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Ultrasonographic Predication of Gestational Age and Expected Whelping Date in Canine (*Canis familiaris*)

Lakshmi Yadav, Chander Shekar Sarswat, Shivani Purohit, Kuldeep Singh Gurjar, Sumit Prakash Yadav and Hitesh Kumar

Dept. of Veterinary Gynaecolgy and Obstetrics, Post Graduate Institute of Veterinary Education and Research Jaipur, Rajasthan (302 031), India

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Corresponding ydvlakshu01@gmail.com

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ABSTRACT

The present study was conducted during August, 2023 to March, 2024 at Veterinary Clinical Complex in department of Veterinary Gynaecology and Obstetrics, Post Graduate Institute of Veterinary Education and Research (PGIVER) Jaipur to study the gestational age during pregnancy in different breeds (*Canis familiaris*) by using various ultrasonographic parameters such as Gestational Sac Diameter (GSD), Head Diameter (HD), Crown Rump Length (CRL) and Body Diameter (BD) and to also study the expected and actual whelping date in different breeds (*Canis familiaris*). Using ultrasound, 30 bitches' pregnancy diagnoses were determined. The bitches were examined during different phases. 11 pregnant bitches were examined during first phase 20 to 30 days' post mating, 6 pregnant bitches were examined during second phase 30 to 45 days' post mating and 13 pregnant bitches were examined in third phase 45 to 60 days' post mating. During first phase Gestational sac diameter was measured, during second phase Crown rump length was measured and during third phase Head diameter and Body diameter were measured. During first phase the mean (±SE) gestational sac diameter was 1.23±0.12 cm. The calculated gestational age by using formula I was found to be 27.4±0.74 days. The difference between expected and actual whelping date during this phase mean (±SE) was -2.36±0.20 days. During the second phase the mean (±SE) CRL measurement was 2.99±0.28 cm. The calculated gestational age by using formula II mean (±SE) found to be 35.96±0.85 days. The difference between expected and actual whelping date during this phase mean (±SE) was -1.5±0.62 days. During the third phase the mean (±SE) BPD was 2.04±0.08 cm and BD was 3.37±0.14 cm. The calculated gestational age by formula III mean (±SE) was 50.57±1.33 days, by formula IV mean (±SE) was 52.59±1.01 days and by formula V mean (±SE) was 52.34±0.88 days. The difference between expected and actual whelping date mean (±SE) was -1.85±0.52 days.

KEYWORDS: Body diameter, crown rump length, gestational sac diameter

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1. INTRODUCTION

The bitch's pregnancy lasts for 63 days from ovulation, which was a short time compared to other domestic animals. In order to prepare for survival outside of the uterus, the major organ systems of the foetus undergo significant development in the latter stages of gestation. The foetuses would not survive postpartum if they were unable to reach full maturity.

Ultrasonography was useful for monitoring high-risk pregnancies, diagnosing canine pregnancy, estimating gestational age, evaluating bitches in dystocia, detecting fetal distress, and determining the gender of the fetus (Lopate, 2023). A recent study (O'Niell et al., 2017) used a new method for gestational ultrasound examination in light of the necessity of scheduling elective cesarean sections for brachycephalic breeds (English Bulldog, Yorkshire, Chihuahua, miniature breeds), that frequently experience obstructive dystocia due to fetal-maternal disproportion.

Ultrasonography (US) has been used more recently to describe and calculate parameters and formulas, the accuracy of these calculations varies mostly depending on the pregnancy phase and litter size (Alonge et al., 2016, Cramer and Nothling, 2018). Different approaches for estimating the date of parturition have been offered by numerous studies (Gil et al., 2014, Rota et al., 2015, Giannico et al., 2015, Beccaglia et., 2016, Lopate, 2018, Gil et al., 2018). The gestational timing evaluation can be done by using ultrasonography where the first appearance of embryonic and foetal structure were evaluated. The gestational sac in the early stages of pregnancy and the biparietal diameter (BPD) formula had been found to be useful in several studies on the prediction of parturition using ultrasonographic measurement (Arlt, 2020). ICC volume did not improve canine gestational ageing accuracy, which supports the continued use of the ICC diameter (Pestelacci et al., 2022)

