STUDIES ON REPRODUCTIVE EFFICIENCY OF FEMALE MUDHOL HOUND DOGS

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By

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CERTIFICATE

This is to certify that the thesis entitled "***STUDIES ON REPRODUCTIVE EFFICIENCY OF FEMALE MUDHOL HOUND DOGS***" submitted by **Dr. MAHESH S. DODAMANI, ID No. DVHK-1212** in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fishers Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar titles.

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This thesis is affectionately dedicated to late. Dr. R. V. PATIL and Family and My Parents for their love, tireless support, Monitoring and encouragement...

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LIST OF ABBREVIATION

SC	Scheduled caste
ST	Scheduled tribal
SCSP	Scheduled caste SubPlan
TSP	Tribal SubPlan
CRIC	Canine Research and Information Centre
g	Grams
Kgs	Kilograms
P4	Serum progesterone
FCI	Federation Cynologea Internationale

Introduction

I. INTRODUCTION

Dogs have always held a special place in the hearts of their human companions. Regardless, historians agree that humans domesticated dogs before any other animal - making dog man's oldest friend, if not his best. Their role among humans was mostly utilitarian until the 18th century, when the term "man's best friend" came into the lexicon. Their working roles are reduced today, but their importance at home has only increased. From pets to sporting companions to service dogs, canines add a measurable degree of quality to the worlds of the humans they share their lives with.

Currently the Federation Cynologae Internationale (FCI) has recognized 332 fully official breeds and 11 provisional breeds. Majority of these recognized breeds are belonging to western Countries. India has got a vast array of many species of domestic and companion animals. Dog has been the important companion animal since time immemorial. In India, 20 dog breeds have been identified, but none of these are internationally recognized. The most well known dog breeds of India are Rajapalayam, Chippiparai, Kombai, Kanni, Mudhol Hound, Rampur Hound, Caravan Hound, Banjara Hound, Bhotia sheep dog, Himalayan sheep dog, Alangu, Kaikadi, Indian Spitz, Bakharwal and Jonangi. The state of Karnataka in southern India is blessed with three such indigenous dog breeds, of which, Mudhol Hound is one among them. The other being Caravan Hound and Pashmi. It is a rare desi dog found in and around Bagalkot and Bijapur districts of Northern part of Karnataka. Even the Kanni, another Indian dog breed found in Tamilnadu, was considered as a further extension of the

Caravan or Mudhol Hound and was also a descendant of the Saluki (Ravimurugan and Kumaravelu, 2008; Srinivasan, 2011).

Mudhol Hounds are native to India, having developed in the western region of the Deccan Plateau, which includes parts of Karnataka, Maharashtra and Andhra Pradesh. For centuries, this breed has served as a companion, hunter and guard dog for both royalty and peasants. Throughout its existence the breed has been known by a variety of names *viz.*, Mudhol Dog, Caravan Hound, Karwani, Lahore Pashmi, Pashmi and Pisuri Hound. In many villages of the Deccan Plateau, the Mudhol Hounds are also known as Karwani or Caravan Hounds. This name originated from when Indian villagers first saw the forerunners of the breed running alongside the caravans of their masters as they travelled from place to place. Some argue that the name of "Caravan Hound" is more appropriate than "Mudhol Hound" because the breed is not only native to Mudhol, but to all of the western Deccan Plateau as well. The Kennel Club of India (KCI) and Indian National Kennel Club (INKC) recognize the breed under different breed names. The KCI registers it as a Caravan Hound while the INKC goes with the name as Mudhol Hound. The INKC's official journal published breed standards for Mudhol Hounds in January, 1995 (Ramakrishna, 1997; Ramakrishna and Yathinder., 2000 and Ramakrishna, 2002).

Regardless of the name, this breed was and is valued for its ability to excel at its tasks, under conditions grueling enough to thwart many other types of working dogs. Mudhol Hounds look much like the Sloughi, the Saluki and the GreyHound, with the graceful gait of the Azawakh. The Mudhol Hound hunts like the Saluki, sprinting after its prey, able to make turns in mid-air and change its course in a heartbeat. Mudhol Hounds

are bred to withstand harsh weather, navigate rugged terrain, while doing demanding work. They have been developed for speed, strength and endurance to chase and kill hares, jackals, blackbucks and chinkaras, while traversing through deep sand or in rocky mountain regions. They have been and are expected to be dependable and efficient guardians of both their owners and their property including livestock (Fig.1).

It is said that Raja of Mudhol, a small princely town in Karnataka, was very fond of hunting with dogs so he crossed his local sight Hounds with Grey Hounds to increase the speed and created the breed called "Mudhol Hound". These 'Mudhol Hounds' are inheriting the speed of Grey Hounds and the stamina of the local sight Hounds. During the past centuries the ruling Ghorpades of the feudal state of Mudhol in the district of Bagalkot in Karnataka have developed the Mudhol Hound by selective breeding. The Chandanshiva family of Mudhol was entrusted with the perpetuation of this breed, their descendants still continuing to do so.

In recognition of this breed Government of India has brought out a postal stamp for propaganda of this breed among citizens in 1995 and Karnataka Government has started an exclusive Canine Research and Information Centre (Mudhol Hound) at Thimmapur of Mudhol Taluk for conservation and development of this breed in 2009 under Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar. Apart from this encouraging the Mudhol dog breeders to participate in dog shows, organsing the exclusive desi dog show at district and state level by agencies like Kennel clubs, NGOs, farm Universities and state department of Animal Husbandry and accessing the quality health services through free dog health check up or vaccination camps in the

breeding tract by government as well non government organizations are ongoing efforts in this direction.

In the recent year though the efforts are going for assessment of desi canine breeds both at phenotypic as well as at molecular level, again those studies are limiting majorly to Rajapalayam, Kanni and Chippiparai dog breeds in Tamilnadu. But, as such no systematic efforts have been carried out to establish reproductive parameters, reproductive efficiency data, biochemical parameters and hematological parameters in Mudhol Hound breed of dogs. Hence, the present study entitled **"Studies on Reproductive Efficiency of Female Mudhol Hound dogs"** is an attempt in this direction with the following Objectives:

1. To record the age at puberty and patterns of estrous cycle in female Mudhol Hound dogs.
2. To define differences between season of the year regarding the distribution of mating and whelping in female Mudhol Hound.
3. To record litter size, sex ratio, incidence of neonatal mortality and frequency of abnormal births in female Mudhol Hound dog.
4. To determine the effects of age and parity of bitch at the time of whelping on litter size as well as the influence of litter size on length of gestation in Mudhol Hound.
5. To compare the reproductive efficiency of Mudhol Hound breed of female dog maintained on a commercially available balanced diet with those maintained on homemade food.

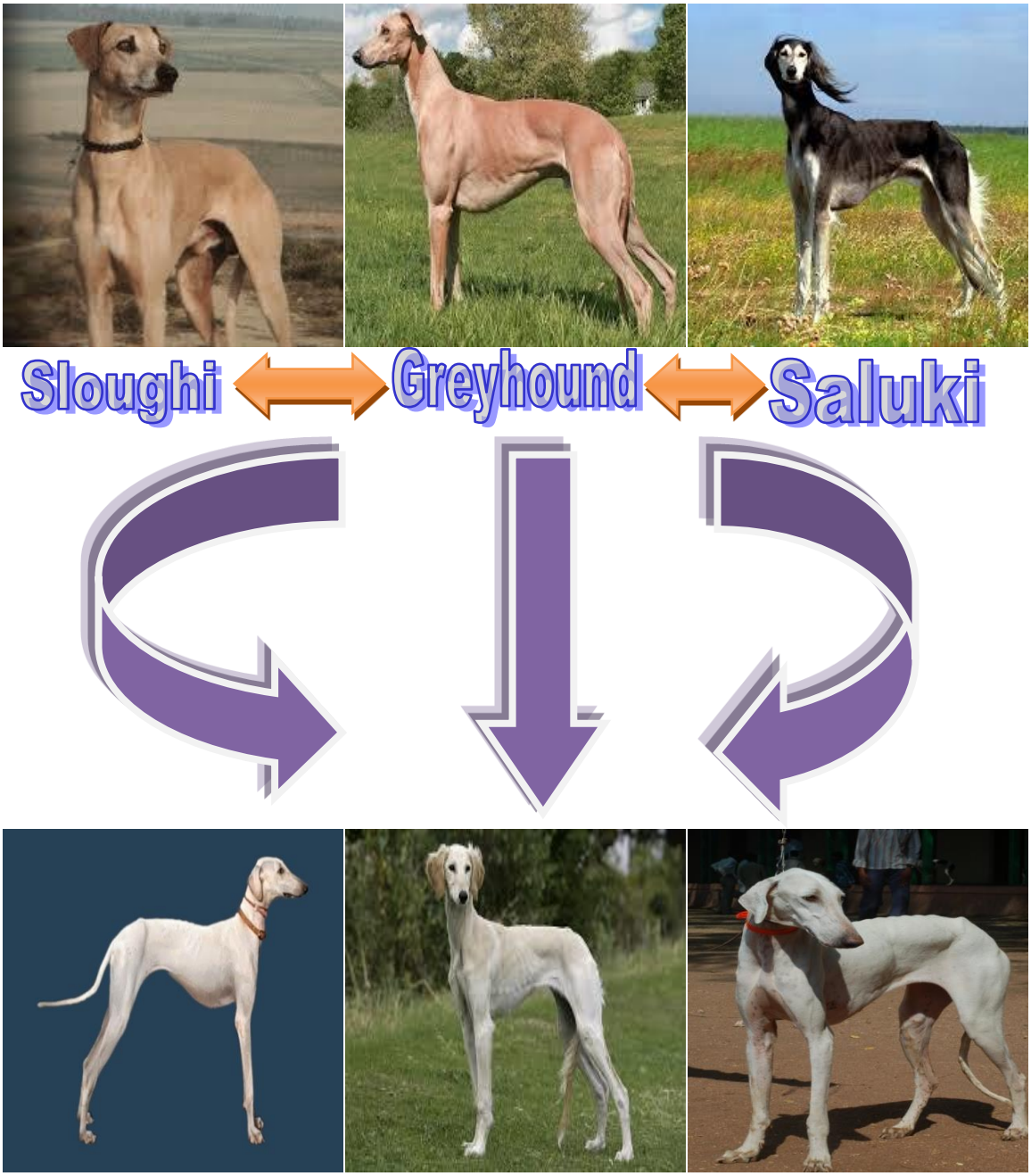


Fig. 01. History and origin of Mudhol Hound and Pashmi breed of dogs - the pride of Karnataka

Review of Literature

II. REVIEW OF LITERATURE

2.1 Origin and history of Mudhol Hound

Mudhol Hounds are native to India, having developed in the western region of the Deccan Plateau, which includes parts of Karnataka, Maharashtra and Andhra Pradesh. For centuries, this breed has served as a companion, hunter and guard dog for both royal families and peasants. Even the Tamil Nadu dog breed, Kanni was considered as a further extension of the Caravan or Mudhol Hound and was also a descendant of the Saluki (Ravimurugan and Kumaravelu, 2008; Srinivasan, 2011) indicating the spread of this Hound across south India long ago.

Accompanying early traders, mercenaries and invaders, the Mudhol Hounds ancestors came from Central and Western Asia, arriving in India via the Himalaya Mountains Khybur Pass, a key trade and military route that linked Central and South Asia. The Khybur Pass provided entry to the Huns, Mongols, Kushans, Persians, Greeks, Turks and other hordes that invaded India since 500 B.C. The Mudhol Hound is a descendent of some of the dogs they brought with them probably the Sloughi (from North Africa), the Saluki (from the Middle East), the Azawakh, the Greyhound and the Afghan Hound (Ramakrishna, 1997; Ramakrishna and Yathinder, 2000 and Ramakrishna, 2002). These breeds were crossed with the local Indian dogs and evolved into India's native Hounds. Over a period of time, these dogs took on the name of the land in which they lived.

Historical evidences suggest that Mudhol Hounds, known for devotion have been used in Guerrilla warfare to guard and sight interpolers during Maratha regime. Marathas,

known for their devotion to canine companions, used Mudhol Hounds in their guerrilla warfare to guard and sight interlopers and used in their fight against British and Muslim invaders. The Mudhol Hounds are fearless and devoted to their masters. “Chatrapati Shivaji”, who led the fight for, founded and ruled the Maratha Empire, had his dogs buried near his own grave at Raighad, which was the capital of Maratha (Ramakrishna, 1997; Ramakrishna and Yathinder, 2000 and Ramakrishna, 2002) indicates the association of this breed with mankind. Sri Chatrapathi Sahu Maharaj, grandson of Chatrapati Shivaji and also a ruler of the Maratha Empire, was saved from a tiger by his faithful Mudhol Hound who attacked and killed the beast. Shri Tulaji Angre fought the British on the high seas, with his dogs on board, to protect Maratha independence. Mudhol Hounds were favored by royal families, these families are treated as the patrons of the Mudhol Hound, but they have never been a pampered breed, always living a rugged existence.

It is said that Raja of Mudhol, a small princely town in Karnataka, was very fond of hunting with dogs. So he crossed his local sight Hounds with Grey Hounds to increase the speed and created the breed now called as "Mudhol Hound”. These 'Mudhol Hounds' are inheriting the speed of grey Hounds and the stamina of the local sight Hounds. The Legends say that Sri Srimanth Raja Malhojirao Ghorphade, who was once ruler of Mudhol, gave King George IV of England a pair of these puppies. King George is said to have inspected them, approved of their conformation as befitting of sight Hounds and started calling them as “**Mudhol Hounds”** (Ramakrishna, 1997; Ramakrishna and Yathinder, 2000 and Ramakrishna, 2002)**.**

This breed is valued for its ability to excel at its tasks, under conditions grueling enough to thwart many other types of working dogs. Mudhol Hounds look much like the Sloughi, the Saluki and the Grey Hound with the graceful gait of the Azawakh. The Mudhol Hound hunts like the Saluki, sprinting after its prey, able to make turns in mid-air and changes its course in a heartbeat. Mudhol Hounds are bred to withstand harsh weather, navigate rugged terrain, while doing demanding work. They have been developed for speed, strength and endurance to chase and kill hares, jackals, blackbucks and chinkaras, while traversing through deep sand or in rocky mountain regions. They have been and are expected to be dependable and efficient guardians of both their owners and their property including livestock (Ramakrishna, 1997; Ramakrishna and Yathinder, 2000 and Ramakrishna, 2002).

During the past centuries the ruling Ghorpades of the feudal state of Mudhol presently in Bagalkot district of Karnataka have developed the Mudhol Hound by selective breeding. The Ghorpades have entrusted the responsibility of conserving this sight Hound to Chandanshiva family in Mudhol and their descendants still continuing to do so. The Mudhol Hound, once the pond of royal families started declining in numbers on one side and faced the problems of apathy as the developmental efforts of government are more directed towards agriculture and livestock on priority.

After independence, though few sporadic attempts have been done to conserve this exclusive canine breed through health and extension service delivery along with other domestic species much has not been achieved. In recognition of this Indian breed Government of India has brought out a postal stamp to promote and popularize among

the citizens. In 2009, the Government of Karnataka finally recognized the importance of conserving this pride canine breed of the state and handed over the responsibility to the veterinary varsity by establishing an exclusive first of its kind institute in nation at Timmapur of Bagalkot District *Viz*., Canine Research and Information Centre –CRIC, (Mudhol Hound), Timmapur, Mudhol, Bagalkot. Now the varsity is carrying out various multifaceted activities like breeding, livelihood support to dog rearers and delivering quality health-extension services to the needy Mudhol Hound breeders in the breeding tract through CRIC.

Mudhol Hounds are a rugged breed; for many centuries these dogs have been expected to live and to work hard in harsh environments. Only the strongest have survived and the result is that Mudhol Hounds are hardy dogs, suffering from no known breed specific health issues. They have a lifespan of approximately 10 to 15 years (Ramakrishna, 1997; Ramakrishna and Yathinder, 2000 and Ramakrishna, 2002).

They are gentle, loyal and affectionate with those they know well, but wary toward strangers to the extent that they do not like to be touched by them. Their aloofness towards people they do not know well, combined with their courage, devotion and loyalty toward their owners and family, make them good watch dogs. This breed cannot tolerate being confined for long periods of time and does not do well when left alone all day (Ramakrishna, 2002). If properly trained, they can do well with livestock because they have been used and bred to protect and sometimes herd of livestock animals (Ramakrishna, 2002).

2.2 Birth weight of new born

The birth weight of new born puppies is reported to be influenced by a variety of factors such as genetic, environment and nutrition, beside fetal uterine position in polytocus species (Bautista *et al*., 2015). In Boxer, also the genetic role in birth weight was reported by Nielen *et al*. (2001).

Groppetti *et al*. (2015) reported that birth weight was related to maternal size, weight and age. Medium-large sized bitches or more than 20 kg in weight whelped pups heavier than 400 g. Birth weight was also related both to litter size, with heavier pups in smaller rather than in larger litters from medium sized bitches, and breed ($P < 0.05$).

Bhagirathi (2008) reported that breeds between 10 to 25 kg of adult body weight are generally categorized as medium sized dog

Bautista *et al.* (2015) reported that besides, the size of the breed, the birth weight of new born is also reported to be influenced by variety of factors such as genetics, environment, nutrition and fetal uterine position

Mila *et al. (*2015) stated that birth weight was related to maternal size, weight and age as well as breed and litter size with heavier puppies in small rather than larger litter from medium sized breeds.

Nielen *et al.* (2001) reported that a definite role of genetics influencing the birth weight of boxer puppies.

Pascal and Gaspar (2015) reported in Romanian Bucovina shepherd dog the male puppies weighed about 52 g more than new born female puppies.

Biglardi *et al.* (2013) also reported that male boxer puppies weighed slightly heavier than female puppies and that differences were significant. The heavier size of male offspring as compared to female offspring has also been described (Gianola and Tyler, 1974; Johnson and Berger, 2003) in cattle.

2.3 Age and Body weight at pubertal estrus

Puberty in the domestic female dogs appears, in most breeds, not to depend on day length, an exception being the Basenji which usually cycles only in the autumn (Concannon, 2011). Puberty seems to be related to size and weight, in that it occurs when the bitch has reached around 85% of the adult weight and consequently, bitches of the smaller breeds in general have their first estrus at an earlier age than those of the larger breeds. Under the influence of the sexual hormones. the growth plates of the long bones close and little further growth will take place after this time. Most bitches, thus, reach puberty between 6 and 15 months of age, but some, especially of the larger breeds not until at 18-20 months of age. During the pubertal estrus circulating hormone levels are often low and fluctuating, causing absence of or incomplete ovulation and the bitch may not show the standing estrus (Chakraborty *et al.*, 1980; Wildt *et al.*, 1981).

In domestic dogs, sexual maturity begins at about 06 to 12 months of age for both male and female (Dewey and Bhagat, 2002). It is positively correlated with breed size (Concannon, 2011). Age at puberty is also influenced likely by genetic as well as

environmental factors, such as nutrition, although, this can be delayed until about 24 months for some large breeds (Nelson and Couto, 2003).

Sokolowaski *et al. (*1977) has stated that, individuals of small breed enter puberty earlier than large breed, with onset of cycle correlating with time the animal reaches growth plateau

Dewey and Bhagat (2002) also stated that in domestic dogs, sexual maturity begins about 6 to 12 months of age for both male and female dogs.

Johnston *et al*. (2001) have stated that the puberty begins between 6 to 10 months age. For bitches of smaller breeds but may not begin for bitches of some larger breeds until they are nearly 24 months old (Nelson and Couto, 2003) and also opined that the age of puberty in dogs could be influenced by the level of nutrition.

Clark and Strainer. (1983) reported that the onset of puberty for some selected breeds was presented as Afghan Hound (7-30 months), Borzoi (15- 18 months), Golden Retriever (9 - 11 months) and Grey Hound (11-30 months and there was strong correlation between age of onset of puberty and breed and also size of bitch.

2.3.1 Body weight at pubertal estrus

Puberty seems to be related to size and weight, in that it occurs when the bitch has reached around 85% of the adult weight and consequently, bitches of the smaller breeds in general have their first oestrus at an earlier age than those of the larger breeds (Chakraborty *et al.*, 1980; Wildt *et al.*, 1981).

Caffaratti *et al.,* (2013) and Concannon (2011) reported that a positive correlation between the onset of puberty and size and weight

2.4 Month of the year during which pubertal estrus and subsequent estrus are observed

Puberty in the domestic female dogs appears, in most breeds, not to depend on day lengnth, an exception being the Basenji which usually cycles only in the autumn (Concannon, 2011). Estrus in female dogs can be seen in all seasons of year, though it is more common in spring and autumn (Arthur *et al.*,, 1989; Johnson *et al.,* 2001). The effect of season on the reproductive cycle and reproductive pattern shows conflicting results and overall probability that an estrus would occur at any month of the year was the same for each month (Bouchard *et al.,* 1991). The season has no influence on the estrus frequencies by month of the year and also stated that a higher frequency of bitches were in estrus during October and a lower frequency in April. (Sokolowski *et al.,* 1977). Inter-estrous intervals of 5–12 months, typically 6–7 months, range from highly variable to regular within bitches, and averages do not vary significantly or consistently between pregnant and non-pregnant bitches (Conconnon, 2011).

Depending on locality and on the month in which some bitches attain puberty which is connected to other factors such as nutrition and breed. Christie and Bell (1971a) stated that high frequency of estrus in the period from February to May compared to June and September and also October to January. Tedor and Reif (1978) observed a distinct, repetitive seasonal distribution of births with primary peak in the spring and early summer. The condition of the rainy season, during September, have been reported to have

a positive effect on conception rate and led to highest whelping rate (Chatdarong *et al.*, 2007). Litter size, too, has been influenced by the climate factors such as temperature and humidity (Chatadarong *et al.*, 2007).

Chatadarong *et al. (*2007) stated that seasonal influence on reproduction in many species, high ambient temperature is reported to cause infertility by compromising ovarian function (Quesnel *et al.*, 1998), estrus and ovulation (Kunavongkrit and Tantasuparuk, 1995), embryonic implantation, seminal and sperm production (Suriyasomboon *et al.*, 2005) in pigs and similarly adverse consequences in cows (Zeron *et al.*, 2001). The adverse effects of estrus in bitch reproduction have not been documented sufficiently.

Gipson (1996) repoted that the bitches are considered non seasonal or mono estrous breeders showing estrus, mating and whelping throughout the year. There are several aspects of seasonal effects on estrus in bitches. The season affects estrous cycle and frequency of prevalence of estrus (Mutembei *et al.*, 2000; Wikstrom and Linde, 2006)

In bitches for instances the highest number of estrus cycles were reported in November in Kenya (Mutembei *et al.*, 2000), between February and May in United Kingdom and March in Swedan (Polat *et al.*, 2015).

Gavrilovic *et al. (*2008) and Chatdarong *et al. (*2007) stated that estrous cycle were also attributed to variation of day light or photo period as shown in the tropical and polar region.

Ortega *et al.* 2007 and Polat *et al.,* 2015 reported that the estrous cycle could be observed in each month of the year.

2.5 Estrous cycle

The normal estrous cycle of the canine can be described in several ways, including behavioral aspects, clinical signs, hormonal profile and cytological changes of the vaginal epithelium. The classical stages of estrous cycle of the bitches introduced by Heape (1900) were proestrus, estrus, metestrus and anestrous. In recent years, the metestrus has largely been replaced by the term diestrus (Olson and Nett, 1986).

Bitches are monoestrus, typically non-seasonal, polytocous, spontaneous ovulators and have a spontaneous luteal phase similar in length to or a bit longer than the 64±1 day luteal phase of the 65±1 days of pregnancy followed by an obligate anoestrus or sexual quiescence before the next heat period irrespective of whether they are pregnant or not (Arthur *et al.,* 1989, Concannon, 2011). It has been demonstrated that domestic dogs housed outdoors cycle year round as do dogs housed under constant lighting conditions of 12 hours light:12 hours dark (Concannon, 1986) with the exception of some fox-Iike çanids (Sheldon,1992) and the bush dog (Porton *et al.,* 1987).

The stages of the cycle of the female dogs are long in comparison with those of other domestic animals (Ibrahim *et al.,* 2011). The estrous cycle is divided into two phases, the estrogenic or follicular phase comprising of proestrus and oestrus. The progesterone or luteal phase comprising the diestrum, while the anoestrous is the phase of sexual quiescence (Wright and Cho Po-Yin, 2001; Nelson and Couto, 2003). In recent years, the term diestrus has largely replaced the term metestrus in the bitch (Olson *et al.,*

1984). The canine cycle is classically divided into 4 phases (Evans and Cole, 1931) a 5–20 day proestrus, 5–15 day estrus, 50–80 day metestrus (post-estrus portion of luteal phase), and anestrus typically lasting 80–240 days. In recent years, the metestrus has largely been replaced by the term diestrus (Olson and Nett, 1986).

2.5.1 Proestrus

The average duration of proestrus in mature bitches is nine days, with a normal range of 0 to 27 days (Bell and Christie., 1971; Olson and Nett, 1986; Alien and England, 1990). The average number of days for proestrus was 9.0 ± 0.0 days (Oluwatoyin *et al.*, 2012). Proestrus is usually simply defined as that stage of the estrous cycle where noticeable signs of serosanguinous (thin, bloody) vaginal discharge are observed. Varying degrees of bloody vaginal discharge are typically seen. The nature of the discharge is typically bright red and voluminous at the onset of proestrus and then becomes less voluminous towards the end. However, it should be noted there is great variation amongst bitches with regard to the amount and nature of their discharge. During proestrus, females are attractive to male dogs, but generally refuse matings. Pheromones, which are present in the female's vaginal secretions, anal sac expressions and urine are responsible for this attractiveness (Goodwin *et al*., 1979; Raymer *et al*., 1986). Sexual reflexes such as flagging of the tail (elevation of the tail away from the vulva and swaying of the hips from side to side) in response to touching the peri-anal region (area between and including the anus and vulva) begin in proestrus (Beach *et al*., 1982). The vulva slowly enlarges throughout proestrus due to edematous (turgid) swelling. As proestrus progresses, this swelling gradually subsides and the vulva is less turgid.

Endocrinologically, the proestrus bitch is under the influence of estrogen and this is the dominant hormone during this stage of the cycle. Estrogen is responsible for most of the clinical signs seen in bitches during proestrus, because this hormone timulates growth and activity of the glandular epithelium of the uterus and promotes swelling and increased vascularity of the lining of the uterus (Jeffcoate, 1998). The serosanguinous vaginal discharge is a direct result of these effects because the junctions between the cells lining the capillaries leak and permit passage of red blood cells into the uterine lumen which is eventually discharged vaginally after passage through the cervix. Serum progesterone concentrations during proestrus are at basal levels (<0.5 ng/mL) and then start a gradual rise at the end of proestrus. Preovulatory follicular luteinization transformation of the estrogen secreting follicle to a progesterone secreting structure), unique to the canine, is responsible for this increase in progesterone concentration (Phemister *et al*., 1973; Wildt *et al*., 1979)

2.5.2 Estrus

Proestrus proceeds into estrus, which is the stage of the cycle characterized by the bitches' acceptance of the male for mounting. The first day the bitch allows mating (standing estrus) is considered as the start of estrus and this phase ends when she no longer accepts the male (Feldman and Nelson, 1996). The average duration of estrus in the bitch has been reported to be nine days, with a range of 4 to 24 days (Bell and Christie, 1971). Concannon (2011) stated that, estrus lasts a variable length of time, wanes slowly or rapidly after 5–10 days (mean 9 days) but can persist to some extent well beyond the day 8 end of "fertile estrus".

Estrous behavior in domestic dogs is a response to a decline or withdrawal of estrogen. and are facilitated by a rise in progesterone (Concannon *et al.,* 1979a; Beach *et al.,* 1982; Feldman and Nelson, 1987). Estrous behaviour, including copulation, also have been observed during declining estrogen and rising progesterone concentrations in serum samples from greywolves (Seal *et al.,* 1979) and fecal samples from African wild dogs (Monfort *et al.,*1997) and maned wolves (Velloso *et al.,*1998*).* Estrous behavior within the genus Canis is characterized by reflex or spontaneous stiffening and deviation of the tail, firmly standing to permit mounting and a reflex lordosis-like presentation of the vulva (Concannon *et al.,* 1979a; Seal *et al.,* 1979; Beach *et al.,* 1982: Pauiraj *et al.,* 1992 and Monfort *et al.,* 1997)**.** In the domestic dogs estrus may be *as* short as 3 days or last for several weeks but, it is generally 4-12 days in length, with an average of 9 days and it can range from 0 to 21 days (Concannon, 1991). The period during which the female dog accepts copulation, the vulva begins to shrink, soften and the duration of this phase (Beijerink, 2007). In the domestic dog it has been reported that intromission and mounting may not occure until a few days after the onset of estrous behavior. During estrus**,** the vulva must progress through a turgid phase to become soft and flaccid. Therefore, no longer providing a difficult barrier for male penetration (Feldman & Nelson. 1987)**.** Mating is usually 5 to 45 minutes in duration and usually results in a copulatory tie during which the male dismounts and stands beside or behind the bitch, with the penis locked in the vagina. The lock withstands tugging and pulling and is terminated by detumescence of the penis.

Endocrinologically, estrus begins with the luteinizing hormone (LH) surge and is characterized by rise in progesterone (P4) and decline in estrogen levels (Goodman,

1998). The bitch appears to become receptive to the male after the serum concentration of estrogen begins to fall and concentration of progesterone begins to rise (Concannon, 1975; Concannon *et al*., 1979; Beach *et al*., 1982). Estradiol continues its decline from peak values of late proestrus to intermediate values of 10 to 20 pg/ml (40 to 90 pmol/l). Serum progesterone rapidly increases above 1 to 3 ng/ml (3 to 6 nmol/l) during the preovulatory LH surge and immediately (or after a 1 to 3 days pause) rapidly increases further, reaching 10 to 25 ng/ml (30 to 80 nmol/l) by day 10, at or shortly after the end of estrus (Concannon, 2011). Although estrogen alone can induce sexual behavior, progesterone seems to further enhance and synchronize this behavior (Leedy, 1988). The decline in serum concentration of estradiol proceeds and may influence the LH surge that occurs near the onset of estrus and lead to ovulation (Concannon *et al*., 1978).

Some investigators have reported that preovulatory LH surge occurred on the first day of standing heat (Nett *et al.,* 1975; Remier *et al.,* 1978), while others have found no such correlation (Mellin *et al.,* 1976; Wildt *et al.,* 1978). In some bitches, the onset of behavioral estrus was found to occur as early as 2 to 3 days before LH surge, while many others opined that it did not occur until 4 to 5 days after LH surge (Johnston *et al.,* 2001). Concannon (1987) stated that in the extreme case an aggressive male might mate a bitch in late proestrus as early as 4 to 5 days before the LH surge, while some bitches may refuse a male until more than six days after the surge.

The duration of preovulatory LH surge in the bitch appears to vary, with range of 24 to 96 hours. (Smith and Mcdonald, 1971; Nett *et al.,* 1975; Concannon *et al.,* 1977; Reimers *et al*., 1978; Wildt *et al*., 1978; Olson *et al*., 1982). Ovulation occurred

approximately 2 to 3 days followi ng preovulatory LH surge (Phemister *et al*., 1973; Concannon *et al*., 1977; Wildt *et al*., 1978; Tsutsui, 1989). The average interval from LH surge to ovulation was two days in 10 Beagle bitches (Phemister *et al*., 1973).

2.5.3 Diestrus

Diestrus is the phase of the cycle that follows estrus and is characterized by progesterone dominance. The duration averages 56 to 58 days in pregnancy and 60 to 100 days in the non-pregnant bitch (Feldman and Nelson, 2004). Diestrus is generally considered to occur clinically when the estrus bitch first refuses a mating. Bitches are also less likely to be attractive to males at this time. Progesterone secretion is maximal approximately 2 to 3 weeks after the beginning of diestrus and reach peaks of 15 to 90 ng/mL at this time (Concannon *et al*., 1989). After this peak, progesterone gradually declines over the remainder of diestrus. It has been suggested that the onset of diestrus be defined by vaginal cytology rather than mating behavior because events such as whelping can be more accurately timed (Holst and Phemister, 1974) and it has also reported that the frequency of first acceptance among 116 bitches, 84per cent of bitch accepted between 5 and 11 days before the onset of diestrus. The mean was 8.5 ± 0.25 days before diestrus. The time of first refusal was compared with the onset of diestrus among 177 bitches, 77per cent of bitch refusal coincided with day one to five of diestrus, the mean day of first refusal was 2.7 ± 0.18 of diestrus and peak incidence of refusal was observed on the 2nd and 3rd days of diestrus.

Endocrinologicaly, diestrus is characterized by progesterone dominance. Concentration of serum progesterone rapidly increase to greater than 1 to 2 ng/ml before

or during the preovulatory LH surge and continue to increase throughout estrus to reach initial peak of 15 to 90 ng/ml by 15 to 30 days after the LH surge, there after begin a gradual decline which continues for 5 to 6 weeks (Concannon *et al*., 1989). Olson *et al*. (1984) reported that the serum progesterone concentration level in the pregnant, nonpregnant and hysterectomized bitches were similar during diestrus (Concannon *et al*., 1977). An abrupt decline in serum progesterone to less than 1 to 2 ng/ml occurs to whelping and is necessary for the normal parturition. Fernandes *et al*. (1987) reported that the mean concentration of progesterone increased from basal levels of approximately 1.0 ng/ml at the onset of cytological estrus to 15.5 ± 5.0 ng/ml on day 1 of diestrus, increased plateau of approximately 20 ng/ml from days 5 to 20.

Concentration of serum testosterone decline during diestrus in the bitch from proestrus phase and remain low (< 0.1 ng/ml) throughout the luteal phase. Serum androstenedione concentration increase during proestrus, reaching a mean of 0.73 ± 0.13 ng/ml near the time of LH surge. Concentration of serum androstenedione decrease in most bitches during estrus, increasing again to reach peak values of 0.4 ± 1.27 ng/ml between 6 to 18 days after the LH surge in both and non-pregnant animals, both androstenedione and progesterone decrease abruptly at parturition (Concannon and Castracane, 1985).

2.5.4 Anoestrus

Anestrus is the quiescent phase of the canine reproductive cycle when defined by behavioral or clinical signs (Johnston *et al*., 2001). Bitches in anestrus are not attractive to males and are not receptive to mating. The vulva is normally small and there is no

discharge present. In the regnant bitch, anestrus is the stage of the cycle when uterine involution occurs, beginning with whelping and ending with proestrus. In the non-pregnant bitch, the onset of anestrus is not readily scernable clinically from the end of diestrus. Similar to other phases of the estrous cycle, there is normal variance in the duration of anestrus. This variation depends on breed, health, age, time of year, environment, and multiple other factors (Feldman and Nelson, 2004). The approximate duration of anestrus in the bitch is 4 to 4.5 months. Following pregnancy the length of anestrus may be extended by 1 to 2 months (Feldman and Nelson, 2004).

Endocrinologically, the anestrus is defined as the time following diestrus when concentration of serum progesterone reaches basal level of less than 1 to 2 ng/ml. However, although concentration of serum estradiol fluctuates during anestrus (Olson *et al*., 1982). They increase again during proestrus with the development of Graaffian follicle. Also, Mellin *et al*. (1976) reported an increase in estrogen level during anestrus in bitches due to the follicular development during anestrus.

2.5.5 LH surge and ovulation

Canine ovulation is reported to occur as early as day five or as late as day 23 after proestrus onset (Phemister *et al.,* 1973, Wildt *et al.,* 1978). On an average, ovulation in female dog occurs approximately 12 days after the onset of proestrus or 2 to 3 days after the onset of estrous (Badinand and Fontbonne, 1993). Ovulation has occurred from 7 to 22 days after the onset of proestrus in 50 female dogs which were presented for assisted breeding and subsequently became pregnant (Johnston *et al.,* 2001). Prediction of the

time of ovulation is also necessary in female dogs with history of failure to show standing or behavioural estrus or small litter size.

Many of the studies have shown that the optimum fertility can be anticipated if the female dog is bred 2 to 4 days after ovulation and mating at appropriate time can result in pregnancy and higher litter size (Van Haaften *et al.,* 1989; Dietrich and Mollar, 1993; Johnston *et al.,* 2001 and Vitasek and Bartoskova, 2006).

The secretory pattern of LH at this period is characterized by frequent increases of short duration (Kooistra *et al.,* 1999). Unlike the other mammalian species, the duration of the LH surge in the female dog is relatively long, ranging from 1 to 5 days (Smith and Macdonald, 1971; Nett *et al.,* 1975; Phemister *et al.,* 1973, Wildt *et al.,* 1978, De Gier *et al,* 2006). Furthermore, Hase *et al.,* (2000) found that the period in which LH values peaked above 10 ng/ml continued for more than 12 hours. De Gier *et al,* (2006) found that this LH peak had a bifurcated aspect in Beagle female dogs. LH is often stated as the ideal technique for determining with accuracy the ovulation period, as the LH peak induces ovulation and is, therefore, generally stated as being the "Day zero" of the sexual cycle of the female dog. Assaying LH is in fact not easy as it requires repeated blood samplings and a specific assay technique. On the other hand, De Gier *et al.* (2006) opined that assaying LH is not a good method to time of ovulation, due to the individual variations among female dogs.

Ovulation occurred approximately 2 to 3 days following the preovulatory LH surge (Phemister *et al.,* 1973; Concannon *et al.,* 1977; Wildt *et al.,* 1978; Tsutsui, 1989). The average interval from LH surge to ovulation was 2 days in 10 Beagle female dogs

(Phemister *et al.*, 1973). However, the day of ovulation in any given female dog may be quite different from that of the average (Johnston, 1995).

Concannon (1986) found that ovulation appeared to occur synchronously in the two ovaries about 36 to 50 hours after the LH peak. Concerning the duration of the ovulation process, Boyd *et al.,* (1993) suggested that the ovulation process seemed to begin in the right ovary and that the whole process may take as long as 36 hours to be completed. After ovulation, oocytes may also remain fertilizable for a significant time, up to 5 days (Tsutsui and Shimizu, 1975) 7 or 8 days (England and Russo, 2006) and even after the closure of the cervix (Verstegen *et al.,* 1997).

Canine oocytes are ovulated as primary oocytes and after ovulation they undergo the first meiotic division to become secondary oocytes (Olson and Nett, 1986; Tsutsui, 1989; Concannon *et al.,* 1989a and Linde, 1991). Oocytes of the female dog are ovulated two days after the LH peak in an immature state and they need to mature at least 48 hours before being able to be fertilized (England and Concannon, 2002). It has been demonstrated that the canine oocytes cannot be penetrated by sperm when they are still immature (Reynaud *et al.,* 2005). Therefore, it is essential for a veterinarian to be as accurate as possible to detect the day of ovulation as this day is reached at least 48 hours before the day of fertilization.

