.23g/dl, 11.91 ±0.22g/dl, 11.0 ±0.18 g/dl respectively and that in non pregnant as 14.19±0.25g/dl, 14.03±0.27g/dl, 14.23 ±0.24g/dl respectively. He found a significant variation in haemoglobin content in pregnant and non pregnant bitches.

2.4.1.3 Erythrocyte Sedimentation Rate (ESR)

Simms (1940) reported that the sedimentation rate varied from one to 52 mm/ hr with an average rate of fall of 15.4 mm from 11 to 56 days of gestation in apparently normal healthy pregnant bitches. According to Doxey (1966) the erythrocyte sedimentation rate in dogs varied from zero to1.5 mm/hr with an average of 0.2mm/hr. Schalm *et al.* (1975) reported erythrocyte sedimentation rate in one hour as one to four millimetre and also found a definite increase above anticipated level at sixth week of gestation with a return to normal anticipated level by eighth week of gestation. Among 20 clinically normal, nonpregnant female dogs, the sedimentation rates ranged between zero to five millimetre per hour. Benjamin(1985) and Sastry (1989) noted 5 to 25mm/hr as the normal range of erythrocyte sedimentation rate in dogs. They also stated that the erythrocyte sedimentation rates in female dogs raised during pregnancy. Henry (1996) opined that pregnancy anaemia increased the ESR because the change in the erythrocyte/plasma ratio, favoured rouleaux formation independent of changes in the concentration of plasma proteins. Thou (1999) found an ESR of pregnant bitches ranging between 8.5 to19.33 mm/hr and it was significantly higher compared to non pregnant bitches, ranging between 5.6 to 5.88mm /hr.

2.4.1.4 Packed Cell Volume (PCV)

Doxey (1966) reported the normal range of packed cell volume as 40 to 58 percent and found a steady fall in packed cell volume in bitches as pregnancy advanced. Hinton and Jones (1978) found the PCV values ranged between 20.7 to 60.4 per cent in normal non pregnant animals. Saror *et al.* (1979) reported a normal mean value of PCV as 42 ± 4.5 per cent in dogs. Benjamin (1985) and Jain (1986) recorded 37 to 55 percent with an average value of 45 per cent for packed cell volume in apparently normal non pregnant dogs. Singh *et al.* (1987) found the average value of packed cell volume as 45.5 per cent. Thou (1999) found a decrease in PCV as pregnancy progresses and found the mean values on days 21 to 25, 30 to 35 and 45 to 50 in pregnant bitches as 41.66 ± 0.333, 39.33±0.333, 35±0.577 per cent respectively and that in non pregnant bitches as 44.5±0.289,44.75± 0.75,45.25±0.25 per cent respectively in Alsatian bitches.

2.4.2 Leucogram

2.4.2.1 Total Leucocyte Count (TLC)

Doxey (1966) noted a rise in white cell counts until eight week of pregnancy and then it fall during lactation. According to Schalm *et al.* (1975) normal value of total leucocytes was eleven thousand/cmm. They opined that during gestation the total leucocyte count increased from 12 thousand to 19 thousand / cmm of blood. Sutton and Johnstone (1977) reported the total leucocyte values range between 10.478±0.223 to 21.102±0.953 thousand /cmm in apparently normal healthy bithces. Swenson (1977) found the total leucocyte ranged from 9 thousand to 13 thousand/cmm. According to Saror *et al.* (1979) normal value of TLC was 12.05±4.59 thousand/cmm in nonpregnant bitches. Benjamin (1985) recorded the normal leucocyte count in dogs ranging between 6.0 and 20 thousand/cmm and 11.8 thousand/cmm as its mean. Singh *et al.* (1987) found a mean leucocyte count in dogs as 8.18 thousand/cmm and noted that total leucocyte count increased during pregnancy. Prabhakaran *et al.* (1996) found no significant difference in leucocyte count during pregnancy and lactation . Thou (1999) found the average count of TLC at 21 to 25 days, 30 to 35 days and 45 to 50 days in pregnant bitches as 9.95 ±0.26, 12.22 ±0.20 and 17.0±0.25 thousand/cmm respectively and that of nonpregnant bitches as 7.95±0.19,7.95±0.27,and 7.99±0.19 thousand /cmm respectively. The variation of count between pregnant and nonpregnant bitches was significant ($P<0.01$).

2.4.2.2 Differential Leucocyte Count

2.4.2.2.a Neutrophils

Schalm *et al.* (1975) reported normal neutrophil proportion in dogs as 70 per cent. Swenson (1977) and Benjamin (1985) recorded the normal range of neutrophils in dogs as 60 to 75 percent and its average values as 69 percent. Singh *et al.* (1987) and Sastry (1989) observed the normal neutrophil count varying between 60 to 80 per cent with an average of 70 per cent.

2.4.2.2.b Band Neutrophil

Doxey (1966) reported that the number of immature neutrophils increased in inflammatory conditions. Schalm *et al.* (1975) reported that band neutrophil was frequently not observed in peripheral blood of healthy dogs and when the cell was present it did not exceed 3 percent of the differential count average being 0.8 per cent in normal dogs. According to Saror *et al.* (1979) normal values of neutrophil band were 0.03±0.16 per cent.

2.4.2.2.c Lymphocytes

Schalm *et al.* (1975) reported a normal value of 20 per cent for the lymphocytes in dogs. According to Prabhakaran *et al.* (1996) the mean lymphocyte count was marginally higher during pregnancy (28± 6.2 per cent) than during lactation (21±4.2 per cent). Thou (1999) found the lymphocyte count in pregnant Alsatian bitches ranged from 22 to 32 per cent. The count in nonpregnant was 17 to 20 per cent .

2.4.2.2.d Monocytes

The value of monocytes in normal healthy dogs ranged between two to twelve per cent with an average of six per cent (Schalm *et al.* 1975, Swenson,1977; Benjamin,1985; Thou 1999). Singh *et al.* (1987) and Sastry

(1989) noted the percentage of monocytes count in dogs as three to nine and three to ten with an average of five percent. However Prabhakaran *et al.* (1996) did not find much change in monocyte count during pregnancy and lactation.

2.4.2.2.e Eosinophil

The eosinophil count in dogs were found varying from two to ten per cent with a mean of five per cent (Schalm *et al.*, 1975; Swenson 1977 and Benjamin 1985). Singh *et al.* (1987) and Sastry (1989) reported the normal eosinophil count in dogs varying from two to 14 per cent and two to 10 per cent with its average being four per cent.

2.4.2.2.f Basophil

Doxey (1966) and Schalm *et al.* (1975) found the basophil concentration as less than one per cent in normal healthy bitches. Benjamin (1985), Singh *et al.* (1987) Sastry (1987) and Thou(1999) reported a basophil count less than one per cent and 0.5 per cent in apparently normal healthy bitches and there was no significant variation of basophil concentration in pregnancy compared to non pregnant animals.

2.4.3 Serum Biochemistry

2.4.3.1 Serum Cholesterol

According to Benjamin (1985) the normal serum cholesterol level in dogs ranged between 150-300 mg/dl and there was marked increase in the level during pregnancy and other conditions like diabetes mellitus and liver diseases. Arosh *et al.* (1999) given the mean value of cholesterol in pregnant bitches as 234.6± 15.53 mg/dl and could not find any significant difference in cholesterol in pregnant and pseudo pregnant bitch. According to Jacobs *et al.* (2000) the normal serum cholesterol level in dogs ranged between 106.2 to 368.2 mg/dl.

2.4.3.2 Total Serum Protein

Schalm *et al.* (1975) reported that the total serum proteins in normal Basenji dog was between 6.0 to 8.0 g/dl, and it varied with age reaching maximum in adult dogs. Swenson (1977) found the total plasma protein as 6.72g/dl and total serum protein as 6.2g/dl. Sutton and Johnstone (1977) recorded the mean total protein value for bitches as 68.8±0.6 g/l to 76.2g/l. Singh *et al.*, (1987) and Sastry (1989) noted the total serum proteins of dog in between 6.1 to 7.8 g/ml. Prabhakaran *et al.* (1996) found the mean total protein value in bitches during pregnancy as 6.8g/dl as against 5.2 g/dl during lactation. According to Jacobs *et al.* (2000) the total serum protein ranged between 5.7 to 7.6 gm/dl in apparently normal nonpregnant dogs. Thou *et al.* (2001) found the serum protein of pregnant bitches as 6.9 to 8.6 g/dl and non pregnant bitches it was 6.3 to 7.0 g/dl. He also noticed that the mean values were higher in pregnant than those in non pregnant ones.

2.4.3.3 Serum Albumin

Swenson (1977) stated the mean value of serum albumin as 3.57g/dl in apparently healthy nonpregnant bitches. Singh *et al.*, (1987) found the serum albumin value ranged from 3.1 to 4.0 g /dl. According to Jacobs *et al.* (2000) the serum albumin in normal healthy dogs ranged between 2.8 to 3.9 gm/dl. Thou *et al.* (2001) reported the range of serum albumin in pregnant bitches as 4.0 to 4.9 g/dl and in non pregnant bitches it was 4.0 to 4.5 g/dl and it did not significantly differ between pregnant and non pregnant bitches.

2.4.3.4 Serum Globulin

Gentry and Liptrap (1977) found a marked alteration in the plasma pro coagulent activity in the bitch during the period of gestation. The mean values of serum globulin in dogs was reported to be 2.63g/100ml (Swenson, 1977and Singh *et al.*, 1987). According to Fisher and Fisher (1981) a decrease in mean serum creatinine and mean gamma globulin could be used in pregnancy diagnosis. Greene (1990) stated that anemia, hyperglobulinemia and hyper fibrinogenemia, and azotemia could be detected in bitches with pyometra. Prabhakaran *et al.* (1996) reported an increased levels of 3.9 ± 0.20 g/dl globulins during gestation than lactation. Arosh *et al.* (1999) found significantly higher levels of globulin in pregnant compared to pseudo pregnant ones. Thou *et al.* (2001) recorded the serum globulin in pregnant bitches in the range of 2.7 to 3.7 g/dl and in non pregnant it was 2.3 to 2.7 g/dl and the mean values were found to be significantly($P > 0.01$) higher in pregnant animals.

2.4.3.5 Plasma Fibrinogen

Fibrinogen is a high molecular weight protein (MW 340,000) that occur in the plasma in quantities of 100 to 700 mg/dl. Fibrinogen is formed in the liver and its level increased during gestation and also in inflammatory conditions of the body (Guyton and Hall, 2000).

Schalm *et al.* (1975) stated that the plasma fibrinogen value ranged from 2.0 to 4.0 mg/dl in normal dogs. Sutton and Johnstone (1977) found a significant increase in plasma fibrinogen values with total leucocyte and neutrophil counts within the defined normal range in diseased dogs.

Gentry and Liptrap (1981) stated that the change in fibrinogen values in gestation appeared to be related to the rise in progesterone which rose from barely detectable levels to a peak concentration of 4.4 ng /ml. Injection of progesterone resulted in elevation of plasma fibrinogen from a pretreatment mean value of 280 mg /dl to 357mg/dl by 24 hours and 454mg/dl by 72 hours and found to be elevated for the next 78 hours.

Ruckebusch et.al., (1991) stated that the relative concentration of fibrinogen in the plasma was between 1.9 and 2.3 g/l. Since serum has a lower concentration of large proteins (fibrinogen and globulin) it's relative viscosity was slightly less than that of plasma.

Parasnis *et al.* (1992) reported a high incidence of hypo fibrinogenemia in cases of abruptio placentae (43.9 per cent) and pregnancy induced hypertension (25 per cent). Hypofibrinogenemia occurred in 10 per cent cases of intrauterine foetal death within 4 weeks of foetal demise.

Eckersall *et al.* (1993) opined that the acute phase proteins elevated between days 30 and 50 days of gestation and could be used in pregnancy diagnosis but incidence of false positive diagnosis likely to be higher, because these proteins are elevated during nonspecific inflammatory states and in inflammatory disease states such as pyometra.

Concannon *et al.* (1995) studied the assay of plasma fibrinogen as a pregnancy test and it was found to be 98 per cent accurate with a value of greater than 280 mg/dl at 21 to 30 days of gestation and nearly 100 per cent accurate with a value of greater than 300 mg/dl at 28 to 30 days of gestation.

Concannon *et al.* (1996) found that the fibrinogen concentration increased between days 21 and 30 after the leutinizing hormone surge, and was more than 280 mg/dl between days 29 and 50 ,with peak values 539± 29mg/dl which was higher than those in nonpregnant dogs(188 ±8mg/dl).

Henry (1996) stated that an accelerated ESR was favoured by elevated levels of fibrinogen and to a lesser extent, $\alpha_{2,}$ β and γ globulins in pregnancy. These asymmetric protein molecules had a greater effect than other proteins in decreasing the negative charge of the erythrocyte that tends to keep them apart. The decreased seta potential promoted the formation of rouleaux which sedimented more rapidly than single cells. Albumin and lecithin retarded sedimentation and cholesterol accelerated the ESR.

Narayanan *et al.* (1996) found the mean fibrinogen level of pseudo pregnant bitches as 2.62g/l and bitches in diestrum (non pregnant) as 2.93g/l and 50 days pregnant bitch as 13.27g/l. They opined that the plasma fibrinogen level estimated even by 17 days after mating could be taken as a parameter for pregnancy detection.

Gunzel-Apel *et al.* (1997) studied the blood coagulation status in 31 bitches of different breeds and found significantly increased concentration of fibrinogen and fibrinogen degradation products during luteal phase of nonpregnant and pregnanct bitches. This activation was more distinct during pregnancy than in normal dogs.

Study conducted by Comeglio *et al.* (1998) revealed that F1+2 plasma levels progressively increased during physiological pregnancy up to values that were several times higher than those observed in non pregnant control women. An increase in fibrinogen during pregnancy is significantly related to F1+2 concentration.

According to Jacobs *et al.* (2000) the normal range of plasma fibrinogen in dogs was 200 to 400 mg/dl. Sridevi (2005) opined that the measurements of fibrinogen greater than 280mg/dl between day 25 to 50 after the LH surge were suggestive of pregnancy in bitches.

2.5. GESTATION LENGTH

According to Sokolowski (1980) the average gestation length for the bitch was approximately 62 days. However, viable foetuses were whelped following a gestation of 58 to 66 days (duration from breeding to parturition).

Concannon *et al.* (1983) reported that gestation length in bitches was 65 days from the serum LH peak (onset of bitches standing behaviour), 60 to 62 days from when the progesterone concentration reaches 5 to 7.5 ng /ml and 56 to 57 days after vaginal cytology changes to the dioestral pattern (noncornified epithelial cells and influx of neutrophils) which corresponds with the cessation of standing behaviour.

Johnson (1986) stated that gestation length in bitch varied from 58 to 72 days after the first breeding date as ovulation occurred at variable and unpredictable times during behavioural or cytologic oestrus and because canine sperm could maintain its ability to fertilize for at least 4 to 6 days in the genital tract. According to Kahn (1994) breeding date in dogs differ considerably from the ovulation date and it was thus difficult to accurately establish gestational age in dogs.

Concannon (2000) opined that using the day of mating or insemination, parturition might occur as early as 56 days later and as late as 68 days in bitches. A large variation in apparent gestation length could be encountered when counting from multiple matings or the last of multiple matings.

Purswell *et al.*(2000) stated that calculating the expected whelping dates from breeding dates was the most common but also the most inaccurate method because of the variability in length of oestrus in bitches.

2.6.LITTERSIZE

Concannon (1986) reported that mean littersize varied among breeds ranging from ten pups in Blood hounds and Pekingese to fewer than three pups per litter in Pomeranians. In most breeds the mean litter size was between four to eight.

Pineda (1989) opined that some toy breeds or miniature breeds had litters of one to three pups, where as larger breeds such as Setters had litters of 10 to 15 pups and concluded that a litterize of five to eight pups could be considered as the average. Perinatal puppy losses were reported to as high as 15 to 30 per cent.

Schroeder and Smith (1995) reported a litter size of two to nine pups with an average of 5.43 pups per bitch in a study on thirty German Shepherd bitches.

Gradil *et al.* (2000) stated that litter size depend on several factors and in general, small sized breeds had two to four, medium sized breeds four to seven and large sized breeds 6 to 10 pups.

Materials and Methods

3. MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Animals for the study consisted of 66 apparently normal healthy bitches selected at random from those presented for detecting optimum time of breeding and also from animals with a known history of mating brought for pregnancy diagnosis at University Veterinary Hospitals at Kokkalai and Mannuthy during the period of October 2003 to May 2005. The bitches brought for breeding advice were subjected to clinicogynaecological examination and exfoliative vaginal cytology. Breeding of bitches at least two times at 72 hour interval was advised based on the history, clinico gynaecological examination and cornification index . The bitches which were bred were subjected to pregnancy diagnosis at various stages of gestation by transabdominal palpation, ultrasonography and haematological studies. Animals presented for pregnancy diagnosis were grouped based on the body weight as large (above 20 kg), medium (between 15 to 20 kg) and small breeds(less than 15 kg).

Table 1. Breeds of bitches subjected to pregnancy diagnosis

	Breed	Number	Total	Grand total
Large breeds	Rottweilers	12	34	66
	Labrador	8		
	Germanshepherd	12		
	Greatdane	2		
Medium breeds	Dalmatian	4	12	
	Doberman	5		
	Basset hound	3		
Small breeds	Dachshunds	12	20	
	Spitz	8		

Bitches subjected to pregnancy diagnosis were allotted to three groups

Group I

Consisted of thirty six animals presented for pregnancy diagnosis at 20 to 30 days post breeding .This include 23 large ,six medium and seven small breeds.

Group II

Consisted of thirty four animals presented for pregnancy diagnosis at 31 to 40 days post breeding. This include18 large, seven medium and nine small breeds

Group III

Consisted of thirty one animals presented for pregnancy diagnosis at 41 to 65 days post breeding .This include 18 large, six medium and seven small breeds

The bitches were repeatedly examined at different stages of gestation thus included in different groups accordingly. Accuracy in diagnosing pregnant and non pregnant status by the above techniques were compared to the actual whelping data and the results were tabulated according to the trimester in which the animal was examined

3.2 ASSESSMENT OF OPTIMAL BREEDING TIME.

3.2.1 Clinicogynaecological Examination

Bitches presented with proestral bleeding were subjected to clinicogynaecological examination and complete breeding history were collected. Clinical parameters such as temperature, pulse, mucous membrane were noted. The bitches were examined for the vulval oedema, nature of discharge, vulval mucosa and abnormalities of external genitalia if any.

3.2.2 Exfoliative Vaginal Cytology

3.2.2.1 Preparation of Vaginal Smear

Vaginal discharge was collected by the technique described by Allen and Dagnell (1982). The animal was controlled in standing position and a sterile pipette with adaptor and syringe at distal end was carefully introduced in to the vagina, directing the pipette craniodorsally to the vestibule and at the vestibulo vaginal junction ,it was redirected cranially to reach the anterior vagina. The fluid aspirated and a small drop of discharge was kept on a slide, a thin smear was prepared and air dried.

3.2.2.2 Staining of Vaginal Smears

The vaginal smears were stained using Modified Wright-Giemsa stains (Post, 1985)

Stain Ingredients

Wright stain	300mg
Giemsa stain	30 mg
Methanol	100ml

Procedure

1. Prepared the vaginal smear on a clean grease free slide and was air dried.
2. Covered the smear with modified wright giemsa stain and allowed to act for 30 seconds.
3. Flooded the slide with buffer, mixed by blowing and allowed to act for 30 seconds.

4. Washed with water, dried and examined under low power then under high power of the microscope.

3.3 CELL TYPES

3.3.1 Epithelial Cells

Epithelial cells were classified in to three major categories. Parabasal cell, intermediate and superficial cells.

3.3.1.1 Parabasal Cells

These are smallest epithelial cells seen in smears and are round or oval in shape. The nucleus occupies about 45 to 90 percent of the cells.

