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Combined thickness of uterus and placenta, foetal heart rate oscillations and progesterone concentration in last week of canine gestation#

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Abstract

This study aimed to evaluate the combined thickness of uterus and placenta, foetal heart rate and serum progesterone concentration in prepartum bitches. Eighteen pregnant female dogs in the last week of gestation were subjected to ultrasonographic examination and progesterone assay to evaluate changes prior to impending whelping. No significant difference was found in the combined thickness of uterus and placenta measurements in the last week of gestation. However, foetal heart rate showed oscillations and progesterone declined 12h prior to whelping.

Keywords: Canine, pregnancy, ultrasonography, whelping.

Ultrasonography is an excellent diagnostic tool in monitoring foetal development, time of gestation and predicting parturition, as well as in the diagnosis of reproductive tract disorders. Among reproductive losses in canines, early placental separation and subsequent abortion has become a major concern. Reproductive loss happening due to uterine inertia is also a major constraint in early obstetric intervention that can be made using tocodynamometry (Magnus *et al.,* 2022). Vasculature of the placenta can be studied by monitoring uteroplacental circulation, however, abnormalities in placental development and separation of the placenta during parturition can be studied by measuring the thickness of the uterus along with the placenta (Gaikwad *et al*., 2017). Any abnormalities in placental development may result in foetal distress and intrauterine growth

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restrictions. Acceleration and deceleration of foetal heart rate indicate impending whelping in canines (Gil *et al*., 2014). Endocrinological factors affecting reproductive loss must also be taken into consideration. In pregnant bitches the sole source of progesterone is the corpus luteum (Concannon *et al*., 1989). A sharp decline in progesterone concentration before impending parturition has been reported. In the current study screening of different dogs in late prepartum for placental abnormalities along with their progesterone concentration was performed. The objective of this study was to evaluate the combined thickness of uterus and placenta, monitor foetal heart rate and progesterone concentration in prepartum female dogs.

Materials and methods

Experimental animals

The study was carried out at the Teaching Veterinary Clinical Complex (TVCC), College of Veterinary and Animal Sciences, Mannuthy. Eighteen pregnant bitches belonging to medium to large breeds with age ranging between 18 months to seven years were the subject of the study. Factors such as age, breed, parity and litter size will not affect gestation length of small breeds such as spitz, pug, beagle, etc. (Vinaykumar *et al.,* 2021). All the animals were in the last week of gestation. Group A consisted of bitches having three to five days before parturition (DBP), Group B contained those having one to two DBP and Group C consisted of bitches that were undergoing whelping.

Vaginoscopic examination

A rigid sigmoidoscope (Welch Allyn, Skaneateles Falls, NY) was used for the examination of the birth canal for the changes associated with whelping. Relaxation of the birth canal, nature and presence of discharge and presentation of foetal membranes were recorded. Animals under examination were either positioned in lateral recumbency or kept standing so that the animal's caudal region was positioned towards the examiner. With proper lubrication using sterile liquid paraffin, sigmoidoscope was inserted craniodorsally into the birth canal at an angle of 45° and the tract was visualised using a fibre optic light source.

Transabdominal ultrasonography

A linear transducer with a frequency ranging from 4-15 MHz was used in measuring combined thickness of uterus and placenta (CTUP) and foetal viability. Foetal heart rate (FHR) was recorded using pulsed wave ultrasonography. After locating the beating heart, pulsed wave Doppler was activated, sample volume or gate was fixed to the beating heart near the valvular opening and switched on the spectral Doppler. Once the uniform wave patterns were formed, the image was acquired, and measured the distance between two consecutive peaks and heart rate was calculated by the inbuilt software of the ultrasound machine. The heart rate of a minimum of three foetuses was measured and the average was calculated.

The CTUP was focused at the thick zonary placental area using a linear transducer, the image was acquired and the distance from the external boarder of the uterine wall to the alanto-chorionic wall bordering foetal fluid was measured. Measurements were taken in centimetres and analysed.

Serum progesterone estimation

Whole blood was collected intravenously into plain clot tubes, centrifuged to separate serum, and stored under refrigeration at 4°C to -18°C. Serum samples were assayed together for progesterone concentration employing chemiluminescent immunoassay (CLIA), (CL-900i, Mindray, Shenzen, China). Commercial CLIA kits were used for serum progesterone assay (PROG) and recorded the measurements in ng/ml.

Statistical analysis

The results obtained were statistically analysed using SPSS version 24.0 software. Progesterone values were not following a normal distribution. Hence comparison was done by Kruskal Walli's ANOVA. The CTUP and FHR were compared using one-way ANOVA

Table 1. Foetal heart rate in different groups of bitches

Variable	Group A	Group B	Group C	F-value (P-value)
Foetal heart rate (bpm)	241.50 ± 5.64[a]	219.17 ± 7.76[ab]	212.00 ± 9.74[b]	3.80[*] (0.046)

** Significant at 0.05 level*

Means having different superscript differ significantly at 5 per cent level

Fig. 1. Vaginoscopic appearance of cervical plug liquefaction of prepartum dogs

followed by Duncan Multiple Range Test (DMRT).

