V. DISCUSSION

Traditionally, breeders have assumed the parturition date as 62-64 days from the day of first mating. In nature, however, the interval from mating to parturition may vary widely (59 to 68 days), making it difficult for breeder to plan the management of parturition. The ability to predict the parturition date at the time of initial mating itself could be of considerable advantage to the breeder as well as to the veterinary obstetrician. However, determination of gestational age and prediction of parturition date presents a challenge.

During the course of the present investigation, 113 bitches were diagnosed pregnant on ultrasound examination and all these bitches had been mated on predetermined dates. These bitches delivered at variable times from the day of first mating and the interval from the first mating to the parturition date ranged between 59 to 72 days and only 9.7% of animals whelped between 62 to 64 day after the first day of mating. The range of the gestation length observed in the present study agrees with the reports of Doak, et al. (1967); Phemister (1973); Wildt et al. (1978); Concannon et al. (1983); Concannon (1986) and Moiser (1986), and emphasize the inaccuracy of predicting the parturition date as the interval from the day of first mating to the day of whelping is quite variable.

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 a., (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 at least 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-Deslanders (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; Okkens et al., 1995). 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 dogs. Furthermore, the number of animals in each breed was
considered too small to draw any conclusions on the influence of breed, litter size, age or parity on the length of gestation in dogs. Nevertheless, if factors such as breed, age, parity or litter size have any effect on the length of gestation in dogs, it is unlikely that it contributes significantly to the high variability (58 to 72 days) observed in the interval from mating to whelping between individual dogs when the matings are unplanned.

A more likely explanation for the apparent variability in the gestation length of bitches appears to be due to the tendency of the female to accept the male from 5 to 6 days before to 2 to 3 days after ovulation. Additional variability may be caused by the prolonged survival of ova (up to 3 days; Holst and Phemister, 1971) and of spermatozoa (up to 6 days; Doak et al., 1967; Concannon et al., 1983).

The rate of canine embryonic development is reported to be synchronous and closely correlated to the time of preovulatory LH surge. Therefore, gestational age and hence, parturition date can be determined more accurately when timed from preovulatory LH surge (Concannon et al., 1983). A gestation length of 65±0.1 day was clinical practice, methods for estimation of LH are not easily available, besides being expensive and time consuming. Therefore, the use of identification of LH surge at the time of mating for prediction of the future parturition date has not found favor with the practicing obstetricians.

Serum progesterone (P₄) concentrations have been reported to increase gradually from basal values with the advancement of proestrus and exhibit a distinct, rapid and detectable increase around the time of LH surge (Concannon, 2000). Identification of this rapid distinct rise has been proposed as an indirect method of predicting the LH surge,
and therefore, the parturition date (Concannon et al., 1983; Goodman, 1998; Wright, 1990; Okkens et al., 1993; Kang et al., 1997; Kutzler et al., 2003; Tsutsi et al., 2006). Elevation of serum P₄ concentration prior to ovulation is reported to be peculiar to bitches and the source of this elevation is reportedly the preovulatory lutinized follicles (Bouchard et al., 1991).

Determination of serum P₄ concentration can be carried out using radioimmunoassay (RIA) or ELISA based techniques. In the present study, serum P₄ concentrations were serially determined during proestrus in an effort to identify the preovulatory rise in serum P₄ concentration using an Enzyme linked florescent assay (ELFA). It has been reported that the pattern of serum P₄ concentration using RIA is similar to that found using ELISA (Dieldman and Blankenstein, 1998). Unlike RIA, detection of serum P₄ concentration on tests based on ELISA techniques are available through many human clinical laboratories. ELFA has the advantage of continuous processing allowing for rapid turn around time without the disadvantages associated with isotopic assay (Radioactivity). The technique is also rapid and detection limit is fairly high.