3.3.1.2 Intermediate Cells

These include cells of varying size and types and represent all stages of maturation between parabasal and fully mature superficial cells. They become more angular, enlarge and flatten as they mature. The relative size of the nucleus decrease as the cells mature. The small intermediate cells are small and polygonal with a relatively large nucleus.

3.3.1.3 Superficial Cells

They are large polygonal cells with irregular or folded borders with or without nucleus. Based on the nuclear characteristics there were four types of superficial epithelial cells

a. Large polygonal dead cells with irregular borders without any nucleus

b. Large polygonal cells with intact nuclear membrane.

c. Large polygonal cells having small remnants of nuclei.

d. Large polygonal cells having pyckotic nuclei.

3.3.2 Cell Index

Cornification index =Number of cornified cells/Total number of epithelial cells X 100

3.4 TRANSABDOMINAL PALPATION

The method described by Sokolowski (1980) was adopted with the bitch in standing position, grasped the abdomen gently, by applying steady pressure up toward to lumbar spine and then gently bringing the fingers together, allowing the abdominal viscera to slip through the fingers to locate pregnant uterus and by identification of discrete round or oval swelling of a size expected to the respective dates in the uterus after mating were assumed as positive (Fig.3.1)

3.5 ULTRASOUND SCANNING

Ultrasound equipment (L&T SYMPHONY 4.0 Ultrasound Scanner) which produces two dimensional gray scale real time image was used for ultrasound scanning (Fig.3.2.). Five to 7.5 MHz transducer was used. Animals were placed in dorsal recumbency and a small strip of hair was shaved on the ventral midline from the pubis to just cranial to the umbilicus. Ultrasonic gel – couplant was applied to the skin and to the transducer to assure good acoustic transmission. Both sagittal and transverse scan planes were used. For scanning purposes, the abdomen was considered as two regions, one right of and the other left of the medial plane. Each region was scanned seperately. Individual foetal sacs or developing fetuses were counted. Gestational sac diameter were taken to calculate the gestational age (Nyland and Mattoon ,1995).

3.6 HAEMATOLOGICAL STUDIES.

3.6.1 Site of Blood Collection

Blood (5ml) was collected from either cephalic vein or saphenous vein. Sodium citrate 3.8 per cent at the rate of 1ml/9ml blood was used as the anticoagulant. Plasma seperated from 2 ml of blood by centrifuging at 3000 rpm for 30 minute and deep freezed at –20°C till the assay. A drop of blood was taken on clean, grease free glass slide to prepare a blood smear (Benjamin,1985)

3.6.2 Haematological Parameters

Estimation of total erythrocyte and leucocyte count, differential leucocyte count, packed cell volume, erythrocyte sedimentation rate and haemoglobin concentration were done as per the standard procedures (Schalm *et al.*, 1975).

3.6.3 Serum Biochemistry

Serum total protein was estimated by modified Biuret method while albumin was estimated by Bromocresol green dye binding method in Merck 200 spectrophotometer using commercially available kits (Agappe Diagnostics). Estimation of serum cholesterol was done by CHOD-PAP method using Merck Ecoline Cholesterol kit.

3.6.4 Plasma Fibrinogen Estimation

By Orion Diagnostica Turbox Fibrinogen Assay (Fig.3.3.).

Principle

The Orion Diagnostica Turbox Fibrinogen assay is a liquid phase immuno precipitation assay with nephelometric end point detection. Antiserum of Fibrinogen was diluted in buffer and added to an aliquot of canine plasma. The light scattering caused by antigen antibody complexes was measured after incubation. The resulting light scattering was directly propotional to the fibrinogen concentration in the sample.

Reagents

Contents

1. Fibrinogen buffer 30ml
2. Fibrinogen blank buffer 30ml
3. Fibrinogen antiserum reagent 2ml
4. Calibrator (Lyophilised) 1ml
5. Magnetic card 2pcs

Preparation of the reagent

1. Antiserum Buffer solution- Pipetted out 2ml antiserum reagent in to the fibrinogen buffer. Mixed gently.
2. Blank buffer -Ready to use.
3. Calibrator-Reconstituted with 1ml distilled water

Sample preparation

Used frozen citrated plasma. Thawed and diluted samples 1:51 (1+50) with 0.9 per cent Sodium Chloride. Turbid Samples centrifuged before assay 2000 X g 15 minutes.

Assay procedure

1. Prepared a separate sample blank for each sample as well as calibrator blank for calibrator.
2. Prepared the Calibrator as a duplicate.
3. Pipetted into the cuvettes (μl) as described below

Table 2. Plasma fibrinogen assay procedure

Particulars	Calibrator(μl)		Sample(μl)	
	Blank	Test	Blank	Test
Sample	-----	----	50	50
Calibrator	50	50	----	----
Blank buffer	500	----	500	----
Antiserum dilution	----	500	-----	500

4. Mixed by shaking gently.

5. Allowed to stand for 30 ± 5 minutes at room temperature (18-----25^{0}C)

Measurement

1. Mixed each cuvette gently before measuring.

2. Measured the calibrators and the samples in Orion Turbox plus Protein Analyser system according to the protocol described by the firm manufacturing the instrument.

3.7 GESTATION LENGTH AND LITTERSIZE

Accuracy in pregnancy status and littersize predicted was compared to actual whelping data determined by regular follow up. For comparative purpose accuracy of results were tabulated according to the trimester in which the bitch was examined. Gestation length was calculated from the first day of breeding to whelping date.

3.8. STATISTICAL ANALYSIS

The results obtained were analysed as per standard procedure suggested by Snedecor and Cochran (1985).

Fig. 3.1. Transabdominal palpation

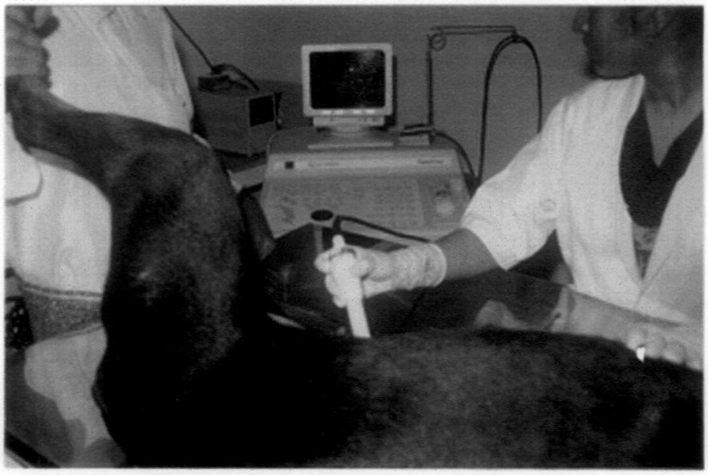

Fig. 3.2. Transabdominal ultrasound scanning

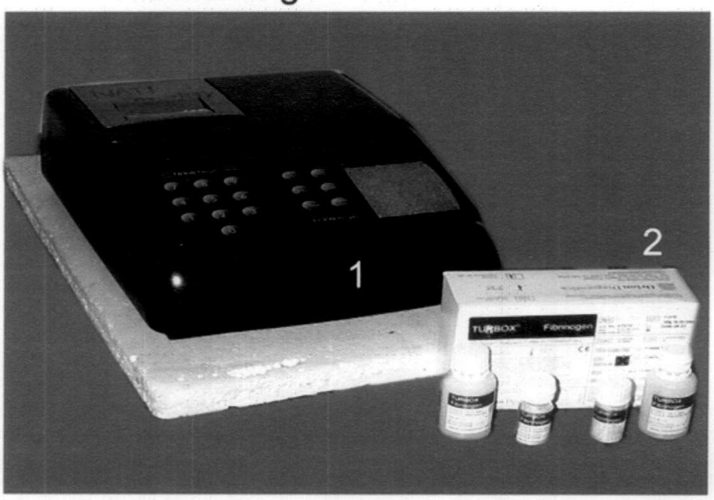

Fig. 3.3. Oriondiagnostica turbox fibrinogen assay

1.Turbox Instrument

Results

4. RESULTS

Pregnancy diagnosis was carried out in 66 bitches comprising of 34 large breeds (>20 kg) which included 12 Rottweilers, eight Labradors, 12 German Shepherd and two Greatdanes. Twelve medium breeds (15 to 20 kg) comprised of four Dalmatian, five Doberman and three Basset hounds. Small breeds (<15 kg) included twelve Dachshunds and eight Spitz. Out of 66 bitches, 36 (54.5 per cent) were bred on predetermined breeding date, based on clinicogynaecological examination and exfoliative vaginal cytology. Rest of 30 (45.45 per cent) animals were selected from cases presented in clinics for pregnancy diagnosis.

4.1 OPTIMAL BREEDING TIME

In 36 animals presented during proestrus and estrus, clinicogynaecological examination and exfoliative vaginal cytology were carried out and optimum time for breeding was advised in animals which have got a cornification index of more than 80 per cent. Among these, 26 (72 .22 per cent) animals became pregnant. Out of 30 animals brought for pregnancy diagnosis, where breeding was done purely based on symptoms of oestrum, 18 (60 per cent) animals conceived (Table 3).

Results of pregnancy diagnosis in bitches by transabdominal palpation, ultrasound scanning and haematological studies are presented in Table 4 to Table 17, and Fig.4.1 to Fig 4.23.

4.2 TRANSABDOMINAL PALPATION

Results of pregnancy diagnosis by transabdominal palpation are presented in Table 4, Table 5 and Fig.4.1 to Fig 4.3

4.2.1 Transabdominal Palpation at 20 to 30 Days Post Breeding

Twenty three large breeds were examined between 20 to 30 days post breeding out of which 21 were confirmed as positive from the whelping data. Out of these 21 true positives 13 (61.9 per cent) were correctly diagnosed by trans abdominal palpation. However, two false negatives were later confirmed as positive by ultrasound scanning. Among large breeds, six (29 per cent) bitches were found to be doubtful out of which, four (67 per cent) were examined in between 20 to 23 days post breeding. More correct diagnosis (85 per cent) were obtained between 26 to 30 days of gestation. Out of five pregnant dogs among medium breeds, 3 (60 per cent) were correctly diagnosed by abdominal palpation. The doubtful cases were later confirmed as positive by ultrasound scanning. Among seven small breeds, out of four pregnant cases 3 (75 per cent) correct positives were obtained by transabdominal palpation.

4.2.2 Transabdominal Palpation at 31 to 40 Days Post Breeding

Out of 18 large breeds examined between 31 to 40 days of gestation 15 were confirmed positive by whelping data out of which ten (66.66 per cent) were correctly diagnosed by abdominal palpation. Five doubtful (33.3 per cent) cases were there among large breeds which were examined at 37 to 40 days of gestation. Among seven medium breeds, out of four pregnant bitches all the four (100 per cent) were correctly diagnosed by abdominal palpation, whereas out of five pregnant small breeds, four (80 per cent) were diagnosed correctly by abdominal palpation.

4.2.3 Transabdominal Palpation at 41 to 65 Days Post Breeding

Out of 18 large breeds, 16 were pregnant out of which 13 (81.25 per cent) cases were correctly declared as positive by abdominal palpation, three cases were doubtful which were examined at 41 to 45 days post breeding. Among six medium breeds, four were pregnant out of which three (75 per cent) were correctly diagnosed as pregnant by abdominal palpation. Among seven small breeds, out of five pregnant bitches 3 (60 per cent) were correctly diagnosed as positive by transabdominal palpation.

4.2.4 Transabdominal Palpation in Non Pregnant Bitches

Out of 7 non pregnant bitches among large breeds 5 (71.42 per cent) were correctly diagnosed by transabdominal palpation. One false positive result was observed in a large breed which was later diagnosed as spleenomegaly by ultrasound scanning. Out of 6 non pregnant medium breeds 5 (83 per cent) were correctly diagnosed. Among nine non pregnant small breeds 8 (88.8 per cent) were diagnosed as positive by abdominal palpation. There was one false positive result obtained among small breed due to accumulation of faecal matter in colon.

4.3 ULTRASOUND SCANNING

All the animals examined for pregnancy by abdominal palpation were subjected to ultrasound scanning. Results of pregnancy diagnosis by ultrasound scanning are presented in Table 4, Table 5 and Fig. 4.1 to Fig. 4. 3. Characteristic echo patterns of foetal appearance during trimesters evaluated in this study are shown in Fig.4.8 to Fig. 4.19

4.3.1 Ultrasound Scanning at 20 to 30 Days Post Breeding

At 20 to 23 days of gestation gestational sacs with black coloured anechoic echotexture could be observed (Fig.4.8 and Fig.4.9). Gestational sacs containing foetal tissue suspended in amniotic fluid were seen between 24 to 28 days post breeding (Fig. 4.11 to Fig.4.16). Out of 21 pregnant cases among large breeds, 19 (90.4 per cent) were correctly diagnosed by ultrasound scanning. The doubtful cases were re examined after 10 days. Out of five pregnant medium breeds, four (60 per cent) diagnosed correctly by ultrasound scanning. Among seven small breeds, four were pregnant out of which three (75 per cent) were declared as pregnant by ultrasound scanning (Table 4). All doubtful cases by ultrasound scanning were re examined to confirm pregnancy. One false negative result was obtained in a small breed (spitz at 22 days of gestation) which was doubtful on abdominal palpation. The same case was diagnosed as positive after 10 days by ultrasound scanning (Table 4.).

4.3.2 Ultrasound Scanning at 31 to 40 Days Post Breeding

All pregnant cases (100 per cent) examined between 31 to 40 days of gestation among large, medium and small breeds were correctly diagnosed by ultrasound scanning (Table 4.). Foetal heart beats could be monitored from 33 days onwards in all pregnant bitches (Fig.4.17 and Fig.4.19).

4.3.3 Ultrasound Scanning at 41 to 65 Days Post Breeding

Among large, medium and small breeds all (100 per cent) were correctly diagnosed as positive by ultrasound scanning (Table 4.). Foetal heart, foetal liver, and foetal urinary bladder could be observed during this period. Vertebrae were observed as hyperechoic (whitish) areas arranged in a segmented pattern dorsal to the liver and heart. They became denser and begin to cast acoustic shadows as mineralization proceeded (Fig. 4. 17.).

4.3.4 Ultrasound Scanning in non pregnant Bitches

Out of 7 non pregnant bitches among large breeds 6 (85.71 per cent) were correctly diagnosed by ultrasound scanning. Among medium and small breeds all (100 per cent) were correctly diagnosed as non pregnant.

4.3.5 Foetal viability

By ultrasound scanning foetal heart beats could be observed in all pregnant animals from 33 days of gestation. Earliest period at which beat could be monitored was at 29 days of gestation. Feable and lowered heart beat could be observed in two cases, which were later aborted at 50 and 55 days of gestation. One pregnant bitch at 65 days post breeding was subjected to ultrasound scanning and foetal heart beats could not be monitored in that case. The sonogram revealed anaechoic ascites fluid in abdomen (Fig.4.21). Later the bitch whelped four dead pups.

4.3.6 Gestational Sac Diameter

Gestational sac diameters (GSD) were taken in large, medium and small breeds between 20 to 35 days of gestation (Fig.4.12). Mean value was used for calculating the gestational age using the formula (GSDx6)+20 and found that coefficient of correlation (r) between predictive value and actual value was 0.792, 0.855 and 0.953 in large, medium and small breeds, respectively (Table 6, Table 7, and Table 8).

4.3.7 Foetal Number

Number of foetuses estimated by ultrasound scanning and actual number of pups born based on whelping data are presented in Table 9. Total per cent of correct counting was 23.52 and wrong counting was 76.46

4.3.8 Gestational Accidents

Three cases of foetal resorption (Fig.4.22), two abortion and one pyometra (Fig.4.23) were encountered during the course of research work. Percentage occurence of foetal resorption and abortion was 4.5 and 3 per cent, respectively. Four (6 per cent) pseudo pregnancies were also diagnosed correctly by ultrasound scanning. Out of 22 negative cases diagnosed, percentage occurence of pyometra and pseudopregnancies were 4.7 and 19 per cent respectively.

4.4 ACCURACY OF ABDOMINAL PALPATION AND ULTRASOUND SCANNING

Accuracy in diagnosing pregnancy in large, medium and small breeds which were confirmed by whelping data are presented in table 4 and Table 5.

4.4.1 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 20 to 30 Days Post Breeding

Among 21 large breeds whelped 13 (61.9 per cent) positive, 6 (28.57 per cent) doubtful and 2 (9.5 per cent) negative results were obtained by abdominal palpation whereas 19 (90.4 per cent) positive and 2 (9.5 per cent) doubtful cases were obtained by ultrasound scanning.

Among 5 pregnant medium breeds 3 (60 per cent) positive and 2 (40 per cent) doubtful results were declared by abdominal palpation where as 4 (80 per cent) positive and one (20 per cent) doubtful results were obtained by ultrasound scanning.

Out of 4 pregnant animals among small breeds 3 (75 per cent) positive and one (25 per cent) doubtful case were obtained by abdominal palpation whereas 3 (75 per cent) positive and one (20 per cent) negative cases were obtained by ultrasound scanning.

The percentage accuracy of diagnosis among large breeds by abdominal palpation and ultrasound scanning was 61.9 and 90.4 per cent respectively (Fig.4.1). The accuracy of correct diagnosis among medium breeds by abdominal palpation and ultrasound scanning was 60 and 80 per cent respectively (Fig.4.2). Among small breeds accuracy was 75 per cent each for abdominal palpation and ultrasound scanning (Table 4 and Fig.4.3).

4.4.2 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 31 to 40 Days Post Breeding

Out of 15 large breeds 10 (66.6 per cent) were correctly diagnosed by abdominal palpation and 5 (33.34 per cent) were doubtful. But on ultrasound scanning all the 15 (100 per cent) were correctly diagnosed (Fig.4.1). Among medium breeds all the positive cases (100 per cent) were correctly diagnosed both by abdominal palpation and ultrasound scanning (Fig.4.2). Among small breeds 4 (80 per cent) correct positive results were obtained by abdominal palpation and one (20 per cent) case was doubtful where as, all (100 per cent) positive cases were correctly diagnosed by ultrasound scanning (Fig.4.3).

4.4.3 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 41 to 65 Days post Breeding

Out of 16 true pregnancies among large breeds, 13 (81.25 per cent) were correctly diagnosed by abdominal palpation and 3 (18.75 per cent) were doubtful, where as all (100 per cent) were correctly diagnosed as positive by ultrasound scanning (Fig.4.1). Among 4 medium breeds 3 (75 per cent) positive and one (25 per cent) doubtful case was obtained by abdominal palpation, while all the 4 (100 per cent) were correctly diagnosed as pregnant by ultrasound scanning (Fig.4.2). Among 5 small breeds whelped 3 (60 per cent) were correctly diagnosed by abdominal palpation and 2 (40 per cent) were doubtful. However all the 5 (100 per cent) were correctly diagnosed by ultrasound scanning (Fig.4.3).

4.4.4 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning in Non Pregnant Bitches

Accuracy of transabdominal palpation and ultrasound scanning in non pregnant bitches are presented in Table 5. Accuracy of abdominal palpation in detecting nonpregnant cases were 71.42, 83 and 88.8 among large, medium and small breeds respectively. Whereas the accuracy of ultrasound scanning was85.71and 100 per cent for large,medium and small breeds.

4.5 HAEMATOLOGICAL STUDIES

4.5.1 Haemogram

Total erythrocyte count in pregnant bitches was 7.62 ± 0.17, 6.99 ± 0.20, 6.70 ± 0.19 million/ cmm at 20 to 30, 31 to 40 and 41 to 65 days of gestation, respectively. In non pregnant it was 8.32 ± 0.28 million /cmm. Statistical analysis revealed significant variation in total erythrocyte count between pregnant and non pregnant animals (Table 10 and Fig.4.4).

Haemoglobin (Hb) concentration at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 11.73 ± 0.18, 10.9 ± 0.2, and 10.69 ± 0.2 g/dl respectively and in non pregnant it was 12.37 ± 0.28 which was higher than the values in pregnant dogs. By statistical analysis there was significant variation observed in haemoglobin values between pregnant and non pregnant (Table 10 and Fig.4.4).

