RESEARCH ARTICLE

Doppler Ultrasonographic Study on Haemodynamic Alterations in Medically Terminated Canine Pregnancies

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Abstract

This study evaluated maternal and fetal haemodynamic changes in medically terminated canine pregnancies using Doppler ultrasonography. Twelve pregnant bitches (35-40 days gestation) were divided into a Group I (control group, n=6) and Group II (treatment group n=6). A combination protocol of cloprostenol (1 µg/kg SC every 48 h) and cabergoline (5 µg/kg PO daily) was used to induce abortion for bitches in group II. Parameters assessed included biparietal diameter (BPD), combined thickness of uterus and placenta (CTUP), fetal heart rate (FHR), progesterone levels, and Doppler parameters (PSV, EDV, RI, PI, S/D ratio) in the uteroplacental artery (UPA), umbilical artery (Uma) and fetal aorta (FA). All group II bitches aborted within 3-5 days, with progesterone dropping below 1 ng/mL by day 5. Significant haemodynamic disruptions were observed post-treatment, including increased UPA resistance (RI >0.61, EDV <15 cm/s) and diminished Uma diastolic flow, signaling placental insufficiency. No structural differences in BPD or CTUP were noted. These findings underscore Doppler's role in high-risk pregnancy management and abortion monitoring in canines.

Key words: Canine pregnancy, Doppler, Progesterone, Resistive index, Umbilical artery, Uteroplacental artery.

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Introduction

Ultrasonography is an essential non-invasive diagnostic tool in veterinary reproduction, widely applied in canine pregnancy detection and fetal monitoring. It enables early pregnancy confirmation and provides insights into gestational age, fetal development, heart rate, and placental health, offering greater accuracy than traditional methods like palpation and radiography (England *et al*., 1990). By employing fetal biometry, ultrasonography helps assess normal fetal growth and detect abnormalities (Beccaglia and Luvoni, 2012). Beyond pregnancy diagnosis, it is useful in identifying reproductive disorders such as pyometra, cystic endometrial hyperplasia, and ovarian tumors (Bigliardi *et al.,* 2004; Troisi *et al*., 2023). Despite advances in reproductive imaging, many high-risk pregnancies go undetected until late-stage complications arise, often leading to fetal loss. The combined thickness of the uterus and placenta (CTUP) is a key marker for detecting placental dysfunction, which can result in intrauterine growth restriction, premature birth, or abortion (Gaikwad *et al.,* 2017). Doppler ultrasonography has further improved fetal assessment by enabling real-time monitoring of blood flow and vascular resistance in key arteries, such as the uteroplacental, umbilical, and fetal aorta (Di Salvo *et al.,* 2006). By measuring peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI), it helps evaluate fetal well-being and detect early signs of distress (Miranda and Domingues, 2010; Blanco *et al.,* 2011). Doppler-based monitoring has been widely used in equine and human obstetrics, yet studies on maternal and fetal haemodynamic indices in canine pregnancies remain limited (Blanco *et al.,* 2009; Gaikwad *et al.,* 2020; Simon *et al.,* 2022). Given its potential to predict pregnancy outcomes and enable timely interventions, this study was planned to evaluate Doppler ultrasonographic alterations in maternal and fetal circulation during medically induced abortion in canines, contributing to improved pregnancy management in veterinary practice.

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Materials and Methods

The study was conducted at the Teaching Veterinary Clinical Complex, College of Veterinary Science, Tirupati,

Andhra Pradesh (India). Bitches brought in for pregnancy diagnosis and pregnancy termination due to misalliance were subjected for ultrasonography. A total of 12 bitches those with gestational age of 35-40 days and no history of other health ailments were enrolled in the present study. Signalment including breed, age, body weight and parity were recorded. Gestational age (GA) was estimated using Nyland and Mattoon's (2002) formula: GA = (15 × BPD) + 20.

Experimental Design

Bitches with a gestational period of 35 to 40 days were divided into two groups: Group I (n=6), received no treatment served as control, and Group II (n=6), received treatment for pregnancy termination with cloprostenol sodium (1 µg/kg SC every 48 h) and cabergoline (5 µg/kg PO daily) until abortion. 15 min before each cloprostenol injection bitches received atropine sulfate (0.04 mg/kg IM) to minimize adverse reactions.