The mean preovulatory serum P₄ concentration in the present study was recorded to range between 2.02 to 3.95 ng/ml with a mean of 3.01 ± 0.03ng/ml. The serum P₄ concentration identified as the preovulatory P₄ concentration in the present study is in close agreement with the reports of Wright (1990) (2to4ng/ml) and Renton et al.(1991) (3ng/ml). In other reports, the preovulatory serum P₄ concentration was determined to be much lower (Johnston and Root, 1995:1to1.9ng/ml; Concannon et al., 1977a:0.8to3ng/ml
and Kutzler et al., 2003:2.02±1.8 ng/ml). To identify accurately, the day of initial rise in
the preovulatory serum $P_4$ concentration, it is important to obtain serum samples every
other day beginning early in proestrus (Kutzler et al., 2003). It is also important to
establish the likely range of serum preovulatory $P_4$ using a particular technique (RIA or
ELISA) in a given laboratory.

In the division of Small Animal Reproduction where the present studies were
carried out, serum $P_4$ concentration estimated by ELFA has been consistently used for
investigation of infertility in bitches and for recommendation of the optimum time for
mating. Research reports from this laboratory indicated that the overall conception rate
was 44.44% when the serum $P_4$ was between 1 to 2 ng/ml and 87.5% when it was more
than 2 ng/ml (Suyash, 2002). In another study from the same laboratory
(Venkatachalapathy, 2005), 93.88% of the bitches conceived where matings were
recommended when the serum $P_4$ was over 4 ng/ml as estimated by ELFA and it was
concluded that serum $P_4$ concentrations over 4 ng/ml are closer to the time of ovulation
rather than the preovulatory concentrations. Mean serum concentration of 3.01 ±
0.03 ng/ml recorded in the present study are therefore, suggestive of a preovulatory LH
surge as progesterone concentrations were determined using the same technique and in
the same laboratory as in studies by Suyash (2002) and Venkatachalapathy (2005).

In the present study, owners had been advised to be getting their bitches mated
once on the day of initial rise in the serum progesterone concentration and second time 48
hrs later. This was partly because of the fact many owners were not to willing to accept
the recommendation of a single mating and in any case would still have gone for a second
mating. However, in the present study, parturition date was determined as 65 days from the day of first mating, which was determined to have coincided with the preovulatory LH surge as estimated by serum progesterone concentrations. Parturition occurring before 65 days was considered from mating after the LH surge/ovulation and parturition beyond 65 days were determined to have occurred from matings earlier to the preovulatory LH surge.

In the present study, parturition occurred over a wide range of 59 to 68 days and the average gestation length was determined as 64.29 ± 0.14 days. These figures are more closer to the predicted date of parturition (65) than those in the (61.4±1.5 days) reports of Okkens et al. (1993). However, in the present study only 18 (16.82%) out of 107 bitches delivered on the predicted date of parturition and almost a similar number of animals (18.69%) delivered one to three days beyond the predicted date of parturition indicating that in these animals the matings were recommended before the onset of preovulatory LH surge. In two third (64.48%) of the animals, the actual delivery date was 1 to 3 days prior to the predicted date suggesting that the recommended mating time was closer to the time of ovulation rather than the preovulatory LH surge.

Many breeders may be reasonably happy if they are provided with a parturition date within ±1 or 2 days from the exact predicted date of parturition (63 to 67 days). In the present study, 65.42% of the animals delivered within ±1 day and the accuracy of predicting parturition within ±2 days was fairly high (87.85%). The results of the present study are in very close agreement with the reports of Kutzler et al. (2003) who studied
the percentage accuracy of predicting the parturition date on the basis of serum progesterone concentrations as 67.90 and 90% for within ± 1 and ± 2 days respectively.

The results of the present study seem to suggest that, prediction of the exact date of parturition using preovulatory serum progesterone concentration could be difficult; part of this problem and could arise from the criteria used for defining the preovulatory P4 concentration. In the present study, serum progesterone concentrations were determined serially and preovulatory progesterone concentration was determined as that concentration which was at least 1ng/ml over the previous reading. It is possible that when this criterion is used, LH surge might either precede or follow by 48 hours in nearly 90% of the animals and hence, parturition can only be predicted to within ±2 days in a majority of the animals.