2.5.6 Fertile period and fertilization period

One very useful way to view the cycle during late proestrus and estrus is to consider the "fertilization period" and the "fertile period" (Concannon *et al.,* 1983). Here in we consider fertilization to mean fusion of the male pronucleus (from the sperm) with

the female pronucleus of the mature secondary oocyte to form the 1-cell zygote. However, sperm can penetrate the immature primary oocyte any time after ovulation (Jeffcoate and Lindsay, 1989; Concannon *et al.*, 2002).

The fertile period is the time during which a mating or insemination can result in a pregnancy (Jeffcoate and Lindsay, 1989; Concannon, 1986). Sperm may survive in the tract for up to 5 or 6 days before ovulation and the opportunity to penetrate recently ovulated singular oocytes and then form a viable male pronucleus and result in fertilization 7 or 8 days after semen deposition (Concannon *et al.*, 2002 and Ibrahim *et al.*, 2011). The fertile period can be considered to extend from three days before the preovulatory LH surge until 7 days after the pre-ovulatory LH surge and may be even longer when using stud dogs with exceptional semen quality or bitches in which the oocytes may survive another day or two beyond the normal (Verstegen *et al.*, 1997 and Concannon *et al.*, 2002).

Fertility for single matings is maximal from the LH surge (day 0) until day 5 and wanes rapidly over the next 3days, in part due to a "cervical closure" at day 7–8 (Concannon, 2011). The termination of the fertilization period may be primarily due to degeneration of oocytes or to closure of the cervix and prevention of sperm entering the reproductive tract in sufficient numbers (Jeffcoate and Lindsay, 1989).

Since fertilizable life span of mature canine ova is short (2 to 3 days), it becomes necessary that an adequate number of spermatozoa must present in the female genital tract during the fertile period of the female dog. The spermatozoa longevity in the female genital tract is reported to extend from 5 to 11 days (Doak *et al.*, 1967; Holst and

Phemister, 1974 and Concannon *et al.*, 1983). This extended spermatozoon longevity in canine allows breeding that occur considerably before fertile period to produce progeny (Goodman, 1998).

Unlike most other mammals, the dog ovulates primary oocytes that are at the beginning of the first meiotic division, at metaphase I (MI) and the germinal vesicle break down (GVBD) takes place shortly thereafter. In-vivo canine oocytes mature in the oviducts and there is a multilayered and tight cumulus mass around the oocyte, which is seen to expand as the oocyte matures, a process that takes 2 to 5 days to complete (Holst and Phemister, 1971; Mahi and Yanagimachi**,** 1976; Tsutsui, 1989; Yamada *et al.*, 1992 and 1993). The oviductal transit takes 5-10 days (Andersen and Simpson, 1973; Tsutsui, 1989). Fertilization occurs during this passage, in the distal part of the oviduct. Mature canine ova may remain alive and fertilizable for 2 to 4.5 days (Concannon *et al.*, 1989a; Tsutsui, 1989). Canine spermatozoa can fuse with immature oocytes (Mahi and Yanagimachi, 1976). Recent data indicate that the interval from fertilization to the 8-cell stage is 5 days if the bitch is inseminated before the oocytes have matured and only 3 days when insemination takes place after maturation, whereas 16-cell embryos were observed at day 11 after the LH surge with either early or late insemination (Concannon *et al.*, 2002). Thus, apparently, embryonic clevage between 2 and 16 cells occurs more rapidly following fertilization of more mature oocytes. Canine spermatozoa have been reported to survive in the uterus of the female for at least 4 to 6 and in one case 11 days, after a single mating (Doak *et al.*, 1967). Theoretically, thus, the bitch could conceive after one mating from about 1 or 2 days before until about 7 or 8 days after the LH peak, a period referred to as ”the fertile period”. Available data point to that the most fertile days are from 2 to 5

days after ovulation, i.e. from 4 to 7 days after the LH peak when the oocytes have all been released and have matured and are ready to be fertilized, a period referred to as "the fertilization period"

2.5.7 Vaginal Exfoliative cytology

The most widespread indirect method to study the estrous cycle of the bitch is by way of vaginal exfoliative cytology. The vaginal epithelial cells respond to the rising estradiol levels during proestrus in a regular manner. The cell layers of the vaginal mucous membrane increase from 2-3 to 20-30 during estradiol stimulation. The cells change during proestrus from small parabasals, with a high nucleus to cytoplasm ratio, over the larger intermediary cells, which still have a large nucleus, to the fully cornified superficial cells, which usually are irregular in shape and sometimes have folded borders and contain either a small pyknotic nucleus or are anuclear. The vaginal epithelial cells respond to the increase in estradiol in peripheral plasma with a lag- time of 3-6 days (Linde and Karlsson, 1984). Maximal cornification in some bitches can be seen for up to 14 days, appearing during late proestrus or early estrus and remaining unchanged period of the abrupt fall in estradiol and rise in progesterone preceding ovulation and throughout estrus. In metoestrus there is a quick shift from merely superficial cells to intermediary cells and parabasals. Very characteristic for diestrus is the appearance of a large number of polymorphonuclear leucocytes. Because the changes of the vaginal cells are caused by estradiol, while no effect has been reported to be caused by the rise in progesterone following ovulation, it cannot with this method be decided whether, or when, the bitch actually ovulates. Vaginal cytology, thus, is not an exact enough method for timing of the bitch for artificial

insemination. The technique is, however, useful in that a smear will show whether the bitch is still in proestrus or already in diestrus.

Holst (1986) documented that the vaginal epithelium is one of the several targets tissues for ovarian hormones. The increasing circulatory levels of estradiol-17β stimulate the growth of vaginal epithelium from a bi-stratified cuboidal epithelium to a stratified squamous epithelium of greater than 30 cells layer with every estrous cycle. Further, estradiol-17β also induces an increase in blood flow and swelling of the vaginal tract, increased mucosal secretion and diapedesis which causes a bloody vaginal discharge (Senger, 2003). The stage of the estrous cycle can be determined by looking at the per centage of cornification which is followed to recommend the appropriate time of mating (Raskin and Meyer, 2001).

Since the classical work of Alien and Doisy (1923) on vaginal cytology, the changes in cellular type within the vaginal smear of female dogs in relation to various stages of estrous cycle has been investigated by many workers (Evans and Cole, 1931; Newberry and Gier, 1952; Harrop, 1960; Schuttle, 1967; Christie *et al.*, 1972; Roszel, 1977; Christiansen, 1984; Olson *et al.*, 1984; Wright and Perry, 1989; Concannon *et al.*,1989; Bouchard *et al.*,1991; Raskin and Meyer, 2001 and Reddy *et al.*, 2011). Johnston *et al.*, (2001) stated that vaginal cytology is a simple technique which can be used by the practitioners to characterize stages of the reproductive cycle of the female dog.

Classical staining techniques such as May-Grunwald Giemsa, Papanicolaou and New methylene blue were used in vaginal cytology in the microscopic assessment of

cellular changes (Olson et. al. 1984; Post 1985; Hiemstra *et al.,* 2001 and Yener *et al.,* 2007).

The vaginal epithelium is a very thin tissue layer in anestrous with only 1 to 2 layers of cuboidal epithelium. Estrogens has a potent mitogenic effect on the cells of the vaginal epithelium, causing marked growth in the thickness of the vaginal wall as the epithelial cells multiply and the myometrium matures, forming prominent muscle bundles (Rehm *et al.,* 2007; Concannon, 2010). Estrogens cause the uterine vasculature to become highly permeable and allow the leakage of red blood cells into the uterine lumen via diapedesis (Bouchard *et al.,* 1991a and Concannon, 2010).

The cytologic characteristic of estrus is the presence of 90 per cent or greater cornified cells with less abundance of mucus and red blood cells and a clear background was noted by Christie *et al.* (1972). Neutrophils are absent and bacteria are usually present. Cell populations change abruptly at the onset of diestrus, at least 20 per cent of superficial cells show a dramatic decrease while the number of intermediate cell shows an increase and neutrophils often reappear (Olson *et al.,* 1983; Concannon *et al.,* 1989a and Bouchard *et al.,*1991a). The cytologic appearance of early proestrus and diestrus can be very similar, so one vaginal smear is not adequate for differentiation of these two stages (Rehm *et al.,* 2007 and Ibrahim, 2011).

In evaluating vaginal smear obtained from 12 beagle female dogs on a daily basis during proestrus, estrus and early diestrus, the day of maximum cornification was variable ranging from 91 to 100 per cent and occurring between -1 to +7 days after the LH surge (Christie *et al.,*1972). The first day, when at least 90 per cent of epithelial cells

were of superficial type; ranged from -6 to +4 days after LH surge. Olson *et al.*, (1983) opined that breeding must occur while the smear is fully cornified regardless of the other observation including behavior.

Linde and Karlsson (1984) studied the correlation between vaginal smear and the time of ovulation in 22 female dogs over 24 estrus periods. In all 24 estrus periods, the estradiol peak preceded the day of maximum keratinization by at least 3 to 6 days. In 70.80 per cent of the estrus periods examined, the plasma progesterone level reached the expected ovulation level at the time of maximum keratinization of vaginal smear. In 25 per cent of estrus periods examined**,** the per centage of keratinization was over 90 per cent and was between 80 to 90 per cent in the rest. Okken *et al.*, (1985) and Wright (1990) recommended the routine use of vaginal exfoliative cytology to monitor the oestrous cycle in female dogs and to suggest the optimum time for mating.

Bouchard *et al.* (1991a) monitored the estrous cycle of 16 mature mongrel female dogs by studying the vaginal cytology twice weekly. The bitch was considered to be in estrus when more than 90 per cent cells were superficial keratinized epithelial cells. Based on vaginal cytology, ovulation was estimated to take place 6.9 ± 1.6 days after 80 per cent of the vaginal epithelial cells were superficially keratinized.

Mshelia *et al.* (2001) examined the vaginal smears from 45 Nigerian Mongrel female dogs in pro-oestrus, estrus and pregnancy. Differential cell count of exfoliated vaginal epithelial cells showed that the mean per centage of superficial cells was 95 in oestrus, 65.5 in proestrus and 15 in pregnant female dogs. The phase of predominance of superficial cells were consistent with high vaginal mucus electrical resistance

measurements and sexual activity. They concluded that vaginal cytology can be applied for detection of estrus in the Nigerian Mongrel female dog.

Hiemstra *et al.* (2001) investigated the reliability of vaginal cytology as compared to plasma progesterone concentrations for determining the optimal mating period in the female dog. The influence of different locations for obtaining the smears and of the staining methods was also examined. The vaginal cytology of 35 female dogs during the follicular phase was examined 3 times a week, until the optimal time for mating was established based on plasma progesterone concentrations. The results indicated that vaginal cytology is not useful for determining the optimal mating period in bitches, since mating according to cytological findings would have resulted in only 28 per cent of the bitches mated at the proper time. The site for obtaining the smear and the staining method did not affect the accuracy.

Suyash (2002) recommended mating of 36 female dogs twice at an interval of three days when a vaginal cornification of at least 90 per cent was observed in the vaginal smear. The average number of days taken for peak cornification was reported as 10.55 ± 0.24 days and range between 7 to 13 days. Clinical sings of oestrous such as relaxed vulva, straw-coloured discharge and flagging of the tail were not well correlated with the optimum vaginal cornification. Mating on the basis of optimum vaginal cornification resulted in conception rate of 50 per cent.

Concannon *et al.*(2002) reported vaginal cytology cornification index on day 7 before LH surge was 30 % to 100%, day of LH surge was 80% to 100%, days 4-5 after LH surge was 80% to 100% and day 8-11after LH surge was 0 to 80%.

During anestrus, parabasal and intermediate cells predominate and are in much lower numbers than during other stages of the cycle when dead cells are sloughing off readily (Christie *et al.,* 1972 and Concannon, 2010). Neutrophils and bacteria are either absent or present in low numbers, further cytological appearance of late diestrus is very similar to anestrus, no cytologic differentiation can be made between these two stages (Raskin and Meyer, 2001).

Feldman and Nelson (2004) documented that the neutrophils could not enter into the vaginal lumen during proestrus due to thickening of vaginal mucosa, but abundant RBCs could enter into lumen by diapedesis due to estrogenic effect. The background on the vaginal smears appeared dirty during anoestrus and proestrus because of the presence of cellular debris and mucin as noticed by Feldman and Nelson (2004). However, during estrus, the background was clear (England, 1998).

Venkatachalapathy (2005) has recorded a peak vaginal cornification as early as day 5 in two female dogs and as late as day 13 in four other female dogs after the onset of proestrus bleeding and the mean number of days taken for optimal cornification was 9.07 ± 0.35 days. Further, on the day of optimum vaginal cornification, only 72.09 per cent of bitches had exhibited behavioral signs of estrus suggesting that vaginal cornification had a poor correlation with genital and behavioral changes. Further, he documented that mating recommended on the basis of vaginal exfoliative cytology resulted in a conception rate of 69.77 per cent.

Reddy *et al.* (2011) documented from their study that superficial cells appeared in higher per cent during proestrus (65.30 ± 1.40) which was further increased to still higher

per cent during estrus (89.94 ± 0.63). Further, superficial cells decreased in numbers during diestrus (18.52 ± 0.94) and anestrus (12 ± 0.70) phases of estrous cycle. The intermediate cells were observed in almost equal number during proestrus (25.17 ± 1.17 %), diestrus (29.77 ± 1.98 %) and anestrus (28.38 ± 1.44 %) phases of estrous cycle. The parabasal cells were predominantly noticed during diestrus (51.71 ± 1.05 %) and anoestrus (58.92 ± 1.37 %). They appeared occasionally during proestrus (9.53 ± 0.29) and estrus (2.76 ± 0.30) phases of the oestrous cycle. Similar exfoliative vaginal cytological changes in female dogs during proestrus were observed by Johnston *et al.* (2001), Arun (2001), Sridevi (2001) and Patil (2006). Reddy *et al.* (2011) reported that high per cent of superficial cells were appeared on day 10.67 ± 1.84 from first day of proestrus. The per cent of female dogs conceived were 66.66 which were bred based on vaginal cytology.

Oluwatoyin *et al.* (2012) identified four distinct phases of the estrous cycle in the Nigerian local bitches using vaginal cytology as proestrus (9.00 ± 0.0 days), estrous (9.00 ± 0.0 days), diestrus (62.00 ± 1.0 days) and anestrus (122.5 ± 2.5 days).

The reliability of vaginal cytology for determining the optimal mating period is controversial. Hiemstra *et al.* (2001) correlated the vaginal cytology of 35 female dogs with the endocrine events (plasma progesterone, LH surge) and concluded that vaginal cytology is not useful for determining the optimal mating period in female dogs, since mating according to cytological findings would have resulted in only 28 per cent of the bitches being mated at the proper time. Christie *et al.* (1972) and Bouchard *et al.* (1991a) reported that the initial day of optimum cornification coincided poorly with the onset of

LH surge and the peak vaginal cornification occurred between 1 to +7 days after the LH surge and therefore, matings timed on the basis of peak vaginal cornification yields poor results.

On the contrary, Venkatachalapathy (2005) has recommended mating time on the basis of exfoliative cytology and observed that such female dogs delivered over a wide range of 58 to 71 days, indicating again the poor correlation of vaginal exfoliative cytology with the LH surge and therefore with the fertility of the animal.

Vaginal cytology cannot be used to detect ovulation prospectively. At the end of the heat period, the "onset of vaginal diestrus", when there is a sudden increase in intermediate cells and parabasal cells, occurs around 5 days after ovulation (Holst and Phemister, 1974). But, it only helps to predict the ovulation retrospectively (Xavier and Fontbome, 2007).

Although, several authors stated that exfoliative cytology is not an accurate predictor of the fertile period in the bitch, they do agree that it could provide a clue as to when to begin the breeding and when to end it and recommend that breeding should commence as soon as vaginal cornification reached 80 per cent or more and continued there after till there was an abrupt decline in the per centage of cornified cells (Holst, 1986; Feldman and Nelson 1996; Johnston *et al.,* 2001).

2.6 Interval between estrous periods

Domestic dogs are monoestrous in each mating season (Christi and Bell, 1971a and Concannon *et al.,* 1989). All female dogs have a prolonged period of anestrus or

sexual quiescence between successive heat irrespective of whether they are pregnant or not (Arthur *et al.*, 1989). The average interval between successive heats irrespective of whether they are pregnant or not is seven months but it may vary (Christi and Bell, 1971b). Several studies indicate that, as a group, Domestic dogs cycle an average every 7 months and are non-seasonal breeders (Christie & Bell 1971a; Feldman & Nelson., 1987; Concannon., 1997 and Concannon, 2011).

Some small breeds may have 3 to 4 estrous cycles in a year while the large breeds have only one. The inter estrous interval are 37 weeks and 26 weeks in Rough Collies and German shepherd breeds respectively (Prole, 1973; Sokolowski and Zimbelman, 1977). An average of 35 weeks was recorded for Grey Hound female dogs (Hancock and Rowlands, 1949) while 31 weeks for Labrador female dogs (Christi and Bell, 1971b).

Roberts, (1971) reported that dog is usually considered as a monoestrus animal and has two estrus cycles a year although some small breeds may have 3 – 4 and large breeds only one and also stated that there is evidence that frequency of estrus is genetically conditioned

Burns and Frazer (1966) stated that the grey Hounds and other larger breeds have one estrus period a year and also quoted first in reporting that in nearly 500 bitches, 338 had an inter-estrus interval of 5- 7 Months, over 100 had interval of over 7 months and only 16 had an interval of less than 5 months.

Anderson (1962) reported that in beagles estrus occurred at every 7 months ±2 Months

2.7 Conception rate

Historically, many owners believe that the fertile period of the female dog is between 9 to 13 days after the onset of proestrus and therefore many breeders routinely breed their female dogs during this period (Reynaud *et al.*, 2005; Xavier and Alain, 2007). However, the conception rate and litter size in female dog routinely bred between 9 to 13 days after the onset of proestrus bleeding has been generally disappointing (England, 1992; Suyash, 2002 and Venkatachalapathy, 2005). However, the duration of proestrus in normal female dog is reported to be highly variable and it can be as short as zero days and as long as 27 days (Bell and Christie, 1971; Olson and Nett, 1986; Alien and England, 1990).

Conception rate is best in female dogs bred from 3 days before to 4 days after ovulation (Holst and Phemister, 1974). Therefore, it becomes necessary to identify the fertile period in a female dog so that mating time can be recommended which can result in conception and above average litter size. Mating earlier than the day of the LH surge have reduced pregnancy rates, suggesting that in most cases sperm are not capable of penetrating oocytes after 2 days in the female reproductive tract (Reynaud *et al.*, 2005).

Concannon (1986) reported in groups of bitches that underwent intrauterine insemination at 8, 9 and 10 days after the LH surge, pregnancy rates were higher (60, 60 and 20% respectively), compared to natural matings on those days (all 0%). Therefore, optimization of the chances for a female dog to become pregnant requires that she has to be bred or inseminated during the fertilization period at a time when it overlaps the

period of peak fertility, preferably days 4 to 7 after the LH surge (Verstegen *et al.*, 1997 and Concannon *et al.*, 2002).

2.8 Duration of gestation length

Duration of gestation length in the bitches has been reported using the following criteria.

a) The interval from the day of mating to parturition.

b) Interval from luteinizing hormone (LH) surge to parturition

c) Interval from estrus to parturition and

d) Interval from preovulatory rise of serum progesterone concentration to parturition

2.8.1 The interval from the day of mating to parturition.

Using the day of mating as a reference point, as observed in cases of just a single mating or insemination, parturition can occur as early as 56 days later and as late as 68 days ater. Similarly, a large variation in apparent gestation length can be encountered when counting from the first of multiple matings or the last of multiple matings, the extremes encountered differing by two weeks. For instance, if a bitch is held for an aggressive stud dog and forced to mate starting 3 to 5 days before the LH surge, the interval from first mating to whelping may be as long as 69 to 70 days. In rare instances where a bitch is still fertile 9 or 10 days after the LH surge and is bred then, the interval from mating to whelping can be as short as 55 or 56 days (Concannon *et al*., 2000). However, full-term gestation, calculated from insemination, is reported to range from 57 to 72 days (Concannon *et al*., 1983). The difference between these measurements was

attributed to the potential 6 day viability of sperm in the female reproductive tract and the long period of receptivity in the bitch of 3 to 21 days (Holst and Phemister, 1974; Concannon *et al*., 1983).

2.8.2 Interval from luteinizing hormone (LH) surge to parturition.

In the vast majority of bitches parturition occurs 64, 65 or 66 days after theovulatory surge in LH (Concannon and Lein, 1989). A 64 to 66 day gestation length measured from the LH surge to parturition is the same as a 62, 63 or 64 day interval between ovulation and parturition, since ovulation has been estimated to occur 2 days after the surge in LH (Concannon and Verstegen, 1998). The actual gestation length in the bitch is 65.00 ± 1.00 days when timed from the preovulatory LH surge in peripheral blood and the period between the preovulatory LH surge and parturition was almost constant (Concannon *et al*., 1983) and the key to timing the duration of canine gestation was neither insemination date nor estrus onset, but rather the preovulatory LH surge and concomitant increase in serum progesterone concentrations (Meyers-Wallen, 1995).

2.8.3 Interval from estrus to parturition

In bitches, identifying the first day of diestrus on the basis of vaginal cytological findings can be used to predict when labor should occur, because most bitches whelp 57.00 ± 3.00 days after Day 1 of cytologic diestrus (Johnson, 2008). Gestation length as measured from the first day of diestrus based on vaginal cytology (Holst and Phemister, 1974), has a large range (51–60 days), although 80per cent of bitches in that study gave birth on the 57th day of diestrus.

2.8.4 Interval from pre ovulatory rise of serum progesterone concentration to parturition.

Prediction of the duration of pregnancy on the basis of LH surge is difficult for a practicing clinician as identification of LH surge require frequent sampling of the blood, sophisticated laboratory facilities, besides being cost effective. Therefore, recommendations have been made for identifications of time of LH surge on the basis of changes in the serum concentration of progesterone (P4) during late proestrus and estrus (Concannon *et al*., 1977a; 1977b; Nett *et al*., 1975). Goodman (1998) opined that if accurate serial quantitative serum P4 concentrations are obtained, the LH surge might be estimated as the day when a distinct rise in levels of serum P4 is seen and that fertile matings should result in whelping 64 to 66 days later. Concannon *et al*. (1983) demonstrated that the changes in the serum P4 concentrations during the later part of proestrus and early estrus characterized by a gradual increase with a sudden sharp rise which coincided with the LH surge. Basal serum P4 levels have been reported to typically range between 0 to 1 ng/ml during an estrus and proestrus (Concannon *et al*., 1977a; 1977b). At the time of LH surge, serum P4 concentration rose rapidly (0.8 to 3 ng/ml), continued to rise at ovulation (1.02 to 8.0 ng/ml), and was even higher (4.02 to 20 ng/ml) towards the end of fertile period (Concannon, 1986 and Jeffcoate and Lindsey, 1989).

Bouchard *et al*. (1991) stated that ovulation was estimated to occur when serum P4 concentration was 4.90 ±1.00 ng/ml (3.4 to 6.6 ng/ml). Wright (1990), reported that LH surge was located to the period when serum P4 concentrations were between 2 to 4 ng/ml and that ovulation occurred when the serum P4 concentrations was 5.4 ng/ml

(range 3 to 8 ng/ml). Renton *et al*. (1991) reported that in bitches, the concentrations of serum P4 reached 3 ng/ml two days before ovulation and recommended the determination of serum P4 concentration for identifying the time of ovulations. Renton *et al*. (1991) reported that serum P4 concentrations were around 3 ng/ml on the day of LH surge in 12 out of 20 bitches and within 1 day in 7 out of 8 bitches. Ultrasound was less accurate in that, only 4 of the 16 estimates agreed. They concluded that the measurements of serum P4 concentration were a better indicator for ovulation than ultrasonography.

Zoldog *et al*. (1994) reported that ovulation in bitches occurred when serum P4 concentrations reached 5 ng/ml. Barr (1995) reported that in all 14 beagle bitches studied, the serum P4 concentration was high, ranging from1.9 to 11.7 ng/ml around the time of ovulation. Johnston and Root (1995) in a retrospective study of 49 bitches reported that serum P4 concentrations began to rise concurrently with LH surge, reaching 1.0 to 1.9 ng/ml on the day of LH surge. The day after LH surge, the serum P4 concentration was 2 to 9 ng/ml. On the day of ovulation, the serum P4 concentration was 4.0 to 10 ng/ml.

Concannon *et al.* (1983) and Yeager and Concannon (1990) stated that a 64 to 66 day gestation length measured from the LH surge to whelping is the same as a 62, 63 or 64 day interval between ovulation and whelping since ovulation has been estimated to occur 2 days after the surge in LH.

In contrast, using the day of mating as a reference point, as observed in cases of just a single mating or insemination, parturition can occur as early as 56 days later and as late as 68 days later (Thatcher *et al.,* 1994). Similarly, a large variation in apparent gestation length can be encountered when counting from the first of multiple mating or

the last of multiple mating, the extremes encountered differing by 2 weeks (Concannon *et al.,* 1989; Thatcher *et al.,* 1994). For instance, if a female dog is held for an aggressive stud dog and forced to mate starting 3 to 5 days before the LH surge, the interval from first mating to whelping may be as long as 69 to 70 days. In rare instances where a female dog is still fertile 9 or 10 days after the LH surge and is bred, then the interval from mating to whelping can be as short as 55 or 56 days (Concannon *et al.,* 2002).

Okkens *et al.* (2003) studied 113 bitches from 6 breeds found a mean gestation length for West Highland White Terriers of 62.8 ± 1.2 days, which was significantly longer than in German Shepherds with 60.4 ± 1.7 days, Labrador Retrievers with 60.9 ± 1.5 days and Dobermanns with 61.4 ± 1.0 days. They also found a negative correlation between mean length of gestation and litter size and concluded that breed was the main factor influencing the length of gestation and that this might be ascribed to breed-related differences in litter size. In contrast, Linde-Forsberg *et al.* (1999) studied the fertility data from 327 frozen-semen AIs found no influence of either breed or litter size on gestation length.

Tsutsui *et al.* (2006) reported that in dogs, the effect of parity on gestation length has not been studied as extensively as litter size, breed, age and season and also reported that gestation length in beagle dogs is not affected by parity or litter size. It is known that the LH surge in dogs occurs approximately 2 d before ovulation (Conconnon *et al.,* 1975). Since the initial elevation in progesterone is strictly associated with the LH surge (Conconnon *et al.,* 1975 and Luvoni and Beccaglia 2006), the measurement of progesterone concentrations has been used to determine the optimal time for successful

mating. It has been demonstrated that the date of parturition can be accurately predicted based on the day of the initial elevation in progesterone concentrations (Kutzler *et al.,* 2003).

There is a large variation in the length of gestation in dogs when gestation is calculated as the interval from mating to parturition. Naaktgeboren (1987), using data obtained from dogs of various breeds, reported a mean gestation of 62.0 days (n = 184), with a variation of 24 days (54 - 77 days); he also observed a negative correlation between the length of gestation and litter size. Concannon *et al.,* (1983) found a mean gestation of 65.3 ± 0.2 (S.E.M.) days (n = 290), with a variation of 16 days (57 - 72 days) in a beagle colony. They showed that the variation in gestation in beagles was reduced to 3 days (64 - 66 days) when gestation (65.1 ± 0.1 (S.E.M.); n = 54) was calculated as the interval from the pre-ovulatory peak of luteinizing hormone (LH) to whelping. They explained the large variation in gestation calculated as the interval from mating to whelping as being caused by the variation in time between mating and fertilization.

The length of gestation observed in one breed in one colony, without consideration of litter size, does not appear to be an adequate reference for other breeds, which probably have a greater variation in litter size and examined the variation in length of gestation in bitches of various breeds, mated at a fixed time after ovulation (Concannon *et al.,* 1983; Wildt *et al.,* 1979). In contrast, Seki Makoto *et al.,* (2010), indicated that gestation length in beagle dogs is not affected by parity or litter size. However, when calculated as the interval between the day of first mating to the day of whelping, gestation length tends to be approximately 1 d shorter in nulliparous bitches

than in multiparous ones. This indicates that ovulation in nulliparous bitches may occur earlier than that in multiparous bitches.

Okkens *et al.* (2001) reported that the number of fetuses and the duration of gestation were negatively correlated. The interval from the first mated to parturition averaged 61.1±1.2 and 60.8±1.9 days for litters of 5-7 (n=19), 8-9 (n=11) pups. Within each breed, litter size had no influence on the gestation length. However, the number of litter size reduced due to age increases in the bitches.

2.9 Incidence and signs of pseudo pregnancy

Canine pseudo-pregnancy is a syndrome, characterized by signs such as nesting, weight gain, mammary enlargement and lactation, which appear in non-pregnant bitches 6–12 weeks after estrus. The intensity of these signs is extremely variable among bitches (Johnston, 1980 and Feldman and Nelson, 1996). Pseudo-pregnancy is now a frequent finding in domestic dogs, with a prevalence estimated to be as high as 50–75% (Johnston, 1980). precise etiophysiology is one of the topics of canine endocrinology that still remains to be explained. It was initially postulated that pseudo-pregnancy was caused either by an overproduction of progesterone (P4) or an abnormal persistence of the corpus luteum (Marshall and Halnan, 1917). Later, it was suggested that clinical signs of Pseudopregnancy were related to the increased concentrations of prolactin (PRL) induced by an abrupt decline of P4 concentrations in the late luteal phase (LP) (Smith and Mc Donald, 1974; Graff *et al.,* 1977; Gerres *et al*., 1988 and Concannon and Lein, 1989).

Feldmen and Nelson, (2007) stated that pseudopregnancy or pseudocyasis is the physiological condition in which female dog shows similar signs of pregnancy. Although its exact prevalence is not known, it is estimated between 50 – 75 per cent.

Caffartti *et al.* (2013) In Dogo Argentenia bitch, the prevalence recorded was 30 % The most frequent sign of pseudopregnancy was exhibited mammary hypertrophy associated with lactation followed by nesting behavior. These signs are also reported to most common signs of pseudo pregnancy exhibited in dogs

2.10 Litter size and sex ratio

Litter size is an important parameter that describes the reproductive potential of an animal. This is the total number of puppies born by a bitch after a particular pregnancy (Mutembei *et al.,* 2000). The number of puppies per litter varies with the size of the dog, toy breeds rarely bear more than two puppies while the large breeds' average close to 10 puppies per litter (Columbia Encyclopedia of Dogs, 2005). The very small litters by small breeds may be due to their very small size which limits their capacity to carry large litters compared to the larger breeds, in which litter size may range from one to twenty one (Battaglia, 2001).

Many factors have been suggested to influence on litter size, such as breed, the size of the dog, the age of the bitch, the age of the sire, the season of the year, the number of previous litters born from the bitch, the number of matings, whether the mating occurred naturally or with AI as well as the quality of the semen [Rowlands 1950; Thamassen *et al.*,2006; Gavrilovic *et al.*,2008; Lyngset and Lyngset. 1970; Tedor and Reif 1978; Strasser and Schumacher, 1968 and Gill 2001).

Mutembei *et al.* (2000) and Chatdarong *et al.* (2007) recorded some litter sizes of 6.6 ± 2.8 German Shepherd Dog and 8.2 ± 1.8 in Rottweiler, while Kelley (2001) in his own report stated litter size of 5.0 ± 2.1 for Beagle dogs, 5.8 ± 2.4 for Boxer, 3.3 ± 1.5 for Chihuahua, 5.0 ± 2.0 for Cocker Spaniel, 3.9 ± 1.5 for Dachshund, 6.6 ± 2.7 for German Shepherd, 7.6 ± 2.7 for Golden Retriever, 7.6 ± 2.6 for Labrador Retriever, 4.6 ± 1.8 for Miniature Schnauzer, 3.0 ± 1.4 for Pomeranian, 3.4 ± 1.8 for Poodle, 6. 6 ± 2.9 for Rottweiler, 4.3 ± 1.9 for Shetland Sheepdog and 3.3 ± 1.5 for Yorkshire Terrier.

Ajala *et al*. (2012) reported the litter size in the Nigerian local breed of bitches used for this study was 7.0 ± 2.0. and Der-Rottweiler (2006) stated that bitches belonging to large working breeds have up to 6-7 puppies and sometimes up to 10 to 12 puppies. Battaglia (2001) stated that most of the small breeds, notably the toys and terriers usually produce very small litters and this might be because of their very small size which limits their capacity to carry large litters.

Further, the physical conditions of the dam at the time of breeding have been also shown to affect the litter size. Nutrition also has been reported as a factor affecting litter size, obese bitches have smaller litter than those that are fit and trim (Russ, 2000). Parity also has a great influence on the litter sizes of bitches; primiparous have smaller litter sizes than multiparous bitches (Schreiber *et al.*, 1975).

Darvelid and Linde-Forsberg (1994) reported that the litter size in dogs varies with breed, or size, ranging from as few as one pup in the miniature breeds to more than 15 in some of the giant breeds. It is smaller in the young bitch, increases up to 3 to 4 years of age; to decrease again as the bitch gets older. A litter size of only 1 or 2 pups predisposes

to dystocia because of insufficient uterine stimulation and large pup size, “the single-pup syndrome”. This can be seen in dog breeds of all sizes. Breeders of the miniature breeds tend to accept small litters, but should be encouraged to breed for litter sizes of at least 3 to 4 pups to avoid these complications.

Seasonal variations in the mean litter size, the litters were grouped according to birth month. Furthermore, a categorical variable called “season” was created, where spring season was February–April, Summer season was May–July, Autumn season was August–October and Winter season was November– January. The reasons for this was that four approximately equally sized groups were desired for the analysis of seasonal effects and reported that the both litter size and the total number of litters were largest in the spring and smallest in the winter season and mean litter size to be largest in the spring (Gavrilovic *et al.,* 2008). The monthly variations in mean litter size were almost negligible (Tedor and Reif, 1978).

Strasser and Schumacher (1968), reported that season has no significant effect on litter size at all. A possible explanation for more litters being born in the spring is that more bitches tend to come into estrus during the winter and spring than during autumn, which is in accordance with the reproductive period of the wolf. Seasonal variations in the number of litters born might also be due to management factors such as mating procedures, housing, exercise, feeding and what is the convenient time of birth for the puppy to reach an optimal age at hunting and competition debuts (Tedor and Reif, 1978).

Tedor and Reif (1978) reported no effects of year, season, or age or parity of the bitch at the time of whelping on the litter size and sex ratio of the pups. The pup death rate was also too low to detect any effects of year, season or parity of the bitch.

The male: female sex ratio of puppies was 1:1.25. This agrees with the report of Cox (2007) that there are usually many more females than males, but sometimes there could be litters with all males or all females. The sex of puppies in a litter, are determined by the stud that served the bitch, where the same stud was used for all the bitches.

Holst and Phemister, (1974) studied that Litter size is another factor which has been studied for its influence on the length of gestation in bitches. Litter of a single Beagle pup had longer gestation duration and litter size was negatively correlated with gestation length.

Okkens *et al.*, (2003) reported the effect of age or parity on gestation length has not been studied extensively in the bitches. A single study found primiparous bitches to have similar gestation duration as multiparous bitch and Okkens *et al.* (1993) also reported within a breed, age had no effect on litter size.

Eilts *et al*. (2005) reported that age or parity had no effect on gestation length

2.11 Neonatal mortality

2.11.1 Incidence

The definition of the neonatal period is generally considered to be the transitional phase from foetal to adult life (Jones, 1987). During this period changes in structure and

function occur as the animal moves from the physically, chemically and microbiologically protected environment of the uterus to face the adaptive requirements for survival in a complex environment. Papich and Davis (1986) have reported that the neonatal period for the dog is approximately the first 30 - 45 days. The hepatic and renal drug elimination mechanisms can be expected to approach adult values after this period.

Andersens (1957) study consisted of 234 consecutive litters and 1157 pups whelped in a Beagle breeding colony. Total mortality (stillborn and pups that died under six weeks of age) was 29.40%. The loss reported at birth was 10.90% of all pups born and 37.00% of the total mortality. The death of pups at birth included stillbirths, malformations and undetermined causes as well as those that died as a result of dystocia, trauma and cannibalism. Early neonatal deaths accounted for 8.30% of all pups born and 28.20% of the total mortality. The total of these losses, that is perinatal mortality, was 19.10% and accounted for 65.30% of all pup deaths.

Maternal Age has significant influence on the reproductive performance. Andersen (1965) studied a colony of 02 years old Beagle bitches (n=65). The number of pups weaned was greatest (4.28 pups) and puppy mortality was lowest (18.50%). In another group of Beagles of age 3.5 years old (n=17), the number of pups weaned was highest (4.19) and neonatal mortality was lowest (15.00%). By five years of age conception failure was more than 50.00%. When these same bitches were seven years old, an average of 1.22 pups were weaned and puppy mortality was 38.90%. By nine years of age, only 0.6 pups were weaned per litter and mortality was 76.30%.

Potkay and Bacher (1977) studied total morbidity and mortality in a FoxHound breeding colony for a period of three-year and reported that total mortality before weaning at six weeks of age was 17.40% (501 pups) and 4.0% died between weaning and 30 weeks of age. The stillborn loss was 2.20% and the early neonatal mortality was 9.70%. These losses combine to give a perinatal mortality of 11.90% which was 68.30% of all pup deaths.

Blunden (1986) conducted studies on Fading Puppy Complex at 30 kennels where neonatal mortality was said to be a major problem. The data from 86 litters and 518 pups were analysed. There were 43 stillbirths (8.30%) and a further 152 pups (29.30%) died in the first three weeks of life. Total mortality was 37.70%.

Pup mortality, both during parturition and neonatal period, is a significant clinical problem and poorly documented. Mortality rates of 10-30% by weaning (Lawler, 1989) and 15-40% in the first 12 weeks of life (Sturgess, 1998) are reported.

The majority of pup losses are stillbirths and deaths within the first week of life, that is, perinatal mortality. Lawler (1989) reported that 65% of the losses occurred in the first week, with about half of these being stillbirths.

2.12 Gestational and parturient complications

Maternal infections are a well-known cause of perinatal morbidity and mortality (Johnson and Raksil, 1987). The dam may show no clinical signs and yet the infectious agent may have significant effects on the foetus or neonate. The type of disease that

results from *in utero* infections depends more on the stage of gestation at which infection occurs than on the etiological agent (Greene, 1984a).

Maternal infections with Canine Parvovirus, Herpes Virus, Distemper, Brucella Canis and toxoplasmosis are all reported to cause foetal and neonatal mortality (Higgins *et al.,* 1981; Lenghaus and Studdert, 1982; Hashimoto *et al.,* 1983; Greene and Kakuk, 1984; Greene, 1984c). Mycoplasmas are common inhabitants of the canine vagina and prepuce but may cause reproductive disease if inoculated into the uterus or vas deferens. The role of mycoplasmas in spontaneous embryonic death, resorption, abortion and stillbirth are unknown (Johnston and Raksil, 1987).