Erythrocyte sedimentation rate (ESR) in pregnant was 12.03 ± 0.97, 17.62 ± 1.11 and 17.07 ± 1.09 mm/hr at 20 to 30, 31 to 40 and 41 to 65 days of gestation, respectively whereas it was 2.5 ± 1.57 mm/hr in non pregnant animals. Statistical analysis revealed significant variation in ESR among pregnant and non pregnant bitches (Table 10 and Fig.4.4).

PCV values in pregnant at 20 to 30, 31 to 40 and 41 to 65 days of gestation were found to be 39.83 ± 0.72, 37.70 ± 0.82 and 36.88 ± 0.8 per cent, respectively, whereas the PCV in non pregnant animals was 45 ± 1.15 per cent. There was significant variation in PCV between pregnant and nonpregnant animals (Table 10 and Fig.4.4)

4.5.2 Leucogram

Total leucocyte count (TLC) in pregnant dogs at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 13.1 ± 0.30, 13.75 ± 0.34, and 13.92 ± 0.34 thousand/cmm respectively and that of nonpregnant it was 12.767 ± 0.49 thousand/cmm. There was slight increase in total leucocyte count in pregnant compared to nonpregnant, however statistical analysis showed no significant variation (Table 11 and Fig.4.5).

Neutrophil count in pregnant at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 60.87 ± 0.57, 61.45 ± 0.65, and 60.92 ± 0.64 per cent respectively and that in nonpregnant was 62.16 ± 0.92 per cent. Lymphocytes count were

29.51 ± 0.55, 28.29 ± 0.62 and 30.24 ± 0.61 per cent ,respectively, in pregnant dogs at 20 to 30, 31 to 40 and 41 to 65 days of gestation, whereas that of nonpregnant it was 27.66 ± 0.88. Even though there were no significant variation obtained by statistical analysis, there was slight increase in lymphocytes in pregnant animals. Monocytes count at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 5.32 ± 0.35, 5.20 ± 0.4 and 5.2 ± 0.39 per cent, respectively and that of nonpregnant bitches it was 5.58 ± 0.56. The eosinophil count at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 4.09 ± 0.28, 4.04 ± 0.32, 3.6 ± 0.31 respectively. In non pregnant animals it was 4.25 ± 0.45. By statistical analysis there were no significant variation observed in leucogram of pregnant and non pregnant bitches (Table 11 and Fig.4.5).

4.5.3 Serum Biochemistry

The total serum protein at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 6.78 ± 0.17, 6.84 ± 0.19 and 6.69 ± 0.19 g/dl, respectively, that of non pregnant it was 6.37 ± 0.37 g/dl. The serum albumin at 20 to 30, 31 to 40, and 41 to 65 days of gestation were 3.88 ± 0.67, 3.84 ± 0.56 and 3.74 ± 0.21 g/dl respectively, while the serum albumin in nonpregnant was 3.64 ± 0.18 g/dl. The serum globulin concentration were 3.08 ± 0.12, 3.08 ± 0.13 and 3.06 ± 0.13 g/dl at 20 to 30, 31 to 40 and 41 to 65 days of gestation and that of nonpregnant was 2.692 ± 0.19 g/dl. There was slight increase in total serum protein and serum globulins in pregnant than nonpregnant bitches. However statistical analysis revealed no significant variation in serum protein concentration. Serum cholesterol concentration in pregnant bitches at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 212.61 ± 12.37, 214.20 ± 13.99 and 218.16 ± 13.7 mg/dl, respectively while that of nonpregnant it was 208.25 ± 19.78. There was slight increase in cholesterol concentration in pregnant bitches compared to nonpregnant bitches, however no significant difference was observed by statistical analysis (Table 12 and Fig.4.6).

4.5.3.1 Plasma Fibrinogen Estimation

Sixty samples were examined for plasma fibrinogen, 6 control, 19 samples at 20 to 30 days of gestation, 18 samples at 31 to 40 days of gestation and 17 samples at 41to 65 days post breeding were included. The (mean ± SE) plasma concentration of fibrinogen in control and pregnant bitches are given in Table 13. Plasma fibrinogen of pregnant bitches at 20 to 30, 31 to 40 and 41 to 65 days were 321.10 ± 20.92, 251.33 ± 21.49, 210.52 ± 22.1 mg/dl respectively. In non pregnant it was 156.33 ± 37.22 mg/dl. Statistical analysis revealed significant difference between pregnant and non pregnant bitches. The mean plasma fibrinogen concentration was found to be highest between 20 to 30 days of gestation (Table 13 and Fig.4.7)

4.5.4 Haematological Parameters in Large, Medium and Small Breeds

Haemogram, leucogram and serum biochemistry of pregnant dogs belonging to small, medium and large breeds are given in Table 14, Table 15, Table 16 and Table 17. By statistical analysis there were no significant variation in leucogram and serum biochemistry between large, medium and small breeds. Significant variation was observed in haemogram between groups (Table 14.). There was significant variation in erythrocyte count at 20 to 30, 31 to 40 and 41 to 65 days post breeding between groups i.e., RBC concentration was highest in large breeds at 20-30 days of gestation (8.00 ± 0.15 million/cmm) and lowest in small breeds at 41 to 65 days post breeding (5.72 ± 0.27 million/cmm). Haemoglobin concentration showed significant variation at 20 to 30 days of gestation and 31 to 40 days of gestation between groups. ESR also showed significant variation at 20 to 30 days of gestation and 31 to 40 days of gestation between groups. PCV showed significant variation at 20 to30 days of gestation in between groups. There were no significant variation observed in plasma fibrinogen concentration, between large, medium and small breeds (Table 17.).

4.6 GESTATION LENGTH

Gestation length calculated from first breeding date to whelping date ranged between 59 to 68 days in large breeds with an average of 63.38 days. And in medium breeds it ranged between 57 to 63 with an average of 59.75 days and among small breeds gestation length varied between 56 to 62 days with an average of 59 days.

4.7 LITTERSIZE

The litter size among large breeds vary between 4 to 11 pups with an average of 5.8 pups per bitch. Medium breeds it ranged between 4 to 8 pups with an average of 5.1 pups per bitch. Small breeds it ranged between one to seven pups with an average of 3.83 pups per bitch.

Table 3. Comparison of conception rate of animals bred based on clinicogynaecological examination and exfoliative vaginal cytology with those bred based on oestrus signs.

Method of breeding	Number of animals	Number of animals became pregnant	Conception rate (%)
Breeding based on vaginal cytology	36	26	72.22
Breeding based on heat signs	30	18	60

Table 4. Comparison of accuracy (%) for abdominal palpation and ultrasound scanning in pregnant bitches

Post breeding days	Animals	True status based on whelping data	Abdominal palpation			Ultrasound scanning			Per cent Accuracy	
			Positive	Doubtful	Negative	Positive	Doubtful	Negative	AP*	US**
20 to 30	Large Breeds (n=23)	21	13▲▲	6	2	19	2	0	61.9	90.4
	Medium Breeds (n=6)	5	3	2	-	4	1	-	60	80
	Small Breeds (n=7)	4	3	1	-	3	-	1	75	75
31 to 40	Large Breeds (n=18)	15	10▲	5	-	15	-	-	66.6	100
	Medium Breeds (n= 7)	4	4	-	-	4	-	-	100	100
	Small Breeds (n=9)	5	4	1	-	5	-	-	80	100
41 to 65	Large Breeds (n= 18)	16	13✹	3	-	16	-	-	81.25	100
	Medium Breeds (n= 6)	4	3✹	1	-	4	-	-	75	100
	Small Breeds (n=7)	5	3	2	-	5	-	-	60	100

*AP-Abdominal palpation **US-Ultrasound scanning

▲Foetal resorption

✹Abortion

Table 5. Comparison of accuracy (%) for abdominal palpation and ultrasound scanning in nonpregnant bitches

Animals	Number	Abdominal palpation(AP)			Ultrasound scanning(US)			Per cent accuracy	
		Positive	Doubtful	Negative	Positive	Doubtful	Negative	AP*	US**
Large breeds	7	1	1	5†◆		1	6	71.42	85.71
Medium breeds	6	-	1	5†	-	-	6	83	100
Small breeds	9	1	-	8††	-	-	9	88.8	100

*AP-Abdominal palpation **US-Ultrasound scanning

† Pseudopregnancy ◆ Pyometra

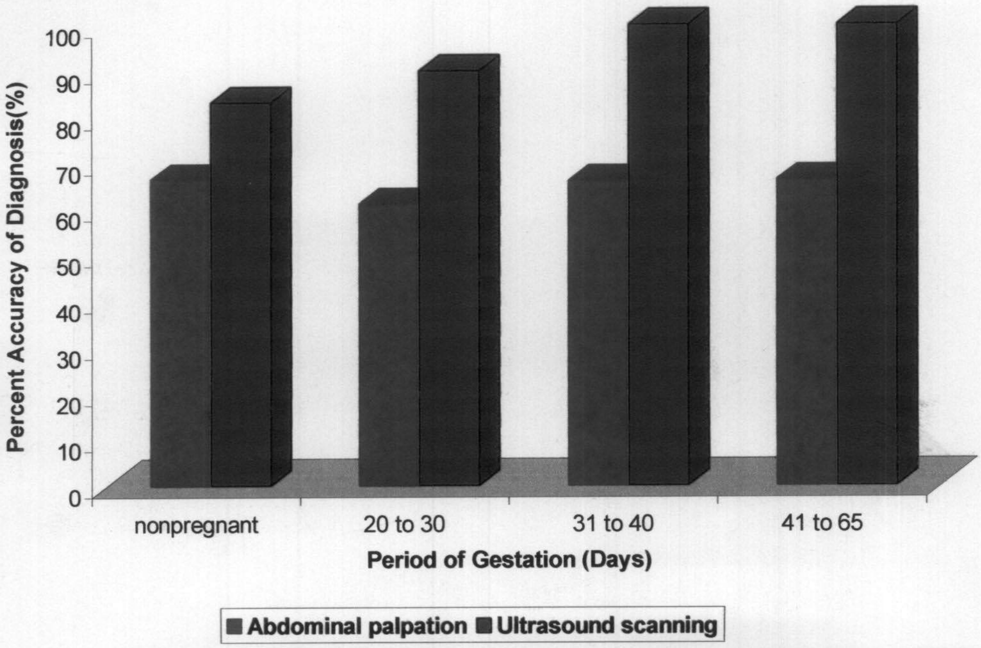

Fig.4.1. Comparison of Transabdominal palpation and Ultrasound scanning in Large Breeds.

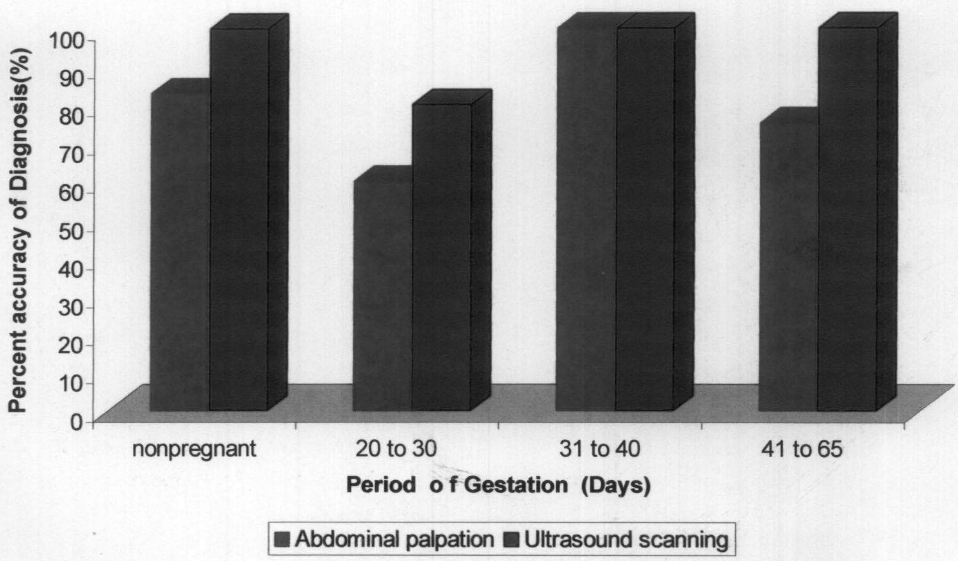

Fig. 4. 2. Comparison of Transabdominal palpation and Ultrasound scanning in Medium Breeds

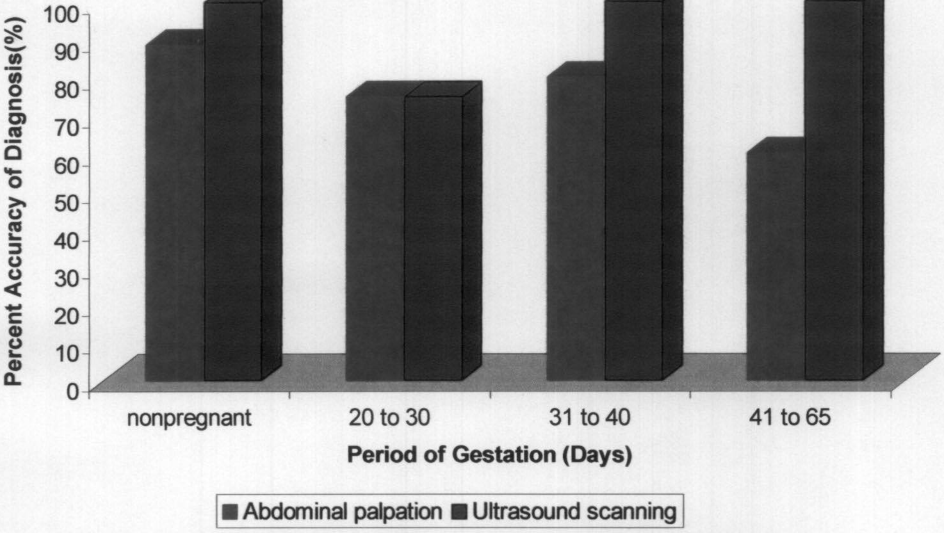

Fig. 4.3. Comparison of Transabdominal palpation and Ultrasound scanning in Small Breeds

Table 6. Assessment of gestational age by gestational sac diameter in large breeds

Post breeding days	Gestational sac diameter(GSD) (Mean) cm	Gestational age calculated by the formula (GSDx6)+20	Coefficient of correlation
20	1.04	26.24	0.792
20	0.63	23.78	
22	1.0	26.0	
23	1.15	26.9	
24	1.365	28.19	
25	1.61	29.66	
25	1.025	26.15	
25	2.145	32.87	
26	1.335	28.01	
26	1.35	28.1	
26	1.315	27.88	
27	1.145	26.87	
29	1.35	28.1	
29	2.175	33.05	
30	1.36	28.16	
30	1.525	29.15	
30	1.48	28.88	
30	2.05	32.3	
31	1.315	27.89	
32	2.15	32.9	
32	1.23	27.38	
32	2.1	32.6	
32	2.3	33.8	
33	1.37	30.2	
35	2.455	34.73	
35	2.825	36.45	
35	2.12	34.58	
35	2.23	33.38	

Table 7. Assessment of gestational age by gestational sac diameter in medium breeds

Post breeding days	Gestational sac diameter(GSD) (Mean) cm	Gestational age calculated by the formula (GSDx6)+20	Co-efficient of correlation
22	0.45	22.7	0.855
27	2.04	32.24	
28	1.40	29.12	
30	2.185	33.11	
32	2.12	32.6	
33	2.3	33.8	
34	2.28	33.68	

Table 8. Assessment of gestational age by gestational sac diameter in small breeds

Post breeding days	Gestational sac diameter(GSD) (Mean) cm	Gestational age calculated by the formula (GSDx6)+20	Co-efficient of correlation
20	0.885	25.31	0.953
23	1.27	27.62	
29	1.90	31.4	
32	2.125	32.75	
33	1.76	30.56	
34	2.225	33.35	
35	2.35	34.1	
35	2.78	36.68	

Table 9. Estimation of foetal number by ultrasound scanning

No	Post breeding day	Foetal sac counted	Whelping data
1	33	4	5
2	29	7	10
3	32	6	7
4	30	5	4
5	38	7	6
6	26	4	4
7	23	6	4
8	25	6	7
9	32	7	1
10	30	4	4
11	30	6	3
12	32	5	4
13	32	10	11
14	32	6	6
15	33	6	7
16	23	5	4
17	29	3	3

Total per cent of correct counting 4/17 = 23.52 %

Wrong counting 13/17 =76.46 %

Table 10. Haemogram of pregnant and non pregnant bitches

Post breeding days	Total erythrocyte count (million/cmm)	Haemoglobin (g/dl)	Erythrocyte sedimentation rate(mm/hr)	Packed cell volume(%)	P value
20 to 30 (n=31)	7.62 ± 0.17^{a}	11.73 ± 0.18^{a}	12.03 ± 0.97^{c}	39.83 ± 0.72^{b}	P<0.05*
31 to 40 (n=24)	6.99 ± 0.20^{ab}	10.90 ± 0.20^{b}	17.62 ± 1.11^{b}	37.70 ± 0.82^{c}	
41 to 65 (n=25)	6.70 ± 0.19^{b}	10.69 ± 0.20^{b}	17.07 ± 1.09^{b}	36.88 ± 0.80^{c}	
Nonpregnant bitches (n=12)	8.32 ± 0.28^{c}	12.37 ± 0.28^{a}	2.50 ± 1.57^{a}	45.00 ± 1.15^{a}	

*Significant
Means having the same superscript are not significantly different

Table 11. Leucogram of pregnant and non pregnant bitches

Post breeding days	Total leucocyte count (thousand/cmm)	Neutrophil (%)	Lymphocyte (%)	Monocytes (%)	Eosinophil (%)	Basophil (%)	P value
20 to 30 (n=31)	13.1 ± 0.30	60.87 ± 0.57	29.51 ±0.55	5.32 ± 0.35	4.09 ± 0.28	-	NS*
31to 40 (n=24)	13.75 ± 0.34	61.45 ± 0.65	28.29 ± 0.62	5.20 ± 0.4	4.04 ± 0.32	-	
41to 65 (n=25)	13.92 ± 0.34	60.92 ± 0.64	30.24 ± 0.61	5.20 ± 0.39	3.6 ± 0.32	-	
Nonpregnant (n=12)	12.76 ± 0.49	62.16 ± 0.92	27.66 ± 0.88	5.58 ± 0.56	4.25 ± 0.45	-	

* Not significant

The total leucocyte count and lymphocyte count in pregnant bitches is slightly higher than non pregnant bitches