Ultrasonography has been widely used as a method of diagnosing early pregnancy and fetal viability in the bitch (Johnston et al., 1983; Shille and Gontarek, 1985; Barr, 1995; and England and Allen, 1990). In the literature, there are only few reports on the use of ultrasonography to determining gestational age and prediction of parturition date. Most of the authors agree that the chorionic cavity diameter (gestational sac diameter; GSD) was a good indicator for the evaluation of embryonic development in early pregnancy and the most accurate predictor of gestational age between days 20 and 37 in the dog (Yeager et al., 1992). From days 38 to 60, fetal head diameter was the most accurate predictor of gestational age (England and Allen, 1990; Yeager et al., 1992) but fetal crown-rump length (CRL) and body diameter (BD) were also significant (Yeager et
al., 1992). It was also noted that fetal CRL was difficult to measure after day 48 because of fetal flexion and fetal size, which exceeded the sector image field.

Although, much has been learnt regarding the application of B-mode ultrasonography in small animal reproduction, concerns have been raised regarding its utility for accurate estimation of fetal age and prediction of parturition date, in view of the great differences in their size, confirmation and litter size. Nevertheless, Mattoon and Nyland (1995) analyzed the data on the measurements of GSD or HD reported by Feldman and Nelson (1987) and Yeager et al. (1992), at different stages of pregnancy in bitches, modified the same and proposed formulas to predict the gestational age and days before parturition in the dog. Presently, these formulae have been commonly used by veterinary obstetricians for estimation of fetal age and prediction of parturition date in dogs, irrespective of their body size or confirmation. The present study also utilized the formulae proposed by Mattoon and Nyland (1995) for calculation of gestational age and prediction of parturition date.

In the present study, the parturition date predicted through measurements of chorionic sac diameter in 44 bitches was same as the actual date of parturition in only 4.5% of the animals. Poor correlations were observed between the parturition date predicted through measurements of head diameter in 69 other animals and actual parturition date. Further the accuracy of predicting the parturition date within ±1 day and ±2 days were also very poor with measurements of both gestational sac diameter as well as head diameter. Although, percentage accuracy of prediction of parturition date using measurements of gestational sac diameter was significantly superior to the predictions
using head diameter, the percentage accuracy was still very low for any sort of recommendations. Similarly, estimation of prediction of litter size on the basis of number of fetal head, visualized through ultrasonography, was significantly better \((p<0.05)\), over the prediction through enumeration of gestational sac diameter, but again no conclusions could be reached regarding the technique that could be recommended for estimation of litter size.

On the other hand, Luvoni and Grioni (2000) reported that, measurements on gestational sac diameter was 90.9\% accurate and that of biparietal diameter was 70.8\% accurate for determining the date of parturition to within ± 1 day. Kutzler et al. (2003) reported that the accuracy of prediction of parturition date to within ± 1 day and ± 2 day was 75 and 87\% respectively, on the basis of measurements of gestational sac diameter. However, parturition date predictions using only biparietal diameter and body diameter were less than 50 \% accurate within ± 2 days.

In the reports of Luvoni and Grioni (2000) and Kutzler et al. (2003) the body size of the bitch was also taken into consideration for predicting parturition date on the basis of measurements of gestational sac diameter or head diameter. These studies predicted the parturition date using previously published tables for bitches with similar body weights. In other studies, accuracy of prediction of parturition date has been reported in a particular breed (Kang et al., 1997; Velocky et al., 1997; Son et al., 2001). In the present study, although prediction of parturition date based on either GSD or HD was attempted in a fairly large number of animals, their body weight varied widely ranging from 10 to 80 kgs. It is therefore, the breed or the body weight of the bitch may have significant
influence on the GSD or HD and that breed or body size of the animal should be taken into account while predicting the parturition date using ultrasonic measurements.

In the present study, the overall accuracy of prediction of litter size based on the enumeration of the number of gestational sacs or fetal heads, was also poor, the percent accuracy for predicting the exact litter size was 13.63% with gestational sacs and only slightly better with that of fetal heads (18.63%). Toal et al. (1986) have similarly reported that ultrasound was only 36% accurate for fetus counting, and in the studies of England and Allen (1990) accuracy of prediction of litter size was 31.8 percent.

