ULTRASONOGRAPHIC ASSESSMENT OF PLACENTAL THICKNESS IN DOGS AND ITS RELATIONSHIP WITH GESTATIONAL AGE

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By

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CERTIFICATE

This is to certify that the thesis entitled ***"ULTRASONOGRAPHIC ASSESSMENT OF PLACENTAL THICKNESS IN DOGS AND ITS RELATIONSHIP WITH GESTATIONAL AGE"*** submitted by **Mr. CHANDRASHEKHAR AYYA SALIMATH., ID No. MVHK-1622** in partial fulfilment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fisheries 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 this thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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(Dr. NAVEEN KUMAR. S.)

Affectionately to My Beloved Parents,

Smt. Lalitha and

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and My lovingly wife,

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LIST OF ABBREVIATIONS

AC	:	Abdominal circumference
HC	:	Head circumference
BD	:	Body diameter
BPD	:	Biparietal diameter
CGA	:	Calculated gestational age
Cm	:	Centimetre
CRL	:	Crown rump length
CTUP	:	Combined thickness of the uterus and the placenta
DBP	:	Days before parturition
DPTV	:	Deep portion of telencephalic vesicle
EVD	:	Embryonic vesicle diameter
FL	:	Femur length
GA	:	Gestational age
GSD	:	Gestational sac diameter
HD	:	Head diameter
HDT	:	Diameter of foetal heart
ICC	:	Inner diameters of the chorionic cavity
IUGR	:	Intra uterine growth restriction
L/S	:	Lecithin/ Sphingomyelin
LH	:	Luteinising hormone

LMP	:	Last menstrual period
MBD	:	Mean body diameter
MHD	:	Mean head diameter
MHz	:	Mega hertz
Mm	:	Millimetres
N	:	Number
OUD	:	Outer uterine diameter
PT	:	Placental thickness
RGA	:	Reported gestational age
SE	:	Standard error
USG	:	Ultrasonography

Introduction

I. INTRODUCTION

Early pregnancy diagnosis and determination of foetal age are of vital importance in companion animal reproductive management. In addition, accurate prediction of day of parturition can help to manage the parturition or plan a caesarean section in pregnant bitches with history of unknown mating or multiple matings (Luvoni and Beccaglia, 2006).

Ultrasonographic biometry of foetus is being applied in both human and veterinary obstetrics. Determination of gestational age, predicting the day of delivery, assessing the weight of foetus and identifying the intra uterine growth retardation (IUGR) of foetus are of critical importance in human obstetrics which involves accurate measurements made by using ultrasonography (USG). Different foetal parameters considered for dating of pregnancy are crown rump length (CRL), biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL). In addition to these parameters, extra foetal structures like placental evaluation by USG has also been put in use to characterise placental position and morphologic changes as it matures (Gottesfield *et al.*, 1966; Kobayashi *et al*., 1970). Detection of placental abnormalities like non-immune hydrops, gestational diabetes and IUGR is possible by evaluation of placental morphology using USG. Further, another aspect of placenta, the placental thickness (PT) is also believed to be of clinical importance which can be measured in a relatively simple way. Measurement of PT can contribute to the management of foetus at risk (Jauniaux, 1992). Recently, several studies were also made

using PT as a parameter in estimating the age of foetus (Tannawattancharoen *et al*., 2000; Jain *et al*., 2001; Mital *et al*., 2002).

In veterinary obstetrics, USG has been put to use as an important imaging modality in pregnancy diagnosis (England and Allen, 1990; England and Yeager, 1993; Luvoni and Grioni, 2000), ennumering the conceptuses (England and Allen, 1990; Luvoni and Grioni, 2000) and assessing the development of foetus (Moriyoshi *et al*., 1996; Son *et al*., 2001). Gestational aging of foetus based on USG examination of foetal and extra foetal structures and prediction of day of parturition were reported by several workers (England *et al.*, 1990; Yeager *et al.*, 1992; Moriyoshi *et al.*, 1996; Luvoni and Grioni, 2000; Son *et al.*, 2001; Luvoni and Beccaglia, 2006).

Dogs belong to the group of species that have zonary type of placenta. In dogs, the placentation is complete zonary type with marginal hematomas (hematophagus zones). These marginal hematomas are the bands of maternal haemorrhage at the margins of zonary placenta. Using USG the echogenic inner layer surrounding the gestational sac can be identified as zonary placenta as early as 16-21 days (Aissi, 2008).

Gestational sac diameter (GSD), crown rump length (CRL), body diameter (BD), biparietal diameter (BPD), deep portion of telencephalic vesicle (DPTV) and diameter of foetal heart (HDT) are the different foetal and extra foetal measurements that are considered during ultrasonography (USG) in determining the age of foetus and predicting the days before parturition (DBP) in canines. However, GSD and BPD are believed to be in high correlation with gestational age (GA) during early and late phases of pregnancy, respectively (Luvoni, 2013). Studies on measurement of placental thickness and its

relation to gestational age are sparse. Since placenta is the first foetal organ to develop and having primordial and critical functions, it seems logical that it should mature in a similar manner to that of other foetal organ systems. Measurement of placental thickness can be an indicator of foetal age in dogs (Maldonado *et al.*, 2012).

In humans, it is reported that placental thickness (mm) increased in a linear fashion with increase in gestational age (weeks) and almost matched it from 11 to 35 weeks of gestation (Abu *et al*., 2009; Adhikari *et al*., 2015). In this study, it is hypothesised that trans-abdominal ultrasonographic evaluation would help to detect parturition date with better accuracy in bitches of different body sizes, as the growth rate of placenta would be similar in all dogs irrespective of their body size. This study was aimed at assessing the progression of placental thickness and its use as a determinant of gestational age in routine USG of pregnant dogs. The objectives of the present study are:

1. To assess the placental thickness in dogs with the normal pregnancies using ultrasonography.

2. To correlate the measured placental thickness with that of the gestational age reported by the owners.

Review of Literature

II. REVIEW OF LITERATURE

2.1 Pregnancy diagnosis

The early determination of pregnancy and gestational age are important for reproductive management in small animal practices. The canine gestational length is highly variable when timed from the day of breeding (58 to 71 days) (Rendano *et al*., 1984; Concannon, 2000; Johnston *et al*., 2001). This wide interval of 13 days makes it difficult to accurately predict the parturition time considering the mating date or breeding. Therefore, a more appropriate event, such as ovulation, should be considered to define day 0 of pregnancy and consequently, to predict the day of parturition in the bitch. When the bitch is first subjected for pregnancy diagnosis and when day 0 cannot be determined, prediction of parturition day or time becomes difficult.

Various techniques have been developed and used to ensure accurate and early pregnancy diagnosis in canines. The similarity in the endocrinology and behaviour of pregnant and non-pregnant dogs are the major constraints in the early diagnosis (Bhardwaj, 2008). Ultrasound has proved to be an accurate and useful imaging modality for determining pregnancy (England and Allen, 1990; England and Yeager, 1993; Luvoni and Grioni, 2000), evaluation of foetal viability (England *et al.*, 1990; Luvoni and Grioni, 2000) and to assess the foetal development (Moriyoshi *et al*., 1996; Son *et al*., 2001). Ultrasound is considered to be the most sensitive and specific method in evaluation of gestational age. It helps in managing parturition or planning a Caesarean section in pregnant dogs with multiple matings or unknown mating time (Luvoni and Beccaglia, 2006).

Day of delivery can be determined by the ultrasonographic measurement of foetal and extra foetal structures like gestational sac diameter (GSD), mean head diameter (MHD) or biparietal diameter (BPD), mean body diameter (MBD), crown rump length (CRL) and placental thickness (PT) and the predictions are expressed as days before parturition (DBP) rather than days of pregnancy (Lopate, 2008).

2.2 Placentation

The formation of placenta, its type and structural arrangement refers to the Placentation. The placenta can be defined as an organ formed by the sustained apposition or fusion of foetal membranes and parental tissue for physiological exchange (Griffith and Wagner, 2017). The placenta is a foetal organ, which acts as a physiologic link between mother and the foetus. It has important metabolic, endocrine and immunologic functions besides being responsible for nutrition, respiration and excretion for the foetus. Placenta has a role in protecting the foetus from noxious agents. Placental size is a reflection of health and size of the foetus (Daftary *et al.,* 1998; Cross, 2005).

Dogs belong to the group of species that have zonary placenta. Other animals like cats, mustelids (ferrets, skunks), bears, seals and elephants also have zonary type of placenta. Histologically, placenta of dogs can be classified as endotheliochorial. In this type of placenta, the endometrial epithelium is not included in implantation and foetal chorionic epithelial cells come to be in contact with maternal endothelial cells. During implantation, cytotrophoblast cells surrounding the central third of chorioallantois proliferate to form syncytium called syncytiotrophoblast. The syncytiotrophoblast erodes through the endometrial epithelium and flows around maternal capillaries. Initially, the

invading foetal cells are in the form of villi, but coalesce soon to form a labyrinthe type of placenta. Canine placenta being zonary has a Marginal hematoma (hematophagus zones) or paraplacenta, characteristic features of dog's placentas which are bands of maternal haemorrhage at the margins of zonary placenta. The distinct green colouration in dogs during parturition is the result of haemoglobin breakdown products (Wooding and Burton, 2008).

