COMPARATIVE EFFICACY OF BROMOCRIPTINE, CABERGOLINE AND THYROXINE IN INDUCING OESTRUS IN BITCHES

By

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DECLARATION

I hereby declare that this thesis, entitled **"COMPARATIVE EFFICACY OF BROMOCRIPTINE, CABERGOLINE AND THYROXINE IN INDUCING OESTRUS IN BITCHES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"COMPARATIVE EFFICACY OF BROMOCRIPTINE, CABERGOLINE AND THYROXINE IN INDUCING OESTRUS IN BITCHES"** is a record of research work done independently by **G. Ajitkumar,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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G. AJITKUMAR

Dedicated to

My parents

&

Teachers

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INTRODUCTION

1. INTRODUCTION

One of the most extensive surveys of the earliest known dog burials in Bonn-Oberkassel, Germany proved beyond doubt that dogs were domesticated between 13000 and 17000 years ago (Darcy, 2006). The ability to understand and respond to human cues as well as the availability of food and companionship might have encouraged the dog to remain dear and near to man. Companion animals can provide the physical and psychological support needed for an individual. They can also reduce the feeling of anxiety and stress. Dog is considered as the best companion in solitude and trouble. In recent years they are being used for a variety of activities by organizations like police, railways, prisons, military etc.

According to the Quinquennial Livestock Census-2003 (AHD, 2004), the domestic dog population in Kerala is 11.31 lakhs. The data over the years reveal an increasing trend in the canine population in tandem with popularization of the nuclear family concept in the State. Eventhough dogs are reared primarily as companion, pet and guard, currently many have identified the potential of dog breeding units as an attractive as well as remunerative vocation.

Domestic bitches are non-seasonally monoestrus and as a result of this unique reproductive physiology, they exhibit cyclicity only once or twice in a year. The interoestrus interval is the time from the onset of one proestrus to the onset of next proestrus, and includes proestrus, oestrus, dioestrus/metoestrus and an obligate anoestrus period.

Canine breeding management has changed a lot over the years. Researches carried out on various aspects of canine reproduction have succeeded in unraveling the mysteries behind the reproductive problems in dogs. Though there is a common saying that dogs are notoriously fertile, reports revealed that a number of infertility problems are encountered in them also. Anoestrus seems to

be an important infertility condition in dogs. Since timely reproduction has got paramount importance in optimizing the returns from dog breeding units, owners of elite bitches with long interoestrus interval often approach veterinarians with the request to shorten it in order to increase the number of litters born per year. Moreover, some control over the cycle of the bitch becomes necessary when artificial reproductive technologies are being applied in canines for intensive breeding programme.

Gobello *et al.* (2002) opined that treatment of anoestrus should be directed towards identifying and treating the underlying cause. However, oestrus induction could be attempted even when the underlying cause was not confirmed. Estrus induction is clinically useful in conjunction with routine breeding management when breeding opportunities are missed or when conception fails. It may also be used for treating delayed puberty, primary and secondary anoestrous conditions including silent oestrus and unobserved oestrus.

According to Johnson *et al.* (1997), hypothyroidism has been identified as a common endocrine disorder in female dogs characterized by obesity, lethargy, alopecia and reproductive abnormalities such as infertility, prolonged or irregular anoestrus, short oestrus, galactorrhoea, foetal death and abortion. Hypercholesterolemia is most commonly recognized secondary to systemic diseases, particularly hypothyroidism in canines (Barrie and Watson, 1995). Prolactin appears to play a role in canine interoestrus interval, possibly by affecting gonadotrophin secretion and/or ovarian responsiveness to gonadotrophins (Gobello *et al.*, 2001).

Previous attempts to induce fertile oestrus in bitches using gonadotrophins and oestrogenic compounds were largely unsuccessful and hence newer methods which involve oral administration of drugs with minimal side effects are under trial. Dopamine agonists or anti-prolactin drugs are ergot derivatives that inhibit prolactin secretion by stimulating secretion of dopamine or suppressing secretion

of serotonin thereby inducing oestrus (Jochle *et al.*, 1987). Bromocriptine and cabergoline are the two dopaminergic agonists used in human medicine primarily to tackle hyperprolactinaemia and associated infertility problems. Since hypothyroidism, hyperprolactinaemia and anoestrus are inter-related, administration of thyroxine may be beneficial in inducing oestrus.

Under these circumstances, the present study was undertaken with the following objectives:

1. Ascertain the prevalence, nature and magnitude of anoestrus among bitches.
2. Estimate the serum prolactin, thyroxine, progesterone, cholesterol, mineral and haematological profile in anoestrous bitches.
3. Assess the efficacy of bromocriptine, cabergoline and thyroxine in inducing oestrus in anoestrous bitches.
4. Study the fertility rate in induced oestrus under different treatments.
5. Evolve the most suitable treatment regimen to tackle anoestrus in dogs.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. BREEDING, FEEDING AND MANAGEMENT OF DOGS AND THE PROFILE OF THE OWNERS

According to the Quinquennial Livestock Census- 2003 (AHD, 2004), the dog population in Kerala was estimated as 14.06 lakhs, which comprised of 11.31 lakhs domesticated and 2.75 lakhs stray dogs. Perusal of literature revealed that information on breeding, feeding and management of dogs from Kerala are sparse.

A survey conducted by Nassar *et al.* (1984) in Kansas revealed that 45 per cent of the dogs were male and 55 per cent female. Margawani and Robertson (1995) reported that in Indonesia 83 per cent of the dogs were male. Analysis of management systems of purebred and crossbred dogs in Central Kerala by Vijayakumar (2001) revealed that the average number of dogs per household was 1.7 and the most preferred breeds were German shepherd and Dachshund. Among the dogs surveyed, 49.6 per cent were male and 50.4 per cent were female.

Chamala and Crouch (1981) reported that companionship (70.4%) seemed to be the most popular motivation for dog keeping followed by companionship and relaxation (15.7%) and safety and companionship (13.9%).

According to Legrand-Defretin and Munday (1993), adult dogs have to be fed once or twice in a day. Pasupathi *et al.* (2000) opined that the exorbitant increase in the price of commercial dog foods resulted in the formulation of economical and nutritionally balanced complete food for dogs under Indian conditions. According to Vijayakumar (2001), the percentage of dogs which received one meal per day was 23.40 only. Most of the dogs (41.90%) received

two meals per day and the percentage of dogs that received three or more meals per day was 34.70. With regard to the type of food, 92.70 per cent of dogs received non-vegetarian and 7.30 per cent received vegetarian food.

Deworming was practiced in 92, 76 and 74 per cent of dogs in Thrissur, Ernakulam and Palakkad districts. Percentage of dogs vaccinated with anti-rabies and multicomponent vaccine was 85.3 and 41.3 respectively (Vijayakumar, 2001).

According to Simon (1997), the average age at puberty in bitches was 11.40 months and the mean interoestrus interval was 6.80 months. Vijayakumar (2001) reported that 89 per cent of the bitches exhibited heat periods twice in a year and the average age at first breeding was 17.20 months. The average duration of proestrual bleeding was 9.60 days. Abortion was noticed at the rate of 3.70 per cent among bitches. The average litter size was found to be 5.30 (Vijayakumar, 2001).

A survey conducted among dog owners of Illinois by Griffiths and Brenner (1977) revealed that female owners (67%) were more than male owners (33%). Vijayakumar (2001) reported that the average age of dog owners in Central Kerala was 47.20 years and in 92 per cent of the households surveyed, the dog owners were male members of the family. The average experience in dog rearing among the owners was found to be 11.20 years.

The negative characteristics of dogs reported by the owners were shedding of hair, annoyance to neighbours, smell and spread of diseases (Selby and Rhoades, 1981). According to Vijayakumar (2001), the major constraints in rearing of dogs were inadequate space, lack of man power, difficulty in looking after the dog while the family was away, restraining difficulties, training and breeding problems and hair shedding.

2.2. OESTROUS CYCLE IN THE BITCH

According to Jones and Joshua (1988), in a complete breeding season which lasted on an average for six months, the bitch exhibited only one oestrus. The oestrous cycle in the bitch was considerably longer than that in most other domestic species and unique in that there was an obligatory anoestrus following the termination of the luteal phase. Arthur *et al.* (1989) categorized the oestrous cycle in bitches into four phases *viz.* proestrus, oestrus, metoestrus/dioestrus and anoestrus.

Okkens (2000) stated that the oestrous cycle of bitch could be divided based on the ovarian function into four phases *viz.* follicular, preovulatory leutinisation and ovulation, luteal and anoestrus phases.

According to Jones and Joshua (1988) and Feldman and Nelson (1996), the period of proestrus started with the onset of blood tinged vulval discharge and ended when the bitch allowed the dog to mount and breed. The other criteria used in describing the onset of proestrus included enlargement of the vulva, attraction of the males to the females and changes in behaviour towards males.

Appreciation of changes of vaginal epithelial cells by exfoliative cytology and visualization of the vaginal mucosa endoscopically could also be employed for the identification of the onset of proestrus and its progress (Feldman and Nelson, 1996; Wilson, 2005).

Feldman and Nelson (1996) opined that no single criterion for finding the onset of proestrus was better than or as simple and reliable as observing the first day of a blood tinged vulval discharge.

According to Olson *et al.* (1987) and Feldman and Nelson (1996), the duration of proestrus averaged nine days in natural oestrous cycles. Jones and Joshua (1988) reported that the duration of proestrus varied from 8 to 13 days. It

was reported to be 4.4 days and 5.4 days respectively in oestrous cycles induced with pregnant mare serum gonadotrophin (PMSG) alone or PMSG and human chorionic gonadotrophin (hCG) in combination (Simon, 1997). Becha (2000) observed shorter duration of proestrus in oestrus induction trials using gonadotrophin releasing hormone (GnRH) in anoestrous bitches. .

Wright (1982) and Prabhakar *et al.* (1989) noticed proestrual bleeding with high intensity in bitches after oestrus induction with PMSG. High intensity of proestrual bleeding was noticed in 83.33 per cent of bitches after oestrus induction using the GnRH analogue, leuprolide acetate (Becha, 2000).

The length of time from the onset of proestrus to the time of first breeding was usually 6 to 11 days with an average of nine days. Variations within what was considered normal could be as brief as 2 or 3 days to as prolonged as 25 days (Feldman and Nelson, 1996).

According to Johnston (1980) and Feldman and Nelson (1996), the duration of oestrus in bitches averaged nine days. Jones and Joshua (1988) reported that the duration of oestrus varied from 4 to 7 days. The duration of oestrus was reported to be 4.2 to 5.6 days in PMSG induced oestrous cycle (Simon, 1997) and eight to nine days in GnRH treated bitches (Inaba *et al.*, 1998). According to Becha (2000), the duration of oestrus averaged eight days in an oestrus induction regimen using leuprolide acetate. However, Gobello *et al.* (2004) reported that the duration of proestrus and oestrus in induced and spontaneous cycles were similar.

The first day of metoestrus was the day characterized by refusal of male and was confirmed by an abrupt 10 to 60 per cent decrease in superficial cells and the reappearance of intermediate and parabasal cells in the vaginal smears (Verstegen *et al.*, 1999; Kutzler, 2007). The end of metoestrus could be appreciated in a number of ways such as when the progesterone secretion of the luteal phase

subsided, mammary development declined or when the plasma progesterone concentration initially fell to a level of 1 ng/ml or less.

2.2.1. Exfoliative Vaginal Cytology (EVC)

Exfoliative vaginal cytology was recommended as a suitable technique to identify the various stages of oestrous cycle in bitches (Schutte, 1967a; Christie *et al.*, 1972; Jones and Joshua, 1988; Arthur *et al.*, 1989; Feldman and Nelson, 1996).

2.2.1.1. EVC During Various Stages of the Oestrous Cycle

According to Feldman and Nelson (1996), the increasing oestrogen concentration during proestrus caused rapid multiplication in the number of cell layers lining the vaginal vault. These increased cell layers kept the luminal lining cells away from the blood supply and resulted in death of those cells. The dead cells functioned as less sensitive and less fragile tissue. Fragility decreased not only because of increased cell layers but also due to the development of keratin precursors within these cells.

Schutte (1967a) opined that for normal cytological studies, vaginal smears taken at every 48 h interval were found to provide sufficient cytological changes for an accurate identification of the various phases of the oestrous cycle. During the first few days of the proestrus, a few cells which has arisen from the deeper layers were found and the percentage of keratinized cells were very low in comparison to those found later and during oestrus. A very high erythrocyte concentration was encountered during the first half of proestrus while only a few were seen during the second half. Leucocytes were encountered during the first few days and normally disappeared completely towards the end of proestrus. In some cases erythrocytes were present throughout the proestrus as well as oestrus.

The smears made during proestrus had a dirty appearance on account of the presence of cellular debris and mucin threads.

Cytology during the oestrus phase was characterized by the absence of erythrocytes as well as leucocytes. The cellular picture consisted exclusively of the more differentiated cellular elements namely anuclear keratinized cells, superficial cells with pyknotic nuclei and large intermediate cells. During the first half of oestrus, the cytoplasm of most of these cells were eosinophilic indicative of keratin and prokeratin deposition. Smears made during oestrus were found to be clean and clear in appearance in contrast to those during proestrus (Schutte, 1967a; Feldman and Nelson, 1996).

The characteristic phenomenon of metoestrus was the very high leucocytic concentration and the predominance of cells from the deeper layers. The number of parabasal and small intermediate cells increased as the metoestrus phase progressed. Parabasal cells infiltrated by leucocytes known as metoestrus cells were characteristic of the phase. The leucocyte concentration decreased during the latter half of metoestrus than remaining more or less constant throughout anoestrus (Schutte, 1967b; Feldman and Nelson, 1996).

According to Christie *et al.* (1972), there was a rise in the proportion of anuclear and superficial cells during proestrus and oestrus associated with a fall in large and small intermediates and parabasal cells, but the magnitude and timing of the highest values for anuclear and superficial cells and the nadir for intermediates varied between bitches.

Dore (1978) studied the vaginal smear in relation to the rest of the genital tract during metoestrus and anoestrus in the bitch and observed that the smear of progressive metoestrus contained superficial, large intermediate, small intermediate and parabasal cells. Cytolysis was absent and leucocytes were abundant during metoestrus. In regressive metoestrus there was a shift towards

more basal type cells and cytolysis was present. In anoestrus, parabasal and small intermediate cells were the dominant cell types, cytolysis was marked and leucocytes were absent or scarce.

According to Post (1985), anoestrus was characterized by presence of intermediate and parabasal cells with or without moderate number of neutrophils. During early proestrus, all epithelial cell types with erythrocytes and neutrophils were encountered and in late proestrus and early oestrus, only superficial cells were encountered. Mid-oestrus was characterized by the presence of superficial cells, most of which were anuclear. The beginning of metoestrus was marked by the subtle appearance of neutrophils, parabasal and small intermediate cells in vaginal smear. Parabasal cells containing cytoplasmic vacuoles could also be present during metoestrus.

During proestrus, Allen (1986) observed an increase in the number of intermediate vaginal epithelial cells with obvious nucleus. Erythrocytes and some polymorphonuclear leucocytes were also observed. As the proestrus proceeded, the neutrophils disappeared and the epithelial cells became polygonal in outline with smaller nuclei. At the beginning of the oestrus, 60 to 80 per cent of the epithelial cells were polygonal without visible nucleus (squames), neutrophils were absent and there were variable numbers of erythrocytes. The epithelial cells became round and nucleated and masses of neutrophils appeared at the beginning of the metoestrus.

Feldman and Nelson (1996) divided the phase of proestrus into early, mid and late and reported that the vaginal smears during early proestrus were similar to that of anestrus except the presence of red blood cells. Along with red blood cells numerous parabasal, small and large intermediates, neutrophils and bacteria were recorded. Mid-proestrus was characterized by the disappearance of neutrophils and appearance of at least 40-60 per cent of superficial cells. Late proestrus was differentiated by the presence of about 60-80 per cent of superficial

cells with either vesiculated nucleus, pyknotic nucleus or no nucleus. Throughout standing oestrus, the cytology remained relatively constant as superficial and anuclear squamous cells together accounted for more than 80 per cent of total cells and often reached 100 percent with absence of neutrophils.

Feldman and Nelson (1996) opined that the onset of diestrus/metoestrus was the day in which a dramatic shift was observed in vaginal cytology, from a phase of 80-100 per cent superficial cells to one with 80-100 per cent parabasal and intermediate cells. Neutrophils reappeared and large amount of debris were observed during the onset of metoestrus.

During anoestrus primarily parabasal and intermediate vaginal epithelial cells were seen. Neutrophils and bacteria could be present or absent whereas red blood cells were usually absent. A clear or granular background appearance after staining was also observed during anoestrus on cytology (Feldman and Nelson, 1996).

England and Concannon (2002) described the status of the various clinical parameters in normal fertile bitches before and after the preovulatory luteinizing hormone (LH) surge. They reported a vaginal cytology cornification index of 30 to 100, 80 to 100, 80 to 100 and 0 to 80 per cent seven days before the LH surge, on the day of LH surge, 4 to 5 days and 8 to 11 days after the LH surge respectively.

2.2.1.2. EVC During Various Stages of the Induced Oestrous Cycle

On treating bitches with PMSG for induction of oestrus, Wright (1980) observed a maximum cornification index of 70 to 95 per cent by 8 to 17 days post-treatment in mature anoestrous bitches. According to Tsutsui (1982), the interval from the first PMSG injection to complete vaginal epithelial cornification was 10 to 13 days. Concannon *et al.* (1989) observed 80 to 100 per cent

cornification in the vaginal smear in bitches within 7 to 12 days of constant infusion of GnRH agonist.

Prabhakar *et al.* (1992) compared the vaginal cytology of natural and induced oestrous cycles and observed that in oestrus induced bitches, the levels of anuclear and superficial cells increased significantly from day five of the oestrous cycle (20.83 ± 4.30 and 17.83 ± 5.02%) and attained peak levels on day eleven (63.33 ± 3.76 and 32.17 ± 3.49%). The mean levels of intermediate cells declined significantly from day five (53.00 ± 7.37%) and reached the lowest on day 11 (4.50 ± 1.61%). Similarly the parabasal cells declined significantly from day five (6.67 ± 2.09%) with a complete disappearance on day 11 to 13. Anuclear cells were lower significantly on all the days preceding oestrus and non-significantly thereafter in oestrus induced group.

Verstegen *et al.* (1997) used purified porcine LH for oestrus induction and observed an increase in superficial epithelial cells to more than 90 per cent in normally responded bitches, 40 to 100 per cent in split oestrus during the initial response and 5 to 40 per cent in bitches with weak proestrus.

Inaba *et al.* (1998) found more than 90 per cent superficial cornified epithelial cells during behavioural oestrus in oestrus induced bitches using a sustained release preparation of leuprolide acetate. Becha (2000) also observed an increase in the mean percentage of superficial cells from the second day of induced proestrus to the day of second mating.

2.2.1.3. 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.*, 1989; Becha, 2000; Asha, 2005; Deepthi, 2007)

2.2.1.3.1. 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 occur 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 using a modified Wright-Giemsa stain. According to the authors, the optimum time for breeding was when the cornification index was above 80 per cent.

2.2.2. Vaginoscopy During Various Stages of the Oestrous Cycle

A number of authors recommended vaginoscopy as a tool to identify the various stages of oestrous cycle in bitches (Olson *et al.*, 1987, Feldman and Nelson, 1996; Sridevi, 2001; Wilson, 2005; Lulich, 2006)

Vaginoscopic examination during anoestrus revealed low mucous membrane folds, which were simple and rounded in outline. The mucosa had a scant mucus coating and was diffuse pink red in colour (Feldman and Nelson, 1996, Hewitt and England, 2000; Wilson, 2005).

During proestrus, the vaginal mucosa appeared rounded, oedematous and smooth. The decreasing oestrogen and increasing progesterone concentrations associated with the last 1 to 3 days of proestrus caused oedema in the vaginal mucosa to subside and the luminal surface became progressively wrinkled and was referred to as crenulation (Feldman and Nelson, 1996; Hewitt and England 2000).

According to Wilson (2005), the endoscopic appearance of the vagina could be divided into four clearly defined phases *viz.* oedematous, shrinkage without angulations, shrinkage with angulations and rounding out. The initial phase of shrinkage without angulations occurred from around the time of the preovulatory LH surge until ovulation. Development of angulations was associated with the period of ovulation and oocyte maturation. The rounding-out phase corresponded to the shift in cell types seen in vaginal smears indicating the onset of dioestrus.

2.2.3. Haematology During Various Stages of the Oestrous Cycle

Jacobs *et al.* (1995) reported the normal haemoglobin level, total erythrocyte count, total leucocyte count and packed cell volume (PCV) in dog as 12 to 18 g/dl, 5.5 to 8.5 millions/mm^3, 6 to 17 x $10^3/mm^3$ and 37 to 55 per cent respectively. In dogs, the erythrocyte sedimentation rate (ESR) by Wintrobe method was reported as 1 to 4 mm/h (Benjamin, 1985). The differential leucocyte counts with respect to neutrophils, lymphocytes, monocytes and eosinophils in canines were 60 to 77, 12 to 30, 3 to 10 and 0 to 10 per cent respectively (Benjamin, 1985).

The haematologic parameters of the Nigerian mongrel bitch at different stages of the oestrous cycle were investigated by Mshelia *et al.* (2005) and reported that RBC counts were highest during anoestrus with a mean of 5.09 ± 0.62 x $10^6/\mu l$, while lowest during pregnancy. White blood cell count, PCV and haemoglobin concentration showed an increasing trend from anoestrus to proestrus and then decreased with transition from proestrus to oestrus and total WBC counts were highest during dioestrus.

2.2.4. Progesterone, Prolactin and Thyroxine Profile During Various Stages of the Oestrous Cycle

2.2.4.1. Progesterone

The average progesterone levels in the blood of Beagle bitches during anoestrus, proestrus and oestrus were 0.6 ± 0.1, 1.7 ± 0.3 and above 2 ng/ml respectively (McDonald, 1989).

Feldman and Nelson (1996) reported that the progesterone concentration throughout the proestrus in bitch was very low (<0.5 ng/ml). The end of proestrus and the beginning of the oestrus could be identified when the plasma progesterone concentration rose above a critical plateau of 1 ng/ml. The serum progesterone concentration was above 5 ng/ml by the first day of dioestrus and by day 10 to 15 of dioestrus, it rose to 25 ng/ml. A zenith in progesterone level was achieved 20 to 30 days after ovulation and the concentrations were in the range of 15 to 60 ng/ml.

According to England (1998), the serum progesterone levels during proestrus and oestrus were less than 1 ng/ml and it rose to 2 ng/ml on the day of LH surge and to 5 ng/ml on the day of ovulation. The serum progesterone level during anoestrus was less than 1ng/ml (England, 1998). Gobello *et al.* (2002) opined that bitches whose progesterone levels were below 1ng/ml could be considered to be in anoestrus.

In naturally occurred cycles in bitches, Becha (2000) observed a mean serum progesterone level of 0.37 ± 0.10 ng/ml on the second day of proestrus and 3.10 ± 0.61 ng/ml on the first day of oestrus. The corresponding values in two groups of bitches treated with the GnRH analogue leuprolide acetate and diethylstilbesterol for induction of oestrus were 0.50 ± 0.23 and 3.37 ± 0.98 and 0.20 and 5.95 ± 0.62 ng/ml respectively. The mean serum progesterone

concentration during anoestrus in both the groups was 0.63 ± 0.28 and 0.50 ± 0.19 ng/ml respectively.

England and Concannon (2002) reported that the serum progesterone levels at seven days before the LH surge, on the day of LH surge, 4 to 5 days and 8 to 11 days after the LH surge were <0.5 ng/ml. 0.9 to 3 ng/ml, 3.5 to 12 ng/ml and 8 to 25 ng/ml respectively.

Levy and Fontbonne (2007) observed a fairly constant level of 6.25 ± 1.55 ng/ml of progesterone at the time of ovulation in bitches.

2.2.4.2. Prolactin

According to Feldman and Nelson (1996), prolactin levels were low during anoestrus, proestrus and oestrus. As progesterone concentrations declined in the latter half of dioestrus, prolactin level increased. An inverse relationship existed between serum concentration of progesterone and prolactin immediately prior to parturition as well as during pregnant and non-pregnant dioestrus.

The mean prolactin concentrations during early, mid and late anoestrous periods in Beagle bitches were 1.8 ± 0.9, 1.6 ± 0.7 and 1.6 ± 0.8 ng/ml respectively (Verstegen *et al.*, 1999). A reduction in serum prolactin concentration was observed in all bitches which were treated with cabergoline for induction of oestrus (from 0.4 ± 0.1 ng/ml on day 2 to 0.3 ± 0.1 ng/ml on day 5). The reduction in serum prolactin concentration observed at the onset of proestrus in control bitches suggested that even in normal cycles prolactin concentration declined towards the onset of proestrus.

A study conducted by Gobello *et al.* (2001) revealed that the serum prolactin level in anoestrous crossbred bitches varied considerably from 2.5 to 12.3 ng/ml and no circadian rhythm appeared to exist in most of them.

2.2.4.3. Thyroxine

Reimers (1984) observed that the serum T_4 and T_3 concentrations were greater in dioestrus bitches than in anoestrus, proestrus and lactacting bitches and in male dogs.

Benjamin (1985) reported the normal serum T_4 level in dog as 1.2 to 3 µg/dl, whereas Jacobs *et al.* (1995) reported a level of 1 to 4 µg/dl in apparently healthy dogs.

According to Feldman and Nelson (1996), the serum T_4 concentration in healthy dogs ranged between 1.5 and 3.5 µg/dl.

2.2.5. Serum Mineral Profile in the Dog

2.2.5.I. Calcium and Phosphorus

According to Jacobs (1995), the normal serum level of calcium and phosphorus in dogs were 8.8 to 10.3 and 2.5 to 5 mg per cent respectively.

Nelson (2003) opined that symptoms of hypocalcaemia were observed in adult dogs when the serum total calcium concentration was less than 7 mg per cent. It was also stated that the serum total calcium concentration between 7 and 9 mg per cent was often clinically silent. Hypocalcaemia in dogs was often associated with conditions like puerperal tetany, primary hypoparathyroidism and malassimilation syndrome. Moreover, acute onset of hyperphosphataemia could also cause hypocalcaemia.

According to Nelson (2003), hyperphosphataemia was present in adult dogs when the serum phosphorus concentration was greater than 6.5 mg per cent. Hyperphosphataemia could result from increased intestinal phosphorus absorption, decreased phosphorus excretion in the urine or shift in phosphorus

from the intracellular to the extracellular compartment. Hypophosphataemia was present when the serum phosphorus concentration was less than 3 mg per cent. Hypophosphataemia resulted from decreased phosphorus absorption in the intestinal tract, increased urinary phosphorus excretion or shift from the extracellular to the intracellular compartment.

Various forms of infertility like anoestrus, suboestrus, irregular cycles and low conception rates had been reported in cattle due to phosphorus deficiency (Arthur *et al.*, 1989). However reports on reproductive problems among bitches due to deficiency of minerals are sparse.

