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Ultrasonographic evaluation of fetal echo biometry and serum progesterone changes in normal and aborted pregnancies in bitches

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Abstract
Thirty bitches of different breeds, weighing 5 to 40 kg and aged between 2 to 6 years presented to the Small Animal Gynaecology unit of Madras Veterinary College, Chennai for pregnancy diagnosis (after 30-35 days of mating) and were divided into three groups of 10 bitches each as Group I (Abortion), II (Foetal Resorption) and III (Normal pregnancy). Foetal echo biometry and serum progesterone estimation were done. Fetal heart rate from the 5th week of pregnancy was significantly lower in abortion (235.2±9.75) and resorption (203±5.49) groups. As gestation age advances, a significant reduction in the fetal heart rate was observed in group I. Gestational sac diameter during the 7th week of gestation was significantly higher ($p<0.05$) in normal pregnancy (G-III) bitches (4.02±0.41 cm) than in G-II and G-I. During 5th week of gestation, bitches with normal pregnancy (G-III) had significantly higher ($p<0.01$) placental diameter (0.49±0.03 cm) than the bitches in abortion and resorption groups. Progesterone concentration in the abortion group was (8.81 ng/ml) found to be significantly lower than that of the normal pregnancy group (10.32 ng/ml). In conclusion, it was found that the bitches in abortion and resorption groups had significantly lower fetal heart rates and lower progesterone concentration than normal pregnant bitches indicating fetal/maternal stress in bitches.

Keywords: Canine, abortion, fetal resorption, pregnancy, ultrasonography, fetal echo biometry, progesterone

1. Introduction
Accurate prediction of pregnancy is clinically useful to prevent or curtail reproductive losses by timely intervention. Traditionally, two-dimensional ultrasonography is considered to be a safe and simple method for tracking foetal growth and assessing viability (Yeager and Concannon, 1990). In Canine, the pregnancy losses are due to embryonic death, foetal resorption, abortion at any stage of pregnancy and infectious causes are the major reasons for it. Apart from infection, hormonal imbalances, hypoluteoidism, trauma, genetic abnormalities, adverse environmental conditions, uterine pathologies, maternal stress, nutritional deficiencies, drugs and toxic agents (Purswell, 1991; Verstegen *et al*., 2008; Gorlinger *et al*., 2005) [1, 2, 3] are responsible for the pregnancy loss in canines. Compared with other reproductive disorders, the incidence of abortion and resorption were reported to be 4.83 per cent and 11 to 25.9 per cent respectively, in canines (Dhurvey *et al.,* 2022) [4]. Progesterone is the hormone of pregnancy and is essentially needed for the maintenance of pregnancy and a drastic reduction in progesterone indicates that the animal is going for abortion and the liveability of the fetus is compromised (Uchanska, *et al.,* 2022) [5]. Considering the above facts, the present study was carried out to assess the ultrasonographic changes in foetal growth, heart rate, and hormonal changes in normal and abortion during pregnancies in bitches.

2. Materials and Methods
Female dogs of different breeds, weighing 5 to 40 kg and aged between 2 to 6 years presented to the Small Animal Gynaecology unit of Madras Veterinary College Teaching Hospital in Chennai for pregnancy diagnosis (after 30-35 days of mating) were utilized for this study.

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2.1 Pregnancy diagnosis by ultrasound
Pregnancy was confirmed by ultrasound examination of bitches from the 5th week of mating/breeding using a sector probe of 5–7.5 MHz. (EDAN). After confirmation of pregnancy, the selected pregnant bitches were monitored for foetal growth and well-being using two-dimensional ultrasonography at weekly intervals (5th week onwards) throughout gestation until abortion/resorption/ normal parturition. Based on the outcome of pregnancy, the selected bitches were divided into three groups of 10 bitches each as Group I (Abortion), II (Foetal Resorption) and III (Normal pregnancy).

2.1.1 Gestational sac diameter
The gestational sac diameter (GSD) or inner chorionic cavity diameter (ICCD) was measured to estimate the age of the foetus during the 5th week of pregnancy as per the formula of Yeager et al. (1992) [6].

2.1.2 Head diameter
The head diameter (HD) or biparietal diameter (BPD) was measured to confirm the age of the foetus during 6th, 7th and 8th week of pregnancy as per the formula of Yeager et al. (1992) [6].

2.1.3 Heart rate
The viability of the foetus was evaluated based on visualization of foetal heartbeat. The frequency of heartbeat was calculated by capturing number of beats per minute or by measuring the distance between two systolic peaks using M-mode ultrasonography.

2.1.4 Placental thickness
The thickness of the zonary placenta within the gestational sac was measured by B-mode ultrasonography and the average placental thickness in centimetres was evaluated (Feliciano et al., 2015) [7].

