Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

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Comparison of different non-invasive techniques for early pregnancy diagnosis in bitches

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Abstract
Pregnancy diagnosis of seventy bitches of various breeds, age and weight was carried out by non invasive techniques. Bitches in group I (n=70) were examined for pregnancy by abdominal palpation. In group II (n=30) bitches, serum progesterone level was estimated where as trans-abdominal ultrasonography was performed for group III (n=30). The accuracy percentage for abdominal palpation was 60 percent. The mean serum progesterone values were found to be 18.01 ± 0.30 ng/ml for pregnant bitches, where as for non pregnant bitches the mean value was 10.15 ± 0.39 ng/ml. Further, a significantly higher value was recorded for pregnant than non pregnant bitches. Trans-abdominal ultrasonography showed gestational sacs and fetal poles as the features of early pregnancy. Echobiometry was carried out for fetal growth and development. In conclusion among three methods It can be concluded that ultrasonography was the most accurate method for early pregnancy diagnosis and in predicting the date of parturition in bitches.

Keywords: Bitch, non invasive technique, pregnancy diagnosis, ultrasonography

Introduction
Understanding the mechanism of ovulation, fertilization, embryonic and fetal development, pregnancy specific changes and maternal physiology is essential while providing clinical services such as breeding management and monitoring of pregnancy in canine. There are different pregnancy diagnosis methods available in dogs, but monitoring of embryonic well-being and diagnosing embryonic death is still a challenge for clinicians. In bitches, abdominal palpation and ultrasound technique remain the most common non invasive methods for pregnancy diagnosis. Some reproductive hormones produced by pregnant bitches also play vital role for confirmation of pregnancy. Keeping all the above facts in mind, the present study was designed to compare the different non invasive techniques for early pregnancy diagnosis and echo-biometry of fetus to predict gestational age through ultrasonography in bitches.

Materials and Methods
The study was conducted for a period of six months in pregnant bitches at Teaching Veterinary Clinical Complex (TVCC) in collaboration with the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar. Seventy (70) bitches irrespective of breed, within the age group of 1-8 years, weighing 5-45 kg were screened for study and divided into three groups. In group I (n=70), bitches were examined for diagnosis of pregnancy through abdominal palpation from 17 to 35 days of post mating. In group II (n=30), bitches were examined for diagnosis of pregnancy through serum progesterone estimation method from 20 -35 days of post mating. In group III (n=30), bitches were examined for diagnosis of pregnancy through ultrasonography. Palpation of discrete round or oval swelling was recorded as positive results for abdominal palpation. In cases, where this swelling was not felt were taken as negative. Serum progesterone concentration was assayed for quantitative estimation by ELISA. Ultrasonographic examinations were performed from day 15 post mating by using Doppler Ultrasonography machine (ALOKA PROSOUND ALPHA 6). Total thirty number of pregnant bitches were selected and divided into three groups (Small, Medium and Large) basing on their body weight. Pregnancy was checked at different day's interval for different parameters starting from 20-30, 30-40, 40-50 and 50-60 days of gestation. For estimation of gestational age, gestational sac diameter (GSD), Latero Lateral External (LLE), Latero Lateral Internal (LLI), Dorso Ventral External (DVE), Dorso Ventral Internal (DVI) and Crown Rump Length (CRL)

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were taken in early pregnancy. Placental thickness was measured from early pregnancy till last stage of gestation. Each echobiometry parameter were analyzed both breed wise and day wise by conducting ANOVA.

Results
a. Abdominal palpation
In this present study, abdominal palpation was conducted in

70 number of bitches starting from day 17 after post mating up to 35 days. Forty four numbers of bitches were presumed to be positive in palpation test, where 24 numbers were actually pregnant. On the other hand, 26 numbers of bitches presumed negative in palpation test, where 18 bitches were actually not pregnant. Therefore, the accuracy of test in palpation method was found to be 60%.