Aralla *et al.* (2013) in their study found that development of placental labyrinth was complete by 18 days of gestation in dogs. They described the morphology and histology of canine placenta at various stages of pregnancy. They observed that foetal and maternal tissues indent with each other in a lamellar shape with growing complexity. The lacunar spaces made by syncytiotrophoblast beneath the labyrinth grow as a spongy structure of glandular chambers. Allantoic vessels penetrate in to the labyrinth by 45 days of gestation resulting in the formation of secondary villi. Placental labyrinth by then continues to expand with involvement of foetal and maternal blood vessels and attains its complete morphological organisation. Glandular chambers in the spongy structure increases only proportionally with increase in the foetal growth. As the pregnancy advances, a thin layer of connective tissue develops between the epithelium and the enormous blood vessels in the yolk sac becoming thicker until the time of parturition. All the extra embryonic tissues increase in their thickness as the pregnancy advances due to increase in the connective tissue component, which is highest at delivery which suggests that morphological modifications in the uterus occur almost daily during pregnancy concurrent with the foetal accommodation and its delivery at the right time (Aralla *et al*., 2013).

2.3. Determination of gestational age of foetus

2.3.1. Gestational sac diameter

During early gestation, the ultrasonographic measurement of extra-foetal structures is a method to assess gestational age and to predict the day of delivery in bitches. The most suitable parameter during this period of pregnancy is the measurement of the inner diameters of the chorionic cavity (ICC) (England *et al.,* 1990; Yeager *et al.,* 1992; Luvoni and Grioni, 2000).

Gestational sac or embryonic yolk sac was identified by ultrasonography as early as 18-19 days after LH surge (Nyland and Mattoon, 1995; Yeager *et al*., 1992; Aissi, 2008). They appeared as spherical anechoic structures of one to two mm diameter within the lumen of the uterus. Although embryonic sac develops as early as day 18-19, Nyland and Mattoon (1995) preferred to wait until day 30, as gestational sac with viable embryo could be identified precisely at that time. Also it was noted that embryonic vesicle diameter (EVD) increased linearly at a rate of one mm per day from days 17 to 30 (England, 1998).

Parturition was predicted with an accuracy of ± one day using gestational sac diameter in early pregnancies (Beccaglia and Luvoni, 2006). The diameters of the gestational sac in medium sized dogs were measured from day 42 to day 21 before parturition (day 0) and in small size dogs the measurements were taken from day 41 up to day 25 before parturition. Luvoni and Beccaglia (2006) also studied that litter size and sex ratio did not affect the prediction of parturition date by measurement of diameters of inner chorionic cavity (ICC). In small and medium sized dogs the measurement of ICC

was significantly related to the gestational age of foetus and was useful in predicting the day of parturition (England *et al.,* 1990; Yeager *et al.,* 1992; Luvoni and Grioni, 2000; Son *et al.,* 2001). However, for giant breeds of dogs prediction of parturition was made by use of correction factor of -2 days (Kutzler *et al.,* 2003). The determination of ICC was made by the mean of two ICC diameters made at 90° angles from one side of the trophoblastic decidual reaction to the other (Luvoni and Grioni, 2000). Multiple ICC measurements were taken during the same examination and mean values were considered. The inner diameters of the gestational sac in medium and small size bitches along with biparietal diameter were found to be most accurate in indicating the gestational age of foetus with adjusted coefficients of determination (R^2) > 0.8. During early pregnancy, the most suitable measurements for predicting the day of parturition were observed to be the inner diameter of the gestational sac (90.9%) in both small and medium sized bitches (Luvoni and Grioni, 2000).

The anechogenic gestational sac was first detected on day 18.0 ± 0.9 (range: 17 - 19) in 9 Miniature Schnauzer bitches with an average gestational duration of 63.0 ± 1.7 (range: 61 - 65) days (Kim and Son, 2007). Similarly, Aissi and Slimani (2008) first detected the gestational sac on day 17.88 ± 1.13 (range: 16-21) indicating that initial detection of the extra-foetal and foetal structures using ultrasonography can be used for early determination of pregnancy and to predict gestational age.

Javeid *et al.* (2013) recorded an average gestational sac diameter (GSD) ranging between 1.2 to 3.3 cm with mean 2.39 ± 0.15 cm, gestational age (GA) ranging between 27.2 to 39.8 days with mean 34.34 ± 0.90 days and days before parturition (DBP) ranging

between 25.2 to 37.8 days with mean 30.66 ± 0.90 days in a study on 20 bitches under 40 days of pregnancy indicating that gestational age before 40 days of gestation in bitches can be determined by measurement of gestational sac diameter (GSD). The gestational vesicles echo biometry performed in brachycephalic pregnant bitches during the 2^{nd}, 5^{th}, 6^{th}, 7^{th} and 8^{th} weeks of pregnancy were efficient, easy to implement and was helpful in improving the early determination of pregnancy (Feliciano *et al.,* 2015).

2.3.2. Biparietal diameter

Determination of gestational age may be influenced by the stage of embryo/foetal development. Recent results demonstrated that the accuracy of the prediction of parturition date in bitches was influenced by the gestational period when ultrasonographic measurements were performed, particularly for BPD. The biparietal diameter (BPD) measured from day 37 to day 1 before parturition in medium sized bitches and day 35 up to day 4 before parturition in small sized bitches showed that the regression was significantly and linearly related to gestational age in all cases. The BPD was considered as a fairly accurate measurement for prediction of parturition date in late pregnancy, during which the foetal head was easily identifiable (Luvoni and Grioni, 2000). Correct distances between the parietal bones of skull are measured when they are parallel to each other. The technique of measurement is relatively simple compared to other parameters and is highly correlated to the gestational age of foetus. The accuracy of prediction of delivery day (±1 day) was 75 percent and 63 percent in small and medium size dogs, while within ±2 days; it was 88 percent and 81 percent, respectively. Further, the accuracy was not affected by litter size and sex ratio (Luvoni, 2013).

Biparietal measurement, which is taken during late gestation, is likely affected by individual variability of growth when few foetuses are present or it may be less accurate when the overlapping of multiple foetuses in the same ultrasonographic image field occurs (Beccaglia and Luvoni, 2006). The foetal head diameter (HD) has been proposed as a predictor of parturition date (Yeager *et al.,* 1992; Moriyoshi *et al.,* 1996; Son *et al.,* 2001).

Beccaglia and Luvoni, (2006) found that measurement of BPD was highly accurate (± 1 day) within the first 6 weeks of pregnancy, afterwards a gradual decrease of the accuracy was observed, thus maintaining a good accuracy at ± 2 days until week 8. On the other hand, ICC measurements lead to a similar accuracy of the prediction at week 4 and 5.

Average head diameter (HD) in 20 bitches was between 1.5 to 2.4 cm with mean 1.92 ± 0.06 cm, gestational age (GA) ranging between 42.5 to 56 days with mean 48.87 ± 1.0 days and days before parturition (DBP) ranged between 9 to 22.5 days with mean 16.12 ± 1.0 days. It was concluded that after 40th day of gestation, GA can be calculated using head diameter (HD) and DBP can be calculated as 65 – GA (Javeid *et al.,* 2013).

2.3.3. Other foetal / extra foetal structures

The deep portion of telencephalic vesicle (DPTV) has been measured ultrasonographically to determine the gestational age of foetus. This was represented by thalamus and basal nuclei primordia and was visualized between the 30th and 8th day before parturition (Beccaglia *et al.,* 2003; Beccaglia and Luvoni, 2004). The reliability of determination of gestational age by measuring the DPTV growth in different body sized

dog breeds was better when combined with measurements of biparietal diameter (BPD). The DPTV gave an accuracy of about 40 percent with ± 1 day, 80 percent with ± 2 days in small breeds and 25 percent with ± 1 day and 62.5 percent with ± 2 days in medium sized dogs in determining the day of delivery. Beccaglia and Luvoni (2004) concluded that DPTV cannot be considered as an optimal parameter to predict the day of delivery when done alone.

CRL is another reliable parameter with high correlation to determine the gestational age of foetus. It is the distance between the most anterior part of crown to the caudal edge of perineum. Because of the flexion of the foetus both laterally and dorso-ventrally after 45 days of gestation, the CRL cannot be measured until delivery. Further, the measurement of CRL was made difficult due to overlying of other foetuses and increased length of foetus than the sector image field (England *et al.,* 1990; Yeager *et al.,* 1992; Son *et al.,* 2001).

Extra foetal structures like gestational sac, zonary placenta and foetal membranes were detected ultrasonographically around 16-21 days, 23-25 days and 24-26 days of gestational age, respectively. Other foetal parameters/structures which were detected initially by ultrasound imaging include embryo, heartbeat, limb bud, stomach, skeleton, lung and liver around 21-24, 22-24, 28-31, 32-35, 34-36, 35-37 and 35-37 days of gestation, respectively as indicated by Aissi and Slimani (2008).

Diameter of foetal heart (HDT) was measured in canines from 25 days before parturition to predict the day of delivery. The growth curve was obtained with the mean values of the major and minor axis of the maximum cross section of heart. However, the

measurements were not as reliable as they were affected by different phases of cardiac cycle (Moriyoshi *et al.,* 1996).

England *et al.* (1990); Yeager *et al.* (1992); Moriyoshi *et al.* (1996) used the body diameter (BD) to assess the gestational age of the foetus. The measurement was taken in the transverse plane at right angles at the level of foetal liver and stomach. The gestational age was related with combined results of the measurements of BD and the biparietal diameter (BPD) to improve the accuracy of prediction. Ultrasonography performed at less than or at day 39 of gestation or at day 30 gave an accurate prediction of parturition date. Accuracy of estimation of gestational age was better when at least two measurements (e.g. foetal crown rump length and body diameter) of more than 2 foetuses were taken. However, prediction was inaccurate when made from foetal measurements in late gestation i.e. more than day 39 (Kim *et al.,* 2007).

Visualization of other foetal organs like kidney, eye, intestine, liver etc ultrasonographically also help in assessing the foetal maturity. However, they cannot predict the date of parturition (Yeager *et al.,* 1992). Similarly, the visualization of foetal skeleton does not help to predict the day of parturition with the accuracy of ultrasonographic measurements (Concannon and Rendano, 1983).