Ultrasonographic Examination

B-mode and Doppler ultrasonography (Esaote MyLab6Vet) was performed every alternate day (day 1, 3 and 5) to assess biparietal diameter (BPD), combined uterine-placental thickness (CTUP), fetal heart rate (FHR), and Doppler parameters (PSV, EDV, RI, PI, S/D ratio) in the uteroplacental artery, umbilical artery, and fetal aorta. The ventral surface of the abdomen was clipped, and bitches were physically restrained and positioned in dorsal recumbency. Acoustic gel was applied to the probe, which was then coupled directly to the skin for scanning. The fetal BPD was measured to estimate the gestational age. The gestational sac was imaged to visualize the characteristic C-shaped placental band in association with the endometrium and CTUP was measured at the center. The beating fetal heart was located within the thoracic cavity using B-mode ultrasonography and pulsed wave Doppler was used to record the FHR.

Haemodynamic Evaluation

The uteroplacental arteries (UPA) were typically located near the junction of the uterine and placental tissues or between two gestational sacs (Nautrup, 1998). The umbilical cord appeared as a free-floating structure in the amniotic fluid connecting the fetal abdomen to the allantois. The fetal aorta (FA) appeared as an anechoic tubular structure originating from the heart and running parallel to the spine. Once the arteries were identified using colour Doppler, pulsed wave Doppler was employed, and the waveforms were obtained. The PSV and EDV were obtained by manual tracing of three consecutive waveforms. The RI and PI were calculated automatically by the machine's inbuilt software.

Estimation of Progesterone

A 2 mL blood sample was collected from each animal (on days 1, 3, and 5) using aseptic technique via cephalic vein puncture into a clot activator vacutainer. The serum separated was stored at -20°C to evaluate progesterone concentration by electrochemiluminescence immunoassay using the Elecsys® Progesterone III kit, Roche Diagnostics with Cobas® autoanalyzer. The measuring range was 0.159-191 nmol/L or 0.05-60 ng/mL.

Statistical Analysis

The data generated was analyzed by using SPSS version 23. The BPD, CTUP, FHR and haemodynamic changes in UPA, Uma, FA between the groups (on day 1 and day 3) were compared with an independent sample 't' test. The serum progesterone concentrations were analyzed by repeated measures ANOVA with Tukey's *post-hoc* test.

Results and Discussion

All six treated bitches successfully terminated pregnancy within 3-5 days of treatment initiation, with a mean duration of 3.42 ± 0.34 days. The number of expelled fetuses ranged from 3 to 6, confirming the efficacy of the combined cloprostenol sodium (1 µg/kg SC every alternate day) and cabergoline (5 µg/kg PO daily) protocol. None required a third cloprostenol injection, supporting its effectiveness. These findings aligned with previous studies demonstrating the success of this protocol for pregnancy termination in bitches (Corrada *et al*., 2006; Reddy *et al.,* 2010; Thangamani *et al*., 2018; Parmar *et al*., 2020). The mean abortion time observed was consistent with Parmar *et al*. (2020), but shorter than findings by Corrada *et al.* (2006), and Reddy *et al.* (2010). This variation may be due to the influence of gestational age on treatment duration, as the CL is more resistant in early gestation (Corrada *et al.,* 2006). Side effects such as panting, salivation, and emesis were noted following prostaglandin administration, similar to reports by Reddy *et al.* (2010) and Parmar *et al.* (2020).

Ultrasonographic assessment revealed no significant variation in BPD between group I and group II ($p>0.05$). On day 1, BPD values in group I and II were 1.11 ± 0.05 cm and 1.21 ± 0.11 cm, respectively, and on day 3, measures were 1.37 ± 0.04 cm in group I and 1.32 ± 0.11 cm in group II. This confirms that BPD measurement was a non-invasive method for determining gestational age and monitoring fetal growth in canines (Valocky *et al.,* 1997; Luvoni and Beccaglia, 2006).