Groppetti et al., 2015, Son et al., 2021, Cecchetto et al., 2017 Socha and Janawosi, 2018, Fernandes et al., 2020, Pederson et al., 2022, de Freitas et al., 2021, Gorka et al., 2021 suggested that both Inner Chorionic Cavity (ICC) and Biparietal Diameter (BPD) were reliable gestational age indicators and also to estimate the parturition date whereas Lopate, 2018, Siena and Milani, 2021 suggested that ICC is more accurate parameter to predict expected whelping date. By measuring biparietal diameter during the second half of pregnancy Reliable results were obtained. Indeed, the findings demonstrate that biparietal diameter served as a reliable predictor of gestational age (Pettina et al., 2023).

In advanced pregnancies, it is crucial to correlate the results of biometric formulas with other ultrasound analyses, such as organogenesis (Pieri et al., 2015) and fetal heart rate measurement (Gil et al., 2014), or more complex studies that use Doppler ultrasound to measure the umbilical artery's resistivity (Giannico et al., 2015), in order to increase the accuracy of predicting the delivery onset and diagnosis of fetal distress.

Fetal measurements using the deep portion of the foetal diencephalo-telencephalic vesicle (DPTV), body diameter (BD), kidney length, femur length, humerus length, tibia length, and kidney length had all been used to gestationally age foetuses if the bitch was over 37 days' gestation (Maronezi et al., 2021).

The gestational timing evaluation can be done by using ultrasonography where the first appearance of embryonic and foetal structure were evaluated. The gestational sac in the early stages of pregnancy and the biparietal diameter (BPD) formula had been found to be useful in several studies on the prediction of parturition using ultrasonographic measurement (Arlt, 2020). Therefore, this study was planned to investigate the different phases of pregnancy in different breeds of bitches by the help of ultrasonography with the following objectives:

1. To study the gestational age during pregnancy in different breeds (*Canis familiaris*) by using various ultrasonographic parameters such as Gestational Sac Diameter (GSD), Head Diameter (HD), Crown Rump Length (CRL) and Body Diameter (BD).

2. To study the expected and actual whelping date in different breeds (*Canis familiaris*).

2. MATERIALS AND METHODS

The present study was conducted during August, 2023 to March, 2024 at VCC in department of Veterinary Gynaecology and Obstetrics, PGIVER Jaipur. This study included 30 pregnant bitches of different breeds aged between 2 to 7 years which were presented to VCC, PGIVER Jaipur. Ultrasonography was carried out in three phases between days 20 to 30, 30 to 45 and 45 to 60 days after last mating. The uterus was scanned using a transabdominal sector transducer and a real-time, B-mode ultrasonography machine (Ultrasound Machine ESAOTE-My Lab Seven Vet) with 3.5 to 5 MHz.

All bitches were scanned in left and right lateral and dorsal recumbency. Ultrasound coupling gel increased ultrasound conductivity on abdomen and probe. A 3.5–5 MHz sector transabdominal transducer had photographed the whole abdomen. Starting from caudal, each horn was scanned in dorsal and lateral decumbency. Ultrasound callipers had measured foetal structures.

Three phases scanned the bitches.

• Phase I Bitches vertical and horizontal gestational sac

diameters (GSD) were measured 20–30 days after mating.

• Phase II Pregnant bitches 30–45 days from last mating were photographed and their crown rump length (CRL) or occipito-sacral length.

• Phase III comprised pregnant bitches 45–60 days after mating. Fetal BPD and BD were measured in these bitches.

The measurements made throughout the several phases that had previously been recorded separately for each bitch, were then correlated with the expected and actual day of whelping.

The ultrasonographic foetal age was calculated using various formulae as suggested by Nyland and Mattoon (1995).

