ULTRASONOGRAPHIC DIAGNOSIS AND MONITORING OF PREGNANCY IN THE BITCH - A REVIEW

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The use of ultrasound as a tool in small animal reproduction has expanded from its initial role in the evaluation of pregnancy in the female to its current use in monitoring fetal development, timing gestation and predicting parturition, diagnosis and management of reproductive tract disease and in supplementing breeding soundness examinations. In fact, B-mode ultrasonography has become an indispensable tool in veterinary practice. When ultrasonographic diagnosis of pregnancy is being performed it is important to remember that all events in canine pregnancy are related to day of the pre-ovulatory LH surge (day 0) or to the time of ovulation (which occurs 2 days after the LH surge) and not to the mating dates. Parturition typically occurs 64 to 66 days after the LH surge. This article aims to review the use of ultrasonography in monitoring pregnancy in the bitch.

A 5-7.5 MHz transducer is adequate for most dogs while for large and giant breeds of dogs a 3.5 MHz transducer is ideal. For diagnosis of mid to late-term pregnancy a 5.0-MHz transducer may be sufficient but may not provide adequate resolution for study of more subtle pathologic changes of the reproductive tract (Yeager and Concannon, 1990, 1995, 1996 and Yeager *et al.,* 1992). Ultrasonography is usually performed with the dog in dorsal or lateral recumbency. Preparation of the abdomen includes clipping the hair and applying acoustic coupling gel on the skin. The ability to ultrasonographically detect or discriminate a developmental feature at one day versus another during gestation is highly dependent on the equipment used (England

et al., 2003). Forced respiration or panting can seriously affect the stillness of the image and thus make its interpretation much more difficult. Temporarily closing either the mouth or the nostrils of the dog can reduce the disturbing effect of respiratory movements, or momentarily remove them (Wolfgang Kahn, 2004).

DIAGNOSIS OF PREGNANCY

Real time ultrasonography has proven to be a valuable tool for diagnosing canine pregnancy and assessing fetal viability (Inaba *et al.,* 1984). The ultrasonographic appearance of a gravid uterus in Beagle bitches at known time of gestation was studied in detail by Yeager and Concannon (1990) and Yeager *et al.* (1992). They detected cardiac activity and fetal movements as early as 25 and 34 days respectively after LH surge. Mattoon and Nyland (1995) reported detection of gestational sac at 20 days post breeding as the first sign of confirming pregnancy using ultrasonography but preferred to wait until day 30 as gestational sac with viable embryo could be identified with high level of confidence at that time. Yeager *et al.* (1992) reported that the gestational sacs or embryonic yolk sacs can be first imaged approximately 18 to 19 days after the LH surge and appear as spherical anechoic structures 1 to 2 mm in diameter within the lumen of the uterus. The embryonic heartbeat has been detected as a bright echogenic flicker as early as 23 days after the LH surge. The embryo has a bipolar shape by day 28 of pregnancy. The head region is identifiable as containing an anechoic area by

day 30; limb buds are usually identifiable from day 32 to 34 onwards. The fetal skeleton is evident by day 34 of pregnancy. The bones of the head appear first, followed by those of the lower body. At this stage the hyperechoic heart valves can be imaged and are seen to be moving and the great vessels can be traced cranially and caudally.

The zonary, circumferential placenta wraps around the central portion of the conceptus like a waistband, and is observed ultrasonographically between the fetus and the uterine walls in all planes. When imaged in the longitudinal plane, the placenta appears as two thick bands one on either side of the fetus, between the fetus and the uterine wall (Concannon *et al.,* 2003).
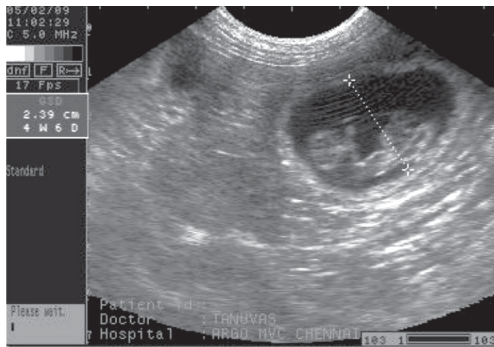