Campylobacter jejuni infection has been documented in association with premature labour and abortion. *Campylobacter jejuni* was cultured from the foetal liver and stomach (Bulgin *et al.,* 1984).

Escherichia coli is a most common aerobic bacteria present in the vagina of the normal bitch. It is commonly cultured from the uterus of bitches with pyometra and from vaginal discharges following foetal death and abortion (Greene and Kakuk, 1984).

Salmonella bacteria have been isolated as a cause of abortion and foetal death in a Boxer. Their role in foetal death like that of *E. coli*, may be that of a primary pathogen or as a secondary ascending invader (Redwood and Bell, 1983).

Beta-haemolytic streptococci are common inhabitants of the vagina of the normal bitch. These bacteria have been isolated from the uterus of some bitches with pyometra and from some puppies with neonatal septicaemia. Mantovani *et al.* (1961) reported that

the presence of *beta-haemolytic streptococci* (type 1) from the vagina and lymph node aspirates of collie bitches with a history of abortion, infertility and neonatal death.

Traditionally, dystocia is classified as being either of maternal or foetal origin, or a combination of both. Maternal causes are reported to account for 75.3% of dystocias occurring in the dog, with 48.9% due to primary complete inertia (maternal primary inertia) and 23.1% due to primary partial inertia (maternal secondary inertia). Foetal causes accounted for 24.7% of dystocias encountered, with malpresentations (15.4%) and foetal oversize (6.6%) the predominant forms occurring (Linde-Forsberg and Eneroth, 1998). Dystocia or prolonged labour and the associated hypoxia or anoxia is very significant cause of early death in the dog (Lawler, 1989). Dystocia is defined as a difficult birth or inability to expel the foetus (es) from the birth canal at the time of parturition (Macintire, 1994). There are, however, limited reports in the literature on the extent of the contribution of dystocia to pup mortality. Andersen (1957) reported the loss of 2.3% of all pups born due to dystocia.

Linde-Forsberg and Eneroth (1998) consider the true incidence of dystocia in the bitch to be around 5% overall, but it may amount to almost 100% in some breeds of dog, especially of the achondroplastic type and those selected for large heads.

The diagnosis of dystocia and acceptable time constraints have been reported by Johnston (1988), Jones and Joshua (1982) and Macintire (1994). The diagnosis of dystocia is dependent on demonstrating failure to start labour on due date (use knowledge of ovulation date, rectal temperature drop), failure to progress normally in labour (more than four to five hours in stage two labour before delivery of the first pup, or more than

two to three hours between puppies), foetal membranes in vulva for greater than 15 minutes, strong contractions lasting more than 30 minutes with no foetus. Jones and Joshua (1982) state that the first pup is usually born within one hour of the onset of meaningful straining, but often appears within as little as 20 minutes; up to 2 hours need not cause anxiety. By six hours placental separation is occurring and the life of the presenting puppy may be in jeopardy, thus six hours should be regarded as the maximal period permissible without investigation; even after two hours some separation may exist. The interval between birth comprises two parts - resting and straining. Rest periods vary from 5 minutes to 3 hours and occasionally even longer, although it is arguable if 4 hours rest falls within normal limits. Provided vigorous straining without progress does not occur, the longer intervals do not call for urgent attention. A common pattern is for a bitch to produce two or three puppies at short intervals e.g. ten to 30 minutes and then go into a rest phase of one to three hours before repeating the process. The birth of a large litter may occupy 24 hours or so but if there is no excessive effort needed for each birth the dam should not become unduly tired. From a veterinary stand point one of the most difficult clinical decisions is the distinction between the end of normal labour and the beginning of abnormal labour. The above time constraints may be excessive and require review. Further, the specific contribution of dystocia to pup mortality and the post mortem and histopathological findings in these pups requires detailed investigation.

2.13 Haemato- Biochemical studies at puberty

Serum biochemical constituents have clinical importance in assessing an animal's growth, health, nutritional status and also for diagnosis and prognosis of diseases in domestic and wide range of captive wildlife (Kalita and Mahapatra, 1998; Smith, 2000;

Fowler and Miller, 2003 and Kaneko *et al.,* 2008). The value of blood picture of the animals varies from region to region (Pandiya *et al.,* 1977). Plasma and/or serum biochemical profiles were used extensively in veterinary medicine for the clinical and metabolic evaluation of individual animals and of groups or herds. When processed and correctly interpreted, these profiles provide relevant information on the animal's clinical condition and nutritional state and also serve to underpin the prescription and monitoring of specific therapeutic protocols and to establish prognoses (Payne and Payne 1987).

Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Maton *et al.,* 1993) with the exception of the fish family, channichthyldae (Sidell and O' Brien, 2006) as well as tissues of invertebrates. Hemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (Ugwuene, 2011; Omiyale *et al.,* 2012; Soetan *et al.,* 2013 and Isaac *et al.,* 2013).

According to Peters *et al.*(2011), previous reports stated that PCV, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals (Awodi *et al.* 2005 and Chineke *et al.,* 2006). Furthermore, Chineke *et al.* (2006) reported that high PCV reading indicated either an increase in number of Red Blood Cells (RBCs) or reduction in circulating plasma volume. Mean corpuscular haemoglobin and mean

corpuscular haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia (Aster, 2004).

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Whippet sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 5.8±1.5, 7.8±0.8, 18.9±1.8, 56.1±5.0, 72.3±3.4, 24.3±1.5, 337±16, 176±42 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 69.9±6.1, 36.9±3.5, 6.8±2.0, 98.3±19.5, 54.5±15.0, 42.3±13.5, 48.2±25.9, 2.5±0.3 and 1.3±0.2 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Grey Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 5.4±1.8, 7.7±0.5, 19.1±1.5, 56.2±4.0, 73.4±1.6, 24.9±0.8, 339±12.0, 173±49.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 69.6±5.8, 35.9±3.1, 6.8±1.4, 137.1±20.5, 57.2±16.5, 46.9±14.2, 57.3±20.4, 2.5±0.2 and 1.3±0.3 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Italian Grey Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 8.1±1.9, 7.6±0.6, 17.4±1.4, 51.9±4.1, 68.1±2.2, 23.1±1.4, 335±16.0, 278±85.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 64.9±6.9, 33.8±1.8, 6.6±1.4, 75.9±8.3, 89.7±45.5, 38.4±14.5, 41.9±17.1, 2.4±0.3 and 1.3±0.6 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Borzoi sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 11.5±2.3, 7.7±0.8, 17.7±1.8, 54.0±5.7, 70.3±2.3, 23.1±1.2, 327±15.0, 209±60.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 70.6±6.7, 34.9±3.6, 6.4±2.6, 107.2±17.2, 50.3±9.6, 43.6±11.1, 78.0±53.2, 2.4±0.4 and 1.5±0.9 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Pharaoh sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 8.8±1.9, 7.5±0.7, 17.7±2.0, 52.8±4.8, 70.4±2.8, 23.6±1.8, 335±20.0, 302±153.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 77.2±6.2, 35.9±3.3, 6.8±1.6, 91.5±14.3, 73.8±32.7, 39.5±9.8, 72.1±33.8, 2.2±0.1 and 1.6±0.4 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Saluki sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 7.6±1.6, 8.0±0.8, 18.5±2.2, 55.0±5.7, 68.9±2.6, 23.2±1.6, 335±15.0, 211±81.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 69.0±8.3, 38.1±3.3, 5.7±1.4, 83.2±10.5, 62.8±21.8, 42.2±12.9, 48.9±40.4, 2.5±0.2 and 1.5±0.4, respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Sloughi Sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 9.8±1.7, 8.1±0.9, 18.9±1.7, 56.5±4.9, 70.2±1.9, 23.5±1.2, 335±15.0, 263±92.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT,

AST, ALP, Ca and P are 67.8±6.4, 35.7±3.2, 7.0±1.4, 92.8±9.3, 56.7±17.5, 39.4±10.6, 52.7±14.0, 2.5±0.2 and 1.5±0.4 respectively.

Uhrilkova *et al.* (2013) reported the haematology and serum biochemistry in the Azawakh sight Hound. The haemtological parameters such as RBC, WBC, Hgb, Hct, MCV, MCH, MCHC and PLT are 6.8±1.1, 8.2±0.9, 19.3±1.6, 55.6±5.9, 68.8±2.1, 23.7±0.9, 334±7.0, 333±107.0 and serum biochemistry are TP, ALB, Urea, CRT, ALT, AST, ALP, Ca and P are 70.2±6.0, 35.3±2.4, 6.4±1.8, 93.5±18.6, 55.8±17.4, 32.4±9.4, 45.4±13.8, 2.4±0.2 and 1.5±0.2 respectively.

2.14 Estimation of serum progesterone concentration.

Based on increase in serum / plasma progesterone concentration, a new method for determination of the best time of mating was proposed by Okken *et al*. (1985).

Determining the mating time using the progesterone assay focuses on using changes in P4 level to identify the occurrence of LH surge. Basal progesterone levels have been reported to typically range from 0.0 to 1.0 ng/ml during anoestrus and proestrus (Concannon *et al.*, 1977). At the time of LH surge, serum progesterone rise rapidly (0.8 to 3.0 ng/ml), continued to rise at ovulation (1.0 to 8.0 ng/ml), and was even higher towards the end of fertile period (4.0 to 20.0 ng/ml), by day 21 of post LH surge, progesterone levels ranged between 15 to 50 ng/ml and remained elevated for 2 to 3 months (Concannon, 1986 and Jeffocate and Lindsay, 1989).

Goodman *et al.* (1998) recommended that if accurate serial quantitative progesterone assay are obtained, the LH surge might be estimated as the day when a

distinct rise in levels of progesterone is seen and timing breeding or mating should begin when progesterone has risen above 2 ng/ml.

Van *et al.* (1989) determined the optimum time for mating bitches by measuring plasma P4 concentrations three times a week after the start of vaginal bleeding. The owners were advised to mate their bitches within 9 hours, if the P4 concentration exceeded 12 ng/ml, within 9 to 33 hours, if it was between 6 to 12 ng/ml and within 33 to 57 hours, if it was between 5 to 6 ng/ml of 104 bitches with reduced fertility, 81 (78 per cent) became pregnant and out of 112 bitches with normal fertility, 105 (94 per cent) became pregnant. The mean interval between start of vaginal bleeding and optimum time for mating in 88 bitches was 11.80 ± 31.00 days. The authors concluded that the progesterone is an excellent indicator of timing of mating in bitches with reduced fertility.

Mestre *et al*. (1990) reported that progesterone concentration remained low throughout proestrus (< 2 ng/ml), but then gradually increase to an average of 16 ng/ml on the 8th day of LH peak and that acceptance of male appeared within 24 hrs. After the LH peak and persisted despite high plasma progesterone concentration. Bouchard *et al*. (1991) stated that the in ovulation was estimated to occur when progesterone concentration was 4.90 ± 1.0 ng/ml (range 3.4 to 6.6 ng/ml).

Arbeiter *et al*. (1993) stated that progesterone concentration of 5 ng/ml in the plasma indicated ovulation.

In the study of Bouchard *et al*. (1991) stated that ovulation was estimated to occur when serum P4 concentration was 4.90±1.0 ng/ml (3.4 to 6.6 ng/ml). In an another study (Conconnon *et al*., 1977) serum progesterone concentration of 5.44 ± 0.93 ng/ml as measured by RIA was found to correspond to ovulation. Recently, the use of RIA for progesterone to improve fertility in bitches has been investigated. Okken *et al.* (1985) reported that the 38 of 41 (93per cent) bitches became pregnant following breeding (single or multiple) after progesterone concentration increased above 5 ng / ml.

Wright (1990) reported that LH surge was located to the period when serum P4 concentrations were between 2 to 4 ng/ml and that ovulation occurred when the serum P4 concentrations was 5.4 ng/ml (range 3 to 8 ng/ml).

Renton *et al*. (1991) reported that in bitches, the concentrations of serum P4 reached 3 ng/ml two days before ovulation, and recommended the determination of serum P4 concentration for identifying the time of ovulations. Renton *et al*. (1991) reported that serum P4 concentrations were around 3 ng/ml on the day of LH surge in 12 out of 20 bitches and within 1 day in 7 out of 8 bitches. Ultrasound was less accurate in that, only 4 of the 16 estimates agreed. It was concluded that the measurements of serum P4 concentration was a better indicator for ovulation than ultrasonography.

Wright (1991) reported that LH surge was located to the period when plasma progesterone concentration were 2 to 4 ng/ml and ovulation occurred when the plasma progesterone concentration was 5.4 ng/ml (range 3 to 8 ng/ml).

Dietrich and Moller (1993) reported that 11 out of 13 bitches became pregnant when mating occurred within 2 to 3 days following serum progesterone concentration of 5 ng/ml or above.

Zoldog *et al*. (1994) reported that ovulation in britches occurred when the plasma progesterone concentration reached 5 ng/ml.

Baar (1995) reported that in all 14 beagle bitches studied, the plasma progesterone concentration was higher ranging from 1.9 to 11.7 ng/ml during ovulation. It was also stated that the ovulation could not be predicted using a single measurement of progesterone.

Johnston and Root (1995) in a retrospective study of 49 bitches reported that serum progesterone concentration began to rise concurrently with LH surge, reach in 1.0 to 1.9 ng/ml (3.1- 5.5 nmol/liter) on that day . the day after LH surge the serum progesterone concentration was 2-3.9 ng/ml on the day of ovulation, serum progesterone concentration measured 4.0 to 10 ng/ml.

Johnston *et al.* (2001) in a retrospective study of 49 female dogs reported that the serum progesterone concentration began to rise concurrently with LH surge, reaching 1.0 to 1.9 ng/ml (3.1 to 5.9 nmol/lit) on that day. The serum progesterone concentration was 2 to 3.9 ng/ml on the day after the LH surge. On the day of ovulation, the serum progesterone concentration measured 4 to 10 ng/ml.

Thomaskovic *et al.* (1997) measured the serum progesterone concentration in 35 female dogs at various stages of oestrous cycle and found that at the onset of proestrus,

the concentration was below 0.1 ng/ml. The serum progesterone concentrations increased to an average of 3.8 ng /ml at oestrus and then ranged from 6.8 to 44 ng/ml during metoestrus.

Dieleman and Blankenstein (1998) found that the pattern of blood concentrations of 82 female dogs of different breeds were examined using RIA and was similar to that found using Enzyme Immuno Assay (EIA). After the concentration exceeded 5.0 ng/ml, the dogs were mated 24 to 48 hours. Pregnancy rate in 28 dogs was 78.6 per cent.

Goodman (1998) recommended that if accurate serial quantitative progesterone assay are obtained, the LH surge might be estimated as the day when a distinct rise in level of progesterone is seen and timing of breeding/mating should begin when progesterone has raised above 2 ng/ml.

Konuk *et al.* (2001) reported that the progesterone level in sera of female dogs was 0.7 ± 0.2 ng/ml 5 days before ovulation and increased by day 5 after ovulation to reach 16 ± 4.2 ng/ml.

England and Cancannon (2002) documented the serum progesterone in female dogs on day 7 before LH surge was < 0.5 ng/ml, day of LH surge was 0.9 - 3.0 ng/ml, days 4 - 5 after LH surge was 3.5 - 12 ng/ml and when oocytes matured, days 8 - 11 after LH surge was 8 - 25 ng/ml.

Suyash (2002) reported that none of the 15 female dogs which had a serum progesterone concentration of less than 1.6 ng/ml conceived when they were advised to be mated on the basis of vaginal exfoliative cytology. Eighteen out of 21 female dogs

which had a progesterone concentration of greater than 1.6 ng/ml at the time of mating conceived and delivered at an average 61.77 ± 0.59 days. The conception rate was 44.44 per cent, when serum progesterone concentration was between 1 to 2 ng/ml and 87.50 per cent when it was greater than 2 ng/ml.

Vitasek *et al.* (2002) conducted a study to assess the rate of conception in female dog mated according to a devised scheme. When serum progesterone concentration was less than 2 ng/ml, the progesterone concentration was determined again after 3 to 5 days. When it was between 2 to 3 ng/ml, either mating was advised or progesterone concentration estimated again. When the progesterone concentration was between 3.1 to 4.5 ng/ml, the mating was advised after 2 to 3 days and after 1 to 2 days when it exceeded 4.6 ng/ml but was within 8 ng/ml. If the serum progesterone concentration exceeded 16 ng/ml, the matings were advised immediately. When female dogs were mated according to this scheme, 139 (77.70 %) out of 179 became pregnant.

Nothling *et al.* (2003) conducted a study to determine whether the first rise above 6 mmol/L of plasma progesterone concentration can be used to predict the best time for artificial insemination. In nine breeds, fourteen female dogs were used during one estrous cycle. Plasma progesterone concentration estimated daily, but sometimes with two intervals starting during late proestrus and ending in estrus. The first decrease in the edema of the vaginal mucosa characterized by shrinking rounded folds, was evaluated as a predictor of the period during which plasma progesterone concentration was likely to start rising. Thirteen female dogs were inseminated with frozen thawed semen or more after the onset of the rise in plasma progesterone concentration and all conceived.

Authors concluded that it is suitable to inseminate female dogs 6 and 7 days after plasma progesterone concentration first exceeds 6 mmol/L.

Venkatachalapathy (2005) recommended breeding as soon as the progesterone concentration in the serum reached a minimum of 4.0 ng/ml. The serum progesterone concentration in those animals had been determined from day 5 of the proestrus bleeding, every second day thereafter, until the levels reached 4 ng/ml. In all the 22 female dogs, the vaginal cytology exhibited a cornification index of over 90 per cent and most animals also exhibited physical and behavioural signs of estrus when the serum progesterone concentration was greater than 4 ng/ml. The average time taken for the serum progesterone concentration to reach greater than 4 ng/ml was 11.32 ± 0.01 days from the onset of proestrus bleeding.

Further, Venkatachalapathy (2005) reported that female dogs conceived and the conception rate achieved with matings on the basis of progesterone concentration was similar to those reported in free roaming dogs (>90%). All the 21 pregnant female dogs delivered at an average of 62.0 ± 0.46 days. Three – fourths of the pregnant female dogs delivered before 64th day suggesting that in most female dogs a progesterone concentration of greater than 4 ng/ml was more closely related to the time of ovulation rather than the LH surge.

On the other hand, female dogs which had a serum progesterone concentration of over 4 ng/ml on the day of recommendation of mating on the basis of vaginal exfoliative cytology conceived, as compared to only 55.55 per cent in animals with less than 4 ng/ml in spite of optimum vaginal cornification. From these results Venkatachalapathy (2005)

strongly reported poor correlation of the vaginal exfoliative cytology with serum progesterone concentration and the fertile period of the female dogs.

Reddy *et al.* (2011) documented that the plasma progesterone level was 0.37 ± 0.07 ng/ml on the first day of proestrus and it was recorded as 1.55 ± 0.19 ng/ml on day 8.00 ± 0.71 of proestrus in eCG treated female dogs of his study. Similar trend in progesterone profile during proestrus was noticed by Johnston *et al.,* (2001) and Sridevi (2001). The per cent of female dogs conceived were 83.33. The litter size was 04.33 ± 0.99.

Oluwatoyin *et al.* (2012) documented the serum progesterone concentration in Nigerian local female dogs during proestrus were 7.80 ± 1.07 ng/ml, oestrus 28.00 ± 8.79 ng/ml, dioestrus 52.36 ± 11.96 ng/ml and at anoestrus phase 13.00 ± 1.14 ng/ml.

Xavier and Fontbonne (2007) stated that in order to reduce the number of progesterone assays during the heat period in a bitch and thereby to reduce the cost of the detection of ovulation, veterinarians should often combine vaginal cytology and progesterone levels.

Materials and Methods

III. MATERIALS AND METHODS

The present study was conducted on Mudhol Hound female dogs belonging to Canine Research and Information Centre (CRIC), Timmapur, Mudhol and to the farmers in and around Bagalkot district. CRIC (MH), Timmapur, Mudhol has been established by Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU), Bidar with the primary objective of popularizing Mudhol breed of dog and to provide technical services to Mudhol dog breeders. In addition CRIC (MH), Timmapur, also breeds the pure bred Mudhol Hound dogs and distributed the puppies to scheduled caste and scheduled tribe (SC-ST) farmers free of cost under scheduled caste sub plan and Tribal sub plan (SCP-TSP) project entitled "Livelihood security through Mudhol Hound dog rearing".

Mudhol is a taluka place located in Bagalkot district of Karnataka which is the northern part of state. It is positioned between 15^0 46' and 16^0 46' North latitude and $79^0$59 and $76^0$20' east longitude. The most elevated position of the district lies between 450 to 800 meters above mean sea level and extends over an area of 6593 Sq. Km. It is surrounded by Vijayapura district towards north, Gadag district towards South, Raichur district towards East, Koppal district towards South East and Belgaum district towards West. Bagalkot district comprises Six talukas, namely Bagalkot, Badami, Beelagi, Hunagund, Jamakhandi and Mudhol.

The climate of Bagalkot district is warm and dry throughout the year and the rain fall is scarce. This district receives lowest rainfall annually in the state. Annual rainfall of the district varies from 562 to 978 mm (the average being 770 mm). The minimum and

maximum relative humidity ranges between 14 and 77 per cent, respectively. The annual mean minimum and maximum temperatures are 21.00°C and 41.30°C, respectively.

The CRIC (MH), Timmapur, Mudhol is being maintained under supervision of qualified veterinarians and animal attendants have been trained in basics of animal handling, feeding and management of dogs. At this centre, the animals are housed individually by segregating male and female animals. At present, the CRIC (MH), Timmapur, Mudhol has maintained 06 adult male and 35 adult female pure bred Mudhol Hound dogs in its premises. The centre strictly follows the following practices.

Following whelping, the puppies are allowed to suckle mother up to 45 days post whelping. However, the puppies are encouraged to consume a nutritionally balanced commercial weaning diet (Royal Canine Maxi starter®) 3 times a day as per body weight of puppy. The body weights were recorded immediately and every second day there after until the puppy was one month old. Subsequently the weight of the puppy is taken at monthly intervals till one year of age.

Following weaning, the puppies were fed with commercial dog food suggested for dogs between 02-09 months old age.(Pedigree –Puppy, Chicken and milk, Waltham India Limited, containing Crude protein 24%, Crude fat 10% , Crude fiber 05% and supplemented with vitamins and minerals. The puppies are fed thrice daily with recommended quantity). After completion of 9th Month of age, the dogs were maintained on commercial dog food meant for adult dogs (Pedigree-Adult Meat and Rice Containing Crude protein 20%, Crude fat 10%, Crude fiber 05% and supplemented with vitamins

and minerals The frequency of feeding and quantity of feeding junior and adult dog food were strictly followed as per the recommendations of manufacturers.

The puppies were dewormed one month after birth with Pyrental palmate liquid (Nimocid®) and subsequently repeated at every month until three months of age. Thereafter, the dogs were dewormed orally with tablets containing combination of Pyrental palmate ± Praziquintol ± Fabental (Tab. Worm trap®). The puppies were vaccinated against Canine Distemper and Parvo virus at the age of one month (Inj. Puppy DP®) followed by combined vaccine against Canine Distemper, Infectious Canine Hepatitis, Parvo virus, Picorno Virus, Influenza Virus and Leptospirosis (Inj. Nobivac DHPPI® ±Nobivac Lepto®) at the age of two month and repeated after one month and also received immunization against rabies (Inj. Nobivac R®) first at the age of three months and 2nd dose at the age of four months. Subsequently, booster vaccination against Canine Distemper, Infectious Canine Hepatitis, Parvo virus, Picorno Virus, Influenza Virus and Leptospirosis (Inj. Nobivac DHPPI® ±Inj. Nobivac Lepto®) and rabies (Inj. Nobivac R®) were carried out annually.

The animal attenders were trained to recognize the various reproductive events such as reporting the day of onset of pro-estrual bleeding, monitoring pregnant animals and supervising the whelping processes. Any issues regarding the health of animals are immediately brought to the attention of practicing veterinarian. As a policy, the female dogs are not mated during their pubertal estrous and matings are carried out when female is in its second estrus.

The optimum time for breeding was determined on the basis of vaginal exfoliative cytology and two matings were carried out once, on day of optimum cornification and second, after 48 hours. All matings were carried out naturally.

Following the mating, the animals were examined around 30-35 days for evidence of pregnancy using Trans-abdominal multiconvex ultrasound probe (Aloka, Prosound alpha-6 model). For this purpose, the animals were restrained in dorsal recumbancy and hair in the caudo-ventral abdomen are clipped. The ventral-caudal abdominal area was cleaned and liberal amount of ultra sound gel was applied to improve the contact between skin and ultra sound probe. All ultra sound studies were generally carried out using 5 mhz frequency. The urinary bladder was first located in caudal part of abdomen. Abdomen anterior and caudal to bladder was scanned for evidence of pregnancy as suggested by presence of spherical anechoic areas with hyper echoic mass. (Fig.3.1)**.** Following the confirmation of pregnancy, the quantity of feed fed to animal was gradually increased at the rate of 10 per cent per week until one month post whelping.

CRIC (MH), Timmapur, Mudhol maintains accurate records of date of birth, weight charts, vaccination, health and reproductive records. The centre also has a follow up policy of distributing male and female pups to pet farmers under SCP-TSP project. The puppies were distributed after weaning. All vaccinations, deworming and health services were provided free of cost by the veterinarians of CRIC (MH), Timmapur, Mudhol. However, the animals maintained by pet farmers were fed with homemade diet comprising of wheat, jawar, rice, maize, milk, egg and chicken/ mutton. The puppies from 2 to 9 months of age were fed 03 times a day, after completion of the 09 month age

the animals were fed twice a day. However, the owners did not always strict to the same diet and frequently changed its composition depending on the availability and cost of ingredients. Hence, Curde protein, Crude fat and crude fiber content of home made diet being fed couldnot assessed. Further,the owners were feeding approximately 400-500 grams per time twice daily. On behalf of pet farmers, the CRIC (MH), Timmapur, Mudhol also maintains records of individual animals similar to those of maintained for animals at CRIC Mudhol.

3.1 Experimental design

This present study was conducted in three phases. The first phase of the study was aimed to record the reproductive efficiency parameters of Mudhol Hound dogs maintained on a nutritionally balanced diet. The second phase of the study was designed to study if the reproductive parameters in Mudhol Hound dogs are influenced by feeding of a homemade diet. The third phase of study was attempted to study the influence of age, parity and litter size on duration of pregnancy by analyzing the reproductive records of the Mudhol Hound dogs maintained at CRIC Mudhol for last six years.

3.2 Phase I: Studies on reproductive efficiency of female Mudhol Hound dogs maintained on a nutritionally balanced diet.

This phase of study was carried out to record the reproductive efficiency of Mudhol Hound dogs maintained on a nutritionally balanced diet. A total of 30 female dogs were utilized for this study and the following reproductive parameters were recorded in each animal.

3.2.1 Birth weight of new born

It was recorded to nearest gram using body weighing scale. (Fig.2).

3.2.2 Body weight and age at puberty

The first day of proestrual bleeding exhibited by animal was considered as the onset of puberty. The age of puberty was determined as the interval between date of birth and first day of onset of pro-estrual bleeding. The age of puberty was recorded in days. The animals were weighed on small animal weighing scale and the weight at puberty was recorded in Kilo Grams.

3.2.3 Month of the year during which puberty was achieved

The Month of the year during which puberty was achieved was also recorded to determine whether the season of year has any influence on the onset of puberty.

3.2.4 Duration of proestrus and estrus

During the first estrus after puberty, the duration of pro-estrus as well as estrus during first heat after attainment of puberty was determined to assess whether there were any differences between duration of proestrus and estrus at puberty and those during subsequent estrus.

Following onset of proestrual bleeding, the animals were examined by vaginal exfoliative cytology technique to record the duration of pro-estrus and estrus in individual animals. For this purpose, a small amount of sterile normal saline was drawn into bovine AI sheath attached to 2 ml syringe. The AI sheath containing sterile normal saline was

gently advanced to the anterior vaginal canal and expelled. The suction force was applied to draw vaginal sample into the AI sheath. The AI sheath was then withdrawn and contents were slowy expelled on to a clean greece free slide. The excess fluid was drained off by tilting slide and slide was allowed to dry at room temperature. The air dried smear was then fixed with methanol for 2 minutes followed by staining with Giemsa stain for 30 minutes. Subsequently, the slide was washed under running water; air dried and examined first under 40x and then under 100x to determine the stage of cycle. The determination of stage of cycle was based on identification of the type of cells and proportion of each type of cell. The identification of different type of cells by vaginal smear was made on the basis of following criteria.

a. **Parabasal cells:** Round or oval cells with a large vesicular nucleus and somewhat dark stained cytoplasm were considered as parabasal cells.

b. **Intermediate cells:** Intermediate cells varied in size and shape, but typically had a diameter two to three times more than that of parabasal cells.

c. **Superficial cells**: Superficial cells were the largest cells seen on a vaginal smear. They were polygonal in shape and distinctly flat, sometimes having the appearance of being rolled up. Their nucleus were either absent or pyknotic (very small and dark). Superficial cells without nucleus are often referred to as being "fully cornified".

The proportion of each type of cell was studied by counting a minimum of 200 cells and expressed in per centage. The study of vaginal exfoliative cytology procedure

was first carried out on first day of proestrual bleeding and was repeated every second day until all the cells examined were superficial cells. The interval between first day of proestrual bleeding and first day on which 100 per cent superficial cells were recorded as duration of pro-estrus.

The first day on which 100% superficial cells were seen was determined as first day of estrus and subsequent vaginal exfoliative cytological examination were carried out every second day from day of onset of estrus and the proportion of cornified cells were determined during each examination. The first day on which the proportion of superficial cells decreases from 100 per cent to atleast 90 per cent was recorded as first day of onset of diestrus and the duration of estrus was determined as the interval between the first day of estrus to last of before the reduction in the number of superficial cells from 100 to 90 per cent. Similarly, the duration of proestrus and estrus was period subsequent to pubertal proestrus and estrus period.

3.2.5 Month of the year during which subsequent heat observed

Following the end of the pubertal estrus, the animals were not bred. The female dogs were carefully examined for the onset of the subsequent estrus and month during which subsequent estrus was exhibited was also recorded.

3.2.6 Interval between estrous periods

The time taken for onset of second estrus after attaining puberty was recorded and the interval between first day of pubertal estrus and first day of subsequent estrus was calculated to determine interval between estrus periods.

3.2.7 Conception rate

The CRIC (MH), Timmapur, Mudhol has a policy of breeding animals at their second estrus. The optimum time for breeding animal was determined by following changes in vaginal exfoliative cytology from day of onset of pro-estrual bleeding and the animals were bred once on the first day when the smear exhibits 100 per cent superficial cells and second time 48 hours later. Following breeding of animals, animals were examined for diagnosis of pregnancy at 30 days post mating using ultrasonography and the animal was determined pregnant or non pregnant. The conception rate was determined using following formula.

$$\textbf{Conception Rate} = \frac{\text{Total number of animals pregnant}}{\text{Total number of animals mated}} \times 100$$

3.2.8 Duration of Gestation length

The duration of gestation length was calculated on the basis of following two criteria.

a. The length gestation was determined as a interval between first day of mating to the day of whelping.

b. The interval between first day on which serum progesterone concentration was estimated to be between 3-5 ng/ml and the day of delivery.

3.2.9 Incidence of pseudopregnancy and signs of pseudopregnancy

The animals were carefully examined from 40 days of onset of proestrual bleeding for various pseudo pregnancy signs in non pregnant animals. The incidence of pseudo pregnancy in non pregnant animals was calculated using following formula:

$$\textbf{Incidence of Pseudo pregnancy} = \frac{\text{The number of animals exhibiting pseudo pregnancy between 45-90 days after } 2^{nd} \text{ estrus}}{\text{The number of non pregnant animals between 45-90 days after the previous estrus}} \times 100$$

The various signs of pseudopregnancy exhibited by pseudopregnant animals were also recorded to determine the most common pseudo pregnancy.

3.2.10 Litter size and sex ratio: Following whelping process, the litter size and sex ratio among new born puppies was recorded.

3.2.11 Frequency of complicated birth

Any abnormality observed in process of parturition was recorded and classified at fetal and maternal dystocia. Following delivery, various post partum complications observed such as retention of fetal membranes, metritis and hypocalcimic tetany were duly recorded to determine the incidence of parturient and post partum complication.

3.2.12 Incidence of neonatal mortality

Neonatal mortality was defined as any puppy mortality occurring from birth to weaning and the incidence of neonatal mortality was determined using following formula.

$$\textbf{Incidence of Neonatal mortality} = \frac{\text{Number of puppies exhibiting mortality upto 40 days}}{\text{Total number of puppies born}} \times 100$$

3.2.13 Studies on hemato- biochemical parameters

3.2.13.1 Blood collection

The blood samples for the studies on haemato-biochemical parameters were collected by cephalic venipuncture from the fore limb and saphenous venipuncture from hind limb separately in to EDTA tubes and serum separating tubes. The blood collected in to EDTA tubes was immediately used for determination of hemoglobin concentration and blood glucose concentration and the blood collected in to serum separating vials was allowed to clot at room temp and subsequently, centrifuged at 3000 rpm for 15 min. The serum so separated was transferred in to plastic vials. A portion of serum was immediately used for determination of total protein concentration and rest of serum stored at -20 ^{o}c for determination of serum progesterone concentration at later date.

3.2.13.2 Schedule of blood collection

Haemoglobin, blood glucose and total protein concentration were determined on first day of pubertal proestrus and first day of the second proestrus period. Blood sample for serum progesterone concentration were obtained on first day of pubertal proestrus as well as first day of subsequent proestrus. Subsequent samples for determination of serum progesterone concentration were obtained on the first day when vaginal exfoliative cytology examination revealed the presence of 100 % superficial cells and again on first day of cytological diestrus.

3.2.13.3 Determination of haemoglobin Concentration

The hemoglobin concentration was determined using automatic haematology analyser. The concentration was expressed as gm%.

3.2.13.4 Random Blood Glucose Concentration

The blood collected in EDTA tubes was also used for estimation of random blood glucose concentration which was carried out using hand held Glucometer. The specificity of assay ranged between 20-600 mg/dl.

3.2.13.5 Total serum protein concentration

The total serum protein concentration was determined using automatic blood analyzer. The concentration was expressed in gram per deciliter (g/dl).

3.2.13.6 Estimation of serum progesterone concentration

The serum progesterone concentration was determined by using Enzyme Linked Immunosorbant Assay (ELISA). The sensitivity of assays was 0.25 ng/ml to 80 ng/ml. The co-efficient variation is 3.527 per cent. The results of serum progesterone analysis were available within 45 minutes of subject into assay.

3.3 Phase II: Studies on reproductive efficiency of female Mudhol Hound dogs maintained on a homemade diet.

This phase of study was conducted on 30 female Mudhol Hound dogs maintained by pet farmers who had been maintaining the female dogs given to them after weaning. These dogs were being maintained on nutrition which was homemade and did not receive any commercial food. However, the record of individual animals had been maintained by

CRIC (MH), Timmapur, Mudhol and out of which 30 animals were randomly selected and fed. These animals were followed from the time to animals were distributed to farmers till completion of present study. The following parameters were recorded in order to assess whether there were any differences in reproductive efficiency of Mudhol Hound dogs maintained under scientific system of feeding and management and those maintained with a homemade diet.

3.3.1 Birth weight of New born

3.3.2 Age and weight at Puberty

3.3.3 Month of the year during which puberty achieved

3.3.4 Month of the year during which subsequent estrus period were observed

3.3.5 Duration of proestrus and estrus.

3.3.6 Inter-estrus interval

3.3.7 Conception rate

3.3.8 Duration of gestation length.

3.3.9 Incidence of pseudopregnancy and signs of pseudopregnancy

3.3.10 Litter size and sex ratio

3.3.11 Frequency of complicated birth.

3.3.12 Frequency of neonatal mortality.

3.3.13 Haemato-biochemical parameters at pubertal age.

All these parameters were recorded in close consultation with the selected beneficiary farmers. Their calendar of events with respect to above parameters as entered in their dairy was taken into consideration for analysis purpose.

3.4 Phase III: Studies on Influence of age, parity and litter size on duration of pregnancy

This study was conducted by analyzing the reproductive records of dogs maintained at CRIC, Timmapur, Mudhol for last 6 years.

3.4.1 Age

In order to determine the influence of age on litter size the animals were categorized into 3 different groups of age less than 2 years, 2-4 years of age and more than 4 years of age. And litter size among the different age group was compared.

3.4.2 Parity

In order to determine the influence of parity on litter size the animals were categorized into 3 different groups of first parity, 2-4 parity and more than 4th parity and litter size among the different age group was compared.

3.4.3 Litter size

In order to determine the influence of litter size on gestation length the animals were categorized into 3 different groups of age less than 2 years, 2-4 years of age and more than 4 years of age and gestation length among the different age group was compared.

3.5 Statistical analysis of data

Data from the schedules was compiled and analyzed by calculating standard statistical parameters. In the present study for phenotypic characterization, the mean and standard error of each parameter were calculated as per standard procedure. The data were subjected to student-t test detailed by Snedecor and Cochran (1989).



Fig. 2: Weighing of Mudhol Hound puppies at CRIC, Timmapur



Fig. 3: Weighing of Female Mudhol Hound dogs at CRIC, Timmapur



Fig. 4: Collection of blood sample from saphenous vein in Female Mudhol hound dog.



Fig. 5: Collection of vaginal fluid sample in Mudhol hound female dog.