2.3.4. Placental thickness

In canines, the uterine wall surrounding the gestational sac, an apparently hyper echoic inner layer gets differentiated into the zonary placenta on day 23.70±0.78 (23-25), which is similar to day 24-28 after ovulation (Ko *et al.,* 2004) and day 27-30 after the preovulatory LH surge (Yeager *et al.,* 1992). By day 21 of gestation, on ultrasound, it

was detected that the echogenic inner layer surrounding the gestational sac develops into zonary placenta. The yolk sac membrane was first detected as echogenic U- shaped foetal membrane around day 23 (Aissi and Slimani, 2008). Placental layers could be detected in the uterine wall by 22-24 days (Yeager *et al*., 1992).

In human obstetrics, on ultrasound, evaluation of BPD was found to be effective in the beginning of pregnancy. However, as foetus develops, BPD values were reported to lose their efficacy. As per the guidelines of American Institute of Ultrasound in Medicine, evaluation of placenta during 2^{nd} and 3^{rd} trimester of pregnancy should be a part of routine obstetrical ultrasonography. The mean placental thickness of 23.1 ± 5.5, 35.0 ± 6.2 and 39.6 ± 7.0 mm was observed at mean gestational age of 18.9 ± 4.2, 33.2 ± 2.9 and 38.1 ± 0.89 weeks, respectively indicating a strong statistical correlation between the placental thickness and gestational age of foetus (Babiker and Eisa, 2014). A placenta of greater than 4 cm has been considered an abnormal indicator during 2^{nd} trimester of pregnancy (Lee *et al.,* 2012). Placental thickness can be used as a new parameter to estimate the gestational age of the foetus and to assess the growth pattern of the placenta with advancing gestational age. It is reported that placental thickness (in mm) increased in a linear fashion with increase in gestational age (in weeks) and almost matched it from 11 to 35 weeks of gestation (Abu *et al.,* 2009; Adhikari *et al.,* 2015). The increase in placental thickness with increasing gestational age has been recorded ultrasonographically approximately 1mm per week. Also, in humans an abnormally increased placental thickness falls under the spectrum of placentomegaly, which indicate various abnormalities on foetal and maternal side, of which foetal hydrops, maternal anaemia and maternal diabetes are important. An abnormally decreased placental

thickness is clinically attributed to pre-eclampsia and intra uterine growth restriction (IUGR). Placental calcification has also been reported in diabetic women, where in placentas are generally much more calcified (Grannum *et al.*, 1979; Petrucha, 1987).

Grannum *et al*. (1979) studied ultrasonic variations in maturation of placenta and graded them into four categories from grade 0 to grade III based on the changes that occur in chorionic plate, placental substance and basal layer. It was observed that a given placenta may have more than one grade when different sections were considered for the study. Correlation of such grading with that of an index of foetal pulmonic maturity and lecithin/sphingomyelin (L/S) ratio was carried out to assess the maturation of placenta. Correlations with mature L/S ratios were 68 percent in grade I, 88 percent in grade II and 100 percent in grade III. Further, it was observed that the placental thickness was gradually decreased after 32 weeks of gestation until term. The average thicknesses of 3.8 cm for grade I, 3.66 cm for grade II and 3.48 cm for grade III were recorded. They concluded that maturational changes in placenta did not progress sequentially through different grades and remained the same until term and abnormal pregnancies showed more rapid changes in maturation of the placenta through the grades.

A linear and direct correlation of placental thickness with femur length in second trimester pregnancy (12 to 24 weeks) was observed in about 100 normal antenatal women. Further, the placental thickness and its growth pattern did not vary relative to the location of placenta (Suresh and Baghavat, 2017). Baghel (2014) observed at 24 weeks of gestation the mean placental thickness as 24.5 mm which closely correlated with biparietal diameter (BPD), femur length (FL) and abdominal circumference (AC). They

concluded that there was a direct and linear correlation of placental thickness with gestational age in 24 weeks. There was a kind of linear relationship between gestational age with placental thickness and FL, which provides correct parameter for estimating foetal gestational age for 21 to 25 weeks. Further, it was concluded that in the absence of the knowledge of last menstrual period (LMP), PT plays an important role as a reliable parameter in the assessment of gestational age.

In equines, the combined thickness of the uterus and the placenta (CTUP) was measured at the placento-cervical junction transrectally and at the uterine body or the uterine horns transabdominally. The CTUP, measured by transrectal ultrasonography did not change between 4 and 8 months of gestation, but increased significantly for each month between 10 and 12 months of gestation (Renaudin *et al.,* 1996). Focal areas of utero-placental thickening and partial separation of the allantochorion from the endometrium in the uterine body and horns can be observed by transabdominal ultrasonography. The combined thickness of the uterus and the placenta (CTUP) should not be more than 12 mm at any site. Using transrectal ultrasonographic evaluation of the placenta, abnormal thickness and partial separation of the allantochorion from the endometrium in mares with clinical signs of placentitis was observed. In advanced stages, the space between the uterus and the placenta was filled with hyper echoic fluid (Troedsson, 2001).

Although there are numerous studies in canines, correlating the size and the gestational age by mathematic formulas based on biparietal diameters, abdominal diameters and on the size of the gestational sac, the simplest way to determine the

approximate age of the foetus is by observing the presence of some anatomic structures (Nyland and Mattoon, 2002; Miglino *et al.,* 2006; Zambelli and Prati, 2006). Comparison of BPD and foetal measurements in English bulldogs and Beagles was highly imperfect for the diagnosis of gestational age, dogs showed a very huge disparity in this parameter (Kutzler *et al.,* 2003; Beccaglia and Luvoni, 2004).

The ultrasonographic measurement of the outer uterine diameter (OUD) at the implantation sites, the placental thickness (PT) and the length of zonary placenta were also significantly and linearly related to gestational age (England *et al.,* 1990; Yeager *et al.,* 1992; Luvoni and Grioni, 2000; Son *et al.,* 2001). However, predictions related to these measurements were less accurate than those obtained by ICC, because of the less defined margins between the uterine wall and foetal membranes than the chorionic cavity (Luvoni and Grioni, 2000).

References relating to the measurement of placental thickness and gestational age of foetus are sparse. Almeida *et al.* (2003) and Maldenado *et al.* (2012) have looked into the possibility of using placental thickness as a predictor of gestational age in canines. Almeida *et al.* (2003) found that the placental thickness was highly correlated with the number of days before delivery relating to the gestational age, while Maldenado *et al.* (2012) verified the placental thickness by ultrasound and correlated it with gestational age in bitches of different breeds and sizes and evaluated the precision of measurement of placental thickness as a foetal morphological parameter. They established a linear expression of curves $y = 0.021x - 0.314$, wherein y is placental thickness and x is gestational age. Placental thickness of 0.34 cm (30.5 days of pregnancy), 0.44 cm (35.2

days of pregnancy), 0.67 cm (46 days of pregnancy) and 0.89 cm (56.3 days of pregnancy) according to linear equation were observed in different breeds of dogs. There was no significant difference between the average reported gestational age and the one calculated by curves adjustment. Placental thickness is a significant parameter in the determination of gestational age, using the linear expression $y = 0.021x - 0.314$.

Materials and Methods

III. MATERIALS AND METHODS

The present study was conducted in the Department of Gynaecology and Obstetrics Veterinary College, Bangalore between April, 2017 and March, 2018. This was a cross sectional observational study conducted in 90 apparently healthy, pregnant female dogs belonging to various breeds and parity with a total of 112 observations. The different breeds of dogs included in the study were Belgium Shepherds, German Shepherds, Rottweilers, Labradors, Golden-Retrievers, Siberian Huskies, Pugs, Beagles, Pomeranians, Cocker Spaniels, Shih Tzu, Pit Bull and Non-descript ones. These dogs were divided into two groups namely, small breeds (Group I) with body weight of < 15 kg and large breeds (Group II) with body weight of > 15 kg. Breeding dates or approximate gestational age according to owners was documented before subjecting the dogs for ultrasonography.

Information pertaining to approximate mating dates was collected from the owners. Ultrasonic evaluation of pregnancy was performed using ALOKA PROSOUND α-6 ultrasound unit. Dogs were subjected to trans-abdominal ultrasonography using convex probe of 3.5 to 5 MHz frequency after positioning in dorsal or lateral decubitus positions. Ventral and lateral abdominal areas were trichotomised and lubricant gel was sufficiently applied before the animals were scanned.

Cross sectional ultrasonic examinations were performed to determine the gestational age of foetuses by measuring gestational sac diameter (GSD) (Plate 1) and biparietal diameter (BPD) (Plate 2) in the early (25-30 days) and late (>30 days) pregnancies. Ultrasonic evaluation of pregnancy was done by identifying gestational sacs

in early pregnancies (25-30 days). The gestational sacs appeared as spherical fluid filled anechoic structures, surrounded by relatively hyperechoic walls with some poorly defined echogenic structures located within the sac. The diameter of gestational sac was measured when the sacs appeared more spherical and two measurements were taken at right angle to one another between inner walls of chorionic cavity. The average of these two measurements was calculated for each conceptus and multiple conceptuses were included for measurements.

In the later stages of pregnancy (>30 days), when foetal ossification was detectable, the gestational age was predicted based on the measurement of biparietal diameter (BPD) which is the distance between two parietal bones in a sagital section. The average gestational age was calculated from the multiple readings of different foetuses and the results were tabulated.

The placental thickness was measured in a longitudinal view perpendicular to the plane of placenta next to its central area (Plate 3). For each foetus average of two to three readings were taken to assess the placental thickness (PT). Average placental thickness (PT) of two to three foetuses were considered per dog in every examination and statistical analysis was done by using correlation and linear regression to assess the relationship between the gestational age as determined by biparietal diameter (BPD) and gestational sac diameter (GSD) and placental thickness (PT). The collected data was subjected to statistical analysis of Pearson's correlation and linear regression along with column statistics as per methods described by Snedecor and Cochron (1989) using Graph Pad prism software (Graph Pad Prism Version 5.00).

