DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR ENHANCING REPRODUCTIVE EFFICIENCY IN FEMALE DOGS

DEEPTHI L.

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

Doctor of Philosophy
In
Veterinary and Animal Sciences

Faculty of Veterinary and Animal Sciences
Kerala Agricultrual University, Thrissur

2010

Department of Animal Reproduction, Gynaecology & Obstetrics

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR – 680651
KERALA, INDIA

DECLARATION

I hereby declare that this thesis, entitled **"DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR ENHANCING REPRODUCTIVE EFFICIENCY IN FEMALE DOGS"** 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.

DEEPTHI. L.

Mannuthy
16-04-2010

CERTIFICATE

Certified that the thesis entitled **"DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR ENHANCING REPRODUCTIVE EFFICIENCY IN FEMALE DOGS"** is a record of research work done independently by **Dr. Deepthi. L**, 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. K.N. Aravinda Ghosh
Chairman, Advisory Committee
Professor and Head
Veterinary College Hospital
Mannuthy, Thrissur

Mannuthy
16-04-2010

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Deepthi. L**, a candidate for the degree of Doctor of Philosophy in Animal Reproduction, Gynaecology and Obstetrics, agree that the thesis entitled **"DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR ENHANCING REPRODUCTIVE EFFICIENCY IN FEMALE DOGS"** may be submitted by Deepthi. L, in partial fulfillment of the requirement for the degree.

Dr. K.N. Aravinda Ghosh
Chairman, Advisory Committee
Professor and Head
Veterinary College Hospital
Mannuthy, Thrissur

Dr. Joseph Mathew
Associate Professor & Head
Department of Animal Reproduction,
Gynaecology and Obstetrics
College of Veterinary and Animal Sciences
Mannuthy
(Member)

Dr. K. Ramachandran
Associate Professor & Head
Regional Cattle Infertility Research Scheme
Calicut
(Member)

Dr. Syam. K. Venugopal
Associate Professor
Department of Veterinary Surgery
and Radiology
College of Veterinary and Animal Sciences
Mannuthy(Member)

Dr. Usha Narayana Pillai
Associate Professor
Department of Veterinary Clinical Medicine
College of Veterinary and
Animal Sciences, Pookot

External Examiner

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

The origin of the domestic dog (*Canis lupus familiaris*) began with the domestication of the gray wolf (*Canis lupus*) several tens of thousands of years ago. Domestication of dog dates back to the history of partnership between humans and dogs. That partnership was based on human needs for help with herding, hunting, an early alarm system, and a source of security in addition to the companionship which many of us enjoy today. Dogs, resulting from domestication enjoy a truly unique status as companion animal. No other domestic animal enjoys the comfort and companionship afforded by the dog. A burial site in Germany called Bonn-Oberkassel has joint human and dog interments dated to 14,000 years ago. The earliest domesticated dog found in China is at the early Neolithic (7000-5800 BC) Jiahu site in Henan Province (Boyko, 2009).

The agricultural revolution and subsequent urban revolution led to an increase in the dog population and a demand for specialization and these circumstances provided the opportunity for selective breeding of dogs. Now dog is considered as the best companion in solitude and periods of trouble. Over the last 10 to 15 years canine breeding has rapidly developed into a highly lucrative business and there has been a tremendous increase in breeding of pedigreed dogs. According to the quinquennial livestock census- 2003 (AHD, 2004) the domestic dog population in Kerala is 11.31 lakhs. Today dog breeding has attained a disciplined way by registering pups with the kennel club, selection of pedigreed dogs, maintenance of highly pedigreed stud dogs and even importing dogs from other countries at fancy prices. It is therefore natural that a canine breeder expects maximum returns for his investments and in this context, the role of a veterinarian right from the selection of puppy to the successful planned reproduction holds upper hand.

In the world of dog breeding, canine infertility means the difference between a pregnancy and a non-pregnancy. Canine infertility is something that every breeder needs to accept and prepare as part of the industry. The reproductive physiology of the bitch is characterized by poor correlation between the behavioural signs of oestrus and hormonal changes, increased plasma progesterone concentration prior to ovulation, gestational length varying between 58 to 72 days and occurrence of pseudo pregnancy (England, 2001).

An important aspect to be considered to achieve a successful mating, is the variation in the oestrus cycle which occurs between bitches and between the cycles of the same bitch. Despite this, many breeders continue to time matings using the standard number of days from the start of bleeding and the bitches are often mated at an inappropriate time, leading to an apparent and usually avoidable infertility (Hewitt and England, 2000). It is very important to note that most of these apparently infertile bitches may be normal and the problems might be related to a poor understanding of canine reproduction.

The duration of proestrus in bitch on an average is nine days but it may be as short as three to as long as 25 and the duration of oestrus on an average is nine days but it may vary between five and 21 days. Bitches normally ovulate 24 to 48 hours after the LH surge and fertilization occurs 2 to 3 days after ovulation when the ova, which are released as primary oocytes, have completed meiotic divisions (Feldman and Nelson 1996). Because of variability in the length of the oestrus cycle and in the duration of the critical phases of proestrus and oestrus, the development of a reliable method for predicting optimal breeding time in bitches have a large impact on canine reproduction.

Various methods are commonly used to monitor the stages oestrous cycle in bitches. Teasing and observing signs like nature and colour of the discharge, degree of vulval oedema and flagging of tail were widely used by breeders to determine optimum breeding time. However, the credibility of these techniques is affected by subjectivity of interpretation and variability among female dogs.

Vaginal exfoliative cytology is a fairly simple method of monitoring the stage of oestrus cycle in female dogs. It serves as a better tool for the accurate identification of various stages of oestrus cycle (Schute, 1967a) whereas according to Purswell *et al*., 2000 vaginoscopy was the most accurate diagnostic breeding tool when compared to vaginal cytology because this technique allows the visualization of vaginal folds. However, Romagnoli (2006) opined that performing vaginal cytology and checking the bitche's behaviour for the onset of male receptivity were the two most practical ways for determining the best time for breeding.

Hormonal assays have been used to determine the time of ovulation in bitches. Leutinizing hormone assays are difficult to perform and expensive, but knowing precisely the concentration of progesterone in plasma would help to determine the time of ovulation in bitches and to decide when to breed it (Concannon, 2006 and Fontbonne *et al*., 2007).

Many techniques are now available to know whether a bitch is pregnant or not, but documented evidence of comparative studies on the efficiency of these techniques in early pregnancy diagnosis are meagre. Looking for behavioural as well as physiological changes and abdominal palpation are some of the earliest routine methods for pregnancy diagnosis.

Acute phase protein estimation like haptoglobin, alkaline phosphatase and globulin (Arosh *et al*. 1999), ultrasound scanning (Kutzler *et al*., 2003), and endocrine assay are some of the recent techniques adopted for pregnancy diagnosis.

Pseudopregnancy can develp in bitches once the serum concentrations of the progestogen falls and a resultant rise in prolactin production occurs. Supression of prolactin causes a rapid resolution of the signs of pseudopregnancy and the antiprolactin antagonists commonly used for the treatment are bromocriptine and cabergoline. The main problem in diagnosing pregnancy in

bitches is that overt pseudo pregnancy is very common which exhibit the behavioural and physical changes simulating pregnancy.

Thus the present study was undertaken with the following objectives:

1. Assessment of optimal breeding time in female dogs using exfoliative vaginal cytology, vaginoscopy and by estimation of serum progesterone profile.
2. Early diagnosis of pregnancy by estimation of serum alkaline phosphatase, globulin and haptoglobin and to compare with ultrasonographic findings.
3. Estimating the progesterone and prolactin profile in non-pregnant and pseudopregnant dogs and to adopt suitable treatment regimen for tackling pseudopregnancy under field conditions.

Review of Literature

2. REVIEW OF LITERATURE

2.1 REPRODUCTIVE CYCLE AND ENDOCRINOLOGY IN THE BITCH

The oestrous cycle of the domestic bitch is unique from other domestic animals and it differed in several aspects- first, each cycle was at least five months in duration; secondly, pregnancy occurred within the normal dioestrus phase rather than prolonging it and thirdly a long period of ovarian inactivity termed anoestrus occurred between the cycles (Jeffcoate, 1998). The bitch is a monoestrous, non-seasonal, polytocous animal that ovulates spontaneous at the end of a variable follicular phase lasting from 4 to 28 days. Each critical phase of the ovarian cycle consisted of proestrus, oestrus, dioestrus and a long anoestrus, when compared to any other species (Hewitt and England, 2000). Reproductive activity in the bitch differs from the polycyclic pattern of other species in that; there are no frequent, recurring periods of heat. During each cycle the bitch has prolonged follicular and luteal phases compared to those of the other cycling species of farm animal (England, 2001).

A rapid increase in plasma oestrogen concentration initiated the onset of proestrus in which the bitch was attractive to the male but did not accept mating. The average length of proestrus was approximately 9 days in mature bitches but ranged from 0 to 25 days (Concannon *et al.*, 1989). The increasing concentration of estradiol caused hyperplasia of vaginal cells and diapedesis of RBC's through uterine capillaries. A subsequent decline in estrogen and increase in plasma progesterone initiated oestrus during which the bitch allowed mating.

Oestrus normally lasted for 9 days on an average and ranged from 0 to 20 days (Feldman and Nelson, 1996 and Jeffcoate, 1998). The increase in plasma progesterone concentration was due to preovulatory luteinization of ovarian follicles (Phemister *et al.*, 1973 and Concannon, 1986) and the low estrogen- progesterone ratio that caused a surge in LH from the pituitary (Concannon, 1980).

2.1.1 Ovulation in Bitches

The greatest incidence of ovulations (77.2 per cent) appeared over an interval of 24 to 72 hours following LH peak (McDonald, 1989). Tsutsui (1989) performed laparotomy in 133 bitches to assess the time of ovulation and reported that none of the bitches had started or completed ovulation when examined at 36 hours after onset of oestrus while all bitches had completed ovulation by 72 hours. The interval from the start of proestal bleeding to ovulation was 7 to 25 days.

The bitch differed from most mammalian species in that the canine oocyte was ovulated as an immature (primary) oocyte which resumed maturation within the fallopian tube. Most ovulations occurred over a span of 2 days and occytes matured over the subsequent two days. Fertilization occurred following the maturation process of these primary oocytes, extrusion of the polar body and completion of the first meiotic division, which were at least 48 hours after ovulation (Feldman and Nelson, 1996). Most ovulations occurred between 24 and 72 hours after peak LH concentration was reached (Hewitt and England, 2000).

Although ova were not released precisely at the same time, they were in similar stage of development ensuring embryonic development of all fetuses progressing similarly (Johnston *et al*., 2001).

Kuztriz (2001) suggested that ovulation was spontaneous in the bitch with luteinization beginning before ovulation. Serum estrogen concentration decreased during late proestrum stimulating a surge in serum leutinizing hormone (LH) concentration and subsequent ovulation. Ovulation occurred $\pm$ 2days after the LH peak. He also observed that bitches ovulated between 2 days prior to and 6 days after standing heat.

2.2 DETERMINATION OF OPTIMAL BREEDING TIME IN BITCHES

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 pre-determined days after onset of proestrus in bitch would result in apparent infertility (Concannon *et al*., 1983). But, England and Allen (1989) opined that the period of peak fertility for natural mating ranged from one day before and until six days after the LH surge. England (2001) reported that for assessing the optimal breeding time for mating in bitches was by observing the timing of vulval softening which often occurs at the time of LH surge, when there was a change from high to low estrogen concentration. Further it was suggested that serial monitoring of plasma progesterone concentration allowed anticipation of ovulation, confirmation of ovulation and detection of fertilization period.

England and Concannon (2002) opined that onset of behavioural estrus, timing of vulval softening and exfoliative vaginal cytology were the cheapest and simple methods used to determine the optimal time for breeding. But Sridevi (2005) had suggested that vaginoscopy was one of the best tools for determining the ovulation in bitches.

2.2.1 Clinico-Gynaecological Examination

Concannon *et al*. (1983) found that some bitches exhibited phantom proestrus by displaying little or no outward signs of bloody discharge, making it difficult to estimate the average date of ovulation.

England (1998) observed that distinct vulval softening occurred at the time of LH surge and suggested that mating should be commenced four days after the onset of vulval softening.

England and Lofstedt (2000) opined that vaginal cytology, vulvar compressibility, ferning and vaginal shrinkage are too loosely correlated with the LH surge and could not be relied for the surge.

Hewitt and England (2000) opined that behaviour of the bitch in response to the male and the softening of vulva could help to estimate the time of ovulation since both of these events occurred approximately two days before ovulation.

Purswell et *al.* (2000) found that vaginoscopy was the most accurate diagnostic breeding tool when compared to vaginal cytology. However, Romagnoli (2006) opined that performing vaginal cytology and checking the bitche's behaviour for the onset of male receptivity were the two most practical ways for determining the best time for breeding.

2.2.2 Vaginal Exfoliative Cytology (VEC)

Vaginal Exfoliative Cytology in the bitch provides a useful marker about the degree of vaginal mucosal proliferation which in turn is influenced by the changes in the plasma estrogen and progesterone concentration. Therefore, vaginal cytology is a fairly simple method of monitoring the stage of oestrous cycle in this species.

Examination of vaginal cytology was commonly used to monitor the oestrous cycle in female dogs and to suggest the optimal time for mating or insemination (Olson, 1989 and Wright, 1990). In human medicine, vaginal cytology was not only of value in hormonal studies, but also utilized to detect uterine malignancy (Schutte, 1967b). Various indices of cornification and keratinisation have been suggested as markers for the stage of oestrus cycle (Allen and Dagnell, 1982).

The vaginal mucosa was a target organ for the ovarian hormones and during oestrus, the vaginal epithelium changed from 2 to 4 layers thick into a multilayered epithelium (Wright and Parry, 1989). Results of vaginal cytology

varied in some bitches with few having only 60 per cent cornification while in others there were two peaks of cornification (Cain, 1995).

Feldman and Nelson (1996) suggested that breeding was to be attempted throughout the period when more than 80 per cent of epithelial cells were superficial and fertility improved with increasing numbers of cornified epithelial cells.

2.2.2.1 Cellular Indices

A number of cellular indices such as superficial cell index, anuclear cell index (cornification index), eosinophilic index and karyopyknotic index were used by various authors to evaluate the cellular changes in the vaginal epithelium (Schutte, 1967c; Arthur *et al*., 1996; Becha, 2000; Asha, 2005; Deepthi, 2007 and Ajitkumar, 2008).

2.2.2.2 Anuclear Cell Index

England (1992) reported that assessment of anuclear cell index before breeding improved the conception rate in bitches.

According to Hewitt and England (2000), anuclear cell index or cornification index was calculated from the cellular changes that occurred during the various phases of the oestrous cycle. It was opined that the fertile period could be predicted by calculating the percentage of epithelial cells that appeared anuclear when modified Wright-Giemsa stain was used. The authors opined that, the optimum time for breeding was when the cornification index was above 80 per cent.

2.2.2.3 Cytological Findings in Different Stages of Oestrus Cycle

Changes in exfoliate cells in smears provided direct evidence of progression of follicular development and estrogen secretion during proestrus and in estimating the fertile period during oestrus. In addition, this would also help to estimate the end of the fertile period and for predicting the parturition (Concannon, 1983).

2.2.2.3.1 Proestrus

Thrall and Olson (1993) opined that the proestrual bleeding occurred mainly due to diapedisis of RBC's through uterine capillaries in response to the increased oestradiol concentration.

The proestrual smears had a abnormal appearance on account of cellular debris and mucin threads (Schutte, 1967a).

Soderberg (1986) reported that during proestrus, the epithelial cells gradually changed from non-cornified (parabasal and intermediate cells) to predominantly cornified (superficial) cells.

Feldman and Nelson (1996) reported that the cytological specimens obtained in early and mid proestrus were characterised by a mixture of epithelial cells, including parabasal, small and large intermediate and superficial cells. Neutrophils and RBC's were also present. They further observed that during late proestrus, neutrophils usually decreased in numbers and large intermediate and superficial cells predominated. Bacteria were often present in large numbers, both free and on the surface of epithelial cells.

2.2.2.3.2 Oestrus

Schutte (1967a) reported that the cellular picture during oestrus consisted of the more differentiated anuclear superficial cells, high concentrations of superficial cells with pyknotic nuceli and less large intermediate cells. During

the first half of oestrus the cytoplasm of most of these cells were eosinophilic, indicative of keratin or pre-keratin deposition.

Linde and Karlsonn (1984) observed absence of neutrophils in an oestrual smear and with or without RBC's.

Brukes (1986) reported that when partially or fully cornified superficial cells comprised 95 to 100 per cent of the cells in a cytology smear with loss of leukocytes and non-superficial epithelial cells, was an indication that estrogen secretions and follicle maturation had reached a point of LH surge resulting in ovulation.

Oestrus was estimated to last 13.8 ± 1.6 days from the first day of bleeding and in oestrual smear more than 80 per cent of the vaginal epithelial cells were superficial keratinized during oestrum (Bouchard *et al.*, 1991b).

England (1992) reported single anuclear cell peak in 58 per cent of bitches while in 42 per cent of bitches, two anuclear cell peaks were identified and the mean interval between the two peaks was 3.6 ± 1.5 days.

2.2.2.3.3 Diestrus

Holst and Phemister (1974) define the onset of dioestrus by observing an abrupt change in relative number of epithelial cells with decreased number of superficial, nucleated and anucleated cells. The superficial cells two days prior to the onset of dioestrus were intact and had well defined borders.

During early proestrus the shift from intermediate and parabasal cells to superficial cells during oestrus was more gradual than the rapid decrease in superficial cells observed at the onset of dioestrus (Dore, 1978).

Thrall and Olson (1993) reported that day 1 of dioestrus occurred 8 days (6 to 10) after LH peak and was characterized by an abrupt change in relative number of superficial epithelial cells. Superficial cell numbers decreased by at

least 20 per cent and often to more than 50 per cent while Feldman and Nelson (1996) reported that with a 24 to 48 hr period at the end of the oestrus the percentage of superficial cells fallen to approximately 20 per cent with the majority of the cells being intermediate and/or parabasal.

Johnston (2001) stated during the first day of dioestrus, epithelial cells changed from predominantly cornified to non cornified cells and could be correlated with conception rate and duration of gestation.

2.2.2.3.4 Anoestrum

Feldman and Nelson (1996) observed the presence of parabasal and intermediate epithelial cells in vaginal cytology of bitches during anoestrum. Neutrophils might or might not be present and the red blood cells were absent.

2.2.2.4 Predicting Optimal Breeding Time Based on Vaginal Exfoliative Cytology

Schutte (1967b) reported individual variation in the time of eosinophilic peak and recommended breeding when the eosinophilic index was approximately 80 per cent.

Linde and Karlsonn (1984) studied the correlation between the vaginal smear and the time of ovulation in 22 bitches. In all the bitches, maximum keratinization of the vaginal epithelial cells was observed at least 3 days after peak estradiol levels and in 17 bitches maximum keratinization was seen when peripheral plasma level of progesterone had increased to ovulatory level of 5.44 ± 0.93 ng/ml.

Olson *et al*. (1986) reported that 90 percent or more of the epithelial cells in a smear from an oestrus bitch would be superficial cells without neutrophils. Erythrocytes diminished in number but could be observed throughout estrous and also during early diestrous in many bitches.

Concannon *et al.* (1989) reported an abrupt decrease in RBC and non cellular debris in smear or decrease in sanguineous discharge occurred at the time of LH surge but such changes were inconsistent and therefore not reliable.

Bouchard *et al.* (1991a) reported that based on vaginal cytology, ovulation took place 6.9 ± 1.6 days after 80 per cent of vaginal epithelial cells were superficial keratinized and 6.8 ± 1.4 days before day 1 of dioestrus. LH surge occurred 8.8 ± 1.4 days before day 1 of dioestrus.

Wright (1991) evolved the relationship between the time of ovulation and the day of the cycle by calculating the vaginal cell eosinophilic index (EI).

England (1992) found the greatest number of superficial cells within the smear between one and seven days before the plasma peak of LH; and mating or insemination was optimal commencing five to 11 days later.

Thrall and Olson (1993) reported that bitches must be bred every 4^{th} day throughout the period when more than 90 per cent of the vaginal epithelial cells were superficial.

Feldman and Nelson (1996) reported that an attempt at breeding should be pursued naturally or artificially throughout the period when 80 per cent or more superficial cells were seen on vaginal cytology and it should be continued until the bitch refused to breed. It was recommended to breed the dog once in every second, third or fourth day of oestrus.

Jeffcoate and England (1997) reported that maximum number of anuclear vaginal epithelial cells was observed 2.4 ± 1.5 days after the plasma LH peak on an average and was of practical value in estimating the fertile period.

Simon and Athman (1998) found an abrupt clearing of background indicating the occurrence of LH surge. Becha (2000) reported that the percentage of superficial cells increased during proestrus until it was nearly 100per cent by the beginning of oestrus.

Hewitt and England (2000) mentioned that the optimal time for breeding was when the cornification index was 80 percent or more and also found that fertile period could be predicted by calculating the percentage of epithelial cells that appeared anuclear when using a modified Wright- Giemsa stain. According to them Anuclear cell index = No: of anuclear cells / Total No: of epithelial cells X 100.

Purswell *et al.* (2000) found that the vaginal cytology had a characteristic feature during oestrous. The superficial cell type predominated while white blood cells were low in number and the RBC present was few in number.

According to Arthur *et al. (*2001) the fertile period could be predicted by calculating the percentage of cornified epithelial cells using a modified Wright-Giemsa stain while England (2001) opined that the fertile period could be predicted by calculating the percentage of epithelial cells that appeared anuclear when using a modified Wright- Giemsa stain. Mating should be allowed throughout the period when more than 80 percent epithelial cells were anuclear.

Johnston *et al.* (2001) also found that greater than 90 percent of the epithelial cells were of superficial type at the time of maximum cornification. The authors also opined that the absence of neutrophils and the presence of numerous bacteria during estrus should be considered normal. Further, England and Concannon (2002) reported that the fertile period could be predicted by recording the percentage of epithelial cells that were superficial when stained using a Wright- Giemsa stain and the percentage of cornified cells reached above 90 per cent.

2.2.3 Vaginoscopy

Vaginal endoscopy (vaginoscopy) was commonly employed for the detailed examination of the vaginal mucosa, using rigid or flexible endoscopes

with fibre optic light source. The procedure was well tolerated in a standing bitch and vaginoscopy helped in the visualization of vaginal mucosal fold contours and profiles (England and Concannon, 2002).

2.2.3.1 Vaginoscopic Findings in Different Stages of Oestrous Cycle

A number of authors recommended vaginoscopy as a tool to identify the various stages of oestrus cycle in bitches (Feldman and Nelson, 1996; Sridevi, 2001 and Lulich, 2006).

2.2.3.1.1 Proestrus

During proestrus large edematous mucosal folds appeared due to rising plasma estradiol concentrations which promoted tissue fluid retention in the mucosa and proliferation of epithelial layers (Lindsay, 1983). The study suggests that that during proestrus, the dorsal median fold and its tubercles presented an even, rounded outline. Clear bright red fluid was seen among the folds or flooding through the external os of the soft, prominent, oedematous tubular cervix. About mid proestrus the "shrinkage" stage became evident. Towards the end of proestrus, transversely disposed complex rugae could be visualized.

Transverse furrows developed and folds appeared as smooth rounded cobble stone pavement surface that appeared in the scope as rounded profiles. The colour varied from pink to cream white and finally to paper white due to progressive thickening and cornification of the epithelium. The serosanguineous uterine discharge appeared as red fluid in the furrows of the folds (Lindsay and Concannon, 1986).

The luminal surface of the vaginal mucosa which appeared rounded and oedematous became progressively wrinkled or crenulated as the phase progressed (Feldman and Nelson, 1996). Similarly Hewitt and England (2000) and Sridevi (2005) reported that the vaginal mucosa was rounded, oedematous and smooth during proestrus. The decreasing oestrogen and increasing progesterone

concentrations associated with the last one to three days of proestrus caused oedema in the vaginal mucosa to subside and the luminal surface became progressively wrinkled and was referred to as crenulation.

2.2.3.1.2 Oestrus

Lindsay (1983) detected four consecutive periods of cyclic vaginoscopic mucosal changes. The cycle began with a proliferative oedematous period with massive oedema of the vaginal mucosa. This was followed by two consecutive periods of progressive shrinkage. In the first shrinkage period, mucosal shrinkage without angulation, mucosal folds shrank but their vaginoscopic profile remained round. In the second shrinkage with angulation, mucosal fold profiles became sharp edged. A period of decline and cessation of mucosal shrinkage, mucosal thinning and sharp profiles becoming rounded marked the end of the oestrus period.

During late proestrus and early oestrus, there was fall in serum estrogen level and the start of the preovulatory LH surge and furrows were formed on the surfaces of the vaginal mucosal folds. Additional wrinkles or indentations became progressively obvious during the preovulatory surge. The large, rounded mucosal folds were subdivided into smaller, rounded crumpled folds and round peaks (Lindsay and Concannon, 1986).

Sridevi (2001) recorded that early oestrus was characterised by the onset of shrinkage without angulation in the vaginal mucosa and progressed to a more obvious shrinkage with angulation corresponding to the fertile period in bitches.

2.2.3.1.3 Dioestrus

In the transition period from oestrus to dioestrus the mucosa thinned out and the profiles became rounded. In the early dioestrus, a patch work of red and white areas was seen (Lindsay and Concannon, 1986; Concannon, 2002 and Ajitkumar, 2008). At the same time intense contraction of the vagina was noticed

in the region of the caudal tubercle of the dorsal fold (Lindsay and Concannon, 1986). Similarly Jeffcoate, (1998) reported that the mucosal folds in the anterior vagina appeared irritable during dioestrus and when provoked they closed rapidly forming a rosette like pattern.

2.2.3.1.4 Anoestrum

Vaginoscopic examination during anoestrus revealed low mucous membrane folds, with simple and rounded outline. The mucosa had a scant mucus coating and was diffuse pink red in colour (Feldman and Nelson, 1996 and Hewitt and England, 2000).

According to England (2001), the vaginal mucosa observed to be relatively flat and red in colour. Arunmozhi (2005) reported absence of mucosal shrinkage, pink coloured mucosa and decreased blood supply in 100, 83.33 and 91.67 per cent of the female dogs and Patil (2006) reported that during anoestrum, vaginal mucous membrane appeared diffuse pink without angulation.

2.2.3.2 Predicting Optimal Breeding Time Based on Vaginoscopy

Lindsay (1983) reported high rate of successful conceptions from single natural matings of bitches having vaginal mucosal shrinkage with maximum angulation. When only a single mating was possible, peak fertility was achieved by mating at the maximum shrinkage of folds with angulations.

There was progressive shrinkage of vaginal mucosa which became very pale and even white in appearance close to the time of LH peak. This progressive wrinkling was referred to as crenulation (Brukes, 1986).

A progressive wrinkling and loss of oedema in the enlarged, white coloured vaginal mucosal folds occurred during the period of LH surge and ovulation. This change was reasonably a good predictor of the time of ovulation if monitored endoscopically every 1 or 3 days (Jeffcoate and Lindsay, 1989).

Initial vaginal crenulation was observed as subtle wrinkling of the mucosa which appeared within 24 hours of the preovulatory LH surge (Lindsay and Concannon, 1986) while Lindsay and Jeffcoate (1993) reported that the stages of obvious and maximum angulation of mucosal folds were reliable indicators of fertilization period.

Obvious and maximum angulations of the vaginal mucosa were striking vaginoscopic features at peak fertilization period. This visible crenulation continued as long as 4 to 9 days after the LH surge and mating could be given during this period (Feldman and Nelson, 1996).

Jeffcoate and England (1997) reported that the onset of peak vaginal mucosal shrinkage with angulation occurred on an average between 2.1 ± 2.4 and 6.1 ± 1.1 days after the LH peak.

Hewitt and England (2000) defined fertilization period as being between 4 and 6 days after vaginal mucosal shrinkage was noted. It was also easy to determine the end of fertile period when mating or insemination could be discontinued.

2.2.4 Serum Progesterone Assay

The bitch was unique in that there was a rise in plasma progesterone concentration which was brought about by preovulatory luteinization of the granulosa cells of the follicle (Concannon, 2002). This led to an increase in progesterone concentration in peripheral blood prior to ovulation.

Estimating the concentration of progesterone in plasma was an important factor in determining the time of ovulation and to decide when to breed the bitch (Olson and Husted, 1986; Van Haaften *et al.*, 1989 and Wright, 1990).

2.2.4.1 Progesterone Levels during Different Phases of Ovarian Cycle

Serum progesterone levels were about 0.5 ng/ml during anoestrus and increased to 0.8 ng/ml during proestrus in bitches. Progesterone increased sharply above 1 ng/ml during the preovulatory LH surge on day 0 (Concannon and Lein, 1983) to reach peak levels of 15 to 80 ng/ml between days 12 and 30 and then slowly declined to 2 to 10ng/ml by day 45, and to less than 1 ng/ml by days 60 to 110 (Concannon *et al.*, 1977 and Feldman and Nelson, 1996). Normal luteal secretion of progesterone in both non pregnant and pregnant dogs was dependent on both LH and prolactin for luteotrophic support throughout the luteal phase (Concannon, 1980 and Olson *et al.*, 1989).

2.2.4.2 Predicting Optimal Breeding Time Based on Progesterone Assay

Van Haaften *et al.* (1994) reported that the blood progesterone concentration appeared to be an excellent indicator for predicting mating in female dogs. They recommended breeding within 9 to 33 hours when the progesterone concentration was between 6 and 12 ng/ml and within 33 to 57 hours when progesterone levels were between 5 and 6 ng/ml. They reported that the mean interval between the start of proestral bleeding and the optimal time of mating was 11.8 ± 3.1 days.

Anderton and Evans (1993) and Deitrich and Moller (1993) reported that the owners were advised to bred their bitches 2 to 3 days after when the progesterone concentration reached approximately 5 ng/ml.

Badinand *et al.* (1993) reported that fertilization occurred when the progesterone concentration was 9 to 26 ng/ml.

Becha (2000) found variation in the level of progesterone ranging from 3.37 to 5.95 ng/ml on first day of oestrous.

Cain (2000) recommended breeding on days two, four and six after LH surge (day 0) on days 3 and 5 if only two breedings were possible. The study

suggested that if natural breeding did not occur on the prescribed dates, artificial insemination should be considered.

Hewit and England (2000) used the commercial ELISA kit (Ovucheck Premate) to predict the breeding time and obtained a conception rate of 87 per cent. The level of progesterone was found to be >10 ng/ml on the day of maximum cornification. They also recommended that bitches should be rebred after 48 hours to achieve maximum conception rate.

Romagnoli (2006) opined that breeding should always be timed using serum progesterone assay and that the bitch should be mated when the serum progesterone exceeds 5 ng/ml.

2.3 METHODS OF PREGNANCY DIAGNOSIS IN BITCHES

A number of techniques were used to diagnose pregnancy in bitches. These include the following:

2.3.1 Behavioural Changes

Jones and Joshua (1982) found that many pregnant bitches showed no change in temperament and behaviour and remained active until and unless gross abdominal enlargement developed. However, England (1998) recorded that both pregnant and pseudopregnant bitches exhibited behavioural changes typical of pregnancy. But, Thou (1999) opined that although no behavioural changes were observed until 30-35 days of gestation, few bitches showed signs of inappetence.

England (2001) stated that pregnant and non-pregnant bitches exhibited behavioural changes typical of pregnancy. Food intake usually increased by approximately 50 per cent in second half of pregnancy, however, it was not uncommon for pregnant bitches to have a brief period of reduced appetite commonly encountered about 3-4 weeks after mating.

2.3.2 Body Weight

Schroeder and Smith (1995) conducted a study on body weight and feed intake of German shepherd bitches during pregnancy and lactation and noted that from the 28th day of pregnancy the bitch maintained a slow and steady increase in body weight, at a rate of 0.0849 kg/day. England (1998) also opined that in pregnant animals, the total body weight increased to 20-55per cent during gestation.

According to Thou (1999), there was a steady increase in the body weight as the pregnancy advanced ranging from 21-36 kg in pregnant Alsatian as against 19-30.5 kg in non-pregnant, and 9-12 kg in pregnant pomeranian as against 8-10.5 kg in non-pregnant.

Arthur *et al*. (2001) stated that gravid uterus and its contents caused no appreciable increase in body weight during the first five weeks of gestation. After five weeks, body weight rapidly increased according to the number of the fetuses. The increase in body weight varied from 1kg in a 5 kg bitch to 7 kg or more in bitch weighing 27 kg.

England (2001) opined that body weight began to increase from day 35 onwards and increased up to 50 per cent of normal weight and observed that these changes might not be obvious in bitch with small litters.

According to Deepthi (2007) there was a slow and steady increase in the body weight of animals as the pregnancy progressed. The body weight before conception was 25.83$\pm$1.8Kg which increased to 26.6$\pm$1.9, 26.78$\pm$1.8 and 29.6$\pm$1.8 at 16 to 20, 21 to 24 and 25 to 30 days of gestation respectively.

2.3.3 Pregnancy Diagnosis by Transabdominal Palpation

Harrop (1960) described in detail about the pregnancy diagnosis by palpation at different stages of gestation and found that the optimum period for

the early diagnosis of pregnancy in the bitch was 24 to 30 days. The embryos were noted to be spherical in shape and about one inch in diameter.

Sokolowski (1980) stated that palpation of the abdomen between day 20 and 28 after breeding was helpful to diagnose pregnancy and noticed that by day 20 the developing uterus had spherical swellings approximately 10 to 15mm in diameter. 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 with an increase in size to 15 to 30 mm in diameter depending on the size of the bitch.

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 percent accurate in diagnosis of pregnancy. Earliest pregnancy detection was 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 palpation. During period from day 25 to 35, the accuracy was 75 per cent.

According to Shille and Gontarek (1985) the early pregnancy detection in the bitch was generally limited to transabdominal palpation of the gravid uterus between days 25 to 36 after breeding since the gestational vesicles were poorly distinguished from other visceral organs.

Tavern (1985) also found that the non-pregnant uterus and the pregnant uterus before day 21 of diestrous were not reliably palpated in most dogs.

Out of 55 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 and stated 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.

Gangadhar (1995) found that the optimum time for pregnancy diagnosis by abdominal palpation was 28 days after breeding and recorded the size of each foetal swelling as two cm in diameter. But Arthur *et al*. (1996) observed that on days 18 to 21 the embryos represented a series of tense and oval distensions in the cornua about twelve mm long and nine mm broad but found difficult to detect embryos in large and obese bitches.

Feldman and Nelson (1996) reported that palpation of abdomen was easy, inexpensive and reliable in recognizing pregnancy between days 20 and 30 of gestation and uterine swellings at individual placental sites were usually palpable at this stage.

England (1998) stated that the optimum time to do abdominal palpation in bitches was one month after mating and at this stage the conceptus was spherical in outline and varied between 15 and 30 mm in diameter and was difficult to do abdominal palpation after day 45 of gestation.