Two-dimensional ultrasonography was used to assess the CTUP. On day 1, CTUP values in group I and group II were 5.46 ± 0.27 mm and 5.23 ± 0.30 mm, respectively, with no significant difference. Day 3, values also showed no significant variation, measuring 6.70 ± 0.31 mm in group I and 6.51 ± 0.56 mm in group II. CTUP increased with gestational progression, aligning with previous reports indicating a rise in placental thickness regardless of breed, age, or weight (Maldonado *et al.,* 2012). In cases of threatened abortion, CTUP values tend to be higher due to pathological changes such as localized edema and inflammation (Gaikwad *et al.,* 2017). The absence of significant day 3 differences in this study suggests that the

administered protocol (cloprostenol sodium and cabergoline) which induced luteolysis, mimicking natural physiological processes that lead to the termination of pregnancy.

In both group I and II on day 1, UPA displayed a biphasic waveform with an early diastolic notch (EDN), consistent with Simon *et al*. (2022), who observed EDN up to 40 days of gestation, disappearing thereafter (Fig. 1). However in group II, on day 3 after initiation of the treatment the waveform exhibited a systolic peak followed by a full diastolic phase with a broadening and gradual flattening of the diastolic upstroke (Fig. 2), aligning with Gaikwad *et al.* (2020). The haemodynamic changes (mean ± SE) of the UPA in group I and II are presented in Table 1. The PSV of UPA showed no significant difference between groups, however it decreased in the group II on day 3 compared to the group I ($p>0.05$), consistent with Gaikwad *et al.* (2020). On day 1, EDV values were similar between groups I and II. However on day 3, EDV significantly decreased in group II compared to the group I ($p<0.01$). From the study it was observed that, EDV values in UPA during 5^{th}-6^{th} week of gestation above 19.5 cm/s were considered normal, while values below 15 cm/s indicated a risk of abortion. Increased PSV and EDV in the group I aligned with Nautrup (1998), highlighting enhanced maternal placental circulation. On day 1, RI of UPA showed no significant difference between group I and II, but significantly increased in group II compared to group I on day 3 ($p<0.01$), suggesting increased placental resistance in response to treatment and potential pregnancy complications. Values in group I were consistent with Nautrup (1998), Di Salvo *et al.* (2006), Umamageswari *et al.* (2018), and Simon *et al.* (2022). On day 3, the RI of UPA in group I ranged between 0.48 and 0.59, whereas group II showed significantly higher values, ranging from 0.61 to 0.70. These results suggest that in canines in UPA an RI below 0.59 after EDV becomes detectable may indicate a normal pregnancy, while values exceeding 0.61 could signal a risk of threatened abortion. The PI and S/D values of day 1 showed no significant differences, however on day 3, PI ($p<0.05$) and S/D ($p<0.01$) were significantly higher in group II compared to group I. These values aligned with Nautrup (1998). The observed decrease in RI and PI during normal pregnancy may be linked to spiral artery remodeling, leading to increased placental blood flow (Nautrup, 1998; Simon *et al.,* 2022).

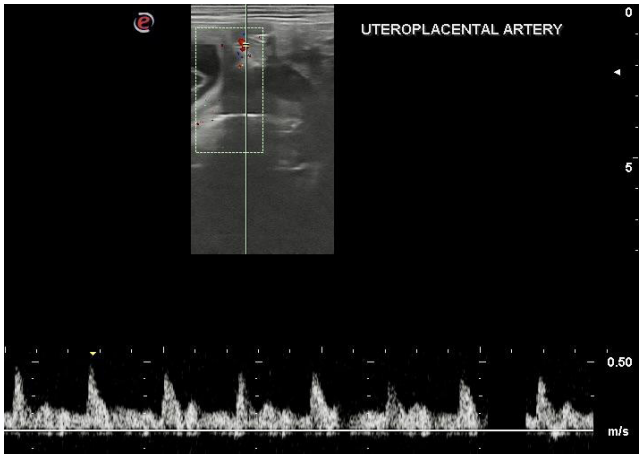

Fig. 1: Ultrasonogram of uteroplacental artery showing early diastolic notch (EDN)