• The formula used for bitches 20 to 30 days' post mating

• Gestational age (days)=[6×Gestational sac diameter]+20

• The formula used for bitches 30 to 45 days' post mating

• Gestational age (days)=[3×Crown rump length]+27

• The formula used for bitches 45 to 65 days' post mating

• Gestational age (days)=[15×head diameter]+20

• Gestational age (days)=[7×body diameter]+29

• Gestational age (days)=[6×head diameter]+[3×body diameter]+30

Statistical analysis: suitable statistical tools were used for meaningful results.

3. RESULTS AND DISCUSSION

3.1. First phase (20 to 30 days)

In the first phase 11 pregnant bitches were scanned. The gestational sac diameter (GSD) observed during first phase was in the range from 0.53 cm to 1.71 cm with the mean (±SE) 1.236±0.12 cm during the observed gestational age ranged from 23 to 30 days with the mean (±SE) 27.73±0.74 (Figure 1).

Formula I was used to estimate the gestational age or foetal age.

Figure 1: Measurement of gestational sac diameter on ultrasound examination (Day 25th day)

The calculated gestational age which was calculated by formula I ranged from 23.18 to 30.26 with the mean (± SE) 27.4±0.74 (Table 1).

Table 1: Showing the Average measurements of canine gestational sacs on ultrasound examination during pregnancy (between days 20 to 30)

Sl. No.	Calculated gestational age	Observed gestational age (days after last mating)	Avg. Gestational sac diameter (GSD (cm)
1.	30.26	30	1.71
2.	30.2	30	1.7
3.	26.6	27	1.1
4.	24.2	24	0.7
5.	23.18	23	0.53
6.	29	29	1.5
7.	30.2	30	1.7
8.	28.4	28	1.4
9.	26.6	27	1.1
10.	25.5	27	0.92
11.	27.26	30	1.21
Mean	27.4	27.73	1.23
SD	2.46	2.45	0.41
SE±	0.74	0.74	0.12

The observations support to Ingawale (2002) who recorded it as 1 cm, England (2003) and Aissi (2008) who recorded it 1 cm at day 28 of gestational age, Gunwan et al. (2019) recorded it as 0.51 cm on 18th day and Maronezi et al. (2021) recorded it as 0.59–0.89cm on 23rd day. Nyland and Mattoon (1995), Alonge et al. (2016) and Socha and Janwoski (2018) have also reported similar findings.

On the other hand, Javeid et al. (2013) noted in less than 40 days of gestation, the mean GA ranged from 27.2 to 39.8 days with mean 34.34±0.90 days,

Using the gestational age determined by formula I, the expected duration for whelping was determined and it was compared with the actual date. Table 2 displays an overview of the findings.

From table 2 the difference between actual and expected whelping date was -2.3±0.20 days ranged from -1 day to -3 days.

Luvoni and Grioni (2000), Son et al. (2001), Kutzler et al. (2003), Beccaglia and Luvoni (2006), Lopate (2008) and other studies had revealed that in large breeds, the accuracy of prediction based on ICC measurement in early pregnancy ranged from 85 to 88% (±2 days).

Table 2: Showing the actual whelping date and expected whelping date and the difference between them

Sl. No.	Expected whelping date	Actual whelping date	Difference between expected and actual whelping date
1.	04/09/23	02/09/23	-2 days
2.	06/11/23	04/11/23	-2 days
3.	16/12/23	14/12/23	-2 days
4.	05/01/24	04/01/24	-1 day
5.	15/01/24	13/01/24	-2 days
6.	27/01/24	23/01/24	-3 days
7.	06/02/24	3/02/24	-3 days
8.	12/02/24	10/02/24	-2 days
9.	6/02/24	03/02/24	-3 days
10.	7/02/24	4/02/24	-3 days
11.	23/03/24	20/03/24	-3 days
Mean			-2.36
SE±			0.20

Between days 23 and 39 of pregnancy, Groppetti et al. (2015) measured the intrauterine circulation (ICC) and found that, at ±1 day and ±2 days, the percentage was reported at 81.8% and 94.5% respectively. Alonge et al. (2016), Cecchetto et al. (2017) and Socha & Janowski (2017) observed that the precision of ICC at ±2 days was considerably greater than that at ±1 day.