2.2 Serum progesterone estimation
Blood samples were collected in all selected pregnant bitches at weekly intervals, beginning from the 5th week of gestation until abortion/resorption/normal delivery of the foetus for hormonal analysis. After separating serum, samples were stored at -20 °C until utilised for progesterone estimation. The estimation of progesterone concentration in group I, II and III bitches were performed using specific commercial Radioimmunoassay kits (RIA, Progesterone, Beckman Coulter, Czech Republic: Cat # IM188) according to the manufacturer's instructions.

2.3 Statistical analysis
The data collected in the study were analysed using one-way analysis of variance (ANOVA) and repeated measures of one-way (ANOVA) for ultrasonography and hormonal parameters between groups. A paired t-test was performed for the echo-biometric parameters to correlate their association with gestational age with the aid of SPSS (20.0 version) statistical software.

3. Results and Discussions
In the present study, foetal eco-biometric parameters like gestational sac diameter (GSD), placental diameter (PD), heart rate (HR) and head diameter (HD) were recorded by 2D ultrasonographic examination at the 5th, 6th and 7th week of gestation in abortion, resorption and normal pregnancy bitches (Table 1 and 2).

3.1 Gestational sac diameter
In the present study, as pregnancy age advances there is a gradual, significant increase in the diameter of gestational sac in normal pregnancy as well as in the abortion group. Gestational sac diameter during the 7th week of gestation was significantly higher ($P<0.05$) in normal pregnancy (G-III) bitches (4.02±0.41 cm) when compared to the bitches (3.75±0.09 cm) which aborted their foetus (G-I). Similarly, Alonge et al., (2016) [8] recorded the gestational sac diameter of 6.9 to 42.4 mm and 8.4 mm to 41.3 mm in large and giant size breeds of dogs from 42 to 26 days and 40 to 25 days respectively, before parturition. Whereas, Mattoon and Nyland (1995) [9] reported that at 20 days of post-breeding detection of a gestational sac was considered as the first sign of confirming pregnancy using ultrasonography.

3.2 Fetal heart rate
The mean heart rate from the 5th week of pregnancy was significantly lower in abortion (235.2±9.75) and resorption (203±5.49) groups when compared to normal pregnancy bitches (277.1±4.07). England (1998) [10] recommended foetal heart rate as a useful parameter to estimate the survival possibilities of foetuses. The cardiac activity was deducted as early as 25 days after LH surge (Yeager et al. 1992) [6].
As pregnancy age advances a gradual significant reduction in the fetal heart rate was observed in normal pregnancy. However, the reduction was more pronounced in abortion groups (235.2±9.75, 197.1±4.43 and 170.9±2.96 at 5th, 6th and 7th week of gestation), indicating fetal and maternal stress. Normal fetal heart rate is 220 to 240 bpm while rates of < 180 bpm are indicative of fetal distress (Zone and Wanke, 2001) [11]. Hypoxia-induced fetal stress, which presents as a drop-in heart rate, can happen during an abnormal pregnancy (Kutzler et al., 2003) [12].

3.3 Placental diameter
During 5th week of gestation, bitches with normal pregnancy (G-III) had significantly higher (P<0.01) placental diameter (0.49±0.03 cm) than the bitches (0.37±0.01 cm) which aborted their foetus (G-I) and bitches (G-II) which shown resorption (0.33±0.02 cm) of the foetus (Table 1). A significant increase in the placental diameter was observed between 5th to 7th week of normal pregnancy and the abortion groups. At any stage of pregnancy, the normal canine placenta should not exceed 1.2 cm at its centremost point regardless of the breed in dogs. The thickness of the placenta may assist in the diagnosis of gestational age in bitches. Maldonado et al., (2012) [13] and Babiker and Eisa (2014) [14] reported that the placental thickness of 0.34 cm, 0.44 cm, 0.67 cm and 0.89 cm would relate to 30.5 days, 35.2 days, 46 days and 56.3 days of pregnancy. The results of the present study were in agreement with them.

3.4 Head diameter
Head diameter observed during the 6th and 7th week of pregnancy in the present study ranged from 1.30±0.06 cm to 2.01±0.03 cm. Alonge et al. (2016) [8] recorded the HD of 5.2 to 28.1mm and 2 mm to 29.4 mm in large and giant size breeds of dogs from 30 to 2 days and 35 to 1 day respectively, before parturition. The head of the fetus showed significant growth during the 6th to 7 weeks of pregnancy in both groups I and III bitches. While in the resorption group, the head

diameter was slightly lower than the groups I and III during the 6th week. Zone and Wanke (2001) [11] found that head diameters of < 2 cm from days 48 to term are indicative of growth retardation, resulting in significantly reduced average birth weight for the breed and are also at risk for abortion or early neonatal loss.