Thou (1999) recorded earliest results of pregnancy diagnosis in bitches by transabdominal palpation on 21st day after breeding in a Pomeranian bitch. By about 30 to 35 days post breeding, the accuracy of palpation in Alsatian and Pomeranian was 88.9 and 100 per cent respectively. But, Gradil *et al*. (2000) palpated pregnancy at 26 to 28 days post breeding and recorded that by day 28 uterine swellings were of three to five cm in diameter for middle-sized bitch. 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) also reported that abdominal palpation was best accomplished by 25 to 28 days when the ‘strings of pearls’ effect on the uterus was most obvious. After 30 to 35 days post breeding, the uterine swellings were elongated, become more oval and more fluctuant. At this time it was difficult to differentiate a gravid uterus from other abdominal contents.

Johnston *et al*. (2001) opined that the bitch should be palpated approximately 31-33days after the LH surge or about 28-30 days after suspected day of ovulation. The embryos and the chorio-allantoic vesicles in the bitch formed a series of ovoid swellings in the early gravid uterus, the most caudal of which could be identified by palpation through the abdominal wall as early as 17-22 days after ovulation.

Deka *et al.* (2004) also 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 percent from 28th day to term.

Arunmozhi (2005) also recorded the earliest correct diagnosis of pregnancy by abdominal palpation by 22 days after first mating.

Asha (2005) found that pregnancy diagnosis by transabdominal palpation at 20- 30 days post breeding was difficult, as the distinct uterine swellings could not be appreciated from the abdominal viscera in bitches. She also reported that among large, medium and small breeds the accuracy obtained by abdominal palpation was 61.9, 60 and 75 per cent respectively. But when the palpation was done in between 31 to 40 days post breeding the accuracy was 66.6, 100 and 80 per cent respectively.

Deepthi (2007) reported that when palpation was done in between 21 to 24 and 25 to 30 days post breeding, the accuracy obtained was 50 per cent and 70 per cent respectively. This study suggests that transabdominal palpation was not useful in diagnosing early pregnancy.

2.3.4 Pregnancy Diagnosis by Ultrasound Scanning

Allen and Meredith (1981) found the optimum period for using A-mode ultra sound scanning to be 32 to 62 days after mating and found that the accuracy of detecting pregnancy was 90 per cent against 83 per cent in non- pregnant bitches.

Bondestam *et al*. (1983) reported difficulty in detecting fetuses by 21 days of gestation and suggested ultrasound scanning from 28-35 days of gestation. The authors recorded that all fetuses detected before 28th day of gestation were small and details of the shape of the foetus could not be demonstrated and suggested that after 40th day details of stomach, urinary bladder and umbilical vein of foetus could be demonstrated by ultra sound scanning.

Cartee and Rowles (1984) observed a semicircular or C shaped embryo first occurred at gestational day of 22-23. They also observed small hyper echoic areas within the lumen on day 10 post breeding and hyper echoic intraluminal embryo averaging 10 mm in length at 17 to 23 days post breeding.

According to Shille and Gontarek (1985) the foetal movements and heartbeat could not be identified until days 28 and 35 post breeding respectively. During the period of 27 to 30 days uterus had an anechoic lumen containing a hyper echoic embryo. However, a low accuracy was observed in predicting the actual foetal number associated with overlapping fetuses or mistaking them as already counted due to the acoustic artifacts.

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

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

According to Barr (1988) pregnancy in the bitch could be consistently diagnosed between day 24 and day 28 after mating when gestational sacs containing fetal tissue suspended in amniotic fluid were seen. By day 28 of gestation, generalized foetal movements and foetal cardiac activity could assess fetal viability. He also pointed that the estimation of litter size by ultrasound scanning was not easy, but the period between 28th and 35th day of gestation was the best time for counting the number of fetuses.

Yeager and Concannon (1990) reported that the embryonic mass and heartbeat were first detected at 23 to 25 days after the LH surge. The diameter of gestational sac at 20 and 25 days of gestation were found to be one to four mm in diameter and one to four mm in length and 8.2 $\pm$ 0.3 mm in diameter and 20.3$\pm$ 1.1 mm length, respectively in beagle bitches.

Gestational sacs at 17 to 20 days after LH surge were found to be 1 to 2 mm in diameter and 1 to 4mm in length and the heartbeats could be observed from day 23 to 25 and a focal anechoic area in the head could be visualized from days 27 to 31 (Yeager *et al*.,1992).

Feldman and Nelson (1996) also found that visualization of the functioning of heart was consistently accomplished by the 25th day of gestation and stated that differentiation of pregnancy from pyometra and early recognition of pregnancy could be established quickly and safely with ultrasonography.

England (1998) found that conceptus might be first imaged from 15 days after ovulation, where they appear as spherical, anechoic structures approximately 2mm in diameter and from day 20 after ovulation the embryo could be imaged having 7mm in diameter and 15mm in length. The efficacy of prediction of litter size was 97 percent in early stages of gestation, which dropped to 20 per cent during later stages of pregnancy.

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

According to study conducted by Johnston *et al.* (2001) in 51 pregnant bitches with B mode ultrasonography, the number of pups could be accurately determined only in 31.8 per cent of the pregnancies.

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 ultra sound scanning. Kutzler *et al*. (2003) opined that the most accurate predictions of parturition date were obtained when fetuses were measured by day 30.

Deka *et al.* (2004) found that the foetal viability could be diagnosed as early as 25 day of pregnancy through ultrasonography as indicated by pulsating cardiac movements. Accuracy of estimating foetal viability and litter size was 97.62 and 96.78 per cent respectively by 25th day of pregnancy. However, the earliest correct diagnosis of pregnancy by ultrasonography was recorded by day 21 after first mating (Arunmozhi *et al*., 2005).

Alacam *et al*. (2005) recorded that allantois along with developing fetuses in individual embryonic vesicles could be monitored from day 22 of gestation.

Deepthi (2007) reported that by ultrasound scanning, the percentage accuracy at 16 to 20 days was 50 per cent which improved to 80 per cent and 100 per cent at 21-24 and 25-30 days post breeding respectively. Foetal heartbeat could be observed in all the positive cases from 24 days of gestation.

2.3.5 Pregnancy Diagnosis by Estimation of Serum Alkaline Phosphatase (ALP)

Kimura *et al*. (1992) reported an ALP activity ranging 24 – 42.6 U/L in Mexican hairless breeds.

Laker (1996) reported high concentration of alkaline phosphatase in liver, bone, intestine, placenta and kidney and suggested physiologically increased ALP levels during the periods of active bone growth and pregnancy where as pathological increase was observed in hepatobiliary disease and bone diseases.

According to the study, level of serum alkaline phosphatase activity rose linearly during pregnancy from a mean of 39 U/L at 19 weeks to 130 U/L at delivery in human beings.

Kahn (2005) reported mean ALP activity in non-pregnant healthy dogs as 10.6-101U/L.

Mohan (2005) recorded elevated ALP levels in diseases of bone, liver and in pregnancy. The study concluded high serum ALP activity could be used as marker of hepatobiliary diseases in the absence of bone diseases and pregnancy in human beings.

According to Divya (2009) among the species cattle, buffalo, goat and dog, the lowest ALP activity was observed in dogs with a mean of 92.9 $\pm$ 7.53 U/L. The study was conducted in adult healthy dogs of age 1.5 to 3 years irrespective of breed and sex.

2.3.6 Pregnancy Diagnosis by Estimation of Serum Haptoglobin (Hp)

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

According to Horadagoda *et al*. (1999) assay of acute phase proteins like estimation of serum haptoglobin and serum amyloid-A could be employed to differentiate chronic and acute inflammation in cattle than haematological tests.

Abate *et al*. (2000) opined that electrophoretic analysis of haptoglobin pattern could be used for evaluating a dog's health status including pregnancy.

According to Berkova *et al.* (2001) haptoglobin was present in human endometrium which was analysed by western blot technique and observed that it

rose in a biphasic pattern with peak in first and third trimester of pregnancy. It was further concluded that women with infertility problems have a decreased level of anti-haptoglobin antibody in their serum which suggested the role of haptoglobin in fertility.

Vannucchi *et al*. (2002) observed that estimation of acute phase proteins could be used as an early pregnancy test for bitches from third week of gestation. The level was found to be 112.42mg/dl by 21 days of gestation. By 30 days of gestation, in pregnant animals the value was found to be 123.53±56.39mg/dl as against 88.79±23.03mg/dl in nonpregnant ones. A maximum level 147.68mg/dl was observed at fifth week of gestation. The study concluded that the Haptoglobin values above 112.42mg/dl could confirm pregnancy in dogs.

Murata *et al*. (2004) defined haptoglobin (Hp) as an alpha-globulin which binds with free haemoglobin and reduces the oxidative damage associated with haemolysis.

Romagnoli (2006) recorded the normal haptoglobin level in non-pregnant healthy dogs as 35 to 50 mg/dl as against 75 to 100 mg/dl in pregnant animals. This rise in the Hp level was observed by 18 to 20 days after ovulation.

Onclin and Verstegen (2008) observed postimplantation increase of acute phase proteins like fibrinogen, C-reactive protein and haptoglobin. The elevation of these acute phase proteins by about 25 to 30 days of gestation was attributed to non-specific inflammatory like responses to the presence of foetoplacental unit.

According to Ulutas *et al*. (2009) acute phase proteins like haptoglobin, ceruloplasmin and fibrinogen rose in pregnant dogs compared to the nonpregnant ones. The level of haptoglobin was found to be 205±0.58mg/dl in pregnant as against 187±0.18mg/dl in anoestrous animals. They found that the elevated level of haptoglobin could be due to the inflammatory reaction produced by embryonic implantation and placental growth. They finally concluded that the acute phase

proteins could not be used alone as pregnancy markers, but could be relied for monitoring the health status of pregnant dogs.

2.3.7 Pregnancy Diagnosis by Estimation of Serum Globulin

Gentry and Liptrap (1977) found a marked alteration in the plasma procoagulent activity in the bitch during the period of gestation. The mean value of serum globulin was reported to be 2.63g/dl.

According to Fisher and Fisher (1981) a decrease in mean gamma globulin could be used in pregnancy diagnosis in bitches.

Prabhakaran *et al*. (1996) reported an increased level of globulin in pregnancy and the value was found to be 3.9±0.2g/dl.

Similarly, Arosh *et al*. (1999) found significant rose in serum globulin level in pregnant animals when compared to nonpregnant ones.

Thou (1999) recorded the serum globulin level in pregnant bitches as 2.7 to 3.7g/dl and in nonpregnant animals it was 2.3 to 2.7g/dl.

Vannucchi *et al*. (2002) recorded the serum globulin value in pregnant dogs as 0.49±0.09g/dl and in non-pregnant as 0.44± 0.13g/dl.

Asha (2005) in her study recorded the serum globulin level in pregnant bitches as 3.08±0.12, 3.08±0.13 and 3.06±0.13g/dl at 20 to 30, 31 to 40 and 41 to 65 days of gestation respectively and that of the nonpregnant was 2.692±0.19g/dl.

2.4 HAEMATOLOGICAL STUDIES

2.4.1 Haemogram

2.4.1.1 Total Erythrocyte count (TEC)

Gentry and Liptrap (1977) recorded the normal RBC value as 5.5 to 8.5 million per cmm in healthy dogs.

England (1998) reported that during gestation in dogs, the erythrocyte number became gradually reduced from a mean of 8.85 million to 4.53 million per cmm.

According to Benjamin (2001) the total erythrocyte values in pregnant animals varied significantly at different gestational age when compared to the non-pregnant animals.

Ajithkumar (2008) recorded the total erythrocyte count on first day of proestrus as 3.06 ± 0.024 million per cmm and on the first day of breeding as 3.02 ± 0.037 million per cmm.

2.4.1.2 Haemoglobin (Hb)

According to Saror *et al.* (1979) normal mean value of haemoglobin in healthy dog was 14.2±1.6g/dl.

Benjamin (1985) reported the value of haemoglobin in the range of 12 to 18g/dl in nonpregnant bitches.

According to Prabhakaran *et al*. (1996) hemoglobin content and packed cell volume did not show much variation between pregnancy and non-pregnancy.

Thou (1999) had 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 and 11.0±0.18g/dl respectively and that in non-pregnant were 14.19±0.25g/dl, 14.03±0.27g/dl and 14.23±0.27g/dl respectively and reported a significant variation in haemoglobin content of pregnant and non-pregnant dogs.

Asha (2005) recorded haemoglobin concentration at 20 to 30, 31-40 and 41 to 65 days of gestation as 11.73±0.18, 10.2±0.2,10.69±0.2g/dl respectively and in non-pregnant as 12.37±0.28 g/dl, which was higher than pregnant dogs. Significant variations in haemoglobin values between pregnant and non-pregnant were also observed.

Deepthi (2007) recorded the haemoglobin concentration on day 0, 16 to 20, 21 to 24 and 25 to 30 days were 11.56±0.27, 10.88±0.31, 10.24±0.22 and 8.7±0.25g/dl, respectively.

2.4.1.3 Packed Cell Volume (PCV)

Saror *et al.* (1979) reported a normal mean value of PCV as 42 - 45 per cent in healthy dogs.

Benjamin (2001) recorded 37 to 55% with an average value of 45 per cent for packed cell volume in apparently normal non-pregnant dogs.

England (1998) opined that packed cell volume were 40 per cent at day 35 of gestation and even less than 35 per cent at term.

Thou (1999) found a decrease in PCV as pregnancy progressed and found the mean values on 21 to 25, 30 to 35 and 45 to 50 in pregnant Alsatian bitches as 41.6± 0.33, 39.33± 0.33 and 35± 0.577 per cent respectively and in non-pregnant bitches were 44.5±0.289, 44.75± 0.75, and 45.25± 0.25 per cent respectively.

Asha (2005) recorded PCV values in pregnant bitches at 20-30,31-40,41-65 days of pregnancy were 39.83±0.72, 37.7±0.82 and 36.88±0.8 per cent respectively, whereas the values in non-pregnant dogs were 45±1.15 per cent. Significant variations in packed cell volume before and after conception were also recorded.

Deepthi (2007) recorded the PCV values on day zero, 16 to 20, 21 to 24 and 25 to 30 days were 34.66±0.9, 30.77±0.94, 28.22±1.02 and 26.0±0.94 per cent respectively which showed a gradual decrease in the PCV values as the pregnancy progressed.

2.4.1.4 Erythrocyte Sedimentation Rate (ESR)

Benjamin (1985) and Sastry (1989) recorded the normal range of erythrocyte sedimentation rate in dogs as 5 to 25 mm/hr and noticed an increase in ESR during pregnancy.

Henry (1996) opined that increase in ESR was because of the change in erythrocyte plasma ratio, which favoured the rouleaux formation independent of changes in the concentration of plasma proteins.

Thou (1999) found an increase in the ESR rate ranging between 8.5 to 19.33mm/hr in pregnant animals and it was higher when compared to non-pregnant bitches, ranging between 5.6 to 5.88 mm/hr.

Asha (2005) recorded the ESR rate in pregnant as 12.03±0.97, 17.62±1.11 and 17.07±1.09mm/hr at 20-30, 31-40, 41-65 days of gestation respectively whereas in non pregnant control animals it was 2.5±1.57mm/hr. Statistical analysis revealed significant variation in ESR between pregnant and non-pregnant animals.

Similar results were obtained in a study conducted by Deepthi (2007) where ESR on day 0, 16 to 20, 21 to 24 and 25 to 30 days of gestation were 4.6±0.33, 14.3±1.09, 17.8±1.28 and 21.76±1.47mm/hr respectively.

2.4.2 Leucogram

2.4.2.1 Total Leucocyte Count (TLC)

Lumsden *et al.* (1979) recorded the normal value of TLC as 6000 to 18,000 per cmm in healthy dogs. While Henry (1996) found that during gestation the total leucocyte count increased from 12,000 to 19,000 per cmm.

Feldman and Nelson (1996) observed mild leucocytosis by 30 to 40 days of gestation and the value ranged between 17,000 to 26,000 cells cmm.

Asha (2005) reported an increase in total leucocyte count as the pregnancy established and the value was 14,000 per cmm as against 12,760 per cmm in nonpregnant dogs.

2.4.2.2 Differential Leucocyte Count (DC)

Schalm *et al*. (1975) reported a marked change in the differential count (viz. neutrophils, lymphocytes, eosinophils and monocytes) in animals suffering from pyometra.

2.4.2.2.a Neutrophils

Benjamin (1985) reported the normal value of neutrophils as 60 to 77 per cent (with an average 70 per cent) which increased during inflammatory conditions.

Asha (2005) reported the neutrophil count between 20 to 30 days of gestation as 59 per cent which rised to 61 per cent by 41 to 65 days of gestation.

2.4.2.2.b Lymphocytes

Schalm *et al.* (1975) reported normal lymphocyte value ranging from 12 to 30 per cent (average 20 per cent).

According to Prabhakaran *et al*. (1996) the mean lymphocyte count was higher during pregnancy than during lactation and corresponded to 28$\pm$6.2 and 21$\pm$4.2 per cent respectively.

Thou (1999) found the lymphocyte count in pregnant Alsatian bitches ranging from 22 to 32 per cent as against 17 to 20 per cent in nonpregnant.

Asha (2005) recorded the lymphocyte count at 21 to 30 days and at 41 to 65 days of gestation of gestation as 29.51$\pm$0.55 and 30.24$\pm$0.61, respectively.

2.4.2.2.c Monocytes

Schalm *et al.* (1975) reported normal value of monocytes in healthy dogs as 3 to 10 per cent (average 4 per cent).

Prabhakaran *et al*. (1996) did not find much change in monocyte count during pregnancy and lactation.

Asha (2005) recorded the monocyte value during 21 to 30 days of gestation as 5 per cent with no appreciable change as the pregnancy advanced.

2.4.2.2.d Eosinophils

According to Schalm *et al.* (1975) the eosinophil count in dogs were found varying from 2 to 10 per cent (average 4 per cent).

Asha (2005) reported the eosinphil count at 21 to 30 days of gestation as 2 per cent with no appreciable increase as the pregnancy advanced.

2.4.2.2.e Basophils

Schalm *et al*. (1975) found the basophil concentration as less than one per cent in normal healthy bitches.

Thou (1999) and Asha (2005) reported no significant variation in basophil concentration between pregnant and non pregnant animals.

2.5 GESTATION LENGTH

According to Sokolowski (1980) the average gestation length for the bitch was approximately 62 days. However, viable fetuses were whelped at gestation of 58 to 66 days.

Concannon *et al*. (1983) conducted studies in a beagle colony in which apparent gestation length was estimated as the interval from the day of first mating

to the day of parturition, which ranged from 57 to 72 days and averaged 65.3±0.2 days.

Johnson (1986) stated that the gestation length in bitch varied from 58 to 72 days after breeding as ovulation occurred at variable and unpredictable times and that the canine sperms could maintain its ability to fertilize for at least 4 to 6 days in the female genital tract.

Kahn (1994) opined that breeding date in bitches differed considerably from the ovulation date and thus made it difficult to accurately establish gestational age in dogs.

According to Feldman and Nelson (1996) the whelping date was likely to be 56 to 58 days after the first day of diestrous as determined by vaginal cytology.

When dogs were bred during estrous, diestrous was replaced by the period of pregnancy, which showed a rather constant gestation length averaging 63 ± 2 days which varied between 57-72 days due to long period of receptivity at oestrus and the extended period of sperm survival in the female genital tract (Hoffmann *et al*., 1999).

Simon (1997) in his study reported average gestation period as 61.9 days while Thou (1999) recorded an average gestation length of 62.43 and 59.50 days from the first day of mating in Alsatian and Pomeranian bitches respectively.

In two group of bitches in which oestrus was induced by the administration of leuprolide acetate and diethylstilbesterol, Becha (2000) recorded an average gestation length of 62.50 ± 0.51 and 62.00 ± 1.15 days respectively.

The average gestation length in the bitch was normally quoted as 63 to 64 days, but the interval from first mating to whelping varied from 56 to 71 days (Noakes *et al*., 2001).

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

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

Gestation length calculated from the day of first breeding to whelping ranged from 59 to 68, 57 to 63 and 56 to 62 days in large, medium and small breeds of dogs respectively (Asha, 2005).

Deepthi (2007) recorded an average gestation length of 63.38 days in large breeds of dogs.

2.6 LITTER SIZE

Bondestam *et al.* (1983) stated that the accurate estimation of litter size by ultrasound scanning was proved to be difficult, especially in large breeds of dogs and reported a figure of 40 percent accuracy at 29 days after mating and 83.3 percent accuracy from 50 days to term.

Concannon (1986) reported that mean litter size 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 and eight.

Schroeder and Smith (1995) in a study of 30 German shepherd bitches recorded a litter size of two to nine pups with an average of 5.43 pups.

Thou (1999) recorded an average litter size of 4.40 and 5.00 respectively, in Alsatian and Pomeranian bitches bred during natural oestrus.

In bitches in which oestrus was induced by the administration of leuprolide acetate, Becha (2000) recorded an average litter size of 5.60 ± 0.75. The corresponding value was 6.00 ± 0.58 when oestrus was induced with diethylstilbesterol.

However, Gradil *et al*. (2000) stated litter size depend on several factors and concluded that small sized breeds had two to four, medium sized breeds four to seven and large sized breeds had six to ten pups.

Asha (2005) observed that the litter size in large, medium and small breeds of dog varied from 4 to 11, 4 to 8 and 1 to 7 pups respectively. The average litter size in the three groups was 5.80, 5.10 and 3.83 respectively.

According to Deepthi (2007), the litter size in large breeds of dog bred during natural oestrus, varied from 3 to 9 with an average of 5.70.

Sathiamoorthy (2007) recorded an average litter size of 5.50 ± 1.30 and 2.00 in bitches inseminated intra-vaginally with fresh and frozen semen respectively and the corresponding values on intrauterine insemination were 6.25 ± 0.10 and 3.00 ± 1.00 respectively.

2.7 PSEUDOPREGNANCY IN FEMALE DOGS

Pseudopregnancy occurs physiologically in non-pregnant bitches which may or may not have mated Beijerink *et al.* (2004). This phenomenon had been described as atavism, of functional importance to the dog's ancestors, especially wolves where non-bred mature bitches had to nurse the litter of the pack-leading she-wolf (Voith, 1980). Tsutsui *et al*. (2007) defined the occurrence of excessive mammary enlargement, lactation and maternal behavior normally associated with the peripartum period when observed in non-pregnant bitches as overt pseudo-pregnancy (PSP). However, the pathophysiology behind this condition was not well documented.

Simon (1997) reported the incidence of pseudopregnancy as 3.2 per cent where as Deepthi (2007) reported the incidence rate as 11 per cent.

2.7.1 Treatment of Pseudopregnancy with Antiprolactin Drugs

Concannon and Lein (1983) found that treatment with prolactin lowering dosages of dopamine agonists bromocriptine and cabergoline induced a premature oestrus in dogs.

Okkens *et al*., 1985 reported that the real pathogenesis of overt pseudopregnancy poorly understood, but he observed a rapid decline in the plasma progesterone concentration as a precipitating factor.

Janssens (1986) observed that brompcriptine was effective for treating pseudopregnancy but, the side effect was emesis. He opined that giving bromocriptine at a low dose or an antiemetic drug given could reduce the chance of emesis.

Chakraborthy (1987) opined that the extent and intensity of overt signs of pseudopregnancy was not related to any specific endocrine changes. Mean progesterone concentration of pseudopreganant bitches on day 0, 1 and 6 were 0.9, 2, 20 ng/ml respectively.

In the study conducted by Arbeiter and Barsch (1988) cabergoline orally at a dose rate of 5µg/kg BW for seven days caused disappearance of morphological, functional and behavioural signs of pseudopregnancy in 95 to 100 per cent of animals treated.

According to Arbeiter and Jochle (1989), bromocriptine a widely used ergoline compound in human medicine could be used as a therapeutic agent for the control of overt clinical and behavioural signs of canine pseudopregnancy.

According to Stienetz *et al*. (1990) in overtly pseudopregnant bitches high plasma prolactin concentration was measured. In six overtly pseudopregnant

Afghan hounds the mean plasma concentration of prolactin was 35ng/ml and was significantly higher when compared to the early luteal phase.

Harvey *et al*. (1997) found that cabergoline is effective in suppressing prolactin release from the pituitary and thus lowering the blood concentration when used at a dose rate of 5µg/kg BW for five days. The mean prolactin concentrations during pre-treatment, post-treatment and after completion of treatment were 6.41, 1.8 and 4.13ng/ml. The progesterone level recorded before treatment was found to be less than 1ng/ml which indicated that the bitches were no longer in the metoestral phase. The side effects reported were less.

Zoldag *et al.* (2001) found that bromocriptine a dopamine agonist was found to be successful in suppressing lactation in pseudopregnant bitches.

According to Gobello *et al.* (2001) the serum prolactin and progesterone concentrations prior to treatment in pseudopregnant bitches were found to be 17.7$\pm$ 2.05 and 1.13$\pm$0.13 ng/ml respectively.

Kooistra *et al.* (1999) found that there was strong association between increase of prolactin release and a decrease of plasma progesterone concentration in overtly pseudopregnant bitches. Elevated prolactin secretions during progression of the luteal phase in the bitch was responsible for mammogenesis and declining plasma progesterone concentration during the second part of the luteal phase appear to influence prolactin secretion.

Concannon (2002) recorded that in pseudopregnant bitches the corpus luteum do not regress and this cause elevated progesterone concentration bringing about the signs consistent with pregnancy. By around day 60 progesterone levels abruptly dropped down and this drop cause serum prolactin to rose. This elevated serum prolactin was responsible for the typical nesting behaviour in pseudopregnant bitches.

Ettinger (2002) found that pseudopregnancy in bitches were mainly attributed to decrease in serum progesterone concentration and increase in serum prolactin concentration.

Beijerink *et al.* (2003) opined that bromocriptine could reduce the interoestrous interval even when used at a low dose. They found that the induction of oestrus in bitch involved a mechanism other than lowering the plasma prolactin concentration.

Rota *et al.* (2003) used cabergoline and buserelin for oestrus induction and it was found that both drugs produced a significant early decline in prolactin concentration ($P< 0.01$) but the effect of cabergoline lasted long.

Gobello *et al*. (2004) studied about the dopaminergic agonists-bromocriptine and cabergoline and concluded that both the agents act directly upon dopamine pituitary receptors to modify the synthesis and release of prolactin by pituitary cells. They also found that cabergoline had fewer side effects with longer duration of action and was more potent than bromocriptine.

According to Gunay *et al.* (2004) the luteal phase in pregnant bitches was about 65 days and 75 to 90 days in non-pregnant ones and they used cabergoline to induce oestrus cycle in bitches with pseudopregnant problem or with prolonged lactation or in prolong anoestrous. No side effects were reported to cabergoline because the drug did not easily penetrate the blood brain barrier.

Corrada *et al.* (2006) studied on the circannual release of prolactin. They found that the secretion of prolactin in both genders might be ascribed to difference in age, breed and stage of oestrus cycle and the prolactin secretion were observed to be in pulsatile fashion.

Cirit *et al*. (2007) and Kutzler (2007) reported that cabergoline had a high specificity for D_2 receptor, long specific activity on pituitary lactotrophic cells

and fewer central nervous system effects than bromocriptine. No side effects were reported in their study when cabergoline was used.

Tsutsui *et al*. (2007) recorded that cabergoline at the dose rate of 5μg/Kg once daily for 5 to 10 days was more effective than bromocriptine for treating pseudopregnancy. The progesterone and prolactin value in pseudopregnant bitches was found to be 1.5±0.2 and 16±1.9 ng/ml respectively while in control dogs it was 2.7±0.4 and 2.9±0.6 ng/ml respectively. They observed that some clinically pseudopregnant bitches had low prolactin concentration which could be related to pulsatile or variable secretion pattern of prolactin.

Materials and Methods

3. MATERIALS AND METHODS

3.1 SELECTION OF ANIMALS

Female dogs belonging to various breeds presented to the Small Animal Obstetrics and Gynaecology unit of Veterinary College Hospital, Mannuthy and University Veterinary Hospital, Kokkalai were utilized for the study. The study conducted consisted of three experimental parts.

3.2 DESIGN OF STUDY

3.2.1 Experiment I

Fifty five female dogs of different breeds showing proestral bleeding brought for the breeding advice formed the animals of this group. A detailed history was obtained on duration of the proestrual bleeding, interoestrus interval and previous mating if any, method for finding the optimal breeding time in previous matings, gestational accidents, data on whelping and any therapeutic measures adopted to enhance reproductive efficiency.

These animals were divided into two groups. In Group I, 30 animals were selected. All the animals were subjected to a detailed clinico-gynaecological examination. The bitches were examined in detail for intensity of vulval oedema (low, medium, and high), nature of proestral discharge (sanguineous, sero-sanguineous and straw coloured) and any abnormalities of external genitalia. Their vaginal secretion was collected aseptically for assessment of pH and fern pattern. These bitches were subjected to vaginal exfoliative cytology (VEC). Breeding was advised based on the anuclear cell index.

In Group II, 25 female dogs were selected and they were subjected to vaginoscopy during proestrum and oestrum using a flexible fibre optic endoscpe. Out of this, ten animals of different breeds of medium size were selected at

random for progesterone estimation on the day of maximum crenulations on vaginoscopic study.

Optimal breeding time was advised based on clinico-gynaecological examination and vaginal exfoliative cytology in Group I and by vaginoscopy in Group II. The fertility rate was assessed and compared between the groups.

3.2.2 Experiment II

Twenty animals presented for the pregnancy diagnosis were subjected to abdominal palpation and ultrasound scanning. Blood was collected for the estimation of serum alkaline phosphatase, serum globulin and serum haptoglobin at 20^{th}, 35^{th} and 50^{th} day of gestation. Body weight and physiological changes were recorded. Blood was collected for haemogram and leucogram studies. All the pregnant animals were monitored till whelping and their litter size was recorded. In five animals, ultrasound scanning was done at to get more sonographic details related to foetal growth and morphological changes.

3.2.3 Experiment III

Animals showing prominent signs of overt pseudopregnancy confirmed by ultrasound scanning were randomly allotted to two different treatment groups.

3.2.3.1 Group I:

Ten bitches with signs of overt pseudopregnancy were treated with antiprolactin drug, cabergoline orally at a dose rate of 5µg/kg body weight daily for seven days.

3.2.3.2 Group II

Ten bitches with signs of overt pseudopregnancy were treated with antiprolactin drug, bromocriptine orally at dose of 50µg /kg body weight daily for seven days.

Blood was collected from animals of Group I and II on first day of treatment, on completion of treatment (on 7th day) and seven days later (on 14th day) to estimate the serum progesterone and prolactin concentration. The values were recorded and the drug response was monitored.

3.2.3.3 Group III

Ten apparently healthy bitches with the history of breeding 70 days prior and which failed to conceive with no signs of overt pseudopregnancy formed the control group. Blood samples were collected for estimation of serum progesterone and prolactin concentration on day one, day 7 and day 14.

3.3 EXPERIMENT I- DETERMINATION OF OPTIMAL BREEDING TIME IN BITCHES

There are several methods for identifying optimal breeding time in bitch.

3.3.1 Vaginal Exfoliative Cytology (VEC)

Vaginal smears were obtained from all the fifty female dogs during proestrum and oestrum.

3.3.1.1 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 into the vagina, directing the pipette cranio-dorsally to the vestibule and at the vestibulo- vaginal junction, and then redirected cranially to reach the anterior vagina. The fluid was aspirated and a small drop of discharge was kept on the slide, a thin smear was prepared and then it was air-dried.

3.3.1.2 Staining of Vaginal Smears

The vaginal smears were stained using **Modified Wright - Giemsa stain.**

Composition of Modified Wright- Giemsa stain.

Wright stain powder	300mg
Giemsa stain powder	30mg
Methanol	100ml

Procedure

1. Prepared the vaginal smear on clean grease free glass slide and air-dried.
2. Covered the smear with modified Wright- Giemsa stain and allowed to act for 30 seconds.
3. Flooded the slide with distilled water, mixed by blowing and allowed to act for 30 seconds.
4. Washed with water, dried and examined under high power of microscope (40X).

3.3.1.3 Types of Cells Observed in Vaginal Exfoliative Cytology

3.3.1.3.a Epithelial Cells

They are classified into three major categories *viz.* parabasal cells, intermediate cells and superficial cells.

3.3.1.3.b *Parabasal Cells*

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

3.3.1.3.c *Intermediate Cells*

These include cells of varying size and types which represent all stages of maturation between parabasal and fully mature superficial cells. The cells become more angular, enlarged and flattened as they mature. The relative size of

the nucleus decreases as they mature. The small intermediate cells are small and polygonal with a relative large nucleus.

3.*3.1.3.d Superficial Cells*

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

- Large polygonal dead cells with irregular borders without any nucleus.
- Large polygonal cells with intact nuclear membrane.
- Large polygonal cells with small remnant of nuclei.
- Large polygonal cells with pyknotic nuclei.

3.3.1.4 Anuclear Cell Index

Anuclear cell index = Number of anuclear cells / Total number of epithelial cells X 100. Breeding advice was given based on history, clinico-gynaecological examination, and anuclear cell index (more than 80 percent cells anuclear).

3.3.2 Vaginoscopy

Vaginoscopy was performed using a flexible fibre optic endoscope (Karl Storz, GmBH, Tiuttlingen, Germany).

3.3.2.1 Vaginoscopic Procedure

The rigid endoscope was 30cm long and had an outside diameter of 4mm which was fitted inside a sheath with the same functional length and an outside diameter of 5mm and working length of 28cm. The fitting was secured by a breech-mount ring near the eyepiece. The outer sheath served as an air insufflation channel through a valve near the breech-lock fitting. Fibre optic

bundles served the eyepiece and the light projecting bundles were served by the fibre optic cable connection port located on the side of the scope near the eyepiece. The fibre optic cable was 250cm long and connected to a dedicated light source via adapter.

The sterilized endoscope lubricated with KY-jelly was introduced into the vagina in a craniodorsal direction after separating the vulval lips. The instrument was inserted as far cranially as it passed freely and the vaginal mucosa was examined for colour, texture and shape of the mucous folds as the instrument was withdrawn 1 to 4cm and then slowly advanced to the original position. Slight amount of air was insufflated during the observation to make visualization easier (Lindsay and Concannon, 1986).The changes in the mucosal fold contours and profile, colour and nature of the fluid present were assessed vaginoscopically. The following characters served as indicators to assess the stage of oestrus cycle (England and Concannon, 2002).

3.3.2.1.1 Anoestrus

The vaginal mucous membranes appeared thin and fragile, relatively flattened, dry and red in appearance.

3.3.2.1.2 Proestrus

At the onset of proestrus, the mucosal folds are greatly enlarged, oedematous and pink or white in colour, with serosanguineous fluid in cervices formed by the folds. The “folds” of vaginal mucosa due to oestrogen dominance give them a rounded, smooth oedematous appearance.

3.3.2.1.3 Estrus

In very late proestrus and early oestrus, there was progressive shrinkage of folds accompanied by pallor. The wrinkling had also been referred to as crenulation of the mucosal folds. The onset of the peak fertile period could be detected by observing the onset of mucosal shrinkage without excessive

angulations, while gross shrinkage of entire mucosal folds with obvious angulations was characteristic of the fertilization period.

3.3.2.1.4 Diestrus

There was cessation or decline of mucosal shrinkage and the mucosa appeared flattened and thin. Rounding out of mucosal profile was the characteristic feature.