3.2. Second phase (30–45 days)

In second phase 6 pregnant bitch were scanned. Their Crown rump length (CRL) was observed which ranged from 2.34 to 4.2 cm with the mean (±SE) 2.99±0.28 and the observed gestational age ranged from 32 to 40 days with the mean (±SE) 36.00±1.21 (Figure 2).

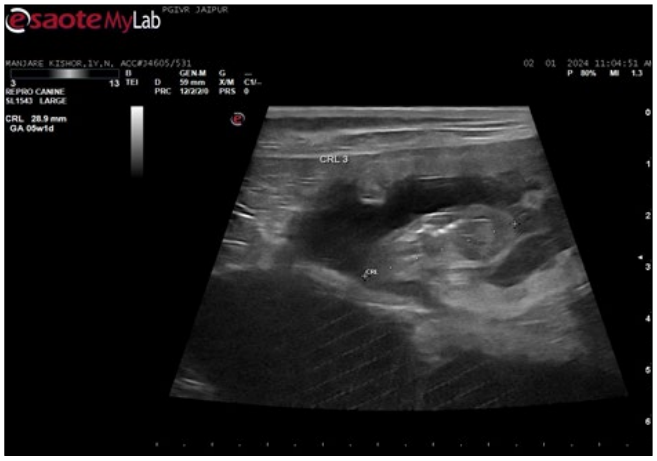

Figure 2: Measurement of crown-rump length on ultrasound examination (Day 36th)

Rajabhau (2007) noted 2.46±0.15 cm had gestational age 34.38±0.47. Kim and Son (2008) noted that from 2.55±0.07 mm on day 22 to 85.25±9.89 mm on day 48. Balaji et al. (2018) noted average CRL to be 18.5 mm from 27th day to 30th day, 25.5mm from 34th to 37th day and 71mm from 38th to 45th day. Formula II was used to estimate the foetal age.

The calculated foetal age ranged from 34.02 to 39.6 with the mean (±SE) 35.96±0.85. The results mentioned above agree with those of Kutzler et al. (2003) and Jayakar (2004) who revealed that the stage of gestation during the 30 to 45 days following mating can be more precisely estimated using CRL measurements with ultrasonography. Rajabhau (2007) noted 34.38±0.47 days using formula II (Table 3).

Table 3: Showing the crown rump length (CRL) on ultrasound examination during pregnancy (between days 30 to 45)

Sl. No.	Calculated gestational age	Observed gestational age (days from last mating)	Crown rump length (CRL)
1.	35.4	35	2.8
2.	39.6	39	4.2
3.	36.84	40	3.28
4.	34.02	32	2.34
5.	34.02	35	2.34
6.	35.85	35	2.95
Mean	35.96	36	2.99
SD	1.91	2.97	0.64
SE±	0.85	1.21	0.28

The gestational age, which was calculated using formula II, was used to determine the expected date of whelping and it was compared with actual whelping date. Table 4 presents an overview of the results. The difference between expected

Table 4: Showing the actual whelping date and expected whelping date and the differ-ence between them (between 30–45 days)

Sl. No.	Expected whelping date	Actual whelping date	Difference
1.	30/12/23	30/12/23	0 day
2.	27/01/24	23/01/24	-4 days
3.	14/03/24	13/03/24	-1 day
4.	17/03/24	17/03/24	0 day
5.	17/03/24	15/03/24	-2 days
6.	28/03/24	26/03/24	-2 days
Mean			-1.5
SE±			0.62

and actual whelping date was -1.5±0.62 days ranged from 0 day to 4 days.