Fig.1. Sonogram of two transverse sections of the uterine horn in a 6 year old cross bred bitch showing marked enlargement of the uterus with anechoic material The uterine wall is mildly thick.
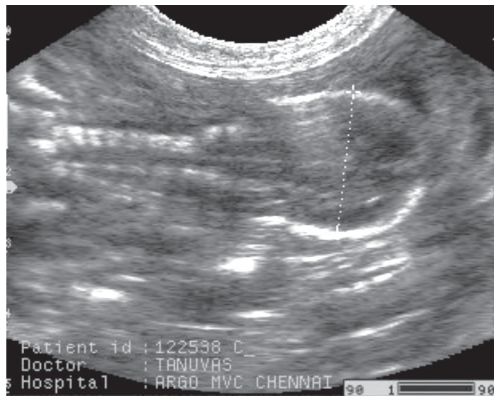
Fig. 2. Fetal head and neck. Sonogram of a day 47 pregnant bitch showing a fetal head and neck in frontal section. The hyperechoic bones (bright white) of the calvarium outline the skull. The bones of the vertebrae are likewise hyperechoic. The bright echogenic area below the head is an acoustical shadow caused by the density of the head bones. The scale on the left is marked in 1.0 cm increments.
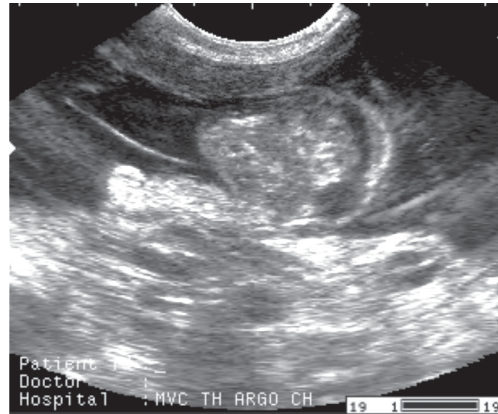

Fig.3. Day 37 yolk sac and placenta. Sonogram showing an uterine gestational sac in longitudinal plane at day 37 of gestation. The long, echogenic remnant of the tubular yolk sac extends the full length of the chorionic sac, being attached at each end to the chorionic poles. The chorionic sac containing the dark, anechoic chorionic fluid extends at each end beyond the margins of the placental girdle.
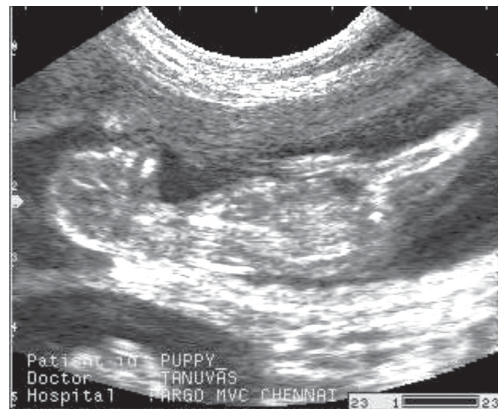
Fig.4. Note the zonary placenta between the fetus and abdominal wall