There has been general acceptance that fetal number cannot be accurately judged with ultrasonography. Mattoon and Nyland (1995) also stated that in their experience, obsolete; accurate determination of fetal number is unreliable, especially at early pregnancy and late in gestation. Similar opinion has also been expressed in other reports (Shille and Gontarek, 1985; England et al., 1990).

The difficulties in predicting the litter size with the use of B-mode ultrasonography has been stated to be due to the fact that only the small portion of the reproductive tract, can be imaged in a given scan plane (Mattoon and Nyland, 1995). Therefore, fetuses may be inadvertently imaged a second time (giving too higher number) or missed when scanning (giving too lower numbers). In the studies of England and Allen (1990) the greatest tendency was to under estimate the number of conceptuses, inaccurate counts due to over estimation of fetal number occurred in 20 percent of the pregnancies. In the present study also, the tendency was to underestimate the number of conceptuses.
The results of the present study, seems to reaffirm that the prediction of litter size with the use of ultrasonography is inaccurate and difficult.

The present study also examined whether, the maternal body weight had any effect on prediction accuracy of the transabdominal ultrasonography. The study revealed that, it was difficult to predict the parturition date based on gestational sac diameter (GSD) in small to medium sized bitches, while, accuracy of prediction of parturition to within ±2days was as high as 80% in giant sized breeds. However, there were only 8 animals in the category of small to medium sized bitch and perhaps ultrasonographic studies on more number of animals under this body weight group may have given more conclusive results. Prediction of parturition date was also very difficult in small to medium sized breeds with measurements of head diameter, and as with GSD measurements, the accuracy of prediction was significantly higher for giant sized breeds (63.63%). However, an accuracy of predicting parturition date to the extent of only 63.63% and that too for predictions for ± 2 days cannot be considered reliable.

There are very few reports on the influence of maternal body weight for prediction of parturition with ultrasonographic measurements. Kutzler et al. (2003) reported that an accurate prediction of parturition date can be made using transabdominal ultrasonography irrespective of the maternal body weight, if correction factors are used in small and giant sized breeds. The study also observed that there was no significant difference in the accurate prediction of parturition between early and mid gestation, using fetal measurement table published by Yeager et al. (1992). Prediction made from ultrasound examinations performed during late gestation using the fetal measurement
table published by England et al. (1990), were significantly less accurate. In contrast to these reports, Luvoni and Grioni (2000) stated that prediction of parturition date was accurate to within one day in both small and medium sized breeds. The study however, did not clearly state the method used for determination of parturition date using ultrasonographic measurements.

The results of the present study, suggest that, even among the breeds with a variation of minor differences in body weight, it may be necessary to develop prediction tables for determination of parturition date using ultrasonographic measurements. The study also establishes that the universal formula suggested by Mattoon and Nyland (1995) cannot be applied uniformly in all breeds.

Prediction of litter size within ±2 fetuses of the actual litter size was possible to the extent of 80 to 87% accuracy in small to medium sized bitches. With large sized breeds, the accuracy of prediction of litter size was 61.90% and 77.77% based on enumeration of gestational sac and head respectively. The accuracy of predicting the litter size in giant size breeds was very poor. There have been no studies to determine whether, the body size makes it easier or tougher to estimate litter size using ultrasonography. The results of the present study again seem to suggest that the accuracy of determining the litter size is perhaps not much influenced by the maternal body size and that variations between the estimated and the actual litter size are probably due to other factors as described earlier.

The present study also made an attempt to construct a table for predicting the parturition date in mongrel bitches. For this purpose, Mongrel bitches of unknown
gestational age were subjected for ultrasonographic measurements of either sac diameter or head diameter, and approximate age was determined retrospectively from the date of delivery. Following construction of the table the gestational age of another 6 mongrel bitches were calculated using the prediction table developed and the parturition date was predicted. All the 6 batches delivered within ±2 days of the predicted date of parturition. This observation emphasize that prediction of date of parturition could be more accurate if prediction tables are developed for each breed of the bitch or for bitches with similar body weight and confirmation. It was also observed that when the gestational age for other breeds was calculated using the prediction table developed for the mongrel breeds, the percentage accuracy was between 50 to 70 percent only.