2.2.5.2. Iron, Copper, Cobalt, Zinc and Manganese

According to Greentree and Hall (1995), the most abundant trace mineral in the body was iron and the use of iron in human pregnancy supplements and general dietary supplements had resulted in it becoming a toxic hazard to pets. It was reported that the normal serum iron concentration in dogs ranged from 85 to 240 µg/dl (0.085 to 0.24 mg%). Jacobs (1995) reported a normal serum iron level of 80 to 180 µg/dl in dogs.

According to Jacobs (1995), the normal serum copper level in dogs ranged from 0.07 to 0.14 mg per cent. Udayasree (2004) reported a mean copper level of 0.087 ± 0.0084 mg per cent in the serum of apparently healthy dogs of Kerala. Jacobs (1995) reported a normal serum zinc level of 0.075 to 0.12 mg per cent in dogs. In apparently healthy dogs of Kerala, Udayasree (2004) observed a mean serum zinc level of 0.0957 ± 0.0161 mg per cent.

Kargin *et al.* (2004) reported that the major part of the zinc present in the body was in the bones and it competed with copper for absorption from the intestinal tract. They opined that the relation between copper and zinc was very much similar to that of calcium and phosphorus.

According to Arthur *et al.* (1989), copper deficiency, either direct or indirect resulted in delayed puberty, anoestrus, suboestrus or poor pregnancy rates. Copper and cobalt deficiencies were associated with depressed oestrus, low fertility and abnormal foetal development (Bearden and Fuquay, 2000).

Bearden and Fuquay (2000) and Tefend and Berryhill (2006) stated that manganese deficiency was rare in dogs and if occurred, it was associated with impaired reproduction characterized by irregular oestrous cycles and anoestrus.

2.3. INFERTILITY IN BITCHES

Allen (1986) opined that most cases of infertility in bitches fell into one of the three main groups *viz.*, as anoestrus, mistiming of mating and possible interference from non-specific bacteria.

2.3.1. Anoestrus

Jones and Joshua (1988) reported anoestrus as a period of sexual quiescence with an average duration of 15 weeks. According to McDonald (1989), the period of anoestrus in bitches lasted for an average of 120 days, but varied from 40 to 270 days. Bouchard *et al.* (1991) and Concannon (1993) reported a non-seasonal anoestrus of variable duration (2 to 10 months) following each oestrous cycle in bitch.

According to Feldman and Nelson (1996), anoestrus could be primary (the bitch that never had an ovarian cycle) or secondary (the bitch that had one or more ovarian cycles but subsequently failed to cycle) and it was opined that secondary anoestrus could occur after the onset of thyroid, other endocrine or non-endocrine diseases. The authors opined that though anoestrus could be considered clinically as a reproductively quiet period, the hormonal activity continued during this phase of the oestrous cycle also.

During anoestrus the follicles that developed in the ovary failed to mature due to improper pituitary hormonal support. The follicles that developed at the time coinciding with gonadotrophin stimulation matured and attained the capacity for oestrogen synthesis and developed to mature eggs. Ovaries of bitches in anoestrus could be visualized on laparotomy as structures with an echogenicity equal to or slightly greater than that of the renal cortex. Follicles appeared as focal hypoechoic or anechoic rounded structures (Feldman and Nelson, 1996).

According to Verstegen *et al.* (1999), the unique feature of the ovarian cycle of the dog was an obligatory anoestrus following the termination of the luteal phase. The anoestrus varied in duration from 3 to 10 months among bitches and varied slightly or greatly among cycles within the same bitch. The normal interoestrous interval ranged from 5 to 12 months with an average of 7 months in both fertile and non-bred cycles. Except in one or two breeds there was no evidence of pronounced seasonality and bitches of most breeds evinced cycle at any month of the year.

Davidson (2006) opined that bitches exhibiting prolonged interoestrous intervals might have prolongation of either anoestrus or dioestrus and that an actual failure to continue to cycle must be differentiated from silent heats. Underlying disease and iatrogenic causes (such as prolonged administration of glucocorticoids) for failure to cycle should be ruled out by careful history, physical examination and database. It was opined that glucocorticoids could feed back on pituitary gonadotrophins *viz.* FSH and LH causing a failure to cycle.

2.3.1.1. Hypothyroidism

Johnston (1980) and Nesbitt *et al.* (1980) reported that hypothyroidism in the bitch was associated with infertility, irregular oestrous cycles, failure to cycle, weak or silent oestrus and prolonged proestrual bleeding.

Nesbitt *et al.* (1980) diagnosed hypothyroidism (T_3 <70 ng/dl or T_4 <1.5 µg/dl or both) in 108 dogs by means of radioimmunoassay techniques and reported that the clinical signs were alopecia (85.2%), coat changes (47.2%), hyperpigmentation (31.5%), pyoderma (27.8%), lethargy (11.1%), cold sensitivity (8.3%), obesity (8.3%) and abnormal oestrus (4.6%). According to Nesbitt *et al.* (1980) and Chastain (1982), galactorrhoea was not a common feature in canine hypothyroidism but when it occurred it was thought to be a response to elevated serum prolactin as in galactorrhoea associated with overt pseudopregnancy.

Benjamin (1985) opined that the clinical features of hypothyroidism in intact females included disturbances in the reproductive cycle, borderline normochromic normocytic anaemia, slight to moderate leptocytosis and hypercholesterolemia. Buckrell and Johnson (1986) reported anoestrus and spontaneous galactorrhoea in a 30 month old hypothyroid Chesapeake Bay retriever bitch. Kaelin *et al.* (1986) observed alopecia, lethargy and obesity as the common clinical signs in a retrospective study involving 16 dogs with confirmed hypothyroidism. Abnormally high plasma cholesterol concentration and anaemia were also noticed.

Peter *et al.* (1989) observed weak oestrual signs and irregular oestrous cycles in a bitch with hypothyroidism and on supplementation with levothyroxine, it became more active, showed improvement in the hair coat and returned to oestrus six months after beginning of treatment. Fontbonne *et al.* (1992) suggested hypothyroidism as a common cause of infertility in bitches causing anoestrus and prolonged interoestrous intervals.

Sullivan *et al.* (1993) opined that anaemia observed in hypothyroid dogs could have resulted from the reduced thyroxine concentration.

According to Panciera (1994), hypothyroidism had been a common endocrine disorder in dogs caused by insufficient production and secretion of thyroid hormones. As thyroid hormone deficiency affected multiple metabolic processes of all body systems, clinical signs were variable and often non-specific. Routine laboratory testing of hypothyroid dogs revealed mild non-regenerative anaemia, hypercholesterolaemia and hypertriglyceridaemia. A detailed investigation carried out in 30336 dogs revealed 0.20 per cent hypothyroidism and reported that the breeds with significantly increased risk were Doberman pinscher and Golden retriever and the most common clinical finding was obesity.

Feldman and Nelson (1996) opined that primary hypothyroidism was the most common cause of naturally occurring thyroid failure in the adult dog, accounting for more than 95 per cent of hypothyroid cases. It was opined that persistent anoestrus could occur after the onset of endocrine diseases like hypothyroidism. Diagnosis of hypothyroidism in dogs was made on the basis of clinical findings, results of routine laboratory and thyroid gland function tests and response to thyroid hormone replacement.

The reproductive abnormalities in female dogs suffering from hypothyroidism included infertility, prolonged or irregular anoestrus, short oestrus, galactorrhoea, foetal death and abortion (Johnson *et al.*, 1997).

According to Sparkes (1997), the signs of hypothyroidism in breeding bitches included skimpy, dry or brittle hair coats, excessive weight gain, lethargy, puffy faces and fertility problems. England (1998) opined that hypothyroidism caused infertility occasionally in the bitch characterized by delayed return to oestrus.

Panciera (1999) reported that measurement of serum total thyroxine (T_4) concentration could be employed as a useful initial screening test for

hypothyroidism since most hypothyroid dogs had T_4 values below the reference range.

Hypothyroidism occurred secondary to increased prolactin secretion in response to pituitary stimulation by thyrotropin releasing hormone. The classic signs of hypothyroidism in dogs included obesity, lethargy, alopecia and reproductive abnormalities (Panciera, 2000). Renju (2005) observed a significant increase in the serum cholesterol concentration in hypothyroid dogs.

Davidson (2006) reported hypothyroidism as a potential cause for failure to cycle in bitch and emphasized that the diagnosis should be well supported by clinical signs and laboratory findings (hypercholesterolemia, non-regenerative anaemia) as well as confirmation of subnormal serum thyroid levels. It was opined that hypothyroid bitches placed on adequate replacement therapy should begin to cycle within six months of becoming euthyroid.

Anoestrus, prolonged interoestrous interval and infertility were not documented by Panciera *et al.* (2007) in bitches with short-term hypothyroidism. However, parturition was prolonged; pups were smaller and more distressed at birth. In addition, hypothyroid bitches predisposed to stillbirth and or birth of weak pups which might suffer perinatal mortality.

2.3.1.2. Obesity

Mason (1970) recorded an incidence of 28 per cent obesity in dogs of England and found Cocker spaniels, Labrador retrievers and Collies as the high-risk breeds.

In an extensive survey involving 8268 dogs of England and Scotland, Edney and Smith (1986) recorded the incidence of obesity as 24.30 per cent and Labradors were found to be the most likely breed to become obese.

Markwell *et al.* (1990) and Kealy *et al.* (2002) opined that obesity could reduce the length and quality of a dog's life.

Obesity associated with hypothyroidism was the result of a low metabolic rate with a relative excess caloric intake (Loeb, 1991). In obese dogs with hypothyroidism, a reduced resting metabolic rate (RMR) contributed to decreased energy expenditure. Thyroid hormone normally increased RMR as a result of increased activity of sodium-potassium-adenosine triphosphatase. It was opined that lack of thyroid hormone resulted in a continued constant energy intake along with decreased energy expenditure and resultant obesity.

Laflamme (1997) opined that approximately 25 to 30 per cent of dogs presented to Veterinarians were overweight and body condition score on a nine point system provided a semi-quantitative assessment of body composition.

Radostits *et al.* (2000) defined obesity as a body weight 15 to 20 per cent or more above the ideal. According to Wolfsheimer (2000), obesity could be arbitrarily defined as a body weight 10 to 20 per cent above the ideal weight for that breed, sex and species and has been estimated to affect 25 to 40 per cent of canine and feline pets. Certain dog breeds, including the Labrador retrievers, Cocker spaniels, Dachshunds, Beagles, Collies, Shetland sheep dogs, Basset hounds and Terriers have been predisposed to obesity, suggesting a genetic component. Obesity has been reported to be more common in older dogs and sedentary lifestyles of contemporary pets and their owners might play a role in the development of obesity. The physical examination was considered as the most practical means of assessing body condition.

Lee *et al.* (2001) reported elevated growth hormone release associated with primary hypothyroidism in dogs and opined that this would bring about physical changes mimicking acromegaly.

A study undertaken by McGreevy *et al.* (2005) to determine the prevalence of obesity in dogs examined by veterinary practices across Australia revealed that out of 2661 dogs surveyed, 54.70 per cent were with ideal body weight, 33.50 per cent were overweight, 7.60 per cent were obese and 4.20 per cent were thin or very thin.

German (2006) opined that obese companion animals might be predisposed to orthopaedic disease, diabetes mellitus, abnormalities in circulating lipid profile, cardio-respiratory, urinary and reproductive disorders, neoplasia, dermatological diseases and anaesthetic complications and stressed the need to increase the awareness of companion animal obesity as a serious medical concern within the veterinary profession.

2.3.1.2.1. Body Condition Scoring

A number of schemes were devised to score the body condition of dogs, with a nine-point scheme being the most widely accepted (Laflamme, 1997). A new seven-point algorithm based approach, specifically designed to be used by owners to assess their own pets was reported by German (2006).

2.3.1.3. Hyperprolactinemia

Prolactin appeared to play a part in canine interoestrous interval, possibly by affecting gonadotrophin secretion and/or ovarian responsiveness to gonadotrophins. Circadian rhythm was not described for prolactin in anoestrous bitches (Gobello *et al.*, 2001). Dopamine was the main endogenous factor inhibiting the release of prolactin and for this reason it was often defined as the prolactin inhibitory factor (Cortese *et al.*, 1997). Chastain and Schmidt (1980) described galactorrhoea associated with primary hypothyroidism in three intact bitches.

Cortese *et al.* (1997) described a case of endocrinopathy characterized by hyperprolactinaemia and galactorrhoea associated with primary hypothyroidism in an entire bitch. Clinical diagnosis was confirmed by lowered baseline T_4 level (0.5 ng/ml), elevated prolactin level (9.64 ng/ml), a low grade normochromic and normocytic anaemia and hypercholesterolaemia (460 mg/dl). The authors also opined that the decreased level of prolactin during proestrus and oestrus might probably be due to the inhibitory effects of oestradiol 17-ß on prolactin release.

According to Spattini *et al.* (2007), the increase in mean plasma concentration of LH noticed without an effect on plasma prolactin level on administration of cabergoline at the rate of 5 µg/kg body weight in anoestrous bitches supported the observation of Olson *et al.* (1982) and Kooistra *et al.* (1999) that in the bitch the induction of follicular development was not initiated by the suppression of prolactin secretion but by other direct or indirect dopaminergic effects on the hypothalamo-hypophyseal-ovarian axis.

2.3.1.4. Hypercholesterolemia

Abnormally high plasma cholesterol concentration was observed by Kaelin *et al.* (1986) in dogs with confirmed hypothyroidism. Hypercholesterolemia associated with hypothyroidism resulted from decreased peripheral lipoprotein lipolysis, reduced hepatic utilization and augmented hepatic production of cholesterol (Weinberg, 1987).

Barrie and Watson (1995) stated that hyperlipidemia was characterized by an increase in plasma concentration of cholesterol or triglyceride or both and the condition might arise as the result of a primary defect in lipoprotein metabolism or as a consequence of an underlying systemic disease.

Hypercholesterolemia was most commonly recognized secondary to systemic diseases in particular hypothyroidism, diabetes mellitus,

hyperadrenocorticism, protein losing nephropathies and obstructive jaundice. Hypercholesterolemia in the dog could be used as an indicator for the identification of endocrine or metabolic disease (Barrie and Watson, 1995). According to Jacobs (1995), the serum cholesterol level in dogs ranged from 100 to 265 mg per cent.

2.4. DRUGS USED FOR INDUCTION OF OESTRUS

2.4.1. Dopamine Agonists/Prolactin Antagonists

According to Okkens *et al.* (1985), shortening of the luteal period achieved by chronic suppression of prolactin suggested that prolactin constituted a part of the luteotrophic complex in the dog.

Suppression of prolactin secretion by administration of dopamine agonists shortened the duration of anoestrus (vanHaaften *et al.*, 1989) or induced oestrus in cases of prolonged anoestrus (Jochle *et al.*, 1987; Arbeiter *et al.*, 1988). England (1994) also reported that prolactin antagonists reduced the interoestrous intervals. Bitches which failed to respond to dopamine agonist therapy did not show a decrease in prolactin concentrations (Concannon, 1993).

Jeukenne and Verstegen (1997) demonstrated that it was possible to induce oestrus not only in anoestrous but also in dioestrous non-pregnant bitches using a D_2 dopamine agonist. Okkens *et al.* (1997) reported that the dopamine agonists did not induce oestrus through suppression of prolactin secretion, but by other direct or indirect dopaminergic effects. Though it was opined that prolactin inhibition alone was not sufficient to terminate anestrous in bitches, it appeared that prolactin inhibition was necessary for oestrus induction to occur.

According to England (1998), apparently normal and fertile oestrus periods could be induced in anoestrous bitches following continued administration of bromocriptine or cabergoline and the time to the onset of

oestrus seemed to relate to the stage of anoestrus; bitches in late anoestrus responded more quickly than those in early anoestrus. It was also opined that the induced oestrus appeared to be physiological and it might be the result of inhibition of remnant progesterone production by the corpora lutea.

Verstegen *et al.* (1999) reported that dopamine agonist for oestrus induction required more than 30 days of treatment and depended upon the stage of anoestrus.

According to Beijerink *et al.* (2004) the prematurely induced oestrus observed during administration of dopamine agonists could not be explained by a decreased plasma prolactin concentration but must be due to some other dopamine agonist effect, probably increased FSH secretion. It was opined that an increase in circulating FSH is essential for ovarian folliculogenesis and consequently the termination of anoestrus in the bitch.

Davidson (2006) opined that prolactin secretion by the pituitary might promote anoestrus and hence dopamine agonists such as cabergoline and bromocriptine could be used to shorten anoestrus in both the normal bitch and in bitches with secondary anoestrus of unknown etiology.

2.4.1.1. Bromocriptine

Bromocriptine, the semi-synthetic ergot alkaloid was used as a dopamine agonist to induce oestrus in bitches (Okkens *et al.*, 1985; vanHaaften *et al.*, 1989). Centrally acting dopamine agonists like bromocriptine commonly induced side effects like vomiting and this could be reduced by using the minimal effective dose and mixing the drug with food (Kutzler, 2005).

Allen and Stockman (1979) observed emesis as a common side effect of bromocriptine administration in bitch. Concannon *et al.* (1987) opined that the side effects of bromocriptine were frequent and proportional to the dose and

consisted of vomiting, anorexia, apathy, increased thirst, depression and behavioural changes. However, habituation to bromocriptine, beginning with lower dose initially, almost completely eliminated emesis, the most common side effect of the treatment (Zoldag *et al.*, 2001).

2.4.1.2. Cabergoline

Cabergoline was found to be a more specific dopamine agonist than bromocriptine. It had a longer duration of action and produced fewer side effects than bromocriptine (Kutzler, 2005).

Jochle *et al.* (1987) and Harvey *et al.* (1997) stated that cabergoline had a more specific action on D_2 dopamine receptors of the anterior pituitary gland and therefore had a greater activity and long lasting effect than bromocriptine. It barely crossed the blood brain barrier and consequently had much less central emetic side effects.

In studies with cabergoline, side effects such as vomiting had been reported in 10 to 25 per cent of dogs (Arbeiter *et al.*, 1988; Gobello *et al.*, 2001; Gunay *et al.*, 2004).

Arbeiter *et al.* (1988) reported development of tolerance to cabergoline with continued therapy and found lesser degree of side effects with lower doses. Rains *et al.* (1995) opined that cabergoline had a high specificity for D_2 receptors, long specific activity on pituitary lactotroph cells and had fewer central nervous system effects than bromocriptine.

Onclin and Verstegen (1997) demonstrated that the effects of cabergoline were mediated through prolactin and not directly through corpus luteum thus confirming the indirect mode of action of dopamine agonist cabergoline on corpus luteum. DelDotto and Bonucelli (2003) reported that cabergoline could

be administered once daily, which was advantageous over other dopaminergic agents in terms of both therapeutic compliance and better symptom control.

Gobello *et al.* (2003) reported transient coat colour changes associated with cabergoline administration for more than two weeks for induction of oestrus in bitches and concluded that the colour shift could be mediated through the inhibition of secretion of melanocyte stimulating hormone. Dattatray (2006) observed anorexia and vomiting in 10 and 20 per cent of bitches treated with cabergoline for induction of oestrus.

2.4.2. Thyroxine

Structural and/or functional abnormalities of the thyroid gland could lead to deficient production of thyroid hormones (Feldman and Nelson, 1996). Thyroid hormone supplementation was recommended by the authors as the treatment of choice in confirmed cases of hypothyroidism with levothyroxine as the initial therapy of choice.

2.5. INDUCTION OF OESTRUS IN BITCHES

The various methods for induction of oestrus in canines included the use of synthetic oestrogens (diethylstilbesterol), dopamine agonists (bromocriptine and cabergoline), GnRH agonists (lutrelin, buserelin, fertirelin, deslorelin and leuprolide) and exogenous gonadotrophins (luteinizing hormone, follicle stimulating hormone, human chorionic gonadotrophin, pregnant mare serum gonadotrophin and human menopausal gonadotrophin) and opiate antagonists (naloxone). According to Kutzler (2005 & 2007), these methods varied widely in the efficacy of inducing oestrus as well as in the fertility of the induced oestrus and the applicability of some of these methods for clinical practice were questionable.

Literature revealed the use of a variety of exogenous hormones and their combinations for induction of oestrus in bitches with varying results. Cain (1995) opined that it is important to thoroughly evaluate all apparent causes of reproductive failure before attempting oestrus induction and that most of the protocols were designed and tested in bitches that were reproductively normal.

2.5.1. Oestrus Induction with GnRH

Different GnRH preparations were used at various dose regimens in pulsatile or continuous infusion, subcutaneous administration and as depots for inducing oestrus in bitches with varying success rate.

In a study, Vanderlip *et al.* (1987) successfully induced oestrus in five out of eight anoestrous Beagle bitches by pulsatile intravenous administration of gonadorelin (40 to 430 ng/kg body weight in every 90 minutes for 6 to 12 days) and emphasized the importance of pulsatile injection of GnRH for induction of oestrus.

Continuous infusion of GnRH at various dose regimens (0.2 to 14.0 ng/kg body weight/minute) and duration of treatment (3 to 11 days) was evaluated by Cain *et al.* (1989), but the response to treatment was poor and only two out of eight treated bitches evinced signs of oestrus and ovulation. Concannon (1992) could induce fertile oestrus in 13 of the 24 anoestrous bitches by continuous subcutaneous infusion of a potent GnRH agonist, lutrelin for 14 days at a dose rate of 1.70 to 2.50 µg/kg body weight/day and of which nine conceived. vanHaaften *et al.* (1994) reported that pituitary sensitivity to GnRH increased from early to late anoestrus and the ovaries responded accordingly in bitches.

Inaba *et al.* (1998) could successfully induce oestrus in Beagle bitches by a single subcutaneous injection of a microencapsulated sustained release

formulation of leuprolide acetate (100 μg/kg body weight), followed by a GnRH analogue, fertirelin (3 μg/kg body weight) on the first day of induced oestrus.

Becha (2000) succeeded in inducing fertile oestrus with high conception rate and normal litter size in anoestrous bitches by administering a sustained release preparation of leuprolide acetate @ 100 μg/kg body weight intramuscularly followed by gonadorelin @ 3 μg/kg body weight intramuscularly on the first day of induced oestrus.

2.5.2. Oestrus Induction with FSH/LH

Olson *et al.* (1982) opined that neither the canine ovary nor pituitary were quiescent during anoestrus. The level of FSH was sufficient during anoestrus for follicular growth, but increase in concentration of LH prior to the onset of proestrus appeared as the triggering factor for inducing a new follicular phase.

Shille *et al.* (1984) treated 14 anoestrous bitches with intramuscular injections of FSH as a single dose, multiple dose regimen and multiple doses of FSH combined with LH. Two out of five bitches and two out of four bitches showed oestrus by single dose and multiple dose regimens of FSH respectively. All the bitches treated with multiple doses of FSH combined with LH failed to evince oestrus.

Sixteen anoestrous Beagle bitches were treated by Verstegen *et al.* (1997) with a highly purified pig LH thrice daily for seven days. Proestrus was observed within seven days in all treated animals and oestrus in 12 animals. Ovulation occurred in seven out of 12 bitches in 16 ± 3 days of treatment and six of them conceived.

According to Kooistra *et al.* (1999), progression from early to late anoestrus was associated with an increase in basal plasma FSH levels without a concomitant rise in basal LH concentration and this increased level of circulating

FSH was critically required for the initiation of ovarian folliculogenesis resulting in the termination of anoestrus.

2.5.3. Oestrus Induction with PMSG (eCG)

The most widely studied gonadotrophin for oestrus induction in the bitch was PMSG and several protocols ranging from daily to weekly injections using either subcutaneous or intramuscular routes of administration were reported. In general, PMSG was found to be more successful in inducing oestrus than gonadotrophins of pituitary origin (Wright, 1980; Renton *et al.,* 1984; Simon, 1997).

Eleven anoestrous bitches were treated by Wright (1980) with PMSG at the rate of 110 IU/kg body weight at weekly intervals until onset of oestrus or for a maximum of three treatments. Oestrus was induced in eight bitches while ovulation occurred in seven of them.

Renton *et al.* (1984) induced oestrus in Beagle bitches by daily injection of 500 IU of PMSG for a maximum of ten days and observed pregnancy failure following mating in induced oestrus.

Simon (1997) succeeded in inducing oestrus in anoestrous bitches with intramuscular injection of PMSG @ 20 IU/kg body weight for five days. Out of the six animals treated, five (83%) came into oestrus and three conceived and whelped normally.

2.5.4. Oestrus Induction with hCG

Raghuwanshi *et al.* (2007) successfully induced oestrus in anoestrous bitches by administering hCG @ 500 IU per animal intramuscularly. Out of 10 bitches treated, eight showed proestrual bleeding and six of them conceived and whelped.

2.5.5. Oestrus Induction with PMSG and hCG

The usefulness of pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) in different dose regimens to induce oestrus and ovulation in anoestrous bitches were evaluated in several studies.

Thun *et al.* (1977) successfully induced oestrus in seven out of 12 bitches with daily administration of PMSG @ 20 IU/kg body weight for 10 days followed by a single dose of hCG on day 10. Wright (1980) found that bitches treated with hCG on the start of oestrus had a better ovulatory response than those treated with PMSG alone.

Archbald *et al.* (1980) treated ten mongrel bitches with PMSG at the rate of 44 IU/kg body weight daily for nine days followed by 50 IU/kg body weight of hCG on day 10 or on the second day of oestrus. Eight bitches ovulated and 78 ova were recovered from six of them.

Successful results were reported by Wright (1982) on administration of 250 IU of PMSG daily for 20 days or until the onset of oestrus followed by 500 IU of hCG. Tsutsui *et al.* (1982) reported successful induction of oestrus in mongrel bitches by daily administration of 200 IU of PMSG followed by 500 IU of hCG after the notice of complete cornification of vaginal smear.

Arnold *et al.* (1989) compared the use of PMSG at the rate of 20 IU/kg body weight consecutively for five days and ten days followed by a single injection of 500 IU of hCG in anoestrous bitches. In the groups treated for five days, a higher rate of pregnancy (50% *vs.* 0%), lower preovulatory oestrogen concentration and reduced incidence of complications from hyperoestrogenism were reported. Similarly, Prabhakar *et al.* (1989) opined that combination of PMSG and hCG was found to be beneficial for inducing ovulatory oestrus in bitches.

England and Allen (1991) could induce oestrus in five out of six bitches using PMSG at the rate of 20 IU/kg body weight for five days followed by hCG at the rate of 50 IU/kg body weight on the fifth day. It was suggested that this schedule of oestrus induction would not allow mating on a predetermined day.

Concannon (1992) opined that PMSG in doses of 20 IU/kg body weight daily for five days or less, followed by 500 IU of hCG was a good regimen for oestrus induction in bitches. Tsuda *et al.* (1995) could successfully induce oestrus in seven out of 11 anoestrous bitches by using PMSG for nine days followed by hCG.

Simon (1997) treated six anoestrous bitches by daily intramuscular administration of 20 IU of PMSG/kg body weight for five days followed by a single injection of 500 IU of hCG on the fifth day. Five out of six treated bitches came into oestrus and on mating two of them conceived and whelped.