All the generated data was tabulated in the increasing order of gestational age (in days) with corresponding placental thickness in millimetres. The tabulated data representing mean placental thickness measured at different gestational ages in different categories was based on the cross- sectional observations made during the study. Statistical analysis of Pearson's correlation was used to establish the relationship between the measured placental thickness (PT), the reported gestational age (RGA) and the calculated gestational age (BPD) in both small breeds (group I) and large breeds (group II). Since the number of sequential observations were more in Pugs (n=27) and in Shepherds (n=27), the data was individually analysed in these breeds. Once a significant correlation was found to exist in each category, a linear regression was applied to further evaluate the reliability of the correlation to arrive at the equation $y=bx+a$, where "x" is the placental thickness in millimetres (mm) and "y" represents the gestational age in days.

Further, reported gestational age (RGA) from the owners and gestational age calculated from BPD was also subjected for similar statistical analysis to assess the type of relationship between both the parameters.

Results

IV. RESULTS

4.1. Assessment of placental thickness in dogs with the normal pregnancies using ultrasonography

Ultrasonographic assessment of placental thickness was done in 90 pregnant female dogs which were presented to the Department of Gynaecology and Obstetrics Veterinary College Bangalore. Animals were categorised into two groups i.e. small breeds (Group I) and large breeds (Group II) based on the body weight. Those breeds of dogs with body weight of <15 kg were considered as small breeds and those with body weight of >15 kg as large breeds. Cross sectional ultrasonic examinations were performed to determine the gestational age of foetuses by measuring gestational sac diameter (GSD) (Plate 1) and biparietal diameter (BPD) (Plate 2) in the early (25-30days) and late (>30 days) pregnancies respectively. Placental thickness (PT) was measured in a longitudinal view perpendicular to the plane of placenta next to its central area (Plate 3). For each foetus average of two to three readings were taken to assess the placental thickness (PT). Average placental thickness (PT) of two to three foetuses were considered per dog in every examination and statistical analysis was done by using correlation and linear regression to assess the relationship between the gestational age as determined by biparietal diameter (BPD) and gestational sac diameter (GSD) and placental thickness (PT).

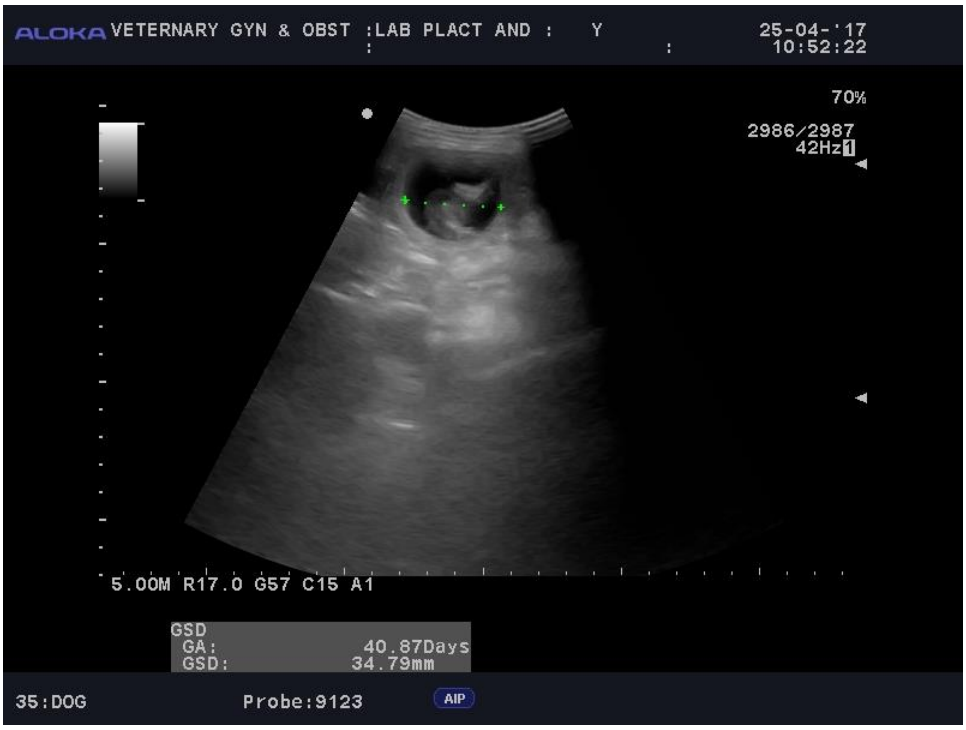


Plate 1: Measurement of gestational sac diameter by ultrasonography

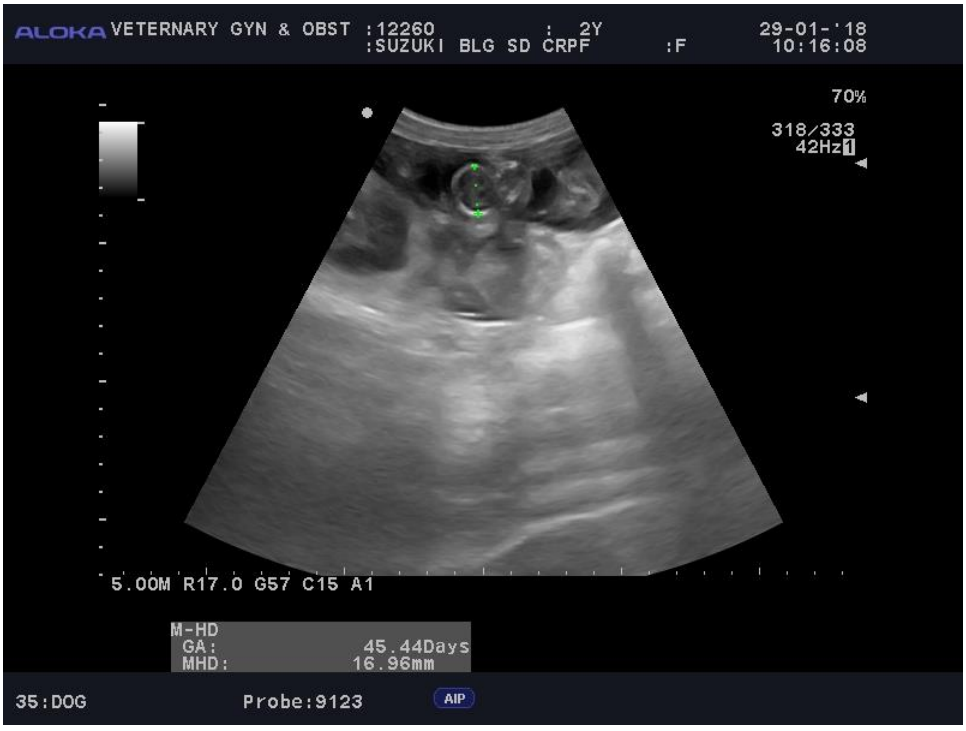
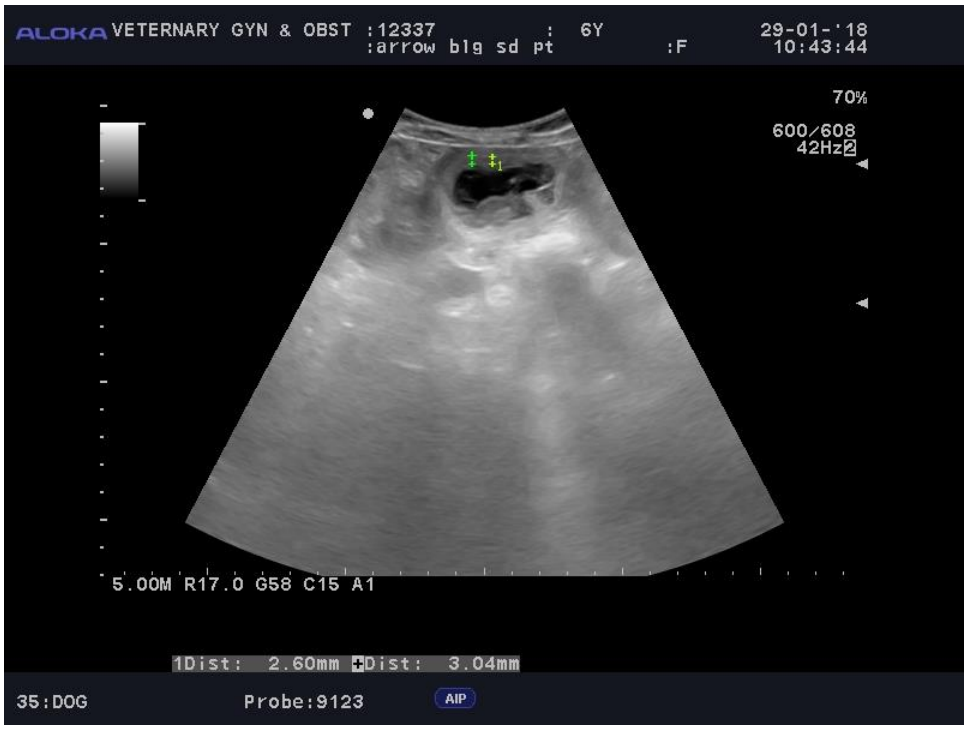
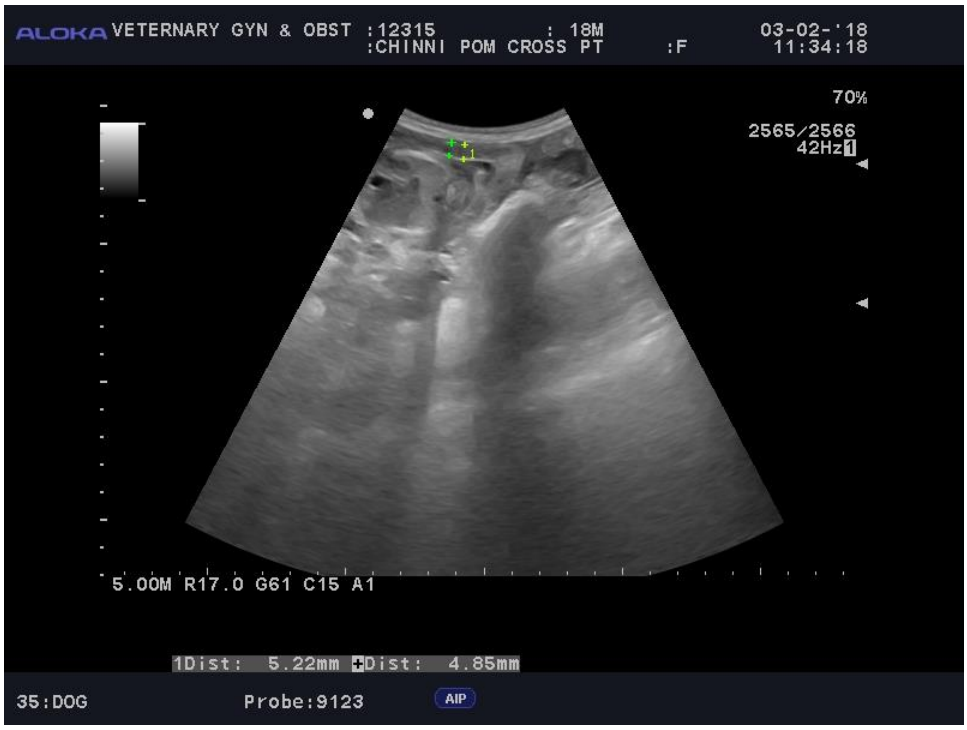