In the present study, the onset of behavioral, mammary and genital changes in relation to parturition date in bitches was most unpredictable. While some bitches exhibited nesting behavior as early as 20 days before parturition, in others, it was either not noticed or noticed only on the day of parturition. Panting commonly believed to be a sign of impending parturition was not observed in all the animals. Similarly, the onset of lactation and mucoid vaginal discharges commenced at variable time before the parturition date.

The behavioral and mammary changes are commonly used by the breeders to predict the parturition date in bitches. The results of the present study, however, indicate that they are not reliable signs for predicting the parturition date. The variability in the time of onset of the mammary and behavioral changes has also been reported earlier (Buckner, 1979; Roberts, 1986a; Johnston et al., 2001).
In the present study, the rectal temperature was monitored in 11 mongrel bitches during the later stages of pregnancy in an attempt to identify the reported prepartum drop in rectal temperature. It has been stated that, a transient and prepartum temperature drop occurred in 78 of 80 pregnancies in which rectal temperature was recorded daily or most frequently (Concannon et al., 1977). It is suggested that drop in rectal temperature was related to the prepartum luteolysis resulting in a rapid decline in serum progesterone and that the decline in serum progesterone which is a thermogenic hormone and is responsible for prepartum drop in rectal temperature (Johnston et al., 2001).

In the present study, rectal temperature ranged from 100 to 102 °F in all the animals until two days prior to whelping. Subsequently, a significant drop in rectal temperature which was characterized by the temperature declining to below 99 °F was recorded in 9 out of 11 animals. In all these 9 animals, the delivery occurred between 12 to 24 hours after the significant decline in rectal temperature. In two animals the prepartum decline in rectal temp could not be identified in spite of frequent recording.

The present study documented that 81.8 percent of the animal showed significant decline in rectal temperature 12 to 24 hrs before whelping. The findings of the present study with regards to prepartum drop in temperature has also been documented in earlier studies (Long et al., 1978; Concannon, 2000; Johnston et al., 2001; Veronesi et al., 2002). However, the drop in temperature was transient and the rectal temperature in all these animals had risen to over 100 °F by the time next recordings were carried out. It is possible that in 2 other animals where, the prepartum decline in rectal temperature could not be identified, the decline did occur but it was too transient.
The present study confirms the utility of recording /monitoring of rectal temperature for predicting the parturition date, the accuracy being fairly high. However; it has its disadvantages in that, (a). the identification of prepartum drop may needs frequent recordings, (b). prepartum drop may be transient and may be missed (c). it is only during the final few hours of parturition that the prepartum drop in rectal temperature can be identified and parturition can be predicted. And the owner may desire that the parturition date is predicted much earlier.

In conclusion, the present study revealed that it is difficult to predict the exact date of parturition with the available techniques. Although, parturition occurs shortly after the prepartum drop in rectal temperature, the technique does not predict the parturition in dogs well in advance; making it difficult for a breeder to plan management of parturition. The onset of physical, behavioral and mammary changes in relation to the parturition date varies too widely between the individual animals making them unreliable. This is attributable to differences in the size of breed as well as its confirmations. Ultrasonography was also not useful for the predicting litter size. There is clearly a need to establish parturition prediction tables using ultrasonographic measurements for each breed of dog. Prediction of parturition date on the basis of initial rise in preovulatory serum progesterone concentration was accurate to the extent of ± 2 days in nearly 90 percent of the animals. Unlike ultrasonography which requires, experience in interpretation, besides the initial cost of equipment, progesterone assays can be easily carried out in many clinical laboratories. It is less expensive, rapid and accurate. Prediction of parturition can be carried out with a fair degree of accuracy at the time of recommendation of breeding of the bitch, allowing the breeder a considerable time to plan the management of whelping.