Table 1: Accuracy test for abdominal palpation for diagnosis of pregnancy

Total number of animal	Total number of animal found positive in palpation test		Total number of animal found negative in palpation test		Accuracy test	
70	44		26		Number	Percentage (%)
	Actual pregnant	Actual not pregnant	Actual pregnant	Actual not pregnant	24+18 =42	60
	24	20	8	18		

b. Serum progesterone estimation
Total 30 numbers of serum samples were examined for progesterone estimation from day 20 -35 of post mating. The mean serum progesterone values were found to be 18.01±0.30 ng/ml for pregnant bitches with a range varied from 15.02 to 19.94 ng/ml. In contrary, the mean serum progesterone value

for non pregnant bitches was found to be 10.15 ± 0.39 ng/ml which ranged from 7.64 to 11.65 ng/ml. A significantly ($P<0.01$) higher serum progesterone value were recorded in pregnant bitches as compared to non pregnant animals as shown below in Table no-2.

Table 2: Mean serum progesterone values in pregnant & non pregnant bitches during 20-35 days of post mating

20-35 days of post mating	Mean ± SE	P value
Non pregnant (11)	10.15±0.39	0.01
Pregnant (19)	18.01±0.30	0.01

c. Echobiometry of foetus through ultrasonography
Gestational sac diameter (GSD) ranged from 10-34 mm in small breed, 11– 38 mm in medium breed and 16– 40 mm in large breed. There was significant difference ($P<0.01$) in the gestational sac diameter (GSD) between small and large sized breed of bitches during 20-30 days of gestation, but no significant difference were observed among small and

medium sized breeds and large and medium sized breeds of bitches. During 30-40 days of gestation, significant difference ($P<0.01$) in GSD found between medium and large breeds of bitches, but no significant difference among small with respect to medium and small with respect to large size breeds of bitches. Their respective mean according to breed was presented in the Table 3.

Table 3: Gestational sac diameter (GSD) of small, medium and large size breed of bitches at different gestational days

Sl no.	No. of animal	Breed	GSD_20-30 Days	GSD_30-40 Days
1	10	Small	$16.05^a \pm 0.33$	$31.70^a \pm 0.517$
2	10	Medium	$17.86^a \pm 0.396$	$35.60^b \pm 0.636$
3	10	Large	$20.42^b \pm 0.358$	$36.70^b \pm 0.700$

Values bearing different superscripts between the row differ significantly ($P<0.01$)

Crown rump length (CRL) ranged from 11-25 mm in small breed, 12-29 mm in medium breed and 20- 39 mm in large breeds of bitches. There was significant difference in the crown rump length (CRL) between small, medium and large

size breeds of bitches during both 20-30 days and 30-40 days of gestation. Their respective mean according to breed was presented in the Table 4.

Table 4: Crown rump length (CRL) of small, medium and large size breed at different gestational days

Sl no.	No. of animal	Breed	CRL_20-30 Days	CRL_30-40 Days
1	10	Small	$13.80^a \pm 0.467$	$22.40^a \pm 0.54$
2	10	Medium	$18.00^b \pm 0.471$	$29.20^b \pm 1.209$
3	10	Large	$22.20^c \pm 0.442$	$34.00^c \pm 1.033$

Values bearing different superscripts between the row differ significantly ($P<0.01$)

Inner chorionic cavity diameter is of 4 types such as Latero lateral external (LLE), Latero lateral internal (LLI), Dorso ventral external (DVE) and Dorso ventral internal (DVI) diameter. These parameters were measured during early stage of gestation between 20-30 days and 30-40 days for small, medium and large size breeds of bitches.
In small breed, LLE, LLI, DVE and DVI ranged from 16-30 mm, 10 -21 mm, 12-30 mm and 8-23 mm respectively. Similarly, in medium breeds, they ranged from 14-39 mm, 12

-31 mm, 15- 30 mm, 10- 23 mm respectively. In large breeds they ranged between 15-42 mm, 11-39 mm, 13-40 mm and 11-37 mm respectively. Statistically, there was significant difference in the LLE (latero-lateral external) diameter between the small and large breeds and medium and large breeds of bitches during 20-30 days and 30-40 days of gestation. Further, there was significant difference in the LLE (latero-lateral external) diameter between the medium and large breeds of bitches during 30-40 days of gestation, but no

significant difference between small with respect to medium and small with respect to large breeds of bitches.