Plate 2: Measurement of biparietal diameter by ultrasonography

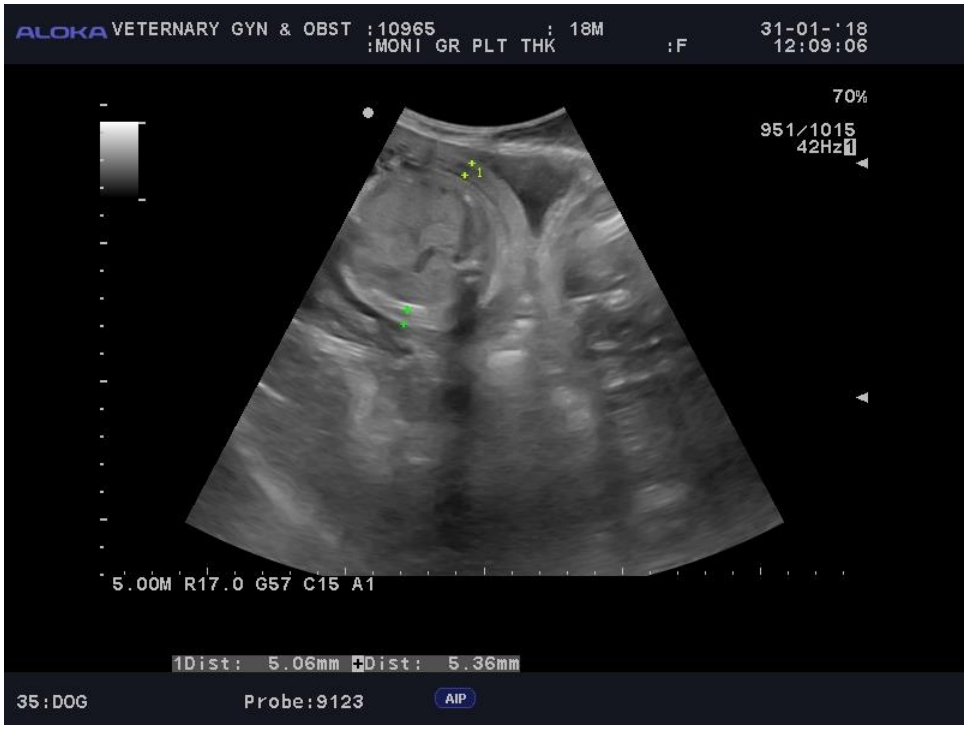


3a. Measurement of PT around 35 days of gestation

3b. Measurement of PT around 45 days of gestation



3c. Measurement of PT around 53 days of gestation



3d. Measurement of PT around 60 days of gestation

Plate 3: Measurement of placental thickness by ultrasonography (a - d)

4.1.2. Mean placental thickness in small breeds

The mean placental thickness in mm as determined by ultrasonography at different gestational ages in days in small breeds were 3.42 ± 0.32, 4.20 ± 0.15 and 4.36 ± 0.12 at 36.11 ± 1.19, 46.11 ± 0.71 and 54.84 ± 0.89 days, respectively (Table 1).

Table 1: Placental thickness (Mean ± SE) measured at different gestational ages in small breeds (n=40)

Gestational Age (days)		Placental Thickness (mm)	
Range	**Mean ± SE**	**Range**	**Mean ± SE**
28.12 – 40.87	36.11 ± 1.19	1.72 – 6.33	3.42 ± 0.32
41.07 – 49.88	46.11 ± 0.71	3.28 – 5.85	4.20 ± 0.15
51.38 – 60.12	54.84 ± 0.89	3.70 – 5.04	4.36 ± 0.12

4.1.3. Mean placental thickness in large breeds

The mean placental thickness in mm as determined by ultrasonography at different gestational ages in days (mean) in large breeds were 2.91 ± 0.24, 5.28 ± 0.15 and 5.88 ± 0.13 at 33.80 ± 0.81, 44.92 ± 0.66 and 57.18 ± 0.98 days, respectively (Table 2).

Table 2: Placental thickness (Mean ± SE) measured at different gestational ages in large breeds (n=72)

Gestational Age (days)		Placental Thickness (mm)	
Range	**Mean ± SE**	**Range**	**Mean ± SE**
26.57 – 38.94	33.80 ± 0.81	0.98 – 5.27	2.91 ± 0.24
40.42 - 49.49	44.92 ± 0.66	3.33 - 6.73	5.28 ± 0.15
50.29 - 67.73	57.18 ± 0.98	4.56 - 7.94	5.88 ± 0.13

4.1.4. Mean placental thickness in German Shepherds and Belgium Shepherds

The mean placental thickness in mm as determined by ultrasonography at different gestational ages in Belgium and German Shepherds collectively were 2.670 ± 0.26, 5.35 ± 0.18 and 6.18 ± 0.39 at 32.73 ± 1.09, 44.09 ± 0.69 and 55.05 ± 2.01 days, respectively (Table 3).

Table 3: Placental thickness (Mean ± SE) measured at different gestational ages in Belgium and German Shepherds (n=27)

Gestational Age (days)		Placental Thickness (mm)	
Range	**Mean ± SE**	**Range**	**Mean ± SE**
26.57 – 37.99	32.73 ± 1.09	1.62 - 3.96	2.67 ± 0.26
41.90 - 49.0	44.09 ± 0.69	4.63 - 6.73	5.35 ± 0.18
50.59 - 64.50	55.05 ± 2.01	5.06 - 7.94	6.18 ± 0.39

4.1.5. Mean placental thickness in Pugs

The mean placental thickness in mm as determined by ultrasonography at different gestational ages (Mean) in Pugs were 3.33 ± 0.31, 4.09 ± 0.10 and 4.16 ± 0.16 at 35.99 ± 1.50, 46.39 ± 0.80 and 54.39 ± 1.16 days, respectively (Table 4).

Table 4: Placental thickness (Mean ± SE) measured at different gestational ages in Pugs (n=20)

Gestational Age (days)		Placental Thickness (mm)	
Range	**Mean ± SE**	**Range**	**Mean ± SE**
29.54 - 40.14	35.99 ± 1.50	1.72 - 4.05	3.33 ± 0.31
41.07 - 49.88	46.39 ± 0.80	3.28 - 4.60	4.09 ± 0.10
51.81 - 60.12	54.39 ± 1.16	3.70 - 5.04	4.16 ± 0.16

Average placental thickness for shepherds and pugs were calculated separately and depicted in Table 3 and Table 4. This was done because the number of dogs represented in these categories of breeds was significant.

4.2. Correlation of reported gestational age (RGA) and calculated gestational age (CGA)

The observations recorded in small breeds (group I) and large breeds (group II) with respect to the reported gestational age (RGA) and calculated gestational age (CGA) based on biparietal diameter (BPD) were analysed statistically by Pearson's coefficient of correlation and linear regression. A highly significant positive correlation was observed between the reported gestational age (RGA) and the calculated gestational age (CGA)

based on biparietal diameter (BPD), in both small and large breeds of dogs with coefficient of correlation (r) = 0.96, R^2= 0.92 and P value of < 0.0001 and r = 0.97, R^2 = 0.94 and P value of < 0.0001, respectively.

The present study recorded a linear regression equations of $y = 0.88x + 3.57$ with R^2= 0.92 for small breeds (Fig. 1) and $y= 0.98x + 0.13$ with R^2= 0.94 for large breeds (Fig. 2) where *x* is the reported gestational age (RGA) and *y* is the calculated gestational age (CGA) based on BPD.