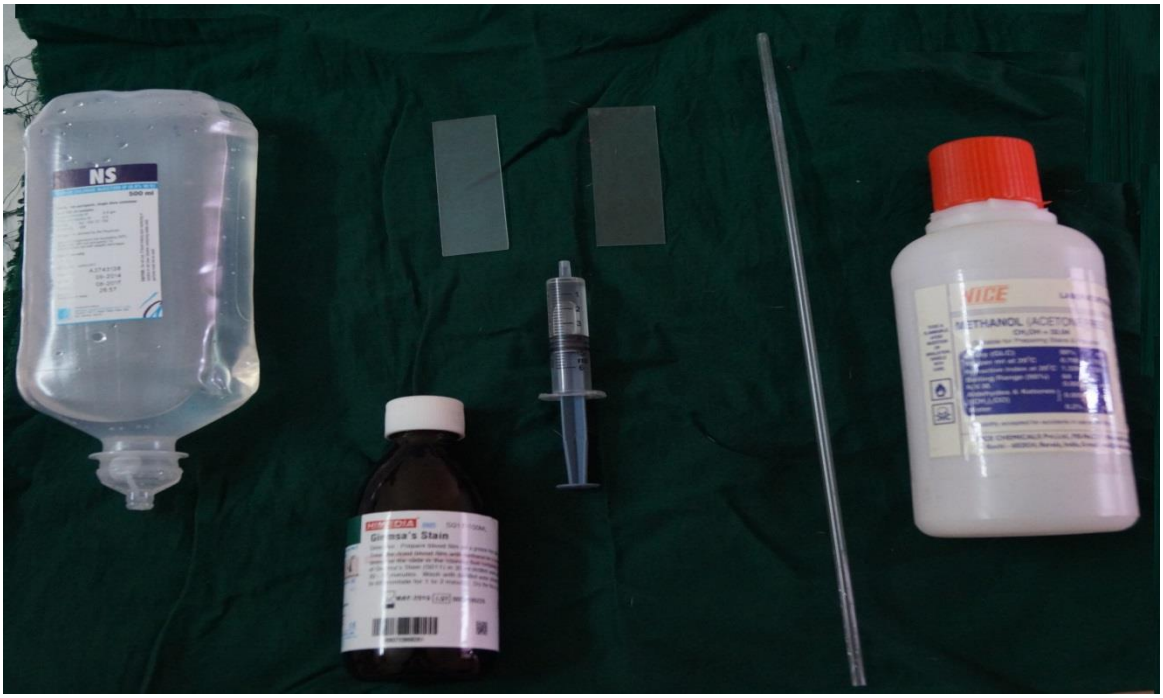


Fig. 6: Instruments used for exfoliative vaginl cytology examination

A) Normal saline B) Microscopic Slide C) Syringe D) Bovine artificial Insemination sheath E) Geimsa Stain F) Methanol

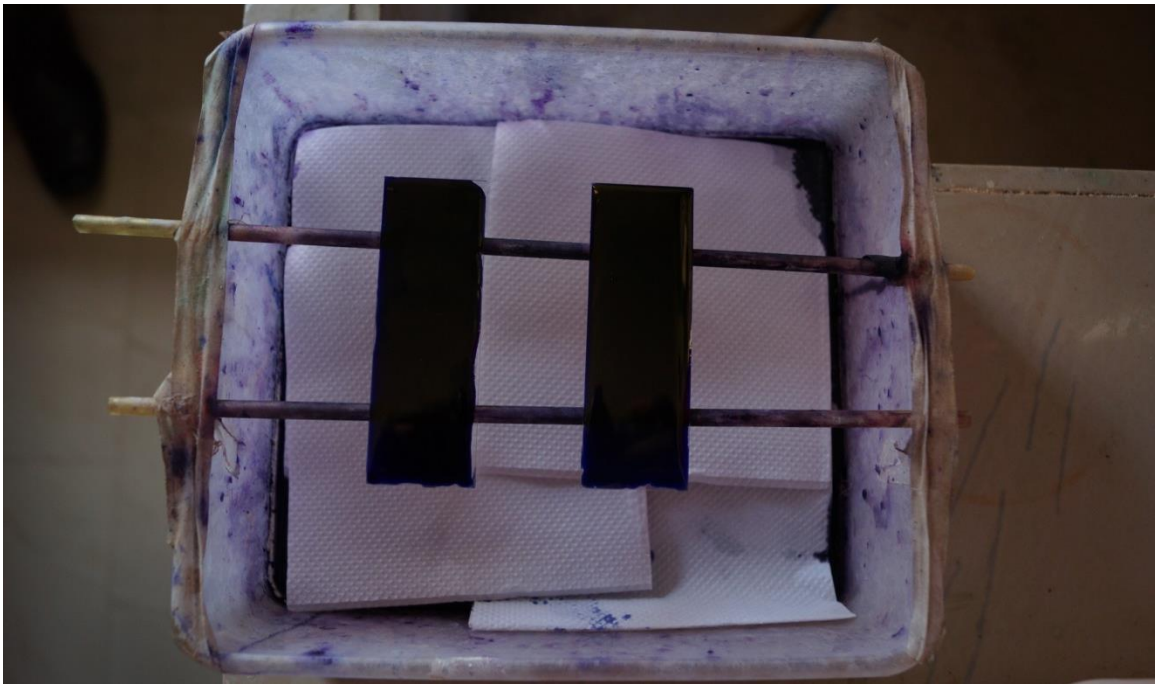


Fig. 7: Staining procedure followed

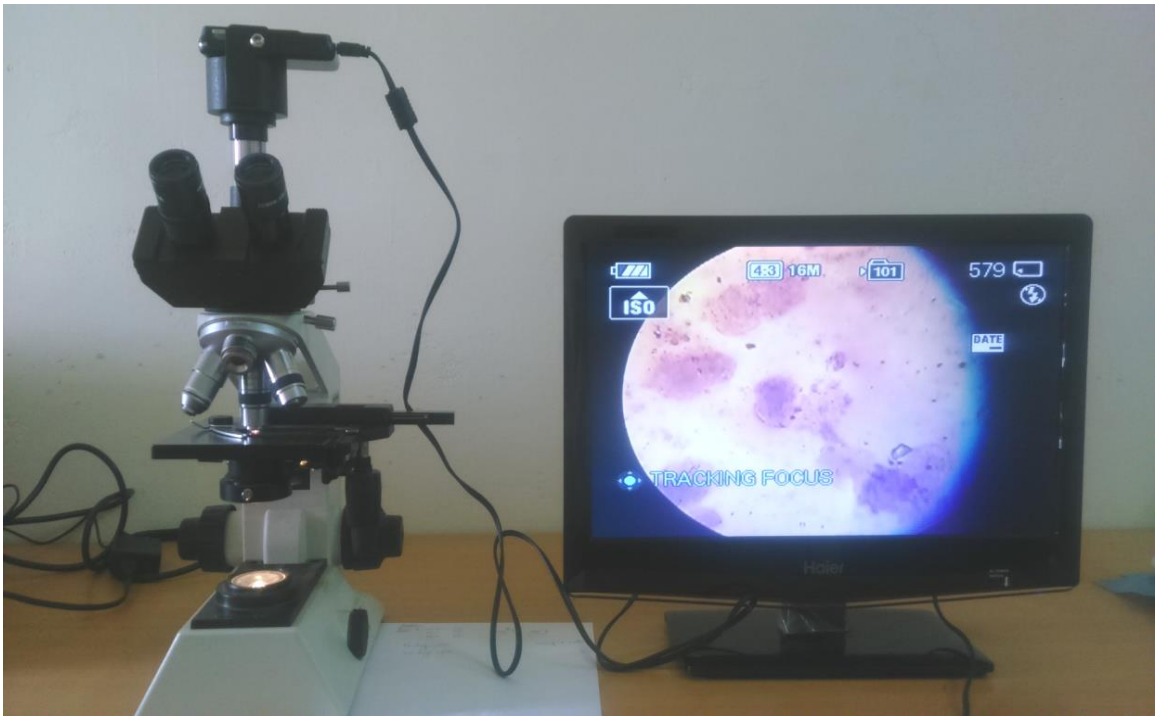


Fig. 8: Evaluation of vaginal exfoliative cytology under the Dark Field Microscope

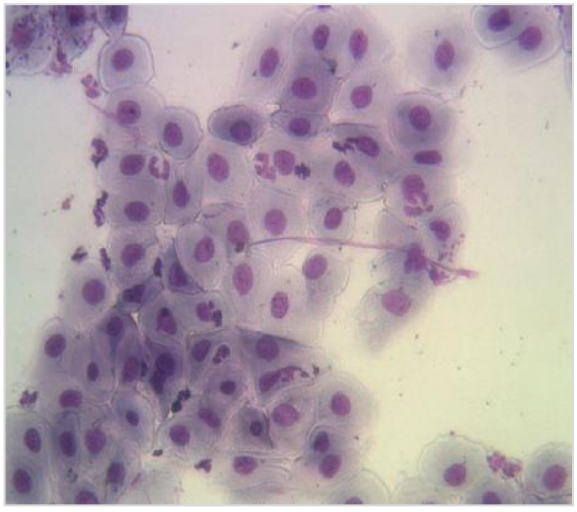


Fig. 9: Parabasal cells, X100, Giemsa stain

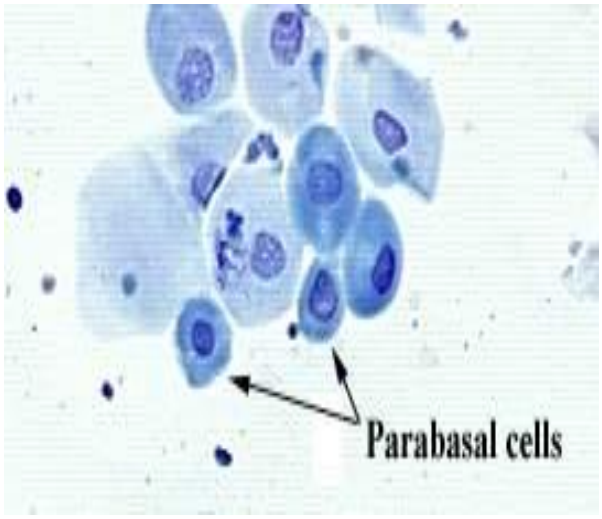


Fig. 10: Parabasal cells, X400, Giemsa

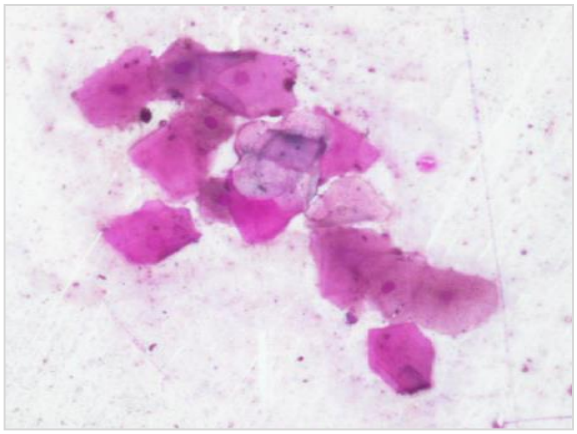


Fig. 11: Intermediate cells, X100, Giemsa stain.

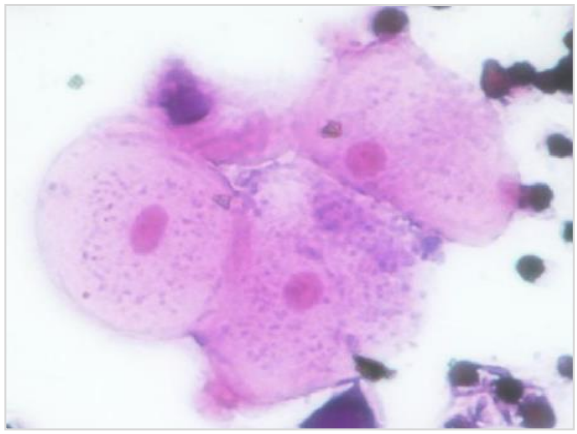


Fig. 12: Intermediate cells, X400, Giemsa stain.

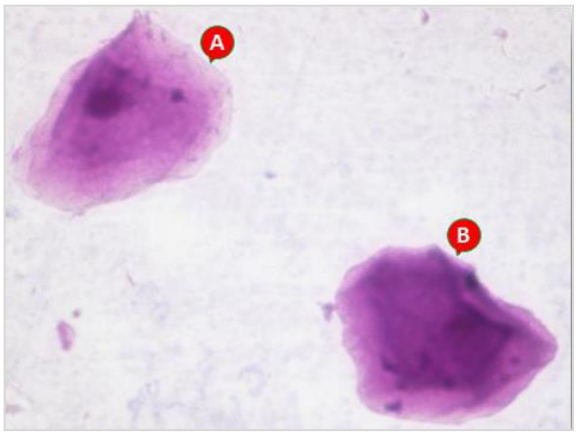


Fig. 13: A) Intermediate cell B) Superficial cell (Anuclear cell), X400, Giemsa stain

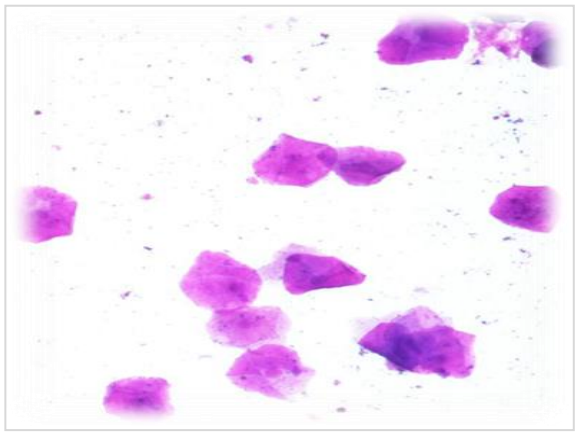


Fig. 14: Superficial cells (Anuclear cells), X100, Giemsa stain

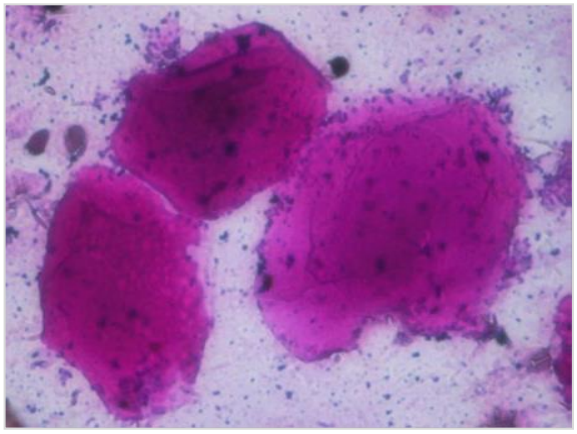


Fig. 15: Superficial cells with dirty back ground (Anuclear cells), X400, Giemsa stain

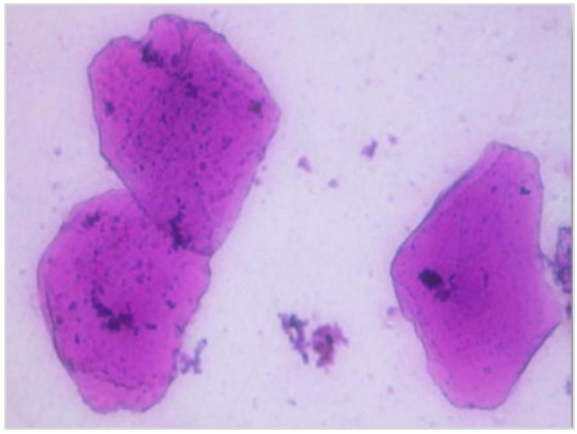


Fig. 16: Superficial cells with clear back ground (Anuclear cells), X400, Giemsa stain

Results

IV. RESULTS

The present study was conducted with the primary objective of determining, if the reproductive efficiency parameters in Mudhol Hound dogs are influenced by the type of diet on which they are maintained. The two methods of feeding systems compared were, feeding of nutritionally balanced dog food and feeding of homemade dog food. The influence of the type of diet fed to dog on the reproductive efficiency was assessed in terms of birth weight of puppies, age and body weight at puberty, month of year during which the animal exhibited pubertal estrus, the duration of pubertal estrus and estrus, inter estrus interval, duration of pro-estrus and estrus during subsequent estrus periods, conception rate, gestation length, litter size and sex ration, incidence of pseudo pregnancy and neonatal mortality and incidence of parturient and post parturient complications. In addition, hematological, biochemical and serum progesterone profiles were also studied.

4.1 Reproductive efficiency of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

4.1.1 Birth weight of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The birth weight of male and female puppies born to Mudhol Hound dogs maintained on nutritionally balanced diet and on homemade diet is presented in Table 1 and Fig. 17. The mean birth weight of male and female puppies born female dog maintained on nutritionally balanced dog food was recorded as 479.11 ± 3.44 and 437.00 ± 5.47 g, respectively. The birth weight of male puppies was found to be significantly higher than female puppies. On the other hand, the weight of male and female puppies

born to mothers maintained on a homemade diet was recorded as 450.11 ± 3.44 and 415.00 ± 4.47g, respectively were found to weigh significantly higher than female puppies. Further, the weight of both male and female puppies born to Mudhol Hound dogs maintained on nutritionally balanced food were significantly better than those born to dams maintained on homemade diet ($p<0.05$).

4.1.2 Age at Puberty of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Table 2 and Fig. 18 depicts the age of puberty recorded in 30 female dogs maintained on a nutritionally balanced food and compares the same with the age of puberty of 30 female dogs maintained on homemade diet. The mean age at puberty of female dogs maintained on nutritionally balanced food was recorded as 12.40± 0.64 months While, it was 16.03 ± 0.06 months in dogs maintained on homemade diet. The dogs maintained on nutritionally balanced diet were found to achieve puberty at a significantly earlier age than those maintained on feeding of homemade diet ($p<0.05$)**.**

4.1.3 Body weight at puberty of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The weight at puberty of female Mudhol Hound dogs maintained on nutritionally balanced food was recorded as 24.32 ± 0.28 Kgs. On the other hand, the body weight at puberty of female dogs maintained on homemade diet was recorded as 23.07 ± 0.17 kgs. However, the body weight at puberty of female dogs maintained under two different system of feeding did not differ significantly (Table 3 / Fig. 19) ($p>0.05$)

4.1.4 Month of the year during which pubertal estrus was achieved in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Table 4 (Fig. 20) exhibits the month of the year during which pubertal estrus was observed in female Mudhol Hound dogs maintained under two different system of feeding. It was observed that the highest no of animals exhibiting pubertal estrus in the month August in female dogs maintained on either, which was diet 23.33 and 33.33 per centage respectively. The number of animals attaining puberty was fairly high and similar during months of January and February irrespective of system of feeding the female dogs were being maintained (16.67% each). The number of animals achieving puberty during hotter months of the year (March, April and May) was either nil or very few under both systems of feeding.

4.1.5 Duration of pro-estrus and estrus in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The mean duration ofpubertal proestrus in 30 dogs maintained on nutritionally balanced food was recorded as 9.53 ± 0.31days and ranged between 7 to 13 days (Table 5). Its duration in dogs maintained on homemade diet was 8.93 ± 0.23 days and was not significantly different from mean duration of proestrus recorded in animals maintained on nutritionally balanced food (Table 5 and Fig. 21) ($p<0.05$).

Table 5a also presents the mean duration of estrus in animals maintained on two different systems of management. In dogs maintained on nutritionally balanced food, the mean duration of second estrus was recorded as 7.40 +0.57 days and ranged between 5 to

9 days. Further, the mean duration of pubertal estrus in dogs maintained on homemade diet did not differ significantly from the mean duration recorded in animals maintained on nutritionally balanced Diet, the mean duration and range of pubertal estrus being 8.27 ± 0.46 and 6 to 10 respectively (Table 5a; Fig. 21a) ($p<0.05$).

4.1.6 Interval between pubertal estrus and subsequent estrous period of Mudhol Hound female dogs maintained on nutritionally balanced diet and on a homemade diet.

In the present study, the interval between pubertal estrus and subsequent estrus was significantly shorter in dogs maintained on nutritionally balanced food as compared to the inter estrous interval of animals maintained on homemade diet (275.67 ± 10.65 v/s 306.03 ± 7.14). The inter estrous interval in animals maintained on nutritionally balanced food ranged between 193 to 401 days while, it was 222 to 423 days in animals maintained on homemade diet. (Table 6 Fig. 22) and the mean inter estrus interval was recorded as 275.67 ± 10.65 days and ranged between 193 to 401 days ($p<0.05$).

4.1.7 Conception rate of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

The conception rate of Mudhol Hound dogs maintained on nutritionally balanced food was about 13.00 % higher than conception rate recorded in animals maintained on homemade diet. 19.00 (63.34%) out of 30.00 animals maintained on nutritionally balanced food were diagnosed pregnant 30 to 35 days after mating, while, only 15.00 (50.00%) out of 30.00 animals maintained on homemade diet conceived. (Table 7 and Fig. 23).

4.1.8 Duration of gestation length determined as Interval from day of 1st mating to day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

The mean duration of pregnancy calculated as interval from first day of mating to day of delivery in dogs maintained on nutritionally balanced food was determined as 67.86 ± 0.80 and in individual animals it ranged between 62 to 70 days. (Table 8 & Fig. 24) This duration of pregnancy was not significantly different from mean duration of pregnancy (67.45 ± 0.42 days) recorded in animals maintained on homemade diet ($p>0.05$).

4.1.9 Duration of gestation length determined as Interval from day on which the serum progesterone concentration was 3-4 ng/ml and the day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The duration of gestation length determined as interval from day on which the serum progesterone concentration was 3-4 ng/ml and the day of delivery in female Mudhol Hound dogs maintained under different system of feeding is presented in Table 9, Fig.25). It was observed that that the mean duration of pregnancy in dogs maintained on nutritionally balanced food was determined as 63.85 ± 0.18 and in individual animals, it ranged between 63 to 66 days. This was not significantly different from mean duration of pregnancy (64.35 ± 0.28 days) recorded in animals maintained on homemade diet ($p>0.05$).

4.1.10 Incidence of pseudo pregnancy in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

Irrespective of the type of feeding, 26 animals were diagnosed as non-pregnant 30 to 35 days after mating and 12 of these animals exhibited one or more clinical signs of pseudopregnancy between 45 to 90 days after mating (Table 10). The overall incidence of pseudopregnancy in Mudhol Hound dogs was therefore determined as 46.15 per cent. The most common sign of pseudopregnancy recorded was mammary gland hypertrophy associated with lactation which was seen in 8 (88.00%) out of 9 animals exhibiting signs of pseudo pregnancy**.** The only signs of pseudopregnancy recorded were nesting behavior (02 animals, 22.23 %). Further, the per cent of animals exhibiting signs of pseudopregnancy in each systems of feeding was almost similar (Table 10 Fig. 26).

4.1.11 Litter size in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

The influence of system of feeding on litter size in female Mudhol Hound dogs is shown in Table 11 and Fig. 27. In the present study, the mean litter size Mudhol Hound dogs maintained under nutritionally balanced food ranged between 4 to 10 and mean litter size was recorded as 7.72 ± 0.28 (Table 11). On the other hand, the mean litter size in dogs maintained of homemade diet ranged between 1-11 and mean litter size was recorded as 5.76 ± 0.31 and this was significantly lower than Litter size observed in dogs maintained on nutritionally balanced food (Table 11 Fig. 27).

4.1.12 Sex ratio of puppies born in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet.

In the present study, the sex ratio of male to female puppies born in dogs maintained on nutritionally balanced food was determined as 1:1.02 (Table 12 Fig. 28).

This sex ratio did not differ significantly from sex ratio (1:1.14) recorded in dogs maintained on homemade diet.

4.1.13 Influence of age, parity and litter size on duration of pregnancy in Mudhol Hound Female dogs

4.1.13.1 Influence of age and parity of dam on litter size in Mudhol Hound female dogs

The influence of age and parity of dam on litter size in Mudhol Hound female dogs is shown in Table 13& 14 and Fig. 29 & 30. In the present study, the mean litter size of Mudhol Hound dogs in different age group of dams is 5.63 ± 0.34 in the age group of less than or equal to 2 years while it is 6.94 ± 0.032 and 6.69 ± 0.38 in age group of 2-4 years and more than 4 years respectively. The mean litter size did not differ significantly irrespective of system of feeding.

The mean litter sizes observed during first parity, 2-4 parity and more than 4th parity in Mudhol Hound female dogs were 6.63 ± 0.34, 6.94 ± 0.032 and 6.69 ± 0.38. The mean values of litter size in Mudhol Hound dogs at Ist 2to 4 parity did not differ But they differ significantly differ significantly More than 4 parity (Table 14 and Fig. 30)

4.1.13.2 Influence of litter size on gestation length in Mudhol Hound female dogs

In the present study, the duration of pregnancy is calculated from the date of mating or last date of mating to the day of parturition. The mean litter size observed in Mudhol Hound dogs with gestation length of 61-62 days or lesser is 8.86 while it is 7.23 (in 63-64 days group), 6.25 (in 65-66 days group), 4.75 (in 67-68 days) and 1.55 (in 68-

70 or more days). The litter size at birth was found to higher in shorter gestation lengths (Table 15, Fig. 31).

4.1.14 Frequency of complicated births in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study irrespective of type of feeding on which Mudhol Hound dogs were being maintained, dystocia was recorded in only two (5.88 %) out 34 deliveries. The two animals in which dystocia were observed were being maintained on homemade diet and both cases were maternal origin. In one animal maternal dystocia was diagnosed to be due to primary uterine inertia and animal was carrying a single fetus. In another animal, also maintained on homemade diet, dystocia was due to partial primary uterine inertia and this animal had a litter size of 11. None of the animals maintained on nutritionally balanced food had any complication at the time of parturition (Table 16).

4.1.15 Gestational and Post parturient complications in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, none of the 19 animals maintained on nutritionally balanced food exhibited any gestational complication or post parturient complication. (Table 15) Further, gestational complications were also not observed in any 15 pregnant animals maintained on homemade diet. However, one animal was diagnosed with mastitis post-delivery (Table 17).

4.1.16 Prevalence of neonatal mortality in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The prevalence of neonatal mortality of puppies born in female Mudhol Hound dogs maintained under different systems of feeding is presented in Table 18 and Fig. 32. In 19 female dogs maintained under nutritionally balanced food delivered a total of 152 puppies and 11 puppies died between days of delivery to day of weaning. The prevalence of neonatal mortality in female Mudhol Hound dogs maintained on nutritionally balanced food was determined as 7.24 per cent. Similarly, 113 puppies were delivered by 15 female dogs maintained on homemade diet and 18 (15.93 %) exhibited neonatal mortality. The prevalence of neonatal mortality was significantly higher in female dogs maintained on homemade diet ($p>0.05$).

4.1.17 Hemoglobin concentration (gm %) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The hemoglobin concentration at puberty in female Mudhol Hound dogs maintained under nutritionally balanced food did not differ significantly from the concentration recorded in dogs maintained on homemade diet. The mean hemoglobin concentration in dogs maintained on nutritionally balanced food was recorded as 13.65 ± 2.86 gm% and ranged between 11.50 to 15.40. The corresponding hemoglobin concentration in homemade diet was recorded as 12.65 ±1.18 gm% and ranged between 9.79 to 14.63 gm% (Table 19, Fig. 33**).**

4.1.18 Random Blood Glucose concentration (mg/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Table 18, depicts random blood glucose concentration recorded at puberty in female Mudhol Hound dogs maintained on either nutritionally balanced or on a

homemade diet. In dogs maintained on nutritionally balanced food, the mean random blood glucose concentration was recorded as 82.00 ± 1.05 mg/dl and ranged between 82 to 139 mg/dl. This mean concentration did not differ significantly from mean concentration of 80.39 ± 0.34 mg/dl (82 to 132 mg/dl) recorded in dogs maintained on homemade diets. (Table 20 Fig. 34).

4.1.19 Serum total protein concentration (mg/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The serum total protein concentration at the time of puberty did not differ significantly between Mudhol Hound dogs maintained under two different systems of feeding (Table 21, Fig. 35). In thirty Mudhol Hound dogs maintained on a nutritionally balanced food, the mean serum total protein concentration on the first day of pubertal proestrus was recorded as 8.85 ± 0.15 mg/dl and ranged between 8.28 to 9.85 mg/dl. The corresponding serum total protein concentration of dogs maintained on a homemade diet was recorded as 8.36 ± 0.12 mg/dl with a range of 7.89 to 9.24 mg/dl.

4.1.20 Serum progesterone profile during proestrus and estrus in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, the mean serum progesterone concentration on the first day of pubertal estrus in animals maintained on nutritionally balanced food was recorded as 0.19 ± 0.07 ng/ml and this concentration did not differ significantly from serum progesterone concentration recorded at puberty in dogs maintained on homemade diets (0.18 ± 0.07 ng/ml). Further, serum progesterone concentration on the first day of

subsequent proestrus also did not differ significantly from concentration recorded on first day of pubertal proestrus irrespective of the system of feeding (Table 22, Fig. 36).

The serum progesterone concentration on first day of pubertal cytological estrus was recorded as 1.75 ± 0.89 ng/ml in dogs maintained on nutritionally balanced food. The corresponding value at similar stage of cycle during subsequent estrus was recorded as 1.82 ± 0.61 ng/ml and this concentration was significantly different from values recorded on first day of cytological estrus. A similar situation was recorded in dogs maintained on homemade diet, the serum progesterone concentration being 1.52 ± 0.87 and 1.61 ± 0.55 ng/ml on the first day of pubertal cytological estrus and subsequent estrus respectively (Table 22, Fig. 36).

The mean serum progesterone concentration on first day of cytological diestrus and first day of subsequent diestrus in dogs maintained on nutritionally balanced food was recorded as 13.16 ± 1.11 ng/ml and 11.22 ± 0.99 ng/ml respectively. The corresponding serum progesterone concentration in dogs maintained on homemade diets was 12.23 ± 1.08 and 11.85 ± 0.94 ng/ml, respectively. The serum progesterone concentration did not differ significantly either between first day of pubertal diestus and first day of subsequent diestrus or between systems of feeding (Table 22).

Table 1. Birth weight (g) of male and female puppies born to Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	System of feeding	Mean Birth weight of puppies (g)	
		Male	Female
1	Feeding of nutritionally balanced food	479.11 ± 3. 44[a] (n =75)	437.00 ± 5.47[b] (n =83)
2	Feeding of homemade diet	450.11 ± 3.44[b] (n=53)	415.00 ± 4.47[b] (n=61)
	Overall (n= 272)	458.00 ± 0.18 gm	

*Mean birth weight with different superscript are significantly different ($p<0.05$)

Fig. 17. Birth weight (g) of male and female puppies born to Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

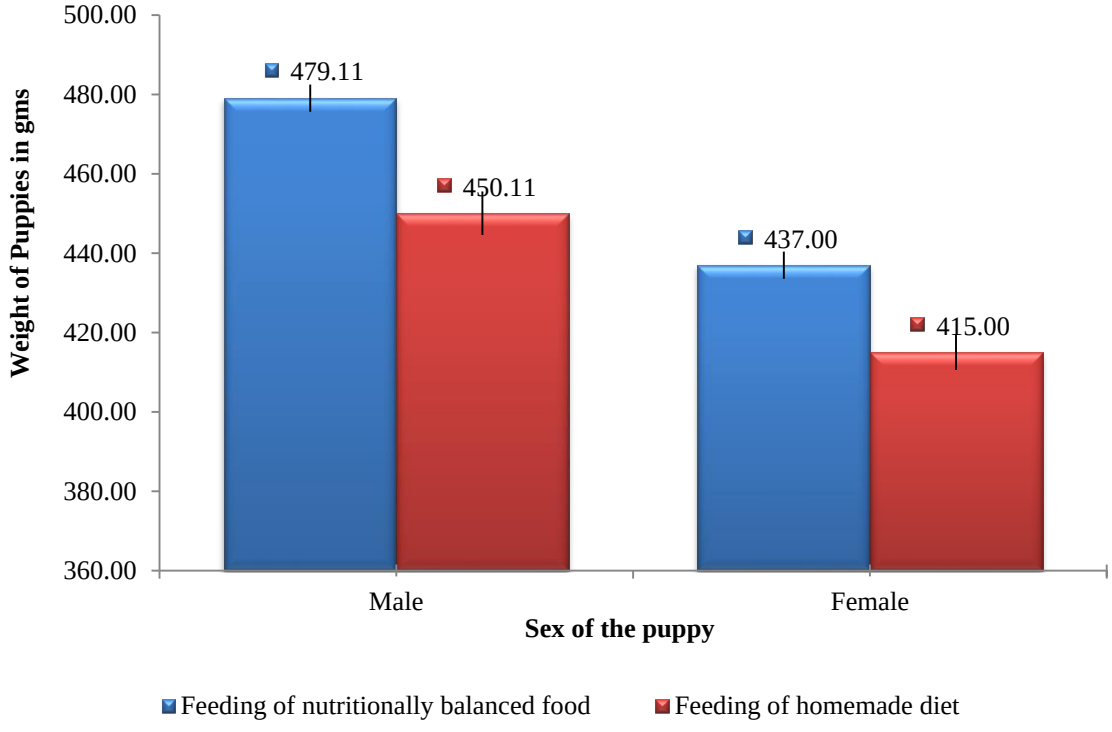


Table 2. Age at puberty (in months) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	System of feeding	No of Animals	Mean Age at Puberty (Months)
1	Feeding of nutritionally balanced food	30	12.40±0. 64[a]
2	Feeding of homemade diet	30	16.03 ± 0.06[b]
	Overall	60	13.80 ± 1.12

*****The mean age at puberty of Mudhol female dogs maintained on nutritionally balanced diet and on homemade diet are significantly different ($p<0.05$)

Fig. 18. Age at puberty (in months) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet (n=30)

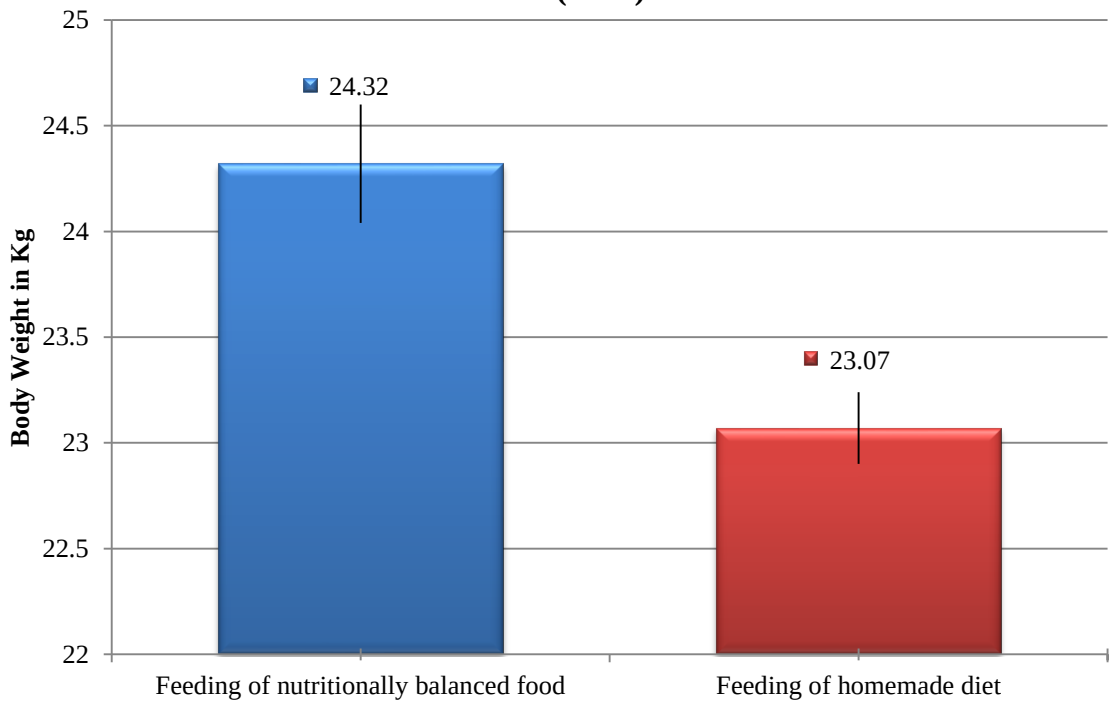


Table 3. Body weight at puberty (Kgs) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	System of feeding	No of Animals	Mean body weight at Puberty (Kgs)
1	Feeding of nutritionally balanced food	30	24.32 ± 0.28[a]
2	Feeding of homemade diet	30	23.07 ± 0.17[a]
	Overall	60	23.70 ± 0.22

*****The mean body weight at puberty of Mudhol female dogs maintained on nutritionally balanced diet and on homemade diet did not differ significantly (p>0.05**)**

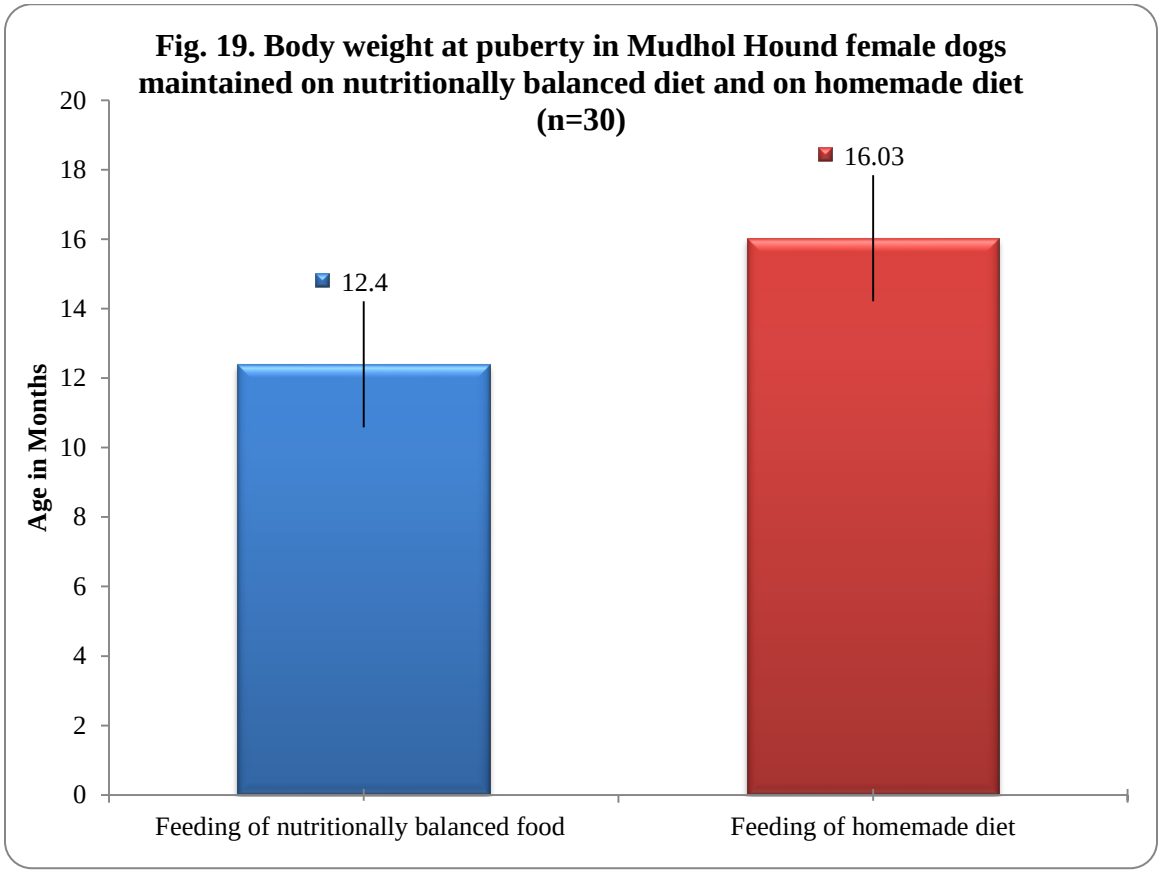


Table 4. Month of the year during which pubertal estrus was exhibited in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet (n=30)

Sl. No	System of feeding and management	No of animals exhibiting pubertal estrus during different months of the year (percentage)											
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**
1.	Nutritionally Balanced food (n=30)	5 16.66	5 16.66	0 0	1 3.33	1 3.33	3 10	4 13.33	7 23.33	1 3.33	1 3.33	1 3.33	1 3.33
2.	Homemade diet (n=30)	5 16.66	5 16.66	0 0	0 0	0 0	3 10	3 10	10 33.33	1 3.33	1 3.33	1 3.33	1 3.33
	Overall (N=60)	10 16.67	10 16.67	0 0.00	1 1.67	1 1.67	6 10.00	7 11.67	17 28.33	2 3.33	2 3.33	2 3.33	2 3.33

Fig. 20. Month of the year during which pubertal estrus was exhibited in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet (n=30)

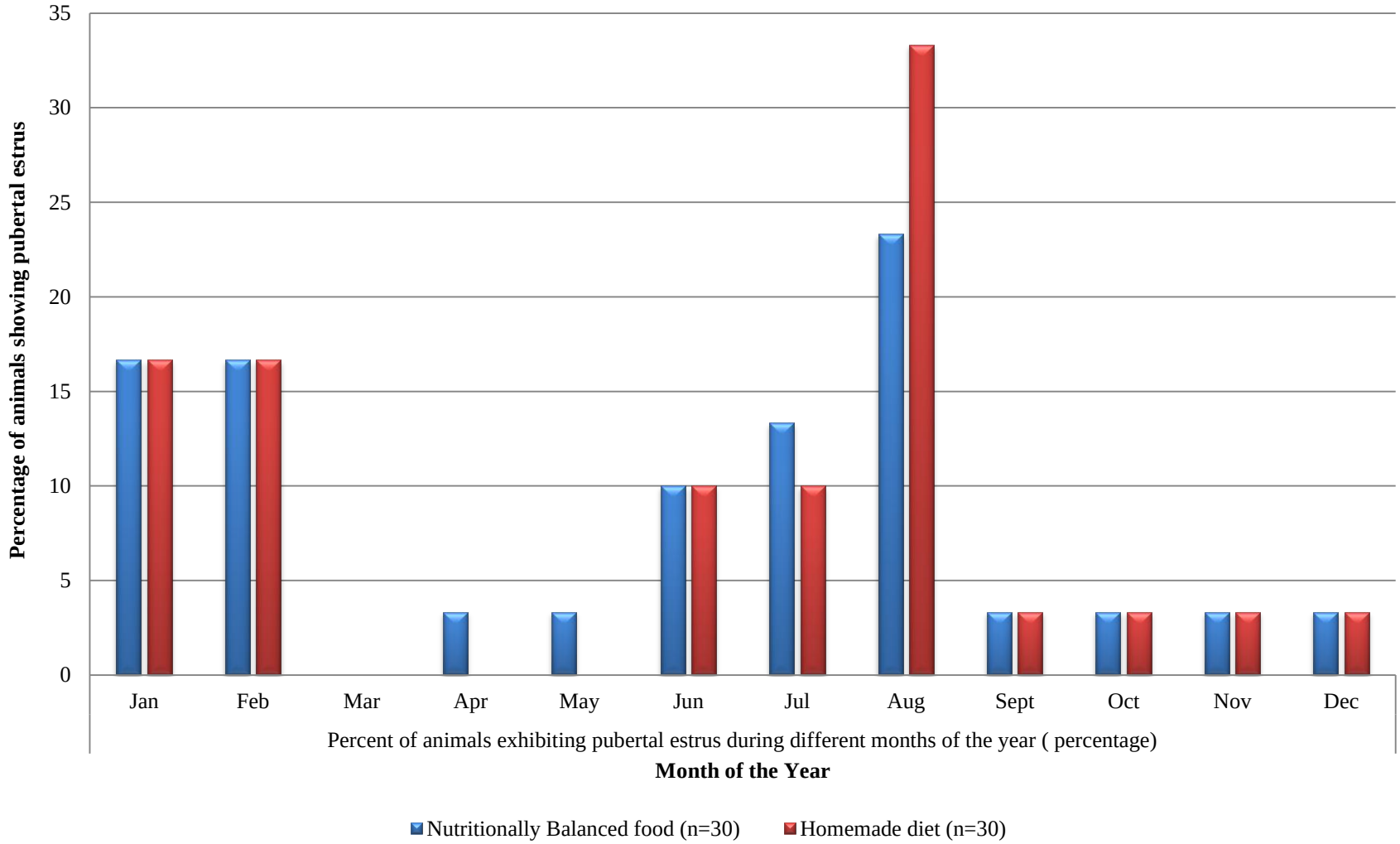


Table 5. Duration of pubertal pro-estrus and second pro estrus (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding and management	Pubertal Proestrus (Days)		Second proestrus (Days)	
		Mean ± SE (Days)	Range (Days)	Mean ± SE (Days)	Range (Days)
1	Nutritionally Balanced food (n=30)	9.53 ± 0.31a	07 to 13	9.23 ± 0.21a	07 to 12
2	Homemade diet (n=30)	8.93 ± 0.32a	07 to 12	9.73 ± 0.34a	07 to 13
	Overall (N=60)	9.22 ± 0.32	07 to 13	9.48 ± 0.28	07 to 13

*Mean values bearing similar superscripts along the column are not significantly different ($p<0.05$).