Due to the increased fetus's flexion of their spines, CRL was less accurate in predicting the timing of parturition when gestation was almost over (England et al., 1990), cecchetto et al. (2017) and Siena and Milani (2021).

3.3. Third phase (45–60 days)

In the third phase 13 pregnant bitches were scanned.

The Biparietal diameter (BPD) / Head diameter (HD) and Body diameter (BD) were measured. The BPD ranged from 1.6–2.75 cm with the mean 2.04±0.08. The BPD measured during the observed gestational age ranged between 49 to 55 days with the mean 51.92±0.64 (Figure 3).

Figure 3: Measurement of head diameter on ultrasound examination (Day 52nd)

Kim and Son (2008) found that on day 27, the foetal head diameter (HD) was 3.43±0.64 mm, and on day 63, it measured 25.06±0.41 mm. Also Alonge et al. (2016) and Socha and Janowski (2018) shows similar result for biparietal diameter (Table 5).

The Body diameter (BD) was ranged between 2.7 to 4 cm having the mean (±SE) 3.37±0.14 cm during the observed gestational age ranged between 49 to 55 days with the mean (±SE) 51.92±0.64 (Figure 4).

Kim and Son (2008) stated that on day 30, the foetal body diameter (BD) was 5.96±0.84 mm; on day 63, it was 43.76±3.36 mm (Table 6).

Using formula III

The calculated gestational age / foetal age ranged between 44 to 61.25 day with the mean (±SE) 50.57±1.33 days. Jayakar (2004) noted the gestational age 45.5, 50.5,51.5 and 51.5 days from the formula III and Rajabhau (2007) noted 49.60±0.90 days.

Using formula IV

The calculated foetal age / gestational age ranged between

Table 5: Showing the Biparietal Diameter (BPD) on ultrasound examination during pregnancy (between days 45 to 60)

Sl. No.	Calculated gestational age (by formula III)	Days from last mating	HD
1.	44	51	1.6
2.	44	48	1.6
3.	54.5	55	2.3
4.	53	54	2.2
5.	47	49	1.8
6.	54.5	55	2.3
7.	51.5	51	2.1
8.	51.5	52	2.1
9.	50	51	2.0
10.	51.5	52	2.1
11.	61.25	55	2.75
12.	48.35	52	1.89
13.	46.25	50	1.75
Mean	50.57	51.92	2.04
SD	4.80	2.29	0.32
SE±	1.33	0.64	0.09

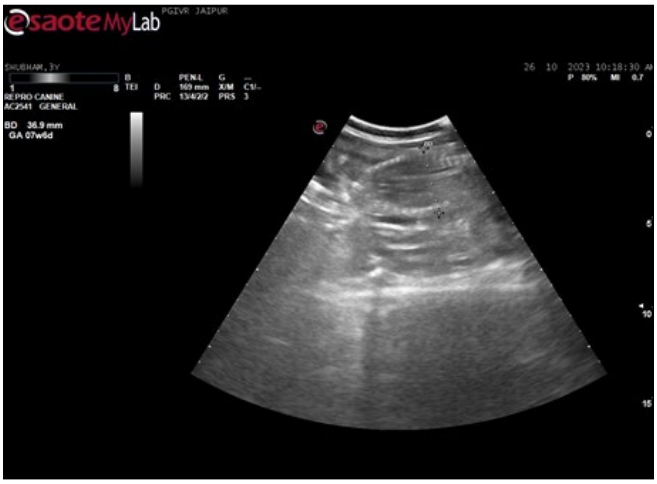

Figure 4: Measurement of body diameter on ultrasound examination (Day 55th)

48.9 to 58.8 days having the mean (±SE) 52.59±1.01. Jayakar (2004) noted the gestational age 47.9, 56.3, 51.4, 52.45 days by using the formula IV and Rajabhau (2007) noted 47.44±0.63 days.