2.5.6. Oestrus Induction with Oestrogens

Shille *et al.* (1984) could successfully induce proestrus and oestrus in Grey hounds using diethylstilbesterol.

Successful induction of ovulatory oestrus and conception in five bitches by oral administration of 5 mg of diethylstilbesterol daily for 6 to 9 days until the onset of proestrus was reported by Bouchard *et al.* (1993). However, England (1994) raised concern over the use of exogenous oestrogens for oestrus induction as it could produce dose related bone marrow suppression, pyometra, alopecia, skin hyperpigmentation and mammary and vulval enlargement. Becha (2000) succeeded in inducing oestrus in four out of six anoestrous bitches by administering diethylstilbesterol at the rate of 0.2 mg/kg body weight orally for nine days.

2.5.7. Oestrus Induction with Clomiphene Citrate

Kodagali *et al.* (1985) successfully induced ovulatory oestrus in an infertile Doberman bitch by administering 75 mg of clomiphene citrate orally on the ninth and eleventh day of the cycle. The bitch gave birth to a normal pup after 61 days of gestation.

Prabhakar *et al.* (1989) and Prabhakar *et al.* (1990) failed to induce oestrus by oral administration of clomiphene citrate in eight anoestrous bitches and observed that four of them exhibited vulval oedema without any sign of proestrual bleeding or oestrus. Simon (1997) failed to induce oestrus in six anoestrous bitches by oral administration of 50 mg of clomiphene citrate daily for five days.

2.5.8. Oestrus Induction with Bromocriptine

Okkens *et al.* (1985) administered bromocriptine in four bitches, orally at the rate of 20 µg /kg body weight twice daily from 1 to 5 days after the LH surge until the beginning of next proestrus and observed that the interoestrus interval was significantly reduced in the treated animals when compared to the control group (123 ± 23 *vs.* 245 ± 8 days).

According to vanHaaften *et al.* (1989), treatment with the dopamine agonist bromocriptine at the rate of 20 µg/kg body weight, twice daily starting from 112 ± 4 days after the onset of proestrus in six bitches reduced the interoestrous interval by 87 days (159 ± 2 *vs.* 246 ± 9 days) and 80 per cent of the treated bitches responded to the treatment by evincing proestrus in 28 days on an average.

Okkens *et al.* (1990) observed that the luteal phase was shortened by 80 per cent of its normal duration on administration of the same dose of

bromocriptine to eight Beagle bitches between days 15 and 20 to 24 of the luteal phase.

Kooistra *et al.* (1999) investigated the secretory pattern of LH and FSH before and during bromocriptine administration in six Beagle bitches and demonstrated that the bromocriptine induced shortening of the interoestrus interval was associated with an increase in plasma FSH concentration without a concomitant increase in plasma LH concentration and that the increased plasma FSH concentration resulted in enhancement of follicle development.

Zoldag *et al.* (2001) administered bromocriptine to 48 anoestrous bitches and recorded 100 per cent oestrus induction and 83 per cent pregnancy with an average litter size of 4.8 ± 1.6. The period between treatment and onset of oestrus varied but averaged 19 days. On comparing the bromocriptine induced and spontaneous oestrous cycles of bitches, it was found that the induced oestrus was characterized by shorter duration, less pronounced vaginal bleeding and clinically fewer oestrus symptoms. It was reported that bromocriptine reduced the duration of luteal phase and time between two oestrous cycles by increasing the concentration of GnRH and FSH in hypothalamus and anterior pituitary respectively along with increased ovarian oestrogen synthesis, which indicated follicular growth.

Beijerink *et al.* (2003) investigated the effect of different doses of bromocriptine on plasma prolactin concentration and interoestrus interval by administering bromocriptine at the rate of 5, 20 and 50 µg/kg body weight twice daily orally to Beagle bitches starting from 28 days after ovulation and reported that the extent of shortening of interoestrus interval was dose dependant; 136 ± 16, 96 ± 6 and 92 ± 11 days respectively. It was observed that bromocriptine shortened the interoestrus interval in the bitch even when the dose was so low without lowering the plasma prolactin concentration. It was opined that the induction of oestrus in the bitch by bromocriptine involved a mechanism other

than *via.,* the lowering of plasma prolactin concentration and that the extent of shortening of the interoestrus interval by bromocriptine was dose dependent.

2.5.9. Oestrus Induction with Cabergoline

Jeukenne and Verstegen (1997) administered cabergoline daily to dioestrous bitches at the rate of 5 µg/kg body weight from day 30 of previous oestrus. Four of the five treated bitches came into proestrus in 29.75 ± 5 days of treatment, but none of them conceived on mating. It was concluded that lack of fertility was related to insufficient uterine involution and regeneration. The study demonstrated that induction of oestrus was possible not only in anoestrous, but also in dioestrous bitches by D_2 dopamine agonist.

Verstegen *et al.* (1999), Gobello *et al.* (2004) and Gunay *et al.* (2004) reported that all cabergoline induced cycles were normal, based on exfoliative vaginal cytology, evidence of ovulation, duration of the stage and physical characteristics compared to the spontaneous oestrous cycles. Jeukenne and Verstegen (1997); Verstegen *et al.* (1999) and Gobello *et al.* (2004) reported that the duration of proestrus and oestrus in induced and spontaneous cycles were similar.

Verstegen *et al.* (1999) administered cabergoline in three groups of five Beagle bitches each, at the rate of 5 µg/kg body weight once daily in early (93-108 days), mid (123-156 days) and late anoestrus (161-192 days) as calculated from the day of preovulatory LH surge and continued until the confirmation of induced proestrus or for 40 days. Out of 15 animals treated, 14 (93.33%) responded by evincing proestrus (80, 100 and 100% in the three groups respectively) and the days taken from the treatment onset to proestrus were 20 ± 3, 14 ± 3 and 6 ± 1 respectively. The duration of induced proestrus in the three treatment groups were 8.5 ± 0.6, 7.3 ± 0.9 and 6.6 ± 0.8 as against 8.4 ± 0.9 days

in control bitches. Out of 14 bitches evinced oestrus, 12 conceived (3, 5 and 4 in the three groups respectively) and produced normal litters.

Gobello *et al.* (2002) successfully induced ooestrus in anoestrous purebred bitches by administering cabergoline at the rate of 5 µg/kg body weight orally and recommended it as a safe and effective treatment regimen for primary and secondary anoestrus. According to the authors, the mean duration of treatment required with cabergoline was 16 days and the range was 4 to 34 days. It was observed that the mean duration of treatment was shorter in secondary anoestrus (11.25 days) than in primary anoestrus (26 days). As administration of cabergoline was effected through food, none of the dogs in their study had any adverse gastrointestinal effects associated with cabergoline administration.

Rota *et al.* (2003) compared two different protocols of oestrus induction, using cabergoline and GnRH agonist in anoestrous bitches. Cabergoline was administered @ 5 µg/kg body weight once daily until the onset of cytological oestrus or for a maximum of 30 days in 12 bitches and buserelin acetate at the rate of 1.5 µg/kg body weight as subcutaneous injection thrice for 11 days and at the rate of 0.75 µg/kg for another three days. It was concluded that cabergoline was more effective in inducing oestrus as 10 out of 12 bitches (83%) showed symptoms of oestrus, conceived and whelped. However, only three out of 10 buserelin treated bitches (30%) showed oestrus, two of which exhibited oestrus seven and 13 days after the end of treatment and the same two bitches accepted mating and conceived.

Gobello *et al.* (2004) recorded two consecutive interoestrus intervals in 23 bitches of different breeds and administered cabergoline at the rate of 5 µg/kg body weight orally once daily from 100^{th} day after the onset of the second proestrus until two days after the onset of induced proestrus. Interoestrus intervals were significantly shortened in cabergoline treated bitches compared to non-treated bitches (184 ± 4.50 *vs.* 239 ± 4.50 days) with a mean of 214 ± 2.90

days. The mean duration of treatment for early, mid and late anoestrus was 27.40 ± 3.70, 17.60 ± 3.80 and 5 ± 3 days respectively.

Cabergoline was administered orally by Gunay *et al.* (2004) to two groups of German shepherd bitches in early (108-124 days) and late anoestrus (160-187 days) as calculated from the beginning of previous proestrus at a dose rate of 6 μg/kg body weight until the onset of proestrus or maximally up to 14 days with the mean duration of treatment being 11.30 ± 2.80 in early and 6.60 ± 0.80 days in late anoestrus. The mean duration of proestrus and oestrus in early and late anoestrus and control groups were 7.60 ± 0.90, 8.70 ± 1.00 and 8.00 ± 1.00 days and 7.00 ± 1.00, 7.00 ± 0.70 and 8.00 ± 1.00 days respectively with a mean litter size of 6.80 ± 1.30, 6.00 ± 0.89 and 7.20 ± 1.50. It was concluded that cabergoline applications were effective in inducing oestrus without deleterious effect in bitches.

DeRensis *et al.* (2006) administered cabergoline to six Grey hound bitches @ 5 μg/kg body weight once daily orally for 20 days beginning from the first day of proestrus and found that there were no differences between the cabergoline treated and control bitches in the duration of proestrus and oestrus. It was concluded that the effect of administration of dopamine agonist was stimulatory in anoestrus but not during oestrus in bitches.

Cirit *et al.* (2007a) reported successful induction of oestrus and ovulation in two groups of breeder bitches with cabergoline @ 5 and 0.60 μg/kg. body weight daily. Oestrus was induced in 80.00 and 81.50 per cent of treated bitches in 23.63 ± 14.33 and 24.41 ± 14.31 days respectively. The average treatment duration of cabergoline at the rate of 0.60 μg/kg. body weight/ day in the early (days 110-120), middle (days 123 -159), late (days 164-198) and very late (days 207-280) phases of anoestrus were 31.70 ± 12.58, 20.80 ± 11.78, 16.20 ± 6.76 and 28.60 ± 13.93 days respectively.

Cirit *et al.* (2007b) recommended administration of cabergoline at the rate of 0.60 µg/kg. body weight/ day than the prolactin lowering dose of 5 µg/kg. body weight/ day as a safe and effective treatment for secondary anoestrus in bitches. In the study none of the dogs had any adverse gastrointestinal effects associated with cabergoline administration.

Spattini *et al.* (2007) investigated the effect of daily administration of cabergoline at the rate of 5 µg/kg body weight for four weeks starting about 95 days after the end of oestrus in anoestrous Beagle and Grey hound bitches and found that 80 and 50 per cent respectively responded to the treatment by evincing proestrus. The mean interoestrous interval of treated animals was 132 ± 5 and 169 ± 7 days and the interval from the beginning of treatment to proestrus was 13.30 ± 1.90 and 20.30 ± 1.70 days respectively. Cabergoline increased the length of proestrus (10.60 ± 0.50 and 11.70 ± 0.50 days) in the induced oestrus compared to the previous cycle (8.40 ± 0.30 and 8.80 ± 0.40 days).

2.5.10. Oestrus Induction with Metergoline

Kusuma and Tainturier (1993) compared induction of oestrus in dogs with metergoline, metergoline plus hCG or PMSG and found that the best result was achieved by giving metergoline alone.

Okkens *et al.* (1997) found that serotonin antagonist metergoline lowered the plasma prolactin level but did not shorten the interoestrus interval.

2.5.11. Oestrus Induction with Thyroxine

Though hypothyroidism had been attributed by many (Johnston, 1980; Nesbitt *et al.*, 1980; Buckrell and Johnson, 1986; Fontbonne *et al.*, 1992; Feldman and Nelson 1996; Johnson *et al.* 1997; England, 1998; Panciera, 2000; Davidson, 2006; Panciera *et al.*, 2007) as a cause of anoestrus in bitches, reports on the use of thyroxine for induction of oestrus were sparse.

Peter *et al.* (1989) reported that a bitch with hypothyroidism returned to oestrus in six months after the beginning of treatment with levothyroxine.

A preliminary trial carried out by Ajitkumar *et al.* (2005) revealed that administration of levothyroxine orally at the rate of 100 µg/animal/day for 10 consecutive days was effective in inducing oestrus in anoestrous bitches. Out of 10 bitches treated, 70 per cent responded by evincing proestrual bleeding and the average period from treatment onset to proestrus was 21 days. Out of seven bitches bred during the induced oestrus, five conceived and whelped uneventfully.

2.5.12. Oestrus Induction with Combination of Hormonal and Non-Hormonal Drugs

Different combinations of gonadotrophic hormones, oestrogens and non-hormonal drugs were tried for induction of oestrus in bitches.

Jensen (1967) induced oestrus by daily oral administration of a synthetic preparation of non-steroidal nature, sexovid [bis(p-acetoxyphenyl) cyclohexylidene methane] at a dose of 4 mg/kg body weight. Oestrus occurred within 3 to 22 days and 71 per cent of the bitches became pregnant.

Ishihara *et al.* (1982) treated 16 anoestrous bitches with intramuscular injection of 0.2 mg oestradiol on alternate days and with 1000 IU of hCG and 400 IU of PMSG subcutaneously on the day of vaginal bleeding. Oestrus occurred in all bitches, but only six of them ovulated.

Chaffaux *et al.* (1984) treated five bitches in mid-anoestrus with 500 IU of PMSG for ten days followed by 50 µg of gonadorelin on the tenth day. Three bitches came into oestrus, but none of them conceived.

Successful oestrus induction was reported by vanHaaften *et al.* (1989) in five out of seven bitches by the combination of PMSG and bromocriptine. Pregnant mare serum gonadotrophin was administered at the rate of 300 IU per day, on alternate days for three days with bromocriptine at the rate of 20 µg/kg body weight, orally twice daily from 17 days prior to the first injection to eight days after the last injection of PMSG.

Oestrus was induced successfully in six out of eight mongrel bitches in late anoestrus by daily oral administration of 5 mg diethylstilbesterol till day two of proestrus and 10 mg of FSH intramuscularly on days five, nine and eleven. Using the same protocol, out of five bitches treated in mid-anoestrus, three came into oestrus. Three animals in late anoestrous and one animal in mid-anoestrus conceived after treatment (Bouchard *et al.*, 1991).

Wanke *et al.* (1997) treated ten healthy anoestrous bitches with intramuscular injection of 75 IU of human menopausal gonadotrophin (hMG) daily for nine days. Nine bitches showed proestrus within three to nine days and four of them produced normal litters.

2.6. PREGNANCY DIAGNOSIS

According to England (1998), the optimum time for pregnancy diagnosis by abdominal palpation was approximately one month after mating as the conceptuses which were spherical in outline during this period could be palpated readily. Though uterine enlargement could be appreciated on radiograph from day 30 onwards, correct pregnancy diagnosis was not possible until after day 45 when mineralization of the foetal skeleton was detectable.

Namsui (1999) succeeded in diagnosing pregnancy by trans-abdominal palpation between 21 to 25 days post-breeding with 66.7 and 75 per cent accuracy in Alsatian and Pomeranian bitches respectively. The corresponding

figures were 88.9 and 100 per cent when conducted between 30 to 35 days post-breeding.

According to Noakes *et al.* (2001), the main problem in confirming pregnancy in the bitch was the higher incidence of overt pseudopregnancy with signs simulating pregnancy.

The optimum period for pregnancy diagnosis by abdominal palpation in bitch was from days 24 to 30 (Arthur *et al.*, 1989). During this period the uterine distensions became spherical in outline and they maintained this form until about day 33. On radiography, at the end of six weeks of gestation, there might be evidence of foetal skeleton with the skull identifiable by 45 days and at the end of seventh week, the whole foetal skeleton could be identified (Noakes *et al.*, 2001).

Asha (2005) reported that more accurate results on diagnosis of pregnancy by trans-abdominal palpation were obtained between 31 and 40 days post-breeding in bitches. The difficulty in confirming pregnancy by trans-abdominal palpation increased between 37 and 46 days post-breeding. It was reported that at 20 to 23 days of gestation, gestational sacs with black coloured anechoic ecotexture and at 24 to 28 days post-breeding, gestational sacs containing foetal tissue suspended in amniotic fluid were observed. Monitoring of foetal heart beat and visualization of foetal skeleton were possible beyond day 40 of gestation.

Deepthi (2007) successfully diagnosed pregnancy in bitches by detecting serum relaxin between 16 and 20 days post-breeding.

2.7. CONCEPTION RATE

In an oestrus induction trial with PMSG in six bitches, Simon (1997) obtained a conception rate of 50 per cent. In bitches in which oestrus was

induced by the administration of leuprolide acetate or diethylstilbesterol, the conception rates were 83.30 and 50 per cent respectively (Becha, 2000).

In an oestrus induction trial in bitches using bromocriptine, Zoldag *et al.* (2001) obtained a conception rate of 83 per cent. Rota *et al.* (2003) also reported a conception rate of 83 per cent in bitches subjected to oestrus induction using cabergoline. Gobello *et al.* (2004) obtained a conception rate of 82.60 per cent in an oestrus induction trial using cabergoline at the rate of 5 µg/kg body weight.

Ajitkumar *et al.* (2005) reported a conception rate of 71.43 per cent in a preliminary trial using levothyroxine for induction of oestrus in bitches.

2.8. GESTATION LENGTH

Namsui (1999) recorded an average gestation length of 62.43 and 59.50 days from the first day of mating during natural cycle in Alsatian and Pomeranian bitches respectively.

In bitches in which oestrus was induced by the administration of leuprolide acetate or 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).

Gestation length calculated from the day of first breeding to whelping ranged from 59 to 68, 57 to 63 and 56 to 62 days respectively in large, medium and small breeds of dog (Asha, 2005). Deepthi (2007) recorded an average gestation length of 63.38 days in large breeds of dog.

2.9. LITTER SIZE

Namsui (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.

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.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. SURVEY ON BREEDING, FEEDING AND MANAGEMENT OF DOGS AND THE PROFILE OF THE OWNERS

A detailed survey on breeding, feeding and management of adult dogs and profile of dog owners was conducted in different parts of the State. A total of 1721 dogs (518 males and 1203 females) belonged to 817 owners were surveyed.

3.2. PREVALENCE, NATURE AND MAGNITUDE OF ANOESTRUS AMONG BITCHES

The prevalence, nature and magnitude of anoestrus among bitches were evaluated based on breeding details of 1203 female dogs surveyed. Nulliparous bitches which failed to exhibit oestrus even after the attainment of 18 months of age and parous bitches which failed to exhibit oestrus beyond six months postpartum were considered as anoestrous animals and were grouped into primary and secondary as per Feldman and Nelson (1996). The data obtained were compiled and analysed using standard statistical procedures.

3.3. HAEMATOLOGICAL, HORMONAL AND MINERAL PROFILE OF ANOESTROUS BITCHES

One hundred healthy anoestrous bitches of different breeds presented at University Veterinary Hospital, Kokkalai and Veterinary College Hospital, Mannuthy during the period from April 2005 to December 2007 formed the material for the present study. They were subjected to detailed clinico-gynaecological examination. The body condition of each bitch was evaluated and scored on a one to nine scale (Laflamme, 1997). In order to confirm anoestrus, they were subjected to exfoliative vaginal cytology and estimation of serum progesterone level. From among them 40 apparently healthy bitches with a

serum progesterone level less than 1 ng/ml were selected as the experimental animals for the oestrus induction trials.

Blood sample (10 ml) from saphenous vein was collected aseptically from all the experimental animals prior to the oestrus induction treatment, 5 ml was transferred to citrated vial for estimation of haematological parameters *viz.*, erythrocyte sedimentation rate (ESR), haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC) and differential leucocyte count (DC) and 5ml was allowed to clot for serum separation. The sera thus separated were stored at -20°C until further analysis. Later serum levels of prolactin, thyroxine, calcium, phosphorous, copper, iron, cobalt, zinc, manganese and total cholesterol were estimated.

3.4. INDUCTION OF OESTRUS

The experimental animals (40 anoestrous bitches) were randomly divided into four groups (treatment groups) *viz.*, Group I, II, III and IV, each consisting 10 animals and ten healthy bitches which exhibited natural oestrus formed Group V (Control Group).

Group I

Bromocriptine[1] at the rate of 50 µg/kg body weight was administered orally after food, once daily for 20 consecutive days to all the animals in this group and observed for side effects such as vomiting and anorexia (Concannon *et al.*, 1987). Ranitidine[2] at the rate of 2 mg/kg body weight, twice daily was administered orally to all the bitches which exhibited vomiting. All the bitches were closely monitored for a period of one month from the date of completion of

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[1] Sicriptin 1.25 mg tablets. Each tablet contains bromocriptine mesylate IP 1.435 mg equivalent to 1.25 mg bromocriptine base. Serum International Limited, Pune.

[2] Rantac 150 mg tablets. Each tablet contains ranitidine hydrochloride 168 mg equivalent to ranitidine 150 mg. J.B. Chemicals and Pharmaceuticals Ltd., Panoli.

the treatment for the onset of proestrual bleeding. Those bitches which responded to the treatment by evincing proestrual bleeding were observed for proestrual response and were subjected to exfoliative vaginal cytology to identify the best time for breeding based on anuclear cell index (Hewitt and England, 2000). The bitches were observed further for oestrual response and bred with apparently healthy, fertile male dogs repeatedly at 72 hour interval till the end of oestrus. Blood samples were collected from all the bitches which evinced proestrual bleeding on the first day of bleeding and on the day of first breeding for the estimation of ESR, Hb, PCV, TEC, TLC and DC. Serum levels of progesterone, prolactin, thyroxine, calcium, phosphorous, copper, iron, cobalt, zinc, manganese and total cholesterol were also estimated. The bitches which were bred during the induced oestrus were subjected to ultrasound scanning and abdominal palpation for pregnancy diagnosis between 20 and 25 days and 30 and 35 days post-breeding. All the bitches which were diagnosed pregnant were closely monitored periodically till whelping and the details pertaining to whelping and litter were collected. Bitches which failed to exhibit proestrual bleeding within one month post-treatment were again subjected to exfoliative vaginal cytology, haematological studies and estimation of serum hormonal and mineral profile. Two animals each from Group I, III and IV and one animal from Group II which failed to respond to the oestrus induction treatment were subjected to vaginoscopy to evaluate the functional status of vaginal epithelium and laparotomy to ascertain the functional status of the ovaries.

Group II

Cabergoline[1] at the rate of 5 µg/kg body weight was administered orally after food, once daily for 20 consecutive days and were closely followed up as in group I.

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[1] Caberlin 0.5 mg tablets. Each tablet contains cabergoline 0.5 mg. Sun Pharmaceutical Industries, Mumbai.

Group III

Levothyroxine[1] at the rate of 10 µg/kg body weight was administered orally once daily for 20 consecutive days and were closely followed up as in group I.

Group IV

Levothyroxine[1] at the rate of 5 µg/kg body weight was administered orally once daily for 20 consecutive days and were closely followed up as in group I.

Group V

All the bitches in this group were subjected to exfoliative vaginal cytology to identify the best time for breeding. Blood samples were collected on the first day of proestrus and on the day of first breeding and ESR, Hb, PCV, TEC, TLC and DC were estimated. Serum levels of progesterone, prolactin, thyroxine, calcium, phosphorous, copper, iron, cobalt, zinc, manganese and total cholesterol were also estimated. They were bred with apparently healthy, fertile male dogs and followed up as in Group I.

3.5. EXFOLIATIVE VAGINAL CYTOLOGY

3.5.1. Preparation of Vaginal Smear

Vaginal discharge was collected aseptically for cytological examination by the technique (Allen, 1986) described hereunder.

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[1] Eltroxin 100 µg tablets. Each tablet contains thyroxine sodium IP 100 µg. Glaxo Smithkline Pharmaceutical Ltd., Nashik.

Sterile glass pipette attached by a rubber adaptor to a 2 ml disposable polythene syringe was employed to aspirate the vaginal discharge. The animal was controlled in standing position and the pipette was carefully introduced through the vulva into the vagina, directing the pipette cranio-dorsally to the vestibule and at the vestibulo-vaginal junction it was redirected cranially to reach the anterior vagina. By gentle traction of the plunger of the syringe, the discharge was aspirated. In situations in which the discharge was scanty, a small quantity of sterile normal saline was infused into the vagina through glass pipette and then aspirated. A drop of the aspirated material was placed on a slide, a thin smear was prepared and air-dried. Two such smears were prepared each time.

3.5.2. Staining of the Vaginal Smear

The vaginal smears were stained using Wright-Giemsa's stain (Post, 1985).

3.5.2.1. Composition of Wright-Giemsa's stain:

Wright stain powder	300 mg
Giemsa stain powder	30 mg
Absolute methanol	100 ml

3.5.2.2. Procedure of staining

The slide was kept on a staining rack and poured the stain.

1. Allowed the stain to remain for 30 seconds (for fixation).
2. Poured a few drops of neutral water on to the slide and mixed it with the stain by blowing.
3. Allowed the stain-water mixture to act for 1-2 minutes.
4. Poured off the stain-water mixture, washed the slide in tap water, dried the smear and evaluated microscopically.

3.5.3. Cell Types

3.5.3.1. Epithelial Cells

The epithelial cells observed were categorized as superficial cells, intermediate cells and parabasal cells.

Superficial cells: Large polygonal cells with irregular or folded borders with or without nucleus were identified as superficial cells and based on the nuclear characteristics they were further divided into four types.

a. Large polygonal dead cells with irregular borders without any nucleus (anuclear keratinized cells).

b. Large polygonal cells with intact nuclear membrane.

c. Large polygonal cells with small nuclear remnants.

d. Large polygonal cells with pyknotic nuclei.

Intermediate cells: Intermediate cells included cells of varying sizes and types and represented all stages of maturation between parabasal and fully mature superficial cells. As they matured, they became more angular, enlarged and flattened. The relative size of the nucleus decreased as the cells matured. The cells observed were small and large intermediate types.

Parabasal cells: The smallest epithelial cells seen in smears which were round or oval in shape were identified as parabasal cells. The nucleus of parabasal cell occupied about 45 to 90 per cent of the cell.

Blood cells

The blood cells identified in smears were erythrocytes and neutrophils.

3.5.3.3. Metoestrum cells

Large intermediate vaginal epithelial cells that appeared to have one or more neutrophils contained within their cytoplasm were identified as metoestrum cells.

3.5.3.4. Foam cells

Parabasal and intermediate cells with obvious cytoplasmic vacuoles were identified as foam cells. .

3.5.4. Anuclear Cell Index

Anuclear cell index (ACI) was calculated based on vaginal cytological observations using the following formula

$$ACI = \frac{\text{Number of anuclear cells}}{\text{Total number of epithelial cells}} \times 100$$

3.6. SERUM PROGESTERONE, PROLACTIN AND THYROXINE ESTIMATION

3.6.1. Estimation of Progesterone

The serum progesterone level was estimated by radioimmunoassay using Progesterone RIA Kit[1] and the value obtained was expressed in ng/ml.

3.6.2. Estimation of Prolactin

The serum prolactin level was estimated by radioimmunoassay using Prolactin RIA Kit[2] and the value obtained was expressed in ng/ml.

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[1] BioMetallics, Princeton, NJ, USA
[2] Radim, Pomezia, Italia

3.6.3. Estimation of Thyroxine

Serum thyroxine level was estimated using Thyroxine Coated Tube RIA Kit[1] and the value obtained was expressed in μg/dl.