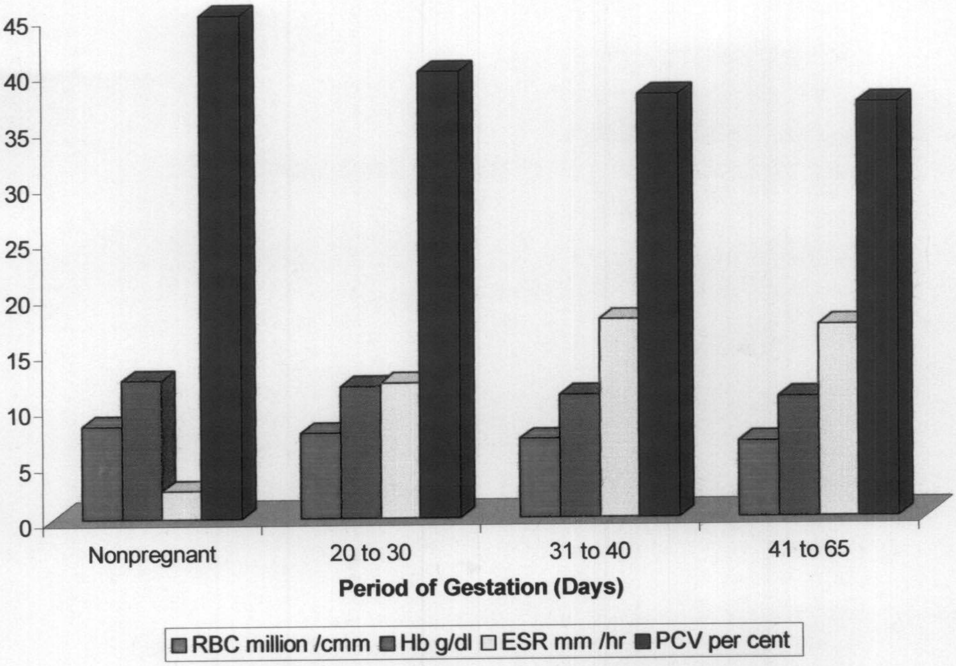

Fig.4.4. Haemogram of Pregnant and Nonpregnant Bitches

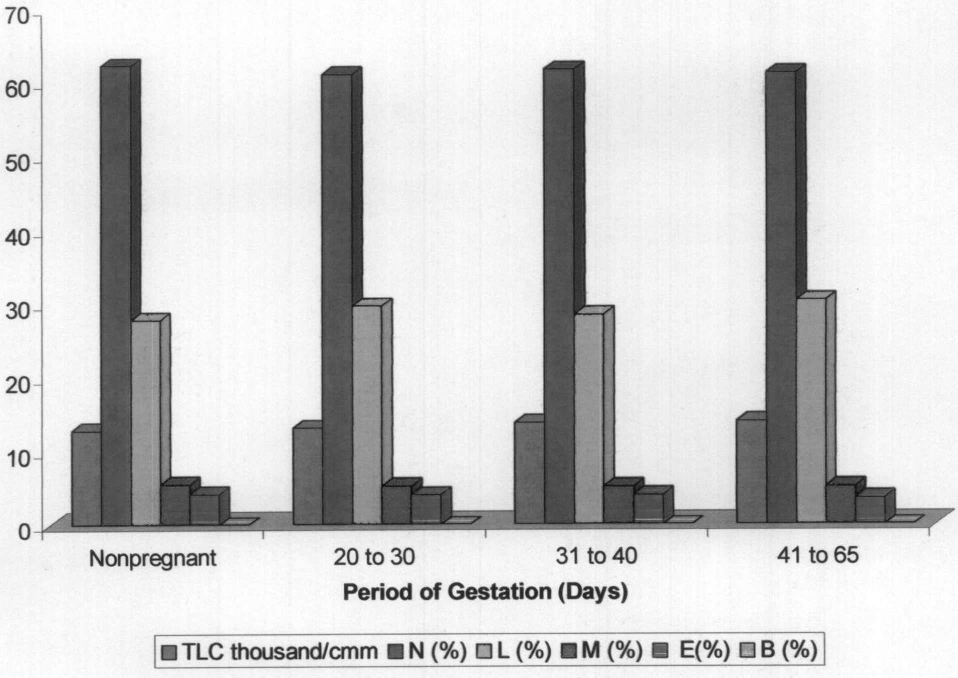

Fig.4.5. Leucogram of Pregnant and Nonpregnant Bitches

Table 12. Serum biochemistry of pregnant and non pregnant bitches

Post breeding days	Total serum protein g/dl	Serum albumin g/dl	Seum globulin g/dl	Serum Cholesterol mg/dl	P value
20 to30 (n=31)	6.78 ± 0.17	3.88 ± 0.67	3.08 ± 0.12	212.61 ± 12.31	NS*
31 to40 (n=24)	6.84 ± 0.19	3.84 ± 0.56	3.08 ± 0.13	214.20 ± 13.99	
41to 65 (n=25)	6.79 ± 0.19	3.74 ± 0.12	3.06 ± 0.13	218.16 ± 13.7	
Nonpregnant (n=12)	6.37 ± 0.23	3.64 ± 0.18	2.69 ± 0.19	208.25 ± 19.78	

*Not significant

Table 13. Plasma fibrinogen in pregnant and non pregnant bitches

Post breeding days	Plasma fibrinogen (mg/dl) Mean ± S.E	P value
20 to30 (n=19)	321.10 ± 20.92 [b]	P<0.05*
31 to 40 (n=18)	251.33 ± 21.49 [c]	
41to 65 (n=17)	210.529 ± 22.11 [d]	
Non pregnant (n =6)	156.33 ± 37.22 [a]	

*Significant
Means having the same superscript are not significantly different

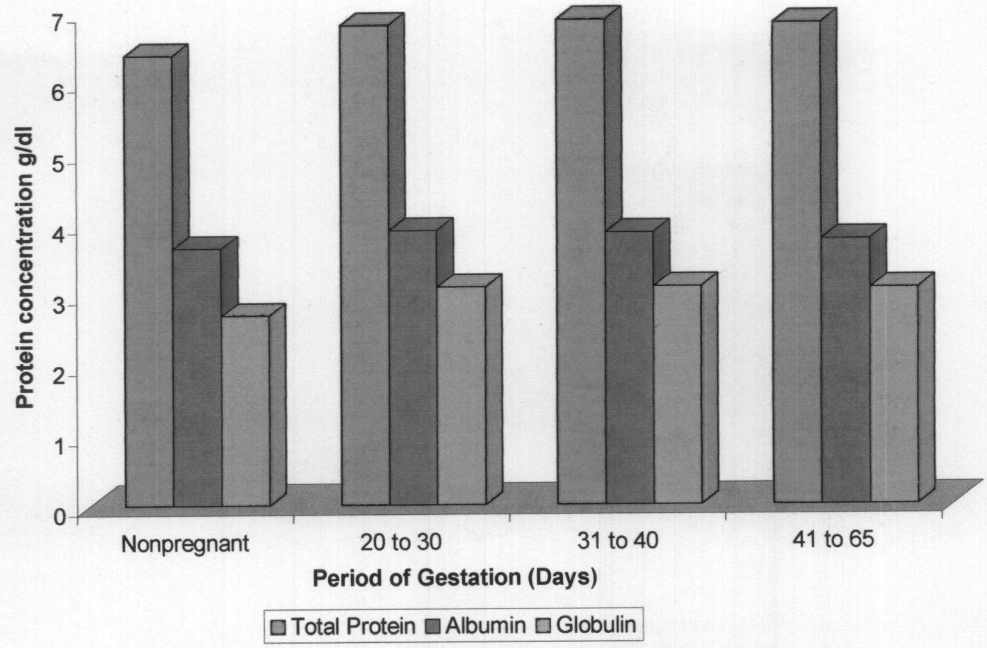

Fig.4.6. Serum Biochemistry of Pregnant and Nonpregnant Bitches

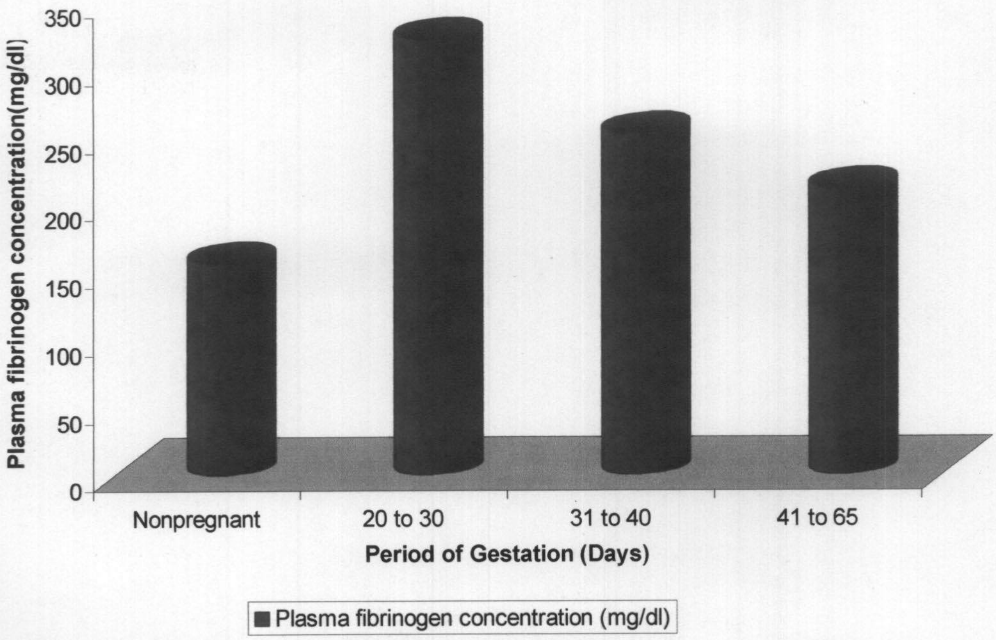

Fig. 4. 7. Plasma Fibrinogen concentration in Pregnant and Non pregnant bitches

Table 14. Haemogram of pregnant bitches (Small, medium and large breeds)

Parameters	Animals	20 to 30 days	31to 40 days	41 to 65 days
RBC (million/cmm)	Small breeds	6.35 ± 0.27^{a}	5.9 ± 0.27^{a}	5.72 ± 0.27^{a}
	Medium breeds	7.66 ± 0.3^{b}	6.25 ± 0.30^{a}	5.57 ± 0.30^{a}
	Large breeds	8.00 ± 0.15^{b}	7.55 ± 0.15^{b}	7.29 ± 0.15^{b}
P value		P<0.05*	P<0.05*	P<0.05*
Hb (g/dl)	Small breeds	10.05 ± 0.43	11.08 ± 0.45	10.12 ± 0.4
	Medium breeds	10.80 ± 0.47	10.5 ± 0.5	10.6 ± 0.45
	Large breeds	11.87 ± 0.24	12.95 ± 0.26	11.58 ± 0.22
P value		P<0.05*	P<0.05*	NS**
ESR (mm/hr)	Small breeds	7.03 ± 1.79^{a}	11.6 ± 1.91^{ab}	14.2 ± 3.09
	Medium breeds	17.6 ± 1.96^{b}	20.5 ± 2.13^{b}	19.7 ± 3.46
	Large breeds	12.15 ± 0.98^{c}	18.86 ± 1.10^{ab}	17.31 ± 1.73
P value		P<0.05*	P<0.05*	NS**
PCV (%)	Small breeds	35.83 ± 1.54^{a}	36 ± 1.95	36.2 ± 1.53
	Medium breeds	40.8 ± 1.69^{a}	37.75 ± 2.18	38.25 ± 1.71
	Large breeds	40.8 ± 0.64^{a}	38.26 ± 1.13	36.76 ± 0.85
P value		P<0.05*	NS**	NS**

*Significant ** Not significant

Small breeds (n=6) Medium breeds (n=5) Large breeds (n=20)

Means having the same superscript are not significantly different

Table 15. Leucogram of pregnant bitches (small, medium and large breeds)

Parameters	Animals	20 to 30 days	31 to 40 days	41 to 65 days
Total leucocyte count (thousand/cmm)	Small breeds	14.6 ± 0.70	14.66 ± 0.63	13.82 ± 0.91
	Medium breeds	12.68 ± 0.77	14.20 ± 0.7	14.02 ± 1.02
	Large breeds	12.75 ± 0.38	13.32 ± 0.36	13.93 ± 0.51
Neutrophils (%)	Small breeds	61.5 ± 1.17	64.0 ± 1.65	60.6 ± 1.49
	Medium breeds	59.6 ± 1.28	62.25 ± 1.85	61.0 ± 1.67
	Large breeds	61.0 ± 0.64	60.4 ± 0.95	61.0 ± 0.83
Lymphocyte (%)	Small breeds	29.5 ± 1.13	25.8 ± 1.43	29.6 ± 1.66
	Medium breeds	30.2 ± 1.23	29.0 ± 1.6	31.25 ± 1.85
	Large breeds	29.35 ± 0.62	28.93 ± 0.83	30.18 ± 0.93
Monocytes (%)	Small breeds	4.66 ± 0.79	5.4 ± 0.88	5.4 ± 1.01
	Medium breeds	5.6 ± 0.87	5.25 ± 0.99	4.0 ± 1.12
	Large breeds	5.45 ± 0.43	5.13 ± 0.51	5.43 ± 0.56
Eosinophils (%)	Small breeds	3.5 ± 0.61	3.8 ± 0.71	3.6 ± 0.88
	Medium breeds	4.4 ± 0.66	4.75 ± 0.79	3.75 ± 0.99
	Large breeds	4.2 ± 0.33	3.93 ± 0.41	3.56 ± 0.49
Basophil (%)	Small breeds	-	-	-
	Medium breeds	-	-	-
	Large breeds	-	--	-

Small breeds (n=6), medium breeds (n=5), large breeds (n=20)
NS. Not significant

Table 16. Serum biochemistry of pregnant bitches (small, medium and large breeds)

Parameters	Animals	20 to 30 days	31 to 40 days	41 to 65 days
Total protein (g/dl)	Small breeds	6.46 ± 0.43	6.64 ± 0.35	6.5 ± 0.47
	Medium breeds	6.68 ± 0.47	7.25 ± 0.39	6.97 ± 0.53
	Large breeds	6.91 ± 0.23	6.8 ± 0.2	6.83 ± 0.26
Albumin (g/dl)	Small breeds	3.7 ± 0.28	3.64 ± 0.26	3.64 ± 0.27
	Medium breeds	3.9 ± 0.31	4.02 ± 0.29	3.77 ± 0.3
	Large breeds	3.93 ± 0.15	3.86 ± 0.15	3.76 ± 0.15
Globulin (g/dl)	Small breeds	2.8 ± 0.28	3 ± 0.27	2.86 ± 0.32
	Medium breeds	2.94 ± 0.3	3.4 ± 0.30	3.2 ± 0.36
	Large breeds	3.20 ± 0.15	3.02 ± 0.15	3.08 ± 0.18
Cholesterol (mg/dl)	Small breeds	211.16 ± 26.79	198 ± 35.41	237.2 ± 31.23
	Medium breeds	217.4 ± 29.35	272 ± 39.59	230.25 ± 34.91
	Large breeds	211.85 ± 14.68	204.2 ± 20.44	209.18 ± 17.46

Small breeds (n=6), medium breeds (n=5), large breeds (n=20)

NS : Not significant

Table 17. Plasma fibrinogen in pregnant bitches (small, medium and large breeds)

Post breeding days	Animals	Plasma fibrinogen (mg/dl) Mean ± S.E
20 to 30	Large breeds (n=8)	284.87± 50.22
	Medium breeds (n=6)	348.66± 57.99
	Small breeds (n=5)	346.0 ± 63.52
31 to 40	Large breeds (n=7)	242.85 ± 20.04
	Medium breeds (n=6)	248.33 ± 21.65
	Small breeds (n=5)	266.8 ± 23.72
41 to 65	Large breeds (n=7)	200.71 ± 26.74
	Medium breeds (n=5)	345.8 ± 31.64
	Small breeds (n=5)	200.00± 31.64

NS : Not significant

Fig. 4.8. Sonogram of uterus of a bitch at 20 days post breeding. The uterine wall in the vicinity of the conceptus (arrows) appears thickened and surrounded a small anaechoic fluid accumulation

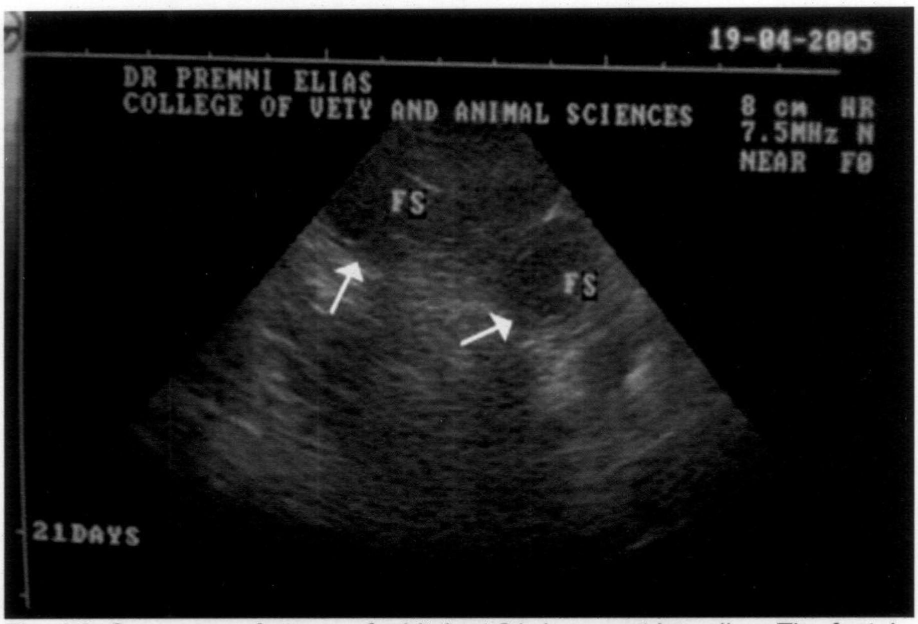

Fig. 4.9. Sonogram of uterus of a bitch at 21 days post breeding. The foetal sacs could be seen as hypoechoic lumen surrounded by an echoic uterine wall. Details of the embryo could not be demonstrated.

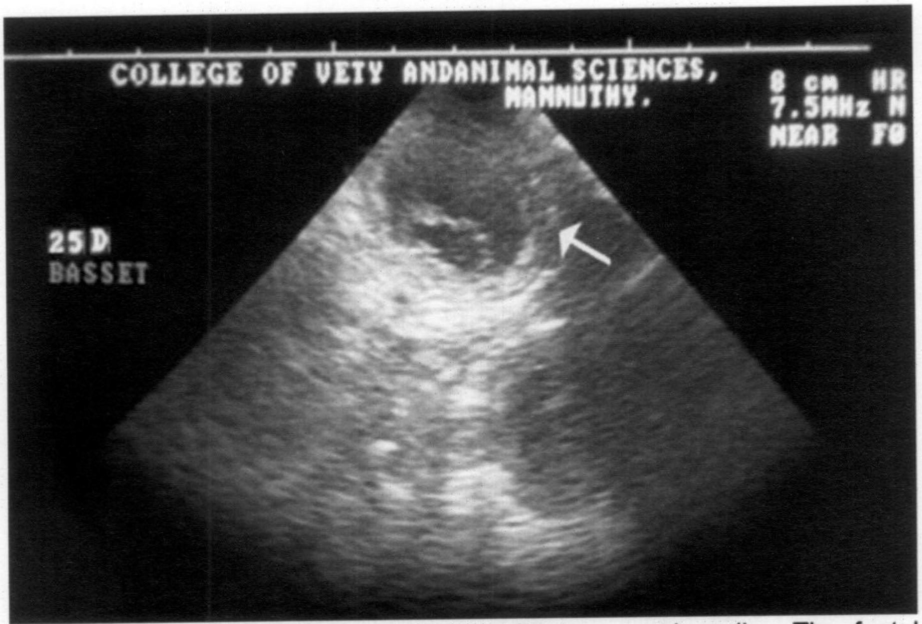

Fig. 4.10. Sonogram of uterus of a bitch at 25 days post breeding. The foetal sac could be seen as a hypoechoic lumen (arrow). A small ill-defined semi circular echoic embryo is seen within the lumen.