There was significant difference ($P<0.01$) in the Latero-lateral internal (LLI) diameter between small with medium and small with large size breeds of bitches during both 20-30 days and 30-40 days of gestation. Similarly, there was significant difference ($P<0.01$) in the Latero-lateral internal (LLI) diameter between small with large and medium with large size breeds of bitches during 30-40 days of gestation.

Statistically, there was significant difference ($P<0.01$) in the Dorso-ventral external (DVE) diameter between the small and large and medium and large breeds of bitches during 20-30 days of gestation, but no significant difference between small and medium breeds of bitches. During 30-40 days of gestation, there was significant difference ($P<0.01$) in the Dorso-ventral external (DVE) diameter between the small, medium and large breeds of bitches. Statistically, there was significant difference ($P<0.01$) in the Dorso-ventral external (DVE) diameter between the small and large and medium and large breeds of bitches during 20-30 days of gestation, but no significance difference was found between small and medium sized breeds of bitches. During 30-40 days of gestation, there was significant difference ($P<0.01$) in the Dorso-ventral external (DVE) diameter between the small, medium and large breeds of bitches. Mean values according to breed was presented in the Table 5 and Table 6.

Table 5: LLE, LLI, DVE and DVI diameter of small, medium and large size breed of bitches at during 20-30 days of gestation

Sl no.	No. of animal	Breed	LLE_20-30 Days	LLI_20-30 Days	DVE_20-30 Days	DVI_20-30 Days
1	10	Small	$16.5^a \pm 0.37$	$13.3^a \pm 0.42$	$15.7^a \pm 0.33$	$13.7^a \pm 0.33$
2	10	Medium	$18.37^a \pm 0.34$	$16.1^b \pm 0.34$	$17.2^a \pm 0.38$	$15.1^a \pm 0.45$
3	10	Large	$21.41^b \pm 0.33$	$18.86^b \pm 0.48$	$20.6^b \pm 0.49$	$18.6^b \pm 0.49$

Values bearing different superscripts between the row differ significantly ($P<0.01$)

Table 6. LLE, LLI, DVE and DVI diameter of small, medium and large size breed of bitches during 30-40 days of gestation

Sl no.	No. of animal	Breed	LLE_30-40 Days	LLI_30-40 Days	DVE_30-40 Days	DVI_30-40 Days
1	10	Small	$24.1^a \pm 0.93$	$22.1^a \pm 0.93$	$25.6^a \pm 0.87$	$23.6^a \pm 0.87$
2	10	Medium	$28.30^a \pm 0.95$	$26.3^a \pm 0.95$	$29.30^b \pm 0.95$	$27.3^b \pm 0.95$
3	10	Large	$38.4^b \pm 0.79$	$36.4^b \pm 0.79$	$39.3^c \pm 0.84$	$37.3^c \pm 0.84$

Values bearing different superscripts between the row differ significantly ($P<0.01$)

Head circumference was also measured from mid stage of gestation between 30-40 days, 40-50 days and 50-60 days in small, medium and large size bitches. In small breeds, it ranged from 19- 42 mm, 21- 56 mm in medium breeds and 22 - 62 mm in large breeds respectively. Their respective mean according to breed was presented in the Table 7. There was significant difference ($P<0.01$) between small with respect to medium and small with respect to large breeds during 30-40 days of gestation, but no significant difference were observed between medium and large breeds of bitches. Statistically, there was significant difference in the head circumference between small, medium and large sized breeds during 40-50 days and 50-60 days of gestation.

Table 7: Head circumference of small, medium and large size breed of bitches at different gestational days

Sl no.	No. of animal	Breed	HC_30-40 Days	HC_40-50 Days	HC_50-60 Days
1	10	Small	$21.33^a \pm 0.524$	$32.70^a \pm 1.146$	$39.40^a \pm 0.733$
2	10	Medium	$27.70^b \pm 1.826$	$40.30^b \pm 0.943$	$48.80^b \pm 1.172$
3	10	Large	$31.40^b \pm 1.564$	$50.00^c \pm 1.211$	$58.20^c \pm 0.854$

Values bearing different superscripts between the row differ significantly ($P<0.01$)

Thickness of the placenta was measured in small, medium and large sized breeds between 30-40 days, 40-50 days and 50-60 days of gestation. In small size breeds, it ranged from 3- 8 mm, 3 - 8.9 mm in medium size breeds and 3.3- 9 mm in large size breeds of bitches.