3.3.3 Serum Progesterone Assay

Blood samples were obtained from ten animals of Group II for the estimation of serum progesterone at mid proestrum and also on the day of peak vaginal crenulation (>90% anuclear cells ie day of breeding). The serum progesterone level was estimated by radioimmunoassay using progesterone RIA kit- Biometallics, Princeton, USA and the value was expressed in ng/ml.

3.4 EXPERIMENT- II : PREGNANCY DIAGNOSIS

Twenty bitches were selected randomly for pregnancy diagnosis by transabdominal palpation and ultrasound scanning on 20^{th}, 35^{th} and 50^{th} day from first mating. Further, blood was collected on these days for the routine haemogram and leucogram. Serum was separated for the estimation of serum alkaline phosphatase, serum haptoglobin and serum globulin estimation. Body weight and the physiological changes of pregnancy were also recorded. In five animals ultrasonography picture was regularly taken for studying more sonographic details of pregnancy.

3.4.1 Transabdominal Palpation

The method described by Sokolowski (1980) was adopted in this study. The bitch was controlled in standing position, grasped the abdomen gently by applying gentle pressure up toward 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.

3.4.2 Ultrasound Scanning

Ultrasound equipment (Honda electronics HS 2000 vet) that produces two dimensional gray scale real time images was used for ultrasound scanning. The frequency used was 5 to 7.5 MHz. Animals were positioned in dorsal recumbency and prepared the mid ventral area from the pubis to just cranial to umbilicus. Coupling medium (Methyl cellulose) was applied to the skin and also to the transducer probe to assure good acoustic transmission. Both sagital and transverse scan planes were used. For scanning, the abdomen was considered as two regions, one right of and the other left of the medial plane. Each region was scanned separately, and the results were monitored.

3.4.3 Estimation of Serum Alkaline Phosphatase (ALP)

The level of serum alkaline phosphatase was estimated using the auto analyzer Cobas and standard kit for ALP.

3.4.4 Estimation of Serum Globulin

The level of globulin was estimated using the auto analyzer Cobas and standard kit for total protein.

3.4.5 Estimation of Serum Haptoglobin (Hp)

The level of haptoglobin was estimated by immunonephelometry on the BN ProSpec® System.

3.4.6 Haematological Studies in Pregnant Dogs

Blood (5ml) was collected from cephalic vein or saphenous vein on the day of breeding as well on different gestational ages. Sodium citrate was used as

the anticoagulant (3.8% at the rate of 1ml/9ml blood). The total erythrocyte count (TEC count), total leucocyte count (TLC), differential count (DC), haemoglobin (Hb), packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) were estimated by standard procedures (Benjamin, 1985).

3.5 DATA ON WHELPING

All the pregnant animals were monitored till whelping. Gestation length, foetal viability and litter size were recorded. Gestation length was calculated from first breeding date to the whelping date.

3.6 EXPERIMENT III- TREATMENT OF PSEUDOPREGNANCY

The treatment regimen falls under two different groups with ten female dogs each in Group I and II and another ten dogs in Group III kept as control.

3.6.1 Group I

Ten non-pregnant bitches with signs of overt pseudopregnancy were treated with antiprolactin drug, cabergoline orally (Tab. Cabgolin*) at dose rate of 5µg/kg body weight daily for seven days.

3.6.2 Group II

Ten non-pregnant bitches with signs of overt pseudopregnancy were treated with antiprolactin drug, bromocriptine orally (Tab. Sicriptin**) at dose of 50µg /kg body weight daily for seven days.

---

* Tab. Cabgolin 0.25mg / Cabgolin 0.5mg are available as strips of 2 tablets.

Company : Sun pharmaceutical industries

** Tab. Sicriptin 1.25mg available as strip of ten tablets (each uncoated tablet contains Bromocriptine mesylate IP 1.435mg equal to 1.25mg of bromocriptine base)

Company : Serum International LTD

Blood was collected from animals of Group I and II on first day of treatment, on completion of treatment (on 7th day) and seven days later (on 14th day) to estimate the prolactin and progesterone concentration. The values were recorded and the drug response was monitored.

3.6.3 Group III

Ten apparently healthy bitches with the history of breeding 70 days prior and which failed to conceive with no signs of overt pseudopregnancy formed the control group. Blood samples were collected for estimation of prolactin and progesterone concentration on day one, day 7 and day 14.

3.7 ESTIMATION OF PROGESTERONE AND PROLACTIN

Blood was collected as per the fixed schedule for the estimation of serum progesterone and prolactin to find out the etiology behind pseudopregnancy.

3.7.1 Estimation of Progesterone

The serum progesterone level was estimated in Group I, II and III by radioimmunoassay using progesterone RIA kit- Biometallics, Princeton, USA and the value was expressed in ng/ml.

3.7.2 Estimation of Prolactin

The serum prolactin level was estimated in Group I, II and III by radioimmunoassay using prolactin RIA kit- Radim, Pomezia, Italia and the value was expressed in ng/ml.

The values obtained pre-treatment and post treatment was compared and drug response was assessed.

3.8 STATISTICAL ANALYSIS

The data obtained were compiled and statistical analysis was carried out using paired ‘T’ test. The results were analyzed as per Snedechor and Cochran (1985).

Results

4. RESULTS

The study comprised of animals that were brought to Veterinary College Hospital, Mannuthy and University Veterinary Hospital, Kokkalai for finding optimal breeding time during the course of study from 2007- 2010. Detailed clinico-gynaecological examination with vaginal exfoliative cytology and vaginoscopy were done in two different groups to assess the optimal breeding time. Out of this, ten animals were selected at random for progesterone estimation on the day of maximum cornification of vaginal mucosal folds observed on vaginoscopic study.

For pregnancy diagnosis, twenty animals were selected and they were subjected to transabdominal palpation and transabdominal ultrasound scanning. Blood was collected for estimation of serum alkaline phosphatase, serum haptoglobin and serum globulin. Whole blood was used for the study of haemogram and leucogram. These animals that were diagnosed as pregnant were monitored till whelping and the gestation length, foetal viability and litter size were recorded.

Animals with prominent signs of pseudopregnancy were grouped into two and treated with two different antiprolactin drugs- cabergoline and bromocriptine. In these animals the serum progesterone and prolactin level were estimated prior to treatment and after the completion of treatment at 7 days interval. Another ten apparently healthy bitches with no signs of pseudopregnancy was taken as control.

4.1 EXPERIMENT I

Group I comprising of 30 animals were subjected to clinicogynaecological examination and vaginal exfoliative cytology.

4.1.1. Optimal Breeding Time Based on Clinico-gynaecological Examination and Vaginal Exfoliative Cytology

The data regarding the intensity of proestrual bleeding, vulval oedema, standing signs and flagging of tail reflex in the animals bred based on EVC and clinico-gynaecological examination are summarized in Table 1. It was observed that in 70 per cent of animals, bleeding was high during proestrum and the discharge decreased at the time of late proestrum. Most of them resisted digital manipulation of vulva during this period. The intensity of vulval oedema (Plate 1) was high in 40 per cent of animals whereas it was medium in 53.3 per cent of animals during proestrum. As the animals reached oestrum phase, 73.3 per cent of animals showed standing signs and flagging of tail in 80 percent of cases. The pH value at the time of proestrum was found to be alkaline whereas the value lies between 5 and 6 during the oestrum phase. Fern pattern could not be obtained from majority of samples. Vaginal hyperplasia and transmissible venereal tumour were also encountered during the clinico-gynaecological examination (Plate 2 and 3).

The vaginal discharge was collected directly from the anterior vagina using a sterile glass pipette for the vaginal cytology study (Plate 4). The most predominant cell encountered during early proestrus was small and large intermediate cells (Plate 5 and 6). Late proestrus was characterized by superficial cell type (Plate 7). During oestrus more than 80 per cent of the cells were anulcear keratinized type (Plate 8). The anuclear cell index during various stages of oestrus cycle were furnished in Table 2. Mean anuclear cell index on the first day of proestrum, mid proestrum and on the recommended day of first breeding was 25.6 $\pm$ 1.33, 79.9 $\pm$ 0.5 and 92.6 $\pm$ 1.29 respectively. There was significant difference in the anuclear cell index as the stage of oestrus cycle progressed form proestrum to oestrum. Breeding advice was given based on anuclear cell index reaching 80 per cent or more. Pregnancy diagnosis was done after 30 days. The oestrual smears suggestive of vaginitis and a smear with presence of spermatozoa were also observed (plates 9 and 10).

4.1.2. Optimal Breeding Time Based on Vaginoscopy

Vaginoscopy was conducted in 25 animals with flexible fibre optic endoscope - Karl Storz, GmBH, Tiuttlingen, Germany (Plate 11). The procedure was carried out in non-sedated standing bitch (Plate 12). The vaginal mucosa during proestrus appeared pink in colour and congested without any shrinkage (Plate 13). The serosanguineous uterine discharge appeared as red fluid in the furrows of the folds (Plate 14). This was considered to be due to the increased oestradiol levels during proestrum causing retention of water in the vaginal mucosa thereby giving the appearance of 'balloons' (Plate 15). The features during oestrus were characterized by two types of shrinkages. In the first shrinkage period, though the mucosal shrinkage appeared without angulation their vaginoscopic picture remained rounded (Plate 16). In the second shrinkage with angulation, mucosal fold profiles became sharp edged (Plate 17). During early dioestrus the mucosal folds in the anterior vagina appeared irritable and when provoked they closed rapidly and formed a rosette like pattern (Plate 18). Breeding advice was advised based on the presence of maximum crenulation with angulation of vaginal mucosal folds. Progesterone estimation was done in ten animals depending on the vaginoscopic study during proestrum and oestrum on the day of maximum crenulation of vaginal mucous folds. Pregnancy diagnosis was done after 30 days.

4.1.3. Comparison of conception rate in animals bred based on vaginal exfoliative cytology and clinicogynaecological examination and vaginoscopy

The results on conception rate are shown in Table 3. In Group I, in which breeding was done based on the findings of clinicogynaecological examination and vaginal exfoliative cytology, a conception rate of 73.3 per cent was obtained whereas in Group II, where the breeding was based on vaginoscopy, the conception rate obtained was 88 per cent. Progesterone assay was done in ten animals and the data pertaining to serum progesterone level are furnished in

Table 4. The serum progesterone value on the day of proestrum and on the day of first breeding was 1.21 $\pm$ 0.18 and 6.58 $\pm$ 0.31 ng/ml respectively. Statistical analysis revealed significant variation in the progesterone value during proestrum and oestrum.

4.2 EXPERIMENT II

The results of pregnancy diagnosis by transabdominal palpation (Plate 19) and transabdominal ultrasound scanning (Plate 20) are given in Table 5.

4.2.1 At 20 Days of Gestation

4.2.1.a Transabdominal Palpation

When transabdominal palpation was carried by 20 days, in four animals slight uterine distension could be felt while three animals were recorded as doubtful and thirteen were categorized under negative. The percentage accuracy at 20 days was found to be 20 per cent.

4.2.1.b Transabdominal Ultrasound Scanning

When these animals were subjected to transabdominal ultrasonography, in three animals embryonic vesicles were observed as spherical structures with anechoic consistency (Plate 21). No foetus or foetal membranes could be visualized at this stage while five animals were recorded as doubtful and twelve were categorized under negative. The percentage accuracy at 20 days was found to be 15 per cent. Doubtful cases were more at this stage.

4.2.2 At 35 Days of Gestation

4.2.2.a Transabdominal Palpation

When subjected to abdominal palpation at 35 days of gestation, in fifteen animals foetus could be appreciated as tense distinct uterine swellings. In three animals a doubtful pregnancy was obtained as the bitches had heavy abdomen

and two were categorized as negative. The percentage accuracy at 35 days was found to be 75 per cent.

4.2.2.b Transabdominal Ultrasound Scanning

In eighteen animals by ultrasound scanning at 35 days of gestation, presence of embryo could be visualized as an anechoic structure with hypo echoic wall (Plate 22). Foetal heart beats as well as placenta could be observed during this stage. The earliest positive result at which heart beat could be visualized was at 24 days of gestation. Two animals were categorized under doubtful as the gestational sac wall was irregular. The percentage accuracy at 35 days was found to be 90 per cent.

By day 25 to 27, echogenic foetal mass and heart beat were detectable (Plate 23 and 24). By day 28 to 40, head and body of the foetus were similar in size and appearance revealing flickering heart beats (Plate 25). Foetal membranes and zonary placenta were prominent (Plate 26). Anatomical features of the foetus became more obvious by about 34 to 39 days of gestation. Shape and size of the foetal head became distinguishable from the body.

4.2.3 At 50 Days of Gestation

4.2.3.a Transabdominal Palpation

Transabdominal palpation was found difficult to perform in later stages of pregnancy. In twelve animals a confluent feeling of the uterus could be felt. The percentage accuracy at 50 days was 60 per cent.

4.2.3.b Transabdominal Ultrasound Scanning

By ultrasound scanning, the percentage accuracy of pregnancy diagnosis at 50 days was found to be 100 per cent. In all the pregnant animals, foetal heart beat along with rib cage could be observed. By day 42 to 55, the foetus appeared longer, surrounded by anechoic foetal fluid. Vertebrae could be observed as

hyper echoic areas arranged in a segmented pattern dorsal to liver and heart (Plate 27 and 28). They became denser and began to cast shadow as the mineralization proceeded. From 55 days to term, the foetal limbs, stomach, urinary bladder, liver, lung and skeleton could be visualized (Plate 29 and 30).

4.2.4 Pregnancy Diagnosis by Estimation of Serum Alkaline Phosphatase (ALP)

The results of serum alkaline phosphatase estimation are furnished in Table 6 and Fig. 1. The level of alkaline phosphatase at 20^{th}, 35^{th} and 50^{th} day of gestation was 67.90 $\pm$ 2.98, 91.85 $\pm$ 2.10 and 139.65 $\pm$ 6.84 U/L respectively. Statistical analysis revealed significant variation between day 20 and day 50 of gestation ($P<0.05$). No significant variation was obtained between day 35 and day 50.

4.2.5 Pregnancy Diagnosis by Estimation of Serum Haptoglobin (Hp)

The results of serum haptoglobin estimation at different gestational periods were given in Table 7 and Fig. 2. The level of haptoglobin at 20^{th} day of gestation was 53.10 $\pm$ 3.22 mg/dl where as the level elevated to 81.12 $\pm$ 3.40 and 119.44 $\pm$ 3.16 mg/dl respectively by 35 and 50 days of gestation. Statistical analysis revealed significant variation between day 20 and day 50 of gestation ($P<0.05$).

4.2.6 Pregnancy Diagnosis by Estimation of Serum Globulin

The levels of serum globulin in pregnant bitches are furnished in Table 8 and Fig. 3. The serum globulin level at 20^{th}, 35^{th} and 50^{th} day of gestation was 2.43 $\pm$ 0.12, 3.05 $\pm$ 0.11 and 3.74 $\pm$ 0.15 g/dl respectively. Statistical analysis revealed significant variation between 20 and 50 days of gestation ($P<0.05$). No significant variation was obtained between day 35 and day 50.

4.3 HAEMATOLOGICAL STUDIES

4.3.1 Haemogram of pregnant animals at different gestational age is furnished in Table 9.

Total erythrocyte count (TEC) at 20th, 35th and 50th days of gestation was 6.07 ± 0.16, 5.58 ± 0.15 and 5.12 ± 0.16 million/cmm respectively (Table 9, Fig. 4). Statistical analysis revealed significant variation in TEC between day 20 and day 50 ($P<0.05$).

Haemoglobin concentration at 20th, 35th and 50th day of gestation was 11.86 ± 0.28, 10.45 ± 0.27 and 9.17 ± 0.30 g/dl respectively. The level of haemoglobin was found to be lowering as pregnancy advanced (Table 9, Fig. 5). Statistical analysis revealed significant reduction in haemoglobin concentration at different periods of gestation ($P<0.05$).

Packed cell volume values were 40.45 ± 0.83, 37.30 ± 0.87and 33.40± 1.10 percent respectively at 20th, 35th and 50th day of gestation. There was gradual reduction in the PCV values during different stages of gestation (Table 9, Fig. 6). Statistical analysis revealed that the variation was significant ($P<0.05$).

Erythrocyte sedimentation rate at 20, 35 and 50th day of gestation were found to be 10.31 ± 0.73, 15.41 ± 0.54 and 21.85 ± 1.04 mm/hr respectively (Table 9, Fig. 7). ESR values were found to increase as pregnancy advanced. Statistical analysis revealed significant variation between the values at different gestational age ($P<0.05$).

4.3.2 Leucogram of pregnant animals at different gestational age is furnished in Table 10.

The total leucocyte count at day 20, day 35 and day 50 of gestation was found to be 13844.90 ± 539.90, 15449.00 ± 569.86 and 17502.50 ± 780.21 cells/cmm respectively. There was slight increase in the values between day 20

and day 35 (Table 10, Fig. 8). Statistical analysis revealed significant variation between day 20 and day 50 ($P<0.05$).

The neutrophil count at 20^{th}, 35^{th} and 50^{th} day of gestation was 67.30 ± 1.11, 70.30 ± 4.95 and 75.35 ± 1.27 per cent respectively (Table 10, Fig. 9). Statistical analysis revealed significant variation between day 20 and day 50 ($P<0.05$).

The lymphocyte count was found to be 27.80 ± 0.87, 31.55 ± 0.88 and 36.95 ± 1.03 per cent respectively at 20^{th}, 35^{th} and 50^{th} day of gestation (Table 10, Fig. 10). Statistical analysis revealed significant variation between day 20 and day 50 ($P<0.05$).

Monocyte count at different periods of gestation did not vary significantly and the values at day 20, day 35 and day 50 were 3.90 ± 0.27, 3.80 ± 0.20 and 4.05 ± 0.22 per cent respectively (Table 10, Fig. 11).

Eosinophil count varied significantly ($P<0.05$) between day 20 and day 50 and the values were recorded as 1.60 ± 0.11 and 3.30 ± 0.13 per cent respectively (Table 10, Fig. 12). There was statistical difference in between the values of day 20 and day 50.

4.4 BODY WEIGHT

Body weight of animals on 20^{th}, 35^{th} and 50^{th} day of gestation was 21.83 ± 1.98, 24.6 ± 1.93 and 36.69 ± 1.45 Kg respectively. It was found that the body weight varied significantly between day 20 and day 50 of gestation ($P<0.05$). It was observed that body weight gradually increased from day 20 to day 50 as the pregnancy progressed (Fig. 13).

4.5 FOETAL VIABILITY

Ultrasound scanning was unreliable for early pregnancy diagnosis as foetus or foetal membranes could not be visualized by 20 days of gestation.

Foetal heart beats could be observed in all pregnant animals from 25 days of gestation. The earliest stage at which heart beat could be visualized was 24 days of gestation. Foetal viability was difficult to be monitored at 20 days of gestation. By day 25 to 27, echogenic foetal mass and heart beat became detectable. By day 28 to 33, head and body of the foetus were similar in size and appearance with flickering heart beats. Foetal membranes and zonary placenta were prominent. Anatomical features of the foetus became more obvious by about 34 to 39 days of gestation. Shape and size of the foetal head became distinguishable from the body. By day 42 to 55, the foetus appeared longer, surrounded by anechoic foetal fluid. From 55 days to term, the foetal limbs, stomach, urinary bladder, liver, lung and skeleton could be visualized.

4.6 GESTATION LENGTH

The results are furnished in Table 11. In the present study gestation length ranged between 58 to 69 days with an average of 63.30 $\pm$ 3.76days.

4.7 LITTER SIZE

The results are furnished in Table 11. Litter size varied between three to eleven with a mean of 6.6 $\pm$ 2.08 pups per bitch (Plate 31).

4.8 EXPERIMENT III

A total of twenty female dogs with prominent signs of pseudopregnancy confirmed by ultrasound scanning formed the animals of this study which were divided in Group I and Group II. The physiological and behavioural changes observed in bitches with overt pseudopregnancy prior to treatment are furnished in Table: 12.

4.8.1 Group I (Cabergoline treated):

Out of ten animals in Group I, six animals showed mild mammary gland enlargement (Plate 32) with presence of fluid and whitish vaginal discharge. Four

animals were presented with abdominal enlargement, engorged mammary glands and abundant milk from teats (Plate 33). Eight animals showed symptoms of anorexia and two with nesting behaviour. Pseudopregnancy was confirmed by ultrasound scanning (Plate 34).

The details regarding the serum progesterone and prolactin concentration before and after treatment with cabergolione are furnished in Table: 13. The perusal of data showed that the level of progesterone prior to treatment and at 7^{th} day and at 14^{th} day post-treatment were 1.11 ± 0.14, 0.85 ± 0.06 and 0.62 ± 0.06 ng/ml respectively (Fig.14). Statistical analysis showed significant difference between the progesterone value prior to treatment and the value obtained on 14^{th} day after treatment ($P<0.05$).

The concentrations of prolactin prior to treatment, post-treatment at 7^{th} and at 14^{th} day were 6.17 ± 2.05, 1.69 ± 0.20 and 0.82 ± 0.13 ng/ml respectively. Statistical analysis showed significant variation in the serum prolactin level prior to treatment and post-treatment at 7^{th} and 14^{th} day ($P<0.05$). There was significant reduction in the serum prolactin value after the treatment with cabergoline.

No side effect other than reduction in food intake was observed. The drug was given in full stomach and no vomiting was reported in this group.

4.8.2 Group II (Bromocriptine treated):

Out of ten animals, seven animals showed anorexia and mild mammary gland enlargement with presence of fluid while five showed abdominal enlargement and whitish vaginal discharge. In three animals, there was prominent engorgement of mammary glands and abundant milk in teats and one animal showed nesting behaviour.

The details regarding the serum progesterone and prolactin concentration before and after treatment with bromocriptine are furnished in Table: 14. The perusal of data showed that the level of progesterone prior to treatment, post-

treatment at 7th day and at 14th day were 1.11 ± 0.14, 0.5 ± 0.01 and 0.5 ± 0.01 ng/ml respectively (Fig.15). Statistical analysis showed significant difference between the progesterone value prior to treatment and 14th day after treatment ($P<0.05$).

The concentrations of prolactin prior to treatment, post-treatment at 7th day and at 14th day were 6.02 ± 1.17, 0.90 ± 0.07 and 0.58 ± 0.08 ng/ml respectively. Statistical analysis showed significant variation ($P<0.05$) in the post-treatment prolactin level. There was significant reduction in the serum prolactin value after the treatment with bromocriptine

Severe vomiting and reduction in food intake was observed in five animals which was managed with antiemetics.

4.8.3 Group III (Control):

Ten apparently healthy bitches with the history of breeding 70 days prior and which failed to conceive with no signs of overt pseudopregnancy formed the control group. Blood samples were collected for estimation of prolactin and progesterone concentration on day one, day 7 and day 14 of visit.

The serum progesterone and prolactin level on day 1, day 7 and day 14 of visit obtained from ten apparently non-pregnant animals with no signs of pseudopregnancy were illustrated in Table: 15. The level of progesterone in these dogs was 1.53 ± 0.48, 0.7 ± 0.11 and 0.44 ± 0.03 ng/ml respectively and no statistical significance was observed.

The level of prolactin in control dogs was 1.16 ± 0.24, 1.02 ± 0.12 and 1.01 ± 0.07 ng/ml respectively. Statistical analysis revealed no significant variation between the serum prolactin value at day one and day 14 (Fig.16).

4.9 RESPONSE TO ANTIPROLACTIN DRUGS

In cabergoline treated Group I, all the animals showed reduction in the size of the mammary gland with decrease in the amount of fluid from the teats

within seven days. The serum prolactin level was found to be reduced and maintained at a low level even after fourteen days. All the dogs were symptomatically cured by 12 days.

In Group II where the treatment was done with bromocriptine, five animals exhibited vomiting as the side effect. The serum prolactin level was found to be reduced and maintained at a low level even after fourteen days. All the dogs were symptomatically cured from pseudopregnancy by about seven days and the animals regained normal feeding habits by fourteen days.

Table 1. Intensity of proestrual bleeding, vulval oedema, standing signs and flagging of tail reflex in the animals bred based on vaginal exfoliative cytology and clinico-gynaecological examination

Stage of cycle	Parameter	Score	No: of animals (n= 30)	Percentage (%)
Proestrus	Intensity of proestral bleeding	High	21	70.0
		Medium	7	23.3
		Low	2	0.06
	Intensity of vulval oedema	High	12	40.0
		Medium	16	53.3
		Low	2	0.06
Oestrus	Standing signs	High	22	73.3
		Medium	7	23.3
		Low	1	0.03
	Flagging of tail	Present	24	80.0
		Absent	6	20.0

Table 2. Anuclear cell index during various stages of oestrus cycle (Mean + S.E)

No: of animals	First day of proestrum	Mid proestrum	Recommended day of first breeding
N= 30	25.6 $\pm$ 1.33	79.9 $\pm$ 0.5	92.6 $\pm$ 1.29

Table 3. Comparison of conception rate in animals based on vaginal exfoliative cytology and Clinico-gynaecological examination with the animals bred based on vaginoscopy

Breeding advice based on	No: of animals bred	No: of animals conceived	Conception rate
Exfoliate vaginal cytology and clinicogynaecological studies	30	22	73.3 %
Vaginoscopy	25	22	88.0 %

Table 4. Serum progesterone profile (ng/ml) in ten female dogs during proestrum and on the day of maximum vaginal mucosal fold crenulation

Number of animals	Serum progesterone level during proestrus (ng/ml)	Serum progesterone level on the day of maximum vaginal mucosal fold crenulation (ng/ml)
N=10	1.21 $\pm$ 0.18	6.58 $\pm$ 0.31

Table 5. Comparison of abdominal palpation and ultrasound scanning for pregnancy diagnosis

Method adopted for pregnancy diagnosis	Gestational age	Positive	Doubtful	Negative	Percentage accuracy
Transabdominal palpation (n=20)	20 days	4	3	13	20 %
	35 days	15	3	2	75 %
	50 days	12	---	---	60 %
Transabdominal ultrasound scanning (n=20)	20 days	3	5	12	15 %
	35 days	18	2	---	90 %
	50 days	20	---	---	100%

Table 6. Serum alkaline phosphatase level (U/L) in pregnant dogs at different gestational age

Day of gestation	Serum alkaline phosphatase (Mean ± S.E)
20th day (n=20)	$67.90^{a} \pm 2.98$
35th day (n=20)	$91.85^{b} \pm 2.10$
50th day (n=20)	$139.65^{b} \pm 6.84$

Mean values having different superscript in a column differ significantly from 20th day ($P < 0.05$)

Table 7. Serum haptoglobin level (mg/dl) in pregnant dogs at different gestational age

Day of gestation	Serum haptoglobin level (Mean ± S.E)
20th day (n=20)	53.10^{a} ± 3.22
35th day (n=20)	81.12^{b} ± 3.40
50th day (n=20)	119.44^{b} ± 3.16

Mean values having different superscript in a column differ significantly from 20th day ($P < 0.05$)

Table 8. Serum globulin level (g/dl) in pregnant dogs at different gestational age

Day of gestation	Serum globulin level Mean ± S.E
20th day (n=20)	$2.43^{a} \pm 0.12$
35th day (n=20)	$3.05^{b} \pm 0.11$
50th day (n=20)	$3.74^{b} \pm 0.15$

Mean values having different superscript in a column differ significantly from 20th day ($P < 0.05$)

Table 9. Haemogram of pregnant bitches at different gestational age

Day of gestation	Total erythrocyte count (million/cmm)	Haemoglobin (g/dl)	Packed cell volume (%)	Erythrocyte sedimentation rate (mm/hr)
20th day (n=20)	$6.07^{a} \pm 0.16$	$11.86^{a} \pm 0.28$	$40.45^{a} \pm 0.83$	$10.31^{a} \pm 0.73$
35th day (n=20)	$5.58^{b} \pm 0.15$	$10.45^{b} \pm 0.27$	$37.30^{b} \pm 0.87$	$15.41^{b} \pm 0.54$
50th day (n=20)	$5.12^{b} \pm 0.16$	$9.17^{b} \pm 0.30$	$33.40^{b} \pm 1.10$	$21.85^{b} \pm 1.04$

Mean values having different superscript in a column differ significantly from 20th day ($P < 0.05$)

Table 10. Leucogram of pregnant bitches at different gestational age

Day of gestation	Total leucocyte count (cells/cmm)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
20th day (n=20)	13844.90 [a] ± 539.90	67.30 [a] ± 1.11	27.80 [a] ± 0.87	3.90 [a] ± 0.27	1.60 [a] ± 0.11	----
35th day (n=20)	15449.00 [b] ± 569.86	70.30 [b] ± 4.95	31.55 [b] ± 0.88	3.80 [a] ± 0.20	1.40 [a] ± 0.11	-----
50th day (n=20)	17502.50 [b]. ± 780.21	75.35 [b] ± 1.27	36.95 [b] ± 1.03	4.05 [a] ± 0.22	3.30 [b] ± 0.13	------

Mean values having different superscript in a column differ significantly from 20th day ($P < 0.05$)

Table 11. Gestation length and mean litter size in animals studied (Mean ± S.E)

No: of animals studied	Gestation length (days)	Litter size (number)
N = 20	63.3 ± 3.76	6.6 ± 2.08

Table 12. Physiological and behavioural changes observed in bitches with overt pseudopregnancy prior to treatment

Group	Mild udder enlargement with presence of fluid	Prominent udder changes with abundant milk	Vaginal discharge	Abdominal enlargement	Nesting behaviour	Anorexia
Group I (n=10)	6	4	6	4	2	8
Group II (n=10)	7	3	5	5	1	7

Table 13. Mean serum progesterone and prolactin level (ng/ml) in overtly pseudopregnant bitches treated with cabergoline

Hormone (n=10)	Pre-treatment	Post-treatment at 7th day	Post-treatment at 14th day
Progesterone	$1.11^{a} \pm 0.14$	$0.85^{b} \pm 0.06$	$0.62^{b} \pm 0.06$
Prolactin	$6.17^{a} \pm 2.05$	$1.69^{b} \pm 0.20$	$0.82^{b} \pm 0.13$

Mean values having different superscript in a row differ significantly from pre-treatment ($P<0.05$)

Table 14. Mean serum progesterone and prolactin level (ng/ml) in overtly pseudopregnant bitches treated with bromocriptine

Hormone (n=10)	Pre-treatment	Post-treatment at 7th day	Post-treatment at 14th day
Progesterone	$1.11^{a} \pm 0.14$	$0.5^{b} \pm 0.01$	$0.5^{b} \pm 0.01$
Prolactin	$6.02^{a} \pm 1.17$	$0.90^{b} \pm 0.07$	$0.58^{b} \pm 0.08$

Mean values having different superscript in a row differ significantly from pre-treatment ($P<0.05$)

Table 15. Mean concentration of progesterone and prolactin (ng/ml) level in control bitches

Hormone (n=10)	1st day	7th day	14th day
Progesterone	$1.53^{a} \pm 0.48$	$0.70^{a} \pm 0.11$	$0.44^{a} \pm 0.03$
Prolactin	$1.16^{a} \pm 0.24$	$1.02^{a} \pm 0.12$	$1.01^{a} \pm 0.07$

Mean values having same superscript in a row did not differ significantly from first day.