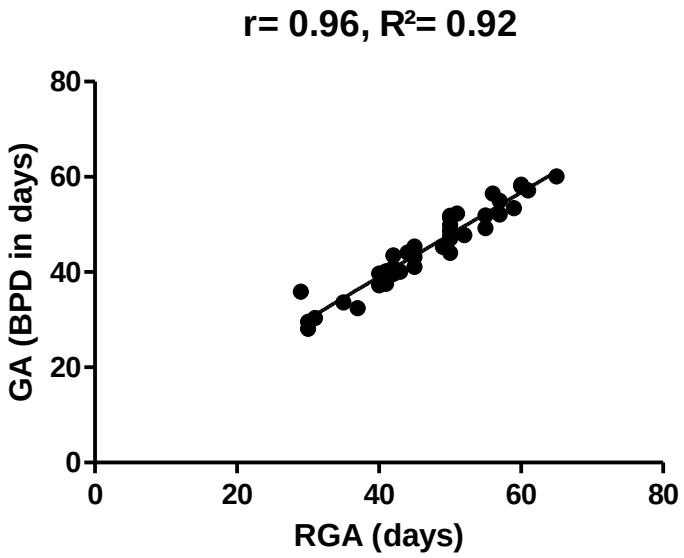


Figure 1: Pearson's correlation between reported gestational age (RGA) and calculated gestational age (CGA) based on BPD in small breeds

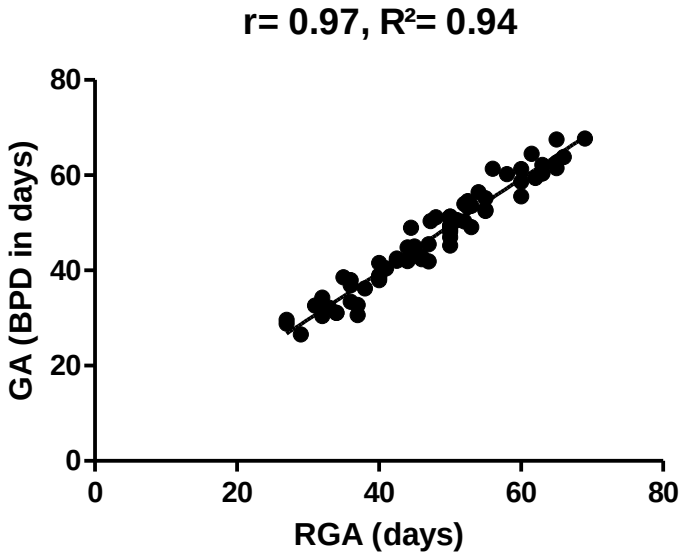


Figure 2: Pearson's correlation between reported gestational age (RGA) and calculated gestational age (BPD) in large breeds

4.3. Correlation between measured placental thickness (PT) and gestational age (GA)

Since results showed a significantly high correlation between RGA and CGA, the placental thickness (PT) was then correlated with calculated gestational age (CGA) which was determined based on biparietal diameter (BPD).

4.3.1. Correlation between placental thickness (PT) and gestational age measured by Biparietal diameter (BPD)

4.3.1.1. Large and small breeds

Pearson's correlation analysis was used to establish the degree of relationship between the measured placental thickness (PT) at different gestational ages and gestational age (GA) as determined by Biparietal diameter (BPD). A significant

correlation was found to exist in both small and large breeds with coefficient of correlation (r) =0.58, R^2=0.34, P value of < 0.0001 and r= 0.82, R^2= 0.67, P value of < 0.0001, respectively (Table 5).

A significant linear regression was observed between the placental thickness (PT) and the gestational age as determined by Biparietal diameter (BPD) in large breeds with linear expression of curves y=5.77x + 18.82 where x represents placental thickness in mm and y represents gestational age in days. The coefficient of determination (R^2) was 0.67 (Fig. 3). Similarly a linear regression was also observed for small breeds with y= 5.30x + 24.12 where x represents placental thickness in mm and y represents gestational age in days. The coefficient of determination (R^2) was 0.34 (Fig. 4).

4.3.1.2. Shepherds

Sequential ultrasonic observations done in pregnant German Shepherds (n=5) and Belgium Shepherds (n=13) at different gestational ages ranging from 32.73 ± 1.09 to 55.05 ± 2.01 showed a significant positive correlation of measured placental thickness (PT) to that of gestational age (GA) determined by Biparietal diameter (BPD) with coefficient of correlation(r)= 0.93, R^2= 0.87 and P value of < 0.0001 with a regression line of y=5.23x + 18.75 and R^2 of 0.87 where x represents placental thickness in mm and y represents the gestational age in days (Fig. 5 and Table 5).

4.3.1.3. Pugs

Similarly, a cross sectional study in 20 pregnant pugs also showed a significant correlation between the placental thickness (PT) and the gestational age (GA) calculated

from biparietal diameter (BPD) with coefficient of correlation r=0.6519, R^2=0.42 and P value of <0.0001 and a linear regression of y=7.45x + 16.36 and R^2=0.42 was arrived with x as placental thickness in millimetres and y as gestational age in days (Fig. 6 and Table 5).

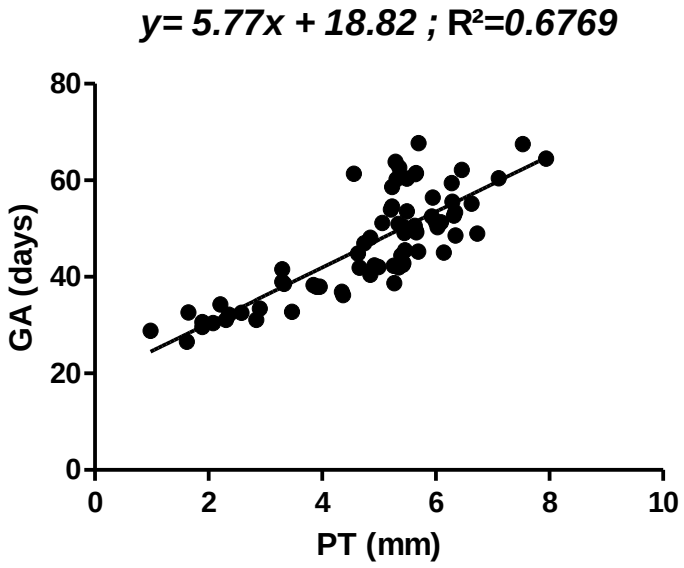


Figure 3: Linear regression between placental thickness (PT) and gestational age (BPD) in large breeds

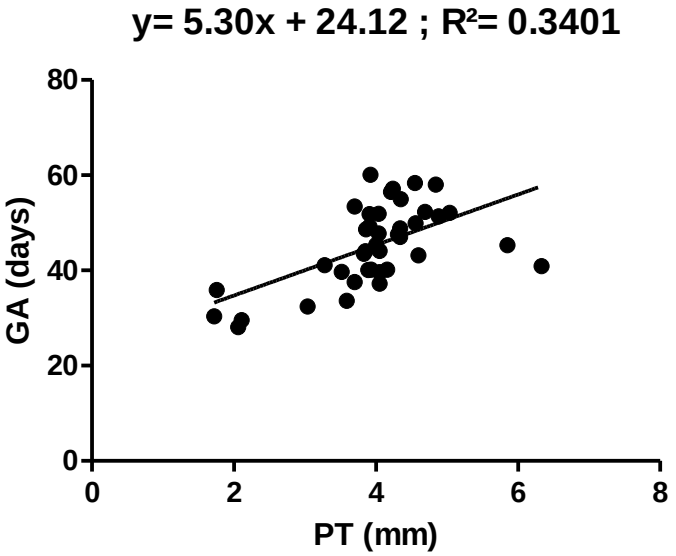


Figure 4: Linear regression between placental thickness (PT) and gestational age (GA) in small breeds

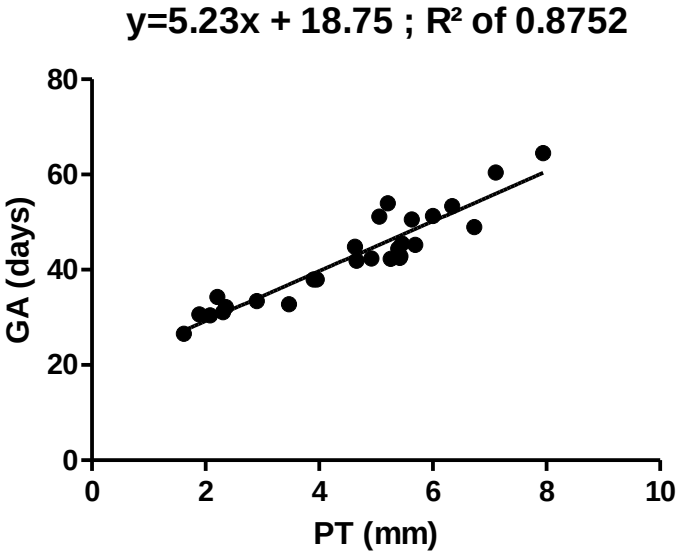


Figure 5: Linear regression between placental thickness (PT) and gestational age (GA) in Belgium and German Shepherds

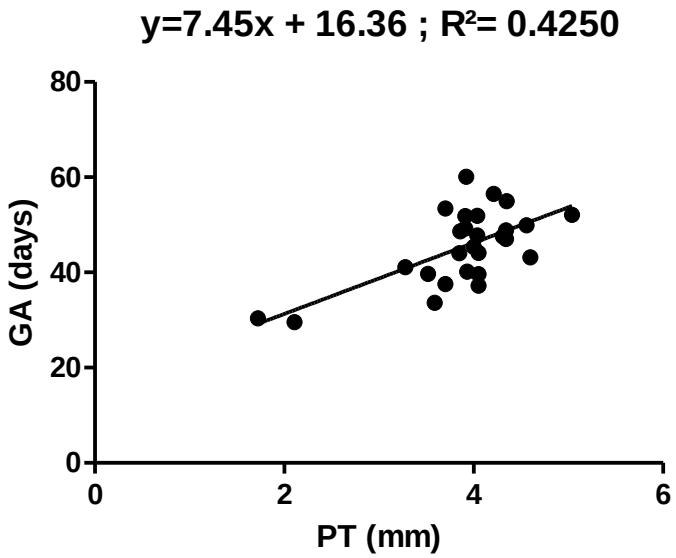


Figure 6: Linear regression between placental thickness (PT) and gestational age (GA) in Pugs