3.7. ESTIMATION OF CHOLESTEROL

The serum cholesterol level was estimated by CHOD-PAP methodology using Cholesterol Kit[2].

3.8. ESTIMATION OF SERUM MINERAL STATUS

The serum level of calcium, copper, iron, cobalt, zinc and manganese was estimated using Atomic Absorption Spectrophotometry[3]. The serum inorganic phosphorus level was estimated by phosphomolybdate methodology using Phosphorus Kit[4].

3.9. PREGNANCY DIAGNOSIS

The experimental bitches which were bred during the induced oestrus and the control bitches which were bred during the natural oestrus were subjected to pregnancy diagnosis by trans-abdominal ultrasound scanning (3.5 and 5 MHz probes) and abdominal palpation between 20 and 25 days and 30 and 35 days post-breeding.

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1 DSL-3200 ACTIVE Thyroxine Coated Tube Radioimmunoassay Kit, Diagnostic Systems Laboratories Inc., Texas.

2 Agappe Diagnostics Ltd. , Agappe Hills, Ernakulam, Kerala

3 Perkin Elmer Atomic Absorption Spectrophotometer-Model 3110

4 Agappe Diagnostics Ltd. , Agappe Hills, Ernakulam, Kerala

3.10. CONCEPTION RATE, GESTATION LENGTH, GESTATIONAL ACCIDENTS AND LITTER SIZE

All the animals which were diagnosed pregnant were followed up till whelping and the conception rate, gestation length, gestational accidents if any and litter size were recorded.

3.11. VAGINOSCOPY AND EXPLORATORY LAPAROTOMY

Two animals each from Group I, III and IV and one animal from Group II which failed to respond to the oestrus induction treatment were subjected to vaginoscopy to evaluate the functional status of vaginal epithelium and laparotomy to ascertain the ovarian status and recording of ovarian biometry.

3.12. STATISTICAL ANALYSIS

The data obtained were compiled and statistical analysis was carried out using Analysis of Variance (ANOVA) to find out significant difference among the five groups and pair-wise comparison was done by Duncan's multiple range test (Snedecor and Cochran, 1989).

RESULTS

4. RESULTS

4.1. BREEDING, FEEDING AND MANAGEMENT OF DOGS AND THE PROFILE OF THE OWNERS

Observations on feeding, management and breeding of dogs and the profile of their owners are summarized in Tables 1 to 7.

4.1.1. System of Dog Rearing

Perusal of data presented in Table 1 revealed that out of the 817 dog owners surveyed, 313 reared male, 657 reared female and 303 reared both male and female dogs. The total number of male dogs reared by 313 owners was 518 and the total number of female dogs reared by 657 owners was 1203.

The number of male and female dogs reared by individual owners ranged from 1 to 24 and 1 to 23 with an average of 1.64 and 1.83 respectively. Majority of the dog owners (78.27%) maintained only one male dog. The corresponding figure with respect to female dogs was 71.39 per cent.

Perusal of data on breed-wise details of dogs maintained by individual owners revealed that even though a number of dog breeds are there in our State, people preferred to rear only a few of them (Table 2 and Fig.1). Out of the 1721 dogs, 357 (20.74%) and 312 (18.13%) were German shepherd dogs and Labrador retrievers. Among the male dogs, 22.20 and 14.09 per cent were German shepherd dogs and Labrador retriever dogs respectively. With regard to female dogs, 20.11 and 19.87 per cent were German shepherd bitches and Labrador retriever bitches respectively. The other exotic breeds of dog commonly reared were Dachshund (11.39%), Rottweiler (9.12%), Spitz (5.52%), Doberman pinscher (3.89%), Pug (2.96%), Pomeranian (2.73%), Dalmatian (2.15%), Great Dane (2.09%), Basset

hound (1.28%) and Boxer (1.00%). Some exotic breeds of dog rarely encountered (4.36%) were Lhasa apso, Bull mastiff, Cocker spaniel, Golden retriever, Neopolitan mastiff, Tibetan spaniel, Pit bull terrier, Jack Russel terrier, Chowchow, Saint Bernard, Caravan hound and Weimaraner. The local non-descript dog reared was 14.64 per cent.

4.1.2. Utility of Dogs Reared

The percentage of dogs reared based on utility are summarized in Table 3. Out of the 1721 dogs, 67.17 per cent was maintained for non-commercial purposes *viz.* as pet and companion (47.47%), to guard house and premises (13.83%), as a hobby and stress buster (4.30%) and on compassionate grounds (1.57%). Only 16.68 per cent of dogs were reared for commercial purposes *viz.* for breeding and sale of pups (14.70%) and as stud dogs (1.98%). Out of the 1721 dogs, 16.15 per cent were reared for both commercial and non-commercial purposes.

4.1.3. Feeding and Management of Dogs

The feeding and management details of dogs are summarized in Table 4. Out of the 1721 dogs surveyed, 94.70 per cent were non-vegetarian and only 5.30 per cent were vegetarian. Majority of the dogs (58.98%) were fed with home made food. The percentage of dogs maintained exclusively on commercial dog food was only 8.77.

With respect to frequency of feeding, the percentage of dogs maintained by one meal per day was only 12.61. However, 49.33 per cent of dogs were fed twice daily and 38.06 per cent three or more times a day.

Deworming as per schedule was carried out in 74.60 per cent and once in a while in 22.49 per cent of dogs. The percentage of dogs which were not at all dewormed was quite low (2.91%).

Perusal of data pertaining to the vaccinations revealed that 45.03 per cent of dogs received prophylactic antirabies (PAR) vaccine as per schedule and 35.10 per cent once in a while. With respect to multi-component vaccine (MCV), the percentage of dogs received it as per schedule was 30.10 as against 40.15 per cent which received it once in a while. The percentage of dogs which failed to receive PAR vaccine and MCV was 19.87 and 29.75 respectively.

4.1.4. Breeding of Dogs

Perusal of data on breeding presented in Table 5 revealed that the percentage of bitches allowed to breed was 92.93. The remaining 7.07 per cent of bitches were not at all bred during their lifetime. The age at first breeding in majority of the bitches (57.85%) was between 1 to 2 years with an average of 16.30 months. The average number of oestrus periods and the average number of breedings per year in bitches, as reported by the owners were 2.14 ± 0.24 and 1.47 ± 0.32 respectively. The average duration of proestrual bleeding was 10.87 ± 1.24 days.

The identification of apt breeding time by observing external signs was followed in 70.39 per cent of bitches. The percentage of bitches in which the apt breeding time was identified based on the signs and veterinary advice was 29.61.

Majority (55.28%) of the bitches were bred by dogs owned by neighbours or friends of the owner. The percentage of bitches bred by commercial stud dogs was 30.32. Regarding the number of matings done in one oestrus, the percentage of bitches bred once, twice, three and more times was 26.65, 51.97 and 21.38 respectively. The period of acceptance ranged from 4 to 22 days with an average of 8.94 ± 2.18 days.

The incentive given for breeding majority of the bitches (61.27%) was one pup born in the litter. Paid breeding was practised only in 24.51 per cent of female dogs and the percentage of bitches bred free of cost was 14.22.

Pregnancy was confirmed by owners on observing the external signs alone in 82.02 per cent of bitches. Pregnancy confirmation based on external signs and veterinary examination was practised in 17.98 per cent of bitches.

Out of the 1118 bitches bred, failure of conception was reported in 211 (18.87%). The incidence of abortion, stillbirth and pseudopregnancy reported were 5.72, 15.56 and 13.69 per cent respectively. However, normal whelping was reported in 93.50 per cent of bitches. The incidence of assisted whelping and caesarean section was 5.18 and 1.32 per cent respectively. The average litter size was 6.94 ± 1.47.

Out of the 907 bitches whelped, problems were encountered during neonatal period in 126 (13.88%) and included agalactia / hypogalactia / mammitis (5.29%), cannibalism (2.65%), puppy fading syndrome (2.09%), puerperal tetany (1.98%) and rejection of young one by dam /poor mothering ability (1.87%).

4.1.5. Profile of Owners Who Maintained Dogs

The profile of owners of adult dogs surveyed is summarized in Table 6. Out of the 817 owners surveyed, 89.11 per cent were male and 10.89 per cent were female. Most of the owners (39.66%) were in the age group of 41 to 50 years and the percentage of them between the age of 21 and 30 was 25.95. With respect to education, the owners were with primary school (6.00%), upper primary school (7.10%), high school (43.08%), pre-degree/plus two (21.91%), degree level (15.06%) and professional (6.85%) qualification.

Data on experience in dog rearing revealed that majority (54.10%) of them were with experience up to 5 years, 23.99 per cent with experience between 6 and 10 years and 18.12 per cent with experience between 11 and 20 years. Only 3.79 per cent of the dog owners were with experience above 20 years.

4.1.6. Constraints in Rearing of Dogs

The constraints faced by dog owners are summarized in Table 7. The major constraints encountered were non-co-operation from family members (21.18%), complaints from neighbours (17.25%) and problems associated with waste disposal (10.65%). The percentage of owners who reported lack of dog care facilities when the family is away as the constraint was 9.18.

4.2. PREVALENCE, NATURE AND MAGNITUDE OF ANOESTRUS

The data pertaining to the prevalence, nature and magnitude of anoestrus are presented in Table 8 to 12. Among the 1203 female dogs, 134 (11.14%) were in the stage of anoestrus. The incidence of anoestrus was high in breeds such as Dachshund (18.18%), Labrador retriever (16.74%), Great Dane (16.67%), Dalmatian (15.38%) and Doberman pinscher (14.00%). It was lowest (2.44%) among local non-descript bitches (Fig.2 and 3).

4.2.1. Primary and Secondary Anoestrus

Perusal of data presented in Table 9 revealed that out of the 134 anoestrous bitches screened, 65.67 per cent was in primary anoestrus whereas the remaining 34.33 per cent was in secondary anoestrus.

4.2.2. Age of Anoestrous Bitches

The average age of bitches of various breeds with anoestrus are presented in Table 10. It was found to be 22.68 and 34.34 months respectively in bitches with primary and secondary anoestrus.

4.2.3. Duration of Secondary Anoestrus

The average duration of secondary anoestrus in various breeds of dog are presented in Table 11. It was found to be longest in Dachshund (19 months) and shortest in Doberman pinscher (6.23 months). The overall average duration of secondary anoestrus was 11.71 months.

4.2.4. Body Condition Score of Anoestrous Bitches

The breed-wise body condition score of 100 anoestrous bitches selected at random are summarized in Table 12. Perusal of data revealed that 42 per cent of the anoestrous bitches were with body score five, 46 per cent with body score more than five and 12 per cent with body score less than five (Plates 1 to 4).

4.3. HAEMATOLOGICAL, HORMONAL, MINERAL AND CHOLESTEROL PROFILE OF ANOESTROUS BITCHES

The haematological, hormonal, mineral and cholesterol profile of 40 anoestrous bitches selected at random for the oestrous induction trials are summarized in Tables 13 to 15.

4.3.1. Haematological Profile

Perusal of data presented in Table 13 revealed that the mean haemoglobin level in 40 anoestrous bitches selected for oestrus induction trials was 8.80 ± 0.28 g per cent. The mean ESR and PCV values were 2.75 ± 0.24 mm/hour and 25.53 ±

0.77 per cent respectively. The TEC and TLC in anoestrous bitches were 3.26 ± 0.10 millions/mm^3 and 14619.50 ± 502.81 cells/mm^3 respectively. The mean percentage of neutrophil, lymphocyte, eosinophil and monocyte counts were 66.99 ± 2.01, 30.22 ± 1.33, 1.79 ± 0.18 and 1.00 ± 0.10 per cent respectively. The mean MCV, MCHC and MCH values were 79.61 ± 2.53 fl, 34.42 ± 0.91 g per cent and 27.38 ± 0.88 pg respectively.

4.3.2. Hormonal Profile

Data presented in Table 14 revealed that the mean serum progesterone level in anoestrous bitches was 0.57 ± 0.03 ng/ml. The mean serum prolactin and thyroxine levels were 0.75 ± 0.05 ng/ml and 1.80 ± 0.06 µg/dl respectively.

4.3.3. Mineral Profile

Perusal of the serum mineral profile of anoestrous bitches summarized in Table 15 revealed that the mean value of serum calcium, phosphorus, iron, copper, cobalt, zinc and manganese were 8.94 ± 0.24, 3.48 ± 0.12, 0.156 ± 0.021, 0.157 ± 0.025, 0.021 ± 0.0239, 0.309 ± 0.031 and 0.0038 ± 0.0243 mg per cent respectively.

4.3.4. Cholesterol Profile

The mean serum cholesterol level in the anoestrous bitches was found to be 223.13 ± 10.12 mg per cent.

4.4. INDUCTION OF OESTRUS

4.4.1. Proestrus and Oestrus Response

Data presented in Table 16 revealed the proestrus and oestrus response in treatment (Groups I, II, III and IV) and control (Group V) groups. Out of 10 animals

treated in each group, five in Group I (50%), nine in Group II (90%), eight in Group III (80%) and seven in Group IV (70%) evinced proestrual bleeding (Fig.4).

The mean treatment onset to proestrus in treatment groups I, II, III and IV was 28.00 ± 3.39, 13.44 ± 3.12, 24.50 ± 3.18 and 33.00 ± 2.21 days respectively. The treatment onset to proestrus in Group II was significantly lower ($P<0.05$) when compared with other groups (Fig.5).

The average duration of proestrus in the treatment groups was 9.80 ± 0.86, 10.11 ± 0.68, 11.25 ± 0.88 and 10.71 ± 0.68 days respectively. In the control group, the average duration of proestrus was 9.70 ± 0.42 days.

The average duration of oestrus in the four treatment groups and the control group were 7.60 ± 0.24, 8.00 ± 0.29, 8.50 ± 0.63, 7.85 ± 0.46 and 8.00 ± 0.39 days respectively.

The data pertaining to the intensity of proestrual bleeding, vulval oedema, interest towards male and tail deviation reflex in induced and natural oestrus are summarized in Table 17. Among the animals which responded to the treatment, 40, 22.22, 25 and 28.57 per cent in the treatment groups respectively exhibited high intensity of proestrual bleeding. High intensity of vulval oedema was observed in 40, 33.33, 25 and 14.28 per cent of animals evinced proestrus in the treatment groups. With respect to the interest towards male, the corresponding figures were 40, 44.44, 37.50 and 28.57 per cent respectively. Tail deviation reflex was present in 80, 88.89, 75 and 71.43 per cent of the animals responded to the treatment. In the control group, high intensity of proestrual bleeding and vulval oedema, interest towards male and tail deviation reflex were exhibited by 30, 30, 50 and 70 per cent of animals respectively.

4.4.2. Vaginal Cytology and Anuclear Cell Index During Various Stages of Oestrous Cycle in Treated and Control Animals

The most preponderant cell type encountered on vaginal cytology during anoestrus was parabasal with a few small intermediate cells. During early proestrus, small and large intermediate cells were observed. Late proestrus was characterized by the presence of superficial cells. During oestrus, more than 80 per cent of the cells were anuclear keratinized type. Metoestrus was characterized by the presence of metoestrus cells, foam cells and a few neutrophils. Microfilaria of *Dirofilaria repens* could be detected on vaginal cytology during proestrus in a pug. The anuclear cell index during various stages of the oestrous cycle in treated and control animals are presented in Table 18. Statistically significant difference could not be detected with respect to anuclear cell index between the treatment groups and control group (Plates 5 to 17).

4.4.3. Side Effects Observed During Drug Therapy for Induction of Oestrus

The side effects observed during drug therapy for induction of oestrus included nausea, vomiting, dehydration and anorexia. Fifty per cent of the animals treated with bromocriptine and 10 per cent of the animals treated with cabergoline exhibited nausea and vomiting. Among the five animals exhibited nausea and vomiting in the bromocriptine treated group, three showed dehydration and anorexia. None of the animals treated with thyroxine exhibited side effects.

4.4.4. Serum Progesterone, Prolactin and Thyroxine Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

Serum progesterone, prolactin and thyroxine levels during various stages of the oestrous cycle in treated and control animals are summarized in Tables 19 to 21.

4.4.4.1. Progesterone profile: Perusal of data presented in Table 19 revealed that the average serum progesterone level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly within and between the treatment groups and the control group (Fig.6).

4.4.4.2. Prolactin Profile: Perusal of data presented in Table 20 revealed that the average serum prolactin level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly with in and between the treatment groups and the control group (Fig.7).

4.4.4.3. Thyroxine Profile: Perusal of data presented in Table 21 revealed that the average serum thyroxine level during anoestrus and on the first day of proestrus did not differ significantly within and between the treatment groups and the control group. Serum thyroxine level on the day of first breeding recorded statistically significant difference ($P<0.05$) between the treatment and control groups, the lowest being 1.56 ± 0.18 µg/dl in Group I and the highest being 2.10 ± 0.13 µg/dl in Group IV (Fig.8).

4.4.5. Haematological Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

The haematological profile during various stages of the oestrous cycle in the treated and control animals are summarized in Tables 22 to 24. The ESR and PCV values during various stages of the oestrous cycle did not exhibit statistically significant difference between the treatment and control groups. The hemoglobin level on the day of first breeding recorded statistically significant difference ($P<0.05$) between treatment and control groups, the lowest being 7.82 ± 0.18 g per cent in Group II and highest being 9.29 ± 0.39 g per cent in Group IV. With regard to TEC, statistically significant difference ($P<0.05$) was observed on the first day of proestrus and on the day of first breeding between treatment and control groups.

Perusal of the MCV, MCHC and MCH values during various stages of the oestrous cycle in the treated and control animals revealed that statistically significant difference ($P<0.05$) in MCV and MCHC values on the day of first breeding was recorded between groups. No statistically significant difference could be observed with regard to MCH values within and between the different groups.

Data on leucogram during the various stages of the oestrous cycle in the treated and control animals revealed that statistically significant difference ($P<0.05$) in TLC on the day of first breeding was observed between groups. Perusal of data on DC revealed that statistically significant difference could not be observed with regard to neutrophils, lymphocytes and monocytes. On the contrary, statistically significant difference ($P<0.05$) in eosinophil count was observed on the first day of proestrus between the treatment and control groups.

4.4.6. Serum Mineral Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

The serum mineral profile during various stages of the oestrous cycle in the treatment and control groups are summarized in Tables 25 to 27.

4.4.6.1 Calcium and Phosphorus: The serum calcium level during anoestrus, on the first day of proestrus and on the day of first breeding did not reveal statistically significant difference within and between groups. On the other hand, the serum phosphorus level on the first day of proestrus and on the day of first breeding in the treatment groups showed statistically significant difference ($P<0.05$) when compared with control group.

4.4.6.2. Iron, Copper and Cobalt: The serum iron and cobalt level during various stages of the oestrous cycle in the treatment and control groups did not differ

significantly. On the other hand statistically significant difference ($P<0.05$) existed between the treatment and control groups with respect to the serum copper level.

4.4.6.3 Zinc and Manganese: The serum zinc and manganese level during various stages of the oestrous cycle in the treatment and control groups did not reveal any significant difference within and between the treatment groups and the control group.

4.4.7. Serum Cholesterol Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

The serum cholesterol level during various stages of the oestrous cycle in the treated and control animals are presented in Table 28. Statistically significant difference could not be detected within and between the treatment groups and the control group (Fig.9).

4.4.8. Pregnancy Diagnosis

The results of pregnancy diagnosis conducted by trans-abdominal ultrasound scanning and abdominal palpation between 20 and 25 days and 30 and 35 days are summarized in Table 29. The accuracy of pregnancy diagnosis done between 20 and 25 days post-breeding by trans-abdominal ultrasound scanning and abdominal palpation was found to be 88.89 and 66.67 per cent respectively. On the other hand, on diagnosing pregnancy by trans-abdominal ultrasound scanning and abdominal palpation between 30 and 35 days post-breeding, the accuracy increased to 100 and 88.89 per cent respectively (Plates 18 to 20).

4.4.9. Conception Rate

Perusal of data presented in Table 30 revealed that the conception rate in relation to the number of animals responded to oestrus induction in the different groups were 80.00, 77.78, 62.50 and 57.14 per cent respectively. However, the

respective overall conception rate in relation to the number of animals subjected to oestrus induction trials in the treatment groups were 40, 70, 50 and 40 per cent only. Out of the 10 animals of the control group which were bred during the natural oestrus, seven (70%) conceived.

4.4.10. Gestation Length, Litter Size and Sex Ratio in Treated and Control Animals

The gestation length, litter size and sex ratio in treatment and control groups are presented in Table 31. Statistically significant difference could not be detected in gestation length, litter size and sex ratio between the treatment and control groups.

4.4.11. Haematological, Hormonal, Mineral and Cholesterol Profile of Anoestrous Bitches Which Failed to Oestrus Induction Treatment

Out of the 40 bitches subjected to oestrus induction treatment, 11 (27.5%) failed to respond. The percentage of treated animals failed to respond in the treatment groups were 50, 10, 20 and 30 respectively (Table 16).

The haematological, hormononal and mineral profile of anoestrous bitches which failed to oestrus induction treatment are summarized in Tables 32 to 34.

4.4.11.1. Haematological Profile: The mean ESR, Hb and PCV during the anoestrus stage were 3.18 ± 0.18 mm/h, 8.61 ± 0.35 g per cent and 26.04 ± 0.83 per cent respectively. The corresponding values one month post-treatment were 3.82 ± 0.38 mm/h, 9.50 ± 0.16 g per cent and 29.55 ± 0.55 per cent respectively.

The total erythrocyte and leucocyte counts during the anoestrus stage were 3.21 ± 0.10 millions/mm^3 and 14025.45 ± 710.33 cells/mm^3 respectively. The corresponding values one month post-treatment were 3.94 ± 0.05 million/mm^3 and 10362.73 ± 474.72 cells/mm^3 respectively. During the anoestrus stage the

differential leucocyte counts with respect to neutrophils, lymphocytes, eosinophils and monocytes were 65.18 ± 1.53, 32.18 ± 1.48, 1.45 ± 0.21 and 1.18 ± 0.23 per cent respectively. The corresponding values one month post-treatment were 68.73 ± 0.69, 28.18 ± 0.54, 1.82 ± 0.18 and 1.18 ± 0.26 per cent respectively.

The mean MCV (gl), MCHC (g %) and MCH (pg) during the anoestrus stage and one month post-treatment were 81.60 ± 2.49, 32.94 ± 0.70 and 26.88 ± 0.98 and 75.17 ± 1.64, 32.25 ± 0.67 and 24.18 ± 0.48 respectively.

4.4.11.2. Hormonal Profile: Data presented in Table 33 revealed that the serum progesterone levels during anoestrus and one month post-treatment in bitches failed to respond to oestrus induction treatment were 0.52 ± 0.07 and 0.61 ± 0.03 ng/ml. With respect to prolactin, the corresponding values were 0.66 ± 0.08 and 0.84 ± 0.06 ng/ml respectively. The serum thyroxine levels during anoestrus and one month post-treatment were 1.90 ± 0.16 and 2.06 ± 0.09 µg/dl respectively.

4.4.11.3. Mineral Profile: Perusal of data on serum mineral profile during the anoestrus stage and one month post-treatment, presented in Table 34 revealed that the mean serum calcium and phosphorus levels were 9.11 ± 0.28 and 3.58 ± 0.23 mg per cent and 9.21 ± 0.14 and 3.84 ± 0.07 mg per cent respectively. The mean serum iron, copper and cobalt level during anoestrus were 0.155 ± 0.007, 0.157 ± 0.033 and 0.0029 ± 0.008 mg per cent respectively. The corresponding values one month post-treatment were 0.148 ± 0.003, 0.133 ± 0.004 and 0.0025 ± 0.0005 mg per cent respectively. The serum zinc and manganese levels during anoestrus and one month post-treatment were 0.018 ± 0.0017 and 0.346 ± 0.071 and 0.020 ± 0.0009 and 0.312 ± 0.024 mg per cent respectively.

4.4.11.4. Cholesterol Profile: The mean serum cholesterol level during the anoestrus stage and one month post-treatment were 221.18 ± 10.72 and 224.13 ± 9.22 mg per cent respectively.

4.4.12. Vaginoscopy and Exploratory Laparotomy

The appearance of anterior vagina on vaginoscopy during anoestrus, induced proestrus and oestrus is presented in Plates 12 to 17. On performing vaginoscopy in seven animals which failed to respond to oestrus induction trials, all of them were found to be continuing in the stage of anoestrus.

The ovarian biometry recorded during anoestrus on exploratory laparotomy in seven animals which failed to respond to oestrus induction trials is presented in Table 35. The ovaries of all the animals were found to be in the anoestrus state (Plates 21 to 23).