Table 1: Foetal eco-biometry (Mean±SE) at 5th, 6th and 7th week of gestation in Abortion, Resorption and Normal pregnancy bitches

Weeks of gestation	Group I	Group II	Group III	'P' value
Gestational sac diameter (cm)				
5th week	1.80±0.07	1.78±0.07	2.05±0.10	0.06
6th week	2.90±0.20	2.65±0.15	3.26±0.08	0.41
7th week	3.75±0.09	-	4.02±0.41	0.02
P value	0.00	0.00	0.00	
Heart rate (bpm)				
5th week	235.21±9.75	203.00±5.49	277.12±4.07	0.00
6th week	197.10±4.43	184.00±12.00	260.70±5.67	0.00
7th week	170.90±2.96	-	241.60±4.93	0.00
P value	0.00	0.00	0.00	
Placental diameter (cm)				
5th week	0.37±0.01	0.33±0.02	0.49±0.03	0.00
6th week	0.58±0.05	0.47±0.12^y	0.74±0.04	0.38
7th week	0.99±0.50	-	0.97±0.09	0.85
P value	0.00	0.00	0.00	
Head diameter (cm)				
6th week	1.47±0.07	1.30±0.06	1.54±0.04	0.27
7th week	1.92±0.08	-	2.01±0.03	0.36
P value	0.00	-	0.00	

3.5 Serum Progesterone

The progesterone concentration was found to be within normal ranges (25.77±1.90, 27.40±2.53 and 28.10±2.30 ng/ml) in all the groups during the 5th week of pregnancy in the present study (Table 2). However, the progesterone concentration during the 6th week in the abortion group was 8.81 ng/ml which was found to be significantly lower than that of the normal pregnancy group (10.32 ng/ml). This decrease in progesterone concentration in the 6th week coincident with clinical signs or signals of threatened or imminent abortion during the 7th week of pregnancy in bitches, wherein the progesterone concentration has further reduced during the 7th week (1.72±0.32 ng/ml).

In pregnant bitches, with the induction of prepartum luteolysis and a steep reduction in P_4 concentration around day 60 of the CL lifespan. This active termination of CL function is associated with increased production of uteroplacental $PGF_{2}\alpha$

(Kowalewski *et al.,* 2020) [15]. The progesterone concentration in the resorption group is drastically (1.72±0.32 ng/ml) reduced than other groups during the 6th week indicating death of the fetus and resorption has started within the 6th week. Similar to the current study, Thuroczy *et al.,* (2016) [16] have reported that a decrease in serum progesterone below 10 ng/ml at 4th week of pregnancy may signal an impending abortion in bitches. According to experimental research, keeping a pregnancy in the bitch requires a plasma progesterone concentration of at least 6–9 nmol/L (2–3 ng/mL), and concentrations below this threshold for longer than 24–48 hours result in pregnancy loss. (Onclin *et. al.,* 2002) [19]. The progesterone concentrations observed in the present study indicate that the animals suspected of hypoluteoidism can have their abortions predicted by serum progesterone estimations.

Table 2: Serum progesterone level ((Mean±SE) at different stages of gestation in Abortion, Resorption and Normal pregnancy bitches

Stages of Pregnancy	Serum level of Progesterone (ng/dl)			P value
	Group I (Abortion)	Group II (Resorption)	Group III (Normal pregnancy)	
5th week	25.77±1.90	27.40±2.53	28.10±2.31	0.75
6th week	8.81±0.55	1.72±0.31	10.32±0.74	0.00
7th week	1.15±0.17	-	5.73±0.60	0.00

In conclusion, ultrasonography has been used as a vital tool to assess low and high-risk pregnancies in canines. A lower heart rate of the fetus and reduction in serum progesterone concentration of dam during pregnancy indicates fetal/maternal stress leading to abortion or fetal resorption in bitches.

Conclusion

Accurate prediction of pregnancy outcomes in canines is crucial for mitigating reproductive losses through timely intervention. Two-dimensional ultrasonography remains a safe and effective method for monitoring foetal development and assessing viability, aiding in early detection of issues such as embryonic death, foetal resorption, and abortion. Factors contributing to pregnancy loss include infections, hormonal

imbalances, trauma, genetic abnormalities, and environmental stressors. This study underscores the utility of ultrasonographic parameters like gestational sac diameter, fetal heart rate, placental thickness, and serum progesterone levels in distinguishing between normal and high-risk pregnancies, offering valuable insights into canine reproductive health management.

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