Results and discussion

In bitches of Group A, on vaginoscopic examination, mild hyperaemia and the presence of a greyish to light greenish mucoid plug, which had started liquefaction could be appreciated (Fig 1). In Group B, oedema and hyperaemia of vaginal mucosa could be appreciated. Cervical plug lysis was almost complete in this stage and hence vaginoscopic observation could reveal a trace or absence of a plug in the birth canal. In Group C, bitches were undergoing the second stage of labour and had dark green colour due to uteroverdin pigments in the vaginal tract, or having a foetal bag or foetus on the anterior vagina. Depending on the colour and nature of mucoid discharge one can detect the impending parturition, while the animals need to be observed. By a single observation of the birth canal, the time of whelping cannot be determined. Nature and colour of discharge should be correlated with gestation age calculated by ultrasonography or from the day of LH surge, in determining the approximate time of whelping. The appearance of the oedematous vaginal mucosa with the presence of yellowish mucus observed in animals a few days before parturition and the appearance of a small amount of greyish-white mucosal plug before whelping was in accordance with McCarthy and Constantinescu (2005).

Pulsed wave Doppler was employed to detect FHR. Detection of FHR was an important tool in analysing foetal distress since a decrease in FHR below 180 bpm indicated the foetus was under heavy distress. As the gestation advances, there will be a fluctuating foetal heart rate known as oscillations. Foetal HR accelerates and decelerates from 72 h to six to one-hour pre-partum (Gil *et al.,* 2014). The study revealed a significant difference in FHR when compared between the groups at P= 0.046 (Table 1). A decreasing trend in FHR was observed as the parturition approached. When observing the same foetus for 30s to one minute, there was a decrease and increase in FHR values in association with uterine contractions. Foetuses under study were having FHR within the normal range. Zone and Wanke (2001) reported that FHR of 200 bpm and above indicated a normal viable foetus without any stress, 180 to 200 bpm indicated moderate stress and less than 180 bpm indicated severe stress. An oscillation of FHR occurred in a posterior foetus from one to two days prepartum while it was profound in most of the foetus 12h prepartum. The significant oscillating HR was due to transient foetal stress that occurred as a result of ongoing uterine contractions. As mentioned by Lopate (2008), with the advancement of gestation, a temporary decline in FHR was caused due to the intermittent uterine contractions over the foetus, which will return to a normal range within 1 to 2 min. In the study, Group C animals were under the parturition process and almost all foetuses showed these oscillating FHR patterns due to the contraction of uterine muscle. Animals in group B exhibited much low oscillating FHR as opined by Blanco *et al.* (2020). Since their FHR ranged within normal values, foetuses were not

Table 2. Combined thickness of uterus and placenta

Variable	Group A	Group B	Group C	F-value (P-value)
CTUP (cm)	0.92 ± 0.03	1.13 ± 0.08	0.99 ± 0.10	1.983^{ns} (0.172)

ns- non-significant (P>0.05)

under any stress. Foetal HR could hence be used in analysing not only foetal distress but also impending parturition when combined with other parameters such as vaginal examination and other ultrasonography findings.

Transabdominal ultrasonography was used for detecting and measuring CTUP (Gaikwad *et al.*, 2017). Measurements were recorded from a minimum of three foetuses in each animal. The average of CTUP measurements of each animal was calculated and statistically analysed between groups and there observed no significant difference (P > 0.05) between CTUP in various groups (Table 2). As stated by Lopate (2008) and Gaikwad *et al.* (2017), the measurements of CTUP did not exceed 1.2 cm and all the animals under the study whelped normally with a healthy viable foetus. The study was in agreement with Troedsson *et al.* (1997) and Gaikwad *et al.* (2017) as the CTUP were within the normal range and did not exceed 1.2 cm (Fig 2) which indicated that neither placentitis nor impending abortion was suspected in the study groups. The mean value of CTUP measured in groups A, B and C were 0.92 ± 0.03 cm, 1.13 ± 0.08 cm and 0.99 ± 0.10 cm, respectively. Puppies born in various study groups were live and active. Placental separation could not be assessed using CTUP since separation happens in the distal layer of the endometrium (Al-Bassam *et al*., 1981) just before whelping or soon after foetal expulsion. Hence continuous monitoring of the canine zonary placenta near the centre and edges were needed to detect placental separation.

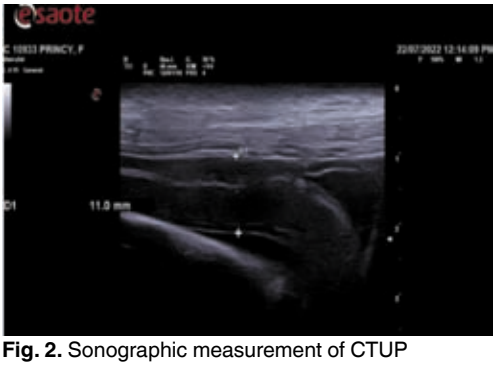

Fig. 2. Sonographic measurement of CTUP

Serum progesterone concentration between groups showed a significant decrease as the parturition approached. Mean progesterone concentrations ranged from 6.16 ± 0.45ng/mL to 7.51 ± 1.28ng/mL in animals with 1 to 5 DBP. While a sudden drop of progesterone to less than 1ng/mL could be seen in animals just before labour. The observations were in accordance with the findings of Concannon *et al.* (1977), Kutzler *et al.* (2003), Kim *et al.* (2007) and Johnson (2008). Analysis of progesterone alone was an indicator to determine the approximate time of whelping in canines since a value less than 2 ng/mL indicated impending labour within 12 to 24 h.

Conclusion

Detection of ultrasonographic FHR oscillations, a non-invasive procedure, helps in assessing foetal viability as well as impending whelping. The combined thickness of the uterus and placenta becomes a promising tool in detecting placental abnormalities and the outcome of pregnancy along with progesterone estimation.

Conflict of interest

The authors declare that they have no conflict of interest.

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