After day 36 - 38 after the LH surge, it is possible to identify the fluid-filled fetal stomach caudal to the liver in more than 90% of fetuses. A day or so later the fetal bladder is identifiable in the caudal abdomen and with careful examination the urachus may be imaged. These changes are obvious by day 40 - 45 after the LH surge. In late gestation the skeleton becomes more obvious in late pregnancy and the skull, spinal column and ribs are easily identifiable Figures 1-5 show the ultrasonographic appearance of various features at different days of gestation.
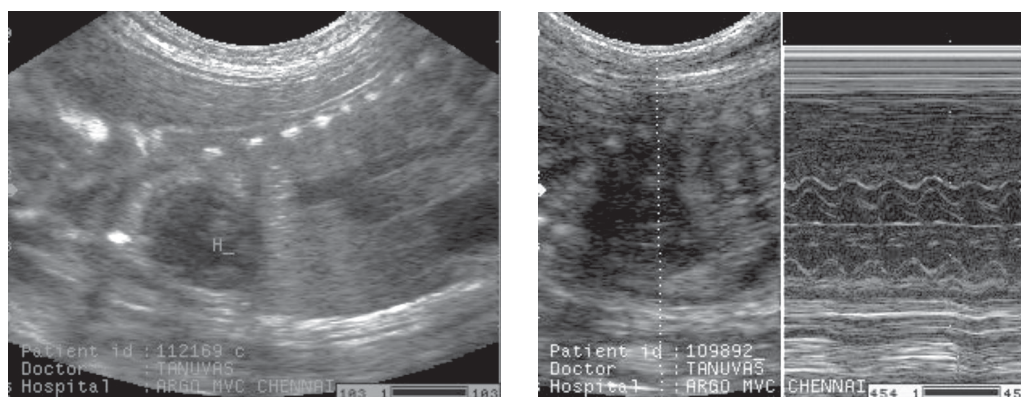
Fig. 5. Day 55 M-mode examination of fetal heart rate. Sonogram of a day 55 canine fetus (Left) and M-mode display of the heartbeat (right). The sonogram is centered on the fetal heart, the chambers and great vessels of which appear as anechoic (dark) areas within the fetal thorax. The thorax is delineated by the two lines of hyperechoic (bright white) ribs, above and below the heart in this view.

PREDICTION OF GESTATIONAL AGE

Shille and Gontarek (1985) in a study involving 23 Grey hound bitches have reported diameter of the gestational vesicle determined by ultrasound technique at seven different stages of gestation. Pregnancy was timed from the calculated date of ovulation to the day when first pup was born. The results revealed that the gestational sac diameter increased as gestation days progressed. On days 27 to 34, 35 to 44 and 47 to 56 after ovulation the average vesicle diameter ranged between 23 and 30mm, 25 and 49mm and 46 and 89 mm respectively with a mean diameter of 26.5mm, 36.1mm and 68.3mm respectively. Yeager *et al.* (1992) used ultrasonography to estimate the gestational age in 8 pregnant Beagle bitches. The gestational age was based from day of preovulatory LH surge which was denoted as "0"day of gestation. Serial ultrasonographic examinations of each pregnant bitch began on day 28 to 37 after the LH surge. It was reported that measurements of chorionic cavity diameter (CD) was the most accurate predictor of gestational age. It had the least variation compared with all other measurements on the fetus. From days 38 to 60, the fetal head diameter was the more accurate predictor of gestational age.

Fetal measurements and estimation of foetal age (Yeager *et al.*, 1992)

·   For pregnancy < 40 days, Gestational Age (GA) is: GA = (6 x GSD or ICC) + 20 where GSD is Gestational sac diameter and ICC is Inner chorionic cavity diameter

·   For pregnancy > 40 days Gestational Age (GA) is: GA = (15 x BPD or HD) + 20 where HD is Head diameter and BPD is Biparietal diameter.

Days Before Parturition (DBP) = 65-GA

The formulae currently used by Luvoni (2013) are as follows:

·ICC in small size bitches: days before parturition = (mm − 68.68)/1.53

·ICC in medium size bitches: days before parturition = (mm - 82.13)/1.8

·BP in small size bitches: days before parturition= (mm - 25.11)/0.61

·BP in medium size bitches: days before parturition=(mm - 29.18)/0.7

A study was undertaken by Luvoni and Grioni (2000) to estimate the gestational age in medium size dogs by ultrasonographic examinations. Formulae were derived to estimate the expected date of delivery by measuring the anatomical fetal structures and the gestational sac was found to be 90.9 per cent accurate and that of biparietal diameter 70.8 per cent accurate for predicting the date of parturition with ±1 day in medium size dogs. The accuracy of prediction of date of parturition for small size dogs was 90.9 per cent using gestational sac diameter and 68.2 per cent using biparietal diameter.