Table 1. Number of male and female dogs reared by individual owners

No. of owners interviewed			817
Number of dogs reared (%)	Male	Female	Total
	518 (30.10)	1203 (69.90)	1721
Number of dogs reared by individual owners	Number of owners (%)		
	With male dogs	With female dogs	
1	245 (78.27)	469 (71.39)	
2-5	56 (17.89)	156 (23.74)	
6-10	7 (2.24)	21 (3.20)	
11-20	4 (1.28)	9 (1.37)	
Above 20	1 (0.32)	2 (0.30)	
Total	313	657	

Table 2. Breed-wise details of dogs reared by individual owners

Breed	Number (%) of dogs reared		
	Male	Female	Total
German shepherd dog	115 (22.20)	242 (20.11)	357 (20.74)
Labrador retriever	73 (14.09)	239 (19.87)	312 (18.13)
Dachshund	64 (12.36)	132 (10.97)	196 (11.39)
Rottweiler	40 (7.72)	117 (9.73)	157 (9.12)
Spitz	29 (5.60)	66 (5.49)	95 (5.52)
Doberman pinscher	17 (3.28)	50 (4.16)	67 (3.89)
Pug	7 (1.35)	44 (3.66)	51 (2.96)
Pomeranian	21 (4.05)	26 (2.16)	47 (2.73)
Dalmatian	11 (2.12)	26 (2.16)	37 (2.15)
Great Dane	12 (2.32)	24 (2.00)	36 (2.09)
Basset hound	3 (0.58)	19 (1.58)	22 (1.28)
Boxer	7 (1.35)	10 (0.83)	17 (1.00)
Other exotic breeds	31 (5.99)	44 (3.65)	75 (4.36)
Non-descript	88 (16.99)	164 (13.63)	252 (14.64)
TOTAL	518	1203	1721

Table 3. Utility of dogs reared by owners surveyed

1.	Non-commercial		Number (%) of dogs
	a.	As pet and companion	817 (47.47)
	b.	To guard house and premises	238 (13.83)
	c.	As a hobby and stress buster	74 (4.30)
	d.	On compassionate grounds	27 (1.57)
		Total	1156 (67.17)
2.	Commercial		
	a.	For breeding and sale of pups	253 (14.70)
	b.	As stud dog	34 (1.98)
		Total	287 (16.68)
3.	Both commercial and non-commercial		278 (16.15)
	Total		1721

Table 4. Details of feeding and management of dogs

Sl. No	Parameter			No. (%)
1.	Feeding habit			
	a.	Vegetarian		91 (5.30)
	b.	Non-vegetarian		1630 (94.70)
2.	Type of food			
	a.	Home made food		1015 (58.98)
	b.	Commercial dog food		151 (8.77)
	c.	Combination of a & b		555 (32.25)
3.	Frequency of feeding			
	a.	1 time daily		217 (12.61)
	b.	2 times daily		849 (49.33)
	c.	3 times or more daily		655 (38.06)
4.	Deworming			
	a.	As per schedule		1284 (74.60)
	b.	Once in a while		387 (22.49)
	c.	Not at all done		50 (2.91)
5.	Vaccination			
			PAR	MCV
	a.	As per schedule	775 (45.03)	518 (30.10)
	b.	Once in a while	604 (35.10)	691 (40.15)
	c.	Not at all done	342 (19.87)	512 (29.75)

Table. 5. Breeding details of female dogs

	Parameter		Number (%)
1.	Total number of adult female dogs surveyed		1203
2.	Number of female dogs in which breeding was		
	a. allowed		1118 (92.93)
	b. not allowed		85 (7.07)
3.	Age at first breeding		
	a.	<1 y	244 (20.28)
	b.	1-2 y	696 (57.85)
	c.	>2 y	178 (14.80)
4.	Average number of oestrus periods/year		2.14 ± 0.24
5.	Average number of breedings/year		1.47 ± 0.32
6.	Average duration of proestrual bleeding		10.87 ± 1.24 days
7.	Identification of apt time for breeding		
	a.	Based on external signs	787 (70.39)
	b.	Based on external signs and veterinary advice	331 (29.61)
8.	Bred by		
	a.	Own male	161 (14.40)
	b.	Neighbour's/friend's male	618 (55.28)
	c.	Commercial stud dogs	339 (30.32)

9.	Number of matings done/oestrus		
	a.	One	298 (26.65)
	b.	Two	581 (51.97)
	c.	Three and above	239 (21.38)
10.	Average period of acceptance of male		8.94 ± 2.18 days
11	Incentive for breeding		
	a.	Free of cost	159 (14.22)
	b.	One pup from the litter born	685 (61.27)
	c.	Cash	
		1. < Rs.1000	164 (14.67)
		2. Rs.1000 to 3000	76 (6.80)
		3. > Rs.3000	34 (3.04)
		Total	274 (24.51)
12.	Method of pregnancy confirmation		
	a.	By external signs	917 (82.02)
	b.	By signs and veterinary examination	201 (17.98)
13.	Failure of conception		211 (18.87)
14.	Gestational accidents		
	a.	Abortion	64 (5.72)
	b.	Stillbirth	174 (15.56)
	c.	Pseudopregnancy	153 (13.69)
	d.	Others	22 (1.97)

14.		Details of whelping	
	a.	Normal whelping	848 (93.50)
	b.	Assisted whelping	47 (5.18)
	c.	Caesarean section	12 (1.32)
15.		Average litter size	6.94 ± 1.47
16.	Problems encountered during neonatal period		
	a.	Rejection of young ones by dam/poor mothering ability	17 (1.87)
	b.	Agalactia / hypogalactia / mammitis	48 (5.29)
	c.	Puppy fading syndrome	19 (2.09)
	d.	Cannibalism	24 (2.65)
	e.	Puerperal tetany	18 (1.98)
		Total	126 (13.88)

Table 6. Profile of owners of adult dogs

Owner's profile		Number (%) of owners
Sex	Male	728 (89.11)
	Female	89 (10.89)
Age (years)	<20	17 (2.08)
	21 - 30	212 (25.95)
	31 - 40	146 (17.87)
	41 - 50	324 (39.66)
	51 - 60	92 (11.26)
	>60	26 (3.18)
Educational qualification	Primary	49 (6.00)
	Upper primary	58 (7.10)
	High school	352 (43.08)
	Pre-degree/Plus two	179 (21.91)
	Degree	123 (15.06)
	Professional	56 (6.85)
Experience in dog rearing	Up to 5 years	442 (54.10)
	6 to 10 years	196 (23.99)
	11 to 20 years	148 (18.12)
	Above 20 years	31 (3.79)

Table 7. Constraints in rearing of dogs

Sl. No.	Constraints in rearing of dogs	Number (%)
1.	Non-co-operation from family	173 (21.18)
2.	Complaint from neighbours	141 (17.25)
3.	Waste disposal problem	87 (10.65)
4.	Lack of dog care facilities when the family is away	75 (9.18)
5.	High maintenance cost	68 (8.32)
6.	Lack of sufficient space	64 (7.83)
7.	Lack of time to spare	54 (6.61)
8.	Difficulty to maintain hygiene in homestead	44 (5.39)
9.	Lack of timely veterinary care	42 (5.39)
10.	Family health hazards	37 (4.53)
11.	Difficulty in sale/disposal of pups	32 (3.92)
	Total	817

Table 8. Prevalence of anoestrus among various breeds of dog

Breed	Number of female dogs	
	Surveyed	Anoestrus (%)
German shepherd dog	242	27 (11.16)
Labrador retriever	239	40 (16.74)
Dachshund	132	24 (18.18)
Rottweiler	117	10 (8.55)
Spitz	66	6 (9.09)
Doberman pinscher	50	7 (14.00)
Pug	44	2 (4.55)
Pomeranian	26	2 (7.69)
Dalmatian	26	4 (15.38)
Great Dane	24	4 (16.67)
Basset hound	19	1 (5.26)
Other exotic breeds	54	3 (6.82)
Non-descript	164	4 (2.44)
TOTAL	1203	134 (11.14)

Table 9. Breed-wise incidence of primary and secondary anoestrus in bitches

Breed	Number of anoestrous bitches		
	Primary anoestrous	Secondary anoestrous	Total
German shepherd dog	18	9	27
Labrador retriever	26	14	40
Dachshund	16	8	24
Rottweiler	5	5	10
Spitz	4	2	6
Doberman pinscher	4	3	7
Pug	1	1	2
Pomeranian	1	1	2
Dalmatian	4	0	4
Great Dane	3	1	4
Basset hound	1	0	1
Other exotic breeds	2	1	3
Non-descript	3	1	4
TOTAL	88 (65.67%)	46 (34.33%)	134

Table 10. Average age of anoestrous bitches

Breed	Average age of anoestrous bitch (months)	
	Primary anoestrus	Secondary anoestrus
German shepherd dog	21.06	37.71
Labrador retriever	22.43	32.62
Dachshund	28.67	39.43
Rottweiler	23.00	24.83
Spitz	19.22	39.00
Doberman pinscher	19.43	48.00
Pug	18.00	33.00
Dalmatian	20.00	--
Pomeranian	21.22	42.56
Great Dane	24.00	39.22
Basset hound	24.01	--
Other exotic breeds	22.12	40.34
Non descript	18.00	37.34
Overall	22.68	34.34

Table 11. Average duration of secondary anoestrus

Breed	Number of animals	Average duration of secondary anoestrus (months)
German shepherd dog	9	10.71
Labrador retriever	14	9.31
Dachshund	8	19.00
Rottweiler	5	10.67
Spitz	2	12.50
Doberman pinscher	3	6.23
Pug	1	10.50
Pomeranian	1	10.54
Great Dane	1	13.54
Other exotic breeds	1	11.12
Non-descript	1	11.21
Overall	46	11.71

Table 12. Breed-wise body condition score of 100 selected anoestrous bitches

Breed	Number of bitches with body condition score								
	1	2	3	4	5	6	7	8	9
German shepherd dog	--	--		1	12	9	1	--	--
Labrador retriever	--	--		2	8	22	3	1	--
Dachshund	--	--		6	7		3	--	--
Rottweiler	--	--			7	4		--	--
Pug	--	--			3		1	--	--
Dalmatian	--	--	1	1	2			--	--
Spitz	--	--			1			--	--
Basset hound	--	--					1	--	--
Bull Mastiff	--	--				1		--	--
Doberman pinscher	--	--			1			--	--
Great Dane	--	--			1			--	--
Non-descript				1					
Total			1	11	42	36	9	1	

Table 13. Haematologial profile of anoestrous bitches (Mean ± SE)

Sl. No.	Parameter		Mean ± SE
1.	ESR (mm/h)		2.75 ± 0.24
2.	Hb (g %)		8.80 ± 0.28
3.	PCV (%)		25.53 ± 0.77
4.	TEC (millions/mm^3)		3.26 ± 0.10
5.	TLC (cells/mm^3)		14619.50 ± 502.81
6.	DC	a. Neutrophils (%)	66.99 ± 2.01
		b. Lymphocytes (%)	30.22 ± 1.33
		c. Eosinophils (%)	1.79 ± 0.18
		d. Monocytes (%)	1.00 ± 0.10
7.	MCV (fl)		79.61 ± 2.53
8.	MCHC (g %)		34.42 ± 0.91
9.	MCH (pg)		27.38 ± 0.88

Table 14. Serum hormonal profile of anoestrous bitches (Mean ± SE)

Sl. No.	Hormone	Mean ± SE
1.	Progesterone (ng/ml)	0.57 ± 0.03
2.	Prolactin (ng/ml)	0.75 ± 0.05
3.	Thyroxine (µg/dl)	1.80 ± 0.06

Table 15. Serum mineral profile of anoestrous bitches (Mean ± SE)

Sl. No.	Parameter	Mean ± SE (mg %)
1.	Calcium	8.94 ± 0.24
2.	Phosphorus	3.48 ± 0.12
3.	Iron	0.156 ± 0.021
4.	Copper	0.157 ± 0.025
5.	Cobalt	0.021 ± 0.0239
6.	Zinc	0.309 ± 0.031
7.	Manganese	0.0038 ± 0.0243

Table 16. Proestrus and oestrus response to oestrus induction treatment

Group	Number of animals treated	Number (%) of animals		Treatment onset to proestrus (days)	Duration of proestrus (days)	Duration of oestrus (days)
		Evinced proestrus and oestrus	Not responded			
I	10	5 (50)	5 (50)	28.00 ± 3.39^{b}	9.80 ± 0.86	7.60 ± 0.24
II	10	9 (90)	1 (10)	13.44 ± 3.12^{a}	10.11 ± 0.68	8.00 ± 0.29
III	10	8 (80)	2 (20)	24.50 ± 3.18^{b}	11.25 ± 0.88	8.50 ± 0.63
IV	10	7 (70)	3 (30)	33.00 ± 2.21^{b}	10.71 ± 0.68	7.85 ± 0.46
Control	10	NA	NA	NA	9.70 ± 0.42	8.00 ± 0.39

Figures bearing different superscripts differ significantly ($P<0.05$)

Table 17. Intensity of proestrual bleeding, vulval oedema, interest towards male and tail deviation reflex in induced and natural oestrus

Stage	Parameters	Score	Number (%) of animals				
			Group I (n=5)	Group II (n=9)	Group III (n=8)	Group IV (n=7)	Control (n=10)
Proestrus	Intensity of proestrual bleeding	High	2 (40)	2 (22.22)	2 (25.00)	2 (28.57)	3 (30)
		Medium	2 (40)	6 (66.67)	5 (62.50)	3 (42.86)	5 (50)
		Low	1 (20)	1 (11.11)	1 (12.50)	2 (28.57)	2 (20)
	Intensity of vulval oedema	High	2 (40)	3 (33.33)	2 (25.00)	1 (14.28)	3 (30)
		Medium	3 (60)	5 (55.56)	4 (50.00)	3 (42.86)	7 (70)
		Low	--	1 (11.11)	2 (25.00)	3 (42.86)	--
Oestrus	Interest towards male	High	2 (40)	4 (44.44)	3 (37.50)	2 (28.57)	5 (50)
		Medium	3 (60)	5 (55.56)	5(62.50)	4 (57.15)	4 (40)
		Low	--	--	--	1 (14.28)	1 (10)
	Tail deviation reflex	Present	4 (80)	8 (88.89)	6 (75.00)	5 (71.43)	7 (70)
		Absent	1 (20)	1 (11.11)	2 (25.00)	2 (28.57)	3 (30)

Table 18. Anuclear cell index during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Group	During anoestrus	First day of proestrus	First day of oestrus	Day of first breeding
I	9.00 ± 1.41	23.60 ± 1.33	83.60 ± 4.39	92.20 ± 1.28
II	9.78 ± 1.02	19.67 ± 1.42	83.78 ± 1.78	92.67 ± 1.07
III	10.50 ± 0.87	21.25 ± 1.71	87.88 ± 1.44	95.75 ± 1.00
IV	12.14 ± 0.96	19.86 ± 1.14	83.57 ± 1.65	93.14 ± 1.14
V	NA	21.50 ± 1.14	79.90 ± 0.50	92.60 ± 1.29

Table 19. Serum progesterone level (ng/ml) during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Group	During anoestrus	First day of proestrus	Day of first breeding
I	0.61 ± 0.053	0.58 ± 0.055	6.45 ± 0.94
II	0.53 ± 0.057	0.60 ± 0.098	5.32 ± 0.19
III	0.64 ± 0.036	0.64 ± 0.032	5.74 ± 0.28
IV	0.59 ± 0.041	0.60 ± 0.051	5.42 ± 0.42
V	--	0.61 ± 0.053	5.79 ± 0.59

Table 20. Serum prolactin level (ng/ml) during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Group	During anoestrus	First day of proestrus	Day of first breeding
I	0.75 ± 0.22	0.56 ± 0.14	0.44 ± 0.93
II	0.90 ± 0.11	0.70 ± 0.078	0.45 ± 0.076
III	0.77 ± 0.088	0.67 ± 0.077	0.50 ± 0.063
IV	0.68 ± 0.095	0.57 ± 0.056	0.40 ± 0.045
V	--	0.56 ± 0.072	0.34 ± 0.054

Table 21. Serum thyroxine level (µg/dl) during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Group	During anoestrus	First day of proestrus	Day of first breeding
I	1.60 ± 0.18	1.59 ± 0.17	1.56 ± 0.18^{a}
II	1.94 ± 0.11	1.99 ± 0.10	2.03 ± 0.096^{b}
III	1.60 ± 0.11	1.80 ± 0.10	1.96 ± 0.078^{b}
IV	1.81 ± 0.11	1.99 ± 0.14	2.10 ± 0.13^{b}
V	--	1.84 ± 0.10	1.85 ± 0.094^{ab}

Figures bearing different superscripts differ significantly ($P<0.05$)

Table 22. Haemogram during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter		Group				
		I	II	III	IV	V
ESR mm/h	During anoestrus	2.30 ± 0.20	2.22 ± 0.52	3.50 ± 0.96	1.86 ± 0.26	--
	First day of proestrus	3.20 ± 0.20	2.22 ± 0.28	3.25 ± 0.70	3.14 ± 1.06	3.43 ± 0.54
	Day of first breeding	3.00 ± 0.00	2.22 ± 0.15	3.25 ± 0.59	2.86 ± 0.55	3.50 ± 0.34
Hb g %	During anoestrus	8.66 ± 0.23	7.89 ± 0.21	9.55 ± 0.66	9.51 ± 0.48	--
	First day of proestrus	8.74 ± 0.19	7.96 ± 0.17	9.56 ± 0.52	9.21 ± 0.43	8.39 ± 0.45^{ab}
	Day of first breeding	8.92 ± 0.17^{b}	7.82 ± 0.18^{a}	9.28 ± 0.55^{b}	9.29 ± 0.39^{b}	8.33 ± 0.19^{ab}
PCV %	During anoestrus	25.00 ± 0.77	26.00 ± 0.60	26.63 ± 1.46	26.14 ± 1.50	--
	First day of proestrus	25.40 ± 0.51	24.11 ± 0.42	27.70 ± 0.96	26.71 ± 1.23	26.12 ± 0.92
	Day of first breeding	25.80 ± 0.58	24.44 ± 0.29	26.25 ± 0.98	26.29 ± 0.92	25.00 ± 0.45
TEC $10^6/mm^3$	During anoestrus	3.00 ± 0.063	2.70 ± 0.075	3.94 ± 0.091	3.44 ± 0.220	--
	First day of proestrus	3.06 ± 0.024^{ab}	2.71 ± 0.056^{a}	3.80 ± 0.080^{c}	3.26 ± 0.250^{b}	2.96 ± 0.078^{ab}
	Day of first breeding	3.02 ± 0.037^{ab}	2.83 ± 0.065^{a}	3.75 ± 0.087^{c}	3.24 ± 0.215^{b}	2.94 ± 0.099^{ab}

ESR- Erythrocyte sedimentation rate Hb- Haemoglobin PCV- Packed cell volume TEC- Total erythrocyte count

Figures bearing different superscripts in each row differ significantly ($P<0.05$)

Table 23. MCV, MCHC and MCH during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter		Group				
		I	II	III	IV	V
MCV (fl)	During anoestrus	83.52 ± 3.44	87.52 ± 1.83	67.85 ± 4.12	76.54 ± 3.17	--
	First day of proestrus	83.03 ± 1.83	89.12 ± 1.72	71.22 ± 2.81	83.42 ± 3.28	86.46 ± 2.01
	Day of first breeding	85.41 ± 1.34^{b}	86.58 ± 1.96^{b}	70.29 ± 3.26^{a}	82.50 ± 4.12^{b}	85.57 ± 1.98^{b}
MCHC (g %)	During anoestrus	34.67 ± 0.55	33.37 ± 0.60	35.61 ± 0.86	36.58 ± 1.12	--
	First day of proestrus	34.43 ± 0.71	33.02 ± 0.61	35.26 ± 1.04	34.52 ± 0.67	34.12 ± 0.72
	Day of first breeding	34.60 ± 0.50^{b}	31.98 ± 0.50^{a}	35.15 ± 1.20^{b}	35.40 ± 1.22^{b}	33.30 ± 0.28^{ab}
MCH (pg)	During anoestrus	28.95 ± 1.18	29.30 ± 0.69	24.35 ± 1.85	28.00 ± 1.09	--
	First day of proestrus	28.55 ± 0.48	29.42 ± 0.74	25.26 ± 1.56	28.84 ± 1.45	28.78 ± 0.73
	Day of first breeding	29.54 ± 0.43	27.70 ± 0.81	24.84 ± 1.66	29.14 ± 1.65	28.48 ± 0.61

MCV- Mean corpuscular volume MCHC- Mean corpuscular haemoglobin concentration MCH- Mean corpuscular haemoglobin

Figures bearing different superscripts in each row differ significantly ($P<0.05$)

Table 24. Leucogram during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter			Group				
			I	II	III	IV	V
TLC (cells/ mm^3)		During anoestrus	13740.00 ± 515.36	16000.00 ± 769.92	13650.00 ± 963.44	15514.29 ± 545.73	--
		First day of proestrus	13100.00 ± 459.35	15077.78 ± 689.56	12762.60 ± 686.69	14971.43 ± 951.87	13140.00 ± 423.08
		Day of first breeding	12780.00 ± 321.56^{ab}	14444.44 ± 615.37^{b}	12086.25 ± 637.11^{a}	14200.00 ± 704.75^{b}	12650.00 ± 420.65^{ab}
DC	N (%)	During anoestrus	67.87 ± 0.97	66.78 ± 0.95	68.98 ± 1.45	69.45 ± 1.43	--
		First day of proestrus	69.45 ± 0.89	69.54 ± 0.99	68.65 ± 1.12	70.12 ± 1.21	68.35 ± 1.12
		Day of first breeding	68.00 ± 0.95	67.78 ± 1.85	67.88 ± 1.93	67.43 ± 1.62	67.20 ± 1.14
	L (%)	During anoestrus	30.40 ± 1.29	29.00 ± 3.10	28.25 ± 3.43	30.57 ± 2.86	--
		First day of proestrus	30.20 ± 0.74	29.89 ± 2.25	28.00 ± 2.53	29.20 ± 1.82	29.28 ± 1.05
		Day of first breeding	29.80 ± 0.86	30.00 ± 1.85	29.00 ± 1.86	29.71 ± 1.51	30.60 ± 1.08
	E (%)	During anoestrus	1.40 ± 0.24	1.33 ± 0.24	2.50 ± 0.53	2.29 ± 0.64	--
		First day of proestrus	1.40 ± 0.24^{a}	1.78 ± 0.22^{ab}	2.25 ± 0.16^{b}	2.43 ± 0.30^{b}	1.63 ± 0.26
		Day of first breeding	1.40 ± 0.24	1.33 ± 0.14	1.63 ± 0.18	1.86 ± 0.34	1.20 ± 0.20
	M (%)	During anoestrus	0.40 ± 0.24	1.00 ± 0.24	1.00 ± 0.19	1.14 ± 0.26	--
		First day of proestrus	0.80 ± 0.20	1.00 ± 0.29	1.00 ± 0.19	1.29 ± 0.18	1.00 ± 0.23
		Day of first breeding	1.00 ± 0.32	0.89 ± 0.11	1.38 ± 0.18	1.00 ± 0.00	1.00 ± 0.21

TLC- Total leucocyte count DC- Differential count N- Neutrophils L- Lymphocytes E- Eosinophils M- Monocytes

Figures bearing different superscripts in each row differ significantly ($P<0.05$)

Table 25. Serum calcium and phosphorus level during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter		Group				
		I	II	III	IV	V
Calcium (mg %)	During anoestrus	8.82 ± 0.15	8.51 ± 0.44	9.19 ± 0.29	9.04 ± 0.27	--
	First day of proestrus	8.90 ± 0.01	8.83 ± 0.33	9.15 ± 0.25	9.22 ± 0.24	8.89 ± 0.24
	Day of first breeding	8.93 ± 0.16	9.03 ± 0.31	9.29 ± 0.23	9.34 ± 0.26	8.75 ± 0.14
Phosphorus (mg %)	During anoestrus	3.57 ± 0.21	3.72 ± 0.19	3.39 ± 0.30	3.03 ± 0.15	--
	First day of proestrus	3.78 ± 0.12^{b}	3.75 ± 0.13^{b}	3.79 ± 0.19^{b}	3.61 ± 0.09^{b}	4.83 ± 0.31^{a}
	Day of first breeding	3.94 ± 0.12^{b}	3.54 ± 0.24^{b}	3.99 ± 0.17^{b}	3.71 ± 0.07^{b}	4.80 ± 0.32^{a}

Figures bearing different superscripts in each row differ significantly ($P<0.05$)

Table 26. Serum iron, copper and cobalt level during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter		Group				
		I	II	III	IV	V
Iron (mg %)	During anoestrus	0.146 ± 0.012	0.158 ± 0.053	0.160 ± 0.094	0.158 ± 0.011	--
	First day of proestrus	0.198 ± 0.086	0.182 ± 0.097	0.180 ± 0.059	0.168 ± 0.012	0.169 ± 0.047
	Day of first breeding	0.180 ± 0.011	0.173 ± 0.079	0.176 ± 0.023	0.166 ± 0.077	0.177 ± 0.070
Copper (mg %)	During anoestrus	0.116 ± 0.034	0.122 ± 0.022	0.188 ± 0.031	0.093 ± 0.017	--
	First day of proestrus	0.134 ± 0.042^{ab}	0.171 ± 0.022^{bc}	0.216 ± 0.026^{c}	0.086 ± 0.010^{b}	0.204 ± 0.015^{c}
	Day of first breeding	0.122 ± 0.030	0.219 ± 0.015	0.223 ± 0.031	0.153 ± 0.043	0.202 ± 0.027
Cobalt (mg %)	During anoestrus	0.018 ± 0.003	0.023 ± 0.002	0.021 ± 0.001	0.023 ± 0.001	--
	First day of proestrus	0.019 ± 0.002	0.024 ± 0.003	0.022 ± 0.001	0.020 ± 0.009	0.022 ± 0.006
	Day of first breeding	0.020 ± 0.002	0.026 ± 0.004	0.022 ± 0.001	0.020 ± 0.008	0.024 ± 0.003

Figures bearing different superscripts in each row differ significantly ($P<0.05$)

Table 27. Serum zinc and manganese level during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Parameter		Group				
		I	II	III	IV	V
Zinc (mg %)	During anoestrus	0.334 ± 0.142	0.317 ± 0.032	0.288 ± 0.028	0.250 ± 0.022	--
	First day of proestrus	0.344 ± 0.139	0.291 ± 0.032	0.280 ± 0.035	0.321 ± 0.024	0.325 ± 0.023
	Day of first breeding	0.308 ± 0.103	0.391 ± 0.028	0.286 ± 0.037	0.340 ± 0.047	0.327 ± 0.055
Manganese (mg %)	During anoestrus	0.004 ± 0.0009	0.005 ± 0.0023	0.005 ± 0.0013	0.002 ± 0.0006	--
	First day of proestrus	0.004 ± 0.0006	0.005 ± 0.0019	0.004 ± 0.0013	0.002 ± 0.0004	0.005 ± 0.0014
	Day of first breeding	0.004 ± 0.0011	0.005 ± 0.0020	0.003 ± 0.0010	0.003 ± 0.0011	0.006 ± 0.0018

Table 28. Serum cholesterol level (mg%) during various stages of oestrous cycle in treated and control animals (Mean ± SE)

Group	During anoestrus	First day of proestrus	Day of first breeding
I	224.20 ± 8.91	219.20 ± 5.67	206.20 ± 4.85
II	215.44 ± 10.77	206.44 ± 9.13	196.33 ± 8.74
III	227.50 ± 10.22	214.50 ± 5.93	201.00 ± 6.97
IV	204.71 ± 10.26	197.71 ± 8.31	193.57 ± 8.32
V	--	200.12 ± 9.14	202.20 ± 4.56

Table 29. Comparative efficacy of trans-abdominal ultrasound scanning and abdominal palpation in pregnancy diagnosis in bitches

No. of animals bred	No. of animals conceived	Technique of pregnancy diagnosis	Between 20 and 25days				Between 30 and 35 days			
39	27		P	D	N	A (%)	P	D	N	A (%)
		Trans-abdominal ultrasound scanning	24	3	12	88.89	27	0	12	100
		Abdominal palpation	18	9	12	66.67	24	3	12	88.89

P- Positive D- Doubtful N- Negative A- Accuracy

Table 30. Conception rate in treated and control animals

Group	Number of animals treated	Number of animals evinced oestrus and bred	Number of animals conceived	Conception rate among responded animals (%)	Overall conception rate Among treated animals (%)
I	10	5	4	80.00	40
II	10	9	7	77.78	70
III	10	8	5	62.50	50
IV	10	7	4	57.14	40
Control	--	10	7	70.00	70

Table 31. Gestation length, litter size and sex ratio in treated and control animals (Mean ± SE)

Group	Number of animals diagnosed pregnant	Number of animals whelped	Gestation length (days)	Litter size	Sex of pups	
					Male	Female
I	4	4	60.50 ± 1.55	6.25 ± 0.48	3.50 ± 0.65	2.75 ± 0.48
II	7	7	61.14 ± 0.77	5.14 ± 0.34	2.57 ± 0.20	2.57 ± 0.30
III	5	5	62.00 ± 0.71	6.40 ± 0.40	3.00 ± 0.48	3.40 ± 0.51
IV	4	4	64.00 ± 0.82	6.00 ± 0.41	3.50 ± 0.29	2.50 ± 0.29
V	7	7	61.43 ± 0.81	6.43 ± 0.78	3.71 ± 0.52	2.71 ± 0.42