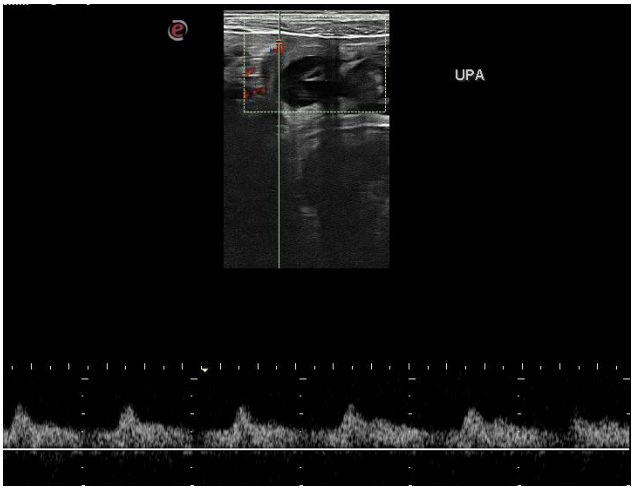

Fig. 2: Ultrasonogram of uteroplacental artery showing a broadening and gradual flattening of the diastolic upstroke

The umbilical artery exhibited a uniphasic waveform with no end-diastolic flow in most cases across both groups (Fig. 3), consistent with Nautrup (1998), Di Salvo *et al.* (2006), and Simon *et al.* (2022). During the 5^{th}-6^{th} week of pregnancy, the Uma typically exhibits a systolic waveform, with diastolic flow emerging later as placental efficiency improves (Fig. 4) (Miranda and Domingues, 2010; Gaikwad *et al.,* 2020). Di Salvo *et al.* (2006) reported that early pregnancy is characterized

Table 1: Haemodynamic changes in the utero-placental artery (UPA) of bitches in group I and group II (Mean ±SE)

S. No	Parameter	Group I		Group II	
		Day 1	Day 3	Day 1	Day 3
1	PSV(cm/s)	37.11±2.59	43.08±4.80	37.83±3.44	33.50±2.59
2	EDV(cm/s)	16.58±1.48	21.98±2.44**	16.31±1.70	11.83±1.13**
3	RI	0.55±0.01	0.53±0.01**	0.56±0.02	0.64±0.01**
4	PI	0.91±0.04	0.90±0.06*	0.99±0.06	1.14±0.04*
5	S/D	2.25±0.09	2.00±0.17**	2.35±0.11	2.88±0.18**

Values within a row differ significantly between groups (*$p<0.05$, **$p<0.01$)

Table 2: Haemodynamic changes in the umbilical artery (Uma) of bitches in group I and group II (Mean ±SE)

S. No	Parameter	Group I		Group II	
		Day 1	Day 3	Day 1	Day 3
1	PSV(cm/s)	19.48±2.11	25.00±1.96*	22.41±2.53	19.26±1.55*
2	EDV(cm/s)	0.73±0.73	4.10±0.35**	1.00±1.00	1.00±0.63**
3	RI	0.97±0.02	0.83±0.00	0.94±0.05	0.92±0.04
4	PI	1.72±0.08	1.89±0.08	1.61±0.10	1.84±0.14
5	S/D	1.00±1.00	6.16±0.38**	0.54±0.54	1.72±1.11**

Values within a row differ significantly between groups (*p<0.05, **p<0.01)

Table 3: Haemodynamic changes in the fetal aorta (FA) of bitches in group I and group II (Mean ±SE)

S. No	Parameter	Group I		Group II	
		Day 1	Day 3	Day 1	Day 3
1	PSV (cm/s)	35.85±2.72	38.48±3.28	34.75±5.71	31.91±3.55
2	EDV (cm/s)	1.15±0.73	4.38±1.00*	1.00±1.00	1.08±0.71*
3	RI	0.97±0.01	0.89±0.02*	0.97±0.01	0.97±0.01*
4	PI	1.86±0.17	1.97±0.13	1.72±0.12	2.05±0.18
5	S/D	3.91±2.48	7.04±1.81	1.55±1.55	3.95±2.50

Values within a row differ significantly between groups (*p<0.05)

Table 4: Fetal heart rates of bitches in group I and group II (Mean ±SE)