Table 5: Relationship between placental thickness (PT) and the gestational age (BPD) and reported gestational age (RGA) and calculated gestational age (CGA)

Group	Measurement	Intercept (a)	Regression coefficient (b)	Pearson's Coefficient of Correlation (r)	R^2 value	n	P
Large breeds	PT vs GA	18.82 ± 2.42	5.77 ± 0.477	0.82	0.67	72	<0.0001
	RGA vs CGA	0.13 ± 1.33	0.98 ± 0.02	0.97	0.94		<0.0001
Small breeds	PT vs GA	24.12 ± 4.89	5.30 ± 1.19	0.58	0.34	40	<0.0001
	RGA vs GA	3.57 ± 1.94	0.88 ± 0.04	0.96	0.92		<0.0001
Shepherds	PT vs GA	18.75 ± 1.92	5.23 ± 0.39	0.93	0.87	27	<0.0001
	RGA vs CGA	-2.75 ± 2.75	1.03 ± 0.06	0.95	0.91		<0.0001
Pugs	PT vs GA	16.36 ± 6.84	7.46 ± 1.73	0.65	0.42	27	0.0002
	RGA vs CGA	3.01 ± 2.16	0.88 ± 0.04	0.96	0.94		<0.0001

Discussion

V. DISCUSSION

The peculiarities in the bitches' breeding behaviour like multiple matings, matings before the ovulation, multiple ovulations over a period of time, time of maturation for ova to be able to get fertilised and availability of viable spermatozoa for several days in the female reproductive tract after mating makes it quite difficult to precisely determine the gestational age in canines. Early determination of pregnancy is important in modulating the diet and management of pregnant bitches for the better outcome of pregnancy. This can be achieved through use of real time B-mode ultrasonography.

5.1. Assessment of placental thickness in dogs during normal pregnancies using ultrasonography

5.1.1. Gestational sac diameter (GSD)

Using the gestational sac diameter (GSD), the earliest determination of pregnancy in the present study was done at an average of 25.8 days, from the last mating. Although, gestational sacs have been visualised by earlier researchers, as early as 21-23 days (Yeager *et al*., 1992; Concannon, 2000), the delayed observation in the present study, was due to the fact that animals were presented for pregnancy diagnosis, only after three to four weeks from the last day of mating. Similarly, by observing gestational sac diameter (GSD), pregnancy was detected ultrasonographically as early as 17-19 days in Miniature Schnauzer breeds (Kim and Son, 2007) and 21 days approximately (after calculated LH peak), by England and Allen (1990) and Moriyoshi *et al.* (1996), from the final day of mating. However, Son *et al.* (2001) opined that the use of measurement of inner

chorionic cavity diameter was a reliable parameter to predict the date of whelping (75% for Maltese and 83.3% for Yorkshire terrier breeds) in dogs when measured between 18-37 days. However, study by Kutzler *et al.* (2003) reported that the accuracy of prediction of parturition date by measurements made during early gestation (near 30 days) using embryonic vesicle diameter (EVD), crown rump length (CRL) and body diameter (BD) were more accurate with a correction factor of +1 day for small breeds and -2 days for giant breeds.

5.1.2. Biparietal diameter (BPD)

In the present study, biparietal diameter (BPD) was found to be a consistent parameter in determining the age of gestation in both small and large breeds of dogs once the foetal ossification was detectable around day 30 of gestation. It also showed a highly significant correlation with the reported gestational age (RGA) by the owners which was based on the mating history. A reliability of 92 percent for small breeds and 94 percent for large breeds, on linear regression was evident during the current study. The observations made during the present study were in accordance with the findings of Ramya *et al.* (2018) and Son *et al*. (2001), who observed a reliability of 99 percent between RGA and BPD in bitches of different breeds and parity. The very high reliability in all the studies could be due to an accurate scan plane of foetal head diameter which was easy to obtain as the foetal ossification progressed linearly, as compared to other foetal or extra foetal structures.

5.1.3. Placental thickness (PT)

In the present study, echogenic inner layer surrounding the gestational sac was identified as the zonary placenta between 26-29 days of gestation as compared to 16-21 days (Aissi, 2008), 22-24 days (Almeida *et al.,* 2003), 23-25 days (Aissi and Slimani, 2008), 23- 26 days (Kim and Son, 2007), 27-30 days (Yeager *et al*., 1992), 28 days (Concannon, 2000) and 24 days (England and Allen, 1990). However, in the current study the PT has been compared with RGA where as in all other studies mentioned above, LH peak had been considered as day 0 of gestation. Such a delay in identification of placenta in our study could be due to the fact that dogs were presented to pregnancy diagnosis only after three to four weeks from the last mating.

5.1.4. Mean placental thickness in small breeds

The present study on small breeds of dogs revealed a significant correlation ($P < 0.0001$) between the PT and gestational age. The mean placental thickness in mm as determined by ultrasonography at different gestational ages in days in small breeds were 3.42 ± 0.32, 4.20 ± 0.15 and 4.36 ± 0.12 at 36.11 ± 1.19, 46.11 ± 0.71 and 54.84 ± 0.89 gestational age, respectively (Table 1). However the reliability was only 34 percent with linear regression of $y=5.30x + 24.12$ where y is gestational age and x is the placental thickness in millimetres was recorded. This is in line with the observations made by Son *et al.* (2001) who assessed the length of zonary placenta in relation to the gestational age and found it to be not significant compared to other extra-foetal structures. Low reliability observed in our study could be due to the wide variations in the measurements of placental thickness at a particular gestational age, among different dogs.

The average placental thickness recorded in pugs in the present study at 35.99 ± 1.50 (5^{th} week), 46.39 ± 0.80 (7^{th} week) and 54.39 ± 1.16 days (8^{th} week) of gestation were 3.33 ± 0.31, 4.09 ± 0.10 and 4.16 ± 0.16 mm, respectively. These observations on PT were similar to that observed by Feliciano *et al. (*2015) wherein they recorded 3.70 ± 0.08 mm in 5^{th} week, 5.0 ± 0.04 mm in 7^{th} week and 5.0 ± 0.12 mm in 8^{th} week. They also recorded a reliability of 34 percent with regression equation of y=41.9x + 24 for brachycephalic pregnant bitches (English bull dog, Pug and Shih Tzu) which was similar to that observed for small breeds in our study which included pugs as a major component (n=20).

5.1.5. Mean placental thickness in large breeds

The mean placental thickness in mm as determined by ultrasonography at different gestational ages (days) in large breeds were 2.91 ± 0.24, 5.28 ± 0.15 and 5.88 ± 0.13 at 33.80 ± 0.81, 44.92 ± 0.66 and 57.18 ± 0.98 days, respectively. The results showed a linear increase in the thickness of placenta with the increase in gestational age.

Observations of placental thickness by Maldonado *et al.* (2012) in different breeds of dogs with varying body sizes revealed a mean thickness (in mm) of 3.4 , 4.4, 6.7 and 8.9 at 30.5, 35.2, 46 and 56.3 days (according to linear equation), respectively. These results were slightly higher than the values in the present study which were around 2.29, 4.28 and 5.32 mm at 31.13, 40.54 and 53.76 days, respectively (pooled data of all breeds of dogs). This variation could be due to the cross sectional study involving different breeds of dogs with varied body sizes.

The present study revealed a PT of 2.91 ± 0.24 mm at 33.80 ± 0.81 days and 5.88 ± 0.13 mm at 57.18 ± 0.98 days of gestation in large breeds and 2.67 ± 0.26 mm at 32.73 ± 1.09 days and 6.18 ± 0.39 mm at 55.05 ± 2.01 days in Belgium and German Shepherds. These readings were lesser than the values of Almeida *et al*. (2003) in boxers with measured a placental thickness of 1 mm at day 43 before parturition (22 days of gestation) to 11 mm at one day before parturition (64 days of gestation). However, the relationship between the thickness of placenta and the gestational age was in linear dispersion in both the studies. In the present study, a reliability of linear regression 87 percent was observed in Shepherds which was close to that observed by Almeida *et al.* (2003) in Boxers with reliability of 91 percent.

5.2. Correlation between placental thickness (PT) and gestational age (GA)

In this study on placental thickness and its correlation with the gestational age, a significant positive correlation was observed in both small and large breeds of dogs with r=0.58 and 0.82, respectively. A linear regression of $y=5.30x + 24.12$ with $R^2= 0.34$ and $y=5.77x + 18.82$ with $R^2= 0.67$, respectively were observed in both small and large breeds of dogs where *x* is the placental thickness in millimetres (mm) and *y* is the gestational age in days. Similar observations were made by Maldonado *et al.* (2012) with $y= 0.021x - 0.314$ where *y* represents the placental thickness (PT) and *x* is the gestational age in days. They concluded that placental thickness can be a significant parameter in the determination of gestational age with application of linear curve adjustments in bitches of different breeds and sizes.

The result of this study in Belgium and German Shepherds with sequential ultrasonic observations at different gestational ages ranging from 32.73 ± 1.09 to 55.05 ± 2.01 showed a very significant positive correlation of measured placental thickness (PT) to that of gestational age (GA) as determined by biparietal diameter (BPD) with coefficient of correlation (r) =0.93, R^2= 0.87 and P value of < 0.0001 with a regression line of y=5.23x + 18.75 and R^2 of 0.87 where x represents placental thickness in mm and y represents the gestational age in days. This showed that assessment of PT can act as a predictor of gestational age similar to that measured by mean head diameter (MHD) or biparietal diameter (BPD) which coincides with the findings of Almeida *et al.* (2003) in Boxers and Maldonado *et al.* (2012) in bitches of different breeds and sizes.