Table 32. Haematological profile of anoestrous bitches failed to respond to oestrus induction treatment (Mean ± SE)

Sl. No.	Parameter		During anoestrus	One month post treatment
1.	ESR (mm/h)		3.18 ± 0.18	3.82 ± 0.38
2.	Hb (g %)		8.61 ± 0.35	9.50 ± 0.16
3.	PCV (%)		26.04 ± 0.83	29.55 ± 0.55
4.	TEC (millions/mm^3)		3.21 ± 0.10	3.94 ± 0.05
5.	TLC (cells/mm^3)		14025.45 ± 710.33	10362.73 ± 474.72
6.	DC	a. Neutrophils (%)	65.18 ± 1.53	68.73 ± 0.69
		b. Lymphocytes (%)	32.18 ± 1.48	28.18 ± 0.54
		c. Eosinophils (%)	1.45 ± 0.21	1.82 ± 0.18
		d. Monocytes (%)	1.18 ± 0.23	1.18 ± 0.26
7.	MCV (fl)		81.60 ± 2.49	75.17 ± 1.64
8.	MCHC (g %)		32.94 ± 0.70	32.25 ± 0.67
9.	MCH (pg)		26.88 ± 0.98	24.18 ± 0.48

Table 33. Serum hormonal profile of anoestrous bitches failed to respond to oestrus induction treatment (Mean ± SE)

Hormone	During anoestrus	One month post-treatment
Progesterone (ng/ml)	0.52 ± 0.07	0.61 ± 0.03
Prolactin (ng/ml)	0.66 ± 0.08	0.84 ± 0.06
Tthyroxine (µg/ml)	1.90 ± 0.16	2.06 ± 0.09

Table 34. Serum mineral profile of anoestrous bitches failed to respond to oestrus induction treatment (Mean ± SE)

Parameter	During anoestrus	One month post-treatment
Calcium (mg %)	9.11 ± 0.28	9.21 ± 0.14
Phosphorus (mg %)	3.58 ± 0.23	3.84 ± 0.07
Iron (mg %)	0.155 ± 0.007	0.148 ± 0.003
Copper (mg %)	0.157 ± 0.033	0.133 ± 0.004
Cobalt (mg %)	0.0029 ± 0.008	0.0025 ± 0.0005
Zinc (mg %)	0.018 ± 0.0017	0.020 ± 0.0009
Manganese (mg %)	0.346 ± 0.071	0.312 ± 0.024

Table 35. Ovarian biometry during anoestrus in bitches failed to oestrus induction trials

Animal No.	Ovarian biometry (cm)					
	Left ovary			Right ovary		
	Length	Width	Thickness	Length	Width	Thickness
1	1.0	0.7	0.4	0.9	0.6	0.4
2	1.1	0.8	0.5	1.0	0.8	0.6
3	0.9	0.5	0.4	0.8	0.5	0.3
4	1.0	0.6	0.4	1.1	0.5	0.4
5	1.2	0.7	0.4	1.0	0.8	0.4
6	1.0	0.8	0.6	0.9	0.7	0.6
7	0.8	0.7	0.4	0.7	0.6	0.3
Mean	1.0 ± 0.05	0.69 ± 0.04	0.44 ± 0.03	0.91 ± 0.05	0.64 ± 0.05	0.43 ± 0.05

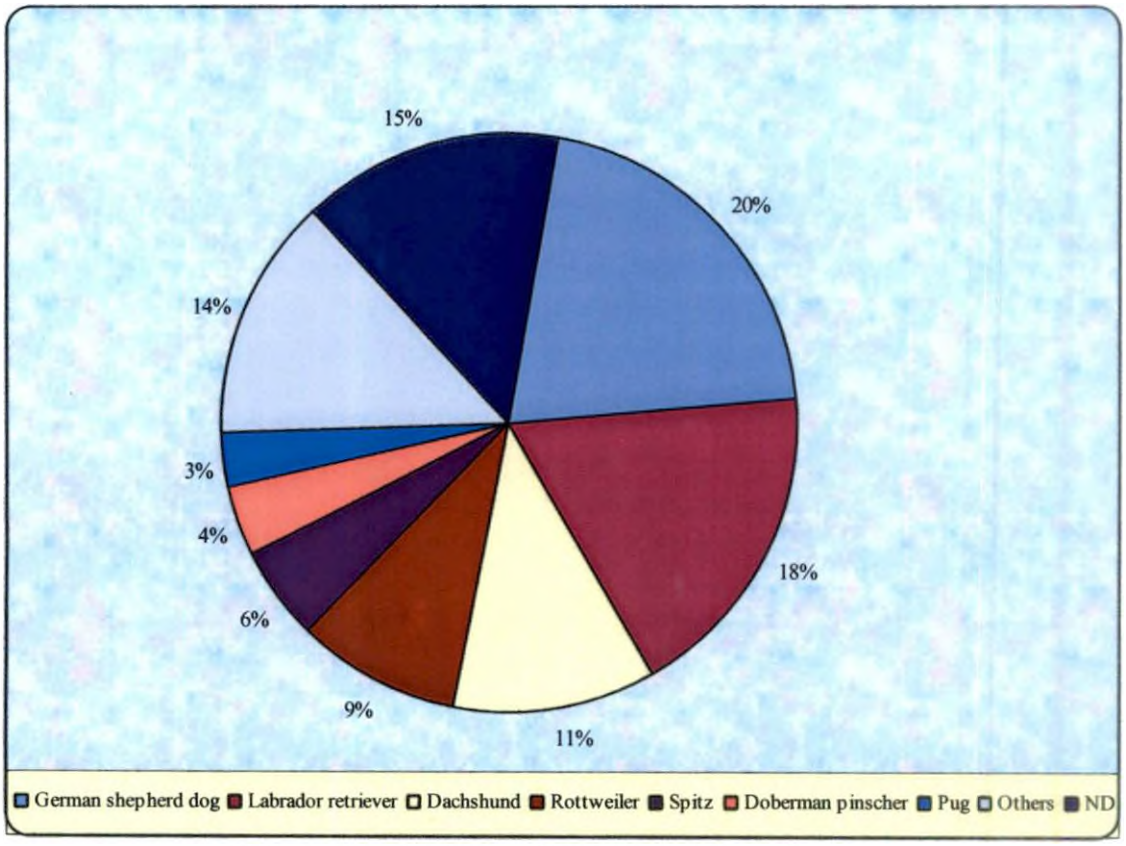


Fig. 1. Breed-wise details of dogs surveyed

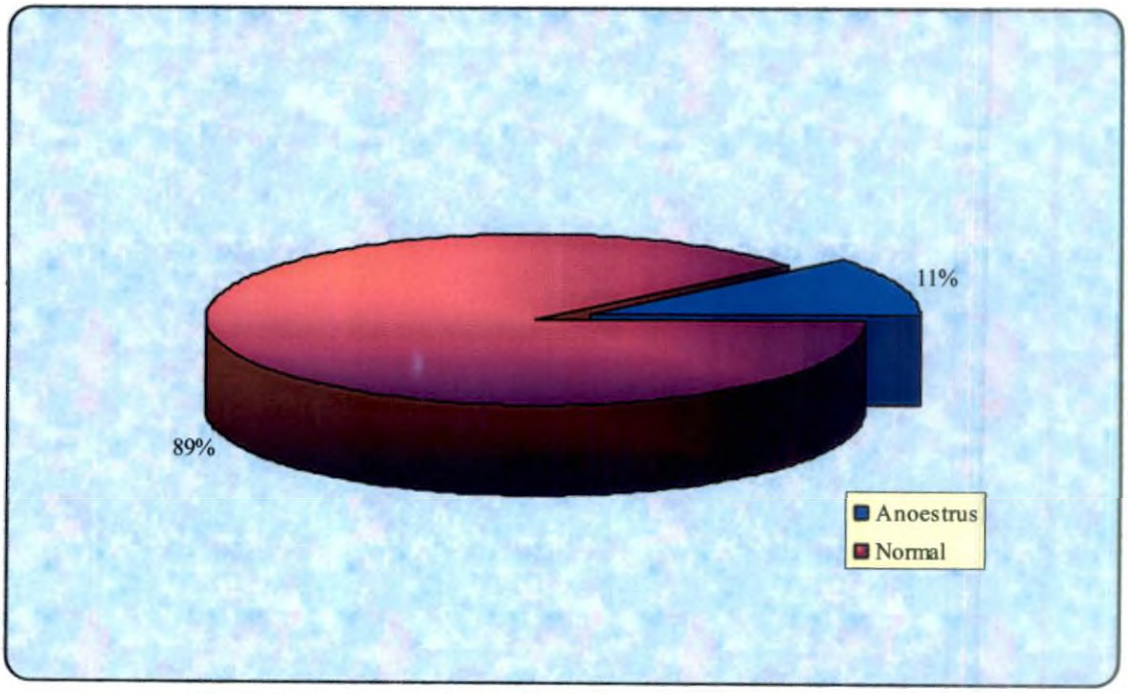


Fig. 2. Prevalence of anoestrus among female dogs

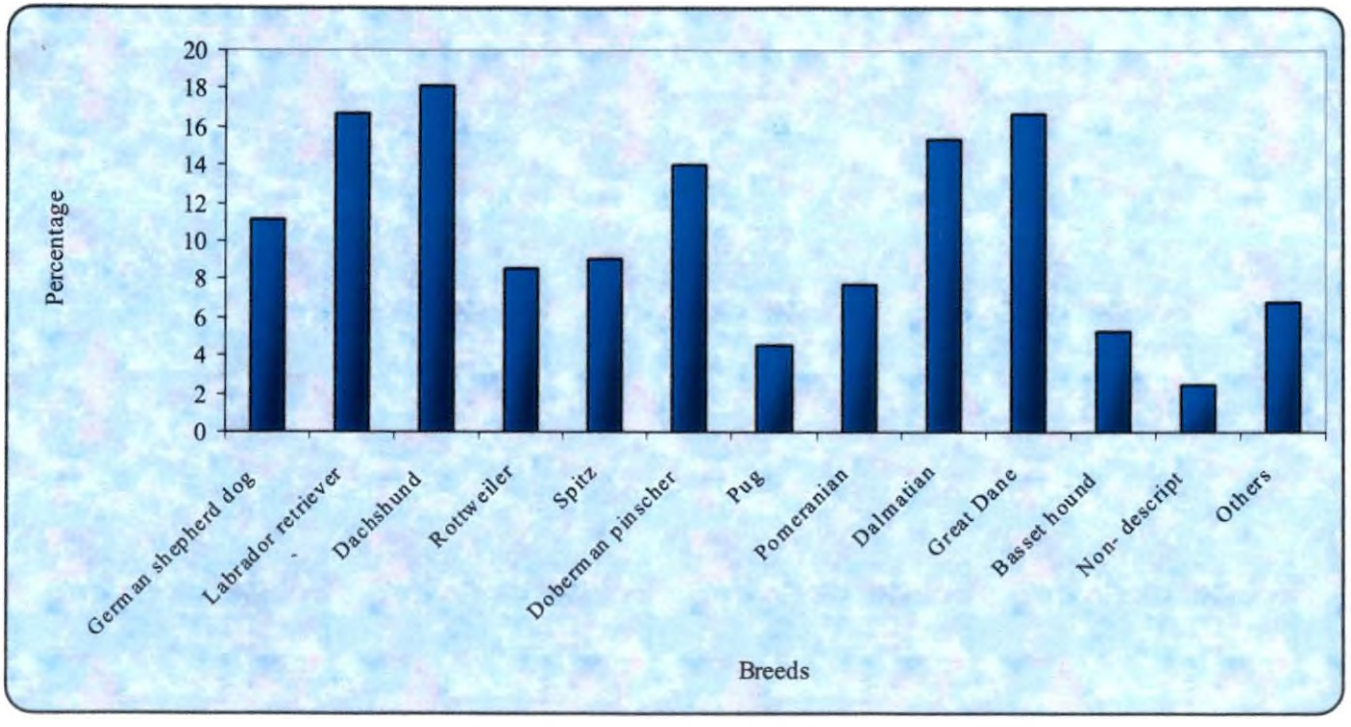


Fig. 3. Prevalence of anoestrus among various breeds of dog

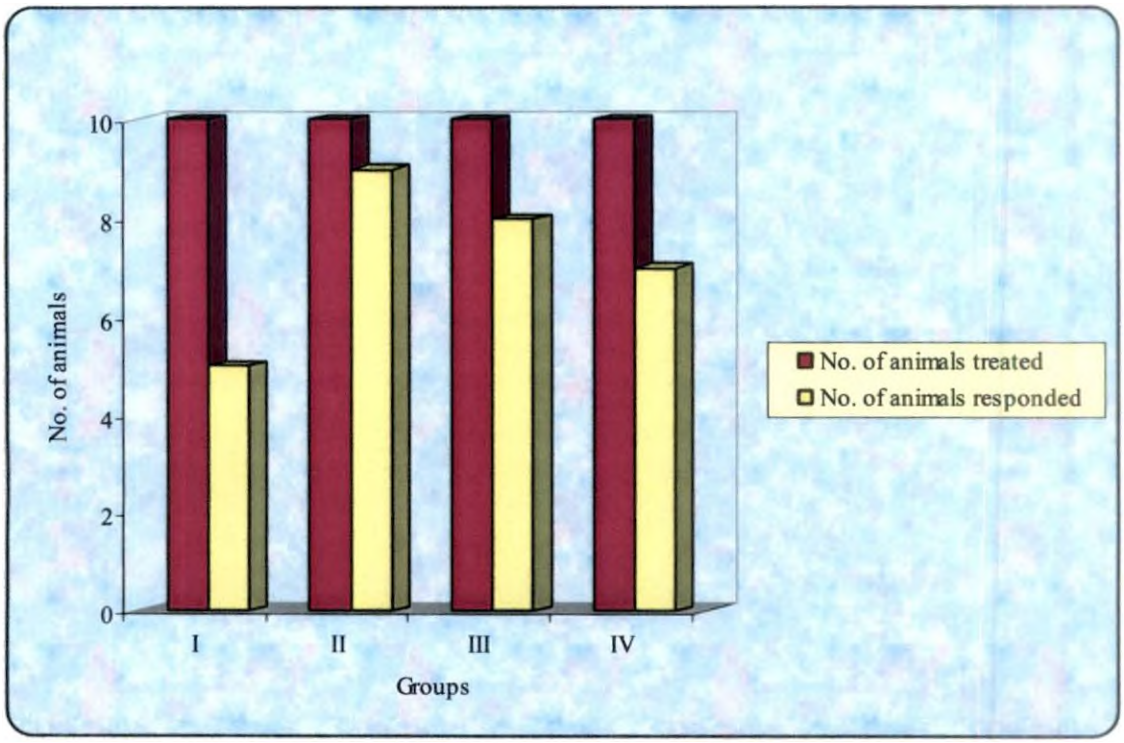


Fig. 4. Response to oestrus induction treatment in treatment groups

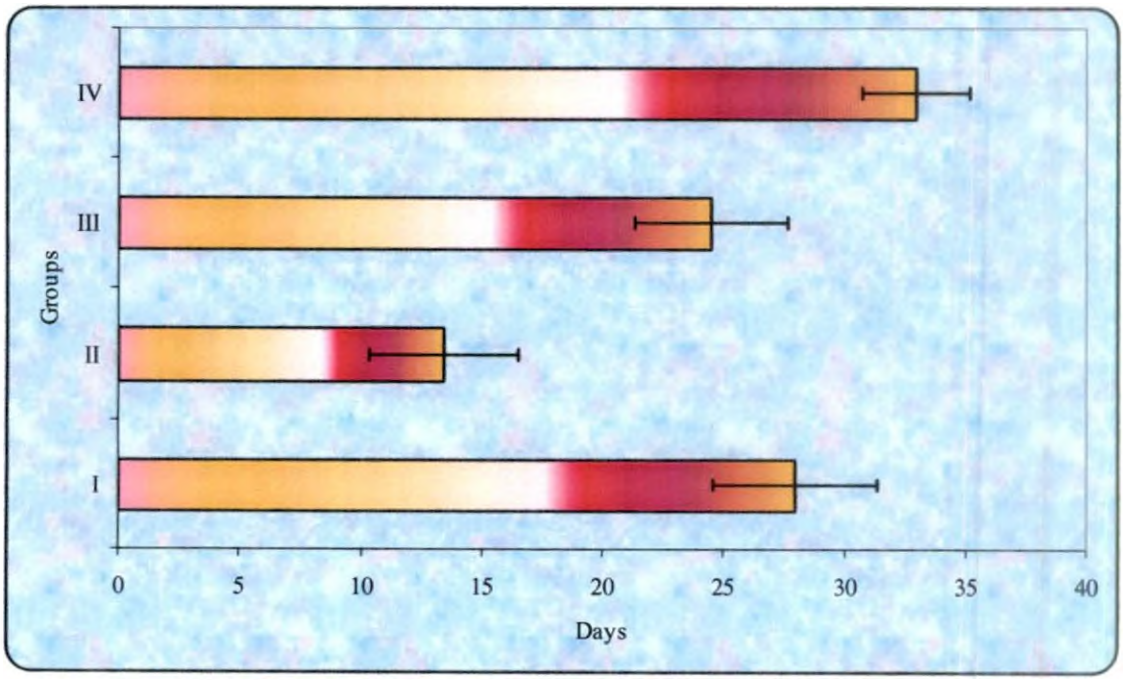


Fig. 5. Treatment onset to proestrus in treatment groups

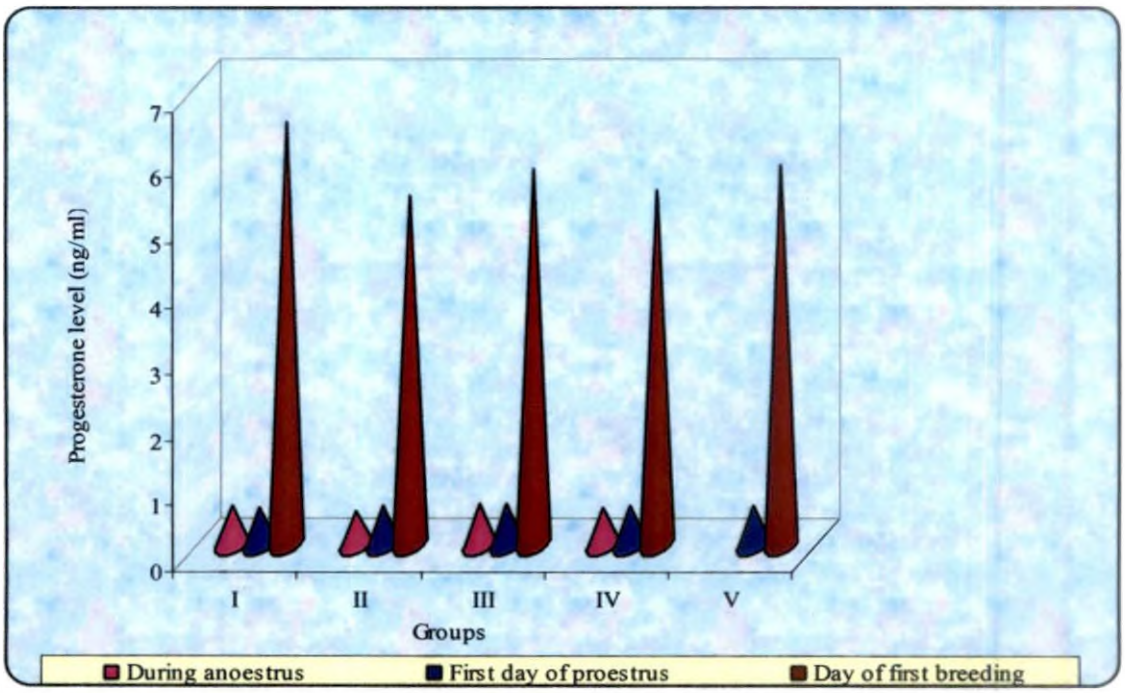


Fig. 6. Serum progesterone level during various stages of oestrous cycle in treated and control animals

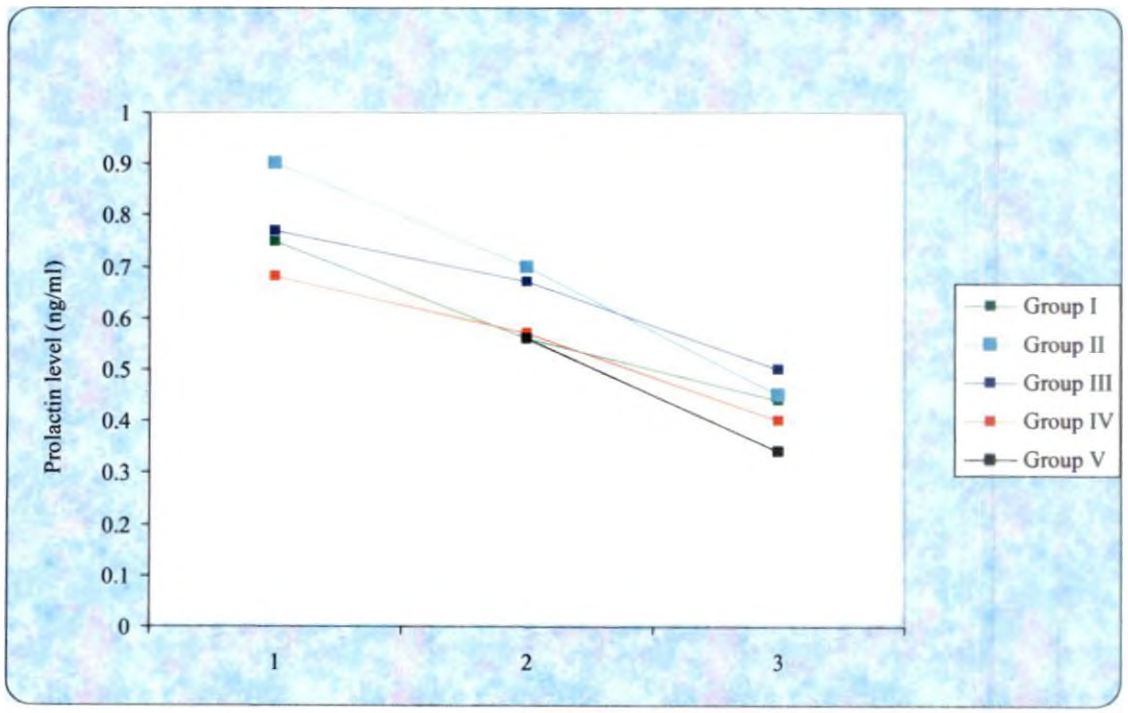
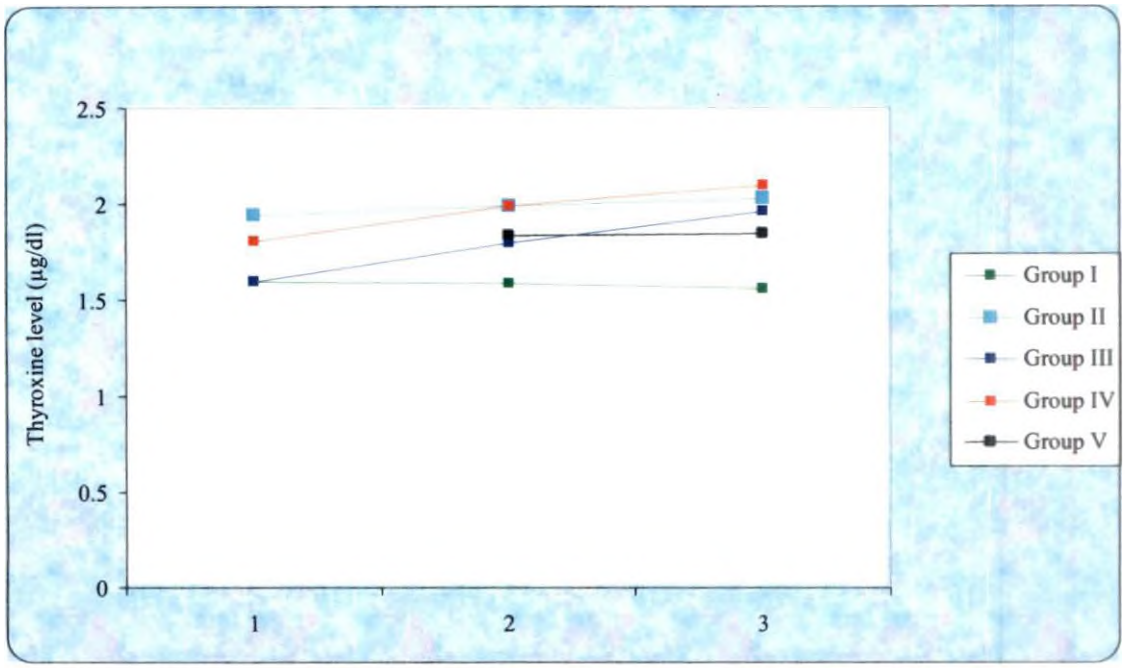
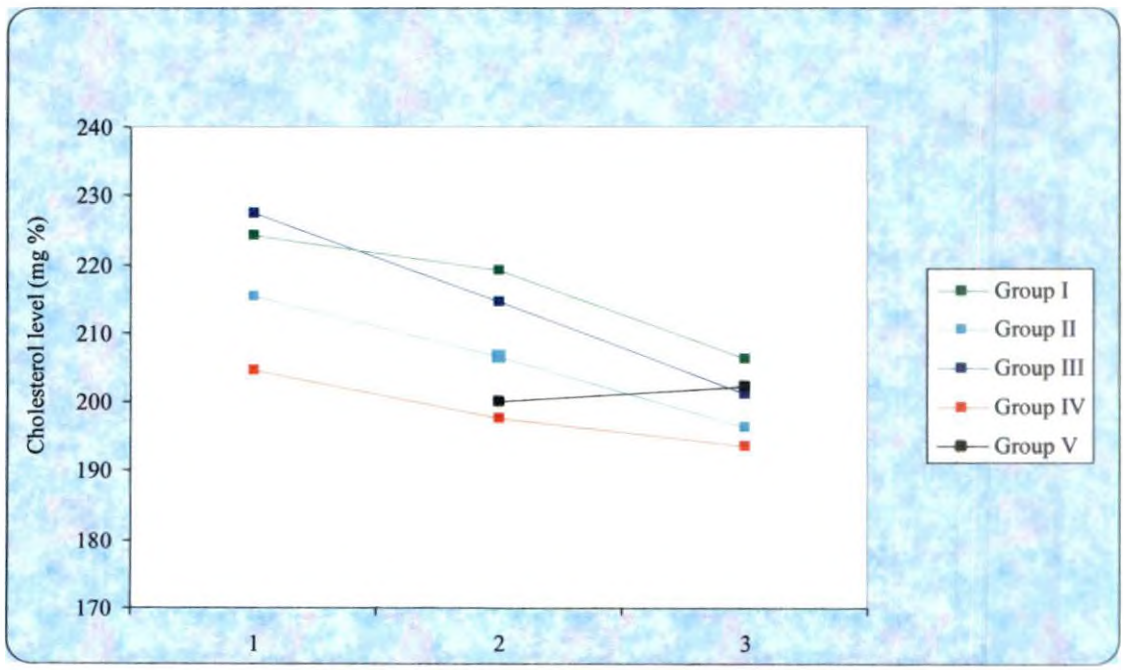