Parameter	Group I		Group II	
	Day 1	Day 3	Day 1	Day 3
Fetal heart rate (bpm)	240.83±3.97	242.33±4.62*	227.67±5.51	223.50±5.90*

Values within a row differ significantly between groups (*p<0.05)

by high placental resistance, reflected by the absence of diastolic flow and elevated RI and PI values, indicating ongoing placental development. As pregnancy advances, diastolic flow appears, and RI and PI decrease, signifying improved placental perfusion and function. These changes are positive markers of placental maturation, essential for maintaining optimal fetal circulation and nutrient exchange. The haemodynamic changes of the Uma (mean ± SE) in group I and II are presented in Table 2. On day 1, PSV of Uma showed no significant difference between both groups, aligning with Di Salvo *et al.* (2006), but higher than Feliciano *et al.* (2014). On day 3, PSV was significantly lower in group II compared to group I (p<0.05), likely due to treatment-induced placental impairment. EDV also decreased significantly in group II (p<0.01), but no absence or reversal of EDV was observed, consistent with Gaikwad *et al.* (2020). RI and PI showed no significant differences on day 1 and 3, though RI remained numerically higher in group II on day 3, reflecting placental insufficiency (Nautrup, 1998; Feliciano *et al.,* 2014). The post-treatment S/D ratio was significantly lower in group II, (p<0.01), aligning with Blanco *et al.* (2009), indicating abnormal placental circulation in abortion-induced cases.

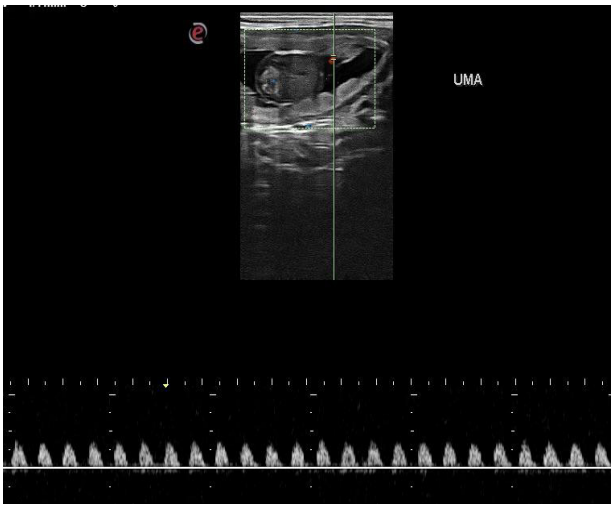

Fig. 3: Ultrasonogram of umbilical artery showing uniphasic waveform

Between 35-40 days of gestation, fetal aorta displayed a uniphasic waveform with no end-diastolic flow, indicating that blood flow was primarily systolic, similar to the pattern observed in the Uma in both the groups (Fig. 5). The

haemodynamic changes (mean ± SE) of the FA in group I and II are presented in Table 3. The PSV of the FA on day 1 and 3 showed no significant difference between group I and group II ($p>0.05$) aligning with Umamageswari *et al.* (2018) and Gaikwad (2018). On day 1, EDV showed no difference between groups, however on day 3, EDV was significantly lower in group II than in group I ($p<0.05$), suggesting treatment-related impacts on fetal circulation. These findings aligned with Gaikwad (2018), who reported reduced EDV in cases of threatened abortion. The day 1, RI was similar between groups, however on day 3, RI was significantly higher in group II than in group I ($p<0.05$). A decrease in RI with advancing gestation reflects improved placental function, as noted by Di Salvo *et al.* (2006), Feliciano *et al.* (2014), and Umamageswari *et al.* (2018). However, increased RI in the treatment group suggests restricted blood flow, aligning with findings by Gaikwad (2018) in cases of threatened abortion. No significant differences were observed in PI or S/D ratio between groups, both day 1 and day 3 ($p>0.05$). Gaikwad (2018) reported that PI was lower in threatened abortion cases during early gestation, but significantly higher later in pregnancy, indicating progressive placental insufficiency.