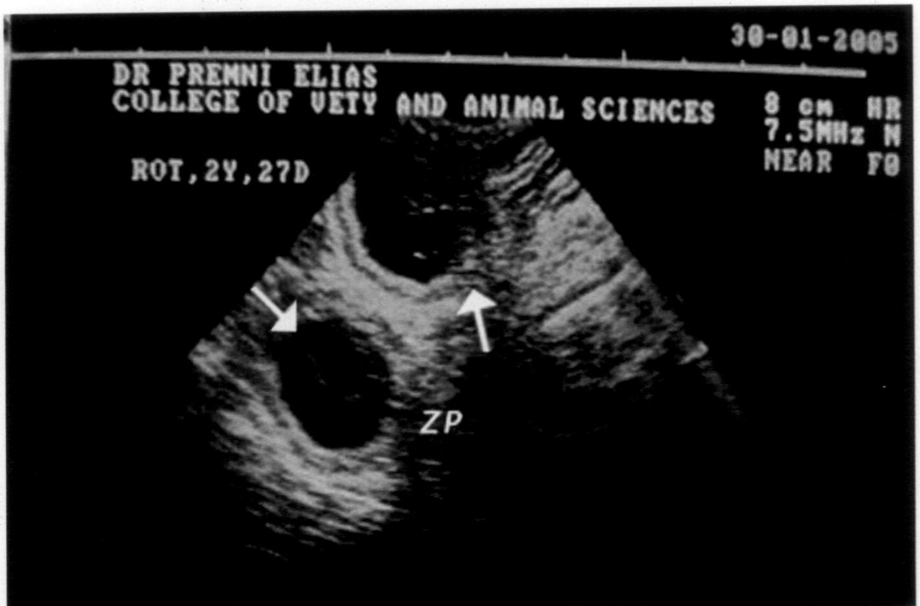

Fig. 4.11. Sonogram of uterus of a bitch at 27 days post breeding. Two gestational sacs (arrows) with ill-defined semicircular embryo. Zonary placentation (ZP) could be seen as hypoechoic area surrounding the anaechoic fluid

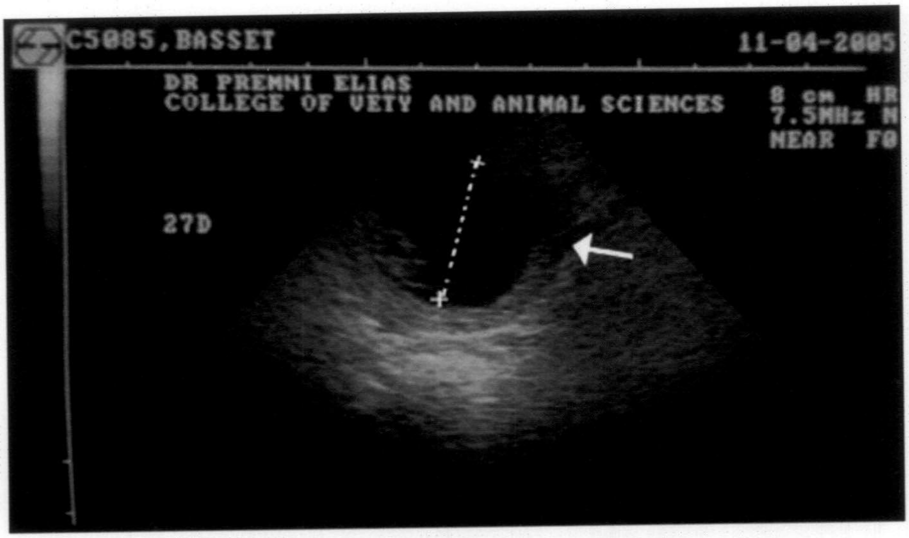

Fig. 4.12. Sonogram of uterus of a bitch at 27 days post breeding. Gestational sac diameter was measured by electronic calipers, which were installed in the scanner indicated by the crosses on the image.

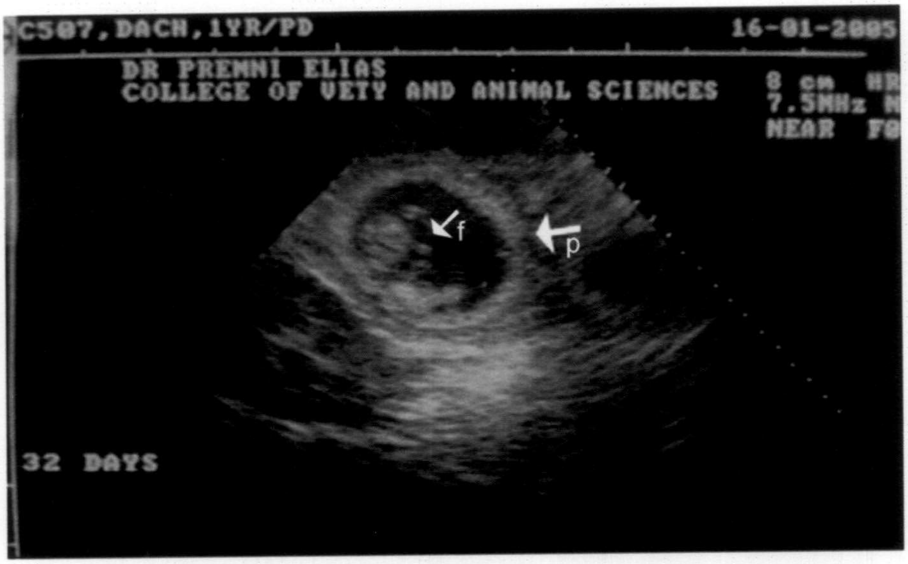

Fig. 4.13. Sonogram of uterus of a bitch at 32 days post breeding. The foetus (arrow-f) lies with its head towards the left within the zonary placenta (arrow-p)

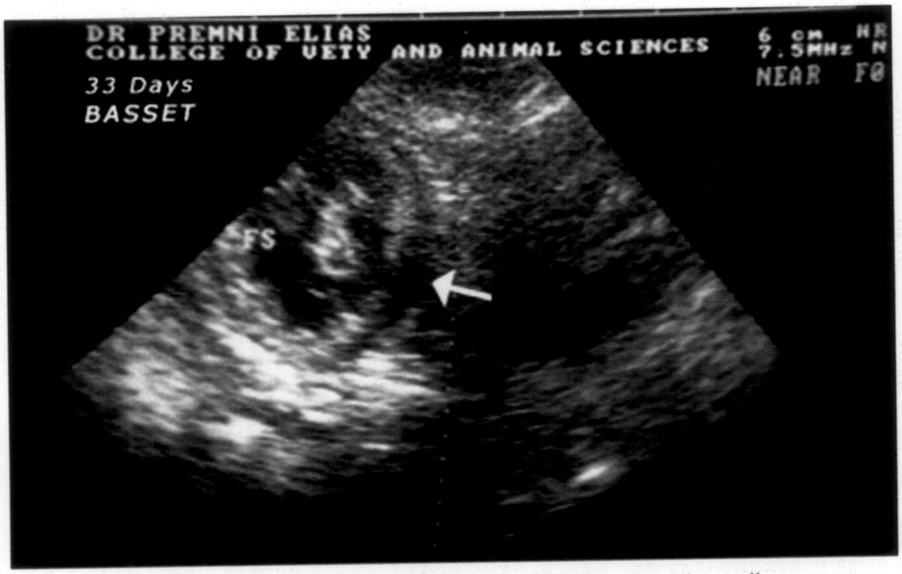

Fig. 4.14. Sonogram of uterus of a bitch at 33 days post breeding.
Gestational sac (arrow) contain developing foetus.

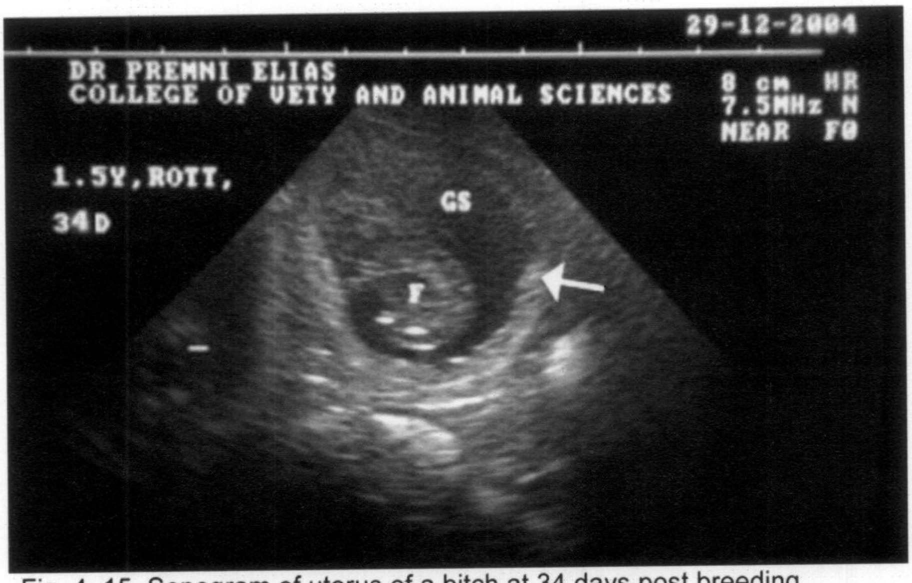

Fig. 4. 15. Sonogram of uterus of a bitch at 34 days post breeding.
Gestational sac (arrow) contain developing foetus.

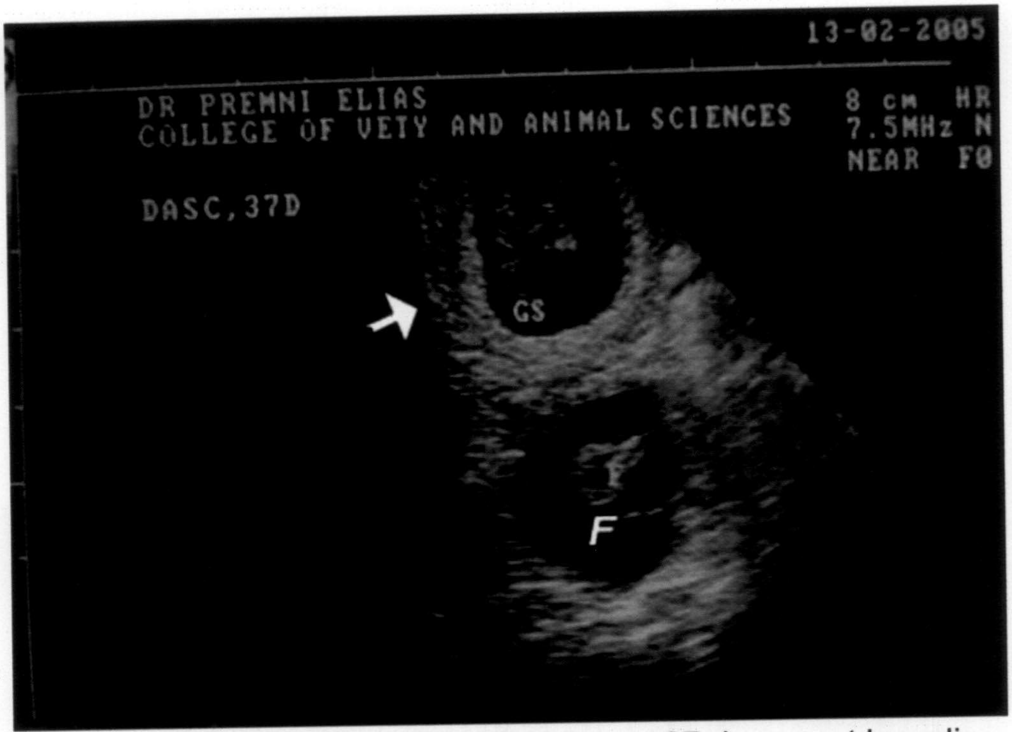

Fig. 4.16. Sonogram of uterus of a bitch at 37 days post breeding. Two gestational sacs contain developing foetus(*F*).

Fig. 4.17. Sonogram of uterus of a bitch at 43 days post breeding. The foetal heart is indicated by the arrow. Vertebrae were observed as whitish hyperechoic areas arranged in a segmented pattern dorsal to the heart (V).

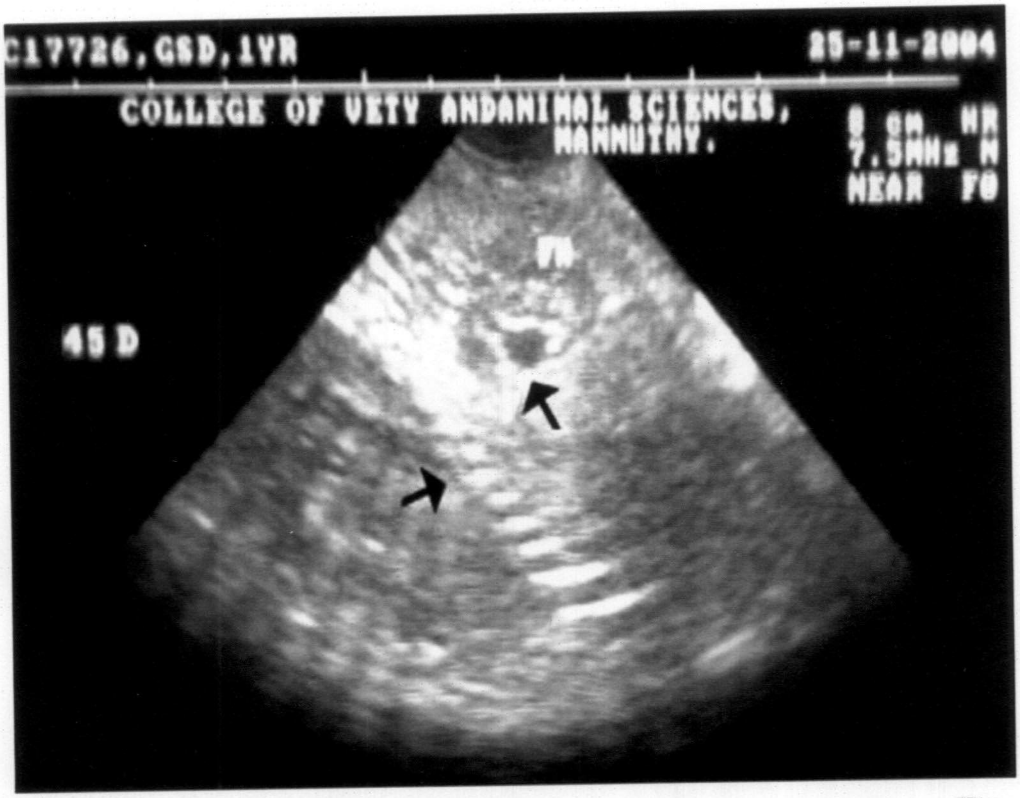

Fig. 4. 18. Longitudinal sonogram of 45 days old foetus. The upper arrow indicates heart of foetus, and the lower arrow indicates vertebrae which became denser and begin to cast acoustic shadows as mineralization proceeds.

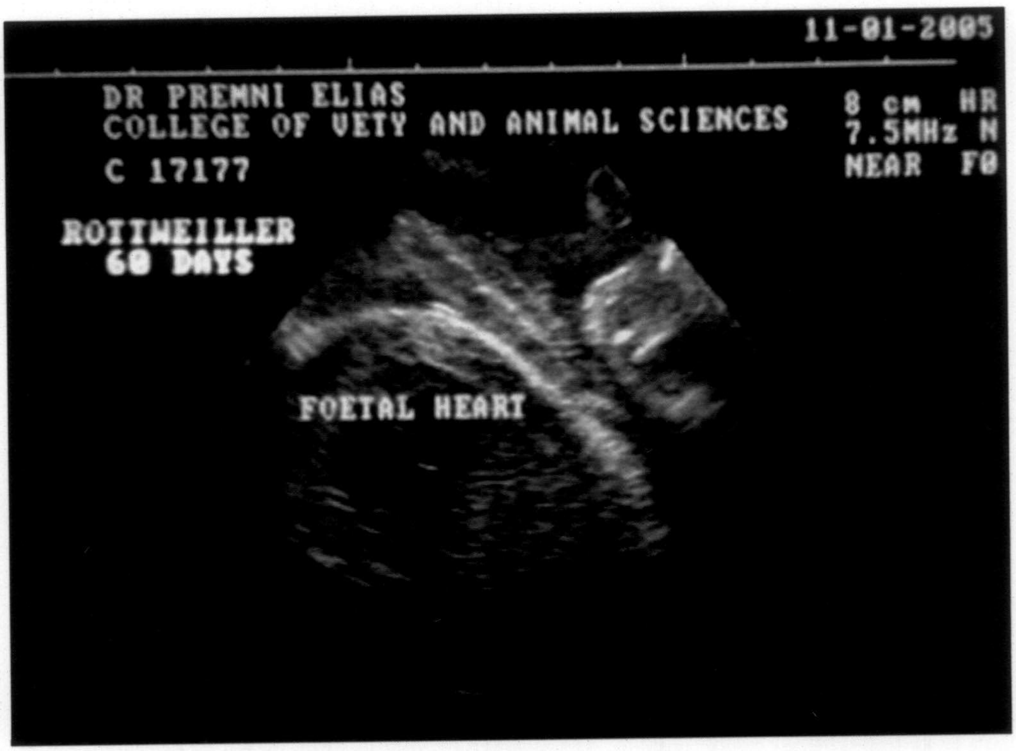

Fig. 4.19. Sonogram of uterus of a bitch at 60 days post breeding

Fig. 4. 20. **A female Dachshund with pups**

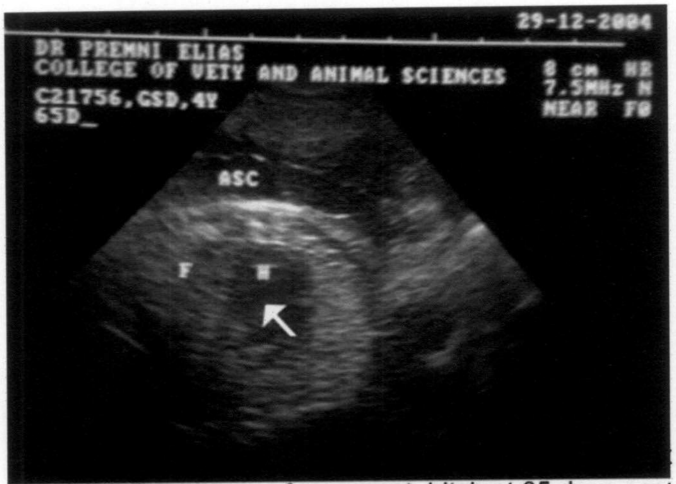

Fig. 4.21. Sonogram of pregnant bitch at 65 days post breeding. Arrow indicates foetal heart. The sonogram revealed anaechoic ascites fluid (ASC).

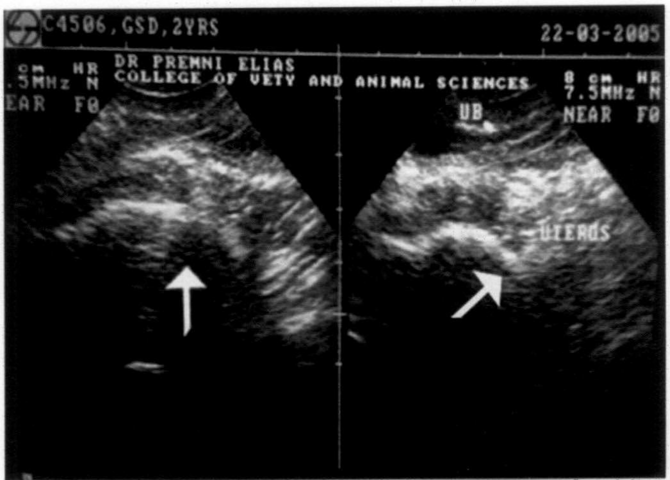

Fig. 4. 22. Longitudinal sonogram of uterus of a bitch after foetal resorption. Uterine walls are thickend and uneven with hyperechoic texture (arrows)

Fig. 4.23. Sonogram of uterus of a bitch suffering from pyometra. Narrow arrow indicates uterine sacculation filled with anaechoic fluids. Broad arrow shows anaechoic urinary bladder.

Discussion

5. DISCUSSION

Early pregnancy diagnosis is one of the most spectacular area which needs considerable studies in order to economise commercial dog breeding. Accurate evaluation of canine pregnancy entails confirmation of pregnancy, estimation of litter size and assessment of foetal viability. Cases of unwanted or accidental matings also require a definite answer of pregnancy status. Many new and old technologies are available for pregnancy diagnosis in bitches. Behavioural and physical changes, abdominal palpation and radiography are some of the routine methods. Ultrasound scanning, estimation of acute phase proteins and endocrine tests are some of the more recent techniques used for pregnancy diagnosis in bitches. Documented evidence of comparative studies on different techniques of early pregnancy diagnosis are meagre. With this perspective comparison of different methods of pregnancy diagnosis such as trans abdominal palpation, ultrasound scanning and haematological studies were carried out and the results were discussed with available literature.