When measuring the gestational sacs, two transverse plane measurements should be taken at 90° angles to each other and the values averaged before using the above

formulas. Head and body diameters are measured to the transverse planes. When taking measurements of fetal or extra-fetal structures at least two distinct fetuses or gestational sac should be measured whenever possible and the measurements averaged before applying them to formulas. This becomes difficult if it is a singleton fetus and measurement of multiple features such as GSD, HD, CRL or body diameter may be carried out to increase the accuracy.

Lenard *et al.* (2007) assessed the accuracy of estimating the gestational age and litter size in 76 bitches using one or two techniques. The first method used the differential features of fetal organ development that occur in early and mid-pregnancy, based on published tables for Beagles. The second method used biparietal head and trunk diameters to predict gestational age based on tables published for late gestational period for Labrador Retrievers. The accuracy of the two methods was compared to evaluate the effect of maternal body weight and litter size. Litter size and maternal body weight did not affect the accuracy of gestational age prediction. Using a combination of both the methods, the overall accuracy of predicting parturition date to within 65 ± 1 day and ± 2 days was 70.8 per cent and 86.1 per cent, respectively. The correct litter size was predicted in 65 per cent of cases and in 89.5 per cent of cases for ± 1 pup. It was concluded that the optimum time for sonographic estimation of fetal age and litter size was early and mid-pregnancy.

PREDICTION OF PARTURITION

Accurate prediction of the date of parturition in the bitch is clinically useful to prevent or minimize reproductive losses by timely intervention. For example, an accurate method of predicting parturition date is necessary to plan an elective caesarean section. Intervening when the pregnancy is full term can reduce losses of offspring from bitches having obstructions of the pelvis or vagina, histories of primary or secondary uterine inertia, or prolonged parturition with resultant puppy mortality. For bitches with histories of pyometra, abortion, embryonic reabsorption, or insufficient luteal phase, accurate assessment of gestational age can assist in therapeutic decision making. Finally, progress in assisted reproductive techniques in this species, such as estrous synchronization and embryo transfer, requires accurate

prediction of ovulation, gestational age, and parturition date (Kim *et al.,* 2007).

The duration of canine gestation, as timed from the preovulatory serum LH peak, is 65 ±1d. However full-term gestation, calculated from insemination, is reported to range from 57 to 72 d (Concannon *et al.,* 1993). The difference between these measurements was attributed to the potential 6-day viability of sperm in the female reproductive tract and the long period of receptivity in the bitch. Therefore the key to timing the duration of canine gestation was the preovulatory LH surge rather than mating / insemination date or estrus onset (Meyers-Wallen, 1995). Ultrasound provides an accurate estimate of parturition date and the most accurate prediction was obtained when the ultrasound examination was conducted at Day 30 (Kutzler *et al.*, 2003). However, prediction was inaccurate when made from fetal measurements in late gestation (>Day 39). Prediction accuracy was also significantly affected by non-pregnant body weight of the dam. Fetal growth was linear from Days 17 to 30 and subsequently became exponential (England, 1998). Kutzler *et al.* (2003) reported that after day 30, fetuses of small bitches (<9 kg) grew slower, and fetuses of giant bitches (>40 kg) grew faster, than those of medium or large bitches. When corrected for body weight of the dam, the overall accuracy for parturition date prediction by the ultrasound method was 75% for the Day 65 ± 1 prediction, 87% for the Day 65 ± 2 prediction, and 100% for the Day 65 ± 3 prediction.