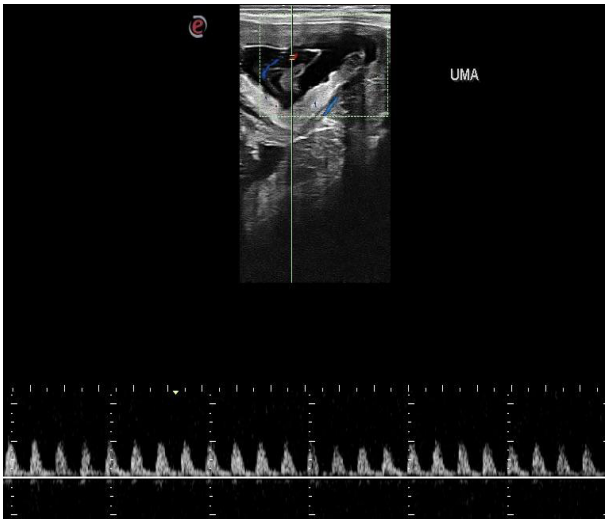

Fig. 4: Ultrasonogram of umbilical artery showing diastolic waveform

The mean fetal heart rates between group I and II are presented in Table 4. The mean FHR showed no significant difference on day 1, between group I and II. However, on day 3, FHR was significantly lower in group II compared to group I ($p<0.05$). Throughout the study, FHR remained above 220 bpm, consistent with England *et al.* (2003), Kustritz (2009), and Simon *et al.* (2022). Alonge *et al.* (2016) reported that FHR increases throughout pregnancy, but declines in the final 20 days before delivery. In this study, the significant FHR reduction in group II post-treatment suggests a treatment-induced impact on fetal cardiac activity.

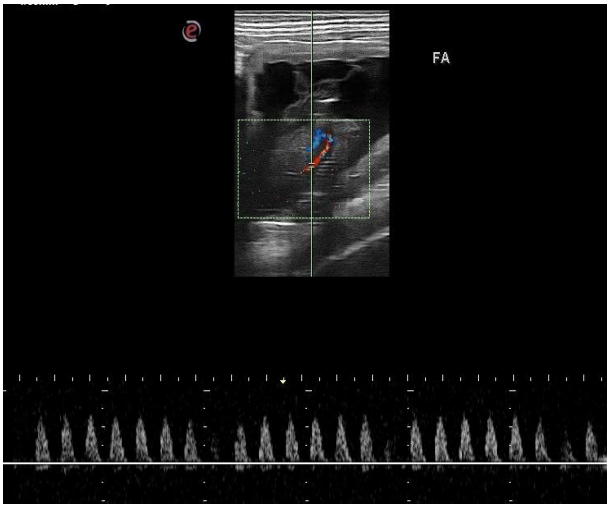

Fig. 5: Ultrasonogram of fetal aorta showing uniphasic waveform

The mean P_4 concentrations in group I were 21.27 ± 2.46, 17.95 ± 1.41, and 11.45 ± 1.36 ng/mL on days 1, 3, and 5, respectively, with a significant decline by day 5 ($p<0.01$). In group II, P_4 levels significantly decreased from 17.97 ± 2.35 ng/mL on day 1 to 1.13 ± 0.18 ng/mL on day 3 and 0.65 ± 0.09 ng/mL on day 5 ($p<0.01$). While day 1, P_4 levels showed no significant difference between groups, values on days 3 and 5 were significantly lower in group II than in group I ($p<0.05$). By day 5, P_4 concentrations in group II fell below 1 ng/mL, aligning with findings by Onclin and Verstegen (1996) and Fieni *et al.* (1997), who reported a rapid decline following pregnancy termination treatment. This confirms that monitoring P_4 levels after treatment initiation is a reliable indicator of pregnancy status.

Conclusion

The study concluded that while anatomical parameters remained unaffected, medical termination led to placental insufficiency, altering maternal-fetal circulation. An RI >0.61 and EDV <15 cm/s in the UPA were identified as markers of compromised pregnancy. The cloprostenol-cabergoline protocol was proved highly effective for pregnancy termination in dogs, and Doppler ultrasonography was validated as a valuable non-invasive tool for monitoring canine pregnancy.

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