Fig. 21. Duration of pubertal pro-estrus and second pro estrus (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

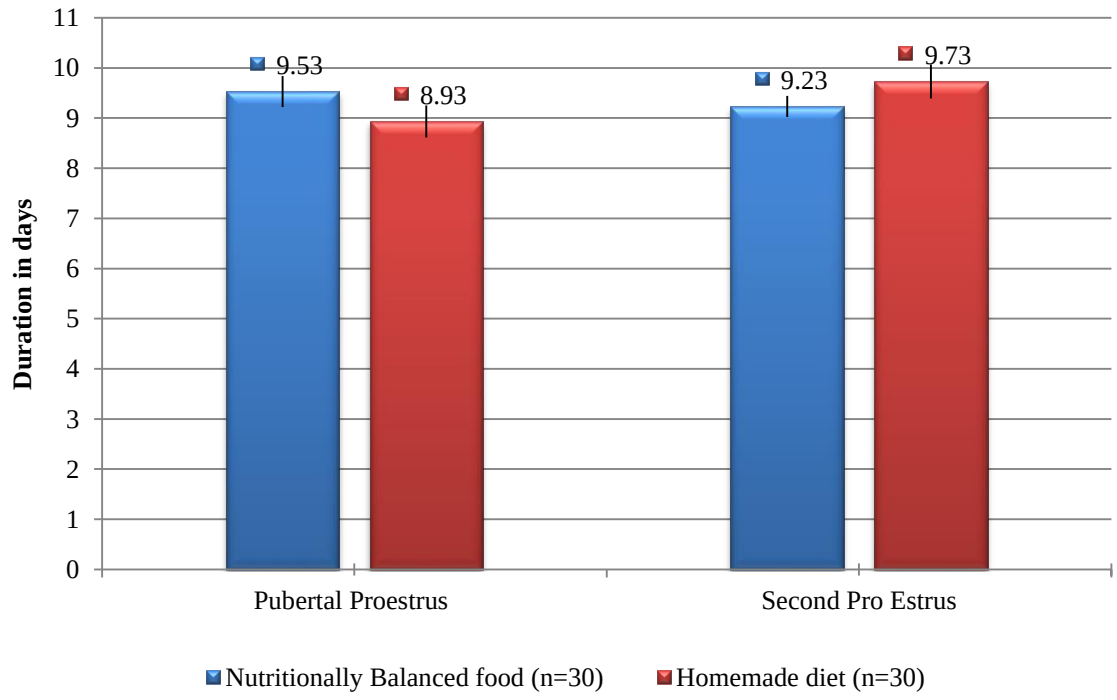


Table 5a. Duration of pubertal estrus and second estrus (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding and management	Pubertal Estrus (Days)		Second Estrus (Days)	
		Mean ± SE (Days)	Range (Days)	Mean ± SE (Days)	Range (Days)
1	Nutritionally Balanced food (n=30)	7.83 ± 0.31a	6 to 10	7.40 ± 0.57a	5 to 9
2	Homemade diet (n=30)	8.23 ± 0.32a	07 to 11	8.27 ± 0.46a	6 to 10
	Overall (N=60)	8.03 ± 0.31	06 to 11	7.84 ± 0.51	5 to 10

*Mean values bearing similar superscripts along the column are not significantly different (p>0.05)

Fig. 21a. Duration of pubertal estrus and second estrus (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

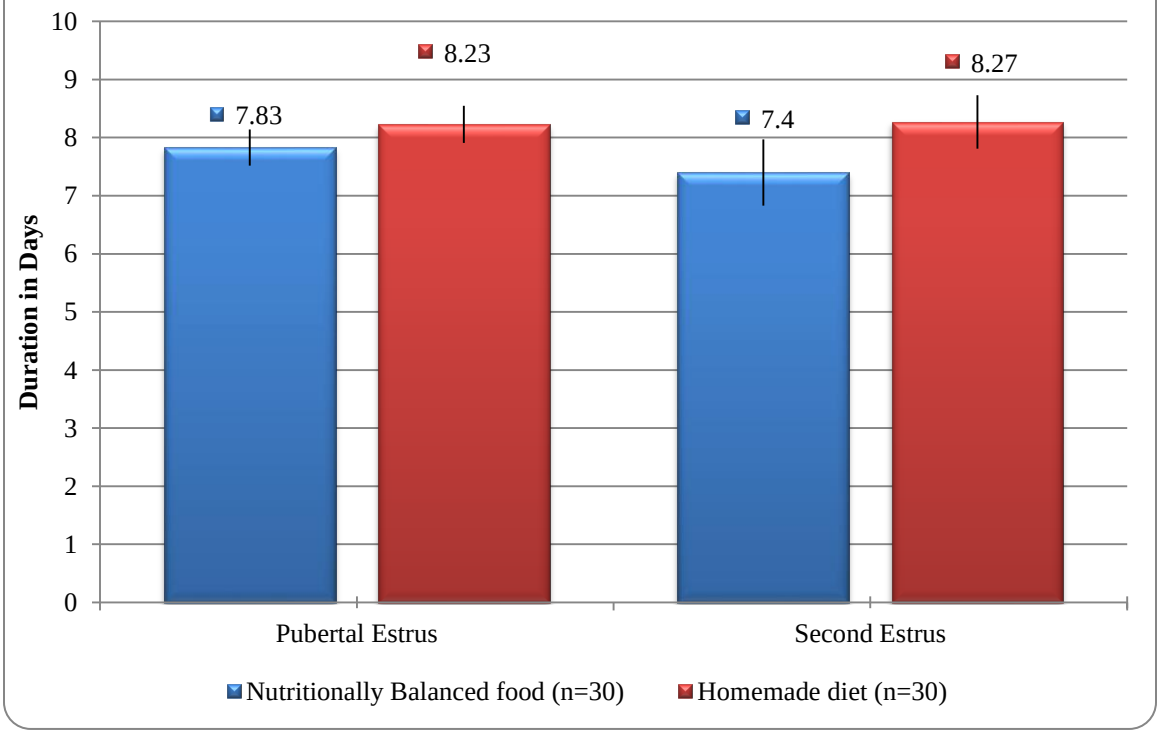


Table 6. Interval between pubertal estrus and subsequent estrus period (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	System of feeding	Inter estrus interval period (Days)	
		Mean± SE (Days)	Range (Days)
1	Nutritionally Balanced food (n=30)	275.67 ± 10.65a	193 to 401
2	Homemade diet (n=30)	306.03 ± 7.14b	222 to 423
	Overall (N=60)	290.85± 9.23	193 to 423

*The mean values with different superscripts are significantly different (p<0.05)

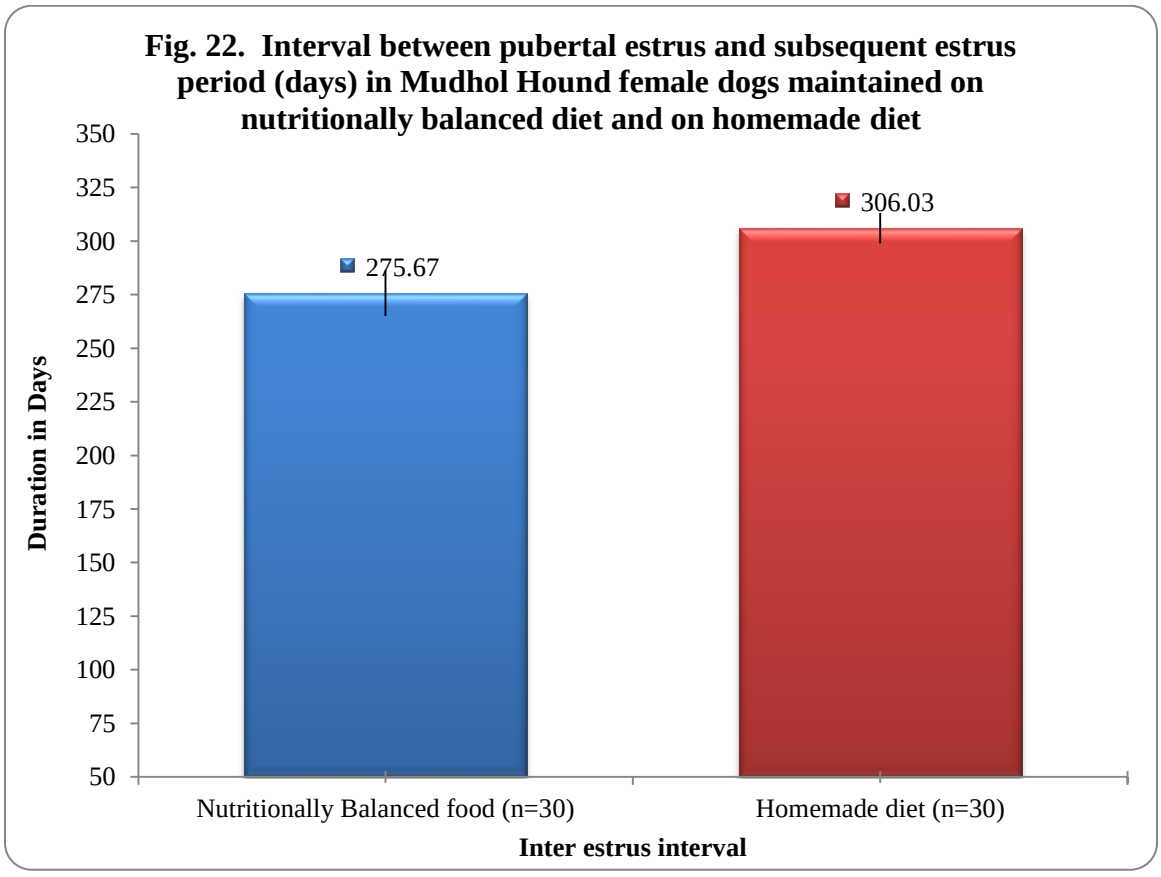


Table 7. Conception rate in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	Particulars	Conception Rate (Per centage)		
		Total no of animals mated	Number of animals conceived	Conception rate (Per cent)
1	Nutritionally Balanced food (n=30)	30	19	63.34
2	Homemade diet (n=30)	30	15	50.00
	Overall (N=60)	60	34	56.67

Fig. 23. Conception rate in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

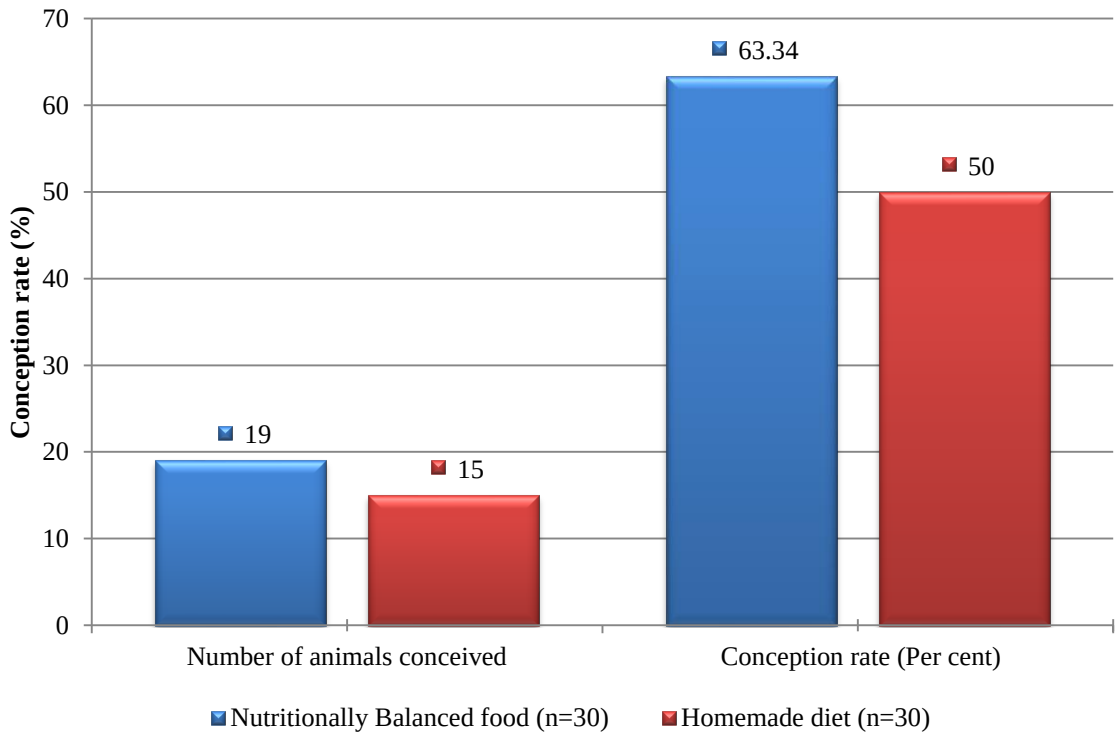


Table 8. Duration of Gestation length determined as interval from day of 1st mating to day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	Mean duration of pregnancy (Days)	Range (Days)
1	Nutritionally Balanced food (n=19)	67.86 ± 0.80a	62 to 70
2	Homemade diet (n=15)	67.45 ± 0.42a	61 to 71
	Overall (N=34)	67.77± 0.35	61to 71

*Mean duration of pregnancy did not differ significantly between dogs maintained on two different systems of feeding (p>0.05)

Fig. 24. Duration of Gestation length determined as interval from day of 1st mating to day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

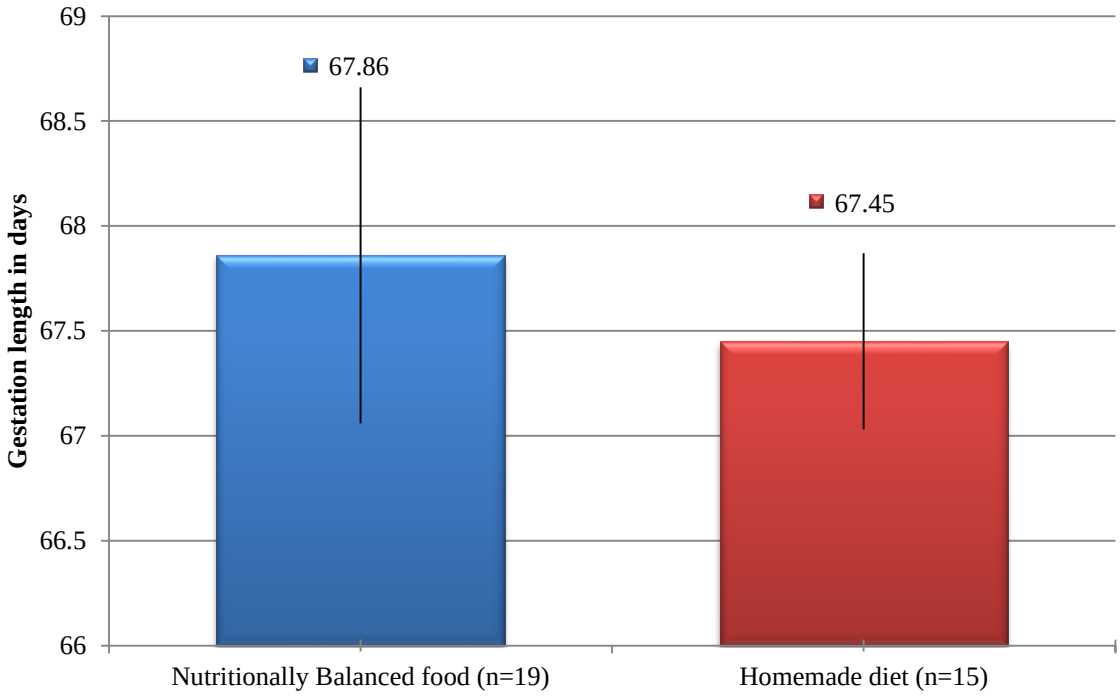


Table 9. Duration of Gestation length determined as interval from day on which the serum progesterone concentration was 3-4 ng/ml and the day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	System of feeding	Mean duration of pregnancy (Days)	Range (Days)
1	Nutritionally Balanced food (n=19)	63.85 ± 0.18a	63 to 66
2	Homemade diet (n=15)	64.35 ± 0.28a	62 to 67
	Overall (N=34)	63.60 ± 0.23	61to 71

*Mean values with similar superscript are not significantly different (p>0.05)

Fig. 25. Duration of Gestation length determined as interval from day on which the serum progesterone concentration was 3-4 ng/ml and the day of delivery in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

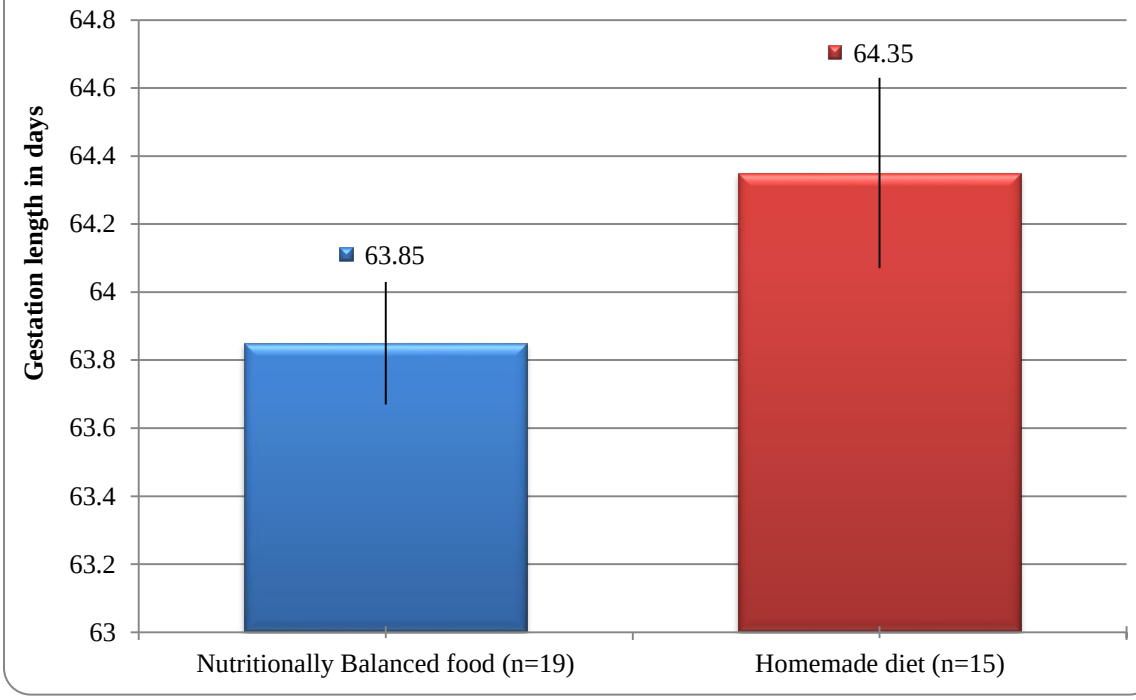


Table 10. Incidence of pseudo pregnancy in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	Number of dogs exhibiting signs of Pseudo pregnancy found	Percentage of incidence
1	Nutritionally Balanced food (n=11)	05	45.46
2	Homemade diet (n=15)	07	46.67
	Overall (N=34)	12	46.15

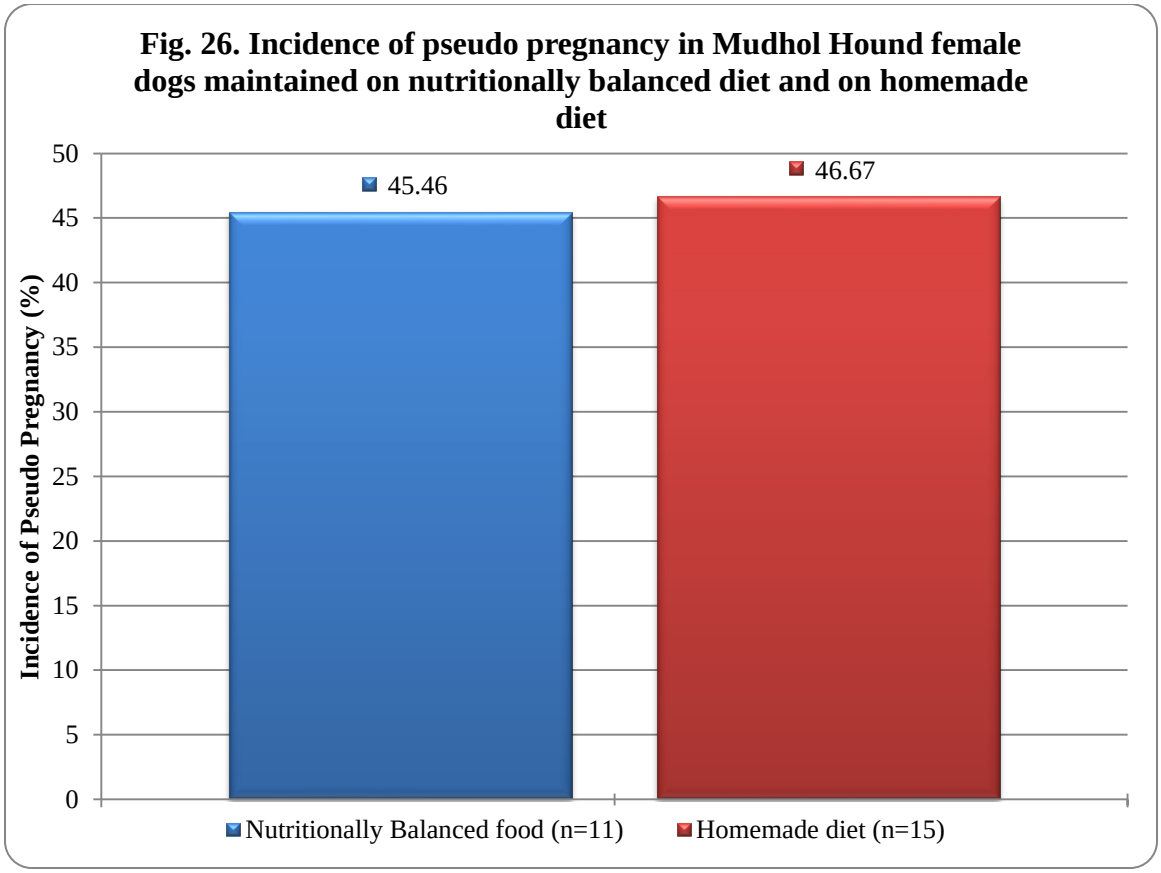


Table 11. Litter size in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	Litter size (in Nos)	
		Mean ± SE	Range
1	Nutritionally Balanced food (n=19)	7.72 ± 0.28a	4-10
2	Homemade diet (n=15)	5.76± 0.31b	1-11
	Overall (N=34)	6.74 ± 0.23	1-11

*Mean value of litter size in Mudhol dogs maintained on nutritionally balanced diet and on homemade diet are significantly different ($p<0.05$)

Fig. 27. Litter size in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

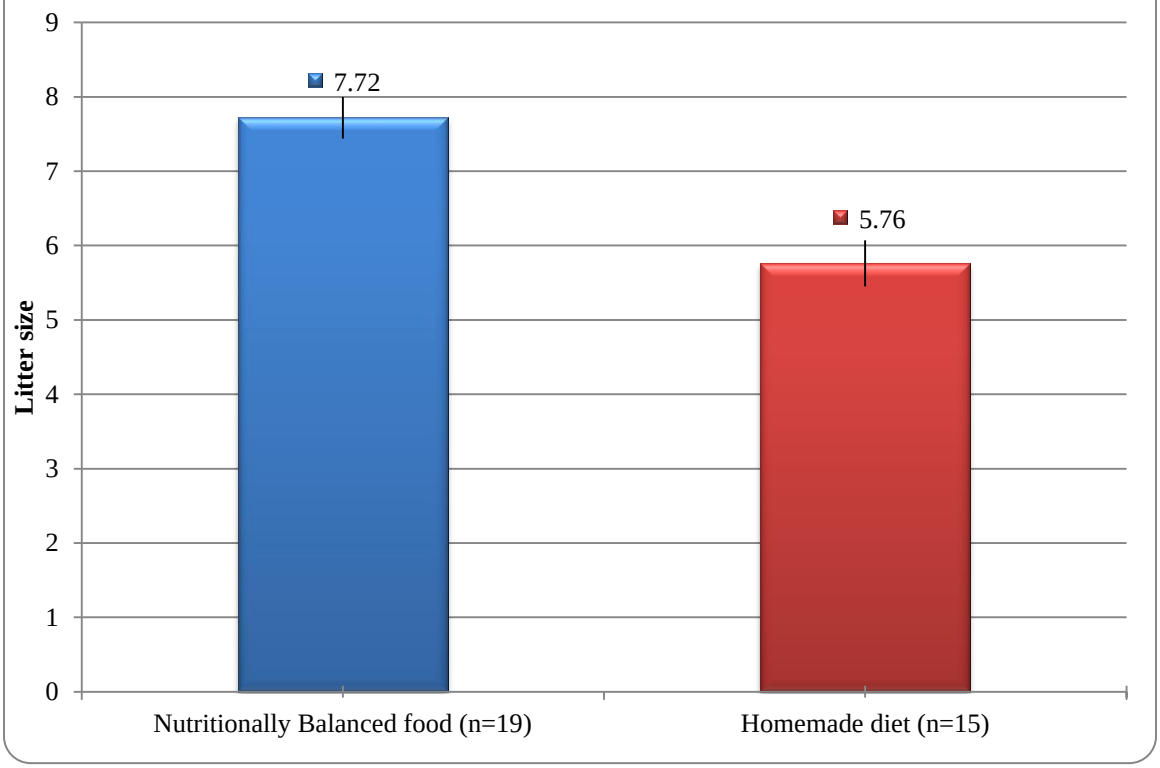


Table 12. Sex ratio of puppies Born to Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	Sex ratio of male to female puppies
1	Nutritionally Balanced food (n=19)	1:1.02a
2	Homemade diet (n=15)	1: 1.14a
	Overall (N=34)	1: 1.08

*****Values with similar superscript are not significantly different (p>0.05)

Fig. 28. Sex ratio of puppies Born to Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

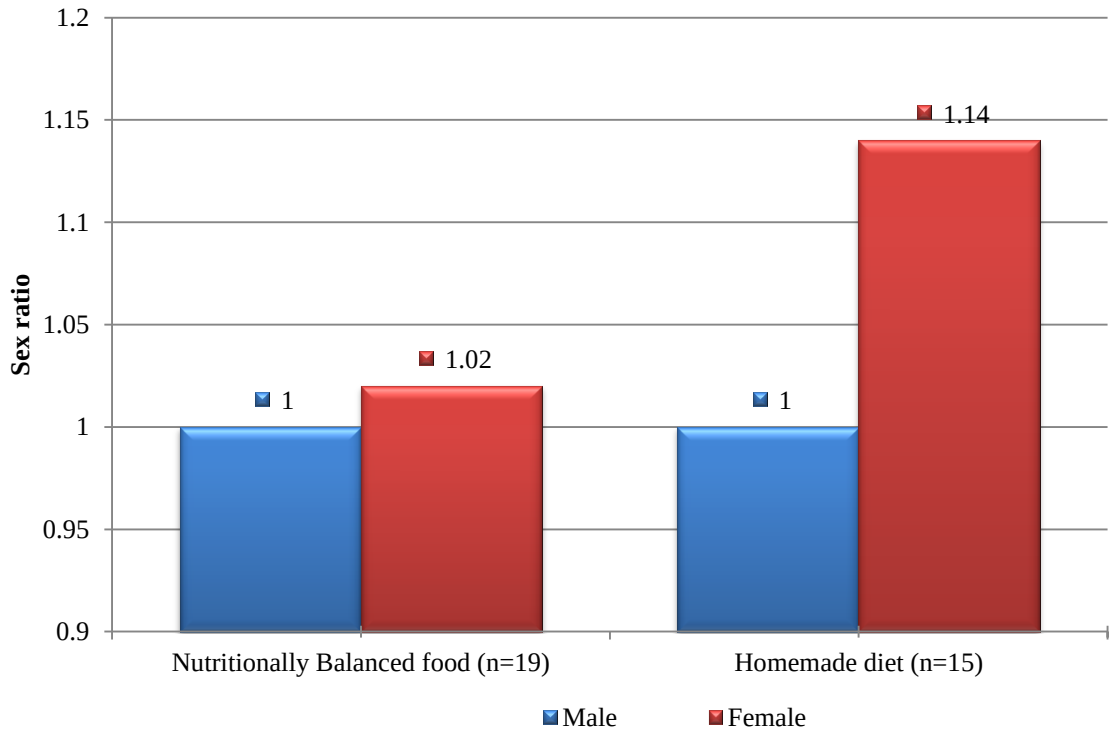


Table 13. Influence of age of dam on litter size of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	Systems of feeding	Litter size		
		Less than 2 years of age	2 to 4 years of age	More than 4 years of age
1	Nutritionally Balanced food (n=30)	7.23 ± 0.32a	7.89 ± 0.28a	7.70 ± 0.43a
2	Homemade diet (n=30)	5.22 ± 0.42a	5.98 ± 0.36a	5.67 ± 0.28a
	Overall (N=60)	5.63 ± 0.34	6.94 ± 0.32	6.69 ± 0.38

***Values with similar superscript along the column are not significantly different (p>0.05)

Fig. 29. Influence of age of dam on litter size of Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

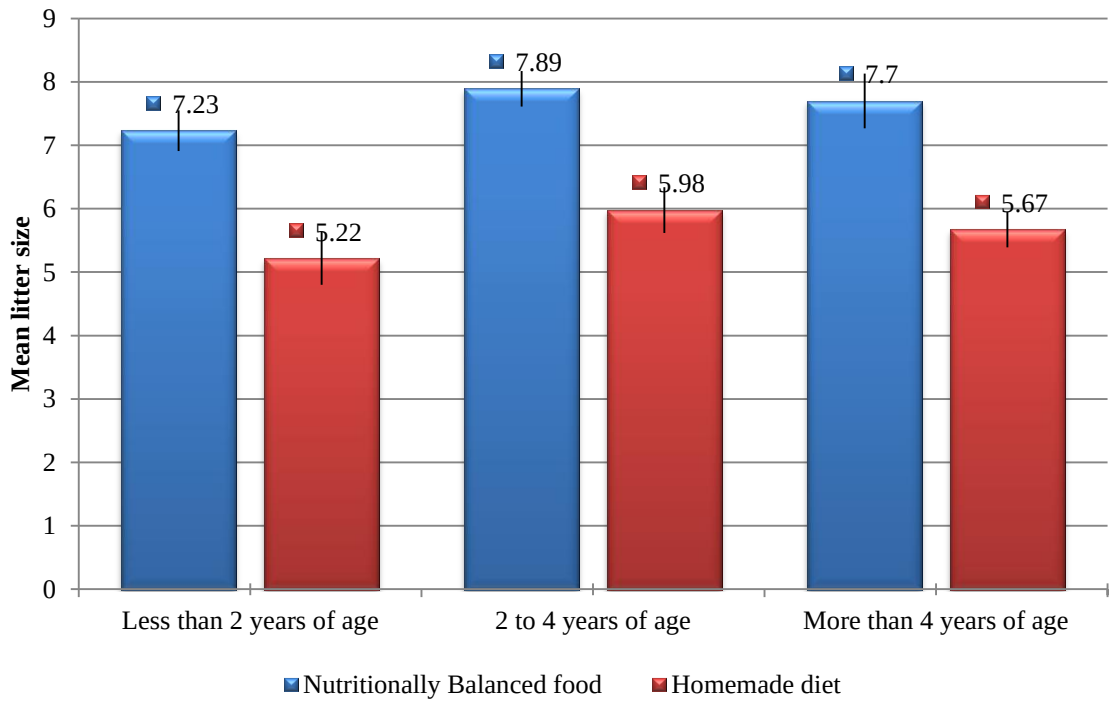


Table 14. Influence of parity on litter size of Mudhol Hound female dogs

Sl. No	Particulars	parity		
		Ist Parity	**2-4 parity**	**>4 parity**
1	Litter size	6.63 ± 0.34^{a}	6.94 ± 0.32^{a}	6.69 ± 0.38^{b}

*Mean value of litter size in Mudhol hound dogs at different parity are significantly different ($p<0.05$)

Fig. 30. Influence of parity on litter size of Mudhol Hound female dogs

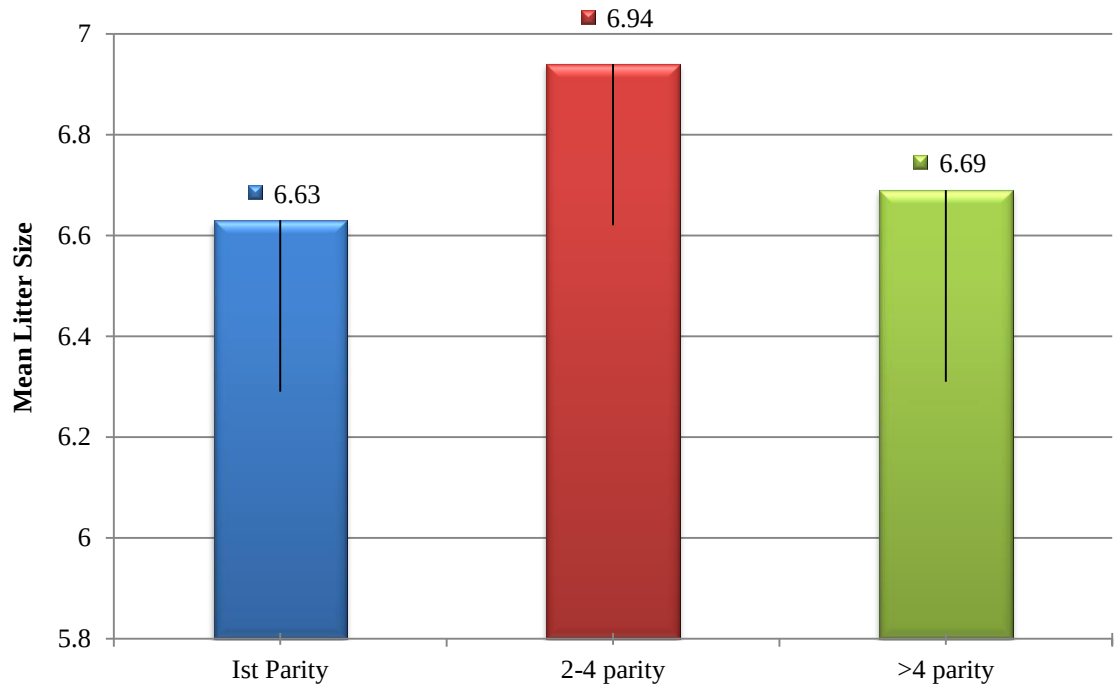


Table 15. Influence of litter size on duration of pregnancy in Mudhol Hound female dogs

