

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

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The measurement of gestational sac diameter and biparietal diameter using ultrasonography remained fairly accurate determinants of gestational age 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 as a determinant of gestational age using ultrasonography in normal pregnancies of Belgium and German Shepherds and then to correlate the same with reported gestational age. A sequential observational study was conducted on 18 pregnant dogs of different parity and a total of 27 observations were made. Gestational age and placental thickness were recorded from 3rd to 4th week of gestation. The mean placental thickness (in mm) of 2.67 ± 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 with a positive correlation between placental thickness vs gestational age and reported gestational age vs calculated gestational age ($r= 0.93$ and 0.95), and respective R^2 of 0.87 and 0.91 was recorded. The present study revealed that there was no significant difference between the calculated gestational age and reported gestational age. It was concluded that the placental thickness was significantly correlated with gestational age and reported gestational age and it can be used to estimate gestational age of foetus along with other parameters like gestational sac diameter and biparietal diameter.

Keywords: Biparietal diameter, Gestational age, Placental thickness, Ultrasonography.

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).

Gestational sac diameter, crown rump length, body diameter, biparietal diameter, deep portion of telencephalic vesicle and diameter of foetal heart are the different foetal and extra foetal measurements that are considered during ultrasonography in determining the age of foetus and predicting the days before parturition in canines. However, Gestational sac diameter and biparietal diameter are believed to be in high correlation with gestational age during early and late phases of pregnancy, respectively. Studies on measurement of placental thickness (PT) 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). This study in was aimed at assessing the progression of placental thickness and its use as a determinant of gestational age in routine ultrasonography of pregnant Belgium and German Shepherds dogs.

Materials and Methods

The present study was conducted on 18 pregnant Belgium and German Shepherd dogs with a total of 27 observations, in the Department of Gynaecology and Obstetrics Veterinary College, Bengaluru between April, 2017 and March, 2018. Breeding dates or approximate gestational age according to owners was documented before subjecting the dogs for ultrasonography. Ultrasonic evaluation of pregnancy was performed. Dogs were subjected to trans-abdominal ultrasonography using convex probe of 3.5 to 5 MHz frequency after positioning in dorsal

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or lateral decubitus positions. Ventral and lateral abdominal areas were trimmed and lubricant gel was sufficiently applied before the animals were scanned.

Sequential ultrasonic examinations were performed to determine the gestational age of foetuses by measuring gestational sac diameter and biparietal diameter in the early (25-30 days) and late (>30 days) pregnancies. The placental thickness was measured in a longitudinal view perpendicular to the plane of placenta next to its central area (Fig.1). Average placental thickness was observed from the multiple readings of different

foetuses. The collected data was subjected to statistical analysis of Pearson's correlation and linear regression along with column statistics. Statistical analysis was done to establish the relationship between the measured placental thickness, the reported gestational age and the calculated gestational age based on biparietal diameter. Once a significant correlation was found to exist, 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, "a" is the intercept and "b" represents regression coefficient.



Figure 1: Measurement of placental thickness by ultrasonography

Results and Discussions

The mean placental thickness in mm as determined by ultrasonography at different gestational ages in Belgium and German

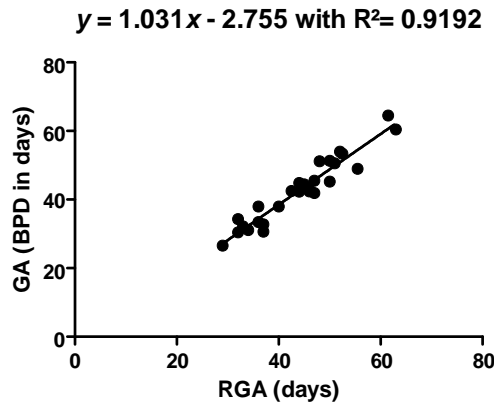
Shepherds collectively were 2.67 ± 0.26 , 5.35 ± 0.18 and 6.18 ± 0.39 at 32.73 ± 1.09 , 44.09 ± 0.69 and 55.05 ± 2.01 days, respectively (Table-1).

Table 1: 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

A significant positive correlation was observed between the reported gestational age and the calculated gestational age based on biparietal diameter with coefficient of correlation ($r = 0.95$, $R^2 = 0.91$ and P value of < 0.0001). The present study recorded a linear

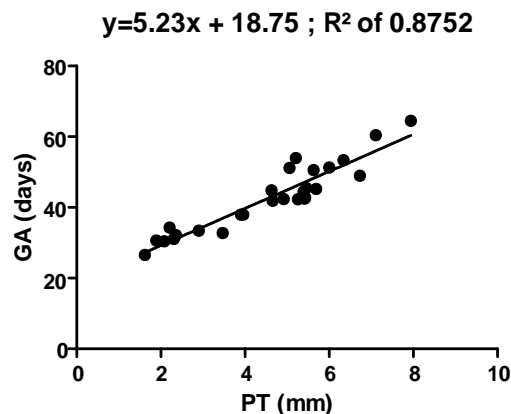
regression equations of $y = 1.031x - 2.755$ with $R^2 = 0.91$ (Graph. 1), where x is the reported gestational age) and y is the calculated gestational age based on biparietal diameter.



Graph 1: Relationship between reported gestational age (RGA) and calculated gestational age (CGA) based on biparietal diameter (BPD) in Belgium and German Shepherds

Sequential ultrasonic observations done in pregnant German Shepherds and Belgium Shepherds at different gestational ages ranging from 32.73 ± 1.09 to 55.05 ± 2.01 showed a significant positive correlation of measured placental thickness to that of gestational age determined by Biparietal

diameter 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 (Graph. 2 and Table 2).



Graph 2: Linear regression between placental thickness (PT) and gestational age (GA) in Belgium and German Shepherds

Table 2: 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
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

In the present study, biparietal diameter was found to be a consistent parameter in determining the age of gestation in Belgium and German Shepherd 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 by the owners

which was based on the mating history. A reliability of 91 percent, on linear regression was evident during the current study. The observations made during the present study were in accordance with the findings of Son *et al.* (2001) and Ramya *et al.* (2018), who observed a reliability of 99 percent between reported gestational age and biparietal diameter 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.

In the present study, echogenic inner layer surrounding the gestational sac was identified as the zonary placenta as early as 27 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) and 27-30 days (Concannon, 2000).

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 2.67, 5.35 and 6.18 mm at 32.73, 44.09 and 55.05 days, respectively. This variation could be due to the cross sectional study involving different breeds of dogs with varied body sizes by Maldonado *et al.* (2012) as compared to the sequential observations made in Shepherds only, in the present study.

The present study revealed a placental thickness of 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.

The study showed that assessment of placental thickness can act as a predictor of gestational age similar to that measured by mean head diameter or biparietal diameter which coincides with the findings of Almeida *et al.* (2003) in Boxers and Maldonado *et al.* (2012) in bitches of different breeds and sizes. 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.

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 as also mentioned by Aralla *et al.* (2013).

Conclusions

It was suggested that the placental thickness is significantly correlated with gestational age and reported gestational age and it can be used to estimate gestational age of foetus along with other parameters like gestational sac diameter and biparietal diameter in large breeds of dogs. Evaluation of placenta may also 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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