Placental thickness of 30 bitches was evaluated. At 30-40 days and 50-60 days of gestation period, the placental thickness varied significantly ($P<0.05$) between small with respect to medium and small with respect to large sized breeds, but there was no significant difference between medium and large sized breeds of bitches. However, at 40-50 days of gestation period, there was no significant difference between the small, medium and large sized breeds of bitches. The average placental thickness at different stages of gestation period of all bitches was presented in the Table 8.

Table 8: Placental thickness of small, medium and large size breeds at different gestational days

Sl no.	No. of animal	Breed	PT_30-40 Days	PT_40-50 Days	PT_50-60 Days
1	10	Small	$3.42^a \pm 0.096$	$5.69^a \pm 0.250$	$7.14^a \pm 0.213$
2	10	Medium	$3.93^b \pm 0.171$	$5.98^a \pm 0.221$	$7.86^b \pm 0.247$
3	10	Large	$4.29^b \pm 0.185$	$6.35^a \pm 0.265$	$8.18^b \pm 0.248$

Values bearing different superscripts between the row differ significantly ($P<0.05$)

Discussion
Abdominal palpation of different breeds of bitches was conducted from 20-35 days of post mating. The accuracy percentage was 60% in the present study which was less than that reported by [2, 9] as 72.7%. The mean serum progesterone concentration was significantly higher as compared to non-pregnant or pseudo-pregnant bitches which were in agreement with [4, 8]. This increase in progesterone concentration often due to secondary increase progesterone concentration between 25th and 40th day of gestation that might reflect a pregnancy

specific mechanism which resulted from additional stimulation of progesterone production.

In the present study, GSD was measured and analyzed both breed wise and also gestational day wise. Gestational Sac Diameter (GSD) measured in small, medium and large size breeds mainly during early stage of gestation between 20-30 days and 30-40 days. It was observed as size of the foetus increased and ossification started, the demarcation of the gestational sac was not clearly visible which was similar to the findings of [1, 7]. During early stage of gestation, the gestational sac diameter was most accurate to evaluate conceptus growth between 20-30 days and 30-40 days of gestation. The present findings were in agreement to the reports of [7].

Among four inner chorionic cavity diameters such as LLE, LLI, DVE and DVI, both LLE and LLI were better than dorso-ventral diameter to assess the conceptus and foetal growth, as the increase in the latero-lateral diameter were closely associated with crown rump length. In the present study, there was also significant difference in the LLE, LLI, DVE and DVI between small, medium and large size breeds and also during different gestational days. The present findings were in agreement to the reports of Miranda et al. [7]. From this study, it was observed that the linear curves adjustments were applicable to bitches of different breeds and sizes. According to Maldonado et al. [5], placental thickness was a significant parameter in the determination of gestational age. The majority of the bitches used in this study had multiple numbers of litters, so that several measurements were taken for observation of homogeneity within each litter, with regard to the evaluated placental thickness.

Parameters usually studied within the period from 20-30 days are gestational sac, fetal adhered to the dorsal gestational sac, heart rate and jaw mineralization [6, 10]. It was found that within this period, placental thickness would measure an average of 0.348 cm and, therefore would refer to 31.5 days of pregnancy, according to the linear equation. Within 30-40 days period of observation, different foetal parameters were visualized as there was significant correlation between the observed and calculated gestational age, hence applied for calculation of gestational length using the measured placental diameter through ultrasonography and individual line for both small, medium and large breeds was found. Among all the echobiometric parameters, the placental thickness showed highest degree of correlation through the gestational days in order to predict the day of whelping. The present findings were in agreement with reports given by Maldonado et al. [5], but contradictory to the findings of Chang et al. [3] who stated that regression of length of zonary placenta was not significantly related to gestational age compared to other extra fetal structures.

Conclusion
It can be concluded that the abdominal palpation for early pregnancy diagnosis in bitches could be adopted where no social equipment are available. Although the difference in progesterone level was not great enough to provide a diagnostic test to determine pregnancy, there was apparently a difference in progesterone level between pregnant and non pregnant bitches. Ultrasound imaging is the most potential tool for early pregnancy diagnosis in bitches among all the three methods studied.

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