Sl. No	Gestation Length (days)	No of Whelping	Litter size
1	61-62 & less	21	8.86
2	63-64	17	7.23
3	65-66	12	6.25
4	67-68	13	4.75
5	69-70 & More	9	1.56

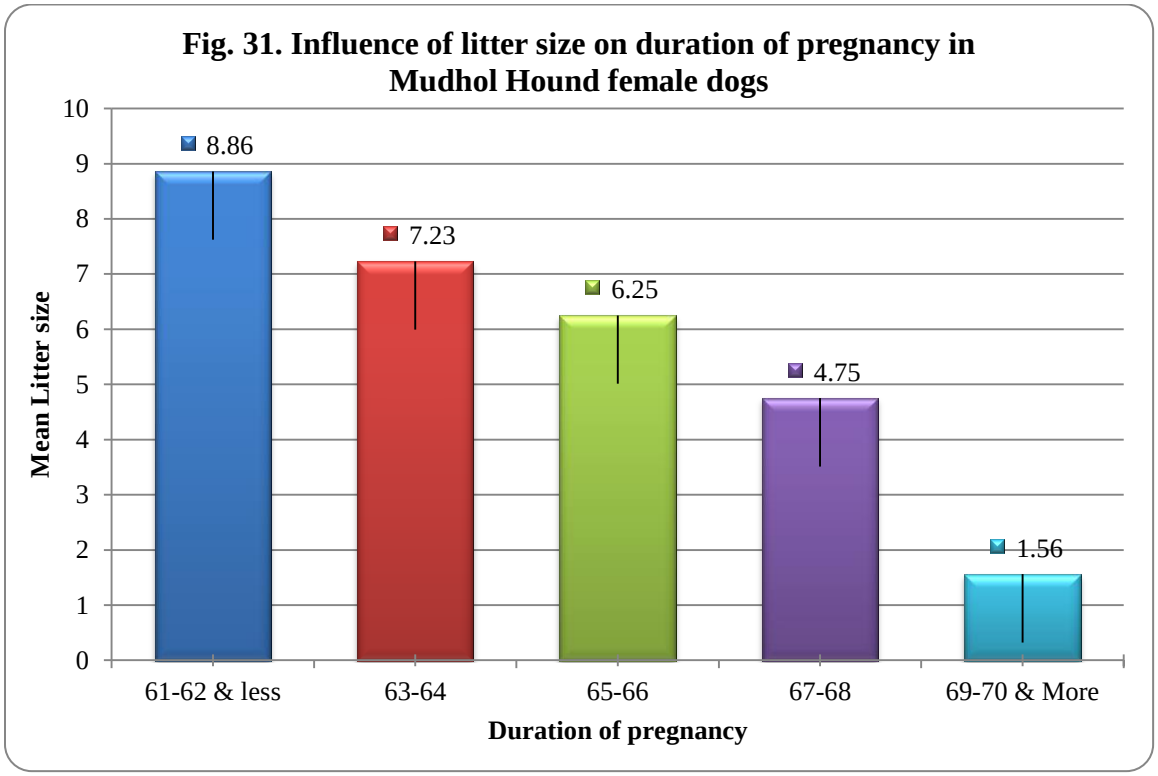


Table 16. Frequency of complicated birth in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of Feeding	Total no of whelpings	Number of complicated births	Per cent
1	Nutritionally Balanced food (n=19)	19	0.00	0.00
2	Homemade diet (n=15)	15	2.0	13.34

Table 17. Gestational and Post parturient complications in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of Feeding	Total no of whelping	Number of dogs exhibiting gestational or parturient complications	Per cent
1	Nutritionally Balanced food (n= 19)	19	nil	0.00
2	Homemade diet (n=15)	15	1.0	6.67

Table 18. Prevalence of neonatal mortality of puppies born in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of Feeding	Total no of puppies born	Number of puppies which died before weaning	Percentage of Neonatal mortality
1	Nutritionally Balanced food	158	11	7.24 a
2	Homemade diet	114	18	15.79b
	Overall	272	29	10.67

*values with similar super script are not significantly different

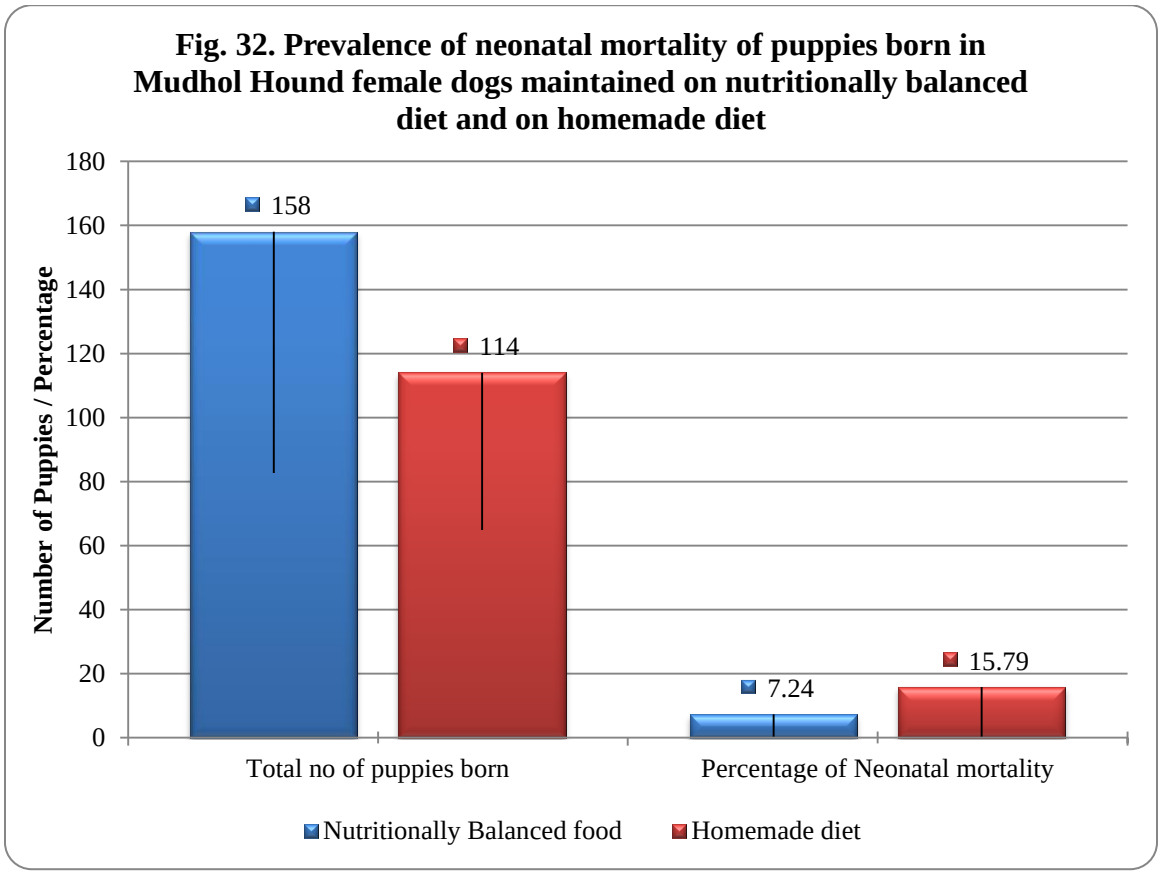


Table 19. Haemoglobin concentration (gm %) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	No of dogs	Mean Hemoglobin concentration on first day of proestrus (gm/%)	Range (gm/dl)
1	Nutritionally Balanced food	30	13 .65 ± 2.84a	11.50 to 15.40
2	Homemade diet	30	12.65 ± 1.18a	10.99 to 14.63.
	overall	60	13.15 ± 2.02	10.99 to 15.40

*mean values with similar superscripts are not significantly different ($p<0.05$)

Fig. 33. Haemoglobin concentration (g %) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

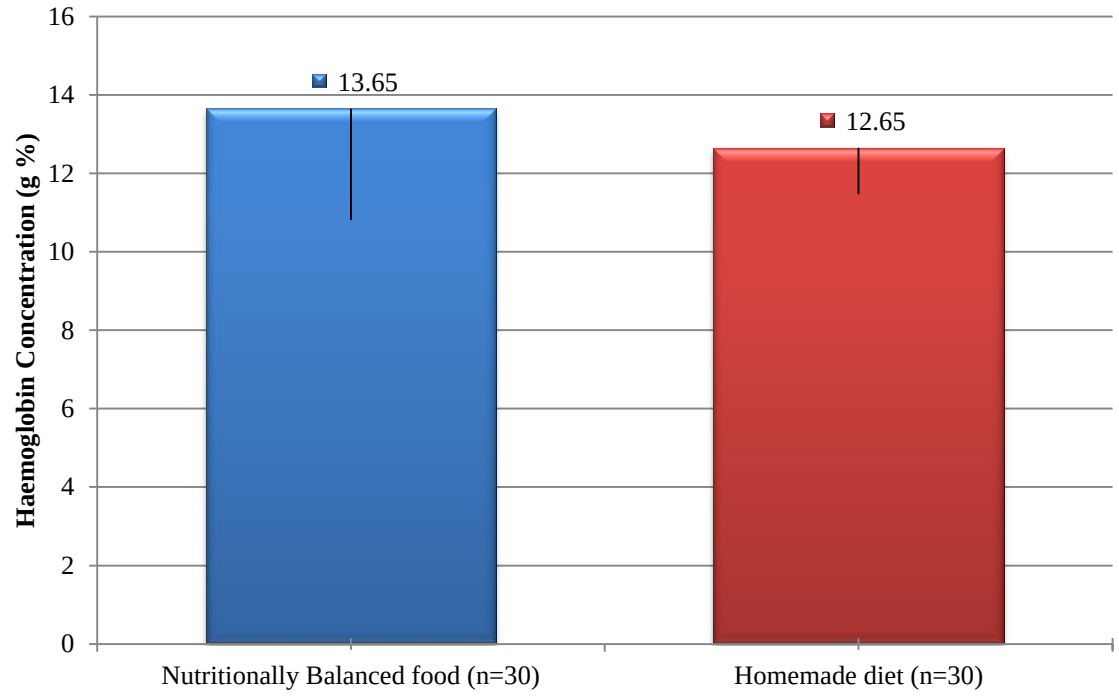


Table 20. Random Blood Glucose concentration (mg/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No	No of dogs	System of feeding	Mean Glucose concentration on first day of proestrus (mg/dl)	Range (mg/dl)
1	30	Nutritionally Balanced food	82.00 ± 1.05a	82 to 139
2	30	Homemade diet	80.39 ± 0.94a	80 to 132
		Overall	81.16 ± 0.98	80 to 139

***** mean values with similar superscripts are not significantly different ($p<0.05$)

Fig. 34. Random Blood Glucose concentration (mg/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

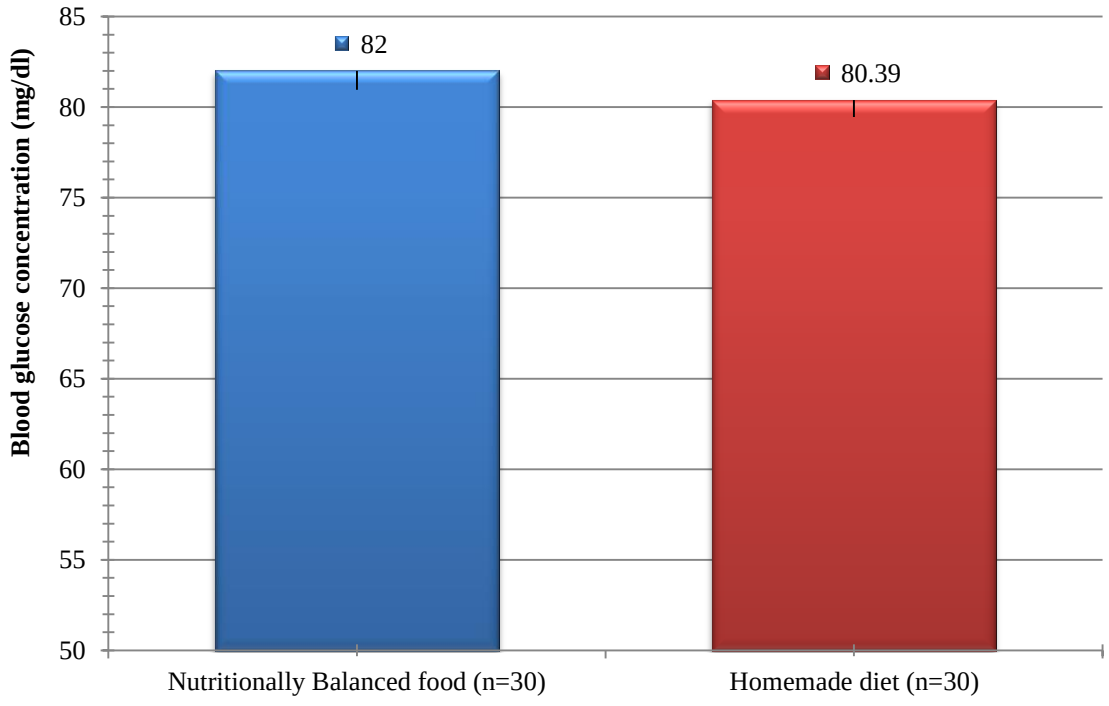


Table 21. Serum total protein concentration (g/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	System of feeding	Mean total protein concentration on first day of proestrus (g/dl)	No of dogs	Range (g/dl)
1	Nutritionally Balanced food	8.85 ± 0.15a	30	8.28 to 9.85
2	Homemade diet	8.36 ±0.12a	30	7.89 to 9.24
	Overall	8.60 ± 0.13	60	7.89 to 9.85

*mean values with similar superscripts are not significantly different ($p<0.05$)

Fig. 35. Serum total protein concentration (g/dl) at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

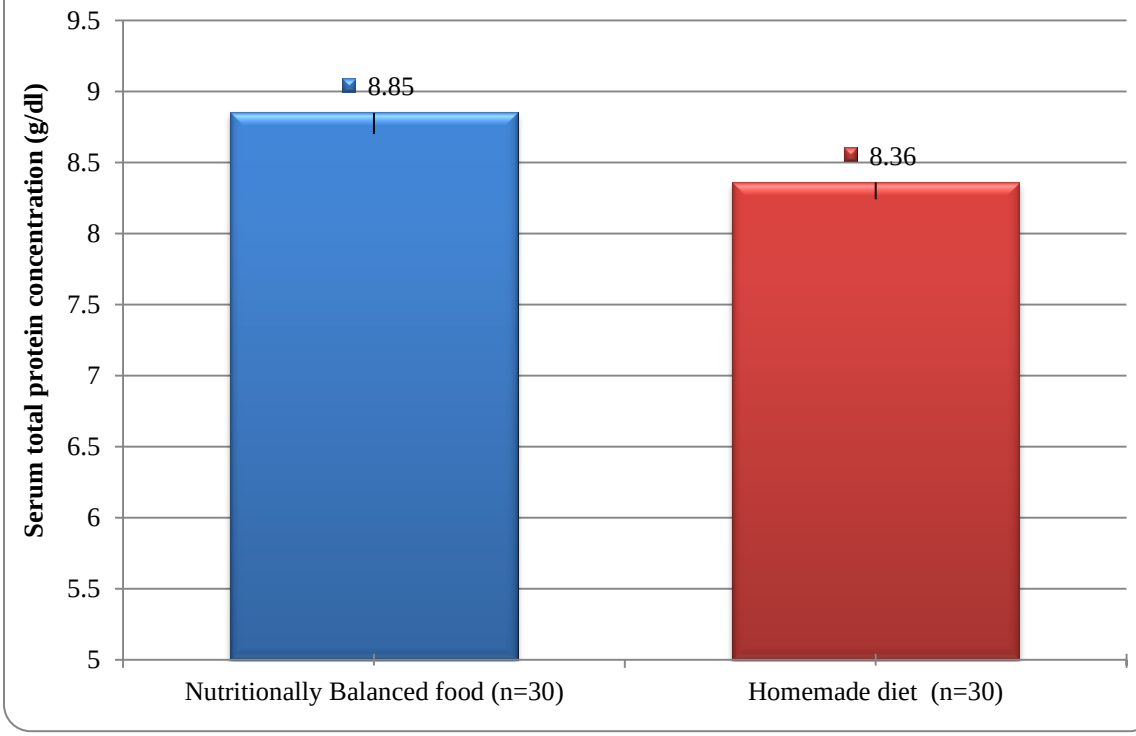
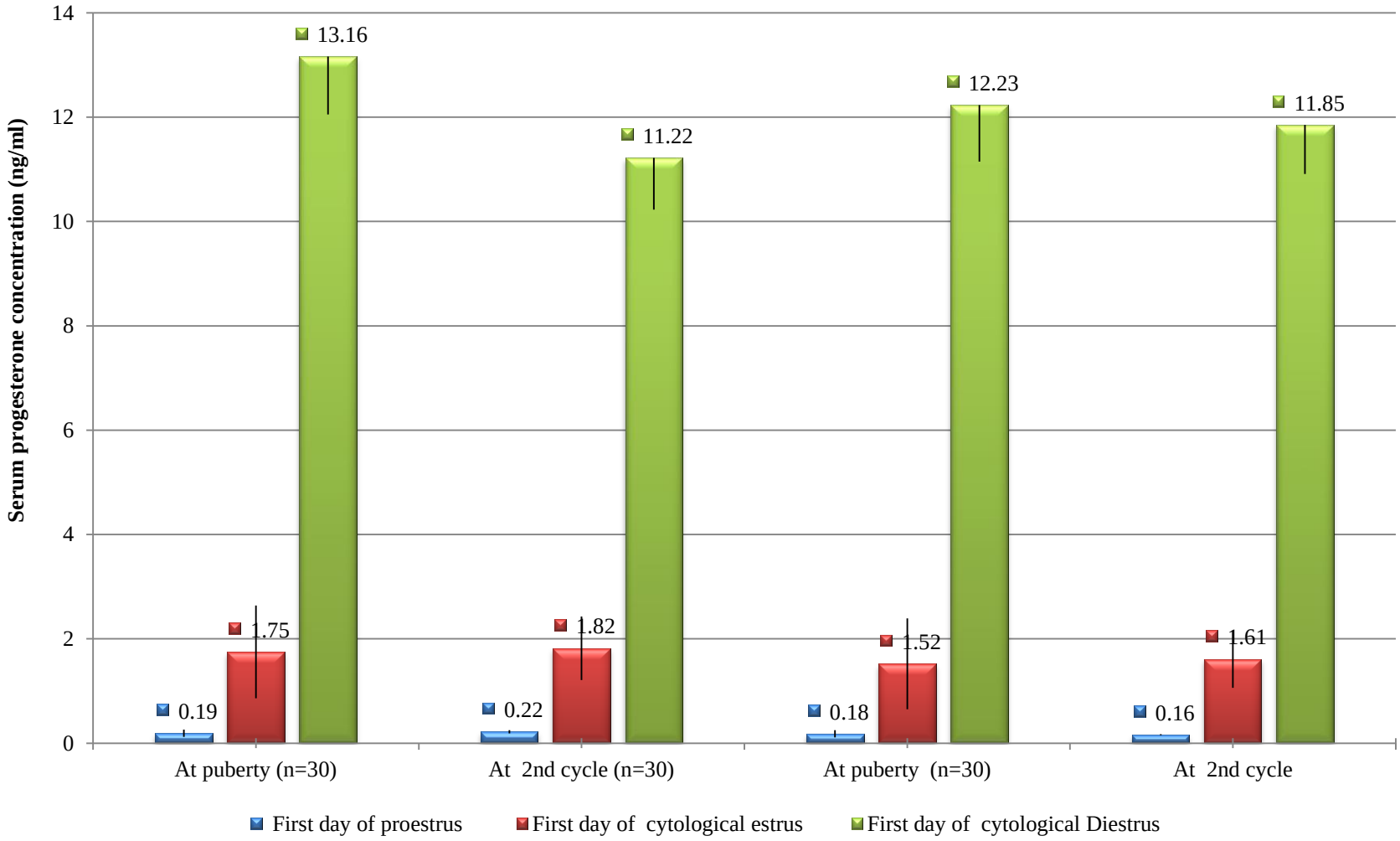


Table 22. Serum Progesterone profile (ng/ml) during proestrus and estrus in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

Sl. No.	Stages of estrus cycle	Nutritionally Balanced food		Homemade diet		Overall (N=60) Mean ± SE (ng/ml)
		At puberty (n=30) Mean ± SE (ng/ml)	At 2nd cycle (n=30) Mean ± SE (ng/ml)	At puberty (n=30) Mean ± SE (ng/ml)	At 2nd cycle (n=30) Mean ± SE (ng/ml)	
1	First day of proestrus	0.19 ± 0.07a	0.22 ±0.03a	0.18 ± 0.07a	0.16 ± 0.01a	0.20 ±0.04
2	First day of cytological estrus	1.75± 0.89a	1.82 ± 0.61a	1.52 ± 0.87a	1.61 ± 0.55a	1.68 ±0.52
3	First day of cytological Diestrus	13.16 ± 1.11a	11.22±0.99a	12.23 ±1. 08a	11.85 ±0.94a	12.12 ± 1.02

*Mean Values with similar superscripts do not differ significantly (p>0.05)

Fig. 36. Serum Progesterone profile (ng/ml) during proestrus and estrus in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet



Discussion

V. DISCUSSION

The Mudhol Hound dog is the only breed from Karnataka recognized so for by Kennel Club of India. Even though, it is known as a hunting dog, it is now also used as companion and guard dogs. Its morphological features have been well established; however, its normal reproductive parameters are not known. This knowledge is essential to achieve a proper reproductive management. The present study is perhaps first comprehensive study on establishing the normal reproductive parameters for any Indian breeds of dogs.

5.1 Birth weight of the new born pups in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, irrespective of the sex of the new born, the mean birth weight of 158 Mudhol Hound puppies was recorded as 458 ± 0.18 g. The adult body weight of Mudhol Hound breed of dog is around 25 kgs and breeds between 10 to 25 kg of adult body weight are generally categorized as medium sized dog (Bhagirathi, 2008). Groppetti *et al.* (2015) reported that the birth weight in dogs was related to the maternal size and the medium size bitches are bitches more than 29 kg in body weight and whelped pups heavier than 400 grams.

Besides, the size of the breed, the birth weight of new born is also reported to be influenced by variety of factors such as genetics, environment, nutrition and fetal uterine position (Bautista *et al.,* 2015). Neilen *et al.* (2001) reported a definite role of genetics influencing the birth weight of Boxer puppies. Groppetti *et a.* (2015) also found that

birth weight was related to maternal size, weight and age as well as breed and litter size with heavier puppies in small rather than larger litter from medium sized breeds

The present study, also found that the Mudhol Hound male puppies weighed significantly heavier than the female puppies, irrespective of the system of feeding on which the dam was being raised (Table 1, Fig.17). On an average, the male were about 35 to 40 g heavier than female puppies. Similarly, Pascal and Gaspar (2015) reported in Romanian Bucovina shepherd dog the male puppies weighed about 52 g more than new born female puppies. Biglardi *et al.* (2013) also found that male boxer puppies weighed slightly heavier than female puppies and the differences were significant. The heavier size of male offspring as compared to female offspring has also been described (Gianola and Tyler, 1974; Johnson and Berger, 2003) in cattle. The heavier birth weight of male offspring is probably due to a heavier muscular and skeletal mass which could be influenced by male hormone.

The present study also revealed that the plane of nutrition status of mother had a significant influence on body weight of new born, with pups of dams maintained on nutritionally balanced diet weighing about 35-40 grams more than the pups from dam maintained on homemade diet.

The effect of plane of nutrition of body weight of new born in bitch appears to have received little attention. However in woman, fetal growth retardation has been documented due to insufficient concentration of nutrition in the mothers' blood stream (Howie, 1982). In pigs, the birth weight of piglets has been related to the size of placenta with heavier piglet associated with a heavier placenta, to date, the relationship between

size and weight of placenta with birth weight of neonates has not been studied in bitches and it is possible the better plane of nutrition may be associated with a heavier placenta and therefore higher body weight in dogs recorded in the study. Gropetti *et al.* (2015) also opined their study could not exclude that the nutritional status of dam may affect the body weight of pups.

5.2 Age and body weight at puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, Mudhol Hound female dogs achieved puberty at an average age of 13.86 ± 1.12 months and in individual bitches, it ranged from 11 to 17 months (Table 3). It has been stated that, individuals of small breed enter puberty earlier than large breed, with onset of cycle correlating with time the animal reaches growth plateu (Sokolowaski *et al.*, 1977). Dewey and Bhagat (2002) also stated that in domestic dogs, sexual maturity begins about 6 to 12 months of age for both male and female dogs. Johnston *et al*. (2001) have stated that the puberty begins between 6 to 10 months age. For bitches of smaller breeds but may not begin for bitches of some larger breeds until they are nearly 24 months old (Nelson and Couto, 2003). The onset of puberty for some selected breeds was presented as Afghan Hound (7-30 months), Borzoi (15- 18 months), Golden Retriever (9 - 11 months) and Grey Hound (11-30 Months) Clark and Strainer, 1983 and there was strong correlation between age of onset of puberty and breed and also size of bitch.

In the study, mean age of puberty in Mudhol Hound female dogs was very much comparable to range in the onset of puberty of dogs. Similarly, body size reported by Clark and Stainer (1983).

In the present study, it was also observed that the system of feeding had a significant influence on the age of onset of puberty in Mudhol Hound female dogs. Mudhol Hound female dogs reached puberty at an average of 4 months earlier than when maintained on homemade diet (Table 3). This observation suggests that besides, genetic factor, nutrition, particularly energy and protein levels in the feed may have a significant impact on the onset of puberty in dogs. Nelson and Couto (2003) have similarly opined that the age of puberty in dogs could be influenced by the level of nutrition.

In the present study, Mudhol Hound female dogs reached puberty at similar body weight irrespective of system of feeding on which they were being maintained (Table 3). The mean body weight of 30 Mudhol Hound female dogs maintained on a nutritionally balanced food was recorded 24.32 ± 0.28 kgs and did not differ significantly from the mean body weight (23.07 ± 0.17 kg) recorded in another group of 30 Mudhol Hound female dogs maintained on the homemade diet. However, the age at which the animals attained the body weight 23-24 kg varied considerably in individual animals. This observation suggest that Mudhol Hound breed of dogs attain puberty when they are around 23-24 kgs and dogs maintained on nutritionally balanced food have better growth rate and gain in body weight and therefore, reached puberty at an earlier age.

The results of the present study are in agreement with findings of Caffaratti *et al.,* (2013) and Concannon (2011) who mentioned a positive correlation between the onset of

puberty and size and weight. However, in one study (Wildt *et al.,* 1981) there were no apparent relationship between weight and age at onset of first proestrus among Labrador Retriver and Beagles and the onset of first puberty appeared quite variable within several small and large breeds of dog. It has also seems to be that size and weight it occurs when the bitch has reached 85% of adult body weight (Concannon, 2011). In view of observation made in the present study, the weight rather than age of animal appears to have definite influence on the onset of puberty in dogs.

5.3 Influence of month of the year on the onset of puberty in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, 50.00 per cent of female dogs were found to achieve puberty between June and August and fairly high number (28.34%) dogs also achieved puberty during months of January and February. These months corresponded to the rainy season and spring season, respectively. Very few number of animals achieved puberty during hotter month of the year and colder month of the year (September, October, November and December). These observations seem to be suggestive of existence of seasonal influence on the onset of puberty in dogs.

The seasonal influence on reproduction in many species, high ambient temperature is reported to cause infertility (Chatdarong *et al.,* 2007) by compromising ovarian function (Quesnel *et al.,* 1998), estrus and ovulation (Kunavongkrit and Tantasuparuk, 1995), embryonic implantation, seminal and sperm production (Suriyasomboon *et al.,* 2005) in pigs and similarly adverse consequences in cows (Zeron *et al.,* 2001). The adverse effects of estrus in bitch reproduction have not been

documented sufficiently. Although the bitches are considered non seasonal or mono estrus breeders showing estrus, mating and whelping throughout the year (Gipson, 1996), there are several aspects of seasonal effects on estrus in bitches. The season affects estrous cycle and frequency of prevalence of estrus (Mutembei *et al.,* 2000; Wikstrom and Linde, 2006). In bitches for instances the highest number of estrous cycles were reported in November in Kenya (Mutembei *et al.,* 2000), between February and May in United Kingdom and March in Swedan (Polat *et al.* 2015). These were also attributed to variation of day light or photo period as shown in the tropical and polar region (Gavrilovic *et al.,* 2008 and Chatdarong *et al.* (2007). Studies reporting a lack of seasonal effects on estrus in bitches (Bouchard *et al.,* 1991) especially those living in temperade climate (Concannon, 1993) are also available. Ortega *et al.,* 2007 and Polat *et al.*, 2015 reported that the estrous cycle could be observed in each month of the year. The results of the present study on the other hand seems to suggest that the month of the year or season of the year has a definite influence on the frequency of estrous cycle observed in dogs. However, its frequency was almost similar between two systems of feeding.

5.4 Duration of Pro-estrus and estrus in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In dogs proestrus has been defined as that stage of reproductive cycle prior to estrus when bitch attract the male, but not responsive to the mating (Olson and Nett, 1986). Cytological proestrus has been defined as period between first day of onset of pro-estrual bleeding to the day on which 100% cornification is seen on study of vaginal exfoliative cytology (Suyash Vardan Bhal, 2002). The second criterion was also used in the present study to define the duration of proestrus. In sixty dogs, irrespective of system

of feeding on which Mudhol Hound female dogs were maintained, the average duration pubertal proestrus recorded as 9.22 ± 0.79 days and individual animal it ranged between 7 to 13 days. Further, the duration of proestrus did not differ between groups of dogs maintained on either nutritionally balanced diet or homemade diet.

The average duration of proestrus recorded in the present study is in agreement with duration of proestrus reported (Suyash Vardan Bhal, 2002). The average duration pubertal proestrus is also stated to be 9 days, ranging from 0 to 27 days (Christe and Bell, 1971a; Alien and England, 1990). However, Chakrabarthy *et al. (*1980) reported range of proestrus to be 0 to 17 days. Further one study (Chakrabarthy *et al.,* 1980) also suggested that the average length of proestrus shorter for pubertal than mature bitches, but subsequent investigation (Wildt *et al.,* 1981) failed to substantiate this finding.

The present study revealed that duration of proestrus in Mudhol Hound female dogs similar to those observed in other breeds of dogs. Although significant individual variations did occur regarding duration of proestrus, it was also interesting to note that the duration of cytological proestrus was not influenced by the system of feeding. Comparable studies on the effect of plane of nutrition on duration of proestrus in dog are not available.

In the present study duration of estrus was defined as interval from first day of which 100% cornification was seen on study of vaginal exfoliative cytology to the first day of diestrus. The mean duration of estrus was recorded as 7.88 ± 0.49 days and the system of feeding did not seem to influence on the duration of estrus. The period of estrus is reported to normally lose for about 10 days but, can range from 3 to 21 days (Olson

and Nett, 1986). Bell and Christie, (1971) also reported that the average duration of estrus, based on behavioural signs is 9 days, ranging from 4 to 24 days. The mean duration of pubertal estrus was recorded in the present study is within the range of estrus reported in earlier studies.

The duration of proestrus and estrus during subsequent estrus also did not differ significantly when compared to pubertal pro-estrus and estrus (Table 5a). However, it has been stated that the duration of proestrus and estrus may differ among pubertal and mature bitches (Johnston *et al.*, 1980), which could not be confirmed in the present study. Further, pubertal bitches are reported to be less likely than mature bitches to demonstrate estrous behavior even when ovulation occurs (Wildt *et al.*, 1989). Additionally, the duration of proestrus and estrus may be shorter, reduced in inconsistent pattern of circulating progesterone have been reported to occur in some pubertal cycles (Wildt *et al.*,1981; Chakraborty *et al.*, 1980) which again was not observed in the present study. The estrous cycle of pubertal bitch is also reported to differ from those of mature animals in that, pubertal bitches are more likely to manifest a split heat or false heat (Jochle and Andersen, 1977). In the present study, the estrous phase at puberty of all 30 bitches progressed to diestrus as revealed by the vaginal exfoliative cytology. These observations suggest that the duration and characters of pubertal proestrus and estrus of Mudhol Hound bitches is similar to the subsequent proestrus and estrus and that the incidence of split heat either nil or extremely low. The results of the present study also suggest that system of feeding has little influence on duration of pubertal and subsequent pro-estrus and estrus.

5.5 Interval between pubertal estrus and subsequent estrous period (days) in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, the mean inter estrous interval of 60 Mudhol Hound female dogs irrespective of system of feeding was recorded as 292.85 ±7.67 days and the interval between first day of pubertal proestrus and first day of subsequent cycle was significantly shorter (275.67 ±10.65 days) in animals maintained on nutritionally balanced food as compared to inter estrous interval recorded in animals maintained on homemade diet (Table 6).

The dog is usually considered as a monoestrous animal and has two estrous cycles a year although some small breeds may have 3 – 4 and large breeds only one (Roberts, 1971). Anderson *et al.* (1962) reported that in beagles estrus occurred at every 7 months ±2 months and Burns and Frazer (1966) stated that the Grey Hounds and other larger breeds have one estrous period a year. Roberts (1986) also stated that there is evidence that frequency of estrus is genetically conditioned. Burns and Frazer (1966) quoted first in reporting that in nearly 500 bitches, 338 had an inter-estrous interval of 5- 7 months, over 100 had interval of over 7 months and only 16 had an interval of less than 5 months. The results of the present study suggests that the average inter estrous interval in Mudhol Hound female dogs is around 9 months and is slightly larger than reported range of 5 to 7 months. This is probably because Mudhol Hound dogs have been developed for hunting purpose and Hounds in general have longer inter estrous interval.

The present study also found the inter estrus interval could be shortened by at least 30 days when maintained on nutritionally balanced food. This observation suggests a definite influence of nutrition on shortening of anestrous period through its stimulatory effects on Hypothalamo pituitary axis.

5.6 Conception rate in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, exfoliative cytology was used as a technique to determine the optimum time of breeding in Mudhol Hound bitches and each bitch was mated twice, once on day of optimum cornification and second time 48 hours later. The overall conception rate in 60 bitches mated on the basis of vaginal exfoliative cytology was determined as 56.67 % (Table 7). This conception rate is significantly lower than 90% reported for mating in free roaming mongrels (Friedman, 1957). The results of present study are however are close to those (50%) reported by Suyash Vardhan Bhal (2002) who recommended mating on the basis of vaginal exfoliative cytology. The conception rate in present study was significantly higher than 30 % reported by Okken *et al.(* 1985), but significantly lower than 92% reported by England (1992) who considered ferning pattern of vaginal fluid in addition to cornification index to recommend at date of mating.

The poor conception rate observed in the present study is probably due to the fact that the initial days of optimum cornification and onset of LH surge (Christie *et al.,* 1972 and Bouchard *et al.,* 1991).

The present study also noted that the conception rate was at least 13% higher in dogs maintained on nutritionally balanced food in comparison to the dogs maintained on

homemade diet. Studies on the effect of nutrition on conception rate of dogs are extremely scanty and results of present study strongly suggests that maintaining dog on nutritionally balanced food may provide a better uterine environment for establishment of pregnancy.

5.7 Duration of gestation length in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet - determined as interval from day of first mating to the day of delivery

The duration of gestation is defined as interval from first day of mating to the day of parturition in 60 female Mudhol Hound dogs, it ranged between 61 to 71 days and the mean duration of gestation was recorded as 67.66 ± 0.35 days (Table 8).

Using the day of mating as a reference point, parturition can occur as early as 56 days later and as later 68 days (Concannon, 1980). Similarly a large variation in apparent gestation length has been encountered when counting from first of multiple mating or last of multiple mating. The extremes encountered differing by two weeks for instances, if bitch is held for an aggressive stud dog and forced to mate starting 3-5 days LH surge. The interval from first mating to whelping may be as long as 69-70 days. In rare instances where a bitch is still fertile 9-10 days after LH surge and is bred then, the interval from mating to whelping can be as short as 55-56 days (Concannon, 2000). However, full term gestation calculated from the day of insemination is reported to range from 57 – 72 days (Concannon, 1983). The difference between the measurements was attributed to potential size, days of viability of sperm in female reproductive tract and long period of receptivity in bitch (Holst and Phemister, 1974; Concannon, 1983).

In the present study, also there was a considerable variability in gestation period of individual bitches when calculated as interval from first day of mating to the day of parturition. This variable may reflect the fact that in some bitches the mating was carried out well before LH surge and in few as well after LH surge, nevertheless system of feeding did not alter the variability observed in the duration of gestation length.

5.8 Duration of gestation length in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet - determined as interval from day on which the serum progesterone concentration was 3-4 ng/ ml and the day of delivery

In the present study when 60 bitches were mated on the basis of serum progesterone concentration, parturition occurred between 63-67 days and mean duration of pregnancy was determined as 63.89 ± 0.04 days (Table 9). Further, the duration of pregnancy was similar between dogs maintained on two different systems of feeding. It is stated that most accurate method determining duration of pregnancy in the interval from LH surge to the day of parturition (Concannon, 1980).

Prediction of the duration of pregnancy on the basis of LH surge is difficult for a practicing clinician as identification of LH surge require frequent sampling of the blood, sophisticated laboratory facilities, besides being cost effective. Therefore, recommendations have been made for identifications of time of LH surge on the basis of changes in the serum concentration of progesterone (P4) during late proestrus and estrus (Concannon *et al.,* 1977a; Concannon *et al.,* 1977b and Nett *et al.,* 1975). Goodman (1998) opined that if accurate serial quantitative serum P4 concentrations are obtained,

the LH surge might be estimated as the day when a distinct rise in levels of serum P4 is seen and that fertile matings should result in whelping 64 to 66 days later. Concannon *et al*. (1983) demonstrated that the changes in the serum P4 concentrations during the later part of proestrus and early estrus characterized by a gradual increase with a sudden sharp rise which coincided with the LH surge. Basal serum P4 levels have been reported to typically range between 0 to 1 ng/ml during an estrus and proestrus (Concannon *et. al.,* 1977a; 1977b). At the time of LH surge, serum P4 concentration rose rapidly (0.8 to 3 ng/ml), continued to rise at ovulation (1.02 to 8.0 ng/ml), and was even higher (4.02 to 20 ng/ml) towards the end of fertile period (Concannon, 1986; Jeffcoate and Lindsey, 1989).