Fig. 7. Serum prolactin level during various stages of oestrous cycle in treated and control animals



1. During anoestrus 2. First day of proestrus 3. Day of first breeding

Fig. 8. Serum thyroxine level during various stages of oestrous cycle in treated and control animals



1. During anoestrus 2. First day of proestrus 3. Day of first breeding

Fig. 9. Serum cholesterol level during various stages of oestrous cycle in treated and control animals

Plate 1. Anoestrous bitch with body condition score 4 (lean)

Plate 2. Anoestrous bitch with body condition score 5 (ideal)

Plate 3. Anoestrous bitch with body condition score 6 (obese)

Plate 4. Anoestrous bitch with body condition score 8 (obese)

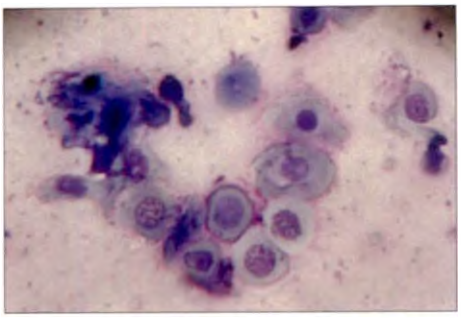


Plate 5. Vaginal cytology during anoestrus revealing more number of parabasal and small intermediate cells (Wright-Giemsa's stain, 400x)

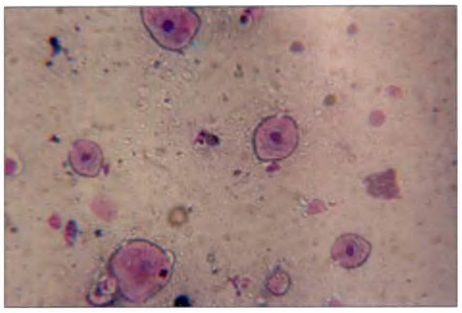


Plate 6. Vaginal cytology during early proestrus revealing more number of intermediate cells and red blood cells (Wright-Giemsa's stain, 400x)

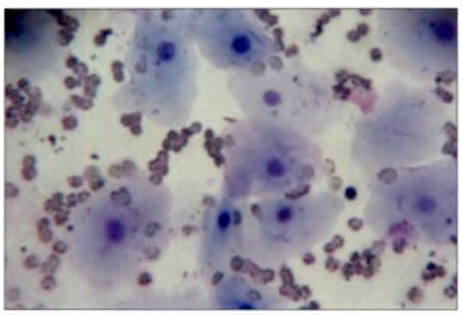


Plate 7. Vaginal cytology during late proestrus revealing more number of superficial cells and a few anuclear keratinized cells (Wright-Giemsa's stain, 400x)

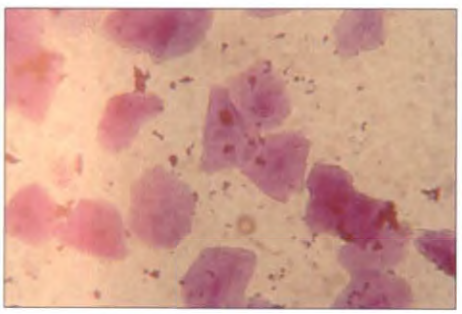


Plate 8. Vaginal cytology during early oestrus revealing more number of anuclear keratinized cells and a few superficial cells (Wright-Giemsa's stain, 400x)

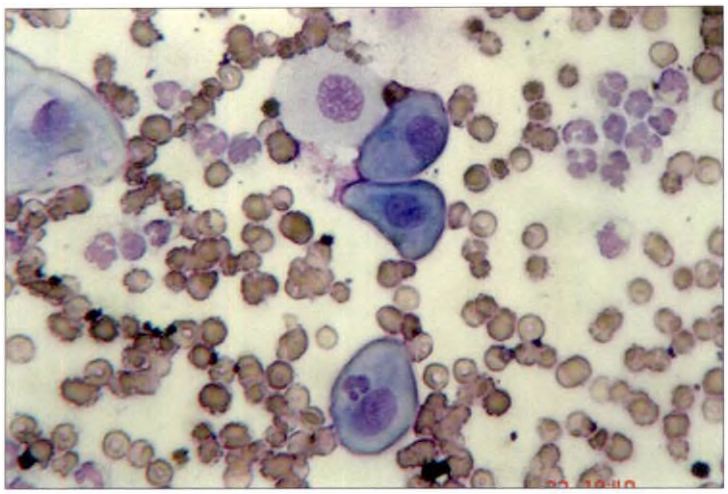


Plate 9. Vaginal cytology during metoestrus revealing metoestrus cells and neutrophils (Wright-Giemsa's stain, 400x)

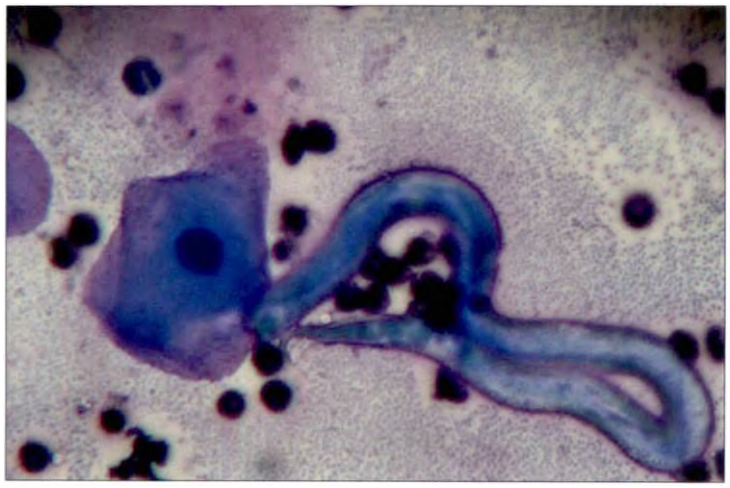


Plate 10. Microfilaria of *Dirofilaria repens* in vaginal smear during late proestrus (Wright-Giemsa's stain, 400x)

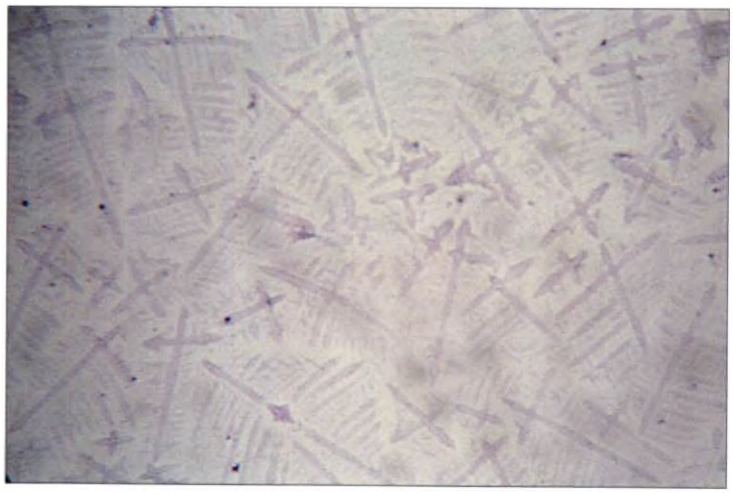


Plate 11. Fern pattern of oestrual mucus (100x)

Plate 12. Performing vaginoscopy in a bitch.

Plate 13. Vaginoscopy- during anoestrus.

Plate 14. Vaginoscopy- during early proestrus.

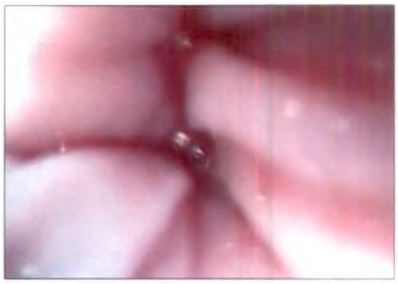


Plate 15. Vaginoscopy- during late proestrus.

Plate 16. Vaginoscopy- during oestrus.

Plate 17. Vaginoscopy- during metoestrus.

Plate 18. Pregnancy diagnosis by ultrasonography -gestational age 20 days.

Plate 19. Pregnancy diagnosis by ultrasonography -gestational age 30 days.

Plate 20. A Labrador retriever bitch with five pups

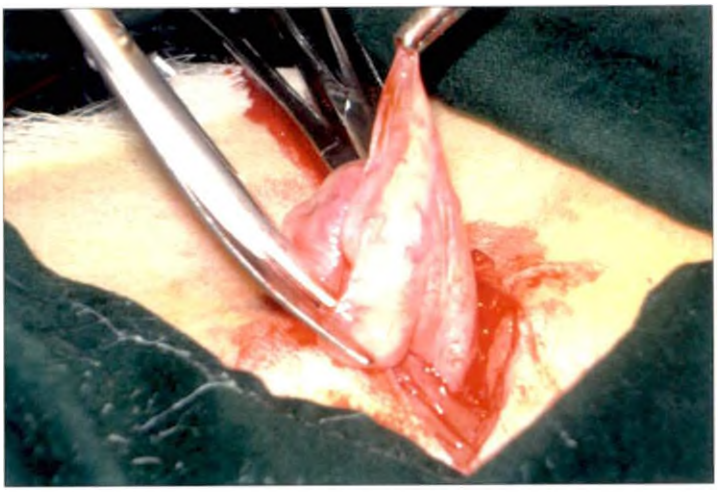


Plate 21. Laparotomy in progress for examination of ovaries

Plate 22. Laparotomy-exposed ovary

Plate 23. Laparotomy-exposed ovary with opened bursa

DISCUSSION

5. DISCUSSION

Detailed survey on breeding, feeding and management of 1721 dogs (518 males and 1203 females) belonging to 817 owners was conducted in different parts of Kerala State. The prevalence, nature and magnitude of anoestrus among the 1203 female dogs were evaluated based on breeding details. Haematological, hormonal, mineral and cholesterol profile of anoestrous bitches were estimated. Oestrus induction trials were carried out using antiprolactin drugs *viz.* bromocriptine and cabergoline and thyroxine at two dose levels, and the data obtained were compiled and compared with that of control.

5.1. BREEDING, FEEDING AND MANAGEMENT OF DOGS AND THE PROFILE OF THE OWNERS

5.1.1. System of Dog Rearing

Among the 1721 dogs surveyed, 518 (30.10%) were male dogs and 1203 (69.90%) were female. The average number of male dogs reared by individual owners was 1.64 as against 1.83 for female. According to Vijayakumar (2001), 49.60 per cent of the dogs in Central Kerala were male and 50.40 per cent were female. The present survey revealed that there is an increasing trend among the dog owners to keep more number of female dogs. This shows the gaining importance of dog breeding units as potential source of income.

Breed-wise details of dogs maintained by individual owners revealed that German shepherd dog (20.74%) was the most popular breed of dog followed by Labrador retriever (18.13%), Dachshund (11.39%), Rottweiler (9.12%) and Spitz (5.52%). This is in agreement with the findings of Vijayakumar (2001), who reported that the breeds of dog popular in Central Kerala were German shepherd dog and Dachshund. The other exotic breeds commonly encountered were

Doberman pinscher (3.89%), Pug (2.96%), Pomeranian (2.73%), Dalmatian (2.15%), Great Dane (2.09%), Basset hound (1.28%) and Boxer (1.00%). The percentage of local non-descript dogs was found to be 14.64 only. The study revealed that even though there is an increasing trend among the dog owners to keep exotic breeds, a few still prefer local non-descript too.

5.1.2. Utility of Dogs Reared

Out of the 1721 dogs surveyed, 67.17 per cent was maintained for non-commercial purposes *viz.* as pet and companion (47.47%), to guard house and premises (13.83%), as a hobby and stress buster (4.30%) and on compassionate grounds (1.57%). Chamala and Crouch (1981) reported companionship (70.40%) as the most popular motivation for dog keeping. The utility of dog as pet and companion seemed to be equally important in Kerala too. The present day nuclear family concept and the changing socio-economic scenario in the State might have influenced this.

Out of the 1721 dogs reared, 16.68 per cent was for commercial purposes *viz.* for breeding and sale of pups (14.70%) and as stud dogs (1.98%) and 16.15 per cent was for both commercial and non-commercial purposes. The pattern of utility revealed that dog breeding and related activities are being identified by many as a potential vocation.

5.1.3. Feeding and Management of Dogs

Data on feeding revealed that 94.70 per cent of the dogs were fed with non-vegetarian and only 5.30 per cent with vegetarian food. Vijayakumar (2001) also reported similar findings; 92.70 per cent of dogs of Central Kerala were fed with non-vegetarian and 7.30 per cent with vegetarian food. Majority of the dogs (58.98%) were fed with home-made food. The percentage of dogs maintained

exclusively on commercial dog food was only 8.77, probably due to its prohibitive cost (Pasupathi *et al.*, 2000) and reduced availability.

With respect to frequency of feeding, the percentage of dogs maintained by one meal per day was only 12.61. However, 49.33 per cent of dogs were fed twice daily and 38.06 per cent three or more times a day. According to Vijayakumar (2001), the percentage of dogs which received one meal per day was 23.40 and that most of the dogs (41.90%) received two meals per day. The present study revealed that dogs in Kerala are fed more times than the recommended schedule of one or two per day (Legrand-Defretin and Munday, 1993). According to German (2006), increased frequency of feeding might predispose to the development of obesity and resultant reproductive disorders.

Deworming as per schedule was carried out in 74.60 per cent and once in a while in 22.49 per cent of dogs. This showed the increased awareness of dog owners on worm infestation and its management.

The vaccination details revealed that 45.03 per cent of dogs received prophylactic antirabies vaccine whereas 30.10 per cent received multi-component vaccine as per standard vaccination schedule. The percentage of dogs which received antirabies vaccine and multicomponent vaccine once in a while was 35.10 and 40.15 respectively. The present study revealed that the awareness among public on prophylactic antirabies vaccination is more, which probably may be due to fear of rabies. However, measures to increase the awareness among public on scientific immunization practice against rabies and other infectious diseases should be initiated.

5.1.4. Breeding of Dogs

Breeding was allowed in 92.93 per cent of bitches and the remaining 7.07 per cent were kept as virgin throughout their lifetime. The age at first breeding in

majority of the bitches (57.85 %) was between 1 to 2 years with an average of 16.30 months. The average number of oestrus periods and the number of breedings per year in bitches were 2.14 ± 0.24 and 1.47 ± 0.32 respectively. The average duration of proestrual bleeding was found to be 10.87 ± 1.24 days. The present findings are in agreement with that of Vijayakumar (2001) who reported that 89 per cent of the bitches exhibited heat periods twice in a year, the age at first mating was 17.20 months and the average duration of proestrual bleeding was 9.60 days.

The identification of apt time for breeding based on external signs was followed in 70.39 per cent of bitches and in 29.61 per cent, it was identified by observing the signs as well as veterinary advice based on exfoliative vaginal cytology and vaginoscopy. The present finding endorses the increasing use of modern diagnostic techniques as tools to identify the most appropriate breeding time in bitches.

The percentage of bitches bred by commercial stud dogs was 30.32. This shows the increasing demand for good quality stud dogs. Most of the bitches (51.97%) were bred twice in one oestrus and the period of acceptance ranged from 4 to 22 days with an average of 8.94 ± 2.18 days. The incentive for breeding of majority of the bitches (61.27 %) was one pup born in the litter and cash payment was practised in 24.51 per cent. This shows the increasing expenditure incurred towards breeding bitches with elite stud dogs.

The present study revealed that pregnancy confirmation based on veterinary examination was practised in 17.98 per cent, highlighting the utilization of modern diagnostic facilities like ultrasonography.

The incidence of failure of conception, abortion, stillbirth and pseudopregnancy were 18.87, 5.72, 15.56 and 13.69 per cent respectively. Moreover, the incidence of assisted whelping and caesarean section were 5.18

and 1.32 per cent respectively. These findings substantiate the importance of sound reproductive healthcare and management in augmenting reproductive efficiency. The average litter size was found to be 6.94 ± 1.47, which was slightly higher than that reported by Vijayakumar (2001).

The neonatal problems encountered in 13.88 per cent of bitches included *agalactia/hypogalactia/mammitis* (5.29%), *cannibalism* (2.65%), *puppy fading* syndrome (2.09%), puerperal tetany (1.98%) and rejection of young one by dam/ poor mothering ability (1.87%). By adopting better management and disease control measures, neonatal problems can be minimized.

5.1.5. Profile of Owners Who Maintained Dogs

Out of the 817 owners surveyed, 89.11 per cent were men and 10.89 per cent were women. According to Vijayakumar (2001), in 92 per cent of the households surveyed, the dog owners were male members of the family. Both studies revealed that the percentage of male owners continued to be very high in Kerala. This necessitates the initiation of programmes to increase the involvement of women, as in other animal husbandry activities, in dog rearing too.

Most of the dog owners (39.66%) were in the age group of 41 to 50 years. The percentage of owners between the age of 21 and 30 was found to be 25.95. This shows that a sizeable number of youngsters are involved in dog rearing activities. With respect to educational qualification of the owners, most of them (86.90%) were with high school level and above. The present finding is in tandem with the high literacy level in Kerala

On analyzing the data on owner's profile, it was found that majority (54.10%) of the owners were with experience up to five years, as against 11.2

years as reported by Vijayakumar (2001). From this it is obvious that the involvement of newcomers in the field of dog rearing is on an increasing trend.

5.1.6. Constraints in Rearing of Dogs

The major constraints faced by the dog owners were non-co-operation from family members, complaints from neighbours and problems associated with waste disposal. Complaints from neighbours and waste disposal problems noted at an increasing rate in the present study might be due to the progress in the urbanization process in the State. The percentage of owners who reported lack of dog care facilities when the family was away as the constraint was 9.18. According to Vijayakumar (2001), the major constraints in rearing of dogs were inadequate space, lack of man power, difficulty in looking after the dog while the family was away, restraining difficulties, training and breeding problems and hair shedding. The present findings are in agreement with the above report.

5.2. PREVALENCE, NATURE AND MAGNITUDE OF ANOESTRUS

Among the 1203 female dogs surveyed, 134 (11.14%) were identified as in the stage of anoestrus. Analysis of breed-wise prevalence revealed that it was high in breeds such as Dachshund (18.18%), Labrador retriever (16.74%), Great Dane (16.67%), Dalmatian (15.38%) and Doberman pinscher (14.00%). It is worth to notice that anoestrus was lowest (2.44%) among local non-descript bitches. The higher rate of prevalence of anoestrus noticed among exotic breeds might probably be due to their low adaptability, poor disease resistance, nutritional deficiencies and/or other stress related conditions.

5.2.1. Primary and Secondary Anoestrus

Out of the 134 anoestrous dogs screened, 65.67 per cent was in primary and 34.33 per cent in secondary anoestrus. From this it was evident that the incidence of anoestrus was more in nulliparous than in parous animals.

5.2.2. Age of Anoestrous Bitches

The average age was found to be 22.68 and 34.34 months respectively for female dogs with primary and secondary anoestrus. According to Arthur *et al.* (1989), the age at puberty in bitches varied from 6 to 20 months. The present study revealed that the age at puberty is much high in bitches thereby delaying return from those kept for breeding.

5.2.3. Duration of Secondary Anoestrus

Secondary anoestrus was found to be longest in Dachshund (19 months) and shortest in Doberman pinscher (6.23 months). The overall average duration of secondary anoestrus was found to be 11.71 months. According to Verstegen *et al.* (1999), the normal interoestrous interval in dogs varied from 5 to 12 months with an average of seven months. Prolonged interoestrus interval noticed in the present study would also badly affect the viability of dog breeding units by delaying returns.

5.2.4. Body Condition Score of Anoestrous Bitches

Perusal of data on body condition revealed that 42 per cent of the anoestrous bitches were with the ideal body score of five, 46 per cent with body score more than five (obese) and 12 per cent with body score less than five (lean) (Laflamme, 1997). The increased frequency of feeding of dogs observed in the present study might have contributed to the development of obesity in a good proportion of bitches. Johnson *et al.* (1997) opined that the classic signs of hypothyroidism in dogs included obesity and reproductive abnormalities. According to German (2006) obese dogs are predisposed to a number of disease conditions including reproductive disorders. However, anoestrus observed in a very few proportion of the dogs with lean body condition might be due to malnutrition.

5.3. HAEMATOLOGICAL, HORMONAL AND MINERAL PROFILE OF ANOESTROUS BITCHES

5.3.1. Haematological Profile

Analysis of haematological profile of anoestrous bitches revealed normochromic normocytic anaemia, which could possibly be associated with mild degree of hypothyroidism. Benjamin (1985) reported borderline normochromic normocytic anaemia as a clinical feature of hypothyroidism in intact females. Leucogram of anoestrous bitches revealed slight leucocytosis also.

5.3.2. Hormonal Profile

5.3.2.1. Progesterone: In the present study, a mean serum progesterone level of 0.57 ± 0.03 ng/ml was obtained in anoestrous bitches. According to England (1998), the serum progesterone level during anoestrus in dogs was less than 1 ng/ml. Gobello *et al.* (2002) opined that female dogs whose progesterone levels were below 1 ng/ml could be considered to be in anoestrus. The progesterone level obtained in the present study confirmed anoestrous condition in the bitches selected for oestrus induction trials.

5.3.2.2. Prolactin: The mean serum prolactin level in anoestrous dogs was found to be 0.75 ± 0.05 ng/ml. According to Verstegen *et al.* (1999), the mean prolactin levels during early, mid and late anoestrous periods in Beagle bitches were 1.8 ± 0.9, 1.6 ± 0.7 and 1.6 ± 0.8 ng/ml respectively. The mean serum prolactin level in anoestrous animals obtained in the present study did not reveal the existence of hyperprolactinaemia.

5.3.2.3. Thyroxine: The present study revealed that the mean serum thyroxine level in anoestrous bitches was 1.80 ± 0.06 µg/dl. According to Feldman and Nelson (1996), the serum thyroxine level in healthy dogs ranged from 1.5 to 3.5 µg/dl. Fontbonne *et al.* (1992) suggested hypothyroidism as a common cause of

infertility in bitches causing anoestrus. The mean serum thyroxine level obtained in anoestrous dogs in the present study was more towards the lower side of the range quoted by Feldman and Nelson (1996), suggesting the possibility of a relationship between low serum thyroxine level and anoestrus.

5.3.3. Mineral Profile

The mean serum calcium and phosphorus levels in anoestrous bitches were found to be 8.94 ± 0.24 and 3.48 ± 0.12 mg per cent respectively. These low values revealed the existence of mild hypocalcaemia and hypophosphataemia suggesting the relationship of deficiency of these minerals with anoestrus condition. However, the serum iron, copper, cobalt, zinc and manganese levels were found to be within the normal range as reported by Jacobs (1995).

5.3.4. Cholesterol Profile

The mean serum cholesterol level in anoestrous dogs was found to be within the normal range, but more towards the upper limit suggesting the possibility of association between anoestrus and mild hypercholesterolemia. Kaelin *et al.* (1986) and Renju (2005) observed abnormally high plasma cholesterol concentration in dogs with confirmed hypothyroidism.

5. 4. INDUCTION OF OESTRUS

5.4.1. Proestrus and Oestrus Response

Out of 10 animals treated in each group, five (50 %) in bromocriptine treated group (Group I), nine (90%) in cabergoline treated group (Group II), eight (80%) in thyroxine (@10 µg/kg. body weight) treated group (Group III) and seven (70%) in thyroxine (@ 5 µg/kg. body weight) treated group (Group IV) evinced proestrual bleeding. The proestrus response was highest in cabergoline treated animals (Group II) and was lowest in bromocriptine treated animals

(Group I). The proestrus response reported by vanHafften *et al.* (1989) and Zoldag *et al.* (2001) in oestrus induction trials using bromocriptine were 80 and 100 per cent respectively. Even though Simon (1997) obtained a proestrus response of 83 per cent on inducing oestrus with PMSG, all the animals treated with clomiphene citrate failed to respond. Verstegen *et al.* (1999) reported a proestrus response of 93.33 per cent in dogs treated with cabergoline. Becha (2000) reported 66.66 and 100 per cent proestrus response on inducing oestrus with diethylstilbesterol and leuprolide acetate respectively. Ajitkumar *et al.* (2005) obtained a proestrus response of 70 per cent in a preliminary study on oestrus induction using levothyroxine. The present investigation revealed that cabergoline @ 5 µg/kg. body weight and thyroxine @ 10 µg/kg. body weight could be successfully used for induction of oestrus in bitches.

The mean treatment onset to proestrus in Groups I, II, III and IV was 28.00 ± 3.39, 13.44 ± 3.12, 24.50 ± 3.18 and 33.00 ± 2.21 days respectively. The treatment onset to proestrus in animals treated with cabergoline (Group II) was found to be significantly lower than that in other groups. vanHaaften *et al.* (1989) reported a mean treatment onset to proestrus of 28 days in bitches treated with bromocriptine. It was found to be 29.75 ± 5 days (Jeukenne and Verstegen, 1997) and 16 days (Gobello *et al.*, 2002) on inducing oestrus with cabergoline. In the present study the mean treatment onset to proestrus in the bromocriptine treated animals was similar to that reported by vanHaaften *et al.* (1989). The findings in the present study revealed that the cabergoline treated animals evinced proestrus earlier than the previous reports.

The average duration of proestrus in the treatment groups was 9.80 ± 0.86, 10.11 ± 0.68, 11.25 ± 0.88 and 10.71 ± 0.68 days respectively as against 9.70 ± 0.42 days in the control group. Jeukenne and Verstegen (1997), Verstegen *et al.* (1999) and Gobello *et al.* (2004) reported that the duration of proestrus and oestrus in induced and spontaneous cycles were similar. The duration of proestrus obtained in the present study are in agreement with the earlier reports.

The average duration of oestrus in the four treatment groups and the control group was 7.60 ± 0.24, 8.00 ± 0.29, 8.5 ± 0.63, 7.85 ± 0.46 and 8.00 ± 0.39 days respectively. The results obtained in the present study with respect to duration of oestrus are in agreement with that of the earlier reports (Jeukenne and Verstegen, 1997; Verstegen *et al.*, 1999; Gobello *et al.*, 2004).

From the findings of the present study, it could be inferred that successful induction of oestrus in bitches could be achieved by the administration of cabergoline @ 5 µg/kg. body weight orally.

5.4.2. Vaginal Cytology and Anuclear Cell Index During Various Stages of Oestrous Cycle in Treated and Control Animals

The various types of cells encountered on vaginal cytology during anoestrus, proestrus, oestrus and metoestrus in the present study were similar to that of the early reports (Feldman and Nelson, 1996; Simon, 1997; Becha, 2000). The anuclear cell index on the day of first breeding in the treatment groups and the control group were 92.20 ± 1.28, 92.67 ± 1.07, 95.75 ± 1.00, 93.14 ± 1.14 and 92.60 ± 1.29 respectively. According to Hewitt and England (2000), the optimum time for breeding was when the anuclear cell index was above 80 per cent. In the present study the anuclear cell index in the treatment and control groups on the day of first breeding was found to be above 90 per cent and that statistically significant difference could not be detected between the treatment and control groups.