5.1 OPTIMAL BREEDING TIME

Optimal breeding time was advised based on clinicogynaecological examination and exfoliative vaginal cytology. In the present study in animals bred based on exfoliative vaginal cytology, more number of animals (72.22 per cent) conceived compared to those bred based upon oestrus signs alone (Table 3). This supports the view that clinicogynaecological examination and exfoliative vaginal cytology will enhance the conception rate in bitches. Similar observations were reported earlier by Simon (1997) and Becha (2000).

5.2 TRANSABDOMINAL PALPATION

Palpation of the abdomen was easily carried out in majority of the bitches. In a few cases some difficulty was caused by excessive fat or tenseness of the abdominal muscles. Among large breeds pregnancy diagnosis by abdominal palpation was more easy in German Shepherd compared to Rottweilers and Labradors. This is due to the lean body confirmation in German Shepherd compared to other large breeds. The temperament of all animals were normal except one Rottweiler which was nervous and it was too difficult to palpate its abdomen. Similar opinion were given by Harrop (1960), Sokolowski (1980), Jones and Joshua (1982), Toal *et al.* (1986) Arthur *et al.* (1996) and Thou (1999) who opined that pregnancy diagnosis by abdominal palpation was difficult in obese and nervous bitches.

5.2.1 Transabdominal Palpation at 20 to 30 Days Post Breeding

Results of pregnancy diagnosis by abdominal palpation is presented in Table 4. Among large, medium and small breeds the percent positive obtained by abdominal palpation were 61.9, 60 and 75 per cent respectively. However, it was lower than the values reported by Thou (1999) who found 67 per cent correct positive by transabdominal palpation, at 20 to 30 days of gestation among large breeds and cent per cent accuracy among small breeds. The accuracy of diagnosis by abdominal palpation was higher in small breeds compared to large breeds. This is in agreement with the findings of Thou (1999) who also found an increased accuracy in small breeds than large breeds. Percentage of animals found positive by abdominal palpation in this study was higher than the findings of Allen and Meredith (1981) who reported 52 per cent accuracy at 20 to 30 days of gestation as against, 85 per cent in the present study. Similar experience were shared by Gradil *et al.* (2000) and Purswell *et al.* (2000) who opined that abdominal palpation is best accomplished at 25 to 28 days. The pregnancy was not confirmed in 29 per cent of large breeds due to obesity and large abdomen of bitches especially in Rottweilers and Labradores. More doubtful cases (67 per cent) were observed among animals which were subjected to abdominal palpation in between 20 to 23 days post breeding. Pregnancy diagnosis by transabdominal palpation was difficult at this stage, as the distinct uterine swellings could not be appreciated from the abdominal viscera in bitches. Similarly Thou (1999) reported more doubtful cases between 20 to 25 days of gestation among large breeds. Arthur *et al.* (1996) and Purswell *et al.* (2000) also observed difficulty to diagnose pregnancy in large and fatty bitches prior to 25 days of gestation.

5.2.2 Transabdominal Palpation at 31 to 40 Days Post Breeding

Animals found positive among large, medium and small breeds at 31 to 40 days of gestation were 66.6, 100 and 80 per cent respectively. It is consistent with the findings of Thou (1999) who reported more per cent positive as 88.9 and 100 per cent, respectively, in large and small breeds. In this study doubtful cases increased at 37 to 40 days of gestation. Similar findings were given by Harrop (1960) Arthur *et al.* (1996) and Purswell *et al.* (2000), who reported that after 35 days post breeding, the uterine swellings elongate, became more oval and more fluctuant. At this time, it was difficult to differentiate a gravid uterus from other abdominal contents. Doubtful cases (33.3%) were more among large breeds. Among small and medium breeds, more correct positive and negative results were obtained compared to large breeds may be due to easiness in palpation in these animals. Similar findings were reported by England, (1998).

5.2.3 Transabdominal Palpation at 41 to 65 Days Post Breeding

The accuracy of diagnosis among large, medium and small breeds in the present study were 81.25, 75 and 60 per cent respectively, at 41 to 65 days post breeding. Similar reports were given by Shille and Gontarek (1985) who found 75 per cent accuracy in diagnosing positive cases by transabdominal palpation during 40 to 50 days of gestation. The accuracy at this stage by abdominal palpation in the present work was lower than the observations given by Deka *et al.* (2004) who found 100 per cent accuracy in detecting pregnancy by abdominal palpation between 41 to 65 days post breeding. Lower values of accuracy in this study were due to deep fat abdomen, tenseness of abdominal muscles especially in large breeds. More doubtful cases were obtained in between 41 to

46 days post breeding, where foetal structures could not be felt but uterine enlargement was suspected. Difficulty of palpation during this period occured due to deep abdomen and uniform enlargement of uterine horns after 35 days post breeding where the swellings became elongated, almost confluent, and more pliable and more difficult to palpate as distinct entities. Similarly, Gradil *et al.* (2000) opined that 35 to 42 days of gestation was the period which was too late for palpation as foetal mass could not get and too early for radiography as foetal mineralization is not complete.

5.2.4 Transabdominal Palpation in Non Pregnant Bitches

The correct diagnosis of non pregnancy by transabdominal palpation among large, medium and small breeds were 71.42, 66.6 and 88.8 per cent respectively. Accuracy of abdominal palpation in the study was found lower than those given by Tavern *et al.* (1985) who reported 93 per cent specificity by trans abdominal palpation. False positive result was observed in a large breed which was later diagnosed as spleenomegaly by ultrasound scanning. Similar reports were given by many research workers, as Harrop (1960), Sokolowski (1980) and Shille and Gontarek (1985) who reported false positive cases by abdominal palpation due to pyometra, spleenic enlargement, neoplasia of liver, uterus etc. There was one false positive among small breed due to accumulation of faecal matter in colon. Similar false positive result was reported by Allen and Meredith (1981). Hence reliability will be much more if the bitch is exposed for rectal enema prior to transabdominal palpation.

5.3 ULTRASOUND SCANNING

All cases examined by abdominal palpation were subjected to ultrasound scanning.

5.3.1 Ultrasound Scanning at 20 to 30 Days Post Breeding

At 20 to 23 days of gestation, gestational sacs with black coloured anechoic echotexture could be observed (Fig.4.8 and Fig.4.9). The fluid is frequently anechoic and at 20 to 23 days of gestation the conceptuses filled with hypoechoic embryonic fluid could be seen (Kahn, 1994). Gestational sacs containing foetal tissue suspended in amniotic fluid were seen between 24 to 28 days post breeding (Fig.4.11 to Fig.4.16). Correct positive diagnosis by ultrasound scanning among large, medium, and small breeds were 90.4, 60 and 75 per cent respectively at 20 to 30 days post breeding. These observations are consistent with those of Toal *et al* (1986) and Barr (1988) who found an overall accuracy of more than 90 per cent by ultrasound scanning between 20 to 30 days of gestation. Positive cases obtained in this study was lower than that reported by Deka *et al* (2004) who found 100 per cent efficacy of diagnosing pregnancy by ultrasound scanning. One false negative result was obtained in a spitz at 22 days of gestation which was doubtful on abdominal palpation. The same case was diagnosed as positive after 10 days by ultrasound scanning. The reasons for false negative result by ultrasound scanning was described by Shille and Gontarek, (1985) as tenseness or restlessness of the bitch, poor transducer to skin contact due to inadequate application of transmission gel, or remnants of sand on the skin.

5.3.2 Ultrasound Scanning at 31 to 40 Days Post Breeding

Among large, medium and small breeds all positive cases (100 per cent) were correctly diagnosed by ultrasound scanning at 31 to 40 days of gestation without false positive and false negatives. Foetal heart beats could be monitored from 33 days onwards in all pregnant bitches (Fig.4.17 and Fig.4.18). Similar reports were given by Allen and Meredith (1981), Bondestam *et al.* (1983), Toal *et al.* (1986) and Deka *et al.* (2004) who found more accurate positive and negative results by ultrasound scanning during 31 to 40 days of gestation.

5.3.3 Ultrasound Scanning at 41 to 65 Days Post Breeding

The efficacy in diagnosing pregnancy at 41 to 65 days post breeding was cent per cent in large, medium and small breeds. Foetal heart, foetal liver, and urinary bladder could be observed during this period. Vertebrae were observed as hyperechoic (whitish) areas arranged in a segmented pattern dorsal to the liver and heart. They became denser and began to cast acoustic shadows as mineraliztion proceeded (Fig.4.17). Similar reports were given by Allen and Meredith (1981), Bondestam *et al.* (1983), Toal *et al.* (1986) and Deka *et al.* (2004) who found 100 per cent accuracy in diagnosing pregnancy at 41 to 65 days of gestation by ultrasound scanning.

5.3.4 Ultrasound Scanning in Non Pregnant Bitches

Correct diagnosis of nonpregnancy in large breeds were 85.71 per cent and in medium and small breeds, it was 100 per cent. The findings were consistent with Allen and Meredith (1981) who had given 92 per cent of correct negative results from day 21 to day 25 and 73 per cent correct negative results between day 26 to 35 and 100 per cent for later stages.

5.3.5 Foetal Viability

In the present study the earliest period at which heart beat could be monitored was at 29 days post breeding. This is in accordance with Bondestam *et al.* (1983) who reported that foetal viability could be assessed by observing heart beats at 28th day of gestation. Cartee and Rowles (1984) observed foetal heart beating at 120 to140 beats/minute from day 28 post breeding and opined that foetal viability could be assessed from this stage onwards by both cardiac and generalized activity. In all positive cases after 33 days post breeding foetal heart beat could be monitored by ultrasound scanning. Similar reports were given by Bhadwal and Mirakhur (2000) and Chandolia *et al.* (2003) who found flickering of heart beat as a sign of foetal viability at day 35 post breeding. However, in one pregnant bitch at 65 days post breeding, by ultrasound scanning the foetal heart beats could not be monitored. The sonogram revealed anaechoic ascites fluid in abdomen (Fig.4.21). Later this bitch whelped four dead pups. Similarly Chandolia *et al* (2003) successfully used ultrasound scanning to assess the foetal viability. Hence ultrasound scanning could be reliably used for assessing the foetal viability.

5.3.6 Gestational Sac Diameter

Gestational sac diameter were taken in large, medium and small breeds between 20 to 35 days of gestation. Mean value were used for calculating the gestational age using the formula (GSD x 6) + 20 given by Nyland and Matoon (1995) and found that coefficient of correlation among large, medium and small breeds were 0.792 , 0.855 and 0.953, respectively. There was linear increase in gestational sac diameter as period of gestation progresses. Similarly Zambelli *et al.* (2002) found highly positive correlation between the anatomic and ultrasonographic measurements of the external diameter of the gestational sacs (r = 0.9967). They also found a linear correlation between the external diameter of the gestational sac and gestational age. There was not much variation on gestation sac diameter between breeds which may be due to the slow growth up to 35 days post breeding. The values stated in this study did not vary appreciably from the measurements given by Cartee and Rowles (1984) including 12 breeds ranging from a Papillon to a Rottweiler. If a scale were established for a particular breed, the vesicle size determined by ultrasound could be used for a rough estimation of fetal age and time of parturition (Shille and Gontarek, 1985). The gestational sac diameter at 20 to 23 days of gestation observed in the present work were similar to those reported by England *et al.* (2003) and hence this could be used for approximate estimation of gestational length.

5.3.7 Foetal Number

Foetal number counted by ultrasound scanning and actual number of pups whelped are presented in Table 9. Correct counting was in 23.52 per cent cases.

Counting disagreed with the whelping data in 76.46 per cent cases. The results suggest that the ultrasound scanning is not a reliable method for foetal counting. Similar opinion were given by Shille and Gontarek (1985), Poffenbarger and Feeney (1986), Toal *et al.* (1986) and Allen and England (1990). According to them the accuracy ranged between 20 to 50 per cent as pregnancy advances. Low accuracy of predicting actual foetal number was associated with overlapping fetuses or mistaking them as already counted due to acoustic artifacts. The optimum period for foetal counting was scheduled between 28 to 33 days of post breeding in this study. It was in accordance with Barr (1988) who opined that the best time for counting of fetuses was between 28 to 35 days of gestation. Foetal counting efficiency in the present study was lower than reports given by England (1998) and Deka *et al.* (2004) probably due to the variation of equipment used.

5.3.8 Gestational Accidents

Three cases of foetal resorption (Fig.4.22), two abortions, four pseudopregnancies and one pyometra (Fig.4.23) case were encountered during the course of work. Percentage occurrence of foetal resorption was 4.5 per cent, pseudopregnancies 6 per cent, abortion 3 per cent and pyometra 1.5 per cent. In this study, among 22 nonpregnant cases the percentage occurrence of pseudopregnancies were 19 per cent. Similar observations were given by (Simon 1997). There were reports about the early embryonic death and foetal resorption and spontaneous abortion in bitches. According to Ettinger and Feldman (2000) resorption of the conceptuses may occur until day 45 of pregnancy without noticeable signs. Hopper *et al.* (2004) reported incidence of foetal resorption, abortion, stillbirth and neonatal mortality in bitches as 7.4, 8.4, 8.15 and 9.6 per cent respectively.

5.4 ACCURACY OF ABDOMINAL PALPATION AND ULTRASOUND SCANNING

5.4.1 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 20 to 30 Days Post Breeding

Among large, medium and small breeds the accuracy obtained by abdominal palpation were 61.9, 60 and 75 per cent respectively. Where as, by ultrasound scanning it was 90.4, 80 and 75 per cent respectively. However, Shille and Gontarek (1985) found 42 per cent accuracy by palpation and 75 per cent accuracy by ultrasonography between 20 to 30 days of gestation. The accuracy of ultrasonography in diagnosing pregnancy from 20 days post breeding was higher (Allen and Meredith, 1981; Shille and Gontarek, 1985; Toal *et al.*, 1986; Deka *et al.*, 2004) than abdominal palpation. In this study two false negatives by abdominal palpation was correctly diagnosed as positive by ultrasound scanning at 20 to 23 days post breeding among large breeds. Similarly England (1998) reported difficulty in diagnosing pregnancy at early stages of gestation by transabdominal palpation especially in large breeds.

5.4.2 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 31 to 40 Days Post Breeding

The accuracy of abdominal palpation among large, medium and small breeds were 66.6, 100 and 80 per cent. Where as, the accuracy by ultrasound scanning was cent per cent for large, medium and small breeds. Similar findings were given by Taverne *et al.* (1985) who found a sensitivity of 89 per cent versus 92.9 per cent for abdominal palpation and ultrasound scanning. They found equal accuracy for abdominal palpation and ultrasound scanning between 25 to35 days of pregnancy and opined that ultrasound was very useful between days 33 to 45, because it was too late to palpate and too early for radiographs. Similarly Deka *et al.* (2004) found accuracy by abdominal palpation between 33 to 40 days of gestation as 83.33 per cent and by ultrasound it was 100 per cent.

5.4.3 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning at 41 to 65 days Post Breeding

Accuracy of diagnosing pregnancy by transabdominal palpation among large, medium and small breeds were 81.25, 75 and 60 per cent,respectively. By ultrasound scanning it was 100 per cent for all animals. Similar observations were given by Shille and Gontarek (1985) who found 75 per cent accuracy in diagnosing pregnancy at 40 days of gestation by trans abdominal palpation and 100 per cent by ultrasound scanning. However, accuracy by abdominal palpation in the present work was lower than the observations given by Deka *et al.* (2004) who found an accuracy of 100 per cent by abdominal palpation between day 41 to term. The difficulty of palpation encountered during 41 to 42 days of gestation in the present study might be due to the uniform enlargement of uterine horns and deep abdomen. Gradil *et al.* (2000) reported that after day 35 post breeding, the uterine swellings became elongated, almost confluent, more pliable and more difficult to palpate as distinct entities. A diffuse distension of the uterus could make diagnosis by palpation unreliable .

5.4.4 Accuracy of Diagnosis by Transabdominal Palpation and Ultrasound Scanning in Non Pregnant Bitches

Accuracy of diagnosing non pregnancy in large ,medium and small breeds by abdominal palpation were 71.42, 83 and 88.8 per cent respectively. Where as, accuracy of detecting non pregnant bitches by ultrasound scanning were 85.71 and 100 per cent respectively. Accuracy results of abdominal palpation in the study was lower than those given by Taverneet *al.* (1985) who reported 93 per cent specificity by trans abdominal palpation. The inaccurate and doubtful decision by abdominal palpation in the present study was due to obesity and in some of the animals having held the abdominal muscles tense. The well developed abdominal musculature of Labrador and Rottweilers also helped to hinder palpation. However, Allen and Meredith (1981) had recorded 92 per cent of correct negative results from day 21 to day 25 and 73 per cent between day 26 to 35 and cent per cent for later stages, which were greater than the observations in this study.

5.5 HAEMATOLOGICAL STUDIES

5.5.1 Haemogram

There were significant variation in haemogram between non pregnant and pregnant bitches in the present study. The mean value of haemogram of nonpregnant animals were similar to that given by Schalm *et al.* (1975), Ishihara (1978) Lumsden (1979), and Jacobs *et al.* (2000). According to Lumsden (1979) haematology and biochemistry observations from an individual or a group of animals compared to reference interval, developed from a corresponding population of animals using similar laboratory techniques. The observations were considered normal, if they fall within the quoted reference interval. From a normal mean control value of 8.32 ± 0.28 million/cmm, it was reduced to 7.62 ± 0.17, 6.99 ± 0.2 and 6.70 ± 0.19 million/cmm in pregnant at 20 to 30, 31 to 40 and 41 to 65 days of gestation respectively. These observations were in agreement with the observations of Prabhakaran *et al.* (1996) who reported mean RBC concentration in pregnant as 6.1 ± 1.1 million/cmm which was lower than 6.8 ± 0.84 in non pregnant dogs. Similar findings were given by Thou (1999) who found the average erythrocyte count as 6.81 ± 0.16 to 5.49 ± 0.11 million/ cmm between 21 to 50 days of gestation. From the above observations it was found that the mean values gradually decreased as pregnancy progressed resulting in anaemic condition. This finding agree with Doxey (1966), Allard *et al.* (1989) and Thou (1999) who reported a steady fall in the count as pregnancy progressed.

In this study, the haemoglobin concentration in pregnant was lower than the non pregnant. From the mean control value of 12.37 ± 0.28g /dl, it was reduced to 11.73 ± 0.18, 10.9 ± 0.2 and 10.69 ± 0.2 g/dl at 20 to 30, 31 to 40 and 41 to term in pregnant bitches. The lower values obtained at advanced stages of gestation was due to haemodilution and increased plasma volume (Sastry, 1989). Similar observations were given by Thou (1999) who found a significant decrease of haemoglobin concentration from non pregnant bitches as 14.19 ± 0.2 g/dl to 11.0 ± 0.18 g/dl as pregnancy advanced.

The erythrocyte sedimentation rate increased in pregnant than non pregnant one. The rapid increase in ESR in pregnant animals is favoured by elevated levels of fibrinogen and to a lesser extent α, β and γ-globulins in pregnancy (Henry, 1996). ESR increased from normal value of 2.5 ± 1.57 mm/hr to 12.03 ± 0.97, 17.62 ± 1.11 and 17.07 ± 1.09 mm/hr at 20 to 30, 31 to 40 and 41-65 days of gestation respectively. Thou (1999) found an ESR of pregnant dog ranging between 8.5 to 19.33 mm/hr and it was significantly higher compared to non pregnant values ranging between 5.6 to 5.88 mm/hr.

The packed cell volume were lower in pregnant compared to non pregnant. The PCV value of control was 45 ± 1.15 per cent, it was reduced to 39.83 ± 0.72, 37.70 ± 0.82 and 36.88 ± 0.8 per cent at 20 to 30, 31 to 40 and 41 to 65 days post breeding. Thou (1999) also found a decrease in PCV as pregnancy progressed and found the mean values on days 21 to 25, 30 to 35 and 45 to 50 in pregnant and non pregnant dogs as 46.33 ± 0.88, 43.33 ± 0.58 and 38.67 ± 0.53 per cent respectively. Schalm *et al.* (1975) also reported a decrease in PCV with increase in ESR in pregnancy.