Fig. 1. Serum alkaline phophatase level in pregnant dogs at different gestational age

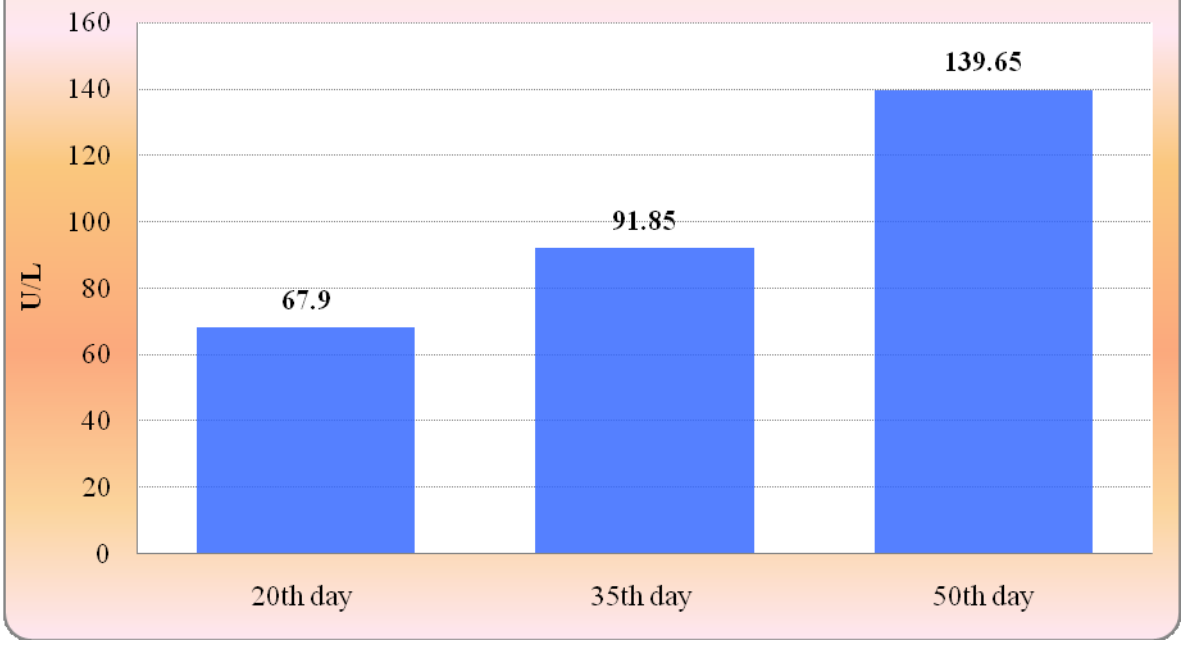
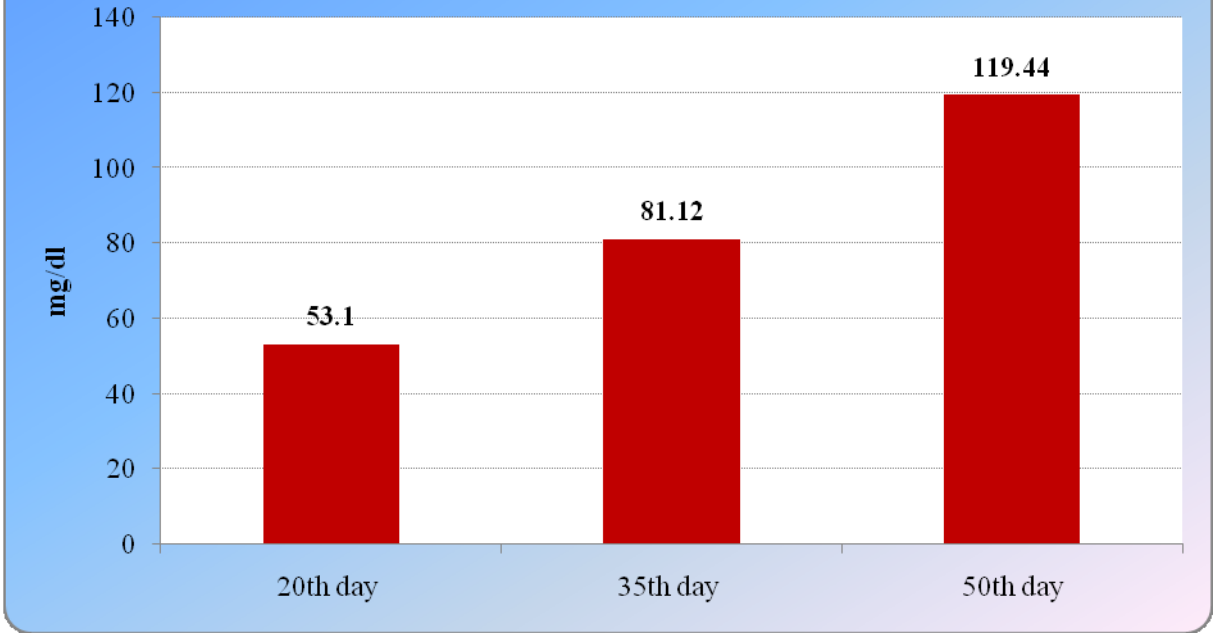


Fig. 2. Serum haptoglobin level in pregnant dogs at different gestational age



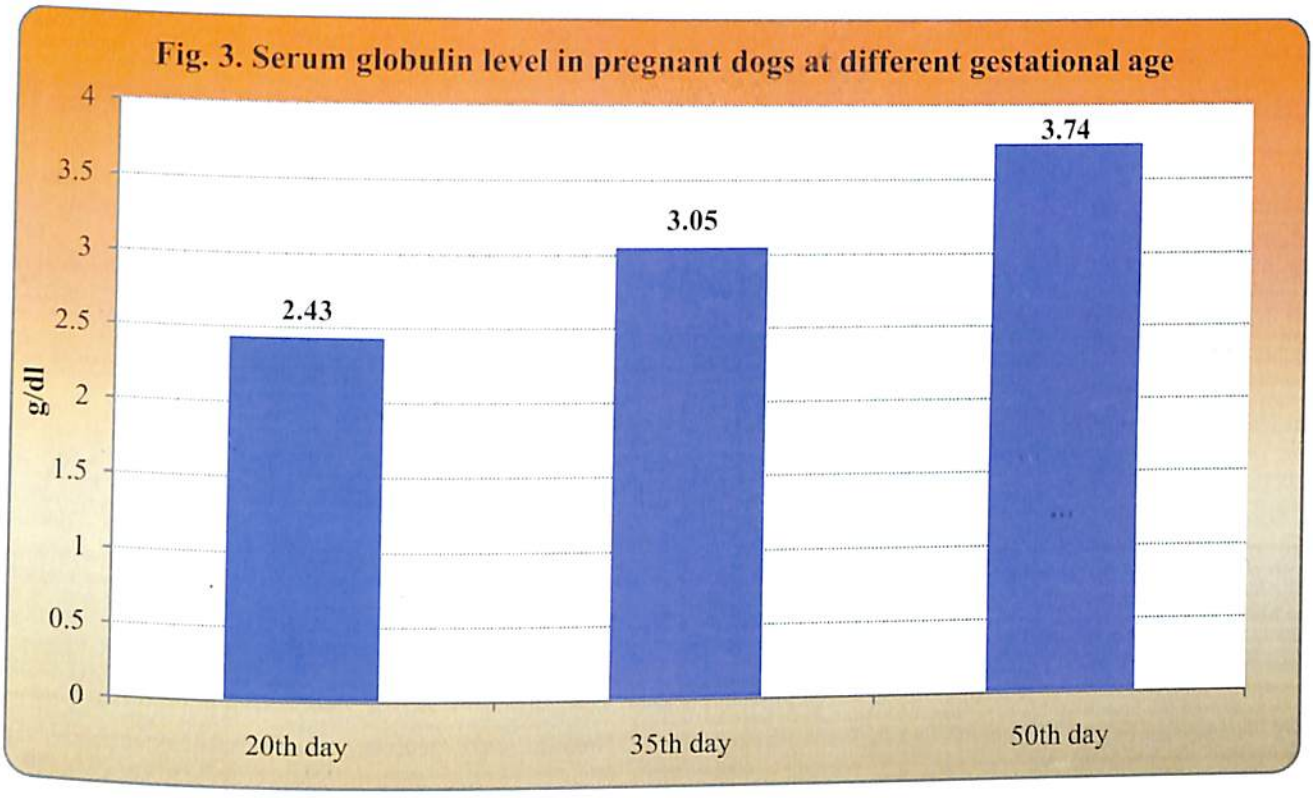


Fig. 3. Serum globulin level in pregnant dogs at different gestational age
g/dl
4
3.5
3
2.5
2
1.5
1
0.5
0
2.43
3.05
3.74
20th day
35th day
50th day

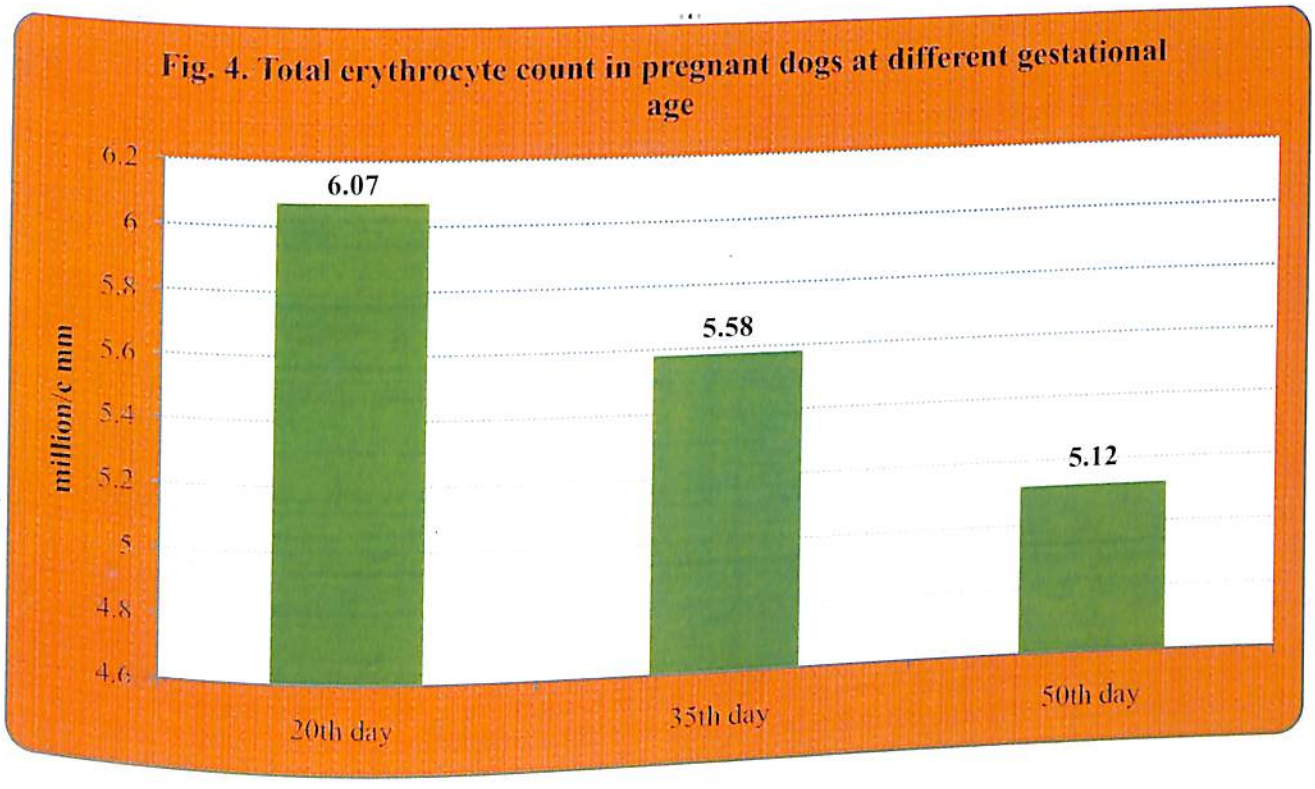


Fig. 4. Total erythrocyte count in pregnant dogs at different gestational age
million/c mm
6.2
6
5.8
5.6
5.4
5.2
5
4.8
4.6
6.07
5.58
5.12
20th day
35th day
50th day

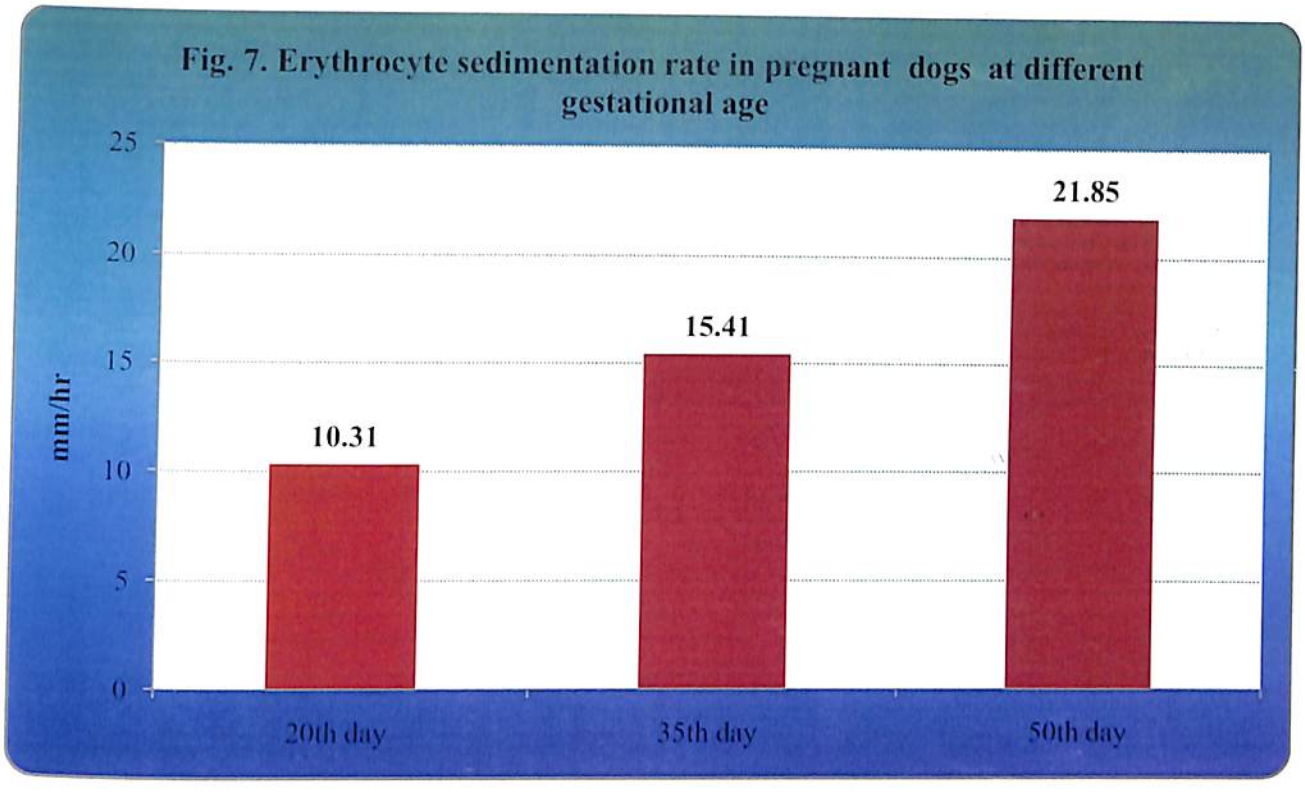


Fig. 7. Erythrocyte sedimentation rate in pregnant dogs at different gestational age
mm/hr
25
20
15
10
5
0
10.31
15.41
21.85
20th day
35th day
50th day

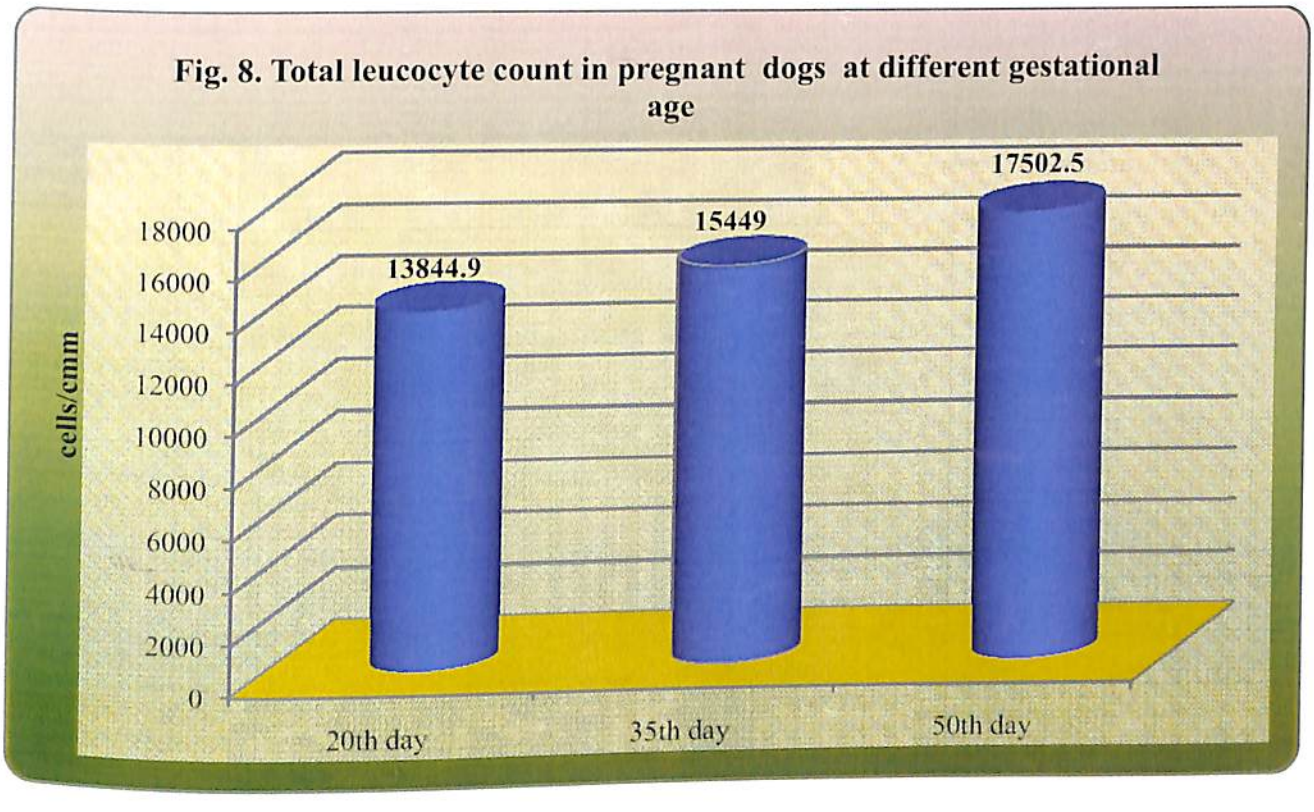


Fig. 8. Total leucocyte count in pregnant dogs at different gestational age
cells/cmm
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
13844.9
15449
17502.5
20th day
35th day
50th day

Fig. 9. Neutrophil count in pregnant dogs at different gestational age

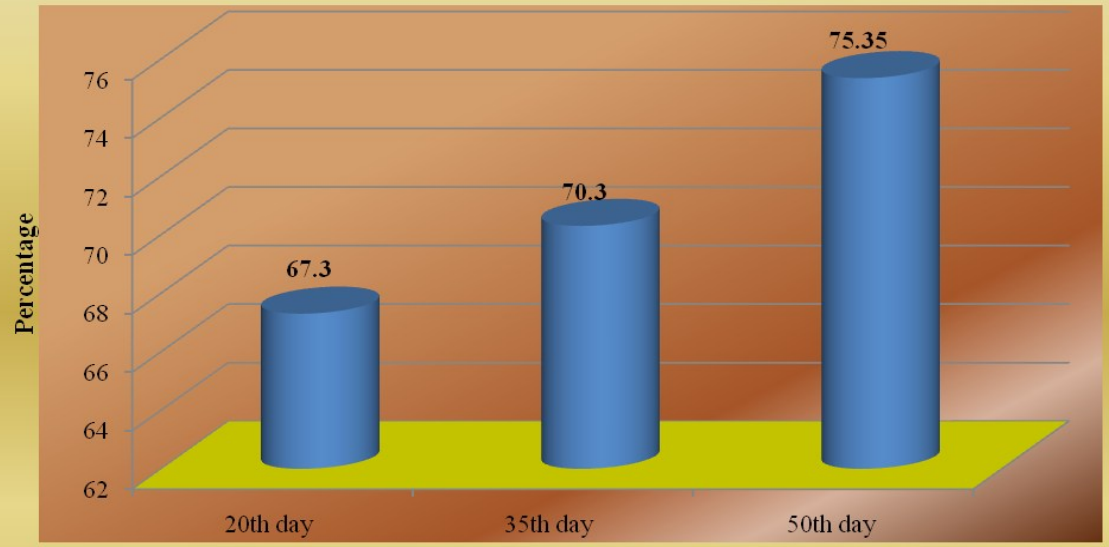


Fig. 10. Lymphocyte count in pregnant dogs at different gestational age

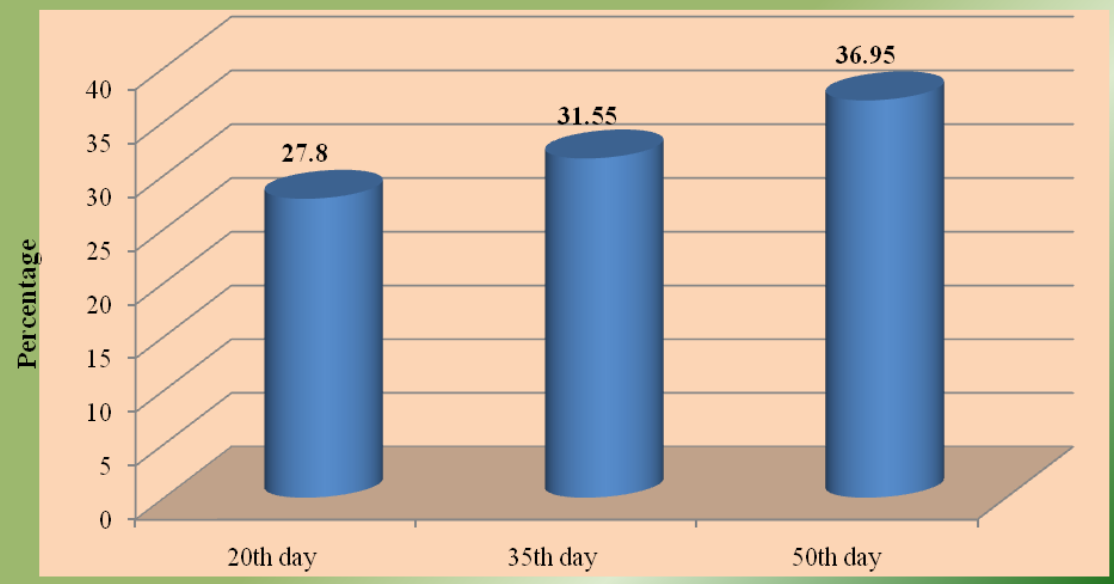


Fig. 11. Monocyte count in pregnant dogs at different gestational age

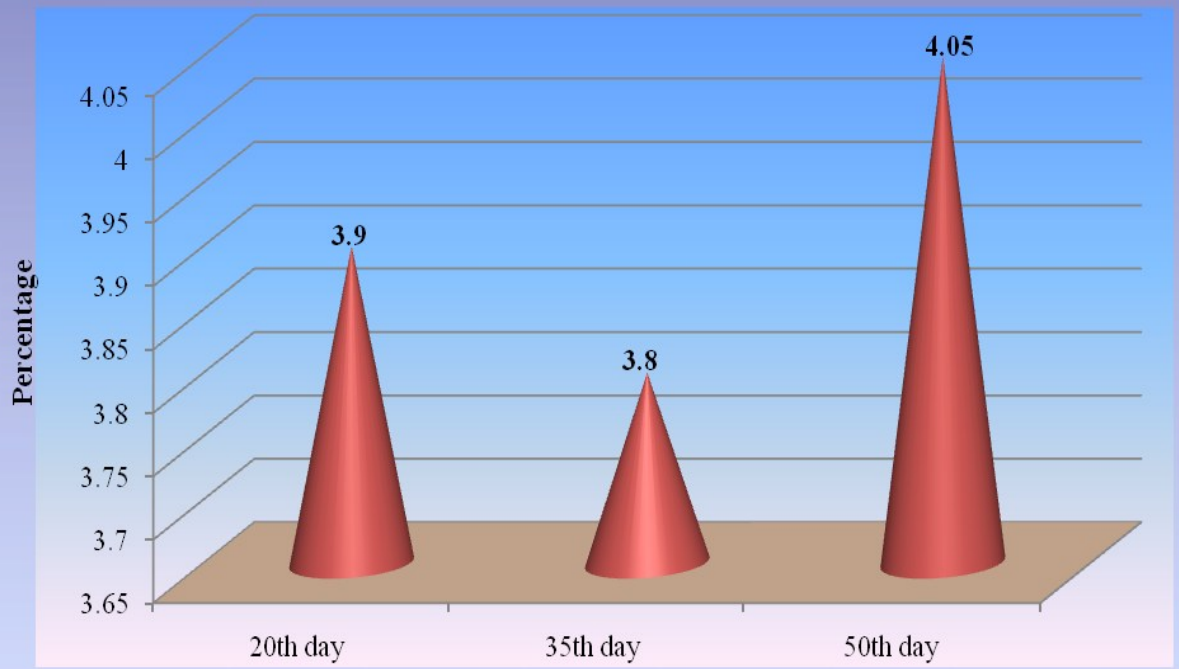
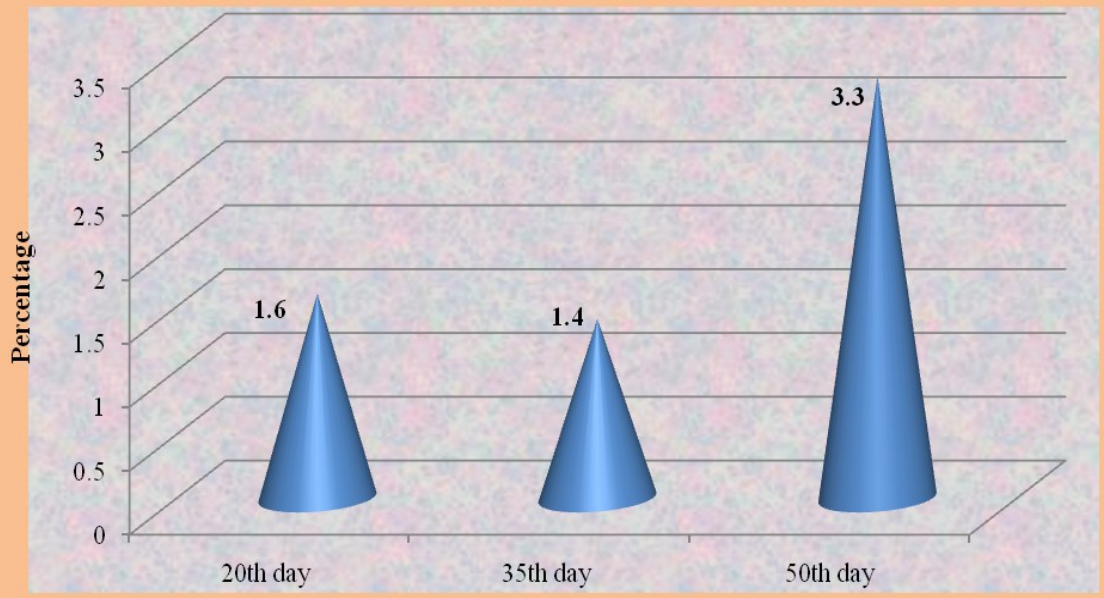


Fig. 12. Eosinophil count in pregnant dogs at different gestational age



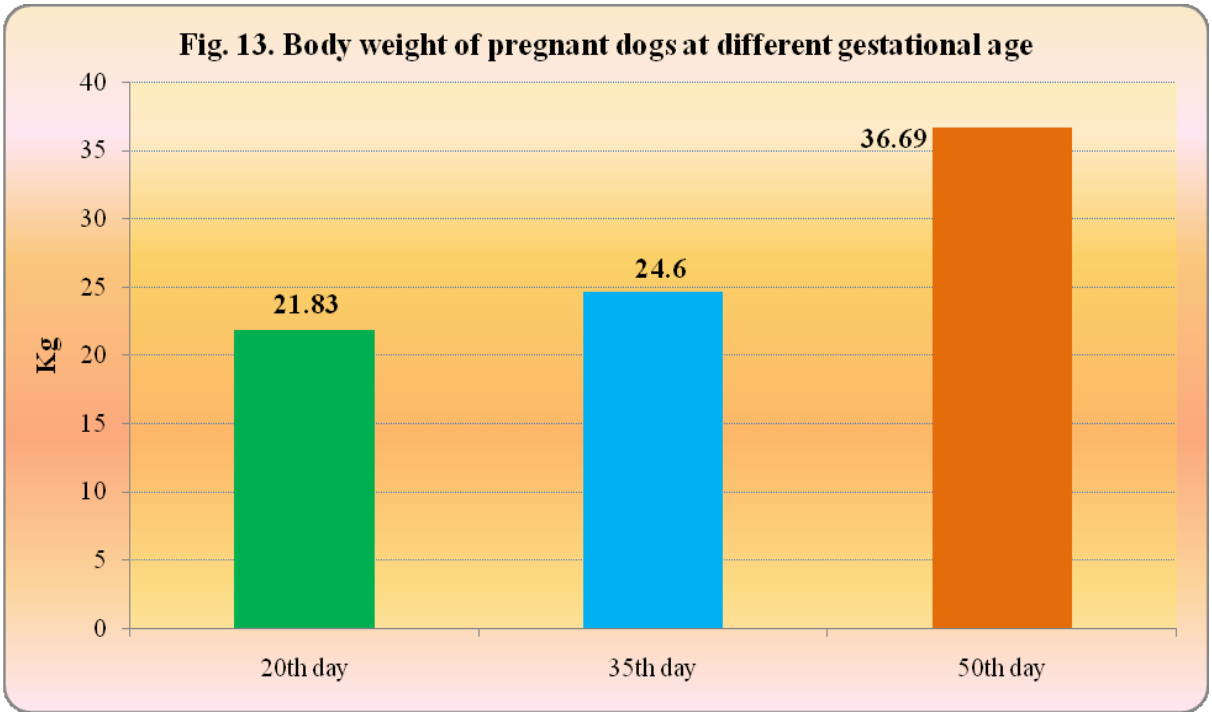


Fig. 13. Body weight of pregnant dogs at different gestational age
Kg
40
35
30
25
20
15
10
5
0
21.83
24.6
36.69
20th day
35th day
50th day

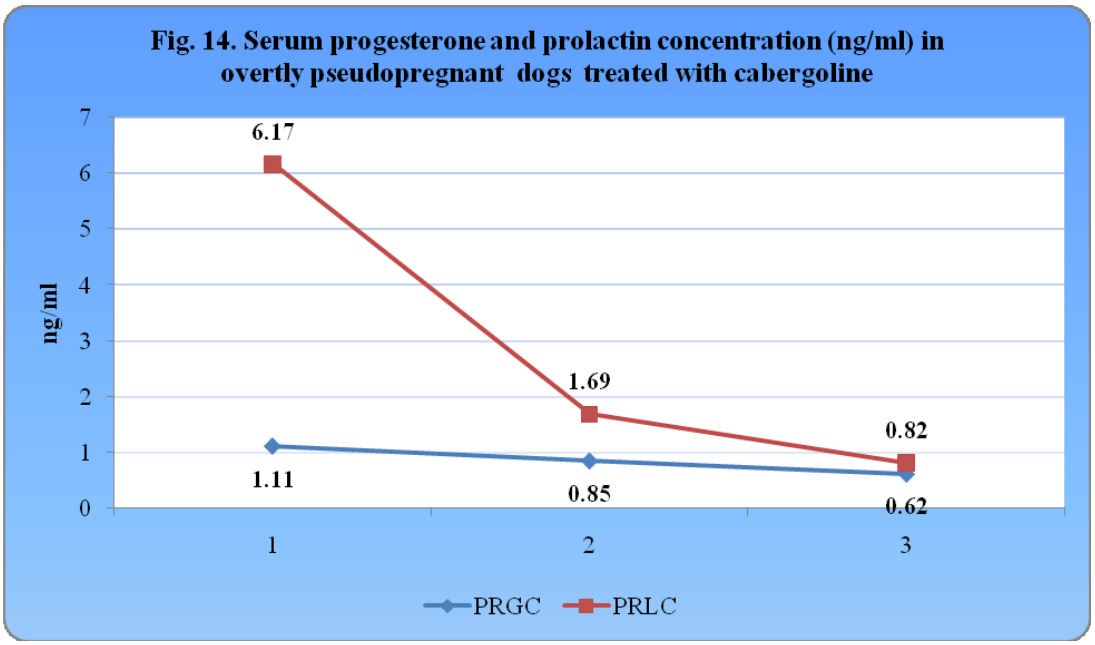


Fig. 14. Serum progesterone and prolactin concentration (ng/ml) in overtly pseudopregnant dogs treated with cabergoline
ng/ml
7
6
5
4
3
2
1
0
6.17
1.69
0.82
1.11
0.85
0.62
1
2
3
PRGC
PRLC

Fig. 15. Serum progesterone and prolactin concentration (ng/ml) in overtly pseudopregnant dogs treated with bromocriptine

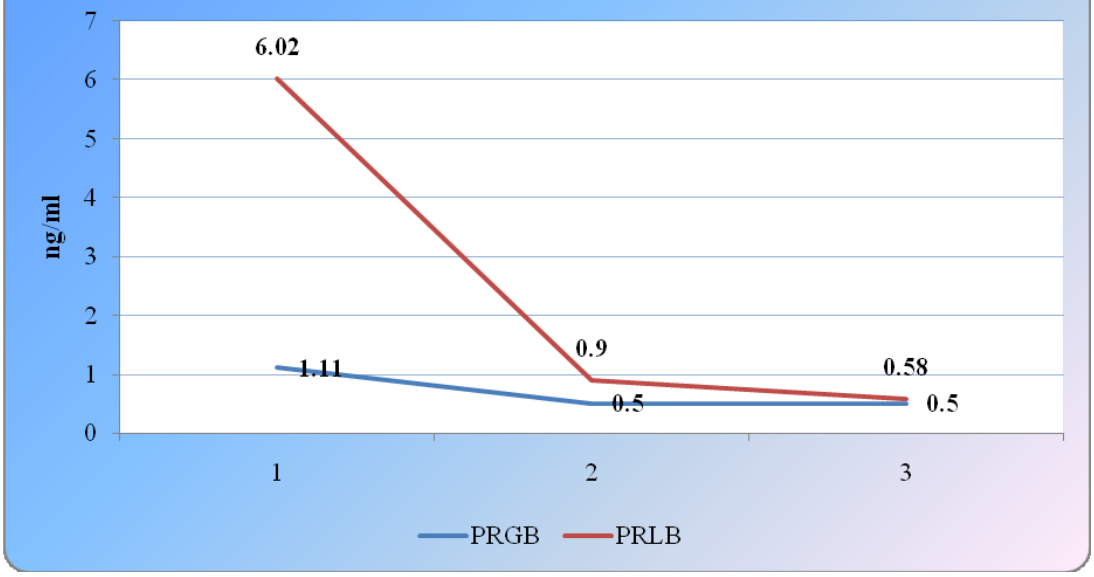
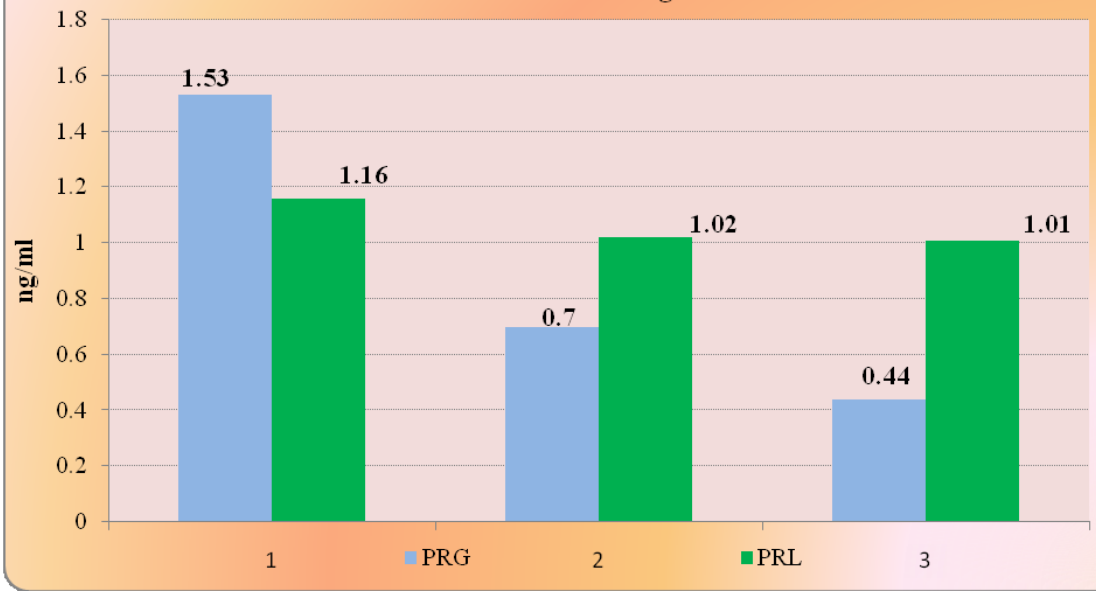


Fig. 16. Mean concentration of progesterone and prolactin (ng/ml) level in control dogs