5.4.3. Side Effects Observed During Drug Therapy for Induction of Oestrus

In the present study, 50 per cent of the animals treated with bromocriptine and 10 per cent of the animals treated with cabergoline exhibited nausea and vomiting. Among the five animals exhibited nausea and vomiting in the bromocriptine treated group, three showed dehydration and anorexia and were

managed by administration of electrolytes. None of the animals treated with thyroxine exhibited side effects.

According to Concannon *et al.* (1987), the side effects of bromocriptine consisted of vomiting, anorexia, apathy, increased thirst, depression and behavioural changes. However, habituation to bromocriptine, beginning with lower dose initially, almost completely eliminated emesis (Zoldag *et al.*, 2001). In studies with cabergoline, side effects such as vomiting had been reported in 10 to 25 per cent of dogs (Arbeiter *et al.*, 1988; Gobello *et al.*, 2001; Gunay *et al.*, 2004). Cabergoline had a longer duration of action and produced fewer side effects than bromocriptine (Kutzler, 2005). Dattatray (2006) observed anorexia and vomiting in 10 to 20 per cent of bitches treated with cabergoline. The side effects observed with bromocriptine and cabergoline treatment in the present study are in agreement with the earlier reports.

5.4.4. Serum Progesterone, Prolactin and Thyroxine Levels During Various Stages of Oestrous Cycle in Treated and Control Animals

5.4.4.1. Serum Progesterone Level: The average serum progesterone level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly within and between the treatment groups and the control group. The serum progesterone levels during anoestrus in the treatment groups and on the first day of proestrus in the treatment and control groups were well below 1 ng/ml. The present findings with respect to the level of progesterone on the first day of proestrus are in agreement with that of Feldman and Nelson (1996) who reported that the progesterone level throughout the proestrus in bitch was very low. In the present study, the serum progesterone level on the day of first breeding was found to be > 5 ng/ml. McDonald (1989) reported that the mean serum progesterone level in Beagle bitches during oestrus was above 2 ng/ml. According to England (1998), the serum progesterone levels increased to 2 ng/ml on the day of LH surge and to 5 ng/ml on the day of ovulation in bitches.

The serum progesterone value on the day of first breeding obtained in the present study was in agreement with that of the previous reports.

5.4.4.2. Serum Prolactin Level: The average serum prolactin level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly between the treatment groups and the control group. How ever, a slight reduction was noticed on the first day of proestrus and on the day of first breeding. According to Feldman and Nelson (1996), prolactin levels were low during anoestrus, proestrus and oestrus. Verstegen *et al.* (1999) reported that a reduction in serum prolactin level was observed in all bitches which were treated with cabergoline for induction of oestrus (from 0.4 ± 0.1 ng/ml on day 2 to 0.3 ± 0.1 ng/ml on day 5). The authors also opined that the reduction in serum prolactin level observed at the onset of proestrus in control bitches suggested that even in normal cycles' prolactin level declined towards the onset of proestrus. In the present study, the rate of reduction in the level of prolactin observed in animals treated with anti-prolactin drugs *viz.* bromocriptine and cabergoline was higher than that in animals treated with thyroxine.

5.4.4.3. Serum Thyroxine Level: The average serum thyroxine level during anoestrus and on the first day of proestrus did not differ significantly within and between the treatment and the control groups. Serum thyroxine level on the day of first breeding recorded statistically significant difference ($P<0.05$) between the treatment and control groups, the lowest being 1.56 ± 0.18 µg/dl in Group I and the highest being 2.10 ± 0.13 µg/dl in Group IV. Animals treated with levothyroxine (Group III & IV) exhibited a slight increase in the mean level of serum thyroxine on the first day of proestrus as well as on the day of first breeding, but the values were found to be within the normal limits (Feldman and Nelson, 1996).

5.4.5. Haematological Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

According to Sullivan *et al.* (1993), anaemia observed in hypothyroid dogs could have resulted from reduced thyroxine concentration as it can directly alter erythroid stimulation. The present study revealed increase in the levels of Hb and TEC on the first day of proestrus and on the day of first breeding in animals of Group III and IV. This might be due to the effect of thyroxine administration for induction of oestrus in these groups.

5.4.6. Serum Mineral Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

The mean serum levels of calcium, iron, cobalt, zinc and manganese during anoestrus, on the first day of proestrus and on the day of first breeding did not reveal statistically significant difference between groups. On the other hand, the mean serum phosphorus level on the first day of proestrus and on the day of first breeding in the treatment groups were found to continue at low levels as in the anoestrous stage. The serum copper level on the first day of proestrus exhibited statistically significant difference between the treatment and control groups.

5.4.7. Serum Cholesterol Profile During Various Stages of Oestrous Cycle in Treated and Control Animals

Even though mild hypercholesterolemia was observed during anoestrus in bitches, the mean serum cholesterol level on the first day of proestrus and on the day of first breeding in the treatment groups fell within the normal range.

5.4.8. Pregnancy Diagnosis

The accuracy of pregnancy diagnosis, when performed between 20 and 25 days post-breeding by trans-abdominal ultrasound scanning and abdominal palpation was found to be 88.89 and 66.67 per cent respectively and it increased to 100 and 88.89 per cent respectively between 30 and 35 days.

According to Namsui (1999), the accuracy of pregnancy diagnosis on trans-abdominal palpation between 21 to 25 days post-breeding in Alsatian and Pomeranian bitches was 66.7 and 75 per cent respectively and the corresponding figures were 88.9 and 100 per cent when performed between 30 to 35 days post-breeding. Asha (2005) reported more accurate results of pregnancy diagnosis by trans-abdominal palpation between 31 and 40 days post-breeding. In the present study, the accuracy of both techniques for pregnancy diagnosis, *viz.* trans-abdominal ultrasound scanning and abdominal palpation improved as the gestational age advanced. Further it was found that trans-abdominal ultrasound scanning was more reliable than abdominal palpation for early pregnancy diagnosis in bitches.

5.4.9. Conception Rate

The conception rate in relation to the number of animals responded to oestrus induction treatment in the treatment groups were 80.00, 77.78, 62.50 and 57.14 per cent respectively. However, the respective overall conception rate in relation to the number of animals subjected to oestrus induction trials in the treatment groups were 40, 70, 50 and 40 per cent only. Out of the 10 animals of the control group which were bred during the natural oestrus, seven (70 %) conceived.

Zoldag *et al.* (2001) and Rota *et al.* (2003) reported a conception rate of 83 per cent each on inducing oestrus in bitches using bromocriptine and

cabergoline respectively. Gobello *et al.* (2004) obtained a conception rate of 82.60 per cent in an oestrus induction trial using cabergoline. Ajitkumar *et al.* (2005) reported a conception rate of 71.43 per cent on inducing oestrus in bitches with levothyroxine.

In the present study, conception rate based on the number of animals responded to the treatment was found to be highest in bromocriptine treated animals and lowest in thyroxine (@ 5 µg/kg. body weight) treated animals. How ever, the overall conception rate based on the number of animals subjected to oestrus induction trials was found to be highest in the cabergoline treated animals and lowest in bromocriptine and thyroxine (@ 5 µg/kg. body weight) treated animals. Since the cabergoline treated animals recorded a conception rate of 77.78 per cent based on the number of animals responded to the treatment and 70 per cent based on the number of animals subjected to oestrus induction trials, it could be concluded that cabergoline @ 5 µg/kg. body weight orally is the treatment of choice for induction of oestrus in bitches.

5.4.10. Gestation Length, Litter Size and Sex Ratio in Treated and Control Animals

In the present study, the average gestation length in the treatment groups varied from 60.50 ± 1.55 to 64.00 ± 0.82 as against 61.43 ± 0.81 in the control group. Namsui (1999) recorded an average gestation length of 62.43 and 59.50 days from the first day of mating during natural cycle in Alsatian and Pomeranian bitches respectively. Becha (2000) recorded an average gestation length of 62.50 ± 0.51 and 62.00 ± 1.15 days respectively in bitches in which oestrus was induced by the administration of leuprolide acetate and diethylstilbesterol. The mean gestation length obtained in various groups in the present study was comparable with the previous reports.

The average litter size in the treatment groups varied from 5.14 ± 0.34 to 6.40 ± 0.40 as against 6.43 ± 0.78 in the control group. Statistically significant difference could not be detected in gestation length, litter size and sex ratio between the treatment and control groups. Becha (2000) recorded an average litter size of 5.60 ± 0.75 and 6.00 ± 0.58 respectively in bitches in which oestrus induction was carried out by the administration of leuprolide acetate and diethylstilbesterol. The average litter size obtained in the present study is comparable with the previous report.

5.4.11. Haematological, Hormonal, Mineral and Cholesterol Profile of Anoestrous Bitches Which Failed to Oestrus Induction Treatment

The percentage of treated animals failed to respond in the treatment groups were 50, 10, 20 and 30 respectively.

5.4.11.1 Haematological Profile: The mean ESR, Hb, PCV and TEC values one month post-treatment recorded a slight increase compared to that in the anoestrous stage. However the mean TLC value showed a slight decrease one month post-treatment.

5.4.11.2 Hormonal Profile: The mean serum progesterone levels during anoestrus and one month post-treatment in bitches failed to respond to oestrus induction treatment were 0.52 ± 0.07 and 0.61 ± 0.03 ng/ml. This showed that all the anoestrous bitches failed to the oestrus induction treatment continued to be in the stage of anoestrus (England, 1998; Gobello *et al.*, 2002). The present finding also ruled out the occurrence of undetected oestrus in these animals. With respect to prolactin, the corresponding values were 0.66 ± 0.08 and 0.84 ± 0.06 ng/ml respectively. The serum thyroxine levels during anoestrus and one month post-treatment were 1.90 ± 0.16 and 2.06 ± 0.09 µg/dl respectively.

5.4.11.3 Mineral Profile: The mean serum calcium, phosphorus, iron, copper, cobalt, zinc and manganese levels during anoestrus stage and one month post-treatment did not differ significantly.

5.4.11.4. Cholesterol Profile: The mean serum cholesterol level during the anoestrus stage and one month post-treatment were suggestive of the continued existence of mild hypercholesterolemia in bitches which failed to respond to the oestrus induction treatment.

5.4.12. Vaginoscopy and Exploratory Laparotomy

Mucosal changes suggestive of a positive response could not be detected on vaginoscopy in bitches which failed to respond to oestrous induction trials. This revealed that all of them were continuing in the stage of anoestrus.

Ovaries with follicular or luteal activity could not be identified on exploratory laparotomy in bitches which failed to respond to oestrous induction trials and all of them were found to be continuing in the anoestrus state.

SUMMARY

6. SUMMARY

Survey on breeding, feeding and management of 1721 dogs belonging to 817 owners was conducted in different parts of Kerala State and the prevalence, nature and magnitude of anoestrus among 1203 female dogs were evaluated based on breeding details. Haematological, hormonal, mineral and cholesterol profile of anoestrous bitches were estimated. Oestrus induction trials were carried out using bromocriptine @ 50 µg/kg. body weight (Group I), cabergoline @ 5 µg/kg. body weight (Group II) and thyroxine at two different dose levels *viz.* @ 10 µg/kg. body weight (Group III) and 5 µg/kg. body weight (Group IV) and the data obtained were compiled and compared with that of control bitches.

Among the dogs surveyed, 518 (30.10%) were male dogs and 1203 (69.90%) were female. Breed-wise details of dogs maintained by individual owners revealed German shepherd dog (20.74%) as the most popular breed of dog followed by Labrador retriever (18.13%), Dachshund (11.39%), Rottweiler (9.12%) and Spitz (5.52%). The other exotic breeds commonly encountered were Doberman pinscher (3.89%), Pug (2.96%), Pomeranian (2.73%), Dalmatian (2.15%), Great Dane (2.09%), Basset hound (1.28%) and Boxer (1.00%). The percentage of local non-descript dogs was found to be 14.64 only.

Breeding data revealed that 92.93 per cent of bitches were allowed to breed and the remaining 7.07 per cent were kept as virgin throughout their lifetime. The age at first breeding in majority of the bitches (57.85%) was between 1 to 2 years with an average of 16.30 months. The average number of oestrus periods and the number of breedings per year in bitches were 2.14 ± 0.24 and 1.47 ± 0.32 respectively. The average duration of proestrual bleeding was found to be 10.87 ± 1.24 days. The identification of apt time for breeding based on external signs was followed in 70.39 per cent of bitches and in 29.61 per cent, it was identified by observing the signs as well as veterinary advice based on exfoliative vaginal cytology and vaginoscopy. The percentage of bitches bred by

commercial stud dogs was 30.32. Most of the bitches (51.97%) were bred twice in one oestrus and the period of acceptance varied from 4 to 22 days with an average of 8.94 ± 2.18 days. The incentive for breeding of majority of the bitches (61.27%) was one pup born in the litter and cash payment was practised in 24.51 per cent. Pregnancy confirmation based on veterinary examination was practised in 17.98 per cent of bitches. The incidence of failure of conception, abortion, stillbirth and pseudopregnancy were 18.87, 5.72, 15.56 and 13.69 per cent respectively. The incidence of assisted whelping and caesarean section were 5.18 and 1.32 per cent respectively. The neonatal problems encountered in 13.88 per cent of bitches included agalactia / hypogalactia / mammitis (5.29%), cannibalism (2.65%), puppy fading syndrome (2.09%), puerperal tetany (1.98%) and rejection of young one by dam / poor mothering ability (1.87%).

Among the 1203 bitches surveyed, 134 (11.14%) were identified as in the stage of anoestrus. Analysis of breed-wise prevalence revealed that it was high in breeds such as Dachshund (18.18%), Labrador retriever (16.74%), Great Dane (16.67%), Dalmatian (15.38%) and Doberman pinscher (14.00%) and was lowest (2.44%) among local non-descript bitches. Out of the 134 anoestrous bitches, 65.67 per cent was in primary and 34.33 per cent in secondary anoestrus and the average age was 22.68 and 34.34 months respectively. The overall average duration of secondary anoestrus was found to be 11.71 months. Data on body condition revealed that 46 per cent of the anoestrous bitches were obese with body score more than five.

The mean serum progesterone, prolactin and thyroxine levels in anoestrous bitches were of 0.57 ± 0.03 ng/ml, 0.75 ± 0.05 ng/ml and 1.80 ± 0.06 µg/dl respectively. Analysis of haematological profile of anoestrous bitches revealed normochromic normocytic anaemia. Mild degree of hypocalcaemia, hypophosphataemia, hypercholesterolemia were also noticed in anoestrous bitches.

Out of 10 animals treated in each group, five (50%) in bromocriptine treated group (Group I), nine (90%) in cabergoline treated group (Group II), eight (80%) in thyroxine (@10 µg/kg. body weight) treated group (Group III) and seven (70%) in thyroxine (@ 5 µg/kg. body weight) treated group (Group IV) evinced proestrual bleeding. The mean treatment onset to proestrus in Groups I, II, III and IV was 28 ± 3.39, 13.44 ± 3.12, 24.50 ± 3.18 and 33 ± 2.21 days respectively. The average duration of proestrus in the treatment groups was 9.80 ± 0.86, 10.11 ± 0.68, 11.25 ± 0.88 and 10.71 ± 0.68 days respectively as against 9.70 ± 0.42 days in the control group. The average duration of oestrus in the four treatment groups and the control group was 7.60 ± 0.24, 8 ± 0.29, 8.5 ± 0.63, 7.85 ± 0.46 and 8 ± 0.39 days respectively. The anuclear cell index on the day of first breeding in the treatment groups and the control group were 92.20 ± 1.28, 92.67 ± 1.07, 95.75 ± 1.00, 93.14 ± 1.14 and 92.60 ± 1.29 respectively. Fifty per cent of the animals treated with bromocriptine and 10 per cent of the animals treated with cabergoline exhibited nausea and vomiting as side effects, whereas none of the animals treated with thyroxine exhibited side effects.

The mean serum progesterone level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly within and between the treatment groups and the control group. The average serum prolactin level during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly between the treatment groups and the control group. How ever, a slight reduction was noticed on the first day of bleeding and on the day of first breeding. The average serum thyroxine level during anoestrus and on the first day of proestrus did not differ significantly within and between the treatment and the control groups. Serum thyroxine level on the day of first breeding recorded statistically significant difference between the treatment and control groups, the lowest being 1.56 ± 0.18 µg/dl in Group I and the highest being 2.10 ± 0.13 µg/dl in Group IV. Animals treated with levothyroxine (Group III and IV) exhibited a slight increase in the mean level of serum thyroxine on the first day of bleeding as well as on the day of first breeding, but

the values were found to be within the normal limits. The Hb and TEC values were found to be increased on the first day of proestrus and on the day of first breeding in animals of Group III and IV.

The mean serum level of calcium, iron, cobalt, zinc and manganese during anoestrus, on the first day of proestrus and on the day of first breeding did not reveal statistically significant difference between groups. On the other hand, the mean serum phosphorus level on the first day of proestrus and on the day of first breeding in the treatment groups were found to continue at low levels as in the anoestrous stage. The serum copper level on the first day of proestrus exhibited statistically significant difference between the treatment and control groups. Even though mild hypercholesterolemia was observed during anoestrus in bitches, the mean serum cholesterol level on the first day of proestrus and on the day of first breeding in the treatment groups fell within the normal range.

The accuracy of pregnancy diagnosis when performed between 20 and 25 days post-breeding by trans-abdominal ultrasound scanning and abdominal palpation was found to be 88.89 and 66.67 per cent respectively and it increased to 100 and 88.89 per cent respectively between 30 and 35 days. The conception rate in relation to the number of animals responded to oestrus induction treatment in the treatment groups were 80.00, 77.78, 62.50 and 57.14 per cent respectively. The overall conception rate in relation to the number of animals subjected to oestrus induction trials in the treatment groups were 40, 70, 50 and 40 per cent respectively. Out of the 10 animals of the control group which were bred during the natural oestrus, seven (70%) conceived. The average gestation length in the treatment groups varied from 60.50 ± 1.55 to 64.00 ± 0.82 as against 61.43 ± 0.81 in the control group. The average litter size in the treatment groups varied from 5.14 ± 0.34 to 6.40 ± 0.40 as against 6.43 ± 0.78 in the control group.

From the present study, it could be concluded that:

1. Among the various breeds of dog, German shepherd dog, Labrador retriever and Dachshund were found to be the most popular breeds in Kerala and the percentage of local non-descript dogs was only 14.64.

2. The dog breeding units are gaining popularity in Kerala and that a sizeable number of youngsters have identified it as a potential vocation.

3. The prevalence of anoestrus among dogs of Kerala was found to be 11.14 per cent and that 65.67 per cent of them were in primary anoestrus and the remaining 34.33 per cent in secondary anoestrus. The incidence of obesity among anoestrous bitches was found to be 46.00 per cent.

4. The average age of primary anoestrous bitches was found to be 22.68 months and the average duration of secondary anoestrus was 11.71 months.

5. The mean serum progesterone level in anoestrous bitches was found to be 0.57 ± 0.03 ng/ml confirming the ovarian inactivity during anoestrus. The mean serum prolactin level during anoestrus was 0.75 ± 0.05 ng/ml. The mean serum thyroxine level of 1.80 ± 0.06 µg/dl obtained in anoestrous bitches was indicative of mild hypothyroidism.

6. Haematological and mineral profile revealed normochromic normocytic anaemia, mild degree of hypocalcaemia, hypophosphataemia and hypercholesterolemia in anoestrous bitches.

7. Oestrus induction trials carried out using antiprolactin drugs *viz.* bromocriptine and cabergoline and thyroxine revealed that cabergoline @ 5 µg/kg body weight orally for a period of two weeks was superior to bromocriptine and thyroxine with satisfactory conception rate.

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COMPARATIVE EFFICACY OF BROMOCRIPTINE, CABERGOLINE AND THYROXINE IN INDUCING OESTRUS IN BITCHES

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ABSTRACT

Detailed survey on breeding, feeding and management of 1721 dogs belonging to 817 owners was conducted in different parts of Kerala State. Among the dogs surveyed, 518 (30.10%) were male and 1203 (69.90%) were female. Breed-wise details of dogs maintained by individual owners revealed German shepherd dog (20.74%) as the most popular breed of dog followed by Labrador retriever (18.13%), Dachshund (11.39%), Rottweiler (9.12%) and Spitz (5.52%). The percentage of local non-descript dogs was found to be 14.64 only. The prevalence, nature and magnitude of anoestrus among the female dogs were evaluated based on breeding details. Haematological, hormonal, mineral and cholesterol profile of anoestrous bitches were estimated.

Data on breeding revealed that 92.93 per cent of bitches were allowed to breed and the remaining 7.07 per cent were kept as virgin throughout their lifetime. The age at first breeding in majority of the bitches (57.85%) was between 1 to 2 years with an average of 16.30 months. The average number of oestrus periods and the number of breeding per year were 2.14 ± 0.24 and 1.47 ± 0.32 respectively. The average duration of proestrual bleeding was found to be 10.87 ± 1.24 days. Most of the bitches (51.97 %) were bred twice in one oestrus and the period of acceptance varied from 4 to 22 days with an average of 8.94 ± 2.18 days. The incidence of failure of conception, abortion, stillbirth and pseudopregnancy were 18.87, 5.72, 15.56 and 13.69 per cent respectively. The neonatal problems encountered in 13.88 per cent of bitches included agalactia / hypogalactia / mammitis (5.29%), cannibalism (2.65%), puppy fading syndrome (2.09%), puerperal tetany (1.98%) and rejection of young one by dam / poor mothering ability (1.87%).

Among the bitches surveyed, 134 (11.14%) were identified as in the stage of anoestrus, 65.67 per cent in primary and 34.33 per cent in secondary anoestrus and the average age was 22.68 and 34.34 months respectively. The average

side effects, whereas none of the animals treated with thyroxine exhibited side effects.

The mean serum progesterone and prolactin levels during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly within and between the treatment groups and the control group. The mean serum thyroxine level during anoestrus and on the first day of proestrus did not differ significantly within and between the treatment and the control groups, whereas on the day of first breeding the mean thyroxine level recorded statistically significant difference between the treatment and control groups, the lowest being 1.56 ± 0.18 µg/dl in Group I and the highest being 2.10 ± 0.13 µg/dl in Group IV. Animals treated with levothyroxine (Group III & IV) exhibited a slight increase in the mean level of serum thyroxine on the first day of bleeding as well as on the day of first breeding, but the values were found to be within the normal limits. The haemoglobin level and total erythrocyte count were found to be increased on the first day of proestrus and on the day of first breeding in animals of Group III and IV.

The mean serum phosphorus level on the first day of proestrus and on the day of first breeding in the treatment groups were found to continue at low levels as in the anoestrous stage. The mean serum copper level on the first day of proestrus exhibited statistically significant difference between the treatment and control groups. The mean serum level of calcium, iron, cobalt, zinc and manganese during anoestrus, on the first day of proestrus and on the day of first breeding did not reveal statistically significant difference between groups.

The overall conception rate in the treatment groups were 40, 70, 50 and 40 per cent respectively as against 70 per cent in the control group. The average gestation length and litter size did not differ significantly between the treatment and control groups.

duration of secondary anoestrus was 11.71 months. Analysis of breed-wise prevalence revealed that it was high in breeds such as Dachshund (18.18%), Labrador retriever (16.74%), Great Dane (16.67%), Dalmatian (15.38%) and Doberman pinscher (14.00%) and was lowest (2.44%) among local non-descript bitches. Data on body condition revealed that 42 per cent of the anoestrous bitches were with the ideal body score of five, 46 per cent with body score more than five (obese) and 12 per cent with body score less than five (lean).

Analysis of haematological profile of anoestrous bitches revealed normochromic normocytic anaemia. The mean serum progesterone, prolactin and thyroxine levels in anoestrous bitches were of 0.57 ± 0.03 ng/ml, 0.75 ± 0.05 ng/ml and 1.80 ± 0.06 µg/dl respectively. The mean serum calcium and phosphorus levels in anoestrous bitches were 8.94 ± 0.24 and 3.48 ± 0.12 mg per cent respectively. The mean serum iron, copper, cobalt, zinc, manganese and cholesterol levels were found to be within the normal range.

Oestrus induction trials were carried out in forty anoestrous bitches and the data obtained were compared with that of control bitches. Out of 10 animals treated in each group, five (50%) in Group I (bromocriptine @ 50 µg/kg. body weight), nine (90%) in Group II (cabergoline @ 5 µg/kg. body weight), eight (80%) in Group III (thyroxine @10 µg/kg. body weight) and seven (70%) in Group IV (thyroxine @ 5 µg/kg. body weight) evinced proestrual bleeding. The mean treatment onset to proestrus in Groups I, II, III and IV was 28 ± 3.39, 13.44 ± 3.12, 24.50 ± 3.18 and 33 ± 2.21 days respectively. The average duration of proestrus in the treatment groups was 9.80 ± 0.86, 10.11 ± 0.68, 11.25 ± 0.88 and 10.71 ± 0.68 days respectively as against 9.70 ± 0.42 days in the control group. The average duration of oestrus in the four treatment groups and the control group was 7.60 ± 0.24, 8 ± 0.29, 8.5 ± 0.63, 7.85 ± 0.46 and 8 ± 0.39 days respectively. Fifty per cent of the animals treated with bromocriptine and 10 per cent of the animals treated with cabergoline exhibited nausea and vomiting as

side effects, whereas none of the animals treated with thyroxine exhibited side effects.

The mean serum progesterone and prolactin levels during anoestrus, on the first day of proestrus and on the day of first breeding did not differ significantly within and between the treatment groups and the control group. The mean serum thyroxine level during anoestrus and on the first day of proestrus did not differ significantly within and between the treatment and the control groups, whereas on the day of first breeding the mean thyroxine level recorded statistically significant difference between the treatment and control groups, the lowest being 1.56 ± 0.18 μg/dl in Group I and the highest being 2.10 ± 0.13 μg/dl in Group IV. Animals treated with levothyroxine (Group III & IV) exhibited a slight increase in the mean level of serum thyroxine on the first day of bleeding as well as on the day of first breeding, but the values were found to be within the normal limits. The haemoglobin level and total erythrocyte count were found to be increased on the first day of proestrus and on the day of first breeding in animals of Group III and IV.

The mean serum phosphorus level on the first day of proestrus and on the day of first breeding in the treatment groups were found to continue at low levels as in the anoestrous stage. The mean serum copper level on the first day of proestrus exhibited statistically significant difference between the treatment and control groups. The mean serum level of calcium, iron, cobalt, zinc and manganese during anoestrus, on the first day of proestrus and on the day of first breeding did not reveal statistically significant difference between groups.

The overall conception rate in the treatment groups were 40, 70, 50 and 40 per cent respectively as against 70 per cent in the control group. The average gestation length and litter size did not differ significantly between the treatment and control groups.