5.5.2 Leucogram

There was slight increase in the total leucocyte count from control value in pregnant bitches. The control mean value was 12.76 ± 0.49 and it was increased to 13.1 ± 0.3, 13.75 ± 0.37 and 13.12 ± 0.34 at 20 to 30, 31 to 40 and 41 to 65 days of post breeding respectively. The results were in agreement with the findings of Thou (1999) who found an increase in total leucocyte count from 9.95 ± 0.26 to 17.0 ± 0.25 thousand/cmm in pregnant dogs. Prabhakaran *et al.* (1996) found no significant difference in leucocyte count during pregnancy and lactation. The slight increase in leucocyte count in pregnant compared to non-pregnant one might be attributed to inflammatory reactions during implantation.

Neutrophil count in pregnant bitches at 20 to 30, 31 to 40 and 41 to 65 days of gestation were 60.87 ± 0.57, 61.45 ± 0.65, and 60.92 ± 0.64 per cent respectively. While the Neutrophil (per cent) in nonpregnant bitches was 62.16 ± 0.92 . There was slight decrease in neutrophil count in pregnant dogs from non pregnant one. Prabhakaran *et al.* (1996) found mean neutrophil per cent in pregnant bitches as 67 ± 9.23 per cent which was higher than the observations in this study. Lymphocytes (per cent) were 29.51 ± 0.55, 28.29 ± 0.62 and 30.24 ± 0.61 at 20 to 30, 31 to 40 and 41 to 65 days of gestation, respectively where as that of non pregnant was 27.66 ± 0.88 in the present study. Lower neutrophil count associated with a higher lymphocyte count was observed in this study. This finding is in accordance with Prabhakaran *et al.* (1996), who found a lymphocyte count of 28 ± 6.2 per cent during pregnancy against 21 ± 4.2 per cent in lactating bitches. The increase in lymphocyte concentration is attributed to the stimulated immune response in pregnant bitch by the foetus. Monocyte and eosinophil count in the present work have not shown any significant variation between pregnant and non pregnant dogs.

5.5.3 Serum Biochemistry

The serum biochemistry of bitches may change related to nutritional status, physiological state and clinical condition. The pregnancy related changes in serum biochemistry especially in total protein, albumin, globulin and cholesterol estimation were done. The total serum protein in control (non pregnant bitches) was 6.37 ± 0.37 g/dl and did not significantly differ from values of pregnant bitches at 20 to 30 days (6.78 ± 0.17), 31 to 40 days (6.84 ± 0.19), and 41 to 65 days post breeding (6.79 ± 0.19). Similar findings were given by Prabhakaran *et al.* (1996) who found the mean total protein value in bitches during pregnancy as 6.8 g/dl as against 5.2 g/dl during lactation. Very slight increase in total serum protein observed in the present study might be due to increased globulins during pregnancy. So also the albumin concentration in both pregnant and non pregnant were similar but there was slight increase in globulin (3.084 ± 0.12) concentration in pregnant bitches than in non pregnant (2.69 ± 0.19) ones. Similar results were given by Prabhakaran *et al.* (1996). Arosh *et al.* (1999) and Thou *et al.* (2001) who reported an increased levels of globulins during gestation than lactation and pseudopregnancy. The higher levels of globulins in pregnant bitches than non pregnant bitches are due to the action of globulins as carrier proteins for metals, lipids, carbohydrates and hormones (Tizard, 2000). In fact that the albumin concentration remained unchanged in pregnancy may be due to the production of other protein (fibrinogen, haptoglobin etc.) for the repair of tissue damage caused by invasive zonary placenta during the process of foetal implantation.

Tizard (2000) reported that in pregnancy, the presence of foetus stimulated the mother immune system and resulted in humoral antibodies (gamma globulins) which helped to carry out the allograft mechanism. Hence the higher level of globulins in pregnant bitches may be attributed to the growth and survival of the foetus and supply of carrier proteins for further biosynthesis during pregnancy.

5.5.3.1 Plasma Fibrinogen Estimation

The acute phase reaction takes place in animals following tissue damage and is particularly associated with inflammation of any cause. During the acute phase reaction the body produces a multifactorial response to remove and replace damaged tissue. Here in this study the plasma fibrinogen was estimated in pregnant bitches at 20 to 30, 31 to 40 and 41to 65 days of gestation. Six non pregnant bitches were taken as control. The plasma fibrinogen concentration at 20 to30, 31 to 40 and 41 to 65 days in pregnant bitch varied as 321.10 ± 20.92, 251.33 ± 21.49 and 210.52 ± 22.11mg/dl respectively. The plasma fibrinogen concentration in non pregnant bitches was 156.33 ± 37.22 mg/dl . The mean plasma concentration of fibrinogen was found to be highest between 20-30 days of gestation. These findings were supported by Concannon *et al.* (1995) who reported that the fibrinogen estimation was found to be 98 per cent accurate with a value higher than 280 mg/dl at 21 to 30 days of gestation and nearly 100 per cent accurate with a value of greater than 300 mg/dl at 28 to 30 days of gestation. Gentry and Liptrap (1981) stated that the change in fibrinogen value in gestation appeared to be related to the rise in progesterone, which rose from barely detectable levels to a peak concentration of 4.4 ng/ml. Injection of progesterone resulted in elevation of plasma fibrinogen from a pre treatment mean value of 280 mg/dl to 357 mg/dl by 24 hour and 454 mg/dl by 72 hours and found to be elevated for the next 78 hours. According to Parasnis *et al.* (1992) plasma fibrinogen value in bitches with foetal resorption, spleenomegaly and pseudopregnancy were found to be lower than the values recorded in pregnancy. He reported hypofibrinogenemia in 10 per cent cases of intrauterine foetal death within a period of four weeks of foetal demise. In the present study peak fibrinogen concentration occurred in between 20 to 30 (321.10 ± 20.92) days of gestation with a peak value of 710 mg/dl at 26 day of post breeding in one case. The mean values of fibrinogen were lower as pregnancy progressed from 31 to 65 days (251.33 ± 21.49). and during later stage of gestation, the mean values were again decreased (210.52 ± 22.1). Gentry and Liptrap (1981) reported that the rise in fibrinogen during gestation are due to increased progesterone level during gestation. They reported two distinct peak in plasma fibrinogen level during gestation, one occurred at 20 to 30 days of gestation and second peak at the time of whelping. Here, in this study, only one peak was observed at 20 to 30 days of gestation. Other stages the values were higher than control but not elevated markedly. The reason for rise in fibrinogen at the time of whelping may be due to increased utilization of fibrinogen and thereby due to increased production of this protein from the liver.

Elevation of plasma fibrinogen concentration elevation during gestation is reported by several workers. In the pregnant women, fibrinogen level increased gradually through out pregnancy and showed a second increase after delivery (Fletcher *et al.* 1979; Comeglio *et al.*, 1998). Similarly, the fibrinogen degradation products and of high molecular weight fibrinogen-fibrin complexes have been shown to rise during pregnancy indicating an increase in fibrinogen catabolism (Comeglio *et al.*, 1998). The reason for increased fibrinogen in human pregnancy is related to both the local utilization of fibrinogen in the inter placental circulation and also to the hormonal changes during gestation (Fletcher *et al.*, 1979). The present results suggests that progesterone is able to alter plasma fibrinogen concentration in bitches and also the zonary placentation may contribute to the local utilization of fibrinogen due to invasive type of placenta. These factors together may be responsible for the rise in fibrinogen especially between 20 to 30 days of gestation, during which time implantation and placentation occur in canine pregnancy. Gentry and Liptrap (1977) opined that progesterone alone were not responsible for increased levels of fibrinogen because, bitches in metestrum had similar progesterone concentration but low levels of fibrinogen than gestation. So it can be concluded that the fibrinogen elevation during gestation especially during 20 to 30 days of gestation are attributed by the implantation and placentation during foetal development which evokes the inflammatory response of body resulting in enhanced production of fibrinogen from liver.

5.5.4 Haematological Parameters in Large, Medium and Small Breeds

In this study there was no significant variation in leucogram, serum biochemistry between large, medium and small breeds. Significant variation observed in RBC, Hb, ESR and PCV values in between groups. RBC concentration was higher among large breeds at 20 to 30, 31 to 40 and 41 to term compared to medium and small breeds. ESR also showed significant variation. PCV values were higher in medium and large breeds compared to small breeds. The increased RBC, Hb, PCV, ESR values in large breeds compared to small breeds may be due to increased body weight. In most mammals blood volume is a constant fraction of body weight. As body weight increases there is more need for a reservoir of oxygen. Thus corresponding increase in heamogram occur as there are reports that racing greyhounds have higher relative blood volume than mongrels (Melbin and Detweiler, 1996). There was no significant variation of plasma fibrinogen observed in between large, medium and small breeds at 20 to 30, 31 to 40 and 41 to 65 days post breeding. Hence the concentration of fibrinogen is not breed specific and so, its elevation during early stages of gestation could be taken as an index for pregnancy diagnosis.

5.6 GESTATION LENGTH

Gestation length calculated from first breeding date to whelping date ranged between 59 to 68 days in large breeds with an average of 63.38 days. And in medium breeds it ranged between 57 to 63 with an average of 59.75 days and among small breeds gestation length varied between 56-62 with an average of 59 days. Similar reports were given by Sokolowski (1980) Concannon *et al.* (1983) Johnson (1986) and Concannon (2000). According to them the gestation length in bitch varied from 58 to 65 days.

5.7 LITTERSIZE

The litter size among large breeds vary between 4 to11 pups with an average of 5.8 pups per bitch. In medium breeds it ranged between 4 to 8 pups with an average of 5.1 puppies per bitch, while in small breeds it ranged between one to seven pups with an average of 3.83 pups per bitch. This is in concurrence with the findings of Simon (1997). Similarly Concannon (1986) reported that mean littersize varied among breeds ranging from ten pups in Blood hounds and Pekingese to fewer than three pups per litter in Pomeranians. According to them in most breeds the mean litter size was between four to eight. Pineda (1989) opined that some toy breeds or miniature breeds had litters of one to three puppies, where as larger breeds such as setters had litters of 10 to 15 puppies and concluded that a litterize of five to eight puppies could be considered as the average.

5.8 CONCLUSION

In this study ultrasound scanning was found to be a more accurate method of pregnancy diagnosis. It is a safe, non invasive technique with which foetal viability could be monitored. Disadvantage observed was the inaccurate estimation of foetal numbers and the cost involved.

Abdominal palpation is a commonly used routine technique for pregnancy diagnosis in the bitch as it is simple, cheap and fairly reliable within the constraints of breed, temperament and stage of gestation provided an experienced technician should interpret the result. However pregnancy could not be confirmed by abdominal palpation, especially in obese nervous bitches. Another disadvantage observed in abdominal palpation was, it provides little information regarding fetal viability.

Haematological studies gave definite picture about the change in haemogram leucogram and serum biochemistry during pregnancy. There was significant variation of haemogram especially ESR and an increase of plasma fibrinogen at all stages of gestation. In this study fibrinogen peak was obtained during early part of gestation and hence this could be taken as an additional index for confirming pregnancy.

Present study showed that ultrasound scanning could be used as a more accurate and scientific method of diagnosing pregnancy even during early stages, especially to confirm the foetal viability. However, it could be summarized that, for routine clinical practice, an approach of transabdominal palpation combined with ultrasound scanning could be used for confirmed pregnancy diagnosis in bitches. Moreover, increase in plasma fibrinogen, at early gestation could be taken as an additional index for pregnancy diagnosis in bitches.

Summary

6. SUMMARY

The current study was taken up to compare different methods for diagnosis of pregnancy in bitches such as trans abdominal palpation, ultrasound scanning and haematological profile at different stages of gestation. Animals for the study consisted of 66 apparently normal healthy bitches, which were subjected to pregnancy diagnosis at various stages of gestation by trans abdominal palpation, ultrasonography and haematological studies. Conception rate were more in animals which were bred based on clinicogynaecological examination and exfoliative vaginal cytology, than those bred based upon estrus signs alone. This supports the view that clinicogynaecological examination and exfoliative vaginal cytology will enhance the conception rate in bitches. Accuracy in diagnosing pregnant and non pregnant status by above techniques were compared to the actual whelping data.

Transabdominal palpation for diagnosis of pregnancy was easily carried out in majority of bitches except in few cases. Percentage accuracy by abdominal palpation was higher among small breeds compared to large and medium breeds. The difficulty in confirming pregnancy was observed in animals which were examined at early stages, prior to 25 days of gestation. More accurate diagnosis by transabdominal palpation were obtained between 31 to 40 days of gestation. Difficulty for confirming pregnancy increased between 37 to 46 days post breeding by abdominal palpation, where foetal structures could not be felt but uterine enlargement suspected. There were two false negatives affirmed by abdominal palpation, of which one was due to spleenomegaly in a large breed and the other due to accumulation of faecal matter in colon. Reliability of abdominal palpation was found to be much more when the bitch was subjected to rectal enema prior to trans abdominal palpation.

All cases examined by abdominal palpation were subjected to ultrasound scanning. At 20 to 23 days of gestation, gestational sacs with black coloured

anechoic echotexture could be observed (Fig.4.8. and Fig.4.9). At 24 to 28 days post breeding gestational sacs containing foetal tissue suspended in amniotic fluid were seen (Fig. 4.11 and Fig.4.16.). In all pregnant bitches after 33 days post breeding foetal heart beat could be monitored by ultrasound scanning (Fig 4.17 and Fig.4.18.) and mineralization of foetal skeleton could be observed beyond 40 days of gestation (Fig.4.17). Correctness of diagnosis by ultrasound scanning was increased at all stages of gestation compared to transabdominal palpation. Ultrasound scanning was very useful between 33 to 45 days post breeding, because it was too late to palpate and too early to expose to radiographs. In one case (Fig 4.21) anaechoic ascites fluid was observed in which foetal heart beat was absent and which subsequently whelped four dead pups. Gestational sac diameter was taken in all pregnant bitches between 20 to 35 days of gestation. There was a linear increase in gestational sac diameter as period of gestation progressed. There was high positive correlation obtained between predicted gestational period and actual gestational period. However, the foetuses counted by ultrasound scanning did not coincided with the actual whelping data. Correct counting was obtained only in 23.52 per cent of cases. Low accuracy of predicting actual foetal number was associated with overlapping fetuses or mistaking them as already counted due to acoustic artifacts. Gestational accidents during the course of study included three cases of foetal resorption (4.5 per cent) and two abortions (3 per cent). Among 22 nonpregnant bitches four (19 per cent) pseudopregnancies and one pyometra (1.5 per cent) (Fig.4.23) were encountered.

Percentage accuracy by abdominal palpation at 20 to 30 days post breeding ranged between 60 to75 percent whereas by ultrasound scanning it was ranged between 75 to 90 per cent. Accuracy of diagnosis by abdominal palpation ranged between 66 to 100 per cent at 31 to 40 days post breeding and it was 100 per cent for ultrasound scanning. The accuracy was 60 to 81.25 per cent for abdominal palpation and cent per cent for ultrasound scanning at 41 to 65 days of gestation.

There was significant variation in haemogram between non pregnant and pregnant animals. The mean value of erythrocyte count, haemoglobin and PCV were decreased as pregnancy progressed, while ESR markedly increased in pregnant bitches compared to non pregnant animals. Statistical analysis revealed no significant variation in leucogram and serum biochemistry in pregnant bitches compared to the non pregnant. The plasma fibrinogen estimation revealed a significant rise in fibrinogen in pregnant especially at early stages of gestation. The mean value at 20 to 30, 31 to 40 and 41 to term in pregnant animals were 321.10 ± 20.92, 251.33 ± 21.79 and 210.52 ± 22.11 mg/dl respectively while in non pregnant it was only 156.33 ± 37.22 mg/dl.

Present study revealed that ultrasound scanning could be used as a more accurate and scientific method of diagnosing pregnancy even during early stages especially to confirm the foetal viability. However, for routine clinical practice, an approach of trans abdominal palpation combined with ultrasound scanning could be used for confirmed pregnancy diagnosis in bitches. Moreover, increase in plasma fibrinogen by haematological studies could be taken as an additional index for early pregnancy diagnosis in bitches.

References

REFERENCES

Allard, R.L., Carlos, A.D. and Fattin, E.C. 1989. Canine haematological changes during gestation and lactation. *Companion Anim. Pract.* 19: 3-6

Allen, W.E. and Dagnall, G.J.R. 1982. Some observations on the aerobic bacterial flora of the genital tract of the dog and bitch. *J. Small Anim. Pract.* 23: 325-335

Allen, W.E., England, G.C.W. and White, K.B. 1989. Hydrops fetalis diagnosed by real- time ultrasonography in a bichon frise bitch. *J. Small Anim. Pract.* 30: 465-467

Allen, W.E. and England, G.C.W. 1990. Studies of canine pregnancy using B-mode ultrasound. Diagnosis of early pregnancy and number of conceptuses. *J. Small Anim. Pract.* 31: 321-323

Allen, W.E. and Meredith, M.J. 1981. Detection of pregnancy in the bitch a study of abdominal palpation, A-mode ultrasound and Doppler ultrasound techniques. *J. Small Anim. Pract.* 22: 609-622

Allen, W.E, Noakes, D.E and Renton, J.P. 1991.The Genital System. *Canine Medicine and Therapeutics.* (eds. Chandler, E.A., Thompson, D.J., Sutton, J.B. and Price, J) Third edition. Blackwell Scientific Publications, London, pp.659-697.

Arosh, A.J., Devanathan, T.G., Kathiresan, D. and Rajasekharan, J. 1999. Serum biochemical profile in pregnant and pseudopregnant bitches. Detection of alkaline phosphatase and globulin as markers of pregnancy. *Indian J. Anim. Sci.* 69: 324-325

Arthur, G.H., Noakes, D.E., Pearson, H. and Parkinson, T.J. 1996. *Veterinary Reproduction and Obstetrics.* Seventh edition. W.B. Saunders Company, London. p.693.

Barr, F.J. 1988. Pregnancy diagnosis and assessment of fetal viability in the dog – A review. *J. Small Anim. Pract.* 29: 647-656

Becha, B.B. 2000. Vaginal cytology and progesterone profile in bitches during induced oestrus cycle. M.V.Sc. thesis. Kerala Agricultural University, Trichur, p.151

Benjamin, M.M. 1985. *Outline of Veterinary Clinical Pathology.* Third edition. Kalyani Publishers, New Delhi, p.351.