Using formula V

The gestational age calculated using formula V ranged between 48.87 to 59.28 days with the mean (±SE) 52.34±0.88. Jayakar (2004) noted the gestational age 48.3,

Table 6: Showing the Body Diameter (BD) on ultrasound examination during pregnancy (between days 45 to 60)

Sl. No.	Calculated gestational age (by formula IV)	Days from last mating	BD
1.	55.6	51	3.8
2.	50.7	48	3.1
3.	57	55	4
4.	56.3	54	3.9
5.	47.9	49	2.7
6.	56.3	55	3.9
7.	50	51	3
8.	50.7	52	3.1
9.	50.98	51	3.14
10.	51.4	52	3.2
11.	58.82	55	4.26
12.	49.51	52	2.93
13.	48.53	50	2.79
Mean	52.59	51.92	3.37
SD	3.66	2.29	0.52
SE±	1.01	0.64	0.14

53.7, 52.2 and 52.65 and Rajabhau (2007) noted 49.87±0.47 days (Table 7).

Table 7: Calculated gestational age on day of ultrasound examination by formula V (between days 45 to 60)

Sl. No.	Days from last mating	Calculated gestational age
1.	51	51
2.	48	48.9
3.	55	55.8
4.	54	54.9
5.	49	48.9
6.	55	55.5
7.	51	51.6
8.	52	51.9
9.	51	51.42
10.	52	52.2
11.	55	59.28
12.	52	50.13
13.	50	48.87
Mean	51.92	52.34
SD	2.29	0.88
SE±	0.64	3.05

The gestational age, which was calculated using formula V, was used to determine the expected date of whelping and it is compared with actual whelping date. Table 8 presents an overview of the results. The difference between actual and expected whelping date was -1.85±0.52 days ranged from 0 to 7 days. Table 8 showing the expected and actual whelping date and the difference between them.

Table 8: showing the actual whelping date and expected whelping date calculated by formula V and the difference between them

Sl. No.	Expected whelping date	Actual whelping date	Difference
1.	03/11/23	01/11/23	-2 days
2.	16/11/23	16/11/23	0 day
3.	18/11/23	18/11/23	0 day
4.	19/11/23	16/11/23	-3 days
5.	11/01/24	4/01/24	-7 days
6.	17/01/24	17/01/24	0 day
7.	18/01/24	17/01/24	-1 day
8.	22/01/24	20/01/24	-2 days
9.	26/01/24	23/01/24	-3 days
10.	31/01/24	30/01/24	-1 day
11.	22/02/24	21/02/24	-1 day
12.	1/03/24	28/02/24	-2 days
13.	2/03/24	29/02/24	-2 days
Mean			-1.85
SD			0.52
SE±	1.01	0.64	0.14

The most reliable predictor of gestational age, according to Nyland and Mattoon (1995), Cecchetto et al. (2017) was BPD. It was discovered that BD and BPD together provide a more accurate predictor of gestational age. Researchers England et al. (1990) and Jayakar (2004) all noted that BPD and BD were the best indicators of gestational age.

From statistical analysis the expected whelping date calculated in Group I, Group II and Group III was found to have non-significant difference (Table 9).

Table 9: ANOVA table for difference in expected whelping date and actual whelping date of all three groups

Source	DF	SS	MSS	F (calcul.)
Group	2	3.89	1.95	0.94
Error	28	58.11	2.08	
Total	30	62		

Table 9: Continue...

Source	F (5%)	F (1%)	F (5%) S/N	F (1%) S/N	*p* value
Group	3.32	5.39	N	N	0.40
Error					
Total					

4. CONCLUSION

Pregnancy was confirmed as early as 23rd day post mating by ultrasonography. Formula I [(6×GSD)+20] was used to predict gestational age at 20 to 30 days post mating. Formula II [(3×CRL)+20] was used to predict gestational age at 30 to 45 days post mating. Formula III, IV and V [(15×HD)+20], [17×body diameter]+29 and [(6×HD) (3×BD)+30] respectively were used to calculate gestational age at 45 to 60 days post mating. 65- gestational age was used to calculate the whelping date.

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