The lesser reliability of regression between the measured placental thickness and the gestational age in both small breeds (34%) and large breeds (67%) of dogs in the present study could be related to wide variations in the measurements of placental thickness at a particular gestational period, among different animals of the same breed and different breeds as well. More such observations may be done in a sequential order with precision among different breeds of dogs to increase the reliability of linear regression with respect to the measurement of placental thickness as observed in Belgium and German shepherds in the present study with R^2= 0.87 for calculated gestational age (BPD) and 0.90 for reported gestational age (RGA).

5.3. Correlation of reported gestational age (RGA) and calculated gestational age (BPD)

There was no significant difference between the reported gestational age and the calculated gestational age in both small and large breeds of dogs in the present study. The coefficient of correlation (r) for small breeds and large breeds was 0.96 and 0.97 and the reliability in both categories of breeds was 92 percent and 94 percent, respectively. These findings were similar to the findings of Maldonado *et al.* (2012) who among all categories of breeds (small, medium large and giant) got a reliability of 75 percent.

Feliciano *et al.* (2015) reported that the biparietal diameter (BPD) served an important foetal developmental parameter which can be used as an estimate for prediction of gestational age in brachycephalic breeds of dogs (English bull dog, pug and Shih Tzu) with P value of < 0.05 and R^2 of 81 percent which was corroborating with our results in Pugs (n=20) where a significant correlation was obtained between the reported gestational age (RGA) and the calculated gestational age based on the biparietal diameter (BPD) with P value <0.0001 and reliability of 94 percent. Similarly, Beccaglia and Luvoni (2004) observed a significant correlation between the biparietal diameter and the prediction of parturition day within ±2 days in small and medium sized dogs which was concurrent with our findings in Belgium shepherds. They also suggested that the measurement of deep portion of telencephalic vesicle (DPTV) may be combined with measurements of biparietal diameter (BPD) to enhance the reliability of estimating the day of parturition in small and medium sized dogs.

The results of the present study can be concluded as, the increase in the thickness of the placenta with the increasing gestational age as observed in the present study could be due to increase in the thickness of all extra embryonic tissues as the pregnancy advances, because of increase in the connective tissue component which is highest at delivery. It was also suggested that the morphological modifications in the uterus occur almost daily during pregnancy concurrent with the foetal accommodation and its delivery at the proper time (Aralla *et al*., 2013).

The increase in placental thickness as observed during the present study in small and large breeds of dogs showed consistency in the early phase of gestation as compared to the later phase. There was no remarkable increase in the thickness of placenta in the later stages of gestation (>40 days) in small breeds as compared to large breeds. Placental size is a reflection of health and size of foetus (Daftary *et al*. (1998); Cross, (2005). This could be due to the rapid growth of foetuses seeking more nourishment from the placenta in large breeds during later stages of gestation. Similar argument was made by Kutzler *et al*. (2003), who suggested that foetal growth rate is different in pregnant bitches' of different body sizes (from ≤9 and >40 kg) during early and later phases of gestation.

Limitations of the present study in measuring the accurate placental thickness (PT) could be due to patient factors (excitation, panting and obesity, irregularity in presentation) and unavailability of separate calm area for ultrasonic examinations. To increase the reliability we suggest that more sequential study, at regular intervals of gestation should be carried out. Further breed wise standardisation has to be done with respect to prediction of parturition date in canines.

Summary

VI. SUMMARY

Early pregnancy diagnosis and determination of foetal age are of vital importance in companion animal reproductive management. In addition, accurate prediction of day of parturition can help to aptly manage the parturition or plan a caesarean section in pregnant bitches with history of unknown mating or multiple matings. In veterinary obstetrics, Ultrasonography (USG) has been put to use as an important imaging modality in pregnancy diagnosiss and assessing the development of foetus. Gestational aging of foetus based on USG examination of foetal and extra foetal structures like GSD, BPD and BD, and prediction of day of parturition has been reported. The present study aimed at assessing the placental thickness (PT) in pregnant bitches of different body sizes and correlating the same with that of the gestational age reported by the owners.

The present cross sectional study was done on 90 different dogs of varied body weights and breeds using ALOKA PROSOUND α-6 ultra sound unit with convex 5 MHz probe. The dogs were grouped into two categories namely, the small breeds (<15 kg) and large breeds (>15kg) based on their body weights. A total of 112 observations were made starting from three to four weeks of gestation and parameters like GSD, BPD and PT were recorded. Reported gestational age (RGA) was noted from the information provided by the owners with respect to the mating dates.

During the present study, echogenic inner layer surrounding the gestational sac was identified as the zonary placenta as early as 26 days of gestation. The mean placental thickness (PT) in mm as determined by ultrasonography at different gestational ages in days (mean) in small breeds were 3.42 ± 0.32, 4.20 ± 0.15 and 4.36 ± 0.12 at 36.11 ±

1.19, 46.11 ± 0.71 and 54.84 ± 0.89 days, respectively. The mean placental thickness (PT) in mm as determined by ultrasonography at different gestational ages in days (mean) in large breeds were 2.91 ± 0.24, 5.28 ± 0.15 and 5.88 ± 0.13 at 33.80 ± 0.81, 44.92 ± 0.66 and 57.18 ± 0.98 days, respectively. The PT in both small and large breeds of dogs showed a significant positive correlation with GA and RGA with coefficient of correlation (r) for small breeds and large breeds as 0.58, 0.59 and 0.82, 0.81, respectively. The increase in the thickness of the placenta with the increasing gestational age as observed in the present study could be due to increase in the thickness of all extra embryonic tissues as the pregnancy advances.

Subsequently, when the data was analysed under linear regression to arrive at the equation $y= bx+ a$ where x represented the PT (mm) and y represented the GA (days), a reliability of 34 percent for small breeds and 67 percent for large breeds was recorded with respect to PT and GA based on BPD. Similarly, reliability of 35 percent for small breeds and 67 percent for large breeds were recorded with respect to PT and RGA. The PT recorded by sequential observations made in 18 pregnant Shepherds (Belgium and German), was found to be significantly correlated with GA (based on BPD) and RGA with coefficient of correlation (r) = 0.93 and 0.94, respectively. The reliability in this breed was found to be significantly higher than other categories in the study with R^2 =0.87 and 0.90 for CGA and RGA, respectively. Pugs being a brachycephalic and a major component of small breeds in the present study also showed a significant positive correlation between PT vs CGA and PT vs RGA with r=0.65, 0.63 and R^2= 0.42 , 0.40, respectively.

The increase in placental thickness as observed during the present study in small and large breeds of dogs showed consistency in the early phase of gestation as compared to the later phase. There was no remarkable increase in the thickness of placenta in the later stages of gestation (>40 days) in small breeds as compared to large breeds. This could be due to the rapid growth of foetuses seeking more nourishment from the placenta in large breeds during later stages of gestation. In this study on placental thickness and its correlation with the gestational age, a significant positive correlation with a regression of $y=5.30x + 24.12$ with $R^2= 0.34$ and $y=5.77x + 18.82$ with $R^2= 0.67$, respectively were observed in both small and large breeds of dogs where x is the placental thickness in millimetres (mm) and y is the gestational age in days. More such observations may be done in a sequential order with precision among different breeds of dogs to increase the reliability of linear regression with respect to the measurement of placental thickness as observed in Belgium and German Shepherds in the present study with $R^2= 0.87$ for calculated gestational age (BPD) and 0.90 for reported gestational age (RGA).

It was suggested that the placental thickness (PT) is significantly correlated with GA and RGA and it can be used to estimate gestational age of foetus along with other parameters like GSD and BPD in both small and large breeds of dogs. Breed wise and sequential evaluation of placental thickness (PT) should be done during ultrasonography to predict the day of parturition with more precision. Also evaluation of placenta may help in diagnosing the pathological conditions relating to changes in the morphology and thickness of placenta (Placentitis) which may end up in abortions.

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Abstract

VIII. ABSTRACT

The measurement of gestational sac diameter (GSD) and biparietal diameter (BPD) using ultrasonography (USG) remained fairly accurate determinants of gestational age (GA) in canines during early and later stages of gestation with precision of ±1 to ± 2 days. The present study looked at the possibility of placental thickness (PT) as a determinant of GA using USG in normal pregnancies of different breeds and body sizes and then to correlate the same with reported gestational age (RGA). A cross sectional observational study was conducted on 90 pregnant dogs of different breeds and parity and a total of 112 observations were made by using ALOKA PRO SOUND α-6 ultra sound unit using 5 MHz convex probe. The dogs were categorised into two groups, namely the large breeds and small breeds, GA and PT were recorded from 3rd to 4th week of gestation. The mean PT in small breeds of dogs was found to be 3.42 ± 0.32, 4.20 ± 0.15 and 4.36 ± 0.12 at 36.11 ± 1.19, 46.11 ± 0.71 and 54.84 ± 0.89 average GA, respectively with a significant positive correlation between PT vs GA and PT vs RGA (r= 0.58 and 0.59), and respective R^2 of 0.34 and 0.35 was recorded. Similarly, for large breeds mean PT of 2.91 ± 0.24, 5.28 ± 0.15 and 5.88 ± 0.13 at 33.80 ± 0.81, 44.92 ± 0.66 and 57.18 ± 0.98 days, respectively with significant positive correlation between PT vs GA and PT vs RGA (r=0.82,0.81) and respective R^2 of 0.67 and 0.67 was recorded. The present study revealed that there was no significant difference between the calculated gestational age (CGA) and reported gestational age (RGA). It was concluded that the placental thickness (PT) was significantly correlated with GA and RGA and it can be used to estimate gestational age of foetus along with other parameters like GSD and BPD.