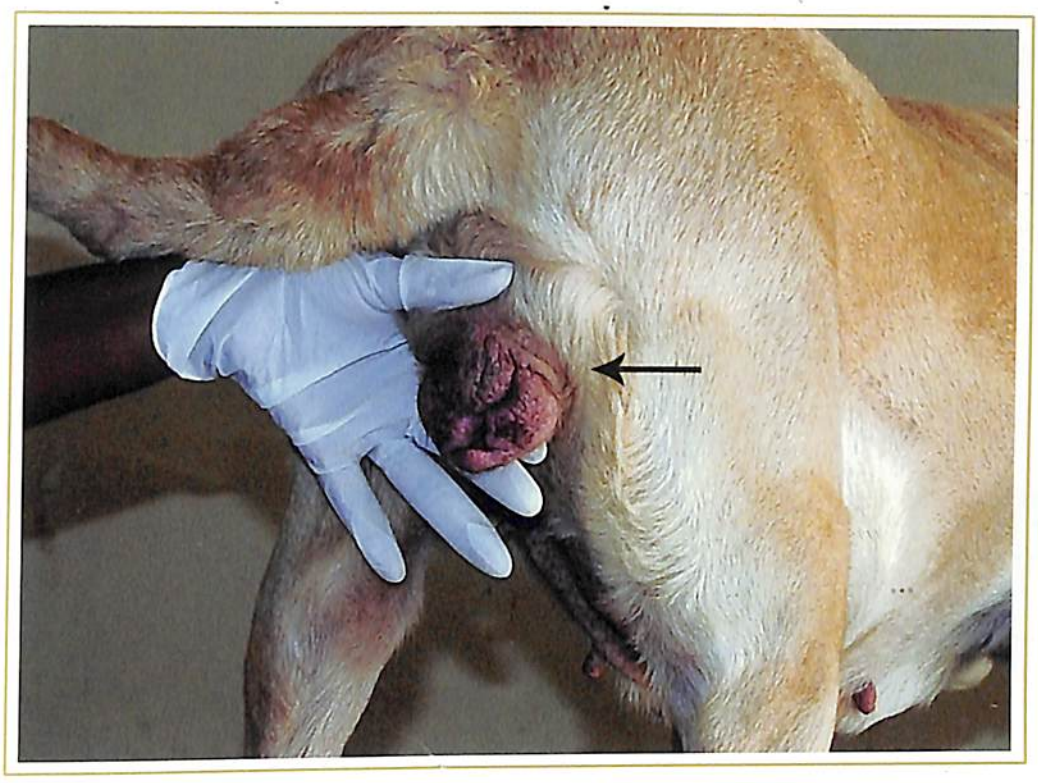


Plate: 1
Bitch in proestrum with highly oedematous vulval lips

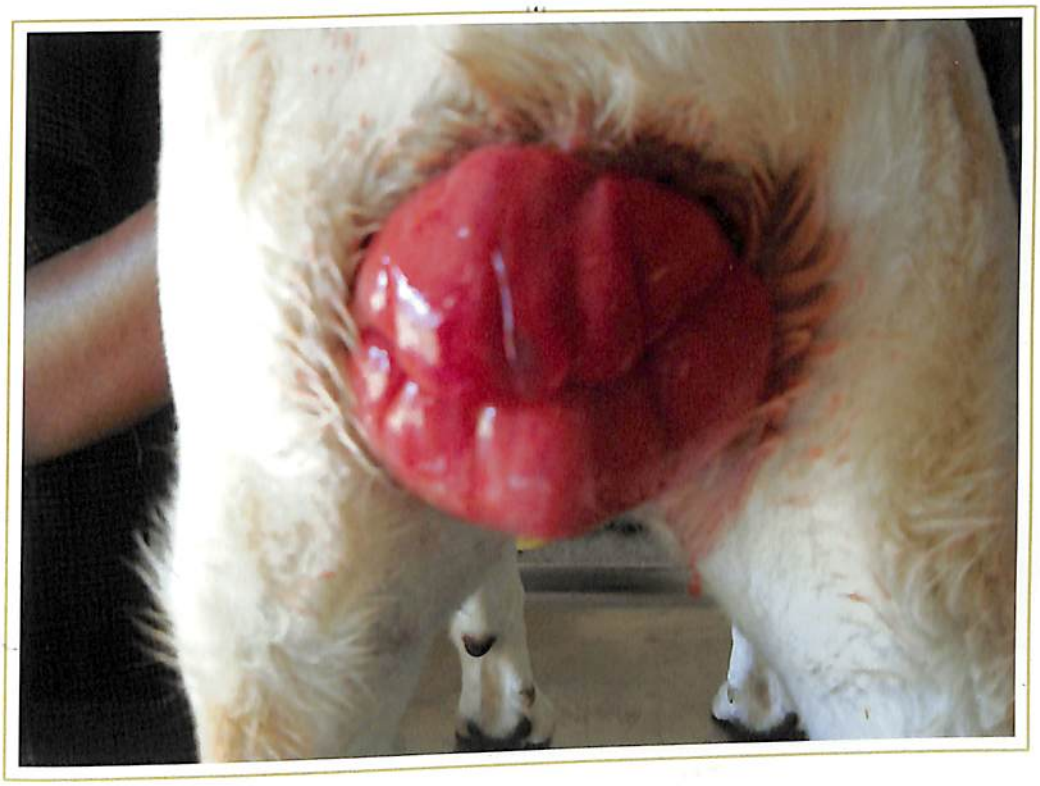


Plate: 2
Vaginal hyperplasia in a bitch due to increased estrogen response observed during clinico-gynaecological examination

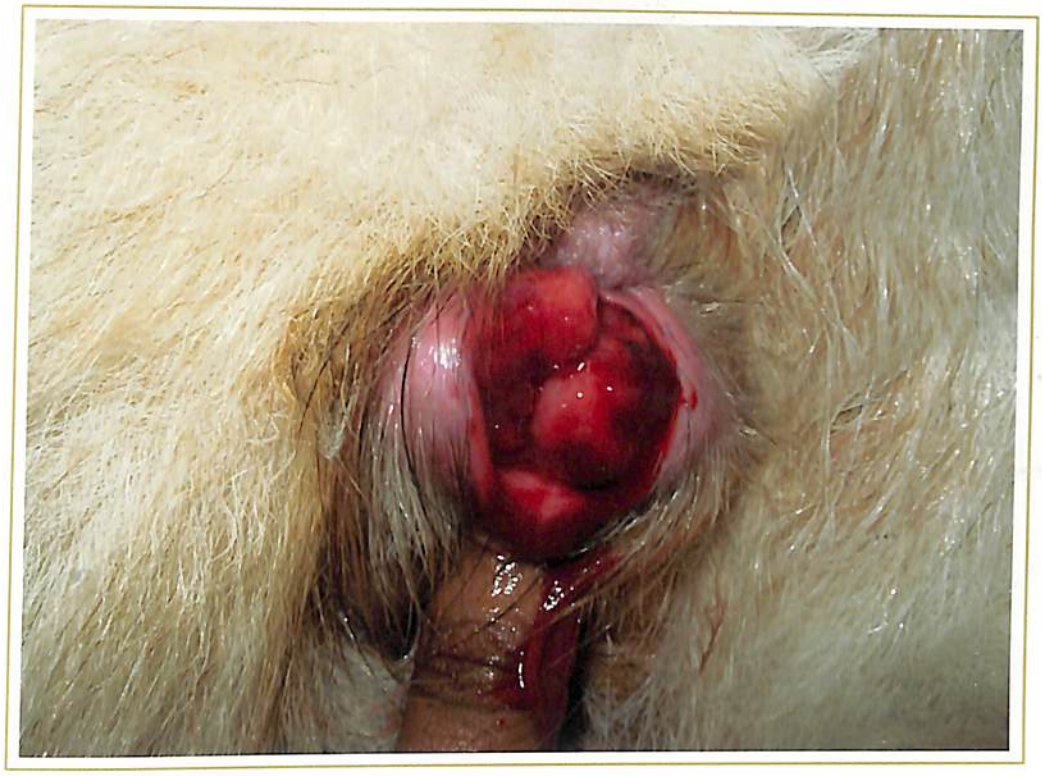


Plate: 3

Transmissible venereal tumour in a bitch observed during clinico-gynaecological examination

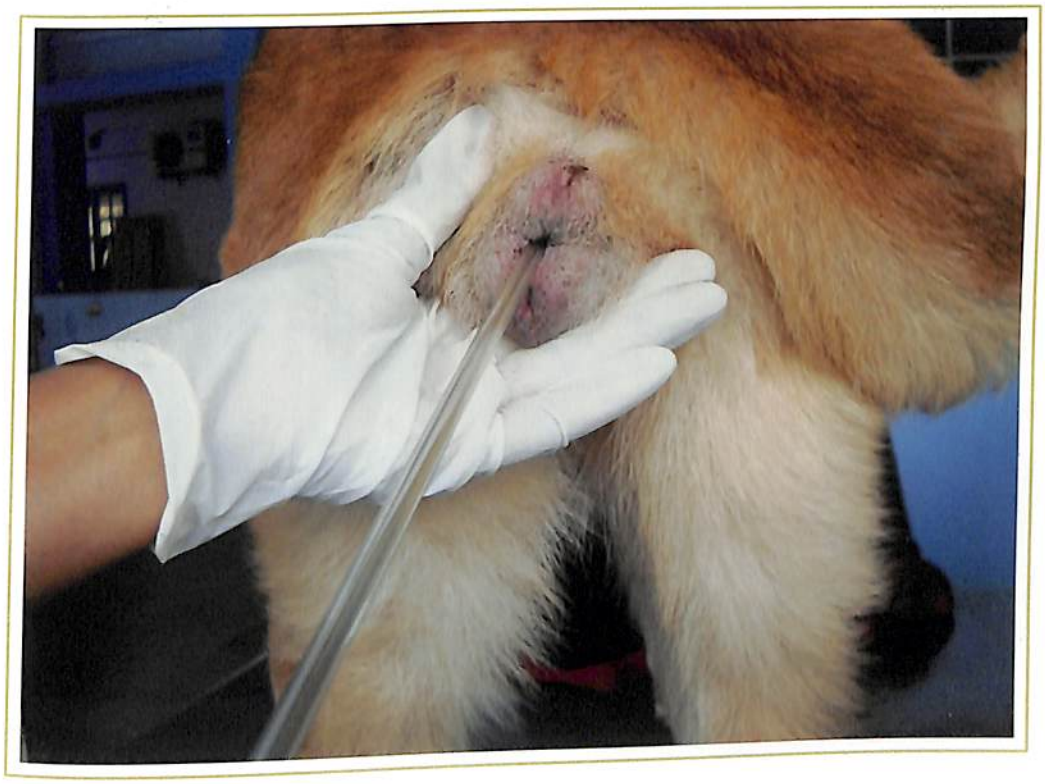


Plate: 4

Collection of vaginal discharge from anterior vagina using a sterile pipette for performing vaginal exfoliate cytology

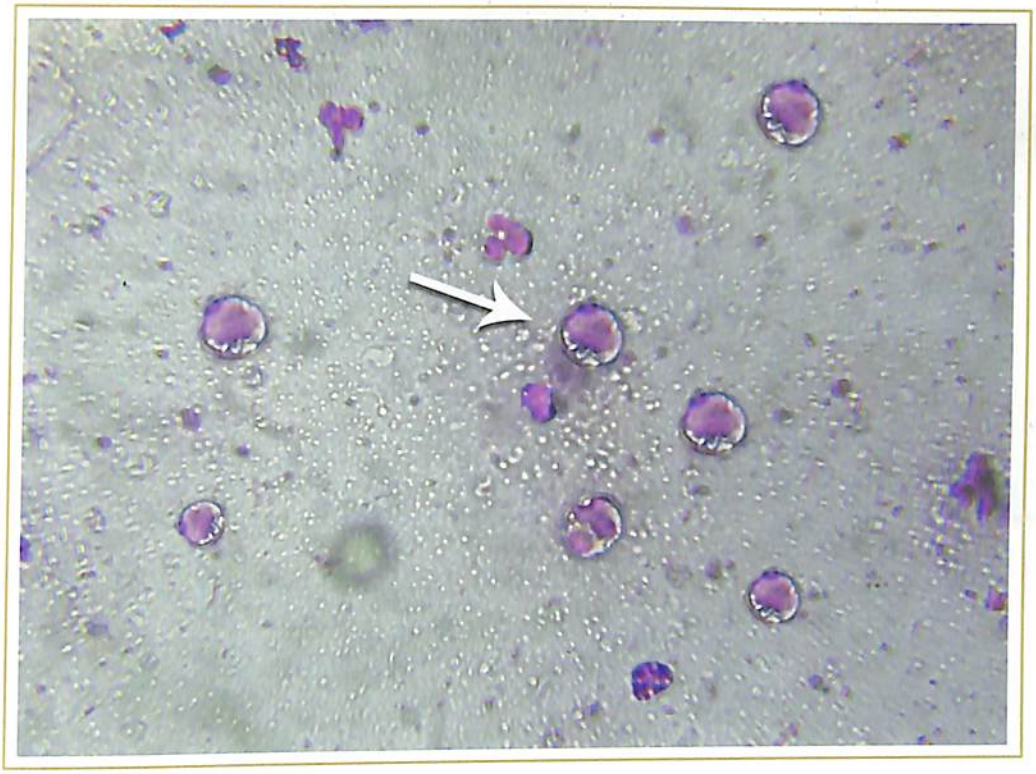


Plate: 5

Vaginal exfoliate cytology illustrating small intermediate cells suggestive of early proestrum (Wright-Giemsa stain, 400x)

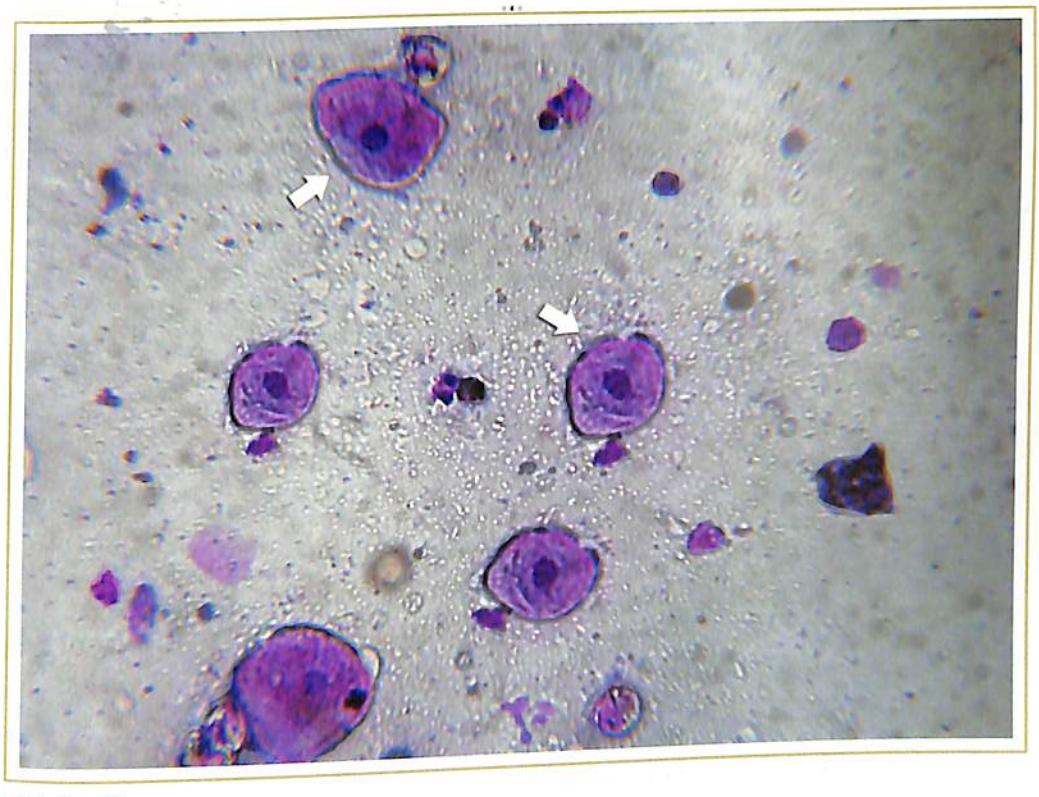


Plate: 6

Vaginal exfoliate cytology illustrating large intermediate cells suggestive of mid proestrum (Wright-Giemsa stain, 400x)

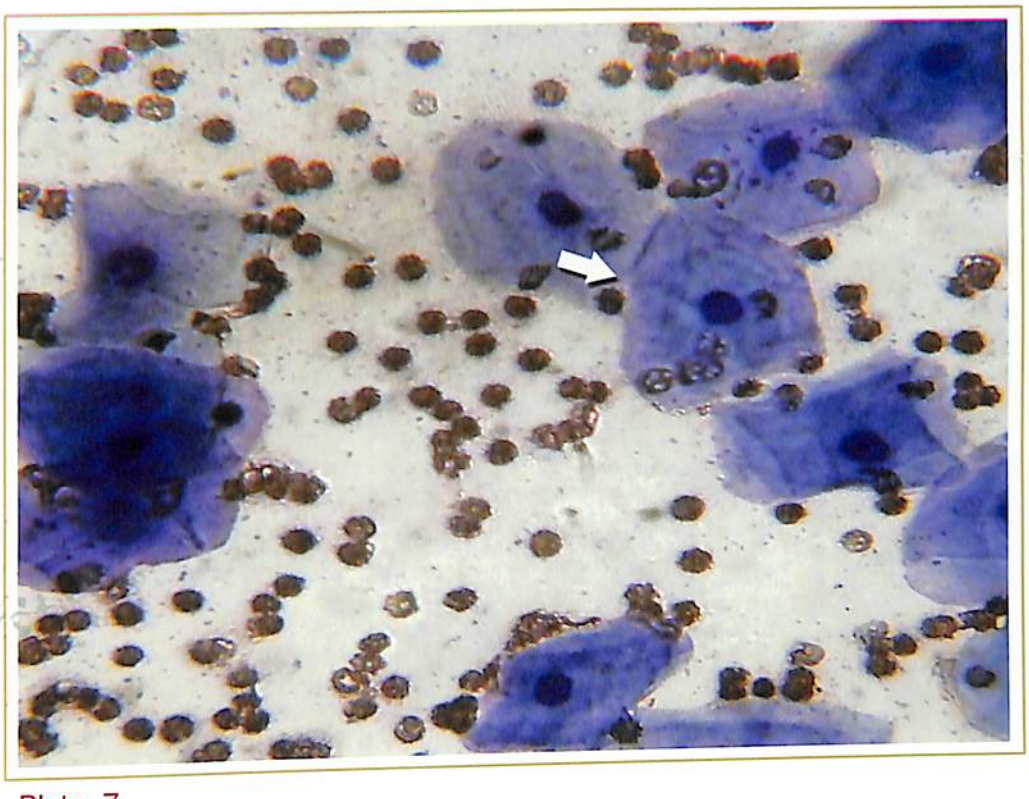


Plate: 7

Vaginal exfoliate cytology illustrating superficial intermediate cells with large number of RBC's distributed throughout suggestive of late proestrum (Wright-Giemsa stain, 400x)

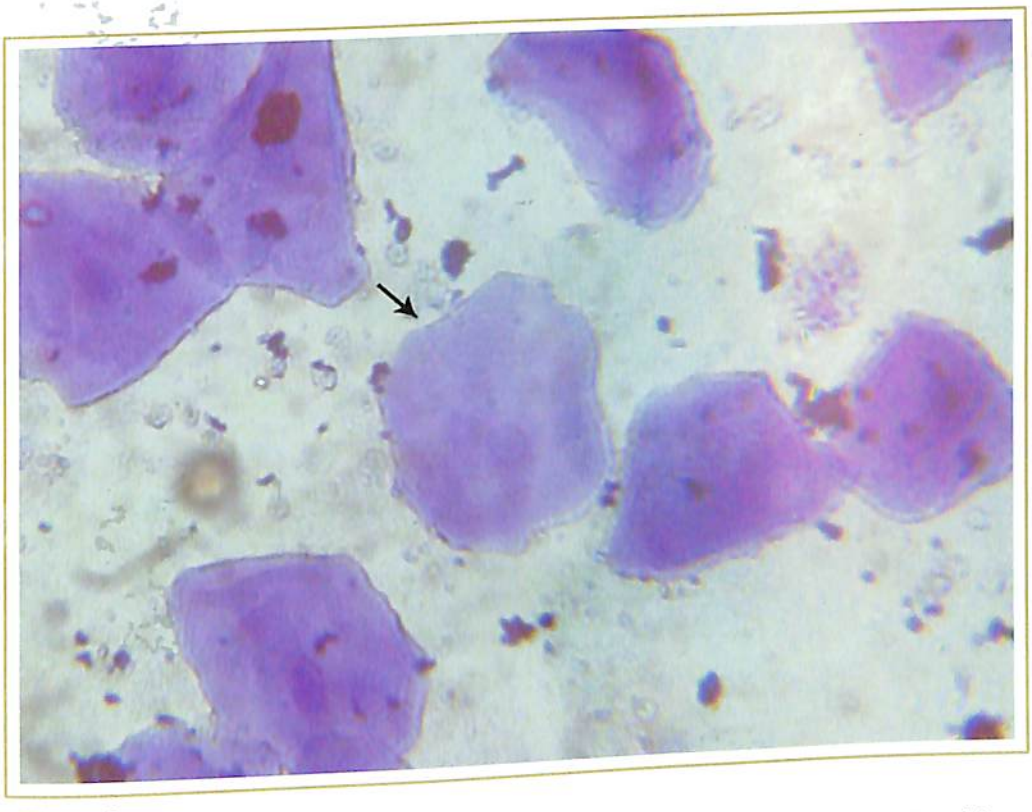


Plate: 8

Vaginal exfoliate cytology illustrating anuclear keratinized cells with less RBC and clear background. Classic picture of oestrus in bitch (Wright-Giemsa stain, 400x)

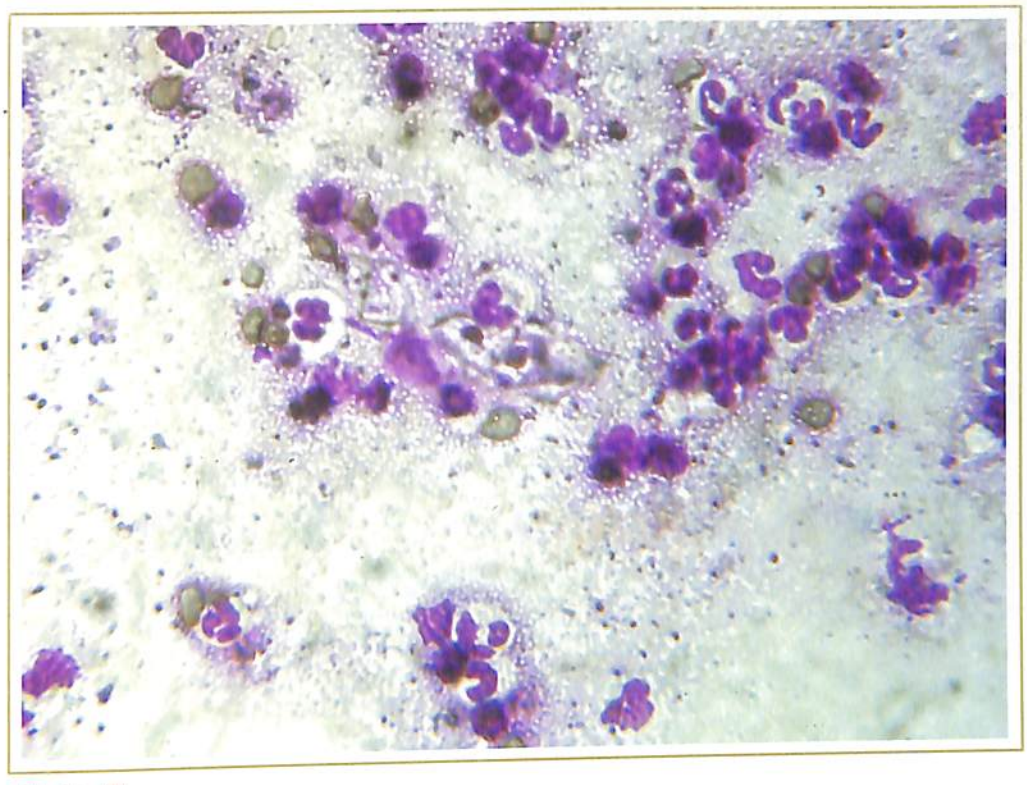


Plate: 9

Vaginal exfoliate cytology illustrating large number of neutrophils suggestive of vaginitis (Wright-Giemsa stain, 400x)

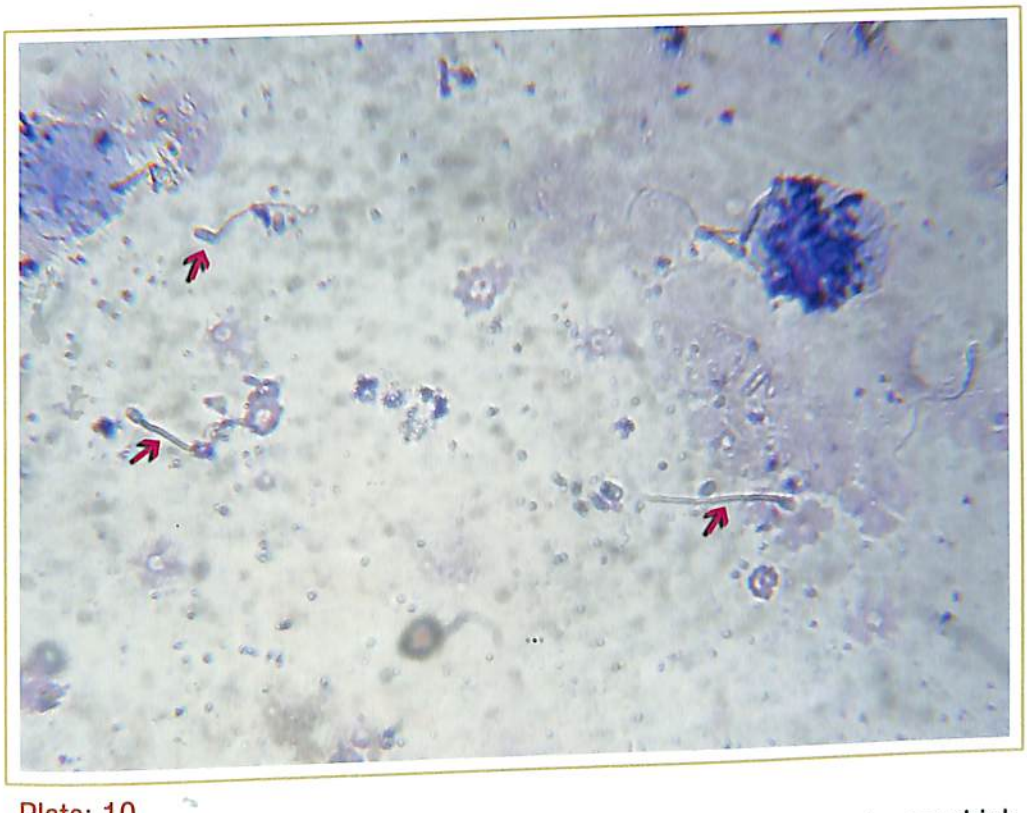


Plate: 10

Vaginal exfoliate cytology illustrating presence of spermatozoa which was bred a day back (Wright-Giemsa stain, 400x)

Plate: 11
Endoscopy unit-Karl Storz, GmBH, Tiuttlingen, Germany

Plate: 12
Performing vaginoscopy in a bitch using flexible fibre optic endoscope

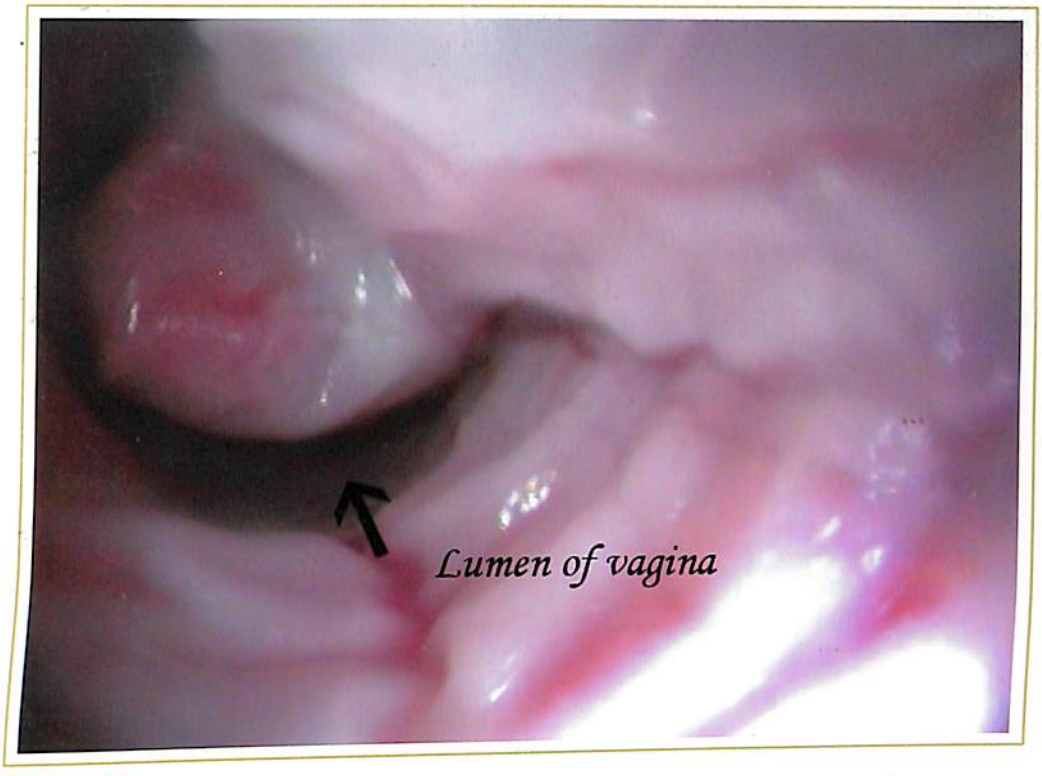


Plate: 13

Vaginoscopic view through lumen of vagina. Primary folds of vagina are seen as large, oedematous balloons with pale pink mucosa

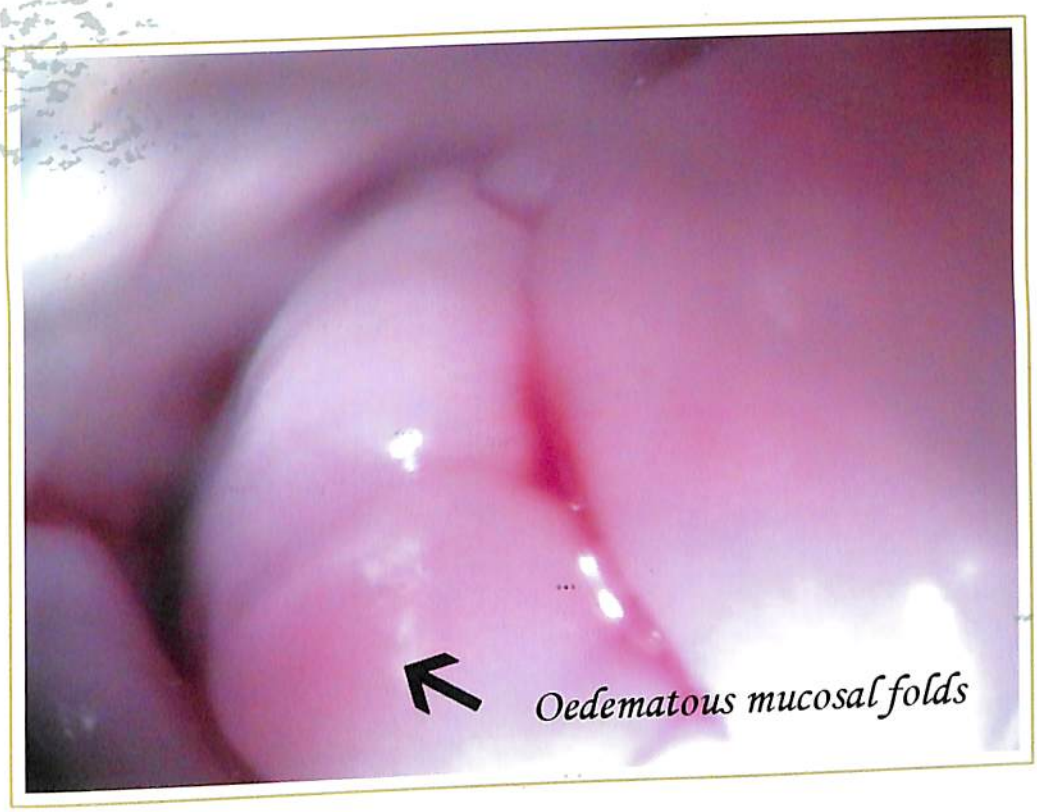


Plate: 14

Vaginoscopic view during early proestrus. Serosanguineous uterine discharge appeared as red fluid in the furrows of the folds

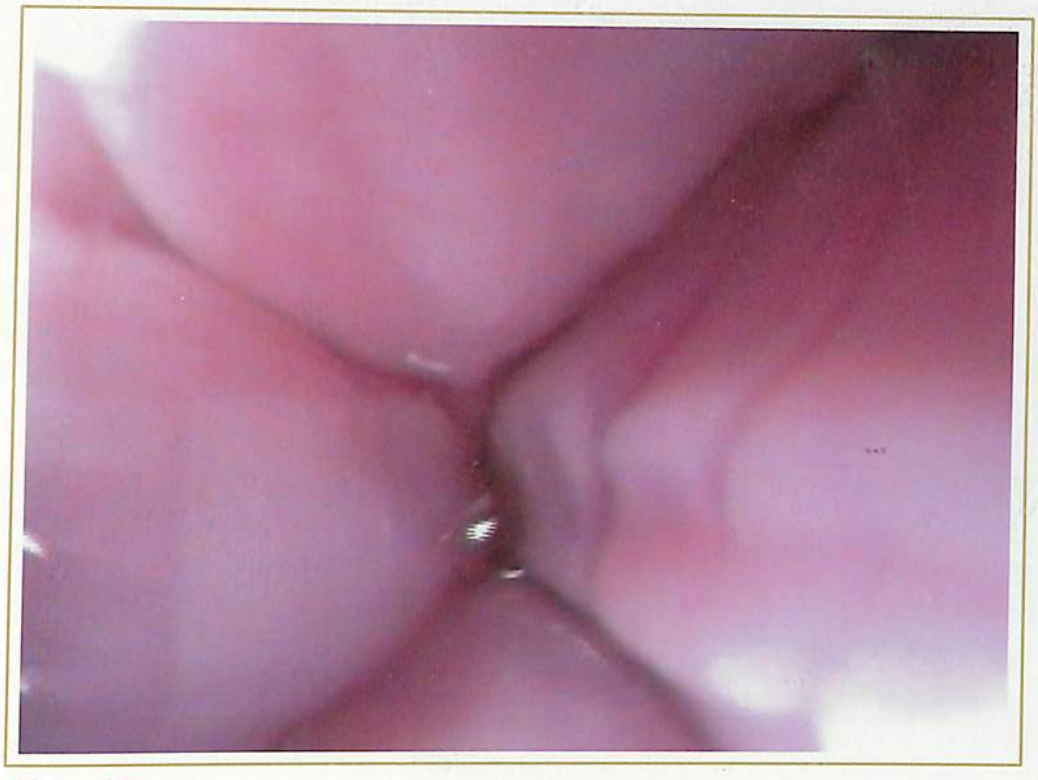


Plate: 15

Vaginoscopic view during mid proestrus. Oedematous proliferated period with unwrinkled and bulbous profiles