MONITORING FETAL WELL BEING
Fetal Death

Recognition of fetal death at or near parturition is of extreme importance to the veterinarian and breeder. As the date of parturition approaches, the following are indications for fetal monitoring with transabdominal ultrasonography:

▪ Failure to initiate parturition as expected.

▪ Unusual vaginal discharge

▪ Vague signs of illness

▪ Delay in parturition after delivery of part of a litter

Fetal heart rate has been recommended as a very useful parameter to estimate the survival possibilities of the

foetuses (England, 1998). Likewise, the evidence of fetal motion can be considered very important to determine fetal survival in the ultrasonographic point of view. Fetal stress is diagnosed by reduced fetal heart rate that is due to hypoxia. Normal fetal heart rate is 220 to 240 bpm while rates of < 180 bpm are indicative of fetal distress (Zone and Wanke, 2001). One should take care to remember that intermittent uterine contractions over a fetus may cause a temporary, substantial reduction in heart rate which would return to normal within 1-2 minutes and would remain within the normal range if there is no fetal distress (Lopate, 2008). Increase in echodensity of fetal fluids is suggestive of passage of meconium (Zone and Wanke, 2001) or haemorrhage (Lopate, 2008) due to premature separation of placenta. Abdominal: biparietal diameters of < 2 from days 48 to term are indicative of growth retardation. Puppies with low abdominal: biparietal diameter ratios tend to weigh < 20 per cent of the average birth weight for the breed and are at risk for early neonatal loss (Zone and Wanke, 2001). Edema and thickening of the placenta indicate abnormalities or alterations in blood flow, reduced ability of placenta to drain fluids or placentitis. At any stage of pregnancy, regardless of the breed the normal canine placenta should not exceed 1.2 cm at its centremost point (Lopate,2008).

Fetal death is recognized by a loss of cardiac activity. Poorly defined organogenesis has been also reported to be an important ultrasonographic feature for the diagnosis of fetal death (Poffenbarger and Feeney, 1986). On assessment of near-term or term fetuses, cardiac activity should immediately be recognized. Fetal movements, such as swallowing, hiccoughs, and body and limb movements, should also be seen. Sonographic recognition of fetal structures rapidly diminishes after death. After a day or two, only mineralized skeletal structures may be recognized by characteristic hyperechogenicity and acoustic shadows. Intrauterine or intra fetal gas may also be identified (Matton and Nyland, 1995).

Should embryonic death occur before 35 days after ovulation, there is usually complete resorption of the conceptus. This can occur without vaginal discharge as late as Day 30. The sonographic aspects of a resorption are generally a reduction in the volume of the conceptus, an increased echogenicity of the embryonic fluid (sometimes particles may be identified free-floating within the allantoic fluid), an absence of the embryonic heartbeat, disintegration of the embryonic mass and ultimately collapse of the conceptus with inward bulging of the uterine wall. The uterus often remains slightly enlarged in this region, and there may be a small volume of free luminal fluid; the uterine wall often appears moderately hyperechoic (Concannon et al., 2003). Konde (1988) has reported poorly defined fetal anatomy with amorphous echodensity, distortion of the gestational sac, and presence of hyperechoic material within the uterus as ultrasonographic signs of fetal death. Gas within the stomach of the fetus observed on ultrasound has been also reported as a sign of fetal death (England, 1998).

Death of fetuses occurring after Day 35 of pregnancy is usually followed by abortion and vaginal discharge and is associated with expulsion of fetal material and fluid. The early features of fetal abortion are an increase in echogenicity of the allantoic and amniotic fluid often with echogenic particles, followed by an absence of the fetal heartbeat and sometimes a thickening of the uterine wall (Fig.6). After expulsion, the uterus assumes an appearance that is similar to that observed in the postpartum bitch (Concannon et al., 2003).