Bouchard *et al*. (1991) stated that ovulation was estimated to occur when serum P4 concentration was 4.90 ±1.00 ng/ml (3.4 to 6.6 ng/ml). Wright (1990) reported that LH surge was located to the period when serum P4 concentrations were between 2 to 4 ng/ml and that ovulation occurred when the serum P4 concentrations was 5.4 ng/ml (range 3 to 8 ng/ml). Renton *et al*. (1991) reported that in bitches, the concentrations of serum P4 reached 3 ng/ml two days before ovulation, and recommended the determination of serum P4 concentration for identifying the time of ovulations. Renton *et al*. (1991) reported that serum P4 concentrations were around 3 ng/ml on the day of LH surge in 12 out of 20 bitches and within 1 day in 7 out of 8 bitches. Ultrasound was less accurate in that, only 4 of the 16 estimates agreed. They concluded that the measurements of serum P4 concentration were a better indicator for ovulation than ultrasonography.

Zoldog *et al.* (1994) reported that ovulation in bitches occurred when serum P4 concentrations reached 5 ng/ml. Barr (1995) reported that in all 14 Beagle bitches

studied, the serum P4 concentration was high, ranging from1.9 to 11.7 ng/ml around the time of ovulation. Johnston and Root (1995) in a retrospective study of 49 bitches reported that serum P4 concentrations began to rise concurrently with LH surge, reaching 1.0 to 1.9 ng/ml on the day of LH surge. The day after LH surge, the serum P4 concentration was 2 to 9 ng/ml. On the day of ovulation, the serum P4 concentration was 4.0 to 10 ng/ml.

Some studies have attempted to determine the factors affecting the variability in the gestational length. The influence of the breed on the length of gestation has been controversial. Okkens *et al.* (1993) reported that breed influenced the duration of gestation in bitches. The most recent study by Okkens *et al*. (2001), had at least 12 bitches representing each breed. They reported that West Highland White Terrier had longer gestation duration than German Shepherd, Labrador Retrievers and Doberman. The same group analyzing atleast 5 bitches from each breed had previously reported that German Shepherd dogs had shorter gestation duration when compared to Boxer, Burmese Mountain dogs, Old English sheep dog and Bouvier-Desflanders (Okkens *et al.*, 1993). Eilts *et al*. (2005) reported that compared to Labrador Retrievers, the German Shepherds, Golden Retrievers and Hounds were more likely to have longer gestation length. However, other investigators have found no breed effect on gestation length (Kutzler *et al.*, 2003).

Litter size is another factor which has been studied for its influence on the length of gestation in bitches. Litter of a single Beagle pup had longer gestation duration in one study (Holst and Phemister, 1974) and litter size was negatively correlated with gestation

length in other studies (Okkens *et al.,* 1993). However, in two other reports (Kutzler *et al.,* 2003) the litter size did not affect the duration of gestation. The effect of age or parity on gestation length has not been studied extensively in the bitches. A single study found primiparous bitches to have similar gestation duration as multiparous bitch (Okkens *et. al.,* 1993). Within breed, age had no effect on litter size (Okkens *et al.*, 2003). Eilts *et al*. (2005) reported that age or parity had no effect on gestation length.

It was not the objective of the present study to investigate the factors influencing the length of gestation in dog. Nevertheless, it appears that the duration of gestation in Mudhol Hound female dogs calculated as interval from initial increase in serum progesterone concentration and the day of parturition, is very close to reported duration of gestation calculated from the time of LH surge. Further, it is very unlikely that factors such as age, parity or litter size and as well as nutrition has any significant influence on gestation length of Mudhol Hound breed of dog.

5.9 Incidence of pseudopregnancy in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study 46.15 % of Mudhol Hound female dogs which did not conceive to the matings exhibited one or more signs of pseudopregnancy between 45 to 90 days post mating and incidence of pseudopregnancy did not appear to be influenced by system of feeding (Table 10)

Pseudopregnancy or pseudocyasis is the physiological condition in which female dog shows similar signs of pregnancy. Although its exact prevalence is not known, it is estimated between 50 – 75 % (Feldman and Nelson, 2007). In Dogo Argentenia bitch, the

prevalence recorded was 30 % (Caffartti *et al.,* 2013). The most frequent sign of pseudopregnancy was exhibited mammary hypertrophy associated with lactation followed by nesting behavior. These signs are also reported to most common signs of pseudo pregnancy exhibited in dogs (Marrow, 1986 and Caffartti *et al.,* 2013) eventhough the occurrence of pseudopregnancy was not so very high, fairly high prevalence mammary enlargement and milk secretion in pseudopregnant bitches should be considered to prevent and treat occasional happening of mastitis.

5.10 Litter size in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In investigations carried out by Okkens *et al.* (1993) on five large breeds, the mean litter size was 6.8 (± 0.4). Similar results were obtained by Feldman and Nelson (2007) who recorded an average of 8 to 10 pups per litter in large breeds. In the study of reproduction parameters in Dogo Argentino (Caffaratti *et al.,* 2013), the average litter size was 8.12 ± 3.44. In medium-sized dog breeds, *e.g*., Nederlandse Kooikerhondje the mean litter size was 5.62 ± 2.08) (Mandigers *et al.*1994) and in the Beagle breed, the average litter size reached 5.7 ± 1.9 (Seki Makato *et.al,* 2010) and 5.5 (Shimatsu *et al.,* 2007). The studies conducted by Borge *et al.* (2011) on 10810 litters of different-size dogs showed an increase in the litter size with the size of the breed; the average number of pups was 3.5 ± 0.04 per litter in miniature breeds (body weight less than 5 kg) and 4.2 ±0.03 in small breeds (body weight 5-10 kg).

Factors influencing litter size are for example season of the year, number of previous litters (Gavrilovic *et al.,* 2008), age of parents (Borge *et al.,* 2011), and breed

(Okkens *et al.,* 2001; Borge *et al.,* 2011). However, results published in various studies are inconsistent (Gavrilovic *et al.,* 2008; Borge *et al.,* 2011), possibly because they are based on reproductive efficiency data sets of bitches pertaining to various breeds (Bouchard *et al.,* 1991; Okkens *et al.,* 2001; Eilts *et al.,* 2005; Chatdarong *et al.,* 2007 and Ortega-Pacheeo *et al.,* 2007 and Borge *et al.,* 2011) and only very few studies deal with reproductive efficiency of only one dog breed, more specifically Drever (Gavrilovic *et al.,* 2008), Irish Hounds (Urfer 2009), German Shepherds (Mutembei *et al.,* 2002), Beagles (Shimatsu *et al.,* 2007), Boxers (Forsberg and Persson 2007), and English bulldog breed (Wydooghe *et al.,* 2013). Moreover, results of some studies are only based on data from a questionnaire (Mutembei *et al.,* 2002; Forsberg and Persson 2007; Wydooghe *et al.,* 2013) and there is no information (or no rigorous information) about reproductive management of the breeders concerned. Other possible factors influencing the reproductive pattern of bitches are said to be different housing, nutritional conditions, and probably management (e.g. proper timing) of mating or insemination (Gavrilovic *et al.,* 2008), i.e. specific environmental influence (Mutembei *et al.,* 2002). Borge *et al.* (2011) mentioned that even though mean litter size is breed-specific, information on the expected number of puppies born for a particular breed is lacking and this knowledge is useful both for breeders and veterinarians. The mean litter size of Mudhol Hound female dog which is medium sized dog, therefore appeared to be similar to litter size of other medium sized breed of dogs.

The present study also confirmed the importance of feeding dogs with nutritionally balanced food for optimizing the litter size. Although several studies have

stated that dogs maintained on poor diet have a lower birth weight, there is little mention on litter size.

5.11 Sex ratio in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study, the ratio of male to female puppies born was 1:1.02 in dogs maintained on nutritionally balanced food and this did not differ significantly from ratio of 1: 1.14 observed in dogs maintained on homemade diet. This ratio on male to female puppies observed in the present study agrees to the ratio of male and female puppies born in Dogo Argentina Bitch (Caffaratti *et al.,* 2013). Similar ratio also has been reported in the Drever breed of dog (Gavrilovic *et al.,* 2008). Tedor and Reif (1978) reported that 50.60% of all puppies born were males. The result of present study suggest that the sex ratio of Mudhol Hound breed of dog is similar to those seen in other breeds of dogs and is not influenced by system of feeding.

5.12 Influence of age and parity of dam on litter size and gestation length of Mudhol Hound female dogs

The influence of age of dam was determined by screening the reproductive records of the individual animals over the last six years. The litter size of seventy two whelpings were available and litter size was compared among animals of age less than two years, 2-4 years of age and more than 4 years of age. It was observed that the litter size of animals aged less than two years and 2-4 years was significantly higher than in bitches aged more than 4 years (Table 13). Similarly the litter size at first parity and second and third parity was significantly higher than in animals at third or more parity or

fourth parity. Thus a significant effect of age and parity of bitch at the time of whelping on the litter size was recorded in the present study. Previous studies have also found an association between reproductive ability and age of bitch (Gavrilovic *et al.,* 2008) also decrease in conception rate and litter size has been reported with increasing age. (Andersen, 1962 and 1965; Mutembei *et al.,* 2000 and 2002; Tsutsui *et al.,* 2006). The effect of parity on litter size was also observed in present study with litter size being more in younger animals. However, one study suggested that parity can have an effect on litter size but not before fifth whelping and that reproductive efficiency decline in dogs after six years of age (Mutembei *et al.,* 2002). Unfortunately, in the present study, litter size in animals older than six years could not be documented as none of the animals over six years of age had been mated.

5.13 Influence of litter size on duration of gestation length in Mudhol Hound female dogs

The duration of gestation length calculated as the interval from first mating to time of delivery was highly variable among individual animals. In group of animals with litter size of 1 to 3, the mean gestation period was determined as 67.68 ±1.27 days and this was with range of 62 to 69 days. A similar range was observed in animals with litter size of 3 to 5 and more than 5. The mean gestation period did not differ significantly. However, it has been reported that litters of 4 or fewer tend to have larger gestation (Kutzler *et al.,* 2003). The litter size was also found to influence the relation of pregnancy by each single pup more or less than the average litter size with significantly shorter gestation period in larger litters and significantly longer in the smaller litters (Gavrivolic

et al., 2008). However, comparison between reports are not possible as criteria used for determining the length of gestation were not uniform.

5.14 Frequency of complicated births in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

In the present study dystocia was recorded in only during whelping process in only two animals out of 34 animals in which the process of parturition was monitored (Table 16). Further the analysis of records of 72 whelping over past six years also revealed that the incidence of complicated birth in Mudhol Hound dogs is extremely low. However due to limited number of dystocia recorded in the present study and analysis of influence of system of feeding on prevalence of dysocia would not be ascertained (Table 14).

The study also revealed that both gestational and parturient complications were almost not existant in Mudhol Hound breed of dog (Table 17).

5.15 Incidence of neonatal mortality of puppies born in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The overall incidence of neonatal mortality defined as the death of puppies occurring from time of delivery to the time of weaning was determined as 10.67% (Table 18). In other reports the incidence of canine neonatal mortality was reported to range 17 - 30% within first 8 weeks of age. (Andersen, 1957; Bowden *et al.,* 1963; Potkay and Bacher, 197; Nielen *et al.,* 1998; Vander Beek *et al.,* 1998 and Hopper *et al.,* 2004) and the death within first week of post partum was responsible for majority of puppy losses in these studies. In comparison to other species, prevalence of canine neonatal mortality is

considerably higher and is related to many factors including prolonged labor, maternal neglect or carelessness, lack of milk, congenital abnormalities and acquired disorders of neonates (Anderen, 1957; Potkay and Bacher, 1977; Nielen *et al.,* 1998 and Hopper *et al.,* 2004).

The etiology for this fading puppy syndrome includes a whole range of causes, like poor mothering, inadequate nutrition, inadequate colostrum, trauma and congenital anomalies, low birth weight and infections [Blunden, 1998 and Sturges, 2006]. The clinical signs of many neonatal diseases are very similar and vague, like restlessness, crying, low body temperature, diarrhoea and breathing difficulties. In the absence of a more specific diagnose the term fading puppy syndrome is often used. Because of the immature status of the newborn puppy, a sick neonate may rapidly become hypothermic, hypoglycemic, dehydrated and hypoxic and then die regardless of the initiating insult (Gunn Moore, 2006).

In the present study the incidence of neonatal mortality was comparatively lower than those reported in other studies. Further, the incidence of neonatal mortality was significantly lower in dogs maintained on a balanced nutrition. The observations show the influence of maintaining the dogs on nutritionally balanced diet to improve the survivability of neonates. The beneficial effects of nutrition could be a better birth weight of new born as observed earlier. It is also possible that dogs maintained on nutritionally balanced diet have a better milk yield and significantly higher immunoglogubulin concentration in the milk which could contribute to the better immunity of neonates.

5.16 Haemato biochemical studies in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

There are no haemato biochemical reports of Mudhol Hound breed of dog. This is surprising as serum biochemical constituents have clinical importance in assessing animal growth, health and nutrition status. In dogs serum biochemical profiles have been used extensively in Veterinary Medicine for clinical and metabolic evaluation of individual animals (Kalita and Mahapatra, 1998; Smith, 2000; Fowler and Miller, 2003; Kaneko *et. al.*, 2008). In the present study, the profiles of hemoglobin, random blood glucose and serum total protein concentration were determined to assess if the system of feeding has any influence on the concentration of these parameters and to assess if they have any role on reproductive efficiency parameters of Mudhol Hound breed of dog. The mean concentration of Hemoglobin of 60 Mudhol hound dogs at the time of onset of puberty is determined as 13.15 ± 2.68 gm/dl irrespective of type of nutrition on which they were being maintained (Table 19). Although further, heamoglobin concentration also did not differ between groups of dogs maintained on different system of feeding. The hemoglobin concentration recorded in the present study is similar to the normal hemoglobin concentration reported for clinically healthy dogs (Kaneko *et al.*, 2008 and Sandeep, 2016).

In the present study, none of the animals were found to be anemic; the absence of significance difference between hemoglobin concentration of 60 dogs maintained on two different systems of feeding and also suggest that the homemade diet was significantly rich in iron and folic acid concentration.

The total serum protein and random blood glucose were also similar and non significantly different between two groups of dogs maintained on two different system of feeding. The total serum protein concentration recorded in 60 dogs at the time of puberty was 8.62 ± 0.18 mg/ dl irrespective of type of nutrition (Table 21) and was similar to reported values of clinically healthy dogs (Kaneko *et al.,* 2008 and Sandeep, 2016). The observations made in the present study none of the animals are hypoproteinemic and also seem to indicate that homemade diet provided to animals was fairly balanced in terms providing protein in the diet.

The random blood glucose concentration at the time of puberty of 60 Mudhol Hound female dogs was also within the normal physiological limits reported for female dog (Kaneko *et al.,* 2008 and Sandeep, 2016). The system of feeding also did not influenced random blood glucose concentration, these observations suggest that Mudhol Hound female dogs fed with homemade diet were hypoglycemic and received adequate amount of glucose to maintain homeostasis.

5.17 Serum progesterone concentration in Mudhol Hound female dogs maintained on nutritionally balanced diet and on homemade diet

The serum progesterone concentration on first day of proestrus of 60 female Mudhol Hound dogs maintained on irrespective of system of feeding was determined as 0.20 ± 0.04 ng / ml (Table 22). Further its concentration did not differ significantly between two groups of dogs maintained on different system of feeding. There are several reports which indicate that serum progesterone concentration remained low (less than 0.5ng/ml) (Phemister *et al.,* 1973and Wildt *et al.,* 1979) prior to and during the first few

days of proestrus (Conconan *et al.*, 1975; Edqvist *et al.*, 1975; Holst and Phemister, 1975; Nett *et al.*, 1975; Olson *et al.*, 1984 and WIldt *et al.*, 1979).

These observations suggest that endocrinologically the events during proestrus in Mudhol Hound breed of dog is similar to other dogs and the serum progesterone concentration is not influenced by system of feeding (Table 22).

It has been reported that during first 2 to 3 days prior to acceptance of male there is slight increase in concentration of serum progesterone (Phemister *et al.*, 1973; Wildt *et al.*, 1979). This increase appeared to be related to pre ovulatory luteinization of follicle (Phemister *et al.*, 1973 and Concannon *et al.*, 1977). The serum progesterone is reported to rapidly increase 1 to 3 ng/ml during preovulatory LH surge. In the present study the mean concentration on the first day of cytological proestrus was determined as 1.76 ± 0.52 ng/ml which is in agreement with reported concentration on first day of cytological estrus in dog (Suyash Vardhan Bhal, 2002).

The serum progesterone concentration is then reported gradually increase reaching concentration of 10-25 ng/ ml by day 10 after onset of estrus (Concannon, 1989). Fernandes *et al.* 1987 reported that the mean concentration of serum progesterone increased from basal level to approximately 1ng/ml at the onset of estrus to 15.5 ± 5.0 ng/ml on first day of estrus which is similar with the observations made in the present study. In conclusion, it appears that the serum progesterone profile during various reproductive phases of Mudhol Hound female dogs is similar to those reported in dogs in general.

The effect of nutrition on the reproductive efficiency parameters in dog was virtually has not been explored. On the other hand, such studies are available in cows and food producing animals (Marrow, 1986). Studies in food producing animals have been documented the definite role of protein, energy, micro and macro elements on various aspects of reproduction. The results of the present study has established the reproductive efficiency parameters of Mudhol Hound female dogs in general which either due to non existence. Many of the reproductive efficiency parameters of Mudhol Hound breed of dog was found similar to those reported in other breeds of similar body weight and size. However a significant advantage was observed in Mudhol Hound breed of dog was very low incidence of gestational, parturient and post parturient complications. Besides Mudhol Hound breed of dog has a comparatively lower incidence of neonatal mortality. The study also established that maintaining dogs on nutritionally balanced food induces earlier puberty, improved rate of puppies at birth, significantly improves conception rate and reduces the incidence of neonatal mortality.

In view of these observations it seems reasonable to recommend that Mudhol Hound breed of dogs kept for breeding and commercial purpose should be maintained on a nutritionally balanced food.

Summary

VI. SUMMARY

An experiment was designed to document the reproductive efficiency parameters in Mudhol Hound breed of dogs and to determine if the reproductive efficiency parameters are influenced by system of feeding, namely feeding with a nutritionally balanced diet or homemade diet.

The mean birth weight of Mudhol Hound puppies was determined as 458 ± 0.18 gm and ranged between 415 to 479 g. The birth weight of Mudhol hound puppies was similar to the birth weight reported for medium sized dogs. Further the ratio of male and female puppies born was determined as 1: 1.08 and male puppies weighed significantly higher (479.11 ± 3.44 g) than new born female puppies (437.00 ± 5.47).

The mean age at puberty of Mudhol Hound female dogs was 13.86 ±1.12 and it ranged between 11 to 17 months. The mean age of puberty of Mudhol Hound female dog was also within the range for onset of puberty in medium sized dogs. The mean body weight at the time of onset of puberty was 23.70 ± 0.22kgs.

The mean duration of proestrus and estrus of 60 Mudhol Hound female dogs was determined as 9.22 ± 0.79 and 9.48 ± 0.28 and ranged between 7 to 13 days. The duration of proestrus and estrus of Mudhol Hound Female dogs was found to be similar to those reported in other breeds of dogs. It was also observed that the frequency of occurrence of pubertal estrus during hotter months of the year was very low suggestive of influence of season

The conception rate of 60 Mudhol Hound female dogs mated on the basis of vaginal exfoliative cytology was only around 56.67 per cent and attributed poor correlation of the cornification index with LH surge. Further the serum progesterone profiles of all the 60 dogs during the first day of pro-estrus, first day of cytological estrus and cytological diestrus are on the expected lines.

The duration of pregnancy when calculated as the interval from the first day of mating to the day of parturition varied widely between individuals (61- 71 days). The mean duration of pregnancy was calculated as 67.77 ± 0.35. This wide range of duration of pregnancy was attributed to the poor correlation between the day of first mating and LH surge. However, when mated on the basis of serum progesterone concentration the duration of pregnancy in 60 Mudhol Hound dogs was less variable (61 to 71) and the mean duration of pregnancy was determined as 63.60 ± 0.23 days.

Mudhol Hound dogs are found to have very low incidence of gestational, parturient and post parturient disorders. The average litter size was determined as 6.74 ± 0.23 with a range of 1-11. Further the incidence of neonatal mortality was comparatively lower (10.67 per cent). The hemoglobin, blood sugar and total serum protein concentration at the time of puberty were within physiological limits.

Two groups of dogs of 30 each were maintained from time of weaning either on a nutritionally balanced diet or homemade diet. It was observed that the pups delivered from dogs maintained on homemade diet were significantly lower (birth weight) and had significantly higher prevalence of neonatal mortality. The litter size was also smaller in

dogs maintained on homemade diet (5.76 ± 0.31). However, the sex ratio was not influenced by the type of feeding.

The body weight was found to be most important factor for determining the time of onset of puberty and all Mudhol Hound dogs attained puberty when they attained body weight of 23 to 25 kg irrespective of system of feeding. However, Mudhol Hound dogs maintained on homemade diet achieved threshold body weight required for attaining puberty of later age (16.03 ± 0.06 months) as compared to those maintained on nutritionally balanced food (12.40 ± 0.64 months).

The duration of pubertal proestrus and estrus was similar to those observed during estrus subsequent to pubertal estrus and type of nutrition did not seem to influence the duration and character of proestrus and estrus. However, the conception rate of female dogs maintained on the homemade diet was significantly lower (50.00 per cent) as compared to conception rate recorded in dogs maintained with nutritionally balanced food (63.34 per cent).

In the present study it was also observed that the duration of pregnancy calculated as the interval from initial rise in serum progesterone concentration to the day of parturition was not influenced by system of feeding. The system of feeding also did not seem to influence the incidence of pseudopregnancy, gestational, parturient and post parturient disorders in Mudhol Hound female dogs. However, pups delivered from dogs maintained on nutritionally balanced diet had a better puppy survival rate.

The blood hemoglobin, random blood glucose, total serum protein and serum progesterone concentration during pubertal estrus were all within the reported range and system of feeding did not seem to alter any of these parameters studied.

It is concluded that when Mudhol Hound dogs maintained for breeding purpose, feeding of nutritionally balanced diet would reduce age of onset of puberty, improve the conception rate and increased puppy birth weight and reduced prevalence of neonatal mortality.

Bibliography

VII. BIBLIOGRAPHY

AJALA OLUWATOYIN, O., FAYEMI, O. E. and OYEYEMI, M., O.2012. Some reproductive indices of the Nigerian local bitches in Ibadan, Nigeria. *Niger. J. Physiol. Sci.* **27**(June 2012) 049 – 05

ALIEN, E.A, and DOISY, E.A., 1923. An Ovarian hormone. *J. Am. Med.* Assoc., **81**:819-821

ALIEN, E.W and ENGLAND, G.C.W., 1990. Reproductive Endocrinology of the bitch. *Manual of Small Animal Endocrinology., BSAVA, London,* 127-129

ANDERSEN A. C. 1962. Reproductive fitness of the female Beagle. *JAVMA* 1962; **141**:1451–4.

ANDERSEN, A.C. 1957. Puppy production to the weaning age. *J. Am. Vet. Med. Assoc.* **130**: 151-158

ANDERSON, A., 1965. Reproductive ability of female Beagles in relation to advancing age. *Exp. Geront. I,* 189-192

ANDERSEN, A. C. and SIMPSON, M. E., 1973: *In* The ovary and reproductive cycle of the dog (Beagle). Los altos, CA, Geron-X: *pp* 266-268

ARBEITER, K., 1993. Anovulatory ovarian cycles in dogs. *J. Reprod. Fertil. Suppl*., **47**: 453-456

ARTHUR, G.H., NOAKES, D.E. and EARSON, H., 1989. Veterinary Reproduction and Obstetrics. 6th Edn, Bailliere Tindal, London, pp 26-30

ARUN, M., 2001. Semi and Non invasive techniques for detection of the stages of estrus cycle and pregnancy in bitches. *Master's thesis. ANG Ranga Agricultural University, India, pp 46-75*

ASTER, J. C., 2004. Anaemia of diminished erythropoiesis. In V. Kumar, A. K. Abbas, N. Fausto, S. L. Robbins, & R. S. Cotran (Eds.), *Robbins and Cotran Pathologic Basis of Disease* (7th ed., p.638-649). Saunders Co. Philadelphia

AWODI, S., AYO, J. O., ATODO, A. D. and DZENDE, T. 2005. Some haematological parameters and the erythrocyte osomotic fragility in the laughing dove *(Streptopella senegalensis*) and the village weaner bird *(Ploceus cucullatus)* (p.384-387). *Proceedings of the 10th Annual Conference of Animal Science Association of Nigeria*

BAAR, C., 1995. Investigations on indirect methods of detection of oestrus for the determination of ovulation in bitches. *Institut fur reproductions medizin,* Tierarztliche Hochschule, Hannover, Germany. 125

BADINAND, F. and FONTBONNE, A., 1993. Repeatability of events during successive estrus periods within bitches: comparison between breeding results and clinical and hormonal data. *J. Reprod. Fertil. Suppl.*, **47**: 548

BATTAGLIA, C. L., 2001. Litter size and singleton of Dogs. http://www. breedingbetterdogs.com. Retrieved 27-01-2001

BAUTISTA, A., RÖDEL, H.G., MONCLÚS, R., JUÁREZ-ROMERO, M., CRUZ-SÁNCHEZ, E., MARTÍNEZ-GÓMEZ, M., and HUDSON, R., 2015**.** Intrauterine position as a predictor of postnatal growth and survival in the rabbit. Physiol. Behav. **138:** 101–106.

BEACH, F.A., DUNBAR, L. F. and BUCHLER, M.G., 1982. Sexual characteristics of female dogs during successive phases of the ovarian cycle, *Horm. Behav.*, **16:** 414-442

BEIJERINK, N., 2007. Endocrinology of physiological and progestin-induced canine anoestrus. *Ph D Thesis. Faculty of Veterinary Medicine, Utrecht University, Netherlands,* 176

BELL, E.T. and CHRISTIE, D.W., 1971. Duration of proestrus, estrus and vulvar bleeding in the Beagle bitch. *Br. Vet. J.*, **127**: 25-27

BHAGIRATHI PUGASHETTI.,2008. Studies on the prediction of parturition date in bitches. *Ph.D thesis submitted to Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar*

BIGLIARDI ENRICO, FRANCESCO DI IANNI, ENRICO PARMIGIANI, GIORGIO MORINI and CARLA BRESCIANI. 2013. Physiological Weight Loss in Newborn Puppies of Boxer Breed, Italian Journal of Animal Science, 12:4, e77. http://dx.doi.org/10.4081/ijas.2013.e77

BLUNDEN, A. S., 1998. The neonate: Congenital defects and fading puppies. In: Manual of Small Animal Reproduction and Neonatology, Simpson, G. M., England, G. C. W. and Harvey, M. (Ed.). *British Small Animal Veterinary Association,* 143 - 152

BLUNDEN, A.S., 1986. A review of the fading puppy syndrome (also known as fading puppy complex). *Veterinary Annual,* **26**:264 – 269

BORGE K.S., TONNESSEN R., NODTVEDT A., and INDREBO A. 2011: Litter size at birth in purebred dogs – a retrospective study of 224 breeds. Theriogenology, 75, 911–919.

BOUCHARD, G.F., SOLORZANO, N., CONCANNON, P.W., YOUNGQUIST, R.S . and BIERSCHWAL, C. W., 1991: Determination of ovulation time in bitches based on teasing, vaginal cytology and ELISA for progesterone. *Theriogenology.,* **35:** 603-611

BOUCHARD, G.F., SOLORZANO, N., CONCANNON, P.W., YOUNGQUIST, R.S. and BIERSCHWAL, C. W., 1991a. Determination of ovulation time in bitches based on teasing, vaginal cytology and ELISA for progesterone. *Theriogenology.,* **35**: 603-611

BOWDEN R. S. T., HOGDMAN S. F. T., HIME J. M. 1963. Neo-natal mortality in dogs. *Proceedings of the 17th World Veterinary Congress, Hannover* **17:**1009-1013.

BOYD, J.S., NICKSONDA. ECKERSALL. P.D., FERGUSON, J.M., HARVEY, M.J.A. and RENTON, J.P., 1993. Molecular biological methods for monitoring oocyte maturation and in vitro fertilization in bitches. *J. Reprod. Fert* (Suppl.)*.*, **47**: 231–240

BULGIN, M. S., WARD, A. C. S. and SRIRANGANATHAN, N., 1984. Abortion in the dog due to Campylobacter species. *Am. J Vet Res,* **45**: 555 - 556.

BURNS, M and FRAZER, M. M. 1966). Genetcs of the dog – the basis of successful breeding*, second edition.* J. B Lippin Cott Co. Phildelphia, Pa.

CAFFARATTI, M., GONZÁLEZ, G., GORLA, N., and GUENDULAIN, C., 2013. Reproductive parameters of the Dogo Argentino bitch. *J. Vet. Med*. 2013:495975.

CHAKRABORTY, P.K., PANKO, W.B. and FLETCHER W.S., 1980 Serum hormone concentrations and their relationship to sexual behaviour at the first and second estrous cycles of the labrador bitch. *Biol - Reprod,* **22**: 227-232

CHATADARONG, K., TUMMARUK, P., SIRIVAIDYPONG, S. and RAKSIL, S., 2007. Season and breed effects on reproductive parameters in bitches in the tropics: a retrospective study. *J.Small Anim.Pract.* **48**: 444-448

CHINEKE, C. A., OLOGUN, A. G. and IKEOBI, C. O. N., 2006. Haematological parameters in rabbit breeds and crosses in humid tropics. *Pak. J. Biol Sci*, **9**(11): 2102-2106

CHRISTIANSEN, I. J., 1984. *Reproduction in the Dog & Cat.* Baillière Tindall, Eastbourne, 309pp

CHRISTIE, D. W., BAILEY, J.B. and BELL, E.T., 1972. Classification of cell types in vaginal smear during the canine oestrous cycle. *Br. Vet. J.*, **128**: 301-309

CHRISTIE, D.W. and BELL, E. T., 1971a. Some observations on the seasonal incidence and frequency of oestrus in breeding bitches in Britain. *J. Small. Anim. Pract*., **12**:159–67

CHRISTIE, D.W. and BELL, E.T., 1971b. Endocrinology of the Oestrous cyde in the bitch. *J. Small. Anim. Pract*., **12**: 383-389.

CLARK, R. D., and STAINER, J R. 1983. Medical and Genetic aspects of purebred dogs. Edwards Villae Kansas, Veterinary Medicine Publishing Company. Page no 19.

COLUMBIA ENCYLOPEDIA 2005. Columbia Encyclopedia – dog – AOL Research and Learn. http://www.a/dog/2005/205232309990025. Retrieved 21-03-2005

CONCANNON, P. W. 1993. Biology of gonadotropin secretion in adult and prepubertal female dogs. *J. Reprod. Fertil.* 1993; 47: 3–27.

CONCANNON, P. W. and CASTRACANE, V. D., 1985. Serum androstenedione and testosterone concentration during pregnancy and non pregnant cycle in dogs., *Biol Reprod*., **33**: 1078-1083

CONCANNON, P. W. and LEIN, D. H., 1989. Hormonal and clinical correlates of ovarian cycles, ovulation, pseudo pregnancy and pregnancy in dogs. In: Kirk RW,ed. Current Veterinary Therapy, Small Animal Practice, Vol. X. W. B. Saunders, Philadelphia: *pp*.1269-1282

CONCANNON, P. W. and VERSTEGEN, J., 1998. Pregnancy in Dogs and Cats. In: Knobil E and Neil JN, eds. Encyclopedia of Reproduction, Vol. 3. Academic Press, New York: - Amazon

CONCANNON, P. W., ENGLAND, G. C.W. and. VERSTEGEN, J., 2000. Canine Pregnancy: Predicting Parturition and Timing Events of Gestation. *International Veterinary Information Service*

CONCANNON, P.W., 1980. Effect of hypophysectomy and of LH administration on the luteal phase plasma progesterone level in the Beagle bitch. *J. Reprod. Fert.,* **58**: 407

CONCANNON, P.W. (1983). Reproductive physiology and endocrine patterns of the bitch. In: Kirk R.W. (ed). *Current Veterinary Therapy VIII. Small Animal Practice.* W.B. Saunders, Philadelphia. pp. 886-901.

CONCANNON, P.W., 1986. Physiology of Reproduction, "*In: Small Animal Reproduction and Infertility" Ed: Burke, T.J, Lea and Febiger, Philadelphia,* 23-77

CONCANNON, P.W., 1987. The physiology of ovarian cycle, pregnancy and parturition in the domestic dogs. *Proc. of the Society for Theriogenol., pp.* 159

CONCANNON, P.W., 1997. A review for breeding management and artificial insemination within chilled or frozen semen, In: *Proceedings of Canine Male Reproduction Symposium Society for Theriogenology.,* Montreal, Quebec, Canada, 1-17

CONCANNON, P. W., (2000). Canine pregnancy. Predicting parturition and timing events of gestation. In: Recent advances in small animal reproduction. International Veterinary information services, (www.wis.org) document No. A1202.0500.

CONCANNON, P.W., 2010. Reproductive cycles of the domestic bitch. *Anim. Reprod. Sci.,* **8**:28

CONCANNON, P.W., 2011. "Reproductive cycles of the domestic bitch," *Animal Reproduction Science,* vol. **124,** no. 3-4, pp. 200–210

CONCANNON, P.W., BUTLER, W.R., HANSEL, W., KNIGHT, P.J. and HAMILTON, J.M., 1978. Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. *Biol. Reprod*., **19:**1113-1118

CONCANNON, P.W., COWEN, R. and HANSEL, W., 1979. LH release in ovariectomised dog in response to estrogen withdrawal and its facilitation by progesterone. *Biol. Reprod.,* **20**: 523-531

CONCANNON, P.W., ENGLAND, G., VERSTEGEN, J. and LINDE FORSBERG, C., 2002. Recent Advances in Small Animal Reproduction. *International Veterinary Information Service*., New York, 1-11

CONCANNON, P.W., HANSEL, W. and MCENTEE, K., 1977. Changes in LH, Progesterone and sexual behaviour associated with preovulatory luteinization in the bitch. *Biol. Reprod.*, **17**: 604- 613

CONCANNON, P.W., HANSEL, W. and MCENTEE, K., 1977a. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. *Biol. Reprod*., **13**:112-121

CONCANNON, P.W., POWERS, M.E., HOLDER, W. and HANSEL, W., 1977b: Pregnancy and parturition in the bitch. *Biol Reprod.,* **16**: 517-526

CONCANNON, P.W., HANSEL, W. and VISEK, W.J., 1975. The ovarian cycle of the bitch: plasma estrogen, LH and progesterone. *Biol. Reprod.*, **13**: 112-121

CONCANNON, P.W., MCCANN, J.P and TEMPLE, M., 1989a: Biology and endocrinology of ovulation pregnancy and parturition in the dog. *J. Reprod. Fert. (Suppl.),*, **39**: 3-25

CONCANNON, P.W., MORTON, D. and WEIR. B., 1989. Dog and Cat Reproduction, Contraception and Artificial Insemination. In: Proceedings of the 1st Int. Symp. Canine Feline Reprod. Cambridge: *J. Reprod. Fert. (Suppl.),* **39**: 350

CONCANNON, P.W., WEIGAND, N., WILSON, S. and HANSEL, W., 1979a. Sexual behaviour in overectomized bitches in response to estrogen and progesterone administration. *Biol. Reprod.,* **20**: 799-809

CONCANNON, P.W., WHALEY, S., LEIN, D. and WISSLER, R., 1983. Canine gestation length: variation related to time of mating and fertile life of sperm. *Am. J.. Vet. Res.,* **44**:1819-1821

DARVELID, A.W. and LINDE-FORSBERG, C., 1994. Dystocia in the bitch: a retrospective study of 182 cases. *J. Small Anim. Pract.*, 35: 402-407

DE GIER, J., KOOISTRA, H.S. and DJAJADININGRAT LAANEN, S.C., 2006. Temporal relations between plasma concentrations of luteinizing hormone, follicle-stimulating hormone, estradiol-17 (beta), progesterone, prolactin, and (alpha) –melanocyte stimulating hormone during the follicular, ovulatory, and early luteal phase in the bitch. *Theriogenology,* **65** :1346- 1359

DER-ROTTWEILER., 2006. Pregnant bitches feeding. Dev – Rottweiler. Com http://www.der-rottweiler .com/menu 3_06html. Retrieved 01-06-2009

DEWEY, T. and BHAGAT., 2002. *Canis Inpus familiaris* Animal. *Diversity Web*

DIELEMAN, S.J. and BLANKENSTEIN, D.M., 1998. Determination of the time of ovulation in the dog by estimation of progesterone in blood: applicability of an EIA method in comparison to RIA. 11th international Congress on Animal Reproduction and Artificial Insemination, University College Dublin, Ireland, **2**(21): 2

DIETRICH, E. and MOLLAR, R., 1993. Use of progesterone enzyme immunoassay to determine the optimal mating time in estrus bitches. *J. Reprod. Fert.,* (Supp1.) **47**: 524

DOAK, R.L., HALL, A. and DALE, H.E., 1967. Longevity of spermatozoa in the reproductive tract of the bitch. *J. Reprod. Fert.,* **13**: 51-58.

EDQVIST, L. E., JOHNSON E. D. B., and KASSTROM, H. (1975). Blood plasma levels of progesterone and estradiol in the dog during the estrus cycle and pregnancy. *Acta Endocrinol* **78:** 554.

EILTS B. E., DAVIDSON A. P., HOSGOOD G., PACCAMONTI D. L. and BAKER DG. 2005. Factors affecting gestation duration in the bitch. *Theriogenology*; 64: 242–251.