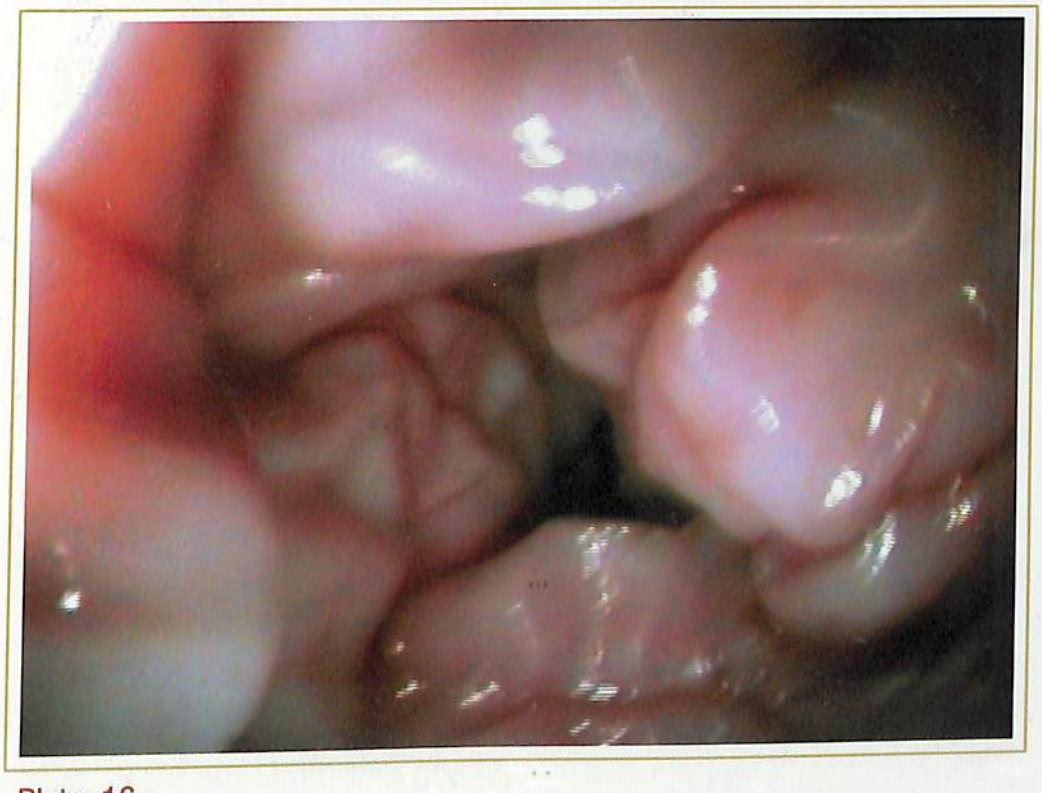


Plate: 16

Vaginoscopic view during early oestrum. Mucosal shrinkage without excessive angulation suggestive of onset of fertile period

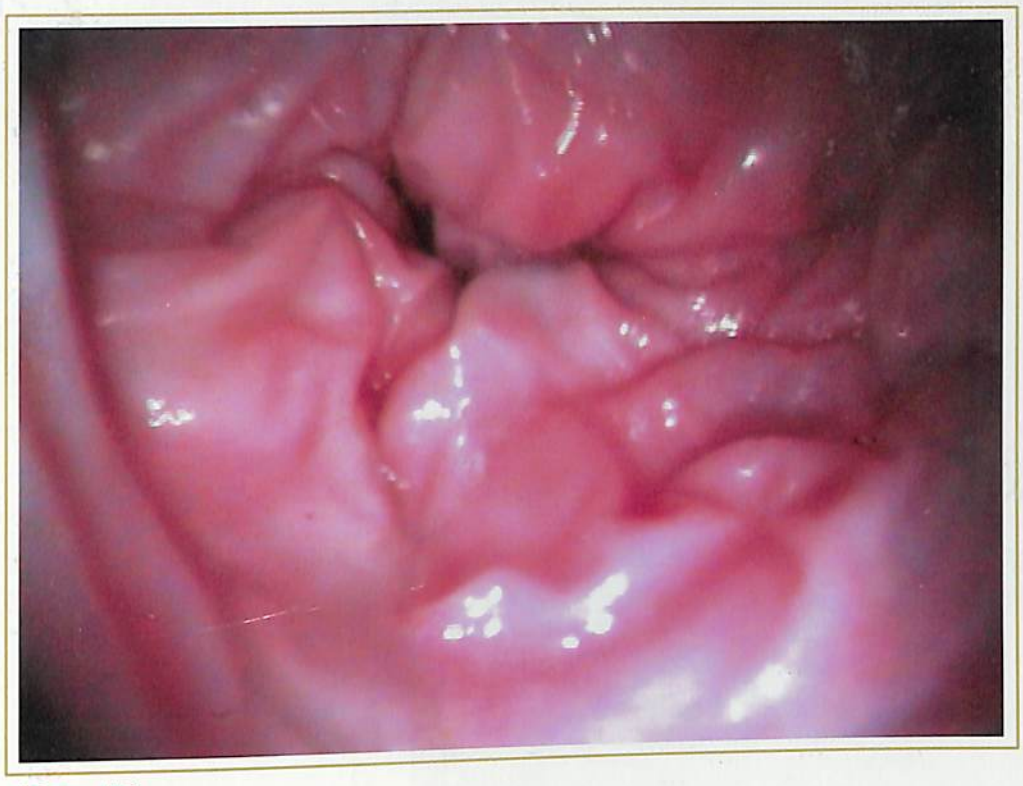


Plate: 17

Gross shrinkage of entire mucosal folds with obvious angulation characteristic of fertilization period

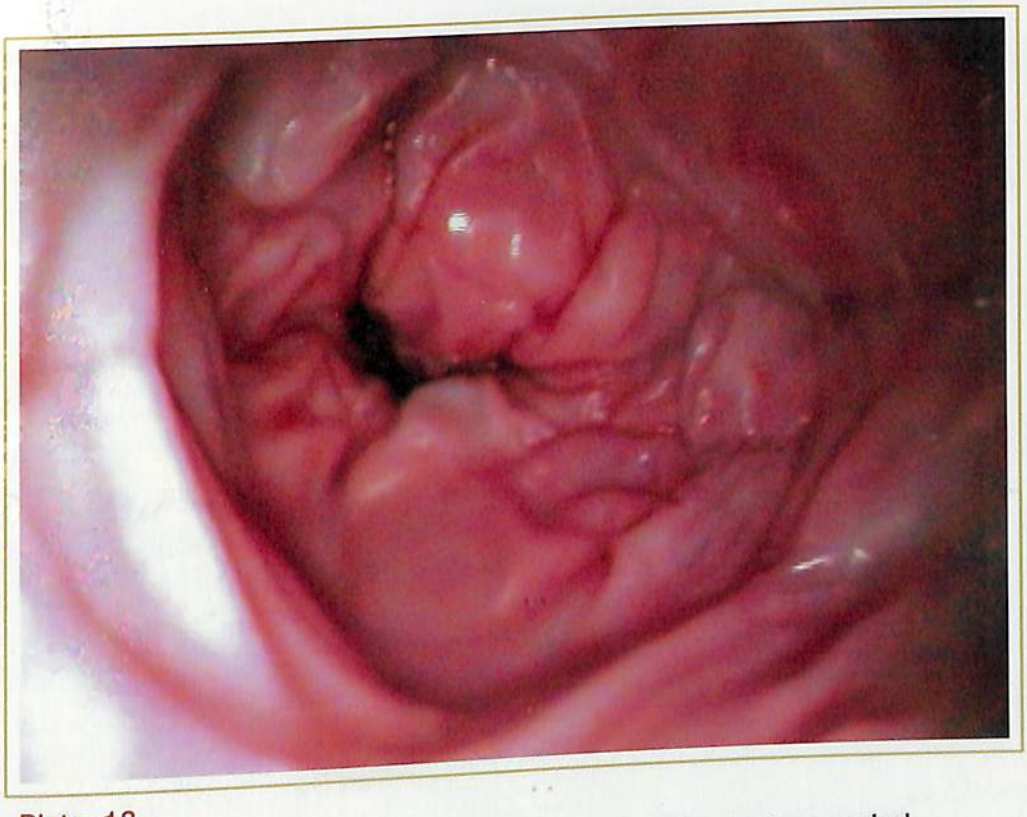


Plate: 18

Crumpled vaginal folds marked the end of the oestrus period

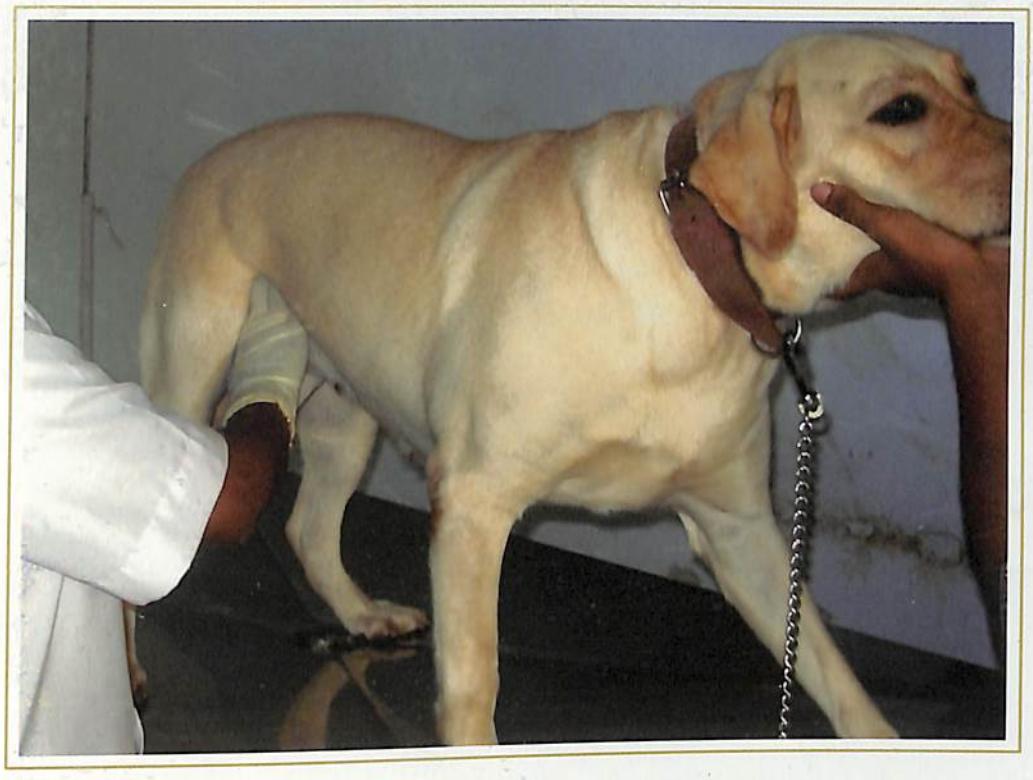


Plate: 19
Performing abdominal palpation in a bitch for pregnancy diagnosis

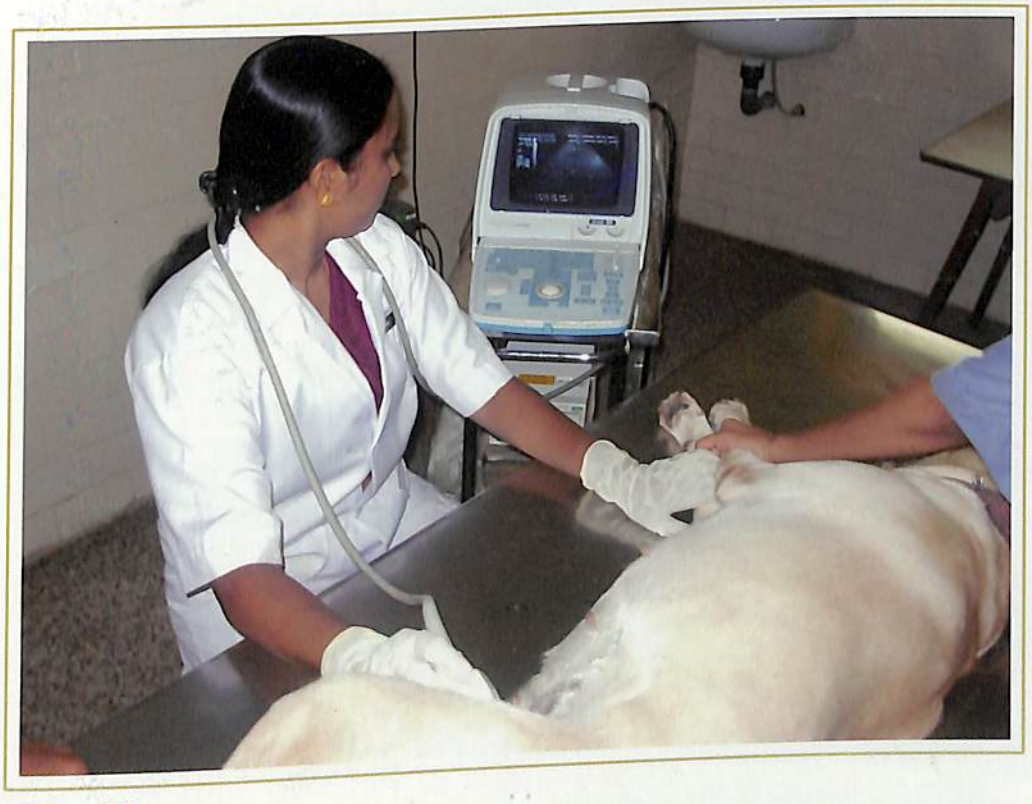


Plate: 20
Performing ultrasound scanning in a bitch for pregnancy diagnosis

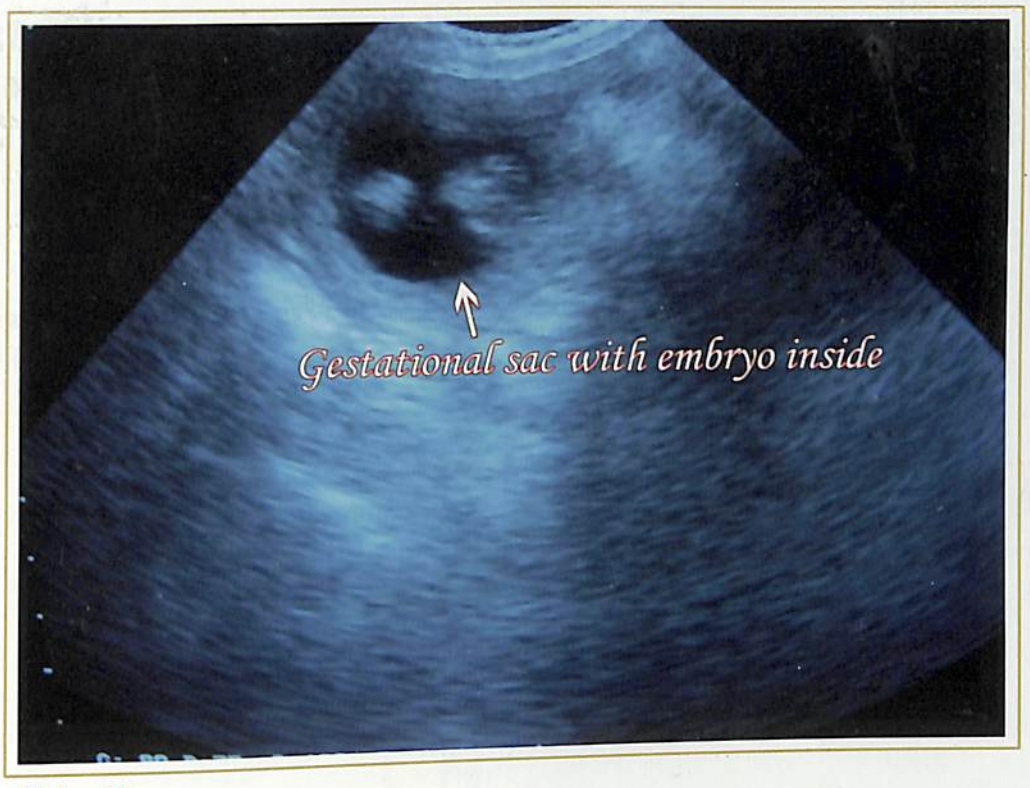


Plate: 21

Sonogram of uterus of a bitch at 20 days of gestation. Conceptus surrounded by small amount of anechoic fluid

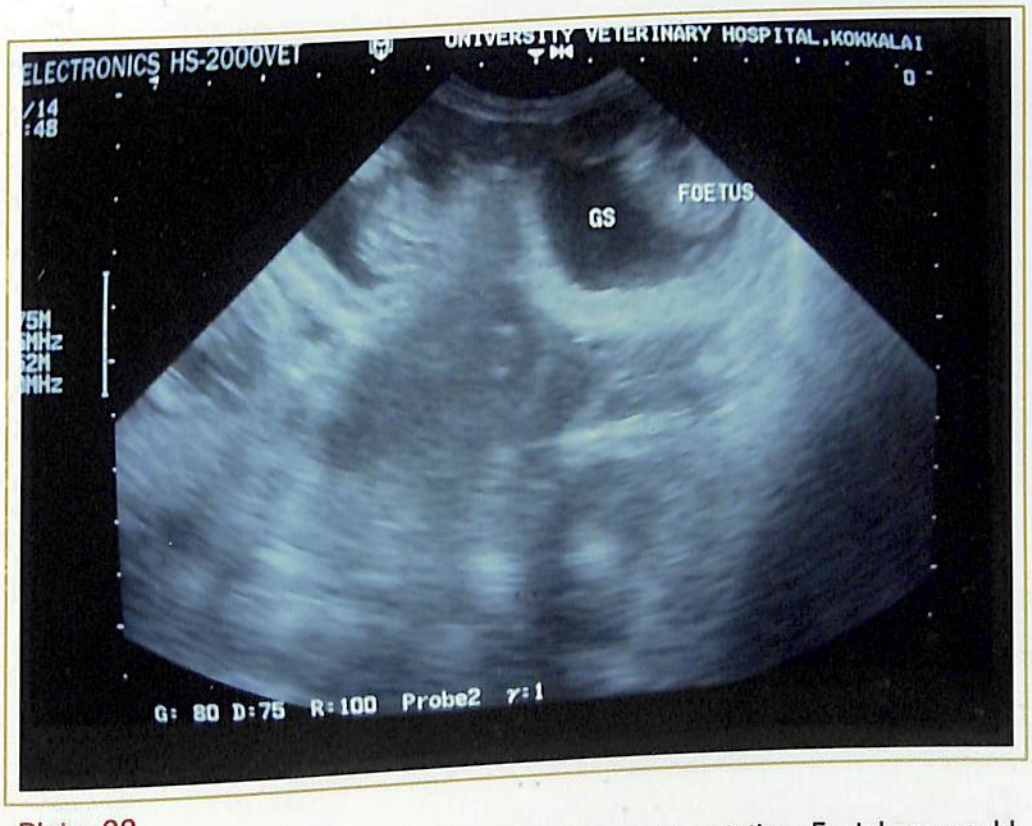


Plate: 22

Sonogram of uterus of a bitch at 23 days of gestation. Foetal sac could be seen as hypo echoic lumen surrounded by echoic uterine wall

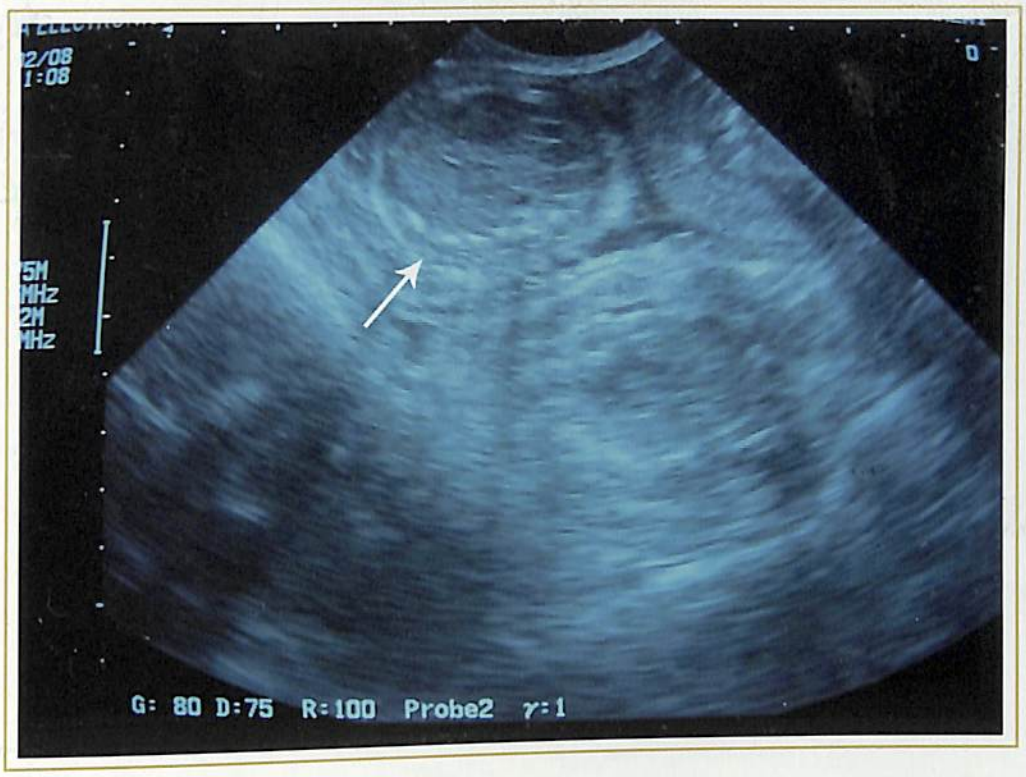


Plate: 23

Sonogram of uterus of a bitch at 25 days of gestation. Three gestational sacs could be observed

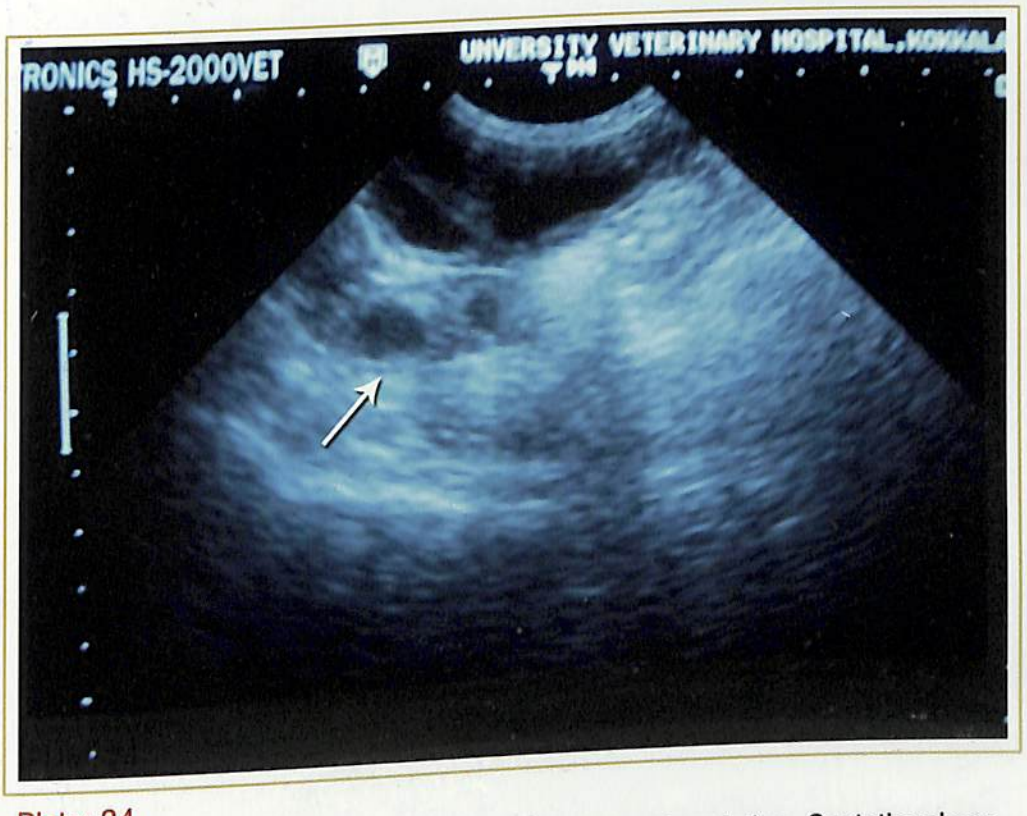


Plate: 24

Sonogram of uterus of a bitch at 27 days of gestation. Gestational sac with developing embryo inside

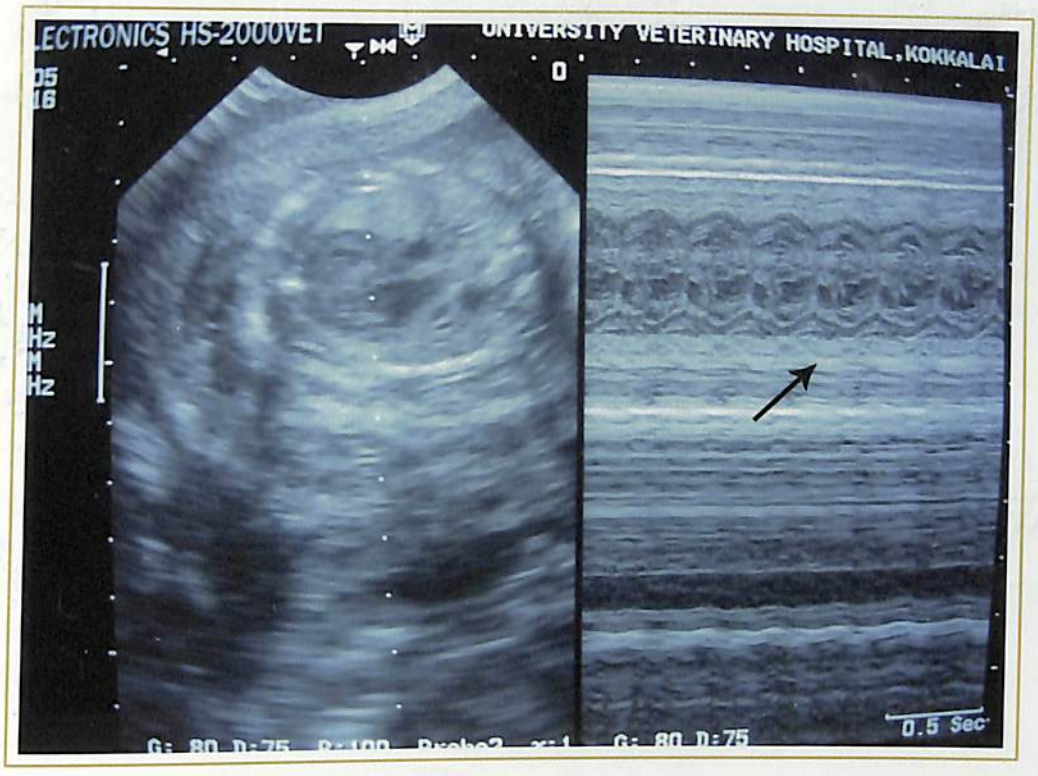


Plate: 25

Sonogram of uterus of a bitch at 35 days of gestation. Foetal heart beat represented as M-mode

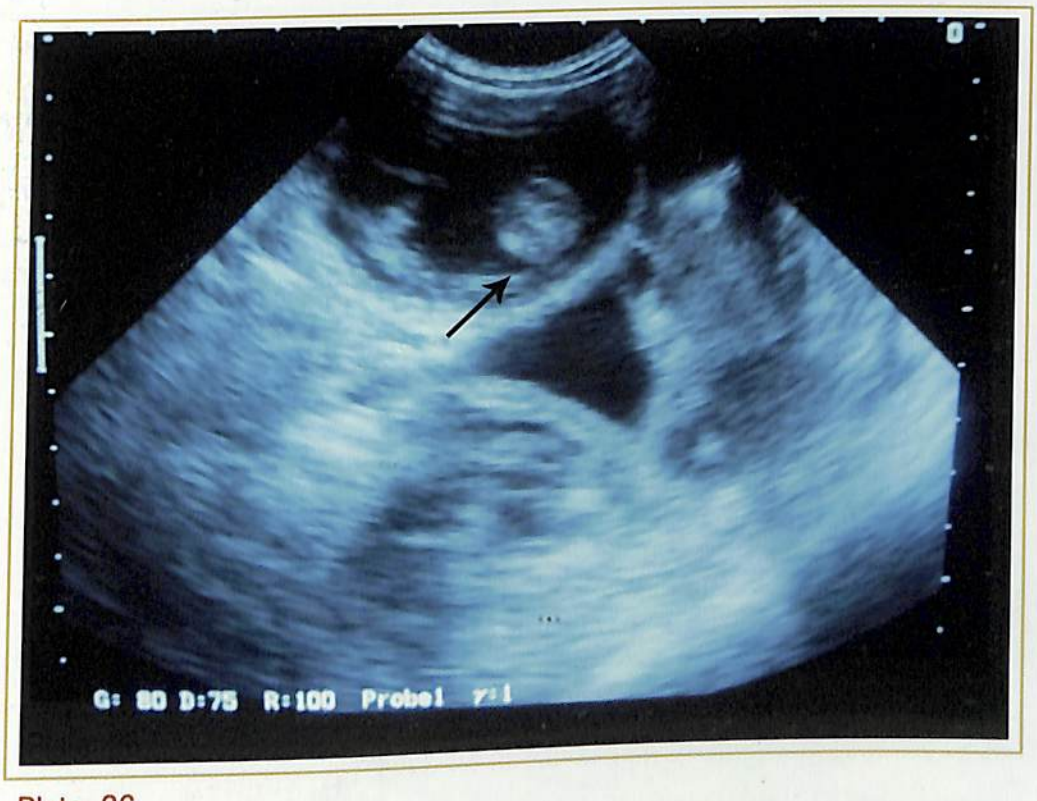


Plate: 26

Sonogram of uterus of a bitch at 38 days of gestation. Gestational sac containing developing foetus

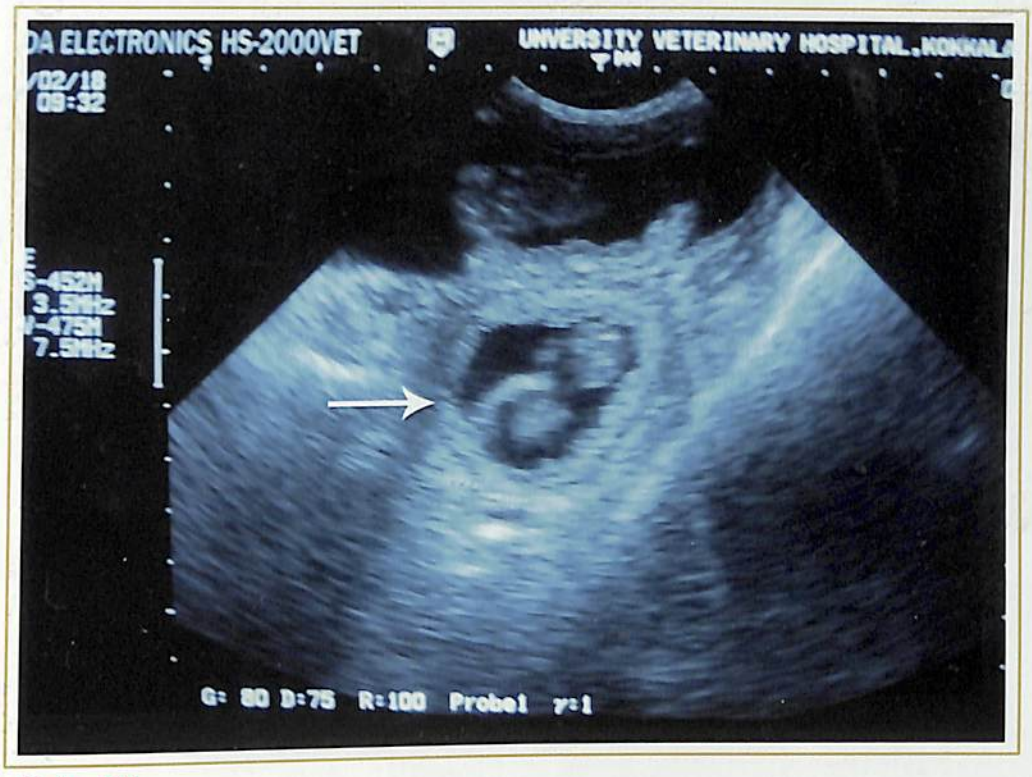


Plate: 27

Sonogram of uterus of a bitch at 45 days of gestation. Foetus could be visualized more clearly

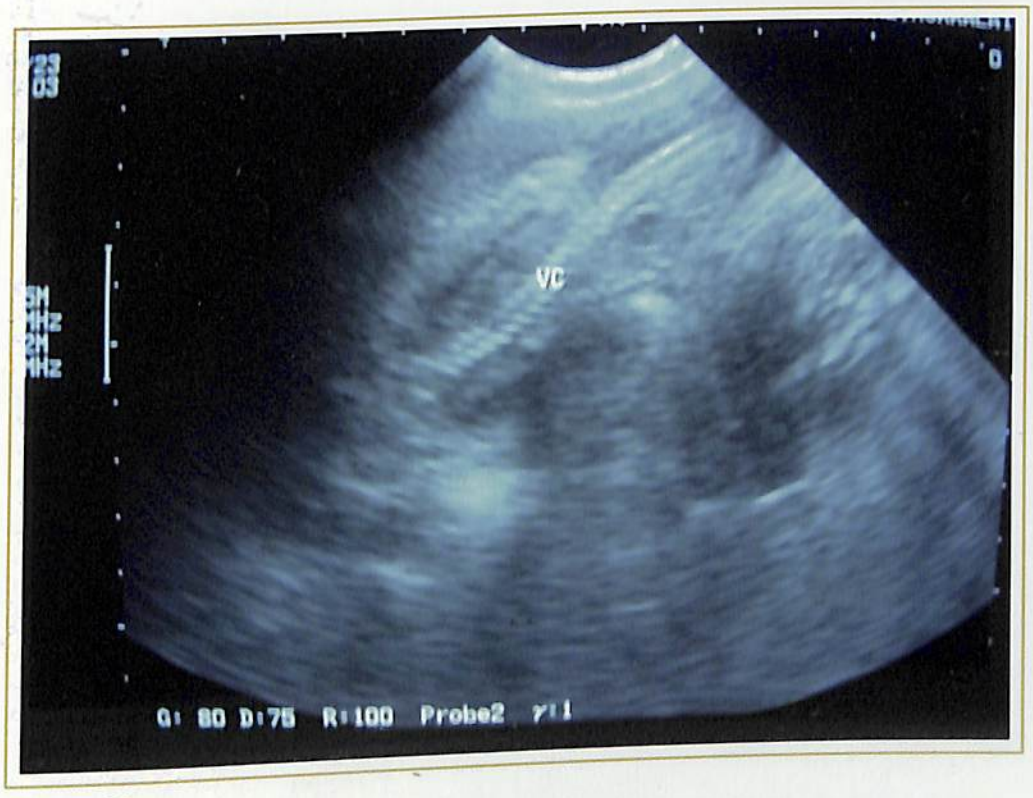


Plate: 28

Sonogram of uterus of a bitch at 50 days of gestation. Foetal vertebrae observed as hyperechoic areas arranged in segmented pattern