Bhadwal, M.S. and Mirakhur, K.K. 2000. Use of grey-scale ultrasonography for evaluating canine uterus. *Indian J. Anim. Sci.* 70: 1143-1144

Bhadwal, M.S. and Mirakhur, K.K. 2001. Ultrasonographic assessment of clinical conditions of the canine uterus. *Indian J. Vet. Surg.* 22: 82-86

Bondestam, S., Alitalo, I. and Karkkainen, M. 1983. Real time ultrasound pregnancy diagnosis in the bitch. *J. Small Anim. Pract.* 24: 145-151

*Bunck, C., Froin, H.R. and Gunzel-Apel, A.R. 2002. Experiences with a commercial Relaxin-Assay for pregnancy diagnosis in the dog. *Kleintierpraxis.* 47: 5

Burke, T.J. and Badertscher, R.R. 1986. Pregnancy Diagnosis. *Small Animal Reproduction and Infertility.* (ed. Burke, T.J.) Lea and Febriger, Philadelphia, pp. 85-92

Cartee, R.E. and Rowles, T. 1984. Preliminary study of the ultrasonographic diagnosis of pregnancy and fetal development in the dog. *Am. J. Vet. Res.* 45: 1259-1265

Chandolia, R.K., Tayal, R., Luthra, R.A. and Behl, S.M. 2003. Ultrasonographic imaging of obstetrical cases in bitches. *Indian Vet. J.* 80: 277-278

Comeglio, P., Fedi, S., Liotta, A.A., Cellai, A.P., Chiarantini, E., Prisco, D., Mecacci, F., Parretti, E., Mello, G. and Abbale, R. 1998. Blood clotting activation during normal pregnancy. *Thromb Res.* 84: 1-6

Concannon, P.W. 1986. Canine pregnancy and parturition. *Vet. Clinics. North. Am. Small Anim. Pract.* 16: 453-475

Concannon, P.W. 2000. Canine pregnancy: Predicting parturition and timing events of gestation. *Recent advances in small animal reproduction* (eds.Concannon P.W., England E. and Verstegen.I). International Veterinary Information Service. Ithaca, New York. http://www.ivis.org/advances/Concannon/concannon/ivis.pdf

Concannon, P.W., Gimpel, P. and Goldsmith, L.T. 1995. Post implantation elevation of plasma fibrinogen as a pregnancy test in dogs. *Biol. Reprod.* 52: 182

Concannon, P.W., Gimpel, I., Newton, L. and Castracane, D. 1996. Post implantation increase in plasma fibrinogen concentration with release in relaxin concentration in pregnant dogs. *Am. J. Vet. Res.* 57: 1382-1385

Concannon, P.W., Whaley, S. and Lein, D. 1983. Canine gestation length. *Am. J. Vet. Res.* 44: 1819

Cupps, P.T. 1991. *Reproduction in Domestic Animals*. Fourth edition. Academic Press, Harcourt Brace Jovanovich Publishers, San Diego, New York, p.670

Deka, H.M., Pandit, R.K. and Shrivastava, O.P. 2004. Comparative efficacy of diagnosing pregnancy through ultrasonography and other techniques in bitches. *Indian Vet. J.* 81: 700-703

Doxey, D.L. 1966. Cellular changes in the blood as an aid to diagnosis. *J. Small Anim. Pract.* 7: 77-89

Eckersall, P.D., Harvey, M.J.A., Ferguson, J.M., Renton, J.P., Nickson, D.A. and Boyd, J.S. 1993. Acute phase proteins in canine pregnancy (*Canis familiaris*) *J. Reprod. Fert. Suppl.* 47: 159-164

England, G.C.W. 1992. Ultrasound evaluation of pregnancy and spontaneous embryonic resorption in the bitch. *J. Small Anim. Pract.* 33: 430-436

England, G.C.W. 1998. *Allen's Fertility and Obstetrics in the Dog.* Second Edition. Blackwell Science Ltd., London, pp. 80-88

England, G.C.W. and Allen, W.E. 1989. Crystallization patterns in anterior vaginal fluid from bitches in oestrus. *J. Reprod. Fertility.* 86: 335-339

England, G.C.W., Allen, W.E. and Porter, D.J. 1990. Studies on canine pregnancy using B-mode ultrasound development of the conceptus and determination of gestational age. *J. Small Anim. Pract.* 31: 324-329

England, G.C.W. and Concannon, P.W. 2002. Determination of optimal breeding time in the bitch. Basic considerations. *Recent Advances in Small Animal Reproduction* (eds Concannon, P.W., England,G. Verstegen J and Linde-Forsbeg). International Veterinary Information Service. Ithaca, New York. http://www.ivis.org/advances/Concannon/england2/IVIS.pdf

England, G.C.W., Yeager, A. and Concannon, P.W. 2003. Ultrasound imaging of the reproductive tract of the bitch. *Recent Advances in Small Animal Reproduction* (eds. Concannon, P.W., England, G., Verstegen, J. and Linda-Forsberg, C.). International Veterinary Information Service, Ithaca, New York, www.ivis.org.

Ettinger, S.J. and Feldman, E.C. 2000. *Textbook of Veterinary Internal Medicine*. Fifth edition. W.B. Saunders Company, Philadelphia, p. 1996

*Ferguson, J.M. 1990. Pregnancy diagnosis in the bitch. *Vet. Annual.* 30: 211-216

Fisher, T.M. and Fisher, D.R. 1981. Serum assay for canine pregnancy testing. *Modern Vet. Pract.* 62: 466

Fletcher, A.P., Alkjaersig, N.K. and Burstein, R. 1979. The influence of pregnancy upon blood coagulation and plasma fibrinolytic enzyme system. *Am. J. Obstet. Gynaecol.* 134: 743

Gangadhar, K.S. 1995. Care and Management of the pregnant bitch. *Pet India* 1: 16-17

Geetha, R. 2000. Effect of different freezing rates on canine spermatozoa. M.V.Sc. thesis. Kerala Agricultural University, Trichur, p 93.

Gentry, P.A. and Liptrap, R.M. 1977. Plasma levels of specific coagulation factors and oestrogen in the bitch during pregnancy. *J. Small Anim. Pract.* 18: 267-275

Gentry, P.A. and Liptrap, R.M. 1981. Influence of progesterone and pregnancy on canine fibrinogen values. *J. Small Anim. Pract.* 22: 185-194

Gradil, C.M., Yeager, A.E. and Concannon, P.W. 2000. Pregnancy diagnosis in the bitch. *Kirk's Current Veterinary Therapy XIII, Small Animal Practice* (ed. Bonagura, J.D.). W.B. Saunders Company, Philadelphia, pp. 918-923

Greene, C.E. 1990. *Infectious Diseases of Dog and Cat.* Second edition. W.B. Saunders Company, Philadelphia, p. 934

Gunzel- Apel A.R., Hayer, M., Mischke, R., Wirth, W. and Hoppen, H.O. 1997. Dynamics of haemostasis during estrous cycle and pregnancy in bitches. *J. Reprod. Fertil. Suppl.* 51: 185-193

Guyton, A.C. and Hall, J.E. 2000. *Text Book of Medical Physiology.* Tenth edition. Gupsons Paper Ltd., Noida, India, p.1148

Harrop, A.E. 1960. *Reproduction in the dog.* Bailliere Tindall and Cox, London, p.203.

*Helper, L.C. 1970. Diagnosis of pregnancy in the bitch with an ultrasonic Doppler instrument. *J. Am. Vet. Med. Assoc.* 156: 60-62

Henry, J.B. 1996. *Clinical Diagnosis and Management by Laboratory Methods.* Nineteenth edition. W.B. Saunders Company, Philadelphia, p.1556

Hewitt, D. and England, G. 2000. Assessment of optimal mating time in the bitch. *In Practice.*22: 24-33

Hinton, M. and Jones, D.R.E. 1978. The haematological examination of canine blood samples received by post the influence of delay in examination on red cell parameters. *J. small Anim. Pract.* 18: 95-99

Hopper, B.I., Richardson, J.L. and Lester, N.V. 2004. Spontaneous antenatal resolution of canine hydrops fetalis diagnosed by ultrasound. *J. Small. Anim. Pract.* 45: 2-8

Ishihara, K. Kitagawa, H., Ojma, M., Yagata, Y and Suganuma. 1978. Clinicopathological studies on Canine Dirofilarial Hemoglobinuria. *Jap. J. Vet. Sci.* 40:525-537

Jacobs, R.M., Lumsden, J.H. and Taylor, T.A. 2000. Canine and Feline Reference Values. *Kirk's current Veterinary Therapy XIII small Animal Practice.* (ed. Bonagura) W.B. Saunders Company, Philadelphia, pp.1207-1218

Jain, N.C. 1986. *Schalm's Veterinary Haematology*. Fourth edition. Lea and Febiger, Philadelphia, p.361

Johnson, C.A. 1986. Disorders of pregnancy. *Vet. Clin. North. Am. Small. Anim. Pract.* 16: 477-482

Jones, D.E. and Joshua, J.O. 1982. *Reproductive clinical problems in the dog.* Second edition. John Wright, London, p.790

Kadirvel, G. 1998. Preservation of dog semen in three extenders at refrigeration temperature. M.V.Sc. thesis. Kerala Agricultural University, Thrissur, p.84.

Kahn, W. 1994. *Veterinary Reproductive Ultrasonography.* Times Mirror International Publishers Limited, London. p. 256

Kutzler, M.A., Yeager, A.E., Mohammed, H.O. and Meyers Wallen, V.N. 2003. Accuracy of canine parturition date prediction using fetal measurements obtained by ultrasonography. *Theriogenology* 60: 1309-1317

Lumsden, J.H., Mullen, K. and McSherry, B.J. 1979. Canine haematology and Biochemistry Reference Values. *Can. J. Comp. Med.* 43: 125-131

Mattoon, J.S. and Nyland, T.G. 1995. *Veterinary Diagnostic Ultrasound.* W.B. Saunders Company, p.357

Melbin, J. and Detweiler, D.K. 1996. The cardio vascular system and blood flow. *Dukes Physiology of Domestic Animals* (eds. Swenson, M.J. and Reece,W.O). Eleventh edition. Panima Publishing Corporation, New Delhi, pp.64-89

Narayanan, P., Prathaban, S., Balasubramanian, S., Dhanapalan, P. and Parthasarathy, K.R. 1996. Plasma fibrinogen value as an index for detecting pregnancy and pseudopregnancy in bitches. *Indian J. Vet. Med.* 16: 72-74

Nyland, T.G., Mattoon, J.S. and Wisner, E.R. 1995. Physical principles, instrumentation and safety of diagnostic ultrasound. *Veterinary Diagnostic Ultrasound* (eds. Mattoon, J.S. and Nyland, T.G.) W.B. Saunders Company, Philadelphia, pp. 3-18

Olson, P.N., Behrendt, M.D. and Weiss, D.E. 1987. Reproductive problems in the bitch finding answers through vaginal cytology. *Vet. Med.* 82: 344-351

Oral, H. and Alacam, E. 2004. Estimation of parturition days with foetal heart diameter measurements by transabdominal ultrasonography in bitches. *Indian Vet. J.* 81: 1116-1118

Parasnis, H., Raje, B. and Hinduja, I.N. 1992. Relevance of plasma fibrinogen in obstetric complications. *J. Postgrad. Med.* 38: 183-185

Pineda, M.H. 1989. Reproductive patterns of dogs. *Veterinary Endocrinology and Reproduction.* Fourth edition. (eds McDonald, L.E. and Pinada, M.H.). Lea and Febiger, London, pp. 460-486

Poffenbarger, E.M. and Feeney, D.A. 1986. Use of grey-scale ultrasonography in the diagnosis of reproductive diseases in the bitch. 18 cases *J. Vet. Med. Assoc.* 189: 90-95

Post, K. 1985. Canine vaginal cytology during the oestrous cycle. *Can. Vet. J.* 26: 101-104

Prabhakaran, V., Kokilaprabhakaran, S. and Appaji Rao, V.N. 1996. Haematological values during gestation and lactation in bitches. *Indian Vet. J.* 73: 935-937

Purswell, B.J., Parker, N.A., Hess, M. and Zajac, A.M. 2000. Managing pregnant and whelping dogs. *Vet. Med.* 95: 793-800

Ruckebusch, Y., Phaneuf, L.P. and Dunlop, R. 1991. *Physiology of small and large animals.* Decker. Inc. Philadelphia, Hamilton, p. 672

Saror, D.I., Schillhorn Van Veen, T.W. and Adeyanju, J.B. 1979. The haemogram of dogs with intestinal parasites in Zaria, Nigeria. *J. Small Anim. Pract.* 20: 243-247

Sastry, G.A. 1989. *Veterinary Clinical Pathology.* CBS Publishers and Distributor, New Delhi, p.121

Schalm, O.W.J., Jain, N.C. and Caroll, E.J. 1975. *Veterinary Haematology.* Third edition. Lea and Febiger, Philadelphia, p. 609.

Schroeder, G.E. and Smith, G.A. 1995. Body weight and feed intake of German shepherd bitches during pregnancy and lactation. *J. Small. Anim. Pract.* 36: 7-11

Shille, V.M. and Gontarek, J. 1985. The use of ultrasonography for pregnancy diagnosis in the bitch. *J. Am. Vet. Med. Assoc.* 187: 1021-1025

*Simms, B.T. 1940. Erythrocyte sedimentation studies in dog. *J. Am. Vet. Med. Assoc.* 96: 77-80

Simon, S. 1997. Hormonal induction of oestrus in bitches. M.V.Sc. thesis. Kerala Agricultural University, Trichur, p.104

Simon, S. and Athman, K.V. 1998. Reproductive problems in the bitch- Finding answers through vaginal cytology. *J. Indian Vet. Assoc.* 3: 20-23

Singh, H., Kumar, A. and Chaudhuri, P.C. 1987. *Veterinary Clinical Guide* Kalyani Publishers, New Delhi, p.215

Snedecor, G.W. and Cochran, W.G. 1985. *Statistical Methods.* Eighth edition. Oxford and IBH Publishing Company, Calcutta, p. 534

Sokolowski, J.H. 1980. Normal events of gestation in the bitch and methods of pregnancy diagnosis. *Current therapy in theriogenology diagnosis, treatment and prevention of reproductive diseases in animals,* (ed. Morrow, D.A.) W.B. Saunders Company. Philadelphia, pp 590-595.

Sridevi, P. 2005. Pregnancy diagnosis in bitches. *Seminar on Canine fertility Management.* 1st May 2005.Food Mall, Puzhakkal, Thrissur Canine Club, Thrissur, pp.13-16

Sutton, R.H. and Johnstone, M. 1977. The value of plasma fibrinogen estimation in dogs A comparison with total leucocyte and neutrophil counts. *J. Small Anim. Pract.* 18: 277-281

Swenson, M.J. 1977. *Duke's Physiology of Domestic Animals.* Ninth edition, Ithaca, London, p.932

* Taverne, M.A., Okkens, A.C. and Van Oord, R. 1985. Pregnancy diagnosis in the dog: a comparison between abdominal palpation and linear-array real time echography. *Vet. Q.* 7: 249-255

Thou, N. 1999. Diagnosis of pregnancy in bitches by transabdominal palpation along with haematological studies. M.V.Sc. thesis, Kerala Agricultural University, Trichur, p. 126

Thou, N., Athman, K.V., Madhavan, E., Balakrishnan, P.P. and Rajankutty, K. 2001. Total serum protein, albumin and globulin in pregnant Alsatian bitches. *Indian J. Anim. Reprod.* 22: 152-153

*Thuroczy, J. and Balogh, L. 2003. The role of ultrasonography in the diagnosis of reproductive problems in dogs II pregnancy and its pathological forms. *Kleintier Praxis.* 4: 61-66

Tizard, I.R. 2000. *Veterinary Immunology.* Sixth edition. W.B. Saunders Company, Philadelphia, p.482

Toal, R.L., Walker, M.A. and Henry, G.A. 1986. A comparison of real time ultrasound, palpation and radiography in pregnancy detection and littersize determination in the bitch. *Vet. Rad.* 27: 102-108

Vyas, S., Deen, A. and Sahani, M.S. 2003. Ultrasound principles and instrumentation. *Vet. Pract.* 4: 55-60

Wright, P.J. 1991. Practical aspects of the estimation of the time of ovulation and of insemination in the bitch. *Aust. Vet. J.* 68: 10-13

Yeager, A.E. and Concannon, P.W. 1990. Association between the preovulatory luteinizing hormone surge and the early ultrasonographic detection of pregnancy and fetal heart beats in beagle dogs. *Theriogenology.* 34: 655-665

Yeager, A.E., Mohammed, H.O., Meyer-Wallen, V., Vannerson, L. and Concannon, P.W. 1992. Ultrasonographic appearance of the uterus, placenta, fetus and fetal membranes throughout accurately timed pregnancy in beagles. *Am. J. Vet. Res.* 53: 342-351

Yuen, S.K. 1996. Pregnancy problems in small breeds of dogs and Perkinese. *Pet. India* 2: 15

Zambelli, D., Castagnetti, C., Belluzzi, S. and Bassi, S. 2002. Correlation between the age of the conceptus and various ultrasonographic measurements during the first 30 days of pregnancy in domestic cats (Felis catus). *Theriogenology* 57: 1981-1987

* Originals not consulted

COMPARISON OF DIFFERENT METHODS OF PREGNANCY DIAGNOSIS IN BITCHES

ASHA MERINA KURIAKOSE

Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2005

Department of Animal Reproduction
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR-680651
KERALA, INDIA

ABSTRACT

With the object of comparing different methods of pregnancy diagnosis and to determine the early and reliable method, a study was undertaken to investigate the efficacy of transabdominal palpation, ultrasound scanning and haematological profile during pregnancy at various stages of gestation in bitches.

Sixty six apparently normal healthy bitches were included in the study. The bitches subjected to pregnancy diagnosis were grouped based on gestational period as 20 to 30, 31 to 40 and 41 to 65 days post breeding. The data obtained were compiled and tabulated.

At 20 to 30 days post breeding the accuracy of abdominal palpation were 61.9, 60 and 75 per cent, respectively, in large, medium and small breeds and by ultrasound scanning it was 90.4, 80 and 75 per cent in that order. Accuracy of abdominal palpation in large, medium and small breeds at 31 to 40 days of gestation were 66.6, 100 and 80 respectively and it was 100 per cent for ultrasound scanning. At 41 to 65 days post breeding the accuracy of diagnosis by abdominal palpation in large, medium and small breeds were 81.25, 75 and 60 per cent respectively, while, by ultrasound scanning it was 100 per cent in all animals.

Foetal heart beat could be observed in all pregnant bitches from 33 days of gestation. There was a high correlation between predicted value and actual value of gestational age by measuring the gestational sac diameter and the coefficient of correlation was 0.792, 0.855 and 0.953 in large, medium and small breeds, respectively. However, the accuracy of estimating the foetal number by ultrasound scanning was only 23.52 per cent.

There was significant variation ($P<0.05$) in haemogram of pregnant bitches compared to non pregnant. Total erythrocyte count at 20 to 30, 31 to 40 and 41 to 65 in pregnant bitches were 7.62 ± 0.17, 6.99 ± 0.2 and 6.70 ± 0.19 million/cmm and that of non pregnant was 8.32 ± 0.28 million/cmm.

Haemoglobin concentration at 20 to 30, 31 to 40 and 41 to 65 days post breeding were 11.73 ± 0.18, 10.9 ± 0.2 and 10.69 ± 0.2 g/dl and that of non pregnant was 12.37 ± 0.28 g/dl. There was significant variation observed in haemoglobin concentration between pregnant and non pregnant dogs.

Erythrocyte sedimentation rate at 20 to 30, 31 to 40 and 41 to 65 days post breeding were 12.03 ± 0.97, 17.62 ± 1.11 and 17.07 ± 1.09 mm/hr respectively and that of non pregnant was 2.5 ± 1.57 mm/hr. There was significant variation observed in ESR between pregnant and non pregnant bitches.

The packed cell volume at 20 to 30, 31 to 40 and 41 to 65 days post breeding in pregnant animals were 39.83 ± 0.72, 37.70 ± 0.82 and 36.88 ± 0.8 per cent respectively. In non pregnant one it was 45 ± 1.15 per cent There was significant variation observed in PCV between pregnant and non pregnant.

There was no significant variation in leucogram and serum biochemistry between pregnant and non pregnant.

There was significant variation of plasma fibrinogen between pregnant and non pregnant animals. The plasma fibrinogen concentration at 20 to 30, 31 to 40 and 41 to 65 days post breeding were 321.10 ± 20.92, 251.33 ± 21.49 and 210.52 ± 22.11 mg/dl respectively and that of non pregnant it was 156.33 ± 37.22 mg/dl. The mean plasma fibrinogen concentration was found to be highest between 20 to 30 days of gestation. Hence estimation of plasma fibrinogen could be taken as an additional index for early pregnancy diagnosis in bitches.

Results of this study indicates that ultrasound scanning could be used as a more accurate and scientific method of diagnosing pregnancy even during early stages especially to confirm the foetal viability. However, for routine clinical practice an approach of transabdominal palpation combined with ultrasound scanning could be used for confirmed pregnancy diagnosis in bitches. Moreover, increase in plasma fibrinogen, by haematological studies could be taken as a supportive index for pregnancy diagnosis in bitches.