ENGALND and CONCANNON, P.W., 2002. Determination of the optimal time of breeding in bitch: Basic considerations. *International Veterinary Information Service, Ithaca New York, Document number* A1231.0602

ENGLAND, G.C.W. and RUSSO, M. 2006. Ultrasonographic characteristics of early pregnancy failure in bitches. *Theriogenology,* **66**: 1694-1698

ENGLAND, G.C.W., 1992. Vaginal cytology and cervico vaginal mucus arborisation in the breeding management of bitches. *J. Small Anim. Pract.,* **33**: 577-582

ENGLAND, G.C.W., 1998. Manual of small animal endocrinology. 2nd ed. *Brazilian. Small Anim. Vet. Assoc.,* 43-46

EVANS, H.M. and COLE, H.H., 1931. An introduction to the study of the estrous cycle in the Dogs. *Mem. Univ. Calif.,* **9**: 65

FELDMAN, E.C. and NELSON, R.W., 1987. *Canine and feline endocrinology and reproduction*. Philadelphia: WB Saunders.

FELDMAN, E.C. and NELSON, R.W., 1996. Canine female reproduction, *Canine and Feline Endocrinology and Reproduction, Philadelphia, W.B. Saunders., 526-547*

FELDMAN, E.C. and NELSON, R.W., 2004. Canine and Feline Endocrinology and Reproduction. 3rd ed. W.B. Saunders, Philadelphia., pp 486–538.

FELDMAN, E.C., and NELSON, R.W., 2007. Reproducción de la hembra canina. In: E.C. Feldman and R.W. Nelson (eds.) Endocrinologıa y Reproducción Canina y Felina, 3rd ed. Intermédica Buenos Aires, Argentina, pp 833-1036.

FERNANDES, P. A., BOWEN, R. A., KOSTAS, A. C., SAWYER, T. M., NETT, T. M. and OLSON, P. N., 1987. Luteal function in the bitch: changes during diestrus in pituitary concentration of and the number of luteal receptors for luteinizing hormone and prolactin. *Biol. Reprod*., **37**: 804-811

FORSBERG C.L., and PERSSON G. 2007. A survey of dystocia in the Boxer breed. *Acta Veterinaria Scandinavica*, 49, 9.

FOWLER, M.E. and MILLER, R.E. (EDS)., 2003. Zoo and Wild Animal Medicine. 5th ed. St. Louis, MO: Saunders, 485-486

FREEMAN, W.E., COUTO, C.G. and GRAY, T.L., 2003. Serum creatinine concentrations in retired racing Greyhounds. *Vet Clin Pathol* 2003., **32**:40–42

FRIEDMAN, M. H., 1957. The effect of O-diazoacteyl (Azaserine) on the pregnancy of the dog. *J. Vet. Med. Assoc*., **130**: 160

GAVRILOVIC, B.B., ANDERSSON, K and LINDE, F.C., 2008. Reproductive patterns in the domestic dog–a retrospective study of the Drever breed. *Theriogenology,* **70:**783–94

GERRES, S., HOEVELER, B., AND HOFFMAN.1988. Canine pseudo pregnancy. *Vet. J. Fuerden. Veterinaer.*, 5 : 29–31.

GIANOLA, D. and TYLER, W. J., 1974 Influences of birth weight and gestation period of Holstein Friesian cattle. *Journal of Dairy Science,* **57 (**2): 235-240.

GILL, M.A., 2001. Perinatal and late neonatal mortality in the dog: *The University of Sidney*, 2001

GIPSON P. S. 1996. Evaluations of behavior of feral dogs in interior Alaska, with control implications. In: *Proceeding of the Vertebrate Pest Control and Management Material: 4th Symposium of the American Society for Testing and Materials. Philadelphia, PA, USA*: pp. 285–294.

GOODMAN, M., 1998. Canine ovulation timing, *In: Canine Reproduction Symposium. Society for Theriogenoly,* 1-6

GOODWIN, M., GOODING, K. and REGNIER, F., 1979. Sex pheromone in the dog. *Science*, **203**: 559-61

GRAFF K., FRIENDREICH, V., MATTHEWS, S., and HASAN, S.H., 1977. Homologous immunoassay for canine prolactin and its application in several physiological states. *J. Endocrinol.,* **75**: 93–103

GREENE, C. E. and KAKUK T. J., 1984. *Dog and Cat. Greene, C. E., W. B. Saunders Co. Philadelphia,* 419 - 429

GREENE, C. E., 1984a. Canine viral enteritis. In: *Clinical Microbiology and Infectious Diseases in the Dog and Cat,* Green. C. E. (Ed) W. B. Saunders Co. Philadelphia, 437 - 460

GREENE, C. E., 1984c. Immunoprophylaxix and immunotherapy. In: *Clinical Microbiology and Infectious Diseases in the Dog and Cat.* Green. C. E. (Ed) W. B. Saunders Co. Philadelphia, 321 - 355

GROPPETTI, D., RAVASIO, G., BRONZO, V. and PECILE, A., 2015. The role of birth weight on litter size and mortality within 24 h of life in purebred dogs: What aspects are involved?. *Animal Reproduction Science,* Volume 163, December 2015, Pages 112–119

GUNN-MOORE D, 2006. Small animal neonatology: They look normal when they are born and then they die. *Proceedings of the 31st WSAVA Congress, Praha,* :714-720.

HANCOCK, J.L. and ROWLANDS, I.W., 1949. The Physiology of Reproduction in Dogs. *Vet. Rec*., **61**: 771-779

HARROP, A.E., 1960. In: Reproduction in the Dog. Bailliere Tindall, London, pp 8-22

HASE, M., HORI, T., KAWAKAMI, E. and TSUTSUI, T., 2000. Plasma LH and progesterone levels before and after ovulation and observation of ovarian follicles by ultrasonographic diagnosis system in dogs. *J. Vet. Med. Sci*., **62**: 243-248

HASHIMOTO, A., HIRAI, K. and SUZUKI, Y., 1983. Experimental transplacental transmission of canine herpesvirus in pregnant bitches during the second trimester of gestation. *Am.. J.Vet. Res.*, **44**:610 - 614

HEAPE, W., 1900. The "Sexual season" of mammals and the relation of the "pro-oestrus" to menstruation. *Quarterly Journal of Microbiological Science* 44: Part I, pp. 1-70

HIEMSTRA, M., SCHAEFERS OKKENS, A.C., TESKE, E. and KOOISTRA, H.S., 2001. The reliability of vaginal cytology in determining the optimal mating time in the bitch. *Tijdschrift. Voor. Diergeneeskunde.* **126**(21): 685-689

HIGGINS, R. J., KRAKOWKA, S. and METZLER, A. E., 1981. Canine Distemper virus-associated cardiac necrosis in the dog. *Veterinary Pathology,* **18**:472 - 486

HOLST P. A. and PHEMISTER R. D. 1975. Temporal sequence of events in the estrus cycle of bitch. *Am .J .Vet. Res.* **36:** 705.

HOLST, P.A. and PHEMISTER, R.D. 1971.The prenatal development of the dog: pre-implantation events. *Biol. Reproduc,* **5**: 194-206

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

HOLST, P.A., 1986. Vaginal cytology in the bitch, "In: *Current therapy in Theriogenology"*. Ed. Marrow. D.A., **2**: 457

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

IBRAHIM AYDIN, EMARAH, TUGBA OZAYDIN and DURSUN ALI DINC, 2011. Determination of the stages of the sexual cycle of the bitch by direct examination. *J. Anim. Vet. Adv.*, **10**(15): 1962-1967

ISAAC, L. J., ABAH, G., AKPAN, B. and EKAETTE, I. U. (2013). Haematological properties of different breeds and sexes of rabbits (p.24-27). *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria*

JEFFCOATE, I., 1998. Physiology and endocrinology of the bitch. In: Manual of Small Animal Reproduction and Neonatology. *British. Small. Anim. Vet. Assoc*., *pp*: 1-9

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

JOCHLE, W and ANDERSEN A C. 1977. The estrus cycle in the dog – a review. *Theriogenology*, **7:** 113-140

JOHNSON J. M. and BERGER P.J. 2003. Birth weight as a predictor of calving ease and perinatal mortality in Holstein Friesian cattle. *Journal of Dairy Science,* **86 (**11): 3745- 55

JOHNSTON, S. D., (1986). Pseudo-pregnancy in the bitch. Current therapy in theriogenology 2nd ed. Morrow. D.A. W.B Saunders Co.Philadelphia. pp:490 – 491.

JOHNSTON, S. D. and RAKSIL, S., 1987. Fetal loss in the dog. *Veterinary Clinics of North America, Small Animal Practice*, **17**: 535 - 554

JOHNSTON, S. D. and ROOT, K. M. V., 1995. Serum progesterone timing of ovulation in the bitch. In : Proceedings of Annual meeting of the Society for Theriogenology, San Antonio, TX, September 13-15. Nashville, Society for Theriogenology., *pp*: 195-203

JOHNSTON, S. D., 1988. Management of normal pregnancy and disorders of pregnancy. In: *Refresher Course For Veterinarians, Proceedings No. 108, Reproduction, Small Companion Animals.* The University of Sydney, Postgraduate Committee in Veterinary Science, 91 – 101

JOHNSON, C. A., 2008. Pregnancy management in the bitch. *Theriogenol*., **70**: 1412–1417

JOHNSTON, S.D., 1980. False pregnancy in the bitch. In: Morrow, D.A. (Ed.), Current Veterinary Theriogenology. Saunders, Philadelphia, pp. 623–624

JOHNSTON, S.D., 1995. Breeding management of the bitch. *Text book of Veterinary internal Medicine, In Ettinger SJ (ed): 4th ed, WB Saunders, Philadelphia, **2***: 1604-1606

JOHNSTON, S.D., KUSTRITZ, M.V.R and OLSON, P.N. S., 2001. The neonate – from birth to weaning. *In Canine and feline theriogenology Philadelphia: WB Saunders;* 2001:146-167

JOHNSTON, S.D., ROOT KUSTRITZ, M.V. and OLSON, P.N., 2001. "In **:** *Canine and Feline Theriogenology*., W.B.Saunders, Philadelphia. pp.16-66

JONES, D. E. and JOSHUA, J. O., 1982. Reproductive Clinical Problems in the Dog. Wright, P.S.G, Bristol

JONES, R. L., 1987. Special considerations for appropriate antimicrobial therapy in neonates. *Veterinary Clinics of North America, Small Animal Practice,* **17**: 577 - 620

KALITA, D.J. and MAHAPATRA, M., 1998. Serum constituents and serum enzyme activities of Black Bengal. *Ind. J. Anim. Res.,* **32**(1): 38-40

KANEKO, J.J., HARVEY, J.W. and BRUSS, M.L., 2008. Clinical Biochemistry of Domestic Animals. 6th ed. San Diego, CA: Academic Press, **493**:889-895

KELLEY, R. L., 2001. Nutritional management of the bitch: pre-breeding to whelping *IAMS "Canine reproduction for breeders from a symposium presented at Westminster K.C. http://www.wood havenlands. Com/feeding-pregnant =does. html. Retrieved* 2-10-2001

KONUK, C.S., KIRSAN, I., GURBULAK, K., TEK, C. and SENUNVER, A., 2001. Determination of ovulation time using the eosinophilic index (El) of vaginal cytology and serum progesterone levels in the bitch. *Veteriner. Fakultesi. Dergisi.* Istanbul, **27**(1): 91-97

KOOISTRA, H.S., OKKENS, A.C., BEVERS, M.M., POPP-SNIJDERS, C., VAN HAAFTEN, B., DIELEMAN, S.J. and SCHOEMAKER, J., 1999. Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrous in beagle bitches. *Biol. Reprod.*, **60**: 65-71

KUNAVONGKRIT A, and TANTASUPARUK W. 1995. Influence of ambient temperature on reproductive efficiency in pigs: 2. Clinical findings and ovarian response in gilts. *Pig Journal*; **35**: 48–53.

KUSTRITZ, M.V., 2001. Use of commercial luteinizing hormone and progesterone assay kits in canine breeding management. *International Veterinary Information Service., New York, USA*

KUTZLER, M.A., YEAGER, A.E., MOHAMMED, H.O. and MEYERS- WALLEN, V. N., 2003. Accuracy of canine parturition date prediction using fetal measurements obtained by Ultrasonography. *Theriogeniology,* **60**: 1309-1317

LAWLER, D. F., 1989. Care and diseases of neonatal puppies and kittens. *In : Current Veterinary Therapy X, Kirk,(Ed).W.B. Saunders Co. Philadelphia,* 1325 - 1333

LENGHAUS, C. and STUDDERT, M. J., 1982. Generalized parvovirus disease in neonatal pups. *J. Am. Vet. Med. Asso.*, **81**: 41 - 45

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

LINDE, F.C., 1991. Achieving canine pregnancy by using frozen or chilled extended semen. *Veterinary Clin . North. Amer.,* **21** (3): 467-485

LINDE-FORSBERG C, STROM-HOLST, B. and GOVETTE, G., 1999. Comparison of fertility data from vaginal vs intrauterine insemination of frozen-thawed dog semen: a retrospective study. *Theriogenology*, **52**: 11-23

LINDE-FORSBERG, L. and ENEROTH, A., 1998. Parturition. In: Manual of Small Animal Reproduction and Neonatology

LUVONI and BECCAGLIA., 2006. The prediction of parturition date in canine pregnancy. *Reprod. Domest. Anim.,* **41**:27–32

LYNGSET, A and LYNGSET, O.,1970. Kullstørrelse hos hund [Litter size in the dog]. *Nord. Vet. Med.* 1970; **22**:186 –91

MACINTIRE, D. M., 1994. Emergencies of the female reproductive tract. In: *The Veterinary Clinics of North America. J. Small Anim. Pract.,* **24** (6): 1173 – 1188

MAHI, C.A. and YANAGIMACHI, R., 1976. Maturation and sperm penetration of canine ovarian oocytes in vitro. *Journal of Experimental Zoology*, **196**: 189-196

MANDIGERS P.J.J., UBBINK G.J., VANDENBROEK J., BOUW J. 1994: Relationship between litter size and other reproductive traits in the Dutch Kooiker dog. Veterinary Quarterly, **16**, 229–232.

MANTOVANI, A., RESTANI, R. and SCIARRA, D., 1961. Streptococcus L infection in the dog. *J. Small Anim. Pract.*, **2**:185 – 194

MARSHALL. F.H and HALNAN. E.T., 1917: On the post oestrus changes occurring in thegenerative organs and mammary glands of the non-pregnant dog. In: Proceedings of the Royal Society of London; **89**:546-559.

MATON, A., HOPKINS, R. L. J., MCLAUGHLIN, C. W., JOHNSON, S., WARNER, C. W., LAHART, D. and WRIGHT, J. D., 1993. *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall

MELLIN, T.N., ORCZYK, G.P., HICHENS, M. and BEHRMAN, H.R., 1976. Serum profiles of luteinizing hormone, progesterone and total estrogen during the canine estrous cycle. *Theriogenology.*, **5**: 175-187

MESTRE, J., WANKE, M. and SUCHEYRE, S., 1990. Exfoliate vaginal cytology and plasma concentrations of progesterone, luteinizing hormone and oestradiol-17 ß during oestrus in the bitch. *J. Small Anim. Prac.*, **31**: *568-570*

MEYERS-WALLEN, V. N., 1995. The elective cesarean section. In: Bonagura JD, Kirk RW, editors. Current veterinary therapy XII. WB Saunders Co. Philadelphia: pp.1085–1089

MONFORT, S.L., WASSER, S.K., MACHBURN, K.L., BURKE, M., BREWER, B.A. and CRE, S.R.,1997. Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (Lvcacon ~ictus) *Zoo. Biol* .1997: **16**: 533-548

MORROW DAVID, A 1986. Current therapy in theriogenology, *published by W. B. Saunders Co.* Harcouit Brace Jovanovich, Inc. Philadelphia.

MSHELIA, G.D., AMIN, J.D. and CHAUDHARI, S.U.R., 2001. Oestrus detection in the Nigerian Mongrel bitch: application of vaginal cytology. *Pakistan Vet. J.*, **21**(1): 44-46

MUTEMBEI H. M., MUTIGA E. R., and TSUMA VT. 2000. A retrospective study on some reproductive parameters of German shepherd bitches in Kenya. *J S Afr Vet Assoc*; **71**: 115–117.

MUTEMBEI H. M., MUTIGA E. R.,and TSUMA V, T. 2002. An epidemiological survey demonstrating decline in reproductive efficiency with age and non-seasonality of reproductive parameters in German shepherd bitches in Kenya. *J. S. Afr. Vet. Assoc*; **73:**36–7.

NAAKTGEBOREN, C., 1987. De geboorte van de hond en zijn Wilde verwanten. *Third revised edition. Naarden, the Netherlands, Strengholt.* 31 -35

NELSON, R.N. and COUTO, C.G., 2003. Small Animal Internal Medicine 3rd Ed. Mosby Westline Industrial Drive St. Louis Missouri, 847-933

NETT, T.M., AKBAR, A.M. and PHEMISTER, R.D., 1975. Levels of luteinizing hormone, estradiol and progesterone in serum during the estrous cycle and pregnancy in the Beagle bitch. *Proc. Soc. Exp. Biol. Med.*, **148** (1): 134

NEWBERRY, W.E. and GIER, H.T., 1952. Determination of breeding time in the bitch from vaginal smears. *Vet. Med.*, **47**: 390- 392

NIELEN ALJ, VAN DER GAAG I, KNOL BW, SCHUKKEN Y. H., 1998 Investigation of mortality and pathological changes in a 14-month birth cohort of boxer puppies. *Vet. Rec*., **142:**602-606.

NIELEN ALJ, VAN DER GAAG I, KNOL BW, SCHUKKEN Y. H., 1999. Investigation of mortality and pathological changes in a 14-month birth preweaning mortality in a birth cohort of puppies. *Am. J. Vet. Res.,* **60:**1106-1110.

NIELEN, A.L.J., JANSS, L.L.G. AND KNOL, B.W., 2001. Heritability estimations for diseases, coat color, body weight, and height in a birth cohort of Boxers. *Am. J. Vet. Res*., **62:**1198–1206

NOTHLING, J.O., IRONS, P.C., GERBER, D. and SCHULMANN, M.L., 2003. The use of plasma progesterone concentration to predict the optimal breeding time in bitches 6 days in advance. *Theriogenology,* **59:** 225

OKKENS A. C., HEKERMAN T. W. M., DEVOGEL J. W. A., VAN HAAFTEN, B. 1993. Influence of litter size and breed on variation in length of gestation in the dog. *Vet Quart*; **15**: 160–161.

OKKENS, A.C, TEUNISSEN, J.M., VAN OSCH W., VAN DEN BROM, W.E., DIELEMAN, S.J., KOOISTRA, H.S., 2001. Influence of litter size and breed on the duration of gestation in dogs. *J. Reprod. Fertil .Suppl*., **57**: 193-197

OKKENS, A.C., DIELEMAN, S.J. and VOGEL, F., 1985. Determination of the ovulation period in the dog, a comparison of the rapid progesterone assay, vaginoscopy and vaginal cytology. *Proc Int Congress of the Royal Neth Vet Assoc 'Voorjaarsdagen' Amsterdam*; 26-27

OKKENS, A.C., VAN OSCH, W., TEUNISSEN, J.M., VAN DEN BROM, W.E., DIELEMAN, S.J. and KOOISTRA, H.S., 2003. The influence of breed on gestation in dogs. *Proceedings 4 International Symposium on Reproduction in Dogs, Cats and Exotic Carnivores, Oslo, Norway,* page 85. *abstract*

OLSON, P.N.M.A., THRALL, P.M., WYKES, P., W HOSTED. and T.M.NETT, 1984. Vaginal cytology (part-i); A useful tool for staging the canine estrous cycle. *Compendium of Continuing Education,* **6**(4): 288-298

OLSON, P. N. and NETT, T. M., 1986. Reproductive endocrinology and Physiology of the bitch, In: "*Current therapy in Theriogenology", ed*. Marrow, D.A, **2**: 453-456

OLSON, P.N., BOWEN, R.A., BEHRENDT, M.D., OLSON, J.D. and NETT, T.M., 1982. Concentrations of reproductive hormones in canine serum throughout late anestrus, proestrus and estrus. *Biol. Reprod.*, **27**(5):1196-1206

OLUWATOYIN, O., AJALA, O.E., FAYEMI. and OYEYEMI, M.O., 2012. The Oestrous Cycle of the Nigerian Local Bitch. *Global Veterinaria*., **8** (6): 574-582

OMIYALE, C. A., YISA, A. G. and ALI-DUNKRAH, L. A., 2012. *Haematological characteristics of Yankasa sheep fed fonio (Digitaria iburua) straw based diets* (p. 87-89). Proceedings of 37th Annual Conference of Nigerian Society for Animal Production

ORTEGA - PACHECO J. C, SEGURA-CORREA JC, JIMENEZ-COELLO M and LINDE-FORSBERG C. 2007. Reproductive patterns and reproductive pathologies of stray bitches in the tropics. *Theriogenology*; **67:** 382–390.

PAPICH, M. G. and DAVIS, L. E., 1986. Drug therapy during pregnancy and in the neonate. *Veterinary Clinics of North America, Small Animal Practice*, **16**: 525 - 538

PASCAL, C. and GAŞPAR, V. 2015. Body and intensity of growth development of specific romanian bucovina shepherd dog. *Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie*, vol. 64

PATIL, M. D., 2006. Induction of estrus in bitches using dopamine agonist Cabergoline. *Masters Thesis*. Tamil Nadu Veterinary and Animal sciences University, Chennai, 26–56

PAUIRAJ, S., SUNDAR RAJAN, N., MANIMOZHI, A. and WALKER, S., 1992. Reproduction of the Indian wild dog (Cuon & inus) in captivity. *Zoo. Biol*., **1**: 235-241

PETERS, S. O., GUNN, H. H., IMUMORIN, I. G., AGAVIEZOR, B. O. and IKEOBI, C. O., 2011. Hematological studies on frizzled and naked neck genotypes of Nigerian native chickens. *Tropical Animal Health Production*, **43**(3): 631-638

PHEMISTER, R.D., HOSLT, P.A. and SPANO, J.S., 1973. Time of ovulation in the beagle bitch. *Biol. Reprod.,* **8**: 74-82

POLAT BULLENT, COLAK ARMAGAN, CENGİZ MEHMET, CANNAZIK ORCUN and HAYIRLI ARMAGAN. 2015. Breed, parity and cycle season affects on life time reproduction in bitches- a retrospective study. *Turk J. Vet. Anim. Sci.,* **39:** 543-549.

PORTON, I. J., KLEIMAN, D. G. and RODDEN, M., 1987. A seasonality of bush dog reproduction and the influence of social factors on the estrous cycle. *Journal of Mammalogy*, **68**: 867–871

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

POTKAY. S and BACHER J.D., 1977. Morbidity and mortality in a closed foxhound breeding colony. *Lab. Anim.Sci.,* **27**: 78 - 84

PROLE, J.H.B., 1973. Some Observations on the. Physiology of Reproduction in the Greyhound Bitch. *J. Small. Anim. Pract.,* **14**: 781-784

QUESNEL HA, PASQUIER A, MOUNIER M, and PRUNIER A. 1998. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. *J. Anim. Sc.,* **76**: 856–863.

RAMAKRISHNA, B.C. and YATHINDER , P.V., 2000. Mudhol hound- An Overview. *Pet India*, **02**:11- 13

RAMAKRISHNA, B.C., 1997. The history and Origin of the Mudhol hound. *Pet India,* **07**:11- 13

RAMAKRISHNA, B.C., 2002. Breed Profile, Mudhol hound- Karnataka's canine pride. *Pet India*, **03**:11- 14

RASKIN, R.E. and MEYER, D.J., 2001. Atlas of Canine and Feline Cytology. *WB Saunders Co. Philadelphia.*, pp 277-312

RAVIMURUGAN, T. and KUMARAVELU, N., 2008. Dog Rearing and Management. *1st Ed., Saradha Publishers*, Chennai.

RAYMER, J., WIESLER, D. and NOVOTNY, M., 1986. Chemical scent constituents in urine of wolf (*Canis lupus*) and their dependence on reproductive hormones. *J. Chem. Ecol*., **12**: 297-314

REDDY, K.C.S., RAJU, K.G.S., RAO, K.S. and RAO, K.B.R., 2011. Vaginal cytology, vaginoscopy and progesterone profile: breeding tools in Bitches. *Iraqi. J. Vet. Sci.,* **25** (2): 51-54

REDWOOD, D. W. and BELL, A., 1983. Salmonella panama: Isolation from aborted and newborn canine fetuses. *Vet. Rec*., **112**: 362 - 365

REHM, S., STANISLAUS, D.J. and WILLIAMS, A.M., 2007. Estrous cycle-dependent histology and review of sex steroid receptor expression in dog reproductive tissues and mammary gland and associated hormone levels. *Birth Defects Res B Dev. Reprod. Toxicol*., **80**: 233-245

REIMER, T.J., PHEMISTER, R.D. and NISWENDER, G.D., 1978. Radioimmunologic measurement of follicle stimulating hormone and prolactin in the dog. *Biol. Reprod.*, **19**:673-679.

RENTON, J.P., BOYD, J.S., ECKERSALL, P.D., FERGUSON, J.M., HARVEY, M.J.A., MULLANEY, J. and PERRY, B., 1991. Ovulation, fertilization and early embryonic development in bitch (*Canis familiariss*). *J. Reprod. Fert.,* **93:** 221-231

REYNAUD, K., FONTBONNE. A., MARSELO, O.N., THOUMIRE, S., CHEBROUT M., DE LESEGNO, C.V. and CHASTANT-MAILLARD, S. 2005. In vivo meiotic resumption, fertilization and early embryonic development in the bitch. *Reproduction*, **130**: 193-201

ROBERTS STEPHEN, J., 1971 Veterinary Obstetrics and Genital diseases (Therioginology), *Second edition, published by Edward brothers, Inc, Ann. Arbor, Michigan.*

ROBERTS, S.J. 1986. *Veterinary Obstetrics and Genital Diseases***.** 3rd Ed, Wood Stock, VT, SJ. Roberts. Pp 245-276.

ROSZEL, J.R., 1977. Normal canine cytology. *Vet. Clin. North. Amer.,* **07:** 667-681

ROWLANDS, I.W., 1950. Some observations on the breeding of dogs. *Proc. Soc. Study. Fertil.,* **2**:40 –55

RUSS, K. 2000, Recent advances in canine and feline nutrition, Vol III. IAMS Nutrition Symposuim Proceedings. Orange Frazer Press, Wilmington pp: 225-239

SANDEEP, K. H, 2016. Studies on pre and post surgical evaluation of female dogs affected with pyometra. *M.V.Sc. Thesis,* Veterinary College, Bangalore. Karnataka veterinary, animal and fisheries sciences university, Bidar

SCHREIBER, R. A., FRRETT, L. K. and HOLBERT, D., 1975. The effect of parity, litter size, season and breeding protocol on the number of DBA/2J Tenmice available for weaning. *Lab. Anim. Sci,.* **25**(5): 602-608

SCHUTTE, A.P., 1967. Canine vaginal cytology – I. Teachinique and cytological morphology. *J. Small Anim. Pract.,* **8**: 301-306

SEAL, U.S., PLOTKA, E.D., PACKARD, J.M. and MECH, L.D., 1979. Endocrine correlates of reproduction in the wolf. I. Serum progesterone, estradiol and LH during the estrous cycle. *Biol. Reprod.,* **21:** 1057-1066

SEKI MAKATO., NORIO WATANABE., KENYO ISHII., YOH-ICHI KINOSHITA., TAKEHIRO AIHARA., SHUJI TAKEIRI and TAKESHIGE OTOI., 2010. Influence of parity and litter size on gestation length in beagle dogs. *Can. J. Vet. Res.*, **74**(1): 78–8

SENGER, P.L., 2003. Pathways to Pregnancy and Parturition. Second edition., 213-143

SIDELL, B. D., and O' BRIEN, K. M., 2006. When bad things happen to good fish: the loss of haemoglobin and myoglobin expression in Antarctic icefishes. *J. Exper. Biol.*, **209**:1791-1802

SHIMATSU Y., YUZAWA H., ARUGA K. and NAKURA M. (2007): Effect of time for mating and gestation length on reproductive efficiency in dogs. *Reproduction in Domestic Animals,* **42:** 664–665

SHELDON, J.W., 1992**:** Wild Dogs. The natural history of the nondomestic canidtie. San Diego: Academic Press Inc..

SMITH, M.S. and MACDONALD, L. E., 1971. Serum levels of luteinizing hormone and progesterone during the estrus cycle, pseudopregnancy and pregnancy in the dog. *Endocrinology*, **94**: 404-412

SMITH, S.A., 2000. Specific species appropriate hematology. In: Feldman B.F., Zinkl J.G., Jain N.C., eds. Schalm's Veterinary Hematology. 5th ed. Baltimore, MD: Lippincott Williams & Wilkins, 1055-1224

SNEDECOR, G.W. and COCHRAN, S.W., 1989. *Statistical methods,* 8th Edition. The Iowa state University Press, Ames, IOWA, U.S.A

SOETAN, K. O., AKINRINDE, A. S. and AJIBADE, T. O., 2013. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor) (p. 49-52). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production*

SOKOLOWSKI J.H, STOVER D.G, and VANRAVENSWAAY F., 1977. Seasonal incidence of estrus and interestrous interval for bitches of seven breeds. *J. Am. Vet. Med. Assoc.*, **171**: 271-273

SOKOLOWSKI, J.H. and ZIMBELMAN, R.G., 1977. Canine reproduction: effects of multiple treatments of medroxyprogesterone acetate on reproductive organs of the bitch. *Am. J. Vet. Res*., **35**:1285-1287

SRIDEVI, P. 2001. Determination of ovulation and fertile breeding time to optimize conception in bitches. Tamil Nadu Veterinary and Animal Science University. India, 62-89

SRINIVASAN, S. R., 2011. Present status of dog genetic resources of Tamil Nadu. In: *Workshop Manual on Conservation of Animal Genetic Resources of Tamil Nadu,* organized by TANUVAS on 23-24, June, 2011 at Chennai, Tamil Nadu. Pp.41-50.

STRASSER, H and SCHUMACHER, W., 1968. Breeding dogs for experimental purposes. II. Assessment of 8-year breeding records for two Beagle strains. *J. Small Anim. Pract.*, **9**:603–612

STURGESS, K., 1998. Infectious diseases of young puppies and kittens. *In: Manual of Small Animal Reproduction and Neonatology, Simpson, G. M., England, G. C. W. and Harvey, M.(Ed.). Brit. Small Anim. Vet. Asso.,* 159-166

STURGESS K., 2006. Feline paediatric medicine**.** *Eur J Comp Anim Pract* 2006, **16:**83-94.

SURIYASOMBOON A, LUNDEHEIM N, KUNAVONGKRIT A, and EINARSSON S. 2005. Effect of temperature and humidity on sperm morphology in Duroc boars under different housing systems in Thailand. *J. Vet. Med .Sci*; **67**: 777–785.

SUYASH VARDHAN BHAL., 2002. Studies on management of mating time in bitches by vaginal exfoliative cytology and vagonoscopy. *M.V.Sc. Thesis,* Veterinary College, University of Agricultural Science, Bengaluru.

TEDOR, J.B. and REIF, J.S.1978.Natal patterns among registered dog in the United States. *J. Am. Vet. Med. Asso.*, **72**: 1179-1185

THAMASSEN, R., SANSON,G., KROGENAES, A., FOUNGER, J.A., BERG, K.A. and FARSTAD, W., 2006. Artificial Insemination with frozen semen in dogs: a retrospective study of 10 years using a non-surgical approach. *Theriogenology*, **66**:1645-1650

THATCHER, M.J.D., SHILLE, V., BUHI, W.C., ALVAREZ, I.M., CONCANNON, P.W., THIBEAULT, D. and COTTON, M., 1994. Canine conceptus appearance and de novo protein synthesis in relation to the time of implantation. *Theriogenology*, **41**:1679-1692

THOMASKOVIC, A., MAKEK, Z., CERGOLJI, M., HERAK, M., KRAMARIC, G., BARAC. and GETZ. I., 1997. Use of Serozyme Progesterone Test in determining the reproductive status of German shepherd bitches. *Vetrinarski. Archiv.* **67**(5):179-185

TSUTSUI, T. and SHIMIZU, T., 1975. Studies on the physiology of reproduction in the dog - I. domestic dogs. *Proc. of the Society for Theriogenol., pp.* 159.

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

TSUTSUI, T., HORI, T., KIRIHARA, N., KAWAKAMI, E. AND CONCANNON, P.W., 2006. Relation between mating or Ovulation and the duration of gestation in dogs. *Theriogeniology,* **66**: 1706-1709

UGWUENE, M. C., 2011. Effect of Dietary Palm Kernel Meal for Maize on the Haematological and Serum Chemistry of Broiler Turkey. *Nigerian Journal of Animal Science,* **13**: 93-103

UHRILKOVA, A LACNAKOVA, K TANDLEROVA, V KUCHAROVA, K REHAKOVA, E JANOVA and J DOUBEKA., 2013. Haematological and biochemical variations among eight sighthound breeds *Aust. Vet. Journal Volume* 91: No 11, November 2013

URFER S. R. 2009: Inbreeding and fertility in Irish Wolfhounds in Sweden: 1976 to 2007. *Acta Veterinaria Scandinavica,* **51:** 12.

VAN DER BEEK, S., NIELEN A. L. J., SCHUKKEN Y.H., and BRASCAMP EW:1998. Evaluation of genetic, common-litter, and within-litter effects on cohort of boxer puppies. *Vet .Rec* **142**:602-606.

VAN HAAFTEN, B., DIELEMAN, S.J. and OKKEN, A.C., 1989. Timing the mating of dogs on the basis of blood progesterone concentrations. *Vet. Rec*., **125**: 524-526

VELLOSO, A.I. WASSER, S.K . MONTIM, S.L. and DIETZ , J.M.,1998. Longitudinal steroid excretion in maned wolves. *Gen Comp Endcrinol.,* **112**: 06- 107

VENKATACHALAPATHY, J.N., 2005. Studies on determination of mating time in bitches. *M.V.Sc. Thesis,* Veterinary College, Bangalore. Karnataka veterinary, animal and fisheries sciences university, Bidar

VERSTEGEN, J., ONCLIN, K., SILVA, L. and CONCANNON, P., 1997. Termination of obligate anestrus and induction of fertile ovarian cycles by administration of purified pig

VITASEK, K., CIHALOVA, P. and ZAJIC, J., 2002. Practical experience with timing the mating of bitches on the basis of progesterone level in the peripheral blood. Folia. *Veterinaria.* **46(**1): 42-43

VITASEK, R. and BARTOSKOVA A., 2006. Influence of body weight and litter size on the duration of gestation in the bitch. Proceedings 5th Biannual EVSSAR Congress Budapest, Hungary : 301

WIKSTROM C, and LINDE-FORSBERG C. 2006. Fertility and fertility problems in the Chow-Chow, mainly an autumn breeder. In: *Proceeding of the 5th Biannual Congress, European Veterinary Society for Small Animal Reproduction (EVSSAR). Budapest, Hungary*: 2006. p. 294.

WILDT, D.E., CHAKRABORTY, P.K., PANKO, W.B. and SEAGER, S.W., 1978. Relationship of reproductive behavior, serum luteinizing hormone and time of ovulation in the bitch. *Biol Reprod.,* **18**: 561-570

WILDT, D.E., PANKO, W.B., CHAKRABORTY, P.K. and SEAGER, S.I., 1979. Relationship of serum estrone, estradiol - 1713 and progesterone to Lii. sexual behavior and time of ovulation in the bitch. *Biol. Reprod.,* **20**: 648-58

WILDT, D.E., SEAGER, S.W. and CHAKARBORTY, P.K., 1981. Behavioral, ovarian and endocrine relationship in the pubertal bitch. *J. Anim. Sci.,* **53**: 182-191

WRIGHT, P. J and PERRY, B. W., 1989. Cytology of canine reproduction system, *vet.Clin. North. Am*., **19**: 862-874

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

WRIGHT, P.J., 1990. Vaginal cytology and plasma progesterone determinations to the management of reproduction in the bitch. *J. Small Anim. Pract.,* **31**: 335-340

WYDOOGHE E., BERGHMANS E., RIJSSELAERE T., and VAN SOOM A. 2013: International breeder inquiry into the reproduction of the English bulldog. *Vlaams Diergeneeskundig Tijdschrift*, **82:** 38–43.

XAVIER LEVY and ALAIN FONTBONNE., 2007. Determining the optimal time of mating in bitches. *Rev. Bras. Reprod. Anim.,* **31**(1): 128-134

YAMADA, S., SHIMAZU, Y., KAWAJI, H., NAKAZAWA, M., NAITO, K. and TOYODA, Y., 1992. Maturation, fertilization, and development of dog oocytes in vitro. *Biol. Reprod,* **46**: 853-858

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

YENER, T.A.T., TUNC, H., ASIAN, H., AYTAN. and CALISKAN, A.C., 2007. Determination of oestrous cycle of the rats by direct examination. *Anat. Histol. Embryo*., **36**: 75-77

ZERON, Y., OCHERETNY, A., KEDAR, O., BOROCHOV, A., SKLAN, D., and ARAV, A. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. *Reproduction*; **121**: 447–454.

ZOLDOG, L., KECSKEMETHY, S., TOLNAI, G. and NAGY, P. 1994. Possibilities of the diagnosis of estrus and ovulation in dogs. *Magyar Allatorvosok Lapja.,* **49**(2): 114-119.

Abstract

VIII. ABSTRACT

An experiment was designed to document the reproductive efficiency parameters in Mudhol Hound breed of dogs and to determine if the reproductive efficiency parameters are influenced by system of feeding, namely feeding with a nutritionally balanced diet or homemade diet. It was observed that the pups delivered from dogs maintained on homemade diet were significantly lower (birth weight) and had significantly higher prevalence of neonatal mortality. The litter size was also smaller in dogs maintained on homemade diet (5.76 ± 0.31). However the sex ratio was not influenced by the type of feeding. Further, Mudhol Hound dogs maintained on homemade diet achieved threshold body weight required for attaining puberty of later age (16.03 ± 0.06 months) as compared to those maintained on nutritionally balanced food (12.40 ± 0.64 months). Type of nutrition did not seem to influence the duration and character of pro estrus and estrus. However, the conception rate of female dogs maintained on the homemade diet was significantly lower (50.00 per cent) as compared to those maintained with nutritionally balanced food (63.34 per cent). The system of feeding also did not seem to influence the incidence of pseudo pregnancy, gestational, parturient and post parturient disorders in Mudhol Hound female dogs. However, pups delivered from dogs maintained on nutritionally balanced diet had a better puppy survival rate. Further, the blood hemoglobin, random blood glucose, total serum protein and serum progesterone concentration during pubertal estrus were all within the reported range and system of feeding did not seem to alter any of these parameters studied. It is concluded that when Mudhol Hound dogs maintained for breeding purpose, feeding of nutritionally balanced diet would reduce age of onset of puberty, improve the conception rate and increased puppy birth weight and reduced prevalence of neonatal mortality.

Key words: Pubertal age, Birth weight, Estrous cycle, Conception rate, Neonatal mortality, Serum progesterone concentration