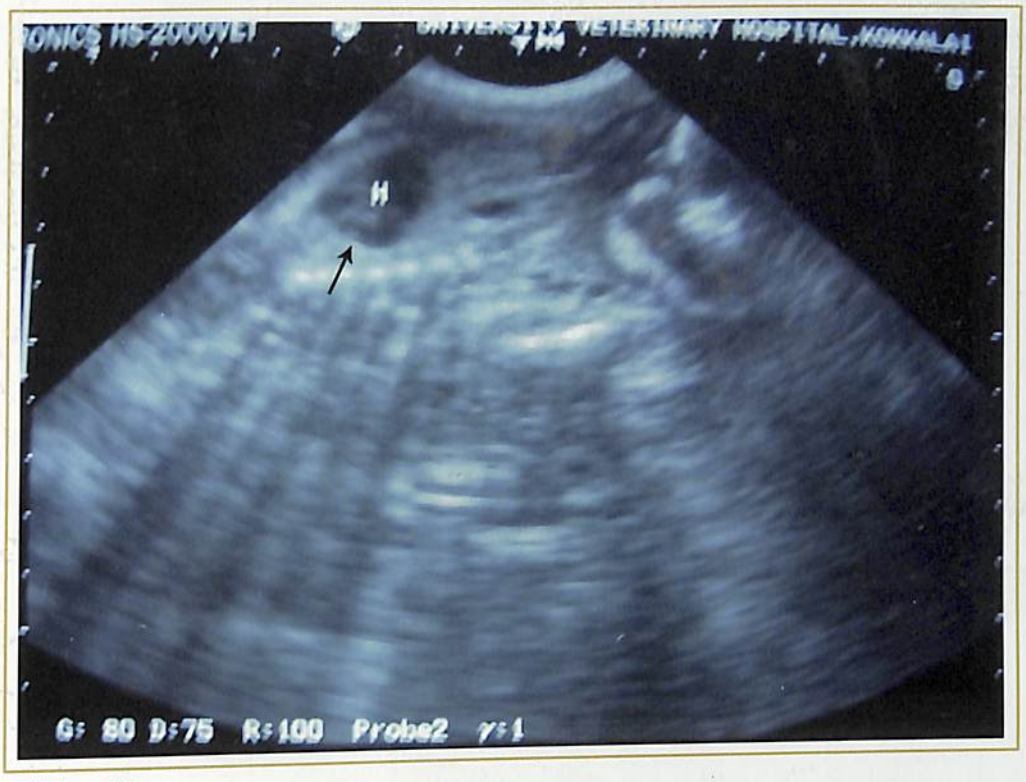


Plate: 29

Sonogram of uterus of a bitch at 55 days of gestation. Foetal heart could be visualized

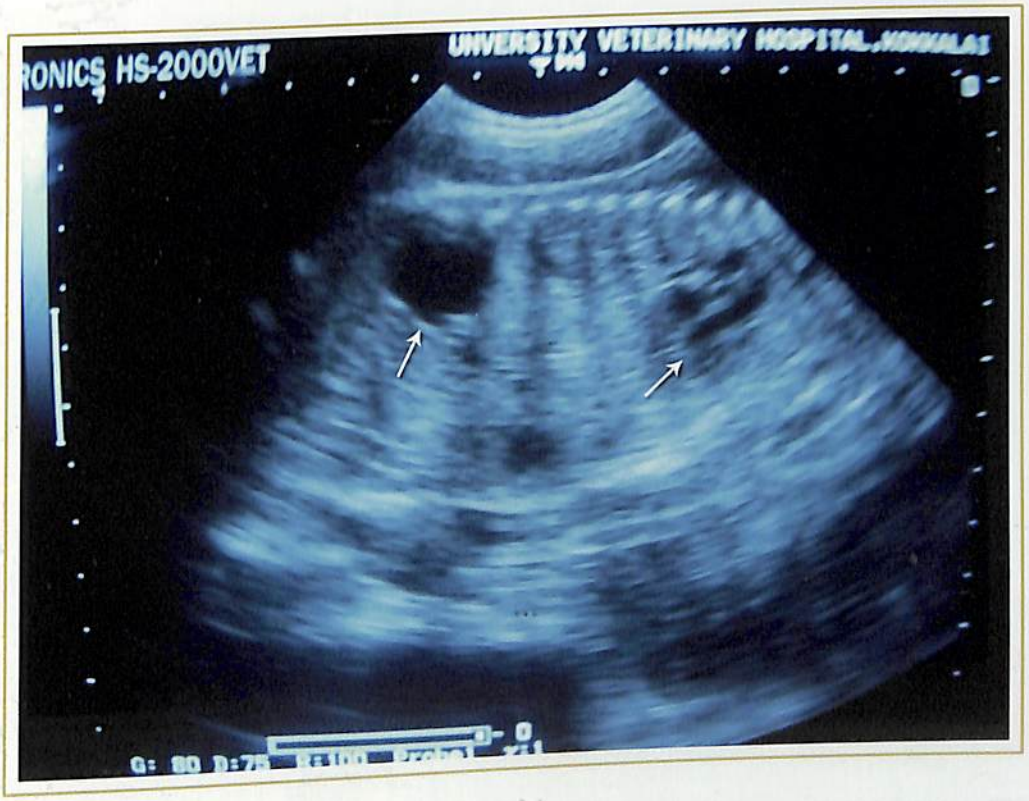


Plate: 30

Sonogram of uterus of a bitch at 64 days of gestation. Foetal heart, urinary bladder and ribs could be visualized

Plate: 31
A Labrador bitch with seven pups

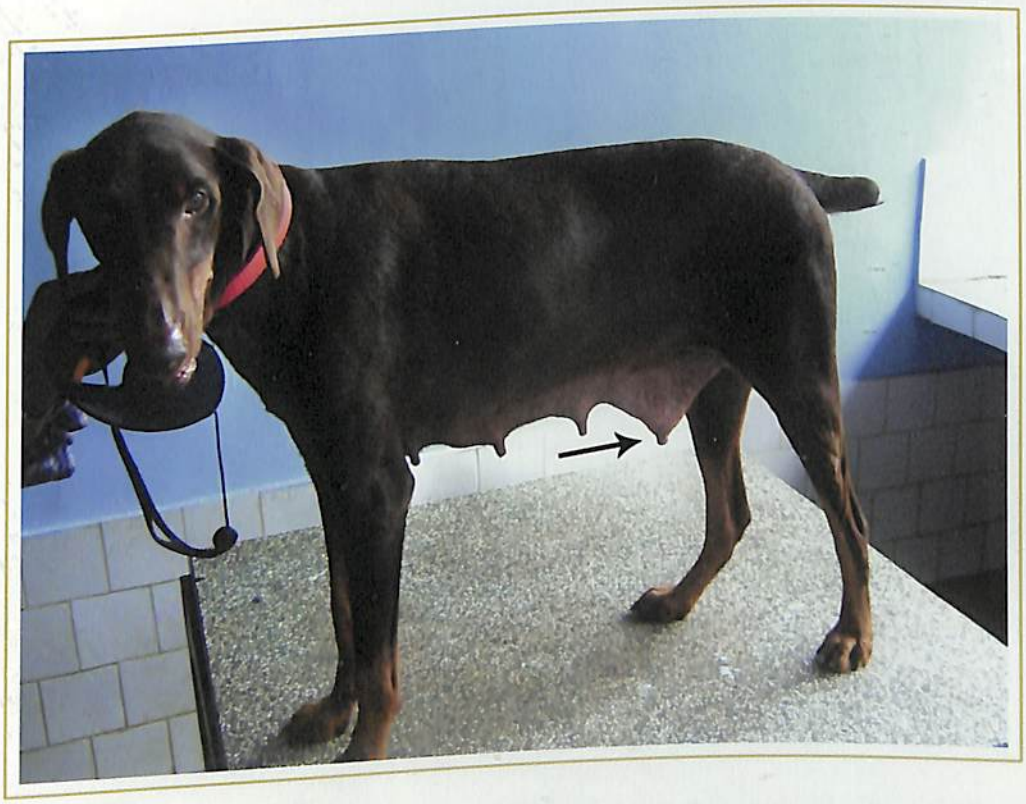


Plate: 32
Overt pseudopregnant bitch with obvious mammary gland enlargement

Plate: 33
Expression of milk in a female dog with overt pseudopregnancy

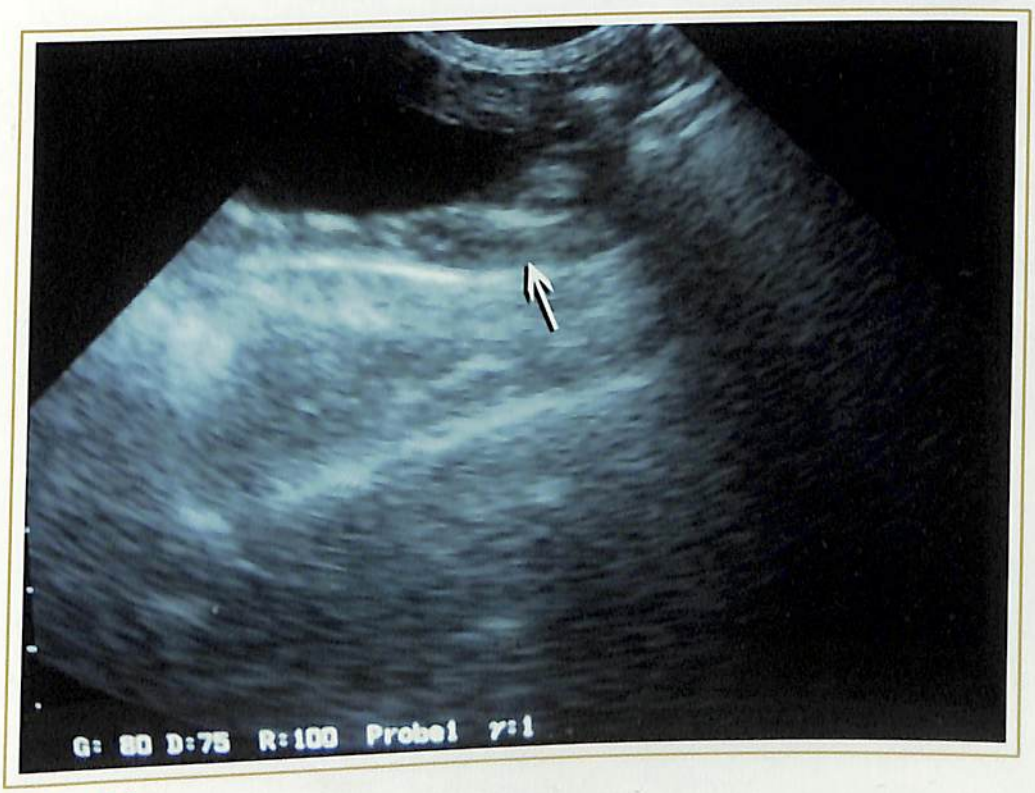


Plate: 34
Sonogram of uterus of bitch with pseudopregnancy. Slight uterine distension could be observed

5. DISCUSSION

5.1 EXPERIMENT I

Optimal breeding time was found based on clinico-gynaecological examination and vaginal exfoliative cytology in group I and by vaginoscopy in group II.

5.1.1 Optimal Breeding Time Based on Clinicogynaecological Examination and Vaginal Exfoliative Cytology

It was observed that the intensity of vulval oedema was high during proestrum and reduced as the animal reached oestrum phase. Similarly bleeding was high during proestrum which turned to a light coloured discharge during oestrum. Standing signs and flagging of tail was prominent during oestrum whereas the animals resisted digital manipulation of vulval lips during proestrum. The pH of vaginal discharge during oestrum was found to be '6' and fern pattern could not be obtained from majority of the samples. These findings are in agreement with Tsutsui (1989); England (2001) and Romagnoli (2006). But England and Lofstedt (2000) and Kutzler (2007) opined that vaginal cytology, vulvar compressibility, ferning and vaginal shrinkage were too loosely correlated with the endocrine profile.

Various type of cells encountered on vaginal cytology during various stages of oestrus cycle in the present study were in agreement with the earlier reports (Feldman and Nelson, 1996; Simon, 1997; Becha, 2000; Deepthi, 2007 and Ajitkumar, 2008). The background on the oestrual smears appeared abnormal during proestrus because of the presence of cellular debris and mucin threads and this observation was in accordance with the findings of Feldman and Nelson (1996). But during oestrus there was clearing of the background and was in agreement with the studies of Simon (1997). The anuclear cell index during the first day of proestrum, mid proestrum and recommended day of breeding were 25.6 $\pm$ 1.33, 79.9 $\pm$ 0.5 and 92.6 $\pm$ 1.29 respectively. According to Hewitt and

England (2000), the optimum time for mating was when the cornification index reached above 80 per cent. In the present study the mean cornification on the day of breeding was 92.6 per cent. There was statistical difference in the anuclear cell index during different stages of oestrus cycle. This supports the view that clinico-gynaecological examination and vaginal cytology could be used as a tool to predict optimal breeding time in bitches. These findings are in agreement with England (2001) and Johnston *et al*. (2001).

5.1.2. Optimal Breeding Time Based on Vaginoscopy

On vaginoscopic examination, the vaginal mucosa appeared pink in colour and congested without any shrinkage during proestrus. The serosanguineous uterine discharge appeared as red fluid in the furrows of the folds. This was considered to be due to the increased oestradiol levels during proestrum which cause retention of water in the vaginal mucosa giving the appearance of 'balloons'. The proestral changes observed in the present study were in agreement with the observations of Lindsay (1983); Lindsay and Concannon (1986) and Sridevi (2001).

The features during oestrus were characterized by two types of shrinkages. In the first shrinkage period, mucosal shrinkage without angulation was observed and the vaginoscopic picture remained rounded. In the second shrinkage with angulation, mucosal fold profiles became sharp edged. These findings revealed that early oestrus was characterized by the onset of shrinkage without angulation in the vaginal mucosa which progressed to a more obvious shrinkage with angulation corresponding to the fertile period in bitches. Similar observations were made by Lindsay (1983); Lindsay and Concannon (1986) and Sridevi (2001). The wrinkles or crenulations correlate to the decreasing levels of oestradiol during oestrum.

During early dioestrus the mucosal folds in the anterior vagina appeared irritable and when provoked they closed rapidly forming a rosette like pattern (Jeffcoate, 1998). Similar observations were recorded in the present study also.

In the present study the observation during anoestrum was the diffuse pink coloured vaginal mucous membrane without angulation. This observation is in accordance with the findings of Hewitt and England, (2000), England (2001) and Ajitkumar (2008).

According to Hewitt and England (2000) the level of progesterone was found to be 10 ng/ml on the day of maximum cornification of vaginal mucosal folds while in the present study it was found to be 6.58 $\pm$ 0.31ng/ml. The present study is in agreement with the findings of Romagnoli (2006) who opined that breeding should always be timed using serum progesterone assay and that the bitch should be bred when the serum progesterone exceeds 5 ng/ml. At the time of ovulation or 2 days later the serum progesterone concentration was in the range of 4 to 10 ng/ml (Feldman and Nelson, 1996). The conception rate was more in group II where the animals were bred based on vaginoscopic findings. This finding is in agreement with Badinand *et al.* (1993).

5.1.3 Comparison of Conception Rate in Animals Bred Based on Vaginal Exfoliative Cytology and Clinicogynaecological Examination and Vaginoscopy

The conception rate was 73.3 per cent in group I and 88.0 per cent in group II. This clearly suggests that performing vaginoscopy to find optimal breeding time could further improve fertility rate in female dogs compared to breeding based on clinico-gynaecological examination and vaginal exfoliative cytology. Similar observations were reported by Jeffcoate and Lindsay (1989), Feldman and Nelson (1996) and Hewitt and England (2000).

5.2 EXPERIMENT II

5.2.1 At 20 Days of Gestation (n=20)

Confirming pregnancy by abdominal palpation was found to be difficult at 20 days of gestation. Pregnancy diagnosis at this stage by transabdominal palpation was very difficult as distinct uterine swellings could not be distinguished clearly from the abdominal viscera in bitches. Further palpation was hindered by tenseness of abdominal muscles and excessive fat over the abdominal region. Similar reports were given by Sokolowski (1980); Arthur *et al.* (1996); Thou (1999) and Asha (2005). They reported that pregnancy diagnosis was too difficult in obese and nervous bitches. In the present study, the percentage accuracy obtained at 20 days of gestation was only 20 percent. More negative results were recorded at this stage because of non-appreciable increase in the size of the uterus. This finding agrees with the findings of Cartee and Rowles (1984); Shille and Gontarek (1985); Thou (1999); Asha (2005); Deepthi (2007) and Ajitkumar (2008).

By ultra sound scanning, embryonic vesicle could be observed as anechoic sac in three animals. The foetus as well as foetal structures remained undetectable at this stage. Accuracy at this stage was only 15 per cent. Hence ultrasound scanning could not be relied completely for too early pregnancy diagnosis. This finding is in agreement with Feldman and Nelson (1996); Kuztritz (2003) and Alacam *et al*. (2005).

By day 42 to 55, the foetus appeared longer and surrounded by anechoic foetal fluid. From 55 days to term, the foetal limbs, stomach, urinary bladder, liver, lung and skeleton could be visualized. These findings are in agreement with earlier observations made by Shille and Gontarek (1985); Yeager *et al*. (1992); Feldman and Nelson (1996); Barr (1998); England (1998) and Bhadwal and Mirakhur (2000).

5.2.2 At 35 Days of Gestation (n=20)

By abdominal palpation, foetus could be appreciated as tense distinct uterine swellings in fifteen animals. The percentage accuracy at 35 days was found to be 75 per cent. Asha (2005) reported more accurate results of pregnancy diagnosis by trans-abdominal palpation between 31 and 40 days post breeding. Similar findings were reported by Gradil *et al.* (2000); Purswell (2001) and Arunmozhi (2005). However Deka *et al*. (2004) reported a higher percentage of accuracy by abdominal palpation between 33 to 40 days of gestation.

In eighteen animals using ultrasound scanning, the presence of embryo could be visualized as an anechoic structure with hypoechoic wall by 35 days of gestation. Foetal heart beats as well as placenta could be observed during this stage. By 30 days foetal skeleton could be visualized and the uterus had an anechoic lumen with hyper echoic embryo. In two animals, gestational sac wall was irregular and pregnancy could not be confirmed. Deka *et al*. (2004) found percentage accuracy by ultrasound scanning between 33 to 40 days of gestation was 100 per cent. In the present study the percentage accuracy at 35 days was found to be 90 per cent. This is in agreement with the earlier reports of Taverne *et al.* (1985) where the percentage accuracy reported at 31 to 40 days of gestation was 92.9 per cent. In the present study, the percentage accuracy of ultrasound scanning by 35^{th} day of gestation was higher than the earlier report of Ajitkumar (2008).

5.2.3 At 50 Days of Gestation (n=20)

Trans-abdominal palpation was found difficult to perform in later stages of pregnancy. In twelve animals, a confluent feeling of the uterus could be felt. The percentage accuracy at 50 days of gestation was only 60 per cent. However the percentage accuracy obtained in the present study was lower than the observation given by Deka *et al*. (2004) where the percentage of accuracy at 41 days of gestation to term was 100 per cent. Gradil *et al.* (2000) reported that after

day 40 post breeding, the uterine swellings became more elongated, confluent more pliable and difficult to palpate as distinct entities. A diffuse distension of uterus could make the diagnosis unreliable.

By ultrasound scanning, the percentage of accuracy in pregnancy diagnosis at 50 days was found to be 100 per cent. In all pregnant animals, foetal heart beat could be observed by 24 days of gestation. By 55 days of gestation foetal heart, vertebral column and urinary bladder could be visualized. Vertebrae could be observed as hyper echoic areas arranged in a segmented pattern dorsal to liver and heart. They became denser and began to cast shadow as the mineralization proceeded. This is in agreement with the findings of Shille and Gontarek (1985); Allen *et al.* (1989); Yeager *et al*. (1992); Feldman and Nelson (1996); Barr (1998); England (1998) and Bhadwal and Mirakhur (2000).

From the present study, it could be inferred that ultrasound scanning was more reliable and could assess foetal viability by 25 days of gestation. Trans-abdominal palpation could not be employed for early pregnancy diagnosis.

5.2.4 Pregnancy Diagnosis by Estimation of Serum Alkaline Phosphatase (ALP)

The level of serum alkaline phosphatase at 20, 35 and 50 days of gestation was found to be 67.90 $\pm$ 2.98, 91.85 $\pm$ 2.10 and 139.65 $\pm$ 6.84 U/L respectively. Statistical analysis revealed significant variation between day 20 and day 50 of gestation ($P<0.05$). The rise in the serum alkaline phosphatase level is due to the placental involvement in the alkaline phosphatase production. Laker (1996) reported high concentration of alkaline phosphatase in liver, bone, intestine, placenta and kidney and suggested physiologically increased ALP levels during the periods of active bone growth and pregnancy. Mohan (2005) recorded elevated ALP levels in diseases of bone, liver and in pregnancy and concluded that high serum ALP activity could be used as marker of hepatobiliary diseases in the absence of bone diseases and pregnancy in human beings. Thus level of

serum alkaline phosphates could be used to assess the health status in pregnant dogs.

5.2.5 Pregnancy Diagnosis by Estimation of Serum Haptoglobin (Hp)

The level of serum haptoglobin at 20th day of gestation was 53.10 $\pm$ 3.22 mg/dl where as the level elevated to 81.12 $\pm$ 3.40 and 119.44 $\pm$ 3.16 mg/dl by 35 days and 50 days respectively. Statistical analysis revealed significant variation between day 20 and day 50 of gestation ($P<0.05$). Vannucchi *et al*. (2002) recorded the serum haptoglobin level by 21 days of gestation as 112.42mg/dl and by 30 days of gestation as 123.53 $\pm$ 56.39 mg/dl whereas 88.79$\pm$23.03 mg/dl in nonpregnant ones. The cause of haptoglobin rise during the third week of gestation could be related to embryonic nidation, placentary formation and pregnancy development. The rise of haptoglobin in the pregnant animals in the present study is in accordance with the reports of Onclin and Verstegen (2008) and Ulutas *et al*. (2009). Hence estimating the serum haptoglobin could be useful to assess the pregnancy status in female dogs.

5.2.6 Pregnancy Diagnosis by Estimation of Serum Globulin

The level of serum globulin in pregnant animals at 20, 35 and 50 days of gestation was 2.43 $\pm$ 0.12, 3.05 $\pm$ 0.11 and 3.74 $\pm$ 0.15 g/dl respectively. Statistical analysis revealed significant variation between day 20 and day 50 of gestation ($P<0.05$). Similar reports were given by Thou (1999) and Asha (2005). Serum globulin acts as a carrier protein for metals, lipids, carbohydrates and hormones. Vannucchi *et al*. (2002) recorded that in pregnancy the presence of foetus stimulated the mother's 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.

5.3 HAEMATOLOGICAL STUDIES

5.3.1 Haemogram

The total erythrocyte counts at 20, 35 and 50 days of gestation were 6.07 ± 0.16, 5.58 ± 0.15 and 5.12 ± 0.16 million/cmm respectively. There was significant reduction in the TEC as the pregnancy advanced ($P<0.05$). This finding is in agreement with the earlier reports of Gentry and Liptrap (1977) where the total erythrocyte count became gradually reduced from a mean of 8.85 million to 4.53 million per cmm during gestation. Similar reports were recorded by and Benjamin (1985) and the reduction could be probably due to increased plasma volume.

Haemoglobin (Hb) concentration at day 20, 35 and 50 days of gestation were found to be 11.86 ± 0.28, 10.45 ± 0.27 and 9.17 ± 0.30 g/dl respectively. The level of haemoglobin was found to be lowering as pregnancy advanced ($P< 0.05$). Similar observations were recorded by Thou (1999); Asha (2005) and Deepthi (2007). The lower values obtained at different stages of gestation were due to haemodilution and increased plasma volume (Sastry, 1989).

Packed cell volume (PCV) values were 40.45 ± 0.83, 37.30 ± 0.87and 33.40± 1.10 per cent at 20, 35 and 50 days of gestation. There was significant reduction in the PCV values during different stages of gestation ($P<0.05$). This finding was in agreement with England (1998) who reported that packed cell volume was 40 per cent at day 35 of gestation and even less than 35 per cent at term. Thou (1999) also found a decrease in packed cell volume as pregnancy progressed and found the mean values on day 21 to 25, 30 to 35 and 45 to 50 in pregnant dogs as 46.33±0.85, 43.33±0.58 and 38.67±0.53 per cent respectively. The decrease in the packed cell volume values could be related to haemodilution.

Erythrocyte sedimentation rate (ESR) at 20, 35 and 50th day of gestation were found to be 10.31 ± 0.73, 15.41 ± 0.54 and 21.85 ± 1.04 mm/hr respectively. ESR values were found to increase as pregnancy advanced.

Statistical analysis revealed significant variation between the values at different gestational age ($P<0.05$). The elevated levels of fibrinogen during pregnancy had resulted in rapid increase in ESR (Henry, 1996). Thou (1999) found ESR in pregnant bitches ranged between 8.5 to 19.33 mm/hr and varied significantly compared to non-pregnant animals whereas Deepthi (2007) recorded mean ESR on day 0, 16 to 20, 21 to 24 and 25 to 30 days of gestation as 4.6 $\pm$ 0.33, 14.3 $\pm$ 1.09, 17.8 $\pm$ 1.28 and 21.76 $\pm$ 1.47 mm/hr respectively.

5.3.2 Leucogram

The total leucocyte count (TLC) at 20, 35 and 50th day of gestation were found to be 13844.90 $\pm$ 539.90, 15449.00 $\pm$ 569.86 and 17502.50 $\pm$ 780.21 cells/cmm respectively. Statistical analysis revealed significant variation between day 20 and day 50 ($P<0.05$). The result is in agreement with the findings of Thou (1999) and Asha (2005).

Gentry and Liptrap (1977) found that during gestation the total leucocyte count increased from 12,000 to 19,000 per cmm while Feldman and Nelson (1996) observed mild leucocytosis by 30 to 40 days of gestation and the value ranged between 17,000 to 26,000 cells cmm. The increase in the leucocyte count was due to the non-specific inflammatory reactions following implantation.

The neutrophil count in pregnant dogs at 20, 35 and 50th day of gestation were 67.30 $\pm$ 1.11, 70.30 $\pm$ 4.95 and 75.35 $\pm$ 1.27 per cent respectively. The lymphocyte count was found to be 27.80 $\pm$ 0.87, 31.55 $\pm$ 0.88 and 36.95 $\pm$ 1.03 per cent at 20, 35 and 50th day of gestation. Statistical analysis revealed significant variation between day 20 and day 50 in the neutrophil as well as lymphocyte count ($P<0.05$). These findings are in agreement with the earlier reports of Thou (1999) and Asha (2005). The increase in the neutrophil and lymphocyte counts could be related to the immune response produced in the bitch by the foetus.

Monocyte count at different gestational age did not vary significantly and the values at 20, 35 and 50th day of gestation were 3.90 ± 0.27, 3.80 ± 0.20 and 4.05 ± 0.22 per cent respectively. Eosinophil count varied significantly ($P<0.05$) between day 20 and day 50 and the values were recorded as 1.60 ± 0.11 and 3.30 ± 0.13 per cent respectively. Similar reports were given by Thou (1999) and Asha (2005).

5.4 BODY WEIGHT

Body weight of animals on 20th, 35th and 50th day of gestation was 21.83±1.98, 24.6±1.93 and 36.69 ± 1.45 Kg respectively. It was found that the body weight varied significantly between day 20 and day 50 of gestation ($P<0.05$). The present study revealed a slow and steady increase in the body weight of animals as the pregnancy progressed. Similar findings were given by Thou (1999), Arthur *et al.* (2001) and Deepthi (2007).

5.5 FOETAL VIABILITY

By ultrasound scanning, no foetus or foetal membranes could be visualized by day 20 after breeding. By day 25 to 27, echogenic foetal mass and heart beat became detectable. The earliest result at which heart beat could be visualized was at 24 days of gestation. By day 28 to 33, head and body of the foetus were similar in size and appearance with flickering heart beats. Foetal membranes and zonary placenta were prominent. Anatomical features of the foetus became more obvious by about 34 to 39 days of gestation. Shape and size of the foetal head became distinguishable from the body. By day 42 to 55, the foetus appeared longer surrounded by anechoic foetal fluid. From 55 days to term, the foetal limbs, stomach, urinary bladder, liver, lung and skeleton could be visualized. These findings are in agreement with earlier observations of Shille and Gontarek (1985); Yeager *et al*. (1992); Feldman and Nelson (1996); Barr (1998), England (1998) and Bhadwal and Mirakhur (2000).

5.6 GESTATION LENGTH

In the present study the gestation length ranged between 58 to 69 days with an average of 63.30 ± 3.76days. Similar findings were reported by Sokolowski (1980), Concannon (2000), Asha (2005), Deepthi (2007) and Ajitkunar (2008). According to them the gestation length in bitches varied from 58 to 65 days. Various methods are in use to calculate gestation length.

According to Yeager and Concannon (1990) the apparent variation in the length of gestation among bitches depends on the event used as the reference point. Counting from the first breeding date, a normal gestation can range from 57 to 72 days. This variation may be influenced by the time between mating and ovulation, the longevity of dog sperm (up to 6 to 7 days), oocyte maturation time (2 to 3 days after ovulation) and the actual time at which the tubo-uterine junction opens (approximately on day 10 after the LH surge) and allows the passage of blastocyst to the uterus. Feldman and Nelson (1996) opined that variation in the timing of ovulation, multiple breeding dates and the inconsistent length of oestrous make it difficult to identify the day of fertilization or the exact due date of a litter.

5.7 LITTER SIZE

Litter size varied between three to eleven pups with a mean of 6.6 ± 2.08 pups per bitch. Concannon (1986) recorded a mean litter size of 4 to 8 pups per bitch while Asha (2005) recorded the mean litter size as 4 to 11 with an average of 5.8 pups per bitch.

5.8 EXPERIMENT- III

The etiology behind pseudopregnancy according to Concannon (2002) was that in bitches that experience pseudopregnancy, the corpus luteum did not regress despite the fact that gestation has not taken place. As a result, increased progesterone levels are maintained bringing about the signs consistent with

pregnancy. Around day 60, progesterone levels will abruptly drop as observed at the end of gestation. This drop in progesterone resulted in the elevation of the hormone prolactin, which was responsible for nesting-behaviour and lactation in bitches. Kooistra and Okken (2002) found that there was strong association between increase of prolactin release and a decrease of plasma progesterone concentration in overtly pseudopregnant bitches. Elevated prolactin secretions during progression of the luteal phase in the bitch was responsible for mammogenesis and declining plasma progesterone concentration during the second part of the luteal phase appear to influence prolactin secretion.

In Group I all the animals showed reduction in the size of the mammary gland with decrease in the amount of fluid from the teats by about seven days of treatment with cabergoline. The serum prolactin level was found to be reduced and maintained a low level even after fourteen days. In Group II, the treatment was done with antiprolactin drug bromocriptine and fifty per cent of animals exhibited vomiting as side effect. The serum prolactin level was found to be reduced and maintained at a low level even after completion of treatment and all the dogs were symptomatically cured from pseudopregnancy by about seven days and the animals regained normal feeding habits by about fourteen days. The above findings are in agreement with the earlier reports of Okkens *et al*. (1997); Zoldag *et al.* (2000); Ettinger (2002); Corrada *et al.* (2003); Rota *et al.* (2003); Gobello *et al*. (2004); Gunay *et al.* (2004); Cirit *et al*. (2006) and Tsutsui *et al*. (2007).

The level of serum progesterone prior to treatment, post-treatment at 7^{th} and 14^{th} day were 1.11 ± 0.14, 0.85 ± 0.06 and 0.62 ± 0.06 ng/ml respectively in the cabergoline treated group while in bromocriptine treated group the values were 1.11 ± 0.14, 0.5 ± 0.01 and 0.5 ± 0.01 ng/ml respectively. Statistical analysis showed significant difference between the serum progesterone values prior to treatment and on 14^{th} day after treatment ($P<0.05$). According to Harvey *et al*. (1997) the progesterone level recorded before treatment was found to be less than 1ng/ml which indicated that the bitches were no longer in the metoestral

phase. The present study is in agreement with the findings of Ettinger (2002) and Tsutsui *et al*. (2006) where the etiology behind pseudopregnancy in bitches was mainly attributed to decrease in serum progesterone concentration and increase in serum prolactin concentration. The level of progesterone in control dogs was 1.53 ± 0.48, 0.7 ± 0.11 and 0.44 ± 0.03 ng/ml respectively and no statistical significance was observed.

The concentration of prolactin in the cabergoline treated group prior to treatment and post-treatment at 7th and 14th day were 6.17 ± 2.05, 1.69 ± 0.20 and 0.82 ± 0.13 ng/ml respectively whereas in the bromocriptine treated group the values were 6.02 ± 1.17, 0.90 ± 0.07 and 0.58 ± 0.08 ng/ml respectively. Statistical analysis showed significant variation ($P<0.05$) in the serum prolactin level prior to treatment and post-treatment at 7th and 14th day. According to Harvey *et al*. (1997) the mean prolactin concentrations during pre-treatment, post-treatment and after completion of treatment were 6.41, 1.8 and 4.13 ng/ml respectively. The study recorded that cabergoline was effective in suppressing prolactin release from the pituitary and thus lowering the blood prolactin concentration when used at a dose rate of 5µg/kg BW for five days. The present study is in agreement with the findings of Onclin and Verstegen (1997) and Ettinger (2002) where the etiology behind pseudopregnancy in bitches was mainly attributed to decrease in serum progesterone concentration and increase in serum prolactin concentration. The level of prolactin in the control dogs at 1, 7 and 14th day was 1.16 ± 0.24, 1.02 ± 0.12 and 1.01 ± 0.07 ng/ml respectively. Statistical analysis revealed no significant variation between the serum prolactin value at day 1 and day 14. This low serum prolactin value is in agreement with Tsutsui *et al*. (2007) where the progesterone and prolactin values in control dogs were 2.7±0.4 and 2.9±0.6 ng/ml respectively.

The side effect observed in cabergoline group was only a reduction in food intake and no vomiting was reported. The absence of vomiting was clearly explained by Cirit *et al*. (2007). The study observed that cabergoline had a high specificity for D_2 receptor, long specific activity on pituitary lactotrophic cells

and fewer central nervous system effects than bromocriptine. No side effects were reported in the study when cabergoline was used. Gobello *et al*. (2004) studied about the dopaminergic agonists-bromocriptine and cabergoline and concluded that cabergoline had longer duration of action with fewer side effects and was more potent than bromocriptine. According to Gunay *et al.* (2004) no side effects were reported to cabergoline as the drug did not easily penetrate the blood brain barrier.

The side effects observed in bromocriptine group were severe vomiting and also reduction in food intake in fifty per cent of animals. These findings were similar to the reports of Janssens (1986) who observed that brompcriptine was effective for treating pseudopregnancy but, the side effect was emesis. He opined that giving bromocriptine at a low dose or an antiemetic drug if given could reduce the chance of emesis.

There was significant reduction in the serum prolactin value and the dogs were symptomatically cured after the treatment with antiprolactin drugs in both groups. This study is in accordance with the findings of Arbeiter and Barsch (1988) where cabergoline orally at a dose rate of 5µg/kg BW for seven days caused disappearance of symptomatic, functional and behavioural signs of pseudopregnancy in 95 per cent of bitches. This is in accordance with Zoldag *et al* (2001) where bromocriptine, a dopamine agonist, was found to be successful in suppressing lactation in pseudopregnant bitches. Gobello *et al*. (2004) studied about the dopaminergic agonists-bromocriptine and cabergoline and concluded that both the agents act directly upon dopamine pituitary receptors to modify the synthesis and release of prolactin by pituitary cells. They also found that cabergoline had longer duration of action with fewer side effects and was more potent than bromocriptine. Thus it could be concluded that antiprolactin drug cabergoline was found to be better than bromocriptine for treating pseudopregnancy at a dose rate of 5µg/kg BW orally for seven days with minimum side effects.