Fetal Abnormalities

It is uncommon to detect fetal abnormalities in the bitch, since there are usually multiple fetuses and it is difficult to fully examine each. However, a number of striking abnormalities have been detected, some of
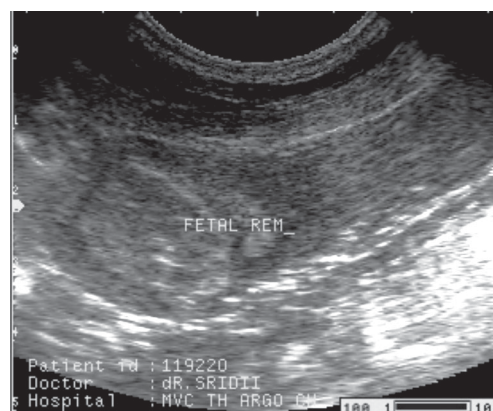
Fig.6. Fetal death and resorption at late gestation. Note the presence of fetal remnants.

Fig.7. Ultrasonographic image of fetal anasarca. Note the increased subcutaneous oedema (arrow) and serous effusions (astrix).

which have necessitated delivery of the litter by caesarean operation. Such abnormalities include hydrocephalus, fetal anasarca (Fig.7), herniation of the ventral abdominal wall and fetal monsters (Poffenbarger and Feeney, 1986).

APPLICATION OF DOPPLER ULTRASONOGRAPHY IN CANINE PREGNANCY

Miranda and Domingues (2010) evaluated blood flow in the uterine (UA) and umbilical (Uma) arteries in the pregnant bitch, by measuring the resistive index (RI) and pulsatility index (PI); and performed conceptus ecobiometry for fetal growth assessment during pregnancy.Triplex Doppler and B-mode ultrasonography were used to assess blood flow and conceptus ecobiometry. All pregnancies ended with a normal whelping and birth of live puppies. Prior to whelping, all conceptus dimensions increased significantly, whereas RI and PI of both the Uma and UA decreased significantly. For the UA, RI and PI were (mean +/- SEM) 0.95 +/- 0.02 and 2.75 +/- 0.41, respectively, on Day -44, and were 0.60 +/- 0.01 and 0.99 +/- 0.03 on Day -4. For the Uma, RI and PI were 0.99 +/- 0.01 and 2.42 +/- 0.03 on Day -31, and were 0.62 +/- 0.01 and 1.15 +/- 0.02 on Day -4. The complete disappearance of the early diastolic notch in the UA, and the appearance of diastolic flow in the Uma occurred on Days -16 +/- 5 and -21 +/- 1. The authors concluded that UA and Uma perfusion were important end points to assess fetal vitality in bitches. Furthermore, the current reference values provided a baseline for monitoring normal and abnormal pregnancies in bitches

Blanco *et al.* (2011) described the changes of uterine artery, umbilical artery and fetal abdominal aorta, renal and internal carotid arteries blood flow in abnormal canine pregnancy. Color and pulsed-wave Doppler examinations of uterine artery were conducted every 10 days from Day 20 to 50 from estimated luteinizing hormone peak. Doppler ultrasonography was also conducted in the fetuses to assess umbilical artery, abdominal aorta, renal and internal carotid arteries from Day 40 to 60 of gestation. Throughout the study, resistance index (RI) of uterine, umbilical and fetal renal arteries decreased up to -15% compared to -36% ($P<0.01$), -11% compared to -23% ($P<0.05$) and 2% compared to -13% ($P<0.05$), respectively in the abnormal and normal bitches. Fetal abdominal aorta and internal carotid did not differ between groups ($P>0.05$). They concluded that in dogs, uterine artery, umbilical artery and fetal renal artery RI differ between normal and abnormal gestation and therefore were useful for the prediction of adverse obstetric outcome.

Thus, to conclude ultrasonographic examination is an indispensable tool for practicing veterinarians for monitoring fetal growth, for assessing gestational age and for prediction of parturition which is particularly valuable for providing clinical assistance during whelping or elective caesarean sections.

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