Summary

6. SUMMARY

The present study entitled “Diagnostic and therapeutic approaches for enhancing reproductive efficiency in female dogs” was undertaken with the objectives of finding optimal breeding time in bitches, early and accurate pregnancy diagnosis and treatment of overt pseudopregnancy using antiprolactin drugs.

Experiment I comprised of 55 apparently healthy bitches presented at Small Animal Obstetrics and Gynaecology unit of Veterinary College Hospital, Mannuthy and University Veterinary Hospital, Kokkalai for breeding advice. Out of this, 30 animals (Group I) were bred based on findings of clinico-gynaecological and vaginal exfoliative cytology studies. Twenty five animals (Group II) were bred based on vaginoscopic findings using a flexible fibre optic endoscope. Blood was collected from ten animals of Group II on day in which maximum crenulation of vaginal mucous folds was observed for the estimation of serum progesterone.

The conception rate was 73.3 per cent in Group I and 88.0 per cent in Group II. Conception rate was more in those animals, which were bred, based on vaginoscopy than those bred based upon clinico-gynaecological examination and vaginal exfoliative cytology. This supported the view that performing vaginoscopy will augment conception rate in bitches. Similarly estimating the progesterone value helped to predict the more accurate breeding time and a higher conception rate was obtained in Group II.

It was observed that clinico-gynaecological findings alone should not be relied for finding optimal breeding time in bitches. The use of vaginal exfoliative cytology along with clinico-gynaecological findings served as a better tool to monitor the progress of different stages of oestrous cycle and helped in the retrospective calculation of fertile period.

The advantage with vaginoscopy was that it could be employed in a non-sedated standing bitch which was the most effective non-invasive method for finding optimal breeding time. Vaginoscopy could predict the fertilization period in female dogs more accurately as visualization of vaginal mucosal folds was obvious and maximum angulations of vaginal mucosa were reliable indicators of fertilization period.

In experiment II, pregnancy diagnosis was carried out in 20 animals at 20^{th}, 35^{th} and 50^{th} day of gestation. It was found that transabdominal palpation was difficult to diagnose pregnancy by 20 days of gestation and the accuracy at this stage was only 20 per cent. When transabdominal palpation was done by 35 days of gestation, uterine swellings could be felt and the accuracy obtained was 75 per cent. On contrary, the accuracy on transabdominal palpation decreased to 60 per cent as the pregnancy progressed to 50 days due to difficulty in palpating tensed abdomen.

By ultrasound scanning at 20 days of gestation, embryonic vesicle could be visualized in 15 per cent of the cases. No foetus or foetal membranes could be visualized at this stage. The percentage accuracy at this period was 90 per cent at 35 days of gestation. By day 50 of gestation, the percentage accuracy was 100 per cent and foetal viability could be monitored by counting the heart beat of the foetus.

By day 25 to 27, echogenic foetal mass and heart beat became detectable. By day 28 to 33, head and body of the foetus were similar in size and appearance with flickering heart beats. Foetal membranes and zonary placenta were prominent during this period. Anatomical features of the foetus became more obvious by about 34 to 39 days of gestation. Shape and size of the foetal head became distinguishable from the body. Thus it could be inferred that ultrasonography was the most modern diagnostic technique for confirming pregnancy from 25 days of gestation and it could be used for assessing the safety of pregnancy.

The level of serum alkaline phosphatase at 20, 35 and 50 days of gestation was 67.90 $\pm$ 2.98, 91.85 $\pm$ 2.10 and 139.65 $\pm$ 6.84 U/L respectively. The level of haptoglobin at 20th day of gestation was 53.10 $\pm$ 3.22 mg/dl where as the level elevated to 81.12 $\pm$ 3.40 and 119.44 $\pm$ 3.16 mg/dl by 35 and 50 days of gestation respectively. The level of serum globulin in these animals at 20, 35 and 50 days of gestation were found to be 2.43 $\pm$ 0.12, 3.05 $\pm$ 0.11 and 3.74 $\pm$ 0.15 g/dl respectively. Statistical analysis revealed significant difference between day 20 and day 50 ($P<0.05$). However, the level of haptoglobin value above 112.42mg/dl could confirm pregnancy in dogs by about 21 days. The level of serum alkaline phosphatase and serum globulin could be used to monitor the health status in pregnant dogs.

Haemogram studies suggests that there was significant reduction in the total erythrocyte count, haemoglobin and packed cell volume at different gestational age ($P<0.05$). These changes were attributed by haemodilution and increased plasma volume. But erythrocyte sedimentation rate has shown an increase which could be attributed to the endometrial implantation by the embryo.

Leucogram study showed that there was statistically significant increase in the total leucocyte count, neutrophil and lymphocyte count as the pregnancy progressed. This was due to non-specific inflammatory response produced by the early embryo during endometrial implantation. No significant variation could be observed in monocyte and eosinophil count.

There was significant variation in body weight of animals as the pregnancy progressed. Body weight of animals steadily increased from day of breeding to various stages of gestation ($P<0.05$).

Foetal viability was difficult to be monitored at 20 days of gestation. The earliest positive result at which heart beat could be visualized was at 24 days of gestation.

The gestation length in the present study ranged between 58 to 69 days with an average of 63.30 ± 3.76 days. Litter size varied between three to eleven pups with a mean of 6.6 ± 2.08 pups per bitch.

Experiment III comprised of twenty animals, ten each in Group I and II showing prominent signs of pseudopregnancy were treated with antiprolactin drug cabergoline and bromocriptine, respectively. Group III comprised of ten apparently healthy bitches with the history of breeding 70 days prior and which failed to conceive with no signs of overt pseudopregnancy formed the control group.

In both the groups, there were significant reductions in the serum prolactin value after the treatment. Side effects observed were reduction in food intake and severe vomiting in bromocriptine treated group but no vomiting was reported in cabergoline treated ones. The serum prolactin level was found to be reduced and maintained a low level even after fourteen days. All the dogs were symptomatically cured from pseudopregnancy by about seven days and the animals regained normal feeding habits by fourteen days. There was no significant variation in the serum progesterone and prolactin value in untreated control animals.

Thus from the present study it could be concluded that,

- Clinico-gynaecological findings along with vaginal exfoliative cytology could be used to monitor the progress of different stages of oestrous cycle which helps to find optimal breeding time in bitches under field conditions.
- Vaginoscopy in addition to being a non-invasive method, had the added advantage of predicting the fertilization period thereby breeding advice could be more accurate as it helps in the visual inspection of vaginal mucosal folds. In the study higher

conception rate was obtained when vaginoscopy was used for finding optimal breeding time.

- Ultrasonography was found to be the most modern diagnostic technique for confirming pregnancy from 25 days of gestation. It has got the added advantage of assessing the safety of pregnancy, monitoring the foetal growth and viability. In addition, it could be employed for differentiating pregnancy from other pathological conditions like pseudopregnancy.
- The level of serum haptoglobin, serum alkaline phosphatase and serum globulin were found to be slowly elevating throughout the gestation period and this could be used to monitor the health status in pregnant dogs.
- There was significant variation in the body weight of animals as the pregnancy progressed.
- The earliest positive result at which heart beat could be visualized was at 24 days of gestation.
- Antiprolactin drug cabergoline was found to be better than bromocriptine for treating pseudopregnancy at a dose rate of 5µg/kg BW orally for seven days with minimum side effects.

Reference

REFERENCES

Abate, O., Zanatta, R., Malisano,T and Dotta, U. 2000. Canine serum protein patterns using high resolution electrophoresis (HRE). *J. Reprod. Fert. Suppl.* 159: 154-160

AHD [Animal Husbandry Department]. 2004. Animal Husbandry Statistics at a Glance. Report of Quinquennial Livestock Census - 2003. Statistical Unit, Animal Husbandry Department, Thiruvananthapuram. pp. 1&2

Ajitkumar, G. 2008. Comparative efficacy of bromocriptine, cabergoline and thyroxine in inducing oestrus in bitches. Ph.D Thesis. Kerala Agricultural University, Thrissur, p.146.

Alacam, E., Oral, H. and Macun, C. 2005. Evaluation of blood relaxin levels in bitches and queens for the diagnosis of pregnancy, false pregnancy and foetal deaths. *Indian Vet. J.,* March, 82: 269-271

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

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. 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., 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

Anderton, D.J. and Evans, J.M. 1993. In practice experiences with the semiquantitative measurement of progesterone in bitch plasma to determine the optimum time to mate. *J. Reprod. Fert. Suppl.* 47: 547.

Arbeiter, K and Barsch, E. 1988. Possibilities of oestrus induction in the bitch with an ergoline derivative. *J. Vet. Med.* 35: 111-117

Arbeiter, K and Jochle, W. 1989. Effects on pseudopregnancy, pregnancy and interoestrus intervals of pharmacological suppression of prolactin secretion in female dogs and cats. *J. Reprod. Fert. Suppl.* 39: 199-207

Arosh, J.A., 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(5): 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.

Arthur, G.H., Noakes, D.E., Pearson, H. and Parkinson, T.J. 2001. *Veterinary Reproduction and Obstetrics.* Eighth edition. W.B. Saunders Company, London. p.693.

Arunmozhi, N. , Venugopal, N.K., Sreenu Makkena, Rao, A.S. 2005. Evaluation of Abdominal palpation, radiography and ultrasonography for pregnancy diagnosis in bitches. *Indian Vet. J*. 82: 1090-1092

Asha, K.M. 2005. Comparison of different methods of pregnancy diagnosis in bitches. M. V. Sc. Thesis, Kerala Agricultural University, Mannuthy, Thrissur, p.108.

Badinand, F., Fontbonne, A., Maurel, M.C. and Siliart, B. 1993. Fertilization time in the bitch in relation to plasma concentration of oestradiol, progesterone and luteinizing hormone and vaginal smears. *J. Reprod. Fert. Suppl*. 47: 63-67.

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, Thrissur, p.151

Beijerink, N.J., Dieleman, S.J., Kooistra, H.S. and Okkens, A.C. 2003. Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. *Theriogenology.* 60: 379-386

Beijerink, N.J., Kooistra, H.S., Dieleman, S.J. and Okkens, A.C. 2004. Serotonin antagonist-induced lowering of prolactin secretion does not affect the pattern of pulsatile secretion of follicle stimulating hormone and luteinizing hormone in the bitch. *Reproduction.* 128:181-188

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

Benjamin, M.M. 2001. *Outline of Veterinary Clinical Pathology.* Third edition. Kalyani Publishers, New Delhi, p.351.

Berkova, Nadia., Lemay, Andre and Fontaine, Jean. 2001. Haptoglobin is present in human endometrium and shows elevated levels in the deciduas during pregnancy. *Mol. Human. Reprod.* 8: 747-754

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

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

Bouchard, G.F., Solorzano, N. Concannon, P.W., Youngquist, R.S. and Bierschwal, C.J. 1991a. Determination of ovulation time in bitches based on teasing, vaginal, cytology, and ELISA for progesterone. *Theriogenology* 35: 603-611.

Bouchard, G.F., Youngquist, R.S., Clark, B., Concannon, P.W. and Braun, W.F. 1991b. Estrus induction in the bitch being a combination diethylstilbesterol and FSH-P. *Theriogenology* 36: 51

Boyko, R.A. 2009. Complex population structure in African village dogs and its implications for inferring dog domestication history. *Proceedings of the National Academy of Sciences (Early Edition)* (in press).

Brukes, T.J. 1986. Canine physiology of reproduction. In Small Animal Reproduction and Infertility. Lea and Febiger, Philadelphia, pp.23-77

Cain, J.L. 1995. The use and misuse of reproductive hormones in canine reproduction. In: *Kirk's Current Veterinary Therapy XII: Small Animal Practice* (eds. Bonagura, J.D. and Kirk, R.W.), W.B. Saunders Company, Philadelphia, pp. 1069-1075

Cain, J.L. 2000. CVT update: Infertility in the bitch. In Current Veterinary Therapy, XIII, Ed. R. Kirk, W.B. Saunders Co., Philadelphia.

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

Chakraborty, P.K. 1987. Reproductive hormone concentrations during estrus, pregnancy and pseudopregnancy in the labrador bitch. *Theriogenology* 27: 827-840.

Cirit, U., Bacinoglu, S., Cangul, I.T., Kaya, H.H., Tas, M. and Ak. K. 2007. The effects of a low dose of cabergoline on induction of oestrus and pregnancy rates in anoestrous bitches. *Anim. Reprod. Sci.*. 101: 134-144

Concannon, P.W. 1980. Effects of hypophysectomy and of LH administration on luteal phase plasma progesterone levels in the beagle bitch. *J. Reprod. Fert*. 58: 407-410.

Concannon, P.W. 1983. Reproductive physiology and endocrine patterns of the bitch. In Current Veterinary Therapy, Vol. VIII, Ed. R.W. Kirk, W.B. Saunders Co., Philadelphia, pp. 886-900.

Concannon, P.W., McCann, J.P. and Temple, M. 1989. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. *J. Reprod. Fert. Suppl*. 39: 3-25.

Concannon, P.W., Hansel, W. and Mckentee, K. 1977. Changes in LH, progesterone and sexual behaviour associated with preovulatory luteinization in the bitch. *Biol. Reprod.* 17: 604-613.

Concannon, P.W., Stevenwhaley, B.S., Lein, D. and Richard Wissler, B.S. 1983. Canine gestation length: Variation related to time of mating and fertile life of sperm. *Amer. J. Vet. Res.* 44: 1819-1821.

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

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

Concannon, P.W. 2002. 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. 2006. Pregnancy management in dogs and cats. Eds. Recent advances in Small Animal reproduction, Publisher. International Veterinary Information Service (www.ivis.org) Ithaca New York A1220.0401

Corrada, Y. Arias., Rodrigez, R and Gobello, C.2006. Combination dopamine agonist and prostaglandin agonist treatment of cystic endometrial hyperplasia-pyometra complex in bitches. Theriogenology. 66:1557-1559

Deepthi, L. 2007. Detection of serum relaxin as a diagnostic tool for early pregnancy diagnosis in bitches. MVSc thesis. Kerala Agricultural University, Thrissur, 63p.

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

Dietrich, E. and Moller, R. 1993. Use of progesterone enzyme immunoassay to determine the optimal mating time in oestrous bitches. *J. Reprod. Fert. Suppl*. 47: 524.

Divya, P.D. 2009. Comparative study and storage stability of hepatobiliary enzymes in ruminants and dogs of humid- tropics. MVSc thesis. Kerala Agricultural University, Thrissur, 102p.

Dore, M.A. 1978. The role of the vaginal smear in the detection of metoestrus and anoestrus in the bitch. *J. Small Anim.Prat*. 19: 561-572.

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. Fertility.* 47: 437-441

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 Lofstedt, V. 2000. Canine physiology of reproduction. A clinical approach to diagnosis and treatment. In Small Animal Reproduction and Fertility. 2nd Edn. Ed. J.J Brukes, Lea and Febiger, Philadelphia, pp. 23-77.

England, G.C.W. 2001. Reproductive physiology of the bitch. Paper present in WSAVA. Eds. Pregnancy diagnosis in the bitch. Publisher: International Veterinary Information Service, www.ivis.org. Ithaca, New York

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. 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.

Ettinger, S.J. and Feldman, E.C. 2002. *Textbook of Veterinary Internal Medicine*. Fifth edition. W.B. Saunders Company, Philadelphia, p. 1996

Feldman, E.C. and Nelson, R.W. 1996. Canine and Feline Endocrinology and Reproduction. Second Edition. W.B. Saunders Company, Philadelphia, p.554

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

Fontbonne, A., Siliart, B. and Dadinand, F. 2007. Hormonal findings in dogs and bitches showing reproductive disorders. *J. Reprod. Fertil. (Suppl.).* 47: 553-554

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

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

Gobello, C., Bolognani, F., Sota, R.D.L and Gaya, R. 2001. Twenty four hour profiles of serum prolactin and luteinising hormone in anoestrous crossbred bitches. *Reprod. Dom. Ani.* 36: 41-45

Gobello, C., Castex, G., Sota, R.D.L. and Corrada, Y. 2004. Shortening of interoestrous intervals with cabergoline in bitches: A clinical trial. *J. Am. Anim. Hosp. Assoc.* 40: 115-119

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

Gunay, A., Gunay,U and Soylee, M.K. 2004. Cabergoline applications in early and late anoestrus periods on german Shepherd dogs. *Revue Med. Vet.* 155: 557-560

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

Harvey, M.J.A., Cauvin, A., Dale, M., Lindley, S. and Ballabio, R. 1997. Effects and mechanism of the anti-prolactin drug cabergoline on pseudopregnancy in the bitch. *J. Small Anim. Pract.* 38: 336-339

Henry, J. B. 1996. Clinical Diagnosis and Management by Laboratory Methods. Nnineteenth edi. 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

Holst, P.A. and Phemister, R.D. 1974. Onset of diestrus in the Beagle bitch: Definition and significance. *Am. J. Vet. Res.* 35: 401-406.

Hoffmann, B., Riesenbeck, A., Schams, D. and Steinetz, B.G. 1999. Aspects on Hormonal control of normal and induced parturition in the dog. *Reprod. Dom. Anim.* 34: 219-226

Horadagoda, N.U., Gibbs, H.A., Reid, S.W.J and Eckersall, P.D. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet. Rec.* 144. 437-441

Janssens, LAA. 1986. Treatment of pseudopregnancy with bromocryptine, an ergot alkaloid. *Vet. Rec.* 119: 172-176

Jeffcoate, I.A. 1998. Physiology and endocrinology of the bitch. In manual of Small Animal Reproduction and Neonatology, Ed. Simpson, G.M., England, G.C.W. and Harvey, M. Cheizenham, BASVA Publications, pp. 1-9.

Jeffcoate, I.A. and England, G.C. 1997. Urinary LH, plasma LH and progesterone and their clinical correlates in the periovulatory period of domestic bitches. *J. Reprod. Fert. Suppl.* 51: 267-275.

Jeffcoate, I.A. and Lindsay, F. 1989.Ovulation detection and timing of insemination based on hormone concentrations, vaginal cytology and endoscopic appearance of the vagina in domestic bitches. *J. Reprod. Fert. Suppl.* 39: 277-287.

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

Johnston, S.D., Root Kustritz, M. and Olson, P.N.S. 2001. Canine and Feline Theriogenology. W.B. Saunders Company, Philadelphia, p. 1204

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

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

Kahn, C. M. 2005. The Merck Veterinary Manual. Ninth edition. Merck and Co., INC. White house station. N.J., USA, 2712p.

Kimura, T., Ohshima, S. and Doi, K. 1992. Haematological and serum biochemical values in hairless and haired descendants of Mexican hairless dogs. *Lab. Anim.* 26: 214-218

Kooistra, H.S. and Okkens, A.C. 2002. Secretion of growth hormone and prolactin during progression of the luteal phase in healthy dogs: a review. *Mol. Cell. Endocrinol.* 197: 167-172

Kooistra, H.S., Okkens, A.C., Bevers, M.M., Popp-Snijders, C., vanHaaften, B., Dieleman, S.J. and Schoemaker, J. 1999. Bromocriptine-induced premature oestrus is associated with changes in the pulsatile secretion pattern of follicle-stimulating hormone in Beagle bitches. *J. Reprod. Fertil.* 117: 387-393

*Kustritz, R. 2001. Updates on canine pregnancy diagnosis. Publisher: International Veterinary Information Service, www.ivis.org. Ithaca, New York

Kutzler, M.A., Mohammed, H.O., Lamb, S.V., Wallen, M.V. 2003. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. *Theriogenology* 60: 1187-1196.

Kutzler, M.A. 2007. Estrus induction and synchronization in canids and felids. *Theriogenology* 68: 354-374

Laker, M.F. 1996. *Clinical Biochemistry for Medical Students.* First edition. W.B. Saunders Company, London, 357p.

Lindsay, F.E.F. 1983. The normal endoscopic appearance of the caudal reproductive tract of the cyclic and non cyclic bitch: Post uterine endoscopy. *J. Small Anim. Pract.* 24: 1.

Lindsay, F.E.F. and Concannon, P.W. 1986. Normal canine vaginoscopy. In Small Animal Reproduction and Infertility. Ed. Burke, T., Lea and Febiger, Philadelphia, 112-120.

Lindsay, F.E.F. and Jeffcoate, I.A. 1993. Clinical methods of estimating the optimum period for natural and artificial insemination in the bitch. *J. Reprod. Fertil.* 47: 556-560.

Linde, C. and Karlsonn, I. 1984. The correlation between the cytology of the vaginal smear and the time of ovulation in the bitch. *J. Small Anim. Pract.* 25: 77-82.

Lulich, J.P., 2006. Endoscopic vaginoscopy in the dog. *Theriogenology.* 66: 588-591

Lumsden, J.H., Mullen, K. and McSherry, B.J. 1979. Canine haematology and biochemistry reference values. *Can.J.Comp.Med*.43: 125- 131

McDonald, 1989. *Veterinary Endocrinology and Reproduction*. Fourth Edition. K.M. Varghese Company, Bombay, pp. 466-472

Mohan, H. 2005. *Textbook of Pathology*. Fifth edition. Jaypee brothers Medical Publishers (p) Ltd., New Delhi, 977p

Murata, H., Shimada, N and Yoshioka, M. 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. *Theriogenology*. 168: 28-40

Noakes, D.E., Parkinson, T.J. and England. G.C.W. 2001. *Arthur's Veterinary Reproduction and Obstetrics*. Eighth edition. Harcourt (India) Private Ltd., New Delhi, 868p

Olson, P.N., Bowen, R.A., Behrendt, M.D., Olson, J.D. and Nett, T.M. 1982. Concentration of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. *Biol. Reprod*. 27: 1196-1206.

Okkens, A.C., Bevers, M., Dieliman, S. and Willemse, S. 1985. Shortening of the anoestrus interval and lifespan of the corpus luteum of the cyclic dog by bromocriptine treatment. *Vet. Quarterly* 7: 173-176.

Olson, P.N. 1989. Exfoliative cytology of the canine reproductive tract. Proceedings of the Annual Meeting of the Society of Theriogenology, 259-263.

Olson, P.N. and Husted, P.W. 1986. Breeding management for optimal reproductive efficiency in the bitch and the stud dog. In Current Therapy in Theriogenology. 2nd Edn. Ed. Morrow, D.A. W.B. Saunders Company, Philadelphia, pp. 463-468.

Olson, P.N., Nett, T.M., Bowen, R.A., Sawyer, H.R. and Niswender, G.D. 1989. Endocrine regulation of the corpus luteum of the bitch as a potential target for altering fertility. *J. Reprod. Fert. Suppl.* 39: 27-40.

Onclin, K. and Verstegen, J. 1997. *In vivo* investigation of luteal function in dogs: effects of cabergoline, a dopamine agonist, and prolactin on progesterone secretion during mid-pregnancy and diestrus. *Domest. Anim. Endocrinol.* 14: 25-38

Onclin, K. and Verstegen, J. 2008. Endocrinology of pregnancy in the dog: A review. *Theriogenology*. 70: 291-299

Patil, M.D. 2006. Induction of oestrus in bitches using dopamine agonist cabergoline. M.V.Sc thesis, Tamilnadu Veterinary and Animal Sciences University, Chennai. 80p

Phemister, R.D., Holst, P.A., Spano, J.S. and Hopwood, M.L. 1973. Time of ovulation in the Beagle bitch. *Biol. Reprod.* 8: 74-82.

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

*Romagnoli, S. 2006. Recent advances in canine female reproduction. Paper presented in wsava/lecture. 24, p. 6

Rota, A., Mollo, A., Marinelli, L., Gabai, G. and Vincenti, L. 2003. Evaluation of cabergoline and buserelin efficacy for oestrous induction in the bitch. *Reprod. Dom. Anim.* 38: 440-443

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

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

Sathiamoorthy, 2007. Fertility in bitches following intravaginal and intrauterine insemination with fresh and frozen semen. PhD thesis. Tamilnadu Veterinary and Animal Sciences University, Chennai. 107p

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

Schutte, A.P. 1967a. Canine vaginal cytology-I. Technique and cytological morphology. *J. Small Anim. Pract*. 8: 301-306

Schutte, A.P. 1967b. Canine vaginal cytology-II. Cyclic changes. *J. Small Anim. Pract*. 8: 307-311

Schutte, A.P. 1967c. Canine vaginal cytology-III. Compilation and evaluation of cellular indices. *J. Small Anim. Pract*. 8: 313-317

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

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

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

Soderberg, S.F. 1986. Canine breeding management. Vet. Clinics of North America, *Small Anim. Prac*. 16: 419-433.

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. 2001. Determination of ovulation and fertile breeding time to optimize conception in bitches. Ph.D. thesis. Tamil Nadu Veterinary and Animal Sciences University, Chennai, 164p

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

Steintez, B.G., Goldsmith, L.T., Harvey, H.J. and Lust, G. 1990. Serum relaxin and progesterone concentrations in pregnant, pseudopregnant and ovariectomized, progestin treated, pregnant bitches. Detection of relaxin as a marker of pregnancy. *Am. J. Vet. Res.* 50: 68-71

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

Thrall, M.A. and Olson, P.N. 1993. In diagnostic cytology of the dog and cat. Ed. R.L. Cowell and R.D. Ryler. American Vet. Publications, Inc., Thornwood Drive.

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

Tsutsui, T. 1989. Gamete physiology and timing of ovulation and fertilization in dog. *J. Reprod. Fert. Suppl.* 39: 269-275.

Tsutsui, T., Kirihara, N., Hori, T and Concannon, P.W. 2006 Treating pseudopregnant bitches. *Aust. Vet. Pract.* 4:164-168

Tsutsui, T., Kirihara, N., Hori, T and Concannon, P.W. 2007. Plasma progesterone and prolactin concentrations in overtly pseudopregnant bitches: A clinical study. *Theriogenology.* 67: 1032-1038

Ulutas, Alkim.Pinar., Musal, Bayazit., Kiral, Funda and Bildik, Aysegul. 2009. Acute phase protein levels in pregnancy and oestrus cycle in bitches. *Res. in. Vet. Sc.* 86: 373-376

VanHaaften, B., Dieleman, S.J., Okkens, A.C., Bevers, M.M. and Willemse, A.H. 1989. Induction of estrus and ovulation in dogs by treatment with PMSG and/or bromocryptine. *J. Reprod. Fertil. (Suppl.).* 39: 330-331

VanHaaften, B., Bevers, M.M., van den Brom, W.E., Okkens, A.C., van Sluijs, F.J., Willemse, F.J. and Dieleman, S.J. 1994. Increasing sensitivity of the pituitary to GnRH from early to late anoestrus in the beagle bitch. *J. Reprod. Fertil.* 101: 221-225

Vannucchi, I. Camila., Mirandola, M.Regina and Oliveira, M.Clair. 2002. Acute phase protein profile during gestation and dioestrus. Proposal for an early pregnancy test in bitches. *Theriogenology*. 74: 87-89

Voith, V.L (1980). Functional significance of pseudocyesis. *Mod. Vet. Prac.* 61: 75-77

Wright, P.J. and Parry, B.W. 1989. Cytology of the canine reproductive system. *Vet. Clinics N. Am. Small Anim. Pract*. 19: 851-874.

Wright, P.J. 1990. Application of vaginal cytology and plasma progesterone determinations to the management of reproduction in the bitch. *J. Small. Anim. Prac*. 31: 335-340.

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

Zoldag, L., Fekete, S., Csaky, I. and Bersenyi, A. 2001. Fertile estrus induced in bitches by bromocriptine, a dopamine agonist: a clinical trial. *Theriogenology.* 55: 1657-1666.

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.

DIAGNOSTIC AND THERAPEUTIC APPROACHES FOR ENHANCING REPRODUCTIVE EFFICIENCY IN FEMALE DOGS

DEEPTHI L.

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

Doctor of Philosophy
In
Veterinary and Animal Sciences

Faculty of Veterinary and Animal Sciences
Kerala Agricultrual University, Thrissur

2010

Department of Animal Reproduction, Gynaecology & Obstetrics

COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR – 680651
KERALA, INDIA

ABSTRACT

The present study was undertaken for finding optimal breeding time in bitches, early pregnancy diagnosis and tackling pseudopregnancy using antiprolactin drugs.

Experiment consisted of 55 apparently healthy bitches which were brought to the Small Animal Obstetrics and Gynaecology unit of Veterinary College Hospital, Mannuthy and University Veterinary Hospital, Kokkalai for getting breeding advice. Fertility rate was assessed in two different groups viz., Group I (n=30) and Group II (n=25). The conception rate was 73.3 per cent in group I and 88.0 per cent in group II. Conception rate was more in those animals, which were bred, based on vaginoscopy than those bred based upon clinico-gynaecological examination and vaginal exfoliative cytology. This supported the view that performing vaginoscopy will augment conception rate in bitches. Clinico-gynaecological examination together with vaginal exfoliative cytology was found to be useful for finding optimal breeding time in bitches under field conditions. Vaginoscopy, in addition to being a non-invasive method, had the added advantage of predicting the fertilization period, thereby breeding advice could be more accurate as it helps in the visual inspection of vaginal mucosal folds.

Pregnancy diagnosis was carried out in 20 animals at 20^{th}, 35^{th} and 50^{th} day of gestation. Abdominal palpation at 20 days after breeding was non-confirmatory of pregnancy but at 35 days of gestation foetus could be appreciated as tense distinct uterine swellings. By ultrasound scanning at 20 days of gestation the image of embryonic vesicles appeared as spherical structures with anechoic consistency. No foetus or foetal membranes could be visualized at this stage. By day 25 to 27, echogenic foetal mass and heart beat became detectable. By day 28 to 33, head and body of the foetus were similar in size and revealed flickering heart beats. Anatomical features of the foetus became more obvious by about 34 .

to 39 days of gestation. Shape and size of the foetal head became distinguishable from the body at this stage.

The percentage accuracy for transabdominal palpation at 20 days of gestation was found to be 20 per cent which improved to 75 per cent by about 35 days. The percentage accuracy at 50 days was 60 per cent. This study suggested that transabdominal palpation was not reliable in early and late gestation. For ultrasound scanning, the percentage accuracy at 20 days of gestation was found to be 15 per cent which improved to 90 per cent by 35 days. The percentage accuracy improved to 100 per cent by 50 days of gestation. Thus ultrasound scanning could be used as reliable tool for assessing the foetal viability.

The level of serum alkaline phosphatase at 20^{th}, 35^{th} and 50^{th} day of gestation was found to be 67.90 $\pm$ 2.98, 91.85 $\pm$ 2.10 and 139.65 $\pm$ 6.84 U/L respectively. The level of haptoglobin at 20^{th} day of gestation was 53.10 $\pm$ 3.22 mg/dl where as the level elevated to 81.12 $\pm$ 3.40 and 119.44 $\pm$ 3.16 mg/dl by 35 days and 50 days of gestation respectively. The level of serum globulin at 20^{th}, 35^{th} and 50^{th} day of gestation was 2.43 $\pm$ 0.12, 3.05 $\pm$ 0.11 and 3.74 $\pm$ 0.15 g/dl respectively. Statistical analysis revealed significant difference between day 20 and day 50 ($P<0.05$). Thus the level of serum alkaline phosphatase, serum haptoglobin and serum globulin was found to be increasing as the pregnancy advanced and this could be used as indicators of healthy pregnancy.

Total erythrocyte count (TEC) at 20, 35 and 50 days of gestation was 6.07 $\pm$ 0.16, 5.58 $\pm$ 0.15 and 5.12 $\pm$ 0.16 million/cmm respectively. Haemoglobin concentration at day 20, 35^{th} day and 50 days of gestation were found to be 11.86 $\pm$ 0.28, 10.45 $\pm$ 0.27 and 9.17 $\pm$ 0.30 g/dl respectively. Packed cell volume values were 40.45 $\pm$ 0.83, 37.30 $\pm$ 0.87and 33.40$\pm$ 1.10 percent respectively at day 20, day 35 and 50 days of gestation. Erythrocyte sedimentation rate at day 20, day 35 and 50^{th} day of gestation were found to be 10.31 $\pm$ 0.73, 15.41 $\pm$ 0.54 and 21.85 $\pm$ 1.04 mm/hr respectively. Haemogram studies showed significant decrease in the total erythrocyte count, haemoglobin

and packed cell volume at different gestational age ($P<0.05$). These changes were attributed by haemodilution and increased plasma volume. But erythrocyte sedimentation rate has shown an increase which could be attributed to the endometrial implantation by the embryo.

The total leucocyte count at day 20, day 35 and day 50 of gestation was found to be 13844.90 $\pm$ 539.90, 15449.00 $\pm$ 569.86 and 17502.50 $\pm$ 780.21 cells/cmm respectively. The neutrophil count at 20^{th}, 35^{th} and 50^{th} day of gestation was 67.30 $\pm$ 1.11, 70.30 $\pm$ 4.95 and 75.35 $\pm$ 1.27 per cent respectively. The lymphocyte count was found to be 27.80 $\pm$ 0.87, 31.55 $\pm$ 0.88 and 36.95 $\pm$ 1.03 per cent at 20^{th}, 35^{th} and 50^{th} days of gestation. Statistical analysis revealed significant variation between day 20 and day 50 in the neutrophil as well as lymphocyte count ($P<0.05$). Eosinophil count varied significantly ($P<0.05$) between day 20 and day 50 and the values were recorded as 1.60 $\pm$ 0.11 and 3.30 $\pm$ 0.13 per cent respectively. This was due to non-specific inflammatory response produced by the early embryo during endometrial implantation.

The body weight of animals varied significantly ($P<0.05$) at different gestational age. Foetal viability was difficult to be monitored at 20 days of gestation. By ultra sound scanning, foetal heart beats could be observed in all pregnant animals from 25 days of gestation. The gestation length ranged between 58 to 69 days with an average of 63.30 $\pm$ 3.76days. Litter size varied between three to eleven pups with a mean of 6.6 $\pm$ 2.08 pups per bitch.

The level of serum progesterone prior to treatment, post-treatment at 7^{th} and 14^{th} day were 1.11 $\pm$ 0.14, 0.85 $\pm$ 0.06 and 0.62 $\pm$ 0.06 ng/ml respectively in cabergoline treated group while in bromocriptine treated group, it was 1.11 $\pm$ 0.14, 0.5 $\pm$ 0.01 and 0.5 $\pm$ 0.01 ng/ml respectively. Statistical analysis showed significant difference between the serum progesterone values prior to treatment and the value obtained on 14^{th} day after treatment ($P<0.05$).

The concentration of prolactin in cabergoline treated group prior to treatment and at 7^{th} and 14^{th} day post-treatment was 6.17 $\pm$ 2.05, 1.69 $\pm$ 0.20 and 0.82 $\pm$ 0.13 ng/ml respectively whereas in bromocriptine treated group the values were 6.02 $\pm$ 1.17, 0.90 $\pm$ 0.07 and 0.58 $\pm$ 0.08 ng/ml respectively. Statistical analysis showed significant variation ($P<0.05$) in the serum prolactin level prior to treatment and at 7^{th} and 14^{th} day post-treatment.

Antiprolactin drug cabergoline was found to be better than bromocriptine for treating pseudopregnancy at a dose rate of 5µg/kg BW orally for seven days with minimum side effects.